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A review on biochar modulated soil condition improvements and nutrient dynamics concerning crop yields: Pathways to climate change mitigation and global food security

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HIGHLIGHTS

- Nutrient value of various biochar is discussed.
- Impact of biochar on improvement of soil pH, CEC and buffering system delineated.
- Major, secondary and micronutrients dynamics in soil is elucidated.
- Effect of biochar on crop yields in different soils across the globe discussed.
- Meta-analysis of data done to explore the influence of biochar on soil chemical properties and crop yields.

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ABSTRACT

The beneficial role of biochar on improvement of soil quality, C sequestration, and enhancing crop yield is widely reported. As such there is not much consolidated information available linking biochar modulated soil condition improvement and soil nutrient availability on crop yields. The present review paper addresses the above issues by compilation of world literature on biochar and a new dimension is introduced in this review by performing a meta-analysis of published data by using multivariate statistical analysis. Hence this review is a new in its kind and is useful to the broad spectrum of readers. Generally, alkalinity in biochar increases with increase in pyrolysis temperature and majority of the biochar is alkaline in nature except a few which are acidic. The N content in many biochar was reported to be more than 4% as well as less than 0.5%. Poultry litter biochar is a rich source of P (3.12%) and K (7.40%), while paper mill sludge biochar is higher in Ca content (31.1%) and swine solids biochar in Zn (49810 mg kg^{-1}), and Fe (74800 mg kg^{-1}) contents. The effect of biochar on enhancing soil pH was higher in Alfisol, Ferrosol and Acrisol. Soil application of biochar could on an average increase (78%), decrease (16%), or show no effect on crop yields under different soil types. Biochar produced at a lower pyrolysis temperature could deliver greater soil nutrient availabilities than that prepared at higher temperature. Principal component analysis (PCA) of available data shows an inverse relationship between [pyrolysis temperature and soil pH], and [biochar application rate and soil cation exchange capacity]. The PCA also suggests that the original soil properties and application rate strongly control crop yield stimulations via biochar amendments. Finally, biochar application shows net soil C gains while also serving for increased plant biomass production that strongly recommends biochar as a useful soil amendment. Therefore, the

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application of biochar to soils emerges as a 'win-win strategy' for sustainable waste management, climate change mitigation and food security.

1. Introduction

During the last decade, biochar has gained importance owing to its roles in climate change mitigation and agronomic benefits among global agriculturists, environmental experts and policy makers. The term "biochar" is referred in recent literature emphasizing its use for atmospheric carbon capture and storage, and soil application differentiating from black carbon (Kookana et al., 2011). The European Commission (Verheijen et al., 2010) comprehensively defined biochar as: "charcoal (biomass that has been pyrolyzed in a zero or low oxygen environment) for which, owing to its inherent properties, scientific consensus exists that application to soil at a specific site is expected to sustainably sequester carbon and concurrently improve soil functions (under current and future management), while avoiding short- and long-term detrimental effects to the wider environment as well as human and animal health".

Biochar is produced by heating organic materials (e.g., plant residues, manures, waste materials) in absence of oxygen or otherwise known as pyrolysis (Lehmann, 2007). During pyrolysis, one-third to half of biomass carbon is converted into biochar. The heat treatment (more often thermochemical treatment) of organic biomass used to produce biochar contributes to its large surface area and its characteristic ability to persist in soils with variable biological decay (Lehmann et al., 2006) having half-life ranging from decades (Nguyen and Lehmann, 2009) to centuries (Zimmerman, 2010). Conceptually, biochar can serve multifaceted roles in soils (Fig. 1). Biochar can act as a soil conditioner or soil amendment to improve the soil quality, enhance plant growth by supplying nutrients, and retain nutrients. In this regard, an obvious positive attribute of biochar is its nutrient value, supplied either directly by providing nutrients to plants or indirectly by improving soil environment, with consequent improvement of fertilizer use efficiency. Nutrient composition and availability from biochar depend upon both the nature of the feedstock and the pyrolysis conditions (Gaskin et al., 2009; Bera et al., 2017). It helps to reduce nutrient leaching (Parvage et al., 2013), and increases crop production. It also provides other services such as improving soil physical and biological properties (Lehmann and Rondon, 2005; Mandal et al., 2016a; Purakayastha et al., 2015; Purakayastha et al., 2016; Bera et al., 2016; Bera et al., 2019). Moreover, biochar can alter the root morphology of crop plants in terms of favoring the fine root proliferation increasing the specific root length and decreasing both root diameter and root tissue density. The improved root conditions help plants to exploit more soil volume even under nutrient-starved soils directing towards biochar's role in increasing the fertilizer use efficiency (Olmo et al., 2016). It also has the capability to improve water retention properties of soil and enhance the soil's ability to retain nutrients (Rens et al., 2018). It could alter various soil properties through changes in pore size distribution, residence time of soil solution and flow paths of nutrients (Major et al., 2009). Overall, biochar can potentially add a holistic dimension for enhancing the soil quality and health which sooner or later is believed to impact crop productivity positively.

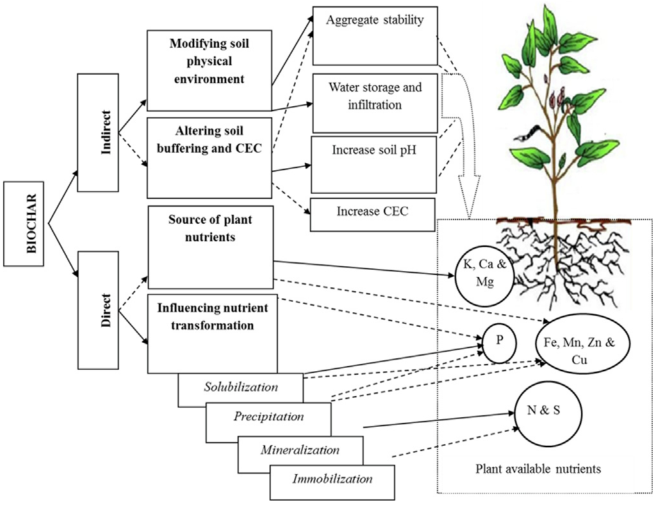

Fig. 1. Pathways of biochar impact in soil for better crop production. ⟶ Indicate primary pathways as evident from previous literature while - - - - → indicated possible pathways which needs to be validated with future research results.

Biochar application in soil for increasing crop production and other benefits including soil carbon sequestration is increasingly being recognized as a win-win strategy. The impact of biochar on crop productivity is largely influenced by the crop type, soil and biochar properties, which in turn depend on feedstock source and pyrolysis temperature. Several recent reviews have discussed the roles of biochar in climate change mitigation (Cayuela et al., 2013; Lehmann et al., 2006; Mandal et al., 2016a; Meyer et al., 2001; Minasny et al., 2017; Purakayastha et al., 2015, 2016; Singh et al., 2010), waste management (Ahmad et al., 2014; Devi and Saroha, 2015; Kookana et al., 2011; Mandal et al., 2018; Mohan et al., 2014), agronomic benefits (Alvarez-Camposa et al., 2018; Atkinson et al., 2010; Clough et al., 2013; Jeffery et al., 2013; Kookana et al., 2011; Lehmann et al., 2015; Liu et al., 2013; Mandal et al., 2016b; Spokas et al., 2000; Woolf et al., 2010), soil quality (Agegnehu et al., 2017; Barrow, 2012; Bera et al., 2016; Huang et al., 2013; Jones et al., 2012; Lehmann et al., 2011; Laird et al., 2010; Sohi et al., 2010), bioenergy production (Laird et al., 2009; Ro et al., 2010), and remediation of polluted soils (O'Connor et al., 2018a,b).

The effectiveness and application of biochar heavily relies on the biomass feedstock and the conditions under which it is produced (Tag et al., 2016; Zhang et al., 2012). Traditional biochar derived from wood or agricultural plant residues may have poor sorption capabilities (Yao et al., 2012), due to the absence of important electrostatic attractions between biochar and the negatively charged ions like phosphate (Vikrant et al., 2018). Several studies have attempted to enhance sorption capacities of anions by developing modified biochar through various coating procedures. Metal oxide-coated biochar, manufactured by bioaccumulation within the feedstock plant itself, including Mg-enriched tomato plants, has proven very successful (Yao et al., 2012). Similarly, co-precipitating metal oxides on the surface of biochar, post pyrolysis, including magnesium-coated oak wood biochar was an effective adsorbent (Takaya et al., 2016). Iron-impregnated orange peel (Chen et al., 2014), corn straw (Liu et al., 2013) and wood chip

(Micháleková-Richveisová et al., 2017) biochars have also been used successfully to remove phosphate from aqueous solutions in laboratory experiments. The biochar based adsorbent production methods recommended for improving contaminant removal efficiency include surface modification (Zhao et al., 2018), chemical group embedding (Zhou et al., 2015), metallic hybridization (Li et al., 2016a,b), and nanomaterial decoration (Inyang et al., 2014). For example, graphenes (Gs) and carbon nanotubes (CNTs) have been used as nanomaterial precursors for the engineered hybrid biochar adsorbent production (Tang et al., 2015). Compared with the pristine biochar, CNT-biochar and G-decorated biochar composites exhibited superior adsorbent properties, e.g., strong affinities for aromatic hydrocarbon and heavy metal pollutants and large specific surface area (Inyang et al., 2014; Sarkar et al., 2018; Zhang et al., 2012). Hybridization of CeO_2–MoS_2 hybrid magnetic biochar greatly improved Pb (II) and humate removal compared to magnetic biochar, with >99% Pb(II) and humate removed within 6 h (Li et al., 2019). In a review, it has been reported that soil amendment with biochar may reduce the bioavailability of a wide range of contaminants, including heavy metal (loids), potentially reclaiming contaminated soils for agricultural use (O'Connor et al., 2018a). The results of this review indicate that biochar application can potentially reduce contaminant bioavailability in the field; for instance, a significant decrease (control normalised mean value = 0.55) in the Cd enrichment of rice crops was observed. Sulphur-modified rice husk biochar increased the biochar's Hg^{2+} adsorptive capacity (Q_{max}) by ~73%, to 67.11 mg g^{-1} (O'Connor et al., 2018b).

However, there is a dearth of recently compiled information on overall impact of biochar properties on crop productivity and soil quality (Liu et al., 2013). There are continuous array of review publications on biochar, but most of them are related to the environment, for example, environmental contamination, water treatment and pollutant remediation. Principally, information on how key parameters, such as biochar feedstock type, pyrolysis temperature, application rate to soil, feedback to soil chemical properties (e.g., pH, cation exchange capacity (CEC) and crop yields are largely inconclusive. Hence, a critical synthesis of information about the above is urgently needed. The current review attempts to reveal biochars' nutrient properties and its role in soil nutrient transformation that influence soil quality and crop productivity in the present context of global climate change. Therefore, this review examines - (i) biochar nutrient value in relation to pyrolysis condition and feedstock types, (ii) biochar roles in soil nutrient availability and transformation, (iii) the potential benefits of biochar in sustainable crop production, and (iv) meta-analysis of the up to date published data for evaluating the effect of biochar on soil condition improvements and crop yield. We believe that this compilation is a useful document highlighting the emerging research needs in this area.

2. Methodology

2.1. Literature search method

Google Scholar was searched for keywords like "biochar", "characteristics", "availability of nutrients", AND "yield" within publication titles. Additional articles were found by searching key words for "biochar" AND "crop yield" with various nutrients, e.g., N, P, K, secondary nutrients and micronutrients. Various online journals, e.g., Science of the Total Environment", "Geoderma", "Soil and Tillage Research", "Bioresource Technology", "Advances in Agronomy", "Agriculture, Ecosystems and Environment", "European Journal of Agronomy", "Soil Biology and Biochemistry", "Biology and Fertility of Soils", "Applied Soil Ecology" etc. were also directly consulted for relevant papers. Only the relevant publications meeting the objectives of this review paper were selected to form the basis of this review. The literature search resulted in various publications relevant to this review paper, are presented in Tables 1–4.

2.2. Data compilation and analysis

In this review paper, we have collected the information on nutrient contents in biochar prepared from various feedstocks at different pyrolysis temperatures, their effects on physical, physicochemical properties of soils, and dynamics of N, P, K, secondary and micronutrient dynamics in soil. The information on the impact of biochar on crop yields was based on various soil orders having dissimilar properties like pH (acidic, neutral to alkaline), texture (silty, sandy clay loam, clay loam), CEC etc. In order to classify biochar, we gathered literature on biochar prepared from various feedstocks, e.g., crop residues, manures, wood, and waste materials. Majority of the information was collected from various peer-reviewed journals of international repute. Two principal component analyses (PCA) were performed in this study using data from published literature: one in which the objective variables were changed in soil chemical properties, e.g., pH and CEC, and the other in which the objective variable was changed in crop yield. Since variables were measured in different units, the variable values were all normalised by subtracting the mean and dividing by the standard deviation of the variable group, and the PCA was computed using the correlation matrix between the variables. All PCAs were performed using the program PAST version 3.18 (Hammer et al., 2001).

3. Role of biochar in mitigating climate change

Any compilation on biochar without mentioning its role in mitigating climate change is incomplete. Thus, it is imperative to briefly mention the role of biochar in negating global warming. In doing so, it is notable to mention that the Paris Climate Agreement in 2015 set a target for participating countries that 'hold the increase in the global average temperature to well below 2 °C above pre-industrial levels and to pursue efforts to limit the temperature increase to 1.5 °C above pre-industrial levels' (IPCC, 2014). While conventional greenhouse gas emission mitigation strategies, such as lowering the consumption of fossil fuels, are needed to achieve the goal of the Paris Agreement, simultaneous actions on negative emissions through sustainable carbon dioxide removal (CDR) technologies and engineered enhancement of natural carbon sinks are also urgently required (Gasser et al., 2015). Recent reports suggest that the goal of holding global warming to well below 2 °C is extremely unlikely unless the emissions gap is not closed by 2030 (UNEP, 2017). In order to achieve large reductions in greenhouse gas emissions, sequestering carbon in the terrestrial sink is needed (Paustian et al., 2016). The global soil has been estimated to hold the largest terrestrial organic carbon pool (~1500 Pg C to a depth of 1 m; 2400 Pg C to 2 m depth) (Batjes, 1996). An increase in organic matter inputs to soil, or a decrease in soil organic matter decomposition rates, or the net carbon gaining effect of the both can increase the carbon stock in soil (Paustian et al., 2016). The recently launched '4 per mille Soils for Food Security and Climate' concept also proposes to increase global soil organic matter stocks by 4 per 1000 (or 0.4%) per year in order to compensate global greenhouse gas emissions due to anthropogenic activities (Minasny et al., 2017). In this connection, the application of biochar to soils has been shown to achieve the net carbon gain in soils while also serving for increased plant biomass production by enhancing the nutrient supply to plants and increasing nutrient and water use efficiencies

Table 1
pH and nutrient composition of various biochar materials produced at different pyrolysis temperatures.

Biochar feedstock	Pyrolysis temp. (°C)	pH	C	N	C/N	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	Mo	Reference
		(%)				(%)					(mg kg^{-1})					
Crop residues																
Corn cob	600 °C	10.1	79.1	4.25	19	–	–	–	–	–	–	–	–	–	–	Mandal et al. (2017)
Macadamia integrifolia	450–480 °C	8.76	78.03	0.43	182	0.24	2.19	0.37	0.17	–	–	–	1211	–	–	Wrobel-Tobiszewska et al. (2015)
Giant reed (*Arundodonax*)		9.45	73.4	0.49	150	–	–	–	–	–	–	–	–	–	–	Zheng et al. (2013)
Switch grass	400 °C	–	73.1	1.35	54	–	–	–	–	0.32						Purakayastha et al. (2016)
Rice straw	450 °C		70.6	0.97		0.218	26.4									Peng et al. (2011)
Wheat straw	400 °C	–	70.5	1.22	58	–	–	–	–	0.29	–	–	–	–	–	Purakayastha et al. (2016)
Corn stover	600 °C	9.95	69.8	1.01	70	0.181	2.461	0.938	0.858	0.08	70	–	1362	226	–	Enders et al. (2012)
Peanut hull	400 °C		65.5	2.0	33	0.00162	0.00153	0.00044	–	–	–	–	–	–	–	Gaskin et al. (2009)
Pearl millet	400 °C	10.6	64	1.10	58	1.60	2.52	1.47	1.06	0.22						Purakayastha et al. (2015)
Soybean straw	500 °C	10.9	62.6	0.37	171	0.44	–	–	–	–	–	–	–	–	–	Yuan et al. (2011)
Canola straw	500 °C	9.39	61.6	0.04	1610	0.27	–	–	–	–	–	–	–	–	–	
Corn stover	300 °C	7.33	59.5	1.16	51	0.137	1.705	0.648	0.588	0.070	132	–	963	142	–	Enders et al. (2012)
Sugarcane bagasse	350 °C	4.96	57	0.34	168	0.058	0.48	–	–	0.032	–	–	–	–	–	
Corn stover	400 °C	–	55.3	1.30	43	–	–	–	–	0.20	–	–	–	–	–	Purakayastha et al. (2016)
Rice hull	400 °C	–	55	0.93	59	–	–	–	–	0.05	–	–	–	–	–	
Peanut straw	500 °C	10.86	48.5	1.51	32	0.95	–	–	–	–	–	–	–	–	–	
Rice husk	800 °C	–	–	–	–	0.044	0.670	0.164	0.084	0.017	29	–	29	22	–	Enders et al. (2012)
Soybean	500 °C	–	–	–	–	0.056	3.779	1.565	1.171	0.112	28	–	699	58	–	
Woods																
Bamboo chip	600 °C	9.59	81.2	4.55	18	–	–	–	–	–	–	–	–	–	–	Mandal et al. (2017)
Eucalyptus bark	600 °C	9.37	79.1	4.20	19	–	–	–	–	–	–	–	–	–	–	
Spruce and pine chips	550–600 °C	10.8	87.8	0.62	142	0.001	3.23	4.44	0.72	0.019	470	10	7190	2570	–	Tammeorg et al. (2014)
Hazelnut	400 °C	6.38	87.6	0.17	510	0.0298	0.429	0.282	0.0554	0.016	10	–	29	13	–	Enders et al. (2012)
Teak and Rose wood	300–400 °C	7.5	87.0	0.31	281	0.0048	0.12	0.044	0.036							Asai et al. (2009)
Eucalyptus deglupta		7.0	82.4	0.573	144	0.6	–	–	–	–	–	–	–	–	–	Rondon et al. (2007)
Eucalyptus camaldulensis L., flash carbonization	800 °C	8.92	81.50			0.086	0.781	1.042	0.059				0.229			Butnan et al. (2015)
Oak	400 °C	4.58	78.8	0.17	468	0.0005	0.147	0.106	0.0061	0.008	33	–	169	15	–	Enders et al. (2012)
Douglas-fir wood pellets	500 °C	7.2	78.2	0.13	602	0.022	0.10	0.20	0.03	0.017	29.1	3.1	250	93.3		Streubel et al. (2011)
Pine	400 °C	4.6	76.3	0.1	763	0.0035	0.037	0.225	0.048	0.010	66	–	1166	258	–	
Wood chips	400–450 °C	10.9	74.8	0.15	499	0.04	0.23	0.59	0.13	0.03	–	–	4200	–	–	Saarnio et al. (2013)
Cooking wood	500–700 °C	9.20	72.9	0.76	121	0.0030	0.046	0.033	0.0048	–	–	–	–	–	–	Major et al. (2012)
Douglas-fir wood bark	500 °C	7.6	72.7	0.35	208	0.047	0.10	1.07	0.048	0.023	40.9	6.8	700	266		Streubel et al. (2011)
Yellow pine chipped	400 °C	5.96	71	0.1	710	0.017	0.18	–	–	0.01	–	–	–	–	–	White Jr. et al. (2015)
Hardwood			70.3	0.30	234	0.0278	0.000241	0.00027	–	–	–	–	–	–	–	Gaskin et al. (2009)
Pine chips			67.0	0.14	479	0.0235	0.000197	0.00017	–	–	–	–	–	–	–	Gaskin et al. (2009)
Eucalyptus camaldulensis L., traditional kiln	300 °C	6.52	61.86	–	–	0.05	0.51	0.541	0.043	–	–	–	0.05	–	–	Butnan et al. (2015)
Sesbaniaroxburghii	400 °C	9.0	57.7	3.50	17	–	–	–	–	–	–	–	–	–	–	Chen et al. (2014)
Manure																
Bull manure	600 °C	9.5[a]	76.0	0.8	95	0.295	3.582	0.938	0.507	0.102	193	–	311	165	–	Enders et al. (2012)
Anaerobic digested fibre	500 °C	9.3	65.8	2.23	30	0.76	1.17	2.40	0.70	0.30	230	163	1280	184	–	Streubel et al. (2011)
Bull manure	300 °C	8.2[a]	60.6	1.3	47	0.301	2.002	0.941	0.395	0.110	162	–	376	137	–	Enders et al. (2012)
Digested dairy manure	600 °C	9.94	59.4	0.225	28	0.827	1.494	2.65	0.850	0.286	200	–	2356	191	–	Enders et al. (2012)
Digested dairy manure	400 °C	9.22	57.7	0.242	26	0.645	1.66	2.2552	0.973	0.272	131	–	1656	145	–	Enders et al. (2012)
Dairy manure	700 °C	9.9	56.7	1.51	38	1.69	2.31	4.48	2.06	0.15	423	163	44800	867	10.0	Cantrell et al. (2012)
Dairy manure	350 °C	9.2	55.8	2.60	22	1.00	1.43	2.67	1.22	0.11	361	99.0	26700	525	7.8	Cantrell et al. (2012)
Paved-feedlot	350 °C	9.1	53.3	3.64	15	1.14	3.20	2.27	0.76	0.45	359	91.7	22600	259	6.2	Cantrell et al. (2012)
Paved-feedlot	700 °C	10.3	52.4	1.70	31	1.76	4.91	3.50	1.22	0.44	448	136	34500	388	6.3	Cantrell et al. (2012)
Swine solids	350 °C	8.4	51.5	3.54	15	3.89	1.78	3.91	2.44	0.80	3181	1538	48400	1453	18.3	Cantrell et al. (2012)
Poultry litter	350 °C	8.7	51.1	4.45	12	2.08	4.85	2.66	0.94	0.61	712	213	13200	640	11.0	Cantrell et al. (2012)
Turkey litter	350 °C	8.0	49.3	4.07	12	2.62	4.01	4.04	0.85	0.55	690	535	27800	710	7.16	Cantrell et al. (2012)
Poultry litter	700 °C	10.3	45.9	2.07	22	3.12	7.40	4.02	1.45	0.63	1010	310	18900	948	13.0	Cantrell et al. (2012)
Turkey litter	700 °C	9.9	44.8	1.94	23	3.63	5.59	5.61	1.24	0.41	909	762	36500	986	10.1	Cantrell et al. (2012)
Swine solids	700 °C	9.5	44.1	2.61	17	5.90	2.57	6.15	3.69	0.85	4981	2446	74800	2240	27.4	Cantrell et al. (2012)
Poultry litter	400 °C	7.7	38.3	2.0	19	0.9	1.0	2.5	0.3	–	238	57	2695	265	5	Macdonald et al. (2014)
Cow manure	500 °C	9.20	33.6	0.15	22	0.814	0.005	0.042	0.034	–	–	–	–	–	–	Uzoma et al. (2011)
Poultry manure	500 °C	10.57	25.4	1.41	18	3.055	2.811	20.42	1.044	0.459	601	–	2034	566	–	Enders et al. (2012)
Poultry manure	600 °C	10.65	23.6	0.94	28	2.359	2.74	24.28	0.877	0.349	595	–	1522	466	–	Enders et al. (2012)
Waste materials																
Brush	500 °C	8.4	84	0.1	840	0.013	0.087	0.756	0.044	0.011	59	–	94	142	–	Enders et al. (2012)
Whole tree residue	600 °C	7.5[b]	78	0.14	557	0.009	0.055	0.140	0.040	0.004	25	3.1	2600	56	<1.2	Van Zwieten et al. (2010)
Orchard pruning biomass	500 °C	9.8	77.8	0.91	63.5	2.33	1.39	2.5	2.87	0.048	.010	.009	.033	.008	–	Baronti et al. (2014)
Leave waste	500 °C	9.0	60.7	1.1	55	0.207	1.084	5.455	0.361	0.103	70	–	1504	555	–	Enders et al. (2012)
Switchgrass	500 °C	9.4	59.2	1.99	30	0.47	3.28	0.87	0.46	0.11	33.7	7.7	620	109		Streubel et al. (2011)

Table 1 (*continued*)

Biochar feedstock	Pyrolysis temp. (°C)	pH	C	N	C/N	P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	Mo	Reference
		(%)				(%)					(mg kg^{-1})					
Grass waste	500 °C	9.6	53.5	4.9	11	1.197	6.129	2.062	0.618	0.629	150	–	1557	360	–	Enders et al. (2012)
Food waste	400 °C	8.27	52.4	3.65	14	0.5007	1.456	5.174	0.534	0.083	39	–	4431	179	–	Enders et al. (2012)
Paper mill waste	550 °C	8.2	50.5	0.31	104	0.009	0.029	–	–	–	–	–	–	–	–	Van Zwieten et al. (2010)
Green waste	450 °C	9.4	36	0.18	200	0.040	0.819	0.008	0.013							Chan et al. (2007)
Paper mill sludge	300 °C	–	23.4	0.22	106.2	–	–	–	–	0.32	–	–	–	–	–	Devi and Saroha (2015)
Paper mill sludge	300 °C	7.8	21.2	0.3	71	0.083	0.278	25.81	0.243	0.031	26	–	4274	136	–	
Paper mill sludge	600 °C	11.5	19.2	0.1	192	0.094	0.385	31.12	0.294	0.031	51	–	6037	160	–	
Waste water sludge	550 °C	8.2	–	2.3		0.110	0.009	0.66	0.043	–	–	–	–	–	–	Hossain et al. (2010)

[a] pH measured in 1 N KCl instead of water.
[b] pH measured in $CaCl_2$.

Table 2
Soil pH and CEC as influenced by feedstock types, temperature and addition rates of biochar.

Feedstock	Temperature (°C)	Application rate (Mg ha^{-1})	Soil type	pH		CEC (cmol$^{(+)}$ kg^{-1})		References
				Control	Treatment	Control	Treatment	
Greenwaste	450	10	Alfisol	4.5	4.75	4.03	10.5	Chan et al. (2007)
		50			5.38			
		100			5.99			
Poultry litter	550	10	Alfisol	4.5	6.66			Chan et al. (2008)
		25			7.29			
		50			7.78			
Sludge + wood chip	550	10	Ferrosol	4.2	5.93			van Zwieten at al. (2010)
			Calcarosol	7.67	7.67	31.0	29.3	
Wheat straw	350–550	10	Anthrosols	5.6	5.70			Cui et al. (2012)
		20			5.81			
		40			5.86			
Spruce + pine chips	550–600	5	Stagnosol	6.6	6.7			Tammeorg et al. (2014)
		10			6.7			
Switch grass	500	10–40	Entisol	7.2	7.9			Streubel et al. (2011)
Wood bark		10–40			8.0			
Digested fibre		10–40			8.0			
Wood pellet		10–40			7.2			
Sludge	550	10		4.0	4.86			Khan et al. (2013)
		20			5.39			
Hardwood	500	22.4	Haplocalcids	7.7	7.7			Lentz and Ippolito (2012)
Wood chip	450	25	Eutric Cambisol	6.8	6.8			Quilliam et al. (2012)
		50			6.8			
Mix wood chips	525	90	Planosol	5.3	6.9	75.1	101.1	Kloss et al. (2014)
Wheat straw					6.5		94.0	
Vineyard pruning					6.6		96.5	
Canola straw	350	4	Acrisol	3.99	4.7	9.1	11.4	Yuan et al. (2011)
Rice straw					4.5		10.7	
Soybean straw					5.2		10.6	
Pea straw					5.0		10.5	
Wood chip	550	72	Chernozem	7.4	7.4	201	208	Karer et al. (2013)
			Cambisol	6.3	6.7	187	214	
Oak + Hickory		5	Hapludolls	6.4	6.4	17.1	19.8	Laird et al. (2010)
		10			6.9		20.7	
		20			7.1		20.8	
Cow manure	500	10		6.40	7.1	0.8	0.9	Uzoma et al. (2011)
		15			7.34		1.2	
		20			8.0		1.3	
Poultry litter	700	4		5.9	9.7			Novak et al. (2009)
Pecan shell	700	4			7.5			
Wheat Straw	350–550	10	Halpudept	6.5	6.75			Zhang et al. (2012)
		20			6.77			
		40			6.77			
Birch wood	500	10	Hapludalf	6.6	6.7			Sun et al. (2014)
		20			6.6			
		50			6.8			
Eucalyptus	350	6	Haplustox	5.0	5.0	108.2	118.5	Rondon et al. (2007)
		12			5.2		131.7	
		18			5.4		131.5	

(NUE and WUE) by plants (Kookana et al., 2011; Lehmann et al., 2015; Minasny et al., 2017). Thus, biochar application to soils has been recommended as an important component of the pathway to ‘climate-smart soil’ management practices in modern global

Table 3
Effect of biochar on nutrient contents and nitrogen transformations in soil at different pyrolysis temperatures.

Biochar	Pyrolysis temperature	Soil	Rate	Nitrogen[a]							P	K	Ca	Mg	S	Zn	Cu	Fe	Mn	Reference
				TSN	AN	MN	IM	N/D	NO_3^-	NH_4^+										
Maize	350 °C	Arable	–	↑	–	↑	–	↑(N)	↑	↓	–	–	–	–	–	–	–	–	–	Nellisen et al. (2012)
	550 °C		–	↑	–	–	–	–	↓	↑	–	–	–	–	–	–	–	–	–	
Cotton stalks	650 °C	Sandy loam	–	–	–	–	–	↑(N)	–	↑	–	–	–	–	–	–	–	–	–	Song et al. (2013)
Corn stalk	450 °C	Clayey Oxisol	–	–	–	↑	↑	–	–	–					↑					Blum et al. (2013)
Rye grass	450 °C	Forest Cambisol	–	↑	–	↑		↑(N)	↓	↓										Maestrini et al. (2014)
Poultry manure	400 °C	Vertisol and Alfisol		–	–	–	–	↑ (D)	–	↓	–	–	–	–	–	–	–	–	–	Clough and Condron (2010)
Douglas fir wood	410 °C	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	–	–	–	–	Pereira et al. (2015)
	510 °C	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	–	–	–	–	
Hog waste wood	600 °C	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	–	–	–	–	
	700 °C	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	–	–	–	–	
Loliumperenne	350 °C	–	–	–	–	–	–	↑ (N)	–	–	–	–	–	–	–	–	–	–	–	Rosa and Knicker (2011)
Oak wood	200 °C	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	–	–	–	–	Zhang et al. (2015)
	400 °C	–	–	–	–	↑	–	↑ (N)	–	–	–	–	–	–	–	–	–	–	–	
	600 °C	–	–	–		No effect	–	↑ (D)	–	–	–	–	–	–	–	–	–	–	–	
Willow (*Salix viminalo*)	470 °C	Flinty clay loam	–	–	–	–	↑	–	↓	↓	–	–	–	–	–	–	–	–	–	Prayogo et al. (2014)
Japanese larch wood (*Larixgmelinii*)				–	–	–	↑	–	–	–	–	–	–	–	–	–	–	–	–	Makoto et al. (2011)
Bamboo (*Bambusa* sp.)	500 °C		–	–	–	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	Asada et al. (2002)
	700 °C	–	–	–	–	–	–	–	–	↓	–	–	–	–	–	–	–	–	–	
Bamboo (*Bambusasp.)*	600 °C	Sandy silt soils	–	–	–	–	–	–	–	↓	–	–	–	–	–	–	–	–	–	Ding et al. (2010)
Pine chips (*Pinussp.*) wood	–	–	–	–	–	–	–	–	↑	↓	–	–	–	–	–	–	–	–	–	Bai et al. (2015)
Eucalyptus	600 °C	Acidic Grey OrthicTenosol	5–25 Mg ha^{-1}	–	–	↓	↑	–	–	–	–	–	–	–	–	–	–	–	–	
Sugarcane bagasse	400 °C	–	–	–	↓	–	–	–	↓	–	–	–	–	–	–	–	–	–	–	Kameyama et al. (2012)
	800 °C	–	–	–	↓	–	–	–	↓	–	–	–	–	–	–	–	–	–	–	
Poultry litter and wheat straw	–	Acidic ferrasol and alkaline calcisol	5 & 10 Mg ha^{-1}	↑	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Macdonald et al. (2014)
Rice husk	–	Acidic Gleysols	41 Mg ha^{-1}	↑	–	–	–	–	–	–	↑	–	–	–	–	–	–	–	–	Haefelea et al. (2011)
Lump biochar	–	Loamy soils		↑	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	Laird et al. (2010)
Maize stover and wheat straw	400 °C	Sandy loam alluvial soil	–	–	↑	–	–	–	–	–	↑	↑	–	–	–	–	–	–	–	Purakayastha et al. (2015)
Charcoal	–	Base rich soils	–	–	–	–	–	–	↑	–	–	–	–	–	–	–	–	–	–	Borchard et al. (2014)
		Extremely acidic soils	–	–	–	–	–	–	↓	–	–	–	–	–	–	–	–	–	–	
Chicken manure	–	–	–	–	↑	–	–	–	–	–	↑	↑	–	–	–	–	–	–	–	Chan and Xu (2009)
Peanut straw	400 °C	Oxisol	–	–	–	–	–	–	–	–	–	–	↑	↑	–	–	–	–	–	Jiang et al. (2015)
Rice straw			–	–	–	–	–	–	–	–	–	–	↓	↓	–	–	–	–	–	
Mixed hardwood	500 °C – 575 °C	–	–	–	–	–	–	–	–	–	–	–	↑	↑	–	↑	↑	↓	↓	Rogovska et al. (2014)

[a] TSN: total soil nitrogen; AN: available nitrogen; MN: mineralization; IM: immobilization; N: nitrification; and D: denitrification.

Table 4
Effect of biochar on crop yield.

Biochar feedstock	Application rate (t ha^{-1})	Soil Type	Test crop	Yield increase/ decrease[a] (%)	Country	Reference
Poultry manure	30	Acidic silty	Wheat	+28.2	Italy	Vaccari et al. (2011)
	60			+28.6		
	12	Alkaline alluvial	Wheat	+38	China	Lashari et al. (2013)
Wheat straw	10–40	Fine loamy Gleysols	Rice	Neutral	China	Huang et al. (2013)
Wheat straw 450 °C	1	Acid Ferrasol		+19	Germany	Macdonald et al. (2014)
	5			+79		
	10			+51		
Wheat (*Triticum aestivum* L.) straw (1 yr + Pyrogallol)	12			+60		
Wheat straw, 350–500 °C	10		Rice	+28	China	Zhang et al. (2012)
	20			+9		
	40			+22		
Biochar 450 °C 1st yr	25	Acidic sandy clay loam, Cambisol	Maize	Neutral	UK	Jones et al. (2012)
2nd yr				Neutral		
3rd yr				+78		
Wood 300 °C, 1st yr		Acidic Oxisol	Maize	+28	Colombia	Major et al. (2010)
2 nd yr				+30		
3rd yr				+140		
Birch wood (*Hordeumvulgare* L.)	20	Acidic sandy loam soil	Oat	Neutral	Denmark	Sun et al. (2014)
			Spring barley	+6		
	50		Maize	−22-24		
Poultry litter 450 °C	1	Acid Ferrasol		+24	Germany	Macdonald et al. (2014)
	5			+101		
	10			+144		
	1	Acidic Aeronosol		Neutral		
	5			Neutral		
	10			−21		
	1, 5, 10	Alkaline Calcisol		Neutral		
		Neutral Vertisol		Neutral		
Domestic green waste biochar 550 °C	25		Wheat	+7.54	Australia	Farrell et al. (2014)
Wheat straw, 400 °C	12	Slightly alkaline sandy loam Inceptisol	Rice	Neutral	China	Xie et al. (2013)
Corn stover 400 °C	12	Acidic clay loam Ultisol				
Maize biochar 400 °C	20	Alkaline sandy loam Inceptisol	Maize	+3.68	India	Purakayastha (2010)
Rice biochar 400 °C	5	Alkaline sandy loam Inceptisol	Rice	+24.3	India	Bera et al. (2015)
	5	Acidic sandy loam Alfisol	Rice	+31.3		
Eucalyptus deglupta 350 °C	90[b]	Neutral clay loam Oxisol	Bean	+46	Colombia	Rondon et al. (2007)
	60[b]		Bean	+39		

[a] Values of yield indicated by '+' and '-' represent yield increase and decrease, respectively.
[b] Biochar application rate in g kg^{-1} soil.

agriculture (Paustian et al., 2016).

4. Carbon and nutrient contents of biochar

Biochar is enriched with C, and contains a range of plant macro, micro and secondary nutrient elements (Chan and Xu, 2009). The composition of biochar depends upon the nature of feedstock and pyrolysis conditions, and published literature suggests a wide variation in biochar compositions (Table 1). Carbon contents ranged from 81.2% in biochar prepared from bamboo chip (Mandal et al., 2017) to 19.2% in biochar prepared from paper mill sludge (Devi and Saroha, 2015) (Table 1). Biochar prepared form crop residues and woody materials contained a higher C content than biochar prepared from manure sources. Waste material biochars had a wide range of C contents (19.2–84.0%) indicating their differential initial constituents. During the pyrolysis process, N in residues is converted to recalcitrant forms, and using nuclear magnetic resonance and near-edge X-ray adsorption fine structure spectroscopy, it was found that both C and N became enriched in aromatic and heterocyclic aromatic structures in biochar (Chen et al., 2014). Manure-derived biochar was undoubtedly the richest source of N among all feedstock types of biochar, showing N content as high as 4.45%. Contrarily, biochar prepared from woody materials was scant in N content. Thus, most of the manure derived biochars had lower C/N ratios ranging between 10 and 30, with few exceptions. Wood-derived biochar had a wider C/N ratio (Atkinson et al., 2010; Rajkovich et al., 2012). The very low N content (0.04%) in canola straw biochar conferred it the highest C/N ratio (160:1). The P content was recorded the highest (5.90) in swine solid biochar, while the lowest (0.017%) in yellow pine chip biochar. On the other hand, the highest (7.40% for poultry litter) and lowest (0.087% for brush) K contents were recorded in manure and waste material derived biochars, respectively (Cantrell et al., 2012; Ro et al., 2010). The paper mill biochar (Devi and Saroha, 2015) and poultry manure biochar (Enders et al., 2012) prepared at 600 °C were reported to be rich sources of Ca (25 and 31%, respectively) and Mg (0.87 and 0.29%, respectively) (Table 1). Data on micronutrient contents in biochar is limited in the literature. Biochar prepared from swine solids contained 74800, 2240, 4981, 2446 and 27.4 mg kg^{-1} of Fe, Mn, Zn, Cu, and Mo, respectively (Table 1; Cantrell et al., 2012). The majority of biochar samples were alkaline in pH with few exceptions such as sugarcane bagasse biochar, yellow pine chip biochar, hazelnut biochar and eucalyptus biochar, which were found to be acidic in solution. Increasing the pyrolysis temperature in general enhances the acid neutralising property of biochar increasing the pH (Bera et al., 2019). The alkalinity of biochar was primarily due to the presence of inorganic alkali salts. The organic COO– and –O– groups that could modify the acid reaction of biochar surface through association with H^+ ions might also contribute to biochar alkalinity (Al-Wabel et al., 2013).

The pyrolysis temperature significantly influenced the pH, C, and nutrient compositions of biochar. Purakayastha et al. (2016) reported that increase in pyrolysis temperature from 400 °C to 600 °C significantly increased the C content, while it decreased the N content in all biochars except that was produced from rice hull. These findings were in agreement with the other studies which also found higher C contents in plant material based biochars, e.g., canola, soybean (Yuan et al., 2011), peanut hull, pine chips (Gaskin et al., 2008), *Eucalyptus saligna* wood and leaf (Singh et al., 2010). Contrastingly, Yuan et al. (2011) reported that the C content decreased in corn and peanut biochar with an increasing pyrolysis temperature from 300 °C to 500 °C. In general, the C/N ratio increased due to an increase in pyrolysis temperature. For example, the C/N ratio of switch grass biochar increased from 54 to 84 when pyrolysis temperature increased from 400 °C to 600 °C (Purakayastha et al., 2016). In contrast, Novak et al. (2009) reported that the C/N of sugarcane bagasse biochar decreased from 129 to 79 when pyrolysis temperature increased from 250 °C to 500 °C. The slow and fast pyrolysis process during heating could also influence the C and N contents, and C/N ratios. Consequently, the C/N ratio of biochar prepared at slow pyrolysis is expected to be greater than that prepared by fast pyrolysis process (Atkinson et al., 2010). For example, Bruun et al. (2012) reported that biochar prepared from wheat straw at slow pyrolysis contained more C (69.6%) than the biochar prepared at fast pyrolysis (49.3%).

5. Interaction of biochar with soils

5.1. Soil physico-chemical properties

5.1.1. Biochar modifying soil physical environment

Biochar amendments were reported to improve soil bulk density, porosity, water retention, and hydraulic conductivity (Abel et al., 2013; Asai et al., 2009; Atkinson et al., 2010; Jeffery et al., 2011; Karhu et al., 2011; Laird et al., 2010). Moreover, biochar application significantly influenced the infiltration capacity in soils (Lehmann et al., 2006; Sohi et al., 2010). Bayabil et al. (2015) reported that incorporation of woody feedstock (Acacia, Croton, and Eucalyptus) charcoals significantly decreased the soil moisture retention at lower tensions (10 and 30 kPa), resulting in an increase in relative hydraulic conductivity at these tensions in a clay soil. Akhtar et al. (2014) found higher water use efficiencies when irrigation was applied through partial root zone drying along with the application of 5% biochars prepared from rice husk or cotton seed mixture, over full irrigation. Addition of 10 Mg ha^{-1} biochar in a sandy soil in Finland increased the available water content in the dry period of the year under *Phleumpratense* growth (Saarnio et al., 2013). In contrast, water holding capacity of Quincy sand soil of Washington State remained unchanged in a laboratory incubation study with the application of biochars prepared from switch grass, anaerobically digested fiber, softwood bark and wood pellet (Streubel et al., 2011). Biochar prepared from black locust (*Robinia pseudoacacia*) when applied at a dose of 20 Mg ha^{-1} increased the available water capacity by 97%, saturated water content by 56%, and reduced the hydraulic conductivity with increasing moisture content in a sandy soil (Uzoma et al., 2011).

Soil aggregation is considered as another important physical property which determines the stability and support of soil, and biochar showed its beneficial impact on that as well. Soinne et al. (2014) reported that biochar had the potential to improve the aggregate stability in clay soils, and thus repeated biochar additions could reduce the deteriorating effect of tillage on soil aggregates. It could even lead to the improvement of the structural stability of cultivated clay soils (Soinne et al., 2014). A study using synchrotron-based X-ray micro-computed tomography revealed that the increased porosity of macroaggregates in biochar-amended soil was jointly contributed by the inherent porosity in the applied biochar as well as the newly formed pores out of soil-biochar interactions (Yu et al., 2016). The authors also reported that wood chip biochar and waste-water sludge biochar were more efficient in increasing the porosities of the products over straw biochar, and hence showed greater effects on soil macroaggregates (Yu et al., 2016). Thus, biochar could improve the physical properties of difficultly manageable clay and sandy soils by changing their air-water relationships through mechanisms like increased aggregate stability, water infiltration and water holding capacity (Fig. 1).

5.1.2. Biochar modifying soil pH, buffering system, CEC

In soil, availability of nutrients for plants is pH dependent. Biochar may alter soil pH, which in turn can change nutrient solubility, thereby modifying the nutrient availability. The impact of biochar addition on soil pH and CEC has been summarized in Table 2. The resultant soil pH values tended to move to the alkaline side when the soil received an increased biochar application rate, and when the biochar was produced at a high temperature (e.g., 700 °C) (Mandal et al., 2016b, 2018). Effect of wood ash or horticultural biochar in modifying soil pH has long been known, and documented by earlier reports (Clarholm, 1994; Glaser et al., 2002; Mahmood et al., 2003). Jeffery et al. (2011) found that biochar could increase soil pH by 0.1–2.0 units in a wide range of soils varying in native pH values. An insight perusal of Table 2 indicated that the magnitude of soil pH change upon biochar addition was inevitably reliant on soil types, biochar properties, and application rates. Chan et al. (2007, 2008) demonstrated that green waste biochar and poultry litter biochar could gradually increase pH by 0.6–2.0 units of an acidic Alfisol at successive application rates ranging from 10 to 100 t ha^{-1} under radish (*Raphanus sativus*) cultivation. Similarly, Van Zwieten et al. (2010) reported increased soil pH values due to sludge biochar addition in an acidic Ferrosol cropped with wheat, radish, and soybean. The plotting of biochar application rate and per cent changes in soil pH provided an interesting observation in segregating various soil types as impacted biochar applications (Fig. 2). The per cent increase in soil pH due to biochar application was the highest (>50%) in Alfisol with biochar application rates ranging from 25–50 Mg ha^{-1}, while the increase was between 4–50% in Alfisol, Anthrosol, Cambisol, Mollisol, Inceptisol and Oxisolis with biochar application rates ranging from 4→72 Mg ha^{-1} (Fig. 2). In Planosol, even at very high rate of biochar application (90–100 Mg ha^{-1}), the per cent increase in soil pH was only between 22 and 33%. Interestingly, in calcareous soils, and some Cambisol and Mollisol, no effect of biochar on soil pH was observed (Fig. 2). Alfisols, Ferrosols and Acrisols are inherently highly acidic in nature, and biochar being alkaline material neutralised the acidity. As there could be variations in active and potential acidity in these soils, the differential impact of biochar on enhancing the soil pH was noticed. Among the biochars, poultry litter biochar being highly alkaline in nature (pH ≈ 10) had the highest impact on the pH of acid soils.

The associated increase in soil pH with biochar addition would result in a greater availability of primary and secondary nutrients like K, P, Ca, Mg (Asai et al., 2009; Glaser et al., 2002; Major et al., 2010). The other advantage of increased pH due to biochar addition is the reduction of Al toxicity in acidic soils. In an acidic Ferrosol, 10 t ha^{-1} biochar addition reduced the ammonium acetate extractable Al from 1.93 cmol (p^+) kg^{-1} soil to an undetectable amount (Van Zwieten et al., 2010). The liming effect of biochar in acid soils, as described above, not only could improve the mineral nutrient supply for plant growth, but also could alleviate Al stress

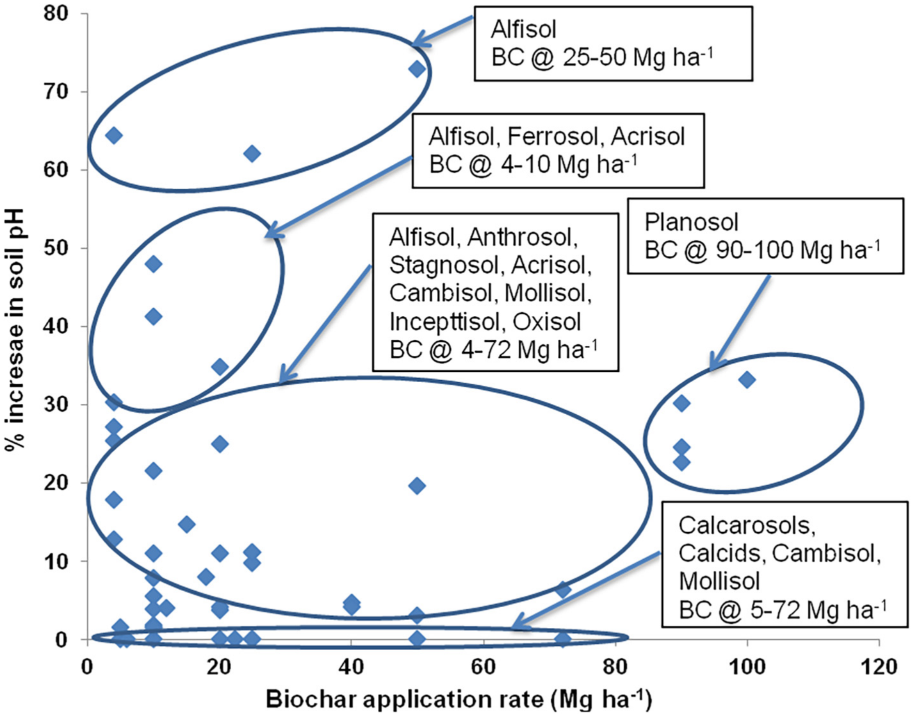

Fig. 2. Effect of biochar (BC) application rate on soil pH.

for better crop production (Liu et al., 2013; Dai et al., 2017). On the contrary, limited information is available on the effects of biochar addition in alkaline soils of arid and semiarid regions. Some studies (Karer et al., 2013; Lentz and Ippolito, 2012; Van Zwieten et al., 2010) did not observe a significant change in soil pH due to biochar addition where initial values were ranging between pH 7.4–7.8. Contrarily, Streubel et al. (2011) found 0.1–0.9 unit pH increase of an alkaline sandy soil. Similarly, Mandal et al. (2018) reported that when biochars produced from poultry manure, green waste compost and wheat straw at various temperatures (250–700 °C) were applied to an alkaline soil (pH 8.01), they could modify the soil pH values by about 0.84 units in both directions ranging from pH 7.37 to 8.23. These discriminating results about pH values, as discussed above, need thorough investigation by conducting biochar application trials in alkaline soils in arid and semiarid regions of the world.

The CEC of soils is an essential property in relation to the soil fertility. A higher CEC soil can hold cationic nutrients in greater amounts and for longer time than a lower CEC soil, preventing the nutrients from leaching loss and increasing their availabilities for plant uptake. As shown in Table 2, CEC increased in all cases except one where the soil was a calcarosol (Van Zwieten et al., 2010). The higher CEC of biochar-amended soils was ascribed to the dominance of negatively charged surface functional groups, increased specific surface area of the products, adsorption of highly oxidized organic matter on biochar surfaces, and the presence of residual volatile matter in the biochar matrix (Glaser et al., 2003; Lehmann and Rondon, 2005; Liang et al., 2006). The increase in total negative charge and charge density on soil applied biochar surfaces was reported due to the biotic and abiotic oxidation of organic functional groups in long-term soil application studies (Cheng et al., 2006; Zimmerman, 2010). Yuan et al. (2011) found a significant increase in soil CEC (15–25%) when canola, rice, soybean and peanut straw biochars (CEC of biochars ranging between 179 and 279 cmol (p^+) kg^{-1} were added to a low CEC Acrisol. Similar findings were reported by previous authors (Kloss et al., 2014; Laird et al., 2010; Van Zwieten et al., 2010). The increase in CEC could affect the retention of phosphate by biochar through anion exchange reaction. However, DeLuca et al. (2009) reported that biochar application to soil increased plant P availability by lowering the activity of soluble Al and Fe. The CEC of biochar is mainly influenced by the feedstock type, pyrolysis temperature and aging time (Heitkötter and Marschner, 2015; Bera et al., 2017). Likewise, biochars produced from non-leguminous straws had a higher CEC than those produced from leguminous straws (Jiang et al., 2014). Thus, a critical decision needs to be made concerning biochar feedstock type, pyrolysis temperature, application rate, and biochar age in order to achieve intended soil pH and CEC values suitable for crop production.

5.2. Soil nutrient dynamics

Fig. 3 shows the mechanisms how biochar potentially can improve the retention of macro- and micronutrients in soils, and consequently may improve their availability to plants. While biochar can interfere with the key carbon and nitrogen cycle processes by interacting with relevant microorganisms, it can also participate in the nutrient cycling processes by physico-chemical interactions, such as surface adsorption of various elements (Agegnehu et al., 2017; Bornø et al., 2018; Mandal et al., 2016b, 2018; Xu et al., 2018a). The unique porous characteristics of biochar along with its heterogeneous surface functional groups can take part in diffusion-controlled adsorption of elements, surface complexation and ligand exchange reactions, which ultimately control the plant-available nutrient dynamics in soils (Mandal et al., 2016a; Liu et al., 2013; Nielsen et al., 2018).

In most of the previous studies, total nutrient contents of biochar were reported rather than the plant available nutrient contents (Table 1). However, the entire amounts of nutrients present in biochar are not readily soluble in water. Nutrients in biochar are

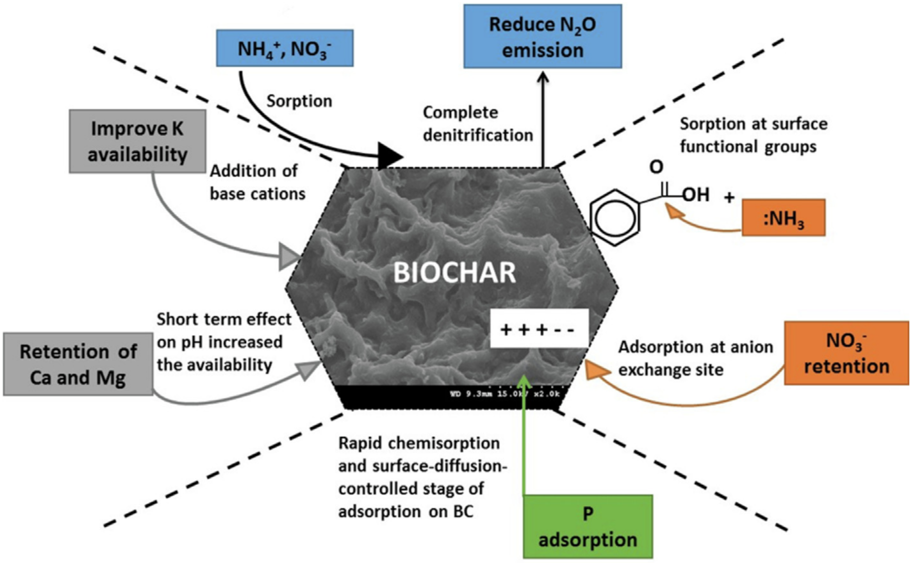

Fig. 3. Schematic diagram representing how biochar improves the retention of macro (N, P, and K) and micronutrients (Ca and Mg) and increases their availabilities in soils.

present either in available or in difficultly accessible forms pertaining to the complex organic and inorganic composition of the material. There is a scarcity of published reports evidencing direct nutrient availability from biochar to crops. The amount of water-soluble nutrients in biochar except K is usually low (Steiner et al., 2010). Bera (2014) reported that water-soluble P, K, Ca and Mg contents in mustard stalk biochar were 13–16%, 65–70%, 14–17% and 23–26% of the individual total nutrient contents, respectively. The remaining amounts of the nutrients existed either as inorganic minerals captivated within the complex organic moiety of C, H, and O, or as an integral component of the organic moiety. Biochar needs to undergo both chemical and microbial decompositions to release these captivated nutrients and subsequently make them available for plant absorption. Gaskin et al. (2010) reported an increased concentration of mineral nutrients (K, Ca and Mg) in maize tissue and soil extracted by Mehlich-1 reagent when peanut hull and pine chip biochar were added to a loamy sand soil in Tifton, Georgia. The impact of peanut hull biochar was more pronounced than pine chip biochar due to the higher contents of K, Ca and Mg in the former, and in the first year of biochar application than the second year (Gaskin et al., 2010). Novak et al. (2009) also found a high concentration of Mehlich-1 extractable P in Norfolk loamy sand soil amended with poultry litter biochar ($4\,\mathrm{Mg\,ha^{-1}}$) containing high total P content (3–4.3%). In another study, soil total N, Olsen-P, exchangeable K, Ca and Mg concentrations increased with cow manure biochar application under maize production in Japan (Uzoma et al., 2011). Following a three years' trial at field conditions, Munda et al. (2018) also reported the possibility of soil fertility enrichment vis-a-vis improved grain yield of rice crop via rice husk biochar application. These are all indirect evidences of enriched nutrient availabilities resulted from biochar addition to soils. Thus, future research needs to be undertaken involving isotopic tracer techniques to measure the availability of plant nutrients directly from biochar, or by comparing the relative contribution of soil and biochar sources with regards to plant-available nutrients.

5.2.1. Effect of biochar on nitrogen dynamics

Application of biochar significantly influences the mineralization-immobilization turnover of nutrients, which is affected by altering both microbial activities and community structure of soils. Since biochar is a C-rich substrate with a high C/N ratio, upon its application to the soil, microorganisms are triggered to decompose the native soil organic matter (SOM) to acquire N via priming effect (Blagodatskaya and Kuzyakov, 2008). Biochar being rich in surface functional groups, including aromatic moieties, can alter cation and anion exchange capacities of soils, which further influences N retention (Clough et al., 2013; Slavich et al., 2013; Mandal et al., 2018). Thus, maize biochar was reported to accelerate soil N transformations by increasing the net N mineralization (Nellisen et al., 2012; Gundale and DeLuca, 2006), accelerating nitrification (Song et al., 2013), affecting denitrification (Cayuela et al., 2013), reducing ammonia volatilization (Mandal et al., 2016b, 2018), and through adsorption of ammonia and increasing NH_4^+ storage in soils (Clough and Condron, 2010).

The transformation of N as impacted by various biochar materials are presented in Table 3. When biochar was added to soil, gross N mineralization, recalcitrant nitrogen fraction and labile N fraction were found to be stimulated (Table 3). This increase was higher in the biochar produced at low temperature (350 °C) than that produced at high temperature (550 °C) (Nellisen et al., 2012). Results showed accelerated soil N cycling following biochar addition, with increased gross N mineralization (185–221%), nitrification (10–69%) and ammonium (NH_4^+) consumption rates (333–508%) (Nellisen et al., 2012). Most of the mineralized NH_4^+ under biochar treatments came from the recalcitrant N in soil, while in the control soil most mineralized NH_4^+ originated from the labile N (Nellisen et al., 2012). This could be due to the biochar induced increase of soil porosity/aeration that stimulates the aerobic/heterotrophic microbial population resulting in the degradation of recalcitrant SOM in the presence of biochar (Anderson et al., 2011). Pereira et al. (2015) reported that the gross N mineralization increased in response to soil-applied biochar materials with high H/C ratios (i.e., Douglas fir wood pyrolyzed at 410 and 510 °C, and hog waste wood pyrolyzed at 600 and 700 °C). The enhancement of N mineralization could be favourable for organic farming systems challenged by insufficient N mineralization during plant growth (Pereira et al., 2015). Studies demonstrated that at least 10% of the ^{15}N added to the soil as ^{15}N labelled pyrogenic organic material (PyOM) (obtained from *Lolium perenne* charred for 4 min at 350 °C) could be utilized by grasses in a Mediterranean agricultural soil within just 72 days of growth (Rosa and Knicker, 2011). This showed a direct evidence that PyOM produced at a low temperature could be easily degraded, and its N would become available to plants (Rosa and Knicker, 2011).

The plausible effects of biochar on soil biological processes can significantly influence soil N transformations. Such effects can be partially explained by biochar properties. For example, biochar could increase the mineralization of recalcitrant soil organic N (Nellisen et al., 2012). The other important mechanisms include an enhanced abundance of ammonia oxidizing microorganisms (Song et al., 2013), and promotion of denitrification by the transfer of electrons to soil denitrifying microbes (Cayuela et al., 2013). For instance, PyOM derived from rye grass pyrolyzed at 450 °C induced a strongly positive priming effect within the first 18 days, and thereafter exhibiting a negative priming effect in a forest Cambisol (Maestrini et al., 2014). The initial increase in organic matter mineralization corresponded to a higher gross N mineralization and NH_4 content in the PyOM-treated soil than in the untreated soil (Maestrini et al., 2014). The effect of biochar on soil denitrification might depend on temperatures at which the product is produced. Compared to the unamended soil, amendment with biochar (produced at 200 °C and 400 °C from oak wood feedstock) significantly increased N_2O emissions, but biochar produced at a higher temperature (600 °C) did not show such effect on N_2O emissions (Zhang et al., 2015).

During the pyrolysis process, N in biomasses get converted to recalcitrant heterocyclic aromatic structures in biochar, and these structural changes may lead to a reduction in C and N mineralization rates (Chen et al., 2014). The mineralized C decreased from 32.7% of the added C of raw biomass to 0.5% in the biochar produced at temperature above 400 °C (Chen et al., 2014). The N dynamics thus shifted from N mineralization in raw biomass to N immobilization in biochar at charring temperature 500 °C (Chen et al., 2014). As such, soil amended with biochar produced at temperatures exceeding 400 °C demonstrated a 25% decrease in dry shoot biomass of water soinach (*Ipomoea aquatica*) compared with unamended soil principally due to N limitation (Chen et al., 2014). Therefore, the C stability of leguminous green manure like *Ipomoea* sp. could be enhanced by converting the raw material into biochar, but the charring process might limit the immediate supply of N. Similarly, corn stalk biochar proved to contain recalcitrant N as indicated by lower decay rate constants (Blum et al., 2013). Application of N-limited biochar may induce microbial immobilization of available N in the soil (Lehmann et al., 2006; Van Zwieten et al., 2009). Soil and biochar mixtures showed evidence of both soil nutrient sorption by biochar, and biochar nutrient sorption by the soil, depending upon the biochar and soil types (Mukherjee and Zimmerman, 2013; Rens et al., 2018). For example, application of willow (*Salix viminalis* L.) branch biochar prepared at 470 °C significantly decreased the available NH_4^+ and NO_3^- levels during 30–90 days in flinty clay loam soils of United Kingdom indicating a net N immobilization (Prayogo et al., 2014). Availability of resin-extractable NH_4^+ and NO_3^- fractions in soil decreased with the addition of wheat straw biochar and olive-tree pruning biochar (Olmo et al., 2016), and this might be governed by the porous nature, high surface area and ion exchange capacity of biochar that can enhance the sorption of NH_4^+ (cation exchange) and NO_3^- (within biochar pores) (Lehmann et al., 2003; Atkinson et al., 2010; Laird et al., 2010; Prendergast-Miller et al., 2014). The rate of N immobilization was significantly higher in the treatment receiving both litter and 2% biochar. Nitrogen deficiency in larch (*Larixgmelinii*) cultivation resulted from the application of Japanese larch wood biochar was also reported (Makoto et al., 2011). The application of hard wood biochar, a mix of white ash (*Fraxinus americana*), oak (*Quercus* sp.), and beech (*Fragus grandifolia*) produced by fast pyrolysis at 500–600 °C with either NPK or digested dairy manure had little effect on N dynamics in Warden silt loam soil of Washington state of USA (Bera et al., 2016).

Leaching of N from soils is a serious problem, especially in light-textured soils, causing environmental pollution and eutrophication. To limit the leaching loss of N from soil, biochars prepared from a variety of feedstocks and at different pyrolysis environments (duration, temperature, heating rate) have been extensively investigated in the recent past (Petersen, 1978; Lehmann et al., 2003; Jones et al., 2012; Zhu et al., 2012). Yao et al. (2012) reported that sugarcane bagasse, peanut hull, Brazilian pepperwood, and bamboo biochars could adsorb 1–12% NH_4^+–N from aqueous solution, and Brazilian pepperwood gave the most effective biochar for NH_4^+ adsorption among these feedstocks. Asada et al. (2002) found a greater adsorption of ammonia (NH_3) by bamboo (*Bambusa* sp.) biochar prepared at 500 °C than that prepared at >700 °C. The NH_4^+ adsorption capacities of commercial coconut shell activated carbon prepared at 600 °C and 400 °C were found to be 2400 and 600–1800 mg NH_3 kg^{-1} carbon, respectively (Rodrigues et al., 2007). Recently Hea et al. (2018) reported that biochar application to soil with urea increased NH_3 volatilization losses by 14.1% in the first rice season, primarily due to increased pH and concentrations of NH_4^+–N in the floodwater, and decreased NH_3 losses in the second rice growth season by 6.8%, probably due to its high adsorption capacity for NH_4^+ and increased nitrification. Application of bamboo charcoal (pyrolyzed at 600 °C) to a variety of sandy silt soils showed a cumulative 15% reduction in NH_4^+–N leaching loss over 70 days (Ding et al., 2010). The adsorption of NH_4^+ on the biochar surfaces was the result of a week van der Waals forces between positively charged NH_4^+ and negatively charged soil or organic matter surfaces (Hale et al., 2013). The adsorbed NH_4^+–N eventually become available to plants or microbes in the long run reducing the loss of mineral N in soils (Taghizadeh-Toosi et al., 2012a, 2012b).

The overall impact of biochar on N transformations in soil is also reflected (positive, negative and neutral) in the post-harvest analysis of soil samples for N contents. Poultry litter biochar and wheat straw biochar, when applied at the rate of 1.0–5.0 Mg ha^{-1} to an acidic Aeronosol and a neutral Vertisol, they did not affect the post-harvest total soil N (Macdonald et al., 2014). However, application of these biochars at 5–10 Mg ha^{-1} to an acidic Ferrasol and alkaline Calcisol increased the total soil N content significantly (Macdonald et al., 2014). Similarly, application of rice husk biochar at 41 Mg ha^{-1} was found to increase total soil N after the harvest of rice crop in an acidic Gleysols of Philippines (Haefelea et al., 2011). The available N content increased in an alkaline sandy loam soil too under the influence of biochar, and the effect was more pronounced for maize stover than wheat straw biochar (Purakayastha et al., 2015). Jones et al. (2012), however, reported that commercially available biochars derived from mechanically chipped trunks and large branches *of Fraxinus excelsior* L., *Fagus sylvatica* L. and *Quercus robur* L. pyrolyzed at 450 °C for 48 h did not affect the dissolved organic N (DON), NO_3^-- or NH_4^+–N contents in the soil. Similarly, biochar addition showed limited effects on the turnover of soil organic carbon, DON and no long-term effect on N mineralization, NH_3 volatilization, denitrification and NH_4 sorption (Clough et al., 2013). In contrast, biochar made from chicken manure increased the available nutrient contents in soils including N (Chan and Xu, 2009; Chan et al., 2008). Peanut shell biochar (5% w/w) promoted the urease activity in a saline soil over short-term laboratory incubation indicating the role of biochar in soil N dynamics (Bhaduri et al., 2016).

Nishio and Okano (1991) reported that biological nitrogen fixation (BNF) at the early stage of alfalfa growth and nodule development stage was 15 and 227% higher, respectively, than the control when biochar (*Eucalyptus deglupta*, 350 °C) was added to the soil. Several studies indicate that biochar serves as an excellent support material for *Rhizobium* inoculants (Pandher et al., 1993; Lal and Mishra, 1998). Rondon et al. (2007) reported that the proportion of fixed N by common bean (*Phaseolus vulgaris* L.) increased from 50% in the control to 72% with 90 g kg^{-1} biochar application. While total N derived from the atmosphere (NdfA) significantly increased by 49 and 78% with 30 and 60 g kg^{-1} biochar applications to the soil, respectively, NdfA decreased by 30% than the control with 90 g kg^{-1} biochar application (Rondon et al., 2007). The primary reason for the higher BNF with biochar additions was the greater B and Mo availability in the amended soil than the unamended control, while a greater K, Ca, and P availability with higher soil pH and lower N availability and Al saturation might have also concurrently occurred (Rondon et al., 2007).

5.2.2. Effect of biochar on phosphorus dynamics

Biochar, produced from common crop residues or unconventional tree species, influences P transformation in soils directly or indirectly by three major mechanisms: (1) being a direct source of soluble P and exchangeable P, (2) modifying the soil pH and ameliorating various elements (e.g., Al^{3+}, Fe^{3+}, Fe^{2+}, Ca^{2+}, Mg^{2+}) that are responsible for making complex with P, and (3) acting as a source of C and energy for enhancing the microbial activities and P mineralization (DeLuca et al., 2009). Many studies reported the increase of P availability via biochar application to soils (Table 3).

Biochar produced at both low and high temperatures (350 °C and 800 °C, respectively) resulted in significant changes in the extractable P pool, with a trend of decreasing extractable P with application of high temperature biochar (Gundale and DeLuca, 2006). Increasing pyrolysis temperature also decreased the water soluble P content in rice, wheat, maize and pearlmillet residue biochars due to the formation of difficultly soluble crystalline P minerals (Bera et al., 2017). The extractable P not only depends on the pyrolysis temperature, but also on the feedstock. For example, Zhang et al. (2016) studied biochars prepared from 9 different residues, and concluded that the Bladygrass (*Imperata cylindrical*) biochar had the greatest amount of extractable P among all the biochars. Similarly, application of biochar (prepared at 400 °C) at the rate of 8.94 g kg^{-1} increased the available P content in a sandy loam alluvial soil (Purakayastha et al., 2015). The application of poultry litter biochar at 20 g kg^{-1} increased Mehlich 1 soil extractable P concentration by 20–28 folds (Novak et al., 2009). Laird et al. (2010) reported that biochar prepared from mixed hardwood feedstock (primarily oak (*Quercus* sp.) and hickory (*Carya* sp.)) increased Mehlich III extractable P in soils (Laird et al., 2010). The total P content ranged 16–9500 mg kg^{-1} for crop residue biochar, 5–6000 mg kg^{-1} for wood biochar, 2950–7.40×10^4 mg kg^{-1} for manure biochar, and 90–23300 mg kg^{-1} for waste material biochar (Table 3). Recently, Xu et al. (2018b) reported that wheat straw biochar application significantly increased (positive effects) various P fractions (except for $NaHCO_3$-extractable P and residual P) in a Haplic Luvisol. The increased soil microbial activity and reduced soil acidity or increased CEC may be accounted for enhanced P transformation in the soil. The reduced $NaHCO_3$-extractable P content may be related to P immobilization with increased soil microbial activity induced by biochar addition because the high C:P ratios of biochar (ranged from 234 to 357) suggested a net P immobilization when biochar was incorporated into the soil (Xu et al., 2018b).

Biochar having high ion exchange capacity might alter P availability by enhancing the anion exchange capacity or by influencing the activity of cations that interact with P (Liang et al., 2006). However, the amount and rate of P adsorption on the surface of ferrihydrite decreased with the presence of biochar (Hao et al., 2011).

The changes in soil P dynamics may vary over time in the presence of biochar. Haefelea et al. (2011) reported that the application of carbonized rice husk biochar increased available P in rice growing soil of International Rice Research Institute (IRRI), Philippines, in the first year, while after three years it did not influence the available P content. In the second cropping year, available P content in the biochar + pyrogallol treated plot was found to increase by 25% over the control (Lashari et al., 2013). Two years after application of biochar prepared from mixed hardwood chips (primarily oak (*Quercus* sp.), elm (*Ulmus* sp.) and hickory (*Carya*spp sp.)) in a fine loamy Hapludols decreased the extractable P at different incubation periods (Rogovska et al., 2014).

Application of 8% maize stover biochar (400 °C) substantially increased soil Olsen-P from 3 to 46 mg kg^{-1} in a Red earth, and from 13 to 137 mg kg^{-1} in a Fluvo-aquic soil in China after a short-term incubation (42 days) (Zhai et al., 2015). These increases were accompanied with a subsequent increase in soil microbial biomass P from 1 to 9 mg kg^{-1} in the Red earth, and from 9 to 21 mg kg^{-1} in the Fluvoaquic soil (Zhai et al., 2015). Researchers indicated that the increase was mainly due to the high concentration of P in the ash fraction of the biochar (77% of total biochar P). Biochar's effect on both soil Olsen-P and microbial biomass-P was increased by higher biochar application rates ensuring lower P-sorption capacity. The maximum concentration of water-soluble P was achieved at the rate of 1% wheat residue biochar (w/w) addition to soils with different textural classes, varying the water-soluble P concentrations from 11 to 253% (Parvage et al., 2013). At higher application rates, P concentrations decreased, which coincided with an increase of soil pH by 0.3–0.7 units (Parvage et al., 2013). The wheat residue biochar can act as a source of soluble P, and low and high additions of biochar showed different effects on soil solution P concentration due to possible reactions of P with Ca and Mg added with biochar. The addition of fresh *Miscanthus* or *Salix* biochar to soil significantly increased soil P contents, but artificially weathered biochars made no such change in sandy loam soil of the Rothamsted Research experimental farm, United Kingdom (Prendergast-Miller et al., 2014). The *Miscanthus* biochar had distinctly larger extractable-P content than the *Salix* biochar (Prendergast-Miller et al., 2014). In sandy soil, addition of biochar produced from mixture of Norway spruce (*Picea abies* (L.) H. Karst) and Scots pine (*Pinus sylvestris* L.) had low P sorption affinity, and thus did not increase the sorption of P in incubated soils (Soinne et al., 2014).

Among different feedstocks, maize biochar showed the highest available P in the soil after one year of incubation followed by rice, pearl millet and wheat biochars (Purakayastha et al., 2015). Rice straw biochar with the higher CEC and the lowest contents of Ca^{2+} and Mg^{2+} showed the greatest inhibition of phosphate adsorption, and thus, could likely be the best choice as an amendment to mobilize phosphate in variably-charged soils (Jiang et al., 2015). The phosphate adsorption in both control and biochar-amended soils decreased with increasing pH. Incorporation of the biochars increased the pH of the amended soils, thereby further mobilizing phosphate in the soil (Jiang et al., 2015). However, Macdonald et al. (2014) reported that both poultry litter and wheat straw biochars applied at the rate of 5 and 10 Mg ha^{-1} did not affect the Olsen's P in an acidic Ferrasol and alkaline Calcisol, but could increase Olsen's P in an acidic aerosol and neutral Vertisol. The interactions between biochar, P fertilizer and P fractionations indicate shifts in potential P availability both as a result of P fertilization and biochar (prepared from green waste at 550 °C) application after harvest of a wheat crop (Farrell et al., 2014). However, in clayey soils, biochar addition increased soil aggregate stability and reduced detachment of colloidal materials, which in turn could be beneficial for erosion control and thereby reducing particulate P losses from agricultural fields.

5.2.3. Effect of biochar on potassium dynamics

Biochar itself is a huge source of K, and it can directly take part in the retention of K in the soil because of having a high CEC (Table 3). Available K contents in both Ultisol and Oxisol after first and second years' of a wheat crop were invariably greater when biochar prepared from Eucalyptus trees (*Eucalyptus camaldulensis* L.) by specialized flash carbonization process was applied to soils (Lashari et al., 2013). Two years of mixed hardwood biochar (primarily oak, elm and hickory) application in fine loamy Hapludols had almost doubled the extractable K content over the unamended soil (Rogovska et al., 2014). In the second cropping year, biochar along with pyrogallol application increased the available K content by 78% over the unamended control (Rogovska et al., 2014). Among the macronutrients (N, P, K), the maximum increase in available pool due to biochar application was observed in the case of K. Purakayastha et al. (2015) found that wheat straw biochar being rich in K contributed in increasing the soil available K. Similarly, Laird et al. (2010) reported that mixed hardwood biochar amendment (oak and hickory) increased the Mehlich III extractable K in soils.

