THE EFFECT OF PLANT PARASITIC NEMATODES ON POST HARVEST QUALITY OF GROUNDNUTS (*Arachis Hypogaea*)

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The effects of plant parasitic nematodes on post harvest quality of groundnuts (Arachis Hypogaea) / Marahaini M. Markam.

THE EFFECT OF PLANT PARASITIC NEMATODES ON POST HARVEST QUALITY OF GROUNDNUTS (*Arachis Hypogaea*)

By

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ENDORSEMENT

The project report entitled **The Effects of Plant Parasitic Nematodes On Postharvest Quality of Groundnuts (*Arachis hypogea*)** by **Marahaini binti M. Markam** , Matric No **UK15413** has been reviewed and corrections have been made according to the recommendations by examiners. This report is submitted to the Department of Agrotechnology in partial fulfillment of the requirement of the degree of Bachelor of Science in Agrotechnology (Post Harvest Technology), Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu.

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DECLARATION

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ABSTRACT

The objective of this study is to investigate the effects of plant parasitic nematode towards groundnut plants and production. This is due to the problem in kenaf plantation by Lembaga Tembakau Negara (LTN) in Telaga Papan, Merang, Terengganu where plant parasitic nematode had been a serious constraint of the field production. The nematode infected soil in the kenaf field was sampled and used for two tests. The first test was bioassay with tomato and kenaf plants where galls and egg masses caused by nematode was found respectively in the plants roots. The number of galls and egg masses occurred in kenaf roots was higher compared to the tomato. The second test was conducted for groundnut plantation where two sets of groundnut plantations were prepared in two separate culverts. One of them filled with sample soil and the other with the control soil. Both culverts were planted with 15 groundnuts variety menglambu. The growth of the plants from sample soil seemed stunted with yellowing leaves and chlorosis compared to the plants from control soil which is healthier. After staining with boiling Fuschin Acid, the observation under compound microscope showed the presence of plant parasitic nematode in the roots of the groundnut plants from sample soil. The yield production by the plants in nematode infected soil, which is estimated by the weight and number of pods produce by plants was lower than the plant production from control soil. The total weight produced from sample soil is 26%, lower than the production in control soil which is 74%. The pods from sample soil also showed lower quality in aspect of colour and sizes and some lesions were observed.

ABSTRAK

Objektif kajian ini adalah untuk mengkaji kesan nematoda perosak tanaman ke atas tanaman dan hasil tanaman kacang tanah. Ini adalah berikutan dengan masalah yang dihadapi oleh penanaman kenaf oleh Lembaga Tembakau Negara (LTN) di mana nematoda perosak tanaman telah menjadi penghalang terhadap hasil tanaman. Sampel tanah telah diambil di ladang penanaman kenaf tersebut untuk digunakan terhadap dua ujikaji. Ujikaji pertama ialah bioassay terhadap tanaman tomato dan kenaf di mana pembengkakkan dan telur didapati pada bahagian akar kedua-dua tanaman tersebut. Bilangan pembengkakkan dan telur pada akar pokok kenaf lebih tinggi berbanding pada akar pokok tomato. Ujikaji kedua pula diadakan pada tanaman kacang tanah di mana dua set penanaman dilakukan pada dua bekas penanaman yang berasingan. Satu daripadanya diisi dengan tanah kawalan. Kedua-duanya ditanam dengan 15 pokok kacang tanah variety menglambu. Tanaman pada tanah sample menunjukkan pertumbuhan yang terbantut beserta dengan kekuningan dan klorosis pada daun berbanding dengan pokok dari tanah kawalan yang lebih sihat. Selepas pewarnaan dengan larutan asid Fuschin mendidih, pemerhatian di bawah mikroskop menunjukkan kehadiran nematoda perosak tanaman di dalam akar pokok dari tanah sampel. Hasil tanaman pokok dari tanah sampel, yang dikaji dari segi jumlah berat dan bilangan biji kacang dihasilkan adalah kurang daripada hasil tanaman dari tanah kawalan. Jumlah berat hasil tanaman dari tanah sampel ialah 26% jauh lebih rendah dari hasil tanaman tanah kawalan iaitu 76%. Biji kacang yang dihasilkan dari tanah sampel juga menunjukkan kualiti yang rendah dari segi warna, saiz, dan juga kehadiran luka-luka pada kulit kacang.