In contrast, application of rice husk biochar at the rate of 41 Mg ha^{-1} did not affect exchangeable K content in soil after harvest of rice crp in an acidic Gleysols of IRRI, Philippines (Haefelea et al., 2011). Nevertheless, evidence showed that excessive application of liming materials including biochar to a coarse-textured low buffering capacity soil might lead to an abrupt increase in soil pH resulting in deficiencies of some plant nutrients (Kamprath, 1971). For example, K deficiency in radish crop due to the application of poultry litter biochar in an acid soil was noticed (Chan et al., 2008).

5.2.4. Effect of biochar on secondary and micronutrient dynamics

Amongst secondary nutrients, S cycle behaves quite similarly as N cycle in the soil (Stevenson and Cole, 1999). Therefore, biochar application could potentially influence the S mineralization in soils like it influences the N transformation (Table 3). Since biochar application influences the pH of soils, S mineralization rates were reported to increase following a fire in a pine forest (biomass converted to biochar by the fire) (Binkley et al., 1992). This effect was probably due to the release of soluble SO_4^{2-} following partial combustion of biomass during the fire or heating event at temperature more than 200 °C (Gray and Dighton, 2006). The maximum leaching of SO_4^{2-} occurred after the application of corn biochar pyrolyzed at 450 °C (11 mg kg^{-1} at the first leaching, corresponding to 29% of the total S added), while the main mechanisms involved in this process were: the abiotic release of mineral S, and the hydrolysis of ester-S mediated by soil enzymes without any observed relationship with CO_2 evolution (Blum et al., 2013). The role of S-forms in the feedstocks (or initial materials) also seemed to drive the S mineralization process (Blum et al., 2013).

Extractable Ca contents increased in both Ultisol and Oxisol after first and second year of wheat crops owing to application of biochar prepared from Eucalyptus tree (*Eucalyptuscamaldulensis* L.) by flash carbonization process (Butnan et al., 2015). However, it showed no impact on extractable soil Mg content when biochar prepared from the same feedstock via traditional kiln or flash carbonization process was applied to the soil (Butnan et al., 2015). Peanut straw biochar pyrolyzed at 400 °C showed significantly higher water soluble Ca and Mg contents in an Oxisol than other straw derived biochars, and rice straw biochar showed the lowest values among various crop straw biochars (Jiang et al., 2015). Rogovska et al. (2014) reported that along with soil available K, soil extractable Ca and Mg also increased in a maize (*Zea mays* L.) crop due to two years application of biochar made from mixed hardwood (oak, elm and hickory) at 500–575 °C. After biochar application, Ca and Mg limiting Savana Oxisol was highly productive due to 77–320% greater Ca and Mg availability, increasing soil pH and decreasing exchangeable acidity (Major et al., 2010).

Slow pyrolysis biochar (550 °C) failed to show any effect on exchangeable Ca content after harvest of a maize crop when applied at the rate of 15.0 g kg^{-1} in a silty Fluvisol, but it became more efficient when the application rate was increased to 100 g kg^{-1} (Borchard et al., 2014). However, the exchangeable Mg content in soil was not influenced by biochar application rate (Borchard et al., 2014). Rice husk biochar applied at the rate of 41 Mg ha^{-1} also did not affect exchangeable Ca and Mg contents after the harvest of rice in an acidic Gleysol in Philippines (Haefelea et al., 2011). Thus, increasing Ca and Mg availability in biochar amended soils would be more realistic in highly acidic Oxisol and Ultisol which are inherently deficient in basic cationic nutrients.

Among the micronutrients, soil extractable Mn and Fe decreased, while Cu and Zn increased due to the application of a mixed wood biochar (Rogovska et al., 2014). Similarly, Borchard et al. (2012) reported that composted charcoal could potentially improve plant available Cu^{2+} in an acidic sandy soil with small organic matter content. Transient effects of biochar on soil pH can overrule the influence of sorption of micronutrient cations on to biochar, resulting in the variable concentrations of trace elements in the soil solution and their availability to plants (Borchard et al., 2012). Biochar prepared from Eucalyptus tree either via traditional kiln process at 350 °C or by flash carbonization at 800 °C significantly increased the soluble Mn concentration (1.39–4.61 mg L^{-1}) in an Oxisol relative to the control (1.12 mg L^{-1}), while they decreased the plant tissue Mn concentration (0.08–0.17 g kg^{-1}) compared to the control (0.41 g kg^{-1}) (Butnan et al., 2015).

6. Pyrolysis conditions, stability and nutrient supplying capacity of biochar

A handful of experimental studies unanimously revealed that the source of feedstock (either plant or animal origin) and pyrolysis environments (duration, heating rate, operating method and temperature) had been the most crucial factors to determine whether the produced biochar would be suitably applied to regulate nutrient dynamics in soils, apart from its other chemical and structural features. Hence, these would decide the applicability of biochar for enhancing crop growth and yield by moderating the soil environment. It is emphasized that temperature generated during pyrolysis define the physical and structural characteristics of biochar (Clough et al., 2013; Zhao et al., 2018). Only few studies concentrated on the characterization of biochar prepared at different ranges of pyrolysis temperatures as well as feedstock materials, and compared the biochar stability and applicability for agricultural uses (Yang and Sheng, 2012; Crombie et al., 2013; Rahman et al., 2014; Zhao et al., 2018). Pyrolysis temperature was also found to be the most influential parameter for obtaining specific characteristics of rapeseed stem biochar, demonstrating a positive relationship of temperature with pH, microporous structure, surface area, fixed C and ash content, whilst showing a negative relationship with material yield, average pore size, functional groups, volatile matter, O and H mass fractions, and the number and density of functional groups (Zhao et al., 2018).

Realising the serious gap of systematically compiled information in published literature about the above, this paper attempted to gather three sets of information after searching across a large number of publications, for: (1) pH and nutrient composition of various biochars produced at different pyrolysis temperatures (Table 1), (2) changes in soil pH and CEC due to application of biochar prepared from various feedstock types, addition rates and pyrolysis temperatures (Table 2), and (3) effects of biochar on nutrient transformations in soil produced at different pyrolysis temperatures (Table 3).

7. Biochar as slow release fertilizer

Fertilizers play a significant role in agricultural production. After application to soils, fertilizers can be lost due to the natural processes occurring in the soil. There has been an increasing interest in using fertilizers, which can release nutrients in soils at a slower and steadier rate over an extended period. Therefore, the use of slow-release fertilizer is a favourable strategy to reduce gaseous and leaching losses of nutrients, especially the losses of macronutrients (N, P, and K) (Wang et al., 2013). Pyrolytic conversion of biomass into biochar has shown an effective impact on reducing nutrient losses (NH_3 volatilization, N_2O emission, CO_2 emission, NO_3 leaching, etc.) from soils, and previous studies found that biochar itself contains nutrients, which help to improve plant growth. It was observed in most studies that nutrients release quickly during the initial period of biochar addition to soils. However, if exogenous nutrients (N, P, and K) was adsorbed on biochar, it could act as a slow-release fertilizer for supplying nutrients (N, P, and K) (Zhou et al., 2015). Kim et al. (2014) observed that lignocellulosic biomass-derived biochar contained low plant nutrients but could be impregnated with additional nutrients and subsequently pelletized, and the final product could control the release of nutrients at a slower rate resulting in a reduced nutrient loss. The slow release was attributed to the physical hindrance in releasing and solubilizing the nutrients through reduced pore size instead of forming any slowly soluble chemical composite (Kim et al., 2014). Wen et al. (2017) prepared biochar based slow release fertilizers (BSRFs) through NH_4^+ absorption on biochar prepared from cotton stalks. Authors found that the application of BSRFs to soil could significantly improve both the water retention and water holding capacity of soils. The BSRFs were also capable of releasing N fertilizer slowly with extended N-longevity, and were more effective in improving total N use efficiency and facilitated cotton plant growth through reducing N loss and improving N retention (Wen et al., 2017). The lowest N-leaching-loss were observed with BSRFs, and the phenomenon was attributed to the fact that BSRFs had better slow-release characteristics and water holding capacity than normal biochar (Gonzalez et al., 2015; Wen et al., 2017). Yao et al. (2011) also found that the phosphate-laden biochar contained valuable nutrients that could act as a slow release fertilizer to enhance soil fertility and sequester C for a longer time in soil. Moreover, physical activation of biochar materials can also make it a slow release fertilizer. For example, Dünisch et al. (2007) found that the mixing of charcoal with ashes and impregnating wood residues with nutrients such as N, P, and K could produce slow release K and N fertilizers. Studies have shown that biochar based slow-release fertilizers with their effective nutrient retention properties can be widely used in sustainable modern agriculture. However, a full assessment of these biochar based slow-release fertilizers, composites, and pellets as slow nutrients (N, P, and K) release fertilizers are needed, for example, field tests are extremely important before the wide application of these materials in soils for supporting plant growth and development.

8. Effect of biochar on crop yield

Researchers observed that biochar application increased, decreased or had a neutral effect(s) on crop yield(s), depending upon soil types, variation in feedstocks and pyrolysis conditions during biochar preparation (Table 4). In majority of the cases, the yield of various crops was enhanced to the tune of 4–144% owing to biochar application, while for few others studies, the yield declined to the extent of 4–24%. Some biochars triggered improved growth with increasing pyrolysis temperatures, though opposite trend was also found (Rajkovich et al., 2012). Therefore, pyrolysis temperature remains an important variable to improve biochar performance for crop yield vis-à-vis soil fertility management. Biochars made from food waste and paper mill waste at lower pyrolysis temperature (300–400 °C) resulted in significant growth reduction of corn (Rajkovich et al., 2012). With increasing pyrolysis temperature, however, the adverse effect of biochar produced from the same feedstock nullified (Rajkovich et al., 2012). On an average, biochar produced at 500 °C showed a better plant growth than those produced at 300–400 °C temperature. Biochar made from poultry litter maintained better plant growth over the control irrespective of application rate and pyrolysis temperature (Macdonald et al., 2014). Across all biochar types, average total biomass production of corn (*Zea mays* L.) was at par for the application rates of 0.2%, 0.5%, and 2%, but reduced to a minimum at the rate of 7% (Rajkovich et al., 2012). Except for the larger application rate (7%), biochar made from corn stover, oak, and pine wood and animal manures exhibited either positive or neutral effect on crop growth, whereas biochar from hazelnut shells did not affect the growth (Enders et al., 2012). Studies emphasized that the positive reflection of agronomic performances under biochar application depends both on soil-biochar interaction and the elemental contents of biochar. However, not only the biochar or soil type, crop choices also can determine the response of biochar as Van Zwieten et al. (2010) found that wheat biomass increased linearly up to a biochar application rate of 10 t ha^{-1}, and decreased with 20 and 50 t ha^{-1}, whereas radish growth did not decrease with high rate of biochar in an acid soil of the tropics. Followed by the increasing macro and micronutrients availability in soil, biochar from mixed hardwood chips (oak, elm and hickory) (pyrolysis temperature: 500–575 °C) increased the grain yield of maize by 11–55% during the first year (Rogovska et al., 2014), presumably because biochar mitigated adverse effects of allelochemicals released from the decomposing maize residues. However, oat (*Avena sativa* L.) yield in an acidic sandy loam soil of Denmark showed no significant response to birch wood biochar application, neither for total biomass nor grain yield (Sun et al., 2014). However, on the same occasion, the total biomass of spring barley (*Hordeum vulgare*) was increased by 11% due to biochar application, though with a non-significant response for grain yield. Maize yield showed a reduction of 22–24% at the single biochar treatment (50 Mg ha^{-1}) which was applied in combination with pig slurry at 21 and 42 Mg ha^{-1} doses (Sun et al., 2014). In acidic sandy soils, the application of rice hull biochar (2% rate) prepared at 350–400 °C increased sugarcane yield in Florida, USA, probably because biochar modulated the nutrient enrichment in the soil (Alvarez-Camposa et al., 2018).

In an acidic aerosol of Australia, both poultry litter biochar and wheat straw biochar demonstrated non-linear trends of biochar application rates with wheat yields (Macdonald et al., 2014). The plant biomass was significantly lower at higher biochar application rates (5 and 10 t ha^{-1}), having a prominent impact on shoot production but also evident in grain yield and root biomass (Macdonald et al., 2014). However, in an acidic ferralsol, a different plant response was evident. The magnitude of plant growth stimulation was more visible by applying poultry litter biochar over wheat straw biochar (Macdonald et al., 2014). More biomass (shoot, root and grain) produced under high rate of poultry litter biochar (10 t ha^{-1}) as compared to wheat straw biochar (Macdonald et al., 2014). Biochar application to a neutral Vertisol had no impact on the plant growth (Macdonald et al., 2014). Besides acidic soils, biochar also proved beneficial in increasing yield of crops cultivated in alkaline soils. Purakayastha (2010) reported that application of biochar at the rate of 1.9 Mg ha^{-1} prepared from wheat straw along with the recommended doses of NPK (180:80:80 kg ha^{-1}) increased the yield of maize in an Inceptisol. Moreover, this treatment was found to be superior for obtaining benefits related to straw reutilization like crop residue incorporation (CRI) and crop residue burning (CRB) in the open field. For both pearl millet and rice, the yields in biochar treatments were at par with those obtained with CRI or CRB treatments (Purakayastha, 2010). In another study, the application of rice straw biochar (prepared at 400 °C) at the rate of 2.25 g kg^{-1} (equivalent to 5.0 t ha^{-1}) along with 100% NPK increased the rice yield by 24.3% in an Inceptisol, and by 31.3% in an Alfisol (Bera et al., 2015). The yield and yield attributing characters of lowland rice was also reported to be enhanced by the combined application of rice husk biochar and flyash supplemented with chemical fertilizers (Munda et al., 2016).

Fertilizer application along with carbonized rice husk (CRH-biochar) improved the grain yields of rice, but the improvement was not always significant and even showed a decline in yield at Nitisol of Siniloan, Philippines (Haefelea et al., 2011). The application of CRH-biochar failed to produce a yield-increasing effect in both anthraquic Gleysols and humic Nitisol in the Philippines (Haefelea et al., 2011). Only in a gleyic Acrisols, the application of CRH-biochar resulted in a higher yield of rice in all four seasons, although the significant increase was only observed in the third and fourth wet seasons (Haefelea et al., 2011). However, Gaskin et al. (2009) found that peanut hull biochar and pine chip biochar failed to show their marks towards crop productivity, and grain yield even decreased for maize crop.

Application of 0, 8 and 20 t ha^{-1} of biochar to a Colombian savanna Oxisol continuously for four years (2003–2006) under a maize-soybean rotation reported that the maize grain yield did not increase in the very first year, but increased in the 20 Mg ha^{-1} plots over the control by 28, 30 and 140%, respectively, in the subsequent years (Major et al., 2010). In that particular experiment, soil pH increased, and exchangeable acidity showed a decreasing trend owing to biochar application. The greater crop yield and nutrient uptake resulted due to more available (77–320%) Ca and Mg in the soil where biochar was applied (Major et al., 2010). Rice yield was increased under biochar treatment in an acidic Anthrosol, and such increase was eventually more (9–28%) in the second cycle than in the first cycle (9–12%) of the crop (Zhang et al., 2012). However, this increment could not be correlated with the biochar amendment rates (Zhang et al., 2012). Biochar can also be composted and be applied in soils for enhancing crop productivity. Application of biochar poultry manure compost and pyroligneous solution to a salt-affected soil for consecutive two years showed an ameliorative effect, decreasing the salinity and pH, and subsequently reflected in increased yield of wheat in a tune of 38% (Lashari et al., 2013).

Biochar behaved differently to crop growth improvement when applied along with fertilizers. Farrell et al. (2014) reported no significant effect on wheat yield at a low application rate (<1.0 Mg ha^{-1}) of biochar in highly P-constrained calcareous soil, but a prominent effect of both biochar and fertilizer on P fractionation was observed. Similarly, applying N fertilizer proved beneficial to rice grain yield when 4.0 and 8.0 Mg ha^{-1} rates of two commercial biochars prepared from wood feedstocks (e.g., teak (*Tectona grandis* L.) and rosewood (*Pterocarpus macrocarpus* Kurz)) were applied in a study reported from northern Laos, but at higher dose of biochar (16 Mg ha^{-1}) with N-fertilizer no positive yield response was observed (Asai et al., 2009). Higher grain yields in biochar treated plots (4.0 and 8.0 Mg ha^{-1}) with N fertilizer resulted due to the combined effects of the improved soil physical properties and the alleviation of biochar induced soil N availability (Asai et al., 2009). Biochar (prepared from 80% varied hardwood and 20% varied coniferous wood chips at 750 °C) and biochar-compost treatments induced only small, economically irrelevant and mostly non-significant effects vine productivity in a poorly fertile, alkaline, temperate soils of Switzerland (Schmidt et al., 2014). However, yield reduction at a high rate of biochar application (16 Mg ha^{-1}) was resonated to N limitation even with N fertilizer application (Asai et al., 2009). Contrary to this observation, Zhang et al. (2012) found maize yield increased by 15.8% and 7.3% without N fertilization, and by 8.8% and 12.1% with N fertilization under biochar amendment at 20 and 40 Mg ha^{-1}, respectively, in a calcareous flavor-aquic loamy soil. In an earlier study, Chan et al. (2007) also found the positive interactive effect of biochar (doses at 50 and 100 Mg ha^{-1}) with N fertilizer (100 Mg ha^{-1}) on radish yield in a hard setting Alfisol. Improvement in soil physical properties along with pH, organic carbon and content of exchangeable cations were the reasons suggested for the higher radish yield. Recently, Ain et al. (2016) reported that application of biochar prepared from a weed (*Parthenium hysterophorus* L.) at 370–417 °C temperature to a rice-wheat cropping system could cut down the cost of fertilizer to half although the yield obtained was just as good as with full application of recommended dose of fertilizers.

In many instances, biochar behaved as a neutral amendment as far as crop yield enhancement is concerned. The bioavailability of N in a wheat-straw biochar prepared at 400 °C was reported to be very low, and did not increase growth of rice crop or nitrogen use efficiency from fertilizer sources during the first year after application (Xie et al., 2013). Biochar was added to an agricultural field at three different doses (0, 25 and 50 t ha^{-1}) and planted with maize (1st year) and grass (2nd and 3rd years) in an acidic sandy loam soil where the biochar addition affected plant performance in the grass crop with significant increase in foliar N (2nd year) and aboveground biomass (3rd year), but biochar treatment behaved neutral towards the maize crop yield (Jones et al., 2012). Another study reported that short-term application of biochar amendment had a positive effect on soil quality in rice cultivation across a wide range of climates and soil types in China, though no significant effect of biochar amendment on rice yield was found (Huang et al., 2013). In contrast to biochar amendment, N fertilizer proved less effective for improving soil quality, but more effective for increasing the rice yield (Huang et al., 2013). More interestingly, the same study further hinted that biochar amendment showed an additional benefit on rice yield under N fertilizer application, and there was a close relationship between the effect of biochar amendment on rice yield and agronomic N use efficiency. Another investigation dealing with large volume application of biochar (30 and 60 Mg ha^{-1}) on durum wheat in the Mediterranean climate showed positive effects (up to 30%) on biomass production and yield, with no significant differences in the nitrogen content of grains (Vaccari et al., 2011). Moreover, no difference between the two biochar treatments were identified, suggesting that even the very high biochar application rate promoted plant growth with a non-detrimental effect (Vaccari et al., 2011).

Biomass production of the N-fixing bean (*Phaseolus vulgaris* L.) was significantly higher than that of the non-N-fixing isoline across all levels of biochar (*Eucalyptus deglupta,* 350 °C) additions. Biochar additions significantly increased total biomass production by 39% at a defined biochar dose of 60 g kg^{-1}, but decreased biomass at par with the control with a higher biochar dose (90 g kg^{-1}). The increase in biomass production by the N-fixing bean was mainly attributed to the greater leaf biomass. Such responses confirmed earlier results with moong bean [*Vigna radiata* (L.) R. Wilczek], soybean [*Glycine* max (L.) Merr.], and pea (*Pisum sativum* L.) (Iswaran et al., 1980), or with cowpea (*Vigna unguiculata* L.) and rice (*Oryza sativa* L.) (Nehls, 2002; Lehmann et al., 2003). Biochar additions at a rate of 15 t ha^{-1} resulted a remarkable difference in plant biomass of bean (*Phaseolus vulgaris* L.) over the control showing an average of 262% increase in shoot biomass, 164% increase in root biomass, 3575% increase in nodule biomass, and 2126% increase in N derived from the atmosphere (Güereña et al., 2015).

9. Principal component analysis to evaluate biochar's effect on soil chemical properties and crop yields

The soil chemistry variables d_pH (change in soil pH) and d_CEC (change in soil CEC) were generated by difference of treatment and control measurements for soil pH and CEC, respectively. Mean value substitution was performed on missing CEC values on some of the measurements, resulting in a total number of cases analysed at 48. The variable representing yield change was generated by difference of treatment and control measurements for crop yield, with yield inhibition represented as negative yield, resulting in a total number of cases analysed at 36.

The PCA scatterplot of points for soil chemical properties in the plane of the first two principal component axes is presented in Fig. 4a. The total variance explained by the first two principal components was 74.3%. The first principal component, accounting for 39.3% of the variance in the dataset, exhibits loadings dominated by biochar application rate and change in CEC (Table 5). The second principal component, accounting for 35% of the variance in the dataset, exhibits loadings dominated by pyrolysis temperature of biochar and pH adjustment of the soil. The latter principal component shows an inverse relationship between [pyrolysis temperature and pH] and [loading rate and CEC].

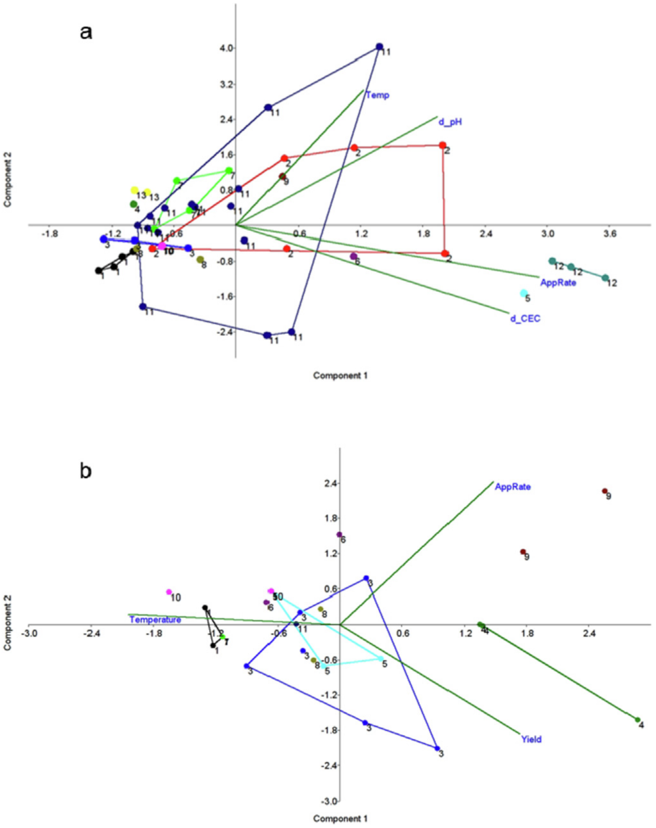

Fig. 4. Principal Component analysis with respect to soil type for effects of biochar on (a) soil chemical properties, and (b) crop yields. Point groups are enclosed by convex hulls. Numbers within the figure represent soil types. In Fig. 4a 1–Acrisol; 2–Alfisol; 3–Anthrosols; 4–Calcarosol; 5–Cambisol; 6–Chernozem; 7–Entisol; 8–Eutric Cambisol; 9–Ferrosol; 10–Halpudept; 11–Haplustox; 12–Planosol; 13–Stagnosol. In Fig. 4b 1–Acidic Aeronosol; 2–Acidic clay loam Ultisol; 3–Acid Ferrasol; 4–Acidic Oxisol; 5–Acidic sandy clay loam; 6–Acidic silty; 7–Alkaline Calcisol; 8–Alkaline sandy loam Inceptisol; 9–Neutral clay loam Oxisol; 10–Neutral Vertisol; 11–Slightly alkaline sandy loam Inceptisol.

The projections of the variable axes onto the plane of the first two principal components (Fig. 3a) reveals that all axes exhibit some positive correlation with each other. The highest pairwise correlations exist between (i) pyrolysis temperature and pH change in soil, and (ii) between biochar loadings and change in CEC of soil. These observations may be explained by increased temperature of biochar pyrolysis resulting in modifications of the types of chemical functional groups (acidic versus ketonic) on the biochar carbon skeletons, which would modify the basicity of the biochar and thus the resulting pH of the soil which was amended by the biochar (Mandal et al., 2016a,b, 2018). The relationship between loading rate and CEC may be explained by noting that the more oxygen-containing functional groups in a soil, the higher the CEC, thus the greater loading of biochar containing the functional groups the greater the CEC (Schmidt and Noack, 2000). The points in Fig. 3 are grouped with respect to soil type, with convex hulls enclosing the groups of points. Points group well with respect to soil type, suggesting that the original chemistry of the soil has a strong component in pH and CEC modification of the soils when amended by biochar.

The PCA scatterplot of points for crop yields in the plane of the first two principal component axes is presented in Fig. 4b. In this case, the total variance explained by the first two principal components was 76.8%. The first principal component, accounting for 45.9% of the variance in the data, was dominated by pyrolysis temperature of biochar, but contained appreciable components of application rate and crop yield modification. The second principal component, accounting for 30.9% of the variance in the data, exhibited no appreciable dependence on pyrolysis temperature, and was instead dominated by application rate and yield, which display an inverse relationship. This suggests an explanation counter to expectations that greater application rates of biochar result in lower stimulation of crop yield. There was some structure evident in the groupings of points in this analysis by soil type, suggesting that plant yield was influenced by soil type also. There was unexplained variance of 23.2% of the dataset that was neglected from the above analysis. It is likely that the low sample numbers and high diversity within the samples is such that not much information may be derived from the temperature-application rate-yield dataset by PCA.

10. Conclusions and future research directions

Biochar can act as a source of nutrient(s) for plants; it has its distinct, physical, physico-chemical and cation exchange properties, which can interact with native soil nutrients and added nutrients in the forms of fertilizer and manures. Therefore, biochar may influence the supply of nutrients to the plants. From the array of published research papers, we discussed in the review, the yield response of crops and nutrient releasing behavior in soil due to biochar application largely depends on the composition of biochar (i.e., feedstock, pyrolysis temperature of biochar preparation) and specific soil type. The majority of biochar is alkaline, except a few like oak and yellow pine chipped biochar, which is acidic.

Many studies showed that biochar significantly influences the mineralization/immobilization turnover of N in soil thereby controlling the N availability without any definite conclusion. However, biochar produced from manure sources being rich in N and other essential nutrients and having narrow C: N ratio could be of higher agronomic value. The majority of the studies showed biochar application increased the P and K availability in soil, and the

Table 5
Eigenvalues, percentage of variation explained by the principal components, and Eigenvectors of Principal Component Analysis (PCA).

Eigenvalues and percentage of variations						
Principal component	Soil chemical properties			Crop yields		
	Eigenvalue	% variance	% variance (cumulative)	Eigenvalue	% variance	% variance (cumulative)
1	1.57	39.3	39.3	1.38	45.9	45.9
2	1.40	35.0	74.3	0.93	30.9	76.8
3	0.62	15.5	89.8	0.70	23.2	100
4	0.41	10.2	100	–	–	–

Eigenvectors							
Principal component	Soil chemical properties				Crop yields		
	Temp	AppRate	d_pH	d_CEC	Temp	AppRate	Yield
1	0.27	0.64	0.43	0.58	−0.67	0.48	0.57
2	0.67	−0.25	0.54	−0.43	0.06	0.79	−0.61
3	0.51	0.48	−0.65	−0.29	0.74	0.37	0.56
4	0.46	−0.54	−0.32	0.63	–	–	–

positive effect was achieved at lower pyrolysis temperature over higher pyrolysis temperature. The mechanism through which the positive impacts of biochars on P and K is not clear yet. Therefore, more research efforts are needed to identify the mechanistic pathway by which soil P and K transformations are being impacted. For other secondary nutrients, there was a mixed response on their availability due to biochar application.

Biochar has positive, negative as well as neutral effects on crop productivity. Biochar showed a positive impact on crop productivity when it was applied to acid soil. However, at a higher rate, biochar might decrease the yield of crops and mostly that could be somewhat complemented by application of fertilizers along with biochar. The biochar application has the potential to improve soil quality, but it is highly dependent on inherent soil properties, fertility and fertilizer management history for that specific piece of land. On the other hand, the negative behavior of biochar towards both nutrient availability and crop productivity demands further insight and thus investigations to find out the most probable reasons for such effect. Therefore, before recommending the application of biochar to a soil under specified crop management, the long-term study is needed along with the clear understanding of the outcome, out of biochar application. Therefore, long-term field scale pilot experiments should be conducted to resolute the following: Impacts of specific biochar properties on crop yield and how these impacts change across soil types, environmental conditions and agronomic management practices with judicious choices of the control treatment. Judicious selection of control is utmost necessary to unify the treatment effects across differential experimental units such as temperate vs. tropical soils; grass land vs. forest soils; or Oxisol vs. Inceptisol, etc. Moreover, the potential of C sequestration benefit and other soil ecosystem services as provided by biochar should be considered while recommending for field applications.

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Author contributions

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Review

Determining Food Stability to Achieve Food Security

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Abstract: Food security, as part as public health protection, constitutes one of the main objectives for countries aiming to ensure the health of all their citizens. However, food security is compromised worldwide by conflict, political instability, or economic crises, both in developed and developing countries. Conversely, because of the importance of agriculture to the economies of rural areas both in developed and developing countries, this sector can contribute to improving food stability, as well as to furthering food security. Thus, livestock and traditional meat products represent a key factor in ensuring food availability. Overall, biosecurity measures improve animal welfare by decreasing the occurrence of diseases that compromise the stability by causing fluctuations in the availability of meat and animal-derived food products such as milk, eggs, or traditional fermented products. As a consequence, an absence of biosecurity measures affects food security (in its quantitative definition, as described above) as well as the productive, sanitary, and environmental sustainability of the rural environment. Products of animal origin support local trade and the regional economy, while contributing to the availability of foods without great external dependence. The manufacture of foods of animal origin aims to create products that are durable and that maintain food availability for long periods of time, even during seasons with scarce resources. Thus, dry-cured or fermented meat products play an important role in food availability. Food security also refers to food access under healthy economic conditions; therefore, knowledge of the main tools that guarantee the safety of these kinds of food products is essential to achieving food stability and further food security.

Keywords: food safety; food security; livestock; biosecurity; food stability; fermented products

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1. Introduction

Food security, as a part of public health protection, constitutes one of the main objectives countries aiming to ensure the health of all citizens. Thus, to achieve this objective, specific policies related to several aspects of food production, such as the hygienic conditions of food processing, packaging, shelf-life, or food labelling information is mandatory in every country worldwide [1]. In developed countries, consumers themselves demand the safety of the foods that they consume, which is associated with the social impact caused by recent food scandals, such as the horse meat scandal, the presence of melamine in milk, or the bovine spongiform encephalopathy [2,3]. Additionally, consumer concerns regarding chemical hazards in food have arisen in the last few years [4].

To address the issue of food security, it is necessary to define the concept, which is complex. The term "food security" has different connotations depending on whether it is referring to rural or urban areas, as well as to developed or developing countries [5]. Thus, food security has a quantitative dimension, which concerns the availability of enough safe and nutritious food, and a qualitative dimension, which concerns the sanitary conditions of food production and processing. Consequently, several concepts arise, such as food security, food safety, food defense, or food nutritional quality. Although these terms have inter-linked aspects, they describe different situations [6].

Food safety refers to handling, preparing, and storing food in a way that best reduces the risk of individuals becoming sick from foodborne diseases [7]. The concept of food security can be defined as the ability to obtain and/or to store basic food products to guarantee that food is available for consumption, saving fluctuations and prices [5]. Food defense refers to the protection of food products against intentional adulteration by biological, chemical, physical, or radioactive agents [8].

Another alternative definition of food safety has been proposed based on the quantification of its effects through the ultimate manifestation of food insecurity, which is malnutrition. Thus, the concept of food and nutritional safety can be defined as the guarantee that individuals, families, and the community as a whole have access at all times to sufficient safe and nutritious food, mainly produced in-country under conditions that are competitive, sustainable, and equitable, so that the consumption and biological use of the foods provides the public with optimal nutrition that supports a healthy and socially productive life, while respecting cultural diversity and consumer preferences [9].

The current review adopted the definition of food security that was established in the Plan of Action of the World Food Summit, which was signed in Rome in 1996 [10]. Food security is achieved when people always have physical and economic access to safe and nutritious food that satisfies the nutritional needs and preferences which are necessary for leading an active life.

According to the concepts provided by the Food and Agriculture Organization (FAO) [11], the term "food security" encompasses four basic pillars: (i) *food availability*: the existence of enough quantities of food of adequate quality, supplied through domestic production or imports (including food aid); (ii) *food access*: people's access to adequate resources to acquire appropriate food and a nutritious diet; (iii) *utilization*: biological use of food through adequate nutrition, drinking water, sanitation, and medical care, to achieve a state of nutritional well-being in which all physiological needs are satisfied, a concept that highlights the importance of non-food inputs in food security; and (iv) *food stability*: a population, a household, or a person must always have access to adequate food in order to have food security. People should not be subjected to the impossibility of being able to acquire foodstuffs due to sudden problems, such as economic crisis, climate crisis, or cyclical events, such as seasonal food inaccessibility. In this way, the concept of stability refers to both the availability and access of people to secure sources of food [12].

It should be noted that access and availability of foodstuffs is not, as a rule, a problem in developed countries. In these societies, the concept of food security would be focused on food safety and relationship between food and health, keeping in mind the growing expectations regarding nutritional quality and the new functional properties of foods. However, the food security concept in developing countries mainly refers to the food supply as mentioned above.

To guarantee the access and availability of foods, the FAO adopts the "two-component approach" to combat hunger, combining agriculture and sustainable rural development throughout specific programs [13]. The objectives of these programs are aimed at increasing direct access to foods (food security) to the most sectors to guarantee a stable supply (food stability). Based on the theoretical framework of the two-component approach, seven principles are the basis of the FAO's overall strategy [14]:

1. *Attention to food security:* ensure that objectives related to food security are incorporated into national strategies to reduce poverty, while considering each strategy's impact at the country-, sub-national-, household-, and community-level;
2. *Promotion of sustainable and broad-based agricultural and rural growth:* foster environmentally and socially sustainable development as a cornerstone of economic growth;
3. *Attend to the entire rural area:* consider, in addition to agricultural production, other opportunities to enhance the economy and income apart from farming;
4. *Attention to the main causes of food insecurity:* improve productivity together with accessibility to the land and human resources;
5. *Attention to the urban dimensions of food insecurity:* address the factors responsible for urban poverty and increase food security in terms of food availability and access, food marketing, management of natural resources, and accessibility to basic services;
6. *Attention to political issues:* take into account national and international policies and issues that impact the implementation and potential results of food security programs, including aspects such as public politics, peace, security, trade, and macroeconomic reforms;
7. *Encouragement of all stakeholders:* engage all in food security-related dialogue that leads to the development of national strategies and ensures broad agreements on common food issues, objectives and solutions.

2. What Measures Can We Apply to Improve Food Stability?

After years of increasing global food security, hunger in the world is rising again. This rise is mainly concentrated in countries affected by conflict and fragility where violent conflict destroys crops and assets, and displaces people. However, the recent economic crisis caused by COVID-19 may lead to a scenario of food insecurity related to a decrease in the economic income of families derived from the increase in unemployment [15]. Conversely, through the importance of agriculture in the economies of rural areas in both developed and developing countries, this sector can contribute to an enabling environment for increased stability. In particular, promoting the resilience of (rural) food systems can serve to increase the food security of households and communities in the face of instability [16]. One measure to ensure food stability is the availability of enough food for local population. Most rural areas have both agricultural and livestock potential, but sometimes the problem of ensuring adequate food availability is associated with a lack of basic infrastructure, human resources and lack of training and education. Additionally, the development of peri-urban landscapes has increased in recent years, contributing not only to aesthetic and environmental sustainability, but also to increasing the networks between rural farms and cities, improving the local economy, and enhancing the value of local (including traditional) products. These strategies also ensure food stability in urban areas [17].

To achieve a better life and sustainable future for everyone, the United Nations, by the resolution adopted in the General Assembly on 25 September 2015, stated 17 sustainable development goals (SDGs), with 169 measurable targets to be achieved by 2030 [18].

In the 2030 agenda, food is one of the most important topics discussed, highlighting the importance of the food sustainability concept. Although concepts such as food security, food availability, or food stability have been described above, the FAO document refers food sustainability in a multidimensional way in which elements such as productive practices, malnutrition or food habits are discussed as a transversal phenomenon related to human health [19].

Food is mentioned, directly or indirectly, in most of the 17 SDGs and their associated specific goals. Thus, SDGs "End of poverty" and SDG 2 "Zero hunger" are those that describe the food sector focused on problems derived from malnutrition and food insecurity. The SDGs do not provide a specific definition of food sustainability; therefore, a lack of guidelines about the implementation of sustainable food policies may hinder food stability.

Previous research has referred to food stability as a set of socio-political aspects (for example, regional conflicts), but specific aspects of animal and/or agricultural production applicable in any production system worldwide have not been defined. Thus, in this paper, two aspects to guarantee the food stability at rural areas will be discussed: livestock production in small farms, and techniques to guarantee food safety in products of animal origin.

2.1. Food Stability at Livestock Production to Achieve the Food Security

Livestock produce a wide range of nutritious, protein-rich foodstuffs such as eggs, meat, milk and cheese, thus diversifying the diets of families. Many animals can be used for weed control and are a vital source of traction power, maximizing the amount of land that can be cultivated and transporting goods to market. In all these different ways, livestock contributes to family health and are part of the family farming business [20].

In developed countries, livestock production is highly technical to obtain high meat or dairy yields. However, this type of production is economically unviable in other regions, due to various factors such as geography, animal adaptation to the environment, technical means or human resources [21]. Although the idea of food stability is associated with developing areas, it is also increasingly important in rural areas of developed countries where small-sized and family farms represents the main work and local economic activity. In addition, increasing green consumerism by consumers implies the implementation of programs to preserve this type of production and guarantee the availability of local foods (i.e., ethnic food) and greater food stability [22].

Thereby, to guarantee food availability, it is necessary to apply the know-how of modern livestock production. However, this knowledge must be adapted to small-sized livestock farms, usually based on extensive management (most of them are familiar farms), both in rural areas of developed and developing countries. The main question is: how do we guarantee the food stability? In order to guarantee livestock in a homogeneous way over time and in a specific geographical region, the application of biosecurity measures is essential to guarantee food stability [23].

Biosecurity can be defined as a set of measures aimed at preventing the entry of a disease into farm [24], as well as preventing the spread of a disease outside a farm in the case of an outbreak. Thus, the concept “biocontainment” (or internal biosafety) or infection control is also related to the concept of biosecurity, and it is defined as a set of measures aimed at preventing the spread of an infectious agent that arises at the farm [25].

Why are biosecurity measures important to guarantee food stability? Biosecurity in animal production represents an important concept because livestock diseases translate into great economic losses. The implementation of biosecurity measures optimizes production costs because it improves animal welfare, reduces the risk of zoonoses, and controls livestock diseases [26]. In general, biosecurity measures improve animal welfare by decreasing the occurrence of diseases that compromise stability by fluctuations in the availability of meat and animal-derived products such as milk or eggs. Consequently, the absence of biosecurity measures affects food security (in its quantitative definition as described above) as well as the productive, sanitary (i.e., health status) and environmental sustainability of the rural environment [27]. Moreover, the lack of biosecurity measures regarding zoonotic diseases may affect farmers’ health, compromising the workforce in a geographical area that may lead to a decrease in animal production and a greater lack of food stability [28]. Additionally, it has been suggested that zoonoses display a greater negative impact on livestock than crops [29].

Biosecurity plans should be considered as a preventive strategy and should be referred to as an investment and not as an expense [30]. Additionally, they should be implemented as part of the daily work and management of the farm. It is important to highlight that food safety is highly dependent on animal health, because certain zoonoses, such as salmonellosis or listeriosis, can be transmitted to humans through food.

The implementation of biosecurity plans in large farms has some advantages due to the existence of specific equipment and facilities that enable keeping specific infectious disease under control easily, as well as the existence of human resources. In contrast, its application in small-sized farms may be conditioned not only by a scarcity of equipment, human resources or facilities, but also by the type of production (extensive management) and the environment.

The implementation of biosecurity measures aims to obtain economic income with lower morbidity (fewer veterinary treatments), less mortality (economic losses due to casualties) and higher meat and/or dairy yield based on correct welfare measures [31]. Biosecurity plans must be specific for each farm; however, some generic measures should be applied in all livestock farms, as described below. Additionally, we discuss how each measure influences food stability.

Farm limits: this control aims to avoid contact with other animals [32]. This measure is easy to implement in intensive farms but almost impossible to apply in extensive livestock management. On those farms that have isolated and fenced pastures near stables, limited contact with other animals can be achieved. However, on small farms whose premises are within villages, this measure is impractical because livestock share paths and roads to move from premises to communal pasture areas. Moreover, sharing males for reproduction in developing countries, where there is scarce veterinary control of reproductive disorders such as brucellosis or Q fever (i.e., Coxiellosis), remains a common practice. Although implementation of the "farm limit" biosecurity measure is impossible in some types of specific livestock management (e. g. extensive management), the implementation of some measures such as avoiding lending males or the geographic distribution of livestock in a village in separate areas to minimize their interactions may contribute to the control of infectious and zoonotic diseases (and the health of farmers), ensuring food stability.

Facilities and equipment—design and hygiene: livestock facilities must be designed according to the species, management (extensive or semi-extensive), production (meat or dairy) and size of the farm. Farm premises must be built with resistant, durable materials that are easy to clean and disinfect to ensure animal welfare. Correct bedding material and maintaining the proper hygiene of premises avoids an excess of insects inside, reducing the risk of disease transmission, decreasing the immunity of animals, and preventing foot problems. Poor hygiene conditions make animals more susceptible to infectious processes with great economic losses. In those geographic areas where extensive management predominates, livestock are reared in communal pastures during the temperate season (spring and summer) and return to the farm in the cold season (autumn and winter). In this type of management, proper premises (including watering points) are located in the communal areas according to the species, livestock population, access paths or climatologic characteristics. The objective of communal premises is to provide and guarantee the necessary welfare conditions to the livestock, such as protection from cold or rain and providing drinking water. Therefore, the implementation of maintenance programs of livestock premises located in communal pasture areas is essential. It is important to remark that the previous biosecurity measure "farm limits" is not applicable here, although this management technique may improve the sustainability of silvopastoral communities [33] and food stability in a specific area.

Control of animal movement: the objective here is to prevent the entry of diseases into farms. In countries with measures such as livestock disease surveillance programs, the existence of traceability systems and veterinary controls, among others, reduces the chance of infectious diseases entering the farm. However, in developing countries in which there is an absence of livestock identification as well as an absence of veterinary controls, and other criteria such as in-breeding, the purchase of livestock with good body conditions, an absence of clinical signs, or the purchase of livestock from the surrounding area, among others, must be considered. Even in the absence of control measures, good livestock practices such as prophylactic treatments (e.g., deworming) or quarantine periods of purchased livestock are always recommended. Livestock movements are essential to guarantee trade [34], but in the case of disease outbreaks, it impacts not only the local economy, but also food stability related to a lack of supply due to the compulsory slaughter of livestock in some cases. Thus, farmers are responsible for the livestock they buy, even in those countries where surveillance programs are implemented (because they only control for specific diseases).

Feed and water: these must be adapted to each animal species and provide enough quantity and quality. Thus, feed shortage seasons such as winter should be considered to avoid an instability of foods of animal origin. Feed can affect the health of the livestock; therefore, it must be stored appropriately. Based on the objective of guaranteeing food stability, farmers must program possible shortages associated with climate changes, price increases, or outbreaks of plant diseases, for example. Thus, new sources of feed such as insects, food waste or plant by-products may be utilized to address the challenge of food security and the health and welfare of livestock while maintaining environmental sustainability [35–37].

Control of external vectors: this represents a potential source of diseases for farm animals and its control is a real challenge. Total control is almost impossible; therefore, the application of some measures contributes to reducing the risk of disease transmission. External vectors are classified as domestic or wild. Domestic vectors include all animals that enter the farm voluntarily, such as dogs, cats, horses or chickens. Wild vectors include all animals that enter the farm involuntarily, such as insects, birds, rodents or wildlife. The presence of only one species in a farm is recommended, although in almost all family farms, the presence of dogs, cats, chickens, rabbits, horses or donkeys is also frequent. What does this mean for avoiding sanitary problems? All species must be subjected to an appropriate veterinary management regimen. Insects represent a source of contamination and disease transmission. Although total control is almost impossible, the application of some measures such as correct manure elimination, installation of mosquito nets on windows, or the application of insecticides can reduce the incidence of insects. Rodent control is also essential on farm, not only to avoid disease transmission, but also due to the contamination of feed and/or water by urine and/or feces [38]. Rodenticide application, as well proper feed storage, can reduce the negative effect on animal health and farm management. As described for rodents, birds can act as vectors of disease transmission through contact with their feet, feathers and/or contamination of food and/or water through feces. Control here involves the correct maintenance of premises and other farm facilities. As previously described, the proper health management of livestock is essential to guarantee food stability [39].

2.2. Food Stability of Products of Animal Origin to Achieve Food Security

Once the importance of the proper management of livestock has been discussed to guarantee the food supply and therefore its stability, the manufacture of products of animal origin maintains local trade and economically supports the region, as well as contributes to the availability of food without great external dependence [40]. Thus, food security can be guaranteed through food stability.

Local and traditional products are increasingly appreciated in developed countries as consumers seek more natural and less processed products. Furthermore, in developing countries, these types of products are part of the daily diet habits [41]. Livestock represent the main source of fresh meat, and other derived products such as eggs, milk or meat-based products are also important in maintaining food availability and play an important role in the nutritional support of the local population.

Regarding the 2030 agenda, food safety aspects are related to SDG 3 "good health and well-being", SDG 6 "clean water and sanitation", and are also related with SDG 2 "zero hunger", because the unlimited access to enough and nutritionally appropriate food to provide the energy and nutrients needed to maintain an active and healthy life must be through the distribution of safe food. In fact, food safety and food security must be considered together [42].

It is important to remark that basic supplies such as electricity or water are not always available in some areas of the world. Thus, the need for food conservation processes with scarce technical resources (e.g., an absence of freezers or fridges) to avoid a lack of food availability must be associated with food processing aiming to extend the shelf-life. Therefore, cured or fermented meat products play an important role in the availability of food.

Meat fermentation is a low-energy, biological acidulation, preservation method, which results in unique and distinctive meat properties, such as flavor and palatability, color, microbiological safety, tenderness, and many other desirable attributes of these products. Changes from raw meat to a fermented product are caused by "cultured" or "wild" microorganisms, which lower the pH [43]. Dry-cured meat products were traditionally produced by the chance contamination of sausages with local microorganisms. Moreover, the manufacture of dry-cured meat products can be achieved even with few technical resources. However, food safety (with its hygienic definition), must be ensured to avoid foodborne outbreaks. Thus, we discuss the different processes and/or techniques commonly used in the manufacture of dry-cured meat products to guarantee safety.

These types of products originated several thousand years ago, in different parts of the world, when microorganisms were introduced incidentally into local foods. It is believed that more than 10 millennia ago, in the Middle East, fermented foods were discovered by keeping milk in animal skins, which produced pleasant, fermented milk drinks and yoghurts [44]. Traditionally, fermented meat processes have been one of the most utilized food conservation methods. The drying of foodstuffs was probably the first development prior to meat fermentation [45]; smoking and salting were also found to be effective. The early manufacture of these products most certainly occurred accidentally [46]. Records showed that Greeks and Romans used this technique thousands of years ago [46,47]. Initially, fermented meat products were produced empirically [45], but it was not until the 1950s that industrialization and developments in the field of microbiology revealed the process which occurs [47]. Initially, fermented meat products were homemade. Nowadays, most fermented meat products are produced industrially, although there are still areas where these products are processed traditionally and represent a significant economic benefit for rural areas [48].

Dry-cured meat products consist of a mixture of comminuted meat and fat, salt, nitrates/nitrites, sugar and spices that are stuffed into casings, subjected to fermentation, and dried; this contributes to its firmness and flavor [43]. Each ingredient has a special role in the fermentation process. Salt solubilizes muscle proteins, increases osmotic pressure in such a way that spoilage bacteria are suppressed, and enhances the flavor. Sodium nitrite promotes the typical color of preserved meats by forming nitric oxide compounds by reactions with the heme group of myoglobin. In addition, it contributes to flavor as well as inhibiting the development of pathogens such as *Clostridium botulinum*. Seasoning with herbs and spices was developed to serve as flavoring agents and also for their antimicrobial properties [49]. Most traditional homemade fermented meat products, both in developed and developing countries, are made without use of technological additives such as nitrate and/or nitrites (NO_x^-). Although the role of NO_x^- has mainly been associated with improving safety against *C. botulinum*, as previously described, other contemporary health concerns such as nitrosamines has emerged in recent years due to their potential carcinogenic effects [50]. Based on the increasing consumption of natural, additive-free products, new research about other natural nitrate-like additives has been studied [51].

Briefly, the manufacture of dry-cured meat products is as follows: comminuted meat and fat, mixed with salt, nitrate/nitrite and spices is stuffed into a casing. Then, the stuffed sausages are held under appropriate conditions to promote the curing process. The sausages are subjected to a drying process, during which, several changes occur regarding the physical, chemical and microbiological composition, which leads to acquiring the final organoleptic characteristics of the product [52].

Dry-cured meat products are considered as safe products due to the development of unfavorable conditions that inhibit the growth of spoilage or pathogenic microorganisms. The low values of pH and water activity (a_w), presence of salt, spices and other ingredients, called hurdle technology, are responsible for the inhibition of pathogenic and spoilage microorganisms [53].

Although fermented meat products are usually safe, these hurdles, in some cases, are not enough and food-borne pathogens can survive, causing outbreaks [54]. There are three hypotheses for the presence or foodborne pathogens in dry-cured meat products: (1) pathogenic microorganisms are present on raw meat and/or ingredients and survive along the fermentation and drying process; (2) pathogenic microorganisms are already present in the production environment and survive the manufacture; and/or (3) the contamination of meat products by improper handling. It highlights the importance of training programs about food safety and good manufacture practices with special relevance in developing countries [55].

2.2.1. Safety of Dry-Cured Meat Products

As stated above, the safety of dry-cured meat products is not only achieved through low pH and a_w values, but also through the role of other ingredients. The most important step in dry-cured meat product manufacture is to decrease the pH of fresh meat (which averages about 5.6 to 5.8 post-rigor) to reduce the growth of spoilage microorganisms. The final pH of fermented sausages typically ranges from 4.8 to 5.2, depending on tanginess, firmness, and other desirable product characteristics [56]. Lactic acid bacteria, which produce lactic acid through glycolysis, can be introduced into meat either by natural fermentation (with relevance in homemade meat products) or by inoculating a starter culture, and their growth and metabolism inhibit the normal spoilage flora of the food material and any bacterial pathogens that it may contain [57].

The production of acid by fermentation plays a significant part in the preservation of foods. In the case of dry-cured meat products, the reduction in pH, and not the production of lactic acid, is mainly responsible for the preservative action [58].

The last hurdle in the stabilization of the dry-cured meat products is the reduction in the a_w. For most meat spoilage microorganisms, a_w values above 0.98 are optimal for growth. Consequently, the inhibition of microbial growth by a_w reduction needs to be engineered either by drying and/or by the addition of solutes such as salt or sugar [43]. The reduction in a_w (from 0.96 to 0.86) during drying also stabilizes the product, by controlling undesirable bacteria. Dry-cured meat products have a_w values below 0.85, inhibiting the most important foodborne pathogens. In general, common spoilage bacteria are inhibited at a_w values of about 0.97 [59].

Smoking represents an ancient technique related to food conservation, consisting of a process that generates some chemical substances with preservative properties that are deposited on the surface of the product. The smoking process reduces the microbiological population due to the heat and the deposits of chemicals which have bacteriostatic action. Many smoke compounds also have bacteriostatic or bactericidal properties; formaldehyde and phenolic compounds are thought to provide much of this effect [60]. The shelf-life of smoked meats depends on the heating time and temperature during the process, on the decrease in a_w, and on the antibacterial and antioxidant activity of smoke components. Moreover, the temperatures used during smoking or smoke concentration may influence the antimicrobial effects [61]. Smoking treatment decreases the number of viable microorganisms in the products by one to two log cycles, although the antimicrobial effect increases with increasing the time and temperature of the smoking process [62].

Herbs and spices in fermented meat products are largely utilized as ingredients to confer specific organoleptic characteristics. They represent a cheap source of substances with antioxidant activity protecting from oxidative deterioration [63], antimicrobial activity [64], or inhibitory effects of biogenic amine contents [65]. Essential oils (EOs) are volatile, natural liquids extracted from plant material that have gained a huge importance in the manufacture of traditional products due to their conservation properties. EOs are extracted from plant material as leaves, roots, bark, seed, or the entire plant. In developing countries, the use of EO may be limited due to a lack of local suppliers or by the need of specific equipment to obtain it. However, in developed countries (in which EO can be purchased from food additive suppliers), the antimicrobial and antioxidant properties of EOs represent an interesting source of natural compounds for food preservation, and thus increasing the shelf-life of foodstuff [66].

The action mechanism of spices and EO is still not totally clear. Its action mechanism is related to alterations in the cell membrane causing permeability and a loss of intracellular material [67]. Additionally, antimicrobial effects could be associated with interactions with membrane proteins and enzymes [68]. Thus, the presence of an outer membrane in Gram-positive bacteria may explain the reduced antimicrobial effect of EOs. The antioxidant effect has been associated with the high reactivity with peroxyl radicals [69]. Thus, both antimicrobial and antioxidant properties may improve the shelf-life of these type of products by the control of foodborne and spoilage bacteria and decreasing unwanted chemical reactions (e.g., rancidity by fat oxidation) that result in organoleptic alterations [70]. Use of local spices may also represent an alternative approach to improve the food safety of food of animal origin in developing countries in which good hygienic practices, food safety knowledge, manufacture and/or storage equipment are not always achievable or available.

As described above, the use of nitrates in the manufacture of dry-cured meat products to improve the organoleptic characteristics and inhibiting *C. botulinum* is a common practice in developed countries. However, its availability from local suppliers in developing countries is not always possible. Thus, the use of spices [71], EO [72] or other vegetables [73] as nitrate replacements, in addition to the antimicrobial and antioxidant effect, has also been suggested.

2.2.2. Contribution of Dry-Cured Meat Products to Food Stability

Regarding food stability, dry-cured meat products represent a source of proteins, vitamins and minerals that are relatively easy to manufacture and allow their preservation without major requirements due to their physical–chemical stability, The shelf-life of these types of products are very importance in geographical areas with fewer resources (such as a reliable electric supply) [40]. Additionally, they contribute to improving the nutritional requirements and protecting against malnutrition in those geographical areas in which access to other types of highly nutritious products is limited or scarce [74]. Fermented meat products can be manufactured from different livestock species [75]; therefore, it may contribute to ecological variety and land sustainability.

In the context of food sustainability, the use of vegetable ingredients such as pulses, cereals, tubers or fruit have been proposed as meat extenders mainly aimed to decrease the meat content and further decrease the impact of livestock on the environment [76]. Although the objective of decreasing livestock production is associated with developed countries in which intensive livestock farming represent an important economic activity, it can also be important to increase the yield of meat products in periods of shortage, contributing to enhancing food stability. In addition, the use of vegetable ingredients may imply greater crop diversity (i.e., not only crops for feed production) which would increase ecological variety due to the potential use of underutilized crops, sustainability improving the land use.

3. Conclusions

Food stability can be defined as the availability of food over time. This concept is linked to another concept, food security, because the latter is considered a measure of access to food population. Thus, to achieve the food security, technical and human resources are necessary to guarantee the food stability.

Food availability is often associated with developing countries, where political conflicts or weak economies lead to food fluctuations. However, recent economic crises may lead to a scenario of food insecurity related to a decrease in the economic income of families derived from increases in unemployment. Conversely, through the importance of agriculture in the economies of rural areas both in developed and developing countries, this sector can contribute to an enabling environment for increased food stability. The promotion of resilience of (rural) food systems can serve to increase household and community food security in the face of instability.

In rural areas, livestock represents not only economic income but also a source of foods and labor. Thus, to guarantee the performance of livestock (and food availability), it is necessary to implement management programs that maintain proper welfare, hygiene, and sanitary conditions. The implementation of biosecurity measures is essential to achieve this objective. Although some measures are difficult to put into practice in small and family farms, their adaptation together with good livestock practices allows farmers achieve healthy and productive animals.

In addition, the manufacture of foods of animal origin aims to obtain more durable products which enables us to guarantee the availability of food over time, even in seasons with scarce resources. Thus, dry-cured meat products play an important role in food availability. Food security refers to food access in healthy conditions; therefore, knowledge of the main tools that guarantee the food safety of these types of products is essential.

As described above, guaranteeing food stability depends on the joint implementation of livestock production and use of their products (either fresh or processed products) in developing countries. This allows a continuous supply of a variety foods that provide adequate nutritional intake which enables the healthy development of populations (food security).

In addition, this productive system implies the use of land to obtain other products, thus contributing to environmental sustainability. It is important to highlight that proper training and education programs regarding livestock and food safety for populations are necessary. In developed countries, in which the concept of food stability as a food supply does not exist, consumers are increasingly concerned about the type of both livestock and agricultural production. Thus, sustainable production must be extensively adapted to satisfy increased consumer demand. Furthermore, the link between livestock and agricultural farming adapted to a specific geographical area may optimize land use, favoring sustainability. Understanding food security, such as the guarantee of access to food by the population and the food availability of products of animal origin (food stability), must adhere to proper livestock management practices (both animal health and welfare) as well as the use of fresh and processed products of animal origin according the technical and geographical conditions of the specific area.

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Emerging microbial biocontrol strategies for plant pathogens

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ABSTRACT

To address food security, agricultural yields must increase to match the growing human population in the near future. There is now a strong push to develop low-input and more sustainable agricultural practices that include alternatives to chemicals for controlling pests and diseases, a major factor of heavy losses in agricultural production. Based on the adverse effects of some chemicals on human health, the environment and living organisms, researchers are focusing on potential biological control microbes as viable alternatives for the management of pests and plant pathogens. There is a growing body of evidence that demonstrates the potential of leaf and root-associated microbiomes to increase plant efficiency and yield in cropping systems. It is important to understand the role of these microbes in promoting growth and controlling diseases, and their application as biofertilizers and biopesticides whose success in the field is still inconsistent. This review focusses on how biocontrol microbes modulate plant defense mechanisms, deploy biocontrol actions in plants and offer new strategies to control plant pathogens. Apart from simply applying individual biocontrol microbes, there are now efforts to improve, facilitate and maintain long-term plant colonization. In particular, great hopes are associated with the new approaches of using "plant-optimized microbiomes" (microbiome engineering) and establishing the genetic basis of beneficial plant-microbe interactions to enable breeding of "microbe-optimized crops".

1. Introduction

It is a persistent issue worldwide that an enormous number of plant pathogens, varying from the smallest viroid consisting solely of a single strand of RNA, to more complex pathogens such as viruses, bacteria, fungi, oomycetes and nematodes, cause many important plant diseases and are responsible for major crop losses. Although there are many causes that can be attributed to the decrease in crop productivity, the loss due to pests and pathogens plays a crucial role in the damages worldwide. Every year, plant diseases cause an estimated 40 billion dollars losses worldwide [1], either directly or indirectly. At least 20–40% of losses in crop yield are caused by pathogenic infections [2]. The consequences of plant diseases range from major devastations to minor nuisances. Some plant diseases can be highly destructive and catastrophic on a large scale. In the 1840s, the potato late blight pathogen *Phytophthora infestans* caused a major destructive disease that had tremendous effects on human history, as it caused food shortages resulting in a million deaths and migration of 1.5 million people from Ireland [3]. Potato is the fourth largest food crop, providing more food on a per hectare basis than any other crop and serving as an important substitute to the major cereal crops for the world population [4]. The annual losses of potato crops due to late blight are conservatively estimated at US$6.7 billion per year [5–7].

Another historic example, brown leaf spot of rice caused by *Helminthosporium oryzae,* had been reported in Asia, Africa, South America and USA. It was not only ranked as one of the major rice fungal diseases, but also one of historical interest [8]. It caused severe devastation by reducing rice yields which caused the death of two million people in Bengal in the 1940s as the direct result of calamitous famine [9,10]. *Helminthosporium maydis* was the causal agent of a severe epidemic of southern corn leaf blight in 1970 in the USA which caused economic hardship that destroyed 15% of the USA corn crop with losses estimated at US$1 billion [9,10]. There are many more historic examples; refer to references [11–15] for a review of the top 10 fungal, oomycete, bacterial and viral plant pathogens, and plant-parasitic nematodes, respectively, that are considered most significant for Molecular Plant Pathology.

Most alarming is the unprecedented recent trend of new fungal and fungal-like plant pathogen alerts that have increased by more than 7-fold since 2000 [16]. This cannot solely be attributed to improved detection methods, but rather agricultural practices, in particular monocultures, increased international trade and the use of only a few cultivars. These practices promote the evolution of more virulent strains, often with increasing pesticide tolerance that not only affect

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agricultural crops but many indigenous wild species.

The control of plant diseases is crucial for the production of food, fibre and biomaterials. As global food production must be increased by at least 70% until 2050, there is growing concern for global food security, which has become one of the most important international issues in recent times [17,18]. It is estimated that the demand for food and global food security will continue to increase for the coming 40 years with the growing human population and consumption. Already at present, food systems are responsible for 60% of global terrestrial biodiversity loss, and around 25% of the global greenhouse gas emissions, hence, sustainable solutions for future food security are urgently needed [19]. The worldwide attempt to expand food production to answer the need for food has become serious [20,21]. The growing world population requires an efficient management and control of diseases in crop production. Crop protection plays a significant role in defending crop productivity against competition from pathogens [22]. The following paragraph introduces *Phytophthora cinnamomi* as an example that has gained global significance for both, food security (crop production) as well as biodiversity of wild species.

Phytophthora cinnamomi, a soil-borne oomycete holds a special interest due to its broad host range, mostly of woody species [23]. Several studies showed that chestnut and oaks are the main species affected in Europe [24–26] as well as ornamental crops and nursery plants such as coniferous and broadleaf plants [27,28]. It is considered as one of the most highly destructive forest pathogens globally [29], and is responsible for severe crop diseases [30]. *P. cinnamomi* is native to Southeast Asia, but it was later found in most temperate to tropical areas [31]. Its global spread has led to devastating consequences. For example, in Australian soils, *P. cinnamomi* is now widely distributed and causes major devastation in horticultural crops such as macadamia, pineapple and avocado [32,33]. It has also been listed as an important threat to the Australian environment and biodiversity by the Federal Government [34]. Depending on the environmental conditions and plant susceptibility, several plant species are at risk of extinction [35,36]. Infected plants exhibit root, collar, and sometimes stem necrosis as the primary symptoms, resulting in declines and stem cankers [23]. Being predominately soil-borne, this pathogen is particularly hard to control.

Beneficial biocontrol microbes may be one of the few options that show potential. These can provide benefits by competing with pathogens or by directly antagonising plant pathogens (e.g. by production of antimicrobial compounds; [37]). The local infection with plant pathogens can lead to systemic acquired resistance (SAR; [38,39]) but of significant interest is also the ability of beneficial non-pathogenic rhizobacteria to prime plants for induced systemic resistance (ISR) against pathogens [40]. This priming offers a better preparedness to plants that are able to respond faster and stronger to pathogen attack. Organic mulches colonized with *Gliocadium virens* (KA 2301) and *Tricoderma harzianum* (KA 159.2), termed "bioenhanced mulches" were found efficient in suppressing *P. cinnamomi* in avocado roots when used as surface mulch [41]. In another study, disease severity and stem lesion length of *Phytophthora capsici* root and crown rot of bell pepper significantly reduced and total microbial population and the biocontrol activity was enhanced when soil was incorporated with compost containing chitosan, crab shell waste, and citrus pulp with molasses [42].

2. Plant disease management

2.1. Resistant varieties

Breeding for resistant varieties is one of the successful options and most reliable management tools for controlling plant diseases [43]. It is one of the most attractive approaches and can be considered as an ideal method if good quality plants are adapted to the growing regions with sufficient levels of tolerance and durable resistance [44]. Resistant varieties were introduced via systematic plant breeding by choosing varieties of high disease resistance level of the same species or genus [45]. The control of plant diseases by means of resistant varieties has been used in many crops and is relatively inexpensive compared to the cost of pesticides [46] but these varieties often take decades to develop and GM-plants suffer from extremely high regulatory approval cost and consumer acceptance. Mixtures of two varieties by the combination of a range of positive characters in a single crop genotype often result in the reduction of yield loss and provide a better disease management [47]. In the USA, disease-resistant plant varieties make up 75% of the land used in crop production [48]. For example, the control of stem rust caused by *Puccinia graminis* f. sp. *tritici* using resistant wheat plants has contributed to the suppression of that disease, whose infection is mainly found on stems and leaf sheaths, as well as on leaf blades and glumes [49–53]. In spite of all the advantages, in many countries the problems began after the breakdown of new varieties' resistance within several years of their release due to several causes, such as mutations of the pathogens toward virulence, sexual and asexual recombination events, issues with variety uniformity in genetics, and decreasing field resistance. Cases of resistance breakdown were observed in many crops, such as blast resistance in rice, cotton leaf curl disease, grapevine downy mildew and yellow wheat of rust [54]. New promising approaches to disease resistant varieties come from genome editing using CRISPR/Cas9 [55] and other intragenic technologies that may be deregulated and considered in par with classical breeding approaches.

2.2. Chemical control

With the growing human population, the need for food has increased as well, resulting in extensive use of agrochemicals targeted to increase crop yields by protecting them from pests and pathogens. Chemicals are one of the main components in Integrated Pest Management (IPM) as they are crucial in preventing losses and damages caused by plant diseases, as demonstrated with the increase in the number of fungicide specifications since the 1960s [56]. It is undeniable that pesticides have brought improvements in crop quality and agricultural output, in terms of market opportunities and facilitation of farm work [57]. However, regardless of the significant importance of chemicals for pest and disease management, fungicide resistance has been one of the unavoidable problems [58,59]. As a result, every year higher costs for fungal disease control applies and higher dosages and new chemicals are introduced to protect crops and plants. As a consequence, this has caused undesirable side effects, such as food contamination, environmental dispersal and higher costs of food production [59]. Many chemicals used in agriculture possibly also destroy the beneficial microbes, such as beneficial endophytic fungi and bacteria [60]. Chemical fungicides are often lethal to beneficial insects and fungi inhabiting the soil and may also enter the food chain [61]. For example, the use of Oryzalin and Trifluralin has been found to inhibit the growth of certain species of mycorrhizal fungi that aid in nutrient uptake in plants roots [62]. Furthermore, Triclopyr, a common landscape herbicide inhibits beneficial ammonia-oxidizing bacteria [63]. Glyphosate, commonly used as weed killer, also greatly reduces the growth and activity of beneficial free-living heterotrophic bacteria that aid in nitrogen fixation [64].

Chemicals used to control pests and diseases are deposited on the crops and their harvests. Reports from FAO-WHO and data provided by the US Food and Drug Administration indicate that persistent organic pollutants (POPs) are present in virtually all types of food, including fruits, vegetables, poultry and dairy products [65]. With the greater concern on the negative effects and continued dependence on toxic chemicals for plant diseases and pest control, more attention should be given to find and develop alternative inputs of less toxic and less disruptive methods to facilitate plant growth in agriculture in controlling pests and pathogens in general and fungal-diseases in particular.

2.3. Biological control

Among the alternatives, biological control of plant pests and pathogens appears to the best option for the development of low cost, eco-friendly and sustainable management approaches for protecting plants and crops. Biologicals, including biocontrol microbes, are now accepted as significant tools for the control of plant diseases in sustainable agriculture [66]. A number of biological control options are available. However, a better understanding of the complex interaction between plants, environment and pathogens is necessary for further exploration on this topic because it may easily fail if plants are already under high disease pressure [67]. In plant pathology, biocontrol can be referred to as the interrelationship of many environmental factors, aiming to diminish the unfavourable effects of detrimental organisms and enhance the growth of useful organisms, such as crops, beneficial insects, and microorganisms [68]. It often results in multiple interactions, such as suppressing the pest organism using other organisms or the application of antagonistic microorganisms to suppress diseases and the introduction of host-specific pathogens. The application of natural products and chemical compounds extracted from different sources, such as plant extracts, natural or modified organisms or gene products are other examples of biological control [68,69]. The primary idea of conducting research on biocontrol is to reduce the reliance on agrochemical use and the risks for human health and the environment [70]. It was developed 20–25 years ago when plant pathology research and education attracted more interest in the application of useful microorganisms for the management of plant diseases, including plant parasitic nematodes [70]. The different types of interactions between the populations are referred to as mutualism [71], protocooperation [72], commensalism [73], neutralism [74], competition [75], amensalism [76], parasitism [77], and predation [78]. All these biological control interactions between plants and microbes occur naturally at a macroscopic and microscopic level [68].

Throughout their life cycle, plants are vulnerable to the various environmental challenges of abiotic and biotic stresses, such as drought, herbivores and potentially pathogenic fungi, oomycetes, nematodes, bacteria and viruses. In response, plants have evolved a broad range of strategies to counter-attack and ward off attackers [79]. Plant defense mechanisms can be categorized as passive defenses; non-host resistance, physical and chemical barriers, rapid active defenses and delayed active defenses. Rapid active defenses involve the changes in membrane function, the initial oxidative burst, cell wall reinforcement, hypersensitive response (HR), resulting in programmed cell death (PCD) and phytoalexins [80]. Delayed active defenses include pathogen containment and wound repair, pathogenesis-related (PR) gene expression and systemic acquired resistance (SAR). Plant defense signaling molecules include salicylic acid (SA), which is generally considered necessary for defense against biotrophic pathogens and SAR, as well as jasmonic acid (JA) and ethylene (ET), both of which are involved in defense against necrotrophic pathogens as well as in beneficial plant-microbe interactions, including priming and induced systemic resistance (ISR) [81]. The latter two terms define a state of higher alertness of the plant that enables a faster and stronger response to pathogen attack. Fig. 1 provides an overview of the various plant-microbe interactions, defense signaling and systemic resistance.

3. Types of pathogens and how some successful pathogens are able to trick plants

Plants are infected by pathogens with different modes of nutrient uptake [82]. These have direct consequences to how the plant needs to respond. Host-pathogen relationships generally comprise three groups according to their mode of infection on plant: necrotrophs, biotrophs, and hemibiotrophs [83]. If this is not recognized early enough, the plant may respond in the wrong manner which can aggravate the damage. A good understanding of the pathogen's lifestyle is therefore important if we wish to assist plants to defend themselves.

3.1. Biotrophic pathogens

Biotrophic pathogens grow, reproduce and obtain nutrients from living plant tissue by engaging in an intimate relationship with living plant cells. Examples are the tomato leaf mold causal agent, *Cladosporium fulvum* and *Ustilago maydis* which is the causal agent for corn smut. Some of them coevolved into obligate biotrophs, and cannot be grown on artificial media, for instance, rusts and powdery mildews [84,85]. On the other hand, the non-obligate biotrophs which limit the damage of host cells can be cultured on artificial media; however, they cannot grow as saprophytes. Biotrophs have a restricted number of host ranges where these pathogens are only fitted to particular types of plants. These pathogens have evolved specialized structures such as nutrient-absorbing haustoria that penetrate into the plasma membrane of the host cells and take up the nutrient sap and release effector molecules that let them grow invasively on particular host genotypes [85–88]. However, the pathogen is unable to initiate a parasitic relationship if the host cells die in advance of invasion by the pathogen. Hence, ROS production, HR, PCD and SAR are the most effective defense responses against biotrophs and in *Arabidopsis* these responses are generally associated with SA signaling.

3.2. Necrotrophic pathogens

Necrotrophic pathogens feed on dead plant tissues by killing the cells before parasitizing the plants. Necrotrophs usually invade the host cell through wound sites or dead cells and secrete toxins and cell wall-degrading enzymes to destroy the host tissue. They can also live as saprophytes outside the host cells and can be grown on synthetic media [89]. The pathogens are unable to kill the host cell if the host genotype is unresponsive to the toxins or the toxins are derivatized, compartmentalized or not released at the right time, place or concentration. Examples of fungal necrotrophs are species of *Cochliobolus, Alternaria* and *Botrytis* [82,85]. It is essential that plants recognize necrotrophs early to counteract the initial oxidative burst and prevent HR and PCD. This role is attributed to JA signaling in *Arabidopsis* that acts as antagonistic pathway to SA signaling in this respect.

3.3. Hemibiotrophic pathogens

Hemibiotrophs such as *Magnaporthe grisea,* the causal agent for rice blast, are pathogens that have an intermediate lifestyle, where they initially have a biotrophic relationship with the host but consequently cause the death of host cells, at which point they switch their lifestyle to necrotrophic nutrient acquisition [90]. *Phytophthora*, *Pythium* and *Fusarium* species have a similar lifestyle as well as some species in the genera *Colletotrichum* and *Venturia* and the well-studied bacterium *P. syringae* [85,91]. Many agronomically important pathogens belong to this group as often crops are not able to respond with plant defense in an appropriate and timely manner.

3.4. How successful pathogens trick plants

According to the feeding behavior of the pathogen, plants activate different post-invasive lines of defense, mediated by phytohormones [92]. When attacked by biotrophs, typically SA activates an HR leading to localized PCD that limits pathogen spread, together with the production of PRs, ROS detoxifying enzymes and antioxidants which exhibit antimicrobial properties and provide protection against oxidative stress, respectively, in the surrounding tissues [92]. SAR can then be triggered in healthy systemic tissues of locally infected plants. Alternatively, necrotrophs and herbivorous insects typically activate the JA defense signaling pathway [92]. This prevents localized cell death and leads to the production of chemical and physical defenses against the

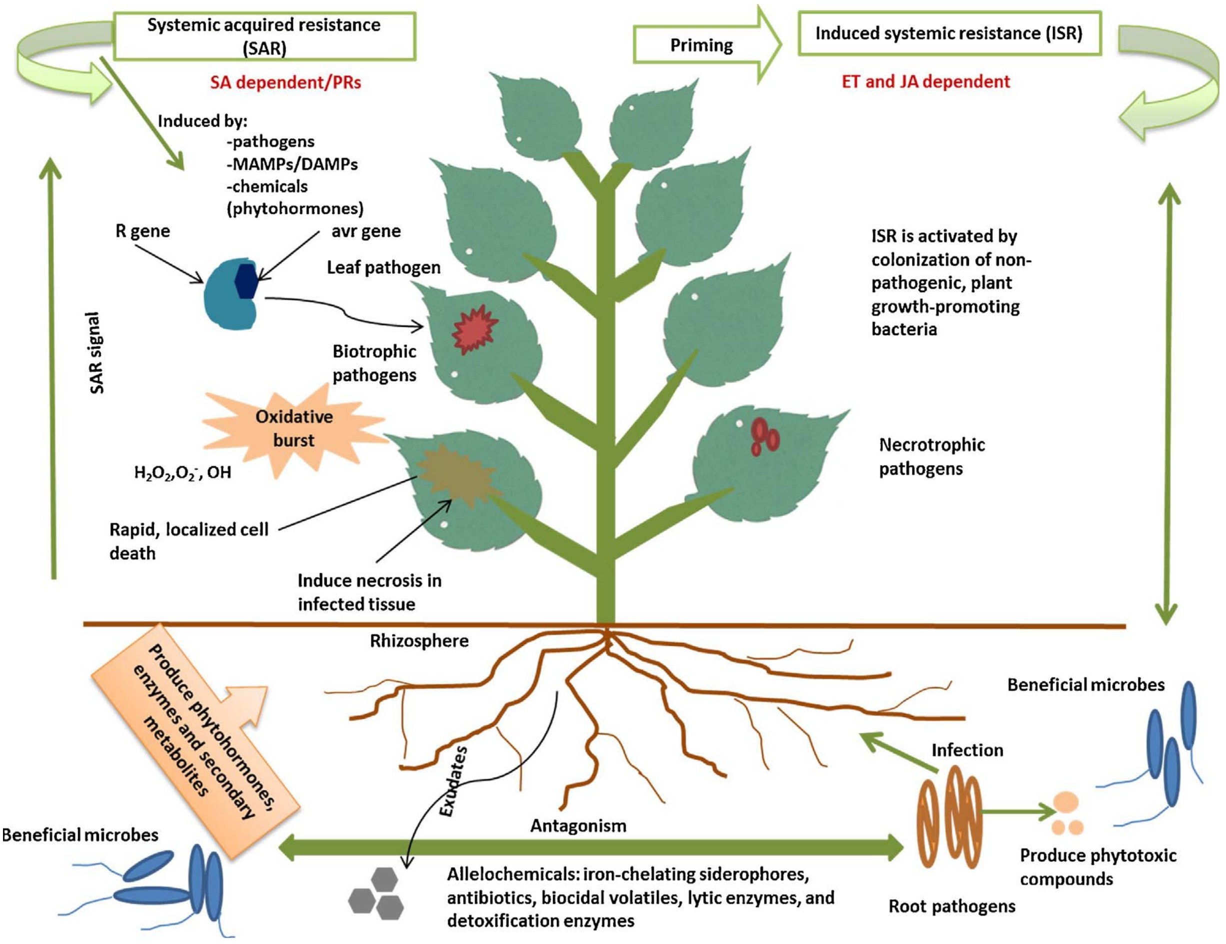

Fig. 1. Schematic diagram showing two different types of systemic resistance in plants. SA-dependent systemic acquired resistance (SAR) is activated upon pathogen attack, foliar treatments of plants with microbe- or damage-associated molecular patterns (MAMPs, DAMPs), phytohormones or certain xenobiotics (e.g. some pesticides). SA-independent induced systemic resistance (ISR) is directed primarily by jasmonic acid (JA) and ethylene (ET). Both SAR and ISR are crucial in plants for defense against biotic and abiotic stresses.

pathogen. However, recently it has become clear (including findings from our own work, [93]) that many severe pathogens hijack the wrong pathway, leading to a further weakening of plants. For example, hemibiotrophs, such as *Fusarium* spp.,

Phytophthora spp., and *P. syringae* as well as plant viruses can suppress the SA pathway by early activation of the antagonistic JA pathway that prevents HR and cell death. This can also be interpreted as pathogens disguising themselves as beneficial microbes. By comparison, root-colonizing beneficial microbes activate ISR that is also mediated by JA and ET signaling. Some of the available microbial products developed for agricultural applications include these microbes in their formulation [94].

4. Microbial biocontrol options

Weller [95] predicted that a big challenge will be faced by microbiologists and plant pathologists who are trying to search for and develop environmentally friendly control agents against plant diseases, to limit the use of large amounts of chemical pesticides. Alternatively, using beneficial microorganisms as biopesticides is one of the most effective methods for safe crop-management practices that works under low to medium disease pressure [69]. For over 65 years, studies on soil-borne pathogens have been conducted by introducing microorganisms into the rhizosphere [70]. Generally, the interest in this research area has gradually increased, evidenced by a number of early books [70,96,97] and reviews that have been published on this topic [98–100]. In the backdrop of food security issues and the alarming increase in recent pathogen alerts [16], there is now significant renewed interest in this topic and all of the large Agbiotech companies are now investing in the development of biological applications [101,102]. It was suggested by the researchers that biological control will continue its significance and play a major role in modern agriculture in the future and present.

4.1. Microbial antagonisms

The microbes that are considered ideal for use as biological control agents are the ones that can grow in rhizospheres, where the soil is described to be microbiologically suppressive to pathogens, as this area provides a frontline defense for the roots against various pathogenic attacks. Root colonization by beneficial microbes delivers their pathogen-antagonising metabolites into the root system where they directly suppress pathogenic bacterial growth [103]. This antagonistic relationship between microbes and pathogens mostly leads to significant disease control, where the established populations of metabolically active beneficial microbes initiate protection either by direct antagonistic activity of pathogens, by outcompeting pathogens or by stimulation of host plant defenses (priming) [104]. It also involves antibiosis which is the secretion of diffusible antibiotics, volatile organic compounds, and toxins, as well as the development of extracellular cell wall degrading enzymes such as chitinase, β-1,3-glucanase, beta-xylosidase, pectin methylesterase and many more [103,105].

4.2. Plant-microbe interactions that assist in biocontrol

Plant roots offer an ecological habitat for the growth of soil bacteria that thrive on root exudates and lysates as nutrients. The mutualistic interaction between plants and beneficial microbes that commonly occurs in nature often leads to either an improvement in plant nutrition and/or enhancement of the plant's ability to prevail over biotic and abiotic stress. In all cases, this provides a competitive advantage leading to improved growth and plant proliferation [101].

A variety of endophytic bacteria and free-living rhizobacteria on the root surface and rhizosphere utilize the nutrients released from the host, as well as secrete metabolite substances to the soil that aid in controlling plant diseases caused by fungi or bacteria [106,107]. This indirect interaction between the microbes and plants causes an increase in the supply of minerals and other nutrients that have an influence on plant growth, for example by atmospheric nitrogen fixation or phosphorous solubilization [108]. The interaction between plants and a group of biocontrol microorganisms also indirectly enhance plant growth by suppressing pathogens' growth and activity [109,110]. Furthermore, microorganisms can be directly involved in plant growth promotion, by acting as agents for stimulation of plant growth and management of soil fitness, for example through the production of auxin [111]. This may also include mitigation of abiotic stress. Despite their different ecological rhizosphere niches, some of the beneficial microbes apply the same mechanisms to stimulate plant growth and suppress deleterious pathogens [112–114]. The following sections provide examples how certain plant-microbe interactions assist in biocontrol against pathogens.

4.3. Production of antimicrobial compounds

The colonization of the rhizosphere niche by plant growth-promoting bacteria (PGPR) is assisted by the production of allelochemicals such as iron-chelating siderophores, antibiotics, biocidal volatiles, lytic enzymes (chitinases and glucanases), and detoxification enzymes [113–115]. For example, rhizobacteria include antibiotic-producing strains such as *Bacillus* sp. producing iturin A and surfactin, *Agrobacterium* sp. producing agrocin 84, *Pseudomonas* spp. producing phenazine derivatives, pyoleutorin and pyrrolnitrin, and *Erwinia* sp. producing herbicolin A [169,170], that are persistent in the rhizosphere [171,172]. The mycoparasitism of phytopathogenic fungi of the *Trichoderma* and *Streptomyces* genera have important roles in secretion of chitinases and glucanases [173]. A common feature of successful biocontrol strains and a crucial factor for plant root pathogen suppression is the production of antibiotic compounds and fluorescent siderophores that enable effective competition for iron [174]. Allelochemicals as secondary metabolites are generated directly or indirectly by plants and secreted into the root zone through abiotic or biochemical reactions [116], but can also be produced by associated fungi and bacteria. Numerous non-pathogenic *Pseudomonas* rhizobacteria have the capability of inducing systemic resistance in plants to protect against a wide range of plant pathogenic fungi, bacteria, and viruses [81]. In addition, they also produce siderophores to suppress soil-borne plant pathogens [117,118]. Bacterial siderophores inhibit plant pathogens by competing for copper, zinc, manganese [119] and especially iron [120]. Solubilization and the competitive acquisition of ferric iron under iron-limiting conditions limit the availability of iron to other soil inhabitants and subsequently limits their growth [121,122]. The scarcity of essential trace elements in the soil ecology results in harsh competition between the soil inhabitants [121].

Pseudomonas is a genus of which many species have been identified to produce antifungal metabolites, such as phenazines, pyrrolnitrin, 2, 4-diacetylphloroglucinol (DAPG), and pyoluteorin [123]. Numerous studies on the plant growth inducer of fluorescent *Pseudomonas* [99,124] have described it as the most promising class of PGPR for biocontrol of plant diseases [125]. This is due to their ability of rapid and aggressive colonization in which this indirectly prevents the invasion of deleterious pathogens from the root surface [126].

Many studies have reported members of the *Bacillus* genus as elicitors of ISR, as well as plant growth promoters. It was indicated that the mechanisms of elicitation are related to the cytochemical alterations and ultrastructural changes in plants during pathogen attack [127]. Some strains of *Bacillus* spp. were found to elicit ISR against *Cucumber mosaic virus* (CMV) on tomato with severity reduction of 32–58% for PGPR-treated plants [128]. Murphy et al. [129] reported the elicitation of systemic protection against CMV by two-strain combinations of *Bacillus* spp. incorporated into the potting mix, resulting in a significant reduction of disease severity and significant increases in plant fresh weight and number of fruits and flowers. Several strains of *Bacillus* sp. have demonstrated an efficacy in reducing disease and promoting plant growth under field conditions. The application of PGPR *Bacillus subtilis* IN937b, *Bacillus pumilus* SE34 and *Bacillus amyloliquefaciens* IN937a as seed treatments for ISR against CMV on tomato has significantly increased the yield and plant height and reduced the disease severity and incidence [128]. Similarly, field trials conducted on cucumber have confirmed the elicitation of systemic protection against cucurbit wilt by *Bacillus* strain INR7 [128].

4.4. Priming and induced systemic resistance (ISR)

Rhizobacteria-induced systemic resistance or ISR, also referred to in its early stage as priming, was first discovered by Van Peer et al. [130]. It is described as an enhanced defensive capacity of the whole plant to multiple pathogens induced by beneficial microbes in the rhizosphere [131] or elicited by specific environmental stimuli which lead to potentiation of the plant's innate defense against biotic challenges [132]. This higher state of alertness then enables the plant to respond faster and stronger against subsequent pathogen attacks. Some selected strains of non-pathogenic PGPR and fungi are able to activate plant defenses in plants to reduce the activity of deleterious microorganisms, and then initiate ISR that is mediated by JA and ET signaling [81,132]. Non-pathogenic rhizobacteria are capable of activating defense mechanisms in plants in a similar way to pathogenic microorganisms, including reinforcement of plant cell walls, production of phytoalexins, synthesis of PR proteins and priming/ISR [133].

The complex ISR system has been partially elucidated in several model plants including *Arabidopsis*, with three general pathways being recognized. Two of the recognized pathways are involved in the direct production of PR proteins with alternate mechanisms for induction. In one of the pathways, PR proteins are produced as the result of pathogen attack while in the other pathway, production of PR proteins are initiated due to wounding or necrosis-inducing plant pathogens. In the pathogen-induced pathway, SA is produced by plants, contrary to the wounding pathway which relies on JA as the signaling molecule [40]. Both pathways antagonize each other, possibly to enable the plant to finely tune its defense response depending on the attacker encountered [92]. The third pathway of induced resistance which leads to systemic resistance is referred to as rhizobacteria-induced systemic resistance (ISR) which is provoked by non-pathogenic root-associated bacteria, and is dependent on the plant hormones JA and ethylene.

Plants with ISR exhibit stronger and/or faster activation of defense mechanisms after a subsequent pathogen or insect attack or as a response to abiotic stress, when inoculated with rhizobacteria [81]. Beneficial microbes play an important role in defense priming of ISR for applied plant protection. Few studies have been conducted on the potential of priming using beneficial microbes in the rhizosphere to induce systemic immunity to airborne pathogens under glasshouse and field conditions. The inducing biocontrol strain *P. fluorescens* WCS374 was found to suppress *Fusarium* wilt and increase radish yield [134]. Similarly, root colonization by the beneficial fungus *Piriformospora indica* increased growth and yield of the medicinal plants *Spilanthes calva* and *Withania somnifera* under field conditions [135]. It was also found to systematically prime barley for tolerance against biotic and abiotic stress [135]. Enhancement in the plant's defense capability by priming is associated with defense gene expression, *de novo* synthesis of PR

Table 1
Some early examples of successful priming of plants by beneficial microbes for ISR-mediated defense mechanisms.

Treatments	Response	References
Tomato roots colonized with mycorrhizal fungus *Glomus mossae*	Fungus systemically safeguards plants against *Phytophthora parasitica*	[140]
Cucumber plants previously inoculated with PGPF *Trichoderma asperellum* T203, then infected with *Pseudomonas syringae* pv. *Lachrymans*	Priming induced in plants triggering expression of PR genes	[166]
Carnation (*Dianthus caryophyllus*) inoculated with *Pseudomonas* sp. WCS417r followed by *Fusarium oxysporum* f. sp. *dianthi* attack	Phytoalexin levels were increased compared to control plants	[130]
ISR induced by *Bacillus pumilus* SE34 against the root-rot fungus *F. oxysporum* f. sp. *pisi* in bean (*Phaseolus vulgaris*)	Appositions of phenolic material and fungal entry successfully prevented by rapid strengthening of root cell wall at penetration site	[167]
ISR triggered by *Pseudomonas fluorescens* WCS417r in *Arabidopsis*	Effective against different types of pathogens without activation of *PR* genes	[168]
Endophytic colonization of *Vitis vinifera* L by plant growth promoting bacteria *Burkholderia phytofirmans* strain PsJN	Accumulation of phenolic compounds and cell walls in the exodermis and cortical cell layers	[169]
ISR activated by *P. fluorescens* EP1 against red rot of sugarcane caused by plant PGPR *Colletotrichum falcatum*	Higher levels in the defenses related enzymes such as chitinase and peroxidase in the treated sugarcane tissue	[170]
Pseudomonas denitrificans and *Pseudomonas putida* evaluated against *Ceratocystis fagacearum* on oak	Oak wilt pathogen significantly reduced crown loss in inoculated containerized live oaks	[171]

Table 2
Examples of bioactive signaling compounds that lead to beneficial plant-microbe interactions.

Exudate/Compound	Beneficial microbe(s) attracted	Function/Benefit to plant/Reference
Flavonoids	Rhizobia	N-fixation to legumes and other plants when free-living; leads to increased yields [144,145]
JA defense signaling-specific exudates (after MeJA treatment)	*Bacillus thuringiensi, Bacillus cereus, Planococcaceae; Paenibacillus amylolyticus; Lysinibacillus sphaericus; Lysinibacillus fusiformis*	Antifungal, antibacterial; antioomycete; insecticidal; assist in defense against root pathogens and herbivorous insects; reduced yield losses [142]
Arabinose, glucose, fructose, ribose, inositol; erythritol (during P deficiency)	To be determined	P solubilization; leads to increased yields [172]
Strigolactone	Mycorrhizae, *Burkholderia, Frateuria, Sphingomonas, Legionella, Talaromyces, Peziza*	P solubilization, water supply, defense; leads to increased yields and resilience against abiotic and biotic stresses (unpublished)
Malate, succinate, fumarate	*Pseudomonas fluorescens*	Suppresses pathogens *Pythium ultimum*, *Gaeumannomyces graminis*, *Fusarium oxysporum*; reduced yield losses from pathogen attacks [173]

proteins and antimicrobial compounds in uninfected tissue as a reaction to the first infection. It has been reported that activation of the inducible resistance and defenses in plants incur a major cost for plant reproduction and growth [136]. However, in other studies, it has been reported that the cost of priming in *Arabidopsis* is much lower than the cost of directly inducing defense against pathogens [137]. Considering the protection priming offers when disease occurs, one can conclude that the benefits of priming outweigh the cost involved during activation.

The first report of ISR being observed was in carnation roots treated with *Pseudomonas* sp. WCS417r which exhibited resistance when inoculated with *Fusarium oxysporum* f. sp. *dianthi* on the stem [130]. In another study, seeds treated with *Pseudomonas fluorescens* caused a reduction in incidence of foliar lesions of *Pseudomonas syringae* pv. *phaseolicola* on bean [138]. A similar incident of induced resistance was observed when *Colletotrichum orbiculare* was inoculated into emerging leaves when PGPR was previously applied to cucumber seeds [139].

Priming is a well-known common feature of induced resistance responses to challenge pathogenic infections by beneficial microorganisms [140]. Table 1 shows some of the first examples of successful priming of plants for defense mechanisms with beneficial microbes. Tomato roots colonized with mycorrhizal fungi showed a similar interaction when systemically protected after infection with *P. parasitica* [140].

5. Emerging biocontrol strategies

Deciphering the “plant-microbiome language” could be a good approach to develop new biocontrol strategies. Plants may have their own language that allows them to communicate with their associated microbiomes by releasing a broad variety of chemicals through their leaves and roots. This language could be what helps the plant to attract and select specific microbes in the rhizosphere and phyllosphere that can provide specific benefits that are needed [141]. In turn, this microbiome will influence plant health and growth, via different mechanisms. Similar like individual plant beneficial microbes, rhizosphere soil microbiomes contain a wide variety of microbes that promote plant growth through direct mechanisms by increasing the availability of nutrients to plants, or by the production of various phytostimulators (modulating plant hormone levels) or through indirect mechanisms by acting as biocontrol agents.

Understanding how plants communicate will give us ideas and may help us fight diseases without the application of chemicals. The attraction of specific microbes in the rhizosphere is associated to the signaling molecules and hormones [142] and specific root exudates [143] secreted by the plants to match their needs. The vast and well-studied symbiosis interaction of legume-rhizobia is a good example of chemical language where secretions of specific compounds by the plants attract specific rhizobacteria [144,145]. Table 2 provides some examples of known plant exudate-microbe interactions that lead to beneficial outcomes.

Comprehensive study of the individual bioactive exudates in the rhizosphere is required to understand the attraction of individual microbes within microbiomes to enable more accurate manipulation of soil microorganisms before it can be adapted into agricultural practices. The following paragraphs outline new promising approaches that may lead to improved crop yields and potentially more resilient plants (Fig. 2).

5.1. Use of exudates as a way to attract beneficial biocontrol microbes

Root exudates are one of the major factors that influence composition and function of rhizosphere microbial populations. Specific root exudates attract specific beneficial microbes that match their specific

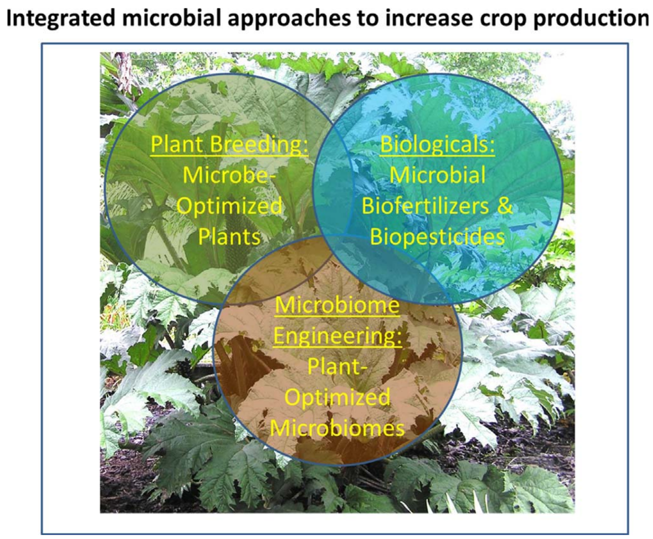

Fig. 2. Proposed integrated strategies for improved crop yields by making use of the plant's microbiome. While the use of microbial biofertilizers and biopesticides for disease control are advancing rapidly in recent years, crop breeding programs have yet to incorporate amenity to beneficial plant-microbe interactions to breed "microbe-optimized plants". Similarly, efforts towards microbiome engineering can in the future lead to microbial consortia that are better suited to support plants. The combination of all three approaches may be integrated to achieve maximum benefits and potentially significantly improved crop yields to address food security.

needs (Table 2). An abundance of evidence has shown that plants provide benefits for the microbes by attracting and maintaining specific microbiomes using chemical exudates. For example, flavonoids released from legumes attract specific nitrogen-fixing rhizobacteria [145] and some beneficial rhizobacteria were found to activate the plant defense responses to prevent foliar diseases [146]. By gathering all these information, we are able to apply this knowledge to use exudates as an approach to attract beneficial microbes to control various plant diseases. The application of soil microbiomes in agriculture has also been practised extensively to improve plant nutrition and/or disease resistance [147,148]. Our recent studies have shown that there is a correlation between hormone-treated plants and defense signaling mutants which resulted in different exudate profiles and an attraction of different microbial populations [142,143]. Furthermore, signaling by the plant hormone strigolactone attracted not only mycorrhiza but also other microbes that aid in phosphate solubilization, water supply and defense (unpublished data). It can be concluded that we can manipulate the rhizosphere microbial population by simply spraying plants with signaling chemicals or altering the genotype (plant breeding) to attract beneficial microbes [143,149].

5.2. *Use of substrates to maintain beneficial biocontrol microbes near crops*

Beneficial biocontrol microbes can be kept by culturing using substrates as medium of growth. Substrates are the composition of nutrients they require for growth, metabolism and activity of microbial cells. Bai et al. [150] showed that the majority of associated microbes can be cultured by employing systematic bacterial isolation approaches. This can be seen as an advantage to recruit the beneficial microbiomes from the existing soil microbiota as well as adding and maintaining beneficial microbes for the biocontrol of plant diseases by providing the right substrates as media of growth. The nutritional versatility of beneficial microbes, especially bacteria, make them adaptable to different types of habitats and environments.

5.3. *Phyllosphere biocontrol*

Foliar diseases are a serious problem for many types of crops [151]. Among the top eight most important fungal plant pathogens worldwide, six of them are the causal agents of serious foliar diseases [11]. It is crucial to gain a better and clear understanding of the role of foliar microbiomes for better insight in crop protection. The application of microbial biocontrol agents is an environmentally friendly and viable alternative method to synthetic chemical control [152]. Biocontrol microorganisms have been tested as spray application on foliar diseases, including powdery mildew, downy mildew, blights and leaf spots [153]. They have also been applied as liquid commercial formulation for controlling stem-end rot pathogen on avocado plants [154]. In another study, several antagonist bacterial was found to inhibit the growth of bacterial stem rot caused by *Erwinia chrysanthemi* on tomato plants under greenhouse condition [155]. The biological products Serenade (*B. substilis* QRD137) suppress floral infection of blueberries and reduces fungal growth in flowers treated with the bacterial strain [156]. Plants defend themselves on the leaf surface by producing antimicrobial compounds or by promoting growth of beneficial microbes through the release of nutrients and/or signals [141]. Scientists have proposed that leaf-colonizing microbes play an essential part in foliar disease progression and prevention in plants [157]. Pre-emptive colonization or niche occupation is suggested as the key factor for development of protection against pathogens [158]. Profiling the phyllosphere microbial [141] and chemical environment, and identifying and making use of important plant-microbe as well as microbe–microbe interactions on the leaf surface will reveal new insights into the shaping of foliar microbiomes by plants and may ultimately lead to new strategies to enhance food security.

5.4. *Breeding microbe-optimized plants*

Different *Arabidopsis* ecotypes have shown up to 4-fold differences in plant yield when inoculated with *Pseudomonas simiae* WCS417r [149]. This demonstrates that the genetic make-up of the plant plays a major role in the outcome of the beneficial interaction [159]. Hence, the aim of this new approach would be to breed plants that are optimized to attract and maintain beneficial biocontrol microbes. Breeding programs have so far not taken this trait into account, but before this can become a major effort it is essential that we better understand how beneficial microbes are attracted and maintained. Genetic engineering and plant breeding would enable us generate microbe-optimized plant that produce the right exudates to attract and maintain beneficial microbes at the right time, either at the root or on the leaf [160]. Plants design their own rhizosphere environment by the secretion of specific exudates to improve nutrient availability and interaction with specific beneficial microbes [160]. A prerequisite for this is that the targeted microbes are present, so this strategy may need to link with the inoculum of the matching biocontrol microbes.

5.5. *Microbiome engineering, plant-optimized microbes and plant-optimized microbiomes*

The aim of this new approach is to engineer or breed individual microbes or entire microbial consortia that harbor beneficial microbes and to maintain them for crop plants in different soil types. As a result, we would produce plant/soil-optimized microbes and plant/soil-optimized microbiomes that can be used as inoculum for different crops in different soils. To our knowledge this strategy has not been deliberately applied anywhere, but there is evidence that soil microbiomes adapt to their crops over time leading to improved plant-microbe interactions [161]. A substantial body of evidence supports the major role of the naturally occurring plant microbiome in disease development and progression in plants [162]. Again, it is essential that we better understand how beneficial microbes are attracted and maintained.

5.6. Matching microbe-optimized plant seed with the optimal microbiome and soil amendment practices for each soil type

Scientists are working hard to find the right microbes that help specific crops grow better. Microbe-coating of seeds with promising microbes for the right soil is one of the best options for optimizing plant-microbe interactions. Seeds laced with the right microbiomes would be the best options compared to other applications like sprays or root soaks when considering the transient nature of the microbiomes. The microbiomes potentially act either as inoculants, which help plants to absorb nutrients, or biocontrol products that aid in protecting the plants against pests and diseases, or both. The microbiomes derived from the soil samples are cultured and cryopreserved and stored up before being applied to the seeds. They will ideally become part of the rhizosphere after the seeds germinate and the plant takes root. To make sure that beneficial microbes are maintained, some soil amendments may be required. Maybe it would be possible to identify “probiotics” to maintain healthy plant microbiomes.

Several powerful inoculants formulated from beneficial bacteria (e.g. *Rhizobium*) for treating legume seeds are available in the market. Apart from stimulating the formation of nitrogen fixing nodules on leguminous plant roots for the growth of healthier plants with better yields, they may also help in suppressing the disease-causing microbes, and hasten nutrient availability and assimilation. Kalra et al. [163] demonstrated the effectiveness of vermicompost-based (granular and aqueous extract) bioformulations from natural microbial growth-promoting compounds which improve the stability and life of the bioformulations. Similarly, Rice et al. [164] had shown a successfully commercialized co-culturing of the phosphate solubilizing fungus *Penicillium bilaii* with *Rhizobium* as a legume inoculant. In another study by Liu and Sinclair [165], co-inoculation of soybean with bradyrhizobia and *Bacillus megaterium* enhanced nodulation of soybean.

The integration of microbial biofertilizers, biocontrol microbes, optimized microbiomes, soil amendments and matching microbe-optimized crops for different soil types would be the penultimate goal to benefit most from positive plant-microbe interactions. Clearly, this is a largely untapped area that deserves major research efforts, as it holds the promise to improve crop yields and address food security in an environmentally-friendly and sustainable manner.

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SPECIAL FEATURE: REVIEW ARTICLE

Synergies and Trade-offs between Sustainable Development Goals and Targets

Impact of Malaysian palm oil on sustainable development goals: co-benefits and trade-offs across mitigation strategies

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Abstract

Palm oil (PO) is an important source of livelihood, but unsustainable practices and widespread consumption may threaten human and planetary health. We reviewed 234 articles and summarized evidence on the impact of PO on health, social and economic aspects, environment, and biodiversity in the Malaysian context, and discuss mitigation strategies based on the sustainable development goals (SDGs). The evidence on health impact of PO is equivocal, with knowledge gaps on whether moderate consumption elevates risk for chronic diseases, but the benefits of phytonutrients (SDG2) and sensory characteristics of PO seem offset by its high proportion of saturated fat (SDG3). While PO contributes to economic growth (SDG9, 12), poverty alleviation (SDG1, 8, 10), enhanced food security (SDG2), alternative energy (SDG9), and long-term employment opportunities (SDG1), human rights issues and inequities attributed to PO production persist (SDG8). Environmental impacts arise through large-scale expansion of monoculture plantations associated with increased greenhouse gas emissions (SDG13), especially from converted carbon-rich peat lands, which can cause forest fires and annual trans-boundary haze; changes in microclimate properties and soil nutrient content (SDG6, 13); increased sedimentation and change of hydrological properties of streams near slopes (SDG6); and increased human wildlife conflicts, increase of invasive species occurrence, and reduced biodiversity (SDG14, 15). Practices such as biological pest control, circular waste management, multi-cropping and certification may mitigate negative impacts on environmental SDGs, without hampering progress of socioeconomic SDGs. While strategies focusing on improving practices within and surrounding plantations offer co-benefits for socioeconomic, environment and biodiversity-related SDGs, several challenges in achieving scalable solutions must be addressed to ensure holistic sustainability of PO in Malaysia for various stakeholders.

Keywords Palm oil · Biodiversity · Poverty · Health · Sustainable Development Goals · Mitigation

Introduction

The African oil palm (OP) *Elaeis guineensis* is a vital source of edible oil, derived from its mesocarp and kernel. Malaysia (26%) and Indonesia (58%) are the largest global palm oil (PO) producers, which together accounted for 84% of globally produced PO in 2021 (USDA 2021). These countries are also global emerging economies that belong to the world's biodiversity hotspots (Myers et al. 2000). Hence, while the rise and expansion of OP as a cash crop has fueled the industry and economy (Qaim et al. 2020; Karki et al. 2018), it has come at significant costs to the region's rainforests and biodiversity (Qaim et al. 2020; Vijay et al. 2016), including violations of land rights towards indigenous peoples (Buckland 2005; Sheil et al. 2009). These drawbacks have led to

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calls for boycotts, anti-PO campaigns and governmental policies, particularly from the European Union, North America, Australia, and New Zealand (Walden 2019). Additionally, the use of PO is debated due to perceived and documented adverse effects on human health, both on nutritional aspects of widespread dietary consumption, and societal aspects in terms of rights of workers and local communities living in plantation landscapes (Qaim et al. 2020).

Arguably, any crop that is cultivated unsustainably inflicts damage to ecosystems and biodiversity due to deforestation, excessive fertilizer usage, pesticide runoffs and negative effect of monocropping on soil and ecosystem resilience (Asher 2019). This was seen in the cultivation of other major crops, such as the rampant deforestation for cattle soy-feed in the Amazon between 1996–2005 or the toxic algae bloom of Lake Erie in 2014 culminating from decades of pesticide and fertilizer runoff from corn crops (Macedo et al. 2012; Nepstad et al. 2014). However, blanket measures to address negative impacts of PO production may undermine the complexity of issues surrounding OP cultivation and the PO industry, and hamper concerted international efforts towards achieving various Sustainable Development Goals (SDGs) (Hinkes and Christoph-Schulz 2020).

While negative impacts of excessive consumption and unsustainable agricultural practices seem indubitable, what constitutes as sustainable practices and their ability to mitigate associated negative issues is less well-defined, exposing significant knowledge gaps in efforts to develop more effective sustainability policies for PO. Thus, this review aims to (1) summarize the available evidence on the impact of PO production and consumption on human health, social and economic aspects, environment, and biodiversity in the Malaysian context, and (2) discuss efforts to mitigate negative impacts and identify SDG-related co-benefits and trade-offs of different mitigation strategies.

Impact of Malaysian palm oil

The impact of PO was summarized based on a systematic search using PRISMA (http://www.prisma-statement.org/) guidelines, with manual addition of relevant contextual articles and reports resulting in a total of 276 full texts reviewed (Supplement, Figure S1). The various impacts of PO were categorized broadly under health, socioeconomics, environment, and biodiversity (in the Malaysian context), and then mapped to specific SDG keywords (Fonseca et al. 2020).

Nutritional impact of palm oil consumption

Palm oil is used ubiquitously as cooking oil in different Asian and West African cuisines because it is tasteless, odorless, and has a high smoke-point, which makes it safe for re-frying (Boateng et al. 2016). Due to its naturally occurring partially hydrogenated fats (Magri et al. 2015), PO results in crunchiness, palatability, and preservation properties in manufacturing (Di Genova et al. 2018), without posing the risk of cardiovascular disease (CVD) associated with industrially produced partially hydrogenated vegetable oils (Odia 2015; Magri et al. 2015). As such, PO-derivatives are commonly found in biscuits, noodles, and bakery products worldwide (Boateng et al. 2016). The health effects of widespread PO consumption remain contentious. Some studies cite adverse effects of saturated fats (Kadandale et al. 2019), while others tout protective effects on cardiovascular health, antidiabetic and anticancer properties, and reproductive health improvement (Giri and Bhatia 2020; Ibrahim et al. 2020a, b) due to PO-derived phytonutrients such as tocotrienols and carotenoids, which is converted into an important micronutrient, Vitamin A.

Phytonutrients and oxidative stability (SDG2)

Red PO (RPO) is known to possess pro-vitamin A activity (Loganathan et al. 2017) and used in vitamin A fortification programs due to its resistance to oxidation and stability (Pignitter et al. 2016). However, RPO trials for preventing Vitamin A deficiency, a key factor in preventable blindness and severe infection in low-income countries, have garnered inconsistent results, with some reporting RPO supplementation effective, and others concluding a lack of significant effects (Dong et al. 2017). Nevertheless, compared to RPO, which retains 80% of its vitamins and carotenoids, most PO consumed as cooking oil or in processed food is refined, bleached, and contains less phytonutrients.

Palm oil also contains relatively high amounts of tocotrienols, a form of vitamin E known to scavenge free radicals for prevention of pathologies (Jegede et al. 2015). Animal studies on PO tocotrienol rich fraction (TRF) suggest beneficial effects such as prevention of bone loss (Wong et al. 2018), amelioration of Alzheimer's related behavior and cognitive impairments (Durani et al. 2018), and improvement in antioxidant levels through modulation activity of antioxidant enzymes (Nor Azman et al. 2018). However, these benefits have not been convincingly observed in human trials such as those on TRF treatment in diabetes (Tan et al. 2018).

Saturated fats and contaminants (SDG3)

Adverse health impacts of PO are related to its saturated fatty acid (SFA) content, whereby the carbon chain "saturated" with hydrogen purportedly increases low-density lipoprotein (LDL) cholesterol, reduces fat oxidation (Yajima et al. 2018), and elevates risk of CVD. However, the majority of SFA in PO is palmitic acid (44%), known to be less potent in raising LDL cholesterol, unlike lauric and myristic acids, which are present in trace amounts (Magri et al. 2015; Boateng et al. 2016).

The association between PO carbon chain saturation and increased risk of CVD is subject to debate. Some claim unsaturation (at the sn-2 position) alters PO characteristics to mimic monounsaturated oils such as olive oil, instead of harmful saturated animal fats (Sin Teh et al. 2018). Others claim that the degree of saturation has a greater effect on blood lipid concentration than the positional distribution of SFAs (Sun et al. 2015). A randomized trial studying the effects of hybrid PO supplementation on human plasma lipid patterns concluded that effects on plasma lipids were comparable to extra virgin olive oil, which is typically consumed for its protective effects on cardiovascular health (Lucci et al. 2016), although notably, the study evaluated crude HPO (from *E. guineensis* and *E. oleifera*), which differs from refined PO in its content of vitamin E and carotenoids.

In animal studies including murids and fish, high-fat diets of PO and/or consumption of polar compounds from deep-frying of PO were associated with elevated levels of LDL, triglycerides (Sales et al. 2019; Larbi et al. 2018), and alkaline phosphatase-induced liver lipid accumulation (Janssens et al. 2015), changes in offspring's adipose tissue in adult life (Magri et al. 2015), and impaired glucose tolerance (Li et al. 2017). Conversely, PO supplementation in sheep feed has been shown to reduce SFA content, thus increasing mono- and polyunsaturated fatty acid content in sheep milk (Bianchi et al. 2017).

The evidence appears more equivocal in humans, especially under conditions of regular consumption. A systematic review by Ismail et al. (2018) challenged the link between PO consumption and the elevation of LDL concluding the lack of strong evidence for increased risk of CVD (Ismail et al. 2018), while an earlier systematic review and meta-analysis of clinical trials by Sun et al. (2015) concluded that PO consumption results in higher LDL cholesterol compared to vegetable oils low in saturated fat, but results in higher HDL cholesterol compared to trans-fat-containing oils in humans. These reviews are notably limited by a small number of studies and publication biases based on funding source, widespread consumption, and difficulty to single out PO from other food items (Ismail et al. 2018; Sun et al. 2015). A recent randomized controlled-feeding trial in healthy normocholesterolemic adults showed that butter raised LDL cholesterol higher than palm stearin regardless of background consumption of carbohydrate and fat (Hyde et al 2021). Thus far, the oft-cited negative effects of saturated fats from PO remains inconclusive.

Finally, processing and manufacturing issues, especially for non-branded oils known to contain less favorable SFA composition (Aung et al. 2018) and increased PO contaminants pose additional health risks. However, such risks may be mitigated with improved industry standards and regulation (Di Genova et al. 2018). Ultimately, consumption of PO, especially in a healthy balanced diet, does not appear to pose significant elevated risk for chronic diseases such as CVD, diabetes, and cancer in adult or pediatric populations (Odia 2015; Di Genova et al. 2018; Mancini et al. 2015; Marangoni et al. 2017; Bronsky et al. 2019). Reducing PO consumption by 50% is predicted to only have a relatively small impact on health (Jensen et al. 2019), unless it is part of a broader dietary and nutritional strategy to achieve significant improvements in health.

Social and economic impacts of the palm oil industry

National economic growth (SDG9, 12)

Most plantations in Malaysia are large-scale plantations with 61% of these plantations belonging to private estates, 22% governed under governmental schemes (half of which, belong to smallholders (van Leeuwen 2019), i.e., family-based plantations of less than 50 ha) and 17% owned by independent smallholders (MPOB 2017). Smallholders produce PO independently and sell their produce directly to a mill or through various governmental schemes (RSPO 2018). More than 300,000 smallholders, including farmers of indigenous tribes both in East and West Malaysia, contribute to more than 18 Mio t of annually exported PO (Hamid et al. 2013). Although, OP agriculture is commonly associated with Borneo, in 2017 Peninsular Malaysia contributed a larger fraction of Malaysia's total PO products (ca. 52%) (MPOB 2018; Shevade and Loboda 2019).

Approximately 30% of all globally produced vegetable oils is PO, accounting for two thirds of exported oils by volume (Shevade and Loboda 2019). Increasing demand from China and India stimulated the rapid growth of the PO industry, with Malaysia supplying 44% of globally exported PO (MPOB 2017; Shevade and Loboda 2019; Brandi et al. 2015). Through a series of policies, including the implementation of New Key Economic Areas for PO (Jomo and Rock 1998; Pemandu 2010), Malaysia has further fostered domestic PO refining businesses, achieving a refining capacity of 26.5 Mio t pa exceeding its annual CPO production (MPOB 2020). The PO sector also draws foreign investments to Malaysia (Mekhilef et al. 2011; Shevade and Loboda 2019) and earnings through corporate taxes (Mahat 2012). Since 2008, the industry contributed ca. MYR65.2bil (ca. USD16bil) in exports (Mekhilef et al. 2011; MPOB 2017), and by 2015, PO contributed to 4.2% of Malaysia's GDP (Shevade and Loboda 2019), and currently 37.7% of agricultural GDP (DOSM 2020).

Poverty alleviation and food security (SDG1, 8, 10)

Since the 1950s, the PO industry has been a catalyst for development (Awang Ali et al. 2011; Arif and Tengku Mohd Ariff 2001; Mahat 2012), becoming a major employer and key contributor to rural growth in Malaysia (Feintrenie et al. 2010; Rist et al. 2010; Lee et al. 2014; Castiblanco et al. 2015; Gatto et al. 2017; Choiruzzad 2019; Arif and Tengku Mohd Ariff 2001). Since 1956, progressive land expansion schemes led by the Federal Land Development Agency (FELDA) aimed to develop plantation land for the landless and rural poor Malays (Teoh 2002) and increased smallholder income from traditional crops (Awang Ali et al. 2011; Mahat 2012). With ca. 85 k ha of developed land and resettling of ca. 110 k families (Ahmad Tarmizi 2008), FELDA has contributed to poverty alleviation amongst settlers with the reported average monthly household income of FELDA smallholders increased from MYR1338 in 2006 to MYR3000 in 2010, exceeding the national poverty limit of MYR720 per month (Ahmad Tarmizi 2008).

The PO industry employs approximately 2.3 million people in Malaysia (Mahat 2012), contributing to Malaysia's low unemployment rate of ca. 3.4%, which only recently increased to 4.6% due to the COVID-19 pandemic (DOSM 2021). Insufficient local labor supply has also led to an influx of one million foreign workers from neighboring developing countries who are mainly employed in plantations and mills (Basiron 2011), including smallholder plantations (Awang Ali et al. 2011) but recruitment activities in the PO industry have stalled since the pandemic. The plantation sector employs more than 70% foreign workers and the ongoing national and international travel restrictions imposed to curb the impacts of the pandemic have caused labor shortages by 500,000 workers, resulting in a 3.8% decrease in PO production between 2019 and 2020. The ongoing COVID-19 pandemic has also led to a decline of PO exports from 18.5 Mio Mt in 2019 to 17.4 Mio Mt in 2020 (MPOC 2020).

In rural areas with limited job opportunities, government incentives enabled farmers to develop their plantations (Asmit and Koesrindartoto 2018) while simultaneously increasing employment opportunities. The labor-intensive plantation sector only has low level mechanization; thus, workers are required for fruit harvesting, collection, and other fieldworks (Ismail 2013). Growing OP is also a lucrative and popular side crop for farmers of other lower-yielding but government-incentivized agricultural crops such as paddy (Alam et al. 2010). Small growers often practice mixed farming by raising livestock and cultivating fruits and vegetables at the OP plantations, enhancing income opportunities (Ashraf et al. 2018). Consequently, OP cultivation along with mixed agricultural activities are associated with improved standard of living throughout the country, narrowing the income gap between rural and urban workforce (Mahat 2012; Hamid et al. 2013; Man et al. 2013). However, smallholders in less accessible areas suffer land shortage and encroachment from nearby estates, rendering them more vulnerable to fluctuations in crude PO prices (Azhar et al. 2020).

Poverty and food security are inextricably linked, and the PO industry generally contributes to both increased employment and food security in Malaysia and globally, as PO remains the world's most affordable edible vegetable oil, and a staple in low-middle income countries across Asia, Africa, and the Middle East (Boateng et al. 2016). Improved income arising from agricultural activities such as rubber and OP cultivation, in combination with traditional hunting and gathering activities, appears to be associated with lower risk of malnutrition among certain indigenous tribes, such as the Jah Hut in Pahang (Law et al. 2020). However, OP plantation expansion has negative impact on rural and indigenous food security when land rights of these rural communities are violated (Nesadurai 2013).

Social conflicts and adverse impact on indigenous peoples (SDG8)

Conflicts are common between indigenous peoples in Malaysia and large companies who were granted development permits in forest reserves, while low wages and/or human rights violations of foreign plantation workers continue to tarnish the reputation of the industry (Wan Daud et al. 2020). Oil palm expansion may also contribute to rising inequality among farmers or between communities (Gatto et al. 2017; Cramb and McCarthy 2016), especially when farmers are forced to sell their land, consequently losing means for their own agricultural production (Bou Dib et al. 2018; McCarthy 2010).

In Sabah and Sarawak, most plantations are on steep hills that were converted from agricultural land with slash-and-burn techniques (Wicke 2011). Traditionally, burning of plant material in old plantations to get rid of unwanted plant waste can enhance nutrient availability in depleted soils (Knicker 2007), but this technique has led to dramatic haze problems throughout Southeast Asia (Padfield et al. 2016), and directly reduced the life quality of farmers living in affected areas (Obidzinski et al. 2012). Many communities that depend on ecosystem services such as clean rivers, natural forest products and/or small-scale agricultural activities for income are highly affected by land conversion, as they lose access to these resources (Martin 2017; Awang Ali et al. 2011; Mahat 2012). Furthermore, chemical fertilizers and pesticides used in large-scale plantations may pollute freshwater resources that are essential to indigenous and rural communities (Obidzinski et al. 2012; Bou Dib et al. 2018; Dudgeon et al. 2006).

The emphasis for commodity crops such as OP and rubber and the rapid expansion of plantations and intensive logging may have indirectly contributed toward poverty and heightened vulnerability of some indigenous peoples in Malaysia (Kari et al. 2016; Wan Daud et al. 2020). Barriers in technology and knowledge transfer to indigenous farmers and small growers renders them less empowered to use best practices that can improve yield while minimizing practices with adverse impacts, such as slash and burn for clearing of old OP trees (Rochmyaningsih 2015). Indigenous small growers also suffer more threats from wild animals, given that they tend to cultivate farms close to forests (Law et al. 2018). Such threats to safety often contribute to agricultural failure, and consequent food insecurity among indigenous people.

Impact of oil palm cultivation on the environment and biodiversity

Land use policies in Malaysia are regulated by the state governments. Half of all plantations are planted on state forest land that was previously used for rubber or other uses (Hansen et al. 2014; Padfield et al. 2019). From 2010 to 2018, OP plantations reportedly increased by 5.06 Mha at a growth rate of 83.5%, with growth rates for East and West Malaysia at 109.5% and 62.1%, respectively (Li et al. 2020). The deforestation for new OP plantations between 2001 and 2017 reached 5.98 Mha accounting for 68.2% of the total amount of deforestation in Malaysia for that period (Li et al. 2020). While west Malaysian plantations mostly expanded on former rubber plantations (Barlow 1997), in Sabah and Sarawak, plantation expansion has driven deforestation with 4.2 Mha of old growth forest cleared between 1973 and 2015 (Gaveau et al. 2016). Compared to Indonesia, Malaysia had a more rapid conversion rate of these cleared lands of ca. 60% within a 5-year time frame (Gaveau et al. 2016).

Specifically for peatlands, plantation area reportedly increased by ca. 200 kha between 2003 and 2008 (Edwards et al. 2010), amounting to one-third of the total new plantations, with the majority occurring in Sarawak (Edwards et al. 2010). However, Gunarso et al. (2013) reported a lower estimate of 13% of OP planted on peatland in 2010. Records from a 250 m spatial resolution map showed that more than 800 kha of Malaysian tropical peat swamp have been converted to OP plantations (Koh et al. 2011). Specifically, Sarawak and Sabah had 49.5% and 34.6% of peatlands covered by OP, respectively, in 2015 (Miettinen et al. 2017). However, since 2015, over 90% of internationally traded PO followed producer commitments for the 'no deforestation and no peat' policy (Austin et al. 2015; Butler 2015). More recently, Wan Mohd Jaafar et al. (2020) reported declined conversion of peatland to plantation by 20.5% and 19.1% in Sarawak and Sabah, respectively.

Land use change for agriculture is associated with adverse impacts on the environment through increased greenhouse gas (GHG) emissions, soil erosion, and microclimate and regional climate changes, i.e., change in temperature and precipitation, and increased risk of flooding (Gaveau et al. 2014; Uning et al. 2020; Wolf 1996).

Emissions, soil, and climate (SDG6, 13).

Malaysia is the fourth highest carbon emitter from forest degradation with over 140 Mio t CO_2 pa after Indonesia, Brazil and India (Harris et al. 2012; Pearson et al. 2017; Begum et al. 2020). The conversion of forest into OP plantations reduced carbon stocks by over 50% and increased GHG emissions by four times, compared to land converted from old rubber plantations (Kusin et al. 2017). Plantations on peat in Malaysia emit GHG ranging from 12.4 up to 76.6 t CO_2-eq ha^{-1} pa (Hashim et al. 2018), with highest emissions recorded in Selangor (65 t C ha^{-1}) in 2006, and the lowest in Sarawak (7 t C ha^{-1}) (Matysek et al. 2018; Melling et al. 2005, 2008, 2013). Cooper et al. (2020) suggest that conversion of peat swamp forest in Malaysia contributes between 16.6 and 27.9% (95% CI) of combined total national GHG emissions. CO_2 release from drained peatland is higher than from mineral soils as peat stocks hold higher quantities of carbon (Choo et al. 2011; Hashim et al. 2018; Page et al. 2011). This loss of stocked carbon increases during the dry season, facilitated by longer and more intense dry seasons associated with current global climate emergency (Matysek et al. 2018). Additionally, plantations on peatlands lead to degradation and fires (Page and Hooijer 2016; Page et al 2009), which are associated with significant air pollution and threats to human health in the region (Uda et al. 2019; Crippa et al. 2016).

While OP plantations and associated cultivation practices emit up to two times more CO_2 than other crops, they also absorb CO_2 and produce around 18 t O_2 ha^{-1} pa (Uning et al. 2020). The emission difference between different croplands depends on the type of soil and amount of carbon stocks, drainage and fertilizer use, and methane use at the mills (Hashim et al. 2018). However, CO_2 uptake above OP canopy of 82 t C ha^{-1} pa was recorded in Sabah and this is reportedly higher than from intact forests (32 t C ha^{-1} pa) (Fowler et al. 2011; Sharvini et al. 2020), suggesting potential carbon neutrality of OP cultivated lands (Kusin et al. 2017).

Plantations' nitrous oxide (N_2O) emissions are lower than those of primary forests as forest soils are a better source of N_2O than plantations (Yashiro et al. 2007). Newly established OP plantations show slightly higher N_2O emissions than older plantations due to the use of fertilizers and other environmental factors (Melling et al. 2007; Yashiro et al. 2008), but overall N_2O emission levels seem similar in OP plantations of different ages (Kusin et al. 2017). Consistent with other studies (Holzinger et al. 2002), emissions of isoprene (C_5H_8), CO_2, and surface ozone (O_3) from OP plantations in Pahang were temperature-dependent, with lower emission during cooler temperatures at night (Uning et al. 2020). Increased NO_x emissions over plantations are caused by fertilizer and PO plant combustion and vehicle exhausts (Uning et al. 2020).

Evapotranspiration processes, but not the amount of precipitation, are impacted by land conversion from forests into OP plantations (Amin et al. 2016). Oil palms planted on steep terrain cause negative hydrological impacts such as increased flooding risk, modification of river ecology, and sedimentation (Nainar et al. 2018; Saadatkhah et al. 2016). Soil mineralization rates are similar between OP plantations and forests in Sabah (Hamilton et al. 2016) with approximately 90% reduction of denitrification and anaerobic ammonium oxidation disrupting nitrogen gas (N_2) production. Consequently, high nitrate concentration occurs in ground water of OP plantations, due to the application of N-fertilizers (Sheikhy et al. 2018).

Changes in microclimate directly impact plant growth and soil nutrient processes, with the temperature inside plantations increased by 6.5 °C compared to primary forest in Sabah (Hardwick et al. 2015). The change in regional climate manifests through increased rainfall, temperature, radiation, atmospheric pressure, cloud cover, and a decrease in evaporation, relative humidity, sunshine hours, and wind speed due to intensive land use change in the Kelantan River Basin between 1984 and 2014, which follows trends found across Malaysia (Nurhidayu and Hakeem 2017).

Biodiversity (SDG14, 15)

There is still poor understanding of how species respond to anthropogenic disturbance in tropical primary forests (Silmi et al. 2013; Fitzherbert et al. 2008) but biodiversity decline of different species of arthropods, fish, amphibians, birds, and mammals in Malaysia due to the expansion of plantations into natural habitats is well documented (Vijay et al. 2016; Brandon-Mong et al. 2018). Some indicator species may predict changes in diversity and geographical distinctness in relation to habitat disturbance. For example, fruit-eating butterfly abundance in Sabah was lowest in OP plantations compared to primary and logged-over forest (Koh and Wilcove 2008), and species richness was lower in areas converted from primary or secondary forest to OP plantations compared to conversion from existing rubber to OP plantations (Koh and Wilcove 2008; Hamer et al. 2003). Oil palm plantations in Sabah showed lower species richness of ants compared to riparian reserves and logged forests (Fayle et al. 2010; Gray et al. 2015). Most ant species in plantations were non-forest species (Brühl and Eltz 2010), suggesting that conversion of forest into plantations results in replacement of native forest ant species with more dominant invasive species. However, when assessing the ratio between regional and local species at a large spatial scale, more common and abundant species of ants were found in OP plantations than forests (Wang and Foster 2015).

Species richness of freshwater fishes was lower in rivers near OP plantations in Bintulu, Sarawak (Kano et al. 2020), and species diversity of amphibians (i.e. order Anura) was lower at OP plantations in Selangor compared to grassland, coconut plantation, and primary forest (Faruk et al. 2013). Similarly, anuran species richness was higher in primary and secondary forest habitats compared with OP plantations in Sabah (Aguilar-León 2020).

Less than 50 species of forest birds were recorded in OP plantations in Sabah demonstrating the lowest diversity compared with primary forest, logged forest and rubber plantation (Koh and Wilcove 2008). The diversity and density of insectivorous and frugivorous bird species were also lowest in OP plantations compared to secondary forests and paddy fields in Kerian River Basin, Peninsular Malaysia, due to lower tree density and basal areas and less fruit availability in plantations (Azman et al. 2011).

A substantial decline of terrestrial mammal abundance in OP plantations compared to nearby forests was reported from Sabah (Wearn et al. 2017; Yue et al. 2015). Although plantations offer food resources to mammals like the Malayan sun bear (Guharajan et al. 2018), camera trap studies recorded their presence in forest patches rather than highly degraded areas such as plantations (Abidin et al. 2018). Sunda clouded leopards appear intolerant to deforestation and forest fragmentation in the Lower Kinabatangan and Kabili-Sepilok areas that are composed of small forest patches embedded with OP plantations (Hearn et al. 2018, 2019) highlighting these as priority areas requiring protection for threatened felid species (Hearn et al. 2016a; b; c).

Conversely, OP plantations hosted the highest number of carnivorous birds including striated, Chinese and Javan pond heron at Kerian River Basin, Perak due to the high availability of prey such as shrews, snakes, and rats (Azman et al. 2011). Plantations may even bring positive effects for selected species, such as the black-shouldered kite, as they provide good vantage positions, shelter from predators and a suitable physical environment for such species (Ramli and Fauzi 2018).

Human–wildlife conflict (SDG15)

Human–wildlife conflict is a main driver of species loss across the globe (Meijaard et al. 2018) usually due to illegal hunting caused by economic constraints, demand for exotic pets and products, road kills, and culling of crop pests (Azhar et al. 2013; Liu et al. 2011). Wildlife poaching is facilitated by habitat conversion to OP plantations and other agricultural crops (Azhar et al. 2013). Terrestrial animals that are highly dependent on forest, such as elephants, tend to forage in the plantations, which is perceived as raiding due to their destructive feeding habits (Guharajan et al. 2018; Cazzolla Gatti and Velichevskaya 2020). Human–elephant conflicts in plantations in the lower Kinabatangan in Sabah are driven by incidental poaching or revenge killings of elephants by plantation workers, although elephants usually actively seek to avoid humans (Evans et al. 2020). While managing elephant populations surrounding plantations via translocation to other areas appears to be an option, studies suggest that this practice is harmful to their populations. A population viability assessment of Asian elephants in the Endau Rompin landscape showed that even translocating only a few individuals poses risks on the population, suggesting the need for an in-situ management plan at OP plantations to curb human–elephant conflicts (Azhar et al. 2013; Asimopoulos 2016; Saaban et al. 2011).

Efforts to enhance benefits and mitigate adverse impacts

As summarized above, the impact of widespread PO production and consumption on different aspects of SDGs are undeniable (Table 1). However, to effectively expand on positive effects of PO while addressing adverse effects, there is a need to holistically evaluate different mitigation strategies and identify co-benefits and trade-offs on different SDGs. We summarize potential co-benefits and trade-offs of specific mitigation strategies in Fig. 1, proposed based on the evidence discussed below.

Replacing palm oil with a different oil (SDG 1–3, 6–10, 12–15)

The negative perception of PO usually relates to deforestation and replacement of more diverse agricultural or agroforestry systems with these monocultures (Meijaard and Sheil 2019). However, debates about PO by supporters and opponents are often highly polarized. Calls for boycotting PO do not consider the fact that (1) OP is the highest yielding available oil crop requiring eight times less land and producing up to 20 times more oil compared to soybean, canola and sunflower (Low 2019; Woittiez et al. 2017); (2) OP crops may alleviate poverty among rural growers who may have little opportunity for other income-generation, especially in Malaysia (Sheil et al. 2009; Pirker et al. 2016); (3) economic reliance on PO in the region is widespread (Shevade and Loboda 2019); and (4) cultivation practices are heterogeneous with varying policies and enforcement practices in producer countries affecting impact (Meijaard et al. 2018). On the other hand, OP supporters often do not weigh in the losses of biodiversity and related ecosystem services but instead argue from nationalistic and socioeconomic perspectives (Liu et al. 2020).

Removing or replacing PO globally would likely adversely impact food security to producer and consumer countries (Gro Intelligence 2016) as restrictions would not only affect the demand, consequently reducing livelihood of producers, but also impact the supply of this edible oil, thus, leading to higher prices of cooking oil and consumer goods that would affect other sectors as well. A comparison of five major vegetable oil crops by Beyer et al. (2020) concluded that better management of future growing areas will be more effective at reducing environmental impacts of global vegetable oil production rather than oil crop substitution. Despite OP having larger impact on range-restricted species, OP was estimated to pose the lowest carbon and species richness loss per-ton-oil (Beyer et al. 2020). Thus, removing or replacing PO may lead to higher biodiversity losses in the future as more land needs to be converted to cultivate lower yielding oil crops leading to further habitat destruction (Meijaard et al. 2018).

Policies and regulations (SDG1,6–10,12–15)

Sustainable certification and 'no deforestation, no peat, no exploitation' (NDPE) policies

Generally, GHG emissions in Malaysia can be reduced by 4.1 t CO_2-eq ha^{-1} pa simply by banning the establishment of new OP plantations on peat soil (Hashim et al. 2018). Additionally, the environmental sustainability of OP can be improved by stopping burning practices, reducing the use of peatland and swamp areas, and replacing fossil fuels by biofuel for plantation activities (Uning et al. 2020). Hence, to fulfil the growing global demand for PO while achieving conservation goals, voluntary certification under the international Roundtable Of Sustainable Palm Oil (RSPO) aims to ensure sustainability through a set of standards, accreditation, and process requirements (RSPO 2017; Abazue et al. 2015), encompassing optimization of productivity and efficiency while adhering to transparency, ethical, and legal principles, respecting communities, supporting smallholders, and protecting workers, while conserving the larger ecosystem.

While Malaysia is already a signatory of the RSPO network (Abazue et al. 2015), the Malaysian Sustainable Palm Oil (MSPO) certification scheme was established in 2015 to improve the PO governance and branding of Malaysian PO through nationwide sustainability initiatives, and transparency throughout the value chain (Pacheco et al. 2018).

Table 1 Summary of impact, mitigation strategies and recommendations related to palm oil production in Malaysia aligned with the SDGs

	Environment	Biodiversity	Health	Socioeconomic
Associated SDGs and their keywords	Deforestation, plantation emissions and waste management impact clean water (SDG6), and climate action (SDG13) SDG6: clean water, ecosystem protection, floods, pollution, rivers SDG13: climate resilience, CO_2 capture, ecosystems, emissions, greenhouse gas emissions, pollution, temperature	Deforestation and human-wildlife conflict compromise on diversity and abundance of native aquatic (SDG14) and terrestrial (SDG15) species SDG14: marine pollution, water resources and policy SDG15: land loss, land use and sustainability, poaching, protected species, species, terrestrial ecosystems, threatened species	Consumption of PO as a cheap, abundant, and phytonutrient-rich oil allows for alleviation of micronutrient deficiencies (SDG2) but may have adverse cardiovascular impact (SDG3) SDG2: food, improved nutrition, nutritional needs SDG3: diseases, health	Diverse impact on human quality of life (SDG10), livelihood (SDG1, SDG8), and industrial (SDG9), alternative energy (SDG7), and national growth and development (SDG12) SDG1: poverty, poverty eradication, resources SDG7: renewable energy SDG8: forced labor, GDP growth, global trade, innovation, job creation, migrant workers, modern slavery, poverty eradication, social policies, sustainable economic growth SDG9: economic development, environmentally sound technologies, industrial diversification, Value chains and markets SDG12: natural resources, reduce waste generation, resource efficiency
Benefits	N_2O emissions are lower than in primary forests (Yashiro et al. 2007) OP plantations absorb CO_2 and produce around 18 t O ha^{-1} pa^{-1} (Uning et al. 2020). CO_2 uptake above OP canopy on average higher than from intact forests (Fowler et al. 2011; Sharvini et al. 2020)		High amounts of phytonutrients (e.g., tocotrienols) in red PO flags potential role in economical supplement and fortification programs (Jegede et al. 2015; Pignitter et al. 2016; Loganathan et al. 2017; Dong et al. 2017; Selby-Pham et al. 2020) Oxidative stability makes PO a safer alternative for long-term storage, repeated use, and high heat cooking (Boateng et al. 2016; Magri et al. 2015)	As a high-yield industry, PO is a key national economic resource, attracting foreign direct investments, employing locals and foreign workers, stimulating biofuel industry, alleviating rural poverty, and contributing to domestic and regional food security (Shevade and Loboda 2019; DOSM 2020; MPOB 2018)

Table 1 (continued)

	Environment	Biodiversity	Health	Socioeconomic
Adverse effects	Conversion of forest into OP plantations were associated with: Increased GHG emissions, reduced carbon stocks (Kusin et al. 2017; Choo et al. 2011; Hashim et al. 2018; Page et al. 2011; Matysek et al. 2018); highly reduced denitrification and enhanced N emissions due to fertilizers, plant combustion and vehicle exhausts (Hamilton et al. 2016; Drewer et al. 2020; Uning et al. 2020); increased flooding risk and sedimentation (Nainar et al. 2018; Saadatkhah et al. 2016); and microclimate and climate changes (Hardwick et al. 2015; Nurhidayu and Hakeem 2017) Peat swamp forests are much more prone to fire than any other forest type (Page and Hooijer 2016; Page et al. 2009) and plantation peatland fire-led haze increases air pollution–related premature mortality at 648 cases per year (26 mortality cases per 100,000 population) due to chronic respiratory and cardiovascular diseases and lung cancer (Uda et al. 2019; Crippa et al. 2016)	Expansion of OP plantations into natural wildlife habitats threatens biodiversity and can result in reduced species abundance, local extinctions, or invasive species (Koh and Wilcove 2008; Brühl and Eltz 2010; Azman et al. 2011; Vijay et al. 2016) Forest fragmentation leads to human wildlife conflicts, poaching, and illegal wildlife trade (Liu et al. 2011; Azhar et al. 2013; Evans et al. 2020)	Saturated fat content of PO increases risk of CVD when consumed excessively (Yajima et al. 2018; Ismail et al. 2018; Sun et al. 2015) Risk of contaminants higher, especially in non-branded oils due to poorly regulated manufacturing processes (Aung et al. 2018; Di Genova et al. 2018)	Social conflicts arising from some practices in the PO industry were linked to "modern slavery" of migrant workers (withholding salaries and passports), land right issues affecting rural and indigenous communities (Wan Daud et al. 2020), deforestation and pollution, which threaten quality of life of local communities Differences in knowledge, technological adoption, and land ownership widens the gap between large companies and small growers and contributes to rising inequality among farmers and between communities (Rochmyaningsih 2015)

Table 1 (continued)

	Environment	Biodiversity	Health	Socioeconomic
Mitigation opportunities	Riparian buffer establishment to mitigate emissions and microclimate changes (Chellaiah and Yule 2018; Luke et al. 2017; Mitchell et al. 2018) Waste conversion of POME residues (Rupani et al. 2010) into biofertilizers (Truckell et al. 2019), PO mills waste into biobutanol and other (Rubinsin et al. 2020; Szulczyk and Cheema 2020). Use of OP shell in cement as an aggregate agent (Hamada et al. 2020) Intercropping to reduce soil erosion, spread of plant diseases and reliance on herbicides, while allowing smallholders to generate supplementary income (Ahmed et al. 2001; Hanafi et al. 2009; Ibrahim et al. 2020a, b; van Leeuwen 2019; Woittiez et al. 2017; Samedani et al. 2015) Use of biological pest control agents to decrease pesticide use and enhance biodiversity in plantations (Salim et al. 2014; Holzner et al. 2019)	Adaptation of some animal species to OP plantations creates opportunities for in situ conservation as opposed to species translocation (Azman et al. 2011) Wildlife corridors & habitat reconnection/reforestation may reduce human-wildlife conflicts and re-establish genetic flow across fragmented forest patches (Faruk et al. 2013; Bernard et al. 2014; Holzner et al. 2019) Education opportunities for habitat protection and enforcement against illegal encroachment, poaching, and wildlife trade (Meijaard et al. 2018) Certification and international agreements such as RSPO, MSPO, Aichi Targets and International Biology Convention may facilitate engagement across multi-stakeholders and stewards (RSPO 2017; Abazue et al. 2015; CBD 2011)	Encouraging moderation and overall healthy diet, instead of focusing only on PO consumption, is more prudent and likely to have a more significant impact on health outcomes (Odia 2015; Di Genova et al. 2018; Mancini et al. 2015; Marangoni et al. 2017; Jensen et al. 2019; Bronsky et al. 2019)	Effective implementation of sustainability certification may ensure protection of worker welfare and level the playing field between small growers and large-scale plantation companies through promotion of technological transfer and best practices (Nesadurai 2013) Addressing issues of inequity and fluctuating prices through governance (reducing reliance on middleman/mills) and certification promote best practices for small growers (Kumaran 2019)
Challenges	Difficulty in harmonizing different and/or competing priorities of stakeholders and jurisdictions of various stewards related to different aspects of OP plantation establishment and management Role of regulation and policies to address transboundary environmental issues (e.g., haze)	Limited funding/lack of political will Extinction cascades/accelerated species loss by multiple factors and climate change	Available data in humans remain inconsistent and equivocal, in part due to widespread presence of PO, and difficulty to tease out independent impact of PO (Sun et al. 2015; Ismail et al. 2018) Seeming publication bias due to source of funding (i.e., industry vs. independent studies) (Sun et al. 2015)	Ensuring transparency of sustainability certification, complex land laws, limited awareness of rights and access to resources for the most vulnerable stakeholders in the PO industry (Nesadurai 2013; Kumaran 2019)

Table 1 (continued)

	Environment	Biodiversity	Health	Socioeconomic
Knowledge gaps and key questions	Gaps in important information at many levels, with available data often limited to specific questions that do not address ecosystem function holistically (Gaveau et al. 2016) Lack of (international) standard framework for conducting LUC assessments and long-term (predictive) modelling of habitat conversion and its impacts on the environment and climate	Literature is widely limited to issues in Indonesia, and most discussions revolve around OP as the main cause of deforestation, but few studies highlight other agricultural activities (e.g., Meijaard et al. 2020) as driver of biodiversity loss or propose feasible mitigation efforts, especially applicable large-scale diversification schemes of existing plantations	Gaps in knowledge related to what is a moderate and safe amount of long-term PO consumption in different groups of people, differences in health impact depending on PO use in diet (cooking oil vs. ingredient) Additionally, the population level health impact of consuming PO, as regular part of diet compared to other oils remains unclear	Not clear how much sustainable certification is actually improving worker welfare, small grower productivity and income; issue with data availability Understanding of main benefits, concerns, and challenges of small growers and vulnerable communities around PO remains limited
Possible recommendations	Developing a standard framework for assessing impact of LUC (especially of peat lands) and identifying feasible management practices Improving communication between stakeholders and awareness on sustainable practices More targeted and comprehensive environmental and bioeconomic impact studies on sustainable vs. conventional PO production to assess the benefits of certification for the environment and local economy, especially for smallholders	Adopt and facilitate national policies on establishing and managing wildlife corridors, habitat protection and diversification of monocultural landscapes Increase funding (national budget) strategies that protect wildlife, habitats and mitigate human-wildlife conflicts Increase education and awareness on the importance of biodiversity and nature conservation in the public and stakeholders of the PO industry	Conduct independently funded multi-site, long-term, community or randomized trials that compare impact of PO vs. other oils as part of a larger diet in real-world setting Broader education on importance of overall balanced and nutritious diet based on standard recommendations, as opposed to overemphasis on single ingredient campaigns	Increased engagement and research to address gaps in equity as well as improving regulations to ensure vulnerable communities are protected

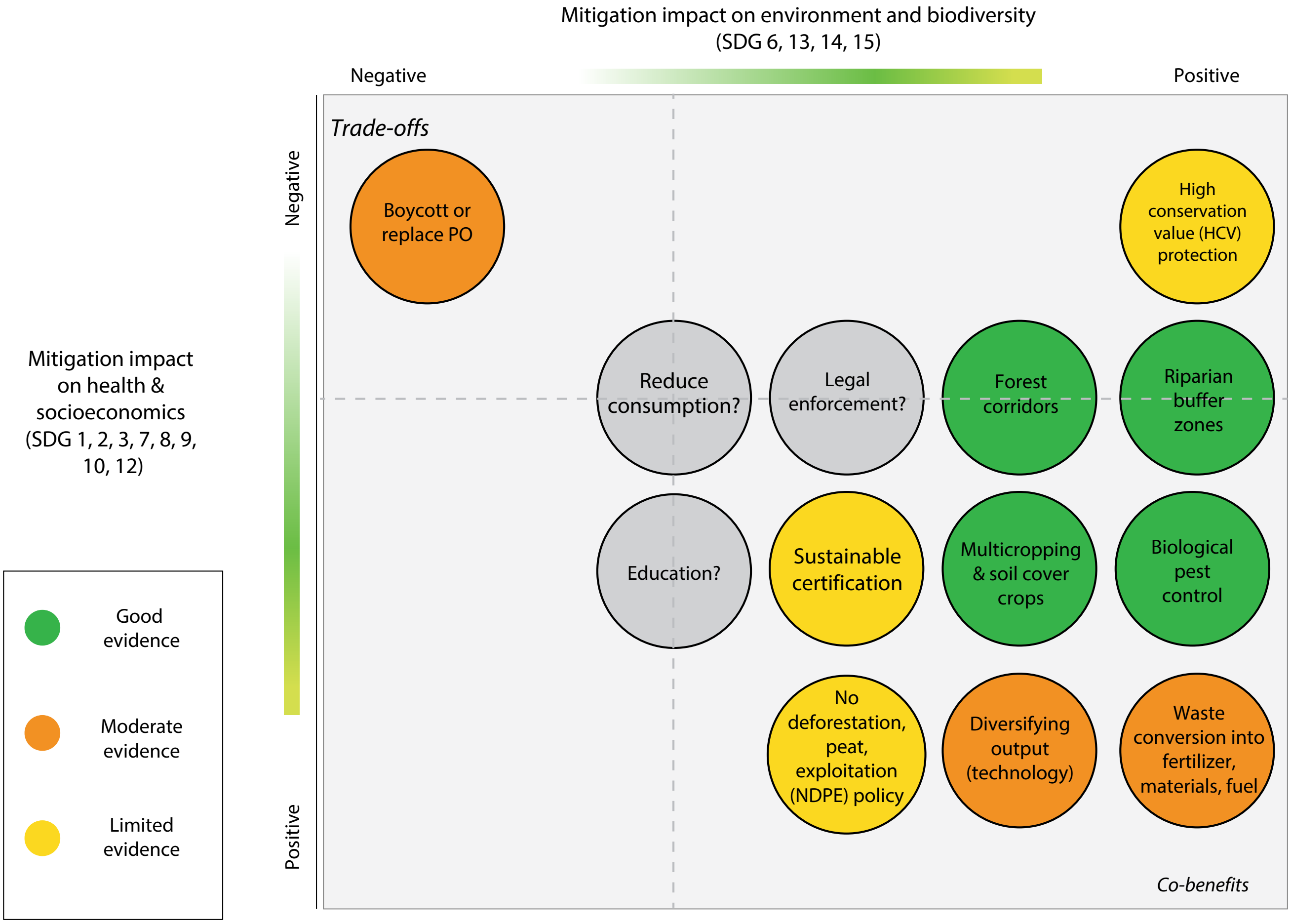

Fig. 1 Proposed interaction plot of mitigation strategies and their impact on health and socioeconomics (*y* axis) and environment and biodiversity (*x* axis) SDGs. The plot suggests that several strategies may have co-benefits, and fewer strategies are either conflicting or trade-offs that detract from efforts to achieve SDGs as a whole. Co-benefit (bottom right): positive for both; one-sided benefit (bottom left or top right): positive for either; or trade-off (top left): negative for both. Level of evidence indicated by color, i.e., green: good evidence; orange: moderate evidence; and yellow: limited evidence. Grey circles indicate potentially important elements that were unable to be directly addressed in the review

Overseen by the Malaysian Palm Oil Board (MPOB) and specifically aimed at supporting small and mid-range PO producers who cannot afford RSPO certification, the MSPO scheme has been made mandatory for OP plantations, independent smallholdings, and PO processing facilities since December 2019 (Kumaran 2019). Similar to the sustainability agenda of the RSPO that demands NDPE policies and 'no slash-and-burn practices' for certified plantations (Padfield et al. 2016), MSPO comprises seven governing principles, with 'Principle 5: Environment, natural resources, biodiversity and ecosystem services' setting the standards for mitigating impacts of OP agriculture on the biosphere.