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Groundnut (*Arachis hypogaea*) is one of important plantation crop in the world. The world's total production of groundnuts is in range of 37.1 million tonnes with average productivity yield in about 1.4 t/ha. The usage of groundnut includes production of edible oil (cooking oil). Major groundnuts producer are mainly India, China, and United States of America. Their total production accounts 70% from world's groundnuts production. In Malaysia, main location of groundnut plantations are Kelantan (514 ha), Kedah (305 ha), Perak (240), and Terengganu (192 ha). In Malaysia, groundnuts are used in variety of ways such as boiled and baked peanuts, or for processed products such as chocolates coated peanuts, biscuits or peanut butters (Ariffin *et al*, 2006). Peanut oil is a mainly monounsaturated fat (50%), much of which (97%) is oleic acid. Saturated fatty acids compose 13% of peanut fat, where palmitic acid is the most present (74%) followed by stearic acid (16%). Peanuts are regarded as an unbalanced source of fat because they have only trace amounts of required Omega-3 fats. Some brands of peanut butter are fortified with Omega-3 in the form of flaxseed oil to balance the ratio of Omega-3 to Omega-6.

Peanuts are a rich source of protein (roughly 30 grams per cup after roasting). Prior to 1990 the PER (Protein Efficiency Ratio) method of protein evaluation considered peanut protein along with soy protein as an incomplete protein, as they contain relatively low amounts of the essential amino acids cystine and methionine (but high in lysine). Peanut is used as a staple food must also include complementary food such as whole grains like corn or wheat, which are adequate in methionine but limited by lysine. Protein combining has been largely discredited. Since 1990, the gold scale of standard for measuring protein quality is the Protein Digestibility Corrected Amino Acid Score (PDCAAS) and upon such criterion, peanut protein and other legume protein such as soy protein is nutritionally equivalent to meat and eggs, essential for human growth and health.

The cultivated groundnut, or peanut (*Arachis hypogaea* L.), is an annual, self pollinating, herbaceous legume native to South America. It is a geotropic plant that produces its pods (fruits) underground. Flowering begins 4 – 6 weeks after planting and extends over a period of several weeks. Within about 1 week after the flowers are fertilized, pointed needle-like structures, carpophores, commonly called 'pegs', develop, elongate, and grew into the soil in a depth of 2-7 cm. Upon entering the soil, the fertilized ovaries located behind the tip of the peg enlarge rapidly and pod growth begins. Two to four seed are formed within a pod; however the number of seeds per pod depends on the groundnut variety. The length of time necessary for pod development to maturity varies with cultivar and environmental condition, as for example, Florunner requires 63-70 days from the time the ovary begins enlarging to maturity.

Figure 1.1.1: Peanut leaves and freshly dug pods.

(Source: en.wikivisual.com/index.php/Arachis)

Groundnut has numerous nematode parasites. Among the most common and economically important nematodes of peanuts are listed as the root knot nematode (*Meloidogyne* spp.), lesion nematode (*Pratylenchus brachyurus*), testa nematode (*Aphelenchoides arachidis*), sting nematode (*Belonolaimus longicaudatus*), pod nematode (*Ditylenchus africauns*), and the *Aphasmatylenchus straturatus* (Bridge and Starr, 2007).

Plant parasitic nematodes are all obligate parasites (only feed on living plants). They are usually found in the soil and in plant roots, but a few species may attack above ground-parts of the plant. Most species are microscopic, with lengths ranging from 300-400 μm and diameters 15-35 μ. Such are within the range of large fungal hayphae. Nematodes undergo four molts during their lifecycles. Most nematodes have a worm-like shape, with the body much longer than it is wide (Trigiano et al, 2008).

The word nematode is derived from Greek word meaning "thread-like". The bodies of these simple animals are arranged in a tube shape contained within the tough, flexible cuticle. Within the outer layer of cuticle, the front part of the nematode contains the intestines and reproductive organs. Each of these organ systems is also arranged as a simple tube-shaped structure. There is no circulatory or respiratory system, but nematodes contain most of the organ system of higher animals, including digestive, reproductive, excretory, nervous systems, and several types of muscles. The adults are larger than juveniles, and in some species may be shaped differently. Most nematodes may be observed easily under a dissecting microscope at 40-60×. Detailed observations needed to identify nematodes species, however require much higher magnification (600-1000×). The amount of time it takes for plant parasitic nematodes to complete their life cycle range from few weeks to more than a year depending on the nematode species, plant host status and ambient temperature.