Approximately 30% of the OP cultivated area globally is covered under voluntary or mandatory certification schemes (Kumaran 2019; RSPO 2017), and certain areas appear to be governed under multiple certification schemes (Barthel et al. 2018). Although, RSPO and MSPO ban establishment of large plantations (> 100 ha) on peatlands, this has not been enforced on the ground and slash-and-burn practices reportedly still exist (Carlson et al. 2018). Despite certification, some areas have sustained deforestation rates with major loss of tree cover and forest fires reported, and in Malaysia, the largest certified plantations have less than 1% of residual forest on their estates (Carlson et al. 2018). These reports highlight the need for routine monitoring of forest cover loss in certified plantations and penalties for members who do not comply (Carlson et al. 2018), to ensure the credibility of such certification schemes.

Although debates on the effectiveness of certification systems to mitigate forest loss and fire remain (Carlson et al. 2018), RSPO certification may reduce illegal deforestation outside of certified supply bases and act as a crucial tool to address negative impacts of PO (Heilmayr et al. 2020). However, more information is needed to determine the effect of certification on OP producers in broader areas, specifically ecological feedbacks and market forces that may improve the effect of certification. Edwards et al. (2010) proposed that funds obtained from certification of existing plantations be channeled into efforts such as biobanking and land sparing schemes that protect wildlife and forest land inside and outside of plantations. Ultimately, strict implementation of guidelines for best practices by governments and regulatory bodies, commitments to zero deforestation from all producer countries, open access of available datasets on global crop production, distribution, coverage, land cover change, and forest loss, tracking of milestones of international agreements, and legal enforcement of best practices embedded in national laws are needed to improve sustainability of PO production practices (Edwards et al. 2011).

Plantation expansion cap and high-conservation value policies

Malaysia recently committed to capping area expansion for OP plantations at 6.5 Mio ha until 2023 (Tan 2019), aiming instead to boost yield (De Pinto et al. 2017) and diversification to reduce dependency on land expansion (MPOB 2018). Besides curbing plantation expansion, high conservation value (HCV) approaches exist (often under certification schemes) to protect biological, ecological, social, or cultural values of outstanding significance, amounting to six HCV categories (HCV Resource Network 2021). HCV areas are identified at a plantation, farm or management unit level through an HCV assessment (Senior et al. 2015; Fleiss et al. 2020), which are then managed and monitored by land developers and other stakeholders. Assessment reports are publicly available and details HCVs present in specific areas as well as the evaluation results (HCV Resource Network 2021). For example, HCV detailed assessment reports for government plantations in Terengganu (Crawshaw 2019) and Sarawak (Sőzer 2016) describe at length the state of identified HCVs, such as specific mapped areas and the vegetation and wildlife species present, threats to different HCVs, and recommendations to manage the threats. While HCVs have yet to show concrete mitigatory impact in Malaysia, they may prove to be effective regulatory tools in the long term if there is commitment for transparent implementation and monitoring. Currently, although HCV assessments have been found to be beneficial, lack of monitoring indicators for plantations managers was identified as a barrier to efficient HCV implementation (Pillai 2020). Encouragingly, the Malaysian Palm Oil Green Conservation Fund (MPOGC) was incorporated on 19 February 2020 to support conservation projects such as the planting of 1 million forest trees in Lahad Datu, Sabah and the Orangutan Population Census and Pygmy Elephants program, a collaboration between the Malaysian Palm Oil Council (MPOC) and the Sabah State Government (Azian et al. 2020), which leverages on data highlighting the need to support these species in fragmented areas (Simon et al. 2019).

Biodiversity-friendly plantation management (SDG6, 13–15)

Forest corridors and riparian buffer zones

Best practice policies and the aim to achieve “biodiversity-friendly” conditions in plantations were among the top questions identified for biodiversity research (Coleman et al. 2019). Creating wildlife corridors by reserving forest patches in and around OP plantations, reforestation of underproductive OP plantation areas, and creation of forest buffer zones along rivers are necessary mitigation measures to enhance fitness of transient and vulnerable wildlife populations in OP landscapes (Wilting et al. 2012; Faruk et al. 2013; Bernard et al. 2014; Hearn et al. 2016a, b, c, 2018; Yamada et al. 2016; Holzner et al. 2019).

Riparian forests near water sources are recognized as important buffers to reduce water contamination from plantations and stabilize riverbanks (Gray et al. 2015; Luke et al. 2019), and to improve hydrology, biodiversity, ecosystem services and landscape connectivity (Marczak et al. 2010). Riparian reserve soils in Sabah release constant low rates of N_2O and NO independently of soil moisture under controlled conditions in contrast to OP plantations and logged forests (Drewer et al. 2020). Streams adjacent to OP plantations with a riparian buffer in Sabah were more shaded with cooler temperature and higher quantity of leaf litter (Chellaiah and Yule 2018; Luke et al. 2017). Carbon stocks in buffers surrounded by OP plantations were similar to intact riparian areas but were highly variable depending on the survey area (Mitchell et al. 2018; Fleiss et al. 2020). Anuran diversity in plantations was enhanced by proper biodiversity management strategies such as maintenance of stream complexity, riparian buffers, and reduced use of mechanical dredging (Faruk et al. 2013), whereby the presence of buffers or patches of forest does not impact OP yield (Edwards et al. 2014).

Additionally, Bornean orangutans show behavioral flexibility and nesting behavior in certified plantation areas with available forest patches (Ancrenaz et al. 2018; Santika et al. 2019). Orangutans of all sex-age groups were found in OP plantations in Kinabatangan as these plantations constitute a source of food and shelter to build nests, as well as travel corridors (Ancrenaz et al. 2015; Sherman et al. 2020). To ensure survival of orangutans, protection of certain tree and canopy attributes of remaining forest patches is crucial due to their large size, far travel distance, and other minimal ecological requirements of these great apes (Davies et al.

2017). Conversely, tree height and canopy cover showed no significant effects for making plantations more hospitable for other (small terrestrial) mammals, but additional land sparing strategies were suggested to tackle space and resource constraints (Yue et al. 2015). Bearded pigs along the Lower Wildlife Kinabatangan Sanctuary, Sabah regularly use OP plantations as habitat although secondary forest fragments are used for a wider range of behaviors such as nesting and wallowing (Love et al. 2018). Hence, although many species can adapt to plantations, forest patches are still important for them to flourish.

Multi-/inter-cropping and soil cover crops

Intercropping of OP plantations with other crops is practiced widely in Malaysia (Corley and Tinker 2016; van Leeuwen 2019), often by smallholders to generate income in the first years of planting before the palm trees produce fruits (Ahmed et al. 2001; Hanafi et al. 2009), and if performed on peat soil, intercropping has positive effects such as protecting the soil from erosion and improving its quality. Intercropping reduces harmful pathogens, such as fungi (Woittiez et al. 2017) that spread from old to new plantations (van Leeuwen 2019) but more studies are needed to confirm the benefits of using intercropping to improve crop disease prevention, productivity, and soil quality (van Leeuwen 2019). Conversely, excessive use of fertilizers and burning of pineapple residues used for intercropping in Johor have negative impacts, but this approach could be improved by removing pineapple waste by hand (van Leeuwen 2019) and valorizing the waste as compost, feed for animals, biogas, or others (Hepton 2003; Seguí and Maupoey 2018). In Pahang, alley cropping in OP plantations facilitated habitat complexity and significantly higher arthropod beta-diversity compared to other traditional monoculture systems (Ashraf et al. 2018). Generally, arthropod orders, but not abundance or composition, were found significantly higher in polyculture than monoculture smallholdings (Ghazali et al. 2016). Planting cover crops such as legumes that form dense low-growing mats also reduces the need to use herbicides to control undergrowth (Samedani et al. 2015).

Modelling studies suggest that intercropping of OP with other crops, in particular cacao, provided high land sparing effects, while also replenishing more ground water and reducing carbon footprint (Migeon 2018; Stomph 2017; Khasanah et al. 2020). Additionally, the implementations of bio farms in Malaysia have been shown to reduce soil nutrients depletion and reduction in chemical use (Howes and Fletcher 2020). This production system improves on standard practices by extending the crop cycle, building soil organic carbon, alternative replanting methods, and minimizing soil loss (Howes and Fletcher 2020).

Biological pest control

Biological pest control agents that prey on rats such as barn owls (Salim et al. 2014; Puan et al. 2011; Saufi et al. 2020), macaques (Holzner et al. 2019), leopard cats (Silmi et al. 2013; Rajaratnam et al. 2007), or snakes and monitor lizards (Lim 1999) may decrease pesticide use and enhance biodiversity in plantations. On top of barn owls that are introduced, high density of other naturally occurring nocturnal bird species recorded in OP smallholdings also posits the potential for these carnivores to act as biological pest controls (Yahya et al. 2020). Ground and epiphytic ferns growing at OP trees constitute nesting sites for insectivorous birds (Koh 2008; Desmier de Chenon and Susanto 2005) that further act as insect pest control (Koh 2008). However, despite their adaptability to the plantations, intact forest patches adjacent to OP plantations remain necessary habitats for the biological pest control agents to rest and breed (Ruppert et al. 2018; Holzner et al. 2019).

Enhanced downstream processing (SDG1, 6–9, 13)

Output diversification

Growing a local palm-based oleochemical industry and moving the local industries up in the commodity value chain, with products spanning from base oleo like fatty acids to end products like polymer and cosmetic products (Salimon et al. 2012), has the potential to further increase the profit margin (Tong 2017), which removes reliance on further plantation expansion. Globally, about 44% of the global vegetable oil (19% from PO and palm kernel oil) was consumed in the chemical industry, with a relatively small amount devoted to biofuel production (Goh 2016). Major oleochemicals produced include fatty acids, fatty alcohols, methyl esters, glycerin, and soap noodles, with prospective markets including highly priced specialty oleochemicals like amino acid esters, and β-carotene that have important applications in the cosmetic, pharmaceutical and food industries (Mba et al. 2015). These specialty oleochemicals are also considered better substitutes for fossil-based chemicals due to their bio-based nature (Basri et al. 2013).

Additionally, there is growing interest in Europe, Japan, and Korea to import OP biomass to substitute fossil fuels for power generation (specifically as an alternative to coal for power generation and district heating in Japan and Korea) (Goh et al. 2019), second generation liquid biofuels, packaging materials as well as drop-in and novel chemicals (Sheldon 2014; Mai-Moulin et al. 2019). Furthermore, these biomass streams can be potentially converted to building blocks (e.g., sugars) for high-value chemicals or substitutes for fossil materials (e.g., bioplastics) (Zahari et al. 2015). Two state-specific strategies were rolled out for Sabah and

Sarawak, under Malaysia's National Biomass Strategy 2020 to develop domestic high value-added biomass-based industries, through valorizing the agricultural residues in combination with municipal solid waste (AIM 2013). The plan was kickstarted with promoting energy pellet production for both local consumption and export, motivated by the Feed-in-Tariff schemes for bioenergy in both Malaysia and overseas markets (Garcia-Nunez et al. 2016).

Waste management and recovery

Waste management practices in plantations impact the air, water, and soil quality of the environment (Gaveau et al. 2014; Truckell et al. 2019). Residues produced in OP mills in Malaysia are around 100 Mio t year-1 (MPOB 2018). Two important residues are the liquid PO mill effluent (POME) and an abundant amount of low value bio-resources in the form of agricultural and forestry residues, such as empty fruit bunches (EFB) and palm kernel shell (PKS) (Truckell et al. 2019). POME disposed from PO mills contains high chemical oxygen demand and biological oxygen demand, thus, it is contained in ponds near to the mills to avoid contamination of water resources (DOE 1974, 1994; MOE 1979). Untreated POME can severely pollute water resources and release large quantities of methane, a major GHG, i.e., 1 t of POME residue can emit 33 kg of methane equivalent to 750 kg of CO_2 (Rupani et al. 2010). However, treated residues composted and neutralized together with EFBs can be used as biofertilizer, whereby they can be returned to the soil to replenish carbon and nutrients through mulching to enhance soil quality in plantations (Truckell et al. 2019; Tao et al. 2017).

Discussion

Increasing recognition of the drawbacks to the rapid expansion of OP as an agricultural sector has led to efforts towards more sustainable practices to mitigate the adverse manifestations of OP industry such as deforestation and human–wildlife conflicts, human rights issues, pollution, and degradation of environmental quality (Tang and Al-Qahtani 2020).

However, perceptions surrounding PO and the OP industry are highly polarized, likely arising from the overemphasis on the negative impacts of irresponsible OP cultivation practices, an anti-PO stance from European and US governing bodies (Choiruzzad 2019; Wahab 2018), and consequently retaliatory stances from PO-producing nations (Liu et al. 2020). Even the literature appears polarized and at times directly conflicting, in particular with regards to nutritional impact of PO consumption on health and the impact of plantation management practices and certification schemes on improving sustainability.

In terms of health, a key limitation in understanding the impact of PO consumption is the fact that most studies are focused on PO as a single oil, or even if compared with other oils, they are rarely designed to observe intake as part of a wider diet. This tends to inflate both negative and positive impacts of consuming PO or its derivatives, depending on study methodology and any underlying biases (Ismail et al. 2018; Di Genova et al. 2018; Sun et al. 2015).

Conflicting views about OP agriculture and impact of sustainable management practices on the environment are often due to missing but important information at various levels making it hard for various stakeholders to understand the complex situation (Gaveau et al. 2016). There is paucity of data on awareness, adoption, and impact of sustainable practices among smallholders and, in particular, indigenous smallholders in Malaysia. Studies of land conversion of peatlands into OP plantations (Edwards et al. 2010; Gunarso et al. 2013) are through estimations or projections that often do not use the same time scale which makes them difficult to compare, highlighting the need for a universal methodological framework for more concerted monitoring of rate of conversion of peatland. Additionally, the literature and most discussions about OP agriculture are skewed towards the Indonesian context and only few studies propose mitigation efforts, especially feasible diversification of existing or rehabilitation schemes of abandoned plantations.

Existing studies aimed at enhancing sustainable practices are widely lacking in depth, long-term data, or demonstrable economic competitiveness, and thus are largely unable to provide applicable and scalable solutions. For example, while Begum et al. (2019) found that millers in Malaysia use efficient and environmentally friendly practices in waste disposal, more research is needed to understand how these practices can be further developed and adopted on a wider scale. Furthermore, the long-term impacts of diverting biomasses for industrial diversification are unclear, and due to logistic constraints, unclear business models, market uncertainties, and fluctuating CPO prices, large-scale mobilization of OP residues and POME treatment systems in rural areas has only been partially realized in the past few years. While data from specific localized studies on reducing forest fragmentation and human–wildlife conflict appear encouraging, the evidence on the broader impact of sustainable certification on improving worker conditions, smallholders and environmental conservation is still being accrued, with effective implementation and transparency being the underlying determinant.

In general, more detailed environmental and bioeconomic impact studies of sustainable vs. conventional PO production are needed to assess the benefits of (certified) sustainable management for the environment and local economy, especially for smallholders. The use of life cycle assessments may better estimate the impact of human activities on the environment, specifically for a commodity chain such as OP (Hashim et al. 2018). As many impacts of PO production on the environment are difficult to quantify, more comprehensive datasets are highly needed to develop better practices for sustainability in the long term (Hashim et al. 2018).

Many studies focus on the impact of OP as a single crop, without comparing these impacts against those arising from other existing and potential crops, which prevents a more contextualized impact assessment. For instance, coconut cultivation practices are less discussed as a driver of biodiversity loss, despite contributing to species loss in many tropical countries (Meijaard et al. 2020). Tackling biodiversity loss in plantation landscapes necessitates a deeper understanding about both positive and negative impacts of OP plantations on different species to develop solutions for environmental, wildlife and human welfare issues. In particular, there is an absence of evidence for mitigating roles of education/awareness, legal enforcement, and general reduced consumption.

Optimistically, the identified key mitigation strategies appear to possess potential co-benefits in advancing efforts for multiple SDG-related goals, with fewer strategies that either are conflicting or trade-offs that detract from efforts to achieve SDGs (Fig. 1). However, the effectiveness and longer-term impact of some mitigation efforts remain unknown.

Conclusion

In many ways, the prevailing conflicting evidence, garnered from single perspectives on this complex issue, further propagates views that are one sided. The different impacts of PO on different aspects of human and planetary health, and the corresponding SDGs, tend to be discussed separately, as are several of the solutions and mitigations efforts proposed. This situation then continues to neglect the complicated aspect of OP agriculture and agriculture in general, which consequently undermines the positive and often necessary socio-economic benefits of these industries to producer countries and the local communities (Gaveau et al. 2016). Moving forward, the varying impacts of PO on sustainability goals must be assessed with the intention to capitalize on the positive interactions and mitigate conflict of goals arising from negative interactions (Nilsson et al. 2018). Improving practices within and surrounding plantations through strategies that combine economic incentives with mitigation of adverse environmental impact may enable farmers and plantations to be part of more scalable holistic sustainable solutions.

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Declarations

Conflict of interest The authors have no conflict of interest to declare that are relevant to the content of this article.

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Review

Intercropping—A Low Input Agricultural Strategy for Food and Environmental Security

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Abstract: Intensive agriculture is based on the use of high-energy inputs and quality planting materials with assured irrigation, but it has failed to assure agricultural sustainability because of creation of ecological imbalance and degradation of natural resources. On the other hand, intercropping systems, also known as mixed cropping or polyculture, a traditional farming practice with diversified crop cultivation, uses comparatively low inputs and improves the quality of the agro-ecosystem. Intensification of crops can be done spatially and temporally by the adoption of the intercropping system targeting future need. Intercropping ensures multiple benefits like enhancement of yield, environmental security, production sustainability and greater ecosystem services. In intercropping, two or more crop species are grown concurrently as they coexist for a significant part of the crop cycle and interact among themselves and agro-ecosystems. Legumes as component crops in the intercropping system play versatile roles like biological N fixation and soil quality improvement, additional yield output including protein yield, and creation of functional diversity. But growing two or more crops together requires additional care and management for the creation of less competition among the crop species and efficient utilization of natural resources. Research evidence showed beneficial impacts of a properly managed intercropping system in terms of resource utilization and combined yield of crops grown with low-input use. The review highlights the principles and management of an intercropping system and its benefits and usefulness as a low-input agriculture for food and environmental security.

Keywords: food; environment; intercropping; security; sustainability

1. Introduction

Agriculture plays a significant role in most of the developing countries. But due to the increased population and development of urban clusters along with industrial growth in the developing world, there is shrinkage in the availability of land for farming because of its non-agricultural uses. Under these circumstances, the adoption of high-intensity cropping systems may be the viable option to increase agricultural productivity and production as a whole [1,2]. Agriculture is a tradition and heritage in most countries. Traditional farming practices are evidenced around the world with the growing of crop mixtures which is nothing but a form of mixed cropping or intercropping. The farming systems of ancient periods in different corners on the planet are documented as having grown crop mixtures which were nurtured by the people for a long time [3]. Early civilizations evidenced the use of intercropping that might be in a different form. South Asian subcontinent experienced growing of diversified crops with environmental diversity [4–6] since the Indus Civilization (c. 2600–1900 BC) in the form of mixed cropping or multi-cropping or intercropping [7,8]. Furthermore, the intercropping system was well known in Greece since about 300 B.C. which indicated growing of cereals and pulses [9] in which pulses were planted at different times during the growing season of cereals like wheat and barley [10]. Traditional mixed cropping has enough potential to contribute as much as 15–20% in the food basket of the world [11]. In Latin America, maize-based intercropping is very common [12]. In Africa, 89% of cowpeas and in Colombia 90% of beans are growing in mixed stands; however, in Malwai intercropping is very common and occupies 94% of cultivated land [13].

Various types of intercropping were known and presumably employed in ancient Greece about 300 B.C. Theophrastus, among the greatest early Greek philosophers and natural scientists, noted that wheat, barley, and certain pulses could be planted at various times during the growing season often integrated with vines and olives, indicating knowledge of the use of intercropping [9]. In tropical countries, intercropping is generally observed with food crop production, but much emphasis has been given in forage production in the temperate world for fulfilment of the high demand for animal feed [14].

Intensive mono-cropping is less complicated for big-scale farmers with the fullest utilization of machines, while smallholder farmers do not have steady access to markets and only produce enough food for their family members under subsistence farming. Intercropping ensures their livelihood. Therefore, intercropping is mainly practiced on small farms. Moreover, intercropping is known to produce stable yields from diversified crops with less use of inputs for nutrient supply and plant protection, focusing on sufficient food under healthy environmental conditions. In organic agriculture, intercropping is useful because less incidence of pest, disease and weed occurs and soil fertility is maintained [15]. During the recent period, the system approach in agriculture has drawn more attention from researchers. A system is comprised of different constituents that are high with interaction among them. The system approach targets greater use of available resources resulting in production sustainability and enhancement of intensity. The cropping systems with a more intense focus on raising of crops and varieties or hybrids having tolerance to biotic and abiotic stresses, capacity to replenish soil for sustainable production and express higher yield. Developing suitable cropping systems is an enormous job for achieving potential yield under any agro-climatic conditions [16]. Actually, some factors like resource availability and management are mainly considered to evolve a cropping system. The competence of a cropping system rests on the productivity of crops, time duration and requirement of land [17,18].

Modern agriculture based on supply with high energy and fossil-fuel-based inputs that are commonly known as Green Revolution Technologies has resulted in a remarkable enhancement in crop yields, but once this flourish was achieved agricultural sustainability disappeared [19]. The modern farming systems infer monoculture, replacing biodiversity with few crops and a limited number of cultivars in vast areas. On the other hand, on-farm biological diversity is maintained by the traditional farmers of developing nations and mixed cropping, intercropping and agroforestry patterns are prominently observed. These farming systems offer the ability to grow a variety of crops, assured production, efficient use of resources, less chance of crop damage by pests and diseases and proper use of the human workforce with a standard income [14]. Different crops grown in an intercropping system may or may not be seeded or harvested simultaneously, however, the crops remain in the same field for a major part of the crop periods. There are various types of plant species that can be included in intercropping, namely, annual crops like cereals, legumes, oilseeds, fodder crops and so on. Low-input and energy-efficient crop production systems are no doubt attractive for sustaining agricultural productivity [11,20], but, modern agriculture imposed less diversified crop production system with the use of high energy inputs and chemicals. Agricultural sustainability can be achieved by creating on-farm diversity and proper use of natural resources with greater ecosystem services [21,22]. Biological diversity in a crop-growing environment can be improved by a recurrent succession of crops in sequential cropping or intercropping systems [11]. Actually, modern agriculture increased crop yields but developed issues related to agricultural sustainability [23]. An intercropping system has enough potential to enable sustainability in agriculture by crop diversification, efficient resource management and soil fertility restoration. The review focuses on essential aspects of intercropping systems as low-input management practices for food and environmental security with agricultural sustainability.

2. Intercropping as Low-Input Agriculture

An economically viable agriculture production system demands a supply of sufficient quantity of inputs. The success of green revolution (GR) in the second half of the previous century greatly focused on the supply of essential inputs and so green revolution technologies (GRTs) were more commonly known as supply-driven technologies. As per the concepts of GRTs, important inputs used in agriculture are high-yielding varieties (HYVs), fossil fuel-based high-energy chemical fertilizers, assured irrigation, use of sufficient plant protection chemicals and so on and interestingly all these inputs need high energy. On the other hand, intercropping systems need comparatively fewer energy inputs like fertilizer, plant protection chemical requirements are less, and diversification of crops is greater creating functional diversity resulting in less pest-disease incidence. Moreover, there is the creation of soothing microclimate with less evaporation [24,25]. Combination of deep-rooted and shallow-rooted crops create the options of bio-irrigation and after all, legume crops in association with non-legumes favour adjustments of nutrients by benefitting non-legumes in the form of nitrogen fixation. The legumes, furthermore, create a congenial environment for harbouring different beneficial microorganisms favouring higher ecosystem services. The cumulative impacts of all factors are reflected in the intercropping system and thus the intercropping system can be considered as a low input agriculture practice with higher output in terms of higher farm output and agricultural sustainability.

3. Concept and Goal of Intercropping

Cultivation of two or more crop species concurrently as they coexist for a significant part of the crop cycle is known as intercropping and it is also sometimes termed as polyculture or mixed cropping [14]. The component crops are neither seeded at the same time nor harvested, but they remain simultaneously in the field for a major portion of the growth periods of component crops. Intercropping is, in general, comprised of the main crop and one or more companion crops, where the production of the main crop is the prime goal. Intercropping is actually the value addition of the cropping system which can ensure higher productivity, efficient use of resources, and more income [26–28].

The history of the adoption of intercropping is not known, but ancient civilizations witnessed cultivation of crop mixture. Intercropping is still adopted in developing countries and it is also observed that intercropping began disappearing from many areas with the advent of high energy-based modern agriculture. The shift from polyculture to so-called ‘modern monoculture’ was driven primarily by commercialization and specialization of industrial agriculture along with the involvement of chemical-based inputs and assured irrigation. Increasing interests in sustainable crop production and ecological issues have distracted consideration back to polyculture as a path of efficient use of available resources with as much care as possible for ecology and leading towards agricultural sustainability. Since the historical period, intercropping activities were noted in different countries of the world with various crop mixtures with cereal mixtures found commonly in temperate regions [12]. Nonetheless, intercropping gained importance and is widely practiced in tropical regions because of extensive genetic diversity in terms of crop choice as well as cropping systems [29–31]. Furthermore, the decline in temperature and rainfall is inversely proportional to adoption in a variety of intercropping systems [32]. With the adoption of industrialized farming, intercropping started to disappear from different parts of developed and industrialized countries as monoculture became popular there. This drift was motivated by the use of high energy inputs, improved farm machinery and specialization and these were considered as the prime strategy for enhancing crop yield. This industrialized agriculture was successful with a single crop or commodity, but the question of higher system products, as well as agricultural sustainability, remained unanswered over time [33].

In intercropping, basic ecological principles are observed in the form of above and belowground diversity, competition, and facilitation, for production of crops [34]. Generally, if the polyculture system of crops is chosen with proper prerequisites, the yield output appears higher than pure stands of individual crops. Moreover, in the intercropping system, different resources are better used by crops from a common pool compared to pure stands of the respective crops which result in greater productivity [34,35]. An intercropping system assures more coverage of the ground area by the canopy of crops, more transpiration takes place by the foliage which may create a cooler microclimate, and this facilitates the ability to minimize the soil temperature [36]. Under moisture stress conditions, in intercropping systems, crops use available water in the form of soil moisture and this microclimate provides a soothing effect at the canopy level of crops [37]. Generally, in intercropping, morphologically dissimilar crops are chosen with different growth habits, so available resources are efficiently utilized and the ultimate gain is the conversion into the crop dry-matter production or crop yield [10]. Different factors like choice of crops and cultivars, sown proportions and agronomic management including water and nutrients and the competitive ability of crops can affect the performance as well as the success of intercropping systems.

4. Types of Intercropping

Intercropping is the raising of two or more crops together as they coexist for some time on the same land. The spatial and temporal crop intensification is done in intercropping and it may be of different combinations of annual and perennial crops as per the choice of the farmers and suitability to the growing conditions [38]. Furthermore, in intercropping, competition is noted among the component species grown during the entire crop period or a part of growing duration for available resources. Different types of intercropping systems are adopted in various countries which can be grouped into the following [39].

4.1. Row Intercropping

The row intercropping is raising of one or more crops sown in regular rows, and growing intercrops in a row or without row at the same time. The row intercropping is a usual practice targeting maximum and judicious use of resources and optimization of productivity [40].

4.2. Mixed Intercropping

In mixed intercropping, two or more crops are grown together without any definite row proportion. Sometimes it is also referred to as mixed cropping [41]. In pasture-based cropping system, grass-legume intercropping is an ideal example of mixed intercropping [42]. The mixed intercropping is commonly observed to fulfil the requirement of food and forage where the land resource is a limiting factor [43]. Furthermore, a review work clearly described perennial polycultures as an agroecological strategy in cropping system with enough potential for the sustainable intensification of agricultural systems spatially and temporally [44].

4.3. Strip-Intercropping

The strip-intercropping is a type of intercropping where two or more crops are cultivated together in strips on sloppy lands. Strip intercropping is known to enhance greater radiation use efficiency in marginal and poor lands [45]. A combination of soil conserving and depleting crops are taken in alternate strips running perpendicular to the slope of the land or the direction of prevailing winds. An important objective of strip cropping is the reduction of soil erosion and harvesting of yield output from poor lands.

4.4. Relay Intercropping

Relay intercropping is raising two or more crops at a time during a portion of the growing period of each. In this system, the second crop is seeded when the first crop completes a major part of its life cycle and reaches reproductive stage or close to maturity but before harvest. The areas with limitation of time and soil moisture are more appropriate for relay cropping [46]. Before harvesting of the preceding crop, the next crop is sown and both the crops remain in the field for some period of their cycle. However, the succeeding crop yields less compared to normal sowing in sequential cropping and more seeds of the succeeding crop are required to obtain a good stand.

5. Crop Geometry in Intercropping

The proportional row arrangement of different crop components crops in an intercropping system ascertains advantage or disadvantage of intercropping compared to pure stands of the respective crops [45]. Based on the arrangement of rows and the proportion of crops sown, the intercropping system is grouped into the following two categories.

5.1. Additive Series

In the additive series, intercrops are added in 100% population of the base crop. The crop sown with 100 per cent density as seeded in the pure stand is called ‘base crop’ and another crop is termed as ‘intercrop’. In this system, ‘intercrops’ are sown within the row space of the ‘base crop’ and sometimes planting geometry of the base crop is modified to create space for intercrops (for example, paired row planting). The proportion of ‘intercrop’ (not base crop) mostly remains less than its optimal population in sole cropping. As the addition of ‘intercrop’ has been considered in 100% population of the ‘base crop’ in additive series, the land equivalent ratio (LER) is mostly observed as more than unity indicating yield advantage. Yield advantage gives extra monetary return and so it is considered as an efficient intercropping system and commonly preferred by small landholders in developing countries.

5.2. Replacement Series

In the replacement series of intercropping, the crops grown together are known as component crops or intercrops. Here, one component crop is introduced by the replacement of the other crop. In the replacement series of the intercropping system, no crop is sown with its fullest population as seeded in respective sole cropping. In this system, a definite proportion of a crop is sacrificed and the component crop is introduced in that place. Sometimes to obtain yield advantage from replacement series of intercropping, plant population is increased compared to their density adopted in the pure stands [47]. In such an intercropping system, competition among species is relatively less than additive series.

6. Consideration for Choosing the Intercropping System

6.1. Crop Choice

The crop choice is an important consideration concerning the growing situation, crop environment of a locality, suitability of the crop as well as demand and availability of a particular variety [41]. The appropriate crop mixtures show complementarity among the species cultivated and yield advantage is observed. Furthermore, in a study in West Bengal India, by Maitra et al. [48] it was noted that finger millet, when intercropped with red gram or groundnut (4:1 ratio), expressed benefits in terms of more net return and benefit: cost ratio than other combinations considered like finger millet + green gram and finger millet + soybean in rainfed conditions. Similarly, Fan et al. [49], recorded more grain output of faba bean + maize; but the yield of fava bean was less in a faba bean + wheat intercrop combination. These are the examples of the importance of crop choice in yield enhancement while selecting the crop in intercropping, generally, crop morphological and physiological characters are considered. For example, combinations of deep and shallow-rooted crops (like finger millet and green gram) or crops with tall and dwarf canopy (like maize + groundnut) are preferred for better utilization of the available resource. Intercropping in maize is very common and legumes are preferably chosen in maize-based intercropping system. In different intercropping studies, it was noted that maize + legume combination registered more yield with greater use of resources [28,48] which are the primary goals of the intercropping system. There are several crop species which may be considered in intercropping, like annuals, perennials and mixture of the both. In alley cropping, a type of agroforestry, perennials are chosen in hedgerows and annual crops are cultivated in alleys. Moreover, the growing period and time of peak demand for resources of different crops selected in the intercropping system is also important to get maximum benefit.

6.2. Crop Maturity

Crop maturity is an important factor for the choice of crops in an intercropping system. The crops preferred in intercropping combination should be of a different kind in terms of their grand growth period, otherwise, there may be a chance of inter-species competition for required resources if it coincides. The complementarity among the species is desirable to obtain the benefits of an intercropping system which are reflected as system productivity. Hence, the crops chosen should be of different duration with dissimilarity in the form of growth and morphology as they can exhibit complementarity among themselves. As an example, it may be stated that maize has been considered a suitable cereal species and also treated as a base crop in the intercropping system in association with preferably dissimilar legumes of shorter lifecycle [48]. Green gram or black gram is of short duration pulse crops when grown as intercrop in association with the base crop of maize, pulses enter into the reproductive stage before maize reaches to the knee-height stage (approximately 6–7 weeks after planting) and thus least competition is observed among the crops. The result of such combination expresses a higher level of mutual benefits in the expression of crop yields of individual species.

6.3. Planting Density

To obtain optimal yield output it is necessary to maintain proper plant stand. But in replacement series, there will be the reduction of plant population of crop species in comparison to sole crops, whereas in additive series, the base crop gets a similar plant stand and other crops that are accommodated may or may not occupy areas like sole cropping. Furthermore, paired-row geometry of planting of the base crop is beneficial because more space for intercrops is created. Sometimes in replacement series of intercropping system, population density is enhanced compared to the pure stand of individual crops to achieve higher system productivity with greater leaf area index (LAI) [50]. In an intercropping system with base crops like maize, cotton, sugarcane and so on, paired row planting in intercropping is commonly practiced [28,51]. The crops with various durations and different growth habit with peak demand for nutrients are chosen to minimize competition among the species.

6.4. Planting Time

In intercropping systems, sowing/planting time of component crops may or may not vary as in relay intercropping system. Intercrops are introduced when the base crop reaches close to its maturity or complete a major period of its growth. The competition among the species is much less in relay intercropping. In south Asian countries, relay cropping of pulses and oilseeds is very common in rainy season rice and by utilizing residual soil moisture and nutrients relay crops yield satisfactorily. When the crops are sown together in intercropping, preferably crops with a different type of growth habit are chosen. For example, in maize-based cropping systems short duration green gram or black gram if sown completely the major part of their growth before maize reaches its peak demand stage. As maize is used as fodder also and maize-legume fodder mixed cropping system is common in different countries. Under this situation, dry matter or biomass production is the ultimate target and competition among crop species does not influence the forage yield. With grain crop maize, legumes generally yield quite reasonably because of wider spacing adopted in maize sowing.

7. Management of Intercropping

Under diverse conditions, farmers adopt different intercropping practices and thus the intercropping system itself becomes complex [52]. There is no doubt that the management of the intercropping system is difficult in terms of requirements of more human labour. But considering the multiple benefits as well as agricultural sustainability intercropping system may be considered as one of the suitable options for food and livelihood security of small farmers.

7.1. Seed-Bed Preparation

A seed-bed is prepared before sowing by physical manipulation of soil and suitable tillage is required for different crops [53]. In intercropping when two or more crops with dissimilar morphological characters are sown together, uniform bed preparation may not be ideal for different crops and it greatly depends on crops [54]. For example, deep-rooted crops need deep tillage, while cereals require shallow tillage. The crops with small seeds (like mustard, sesame and jute) require pulverized soil and fine beds. Some crops are sown on ridges (cotton, maize) whereas others such as green gram, black gram and mustard prefer flat-beds. In additive series, the bed is prepared as per the requirement of the base crop. In maize + green gram/black gram intercropping, generally, crops are sown on a flat-bed [28]. In a sugarcane-based intercropping system, sets are planted in furrows, but intercrops are sown on ridges [50,55].

7.2. Varieties

The varieties of crops chosen in intercropping should have some desired characteristics as the highest level of complementarity and least competition occur [54,56]. The crop varieties are required to be photoperiod insensitive as these can be cultivated at any time of the year [57,58]. The short duration sorghum hybrids like CSH 17 (103 days) and CSH 23 (105 days) are suitable for intercropping long-duration pigeon pea varieties like GAN 1 and WRP 1 (both are of 160–165 days). The varieties selected for intercropping should have some morphological and physiological characteristics like thin leaves, less branching and be tolerant to shading.

7.3. Sowing and Plant Stand

In the intercropping system, modification or alteration is done in planting geometry, spacing and thus plant stand [59]. Paired row planting is one of the modifications, where two rows of the base crop are sown in close spacing followed by a wide gap between two pairs and spaces between two pairs are used to accommodate intercrops. In additive series of an intercropping system, for widely spaced crops like cotton, maize and red gram, paired row planting is beneficial and higher total yields are obtained from the crops grown in the mixture. Furthermore, the base crop population is maintained equal to the plant population of sole cropping. Paired row cotton yielded more than uniform row cotton in entisols of Sundarbans when intercropped with a single row of green gram and groundnut and paired row planting registered higher monetary advantage than uniform row planting of cotton with the same combination of legumes [51]. In a pearl millet + green gram intercropping system, paired row planting resulted in more yield than uniform row planting, and intercropping of paired row maize + pigeon pea performed well in the southern dry zone of Karnataka [60]. In replacement series after sowing of a crop with some uniform rows, replacement is done by another crop of some rows and row proportion is determined mainly by the farmers or as per the recommendations. Sometimes closer spacing within rows is also followed to accommodate more number of plants and generally a greater number of plants is accommodated in intercropping. Other factors related to optimal and uniform plant stands include seed treatment, bed preparation, sowing at the proper depth and so on, are maintained as per the standard procedure. Further, gap filling is an important operation in drylands, which can also be taken care of to obtain the desired plant population.

7.4. Fertilizer Application

The nutrient removal by the crop is greater because of more dry matter production or biological yield in intercropping. In a cereal–legume combination of intercropping, legumes use less N from the soil and it may be either from inherent soil fertility or in the form of applied fertilizer. On the other hand, cereals are more N demanding and use a major portion of applied N. Nevertheless, legumes initially use P for better nodulation, but after nodulation, the root exudate of legumes and other rhizospheric micro-organisms make P available to both legumes and the companion cereals. In N-deficient soils, legume fixes a considerable quantity of N, but when sufficient N is supplied as fertilizer, biological N fixation is reduced. Moreover, sufficient supply of N fertilizer promotes the growth of cereals because cereals are more aggressive in nature and the growth of legumes is suppressed. Considering the above, it is advisable to apply N as basal and topdressing to cereal rows and P and K to the whole plot. In the molybdenum-deficient soils, the micronutrient application should be done as basal or by foliar spray, because sufficient molybdenum enhances nodulation as well as biological N-fixation by legumes [61]. The mutual benefit or complementarity observed in the cereal–legume combination is the result of below-ground chemical and biological processes which can assure the availability of some micro-nutrients like iron and zinc [62].

7.5. Water Management

Water is the most valuable resource and it has a great impact on national development which needs special attention for enhancing higher output from land, better efficiency, increased earnings, and maintaining the ecological balance. Sustainable agricultural production can be achieved if different natural resources, more especially water resource are utilized efficiently [63]. Management of water resource in an integrated manner is a concept where water is used judiciously and in agriculture the optimal use of irrigation water for enhancing water productivity per unit area. There is no basic difference in the management of water in multiple cropping, sequential cropping and intercropping so as to provide irrigation for crop needs. However, when two dissimilar types of crop are taken into consideration in intercropping, special management techniques are to be followed in intercropping. Moreover, in additive series of intercropping, the combined plant population appears as more than 100%, which means soil moisture use in the form of evapotranspiration increases. Under rainfed, moisture-stress conditions and dryland situations, the extra need for water for crop mixture in intercropping will be an additional burden to resource-poor farmers. On the other side, more coverage of ground area restricts evaporation loss and, generally, it is observed that the water needs of intercropping do not exceed that of pure stands. Nyawade et al. [25] in upper midland of Kenya observed that in rainfed potato + legume intercropping system more LAI was noted which was indicative of more coverage of the ground area that lowered soil temperature by 7.3 °C at 0–30 cm depth and ultimately increased soil water content and crop water productivity than sole cropping of potato. In an oasis of arid north-west China, in wheat + maize intercropping system, alternate irrigation exhibited higher water use efficiency (WUE) than conventional irrigation of either of the crops [64]. In China, in a maize + soybean intercropping system maximum water use efficiency and water equivalent ratio were noted with 40:160 cm planting geometry using 200 cm bandwidth [65]. Similarly, in another experiment, [66] noted that strip-intercropping with pea and maize in China showed complementarity in sharing water and both the crops gave more grain yield by 25% and enhanced water use efficiency by 24% than solely maize. However, in irrigated conditions when sufficient water is available, the crop combinations may suffer due to the difference in water requirements. For example, in cotton + green gram / black gram intercropping, irrigation should be given to cotton at an interval of two to three weeks, but these legumes do not require the same frequency and well as quantity and legumes may suffer due to excess water. In most of the intercropping systems, it is better to schedule irrigation by following IW/CPE ratio. Bio-irrigation is another beneficial phenomenon by which shallow-rooted crops obtain support from deep-rooted ones in intercropping under limited moisture conditions. The experimental results clearly indicated that deep-rooted pigeon pea played the role of bio-irrigators and shared moisture for shallow-rooted finger millet [67,68].

7.6. Weed Management

In intercropping systems, chemical herbicide application is difficult once crops have emerged particularly when a combination of dicotyledonous and monocotyledonous plants are chosen in combination [69]. However, as the greater portion of the land area is covered by the crops in intercropping, there will be fewer weeds. Fast-growing crops like mung and black gram under intercropping cover maximum land area and suppress weed growth. The weed suppression ability in the intercropping system depends on some factors like selection of crop, the genotypes used, plant population, the ratio of crops considered in the intercropping and spatial arrangement, fertility and soil moisture. Mostly hand weeding is practiced in intercropping. Weed control by the application of chemical herbicides is difficult as most of the herbicides are crop-specific. The more complex the intercropping system, the less likelihood of a finding of herbicides. Earlier Reddy [70] mentioned that Isoproturon (1.0 kg ai ha^{-1}) was effective in intercropping wheat + chickpea and wheat + mustard. Likewise, Alachlor (1.5 kg ai ha^{-1}) was beneficial in maize + cowpea and sorghum + black gram intercropping systems. Further, Butachlor (1.25) resulted in successful control of weeds in maize + mung intercropping system [70].

Furthermore, allelopathy may reduce weed population. Researchers noted less weed growth in different intercropping combinations like wheat and chickpea [71], maize + legume [72] and so on. Paired row planted maize + 2 rows of soybean/2 rows of sesame reduced weed growth in Nagaland [73]. Experimental results showed that intercropping of maize + soybean and maize + cowpea significantly reduced weed growth than sole cropping and the pre-emergence application of alachlor 2 kg ha^{-1} or metolachlor 1.0 kg ha^{-1} controlled weeds successfully [74]. Similarly, maize + cowpea and maize + black gram suppressed weeds more than pure stands of maize, but maximum weed control efficiency and yield of maize were noted by the herbicide Pendimethalin at 0.75 kg ha^{-1} and mechanical hoeing at five weeks after sowing [60,75]. Moreover, in intercropping systems available resources are efficiently utilized by two or more crops in combination and thus weeds do not get exploitable resources and growth of weeds is suppressed [76]. In a study, Divya et al. [77] noted that less density of grassy weeds and sedges were observed when runner bean (*Phaseolus coccineus* L.) was intercropped in maize than pure stands of maize.

7.7. Pest and Disease Management

The insect-pest population is regulated by the intercropping system itself. In marginal farming mixed cropping is chosen because of the low incidence of insect pests [78]. The crop mixture attracts beneficial insects which have the potential to maintain the harmful pest population below the threshold level [22]. Researchers noted less incidence of insect pests in intercropping and the presence of natural enemies as observed in maize-based intercropping with beans and cowpea [79]. In Nigeria, the weboorm (*Antigostra* sp.) caused less damage to sesamum when intercropped with sorghum [80]. Polyculture reduced the population of *Empoasca krameri* by 26% in beans and *Spodoptera* by 14% in maize in intercropping compared with their pure stands [81]. When cowpea was intercropped with cotton, sucking pest population was reduced [82] and stem borer (*Chilo zacconius*) and green stink bug (*Nezara viridula*) were checked when upland rice + groundnut was intercropped [83]. Adoption of integrated pest management is advisable to keep the pest population below the threshold level. In general, adoption of cultural measures reduces the chance of pest attack and when the attack is noticed mechanical, biological and chemical methods should be applied for the protection of crops. Intercropping also checks plant diseases by creating a functional diversity which limits the population of harmful micro-organisms [84]. For the management of diseases, also the application of the integrated approach is the best. Management of seed-borne pathogens can be done by treating seeds with chemicals or bio-fungicides. Removal of diseased plants will reduce the inoculum source. Planting of disease-resistant cultivars is also a suitable measure to reduce disease incidence.

8. Indices for Measuring the Efficiency of Intercropping

In intercropping systems, most of the competition studies have examined growing two crop species and also in a 'replacement series'. The component crops involved in the system may be related to each other in the manners mentioned below:

(i) Competitive: In this relationship, the output of one crop would be increased through the decline in the production of the other. This is also known as 'compensation'. Willey [85] referred to the two species as 'dominant' and 'dominated' species.

(ii) Complementary: This is another type of relationship in which an increase in output of one crop helps to bring about an increase in output of the other species. This is termed as 'mutual cooperation' [85] and is not very common.

(iii) Supplementary: In this case, the output of one crop may be increased without having any influence on the output of the other. This situation commonly occurs when the maturity of two crop species differ widely.

(iv) Mutual Inhibition: Mutual inhibition happens when the actual productivity of each component of crops harvested is less than the expected yield. The competitive and supplementary relationship is very common in different intercropping systems. The majority of research works carried out that for value assessment of variation among pure stand and the intercropping system was developed during the period from 1970 to 1980. Most remarkable was the proposal of the land equivalent ratio (LER) and afterwards, widespread application of the LER was noted to evaluate the performance of an intercropping system) [85–87]. Later various researchers reviewed these studies and validated the concept of LER [12,39]. The focus of these studies was mostly on the use of replacement series of intercropping (mainly with two crops) and productivity of intercropping is compared with pure stands of each crop species. A major problem is that additive series of intercropping the LER exhibits the combined value of base crop with 100% plant density and the additional value of intercrops which ultimately results in the combined LER value with more than unity [86,88,89]. However, researchers concluded that the derivation of LER values is the concerned researchers' concern in estimating the efficiency of an intercropping system over pure stand [33].

There are also other concepts developed over time by different researchers to describe the competitive relationship and of them, some are also described below:

8.1. Land Equivalent Ratio (LER)

Willey and Osiru [86] gave the idea of the LER and it is described as the proportionate land area required under a pure stand of crop species to yield the same product as obtained under an intercropping at the same management level [48]. The LER of intercropped plots are estimated for each component crops separately by adding the estimated total of two varieties; the LER of the sole crop is taken as unity (1). In a replacement series of intercropping with a combination of two crops at the ratio of 50:50, the LER can be calculated by the following expression.

$$\text{LER} = \frac{Yab}{Yaa} + \frac{Yba}{Ybb} = \text{L}a + \text{L}b \tag{1}$$

where, Y*ab* is the yield of "*a*" crop grown in association with "*b*" crop and Y*ba* is the yield of "*b*" crop grown in association with "*a*" crop. Y*aa* and Y*bb* represent the yields of "*a*" and "*b*" crops grown in a pure stand, respectively.

The modified formula for any other situation is:

$$\text{LER} = \frac{Yab}{Yaa \times Zab} + \frac{Yba}{Ybb \times Zba} = \text{L}a + \text{L}b \tag{2}$$

The LER denotes the benefits of an intercropping system to utilize the resources as against their pure stands [88]. The LER value greater than unity (1.0) indicates the advantages of the intercropping system [39] and less than one (1.0) is considered as a poor performance of the intercrops [90]. The LER value of some intercropping system with major crops is presented below (Table 1).

Table 1. Land equivalent ratio (LER) in intercropping systems.

Intercropping System	Ratio	LER	Country	References
Sorghum + Sesbania	2:1	1.06	Syria	[91]
Wheat + Faba bean	1:1	5.24	UK	[92]
Sorghum + Cowpea	2:1	1.08	Nigeria	[93]
Wheat + Mustard	1:1	1.46	Bangladesh	[94]
Wheat + Fenugreek	1:3	1.4	Pakistan	[95]
Wheat + Maize	1:1	1.19	China	[96]
Sorghum + Soyabean	1:1	1.40	Nigeria	[97]
Pearlmillet + Soybean	-	2.77	Nigeria	[98]
Sorghum + Ground nut	1:1	2.10	Ethiopia	[99]
Maize + Soybean	1:1	1.54	Nigeria	[100]
Maize + Groundnut	2:2	1.42	Ghana	[101]
Maize + Potato	1:2	1.58	Ethiopia	[102]
Maize + Garden pea	1:2	1.56	Bangladesh	[103]
Maize + Groundnut	2:2	1.82	India	[28]
Maize + Soybean	2:2	1.90	China	[104]
Wheat + Lentil	2:2	1.34	India	[105]
Potato + Dolichos	1:2.4	1.24	Kenya	[106]
Potato + Vetch	1:2	1.75	Kenya	[16]
Pearlmillet + Green gram	1:1	2.03	India	[107]

8.2. Area Time Equivalent Ratio (ATER)

The LER emphasizes on the only land area without considering the time factor for which the crop occupies the field. As time factor is not a part in the LER, researchers needed another expression considering the field occupancy by the crops in an intercropping to correct this constraint of the LER. Hiebsch [108] developed the concept of Area Time Equivalent Ratio (ATER) in which the duration of crops (starting from seeding to harvest) was considered. The ATER is calculated by the following formula.

$$\text{ATER} = \frac{(\text{RYc} \times \text{tc}) + (\text{RYp} \times \text{tp})}{\text{T}} \tag{3}$$

where, RY = Relative yields of crop species "c" and "p" = Yield of intercrop ha^{-1}/ Yield of sole crop ha^{-1}, t = duration (in days) for species "c" and "p" and T = duration (in days) for the intercropping system. However, the LER generally overemphasizes and the ATER undervalues the land-use efficiency [27]. Researchers revealed the advantageous ATER values in different intercropping systems (Table 2).

Table 2. Area time equivalent ratio (ATER) in maize-legume intercropping systems.

Intercropping System	Proportion	ATER	Country	References
Cotton + Cowpea	-	1.13	Pakistan	[109]
Lupine + Wheat	75% + 100%	1.31	Ethiopia	[110]
Maize + Soybean	2:6	1.32	India	[111]
Maize + Black cowpea	2:2	1.51	India	[112]
Pearlmillet + Green gram	2:1	1.25	India	[113]
Wheat + Faba bean	-	1.28	Pakistan	[114]
Potato + Dolichos	1:2.4	1.13	Kenya	[106]

8.3. Aggressivity

This was proposed by [115]. Aggressivity denotes a simple measurement of the quantity of the relative yield increases in crop species "a" than crop species "b". Aggressivity is expressed as "A". For a replacement series of treatment, aggressivity is measured by the following formula.

$$\text{Aab} = \frac{\text{Mixture yield of } a}{\text{Expected yield of } a} - \frac{\text{Mixture yield of } b}{\text{Expected yield of } b} \tag{4}$$

For other than replacement series of intercropping, the aggressivity is calculated by the following expression.

$$\text{Aab} = \frac{\text{Y}ab}{\text{Y}aa \times \text{Z}ab} - \frac{\text{Y}bb}{\text{Y}aa \times \text{Z}ab} \tag{5}$$

where, Y*ab* = yield of crop "*a*" in intercropping system; Y*aa* = yield of crop "*a*" in pure stand (sole cropping); Z*ab* = sown proportion of crop "*a*" in intercropping; Y*ba* = yield of crop "*b*" in intercropping system; Y*bb* = yield of crop "*b*" in pure stand (sole cropping); Z*ba* = sown proportion of crop "*b*" in intercropping.

The value of aggressivity (A) zero means none of the crops are considered as aggressive or both crops have equal competitive ability. But, when the aggressivity value becomes positive, then "a" crop is considered as aggressive or dominant over intercropped "b" crop. If 'A' value becomes negative, then intercropped "b" is considered as aggressive or dominant over "a" crop.

8.4. Competitive Ratio (CR)

In an intercropping system, competitive ratio (CR) denotes the competitive ability of the component species [116]. The CR expresses the number of times by which one component crop is more competitive than other [116] and CR actually represents the proportion of individual LERs of the crops considered in intercropping and also takes into account the ratio of the crops sown in a mixed stand. The CR can be calculated by the following formulae.

$$\text{CR}a = (\text{LER}a/\text{LER}b) \times \text{Z}ba/\text{Z}ab) \tag{6}$$

$$\text{CR}b = (\text{LER}b/\text{LER}a) \times (\text{Z}ab/\text{Z}ba) \tag{7}$$

where, CR*a* and CR*b* are indicative of the competitive ratios of the crop species "*a*" and "*b*" and LER*a* and LER*b* are the LERs of the crop species "*a*" and "*b*" respectively. Z*ab* is the sown ratio of species "*a*" in mixture with "*b*" and Z*ba* is the sown proportion of the species "*b*" in mixture with "*a*". If the value of CR is <1, there is a positive benefit and it means there is limited competition between component crops and therefore they can be grown as intercrops. If the CR value is more than one (CR > 1), there is a negative impact.

In this condition, the competition between intercrops in mixture is too high, and they are not recommended to grow as intercrops.

In Table 3, the values of CR of legumes appeared as >1, representing that legumes were more competitive than finger millet. Among the legumes, green-gram was found to be the least aggressive on affecting the growth of finger millet and thus it provided a balanced competition with finger millet.

Table 3. Competitive ratio (CR) of finger millet + legume intercropping (4:1) systems [27].

Intercropping Systems	Competitive Ratio (CR)	
	Finger Millet	**Legumes**
Finger millet + Red gram (4:1)	0.28	3.59
Finger millet + Green gram (4:1)	0.71	1.41
Finger millet + Groundnut (4:1)	0.58	1.73
Finger millet + Soybean (4:1)	0.68	1.48

8.5. Relative Crowding Coefficient (RCC)

In intercropping system, the relative crowding coefficient (RCC) indicates relative dominance of one component crop over another. The concept of RCC has been proposed by De Wit [117] and examined in detail by Hall [118,119]. It assumes mixture treatments from a replacement series. Each species in an intercropping system has its own co-efficient (K) which gives the measure of whether that species has produced more or less than expected yield. The RCC is calculated by the following formulae. For species "*a*" in a 50:50 mixture with species "*b*", RCC is measured as:

$$\text{Product of RCC (K)} = \mathrm{K}ab \times \mathrm{K}ba \tag{8}$$

where, K*ab* = (Y*ab*)/(Y*aa*−Y*ab*) = (Mixture yield of *a*)/(Pure yield of *a*—Mixture yield of *a*); K*ba* = (Y*ba*)/(Y*bb*−Y*ba*) = (Mixture yield of *b*)/(Pure yield of *b*—Mixture yield of *b*).

Furthermore, for a mixture differing from 50:50 proportion, RCC can be generalized as:

$$\mathrm{K}ab = (\mathrm{Y}ab \times \mathrm{Z}ba)/(\mathrm{Y}aa - \mathrm{Y}ab) \times \mathrm{Z}ab \tag{9}$$

$$\mathrm{K}ba = (\mathrm{Y}ba \times \mathrm{Z}ab)/(\mathrm{Y}bb - \mathrm{Y}ba) \times \mathrm{Z}ba \tag{10}$$

where K is the product of RCC; K*ab* and K*ba* are RCC for the crop species "*a*" and "*b*" respectively; Y*ab* = yield of crop "*a*" in intercropping, Z*ba* = sown proportion of crop "*b*" in intercropping; Y*aa* = yield of crop "*a*" in sole cropping, Z*ab* = sown proportion of "*a*" in intercropping and Y*ba* = yield of crop "*b*" in intercropping, Y*bb* = yield of legume "*b*" in the sole cropping.

When the RCC (K) value, that is, the product of two coefficients (K*ab* × K*ba*) is more than one (>1), there will be yield advantage in the intercropping. When K appears as one (1), there is no yield advantage/disadvantage. However, when the value of K appears as less than one (<1), there is a competition between intercrops indicating disadvantage in intercropping.

9. Benefits of Intercropping

Agricultural sustainability is a countless task for all developing countries and more precisely for a populous nation like India to produce bumper for continuously increasing needs. The accessibility of farm-land is contracting each day because of other uses. In this context, one of the significant approaches to enhance farm output is the system approach which is considered a holistic approach too. A system is comprised of some inter-related and interacting components and system approach enhances the efficiency of use of available resources. Developing appropriate cropping systems based on agro-climatic conditions and resources is a huge task for realizing potential output. The consequence of a cropping system is estimated by the productivity of the crops grown by using the resources efficiently.

However, the latest concepts of agronomy suggest not only measuring the productivity of an individual and/or component crops of the system but another two dimensions, namely, time and space.

The cropping system consists of sequential cropping and intercropping. The potential of sequential cropping is already exploited, but the scope for achieving benefits from intercropping is still untapped. There are numerous reports concerning the beneficial outcomes and predominance of intercropping over the pure stand. A few specialists express their idea that intercropping is reasonable just for the smallholders who are engaged with subsistence cultivation. However, it has been observed in various parts of the world that polycultures in farming have enough potential to achieve agricultural sustainability because of diversification due to crop mixture [41,120,121]. Intercropping is beneficial in many ways as it assures greater resource use, reduction of the population of harmful biotic agents, higher resource conservation and soil health and more production and sustainable output of the system [2,48]. In an intercropping system, more than one crop is grown together on the same land and utilizes the soil nutrients, soil moisture, atmospheric CO_2 and sunlight. The resource conservation and soil health aspects are also positive effects of an intercropping system as it checks run-off, soil erosion and less nutrient loss from the soil [122,123]. Moreover, it facilitates soil fertility enhancement when small millets are intercropped with legumes and enables the diversity of beneficial soil microorganisms. In an intercropping system, complementarity among the species cultivated is very important for increasing crop yields.

In drylands, the intercropping system offers natural insurance against the failure of a crop. Different crops grown in an intercropping system require dissimilar agronomic management including post-harvest care. Mechanization is difficult in this situation and so more employment generation is created. In dryland regions, farming is not practiced throughout the year and unemployment of the farm workforce is an issue which can be minimized to some extent as intercropping is a labour-intensive practice. Cereal-legume combination provides food and nutritional security to smallholders of drylands. The population dynamics of different biotic agents namely, weeds, insect-pests and pathogen are changed. In small millet + legume intercropping system, cereal component gets benefit due to legume effect. Ultimately, less (chemical) inputs are involved in agriculture based on intercropping. Growing of two or more crops not only creates crop diversity but also makes the ecology favourable for predators. In other words, it may be stated that a better ecosystem service is achieved by the intercropping system which leads agriculture towards sustainability (Figure 1).

9.1. Yield Advantage

In the intercropping system, the same land area or unit area is provided to two or more crops in association and preferably more total yield is obtained from crops. The crop species grown in the mixture may show complementarity and less competition among crops result in certain yield advantage [85]. In additive series of intercropping, when additional crop or crops include the normal population of the base crop, there will be assured yield advantage [28]. In replacement series, yield advantage is also obtained and complementarity among the crop species matters [27,51,124]. The yield advantage in intercropping is measured by using some competition functions like relative yield total (RYT), relative value total (RVT), and monetary advantage and base crop equivalent yield may be considered. In an experiment, Mandal et al. [125] noted 5.48 t ha^{-1} of maize equivalent yield (MEY) in maize and soybean (1:2) intercropping against 2.48 t ha^{-1} in sole maize. Relative yield total (RYT) values of intercropping were higher than unity in different experiments indicating yield advantage [126]. Moreover, Manasa et al. [127] mentioned that MEY was 7.6 t ha^{-1} when paired row maize was intercropped with groundnut (2:2) as against sole maize yield of 5.7 t ha^{-1} and RYT was 1.47.

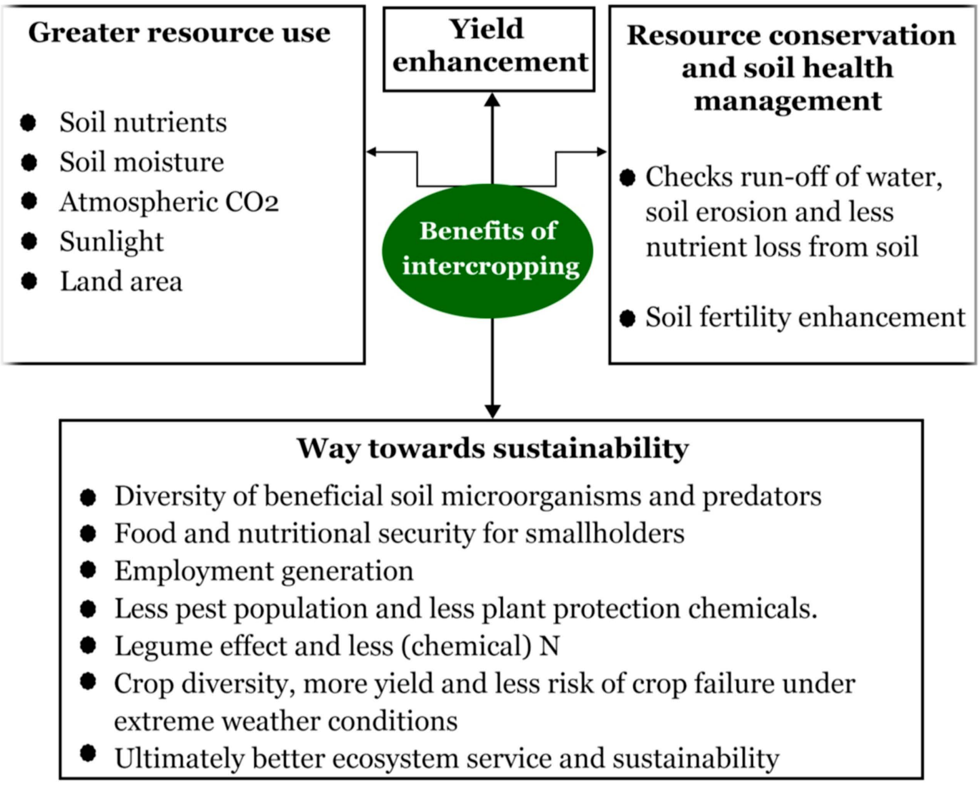

Figure 1. Benefits of intercropping system.

9.2. *Greater Use of Resources*

The intercropping system ensures a greater use of available resources [2], namely, land area, soil moisture, soil nutrients, sunlight and carbon dioxide used by a greater number of plants or crop species. Efficient utilization of these resources is reflected in higher biomass production and yield of crops. Higher yield is generally observed when the crops cultivated in association do not compete among themselves for the same resources. In other words, it may be stated that weaker intraspecific competition and greater complementarity among the crop species chosen are important for greater use of resources. Therefore, the combination of short and long duration crops or shallow and deep-rooted crops are preferred. The crops should be selected based on their resource use capability and competitive ability in time or space. Moreover, plant stand and planting geometry also influence resource use efficiency by crops in the mixture [48].

9.2.1. Soil Nutrients

The combination of cereal and legume in intercropping system triggers the soil fertility as legumes biologically fix N of about 80 to 350 kg ha^{-1} [128]. Intercropping legumes changes the micro-organism colony dynamics in rhizosphere which facilitates increased mineralization of nutrients [59]. Different studies indicated that there were variations in physicochemical properties in the soil of rhizosphere by the adoption of the legume-based intercropping system [129] due to the addition of fresh organic matter and changed microbial population which increased the availability of organic carbon, nitrogen and phosphorus in the soil [130]. In the combination of cereal-legume mixed stands, root exudates alter the availability of nutrients [131]. Furthermore, the root exudates might be comprised of organic acids and enzymes. In a pea + barley intercropping system P, K and S accumulation were positively influenced which ultimately was reflected in the productivity of crops [132]. The intercropping system assures more utilization and removal of available soil nutrients. In the legume-based intercropping system, atmospheric N is fixed biologically and used by legumes as well as associated non-legumes as legumes share up to 15%

of N to cereals [133]. Generally, the combined biomass yield is more in intercropping and thus uptake by the crops is more. Not only N, but P and K uptake in intercrops were also more by 43% and 35% compared to pure stands [134] and such enhancement was due to more dry matter production. Enhanced P uptake was reported by different researchers under a varied intercropping system where legume was considered as a component, for example, pigeon pea + sorghum intercropping [135] and lupin + wheat intercropping [136]. Mobasser et al. [59] also suggested more P uptake in intercropping. In acid soils, P is a limiting factor and root of some crops like groundnut, cowpea, maize and beans secrete organic acids and phosphatases into the rhizosphere and enhance P availability [137]; hence intercrops grown in combination with these crops get more P. Furthermore, in acid soils, Al toxicity may be harmful to crops and the release of organic acids protects the roots from Al toxicity [138]. The soil microbiome plays a vital role in making soil nutrients available to crops and it happens more prominently in a cereal–legume intercropping system. Dai et al. [139] mentioned greater Fe foraging in maize + groundnut intercropping system in calcareous soil. In an intercropping system, the competition for nutrients among the crop species can be reduced by selecting appropriate crops with dissimilarity in nutrient needs, root morphology and time of peak requirements. Furthermore, use rhizobia inoculants in addition to the recommended fertilizer inputs applied in intercropping systems may boost the availability of nutrients to crops and thus enhance the productivity of the intercropping system [140]. In maize + pea intercropping system when crops were fertilized with low N with a higher density of maize, there was more corn yield in the oasis region of northwest China [141]. Actually, the mutual benefit or complementarity observed in mixed stands of cereal and legume in the intercropping system is the result of below-ground chemical and biological processes which can ensure the availability of some micro-nutrients like iron and zinc [62].

9.2.2. Available Soil Moisture

In intercropping systems, plants grown simultaneously use the common pool of soil moisture. The combination of shallow and deep-rooted crops is beneficial for efficient utilization of available soil moisture [24]. Chen et al. [66] observed that strip intercropping with shallow-rooted pea and maize showed complementarity in sharing water and both the crops performed well and enhanced water use efficiency. Bio-irrigation is another beneficial phenomenon, which takes place in the intercrop combination of deep and shallow-rooted crops. Bio-irrigation is a hydraulic lift of the redistribution process of moisture. The deep-rooted crops penetrate taproot into the deeper layer of the soil to such water under moisture deficit conditions to cater for their physiological and metabolic activities and deposit a small quantity of water in the comparatively dry upper layer of the soil at night time when photosynthate production stops. The transported water to the upper layer of the soil is utilized by lateral secondary branches of the roots of the same crop and neighbouring crops also share it. The experimental results clearly indicated that deep-rooted pigeon pea shared played the role of bio-irrigators and shared moisture for shallow-rooted finger millet [67,68]. Moreover, Chai et al. [142] mentioned that WUE was increased by 95% in a maize + pea intercropping system over the pure stand of pea.

9.2.3. Atmospheric Carbon Dioxide

Plants use atmospheric carbon dioxide for production of assimilate or photosynthate by a biochemical process known as photosynthesis and release oxygen to the atmosphere. In an intercropping system sometimes more plant population is arranged and allowed for better utilization in space. In particular, in the additive series of intercropping, 100% of the population of the base crop is maintained as it remains in a pure stand and additional plant species are included as an intercrop with less population than the pure stand of the intercrop. In this way, the total population exceeds the population of the base crop in the pure stand and more plants exploit the available resources. If the crops and varieties are chosen properly, the complementarity effect is observed among the intercropped species and more biomass is produced in this way as reported by earlier workers [2]. More biomass production is caused due to more assimilated production by the crops together and in this way, more quantity of greenhouse gas is used in the process [143–145].

9.2.4. Sunlight

Crops with a preferably dissimilar type of morphological characters are chosen in intercropping to assure complementarity among the species. Generally, for crops with shorter canopy structure, if selected with tall crops, dwarf species will certainly be affected by shade, but overall more sunlight will be used by the crops together in association. The combination of maize + cowpea was reported to enhance more light interception than sole cropping of maize [146]. Mahallati et al. [147,148] suggested that maize–bean strip cropping showed higher radiation absorption and system output than pure stands of either maize or bean. Sole cowpea and soybean used more photosynthetically active radiation (PAR) than intercropping when these legumes were intercropped with maize at different proportions, but maize + legume intercropping combination intercepted higher PAR over a pure stand of maize [101]. Experimental results indicated that defoliation of the top two leaves of maize at silking stage enhanced the productivity of maize + soybean intercropping system probably due to better light interception and partitioning of dry matter to reproductive parts [104].

9.2.5. Land Area

Generally, in the unit area, more plants of two or more crop species are sown in intercropping. The component crops may compete among themselves for land area. The component crops if differing in duration or canopy structure or morphological characters may show less competition [2] and preferably dissimilar crops are chosen in intercropping. To measure the efficiency of land area by intercrops, Willey and Osiru [86] proposed the concept of the LER. But interestingly if the component crops with similar durations may show their maximum need for growth resources almost at the same period and compete for the same. Therefore, crops with dissimilar growth habit are selected in intercropping to achieve less competition among species. In LER, the time factor or duration of the crops is not considered and that may be considered as a limitation of the expression to evaluate the efficiency of the intercropping system. Hiebsch [108] suggested another competition function named the area time equivalent ratio (ATER) where land area and time both are considered. For both the competition functions if the expression value exceeds unity (more than 1), the intercropping system is considered efficient.

9.3. Resource Conservation and Soil Health Management

Conservation of resources is also an important advantage of intercropping. Soil and water are efficiently managed and more coverage of ground area enhances the possibility of effective soil and water conservation. Furthermore, topsoil erosion is checked by more coverage of the ground area and thus loss of nutrients is also restricted.

9.3.1. Reduced Run-Off of Water, Soil Erosion and Nutrient Loss

In intercropping, the maximum ground area is covered; hence there will be a minimum chance of run-off, soil erosion and nutrient loss [123,148]. In an agroforestry system, *Gliricidia* alley cropping can reduce run-off by 28.2% and soil loss by 49.3–51.1% over no alley cropping system. Furthermore, *Gliricidia* alley can conserve soil organic carbon, N, P and K by 63.4, 5.0, 0.3 and 2.4 kg ha^{-1}. Similarly, the *Leucaena*-based alley cropping system is also effective in terms of checking run-off of water, conserving soil and preventing nutrient loss. *Leucaena* alley with a miniature trench can reduce run-off by 18.3–18.7% and soil loss by 37.2–43.0%. The alley cropping of *Leucaena* can conserve organic C, N, P and K by 57.7, 4.6, 0.3 and 2.2 kg ha^{-1} [149]. The intercropping combination of finger millet + black gram recorded the lowest runoff (10.2%) and losses of soil and nitrogen, phosphorus and potassium through erosion over sole when sown in contour because black gram covered enough ground area in intercropping with finger millet [150].

9.3.2. Soil Fertility Enhancement

The soil fertility status was also improved in finger millet + pulses intercropping which was due to contribution of leaf fall and biological nitrogen fixation by legumes [150]. The cereal–legume combination of intercropping is known to enable long term immobilization of N [151]. Nutrient balance studies indicated that cereal–legume intercropping enhanced N fertility of the soil. Among legumes, groundnut in combination with maize added N to the soil because of above- and below-ground architecture of groundnut and more soil coverage [152,153]. However, a study conducted at Basar, Arunachal Pradesh showed that a greater proportion of legumes in maize, either soybean or groundnut (1-row maize: 4 rows legume) enhanced P balance of the soil. However, irrespective of row proportion, legumes in intercropping with maize added K in the soil in the two years' trial [153]. The legume factor is responsible for such enhancement of soil fertility in a cereal legume intercropping system.

9.4. Sustainability

The introduction of legumes as a component crop in the intercropping system can reduce the use of chemical inputs and thus minimize the emissions of greenhouse gases (GHGs). In the process of chemical N fertilizer production, CO_2 is generated. To meet the present need of chemical N fertilizers, annually 300 Tg of CO_2 is released to the atmosphere [15]. On the other hand, legumes fix N biologically and share the fixed N to non-legume crops in the mixture [48,154,155] and thus benefit not only the N economy of the crop production but also checking atmospheric pollution. Moreover, enhancement of above and below-ground diversity and change in pest population dynamics created a favourable environment for crop growth. Intercropping gives food and nutritional security to smallholders in drylands and natural insurance against crop failure. A lesser quantity of fertilizer is required in the legume-based intercropping system and thus use of outsourced chemicals in agriculture is reduced. Soil organic carbon (SOC) is a key factor for the enhancement of soil fertility and agricultural sustainability. In an intercropping system, total biomass production is obtained more than in sole cropping if the component crops are chosen wisely. More biomass production and biomass return in the form of leaf fall during the cropping period and stubble incorporation after harvest increase the organic content of the soil which is synonymous with SOC sequestration. All the benefits lead farming towards agricultural and environmental sustainability. Furthermore, the involvement of more labour inputs in intercropping assure engagement of family labourers including women in active participation in farming integrating gender equity and feminization [156,157] and an unemployed workforce in agriculture for stallholders [10] in the developing countries; thus, it ensures social sustainability.

9.4.1. Biotic Diversity

In the intercropping system, above-ground diversity is not only pronounced as the growing of more crops than one but also the crop mixtures increase the population of different arthropods, insects and birds. Omaliko [158] found a greater diversity of pollinators when cowpea was considered as a legume crop in intercropping. Furthermore, below-ground diversity is increased in the form of diversity in micro-organisms [22]. In legumes, when considered as a component crop in intercropping, the Rhizobium population is increased along with other beneficial micro-organisms like *Pseudomonas* sp., Alphaproteobacteria, Betaproteo bacteria and Cyanobacteria [159–161]. All these ultimately create a healthy ecosystem. Moreover, intercropping limits the population of harmful soil micro-organisms [84,162].

9.4.2. Food and Nutritional Security

For smallholders in developing countries, food and nutritional security is a huge task under subsistence farming. Mixture crops, especially, cereal and legume/oilseed combinations provide a large portion of the family requirement (calorie intake) and thus intercropping systems play a vital role in the alleviation of hunger.

9.4.3. Pest Population Dynamics

Intercropping systems can regulate the insects, diseases and weed population dynamics. Above ground, diversity is caused by the inclusion of two or more crops in intercropping. These crop mixtures attract pollinating bees and other predators which has a significant impact on production enhancement and insect population dynamics [2,22,163]. Intercropping of brassicas with various taxonomically unrelated crops increased the number of predators [164] and correspondingly reduced infestation of cabbage root fly and other Lepidopteran pests in comparison to the pure stand of brassicas [165]. When cowpea was grown in an intercropping system, the cotton sucking pest population was reduced [82] and green stink bug (*Nezara viridula*) and stem borer (*Chilo zacconius*) were checked when upland rice + groundnut was intercropped [83]. Intercropping also checks plant diseases. Actually, in intercropping a functional diversity is created which limits the population of harmful micro-organisms [84]. Growing of sorghum as an intercrop significantly reduced the incidence of bud necrosis disease of groundnut [166]. The following table (Table 4) indicates the restricted disease incidence in intercropping as evidenced by researchers. In intercropping, chemical herbicide application is difficult once crops emerge particularly when a combination of dicotyledonous and monocotyledonous plants is chosen in combination [76,167]. There is no reference available for applying certain types of pesticide to crop mixtures in the intercropping system. However, as the greater portion of the land area is covered by the crops in intercropping, there will be fewer weeds. Furthermore, allelopathy may reduce the weed population. Researchers noted less weed growth in different intercropping combinations like wheat and chickpea [71], maize + legume [72], and so on.

Table 4. Reduction of disease by the adoption of the intercropping system.

Crop	Name of the Restricted Disease	Intercropping Combination	References
Potato	Bbacterial wilt (*Pseudomonas solanacearum*)	Maize + potato	[168]
Faba bean	Chocolate spot (*Botrytis fabae*)	Maize + faba bean and barley + faba bean	[169]
Beans	Angular leaf spot (*Phaeoisariopsis griseola*)	Maize + bean	[170]
Pea	Ascochyta blight (*Mycosphaerella pinodes*)	Cereal + pea	[171]

9.4.4. Legume Effect and Less Chemical Fertilizers

The legume effect is pronounced as N benefits when these crops are considered in the intercropping system. Legumes are less N-demanding crops and these can fix atmospheric N biologically. The fixed N by legumes is used for their own nutrition and a portion is transferred to the associated non-legume in intercropping. A study on ^{15}N labelling clearly indicated that N was transferred from soybean to corn when these crops were intercropped and seed inoculation of *Glomus mossacae* and *Rhizobium* facilitated the process [172]. Furthermore, P and K balance of the soil also increased due to the legume factor in cereal–legume association [153]. Such enhancement of soil fertility leads to less use of chemical fertilizers.

9.4.5. Crop Diversity and Natural Insurance

Biodiversity is enriched in intercropping and diversity in crop ecosystem assures sustainability. Under fragile ecological conditions in drylands, crop failure due to biotic and abiotic factors is a very common phenomenon and monoculture may be severely affected. But intercropping or polyculture is by nature diversified. As a result, total crop failure is much less likely and, thus, intercropping provides security and natural insurance to the farmers [2].

9.4.6. Ecosystem Services

The ecosystem is comprised of the biological community in the physical environment (all flora and fauna in the ecology) and their healthy interaction. When the ecology is congenial for proper nourishment of the biological community, healthy interaction will be observed. There is no doubt intercropping creates favourable ecological conditions in many ways to nurture the biological community as discussed earlier. Intercropping is beneficial for low C emission from the field and in a study it was noted that maize + pea and soybean + wheat emitted less C from the crop field [142]. Soybean minimizes NO_3 in the soil compared to other crops [173]. The presence of beneficial insects and micro-organisms and a lower population of weeds assures a healthy ecosystem in intercropping. Ecosystem services include provisioning good and services (food, forage and feed, biofuel and fuel), supporting services (pollination, biocontrol, C-sequestration, nutrient cycling, soil improvement), regulating and cultural services [174]. Among these, the first two ecosystem services are prominently observed in different types of the intercropping system adopted around the world.

10. Limitations of the Intercropping System

There are benefits of intercropping as stated earlier, but some limitations are also observed over mono-cropping (Table 5. These are mainly due to inter-species competition for limited resources, expression of allelopathic effects and difficulty in agronomic management of crops in the mixture. Generally, crops are chosen in such a way that they show complementarity or mutual sharing of nutrients, light and water and thus, advantages of intercropping are recorded [54,175]. However, if the choice of crop species is not appropriate, due to competition only the adverse effect may be noted in the productivity of crop mixture [176,177]. The selection of appropriate crops and suitable varieties, seeding rates and plant population, and manipulation in planting geometry of the crops can minimize inter-species competition among crops.

Table 5. Limitation of different intercropping system.

Intercropping System	Limitation and Comments	References
Row intercropping	Preferably crops of dissimilar growth habits are grown to obtain higher level of complementarity and crops attain maturity at different times that make harvesting laborious. If crops are not chosen properly, inter-species competition may limit yields.	[48,54]
Mixed intercropping	Grass-legume is most common and harvested mainly as forage that creates no complexity and any limitation. But if crops are harvested separately, it will be labour intensive.	[54]
Relay intercropping	Succeeding crops may yield less compared to normal crops grown in sequential cropping and more seed rate of relay crop is required.	[178]
Strip intercropping	A combination of soil conserving and depleting crops are grown simultaneously in alternate strips. If perennial crops are grown in combination, may create shade problem to annuals.	[138]

Plants release special bioactive chemicals (allelochemicals) which interrelate with the environment and both positive and negative impacts are observed. Allelopathy may be a negative issue in intercropping as allelochemicals produced by one species may hamper growth and productivity of another crop. For example, black walnut (*Juglans nigra* L.), a popular planted species in alley cropping, silvopastoral, and mixed-species systems, produces the chemical *juglone* that has an allelopathic effect on different crop species [179]. The aqueous leaf extracts of *Jatropha curcas* inhibits germination and retards shoot and root length in *Capsicum annum* L. [180].

A major disadvantage in intercropping is the difficulty in practical management of essential agronomic operations, particularly where farm mechanization is adopted or when the component crops grown in intercropping have dissimilar requirements for fertilizers, water and plant protection requirements. During or after harvest, mixed grains are separated which incur additional cost and, at harvest of the early maturing crop, there may be some mechanical disturbance to the long duration crop. Farm mechanization is really difficult in intercropping because machinery used for different faring operations like seeding, weed management, harvesting and threshing are made for big uniform fields. Further, during the harvest of one crop, there may be some kind of damage to other crops in combination. However, in intercropping of cereal + legume forage crops, there is no problem because both can be harvested or grazed at the same time [2]. In developing countries, the human workforce is available and farm mechanization is not fully adopted and under these conditions intercropping will not show any limitations. In many cases, it was further noted that intercropping caused yield reduction of the main/base crop than its pure stand because of competition among intercropped plants for light, soil nutrients and water [85]. This yield reduction may be economically meaningful if that particular main crop has a more attractive market price than the other intercropped plants. Furthermore, the intercropped canopy cover may cause a microclimate with a higher relative humidity conducive to disease incidence, especially of fungal pathogens [181].

11. Conclusions

Food and environmental security as well as enhancement of input use efficiency are global concerns in agriculture. Both the developed and developing nations are in a quest for a low carbon footprint in agriculture and thus there is an urgent need for a reduction of high energy chemical fertilizers, plant protection chemicals and energy use in farm mechanization. Furthermore, intensive agriculture caused a gradual degradation of natural resources and the enhancement of farm productivity is a tough job for all targeting future demand. Intensification of crops can be undertaken spatially and temporally by the adoption of the intercropping system. Intercropping ensures multiple benefits like enhancement of yield, environmental security, income as well as production sustainability and some ecosystem services. Among different ecosystem services, provisioning goods and services and supporting services are prominently observed in different types of intercropping system. In intercropping, two or more crop species are grown concurrently as they coexist for a significant part of the crop cycle and interact among themselves and with their related agro-ecosystem. Legumes as component crops in the intercropping system play versatile roles like biological N fixation and soil quality improvement, enhancement of environmental quality by reducing the use of chemical N fertilizer, additional yield output including protein yield, and creation of functional diversity. But growing two or more crops together requires additional care and management for the creation of less competition among the crop species and efficient utilization of natural resources. The choice of a proper intercropping system and appropriate management practices like the choice of crops, planting geometry, intercultural operation and plant protection are major concerns to obtain advantages from the intercropping system. The review provided an overview of earlier evidence indicating beneficial impacts of the properly managed intercropping system in terms of resource utilization and higher combined yield of crops grown with low inputs. In developing countries, resource-poor smallholders prefer to adopt low input agriculture with the employment of the family workforce. Under subsistence farming where the production of sufficient food grain is a great challenge, beneficial impacts of the intercropping system are very common and proper utilization of resources by the adoption of intercropping ensures higher productivity as well as food security for a large number of smallholders in the world. Thus, the advantages of intercropping clearly derive from its usefulness as a low-input agriculture for food and environmental security in the present context.

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Chemical and Biological Technologies in Agriculture

REVIEW Open Access

Multiple benefits of legumes for agriculture sustainability: an overview

Fabio Stagnari[1*], Albino Maggio[2], Angelica Galieni[1] and Michele Pisante[1]

Abstract

Food security, lowering the risk of climate change and meeting the increasing demand for energy will increasingly be critical challenges in the years to come. Producing sustainably is therefore becoming central in agriculture and food systems. Legume crops could play an important role in this context by delivering multiple services in line with sustainability principles. In addition to serving as fundamental, worldwide source of high-quality food and feed, legumes contribute to reduce the emission of greenhouse gases, as they release 5–7 times less GHG per unit area compared with other crops; allow the sequestration of carbon in soils with values estimated from 7.21 g kg^{-1} DM, 23.6 versus 21.8 g C kg^{-1} year; and induce a saving of fossil energy inputs in the system thanks to N fertilizer reduction, corresponding to 277 kg ha^{-1} of CO_2 per year. Legumes could also be competitive crops and, due to their environmental and socioeconomic benefits, could be introduced in modern cropping systems to increase crop diversity and reduce use of external inputs. They also perform well in conservation systems, intercropping systems, which are very important in developing countries as well as in low-input and low-yield farming systems. Legumes fix the atmospheric nitrogen, release in the soil high-quality organic matter and facilitate soil nutrients' circulation and water retention. Based on these multiple functions, legume crops have high potential for conservation agriculture, being functional either as growing crop or as crop residue.

Keywords: Soil fertility, Conservation agriculture, Sustainable agricultural systems, Food security, Climate change, Greenhouse gas, Energy

Introduction

Global population will hit 9.6 billion people by 2050 [108] and will face global challenges among which achieving food security, lowering the risk of climate change by reducing the net release of greenhouse gases into the atmosphere and meeting the increasing demand for energy are the most critical ones. In particular, the impact of climate change and associated biotic and abiotic stresses to which crop systems will be increasingly exposed pose serious implications for global food production [119].

To meet these challenges, a policy framework needs to be developed in which the *sustainability* of production/consumption patterns becomes central. In this context, food legumes and legume-inclusive production systems can play important roles by delivering multiple services in line with sustainability principles. Indeed, legumes play central roles [112]: (1) at food-system level, both for human and animal consumption, as a source of plant proteins and with an increasingly importance in improving humans health [106]; (2) at production-system level, due to the capacity to fix atmospheric nitrogen making them potentially highly suitable for inclusion in low-input cropping systems, and due to their role in mitigating greenhouse gases emissions [53]; and (3) at cropping-system levels, as diversification crops in agroecosystems based on few major species, breaking the cycles of pests and diseases and contributing to balance the deficit in plant protein production in many areas of the world, including Europe [43, 48, 72, 78, 116].

Leguminosae family comprises 800 genera and 20,000 species [54] and represents the third largest family of flowering plants. Some legumes are considered weeds of

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Table 1 Trends for word acreage (million ha) and yield (t ha^{-1}) for legume crops included in FAOSTAT classification starting from 1974 to 2014 [23]; the major three cereal crops are also reported, for comparison

	Harvested area (Million ha)					Yield (t ha^{-1})				
	1974	**1984**	**1994**	**2004**	**2014**	**1974**	**1984**	**1994**	**2004**	**2014**
Legume crops										
Bambara bean	0.05	0.05	0.09	0.12	0.37	0.67	0.66	0.64	0.65	0.77
Dry bean	23.9	26.3	26.7	27.3	30.14	0.53	0.6	0.65	0.67	0.83
Faba bean	3.98	3.32	2.48	2.65	2.37	1.07	1.29	1.45	1.62	1.82
Chickpea	10.6	9.85	9.96	10.5	14.8	0.56	0.67	0.71	0.8	0.96
Cowpea	4.7	3.66	7.35	9.18	12.52	0.35	0.31	0.38	0.45	0.45
Groundnut	19.9	18.2	22	23.7	25.68	0.94	1.1	1.3	1.54	1.65
Lentil	2.03	2.56	3.43	3.85	4.52	0.61	0.68	0.81	0.93	1.08
Lupin	0.76	1.06	1.56	1.05	0.76	0.84	1.05	0.78	1.18	1.3
Pea	8.13	8.91	7.65	6.34	6.87	1.22	1.3	1.88	1.85	1.65
Pigeon pea	3.04	3.61	4.24	4.72	6.67	0.54	0.78	0.74	0.7	0.73
Soybean	37.4	52.9	62.5	91.6	117.72	1.41	1.71	2.18	2.24	2.62
French bean	0.22	0.17	0.22	0.23	0.20[a]	5.76	6.93	7.44	9.04	9.32[a]
Vetch	1.52	1.29	0.93	0.89	0.52	1.24	1.21	1.12	1.43	1.71
Pulses, nes	5.67	5.65	5.33	4.27	6.1	0.54	0.58	0.65	0.82	0.84
Vegetables, leguminous nes	0.14	0.18	0.18	0.25	0.24[a]	5.41	5.09	5.18	6.54	6.86[a]
Major cereal crops										
Wheat	222.12	230.77	215.12	216.57	221.62	1.62	2.22	2.45	2.92	3.29
Maize	119.86	127.76	137.99	147.45	183.32	2.56	3.53	4.12	4.94	5.66
Rice (paddy)	136.89	144.24	147.29	150.58	163.25	2.43	3.23	3.66	4.03	4.54

In Table 2, for each legume crop, item name and code as well as FAO definitions are reported

[a] Data are referred to year 2013 (2014 data not available)

cereal crops, while others are major grain crops; these latter species are known as grain legumes, or pulses,[1] and represent the focus of this review. For some of these species, the trends for word acreage and yield are available, as reported in Table 1.

Despite the growing trends observed during the 50-year period between 1974 and 2014 for some warm-season legumes (e.g. soybean, cowpea, dry bean, groundnut, and pigeon pea), the acreages of several temperate legumes (e.g. pea, faba bean, lupin, french bean and vetch) have declined worldwide with differences between world Regions (Table 3). In any case, food legumes occupy a minimal part of arable land, mostly dominated by cereal crops [99]; soybean represents the most important and cultivated legume, acreage of which reached 117.72 million ha in 2014 (steadily increased over years, see also Table 3), which is about that of the other grain legumes, but still far below the major cereals (e.g. rice, wheat, maize). Such trend is mainly associated to the expansion of more specialized and intensive production systems [82]. Market forces stimulating specialization of cropping systems as non-marketable benefits of diversification, like cultivation/introduction of legumes in the farming system, do not deliver immediate and/or apparent profits [82]. This is, however, not equally perceived throughout the globe, and there is indeed a remarkable diversity in grain legumes' production trends across the world (Table 3).

The European decline in grain legume's production is not mirrored by other regions of the world such as Canada or Australia, where legume's cultivation has been increasing over the last few decades. In these areas, monoculture of cereals, which relies on frequent summer-fallowing and use of mechanical tillage, has been replaced by extended and diversified crop rotations together with the use of conservation tillage [122]. Furthermore, supply chains and markets are inadequately developed for most legume crops (see also [66], for France) with the exception of soybean, for which the global market is well developed [85]. Nevertheless, soybean areas in Europe are constrained by climatic factors although there is considerable potential to develop new varieties suitable to flourish under cool growing conditions [123].

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[1] Soybean and groundnuts are not defined by FAO as 'pulse crops'.

Table 2 Definition of legume crops focused in Table 1 and corresponding item name in FAOSTAT

Legume crop	Scientific name	Corresponding FAO item name and code	FAO definition
Bambara bean	*Voandzeia subterranea*	Bambara bean [203]	Bambara groundnut, earth pea. These beans are grown underground in a similar way to groundnuts
Dry bean	–	Beans, dry [176]	*Phaseolus* spp.: kidney, haricot bean (*Ph. vulgaris*); lima, butter bean (*Ph. lunatus*); adzuki bean (*Ph. angularis*); mungo bean, golden, green gram (*Ph. aureus*); black gram, urd (*Ph. mungo*); scarlet runner bean (*Ph. coccineus*); rice bean (*Ph. calcaratus*); moth bean (*Ph. aconitifolius*); tepary bean (*Ph. acutifolius*). Several countries also include some types of beans commonly classified as *Vigna* (*angularis, mungo, radiata, aconitifolia*)
Faba bean	*Vicia faba*	Broad beans, horse beans, dry [181]	*Vicia faba*: horse-bean (var. *equina*); broad bean (var. *major*); field bean (var. *minor*)
Chickpea	*Cicer arietinum*	Chick peas [191]	Chickpea, Bengal gram, garbanzos (*Cicer arietinum*).
Cowpea	*Vigna ungiculanta*	Cow peas, dry [195]	Cowpea, blackeye pea/bean (*Vigna sinensis*; *Dolichos sinensis*)
Groundnut	*Arachis hypogaea*	Groundnuts, with shell [242]	*Arachis hypogaea*. For trade data, groundnuts in shell are converted at 70% and reported on a shelled basis
Lentil	*Lens esculenta*	Lentils [201]	*Lens esculenta; Ervum lens*
Lupin	–	Lupins [210]	*Lupinus spp.* Used primarily for feed, though in some parts of Africa and in Latin America some varieties are cultivated for human food
Pea	–	Peas, dry [187]	Garden pea *(Pisum sativum);* field pea *(P. arvense)*
Pigeon pea	*Cajanus cajan*	Pigeon peas [197]	Pigeon pea, cajan pea, Congo bean *(Cajanus cajan)*
Soybean	*Glycine max*	Soybeans [236]	*Glycine soja*
French bean	–	String beans [423]	*Phaseolus vulgaris*; *Vigna* spp. Not for shelling
Vetches	*Vicia sativa*	Vetches [205]	Spring/common vetch (*Vicia sativa*). Used mainly for animal feed
Pulses, nes	–	Pulses, nes [211]	Including inter alia: lablab or hyacinth bean (*Dolichos* spp.); jack or sword bean (*Canavalia* spp.); winged bean (*Psophocarpus tetragonolobus*); guar bean (*Cyamopsis tetragonoloba*); velvet bean (*Stizolobium* spp.); yam bean *(Pachyrrhizus erosus)*; *Vigna* spp. other than those included in 176 and 195; other pulses that are not identified separately because of their minor relevance at the international level. Because of their limited local importance, some countries report pulses under this heading that are classified individually by FAO
Vegetables, leguminous nes	–	Vegetables, leguminous nes [420]	*Vicia faba*. For shelling

The low diffusion of legumes' cultivation is also due to reduced and unstable yields and susceptibility to biotic and abiotic stress conditions; the average yields for unit area have increased (soybean +86%, lentil +77%, groundnut +75%, chickpea +70%) less than cereal crops (+104%, on average) (Table 1). Moreover, legume cultivation depends not only on the effect of farmers' choices, although they play a central role for such decision, but also on policymakers who have the responsibility to provide effective strategies to support the integration of legumes into cropping systems. This aspect is particularly relevant if the overall objective for future agricultural systems is to promote sustainability, improve resource use efficiency and preserve the environment [82].

Grain legumes impacts on atmosphere and soil quality

Among the many important benefits that legumes deliver to society, their role in contributing to climate change mitigation has been rarely addressed. Legumes can (1) lower the emission of greenhouse gases (GHG) such as carbon dioxide (CO_2) and nitrous oxide (N_2O) compared

Table 3 Trend for Region Δ acreage (%) during the 50-year period starting from 1974 to 2014 for legume crops included in FAOSTAT classification [23]; the major three cereal crops are also reported, for comparison

	Δ harvested area 1974–2014 (%)					
	Africa	Northern America	South America	Asia	Europe	Oceania
Legume crops						
Bambara bean	+612	–	–	–	–	–
Dry bean	+207	+16	−20	+25	−84	+1778
Faba bean	+7	Disappeared	−53	−59	−54	+75,085
Chickpea	+30	Appeared	+1	+37	−35	–
Cowpea	+168	Appeared	Appeared	+402	+153	–
Groundnut	+69	−10	−22	+6	+16	−39
Lentil	−20	+3376	−75	+72	−45	Appeared
Lupin	−82	–	+577	−89	−64	+315
Pea	+49	+1119	+7	−21	−63	+578
Pigeon pea	+226	–	−83	+108	–	–
Soybean	+642	+71	+882	+116	+291	−10
French bean	Appeared[a]	−39[a]	+129[a]	+66[a]	−18[a]	+122[a]
Vetch	+109	–	–	−73	−80	+4757
Pulses, nes	+20	–	−69	−15	+73	+7648
Vegetables, leguminous nes	+180[a]	Appeared[a]	+118[a]	+23[a]	−31[a]	−52[a]
Major cereal crops						
Wheat	+11	−20	+16	+39	−33	+51
Maize	+98	+29	+45	+76	+21	+31
Rice (paddy)	+185	+15	−16	+16	−28	+2

In Table 2, for each legume crop, item name and code as well as FAO definitions are reported

[a] Data are referred to year 2013 (2014 data not available)

with agricultural systems based on mineral N fertilization, (2) have an important role in the sequestration of carbon in soils, and (3) reduce the overall fossil energy inputs in the system.

Greenhouse gas emissions

The introduction of legumes into agricultural rotations help in reducing the use of fertilizers and energy in arable systems and consequently lowering the GHG emissions [52]. N fertilizer savings across Europe [51], in rotations including leguminous crops, range around 277 kg ha^{-1} of CO_2 per year (1 kg $N = 3.15$ kg CO_2, [42]. It has been reported that half of the CO_2 generated during NH3 production would be reused if the NH3 was converted to urea. This is, however, only a time shift of CO_2 release in the atmosphere since, once the urea is applied to the soil, the hydrolyzation activity by urease will release CO_2 originally captured during urea production [39]. Considering an efficiency of 2.6–3.7 kg CO_2 generated per kilogram of N synthesized, the annual global fertilizer leads to a release of 300 Tg of CO_2 into the atmosphere each year [42]. Some studies indicate that at global scale, the amount of CO_2 respired from the root systems of N_2-fixing legumes could be higher than the CO_2 generated during N-fertilizer production [42]. However, it is important to emphasize that the CO_2 respired from nodulated roots of legumes comes from the atmosphere through the photosynthesis activity. Conversely, all the CO_2 released during the process of N-fertilizer synthesis derives from fossil energy, thus determining a net contribution to atmospheric amount of CO_2 [42].

N_2O represents about 5–6% of the total atmospheric GHG, but it is much more active[2] than CO_2 [21]. Agriculture represents the main source of anthropogenic N_2O emissions (about 60%; [84], due to both animal and crop production [38]). A majority of these emissions result from the application of nitrogen fertilizers [84]: every 100 kg of N fertilizer about 1.0 kg of N is emitted as N_2O [42], although different amounts depend on several factors including N application rate, soil organic C content, soil pH, and texture [78, 88]. Denitrification processes are the most important source of N_2O in most cropping and pasture systems [76, 88, 102].

In the recent years, several studies have focalized on the role of legumes in the reduction of GHG emissions. Jeuffroy et al. [44] demonstrated that legume crops

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[2] N_2O absorbs approximately 292 times as much infra-red radiation per kilogram as CO_2.

emit around 5–7 times less GHG per unit area compared with other crops. Measuring N_2O fluxes, they showed that peas emitted 69 kg N_2O ha^{-1}, far less than winter wheat (368 kg N_2O ha^{-1}) and rape (534 kg N_2O ha^{-1}). Clune et al. [19] reviewed different life cycle-assessment (LCA) studies on GHG emissions carried out from 2000 to 2015 around the world (despite the used literature was predominately European centric) highlighting that pulses have a very low Global Warming Potential (GWP) values (0.50–0.51 kg CO_2 eq kg^{-1} produce or bone-free meat[3]). In a comparison between vetch and barley under Mediterranean environments and alkaline soil, N_2O emissions were higher for barley than vetch; furthermore, the N_2O fluxes derived from the synthetic fertilizers added to the crops were 2.5 times higher in barley compared with vetch [29]. In two field experiments conducted in a black Vertosol in sub-tropical Australia, Schwenke et al. [95] demonstrated that the cumulative N_2O emissions from N-fertilized canola greatly exceeded those from chickpea, faba bean and field pea (385 vs. 166, 166 and 135 g N_2O-N ha^{-1}, respectively). The same authors highlighted that grain legumes significantly reduced their emission factors suggesting that legume-fixed N is a less-emissive form of N input to the soil than fertilizer N.

Nevertheless, it is important to highlight that the influence of legumes in reducing GHG depends also on the management of agro-ecosystems in which they are included. When faba bean was grown as mono cropping, it led to threefold higher cumulative N_2O emissions than that of unfertilized wheat (441 vs. 152 g N_2O ha^{-1}, respectively); conversely, when faba bean was mixed with wheat (intercropping system), cumulative N_2O emissions fluxes were 31% lower than that of N-fertilized wheat [96]. Anyway, the benefits derived from the introduction of legumes in crop rotations become significant when commercially relevant rates of N fertilizer are applied [42].

The mitigation in terms of GHG emissions is also obtained by adopting sustainable agricultural systems, such as conservation tillage and conservation agriculture systems, which are suitable for the cultivation of both grain and green-manure legumes (see "Grain legumes and conservation agriculture" section).

In conclusion, it is noteworthy to underline that field tests and experimental analyses on GHG emissions, and in particular on N_2O, provided quite different results [89] due to the influences of differences of several variables, including climatic, soil and management conditions [45, 78, 88].

In general, N_2O losses from soils covered with legumes are certainly lower than those from both N_2O fertilized grasslands and non-legume crops, as also indicated by Jensen et al. [42] who report a mean of 3.22 kg N_2O-N ha^{-1}, calculated from 67 site years of data. In addition, there is no direct association between N_2O emissions and biological nitrogen fixation [42], since organic N from legume residues is decomposed, mineralized and rapidly immobilized by microorganisms [78]. Emissions of N_2O could occur either during nitrification or due to denitrification, being affected by timing of mineralized N supply [20]: the asynchrony between N supply and utilization from the following crops enhances N loss, especially in winter/early spring in cold wet soils [64].

Soil properties

Cultivation and cropping may cause significant SOC losses through decomposition of humus [18]. Shifting from pasture to cropping systems may result in loss of soil C stocks between 25 and 43% [101].

Legume-based systems improve several aspects of soil fertility, such as SOC and humus content, N and P availability [42]. With respect to SOC, grain legumes can increase it in several ways, by supplying biomass, organic C, and N [27, 53], as well as releasing hydrogen gas as by-product of BNF, which promotes bacterial legume nodules' development in the rhizosphere [49].

In sandy soils, the beneficial effect of grain legumes after three years of study was registered in terms of higher content of SOC compared with soils with oats (7.21 g kg^{-1} DM, on average). Specifically, cultivation of pea exerted the most positive action to organic carbon content (7.58 g kg^{-1}, after harvest, on average), whereas narrow-leaved lupin had the least effect (7.23 g kg^{-1}, on average) [30]. In southern America (Argentina), the intercropping of soybean with maize at different rates favoured a SOC accumulation of 23.6 g C kg^{-1} versus 21.8 g C kg^{-1} of the sole maize; the greatest potential for enhancing SOC stocks occurred in the 2:3 (maize:soybean) intercrop configuration [11]. Furthermore, just only amending the soil with soybean residues allows to obtain an increase of 38.5% in SOC [11].

Thanks to BNF, legumes also affect significantly soil N availability; by using legumes as winter crops in rice–bean and rice–vetch combination, rice residue N content is enhanced by 9.7–20.5%, with values ranging from 1.87 to 1.93 g N kg^{-1} soil [120]. It needs to be underlines that a majority of studies on the role of legumes for soil

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[3] In the study of Clune et al. [19], each GWP value recorded from the literature data was converted into a common functional unit and system boundary in kg CO_2 eq kg^{-1} bone-free meat (BFM), using the conversion ratios identified in the literature.

N fertility have investigated the shoot N content. In this regard, Carranca et al. [15] found that 7–11% of total legume N was associated with root and nodules and an allocation of 11–14 kg N fixed t^{-1} belowground dry matter, representing half the amount of total aboveground plant.

In intercropping cowpea–maize, Latati et al. [50] found an increase in P availability at rhizosphere level associated with significant acidification (−0.73 U) than in sole cropping. Wang et al. [115], assessing properties related to N and P cycling in the rhizosphere of wheat and grain legumes (faba bean and white lupin) grown in monoculture or in wheat/legume mixtures, found that the less-labile organic P pools (i.e. NaOH-extractable P pools and acid-extractable P pools) significantly accumulated in the rhizosphere of legumes. However, the P uptake and the changes in rhizosphere soil P pools seem to depend also on legume species. Compared with the unplanted soil, the depletion of labile P pools (resin P and $NaHCO_3$-P inorganic) was the greatest in the rhizosphere of faba bean (54 and 39%) with respect to chickpea, white lupin, yellow lupin and narrow-leafed lupin [31]. Of the less-labile P pools, NaOH-P inorganic was depleted in the rhizosphere of faba bean, while NaOH-P organic and residual P were most strongly depleted in the rhizosphere of white lupin [31].

Also in North Rift, Kenya Region, in well-drained, extremely deep, friable clay, acid humic top soil, the effects of cultivation and incorporation of lupine and garden pea were significant in terms of soil-available P with respect to fallow, with lupine showing higher P availability than pea (from 20.3 to 31.0% higher).

Although there is a general agreement on the influence of grain legumes on rhizosphere properties in terms of N supply, SOC and P availability, the magnitude of the impact varied across legume species, soil properties and climatic conditions. Among these, soil type represents the major factor determining plant growth, rhizosphere nutrient dynamics and microbial community structure. The pattern of depletion and accumulation of some macro- and micronutrients differed also between cropping systems (i.e. monoculture, mixed culture, narrow crop rotations) as well as among soil management strategies (i.e. tillage, no-tillage).

Role of grain legumes in cropping systems

Legumes could be competitive crops, in terms of environmental and socioeconomic benefits, with potential to be introduced in modern cropping systems, which are characterized by a decreasing crop diversity [24, 80] and an excessive use of external inputs (i.e. fertilizers and agrochemicals).

Grain legumes into crop-sequences

In the recent years, many studies have focused on the sustainable re-introduction of grain legumes into crop rotations,[4] based on their positive effects on yield and quality characteristics on subsequent crops [46, 82, 103]. However, assessment of the rotational advantages/disadvantages should be based on a pairwise comparison between legume and non-legume pre-crops [82]. Some experimental designs involving multi-year and multispecies rotations do not provide information on yield benefits to the subsequent species in the rotation sequence. Therefore, it is difficult to formulate adequate conclusions [2].

The agronomic pre-crop benefits of grain legumes can be divided into a 'nitrogen effect' component and 'break crop effect' component. The 'nitrogen effect' component is a result of the N provision from BNF [77], which is highest in situations of low N fertilization to subsequent crop cycles [82]. The second one (break crop effect) includes non-legume-specific benefits, such as improvements of soil organic matter and structure [34], phosphorus mobilization [98], soil water retention and availability [2], and reduced pressure from diseases and weeds [87]. In this case, benefits are highest in cereal-dominated rotations [82].

Several authors have reviewed the yield benefits of legumes for subsequent cereal crops.

In Australia, Angus et al. [2] reported higher yield of wheat after legumes (field peas, lupins, faba beans, chickpeas and lentils) than those of wheat after wheat. In particular for a wheat–wheat yield of 4.0 t ha^{-1}, the mean grain legume-wheat yield was 5.2 t ha^{-1} (+30% on average). Other studies from Australia quantified yield benefits compared to pure cereal crop sequences at 40–50% for low N levels and 10–17% for high N levels [3].

In Europe yields benefits of grain legumes have been shown to strongly depend on climatic factors which affect N dynamics in soils [52]. In temperate environments, cereals yield is on average 17 and 21% higher in grain-legume based systems than wheat monocropping, under standard and moderate fertilization levels, respectively [40]. Conversely, yield benefits are lower in Mediterranean climates where water availability is the limiting factor to cereal yields [46, 61, 62].

The yield advantage to subsequent cereal crops provided by legumes depends also on the species and amounts of fixed N [114, 121]. Field pea and faba bean

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[4] According to Angus et al. [2], crop-sequences experiments can be classified into rotation experiments and break crop experiments. Rotation strictly defined, refers to a recurring sequence of crops, forages and fallows, or more loosely defined, to a cropping sequence that contains fallows, or crops and forages in addition to the locally dominant species. A break crop generally refers to a single alternative crop followed by the dominant species.

accumulate about 130 and 153 kg N ha^{-1} in their aboveground biomass, respectively [77] and significant quantities (30–60% of the accumulated total N) may also be stored in belowground biomass [77]. Differences in BNF patterns are also found between the same species. For example, Mokgehle et al. [69] compared 25 groundnut varieties for plant BNF at three differing agro-ecologies in South Africa, highlighting N-fixed range between 76 and 188 kg ha^{-1}, depending also on soil and environmental conditions as well as on N-uptake. Other factors influencing BNF include salinity and sodicity (alkalinity) of soils, as observed in chickpea [83], common bean [22] and faba bean [109].

It is, however, rather difficult to quantify the legume dependent increase in N uptake in subsequent crops, versus other sources of N [46, 77]. In temperate environments of Australia, measurements of the additional N-nitrate available to wheat crops following legumes instead of cereals, averaged around 37 kg N ha^{-1} [17]. In Denmark, nitrogen uptake in crops that follow legume crops has been reported to increase by 23–59% after field pea and narrow-leafed lupin on different soil types [40], but only 14–15% for durum wheat following vetch in a semi-arid Mediterranean environment [28]. Increased N uptake of crops after grain legumes reached up to 61% or 36 kg ha^{-1} for a vetch-barley rotation in Cyprus [74]. Further, some legume residues have beneficial effects on some quality aspects of the subsequent crops in southern Italy [104].

Among other beneficial effects brought about by legumes, the production of hydrogen gas (H_2) as a by-product of BNF greatly affects the composition of the soil microbial population, further favouring the development of plant growth-promoting bacteria [2].

Some grain legumes, including chickpea, pigeon pea and white lupin can mobilize fixed forms of soil P through the secretion of organic acids such as citrate and malate and other P mobilizing compounds from their roots [36]. Among grain legumes, white lupin most strongly solubilize P, a function that can be facilitated by its proteoid roots that may englobe small portions of soil [2]. Glasshouse experiments using a highly P-fixing soil showed better wheat growth following white lupin than soybean [37], suggesting that the cereal was able to access P made available by the previous white lupin break crop. ‘Break crop’ effects also include increased soil water content, since the break-crop stubble can affect retention of soil water and infiltration and retention of rain water [47]. A species-specific response has also been documented. Soil profiles after pea field can be wetter than after a wheat crop [2]. In Saskatchewan, Canada, Miller et al. [68] reported that post-harvest soil water status up to 122 cm-depth was 31 and 49 mm greater for all legumes (field pea, lentil and chick pea) with respect of wheat under loam and clay soils, respectively. This was primarily due to increased plant water use efficiency. Lentil in rotation with cereals has been shown to increase total grain production by increasing residual soil water in dry areas of Saskatchewan [25].

In general, grain legumes are not susceptible to the same pests and diseases as the main cereal crops (non-host), resulting suitable as break crops in wheat-based rotations [121]. Grain legumes as break crops can also contribute to weed control [97] by contrasting their specialization and helping stabilizing the agricultural crop weed community composition [7].

Despite the described beneficial effects, there are still concerns on the introduction of grain legumes into cropping sequences. Cropping systems that include legume crops in farm rotations must be supported by best crop-management practices (e.g. N fertilization rates and timing, soil management, weeding, irrigation), which often do not match standard techniques normally applied by farmers. For example, some possible risks in terms of nitrate leaching associated to grain legumes cultivation can be counteracted by including cover crops in the system [33, 81]. Additional reasons may explain why grain legumes are not very common in high-input cropping systems. These include (1) their low and unstable yields [16, 86]; (2) inadequate policy support [14]; (3) lack of proper quantification (and recognition) of long-term benefits of legumes within cropping systems [82]. However, other efforts could be addressed, for example, to breeding programs for improved crop cultivars, to better sustain livelihood and increase the economic return to farmers. Indeed, during last years significant progresses in breeding for quality traits for food [110] and feed uses [79], as well as for resistances to biotic [91] and abiotic stresses [4] are being achieved, but several others, many of which are controlled quantitatively by multiple genes, have been more difficult to achieve.

Grain legumes in intercropping

Intercropping systems consist in simultaneous growth of two or more crop species on the same area and at the same time [13]. Intercropping is widely used in developing countries or in low-input and low-yield farming systems [73]. Despite several recognized beneficial aspects of intercropping such as better pest control [60], competitive yields with reduced inputs [70, 107], pollution mitigation [63], more stable aggregate food or forage yields per unit area [100], there are a number of constrains that make intercropping not common in modern agriculture, such as example the request of a single and standardized product and the suitability for mechanization or use of other inputs as a prerogative in intensive farming system [13]. It is therefore necessary to optimize intercropping systems to enhance resource-use efficiency and crop yield simultaneously [55], while also promoting multiple ecosystem services (see also [13]). Most recent research has focalized on the potential of intercropping in sustainable productions and in particular on grain legumes that can fix N_2 through biological mechanisms (BNF). Indeed, legumes are pivotal in many intercropping systems, and of the top 10 most frequently used intercrop species listed by Hauggaard-Nielsen and Jensen [32], seven are legumes One of the basic spatial arrangements used in intercropping is strip intercropping, in which two or more crops grow together in strips wide enough to permit separate crop production using inputs but close enough for the crops to interact. The current challenge is how to determine an optimal intercropping width to maximise the resources use efficiency and, consequently, the crop productivity. In a maize-bean strip intercropping, Mahallati et al. [65] suggested that strip width of 2 and 3 rows was superior compared with monoculture and other strip intercropping combinations in terms of radiation absorption, radiation use efficiency and biological yields of both species, also allowing to an improve of total land productivity and land equivalent ratio (1.39 and 1.37). Gao et al. [26] showed a total yield increase of 65 and 71% in a system of 1 and 2 rows of maize (planted at a higher density in intercropping) alternated with 3 rows of soybean compared with both crops grown as monoculture. However, Liu et al. [59] showed a reduction in the photosynthetically active radiation and R:FR ratio at the top of soybean canopy intercropped with maize - under two intercropping patterns: 1 row of maize with 1 row of soybean; 2 rows of maize with 2 rows of soybean - leading to increased internode lengths, plant height and specific leaf area (SLA), but reduced branching of soybean plants. In order to gain sufficient light in the most shaded border rows of the neighbouring, shorter crops, efforts could be addressed to (i) the selection of highly productive maize cultivars with reduced canopy height and LAI; (ii) the increase of the strip width under a higher fraction of direct PAR; (iii) the selection of crops and cultivars suitable under the shade levels that likely occur in strip-intercropping systems with maize [71].

The increase in N availability in intercrops hosting legumes occurs because the competition for soil N from legumes is weaker than from other plants. Moreover, non-legumes obtain additional N from that released by legumes into the soil [56, 117] or via mycorrhizal fungi [113]. Legumes can contribute up to 15% of the N in an intercropped cereal [57], thus increasing biomass production and carry-over effects [75], reducing synthetic mineral N-fertilizer use and mitigating N_2O fluxes [9, 96]. However, the adoption of grain legume intercropping systems should benefit from the identification of suitable legumes that are less susceptible to N fertilizer-induced inhibition of BNF—that is, legumes that sustain higher %BNF in the presence of increasing soil mineral N. To this purpose, Rose et al. [90] indicated that faba bean is more suitable as intercrop than chickpea when supplementary N fertilizer additions are required, with about 40%BNF and 29%BNF maintained in faba bean and chickpea, respectively, supplying both crops with 150 kg N ha^{-1}.

BNF represents the most common plant growth stimulating factor that can also improve crop competition with respect to weeds in both organic and sustainable farming systems [10]. Grain legumes are weak suppressors of weeds, but mixing species in the same cropping system could represent a valid way to improve the ability of the crop itself to suppress weeds [41, 94]. In a wheat-chickpea intercropping system (20 cm spacing without weeding treatment) it was observed a 69.7% reduction in weed biomass and 70% in weed population as compared to un-weeded monocrop wheat at 20 cm spacing [6]. Similar results on weed smothering have been obtained by Midya et al. [67] in rice-blackgram (20 cm) intercropping system although the deferred seeding of blackgram in rice field (30 cm) with one weeding may be recommended for both better yield and weed suppression.

Direct mutual benefits in cereal-legumes intercropping involve below-ground processes in which cereals while benefiting of legumes-fixed N, increase Fe and Zn bioavailability to the companion legumes [118].

Physiology, agronomy and ecology can simultaneously contribute to the improvement of intercropping systems, allowing to enhance crop productivity and resource-use efficiency, so making intercropping a viable approach for sustainable intensification, particularly in regions with impoverished soils and economies where measured benefits have been greatest [93]. But to realize these goals, major efforts in research programs still remain. For example: (1) breeding for intercrops; (2) better understanding of the interactions between plants and other organisms in crop systems, focusing on the roles of above- and below-ground interactions of plants with other organisms; (3) improving agricultural engineering and management, i.e. developing new machinery that can till, weed and harvest at small spatial scales and in complex configurations to encourage the uptake of intercropping without greater demands for labour [58]; (4) adoption of a wider 'systems thinking' through the enactment of schemes, including payment for ecosystem services [105].

Grain legumes and conservation agriculture

Legumes have some characteristics particularly suitable for sustainable cropping systems and conservation agriculture, and making them functional either as growing crop or as crop residue. Conservation agriculture is based on minimal soil disturbance and permanent soil cover combined with rotations [35]. As previously described, major advantages of legumes include the amount of nitrogen fixed into the soil and the high quality of the organic matter released to the soil in term of C/N ratio. Some legume species have also deep root systems, which facilitate nutrients solubilization by root exudates and their uptake/recycling as well as water infiltration in deeper soil layers.

Many countries already rely on conservation agriculture. Brazil has implemented conservation agriculture systems using soybean as legume crop. Grain legumes like lentil, chickpea, pea and faba bean play a major role in conservation agriculture in North America, Australia, and Turkey. In Australia, some advantages of minimum tillage for grain legumes have been quantified for water-limited environments. Some studies indicate that the majority of grain-legumes producers use direct seeding after a legume pre-crop [1]. This change from conventional tillage (CT) to reduced or no tillage (NT) systems (with at least 30% of the soil surface covered) would lead to significant positive impacts on SOC [18]. In contrast, other results indicate that such positive effects are limited to the first 20 cm depth, while little or no difference between CT and NT in total SOC can be seen lower down the soil profile [5, 111]. Such findings suggest that C stock changes in the soil are mainly dependent to the net N-balance in the system. With high N harvest index legumes, SOC stocks are not preserved due to the high amount of N taken off from the field into the grain [42]. Conversely, the effect of legumes on soil carbon sequestration is more detectable for forage, green-manures and cover-crops which return to the soil large amounts of organic C and N [52]. Boddey et al. [12] indicate that vetch under no tillage may increase SOC stocks under NT (0–100 cm) at a rate between 0.48 and 1.53 Mg C ha^{-1} per year [42].

The implementation of practices of conservation tillage could significantly reduce the GWP, especially when a grain legumes is added to the rotation. In Mediterranean agro-ecosystems, Guardia et al. [29] compared three tillage treatments (i.e. no tillage: NT, minimum tillage: MT, conventional tillage: CT) and two crops (i.e. vetch, barley) and recorded the emission of N_2O, CH_4 and CO_2 during one year. Authors found a significant 'tillage × crop' interaction on cumulative N_2O emissions with vetch releasing higher N_2O amount than barley only in CT and MT, whereas similar fluxes were observed under NT. This was attributable to the soil water-filled pore space, dissolved organic carbon content and denitrification losses, in spite of the presumable predominance of nitrification. In any case, the most sustainable crop and tillage treatments in terms of GWP were represented by the non-fertilized vetch and NT, due to higher carbon sequestration, lower fuel consumption and the absence of mineral N fertilizers [29]. In subtropical Ultisol, under legume cover crops, NT soil exhibited increased N_2O emissions with respect to CT soil (531 vs. 217 kg CO_2 eq ha^{-1} $year^{-1}$); however, emissions of this gas from NT soil were fully offset by CO_2 retention in soil organic matter (-2063 to -3940 kg CO_2 ha^{-1} $year^{-1}$) [8]. Moreover, NT soil under legume cover crops behaved as a net sink for GHG (GWP ranged from -971 to -2818 kg CO_2 eq ha^{-1} $year^{-1}$) [8].

The expansion of ecological-based approaches like conservation agriculture opens opportunities to food legumes to be profitably included in sustainable cropping systems. There are still major challenges for conservation agriculture that need to be overcome, including the development of effective methods for weed control (see also [92]) that can avoid the use of herbicides or tillage. Overall conservation agriculture is an environmentally sustainable production system that may boost the incorporation of grain legumes within large and small-scale farming.

Conclusion

The roles and importance of grain legumes in a context of sustainability in agriculture could be enhanced by the emerging research opportunities for the major topics discussed above.

A major task in the future will be the selection of legume species and cultivars which could be effectively introduced across different cropping systems. An important point concerns balancing yield, which gives economic return, with the environmental and agronomic benefits.

Some priority areas seem emerge. Nitrogen fixation activity of grain legumes should be evaluated in relation with soil, climatic, plant characteristics and management conditions to find the suitable approach to achieve the best improvements. With this respect, the ability of the host plant to store fixed nitrogen appears to be a major component of increasing nitrogen fixation input. A particular focus should be paid also to the study of abiotic stress limitations and in particular water deficit, salinity and thermal shocks require extensive investigation.

Legumes that can recover unavailable forms of soil phosphorus could be major assets in future cropping systems. Consequently, those legumes which are able to accumulate phosphorus from forms normally unavailable need to be further studied, since phosphorus represents an expensive and limiting resource in several cropping systems.

Because of the growing request for plant products, i.e. protein and oils, and to the increased economic and environmental pressures on agro-eco systems, it emerges that grain legumes would play a major role in future cropping systems.

Abbreviations

GHG: greenhouse gases; CO_2: carbon dioxide; N_2O: nitrous oxide; LCA: life cycle assessment; GWP: global warming potential; BNF: biological nitrogen fixation; NT: no tillage; CT: conventional tillage.

Authors' contributions

FS participated in the topic literature view and selection, and in drafting of the manuscript. AM drafted the manuscript and revised it critically. AG participated in the topic literature view and selection, and in the drafting of the manuscript. MP drafted the manuscript and revised it critically. All authors read and approved the final manuscript.

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Review

Probiotics, prebiotics, and synbiotics used to control vibriosis in fish: A review

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ABSTRACT

The aquaculture industry is still expanding to provide human beings with healthy and nutritious protein sources. Infectious diseases, deteriorated water quality parameters, and other environmental stressors are from the main obstacles that threaten fish farming and reduce its profitability. Vibriosis is one of the most common bacterial diseases that negatively affect shrimp, marine fishes, and some freshwater fish causing high mortalities and severe economic loss. Chemotherapeutic agents as antibiotics are commonly applied for treatment strategies; however, their numerous drawbacks to fish and the aquatic environment have limited their use. Moreover, FDA has prohibited certain antibiotics from being used for food fish to avoid their negative consequences on human consumers. Water quality control and biosecurity protocols are traditionally applied to combat vibriosis. Nowadays, immunomodulators are greatly used and described throughout the globe to enhance the fish immunity. In this concern, probiotics, prebiotics, and synbiotics have become common solutions to increase the fish resistance against vibriosis. They were approved to be current alternatives to limit the usage of antibiotics in aquaculture resulting in less mortality and increased health and welfare of the aquatic organisms. Many studies speculated that probiotics, prebiotics, and synbiotics could be efficiently and effectively used as food or water additives to increase immunity, thereby reducing the mortality caused by several fish pathogens such as *Vibrio* species. Comprehensively, this review article presents the latest knowledge on the potential roles of probiotics, prebiotics, and synbiotics to boost immunity and reduce the impacts of vibriosis in several finfish species. This review article will also provide new findings and possible mechanisms of action of probiotics, prebiotics, and synbiotics to control vibriosis in fish. These literature will be helpful to increase the sustainability of aquaculture and health welfare of farmed fish.

1. Introduction

The aquaculture industry is the fastest-growing food production sector supporting almost 50% of all aquatic foods for human consumption (FAO, 2021). The ultimate goal of aquaculture is to increase the production rates to maximize profitability. However, in the large-scale production system, fish may be exposed to various diseases especially if they were stressed by deteriorated water quality and exposed to stressful conditions resulting in severe economic losses (Abdel-Latif and Khafaga, 2020; Abdel-Latif et al., 2020a).

Bacterial fish diseases cause severe economic losses in many countries worldwide (Austin, 1999; Austin and Austin, 2016). Vibriosis is one of the most common bacterial fish diseases affecting a wide range of economically important farmed shrimp, marine fish species and some freshwater fish around the globe (Toranzo et al., 2005; Mohamad et al., 2019). Moreover, it was recorded that vibriosis is responsible for significant economic losses and mass mortality events in aquaculture (Ina-Salwany et al., 2019). *Vibrio anguillarum*, *V. parahaemolyticus*,

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V. damsela, *V. vulnificus*, *V. alginolyticus*, *V. harveyi*, *V. ordalli*, *V. owensii*, and *V. campbellii* are the most common *Vibrio* species that cause vibriosis in several finfish species (Austin and Austin, 2012).

For a long time, several antibiotics and antimicrobials have been used to control vibriosis (Laganà et al., 2011; Yano et al., 2014). Nonetheless, the use of antibiotics in aquaculture for the treatment of aquatic animals can lead to several hazardous problems such as the existence of antibiotic-resistant bacterial strains, antibiotic residues in fish muscles which will induce serious public health effects on the human consumers, and also the occurrence of environmental pollution (Alderman and Hastings, 1998; Adel et al., 2017). Thus, finding promising safe and environmentally friendly alternatives are necessary to avoid the negative consequences of antibiotic usages (Abdel-Latif et al., 2020b; Ahmadifar et al., 2020; Abdel-Tawwab et al., 2020a; Dawood et al., 2021; Abdel-Tawwab et al., 2021).

Probiotics, prebiotics, and synbiotics have gained significant interests from several researchers because of their unique beneficial applications in aquaculture (Dawood et al., 2020b). Probiotics can be defined as live organisms that can benefit the host when administered in the appropriate amounts. There were a wide range of probiotics that have been used in aquaculture such as probiotic bacteria (like *Pediococcus*, *Lactobacillus*, *Bacillus*, *Enterococcus*, *Micrococcus*, *Lactococcus*, *Roseobacter*, *Pseudomonas*) and probiotic yeasts (like *Aspergillus oryzae* and *Saccharomyces cerevisiae*) (Kuebutornye et al., 2019). These probiotics can be used to boost the immune responses and increase the resistance of fish to diseases (Ringø, 2020). Probiotics could also improve the beneficial intestinal microbial populations, intestinal morphology, and increase the digestive enzyme activities which help to improve nutrients absorption and feed utilization (Gatesoupe, 1999; Hai, 2015; El-Saadony et al., 2021). Prebiotics such as inulin, oligofructose, galactooligosaccharides, isomaltooligosaccharides, fructooligosaccharides, and mannan oligosaccharides have been applied in feed of several finfish species with significant and profitable roles (Ringø et al., 2010; Ringø et al., 2014). The mixture of prebiotics and probiotics is known as "synbiotic" (Kolida and Gibson, 2011), which has numerous beneficial effects for fish such as improved gut microbiota, immunological responses, growth rates, and overall health status (Huynh et al., 2017).

Researchers reported the beneficial roles of single or multiple strain probiotics in reducing the cumulative mortality rate of fish after being experimentally challenged with *Vibrio* species (Gildberg et al., 1997; Gildberg and Mikkelsen, 1998; Balcázar et al., 2008). Moreover, new research studies also found that "autochthonous" or host-derived probiotics can also be used as quorum quenching probiotics which conferred health benefits, promoted the defense mechanisms, and protected fish against vibriosis (Ghanei-Motlagh et al., 2021a; Ghanei-Motlagh et al., 2021b). Another research work found that *Lactococcus lactis* BFE920 can be used as a vaccine vehicle that helps provides protection against several *Vibrio* species (Lee et al., 2021). On the other hand, prebiotics can confer the protection of fish against challenges with *Vibrio* species (Castro-Osses et al., 2017; Serradell et al., 2020). Synbiotics can boost immunity and enhanced the survival rates of fish experimentally challenged with *Vibrio* species (Rodriguez-Estrada et al., 2009; Ai et al., 2011; Geng et al., 2011). This review article will highlight the most commonly used probiotics, prebiotics, and synbiotics for controlling vibriosis. Their application, modes of action, and beneficial effects on the aquatic organisms will also be described.

2. Vibriosis in fish

Vibriosis is one of the most common bacterial diseases affecting many farmed and wild fish species and caused by *Vibrio* species (family vibrionaceae), which are a group of gram-negative rod-shaped bacteria inhabiting freshwater, estuarine and marine environments (Noga, 2010). *V. anguillarum*, *V. ordalii*, *V. alginolyticus*, *V. carchanae*, *V. cholera*, *V. damsela*, *V. vulnificus*, *V. parahemolyticus*, and *V. salmonicida* are from the commonly isolated *Vibrio* species and caused significant economic losses in the diseased fish (Ina-Salwany et al., 2019; Mohamad et al., 2019). Table 1 shows the clinical picture (clinical signs and postmortem lesions), locality, and name of *Vibrio* species that are involved in the occurrence of vibriosis in a wide range of finfish species.

Vibriosis has been named as red pest, red disease, cold pest, ulcer disease, or eye disease. This disease generally occurs in warm water, especially when stocking density, salinity, and organic loads are high (El-Son et al., 2021). It is well known that chemical, biological, and physical stressors are the principal factors that contribute to the incidence of vibriosis (Austin and Austin, 2016). It has been reported that waterborne transmission is the primary mode of transmission of vibriosis through direct contact of the pathogen with the skin, fins, anus, and gills. Moreover, oral route infection can be also reported, whereas vibriosis outbreaks have occured after feeding with contaminated leftover fish, waste seafood, and non-thermally processed leftover fish (Noga, 2010; Austin and Austin, 2016).

V. anguillarum is a halophilic Gram-negative, non-spore forming, comma-shaped bacterium and motile with a polar flagellum (Frans et al., 2011; Austin and Austin, 2012). It was found that eels (family Anguillidae) infected with *V. anguillarum* typically had skin discoloration, red necrotic lesions on the abdominal muscles, red spots on the bottom of the fins, around the anal region, and inside the mouth (Noga, 2010). Reports showed that *V. anguillarum* had caused vibriosis in more than 50 freshwater and sea fish species such as Pacific salmon, Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), turbot (*Scophthalmus maximus*), sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), striped bass (*Morone americanus*), cod (*Gadus morhua*), Japanese eels (*Anguilla japonica*), European eel (*Anguilla anguilla*) and ayu (*Seriola quinqueradiata*) leading to economic loss (Toranzo and Barja, 1990). Being deadly hemorrhagic septicemia in marine fish, *V. anguillarum* causes significant economic losses to fish farming (Santos et al., 1996; Mikkelsen et al., 2011).

V. ordalii induced heavy losses in Atlantic salmon in southern Chile (Colquhoun et al., 2004; Silva-Rubio et al., 2008) and anorexia, deep skin ulcers, fin rot, jaw erosion, and surface hemorrhages in gilthead seabream in Turkey (Akayli et al., 2010). *V. vulnificus* caused histopathological lesions and heavy mortalities in grass carp (*Ctenopharyngodon idellus*) (Liu et al., 2019), and skin ulcers of GIFT tilapia (genetically improved farmed tilapia) (*Oreochromis niloticus*) (Sumithra et al., 2019).

V. alginolyticus caused typical bacterial septicemia in the infected fish which characterized by darkened skin color, skin ulcers and hyperemic capillary vessels of the intestinal wall (Balebona et al., 1995). The disease caused by *V. alginolyticus* also characterized by exophthalmia and corneal opaqueness in grouper (*Epinephelus malabaricus*) (Lee, 1995), hemorrhagic fins and ulcers in gilthead seabream (Balebona et al., 1998), and high mortalities in cage-cultured gilthead seabream and European seabass in Tunisia (Ben Kahla-Nakbi et al., 2006; Ben Kahla-Nakbi et al., 2009).

V. harveyii induced high mortalities of silvery black porgy (*Acanthopagrus cuvieri*) and brown-spotted grouper (*Epinephelus tauvina*) in Kuwait (Saeed, 1995), ascites, gastroenteritis, and high mortality of cobia (*Rachycentron canadum*) (Liu et al., 2004), tail rot in gilthead seabream (Haldar et al., 2010), hemorrhagic skin ulcers in head and body, hemorrhagic spots, and fin rot in Arabian Surgeon fish (*Acanthurus sohal*) (Hashem and El-Barbary, 2013), high mortalities of large yellow croaker (*Pseudosciaena crocea*) (Liu et al., 2016), and muscle necrosis disease in Asian seabass (barramundi) (*Lates calcarifer*) (Dong et al., 2017).

V. damsela has been isolated from rainbow trout during outbreaks (Pedersen et al., 1997). It has also retrieved from the skin ulcers from several fish species such as blacksmith damselfish, yellowtail, turbot, gilthead seabream, and red-banded seabream (Noga, 2010). *V. parahaemolyticus* also induced septicemic signs in European seabass (Khouadja et al., 2013), Sebae clownfish (*Amphiprion sebae*)

Table 1
Some *Vibrio* species identified from diseased fish species in different localities in the world.

Fish species	Locality	*Vibrio* species	Common clinical signs and lesions	References
Grouper (*Epinephelus malabaricus*)	Taiwan	*V. alginolyticus*	Exophthalmia with corneal opaqueness	(Lee, 1995)
Dark-spotted stingrays (*Himantura uarnak*) Tahitian stingrays (*Himantura fai*)	Egypt	*V. alginolyticus*	Dorsal disc surface skin lesions with whitish necrotic tissues	(Emam et al., 2019)
Silvery black porgy (*Acanthopagrus cuvieri*) Brown-spotted grouper (*Epinephelus tauvina*)	Kuwait	*V. harveyi*	High mortalities	(Saeed, 1995)
Red conger eel (*Genypterus chilensis*)	Chile	*V. toranzoniae*	Gill, fin, and skin lesions	(Lasa et al., 2015)
Rainbow trout (*Oncorhynchus mykiss*)	Denmark	*V. damsela*	Vibriosis outbreaks	(Pedersen et al., 1997)
Blacksmith damselfish (*Chromis punctipinnis*)	USA	*V. damsela*	Skin ulcers	(Love et al., 1981)
Gilthead seabream (*Sparus aurata*)	Spain	*V. alginolyticus*	Hemorrhagic fins and ulcers associated with high mortality	(Balebona et al., 1998)
Orange-spotted grouper (*Epinephelus coioides*)	Taiwan	*V. carchariae*	Gastroenteritis	(Lee et al., 2002)
Cobia (*Rachycentron canadum*)	Taiwan	*V. harveyi*	Ascites, gastroenteritis, and high mortalities	(Liu et al., 2004)
Gilthead seabream (*S. aurata*) European seabass (*Dicentrarchus labrax*)	Tunisia	*V. alginolyticus*	High mortalities	(Ben Kahla-Nakbi et al., 2006) (Ben Kahla-Nakbi et al., 2009)
European seabass (*D. labrax*)	Spain	*V. anguillarum*	Immune suppression	(Sepulcre et al., 2007)
Gilthead seabream (*S. aurata*)	Malta	*V. harveyi*	Tail rot disease	(Haldar et al., 2010)
Gilthead seabream (*S. aurata*)	Turkey	*V. ordalii*	Anorexia, deep skin ulcers, fin rot, jaw erosion, and surface hemorrhages	(Akayli et al., 2010)
European seabass (*D. labrax*)	Tunisia	*V. parahaemolyticus*	Outbreaks of high mortalities	(Khouadja et al., 2013)
Atlantic salmon (*Salmo salar*)	Chile	*V. ordalii*	Disease outbreaks	(Colquhoun et al., 2004) (Silva-Rubio et al., 2008)
Gilthead seabream (*S. aurata*)	Egypt	*V. parahaemolyticus*	Severe bilateral abdominal distention, vent prolapse, and skin hemorrhages	(Aly et al., 2020)
Arabian Surgeon fish (*Acanthurus sohal*)	Egypt	*V. harveyi*	Hemorrhagic skin ulcers in head and body, hemorrhagic spots, and fin rot	(Hashem and El-Barbary, 2013)
Freshwater catfish	China	*V. mimicus*	Mass mortalities (80%–100%)	(Geng et al., 2014)
Sebae clownfish (*Amphiprion sebae*)	India	*V. parahaemolyticus*	Tail rot disease	(Marudhupandi et al., 2017)
GIFT tilapia (*Oreochromis niloticus*)	India	*V. vulnificus*	Skin ulcers	(Sumithra et al., 2019)
Grass carp (*Ctenopharyngodon idellus*)	China	*V. vulnificus*	Histopathological alterations and mortalities	(Liu et al., 2019)
Egyptian Sole (*Solea aegyptiaca*) Dusky grouper (*Epinephelus marginatus*) Striped mullet (*Mugil cephalus*)	Egypt	*V. alginolyticus* *V. parahaemolyticus* *V. vulnificus*	Hemorrhagic septicemia	(Abdelaziz et al., 2017)
Barramundi (*Lates calcarifer*)	Vietnam	*V. harveyi*	Scale drops and muscle necrosis disease	(Dong et al., 2017)
Large yellow croaker (*Pseudosciaena crocea*)	China	*V. alginolyticus* *V. harveyi* *V. parahaemolyticus*	High mortalities	(Liu et al., 2016)
Golden pompano (*Trachinotus ovatus*)	China	*V. ponticus*	Swollen and hyperemic liver and spleen, yellowish effusion in the gut, and ulcerations on the base of the dorsal and pelvic fins	(Liu et al., 2018a)
Striped mullet (*M. cephalus*)	Egypt	*V. parahaemolyticus*	Massive skin hemorrhages and high mortalities	(El-Son et al., 2021)

(Marudhupandi et al., 2017), gilthead seabream (Aly et al., 2020), and striped mullet (*Mugil cephalus*) (El-Son et al., 2021).

Cold-water vibriosis (Hitra disease) is caused by *V. salmonicida* (Noga, 2010). It is clinically characterized by anorexia, unorganized swimming movements, pale gills, hemorrhage at the base of fins, redness of the anus, swelling, and the presence of petechial hemorrhages in the abdominal wall. Moreover, abdominal dropsy and hemorrhages were distributed over the abdominal fat, liver, and other visceral organs (Egidius et al., 1981). There are also several other *Vibrio* species such as *V. carchariae*, which caused gastroenteritis in orange-spotted grouper (*Epinephelus coioides*) (Lee et al., 2002), *V. mimicus,* which caused mass mortalities in freshwater catfish (Geng et al., 2014), and *V. ponticus*, which induced ulcerations on the bases of the dorsal and pelvic fins in golden pompano (*Trachinotus ovatus*) (Liu et al., 2018a).

3. Control of vibriosis using chemotherapeutics and vaccines

3.1. Chemotherapeutics

Fish farmers are haphazardly utilized antibiotics to control diseases as antibiotics can be defined as “readily available over the counter”. Although there are a lot of demand to eliminate antibiotics from aquaculture needs, it is predicted that there will be growing investments in the research and development of antibiotics (Bondad-Reantaso et al., 2005). In the past 50 years, antibiotics have been demonstrated as a very effective method in controlling bacterial infections in animals and fish (Alderman and Hastings, 1998). Some antibiotics have proven to fight vibriosis, such as oxytetracycline, tetracycline, quinolones, nitrofurans, potentiated sulfonamides, trimethoprim, sarafloxacin, flumequine, and oxolinic acid (Laganà et al., 2011; Yano et al., 2014).

The emergence of antibiotic-resistant bacterial strains was observed due to overuse and/or misuse of antibiotics to control infections in aquaculture, which will render the antibiotics to be an ineffective therapy (Defoirdt et al., 2007). The frequent use of chemotherapeutic agents will also increase the treatment costs and antibiotic residues in fish muscles, which will negatively affect the health status of human consumers and will pollute the aquatic environment (Alderman and Hastings, 1998). For these reasons, vaccines have been significantly developed worldwide and directed towards prevention rather than treatment of vibriosis (Ji et al., 2020).

3.2. *Vaccination against fish Vibrio species*

Fish vaccination, generally, means administeration of the antigen of a particular pathogen to the fish to obtain particular immunoprotective responses (Gudding et al., 1999). The most reliable immunoprophylactic precaution to fight diseases is vaccination as it can help fish to survive against the challenging pathogens. An ideal vaccine should not pose a danger to fish, the person administering it, and also human consumers. The vaccine should induce a significant protection level against many pathogenic strains (or serotypes of a single pathogen), cost-efficient, have a prolonged impact, and preserve its effectiveness throughout the production period. It should also easy during application and suitable for several fish species (Wali and Balkhi, 2016).

Significant progress has been made in the development of vaccines against *Vibrio* species infecting fish. Several types of fish vaccines have been developed such as traditional inactivated bacterins, live attenuated vaccines, DNA vaccines, subunit vaccines, and live vector vaccines (Ji et al., 2020). Vaccines against *Vibrio* species are relatively safe and effective for preventing vibriosis in aquaculture because they can induce potent immune responses for the vaccinated fish (Toranzo et al., 1997). However, there are some problems associated with vaccination are observed such as difficult administration, higher labor force, and expense. For these reasons, scientists and aquaculturists resorted to novel, practical biological control methods, including probiotics, biovaccination, and bacteriophage therapy, to achieve a solid and sustainable aquaculture production.

4. Use of probiotics, prebiotics, and synbiotics to control vibriosis

4.1. *Probiotic applications*

Probiotics are live microbial feed supplements that beneficially maintain the host intestinal microbial balance (Fuller, 1989; Skjermo and Vadstein, 1999). Probiotics can also be described as microbial cells administered through the gastrointestinal tract (GIT) to enhance the overall health of the treated fish (Gatesoupe, 1999). A probiotic can also be defined as a live microbial adjunct that induces numerous beneficial effects in several ways, such as modifying the host ambient microbial community, enhancing the nutritional values of feed ingredients, and upgrading the immune responses of the host towards the challenging pathogens (Verschuere et al., 2000). These microbial products can be administered either as feed or water supplements for increasing the fish disease resistance, health status, growth performance, and also modulation of stress responses. Their main effects can be occurred via improving the host-microbial balance (Merrifield et al., 2010; Akhter et al., 2015).

In aquaculture, probiotics can be administered either as mono (single) strain probiotic or multiple (multi) strains or even combined with prebiotic substances to be used as "synbiotics" (Hai, 2015). Several types of probiotic bacterial strains have been proven to be efficiently used in aquafeed with unique and beneficial properties such as *Bacillus*, *Lactococcus*, *Lactobacillus*, *Pseudomonas*, *Enterococcus*, *Aeromonas*, *Alteromonas*, *Bifidobacterium*, *Clostridium*, *Phaeobacter*, *Pseudoalteromonas*, *Rhodosporidium*, *Roseobacter*, *Streptomyces*, and some several other bacterial species (Hai, 2015; Ringø, 2020; Butt et al., 2021). Besides, some yeast species also can possess beneficial probiotic effects in the treated fish (Caruffo et al., 2015; Shukry et al., 2021).

Host-associated probiotics can also be successfully used to maintain the sustainability of the aquaculture industry (Amenyogbe et al., 2020; Van Doan et al., 2020). There are numerous beneficial uses of probiotics in aquaculture such as the control of fish diseases (Hoseinifar et al., 2018) and reducing the negative impacts of antibiotics and other antimicrobials on fish (Dawood and Koshio, 2016). Moreover, probiotics can modulate the transcription of immune-related genes which confer strong immunity and protection against challenging pathogens (Dawood et al., 2020b). They also can increase the abundance of beneficial bacteria in water and therfore enhance the application of biofloc technology (Mohammadi et al., 2021; Romano, 2021). From the widely used probiotics, *Bacillus* spp. have also been extensively used in aquaculture for modulating toxicity signs (El Euony et al., 2020) and improving immunity and antioxidant capacity (Abdel-Tawwab et al., 2020b). Table 2 shows the beneficial effects of probiotics in control of vibriosis in several finfish species.

Probiotics used to control fish vibriosis can be classified into 4 main items:-.

4.1.1. *Single strain probiotics to control vibriosis*

It was found that the probiotic *Carnobacterium divergens* isolated from intestines of Atlantic cod significantly reduced cumulative mortality rate (CMR) % of Atlantic cod fries that experimentally infected with *V. anguillarum* (Gildberg et al., 1997; Gildberg and Mikkelsen, 1998). The dietary application of *Carnobacterium* sp. strain K1 retrieved from the GIT of Atlantic salmon provided protection and reduced the mortalities of Atlantic salmon after cohabitation with *V. ordalii* but not *V. anguillarum* (Robertson et al., 2000). Moreover, the water application of *Pseudomonas fluorescens* AH2 reduced the mortality rate of rainbow trout challenged with *V. anguillarum* (Gram et al., 1999).

Brunt et al. (2007) found that dietary application of *Bacillus* sp. JB-1 or *Aeromonas sobria* GC2 significantly enhanced the immune responses of rainbow trout. Those authors also reported that these probiotic bacteria increased the production of siderophore and chitinase enzyme, enhanced lysozyme (LYZ), phagocytic (PA), and respiratory burst activities (RBA), and reduced the CMR% after challenge with *V. anguillarum* to 13% and 6%, respectively compared to the controls (92%) (Brunt et al., 2007).

Notably, the dietary supplementation with live or dead *Clostridium butyrium* strain CB2 improved the immune responses of Chinese drum (*Miichthys miiuy*). These strains increased PA of head kidney macrophages, serum LYZ, total IgM levels, and SR % of fish experimentally challenged with *V. anguillarum* (Pan et al., 2008). Those authors also found that the SR% was 70% and 78% in dead and live CB2, respectively, compared with 40% in the control group without probiotic supplementation. The dietary supplementation with 1% *Enterococcus faecalis* significantly improved the immunity and reduced the mortality rate of rainbow trout experimentally infected with *V. anguillarum* to 22.9% compared with 62.5% in the control (Rodriguez-Estrada et al., 2009).

Sharifuzzaman and Austin (2010) found that dietary application of *Kocuria* SM1 isolated from the GIT of rainbow trout increased the hemato-immunological responses of rainbow trout (increased WBCs, RBCs, total protein, globulin, albumin, RBA, complement, LYZ, and bacterial killing activities), and also reduced the CMR% of fish after being challenged with *V. anguillarum* and *V. ordalii*. Another study showed that the IP injection of extracellular proteins (ECPs), cell wall proteins (CWPs), and whole-cell proteins (WCPs) of *Kocuria* SM1 and *Rhodococcus* SM2 reduced the mortalities of rainbow trout experimentally challenged with *V. anguillarum* (Sharifuzzaman et al., 2011).

The probiotic strain *Vagococcus fluvialis* protected European seabass against challenge with *V. anguillarum* and significantly reduced the CMR % compared to the control group without probiotics (Sorroza et al., 2012). Another single probiotic *B. coagulans* upregulated the expression of immune-related genes and enhanced the SR% of orange-spotted grouper and zebrafish (*Danio rerio*) experimentally infected with *V. vulnificus* (Pan et al., 2013).

Table 2

Probiotic applications used for protection against challenge with *Vibrio* species in various fish species.