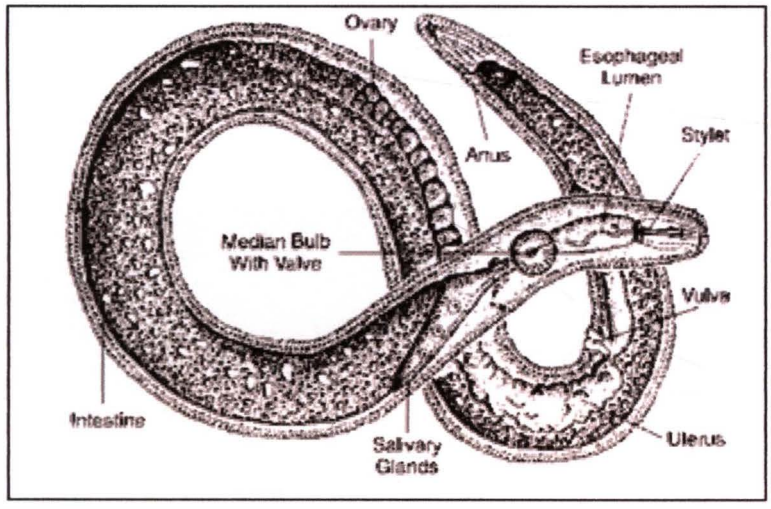

Figure 1.1.2: General morphology of plant parasitic nematodes. (Source: Todd, 1993)

Plant pathogenic nematodes either enter their hosts and feed from within (endoparasites e.g. *Meloidogyne* spp.), feed externally without entering the host (ectoparasites e.g. *Tylenchorhynchus* spp.), or may feed by remaining partially

embedded in the host (semi endoparasites e.g. *Tylenchulus semipenetrans*) (Pathak et al, 2006).

1.2 Problem Statement

Pests and disease are important constraints on crop yields in most countries. When compared with insect pests and fungal diseases, very little research has been done on nematode problems of certain crop such as groundnut especially in Malaysia.

Root-knot nematodes *(Meloidogyne* spp.) are reported to cause serious losses in various crops in the temperate, tropical and subtropical regions of the world. Plant nematodes are primary parasites of groundnuts in all production regions of the world. Based on a worldwide survey of nematologist, annual losses caused by all nematodes to groundnuts were estimated at 12% and monetary losses were estimated at US$1.03 billion (Trigiano, 2008).

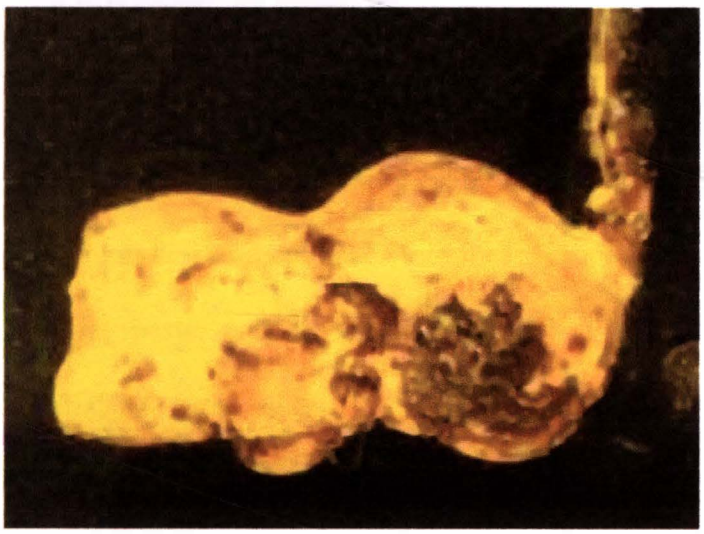

Figure 1.2.1 Affect of root-knot nematode on groundnut.

(Source: www.icrisat.org)

The lesion nematode, *Pratylenchus brachyurus*, is also a major nematode parasite of groundnut, with distribution mainly in the warmer groundnut production regions of the world.

1.3 Significance of Study

Recently, problem about plant parasitic nematodes had appeared in kenaf plantation reported by Lembaga Tembakau Negara in Bris soil located in Telaga Papan, Terengganu. The above ground symptoms showed by the plants are uneven growth, yellowing colour of the leaves, and there are areas of dead plants fully covered by weeds. This gives a huge crisis to the farmers where the yield had been affected seriously. We would not realize that the cause of the problems came from plant parasitic nematodes just by observing the above ground symptoms of those plants. After carefully inspecting the roots of the plants with many galls of root, planters realize that it was caused by nematodes of *Meloidogyne* species.