Probiotics	Dosages	Period	Fish species	General effects	Protective effects against vibriosis						References
					Vibrio spp.	Adminstration route	Count	Period	CMR% or SR% in control	CMR% or SR% in probiotic	
Bacillus coagulans (BC) *Bacillus licheniformis* (BL) *Paenibacillus polymyxa* (PP)	10^{12} CFU/kg diet	56 d	Northern whiting fish	↑ in SR%, FBW, WG, SGR, PER, HSI, VSI, CP and CL in the body, SOD, ALP, ACP, GPX Significant ↓ in the FCR, AST, ALT, MDA Enhance intestinal morphometry	*V. harveyi*	0.2 mL/IP injection	1×10^8 CFU/mL/Fish	14 d	CMR % = 78%	CMR% BC = 58% BL = 53% PP = 43%	(Amoah et al., 2021)
Bacillus sp. JB-1 *Aeromonas sobria* GC2	2×10^8 cells/g diet	14 d	Rainbow trout	↑ siderophore and chitinase production, LYZ, PA and RBA	*V. anguillarum*	0.1 mL/IP injection	3×10^7 cells/mL	14 d	CMR % = 92%	CMR % JB-1 = 13% GC2 = 6%	(Brunt et al., 2007)
Bacillus spp. M001	10^8 CFU/g diet	42 d	Turbot	No significant differences of WG, SGR, FCR Significant ↑ protease and amylase activities in hepatopancreas, intestinal protease activity and lipase activity in stomach Significant ↑ Serum SOD and TP	*V. anguillarum*	0.2 mL/IP injection	4.89×10^4 CFU/Fish	15 d	CMR % = 89.3%	CMR % = 33.3%	(Chen et al., 2016)
B. subtilis (7×10^9 CFU/g) *B. licheniformis* (3×10^9 CFU/g) *Lactobacillus* spp. (5×10^8 CFU/g) *Arthrobacter* spp. (1×10^8 CFU/g)	1, 2, 3, 4 and 5 g/kg diet	8 Wks	Cobia	Significant ↑ SGR, LYZ, ACH50, phagocytosis, and RBA of head-kidney macrophages	*V. harveyi*	0.1 mL/IP injection	4.8×10^7 CFU/Fish	7 d	Highest mortalities	Lowest cummulative mortality	(Geng et al., 2012)
Bacillus thuringiensis QQ1 *B. cereus* QQ2	1×10^9 CFU/g diet	35 d	Asian seabass	Significant ↑ HTC and TLC in QQ1 group Significant ↑ RBA in probiotic groups Significant ↓ serum TC in probiotic groups	*V. harveyi*	0.2 mL/IP injection	1×10^8 CFU/mL	7 d	CMR % = 71.5%	CMR % QQ1 = 23.8% QQ2 = 16%	(Ghanei-Motlagh et al., 2021a)
B. thuringiensis QQ1 *B. cereus* QQ2	1×10^9 CFU/g diet	42 d	Asian seabass	↑ CAT, SOD, LYZ, antiprotease, MPO, RBA and bactericidal activities in probiotic	*V. alginolyticus*	0.1 mL/IP injection	6×10^8 CFU/mL	7 d	SR% = 36%	SR% QQ1 = 86.8% QQ2 = 97.1%	(Ghanei-Motlagh et al., 2021b)
Carnobacterium divergens isolated from intestines of Atlantic salmon	10^8 viable cells/g diet	3 Wks	Atlantic cod	Reduced CMR% of infected fish	*V. anguillarum*	Water bath	10^7 CFU/mL	12 d	High CMR%	Reduced CMR%	(Gildberg and Mikkelsen, 1998)
C. divergens isolated from intestines of Atlantic cod	2×10^9 CFU/g diet	3 Wks	Atlantic cod	Reduced CMR% of infected fish	*V. anguillarum*	Water bath	7×10^6 CFU/mL	22 d	MR % = 60%	CMR % = 40%	(Gildberg et al., 1997)
Pseudomonas fluorescens AH2	10^5 CFU/mL 10^7 CFU/mL	5 d During infection	Rainbow trout	Reduced CMR% of infected fish	*V. anguillarum*	Water bath	10^4–10^5 CFU/mL	7 d	CMR% = 47%	CMR % = 25%	(Gram et al., 1999)
Pediococcus pentosaceus isolated from cobia intestine	10^9 CFU/g diet	3 Wks	Orange-spotted grouper	Significant ↑ leukocyte numbers in the peripheral blood and PA of the head-	*V. anguillarum*	IP injection	5×10^5 CFU/Fish 6×10^5 CFU/Fish	7 d	CMR% = 40% CMR% = 65%	CMR% = 10% CMR% = 15%	(Huang et al., 2014)

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Table 2 (*continued*)

Probiotics	Dosages	Period	Fish species	General effects	Protective effects against vibriosis						References
					Vibrio spp.	Adminstration route	Count	Period	CMR% or SR% in control	CMR% or SR% in probiotic	
				kidney phagocytes 1-day post-infection							
L. plantarum 1KMT	1×10^8 CFU/g diet	60 d	Nile tilapia	Improvement of intestinal microflora, SGR, ACH50, IgM, and LYZ SR% = 96.5% in probiotics and 86.0% in control	*V. parahaemolyticus*	IP injection	10^5 CFU/mL	21 d	CMR% = 77.7%	CMR% = 66.6%	(Jules-Bocamdé et al., 2020)
B. subtilis WB60 (BS) *L. plantarum* KCTC3928 (LP)	BS = 10^6, 10^7, and 10^8 CFU/g diet LP = 10^6, 10^7, and 10^8 CFU/g diet	8 Wks	Japanese eel	↑ WG, FER, PER, serum LYZ, SOD, and MPO, and expression of intestinal IgM, HSP70, HSP90 and GAPDH genes in BS at 10^7 and 10^8 CFU/g diet	*V. anguillarum*	0.1 mL IP injection	5×10^7 CFU/mL	10 d	Lowest SR%	Highest SR% only in BS or LP at 10^7 and 10^8 CFU/g diet	(Lee et al., 2017)
Vaccine generated by *Lactococcus lactis* BFE920 expressing fusion antigen of Vibrio OmpK and FlaB	1×10^7 CFU/g diet	3 Wks (1Wk interval)	Olive flounder	↑ antigen-specific antibodies, T cell numbers, cytokine production, and innate immune responses	*V. anguillarum* *V. alginolyticus* *V. harveyi*	IP injection	2×10^7 1×10^9 1×10^7 CFU/Fish	3 Wks	Lowest SR%	Highest SR% in vaccinated fish	(Lee et al., 2021)
Bacillus velezensis K2	1×10^7 CFU/g diet	28 d	Hybrid groupers	↑ serum ACP activity ↑ expression of immune-related genes in the head kidney	*V. harveyi*	0.1 mL IP injection	1×10^7 cells/mL	8 d	SR% = 25%	SR% = 55%	(Li et al., 2019)
B. subtilis E20 (BS)	10^8, 10^9, and 10^{10} CFU/kg diet defined as BS8, BS9 and BS10	56 d	Parrot fish	Enhancement of growth in BS 10^8 CFU/kg diet No significant differences in SR% and dorsal muscle composition	*V. alginolyticus*	IP injection	5×10^6 CFU/g Fish	7 d	MR% = 96.67%	MR% BS8 = 96.67% BS9 = 90% BS10 = 46.67%	(Liu et al., 2018a)
B. pumilus A97 derived from golden pompano	1×10^8 CFU/g diet	56 d	Golden pompano	↑ WG, SGR, FER ↑ expression of TLR8 in the intestines and TLR9 in the kidneys	*V. ponticus*	0.1 mL IP injection	1.75×10^2 CFU/g Fish	7 d	SR% = 23.68%	SR% = 59.65%	(Liu et al., 2020)
Clostridium butyrium CB2	Live or dead CB2 (10^8 cells/g diet)	30 d	Chinese drum	↑ PA of head kidney macrophages, serum LYZ and IgM levels	*V. anguillarum*	0.1 mL IP injection	1×10^6 cell/mL/Fish	14 d	SR% = 40%	SR% Live CB2 = 78% Dead CB2 = 70%	(Pan et al., 2008)
B. coagulans	10^4, 10^6, 10^8 and 10^{10} in 50 mL media mixed with 50 g eel powder	30 d	Groupers Zebrafish	↑ expression of MyD88, IL-1β, and TNF-α (in groupers) ↑ expression of TLR4, TNF-α, and NF-κB (in zebrafish)	*V. vulnificus*	Injected into caudal peduncle	10 μL (zebrafish) (10^4 CFU/Fish) 50 μL (groupers) (10^3 CFU/Fish)	13 d	Lowest SR%	Dose-dependent manner in SR% over the control	(Pan et al., 2013)
Enterococcus faecalis	1%	12 Wks	Rainbow trout	↑ HTC, PI, PA, mucous production	*V. anguillarum*	IP injection	10^5 CFU/mL	14 d	MR% = 62.5%	MR% = 22.9	(Rodriguez-Estrada et al., 2009)
Kocuria SM1 isolated from digestive tract of rainbow trout	10^8 cells/g diet	2 Wks	Rainbow trout	↑ WBCs, RBCs, TP, GLO, ALB, upregulation of RBA, complement, LYZ and bacterial killing activities	*V. anguillarum* *V. ordalii*	0.1 mL IP injection	3×10^5 cells/mL 5×10^4 cells/mL	14 d	MR% = 80% MR% = 74%	MR% = 15% MR% = 20%	(Sharifuzzaman and Austin, 2009)
		7 d			*V. anguillarum*			7 d			

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Table 2 (*continued*)

Probiotics	Dosages	Period	Fish species	General effects	Protective effects against vibriosis						References
					Vibrio spp.	Adminstration route	Count	Period	CMR% or SR% in control	CMR% or SR% in probiotic	
Kocuria SM1 *Rhodococcus* SM2	0.1 mL IP injection of ECPs, CWPs and WCPs (2.0 ± 0.5 mg/mL)		Rainbow trout	Protection against *V. anguillarum*		0.1 mL IP injection	3×10^5 cells/mL		MR% = 86%	MR% CWPs and WCPs = 11–17% ECPs = 33–38%	(Sharifuzzaman et al., 2011)
Vagococcus fluvialis	10^9 CFU/g diet	20 d	European sea bass	Protection against *V. anguillarum*	*V. anguillarum*	0.1 mL IP injection	10^8 CFU/mL	20 d	MR% = 30%	MR% = 17.3%	(Sorroza et al., 2012)
Lactobacillus acidophilus (LA) *L. plantarum* (LP) *L. sporogenes* (LS)	10^6 CFU/g diet	60 d	*Carassius auratus*	Maximum WG in probiotic groups	*V. parahaemolyticus*	0.1 mL IP injection	3×10^9 cells/mL	15 d	SR% = 33.33%	SR% LA = 50.0% LP = 83.33% LS = 66.66%	(Subharanjani et al., 2015)
L. lactis HNL12	10^6, 10^8, and 10^{10} CFU/kg diet	4 Wks	Humpback grouper	Significant ↑ WG% in 10^8 CFU/kg Enhanced RBA of head kidney macrophages, serum SOD, ACP, LYZ	*V. harveyi*	IP injection	1×10^6 CFU/mL	15 d	SR% = 36%	SR% = 56%, 70%, and 68%, respectively	(Sun et al., 2018)
B. subtilis *L. acidophilus* *Clostridium butyricum* *Saccharomyces cerevisiae*	$>1.6 \times 10^7$ CFU/g diet $>1.2 \times 10^8$ CFU/g diet $>2.0 \times 10^7$ CFU/g diet $>2.0 \times 10^7$ CFU/g diet	50 d	Japanese flounder	Protection against *V. anguillarum*	*V. anguillarum*	Water bath for 1 h	2×10^7 CFU/mL	14 d	Lowest SR%	Highest SR% over controls	(Taoka et al., 2006)
B. subtilis	1.05×10^7 CFU/g diet 5.62×10^7 CFU/g diet	8 Wks	Ovate pompano	Significant ↑ RBA, PA, ACH50, LYZ	*V. vulnificus*	0.2 mL IP injection	1.9×10^6 CFU/mL	10 d	MR% = 75.93%	MR% = 51.85% and 40.74%	(Zhang et al., 2014)

Abbreviations: ACH50: Alternative complement pathway; ACP: Acid phosphatase; ALB: Albumin; ALP: Alkaline phosphatase; ALT: Alanine aminotransaminase; AST: Aspartate aminotransaminase; CAT: Catalase; CL: Crude lipid content; CMR%: Cumulative mortality percent; CP: Crude protein content; CWPs: Cell wall proteins; ECPs: Extracellular proteins; FBW: Final body weight; FCR: Feed conversion ratio; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; GLO: Globulin; GPX: Glutathione peroxidase; HSI: Hepatosomatic index; HSP70: Heat shock protein 70; HSP90: Heat shock protein 90; HTC: Hematocrit values; IgM: Immunoglobulin M; IL-1β: Interleukin 1 beta; IP: Intraperitoneal injection; LYZ: Lysozyme activity; MDA: Malondialdehyde levels; MPO: Myeloperoxidase; MyD88: Myeloid differentiation factor 88; NF-κB: Nuclear Factor Kappa B; PA: Phagocytic activity; PER: Protein efficiency ratio; RBA: Respiratory burst activity; RBCs: Red blood cells; SGR: Specific growth rate; SOD: Superoxide dismutase; SR%: Survival rate percent; TC: Total cholesterol; TLC: Total leucocytic count; TLR4: Toll like receptor 4; TLR8: Toll-like receptor 8; TLR9: Toll-like receptor 9; TNF-α: Tumor necrosis factor alpha; TP: Total protein; VSI: Viscerosomatic index; WCPs: Whole cell proteins; WBCs: White blood cells; WG%: Weight gain percent; WG: Weight gain.

The probiotic *Phaeobacter* 27–4 strain grown in a ceramic biofilter significantly reduced the CMR% of 10-day-old turbot larvae (*Psetta maxima*) to 35–40% after experimental challenge with *V. anguillarum* compared with 76% in the control group (Prol-García and Pintado, 2013). Furthermore, the administration of *Artemia franciscana* nauplii enriched with *Lac. lactis* subsp. *lactis* in diets of European seabass larvae significantly increased their SR% to 81% compared with 24% in the control group after experimental challenge with *V. anguillarum* (Touraki et al., 2013).

Dietary administration of *Pediococcus pentosaceus* derived from the GIT of cobia increased the PA, leucocytic count and enhanced the resistance of orange-spotted grouper against challenge with *V. anguillarum* (Huang et al., 2014). Also, dietary application of the probiotic *B. subtilis* (in a dose rate 1.05×10^7 or 5.62×10^7 CFU/g diet) for 8 weeks reduced the CMR% of ovate pompano juveniles experimentally infected with *V. vulnificus* to 51.85% and 40.74%, respectively compared with 75.93% in the control group (Zhang et al., 2014). Also, supplementing diets with either *Lactobacillus acidophilus*, *L. plantarum*, or *L. sporogenes* significantly increased the SR% of *Carassius auratus* experimentally challenged with *V. parahaemolyticus* (Subharanjani et al., 2015). Likewise, supplementing the probiotic *Bacillus* spp. strain M001 in diets of turbot reduced the CMR% to 33.3% after being experimentally challenged with *V. anguillarum* compared with 89.3% in the non-probiotic control group (Chen et al., 2016).

The dietary probiotic yeast *S. cerevisiae* significantly modulated the immune responses of rainbow trout after a challenge with *V. anguillarum* (Castro-Osses et al., 2017). Moreover, *B. subtilis* WB60 and L. *plantarum* KCTC3928 in a dose rate of 10^7 and 10^8 CFU/g diets significantly increased the SR% of Japanese eel after challenge with *V. angulillarum* (Lee et al., 2017). Another study showed that dietary *B. subtilis* E20 (10^{10} CFU/kg for 56 days) significantly decreased the CMR% to 46.67% in parrotfish (*Oplegnathus fasciatus*) challenged with *V. alginolyticus* compared with 96.67% in the control group (Liu et al., 2018b).

Sun and coauthors found that dietary *Lac. lactis* HNL12 enhanced the RBA of head kidney macrophages, superoxide dismutase (SOD), acid phosphatase (ACP), LYZ activities, and disease resistance of humpback grouper (*Cromileptes altivelis*) against *V. harveyi* infection (Sun et al., 2018). Moreover, the probiotic *Bacillus velezensis* K2 (1×10^7 CFU/g diet) significantly increased the expression of immune-related genes in the head kidney of hybrid groupers (*E. lanceolatus* ♂ × *E. fuscoguttatus* ♀) and increase their resistance against the challenge with *V. harveyi* (Li et al., 2019). The host-derived *B. pumilus* A97 (1×10^8 CFU/g diets for 56 days) increased the growth performance, non-specific immune responses, and disease resistance of golden pompano against challenge with *V. ponticus* (Liu et al., 2020). Furthermore, the probiont L. *plantarum* 1KMT significantly improved the non-specific immunity and resistance of Nile tilapia against the challenge with *V. parahaemolyticus* (Jules-Bocamdé et al., 2020).

Recently, dietary supplementation with *B. coagulans*, *B. licheniformis,* and *Paenibacillus polymyxa* improved the growth performance, body somatic indexes, antioxidant capacity, and enhanced the intestinal morphometry of Northern whitings (*Sillago sihama*) (Amoah et al., 2021). Those authors also reported significantly reduced CMR% after a challenge with *V. harveyi* in probiotic groups over the controls. Another study showed that supplementation of *A. franciscana* with the probiotic *C. divergens* considerably enhanced the growth, SR%, and resistance of larval stages of Atlantic cod after challenge with *V. anguillarum* (Puvanendran et al., 2021).

4.1.2. Multi- (multiple) strain probiotics to control vibriosis

Dietary supplementation with multiple strain probiotics contains *B. subtilis*, *L. acidophilus*, *Cl. butyricum*, and *S. cerevisiae* increased the SR % of Japanese flounder (*Paralichthys olivaceus*) against challenge with *V. anguillarum* (Taoka et al., 2006). Moreover, supplementing a probiotic mixture composed of *B. subtilis*, *Lactobacillus* spp., and *Arthrobacter* spp. in diets of cobia significantly increased specific growth rate, immune responses (LYZ, alternate complement pathway, PA, and RBA of head-kidney macrophages), and decreased the CMR% after challenge with *V. harveyi* infection (Geng et al., 2012).

4.1.3. Quorum quenching probiotics to control vibriosis

The use of quorum quenching (QQ) probiotics is a new trend with potential efficacy for control vibriosis in Aquaculture. It was found that autochthonous QQ probiotics showed increased degrading activities against *N*-acyl-homoserine lactone (AHL)-associated virulence factors in several *Vibrio* species affecting fish (Ghanei-Motlagh et al., 2020). In this regard, it was found that dietary supplementation with *B. thuringiensis* QQ1 and *B. cereus* QQ2 significantly reduced the CMR% of Asian seabass experimentally infected with *V. harveyi* to 23.8% and 16%, respectively, compared with 71.5% in the control group (Ghanei-Motlagh et al., 2021a). Moreover, dietary QQ1 and QQ2 noticeably enhanced the SR % of Asian seabass experimentally infected with *V. alginolyticus* to 86.8% and 97.1%, respectively, compared with 36% in the control group (Ghanei-Motlagh et al., 2021b).

4.1.4. Probiotics as vaccine delivery vehicle against vibriosis

Studies showed that the probiotic lactic acid bacteria known as *Lac. lactis* BFE920 could be efficiently used as a novel vaccine delivery vehicle. These studies provided new ideas and elucidated mechanisms for protection of fish against several pathogens such as *Streptococcus iniae* (Kim et al., 2016) and *Edwardsiella tarda* (Beck et al., 2017). Lee et al. (2021) prepared a vaccine generated by the recombinant *Lac. lactis* BFE920 expressing *Vibrio* fusion antigens of the outer membrane protein K (OmpK) and flagellin B subunit (FlaB). Those authors indicated that this vaccine could robust the innate immune responses of olive flounder and provide protection against *V. anguillarum*, *V. alginolyticus*, and *V. harveyi* infections (Lee et al., 2021).

From the aforementioned research studies, we can conclude that probiotics could enhance the immune responses of the treated fish and potentiate their resistance to the challenging pathogens (Hoseinifar et al., 2018). Reports illustrated the potential efficacy of host-derived probiotics for controlling several other fish bacterial diseases such as furunculosis (caused by *Aeromonas salmonicida*) and enteric red mouth disease (caused by *Yersinia ruckeri*) in rainbow trout (Irianto and Austin, 2002; Kim and Austin, 2006). The possible mechanisms of action of probiotics to control bacterial infections of fish may include the following:-.

a) *Enhancement of fish immune responses:* - The ability of probiotics to enhance the cellular and humoral immunity of the treated fish (Brunt et al., 2007).
b) *Competition for nutrients:* - Some probiotics can produce siderophores which exert inhibitory effects against the other microflora through iron depletion from their microbial environment (Brunt et al., 2007).
c) *Antagonistic activity:* - For instance, reports showed that *B. amyloliquefaciens* can produce secondary metabolites which have potent antimicrobial activities (Chen et al., 2009).
d) *Colonization capacity:* - The ability of probiotics to colonize of the fish GIT and prevent the invasion with pathogenic bacteria through competitive exclusion mechanisms (Verschuere et al., 2000). They also exerts competition for the adhesion to receptors with pathogenic agents (Hai, 2015).
e) *Modulation of the transcriptome:*- The modulatory roles of dietary probiotics on the transcriptomic profile of immune-related genes also contribute to the mechanisms of action of probiotics (Dawood et al., 2020b).
f) *Potentiation of the antioxidant defense mechanisms of fish:*- Several studies proposed the potential efficacy of probiotics in modulation of the fish antioxidant mechanisms (Hoseinifar et al., 2021).

Although the previously mentioned hypotheses of the modes of action of probiotics, the actual mechanisms on fish are still unknown and

Table 3
Prebiotic applications used for protection against challenge with *Vibrio* spp. in various fish species.

Prebiotics	Dosages	Period	Fish species	General effects	Protective effects against vibriosis						References
					Vibrio species	Adminstration route	count	Period	CMR% or SR% in control	CMR% or SR% in prebiotic	
Mannanoligosaccharides (MOS)	2 and 4 g/kg diet	9 Wks	European seabass	Significant ↑ LYZ, and ACH50 Significant ↓ No. of dead fish after 21 days of experimental challenge	*V. alginolyticus*	Gut canalization	2.4×10^8 CFU/mL/Fish	21 d	–	–	(Torrecillas et al., 2007)
Mannanoligosaccharides (MOS)	4 g/kg diet	8 Wks	European seabass	↑FBW, SGR, Final relative growth, CF	*V. anguillarum*	Anal inoculation	10^7 CFU/mL/Fish	6 d	CMR% = 66%	CMR% = 12.5%	(Torrecillas et al., 2012)
Mannanoligosaccharide (MOS) Polyhydroxybutyrate acid (PHB)	MOS 0.4% PHB 1%	12 Wks	Rainbow trout	↑ FBW, WG, SGR and FGR in MOS group ↑ HTC, PI, PA, and mucous production in MOS and PHB groups compared with controls	*V. anguillarum*	IP injection	10^5 CFU/mL	14 d	CMR% = 62.5%	CMR% MOS = 27.0% PHB = 50.0%	(Rodriguez-Estrada et al., 2009)
Levan produced by *Bacillus licheniformis* FRI MY-55	5, 10, 25 and 50 g/kg diet	12 Wks	Orange spotted grouper	Significant ↑ FBW and WG% in the 25 g/kg group than controls Significant ↓ total viable aerobic bacteria and *Vibrio* spp. counts in the intestines Significant ↑ TP, GLO, LYZ (25 g/kg group)	*V. harveyi*	IP injection	5×10^7 CFU/Fish	9 d	Lowest SR%	Highest SR% in 25 g/kg diet	(Huang et al., 2015)
Fructooligosaccharide (FOS)	0.2% or 0.4%	8 Wks	Ovate pompano	↑ SGR, FER in 0.4% FOS group ↑ RBA, PA, ACH50, and LYZ in 0.4% FOS group	*V. vulnificus*	0.2 mL IP injection	1.9×10^6 CFU/Fish	10 d	CMR% = 75.93%	CMR% = 0.2% FOS = (62.96%) 0.4% FOS = (51.85%)	(Zhang et al., 2014)
Mushroom beta-glucan mixture (MBG)	0.1% and 0.2%	15 d	Orange spotted grouper	Significant ↑ LYZ, ACH50, PA and RBA	*V. alginolyticus*	IP injection	1×10^6 CFU/Fish	15 d	Highest CMR%	Significant ↑ of SR% up to 16%	(Chang et al., 2013)
Ecklonia cava	1%	16 Wks	Olive flounder	Improved the growth and FBW ↑ RBA, serum LYZ and MPO activity	*V. harveyi*	IP injection	3×10^5 cells/mL	2 Wks	Highest CMR%	Significant ↓ CMR%	(Lee et al., 2016)
Galactomannanoligosaccharides (GMOS)	0.5% GMOS	9 Wks	European seabass	↓ plasma cortisol after confinement stress ↑ serum LYZ after experimental infection	*V. anguillarum*	Anal inoculation	10^5 CFU/Fish	7 d	–	–	(Serradell et al., 2020)
β-glucan	5 g/kg diet	28 d	Hybrid groupers	β-glucan restored oxytetracycline-induced immune suppression	*V. alginolyticus*	IP injection	6.5×10^9 CFU/Fish	5 d	Highest CMR%	Significant ↑ of SR%	(Lee et al., 2020)

Abbreviations: ACH50: Alternative complement activity; CF: Condition factor; FBW: Final body weight; FER: Feed effeciency ratio; FGR: Feed gain ratio; GLO: Globulin; LYZ: Lysozyme activity; CMR%: Cumulative mortality rate percent; MPO: Myeloperoxidase; PA: Phagocytic activity; RBA: Respiratory burst activity; SGR: Specific growth rate; SR%: Survival rate percent; TP: Total protein; WG%: Weight gain percent; WG: Weight gain.

Table 4

Synbiotic applications used for protection against challenge with *Vibrio* spp. in various fish species.

Synbiotic	Dosage	Period	Fish species	General effects	Protective effects against vibriosis						References
					Vibrio spp.	Adminstration route	Count	Period	CMR% & SR% in control	CMR% & SR% in synbiotic	
Enterococcus faecalis (EF) Mannanoligosaccharide (MOS) Polyhydroxybutyrate acid (P)	EF 1% MOS 0.4% Polyhydroxybutyrate 1%	12 Wks	Rainbow trout	↑ WG, SGR, and FGR ↑ HTC, PA, PI, and mucus production	*V. anguillarum*	IP injection	10^5 CFU/mL	14 d	CMR% = 62.5%	CMR% EF + MOS = 10.4% EF + MOS + *P* = 18.7%	(Rodriguez-Estrada et al., 2009)
Bacillus subtilis (BS) Fructooligosaccharide (FOS)	BS = (0.42 × 10^7 CFU/g and 1.35 × 10^7 CFU/g) FOS = (0.2% and 0.4%)	10 Wks	Large yellow croaker	FOS levels and 1.35 × 10^7 CFU/g BS significantly ↑SGR, FER, LYZ and SOD	*V. harveyi*	0.2 mL IP injection	2.1 × 10^8 CFU/Fish	10 d	CMR% = 70–80%	CMR% = 30–40% (FOS levels and 1.35 × 10^7 CFU/g BS)	(Ai et al., 2011)
B. subtilis (BS) Chitosan (CH)	BS = (2 × 10^{10} CFU/g) (1.0 and 2.0 g/kg diet) CH = (3.0 and 6.0 g/kg diet)	8 Wks	Cobia	Significant ↑ serum ACP activities in BS (1.0 g/kg diet) + CH (3.0 g/kg diet) BS (2.0 g/kg diet) + CH (6.0 g/kg diet) Significant ↑ PA & RBA in combined groups	*V. harveyi*	0.1 mL IP injection	1.2 × 10^7 CFU/Fish	7 d	Highest CMR%	Lowest CMR% in combined treatment BS (2.0 g/kg diet) + CH (6.0 g/kg diet)	(Geng et al., 2011)
B. subtilis (BS) Fructooligosaccharide (FOS)	BS = (1.05 × 10^7 or 5.62 × 10^7 CFU/g diet) FOS = (0.2% or 0.4%)	8 Wks	Ovate pompano	Highest SGR in BS (5.62 × 10^7 CFU/g diet) + 0.2% FOS Highest RBA in BS groups +0.2% FOS Highest ACP in BS (5.62 × 10^7 CFU/g diet) + FOS groups	*V. vulnificus*	0.2 mL IP injection	1.9 × 10^6 CFU/Fish	10 d	CMR% = 75.93%	CMR% = (29.63%) in BS (5.62 × 10^7 CFU/g) + 0.2% FOS (33.33%) in BS (5.62 × 10^7 CFU/g) + 0.4% FOS	(Zhang et al., 2014)
B. subtilis WB60 (BS) Mannanoligosaccharide (MOS)	BS = (0.5 and 1.0 × 10^7 CFU/g diet) MOS = (5 g/kg diet)	8 Wks	Japanese eel	↑ AWG, FER, SGR, PER, serum LYZ, MPO and expression of IgM in BS (0.5 × 10^7 CFU/g diet) + MOS (5 g/kg diet) BS (1.0 × 10^7 CFU/g diet) + MOS (5 g/kg diet)	*V. anguilarum*	0.1 mL IP injection	5 × 10^7 CFU/Fish	10 d	Highest CMR%	Highest SR% in combined treatments	(Lee et al., 2018)
Mannanoligosaccharide (MOS) *Pediococcus acidilactici*	MOS 0.3% and 0.6%	90 d	European seabass	↑ WG in combined treatments No effects on somatic indexes and intestinal histomorphology	*V. anguillarum*	IP injection	10^6 CFU/mL/Fish	6 d	Highest CMR%	Highest SR% in 0.3% MOS and probiotic	(Torrecillas et al., 2018)

Abbreviations: ACP: Alternative complement pathway; AWG: Average weight gain; FER: Feed efficiency ratio; FGR: Feed gain ratio; HTC: Hematocrit value; IgM: Immunoglobulin M; LYZ: Lysozyme; CMR%: Cumulative mortality rate percent; MPO: Myeloperoxidase; PA: Phagocytic activity; PER: Protein efficiency ratio; PI: Phagocytic index; RBA: Respiratory burst activity; SGR: Specific growth rate; SOD: Superoxide dismutase; SR%: Survival rate percent; WG: Weight gain.

complicated. Accordingly, more comprehensive, and specified knowledge should be identified, and further research studies are still necessitated. Further research points should be focused on the possibility of encapsulating the probiotic products to be a suitable approach for better convey the probiotics to the GIT of aquatic animals (Hai, 2015). Moreover, the dosage of the probiotics and time duration to reach the GIT of fish are significant factors that should also be considered for providing desired results. Suggestions for further studies on the effects of probiotics in aquaculture are proposed.

4.2. *Prebiotic applications*

Prebiotics have a broad definition as the indigestible fibers that improve the count of beneficial gut commensal microbiota resulting in the improvement of the overall health status of the host (Song et al., 2014). Several prebiotics such as mannan oligosaccharides (MOS), fructooligosaccharide (FOS), inulin, β-glucan (BG), galactomannan oligosaccharides (GMOS), and several others are efficiently used and increasingly applied in aquaculture as immunomodulatory agents (Ringø et al., 2010; Ringø et al., 2014; Hoseinifar et al., 2015; Dawood and Koshio, 2016; Dawood et al., 2020a). The enhancement in the humoral and innate immune responses in fish is responsible for high tolerance against infection with infectious pathogens (Dawood et al., 2020b).

In general, studies showed that prebiotics markedly enhanced the growth performance (Lv et al., 2007; Mahious et al., 2006), feed efficiency (Huang et al., 2015), and hemato-biochemical traits (Castro-Osses et al., 2017). They also can modulate the physiological responses of fish against stressors (Torrecillas et al., 2012). They have also significant roles in improving the humoral responses (Chang et al., 2013) and innate immunity of fish (Serradell et al., 2020; Zhang et al., 2014).

Mahious et al. (2006) found that the use of inulin and oligofructoses as prebiotics resulted in a marked decrease in the dominance of *Vibrio* spp. in the intestinal microbiota of weaning turbot. In a similar trend, dietary supplementation with MOS induced a marked decrease of *Aeromonas* and *Vibrio* counts in the intestinal microbial population of rainbow trout (Dimitroglou et al., 2009). The study conducted by Torrecillas and coauthors showed that supplementation of MOS derived from the outer cell wall of *S. cerevisiae* in diets of European seabass for 8 weeks (4 g/kg diet) resulted in a significant reduction of *V. anguillarum* count in the gut after *ex vivo* exposure, and its absence from head kidney and liver specimens after *in vivo* exposure (Torrecillas et al., 2011). Another study showed that dietary short-chain FOS decreased *V. parahaemolyticus* and *A. hydrophila* counts in the intestinal microbiota of hybrid tilapia (*O. aureus* ♂ × *O. niloticus*♀) (Lv et al., 2007).

Reports showed that dietary prebiotics considerably improved the SR % of fish experimentally challenged with *Vibrio* spp. (Rodriguez-Estrada et al., 2009; Torrecillas et al., 2012; Chang et al., 2013; Zhang et al., 2014; Huang et al., 2015; Lee et al., 2020; Serradell et al., 2020). The potential effects of some selected prebiotics on enhancing the immune responses and improving the resistance of several finfish species against challenge with *Vibrio* spp. are presented in Table 3.

It was found that dietary supplementation with MOS derived from the outer cell wall of *S. cerevisiae* in diets of European seabass for 9 weeks increased the non-specific immunity and decreased the number of infected fish after 21 days of experimental challenge with *V. alginolyticus* (Torrecillas et al., 2007). Moreover, 0.4% MOS considerably improved the growth performance, immune responses and reduced the CMR% in rainbow trout after challenge with *V. anguillarum* (Rodriguez-Estrada et al., 2009). Furthermore, using a yeast-derived MOS or BG in diets of Atlantic cod for 5 weeks led to increased resistance of fish against *V. anguillarum* infection through modulation of the transcriptomic profile of cytokine genes in the intestinal tract of the challenged fish (Lokesh et al., 2012). After an 8-week feeding experiment, Torrecillas et al. (2012) illustrated that dietary MOS (4 g/kg diet) significantly decreased the CMR% of European seabass after anal inoculation with *V. anguillarum*. Another study concluded that dietary supplementation with 0.6% MOS markedly modulated the expression of immune-related genes and inflammatory responses in rainbow trout following challenge with *V. anguillarum* (Castro-Osses et al., 2017).

Dietary supplementation with 0.4% FOS for 8 weeks noticeably increased the growth performance, immunity and reduced the CMR% in ovate pompano experimentally challenged with *V. vulnificus* (Zhang et al., 2014). Recently, 0.5% GMOS-supplemented diets increased the immune responses in European seabass after anal inoculation with *V. anguillarum* (Serradell et al., 2020).

Another study demonstrated by Chang et al. (2013) indicated that dietary mushroom BG mixture in a supplementation rate of 0.1% and 0.2% potentiate the immunity and increased the resistance of orange-spotted grouper against *V. alginolyticus* infection with increased SR% up to 16%. Interestingly, it was found that dietary levan produced by *B. licheniformis* (at 25 g/kg diet) significantly enhanced growth, decreased the intestinal counts of total viable aerobic bacteria and *Vibrio* spp. and increased the SR % of orange-spotted grouper intraperitoneally injected with *V. harveyi* (Huang et al., 2015).

Ecklonia cava is a brown alga and contains high amounts of polysaccharides that can be isolated from *L. brevis*-fermented *E. cava* (Lee et al., 2013). Importantly, it was found that dietary supplementation with *E. cava* induced significant prebiotic effects in olive flounder (Lee et al., 2016). These authors clarified that the inclusion of 1% fermented *E. cava* in diets of olive flounder for 16 weeks resulted in improvement of the growth performance, non-specific immunity, and resistance against *V. harveyi* infection. Lee et al. (2020) illustrated that dietary BG considerably alleviated the oxytetracycline-induced immune suppression and increased the survivability of hybrid groupers (*E. fuscoguttatus* × *E. lanceolatus*) after experimental challenge with pathogenic *V. alginolyticus*.

From the previously mentioned studies, we can conclude that dietary prebiotics can reduce the mortalities of vibriosis-infected fish because of their potential ability to enhance the fish immune responses, which will subsequently help fish resist the challenge with *Vibrio* spp. The immunomodulatory roles of prebiotics have been extensively reported and discussed by several authors (Akhter et al., 2015; Hoseinifar et al., 2015; Carbone and Faggio, 2016). These products can boost the fish innate immunity by increasing phagocytic cell activation, stimulating the alternative complement system, augmented the lysozyme activities, and interacting with the pattern recognition receptors expressed on the immune cells (Song et al., 2014). Furthermore, they can modulate the transcriptomic profile of treated fish (Dawood et al., 2020b). Although the positive impacts of the prebiotics in increasing the resistance of fish against vibriosis, further research studies should be demonstrated to understand better the actual modes of action of these functional products to enhance fish immunity and resistance against the challenging pathogens.

4.3. *Synbiotic applications*

Synbiotics are firstly defined as a mixture of prebiotics and probiotics that positively affect the overall health status and welfare of the hosts by increasing the survival rates and implant of live microbial supplements in the GIT of the host (Gibson and Roberfroid, 1995). The synergistic combination of synbiotics can be efficiently used in animal feeds (Dawood et al., 2018). The term “synbiotics” also refers to a dietary supplement that contains a mixture of both probiotics and prebiotics and produces synergistic effects (Huynh et al., 2017; Dawood et al., 2020b; Butt et al., 2021). The potential roles of dietary synbiotics on the overall health status and resistance of finfish species to the challenge with *Vibrio* spp. are illustrated in Table 4.

Rodriguez-Estrada and colleagues found that dietary inclusion of a synbiotic mixture of 1% *E. faecalis* (EF), 0.4% MOS, and 1% polyhydroxybutyrate (PHB) significantly improved the growth performance, haemato-immunological responses and decreased the CMR% in rainbow trout after experimental challenge with pathogenic *V. anguillarum* (Rodriguez-Estrada et al., 2009). Those authors also found that the CMR % was 62.5%, 10.4%, and 18.7% in control, EF + MOS, and EF + MOS + PHB groups, respectively.

Another study demonstrated by Ai et al. (2011) showed that dietary supplementation with a synbiotic mixture of *B. subtilis* (BS) (1.35×10^7 CFU/g diet) plus FOS (0.2% or 0.4% of dry weight) significantly improved the growth rates, immunity, and reduced mortalities of large yellow croaker (*Larimichthys crocea*) juveniles after being challenged with *V. harveyi*. In a similar trend, it was found that combined supplementation with BS in a dose rate of 5.62×10^7 CFU/g diet +0.2% FOS significantly enhanced specific growth rate, RBA, ACP and reduced the CMR% of ovate pompano after challenge with *V. vulnificus* (Zhang et al., 2014).

The mixture of BS and chitosan (CH) noticeably enhanced ACP, PA and RBA of cobia (Geng et al., 2011). Those authors also found the lowest mortalities were recorded in cobia previously fed diet supplemented with BS (2.0 g/kg diet) + CH (6.0 g/kg diet) and then experimentally infected with *V. harveyi* (Geng et al., 2011). Moreover, dietary supplementation with a synbiotic mixture of *Weissella cibaria* and 0.5% inulin resulted in a significant decrease of the *Vibrio* spp. count in the intestinal microbial populations of hybrid surubim (*Pseudoplatystoma* sp) (MouriÑO et al., 2012).

Asynbiotic mixture of *B. subtilis* strain WB60 and MOS improved the growth and boosted the immunity of Japanese eel (Lee et al., 2018). After experimental infection with *V. anguillarum*, those authors also found that the highest SR% was noticed in Japanese eels fed diet supplemented with the synbiotic mixture (Lee et al., 2018). The study by Torrecillas et al. (2018) illustrated that the dietary mixture of MOS (0.3% or 0.6%) and *Pediococcus acidilactici* successfully displayed as a functional synbiotic preparation in enhancing the overall health status of European seabass. Those authors also indicated that this synbiotic mixture enhanced the resistance of seabass against challenge with *V. anguillarum*.

Therefore, from the studies mentioned above, we can conclude that the use of synbiotics in aquaculture resulted in greater benefits than probiotics and prebiotics alone in terms of the growth, non-specific immunity, and resistance against the challenge with *Vibrio* spp. The proposed mechanisms of action of synbiotics in improving the growth, immunity, disease resistance, and overall health status of fish have been previously declared (Huynh et al., 2017). Those authors illustrated that synbiotics could promote fish growth by releasing extracellular bacterial enzymes and bioactive products from synbiotic metabolic processes, which will, in turn, activate precursors of the host digestive enzymes and thus can augment the absorption of nutrients, which positively contributes to the better feed utilization. Those authors also illustrated that synbiotics stimulate the intestinal epithelial cells to secrete cytokines which modulate immune cells such as T cells and B cells, and increase the ability of lipopolysaccharides to trigger the transcription of tumor necrosis factor-alpha and Toll-like receptor 2 genes leading to increased respiratory burst activity, phagocytosis, and nitric oxide production (Huynh et al., 2017).

The beneficial impacts of synbiotics may be of great interest in the aquaculture industry and fish farming activities. Nevertheless, several factors should be considered when selecting a “synbiont” such as the interactive effects between the probiotic strain and prebiotic substance present in the synbiotic product. Synergism or synergistic interaction is significantly required to establish the use of synbiont in aquaculture. Further research studies should be accomplished to determine the proper relationship and mechanisms of synbiotics on the immunity and disease resistance of fish against the challenge with pathogenic microorganisms.

5. Concluding remarks and future perspectives

Vibriosis is a dangerous bacterial disease that attacks a wide range of fish species especially the immunosuppressed one, resulting in high mortality rates. It endangers the fresh and marine fish industry with expected high economic loss. Besides the traditional remedies (e.g., vaccines and antibiotics), probiotics, prebiotics, and synbiotics are illustrated as practical solutions to combate vibriosis in several fish species. Probiotics, prebiotics and synbiotics can be applied orally as feed additives or in the water depending on the farming conditions, fish species, size, type, and concentration of additives. This review presents more knowledge about the current research about the roles of probiotics, prebiotics, and synbiotics against vibriosis to update academic researchers and fish farmers with a comprehensive guide. Future studies are encouraged to develop novel probiotics for practical use in fish farming. Alongside, high purity prebiotic additives should be developed for efficient usage in aquaculture. It is also necessary to evaluate more synbiotic mixtures that combine probiotics and prebiotics properties to activate fish immunity and combat vibriosis. The feasibility of using probiotics, prebiotics, and synbiotics has to be considered to ensure a profitable and sustainable aquaculture industry.

From a research point of view, the influential roles of probiotics, prebiotics, and synbiotics against vibriosis have to be evaluated using more advanced techniques to build up a transparent mode of action about the actual function of these additives. Furthermore, other functional bioactive alternatives, such as paraprobiotics (non-biological parts of the probiotics), herbal extracts, algae, and plant-based diets with potential prebiotic characteristics are highly recommended because the natural products can yield similar profitable and beneficial outcomes.

Further investigations on probiotics, prebiotics, and synbiotics will produce new findings and several beneficial effects to increase their use in the field of aquaculture. Additional research studies should be done to evaluate the factors that may affect the use of these beneficial products in aquatic organisms such as the feeding habits and living environmental conditions (e.g., temperature). Supplementary research experiments should be done also to better understand their mechanisms of action inside the host fish. Moreover, the application of probiotics, prebiotics, and synbiotics in aquaculture in a large-scale will also increase the necessity to be manufactured under suitable industrial circumstances that strictly follow the quality control measures. These works will be helpful to produce safe and organic fish products that will be free from antibiotic residues and therefore, ensure the health status of human beings.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Review

Silver nanoparticles: Synthesis, medical applications and biosafety

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Abstract

Silver nanoparticles (AgNPs) have been one of the most attractive nanomaterials in biomedicine due to their unique physicochemical properties. In this paper, we review the state-of-the-art advances of AgNPs in the synthesis methods, medical applications and biosafety of AgNPs. The synthesis methods of AgNPs include physical, chemical and biological routes. AgNPs are mainly used for antimicrobial and anticancer therapy, and also applied in the promotion of wound repair and bone healing, or as the vaccine adjuvant, anti-diabetic agent and biosensors. This review also summarizes the biological action mechanisms of AgNPs, which mainly involve the release of silver ions (Ag^+), generation of reactive oxygen species (ROS), destruction of membrane structure. Despite these therapeutic benefits, their biological safety problems such as potential toxicity on cells, tissue, and organs should be paid enough attention. Besides, we briefly introduce a new type of Ag particles smaller than AgNPs, silver Ångstrom (Å, 1 Å = 0.1 nm) particles (AgÅPs), which exhibit better biological activity and lower toxicity compared with AgNPs. Finally, we conclude the current challenges and point out the future development direction of AgNPs.

Key words: Silver nanoparticles; Silver Ångstrom particles; Synthesis; Antimicrobial; Anticancer; Toxicity; Mechanisms

Introduction

Silver and its compounds have been used for antibacterial and therapeutic applications for thousands of years [1, 2]. Ancient Greeks and Romans used silverwares to store water, food, and wine to avoid spoilage. Hippocrates used silver preparations to treat ulcers and promote wound healing. Silver nitrate was also used for wound care and instrument disinfection. In 1852, Sims sutured the vesicovaginal fistulas caused by delivery with fine silver wires which significantly decreased infection. At the beginning of the 19th century, silver preparations were developed for wound infection and burn care. However, in the 1940s, the medical applications of silver gave way to the clinical introduction of antibiotics [1]. With the abuse of antibiotics, bacterial resistance has become a worldwide problem especially since the 1980s, and silver began to receive attention again especially with the development of nanotechnology in the early of this century.

Nanomaterials (1–100 nm materials) have been attracting much attention in the past few decades in many fields such as biomedicine, catalysis, energy storage, and sensors, due to their unique physicochemical properties as compared to their bulk forms. Silver nanoparticles (AgNPs) have received special interest, especially in biomedicine. AgNPs are famous for their broad-spectrum and highly efficient antimicrobial and anticancer activities. Other biological activities of AgNPs have been also explored, including promoting bone healing and wound repair, enhancing the immunogenicity of vaccines [3], and anti-diabetic effects [4]. Deciphering the biological mechanisms and potential cytotoxicity of AgNPs will facilitate their better medical applications.

Herein, we review the achievements of AgNPs in the past decade, especially focused on the past five years. This review intends to provide a valuable reference for researchers who are interested in the biomedical applications of AgNPs. The main contents include:

- Synthesis of AgNPs, including physical, chemical and biological synthesis methods;
- Medical applications of AgNPs, focusing on antimicrobial and anticancer properties and potential mechanisms, as well as other medical applications, including wound repair, bone healing, dental applications, vaccine adjuvant, antidiabetic agent, and biosensing;
- The potential toxicity of AgNPs, including potential damages of AgNPs to many systems and organs *in vivo*, including skin, eyes, respiratory system, hepatobiliary system, central nervous system, urinary system, immune system and reproductive system.

Numerous studies focus on the synthesis of AgNPs with controlled size and shape, and a variety of specific synthetic methods have been developed, including physical, chemical, and biological methods [5]. The predominant processes of the physical methods are classified into two parts: mechanical and vapor-based processes [6]. Conventional physical methods may involve mill, pyrolysis, and spark discharging [7]. Physical synthesis can obtain AgNPs with uniform size distribution and high purity [8]. Chemical synthesis is the most commonly used method to obtain AgNPs [8]. This method involves reducing silver ions to silver atoms [9], and the process can be divided into two steps, nucleation and growth [10]. Size- and shape-controlled AgNPs can be obtained by regulating the growth rate of nucleation. Besides reducing agents, capping agents and stabilizers also play important roles in obtaining AgNPs with good dispersion stability and uniform size distribution [11]. In addition, external energy can synergistically synthesize AgNPs, such as microwave, light, heat, and sound [12-15]. Although chemical synthesis methods of AgNPs are widely used, the toxicity and pollution caused by chemicals must be highlighted and more attention should be given. Compared with physical and chemical methods, the biological method proves an economical and environmental approach for AgNPs [8]. Microorganisms include bacteria, fungi, and algae, as well as plant parts, include bark, peel, callus, leaves, flower, fruit, stem, seed, and rhizome are widely used in biological synthesis [16]. The organics, like enzymes, alkaloids, phenolic compounds and terpenoids, are abundant in extracts of microorganisms and plants, which can be available to reduce silver salts [16, 17]. Furthermore, some organic substances among these can also be used as stabilizers and capping agents [17]. Among the different methods, the additives mentioned may influence the subsequent medical applications of AgNPs.

AgNPs are recognized for wide-spectrum and high antimicrobial activity, they can effectively kill a variety of pathogens even at very low concentrations [18], including (i) bacteria, such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*; (ii) fungi, such as *Candida albicans*, *Aspergillus niger*; (iii) virus, such as Hepatitis B virus (HBV) and human immunodeficiency virus (HIV). Besides, some studies have shown that AgNPs have nematicidal and anthelmintic activity. The mainstream recognition of the antimicrobial mechanisms of AgNPs includes destructing bacterial cell walls, producing reactive oxygen species (ROS) and damaging DNA structure [18, 19]. Unlike the risk of antibiotic resistance which may limit medical applications, rare AgNPs resistance of bacteria is observed [20]. This may be attributed to the simultaneous multiple antibacterial mechanisms of AgNPs.

In recent years, the anticancer effect of AgNPs has been widely studied. AgNPs play an efficient role against a variety of cancer both *in vitro* and *in vivo*, including cervical cancer, breast cancer, lung cancer, hepatocellular carcinoma, nasopharyngeal carcinoma, hepatocellular carcinoma, glioblastoma, colorectal adenocarcinoma, and prostate carcinoma [21-23]. The anticancer activity of AgNPs is affected by inherent properties, including size, shape and surface charge [24-26]. Generally speaking, the smaller the particle size, the higher the biological activity. To obtain an ideal anticancer agent with high biological activity, our team successfully synthesized a kind of very small silver particles which reached up to Ångstrom (Å; one-tenth of a nanometer) scale and determined the stronger anticancer activities of silver Ångstrom particles (AgÅPs) compared with AgNPs [21]. In addition, exposure time and dose are also considered as crucial factors. Longer exposure time and higher dosage will trigger stronger anticancer effects. Some possible mechanisms involving the anticancer effects of AgNPs have been proposed. AgNPs can cause apoptosis or necrosis by destroying the ultrastructure of cancer cells, inducing ROS production and DNA damage, inactivating enzymes, as well as regulating signaling pathways [27-29]. In addition, AgNPs can also block the invasion and metastasis of tumor cells by inhibiting angiogenesis [30-32]. Due to the enhanced permeability and retention (EPR) effect, tumor cells preferentially absorb NPs-sized bodies than normal tissues [33, 34]. While the poor lymphatic drainage in the tumor enables nanoparticles to stay and penetrate [35]. This may enhance the targeted drug delivery of AgNPs. Further studies of anticancer mechanisms of AgNPs are essential to develop economical, reliable, and broad-spectrum anticancer agents.

Besides the most studied antimicrobial and anticancer activities, AgNPs have also received attention in other cutting-edge medical applications, including wound repair, bone healing, dental material filling, vaccine adjuvants, antidiabetic agents, and bioimaging. In this review, we will also briefly introduce these biomedical applications.

Considering various products containing AgNPs, such as dressings, creams, solvents, and scaffolds, it seems necessary to assess the potential toxicity of AgNPs in cells, tissues, and organs. Generally speaking, primary exposure routes include skin contact, inhalation, ingestion, and injection [36-38]. These routes will distribute AgNPs to different tissues and organs, such as skin, respiratory, circulatory, nervous, hepatobiliary, urinary and reproductive systems [36-41]. The deposited AgNPs may be potentially toxic to these tissues or organs by inducing cell necrosis, apoptosis or genetic mutations [42-45]. For example, AgNPs deposited in the lungs can cause pneumonia and asthma [46]. AgNPs may cross the blood testis barrier (BTB) and reduce the fertility of model animals and cause teratogenicity in offspring [42]. Some toxicological studies on model animals have shown that the potential toxicity of AgNPs is related to the inherent properties [25, 47]. Large surface area may lead to increased silver ions (Ag^+) released from AgNPs, which may enhance the toxicity of nanoparticles. Besides the unique properties, the potential toxicity of AgNPs is closely related to dose, concentration and exposure time [24, 48-50]. Exploring the pharmacodynamics of AgNPs *in vivo* may contribute to the development of bio-friendly and safe agents.

In recent years, a considerable amount of researches involving AgNPs prove enough evidence of promising medical applications of silver nanomaterials. However, the potential toxicities of AgNPs to mammals *in vivo* and cell lines *in vitro* alert us to be cautious about its utilization. This reminds us to carry out more researches to obtain safe, bio-friendly agents containing AgNPs. This article provides a review of the applications of AgNPs and potential toxicology from an objective stance with insights toward understanding deep implications for medicine.

Synthesis of AgNPs

The synthesis methods of nanoparticles (NPs) are mainly divided into two processes: top-down and bottom-up (**Figure 1**). The top-down approach refers to the formation of metal NPs from bulk materials using various physical forces to synthesis NPs, such as mechanical energy used in ball milling, crushing and grinding; electrical energy used in the electrical arc-discharge method and laser ablation method; and thermal energy used in vapor condensation method [51]. These approaches can obtain NPs between 10 and 100 nm in size. The top-down approach, mainly the physical method, may acquire pure nanoparticles without chemical additives. NPs synthesized by physical method may exhibit uniform particle size distribution and high purity. Though the physical approach does not contain chemical reagents which may harm human and environment, it brings a great challenge to prevent agglomeration due to absence of stabilizer or capping agents. Furthermore, these methods need complex equipment and external energy in the process. The bottom-up approach involves the construction of complex clusters to obtain NPs from molecular components by employing nucleation and growth processes [51, 52]. The commonly used bottom-up approaches include chemical synthesis and biological synthesis, both can obtain NPs by reducing the precursor salt [52]. The chemical synthesis can be coupled with alternative energies, such as photochemical [53], electrochemical [54], microwave-assisted [55] and sonochemical methods [12]. Though the chemical method is carried out to quickly obtain various shapes of NPs, the use of harmful chemical additives may limit the medical applications of NPs. To overcome the shortcomings of the chemical method, the biological method has been regarded as an alternative option. The biological method usually relies on macromolecular substances in bacteria, fungi, and algae [16], such as exopolysaccharide, cellulose, and enzymes, and organic components in plant extracts such as enzymes, alcohol, flavonoids, alkaloids, quinines, terpenoids, phenolic compounds [16, 56-59]. Biological synthesis is an economical, environmentally friendly, simple and reliable approach, but the components on the surface of nanoparticles must be adequately considered in the application. Based on these two approaches, frequently used methods for synthesizing AgNPs, including physical, chemical and biological methods are discussed herein.

Physical Method

The physical synthesis of AgNPs involves mechanical processes and vapor-based processes. Energies are used to reduce particle size, including mechanical energy (ball milling method) [60], electrical energy (electrical arc-discharge method) [61], light energy (laser ablation method) [62], and thermal energy (physical vapor deposition) [6] (**Table 1**). During the ball milling progress, high-speed collisions between rigid balls, such as ceramics, flint pebbles, and stainless steels, can produce localized high pressures, which grind the metal into very fine powders [60]. The electrical arc-discharge method can obtain NPs via arc discharge device under direct current (DC) power [63]. The device uses the powder reagent layer as the anode and the electrodes are immersed in dielectric liquids such as hydrocarbons, liquid inert gas, and deionized water. Laser ablation method refers to the ablation of a metal plate by a high-power laser, the metal target absorbs the laser beam energy and photoions, followed by nucleation and growth of metal particles during the plasma plume cooling process and eventually synthesize NPs [62, 64]. Sputtering and evaporation are two processes in physical vapor deposition. Sputtering refers to bombarding a target coating material with a high-energy electrical charge to sputter off atom or molecule that can be deposited on the substrate. While evaporation refers to heating the coating material to the boiling point in a vacuum environment and evaporating, and the evaporated material rises in the vacuum chamber and condenses on the substrate. Although physical synthesis can produce AgNPs on a large scale, AgNPs may aggregate and form large-sized particles which will affect subsequent applications. In order to avoid the re-aggregation of AgNPs, some stabilizers are used to obtain stable colloids AgNPs. For example, polyvinyl pyrrolidone (PVP) may be used as both the electrolyte and stabilizer in the synthesis of AgNPs by laser ablation method [65]. Our team prepared Ångstrom silver particles, capped with fructose as stabilizer, can be stable for a long time [21]. In summary, the physical method can quickly produce NPs with uniform size distribution and high purity, but complex equipment and external energy are required.

Ball Milling Method

Mechanical ball milling technique is to put milling balls and metal materials with a specific mass ratio as well as gas (air or inert gas) in a container rotated at a high speed. The milling time, rotating speed and the atmospheric medium in the process of ball milling are playing essential roles in the morphology of metal materials. A suitable milling time is closely related to the production of particles with a satisfactory size. The smaller size of particles, the higher surface energy, therefore particles prefer to aggregate. The temperature of the powder in the ball milling process influences the diffusivity and phase of nanoparticles [60]. Generally speaking, a higher temperature of powder tends to synthesize intermetallic compounds, while lower temperature tends to obtain amorphous and nanocrystalline phases [52].

Electrical Arc-Discharge Method

The electrical arc-discharge apparatus consists of DC power between two silver rods, which are immersed in dielectric liquids [61, 66]. During the process of arc discharge, the silver electrode is etched in the dielectric medium, and the surface of the silver electrode is vaporized because of the high temperature near the electrode. Subsequently, the silver vapor is condensed into AgNPs and suspended in the dielectric liquid. This apparatus can obtain pure AgNPs with a simple and low-cost device.

Table 1. Synthesis of Silver Nanoparticles by Physical Methods

Method	Silver precursor	Stabilizer/Surfactant/Dispersant	Operating conditions	Size (nm)	Shape	Reference
Ball milling method	Silver powder	–	Dry, under protective Ar gas atmosphere, below –160 ± 10°C	4–8	Spherical	[85]
	Silver wire	–	Multi-walled carbon nanotubes-aqueous nanofluids, 15–40°C, DC power	About 100	Spherical	[86]
Electrical arc-discharge method	Silver wire	–	25°C, current, voltage, deionized water	–	–	[66]
	Silver wire	–	DC arc-discharge system, 70°C, stirring	72	Spherical	[63]
	Silver wire	–	DC arc-discharge system, room temp.	19	Cubic	[87]
	Silver wire	–	DC arc-discharge system, deionized water, stirring	20–30	Spherical	[61]
Laser ablation method	Silver plate	–	Laser pulses, organic solvent	4–10	Spherical	[88]
	Silver plate	PVP	Laser pulse, stirring	20–50	Spherical	[65]
	Silver plate	–	Laser pulse, solution of chlorobenzene, stirring	25–40	Spherical	[89]
Physical vapour condensation	Silver wire	Fructose	High voltage power, rapid cooling	19.2±3.8 Ång	Spherical	[21]

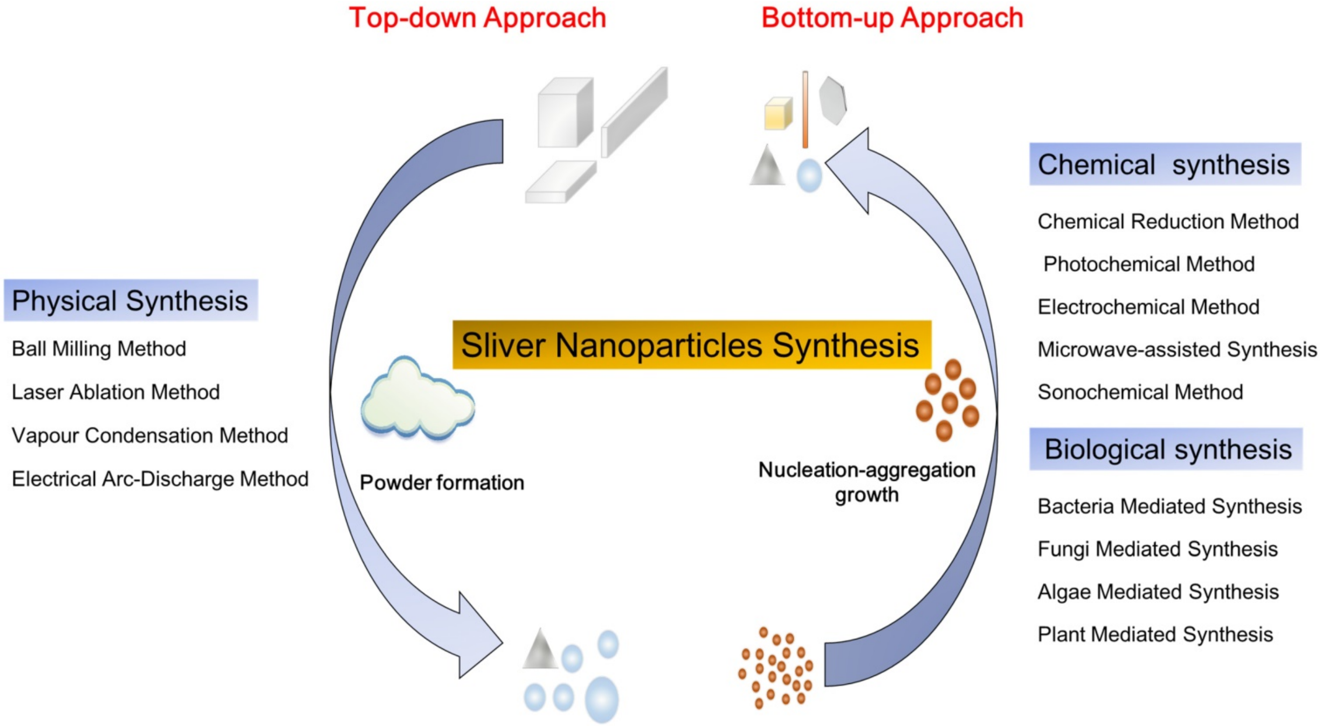

Figure 1. Silver nanoparticles synthesis: top-down approach and bottom-up approach, i.e. physical synthesis method, chemical and biological synthesis methods, separately. The top-down approach refers to the formation of metal nanoparticles from bulk materials, while the bottom-up approach refers to the growth of complex clusters and obtained nanoparticles from molecular components.

Laser Ablation Method

Laser ablation method refers to a pulsed laser instantaneously heat the target bulk metal immersed in water or an organic solvent to form plasma plume, followed by nucleation and growth of metal particles during the plasma plume cooling process and eventually form nanoscale clusters [62, 64]. During the process of laser ablation, nanoparticles can absorb photons through multiple pathways, including plasmon excitations, interband transitions, and multiphoton absorption, which are closely related to pulse time, laser wavelength, and laser fluence. These factors, as well as the type of aqueous medium, may affect the characteristics of NPs [62]. Different synthesis conditions, such as laser fluences, pulse wavelength, as well as solvent type, may affect the size of the NPs. The addition of organic stabilizers such as cetyltrimethylammonium bromide (CTAB) and PVP can enhance the dispersibility of AgNPs [11]. However, it is difficult for laser ablation method to control the size distribution of NPs [62].

Physical Vapor Deposition Method

The basic and most commonly used physical vapor deposition processes are divided into two general categories: arc evaporation and sputtering [67]. The former refers to the utilization of a cathodic arc source in a vacuum chamber or protective gases to obtain metal vapor and deposit it on a target coating material to form a thin, adherent pure metal or alloy coating. During this process, highly ionized metal vapor generates plasma [68]. And the latter refers to using a high-energy electrical charge to bombard the target coating material and deposit metal on the substrate. In this process, ions and energetic atoms impact atoms and mechanically eject them from the target material. Recently, our team successfully synthesized a kind of very small silver particles which reached up to Ångstrom (Ång) scale for the first time with a self-developed evaporation–condensation system [21]. A pure silver wire was fed into an explosion chamber filled with protective gas Argon, following by a high voltage of 25 kV when the wire contacted the positive electrode plate. The silver wire was exploded and evaporated to yield silver vapor plasma. Then the silver vapor was rapidly cooled and coagulated to form Ag particles in the rapid cooling chamber with a water chiller at 0-4 °C. High-intensity ultrasonic and demagnetization devices were used successively to prevent re-agglomeration of Ag particles. In conclusion, the physical vapor deposition method can obtain pure and dispersible AgNPs with small particle size, but complicated devices and external energy are required.

Chemical Method

Chemical synthesis is currently the most common method to synthesize AgNPs (**Table 2**). The process involves the reduction of Ag^+ (supply by silver salt precursor) to elemental silver (AgNPs) through electron transfer under certain conditions [8, 69]. In general, chemical synthesis can be promoted by reducing agents such as sodium borohydride (NaBH4) and sodium citrate (TSC). The chemical method can be combined with external energy sources to prepare AgNPs, such as photochemical, electrochemical, microwave-assisted and sono-chemical methods. Among these methods, the generation process of AgNPs can be divided into two parts: nucleation and growth. The monomer concentration in the solution rapid rises above the critical level of supersaturation, and triggers "burst-nucleation" and precipitation [70, 71]. The precipitation of the monomer forms the nucleus, and the repetitive nucleation process promotes the continuous birth of new seeds. As the seed formation, monomer concentration drops below the critical level of supersaturation. After nucleation, the increased addition of monomer induces the growth of nuclei and forms NPs with a larger size. During the synthesis process, stabilizers such as PVP and CTAB are usually used to stabilize and disperse AgNPs. Even though the chemical method of AgNPs is a reliable, high-yield, time-saving and controllable route, it must be noted that chemicals used in this method may cause environmental pollution.

Chemical Reduction Method

Chemical reduction is a reliable method for preparing colloidal AgNPs in organic solutions or water. AgNPs with desired shapes can be obtained by chemical reduction method, such as nanosphere, nanoprism, nanoplate, nanowire, nanocube, and nanorod. The chemical reduction method includes three components: salt precursor, reducing agent, and stabilizer. Silver precursors can be effectively reduced to AgNPs by different reducing agents with the presence of a stabilizer. There are several alternative silver precursors continuously providing monomers for nucleation, such as silver nitrate [69], silver ammonia (ie. Tollens reagent) [72], silver sulfate [73], and silver chlorate [74]. Frequently used reducing agents may affect the growth of nuclei, including NaBH4, hydrazine, N, N-dimethylformamide, TSC, ascorbic acid, ethylene glycol, polysaccharides, and formaldehyde. The types and ratio of precursors and reducers, as well as the temperature and pH of the solution, may influence the characteristics of AgNPs [75-78]. The nucleation and subsequent growth of the particles in the chemical reduction process can be controlled by alternating the components and adjusting the reaction parameters. For example, Jiang et al. [78] studied the role of temperature in the synthesis of AgNPs by chemical reduction method (**Figure 2**). At the reaction temperature range of 0 to 55 °C, the low temperature significantly slowed down the generation of nuclei and growth, therefore, it took a longer time to complete the reduction of precursors. From 17 to 55 °C, the reaction rate increased with

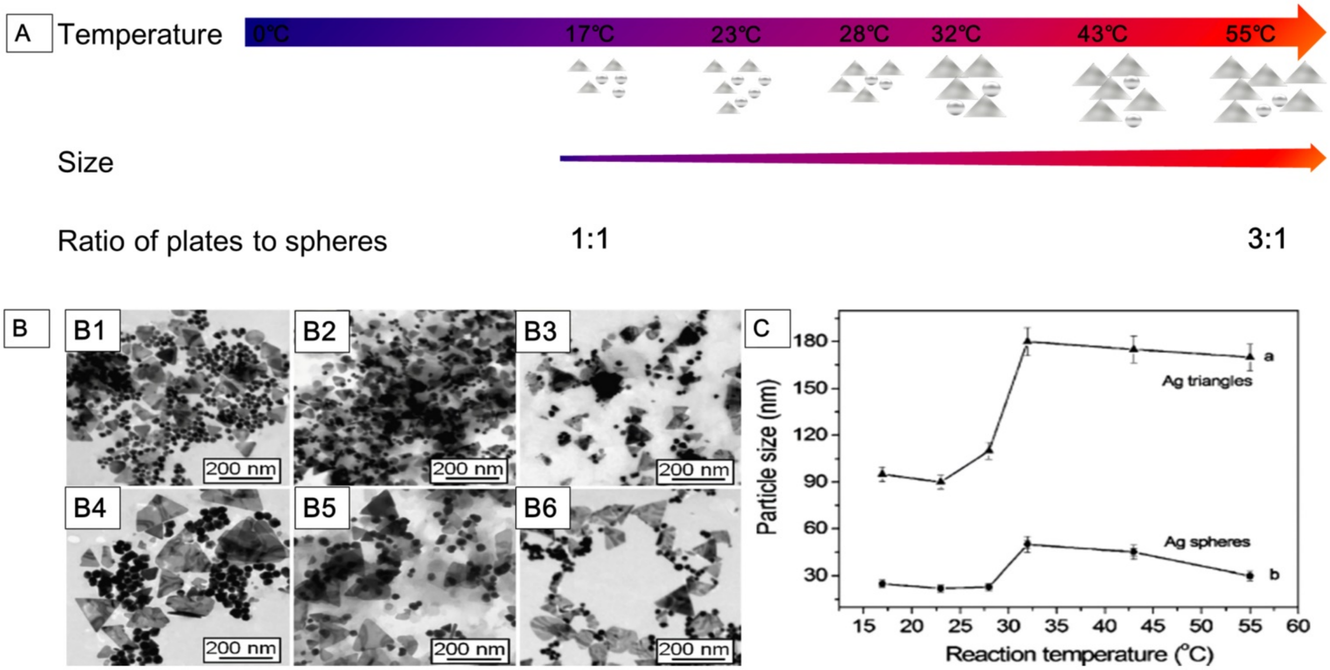

Figure 2. AgNPs synthesized at various temperatures. (**A**) The shape and size of AgNPs obtained in reaction systems at different temperatures ranged from 17 °C to 55 °C. (**B**) Transmission electron microscope (TEM) images of the AgNPs synthesized at different temperatures: (B1) 17 °C; (B2) 23 °C; (B3) 28 °C; (B4) 32 °C; (B5) 43 °C; (B6) 55 °C. (**C**) The average size of AgNPs (curve a: silver nanoplates; curve b: silver nanospheres) synthesized at different temperatures. Adapted with permission from [78], copyright 2011 Nanoscale Research Letters.

rising temperature, as well as the size of nanoparticle (**Figure 2A, 2B**). There was a size jump in the reaction synthesis at around 32 °C, i.e., the size of nanoparticles increased rapidly from around 90 nm to 180 nm for the edge length of plate AgNPs and from around 25 nm to 48 nm for the diameter of spherical AgNPs (**Figure 2C**). The ratio of the plate to spherical nanoparticles might be fundamentally dependent on the amount of single-crystal and twin structures formed at the nucleation process. In this experiment, the amount of spherical AgNPs decreased while the plate ones increased with the temperature rising. The ratio of the plate to spherical AgNPs was 1:1 at 17 °C, while 3:1 at 55 °C (**Figure 2A**).

Photochemical Method

The photochemical method refers to reduce the precursors to AgNPs under the illumination. The silver precursors and solution in the luminescent region produce reduced free radicals and hydrated ions, which can reduce Ag^+ to Ag^0 *in situ* directly. Light sources involving in the photochemical method include ultraviolet light, sunlight, and laser light, among which ultraviolet light is most commonly used. The source, intensity and wavelength of the light, and the irradiation time may affect the synthesis of AgNPs [79]. For example, prolonging the irradiation time and increasing the irradiation intensity during photochemical synthesis may promote the reduction of Ag^+ [79]. The photochemical method has the unique advantage of synthesizing highly dispersible nanoparticles *in situ* in the illumination region. Therefore, the photochemical method can obtain AgNPs on the surface of various media, such as polymeric films, glass, and cells which are illuminated. The photochemical method typically requires relatively simple equipment and can be carried out at room temperature without harmful or strong reducing agents. The reactions can be terminated or attenuated by stopping the illumination.

Table 2. Synthesis of Silver Nanoparticles by Chemical Methods

Method	Silver precursor	Reducing agent	Stabilizer/Surfactant/ Dispersant	Operating conditions	Size (nm)	Shape	Reference
Chemical reduction	Tollens reagent	Triazole sugar	–	Room temp.	9.7 ± 1.9	Spherical	[72]
	$AgNO_3$	Sodium citrate and tannic acid	–	Room temp., 100°C	About 30	Spherical	[90]
	$AgNO_3$	Trisodium citrate/sodium borohydride/ascorbic acid	Sodium borohydride	Heat	–	–	[91]
	$AgNO_3$	Hydrazine hydrate	Sodium dodecyl sulphate	Room temp.	40–60	Spherical	[92]
Photochemical method	$AgNO_3$	Sodium borohydride	Trisodium citrate	Room temp., LED of specific wavelength	40–220	Decahedron, plate, prism	[93]
	$AgNO_3$	NaCl	–	Room temp., UV light, stirring	About 8.6	Spherical	[13]
	$AgNO_3$	Sodium borohydride	Trisodium citrate	Mixed light irradiation, DC power	31.4 ± 1.4	Triangular plate	[53]
	$AgNO_3$	2-hydroxy-2-methylpropiophenone	–	Polychromatic Xe−Hg lamp, stirring	0.74–1.12	Spherical	[79]
	$AgNO_3$	Sodium borohydride, tri-sodium citrate dihydrate	Polyvinylpyrrolidone	LED of different wavelength	4–20	Spherical, rod, polyhedrons	[94]
	$AgNO_3$	Sodium citrate	–	25°C, Hg-halide floodlight	4.92±1.17	–	[95]
Electrochemical method	Silver plates	–	–	Room temp., galvanostatic	20	–	[96]
	Ag electrodes	–	–	20-95°C, 20 V, direct current, stirring	2–20	Spherical	[80]
	Ag electrodes	–	N-vinyl-2-pyrrolidone and sodium lauryl sulfate	Room temp., alternating polarity, 5-10 mA direct current, stirring	10–55	Spherical	[97]
	Silver plate	–	Chitosan	25°C, constant potential, UV irradiation, stirring	2 – 16	Spherical	[98]
	$AgNO_3$	Sodium borohydride	Chitosan	Room temp., voltalab potentiostat/galvanostat	About 50	–	[69]
Microwave-assisted synthesis	$AgNO_3$	Apple extract	–	Microwave, 100 °C	22.05 ± 1.05	Elongated and spherical-like	[99]
Sonochemical method	$AgNO_3$	Glucose	Gelatin	High-intensity ultrasound irradiation, ambient conditions	About 5.3	Spherical	[84]
	$AgNO_3$	–	J-carrageenan	Room temp., ultrasound irradiation	7.07 ± 2.54; 4.08 ± 2.09; 5.01 ± 6.48	Spherical	[12]
	$AgNO_3$	–	Dihydrolipoic acid	Ultrasound irradiation, 50°C	5–10	Nanocluster	[100]
	$AgNO_3$		Dihydrolipoic acid	Ultrasound irradiation, UV, room temp.	2 – 4	Nanocluster	[101]
	$AgNO_3$	Polyacrylic acid	Acrylate	Ultrasound irradiation, 50°C	10–15	Spherical	[102]
	$AgNO_3$	Sodium borohydride	Poly(vinyl alcohol)	Ultrasound irradiation, 60°C	13, 15, 18	Spherical	[103]
	$AgNO_3$		Dihydrolipoic acid	Ultrasound irradiation, room temp.	2–3; 5–10	Nanocluster	[104]

Electrochemical Method

Electrochemical method can form an electric potential in the electrolyte and reduce Ag^+ to Ag^0 [80]. The nucleation and growth of AgNPs occur almost simultaneously under the external electric field. Electrochemical method can synthesize AgNPs with different sizes by adjusting the current density. Besides, electrode types, electrolytes, and solvents are also important in the synthesis of size-controlled AgNPs. In the synthesis process, increased precursor concentration and enhanced current intensity, as well as prolonged implementation time, will obtain more AgNPs with smaller size [81]. To obtain dispersed and stable AgNPs, stabilizers and capping agents are optional additives. The steric hindrance formed by these additives will prevent the aggregation of AgNPs. The electrochemical method has the advantages of easy reaction control, mild reaction conditions, and less environmental pollution.

Microwave-assisted Method

Microwave-assisted method refers to rapid heating the silver precursor by microwave irradiation, which may promote the generation of nuclei *in site* [82]. Several factors may influence the microwave-assisted synthesis of AgNPs, including the concentration of precursor and the type of stabilizer, power input and irradiation time of the microwave, dielectric constant, refractive index of the medium and chirality of reducing agents [55]. Water and alcohol are ideal media for microwave heating stabilizer because of their high dielectric losses [83]. For example, polar molecules such as H_2O attempt to orient the electric field in the microwave. When dipolar molecules attempt to reorient relative to an alternating electric field, they lose energy in the form of heat which may contribute to the reduction of Ag^+. Microwave-assisted method has the advantages of high energy conversion efficiency, time-saving, cleanliness, and convenience, most importantly, it can be used to obtain large-scale production of high dispersive AgNPs.

Sonochemical Method

Sonochemical method refers to the cavitation effect generated by ultrasonic irradiation, which produces a local hot spot and promotes the synthesis of AgNPs [84]. The instantaneous high pressure and microjet generated by ultrasonic irradiation can uniformly mix the solution and generate bubbles, which may suddenly collapse when the bubbles grow. The adiabatic compression of the gas phase in the bubble creates a local hot spot, which accelerates the contact of Ag^+ with the reducing agent and rapidly reduces it to AgNPs. Ultrasound prevents the agglomeration of nanoparticles in the aqueous solution to decrease the size of AgNPs. Besides the high temperature, other factors such as pressure, pH, high-speed microjet, and high cooling rate may also contribute to the synthesis process. In summary, the sonochemical method is a simple, economical, and environment-friendly technique for preparing colloidal silver nanoparticles.

Biological Method

In recent decades, a variety of microorganism- and plant-mediated biological syntheses of AgNPs are developed. The microorganisms can evolve metal tolerance genes and metal bioconcentration capability to survive in an extreme silver-rich environment [105, 106]. These adaptive evolutionary mechanisms include altering and decreasing the cytotoxicity of metal and resulting formation of AgNPs. AgNPs can be regarded as "by-product" of the resistance mechanism of microorganisms against free Ag^+. Plant mediated synthesis can reduce Ag^+ to Ag^0 using functional groups such as O-H and =C-H in organic components and their derivatives contained in the extract of plant parts [107]. Commonly used plant parts include bark, peel, callus, leaves, flower, fruit, stem, seed, and rhizome. In the process of biosynthesis, various biological components act as reducing agents, such as exopolysaccharide, peptides, nitrate reductase, reducing cofactors, c-type cytochromes, separated from microorganisms, and starch, cellulose, chitin, dextran, alginates, separated from plants. However, the organic components in the biosynthesis of AgNPs require to be further studied due to their complex interaction with AgNPs and the diversity of plants. Compared with physical or chemical methods, biological method can be carried out at normal temperature and pressure and avoid the use of toxic or hazardous additives. In this part, we will introduce several microbial and plant synthesis approaches of AgNPs, as well as the mechanisms involved in these processes.

Bacteria-Mediated Synthesis

Since Tanja Klaus et al. firstly reported the phenomenon of aggregation of AgNPs in *Pseudomonas stutzeri* AG259 in 1999 [105], series of bacteria, both Gram-negative and Gram-positive, are been screened for the synthesis of AgNPs (**Table 3**). The property of bacteria to survive in an extreme silver-rich environment might contribute to the accumulation of AgNPs [105, 108]. Depending upon the location of the nanoparticles distribution, AgNPs may be synthesized intracellularly or extracellularly using biomass, supernatant, cell-free extracts, and derived components of the bacteria. Among these two modes, extracellular method is advantageous over intracellular method due to the convenience of recovery of AgNPs. The abilities and mechanisms of strains used in the biosynthesis of AgNPs are different from each other due to the organic substances. Various organic substances in bacteria can be used as reducing agents, such as exopolysaccharide, peptides, reductase, cofactors, c-type cytochromes, and silver-resistant genes. Among these, several enzymes have been involved in synthesizing AgNPs, such as nitrate reductase and lactate dehydrogenase; and peptides with special amino acid, such as methionine, cysteine, lysine, and arginine, may attach on the surface of nuclei and act as reducing agents [109]. Nitrate reductase, a kind of NADH-dependent enzymes, has gained more attention in the bacteria-mediated synthesis of AgNPs. Nitrate reductase can participate in the electron transport chain, and subsequently creates a miniature reducing environment by transferring hydrogen atoms. The enzyme gains electron from NADH, oxidizes it to NAD^+, and undergoes oxidation to reduce silver ions to AgNPs [18, 109]. Some organic substances can also act as stabilizers and capping agents for AgNPs to prevent particle aggregation [18, 110]. The mechanisms of bacteria-mediated synthesis of AgNPs still need to be further explored. In conclusion, bacterial-mediated synthesis of AgNPs is a simple, effective, and environmentally friendly method.

Fungi-Mediated Synthesis

Fungi-mediated synthesis of AgNPs is an effective and straightforward approach [111, 112]. According to the location of nanoparticles, fungi-mediated synthesis can obtain intracellular and extracellular AgNPs using mycelia and fungal cell-free filtrate, respectively [113, 114] (**Table 3**). Compared with intracellular synthesis, the extracellular synthesis of AgNPs using fungi is preferred due to the advantages of convenient collection and downstream processing. Plenty of fungi, due to their unique abilities of metal bioconcentration, high tolerance in the metal-rich environment, rapid mycelial growth, various extracellular enzymes secretion, and economic viability, are selected for biosynthesis of AgNPs [115], such as *Fusarium oxysporum* [116], *Trichoderma harzianum* [57], *Penicillium polonicum* [117], *Phomopsis liquidambaris* [118]. However, some fungi, such as *F. oxysporum* [111], are recognized to be potentially pathogenic, which may result in health risk in subsequent applications. While the AgNPs synthesized by extracellular method using the fungal extract can be purified by washing or precipitating unnecessary fungal components. Various organic components of fungi play an important role in the synthesis of AgNPs, such as nitrate-dependent reductase, xylanases [119], naphthoquinones and anthraquinones, and quinine derivates of the latter two, are involved in the reduction of silver precursor. In addition, some proteins secreted by fungi can be used as capping agents to form shape-controlled AgNPs [120]. Various incubation conditions might influence the characteristics of AgNPs, such as the types of carbon and nitrogen sources, temperature and light source [56]**.** In conclusion, fungi mediated synthesis of AgNPs is a convenient, effective, low-cost and energy-saving biological method. However, reducing potential pathogens on the surface of AgNPs should be considered to obtain safe products.

Algae-Mediated Synthesis

Algae, as one of the most potential coastal renewable living resources, have received more attention in the biosynthesis of nanometer materials in recent years (**Table 3**). Algae contain a variety of biologically active organic matters, such as carbohydrates, polysaccharides, enzymes, proteins, vitamins, pigments and secondary metabolites [17, 121, 122]. These abundant organic compounds make algae an ideal candidate for biosynthesis of AgNPs. These active organic matters may be used as reducing agents to form size- and shape-controlled AgNPs, including spheres, triangles, cubes, rods, wires, hexagons, pentagons and wires. The roles of many algae in biosynthesis of AgNPs are verified, including *Cyanophyceae*, *Chlorophyceae*, *Phaeophyceae*, *Rhodophyceae* [123]. These studies support algae as a promising bioresource for the synthesis of AgNPs with various shapes and sizes. Biomolecules in algae extracts, such as amino acids, proteins and sulfated polysaccharides, can also act as stabilizers or capping agents in the biosynthesis of AgNPs with variable properties [124]. The specific factors involved in the algae-mediated synthesis of AgNPs are necessary to be identified and determined, including the ratio of silver precursor to algae extract, mixture pH, incubation time and temperature [125]. In conclusion, the biosynthesis of AgNPs using algae extract provides a facile, sustainable and eco-friendly method. Various algae can be considered as candidates in the biosynthesis of AgNPs due to their unique properties of rapid growth, high metal accumulation ability and abundant organic content.

Plant-Mediated Synthesis

Plant-mediated synthesis of AgNPs, as a promising approach, has received great attention in recent years. Extracts from different parts of the plants, including bark, peel, callus, leaves, flower, fruit, stem, seed and rhizome, are involved in biosynthesis of AgNPs with various sizes and shapes [59] (**Table 4**). These extracts from different plant parts contain organic components such as enzymes, alcohols, flavonoids, alkaloids, quinines, oils, terpenoids and phenolic compounds [126, 127]. There are different functional groups in these organic components [58], like hydroxyl, carbonyl, amidogen, which may contribute to the reduction of Ag^+ to Ag^0. A variety of plant extracts, including the components mentioned above and plant derivatives such as starch, cellulose, chitin, dextran and alginates, act simultaneously as reducing agents and stabilizers [128]. The plant-mediated synthesis of AgNPs is influenced by different reaction parameters such as temperature, reaction time, pH and concentration of plant extracts and precursors [129, 130]. The AgNPs with different size and shape can be obtained by changing the synthesis parameters [129]. In summary, plant-mediated synthesis of AgNPs can be controlled by a variety of reaction conditions. In addition, different parts of plant exhibit various abilities in the synthesis of AgNPs [131]. The mechanisms of plant-mediated synthesis of AgNPs need more exploration. In conclusion, the plant-mediated synthesis of AgNPs using plant extract is a promising method due to its easy availability, nontoxicity, simplicity, cost-effectiveness and high reducing potential.

Medical Applications of AgNPs

Antimicrobial and anticancer properties of AgNPs have been widely studied. Studies have shown that AgNPs have broad-spectrum antimicrobial properties against pathogens including bacteria, fungi and viruses [19, 49]. Besides, AgNPs can effectively damage or kill nematodes [152] and worms [153]. A variety of factors affect the antimicrobial activities of AgNPs, including size, shape, dose and stabilizer of AgNPs [49, 154, 155]. Interestingly, AgNPs may have different antibacterial effects against Gram-positive and Gram-negative bacteria [156]. AgNPs exhibit broad-spectrum anticancer properties. Anticancer activity of AgNPs is also affected by a variety of factors, including size, shape, dose, and exposure time [22, 157, 158]. It is also realized that the surface charge of AgNPs is a potential factor. Although current specific mechanisms of antimicrobial and anticancer properties of AgNPs are still unclear, many studies have carried out hypothesis. AgNPs can inhibit the growth of bacteria or kill them by inducing membrane destruction, ROS generation, DNA damage, enzyme inactivation and protein denaturation [4, 56, 159]. However, the anticancer mechanisms of AgNPs are much more complicated. Until now, it has been approved that AgNPs can inhibit the growth of tumor cells by destroying the cellular ultrastructures, inducing ROS production and DNA damage [21-23, 160]. In addition, AgNPs can induce tumor cell apoptosis through inactivating proteins and regulating signaling pathways, or blocking tumor cell metastasis by inhibiting angiogenesis within lesion [31, 161]. Besides antimicrobial and anticancer properties, AgNPs can also be used in other medical applications, such as bone repair [162] and wounding repair [163]. And AgNPs can be regarded as an additive in dental materials or an adjuvant in vaccine. In this part, we will discuss the antimicrobial and anticancer properties and possible mechanisms of AgNPs, as well as other promising medical applications.

Antimicrobial Application of AgNPs

Antibacterial Properties of AgNPs

AgNPs have been proven to effectively inhibit various pathogenic bacteria, fungi and viruses, including *Staphylococcus aureus* [164], *Escherichia coli* [165], *Pseudomonas aeruginosa* [166], *dermatophyte* [167], HIV-1, etc. [168, 169]. The antibacterial effect of AgNPs against various strains of bacteria is different [156]. Rather than Gram-positive bacteria, AgNPs show a stronger effect on the Gram-negative ones. This may be due to the different thickness of cell wall between two kinds of bacteria [170]. Besides the bacteria strains, AgNPs may also exhibit size-, shape-, concentration-, time-, and charge-dependent antibacterial activity. In general, as particle size decreases, the antibacterial effect of AgNPs increases significantly [171]. Especially when the size is less than 10 nm, AgNPs show better antibacterial activity [172]. The antibacterial effect can be significantly enhanced by prolonging the treatment time of AgNPs [173]. The increased bacterial mortality may be ascribed to the accumulation of AgNPs and silver ions during the exposure period. Besides, the shape of AgNPs may also influence the antibacterial activity [171, 174]. By comparing the antibacterial activity of spherical, triangular, linear and cubic AgNPs, it is observed that spherical shaped AgNPs exhibit superior antibacterial effect. This phenomenon suggests that AgNPs with larger surface to volume ratio, which relates to both higher effective contact and larger reaction surface, may show stronger antibacterial activity [174]. In addition, the antimicrobial activity of AgNPs is also affected by the surface charge [156, 175]. Due to the presence of lipopolysaccharide, peptidoglycan and multiple groups, including carboxyl, amino and phosphate groups, bacterial membranes are primarily loaded with negative charges [170, 176]. Positive charge can facilitate the adherence of AgNPs on bacterial membranes through electrostatic attraction [156]. Therefore, adjusting the surface charges of AgNPs may contribute to the enhanced antibacterial effect [175]. The stabilizers may influence the size, dispersion, and surface charge of AgNPs, which may involve in the antibacterial effect of AgNPs [154, 177]. Some stabilizers, such as citrates, PVP [154] and polyvinylalcohol [177], have been shown to influence the bacterial effect by adjusting the characteristics of AgNPs.

Although AgNPs exhibit good antibacterial activity, the specific mechanisms have not been completely clarified. Many hypotheses have been proposed to explain the antibacterial mechanisms of AgNPs, including i) Destructing the bacterial membrane and leaking cellular contents; ii) Generating ROS and disabling the respiratory chains; iii) Destructing the DNA structure and blocking the DNA replication; iv) Inactivating enzymes and denaturing proteins. Due to these mechanisms, AgNPs exhibit broad-spectrum and effective antibacterial properties. These make AgNPs an alternative for the implementation of novel biomedical strategies, such as catheter modification, dental application, wound healing and bone healing.

Antifungal and Antiviral Activities of AgNPs

Some studies confirm that AgNPs exhibit good antifungal properties against *Colletotrichum coccodes*, *Monilinia sp.* [178], *Candida spp.* [179] and various plant pathogenic fungi in size- and dose-dependent manners [180]. Some studies also point out that the type of culture media used in their experiments may also affect the inhibition activity [180]. Besides, AgNPs also show good antiviral activity against hepatitis B virus (HBV) [181], human parainfluenza virus (HPIV) [182], herpes simplex virus (HSV) [183] and influenza A (H1N1) virus [184]. AgNPs with less than 10 nm size exhibit good antiviral activity [185, 186], which may be due to their large reaction area and strong adhesion to the virus surface.

Table 3. Bacteria-, Fungi-, Algae-mediated Synthesis of Silver Nanoparticles

Bacteria/Fungi/Algae	Position	Precursor	Responsible organic components/functional groups	Operating condition	Size (nm)	Shape	Reference
Streptomyces violaceus	Extracellular	$AgNO_3$	Exopolysaccharide	37°C; shaking; pH 7.0;	10–60	Cubic; crystalline; spherical	[132]
Penicillium polonicum	Extracellular	$AgNO_3$	Proteins	Room temp.; shaking; light	10–15	Spherical; near spherical	[133]
Falcaria vulgaris	Extracellular	$AgNO_3$	Hydroxyl group	50°C	10–30	Spherical	[134]
Pseudomonas	Extracellular	$AgNO_3$	Aromatic and aliphatic amines	28°C; shaking	10–40	Irregular	[135]
Pantoea ananatis	Extracellular	$AgNO_3$	Proteins or amino acids	37°C; shaking	8–90	Spherical	[136]
Fusarium oxysporum	Extracellular	$AgNO_3$	Proteins	28°C; shaking	21.3–37.3	Spherical; oval	[111]
Botryosphaeria rhodina	Extracellular	$AgNO_3$	NADH-dependent nitrate reductase	Room temp.; dark	below 20	Spherical	[137]
Monascus	Extracellular	$AgNO_3$	Lactone ring	28–30°C; shaking	10–30; 15–40	Spherical	[138]
Aspergillus tamarii	Extracellular	$AgNO_3$	NADH-dependent nitrate reductase	25±2°C; shaking	3.5 ± 3	Spherical	[120]
Nostoc linckia	Extracellular	$AgNO_3$	Phycocyanin	Room temp.; pH 10.0	9.39–25.89	Spherical	[139]
Caulerpa serrulata	Extracellular	$AgNO_3$	Caulerpenyne; caulerpin	27–95°C; pH 4.1–9.5	10 ± 2	Crystalline; spherical	[125]
Laurencia aldingensis	Extracellular	$AgNO_3$	Proteins	Dark; shaking	5–10	Spherical	[140]

Table 4. Plant-mediated Synthesis of Silver Nanoparticles

Plant	Plant part	Precursor	Responsible phytoconstituent	Operating condition	Size (nm)	Shape	Reference
Coptis chinensis	Leaf	$AgNO_3$	–	Room temp.; dark	6–45	Spherical	[23]
Phyllanthus pinnatus	Stem	$AgNO_3$	Phytochemicals	Room temp.; sterility	below 100	Cubical	[141]
Parkia speciosa	Leaf	$AgNO_3$	Polyphenols	60°C; pH 11.0	26–39	Spherical	[142]
Plantago major	All	$AgNO_3$	–	85°C; dark	10–20	Spherical	[130]
Avicennia marina	Leaf, stem and root	$AgNO_3$	–	Room temp.; shaking	About 75	Spherical	[131]
Origanum vulgare L.	Aerial part	$AgNO_3$	–	–	2–25	Cubic	[143]
Gossypium hirsutum	Shoot	$AgNO_3$	–	60°C; shaking	20–100	Spherical	[144]
Flacourtia indica	Leaf	$AgNO_3$	Phenolic, lignin and sterols	50°C	14–24	Spheroid	[145]
Walnut	Fruit	$AgNO_3$	–	37–40°C; shaking; dark	About 31.4	Spherical	[128]
Cleome viscosa L.	Fruit	$AgNO_3$	–	Room temp.; dark	20–50	Spherical	[146]
Alpinia katsumadai	Seed	$AgNO_3$	Phytochemicals	Room temp.; shaking; dark; pH 10	About 12.6	Quasispherical	[147]
Ocimum Sanctum	Leaf	$AgNO_3$	Quercetin	–	250–600	Spherical	[129]
Mimosa Pudica	Root	$AgNO_3$	–	Room temp.	35–42.5	Spherical	[148]
Aloe vera	Leaf	$AgNO_3$	Lignin, hemicellulose, and pectins	100°C or 200°C; shaking	70.70 ± 22, 192.02 ± 53	Spherical	[149]
Carambola	Fruit	$AgNO_3$	Polysaccharide and ascorbic acid	Stirring at 40°C	10–40	Face-centered-cubic	[150]
Anogeissus latifolia, Boswellia serrata	Gum ghatti, gum olibanum	$AgNO_3$	–	121°C, 15 psi	5.7 ± 0.2; 7.5 ± 3.8	–	[151]

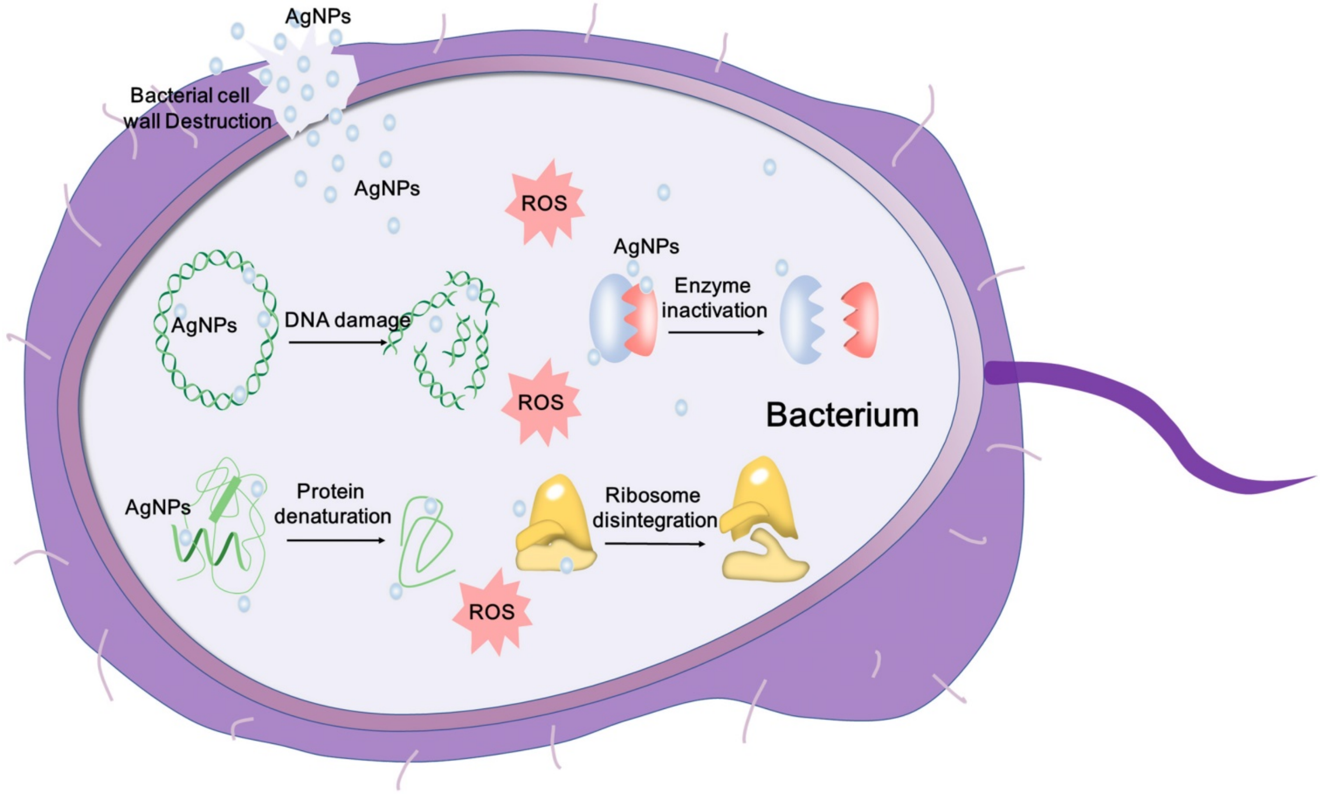

Figure 3. Schematic representation of the mechanisms of AgNPs against bacteria, depicting ROS-dependent pathway, DNA damage, protein denaturation and enzyme inactivation for antibacterial action of AgNPs.

For example, AgNPs can bind to the glycoprotein knobs and inhibit the reverse transcriptase (RT) of HIV-1 and interact with the virus in size- and dose-dependent manner [169, 185]. To develop AgNPs for antimicrobial applications, the detailed mechanism needs to be further studied.

Antimicrobial Mechanisms of AgNPs

The antimicrobial effect of AgNPs has been widely studied, and the mechanisms are being explored. It is observed that AgNPs can anchor and then penetrate the bacterial membrane, and subsequently trigger the destruction of cell membrane and leakage of content [187]. Besides, AgNPs can influence crucial intracellular activities, such as attacking the respiratory chain, disturbing DNA replication and inhibiting cell division [188]. The antibacterial mechanisms of AgNPs are illustrated in **Figure 3**. AgNPs also have a good microbicidal effect in drug-resistant fungi via influencing the cellular targets, which are involved in the drug resistance and pathogenicity. For example, Venkatraman et al. [189] demonstrated that AgNPs could affect drug sensitivities by acting on multiple cellular targets of *Candida albicans*, including fatty acids like oleic acid, which were important in the hyphal morphogenesis involved in the pathogenicity. Some studies speculate that AgNPs can saturate and adhere to the fungal hypha and eventually inactivate the fungus [180]. The antiviral mechanism of AgNPs has also been deeply explored. AgNPs can be used to prevent viral infection against several virus by blocking virus contact with cells and entry steps, or directly inactivating the virus, including herpes simplex virus (HSV), human parainfluenza virus 3, vaccinia virus, chikungunya virus and respiratory syncytial virus [182, 190-192]. These studies indicate that AgNPs can be used as a novel promising virucide agent. In order to develop safe and effective antimicrobial agents, the yet-to-be-determined mechanisms of antimicrobial properties of AgNPs are needed to be further studied.

Nematicidal and Anthelmintic Activity

Worm infection via contact with contaminated soil is one of the most common diseases among children from middle and low-income countries [193]. Worm infections often lead to stunted growth, malnutrition and lagging academic performance [193, 194]. According to recent studies, AgNPs may become a candidate as a novel insecticide. Saha et al. [195] confirmed that AgNPs were effective in killing filaria and larvae. AgNPs induced the cell apoptosis and destroyed parasites mainly through the generation of ROS. It was worth noting that the carbohydrate polymer not only participated in the synthesis of AgNPs, but also enhanced the filaricide activity of AgNPs. This suggested that AgNPs may be a potential preparation for filariasis control. In addition, they also tried to use AgNPs synthesized by *Acacia auriculiformis* to kill filaria, and also achieved impressive results [196]. Tomar et al. [197] realized the biologically synthesized AgNPs might inhibit both egg hatch and adult motility in dose-dependent manner. That was, a higher dose of AgNPs might exhibit better anthelmintic activity. Shabad et al. [198] confirmed the AgNPs synthesized by *Ziziphus jujuba* leaf extract showed ideal ovicidal and anthelmintic activity against *Haemonchus contortus* via nutrient depletion. The combination of AgNPs and organic components separated from plants can produce a synergistic effect which may enhance anthelmintic activity. Mamun et al. [199] speculated that organic substances in *M. charantia* fruit extracts, such as glycosides, alkaloids, reducing sugars and free acids, can help biosynthetic AgNPs to protect against parasitic infections. The phytochemicals might exert effect by adhering to the gastrointestinal tract or parasite cuticles. AgNPs exhibited larvicidal activities against larvae of Anopheles stephensi and Culex quinquefasciatus, thus contributed to the prevention of malaria and filariasis [200]. In conclusion, AgNPs may be used as an effective insecticidal agent to kill eggs, larvae and adult parasites. However, the mechanisms still need to be further explored.

Anticancer Application of AgNPs

Anticancer Properties of AgNPs

Cancer is currently considered an important factor in morbidity and mortality worldwide [201]. About 14 million new cancer cases are predicted by 2035, which will lead to a substantial impact on the economy and society around the world [202]. Therefore, there is an urgent need to develop effective and advanced treatment methods to reduce the adverse effects of cancer incidence. Common treatments of cancer or tumor include surgery, chemotherapy and radiotherapy. However, side effects and limitations of conventional treatments influence the outcomes. For example, standard chemotherapy may cause serious side effects, including local reactions, such as thrombophlebitis and tissue necrosis, and systemic reactions, including myelosuppression, dysfunction of liver and kidney and immunosuppression [203]. In addition, malignant tumors can develop multi-drug resistance (MDR), which may lead to chemotherapy failure [204]. Therefore, it is essential to develop novel drugs to improve the therapeutic effects. In recent years, nanoparticles have attracted more attention in cancer therapeutics due to their special physical and chemical properties, which gives rise to a new field of anticancer − cancer nanomedicine [205, 206]. Compared to traditional anticancer agents, metallic nanoparticles (MNPs) can be used as novel therapeutic agents or drug carriers in combination with drug candidates, and undesirable side-effects can be prevented by providing a targeted approach [207]. Among these nanoparticles, AgNPs represent an ideal one in the search for anticancer or antitumor therapeutic agents [207].

AgNPs have been observed to exhibit good anticancer activities in breast cancer [158], cervical cancer [208], colon cancer [209], ovarian cancer [210], pancreatic ductal adenocarcinoma [211], lung cancer [212], hepatocellular carcinoma [213], melanoma [214], osteosarcoma [215], etc. (**Table 5**). Several studies confirm that the anticancer activities of AgNPs with various sizes, shapes and doses/concentrations are discrepant in different cancer cells [210-212, 215]. In addition, other factors, such as pH of lesions, exposure time, cell lines and tumor microenvironment, also influence the anticancer activity of AgNPs [210, 211, 214]. Generally speaking, AgNPs exhibit wide spectrum anticancer activity in size-, dose-/concentration- and time-dependent manners. AgNPs with smaller size can elicit enhanced endocytosis, and induce more significant cytotoxicity and genotoxicity. Compared with other shapes, spherical AgNPs exhibit better cytotoxicity due to the higher surface-to-volume ratio [216]. And higher dose of AgNPs usually leads to more apoptosis than lower one. In this section, we highlight these factors.

Size- and Shape-dependent Manners

Nanoparticles motility in capillaries, as well as endocytosis and metabolism in tumor cells, are significantly affected by the size of AgNPs [217, 218]. It has been found that the kinetics of uptake, intracellular accumulation and excretion, and the resulting cytotoxicity and genotoxicity, varied with the different sized AgNPs. In general, smaller AgNPs have higher endocytosis and exocytosis efficiency, therefore are supposed to produce greater cytotoxicity than larger particles [49, 217]. To investigate the effect of nanoparticle size on distribution within tumor, Gavin Fullstone et al. [219] simulated the transport of nanoparticles in blood flow using an agent-based approach, testing the ability of 10 nm, 20 nm, 50 nm, 70 nm, 80 nm, 100 nm and 160 nm nanoparticles to traverse fenestrations with pore size of normal blood vessels and tumor-associated blood vessels.

Table 5. Anticancer Mechanisms of Silver Nanoparticles

Cancer cell lines	AgNPs		Concentration, IC_{50}, exposure time	Manners	Mode of action	References
	Synthesis methods	Size; Shape				
HeLa cells	Plant	40 nm; spherical and pentagonal	25, 50, 100, 250 µg/mL; 24 h	Dose-dependent	ROS generation; ultrastructural changes; mitochondrial dysfunction	[229]
HeLa cells	Plant	33 nm; face-centered-cubic	0–100 µg/ml; 24 h	Concentration-dependent	Sub G1 cell cycle arrest; ROS generation; down-regulation of MMP	[261]
HeLa cells	Chemical	20–40 nm; spherical	1.35 µg/mL and 13.5 µg/mL; 24 h and 48 h	Dose-, concentration- and time-dependent	Decreased the number of cells at S and G2/M phase; increased the number of cells at sub-G1 phase	[244]
HeLa cells	Chemical	26.5 ± 8.4 nm; spherical	10, 20, 50 µg/mL; 10 h and 24 h	Dose- and time-dependent	Regulation of PtdIns3K signaling pathway	[22]
A549	Fungi	25 nm; round and triangular	1–10 µg/mL; 48 h	–	ROS generation; nucleus damage	[273]
A549	Plant	17–25.8 nm	25 µg/ml	–	Activation of apoptotic gene; inhibition of cell migration and invasion	[274]
MCF-7	Plant	22 nm; spherical	IC_{50}: 20 µg/ml; 24 h and 48 h	Dose- and time-dependent	ROS generation; DNA damage; disruption of the cell membrane	[275]
MCF-7	Plant	12 nm; different shapes	IC_{50}: 20 µg/mL; 24 h	Dose-dependent	Regulation of Bax and Bcl-2 gene expression	[276]
MCF-7	Peptides	31.61 nm; spherical	IC_{50}: 104.1 µg/mL; 24 h	Dose-dependent	ROS generation; disruption of mitochondrial respiratory chain	[277]
MCF-7, EAC	Algae	7.1–26.68 nm; spherical	IC50: 13.07 ± 1.1 µg/mL; 48h	Dose-dependent	Inhibition of proliferation; mitochondria dysfunction	[278]
A549	Plant	6–45 nm; spherical	10 µg/mL and 25 µg/mL; 24h	Dose-dependent	Inhibition of proliferation, migration and invasion	[23]
MCF-7; MDA-MB-231	Plant	15–30 nm; spherical	IC_{50}: 20 µg/mL (MCF-7), 30 µg/mL (MDA-MB-231)	Dose-dependent	Regulation of p53, Bax and Bcl-2 expressions	[279]
A549	Plant	45.12 nm; spherical	IC_{50}: 62.82 µg/mL (24 h) and 42.44 µg/mL (48 h)	Dose- and time-dependent	S phase cycle arrest; decrease of cell population in sub G1 phase	[280]
A549; Hep G2	Purchased	21 ± 8 and 72 ± 11 nm; spherical	1–20 µg/mL; 48 h	Concentration- and dose-dependent	Inhibition of telomerase activity and telomere dysfunction	[240]
HT29	Plant	9.13 ± 4.86 nm; spherical	IC_{50}: 38.55 µg/mL; 24 h	Dose-dependent	Induction of apoptosis pathway	[281]
HCT116	Bacteria	15 nm; spherical	IC_{50}: 0.069 µg/mL; 24 h	Dose- and time-dependent	Induction of nuclear condensation and fragmentation	[159]
HCT-116	Plant	24–150 nm; spherical, triangular	IC_{50}: 100 µg/ml; 24 h	Dose-dependent	Up-regulated modulators of apoptosis, Caspase-3, Caspase-8 and Caspase-9; mitotic arrest; DNA fragmentation	[282]
PANC-1	Purchased	2.6 and 18 nm; spherical	IC_{50}: 1.67 µg/mL (2.6 nm), and 26.81 µg/mL (18 nm); 1 h, 24 h	Size- and concentration-dependent	Ultrastructural change; regulation of p53, Bax, Bcl-2, RIP-1, RIP-3, MLKL and LC3-II expression,	[211]
SCC-25	Purchased	10 ± 4 nm; spherical, cubic	IC_{50}: 37.87 µg/mL; 24 h	Dose-dependent	Chromosome instability; mitotic arrest; regulation of gene expression	[24]
HOS; HCC	Fungi	8 ± 2.7 nm; spherical	IC_{50}: < 5 µg/mL (Huh7 cells), 10 µg/mL (OS cells)	Dose-dependent	ROS generation; activation of JNK signaling	[283]
CNE; HEp-2	Chemical	20 nm; spherical	IC_{50}: 9.909 µg/mL; 24 h	Dose-dependent	Mitotic arrest; regulation of Bax and P21 and Bcl-2 expression	[284]
PC-3	Plant	9 – 32 nm; spherical	IC_{50}: < 10 µg/mL; 24 h	Dose-dependent	Decrease of stat-3 and bcl-2 expression; increase of caspase-3 expression	[285]
DU145; PC-3; SKOV3; A549	Plant	10 – 30 nm; spherical	IC_{50}: 4.35 µg/mL (DU145); 7.72 µg/mL (PC-3); 4.2 µg/mL (SKOV3); 24.7 µg/mL (A549)	Dose-dependent	ROS generation; regulation of LPO and GSH level; regelation of caspase, p53 and Bax and Bcl-2	[29]
DLA	Bacteria	50 nm; spherical	IC_{50}: 500 nmol/L; 6 h	Dose- and time-dependent	Activation of caspase 3; DNA fragmentation	[286]
SKBR3; 8701-BC; HT-29; HCT 116; Caco-2	Bacteria	11 ± 5 nm; spherical	IC_{50}: 5 µg/ml (SKBR3); 8 µg/ml (8701-BC); 20 µg/ml (HT-29); 26 µg/ml (HCT116); 34 µg/ml (Caco-2)	Dose- and time-dependent	Decrease of MMP-2 and MMP-9 activities; ROS generation	[269]
Murine fibrosarcoma	Chemical	10 nm; spherical	IC_{50}: 6.15 mg/kg	Dose-dependent	ROS generation; alteration of the IL-1b function	[287]
BxPC-3; A549; PC-3; Hep G2; CNE1; AsPC-1; U-87 MG; SW480; EC109; MDA-MB-231	Physical	19.2 ± 3.8 Ång; spherical	IC50: 10.36–25.85 µg/ml; 0 – 400 min, 24h	Dose- and time-dependent	Ultrastructure change; ROS generation; mitochondrial dysfunction; cell cycle arrest	[21]

*NOTE: ROS, reactive oxygen species; MMP, matrix metalloproteinase; LPO, lipid peroxidation; GSH, glutathione; JNK, c-jun N-terminal kinase; MCF-7, human breast cancer cell line; EAC, ehrlich ascites carcinoma; A549, human lung carcinoma cells; BxPC-3, human pancreas adenocarcinoma cells; PC3, prostate adenocarcinoma cells; HepG2, hepatocellular carcinoma cells; CNE1, nasopharyngeal carcinoma cells; AsPC-1, pancreas adenocarcinoma cells; U-87 MG, glioblastoma cells; SW480, colorectal adenocarcinoma cells; EC109, esophageal cancer cells; MDA-MB-231, breast adenocarcinoma cells; HT29, human colorectal adenocarcinoma cell line; HCT-116, human colon cancer cell line; PANC-1, human pancreatic ductal cell line; SCC25, human tongue squamous carcinoma; DU145 and PC-3, human prostate carcinoma cell lines; SKOV3, human ovarian carcinoma; CNE, human nasopharyngeal carcinoma cell line; HEp-2, laryngeal carcinoma cell line; DLA, Dalton's lymphoma ascites cell lines; SKBR3, human breast cancer cell line; Caco-2, heterogeneous human epithelial colorectal adenocarcinoma cells; HCC, human hepatocellular carcinoma cells; HOS, human osteosarcoma cells; MDA-MB-231, triple-negative breast cancer cell line.

Although 50 nm, 70 nm and 80 nm nanoparticles can effectively penetrate both, 100 nm nanoparticles cannot penetrate normal fenestrations, suggesting that there might be an optimal size for effective leakage of nanoparticles from the microvasculature into the tissue. Rona et al. [41] demonstrated that size of AgNPs could influence cellular uptake and toxicity. Smaller particles (10 nm, 20 nm) easily penetrate LoVo cells and then significantly increase intracellular ROS levels, while larger particles (100 nm) appeared mainly on the cell surface. Alicia et al. [220] also found that smaller AgNPs were more cytotoxic than larger AgNPs when studying the therapeutic effects of AgNPs on human hepatoma and leukemia. Our team [21] used an evaporation–condensation system to obtain silver particles approaching the Ångstrom dimension. By comparing AgNPs with larger size, we found Ångstrom-scale silver particles had greater cytotoxicity to tumor cells, but did not induce notable toxicity on normal tissues.

The applications of AgNPs can be extended by tailoring the shape of nanoparticle, which may optimize the physicochemical and biological properties of AgNPs [26, 221]. The shape-controlled AgNPs can be obtained by changing the parameters in different synthesis methods. Though AgNPs with various shapes are prepared, such as sphere, triangle, cuboid, rod, tube, disk and wire, only a few among these are chosen for anticancer therapy. The cellular uptakes of AgNPs, as well as particle-to-cell or particle-to-protein interactions, are partly dependent on the shape of nanoparticles [216, 222]. In general, spherical AgNPs may display stronger endocytosis and more active anticancer effect than other shapes. Because it is more efficient for spherical AgNPs than non-spherical nanoparticles to pass through vascular endothelium, and their higher specific surface area is more beneficial for them to enter cancer cells [216, 222]. In addition, the active or weak endocytosis may be related to the different membrane bending energies of various shaped AgNPs. Ying Li et al. [223] compared the internalization rates of spherical-, cubic-, disk- and rod-shaped nanoparticles to find out the shape effect on endocytosis. They realized that the spherical nanoparticles exhibited the fastest internalization rate, followed by the cubic nanoparticles, while the disk- and rod-shaped nanoparticles exhibited the slowest internalization rate. After analyzing the free energies of four shaped nanoparticles, they speculated that the membrane bending energy of nanoparticles during endocytosis might be the main factor inducing the shape effect of the nanoparticles. Among these four shaped nanoparticles, compared with the non-spherical, the spherical nanoparticles only needed to overcome a minimal membrane bending energy barrier, while the disk shaped nanoparticles faced a larger free energy barrier caused by stronger membrane deformation. In order to understand the effect of more complex shaped particles on cellular uptake, Yuanzu He et al. [224] treated LnCAP cells with particles of different keyboard character shapes and compared the cell endocytosis. Compared with shapes without sharp features, like number 0, letter O and pound key, the rod-like microparticles, such as number 1, letter I, and arrow key, were more likely to adhere, penetrate and enter the cancer cells. The results explained that the shapes of microparticles with sharper angular features and higher aspect ratio might have a higher chance to contact and be internalized by cancer cells.

Dose and Exposure Time

The AgNPs exhibit dose- and time-dependent cytotoxicity against cancer [21, 225-227]. In general, increased dose and prolonged exposure time can cause more tumor cell apoptosis [228, 229]. Increasing dosage and prolonged exposure time can provide more opportunities for AgNPs to enter cells and trigger multiple anticancer mechanisms. Muthu et al. [226] studied the anticancer effect of AgNPs on Dalton’s lymphoma ascites (DLA) cell lines and found that AgNPs showed dose-dependent cytotoxicity to DLA cells through activation of caspase 3 enzyme, ultimately inducing apoptosis. Bita Mousavi et al. [230] found that AgNPs synthesized by *Artemisia turcomanica* leaf extract showed both dose- and time-dependent anticancer effect on gastric cancer cell line. Although increased dose of AgNPs and prolonged exposure time can result in better anticancer effects, the potential toxicity to normal tissues needs to be carefully considered.

Surface Charge and Protein Corona

Surface charges participate in the formation of AgNPs surface chemistry, which play an important role in cytotoxicity [231-233]. The surface charges of AgNPs determine the binding with serum albumin, as well as the adhesion and uptake of cells [25]. Negatively charged and neutrally charged AgNPs can adhere to cell membranes but internalize in small amounts, while positively charged AgNPs exhibit more efficient cell membrane penetration and internalization [25]. Besides, the positively charged AgNPs tend to stagnate on the surface of the tissue and the lumen of the blood vessels for a long time, which may be beneficial for the targeted delivery of anticancer agents [234]. AgNPs with opposite surface charges exhibit different cytotoxicity in tumor cells. The greater cytotoxicity and more ROS production are observed in tumor cells exposed to high positive charged AgNPs [234]. Nanoparticles exposed to a protein-containing medium are covered with a layer of mixed protein called protein corona [235]. The electrostatic interactions between proteins and nanoparticles contribute to the formation of protein corona [236]. Some proteins may undergo conformational changes during the formation of protein corona [235]. Protein corona has an important effect on the absorption, accumulation and subsequent behaviors of nanoparticles in cells [237]. It is proved that AgNPs with protein coronas enter cells via receptor-mediated endocytosis and subsequently induce mitochondrial dysfunction and cell apoptosis [238]. By comparing nanoparticles without protein coronas, it is realized that the formation of protein coronas around AgNPs can be a prerequisite for their cytotoxicity.

Anticancer Mechanisms

AgNPs have broad-spectrum anticancer activity via multiple mechanisms [21, 239, 240]. Numerous experiments *in vitro* and *in vivo* have proved that AgNPs can decrease the proliferation and viability of cancer cells. AgNPs can cause apoptosis and necrosis by destroying the ultrastructure of cancer cells, inducing the production of ROS and DNA damage [21, 241]. AgNPs can promote apoptosis by up- or down-regulating expression of key genes, such as p53 [242], and regulating essential signaling pathways, such as hypoxia-inducible factor (HIF) pathway [243]. Cancer cells treated with AgNPs may also show cell cycle arrest [160, 244]. Several cancer cells exposed to AgNPs undergo sub-G1 arrest and apoptosis. Besides, AgNPs can also reduce distant metastasis by inhibiting tumor cell migration and angiogenesis [28, 245]. Multiple anticancer mechanisms of AgNPs are described in **Figure 4**. In order to develop safe and effective anticancer agent, more mechanisms for anti-cancer effects of AgNPs remain to be explored. Here, we summarize the possible anticancer mechanisms of AgNPs both *in vitro* and *in vivo*.

Ultrastructural Destructions of Cancer Cells

Destruction of ultrastructures such as cell membranes and intracellular organelles leads to cell apoptosis and necrosis [21]. Tumor cells exhibit intact cell structure under light microscope, such as round nuclei, intact nuclear membrane, homogeneous chromatin, normal mitochondria and rough endoplasmic reticulum [40]. The ultrastructural changes of AgNPs-exposed tumor cells are in a dose- and time-dependent manner [246]. Generally, the higher the concentration of AgNPs and the longer the exposure time, the more serious the damage of cell ultrastructure. TEM observation showed that AgNPs-exposed cells are suffering morphological change or cytoplasmic organelle damage, and undergoing different death patterns: apoptosis, necrosis and autophagy [40]. Autophagosomes associated with apoptosis and necrosis are formed in the cytoplasm of AgNPs-treated tumor cells [247]. AgNPs promote autophagosome formation through the PtdIns3K pathway, and induce autophagy in tumor cells without inhibiting lysosomal function [22]. Structural and functional disruption of the actin cytoskeleton may be the cause of morphological deterioration of tumor cells exposed to AgNPs, and may be involved in inhibiting migration and invasion of tumor cells [248]. Free Ag^+ released from AgNPs is involved in the destruction of cellular membranes. Ag^+ released by AgNPs induces oxidation of glutathione, and increases lipid peroxidation in cellular membranes, resulting in cytoplasmic constituents leaking from damaged cells [249]. Our team found time-dependent morphological changes in cancer cells treated with F-AgÅPs, such as organelle compaction, nuclear fragmentation and cell blebbing [21]. Tumor cells exposed to AgNPs lose their typical shape due to pseudopod contraction, decreased cell adhesion and reduced cell density. Scanning electron microscopy analysis of AgNPs-treated tumor cells reveal spherical appearance, foamed membrane and shorten filopodia [248]. Tumor cells exposed to AgNPs show apoptotic cell characteristics such as loss of intact membrane, decreased contact with adjacent cells, condensed and detached from the culture plate [250].

ROS Production

ROS are by-products of biological aerobic metabolism, including oxygen ions, peroxides and oxygenated free radicals [251]. ROS are highly active due to the presence of unpaired free electrons. ROS are controlled at a low level by normal cellular antioxidant defense mechanisms and do not affect the normal physiological activities of the cells. However, excessive ROS can produce oxidative stress that reduces the activity of biological macromolecules and damages subcellular organelles and DNA structures [252, 253]. Oxidative stress trigger lipid peroxidation, impaired mitochondrial function, amino acid oxidation in proteins, enzyme inactivation and DNA/RNA damage [233], which may lead to autophagy, apoptosis and necrosis of cancer cells. AgNPs distributed in tumor cells via endocytosis can result in autophagy and apoptosis through a variety of ROS-mediated stress responses. In addition, AgNPs-induced formation of ROS may affect cellular signal transduction pathways, which may participate in the activation of apoptosis [254]. For example, the mitochondrial function can be inhibited by AgNPs via disrupting mitochondrial respiratory chain, suppressing ATP production. Besides, ROS induced by AgNPs may ultimately lead to DNA damage [255]. Superoxide radicals directed to mitochondria may enhance mitochondrial outer membrane permeabilization (MOMP) and the release of Cyt *c*, destroy the electron transport chain, and impair mitochondrial function [256]. Some factors influence the generation of ROS induced by AgNPs. Smaller size and higher concentration of AgNPs exhibit higher induction of ROS and stronger cytotoxicity, and sharp increased ROS appear in different cancer cells treated with AgNPs [220].

DNA Damage

AgNPs can induce ROS production to disrupt DNA structure, or directly contact with DNA to cause DNA mutations [209, 241, 248]. High levels of ROS can generate damage to DNA double helix in a concentration-dependent manner, including breaking the single or double-stranded DNA, affecting base modifications and DNA cross-links [241, 253, 257]. AgNPs-treated cancer cells may exhibit DNA methylation, DNA base pairing errors, DNA repair defects and increased chromosomal aberrations [209, 248, 258]. AgNPs may play an important role in the regulation of gene expression of cells. AgNPs inhibit the proliferation of cells and trigger DNA repair defects by down-regulating the functions of proteins involved in cell cycle progression and DNA repair [259]. For example, proliferating cell nuclear antigen (PCNA) gene plays an important role in DNA synthesis and repair as a cofactor for DNA polymerase. PCNA is down-regulated in AgNPs-exposed cells. While the up-regulation of the apoptotic precursor protein Bax suggests that AgNPs initiate apoptosis via the mitochondrial pathway [260]. AgNPs-treated cells may undergo S phase, G2/M phase and sub-G1 cell cycle arrests in a

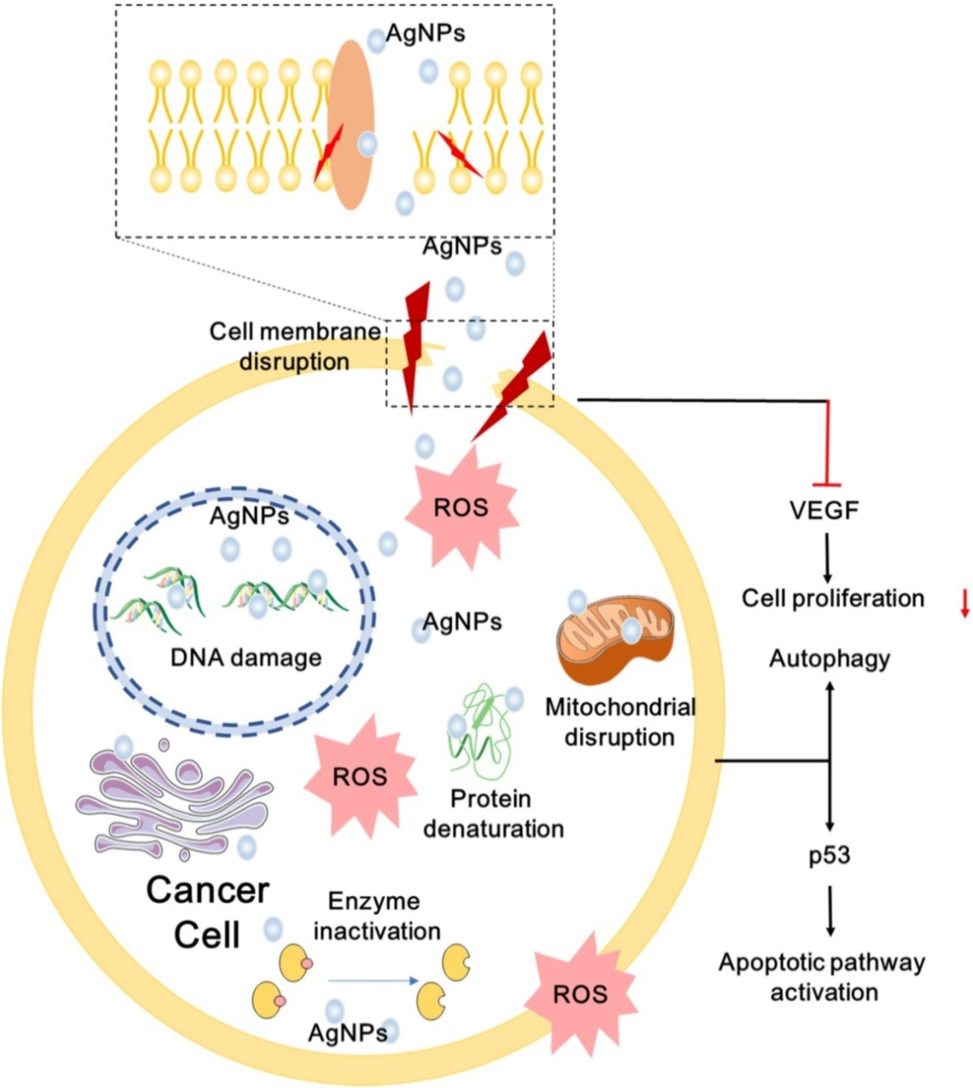

Figure 4. A schematic diagram of anticancer mechanisms of AgNPs. AgNPs can destroy the ultrastructure of cancer cell, induce ROS generation and DNA damage, promote apoptosis and autophagy by regulating multiple signaling pathways.

concentration-dependent manner, as well as the increased number of G0/G1 phase cells, which may be prone to apoptosis [244, 258, 261]. AgNPs can not only induce apoptosis through ROS-mediated DNA damage, but also destroy DNA structure directly via Ag^0 and Ag^+ released by AgNPs [157]. The DNA double helix structure is composed of four bases of adenine, guanine, cytosine and thymine by strictly complementary base pairing. Base pairs are bounded by hydrogen bonds to form a unit of DNA double helix. The destruction of hydrogen bonds decreases the stability of DNA structure. Tsuneo Ishida [157] analyzed the activities of AgNPs in the nucleus. Silver could form a complex containing silver within DNA. Ag^+ caused DNA damage by replacing the hydrogen bonds in the G≡C and A=T base pairs. The Ag atom was twofold coordinated by two N atoms to form $N\text{-}Ag^+\text{-}N$ complex in G≡C base pair, and other complex structures appearing in the base pair were $O\text{-}Ag^+\text{-}N$ (G≡C base pair), $N\text{-}Ag^+\text{-}O$ (both G≡C and A=T base pairs). DNA damage caused by these complexes might be a factor in triggering cancer cell apoptosis.

Generally speaking, AgNPs can exert anti-cancer effects through multiple pathways. Bandyopadhyay et al. [262] confirmed that AgNPs could exhibit antitumor properties through multiple channels, including triggering cell morphological changes, ROS generation, and nuclear fragmentation, while exhibited minimum toxicity in normal peripheral blood lymphocytes. The considerable anticancer activity and histocompatibility might relate to the types of reducing agent and stabilizer.

Inactivate Proteins and Regulate Signaling Pathways

In the development and progression of tumors, many signaling pathways are involved in controlling cell growth and proliferation, apoptosis and viability, and can participate in more complex signaling networks that contribute to tumor progression, such as tumor microenvironment (TME), angiogenesis and inflammation [263]. Some proteases and cytokines are also involved in these regulations, such as vascular endothelial growth factor (VEGF), matrix metalloproteinase (MMPs) and fibroblast growth factor 2 (FGF-2), etc. AgNPs have been confirmed to inhibit tumor proliferation, invasion and angiogenesis by regulating the associated signaling pathways or inactivating proteins. For example, AgNPs can regulate the HIF signaling pathway [161]. In general, rapid proliferation of tumor cells and irregular vasculature cause hypoxic TME [264-266]. HIF-1 level is up-regulated in hypoxic TME, followed by activation of target genes that in response to hypoxia. These genes contribute to cellular activities, such as cell proliferation, angiogenesis, and eventually lead to failure of cancer treatment [161]. Therefore, HIF-1 is a potential target for cancer treatment. It has been demonstrated that hypoxia can weaken HIF-1α-mediated autophagy [247]. Tieshan Yang et al. found that AgNPs could disrupt the HIF signaling pathway by attenuating HIF-1 protein accumulation and downstream target genes expression [161]. AgNPs can also inhibit the progression of tumors by inhibiting MMPs activity. MMPs are known as protein family and classified into different evolutionary groups according to their primary sequences [267]. MMPs play a dominant role in tumor progressions, such as tumor cell proliferation, invasiveness and distant metastasis, evasion of immune surveillance, and angiogenesis [267, 268]. Therefore, MMPs are considered as potential targets for cancer therapy [31]. In order to obtain antitumor drugs with targeting capabilities, some teams have attempted to develop inhibitors against members of MMPs.

Other signaling pathways and proteases involved in tumor progression have also been highlighted. Melissa M Kemp et al. [245] found that AgNPs could effectively inhibit FGF-2-induced angiogenesis. Their results suggested that AgNPs may have great potential for inhibiting pathological angiogenesis in cancer. Eom et al. [27] indicated that AgNPs induced cytotoxicity, including DNA damage, cell cycle arrest and apoptosis, by activating the p38 MAPK signaling cascades. These studies may inspire the development of anticancer agents containing AgNPs. In view of the complex signaling pathways and various proteins involved in the regulation of tumor development and progression, anticancer mechanisms of AgNPs by regulating intracellular signaling pathways and inactivating proteins still need to be further explored.

Inhibit Migration and Angiogenesis

Numerous studies have confirmed that AgNPs can inhibit migration and invasion of tumor cells in concentration- and dose-dependent manners [23, 30, 32, 269]. Migration and invasion are important hallmarks of cancer progression and deterioration [270]. Although it has been observed that AgNPs can inhibit tumor invasion [269], the specific mechanism is still unclear. It is hypothesized that AgNPs may decrease the protein expression of cytokines and growth factors within cancer cells, or reduce the enzymatic activity of MMPs. VEGF is an important signaling protein involved in vasculogenesis and angiogenesis, which plays a crucial role in tumor growth and metastasis [32]. Various studies support that AgNPs can deprive cancer cells of both nutrients and oxygen via inhibiting angiogenesis. It has been demonstrated that AgNPs can inhibit VEGF-induced angiogenesis by inactivating PI3K/AKT pathway [271]. Besides, AgNPs can block VEGF-induced Akt phosphorylation, this may influence the proliferation and migration of cells [272]. Another study has proved that AgNPs can disrupt the HIF-1 signaling pathway, thus lead to inhibition of angiogenesis [161].

Other Medical Applications

The special physicochemical properties of AgNPs make the nanoparticles and composites having considerable application prospects in the biomedical field. Besides the antimicrobial and anticancer applications mentioned above, AgNPs exhibit good properties in promoting wound repair and bone healing, as well as inhibition of inflammation. AgNPs can also be used as an additive in dental materials and adjuvant in vaccines.

Wound Repair

The wound healing is closely related to the prognosis of surgical treatment. The rapid development of nanotechnology in recent years has provided a new therapeutic strategy for healing wounds, but the specific mechanisms of AgNPs on wound healing still need more exploration. Jun Tian et al. [288] found that AgNPs could increase wound healing rate with less hypertrophic scarring in the thermal injury model. Compared with the healing time of deep partial-thickness wounds treated with silver sulfadiazine, the AgNPs treated group could heal in a shorter period and a superior cosmetic appearance was observed, including nearly normal hair growth and less hypertrophic scarring. In the healing process, lower level of TGF-β and increased level of interferon-γ were detected at the same time in AgNPs treated group, while the former was related to keloids and hypertrophic scars, and the later was involved in the inhibition of fibroblast proliferation. In addition, higher level of VEGF mRNA detected in keratinocytes at the edge of the wound suggested that AgNPs might promote wound healing by inducing angiogenesis. These results indicated that AgNPs could participate in wound healing by regulating various cytokines and achieve cosmetic effects. Other mechanisms of AgNPs in wound repair are being explored. AgNPs can remain in the cytoplasm of fibroblasts in skin biopsies, and promote the reconstruction of dermis and epidermis [289]. Some studies prove that AgNPs can induce the proliferation and migration of keratinocytes, decrease the amounts of collagen and hydroxyproline, and promote the differentiation of fibroblasts into myofibroblasts, which may help wound early adhesion, contraction and closure [290]. Besides, AgNPs can promote wound healing by regulating the production of cytokines or proteins, such as inflammatory cytokines, VEGF and MMPs [163, 291]. The above studies of AgNPs on wound repair broaden our understanding of the activity of AgNPs in cellular events. The role of AgNPs in wound repair is positive for clinical wound care and postoperative results.

Bone Healing

Bone is an active tissue with regenerative and restorative capabilities. The self-repairing capability of bone is usually compromised when bacterial infection occurs in bone defects. Bone grafts are commonly implanted to replace or restore large defects that usually resulted from severe trauma, tumor resection or genetic malformation. Orthopedic infections are usually related to bone destruction and implant loose [292]. AgNPs can be used as doping materials for synthetic bone scaffolds. AgNPs-implanted crystallized hydroxyapatite (HA) or titanium scaffolds display strong antibacterial ability against both Gram-positive and Gram-negative bacterial strains [162]. AgNPs can promote fracture healing as an osteoconductive biomaterial. For example, AgNPs can naturally stimulate the osteogenic differentiation and matrix mineralization of MC3T3-1 cells [293]. In a mouse model, AgNPs has been proved to stimulate proliferation and osteogenic differentiation of mesenchymal stem cells (MSCs) *in vitro*, and promote the healing process of bone fracture [294].

Dental Applications

Plaque biofilm formation is one of the causes of dental diseases. AgNPs have been incorporated into some dental biomaterials for reducing biofilm formation due to its antibacterial activity. Polymethyl methacrylate (PMMA), also known as acrylic resins, and composite resins are applied for the fabrication of dentures, but potential harmful organisms are likely to adhere to the rough surface of dentures [155]. AgNPs incorporated in PMMA can improve the antibacterial effect of dental material. It is proved that PMMA-AgNPs showed great anti-bacterial effect by continuous releasing of Ag^+ even in 28 days. It is highlighted that increased loading of AgNPs in PMMA also improved the mechanical properties [155]. While Acosta-Torres et al. demonstrated PMM-AgNPs could efficiently decrease the adherence of Candida albicans and exhibit no obvious genotoxicity or cytotoxicity. Comparison study of the anti-bacterial and anti-biofilm efficacies of AgNPs capped with carboxymethyl cellulose and sodium alginate, respectively, showed that carboxymethyl cellulose-capped AgNPs exhibited stronger inhibition to Gram-negative organisms, which were primarily responsible for periodontal infection [295].

Vaccine Adjuvant

Vaccination is one of the most effective methods to prevent infectious diseases and manage healthcare costs [296]. Traditional vaccines have good immunogenicity due to the complex nature of the formulation and the presence of adjuvants. However, purified preparations lack immunogenicity, which makes the addition of adjuvants essential. Adjuvants can simultaneously reduce the amount of antigen required, shorten the time needed for a protective threshold of antibody production and improve the intensity of the elicited responses, stimulate long-term memory responses to reduce the requirement of repeated vaccinations. Yingying Xu et al. [297] firstly reported the remarkable immunological adjuvant effect of AgNPs both *in vitro* and *in vivo* using model antigens ovalbumin and bovine serum albumin in 2013. After intraperitoneal or subcutaneous immunization of mice, AgNPs increased the production of serum antigen-specific IgG, as well as antigen-specific IgE, indicating that AgNPs stimulated Th2-biased immune responses. Further study of the mechanism of adjuvant revealed that AgNPs could recruit and activate local leukocytes and macrophages. Vahid Asgary et al. [298] evaluated AgNPs as an adjuvant for the rabies vaccine in 2014 and 2016, respectively. They found that although the load of AgNPs could significantly increase the immune responses by arising neutralizing antibody against rabies virus in mice, the lowest concentration of virus-loaded AgNPs decreased cell viability. This limited the use of AgNPs as an adjuvant in rabies virus. They then challenged the green synthesis of AgNPs using leaf extract of *Eucalyptus procera* and added AgNPs as an adjuvant in rabies veterinary vaccine, following by estimating vaccine efficacy in mice and dogs. They confirmed that the vaccine loaded with a suitable concentration of AgNPs was nontoxic [299].

Antidiabetic Agent

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia. DM is due to either insufficient insulin secretion or insulin resistance of the cell. Commonly used hypoglycemic agents can lower blood sugar by promoting secretion of insulin or increasing cell sensitivity [300]. In recent studies, it is noticed that AgNPs synthesized by plant extracts exhibit antidiabetic potential. Arumugam et al. [301] synthesized AgNPs using leaf extract of *Solanum nigrum* and evaluated the anti-hyperglycemic effect in alloxan-induced diabetic rats. They found that the blood glucose level of diabetic rats decreased when treated with AgNPs for 14 days and 21 days without significant acute toxicity. And they assessed glucose tolerance of AgNPs in diabetic rats. The results showed that AgNPs exhibited a good hypoglycemic effect compared to glibenclamide, a standard antidiabetic drug. Saratale et al. [4] demonstrated that AgNPs synthesized by leaf extract of *Argyreia nervosa* exhibited antidiabetic activity *via* inhibiting α-amylase and α-glucosidase. These two carbohydrate digestive enzymes contribute to decompose carbohydrates into monosaccharides. The antidiabetic mechanism of AgNPs is still unclear. Jihan Hussein et al. [302] hypothesized that AgNPs might influence insulin signaling pathway or insulin sensitivity in diabetic rats. The results supposed that AgNPs could activate protein kinase C and PI_3K pathway at the insulin receptor substrate level, as well as inhibit protein kinase C isozymes, thus effectively enhance insulin secretion and sensitivity. It was highlighted that AgNPs were effective in reducing insulin resistance and DNA damage.

Biosensing and Imaging

Surface-enhanced Raman scattering (SERS) has attracted the attention of noble metals with Raman signals in many application strategies, including biochemical sensing, analytical chemistry, and materials science [303]. Among these nanomaterials, AgNPs can be used as a cost-effective surface-enhanced Raman scattering substrate. Nanoparticles containing AgNPs can be used as biosensors to detect blood glucose, enzymes, molecular markers of tumor cells, pathogens, etc. For example, Jiang et al. [304] prepared silver-containing nanocomposites as acetylcholinesterase biosensors for electrochemical detection of organophosphorus pesticides. AgNPs improved the electrical conductivity and biocompatibility of nanocomposites and made them more suitable for enzyme activity and stability. Anderson et al. [305] prepared a high-sensitivity nonenzymatic biosensor for the detection of glucose using AgNPs as a conductive additive. Both the porous nanostructures of AgNPs and large surface areas of carriers enhanced the interaction sites between AgNPs and electrode/glucose, which could accelerate the electron transfer of AgNPs and therefore improve the sensitivity of the biosensor. Although the electrochemical characteristics and Raman scattering make AgNPs exhibit good application prospects in the field of biosensing, the matrix composition may affect their SERS and reduce the detection sensitivity. Therefore, it is necessary to modify AgNPs in order to improve the sensitivity of re-creating platforms. For example, Zeng et al. [306] synthesized hybrid Ag@NGO nanoparticles by a one-step reduction method. Among these platforms, the nanosized graphene oxide (NGO) worked as inert protective layers and provided an ultrathin protective layer for AgNPs. Ag@NGO exhibited the advantages of both SERS biosensing and drug delivery, ie, monitoring biomolecule signals in tumor cells through SERS biosensing and interacting with the anticancer drug doxorubicin through formation of π-π bonds. These results prove that AgNPs hold great application potential with capabilities of SERS biosensing.

Silver nanoclusters have unique optical and electrical properties and can be used as materials for synthetic probes. While proteins have multiple chelating and functional groups, therefore, they have unique advantages as ligands in biological imaging. Cunlan Guo and Joseph Irudayaraj [307] used denatured bovine serum albumin as a stabilizer to synthesize silver clusters, which could sensitively and selectively detect the content of mercury. The probe had important application value for detecting mercury content in water, soil and food. Sun et al. [308] used glutathione as a ligand to passivate silver nanoclusters and obtained highly sensitive fluorescent probes. During the passivation of glutathione, the specific recognition of silver nanoclusters modulated from Hg^{2+} to Cu^{2+}. This fluorescent probe was highly sensitive and selective in detecting Cu^{2+} in blood samples. The synthesis of silver nanoclusters with DNA as the backbone has excellent spectral and photophysical properties. The generation of this fluorophore is highly dependent on the DNA sequence. Oligonucleotide sequence changes may trigger the adjustment of the photoluminescence emission band, thus identifying the mutant nucleotide sequence. Guo et al. [309] designed a double-stranded DNA scaffold that hybridizes probe DNA strands and sickle cell anemia mutation target DNA to generate fluorescent silver nanoclusters. The fluorescent silver nanoclusters specifically recognized sickle cell anemia mutations. The research extended from DNA scaffold single-stranded oligonucleotide to hybrid DNA double-stranded mutation site recognition, which may have more applications in the field of biological analysis. These studies suggest that silver nanoclusters have great clinical application potential.

Potential Toxicity of AgNPs

The potential harm of nanomaterials to organs and systems in the body has been gradually observed [310-312], which may influence the biomedical application of nanomaterial. Therefore, it is necessary to review the dynamics of AgNPs *in vivo*. AgNPs can be taken and distributed to different organs through a variety of routes of administration, mainly include inhalation, ingestion, skin contact, and subcutaneous or intravenous injection (**Figure 5**). The absorbed AgNPs are distributed in many systems [310, 311], such as the dermis, respiratory, spleen, digestive, urinary, nervous, immune and reproductive system, and mainly distributed in the spleen, liver, kidney and lung, while little deposition of AgNPs is observed in teeth and bones. The small-sized AgNPs are easy to penetrate the body and cross biological barriers like the blood-brain barrier and the blood-testis barrier, and subsequently induce potential cytotoxicity. Besides the directly exposed tissues, AgNPs can also be transported to different organs via blood circulation. Therefore, the non-specific distribution of AgNPs may produce cytotoxicities such as dermal toxicity, ocular toxicity, respiratory toxicity, hepatobiliary toxicity, neurotoxicity and reproductive toxicity, which limit the applications of AgNPs. The potential cytotoxicity of AgNPs depends on the routes of administration and the properties or characteristics of the AgNPs, such as the size, shape, and concentration. At the cellular level, Wang et al. [313] used TEM and integrating synchrotron radiation-beam transmission X-ray microscopy (SR-TXM) with 3D tomographic imaging to capture the information of the cellular uptake, accumulation, degradation, chemical transformation, and removal of AgNPs. The experiment revealed that the cytotoxicity was caused by the chemical transformation of AgNPs, ie. Ag^0 transformed into Ag^+, Ag-O-, and Ag-S- species, which might induce the cellular biochemical changes. However, there is still inadequate acknowledge of the potential cytotoxicity, long-term adverse health effects, and the specific mechanisms of AgNPs accumulated in the different tissues and organs. In order to develop AgNPs with better biocompatibility for medical applications, it is urgent to systematically study their potential cytotoxicity. This chapter provides a brief overview of the potential toxicity and possible mechanisms of AgNPs in different organs, including skin, eye, kidney, respiratory system, hepatobiliary system, central nervous system, immune system and reproductive system (**Table 6**).

Skin Toxicity

Even as early as in 1614, Angelo Sala reported the first case of a definitive diagnosis of argyria, a kind of disease induced by the deposition of silver in tissues [314]. Since the mid-19th century, it has been recognized that silver or silver compounds may induce some tissues to turn gray or blue-grey, especially involving the skin. The skin, as the largest organ and the first-line barrier of the human body, can isolate the external pathogens from the internal environment. Topically applied AgNPs may induce cytotoxicity in the site and penetrate the skin and subsequently access the systemic circulation followed by adverse effects on other organs. For example, applying AgNPs gel and covering dressings will allow particles to penetrate and accumulate in the skin and produce potential cytotoxicity [315]. Before AgNPs, there are several reports on the skin toxicity of elemental silver, known as Argyria [316]. Argyria is a disease characterized by permanent gray-blue pigmentation of mucous membranes, eyes and skin, occurring in individuals exposed to high concentrations of silver for a long period. G D DiVincenzo et al. [317] previously reported that the skin of workers exposed to silver aerosols showed a distinctive gray bluish hue change, and deposited silver was also detected in worker's hair, urine and feces. Jennifer et al. [318] reported a Argyria case. The patient showed uniform accumulation of silver on the skin after long-term consumption of silver solution. Current studies show that AgNPs can enter the hair follicles to induce local deposition and deposit into the subcutaneous structure by penetration pathways. The follicular penetration pathway is most commonly used to explain the penetration of particles into the skin [319-322]. Yu Kyung Tak et al. observed that AgNPs of different shapes would remain at different layers of skin. Rod-shaped, spherical, and triangular AgNPs penetrated the dermis, epidermis and stratum corneum layers, respectively. They observed the behavior of AgNPs in subcutaneous capillaries. And prolonged exposure time would increase the amount of nanoparticles. Notably, they found that the penetration of AgNPs was achieved by the follicular penetration pathway and intercellular penetration pathway [323]. Francesca et al. [324] attempted to use AgNPs to act on intact or damaged skin. They demonstrated a significantly higher penetration of AgNPs used on damaged skin as compared with intact skin, and they speculated that a small part of the particles would diffuse into the skin as silver ions. Radoslaw et al. [325] explored the cytotoxicity of AgNPs on epidermal keratinocytes (NHEK). The results showed that AgNPs inhibited cell proliferation and migration, induced activation of caspase 3 and caspase 7, and damaged DNA. In addition, by measuring the ATP content of cells treated with different concentrations (6.25, 12.5, 25 and 50 μg/ml), it was found that a high concentration of AgNPs significantly decreased the ATP production, and this phenomenon worsened with prolonging exposure time.

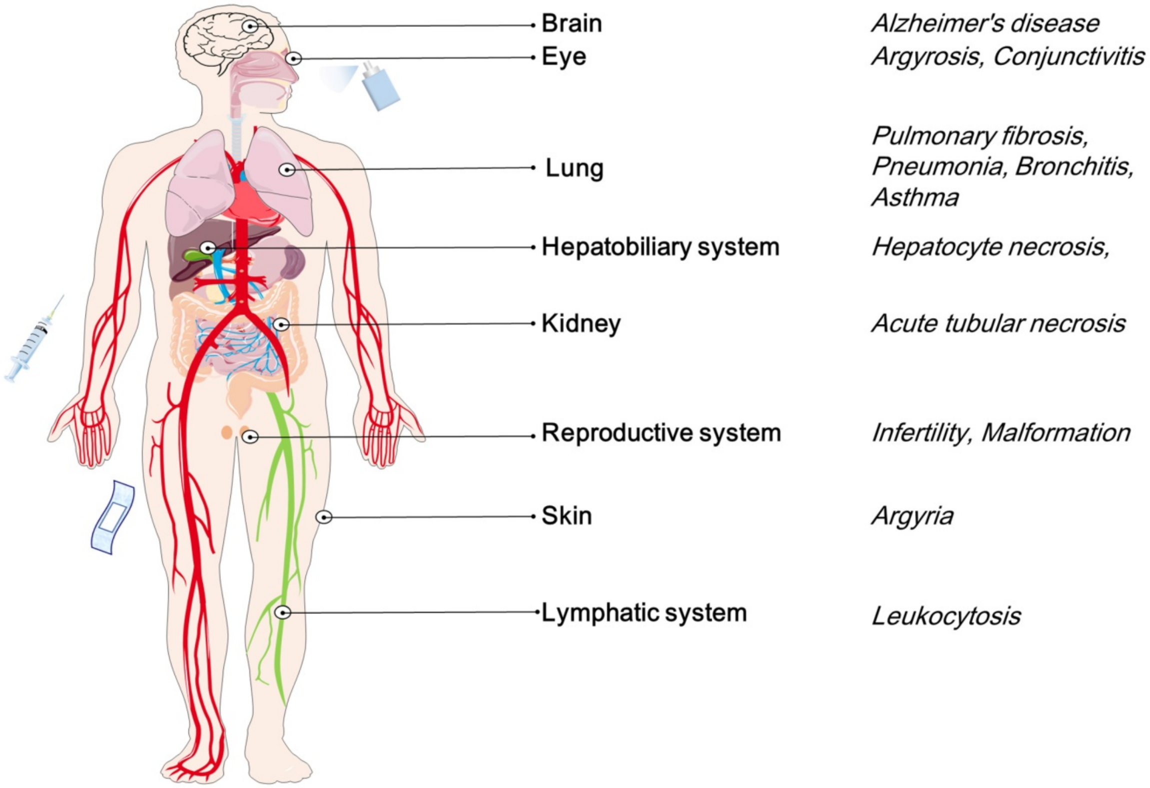

Figure 5. A schematic of potential toxicities of AgNPs in the human body. The exposure patterns of AgNPs include respiratory inhalation, intravenous injection and skin contact. Affected organs include the eye, kidney, skin, and nerves, respiratory, immune, hepatobiliary and reproductive systems. Diseases or pathologic changes induced by AgNPs are listed.