However, it is obvious that not all farmers and planters are adequately conscious about the danger of plant parasitic nematodes. They acknowledge other form of threats which may derive themselves by other factors such as plant diseases, viruses, fungi, bacteria, pests, and weeds but then, such nematodes problem is somehow being ignored as it may due to lack of acquired information regarding to such. It is the significant of this study to help Malaysian farmers and planters to gain much more understandings about plant parasitic nematodes and how they can recognize such symptoms.

Some species of nematodes affected groundnut plantation such as *Meloidogyne* and *Pratylenchus* species in major countries like United States of America, Mexico, Australia, Brazil, and some Asian countries like India and Thailand (Sharma and McDonald,1987). While nematodes problems and studies are not commonly heard locally, this project hopefully can help to study this problem towards groundnut plantation in Malaysian condition.

There are studies that recommend ways of controlling nematodes problems such as sanitation, crop rotation, flooding, heat, chemicals, and by use of some resistant varieties. But it is imperative for farmers to get most effective yet efficient way to manage nematode problems. Chemical substances usage may be effective to kill all the nematodes in plantation area but in terms of financial issue, it may not be cost-wise method. Accordingly, alternative ways can be used like crop rotation which proves to be financially sustainable in long term. This study can help to find out whether groundnut is a suitable plant for crop rotation as nematode management.

1.4 Objective

The objective of this study is to evaluate the susceptibility of groundnuts to plant parasitic nematodes. The effect of nematodes will be observed on plants' growths during pre and post harvest. The quality of the groundnuts production will be evaluated and be compared with the control treatment. The effects and most importantly, the danger of plant parasitic nematodes will be observed and be greatly evaluated.

CHAPTER 2

LITERATURE RIVIEW

2.1 Plant Parasitic Nematodes in Groundnut Plantation

Groundnut is a good host of several nematodes species such as the root knot nematode (*Meloidogyne* spp.), the lesion nematode (*Pratylenchus brachyurus*), the testa nematode (*Aphelenchoides arachidis*), the sting nematode (*Belonolaimus longicaudatus*), the pod nematode (*Ditylenchus africauns*), and *Aphasmatylenchus straturatus* (Bridge and Starr, 2007). Each of the species has specific symptoms and effects on groundnut plant and effect yield production.

The root gall nematodes (*Meloidogyne arenaria*, *M. hapla*, and *M. Javanica*) are among the prevalent species affected groundnut plantation worldwide. (Bridge and Starr, 2007). *M. arenaria* causes the most significant losses on groundnuts, especially in West Africa. This species occur throughout the world, especially in areas with warm or hot climates and short or mild winters, and in green houses everywhere (Agrios, 2005). They are also found in sandier soil (Trigiano, 2008). They attack more than 2000 species of plants, including almost all cultivated plants, and reduce world crop production by about 5% (Agrios, 2005).

The second-stage juvenile can be identified to genus based on its acute tail, overall length of 0.36-0.56 mm, with *M. hapla* being typically shorter than M. *javanica*, which in turn is shorter than *M. arenaria*. The juveniles are also characterized by having a slender body, a distinct oesophagus, overlap of intestine, and an acute tail terminus. The stylets are delicate with a length of 10-12 μm. The mature females dissected from roots have distinctly rounded to pear-shaped bodies. The perineal patterns (cuticular markings surroundings the anus and vulva) are helpful in the identification of this species (Bridge and Starr, 2007).

Meloidogyne spp. is all sedentary endoparasites based on their life cycle and the way they feed (Trigiano, 2008). This species penetrate and live inside root tissue in the way to consume all the plants nutrients. They have several juvenile stages before mature. The first stage juvenile undergoes one molt while still in egg mass. The newly hatched, which have a worm shaped is in second stage juvenile. This species are harmful in this stage where they are starting to penetrate suitable host. They will begin feeding on several cells near endodermis right after they had migrated to developing vascular cylinder of the root.

The nematode will inject secretions from esophageal glands through stylet into nearby cells. The cells then become enlarged and multinucleate therefore serve as feeding cells for the rest of the nematode life cycle. These enlarge cells for root-knot nematodes are called the giant cells. These specialized feeding cells have extensive cell wall in-growths which increase the surface area of the cellular membrane. The giant cells function as a large siphon, diverting the downward flow of nutrients from the phloem into feeding the nematodes. A gall rapidly begins to develop around the feeding juvenile as a result of cell division enlarged, stimulated by the nematode activities.