Table 6. Potential toxicity of AgNPs *in vivo* and *in vitro*

Objects			Exposure		Toxicity				References
Animal model	*In vitro/ vivo*	Cell lines/Tissues	Size; Shape	Dosages	Route	Time	Effect	Toxicity manners	
Pig	*In vitro* and *vivo*	HEKs and porcine skin	20, 50 and 80 nm	0.34, 1.7 µg/mL	Incubation; skin contact	Acute: 18 and 24 h; chronic: 14 d	Focal inflammation	Dose-dependent	[350]
Mice	*In vivo*	Liver	Less than 30 nm	10 ppm	Skin contact	2, 7 and 14 d	Central venous dilation; hyperemia, cell swelling, Kupffer and inflammatory cells increase	Time-dependent	[332]
Mice	*In vivo*	Spleen, liver, lung and kidney	12–20 nm	7.5, 30 and 120 mg/kg	Intravenous administration	7 and 14 d	Induction of inflammatory reactions in lung and liver cells	Gender-, concentration- and time-dependent	[346]
Mice	*In vivo*	Lung	10–20 nm; spherical	10, 100, 1000 and 10,000 ppm	Intratracheally administration	1, 3, 7 and 15 d	Acute lung inflammation and bronchitis; hyperplasia of alveolar epithelial cells	Dose-dependent	[330]
Mice	*In vivo*	Liver, spleen, kidneys, heart, lungs, testes, stomach, intestine and seminal vesicles	3±1.57 nm; spherical	11.4–13.3 mg/kg	Intravenous injection	1, 28 d	Inflammatory response; alteration of hematological factors; change of gene expression; ROS generation	Dose-dependent	[351]
Mice	*In vivo*	Liver, kidneys and lung	10, 75 and 110 nm; spherical	25 µg/mice	Intravenous administration	1, 3 and 7 d	Peripheral inflammation in liver, kidneys and lungs	Time-, concentration- and size-dependent	[343]
Mice	*In vivo*	Lung	20 and 110 nm	0.05, 0.15, 0.45 and 1.35 mg/kg	Intratracheal instillation	1, 7 and 21 d	Alter SP-D level; deficit immune defense function	Size- and stabilization-dependent	[44]
Mice	*In vivo*	Brain, lung, liver, kidney and testis	22, 42 and 71 nm	0.25 mg/kg, 0.50 mg/kg, 1.00 mg/kg	Oral administration	14 and 28 d	Induce organ toxicity and inflammatory responses	Dose-dependent	[352]
Mice	*In vivo*	heart, lung, kidney, liver and blood	1.4–250nm	11.4–13.3mg/kg body weight	Intravenous administration	28 d	Induce gene expression; ROS generation; apoptosis	Dose-dependent	[351]
Mice	*In vitro* and *in vivo*	A549, BxPC-3; PC-3; Hep G2, CNE; AsPC-1; U-87 MG; SW480; EC109; MDA-MB-231; VSMC; HMEC; LO2; 293FT; tumor, brain, heart, kidney, lung, spleen, and liver	19.2±3.8 Ång, spherical or ellipsoidal	0–32ng/µl, 1.875 mg/kg	Intravenous administration	Acute: 24 h; chronic: 28 d	None	Dose- and time-dependent	[21]
Mice	*in vivo*	Lung	20 and 110 nm, spherical	0.1, 0.5 and 1.0 mg/kg	Inhalation	Acute: 40 h; chronic: 21 d	Pulmonary fibrosis	Size- and coating-dependent	[353]
Mice	*in vivo*	Kidney, liver and spleen	2.45–19.53 nm	0.37, 0.65, 13 and 21 mg/kg	Oral administration	27 d	Tissue destruction; cell necrosis and apoptosis	dose-dependent	[354]
Rat	*In vivo*	Brain	>100 nm	5 and 50 mg/kg	Oral administration	79 d	Cell death, disturbed neurotransmitter and cytokine production, ROS generation	--	[355]
Rat	*In vivo*	Sperm and testicular tissue	60–80 nm	30, 125 and 300 mg/kg	Intraperitoneal injection	28 d	Decrease normal sperm morphology, sperm vitality and sperm count	Dose-dependent	[348]
Rat	*In vivo*	Kidneys, liver and blood	20–65 nm	2,000 mg/kg	Intraperitoneal administration	3 d	Liver and kidney damage	Time- and dose-dependent	[356]
Rat	*In vivo*	Lung, spleen, liver, kidney, thymus and heart	6.3–629 nm	0.5 mg/kg	Intravenous administration	24 h	Liver and kidney damage; chromosome breakage; genotoxicity	Dose-dependent	[357]
Rat	*In vivo*	Epididymal sperm	20–30 nm	50, 100 and 200 mg/kg	Oral administration	90 d	Sperm anomalies; decrease sperm viability	Dose-dependent	[37]
Rat	*In vivo*	Brain	3–10 nm, spherical	1 and 10 mg/kg	Intragastric administration	14 d	Neuron shrinkage; cytoplasmic or foot swelling of astrocytes	Dose-dependent	[38]
Rats	*In vivo*	Kidney, liver and blood	20–60 nm, spherical	2,000 mg/kg bw, twice injections	Intraperitoneal injection	5 d	Liver and kidney damage; blood parameters disrupt	Dose- and time-dependent	[356]
Rat	*In vivo*	Spleen, liver, and lymph nodes and blood	20 nm and 100 nm	6 mg/kg	Intravenous administration	28 d	Suppression of the natural killer cell activity; stimulate LPS mitogen; increase cytokine production	Dose-dependent	[358]
Rat	*In vivo*	Liver and kidney	56 nm	30, 125 and 500 mg/kg	Oral administration	90 d	Liver damage; bile-duct hyperplasia	Dose- and gender-dependent	[341]

Objects			Exposure		Toxicity				References
Animal model	*In vitro/ vivo*	Cell lines/Tissues	Size; Shape	Dosages	Route	Time	Effect	Toxicity manners	
Rat	*In vivo*	Kidney	52.7–70.9 nm	10 ml/kg	Oral administration	90 d	Deposite in kidneys	Dose-dependent	[340]
Female ICR mice; male guinea pigs	*In vivo*	Oral, skin and eye	10–20 nm, spherical	5,000 mg/kg (oral); 50 and 5,000 ppm (eye); 50 and 100,000 ppm (skin)	Oral administration; eye drops; skin contact	1, 2 and 3 day	Conjunctivae irritation	—	[326]
Male ICR mice	*In vivo*	Blood, liver, spleen, kidney, lungs and brain	10, 40 and 100 nm, spherical	10 mg/kg	Intravenous injection	24 h	Bleeding or necrosis of multiple internal organs	Size- and tissue-dependent	[331]
BN and SD rats	*In vivo*	Lung	20, 110 nm, spherical	0.1 mg/kg or 90 breaths/minute	Intratracheal administration	1, 7 and 21 d	Lung eosinophilia and bronchial hyperresponsiveness; distruction of blood/alveolar epithelial permeability barrier	Dose- and size-dependent; rat strains related	[46]
Mice and guinea pigs	*in vivo*	Lung, lymph node, heart, liver and kidney	10–20 nm, spherical	5,000 mg/kg, 5000 ppm	Oral administration, eye and skin contact	14 d	No mortality and toxic signs		[326]
Freshwater fish	*In vivo*	Embryo	25.9–36.7 nm, spherical	Acute: 0.3, 0.6, 1.2, 2.4 and 4.8 mg/L; subchronic: 0.05, 0.1, 0.25 and 0.5 mg/L	Incubation	14 d	Liver damage; deplete glutathione; deactivate lactate dehydrogenase and antioxidant enzymes	Time- and dose-dependent	[39]
Japanese medaka	*in vivo*	Embryo	20–37 nm, spherical	0, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L	Oral administration	48 h	death	dose-dependent	[327]
Zebrafish	*In vivo*	Embryo	20 and 110 nm, spherical	0.08, 0.4, 2, 1,0 and 50 mg/L	Hatch	5 d	Multiple developmental abnormalities	Size- and surface coating-dependent	[359]
Zebrafifish	*In vitro* and *in vivo*	Brain, heart, yolk and blood of embryo	5–20 nm	5, 10, 25, 50 and 100 µg/mL	Hatch	24, 48 and 72 h	Multiple developmental abnormalities	Concentration-dependent	[43]
Drosophila melanogaster	*in vivo*	Parents, egg and offspring	2–20 nm	10, 20, 30, 40, 50 and 100 mg/L	Oral administration	5,10, 15, 30, 45, 60 and 95 min	Abdominal pigmentation	Dose-dependent	[360]
Drosophila melanogaster	*In vivo*	Germline stem cell; testis	20 nm	2, 3.5 and 5 mg/L	Oral administration	24h, 5 d	Delay the development of the F1 offsprings; ROS generation; premature GSC differentiation	Dose-dependent	[42]
Caenorhabditis elegans	*In vivo*	The worms' body	96.4±35.6 nm, spherical	0–1mg/L	Culture	6 and 24 h	DNA damage, ROS generation, inhibition of growth	Dose- and time-dependent	[361]
Caenorhabditis elegans	*In vitro*	The worms' body	< 100 nm, spherical	0.025, 0.05 and 0.075 µg/mL	Culture	24 h	ROS generation; DNA damage	Size-dependent	[152]
—	*In vitro*	rat brain microvascular endothelial cells, pericytes, and astrocytes	7±2 nm	1 and 10 µg/mL	Incubation	24 h	Trx system, Nr4a1 and Dusp1 regulaion , inflammation and apoptosis	Dose-dependent	[338]
—	*In vitro*	Mouse ESCs	20.2 ±4.1 nm, spherical	5.0 µg/ml	—	24 h	Heat shock protein and the metallothionein families regulation, induce oxidative stress and apoptosis	Dose-dependent	[362]
—	*In vitro*	Mouse microglia N9 cell line, N27 neuronal cells	49.7±10.5 nm, spherical	50 µg/mL	Incubation	24 h	Nitric oxide and TNFα production	Dose-dependent	[363]
—	*In vitro*	Mouse lymphoma cell line, human lymphoblastoid cells	20, 50 and 100 nm, spherical	0–400 µg/mL	Incubation	4, 8, and 24 h	DNA mutants	Size-, concentration- and coating-dependent	[47]
—	*In vitro*	Rat primary cerebral astrocytes	24.18±4.14 nm, spherical	0.01, 0.1, 1 and 10 mg/mL	Incubation	24 h	Neuroinflammation and apoptosis; increase caspase activities	Dose-dependent	[337]
—	*In vitro*	Primary astrocyte cell, rat glioma C6 cell line	6.9–8.7 nm, spherical	0.1, 1, 10, 50, 75 and 100 µg/mL	Incubation	24 h	Necrosis and apoptosis	Dose-dependent	[364]
—	*In vitro*	Murine brain ALT astrocytes, murine microglial BV-2 cells and mouse neuroblastoma Neuro-2a (N2a) cells	3–5 nm	0.5, 1, 5, 10 and 12.5µg/mL	Incubation	24 h	Cytokine secretion, Aβ amyloid deposition, inflflammatory response	Dose-dependent	[339]
—	*In vitro*	UMR-106	6 nm, cubic	10, 25, 50, 100, 150 and 225 µM	Incubation	24h	Decrease lysosomal and mitochondrial activity	Dose-dependent	[365]
—	*In vitro*	U937 cell	4,20 and 70 nm, round	1.56, 3.12, 6.25, 12.5, 25 and 50 µg/mL	Incubation	24 h	Oxidative stress; cytokines release	Size-dependent	[50]
—	*In vitro*	HepG2 cell line	20 nm	2.5 to 50 µg/cm^3	Incubation	24 h	Endogenous antioxidant defence regulation	Dose-dependent	[366]

Objects			Exposure		Toxicity				References
Animal model	*In vitro/ vivo*	Cell lines/Tissues	Size; Shape	Dosages	Route	Time	Effect	Toxicity manners	
–	*In vitro*	Jurkat T, NCI-H460, HeLa cells, HepG2, MCF-7, Beas-2B	5–10 nm	0.2, 0.5 and 1 mg/L	Incubation	4, 12 and 24 h	DNA damage; p38 MAPK activation; cell arrest; apoptosis	Time- and concentration-dependent	[27]
–	*In vivo*	Human lymphocytes and sperms	8–10 nm	Density gradient: 1:9, 1:3, 1:1	–	30 and 60 mins	Cell viability decrease	Concentration- and time-dependent	[367]
–	*In vitro*	Pk15	61.2±33.9 nm, nonuniform	50 mg/L	Incubation	24 and 48 h	Genotoxicity in Pk15 cells	Dose-dependent	[342]
–	*In vitro*	Zebrafish ovarian follicle cells	30–55 nm	30 μg/mL	Incubation	2 h	Apoptosis of ovarian follicle cells; germinal vesicle breakdown	Concentration-dependent	[349]

*NOTE: UMR 106, rat osteosarcoma cells; MDA-MB-231, triple negative breast cancer cell line; PMBC, peripheral blood mononuclear cell; HepG2, human liver cancer cell line; Jurkat T, human T lymphocyte cell line; NCI-H460, human lung cancer cell line; MCF-7, human breast cancer cell line; Beas-2B: human bronchial epithelial cells; SPD, surfactant protein-D; U937, human histiocytic lymphoma cell line; Pk15, pig kidney cell line; BV-2, murine microglial cell line; N2a cells, mouse neuroblastoma; HEKs, human embryonic kidney cells; A549, human lung carcinoma; BxPC-3, human pancreas adenocarcinoma cells; PC3, prostate adenocarcinoma cells; HepG2, hepatocellular carcinoma cells; ESCs, embryonic stem cell; CNE, nasopharyngeal carcinoma cells; AsPC-1, pancreas adenocarcinoma cells; U-87 MG, glioblastoma cells; SW480, colorectal adenocarcinoma cells; EC109, esophageal cancer cells; VSMC, vascular smooth muscle cells; HMEC, human microvascular endothelial cells; LO2, hepatocytes; 293FT, embryonic kidney cells.

Eye Toxicity

AgNPs agent may cause concentration-dependent acute conjunctival irritation, but there is still no reliable evidence for toxicological effects. Pattwat et al. [326] dripped 50 ppm and 2,5000 ppm colloidal AgNPs into the eyes of guinea pig and explored whether there were acute eye irritation or corrosion throughout the 78 hours observation period. Although transient mild conjunctival irritation, i.e. blood vessel hyperemia in conjunctivae, was observed within 24 hours after 5000 ppm AgNPs treatment, neither low-dose nor high-dose colloidal AgNPs caused any acute toxicological effects in guinea pigs. AgNPs may have developmental toxicity in the eyes of early-stage individuals, which can eventually result in multiple types of eye defects. Yuan Wu et al. [327] studied the developmental toxicity of AgNPs by using Japanese medaka at early-life stages as experimental models, including embryonic, larval and juvenile stages. The Japanese medaka was exposed to 100–1000 mg/mL AgNPs for 70 days and various morphological malformations were described and analyzed, such as edema, visceral deformities, heart malformations, spinal abnormality, especially eye defects. AgNPs-treated group showed different eye defects, such as microphthalmia, exophthalmia, cyclopia and anophthalmia. Histopathological examinations of 2-day-old larvae showed increased thickness of retinal pigment epithelium and missing of the retina in inner segments. Interestingly, comparing with the high-dose groups, the types and numbers of eye malformations in the low-dose groups were significantly higher. These morphological abnormalities and non-linear dose-response pattern suggest that the developmental toxicity of AgNPs may exhibit complex toxicological mechanisms.

Respiratory toxicity

AgNPs can induce acute lung toxicity and therefore impair lung function, and the damage severity is related to particle accumulation and clearance. Akinori [328] et al. studied the pulmonary toxicity of nanometer particles in mouse models. Ultrafine particles may pass the air-blood barrier through the gap between alveolar epithelial cells, induce vacuolation and necrosis of bronchiolar epithelial cells, resulting in transient acute lung inflammation and tissue damage. The oxidative stress and apoptosis induced by ultrafine particles may contribute to lung damage. In addition, nanoparticles showed size-dependent pulmonary toxicity, i.e. the particles in smaller size exhibit higher capacity for inducing lung inflammation and tissue damage than larger size [36, 329]. On the other hand, AgNPs may induce dose-dependent lung toxicity. Kaewamatawong et al. [330] demonstrated dose-dependent acute lung toxicity in mice induced by AgNPs using a single intratracheal instillation of 0, 10, 100, 1000 or 10000 ppm of colloidal AgNPs. And they observed moderate to severe bronchitis and multifocal alveolitis in 100, 1000 and 10,000 ppm AgNPs treated groups. Proinflammatory cytokines such as IL-1β and TNF-α released by alveolar macrophages and airway epithelial cells might involve in the inflammatory lesions in mice. The aggregation of AgNPs had a direct effect on the basement membrane, and disrupted equilibrium between the synthesis and degradation of the extracellular matrix, thus may cause pulmonary fibrosis. Similarly, they also speculated that AgNPs induced oxidative stress in the lung. Furthermore, they recognized that metallothionein (MT) expression induced by AgNPs might be regarded as one of the possible protective mechanisms of lung. Different concentrations of AgNPs, which induce lung damage, may also accumulate in peripheral organs and cause potential health risks. Joanna et al. [46] found that AgNPs disrupted the blood/alveolar epithelial permeability barrier, elicited oxidative stress, activated eosinophils and promoted the release of multiple cytokines. Most importantly, their results showed that AgNPs induced eosinophilic and neutrophilic inflammation, which was an important pathological change in asthma. This might suggest that exposure to AgNPs could trigger asthma.

Hepatobiliary System Toxicity

Part of the ingested nanoparticles tend to be sequestered, degraded and accumulated in the liver, which means the liver may be responsible for the metabolism of nanoparticles as well as one of the most frequently attacked organs. On the other hand, the gallbladder collects, stores and excretes bile or biological waste to the intestine. Various metal nanoparticles, including AgNPs, are recognized to be exported from the liver through this pathway. Therefore, hepatocytes are widely studied in the liver toxicity of AgNPs. Maglie et al. [48] found that AgNPs induced severe hepatobiliary damage in mice, including significant hepatocyte necrosis and gallbladder hemorrhage. In this study, AgNPs exhibited size- and dose-dependent hepatobiliary toxicity, i.e. AgNPs in smaller size produced more serious toxic effects, and higher dose of AgNPs induced severer hepatobiliary damages. Camilla et al. [331] observed multi-system acute toxicities in mice with a single intravenous injection of AgNPs. First of all, significant hepatobiliary damages were recognized, including hepatocyte necrosis, micro-hemorrhage around the biliary tract, and portal vein injury. Secondly, they also observed that AgNPs could induce acute tubular necrosis and apoptosis, and moderate splenomegaly. The results of Mohammad et al. [332] showed that AgNPs penetrating via the skin induced time-dependent liver damage such as hyperemia, dilatation in central venous, swelling hepatocytes and increased inflammatory cells. Besides hepatocytes, Kupffer cells (KCs) are also responsible for the removal of AgNPs [333]. KCs are macrophages that reside in the hepatic sinusoids and have the active ability of phagocytosis, maintaining the normal immune response and removing nanoparticles from organisms [333, 334]. Therefore, KCs become the focus of research on liver toxicity and metabolism of AgNPs.

Central Nervous System Toxicity

The central nervous system consists of two parts: the brain and the spinal cord. Lots of supporting non-nervous cells, i.e. neuroglial cells fill the interneuronal space within the central nervous system. In recent years, some articles point out that AgNPs may penetrate the brain and subsequently induce neuronal death. Due to the limited self-repairing ability of nerve cells, the potential neurotoxicity of AgNPs is receiving more attention. Different exposure patterns can lead to the accumulation of AgNPs in the brain. Injected AgNPs cross blood-brain barrier (BBB) and then penetrate the brain, while inhaled AgNPs reach the central nervous system through the olfactory and/or BBB [335, 336]. Due to the unique physicochemical properties of AgNPs, deposited AgNPs in nerve cells, astrocytes and extravascular lymphocytes may cause and aggravate neurotoxicity and inflammation, and increase the permeability of BBB. In the study of the cytotoxicity of AgNPs on rat cerebral astrocytes, Cheng et al. [337] investigated the neurotoxicological effects of AgNPs and Ag^+ and compared the mechanisms. Both AgNPs and Ag^+ exposure could internalize silver in astrocytes in dose- and time-dependent manners. The AgNPs had higher bioaccumulation ability than Ag^+ after 24 h treatment. After the same treatment time, they found that AgNPs might induce intracellular ROS generation in rat cerebral astrocytes and caused cell apoptosis, however, there were undetectable alterations in Ag^+ group. More importantly, they confirmed that AgNPs could increase the level of phosphorylated JNK, a kind of kinase involved in mediating apoptosis. The non-cytotoxic dose of AgNPs, rather than Ag+, might induce neuroinflammation by promoting the secretion of multiple cytokines of astrocytes, including CINC-2a/b, CINC-3, IL-10, IP-10, L-selectin and thymus chemokine. Liming et al. [38] investigated the neurotoxicity of AgNPs in the rat after intragastric administration of low-dose (1 mg/kg, body weight) or high-dose (10 mg/kg, body weight) for two weeks. They observed a variety of cell morphological changes in the nervous system, including neuron shrinkage, astrocytes swelling and extravascular lymphocytes. They also observed significantly increased inflammatory factors such as IL-4 in the serum. These data supported the proinflammatory effects of AgNPs in the nervous system. Then they focused on the possible mechanisms for AgNPs or Ag^+ transporting across the blood-brain barrier. AgNPs or released Ag^+ might cross through the blood-brain barrier via ionic pores or channels and subsequently damage the nerve cells. Besides, AgNPs could enter the central nervous system via vesicular transport of endothelial cells and subsequently induced neuroinflammation. At the same time, they demonstrated the increased permeability of the blood-brain barrier in a rat model after AgNPs treatment. They also observed that

AgNPs might inhibit the antioxidant defense of astrocytes by increasing thioredoxin interacting protein, thus lead to the central neurotoxicity. AgNPs might induce ROS, inflammation and apoptosis through regulating the MAPK pathway, mTOR activity and Bcl-2 expression in astrocytes. AgNPs could cause severe ultrastructural changes in astrocytes, including mitochondrial contraction, endoplasmic reticulum expansion and nuclear atypia. Furthermore, AgNPs regulated the expression of multiple genes, inhibited metabolic and biosynthetic processes, thus affect astrocytes function and increase the neurotoxicity. More importantly, the impairing of learning, memory and cognition processes by AgNPs reduced the learning ability and cognition function of rats [338]. AgNPs may induce neurological diseases such as Alzheimer's disease by altering gene expression. Chin et al. [339] reported that AgNPs could induce the expression of amyloid precursor protein (APP) gene in nerve cells. APP gene promoted the deposition of amyloid-β (Aβ) protein, a key pathological feature of Alzheimer's disease.

Kidney Toxicity

The kidney participates in balancing body fluid volume and pH, regulating osmotic pressure and electrolyte concentration, drug metabolism, and toxic emissions. Abnormal renal function may occur in AgNPs-treated mammalian kidneys. AgNPs exhibits a dose-dependent accumulation in most examined tissues, such as the brain, lung, liver, dermis, blood and testes. However, there is a gender-related difference in silver accumulation in the kidney. Wan et al. [340] observed that female rats treated with AgNPs had a twofold higher concentration of silver in kidneys than male rats. Ag enhancement staining of the kidneys showed that AgNPs preferentially accumulated in the basement membrane of the glomerulus as well as renal tubules, while mildly accumulated in the adrenal capsule and cortex. There were two possible mechanisms of gender difference in the accumulation of AgNPs: the gender difference in the expression of organic cation transporters, and hormonal regulation. Renal metallothionein and zinc-binding protein, a kind of transporter or binding protein molecules in the kidney, might contribute to the silver accumulation. While organic anions secreted by kidneys might influence the clearance and accumulation of silver [341]. Mirta et al. [342] studied the uptake mechanism and potential cytotoxicity of AgNPs in porcine kidney (Pk15) cells *in vitro*. TEM results showed that there were aggregates in the lysosome and early endosomes. In addition to micropinocytosis, as an uptake pattern, clathrin- and caveolin-mediated endocytosis could also be the possible endocytotic mechanisms. AgNPs could decrease the number of viable Pk15 cells *in vitro* in a dose-dependent manner. Hua et al. [343] studied the distribution, accumulation and potential toxicity of AgNPs in different sizes in liver, lung and kidney of mice. They found that AgNPs could be taken up by vascular endothelial cells, then induced the generation of intracellular ROS and down-regulated the expression of vein endothelial cadherin. Therefore, AgNPs destroyed the conjunction between endothelial cells, allowing AgNPs to cross the endothelial layer and accumulate in organs. Besides, the leaking AgNPs could also result in peripheral inflammation in a size-dependent manner. Mice receiving single or multiple intravenous injections of AgNPs showed basement membrane injury in glomeruli.

Immune System Toxicity

Our immune system, a natural host defense barrier, is composed of immune cells, tissues and organs, can constantly interact with the internal environment and protect us from pathogens in the external environment, and provide the inherent knowledge to separate the friend and foe within our body [344]. Seung et al. [345] found that AgNPs inhibited the proliferation and the production of cytokines, including IL-5, INF-γ and TNF-α, and induced cytotoxicity in peripheral blood mononuclear cells in a concentration-dependent manner. AgNPs may deposit in the immune organs and influence the number of immune cells and the production of cytokines. Wim et al. [45] investigated the effects of AgNPs on the immune system of rats by repeated intravenous administration of AgNPs with different sizes (20 nm and 100 nm) for 28 days. They found that AgNPs administered at the maximum dose (6 mg/kg) were still well tolerated by the rats. The size and weight of the spleen increased significantly, possibly due to the increased cell number of T cells and B cells. However, the cytotoxic activity of NK cells in the spleen was almost completely inhibited. For multiple immune-related cytokines in serum, levels of interferon-γ, IL-10, IL-6 and TNF-α declined, while levels of IL-1β, IgM and IgE increased. The number of neutrophilic granulocytes in peripheral blood also increased. Besides, brown and black pigments were observed in histopathological sections of spleen and lymph nodes, indicating the accumulation of AgNPs in these immune organs. This study suggested that the immune system was sensitive to the potential adverse effects of AgNPs. The spleen may be one of the main organs for the accumulation and elimination of AgNPs, and both processes are in a sex-dependent manner. Yuying et al. [346] observed the potential acute toxicity and biokinetics after repeated intravenous administration of AgNPs in mice. During the 14-day observation period, both the survival and behavior of the mice were normal. They found that AgNPs were widely distributed in tissues, especially in the spleen, followed by the liver. The biokinetics of AgNPs in the kidney and lung seem to show gender-related differences, i.e. the accumulation of silver in kidney and lung of female mice was higher than that of male mice, the longer elimination half-life and slower clearance of AgNPs in female mice than male mice. Besides, the KCs in the liver were mainly responsible for the retention and elimination of AgNPs. The silver content in the liver significantly decreased after one day. While in the spleen, the marginal zone and the red pulp macrophages contributed to the clearance of silver.

Reproductive System Toxicity

Biological barriers, such as the blood-testis barrier, placental barrier and epithelial barrier, can protect the reproductive system from infection and toxicity. AgNPs can cross the biological barriers to deposit in reproductive organs including testis, epididymis, ovary and uterus. Thus, AgNPs may damage germ cells and related cells, such as primary and secondary follicles, germline stem cells, Sertoli cells and Leydig cells [42, 347]. Besides, AgNPs can also cause changes in sexual behavior by affecting the secretion of hormones within the reproductive organs and glands. Further studies confirmed that the reproductive toxicities of AgNPs are achieved by increasing inflammation, disrupting DNA structure, down-regulating gene expression, decreasing mitochondrial function, inducing ROS production and apoptosis. These toxicities of AgNPs to the reproductive system are size-, time- and dose-dependent [347, 348]. Zhang et al. [347] investigated the effects of AgNPs with different sizes (10 nm and 20 nm) on male somatic Leydig cells and Sertoli cells, and found that cell viability was inhibited by AgNPs in size- and concentration-dependent manners. The 10 nm AgNPs showed more cytotoxicity than the 20 nm AgNPs. and cell proliferation was significantly decreased as the concentration of AgNPs increased from 0 to 100 μg/ml. AgNPs-treated Sertoli cells showed decreased mRNA levels of ZO-1 and Cx43, both are involved in encoding tight junction proteins which playing a crucial role in the formation of BTB. As well as AgNPs-treated somatic Leydig cells showed decreased mRNA levels of StAR, 3β-HSD and 17β-HSD, which are involved in the production of testosterone. It is widely acknowledged that spermatogonial stem cells (SSCs) can continuously proliferate, renew and produce sperms throughout male's postnatal life. Cytokines secreted by Sertoli cells play an important role in the proliferation and renewal of SSCs. In this study, AgNPs-treated Leydig cells secreted decreased level of testosterone, which was responsible for inducing spermatogenesis and maintaining normal functions of Sertoli cells. These results suggest that AgNPs can impair the function of Leydig cells and Sertoli cells, then worsen the function of SSCs, ultimately suppress male fertility. Cynthia et al. [42] evaluated the fecundity and development of Drosophila fed with AgNPs at various concentrations from 0 to 5 μg/mL. AgNPs decreased the viability and delayed the development of Drosophila in a dose-dependent manner. Germline stem cells (GSCs) and early germ cells were concentrated at the apical tip of the testis. Among different treated groups, a significantly increased ROS level was observed at this tip area of Drosophila treated with 5 μg/mL AgNPs. They also proved that AgNPs might disrupt GSCs maintenance by triggering precocious differentiation of GSCs, thereby decreased the number of sperms. Besides, the first generation of Drosophila fed with a higher concentration of AgNPs showed delayed eclosion and decreased male offsprings as compared to control or lower concentration group. The mating success of Drosophila and the number of their second or third generations decreased in AgNPs-treated groups than the control group. This might suggest that AgNPs accumulated in GSCs could be passed onto offspring and affect the development and fecundity of the offspring. Lafuente et al. [37] studied parameters of epididymal sperm of rat fed with different doses of PVP-AgNPs (50, 100 and 200 mg/kg/day), including sperm morphology, motility and viability. PVP-AgNPs induced sperm morphology abnormalities in a dose-dependent manner. In their study, 100 mg/kg/day of PVP-AgNPs significantly increased abnormal morphologies in epididymal sperms, such as banana head, tail bending, head loss and neck abnormalities. Abnormal sperm morphology reduced sperm motility and vitality. Some studies focus on the effects of AgNPs on female reproduction. Chen et al. [349] evaluated the potential toxicity of AgNPs and Ag^+ on zebrafish oocytes. Vacuolation or swollen mitochondria, and condensed nucleus were observed in AgNPs- and Ag^+-treated follicular cells. Zebrafish oocytes treated with AgNPs or Ag^+ showed a decreased concentration of cAMP, which plays a key role in the maintenance of meiosis arrest, and results in meiosis resumption and subsequent oocyte maturation. Besides, AgNPs and Ag^+ up-regulated caspase 3 and caspase 9, respectively, both of which play important roles in the initiation and execution of apoptosis, ultimately leading to apoptosis in ovarian follicle cells.

Conclusion and prospect

Over decades, AgNPs have been studied rapidly and extensively due to the unique physical, chemical, optical, electronic and catalytic properties. These properties are closely related to characteristics of AgNPs, especially the size and shape. AgNPs with different characteristics can be produced by physical, chemical and biological routes. External energy sources such as light, heat, electricity, sound and microwave can be used in the synthesis process. Various factors should be considered in the synthesis of AgNPs with expected size and shape. Besides the types of precursor salts, additives such as reducing agents, capping agents and stabilizers, as well as the importance of reaction parameters, including reaction temperature, time, pH and extra energy sources should be recognized in the production process. Among these methods, biological synthesis using bacteria, fungi and plant extract proves a simple, environmentally friendly, cost-effective and reliable approach. Compared with physical and chemical methods, biological method does not require high temperature or toxic/hazardous additives, but the potential pathogens need to be carefully considered. We review the synthesis methods of AgNPs and compare the advantages and disadvantages to help understand how to obtain nanoparticles with controlled size and shape.

AgNPs have broad prospects in medical applications. Among them, antimicrobial and anticancer properties have received more attention. A variety of factors influence the antimicrobial and anticancer effects of AgNPs, including size, concentration/dose, exposure time, stabilizer and surface charges. The proposed mechanisms for antimicrobial activity of AgNPs involve destroying the structure of cell wall, inducing ROS production and DNA damage. Anticancer mechanisms of AgNPs are more complicated. AgNPs can induce apoptosis and necrosis of cancer cells by destroying cell ultrastructure, inducing ROS production and DNA damage, inactivating proteins and regulating multiple signaling pathways. Besides, AgNPs may block invasion and migration of cancer cells by inhibiting angiogenesis within the lesion. However, the potential cytotoxicity of AgNPs may limit their medical applications. In order to improve the compatibility of AgNPs, proper surface functionalization is widely concerned. The AgNPs surface allows coordination of multiple ligands and thus can be functionalized. The surface functionalization of AgNPs can simultaneously improve their biological safety and challenge their drug delivery, which is conducive to the development of more antibacterial and antitumor agents involving AgNPs. AgNPs can also be used as an additive or adjuvant in bone scaffolds, dental materials and vaccines. The antidiabetic effect of AgNPs is also explored. Besides the impressive antimicrobial and anticancer activities, the unique optical properties of AgNPs make them great clinical potential in the field of biosensing and imaging. The AgNPs surface allows coordination of multiple ligands and thus can be functionalized.

Although most studies focus on the therapeutic purposes of AgNPs, the potential toxicities of AgNPs in multiple systems including skin, eyes, kidney, respiratory system, hepatobiliary system, immune and reproductive systems have been discussed. Further in-depth studies are required to evaluate the biocompatibility and potential cytotoxicity of AgNPs, which may help to develop safer and biocompatible AgNPs-based agents.

In this review, we separately introduce the synthesis method and anticancer properties of Ång-scale silver particles in the relevant sections. Compared with AgNPs mentioned in this review, we prepared pure and fine silver particles with Ångstrom size. This ultra-fine size may be a threshold for silver particles in the medical applications, that is, Ång-scale silver particles exhibit broad-spectrum anticancer activities without obvious cytotoxicity. This exciting discovery inspires us to explore more promising applications of Ång-scale silver particles in nanomedicine.

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Author Contributions

H.X. guided the planning and writing of the review manuscript. L.X., H.X. and Y.-Y.W. wrote the manuscript. L.X. and J.H. prepared the figures. H.X., C.-Y. C., Z.-X.W., L.X. and Y.-Y.W. amended the manuscript.

Competing Interests

The authors have declared that no competing interest exists.

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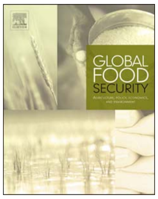

Small-scale poultry and food security in resource-poor settings: A review

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ABSTRACT

Small-scale poultry production systems are mostly found in rural, resource-poor areas that often also experience food insecurity. They are accessible to vulnerable groups of society, and provide households with income and nutritionally-rich food sources. However, they also improve food security in indirect ways, such as enhancing nutrient utilisation and recycling in the environment, contributing to mixed farming practices, contributing to women's empowerment, and enabling access to healthcare and education. Further, they may contribute to several of the Sustainable Development Goals, and to future food security through maintaining biodiverse genomes. In extensive small-scale poultry production systems, significant impediments to achieving these contributions are disease and predation, which can be reduced through improved agricultural and livestock extension and community animal health networks. For small-scale intensive systems, feed price fluctuations and inadequate biosecurity are major constraints.

1. Introduction

Small-scale poultry (SSP) production systems have been integrated with human livelihoods for thousands of years, enhancing diet, income, and food and nutrition security of the rural poor (Alders and Pym, 2009). Currently, global livestock production systems are under scrutiny, given the projected environmental and food system impacts of increasing livestock production to meet the growing demand for animal-source foods (ASFs) (Delgado, 2003). This review highlights literature that demonstrates and describes linkages between SSP production and food security in low- and middle-income countries (LMICs) with limited resources (resource-poor settings). The potential contributions and impacts of extensive, small-scale scavenging poultry production systems in rural, resource-poor areas differs significantly from more intensive systems in urbanised settings; these differences are highlighted while the contributions of SPP to each dimension of food security – availability, access, utilisation and stability – are explored. Lastly, common constraints to small-scale poultry production in resource-poor areas, and, should these be addressed, their potential contributions towards achieving the United Nations' (UN's) Sustainable Development Goals (SDGs) are presented.

2. Methods

2.1. Review of literature

The terms "small-scale poultry", "scavenging chickens", "village chickens", and "backyard poultry" were searched in Web of Science, BIOSIS Previews, CAB abstracts, and Medline, yielding 1176 results. The search was refined by research area (eliminating 254 results), then assessed for relevance to SSP production, resource-poor areas, and food and nutrition security by article title (eliminating 749 results), then abstract (eliminating 141 results), leaving 32 articles reviewed in full. The Food and Agriculture Organization of the United Nations (FAO) document repository, reference lists from selected documents, and the knowledge of co-authors were also utilised to source relevant publications. Information related to rural, family, or backyard poultry were included if they were relevant to SSP production. Results are grouped by relevance to each dimension of food security. As the majority of SSP production systems raise chickens, this review will use the terms "poultry" and "chickens" interchangeably.

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2.2. Poultry system definitions

Small-scale poultry production systems, largely comprised of chickens, account for the majority of the poultry population in LMICs (Gilbert et al., 2015). The term "family poultry" is used for systems which rely on family labour and, generally, locally available feed resources (FAO, 2004; Thieme et al., 2014). Thieme et al. (2014) describes four categories of family poultry production: small extensive scavenging (1–5 adult birds), extensive scavenging (5–50 birds), semi-intensive (50–200 birds), and small-scale intensive production (> 200 broilers or > 100 layers). Although this spectrum of systems may be viewed as a continuum, SSP farmers utilise the production system that best suits their situation and objectives (Rota et al., 2014). More intensive poultry raising systems require reliable access to inputs, including commercial stock, feed, labour, and health services as well as efficient marketing channels (Branckaert and Guèye, 2000; Mack et al., 2005; Thieme et al., 2014). In rural areas, access to markets, cold chains, and veterinary services is typically limited (Thieme et al., 2014).

The largest number of households worldwide are engaged in "village poultry" production, which encompasses the first two systems, and are comprised of mostly indigenous or sometimes crossbred species (Alders and Pym, 2009). In these free-ranging systems, birds largely scavenge for feed, although supplementary feed may be given, and housing, if provided, is simple and made from locally-available materials (Sonaiya, 2004; Thieme et al., 2014). Small-scale poultry production is commonly incorporated into mixed production systems with crops and other livestock, and are a way for vulnerable households to spread risks (Alders et al., 2013; Thieme et al., 2014). Flocks are self-propagating, with broody hens laying 30–80 eggs per year in 2–4 clutches, and spending time between clutches to rear chicks (Fotsa et al., 2014; Mapiye et al., 2008).

3. Dimension one: availability

The availability dimension of food security generally refers to national food availability, taking into account domestic food production, stores, imports, and aid (WFP, 2009), however, it is also considered at the household level. Food availability refers to foods of "appropriate quality", and those which are culturally and socially acceptable by a given population (FAO, 2006). Poultry are generally the most numerous livestock in resource-poor areas, where their contributions to food availability are both direct, through supplying nutrient-rich and culturally acceptable products for human utilisation, and indirect, through enhancing crop, vegetable and other livestock production with the provision of manure and pest control.

3.1. Availability in vulnerable areas

Despite small flock sizes, in aggregate, rural poultry flocks account for 60–90% of the poultry population in many LMICs across Africa and Asia (Akinola and Essien, 2011; Guèye, 2000a; Mapiye et al., 2008). Dolberg (2007) and Gilbert et al. (2015) noted the relationship between income and poultry production systems, showing that extensive, scavenging poultry systems are most commonly found in rural, resource-poor areas. Fig. 1 highlights the overlapping distribution of extensive poultry production systems and food insecure areas.

It is common for livestock to fulfill multiple roles within households in resource-poor settings, and livestock ownership does not necessarily translate to increased utilisation of ASFs (Turk, 2013). However, Azzarri et al. (2014) found that ownership of poultry is associated with increased chicken utilisation. This is likely due to their small size and short production cycles, factors which make households more likely to decide to slaughter or sell in times of need, compared to larger livestock (Kariuki et al., 2013). Rural poultry supply 70–90% of poultry products in Africa (Alabi et al., 2006; Branckaert and Guèye, 2000; Kitalyi, 1998; Mack et al., 2005), and contribute 20–32% of total animal protein intake (Kitalyi, 1998; Tadelle et al., 2003).

There is high demand for meat from indigenous chicken breeds, due to their suitability to local taste preferences and cooking methods (Aini, 1990; Choprakarn and Wongpichet, 2008; Kitalyi, 1998; Umaya Suganthi, 2014). The persistence of SSP production systems in regions where large-scale commercially-produced poultry products are available is an example of food sovereignty, where communities have chosen a sustainable production system that produces healthy, culturally appropriate food.

3.2. As a food source

Meat (both muscle and organ meat) and eggs from indigenous chickens constitute a high-quality food source, densely packed with essential macro- and micronutrients. Animal-source foods are particularly concentrated in highly bioavailable iron, vitamin A, vitamin B12, zinc, and riboflavin - nutrients that are often deficient or absent in the largely vegetarian diets common in rural, resource-poor settings (Bwibo and Neumann, 2003; de Bruyn et al., 2015; Demment et al., 2003; Murphy and Allen, 2003; Turk, 2013). Slaughter of livestock for home consumption is conducive to use of the entire carcass, including organ meats and bones, which are good sources of high bioavailable vitamin A, vitamin B12, iron, riboflavin, niacin, thiamin and folate (Williams, 2007). Consuming foods with high concentrations of bioavailable nutrients is particularly important for infants and young children, with limited gastric volume, pregnant and lactating women who have increased nutrient requirements, elderly people who may have decreased intestinal absorption capacity, and those who are ill (Olaoye, 2011).

Eggs, containing all nutrients required to support the development of a chick, have a "nearly perfect balance of nutrients" (Vizard, 2000) to meet human nutrition requirements. Eggs have been recognised as the lowest-cost source of protein, vitamin A, vitamin B12, riboflavin, iron and zinc (Drewnowski, 2010), and are also a good source of folate, selenium, vitamin D, and vitamin K (Applegate, 2000). Liver and eggs are among the best sources of vitamin A available (Vizard, 2000). Although ASFs are significant contributors to dietary energy and protein, it is their concentration of micronutrients and their ability to counter multiple micronutrient deficiencies that make them particularly valuable food sources. It has been shown that regular ASF consumption has significant positive benefits for children's nutritional status, linear growth, and educational outcomes, leading to increased income and productivity in adulthood (Bwibo and Neumann, 2003; Demment et al., 2003; Murphy and Allen, 2003). Thus, the cumulative benefits of SSP product utilisation are far greater than being an available food source alone. Dolberg (2007) stressed that the consumption of ASFs in LMICs should not be tempered by the known health risks associated with overconsumption of ASFs seen in high-income countries.

3.3. Enhancing food availability and production

One of the major food security concerns related to livestock production is the diversion of potential human food sources to livestock feed, particularly in the case of monogastric livestock (Flachowsky, 2002). However, the scavenging feed resource base (SFRB) utilised in extensive and semi-intensive poultry production transforms feed ingredients in the environment that are less suitable or unavailable for human consumption, including plant seeds, earthworms, and insects, into palatable and nutrient-rich food products for people (Sonaiya, 2004, 2014a).

Small-scale poultry production is commonly used as part of mixed or integrated farming systems, which allows farmers to use resources efficiently, spread risk and protect against shocks (Alders et al., 2013; Prein, 2002). In Bangladesh, Helen Keller International reported great

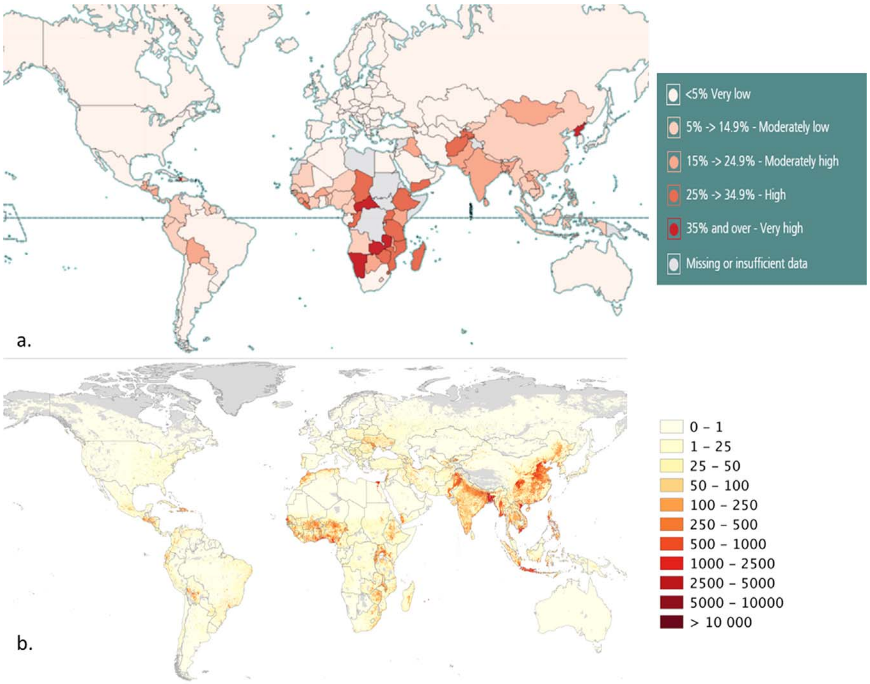

Fig. 1. Globally, the regions of a. higher hunger and food insecurity correlates with b. the distribution and density of extensive poultry producers, showing their importance as a food and income source to these resource-poor areas. Images from a. FAO (2015) and b. Gilbert et al. (2015).

success in the introduction of village poultry to home gardens to enhance homestead food production (Talukder et al., 2010). In other parts of Asia, integrated farming systems make use of chicken manure from overnight housing to fertilise underlying fish ponds, sediment from which is then used as organic fertiliser for crop production (Udo et al., 2006).

Aside from the use of manure, SSP production enhances food production by controlling pest species. Controlled access to crops and vegetables allow chickens to remove pests (Guèye, 2000a), and for livestock species such as cattle, mixed farming with chickens has been shown to decrease the numbers of tick species in the environment and on animals (Dreyer et al., 1997).

4. Dimension two: access

The access dimension of food security refers to the ability of people to obtain available food, which is more difficult for economically, physically, or socially disadvantaged population groups (WFP, 2009). The low-input nature of extensive and semi-intensive SSP production makes it accessible to these vulnerable or marginalised groups who are at a higher risk of food insecurity, in contrast to large-scale or intensive systems which require greater inputs and are often kept by wealthier households (Dolberg, 2007). Village chickens can make significant economical contributions to households, both as a small source of regular income, or as a liquid asset, which can be used by households to access food. When SSP are reared by women, it is possible for this income to be under the complete control of women, increasing their empowerment, which in turn enhances household food security.

4.1. Village chickens and vulnerable groups

Access to a year-round adequate, nutritious diet can be a challenge for all members of resource-poor communities. However increased difficulties are often faced by poorer subsets of society, along with women, children under five years of age, people who are chronically ill, and the elderly (FAO, 2011a). When extensive management systems are used, small-scale poultry production requires few inputs and no land, making it particularly accessible to those with limited income sources (Riise et al., 2005). Alabi et al. (2006) found that 35% of women's income is derived from family poultry, while Dolberg (2007) found that poorer households more commonly kept poultry and pigs than wealthy households. Small-scale poultry have minimal care requirements, which is important for people living with a disability or those affected by chronic illness, such as HIV & AIDS. They are accessible to people living in remote areas, and to disadvantaged cultural groups (Ahlers et al., 2009; Alders et al., 2010; Copland and Alders, 2005; Mutenje et al., 2007). “Chickens are the most popular and the most democratic livestock species raised by households” (Bagnol, 2009), because all members of the household - even children - can own them. For people with limited resources, poultry may be the only livestock they can keep, with Aklilu et al. (2008) reporting that “poultry are the first and the last resource a poor household owns”.

4.2. A liquid asset

One of the central roles of livestock is income generation. In resource-poor settings, chickens are amongst the most affordable livestock, and they may be sold or exchanged for sequentially larger and higher-value species, building a household's asset base, or used to provide income in times of need. As such, they are the first rung on the livestock ladder – “Poultry are the seeds you sow to get the fruits, cattle” (Aklilu et al., 2008). For larger livestock species the highest market price is earned when a mature animal is sold, requiring households to commit much time and resources to raise these animals to the point where they are most profitable. Therefore, the sale, consumption, or offering as gifts of large animal species is infrequent. When households are faced with food insecurity, the decision to sell large livestock at a sub-optimal price is a difficult one, however, small livestock species including poultry mature rapidly, and are consistently available, so they are often the first livestock species sold by households to meet their immediate needs (Mapiye et al., 2008; Maxwell and Smith, 1992).

The economic contributions of SSP to households vary by production system and location. Village poultry brought an average annual income of USD13 to households in Ethiopia (Muhiye, 2007); USD27 in Haiti (Nchinda et al., 2001), USD55 in Mozambique (Woolcock et al.,

2004) and USD124, or 50% of the per capita income, to households in Nigeria (Alabi et al., 2006). Alabi et al. (2006) estimated that ten laying hens can earn Nigerian households USD100 per annum, and in Indonesia, ten laying hens vaccinated against Newcastle disease can generate more than 25% of the monthly household income (Moerad, 1987).

In Lao PDR, village poultry production produced a net household annual income of USD67. With control of Newcastle disease and Fowl Cholera, net household incomes from meat production were between USD97 in remote areas, USD120 in rural villages, and USD108 in regions with access to the cold chain (Alders, 2004). In India, net income from indigenous poultry production differs by socioeconomic status: households with incomes in the middle and upper 20% earned a greater profit from their poultry, however they delivered a lower net profit margin ratio than producers in the lower 20% income group due to higher expenditures on inputs (Ahuja et al., 2008). Wantasen et al. (2014) found that purchased feed was the greatest cost in small-scale semi-intensive chicken production in Indonesia, although farmers earned a net annual income of IDR4,329,038.

There is typically a consumer preference for local chicken meat due to suitability of taste and texture, and the minimal use of pharmaceuticals during production (FAO, 2010; Guèye, 2000a; Melesse, 2014; Umaya Suganthi, 2014). Although production is low and markets are limited, local chickens and eggs fetch a significantly higher market price, from 1.5 to 3 times the cost of a commercial product, (Barua and Yoshimura, 1997; Choprakarn and Wongipechet, 2008). Income from the sale of SSP products allow households to purchase a greater variety of food, and cover schooling and healthcare costs (Alam, 1997; Mapiye et al., 2008). Small-scale poultry products have an advantage over larger livestock species in that although market access greatly aids the sale of SSP products, they are mostly sold or bartered locally and do not require the presence of formal markets (Akinola and Essien, 2011).

4.3. *Women's empowerment*

In many LMICs, poultry are often the only livestock under the independent control of women (Bagnol, 2009; Thieme et al., 2014). In Zimbabwe and Ethiopia, it was found that village chickens belong almost exclusively to women (Mapiye et al., 2008; Tadelle and Ogle, 2001), who are responsible for the care of the birds, and for selling chickens and eggs. Men are often involved in the construction of shelter for the chickens, or in their treatment or slaughter, however even in male-headed households, women are often responsible for decision-making on issues related to chicken production (Dolberg, 2007; FAO, 2010; Kusina et al., 2001; Muchadeyi et al., 2004).

Income from the sale of poultry products is often the main source of income for female-headed households, whereas male-headed households usually have multiple income sources (Aklilu et al., 2008; Muchadeyi et al., 2004). It has been found that 90% of income under the control of women is channeled back into their households or local communities, in contrast with only 30–40% for men (OECD, 2009), and that women use their income to increase the quantity and variety of foods purchased, on medical care, and on schooling for children (Meinzen-Dick et al., 2011). In this way, women's income leads to greater improvements in household health, education and nutritional status than men's income, and has a positive impact on household food security (Guèye, 2000b; Meinzen-Dick et al., 2011; Muchadeyi et al., 2004). Village poultry production systems are a particularly important income-generating activity for women, as they place little demand on mothers' time, allowing adequate time allocation to child care, a crucial element to achieving good nutrition (Quisumbing et al., 1995).

The inclusion of women in SSP training programs to become community vaccinators increases the knowledge and standing of women within their household and the wider community (Alders et al., 2010; Bagnol, 2012). Livestock interventions that target species under the control of women, including SSP, may enhance the impact upon household food and nutrition security through the empowerment of women.

5. Dimension three: utilisation

This dimension refers to both household and individual utilisation of nutritious and safe diets, acknowledging elements such as health status, sanitation, feeding practices, and food safety, which can all impact utilisation and nutrient waste (WFP, 2009). It is found that in many contexts taboos prohibiting the consumption of eggs by children and pregnant women exist (Bagnol, 2001), meaning that even if nutrient-rich food items exist in the household, it might not be utilised by all the members in the same way. In the absence of Newcastle disease control, it has been observed that households very rarely utilise chicken and eggs, preferring to keep the eggs to produce chickens that can be sold, often to allow the purchase of staple foods and other less nutritious food (Bagnol, 2001). Control of Newcastle disease increases the availability of eggs and healthy chickens for consumption (Alders et al., 2010). Poultry meat can be consumed on the day of slaughter, and eggs do not require storage facilities, although care needs to be taken with zoonotic diseases. The concentration of *haem* iron increases the bioavailability of other nutrients in food, and income from the sale of SSP products can also be used to provide access to healthcare and improved sanitary environments.

5.1. *Nutrient bioavailability*

Due to similarities between human and animal physiology, ASFs contain a variety of nutrients in a readily available form. Animal proteins have a digestibility of up to 98%, compared to 75–85% for foods from plant sources (Bhutta, 2005). Iron is pre-bound in a *haem* molecule, allowing direct absorption, whereas iron from plant sources requires transformation within the gut before absorption is possible (Allen, 2003; Murphy and Allen, 2003). Similarly, vitamin A is found as pre-formed retinol, ready for direct absorption (Allen, 2003; Murphy and Allen, 2003). As iron and vitamin A are two of the most widespread micronutrient deficiencies (Ahmed et al., 2013), particularly in LMICs, regular utilisation of highly bioavailable micronutrient source foods can help to ameliorate these deficiencies.

Micronutrient absorption is of particular concern when diets largely consist of cereal and tuber staple foods and vegetables. High levels of fibre, phytate and oxalate decrease micronutrient bioavailability from plant-based diets, with absorption of iron, zinc and calcium being particularly affected (Allen et al., 1991; Gibson, 1994; Libert and Franceschi, 1987). The addition of even small amounts of ASFs to a largely vegetarian diet counteracts this inhibition, thereby enhancing overall micronutrient absorption (Allen et al., 1991; Fairweather-Tait and Hurrell, 1996; Hallberg and Hulthén, 2000; Leroy and Frongillo, 2007; Miller and Welch, 2013; Welch and Graham, 2000).

A final contribution of SSP to utilisation is through the use of income generated from poultry and egg sales allowing greater access to health services, household sanitation and hygiene and providing opportunities to purchase a greater quantity and variety of foods (Guèye, 2000b).

5.2. *Food safety*

Poultry products are well-sized for immediate utilisation. Eggs, when their shell is intact and when stored under appropriate conditions, are sterile and easy to cook (Board et al., 1994). Chickens can be slaughtered and consumed by households in a single meal, eliminating the need for meat storage, which is required for larger livestock species (Aklilu et al., 2007). However, zoonoses such as highly pathogenic avian influenza (HPAI) and bacterial contamination with *Salmonella* and *Campylobacter* species are potential public health risks, especially given the common practice of slaughtering and consuming sick birds or recently dead birds (Conan et al., 2012; Alders et al., 2014). Effective husbandry and disease control leads to increased flock sizes and provides assurance of stability of supply of poultry products. Such strategies increase the availability of healthy chickens, and can help to reduce the likelihood that food-insecure households will resort to eating diseased or dead chickens (Alders et al., 2013).

5.3. Environmental interactions between humans and livestock

Recent literature has raised concerns regarding the potential for shared environments with poultry to have adverse effects on child growth outcomes. In resource-poor settings, chickens are often housed within human dwellings overnight, to prevent predation and theft. There is evidence to indicate a positive association between exposure to livestock faeces and diarrhoea in children (Zambrano et al., 2014), and suggestions that even non-pathogenic bacteria can contribute to a subclinical condition of the gastrointestinal tract (environmental enteric disorder; Mbuya and Humphrey, 2015) – potentially contributing to poor nutrient absorption and a heightened risk of chronic growth restriction or stunting (Korpe and Petri, 2012; The SHINE Trial Team, 2015).

A cross-sectional study of rural households in Ethiopia reported an overall positive association between poultry ownership and height-for-age *Z*-scores in children (with stunting defined as Z-scores below −2), but a negative association if poultry were housed indoors (Headey and Hirvonen, 2016). By contrast, longitudinal findings from Tanzania indicate no significant association between poultry ownership or the practice of keeping chickens indoors overnight and the height-for-age or incidence of diarrhoea in children (de Bruyn et al., 2016). Elsewhere, studies have found no relationship between livestock ownership and growth outcomes in children (Headey and Hirvonen, 2016; Mosites et al., 2016). For children over six years of age, exposure to livestock and higher levels of microbial endotoxin in the home environment has been shown to enhance innate immunity and associated with decreased levels of respiratory disease (Stein et al., 2016).

Clearly, the interactions between human immune systems, livestock and the environment are complex and further research is needed in this sphere.

6. Dimension four: stability

The fourth dimension in food security is stability in food availability and accessibility, and resilience in adapting to economic or environmental shocks or changes (FAO, 2006). Small-scale poultry can be a year-round source of food when threats to production, particularly Newcastle disease, are addressed and controlled. Village chickens are hardy, well-adapted to their environments, and genetically diverse; inherent characteristics that, when combined with their extensive production system, can improve survival rates in the short term during disease outbreaks, and in the long term, through climate change. As extensively-raised monogastrics that do not require land clearing for production, their carbon and water footprints are low, and their manure contributes to soil health (Chantalakhana, 2000) – in contrast to intensively-raised poultry whose largest greenhouse gas contribution is from feed production (Gerber et al., 2013). Increasing the availability of SSP products for consumption may also play a role in wildlife conservation. Lastly, income earned through SSP production is often used to pay for children's education. Through increased opportunities for schooling and the empowerment of women, SSP production systems can contribute to the food security of future generations.

6.1. Genetic diversity of indigenous chickens

Biodiversity loss is a great threat to ecosystems, with genetic losses having strong negative impacts on ecosystem function, efficiency and stability (Cardinale et al., 2012). At present, up to 70% of the word's livestock breeds are found in LMICs (Rege and Gibson, 2003). A review (Eltanany and Distl, 2010) of the genetic diversity within and between chicken breeds and populations found higher genetic diversity within indigenous and village chickens compared to commercial breeds, a finding shared by Elkhaiat et al. (2014), Lyimo et al. (2014), Mahammi et al. (2016), and Mercan and Okumus (2015) highlighting their value in genetic conservation.

Indigenous poultry breeds have co-evolved with their environments, and have the highest likelihood of survival in harsh conditions due to their disease resistance, ability to scavenge and avoid predators, and their broodiness (Besbes, 2009; Fotsa et al., 2014; Guèye, 1998; Umaya Suganthi, 2014). The introduction of commercial breeds to these environments has often been hampered by high mortality rates, and crossbreeding between local and commercial breeds can have negative effects on broodiness and mortality rates (Pym, 2013; Udo et al., 2011), although some successes are reported in Section 8.1.

An assessment of heat tolerance found that commercial chicken breeds have lost the coping mechanisms that allow village and red jungle fowl to tolerate higher ambient temperatures (Soleimani and Zulkifli, 2010), although genetic feather variations including Naked-neck allow a greater degree of heat tolerance (Melesse et al., 2011). With increasing climate variability, extremes in weather conditions are expected to become more frequent, feed quality and water availability will decline, and the incidence of illness in livestock increase (Thornton et al., 2009). Future food production challenges are unpredictable and likely will include new diseases or more virulent recurrent diseases, and environmental changes necessitating alternatives. Therefore, a healthy and diverse genetic reservoir in food-producing animals remains as crucial as ever (Pym and Alders, 2016). Muir et al. (2008) suggested that non-commercial flocks, including those found in many LMICs, could potentially represent the reservoir opportunity for alleles 'missing' from commercial pure line stocks.

Thus, conservation of the indigenous genepools raised under SSP production systems may not only contribute to ecosystem health, but may ensure the long-term survival and productivity of poultry as livestock.

6.2. Environmental footprint

Adverse environmental impact is a predominant concern in agriculture, particularly in livestock production, which accounts for around 15% of greenhouse gases (GHGs) arising from human activity (Gerber et al., 2013). For intensively raised livestock, 61% of emissions are attributed to beef and dairy cattle production, nine percent to pig meat production, and eight percent to poultry meat and egg production (Gerber et al., 2013), with the most significant emissions arising from ruminant enteric methane production, nitrous oxide from feed production, and land use (Herrero et al., 2015). Gerber et al. (2013) reported that subsistence-driven chicken production is less efficient than intensive production, so emission intensities are higher in the former systems, however they account for less than 10% of poultry's greenhouse gas emissions.

Despite inefficiencies of production, SSP contribute to environmental health in ways that have not yet been adequately understood. The interaction between crop and livestock production in mixed farming systems allows nutrient cycling to improve soil fertility, and the use of manure adds organic matter to soils, improving water-holding capacity and structure (Chantalakhana, 2000; Devendra and Chantalakhana, 2002).

Despite a distinct lack of published data on the nutrient content of ASFs produced in resource-poor settings (de Bruyn et al., 2016), available data indicates protein production to be more nutritionally and ecologically efficient in poultry meat and eggs than in beef or pork (Flachowsky, 2002; Pelletier and Tyedmers, 2010). Improvements in SSP management systems that increase production efficiency could lower emission intensities while maximising ecosystem benefits. With increasing urbanisation, and population and income growth in LMICs, the increasing demand for ASFs is predicted to continue (Pica-Ciamarra and Otte, 2011). Sourcing ASFs from livestock species and production systems with lower GHGs emissions would be the optimal choice.

6.3. *Role in wildlife conservation*

The hunting and consumption of non-domesticated animals (bushmeat) can be a significant source of protein and micronutrients for rural families (Alders and Kock, 2017; Foerster et al., 2012; Golden et al., 2011), however, hunting of threatened or endangered species is unsustainable and leads to loss of biodiversity (Foerster et al., 2012; Harrison et al., 2016). Studies have found that the primary goal of hunting by rural, resource-poor households is for consumption (Albrechtsen et al., 2005; Brashares et al., 2011; Foerster et al., 2012). These studies concluded that increasing access to affordable alternate protein sources would decrease hunting pressure on wildlife, and contribute to the conservation of endangered species. Increasing the output and efficiency of SSP production in communities living in close proximity to protected environments increases the availability of ASFs, and has the potential to decrease other income-generating activities such as tree felling (Ahlers et al., 2009; Chantalakhana, 2000; Dumas et al., 2016).

7. Constraints to small-scale poultry production

The ability of SSP production to achieve maximal contributions to food and nutrition security can only be realised if the constraints to production are addressed. Major constraints include inherent low production, disease, and predation in extensive systems, and fluctuations in feed prices and inadequate biosecurity in intensive systems. In addition, inadequate nutrition, housing, and access to veterinary services and appropriate extension materials all present potential barriers to production. To successfully address these barriers, management practices need to be assessed for local sustainability, cost-effectiveness and the greater involvement of women.

7.1. *Low productivity*

When judged by conventional measures of productivity commonly used in the commercial poultry sector such as feed conversion ratios or daily weight gain, local chicken breeds are low and slow producers of eggs and meat. Even under ideal housing and feeding conditions, productivity in indigenous breed chickens is much lower than in their commercial counterparts (Sørensen, 2010). Indigenous birds kept under village settings expend a significant amount of energy scavenging for feed and evading predators, lowering their growth rate and egg production, and hens spend up to 75% of their time hatching eggs and rearing chicks (Pym and Alders, 2012). Notwithstanding this, these birds survive and reproduce in the harsh village environment where commercial breeds perform very poorly. In conjunction with their other contributions to the household, comparative productivity as measured by benefit-cost ratio or net profit margin ratio, is higher in the indigenous breeds (Ahuja et al., 2008).

7.2. *Disease*

The most common cause of the high mortality rates observed in SSP flocks, particularly in tropical countries, is Newcastle disease (ND) (Alders et al., 2010; FAO, 2014; Spradbrow, 1993). Newcastle disease virus is highly infectious among chickens, and virulent strains can cause up to 100% mortality annually (Samal, 2011). Alders et al. (2010) found that the training of community vaccinators to administer thermotolerant ND vaccines every four months was effective in controlling ND, and greater sustainability was associated with the involvement of women as community vaccinators and farmers paying a small fee for vaccination services to assist in cost-recovery. The benefits of ND control included increased income for female-headed households, increased utilisation of poultry products, and increased trust between community and government (Alders et al., 2010). Aklilu et al. (2007) found that ND control in SSP production systems can enable incomes to be doubled and nutrient intake to improve. Fundamental in the success of ND control through vaccination is the establishment of a network of community vaccinators who receive payments from farmers to compensate them for their efforts (Bagnol et al., 2013).

The control of ND is particularly important in the face of an HPAI outbreak, as the two diseases are clinically indistinguishable (Gardner and Alders, 2014). The emergence of and response to HPAI placed a heavy burden on SSP producers, directly, due to the loss of birds, and indirectly, as initial control measures resulted in massive depopulation, often with inadequate or no compensation (Mack et al., 2005). These measures lead to decreased trust between SSP farmers and authorities, and discouraged many farmers from continuing with SSP production (Mack et al., 2005; Otte et al., 2008). Studies examining the impact of HPAI and its control found devastating effects on smallholder flock sizes, livelihoods and children's nutritional and educational status, with women and poor to very poor households most affected (Alders et al., 2013; Bagnol, 2009; FAO, 2009). However, the rapidly fatal course of disease, relative fragility of the virus and lack of interconnectedness between SSP inputs and outputs means that SSP farms are at lower risk of exposure and propagation of HPAI (FAO, 2008, 2011b; Otte et al., 2008). These factors and the importance of SSP flocks to their farmers are now recognised, with the FAO stating that although achieving biosecurity in SSP systems is difficult, “it is neither feasible nor desirable to limit scavenging poultry as a livelihood option for the poor” (FAO, 2008).

Importantly, the control of ND facilitates the early detection of HPAI, allowing a more rapid response in order to control losses. Once ND control is established, other diseases may rise to significance. Currently, the economically significant diseases include fowl cholera in Southeast Asia, and fowl pox in south-eastern Africa (FAO, 2014).

7.3. *Predation, housing and nutrition*

In addition to disease outbreaks, a significant constraint to SSP production is the loss of chicks through predation, inadequate nutrition, and environmental stress (Ahlers et al., 2009). These issues can also affect adult chickens, however, chicks are more vulnerable and typical attrition rates range from 50% to 70% (Ahlers et al., 2009; FAO, 2010). The provision of dedicated nests for hens to brood, more intensive management of chicks including the provision of protective housing, and food and water supplementation, can help to reduce losses and contribute to increased flock size (Ahlers et al., 2009; Melesse, 2014).

Adult chickens can be protected from predation through the provision of simple night-time housing designed to minimise predator access (Ahlers et al., 2009; Melesse, 2014). Housing at night time also protects chickens from weather extremes, while still allowing full use of the SFRB during the day, and facilitates feed supplementation, inspection or vaccination of chickens as required (Ahlers et al., 2009).

The SFRB provides the greatest input for extensively-raised chickens, and consists of plants and insects found in gardens, fields and forests, household wastes, and crop by-products (Roberts, 1992). It is subject to seasonal change, may result in suboptimal nutrition during particular months of the year (Ahlers et al., 2009; Ncobela and Chimonyo, 2016; Raphulu et al., 2015). Supplementary foods from local ingredients that include protein and mineral sources can improve nutritional status during these times (Ahlers et al., 2009), and is particularly important for chicks. The SFRB is a limiting factor in SSP production, as exceeding maximal capacity will lead to undernutrition of all birds utilising the resource (Roberts, 1992), sometimes resulting in clinical manifestations of hypovitaminosis A, amongst other micronutrient deficiencies. With increased SSP production, farmers will need to actively manage their flock, planning to sell chickens or eggs at times when prices are higher or when the SFRB is insufficient (Ahlers et al., 2009).

For small-scale semi-intensive systems, especially in rural areas, availability of nutritionally-balanced, age-appropriate feeds is a major constraint. Wantasen et al. (2014) found that poultry feeds were the greatest expense for farmers using this system. Fluctuations in feed costs can have a major impact on economic return and sustainability of production, and to manage this risk, farmers would need to have access to credit or microfinance (Sonaiya, 2014b).

7.4. Veterinary and extension services

In many rural areas in LMICs, the widespread area and a lack of resources and infrastructure can result in limited veterinary and extension services (FAO, 2014). Where they exist, they are often focused on crop or ruminant production, with little health care or advice accessible to SSP keepers (Bagnol, 2009). This negatively impacts farmer access to information, including about adequate biosecurity practices, a major concern for small-scale intensive poultry producers (Alders et al., 2014). However, the formation of networks of community-based animal health workers, where training and knowledge is passed between veterinarians, governments and communities, has been found to be effective in both delivering services such as vaccination or health care, and reporting, investigating, or controlling animal diseases (FAO, 2010; Leyland et al., 2014).

A lack of consideration of gender issues can also limit the effectiveness of extension services. Data from the FAO indicate that female farmers receive only 5% of agricultural extension services; that only 15% of extension workers are women; and that only 10% of agricultural aid goes to women (FAO, 2016). This situation indicates the current bias towards men in the agriculture sector. Much of the training, communication and extension materials are directed at men, and women, who are the main carers in SSP production systems, may not receive the information they need (Bagnol, 2012; Guèye, 2000a). Lower literacy levels among women also decrease the utility of written communication materials, with oral or visual materials being more effective in these settings (Bagnol, 2012). Ensuring gender equity in the selection of community animal health workers can allow more effective communication with male and female poultry keepers (Bagnol, 2012), and a gender sensitive approach at all levels of the intervention is necessary to ensure that women benefit from interventions involving poultry-raising activities (Bagnol et al., 2013; Guèye, 2000a).

8. Looking ahead

8.1. Innovations towards market orientation

For many farmers, village poultry are raised for home utilisation and for emergency income, requiring minimal inputs, often as part of mixed farming systems. Choprakarn and Wongipechet (2008) stated that these systems are still appropriate for most SSP farmers in Thailand, and changes in management systems are not always suitable. However, as economies develop and access to inputs and markets improve, use of high-value SSP stock can enable some households to move to more market-oriented production (Ahuja et al., 2008).

Selective crossbreeding can combine desired characteristics of indigenous and commercial chicken breeds. The Kuroiler chicken was developed in India in 1993 as a high-yielding, fast-growing dual-purpose bird that retains its indigenous feather colours, ability to evade predators, disease resistance, and suitability to rural environments (Ahuja et al., 2008; Sharma et al., 2015). Kuroiler chickens are produced at a parent farm, with hatchery units then supplying day-old chicks to “Mother Units” to rear until two to three weeks of age. A network of mobile vendors then sell these chicks door-to-door in villages, where households rear the Kuroiler chickens mostly for sale in village markets, but also for home utilisation (Ahuja et al., 2008). Kuroiler households could earn more than 5 times the income from poultry production than non-Kuroiler househlds, although net profit margin ratios were lower due to higher production costs (Ahuja et al., 2008).

In Thailand, the Kai Baan Thai (Thai Village chicken) has been developed as a fast-growing broiler that retains the meat texture and flavour characteristics of indigenous chickens (Choprakarn and Wongipechet, 2008). This is an example of the commercial adoption of indigenous poultry genetics to supply a high-end, niche market.

8.2. Small-scale poultry for sustainable development

When considering the increasing demand for food by a growing global population and the challenges facing agriculture in the near future, including the impact of climate change, and decreasing land and water availability, the outlook may seem bleak. However, the UN's SDGs have been developed as a set of globally applicable guidelines and targets aimed at improving human life, eradicating poverty, promoting peace and prosperity, and protecting the planet (UNDP, 2015). Although SSP production will not be the only solution to increasing global food production, they can have a strong impact on the most vulnerable sectors of society and play a role in the sustainable development of communities. Table 1 summarises the potential contribution of SSP production systems to eight of the 17 SDGs. By tackling the constraints to SSP production efficiently, their potential contribu-

Table 1

Contributions of small-scale poultry to the UN Sustainable Development Goals (Alders and Pym, 2009; de Bruyn et al., 2015; UN, 2015).

Contribution pathway of small-scale poultry	Sustainable Development Goal
Increasing the availability, accessibility, utilisation and stability of supply of food and nutrients.	2: Zero hunger 3: Good health and well-being
Small-scale poultry are able to be kept by vulnerable groups, giving them access to a source of income. Community-supported models for Newcastle disease prevention can provide employment, including for women, and increased production can promote rural economic growth.	1: No poverty 8: Decent work and economic growth
By targeting a livestock species and production system that is largely under the control of women, improvements to the SSP production systems can preferentially benefit women, promoting their empowerment. Income under the control of women is also more likely to be used to support the education of their children.	5: Gender equality 4: Quality education
Efficient and sustainable use of natural resources while achieving adequate nutrition globally requires high-income countries to decrease food wastage and consumption of calorie-dense, nutrient-poor foods, while low-and-middle-income countries need to increase their consumption of nutrient-rich foods. Small-scale poultry are nutritious and locally-available, typically with a short supply chain, and measures to improve health and welfare will improve production efficiency and ensure sustainability.	12: Responsible consumption and production
Production of SSP does not require land clearing, contributes positively to ecosystem health, and can reduce loss of biodiversity by being a rich pool of genetic diversity and by being an alternate protein source to bushmeat.	15: Life on land

tion to sustainable development can be enhanced.

9. Conclusion

The roles of SSP in LMICs are many, and this review highlights the multitude of avenues through which they can contribute to improved household food and nutrition security. As a highly available and accessible form of livestock in rural, resource-poor areas that often experience food insecurity, SSP are a significant source of income, nutrition and security for the poorest of households. In particular, the importance of these systems to the livelihoods of women, children, the elderly, and the chronically ill should not be overlooked. Barriers to maximising the potential impact of SSP production systems are significant, with high burdens of disease and predation limiting production and utilisation of poultry products, but many of these constraints can be addressed with local adaptations of management strategies, including the development of gender-sensitive training and extension materials (Bagnol et al., 2013). Finally, SSP production systems have persisted for thousands of years, and the local chickens within these systems are well-adapted to harsh environments. Recognition of their ability to survive and reproduce in these conditions, their value as a rich source of genetic biodiversity, and their potential to contribute to sustainable development should promote interest in investing in the protection and conservation of local breeds kept in SSP systems.

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