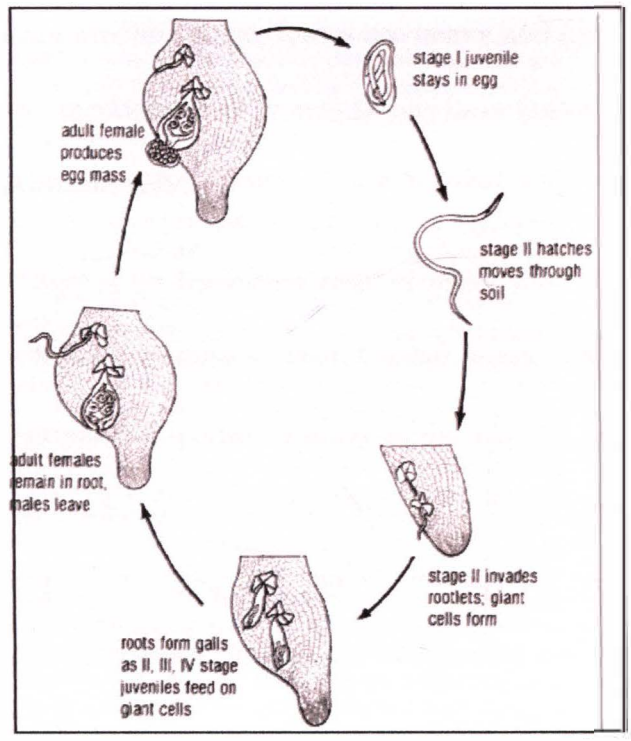

Figure 2.1.2 Life cycle of *Meloidogyne* sp.

(Source: http://www.ctahr.hawaii.edu/nelsons/coffee/coffee.html)

All the juveniles starting to undergo another three additional molts after feeding process had begun.The third and fourth stages juvenile, are slightly swollen. At maturity, root-knot nematodes exhibit sexual dimorphism. Females will enlarge and become spherical (400-µm diameter, 700-µm length), having a flask shape called pyriform. While males molt to moderately large vermiform shape (30-µm diameter, 1400-µm length) and migrate from the root. The posterior end of the female's adults usually protrudes from the surface of the root gall, where an egg mass containing 300-500 eggs is produced. The life cycle typically requires 2 to 50 days depending on root-knot species, plant host, and environment (Trigiano, 2008).

Root – knot nematodes damage plants by devitalizing root tips and causing the formation of swellings of the roots. These effects not only deprive plants nutrients, but also disfigure and reduce the market value of many root crops. When susceptible

plants are infected at the seedling stage, losses are heavy and may result in complete destruction of the crop. Infections of older plants may have only slight effects on yield or may reduce yields considerably.

Other species that is an important pest in groundnut plantation throughout worldwide is the root lesion nematodes (*Pratylenchus* spp.). This species is a major nematode parasite of groundnut, present mainly in the warmer groundnut production regions of the world and attack the roots of all kinds of crops such as cereals and other field crops, vegetables, fruit trees, and many ornamentals (Agrios, 2005). Most common species in groundnut is *Pratylenchus brachyurus* and generally present in warm and tropical region. This species has been specifically reported attacking groundnuts in Australia, Egypt, USA, and Zimbabwe (Bridge and Starr, 2007).

P. *brachyurus* can be identified based on its distinct lip region with two annules, a robust stylet (17-22μm), and an overall length of 0.75 mm. Adult females, as with all Pratylenchus, have a single ovary, with the vulva located posteriorly (80% of body length). The tail terminus is typically blunt. The oesophagus overlaps the intestine ventrally. Males are rarely observed (Bridge and Starr, 2007). They are migratory, endoparasitic nematodes (Agrios, 2005).

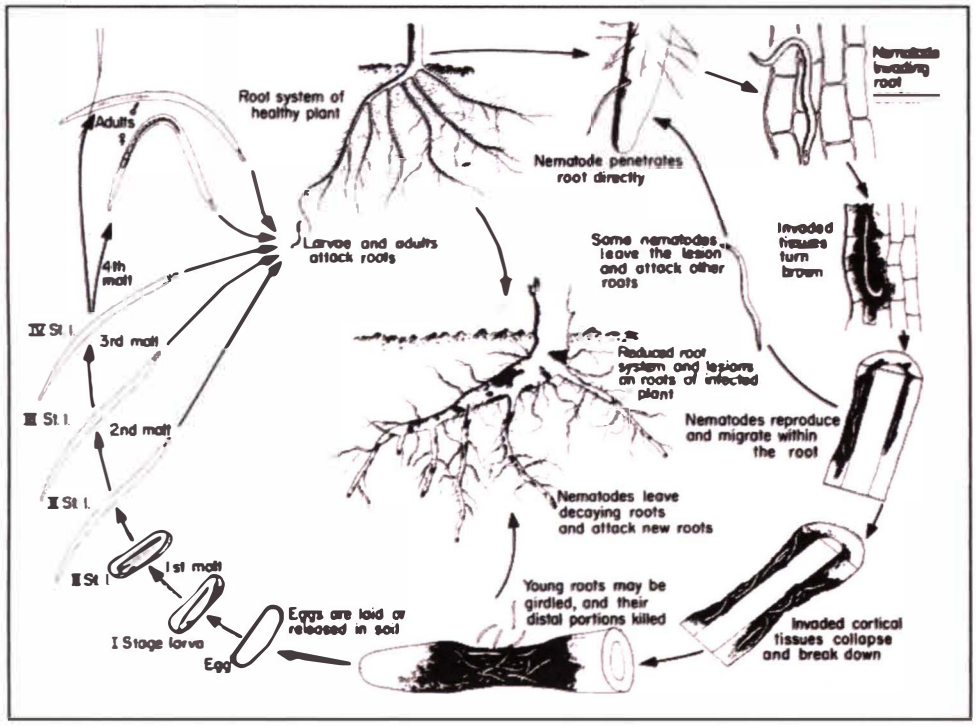

Figure 2.1.4 Life cycle of *P. Branchyrus*

(Source: http://www.sardi.sa.gov.au/pestsdiseases/)

The life cycle of lesion nematodes is typical for many plant-parasitic nematodes. Adult female lay eggs singly in root tissue or in the soil. The first-stage juvenile molts to the second-stage within the egg. The second-stage juvenile hatches from the egg and then molts three more times to become an adult. The presence of host roots has been shown to stimulus egg hatch. All juvenile stages outside the egg and adults can infect host roots. Lesion nematodes are classified as migratory endoparasites because they enter and exit roots numerous times to feed during a growing season. Invasion of roots may occur at the root tip, root hair region, and occasionally in young lateral root junction. As these nematodes migrate through the cortex of host roots, using their stout, well-developed stylets to destroy cells in their path, they briefly feed on nearby cells and then continue moving. Death of nearby cells is caused by the nematode movement and feeding activities, resulting in elongated, spreading lesions just below the root surfaces. With large number of lesion

nematodes, the lesions may completely encircle the roots, causing death of the distal portion of the root segment (Trigiano et al. 2008).

2.2 Symptoms of Plant Parasitic Infected Groundnut Plantation

Symptoms of plant parasitic infected plants can be found obviously through observation on both above ground and underground characteristics of the plants. Roughly, the plant that has been infected with plant parasitic nematodes became stunted and chlorotic, with uneven growth, as a result of suffering from mineral deficiencies that taken by the nematodes. Some plants however, produce patches of yellowish green plants that grow poorly.

Figure 2.2.1 Effects of *Meloidogyne sp.* on groundnuts (Source: www.sardi.sa.gov.au)

Specific symptoms (*Meloidogyne* spp.) include knots on roots which look like beaded roots. By observing the root one cannot see the adult female since it is found completely inside the roots. The egg mass can be seen within the knots which contains

200-500 eggs. The above ground general symptoms comprise chlorosis, wilting, stunting and smalling of leaf and fruits (Pathak et al, 2006)

Both *M. arenaria* and M. javanica can cause severe galling of the pegs and the pods, with the adventitious root development from the pod galls while *M. hapla* rarely forms galls on the pods. It is important that there will be no mistake with *Rhizobium* root nodules as nematode-induced galls while diagnosis is done. In the middle to the later portion of the growing season, most of the nematodes are developing inside the galled roots or are present as eggs in the egg masses on the root surface. Number of J2 (second-stage juvenile) in the soil are the highest in the later half of the growing season and may exceed 1000 J2/100 cm^3 soil (Bridge and Starr, 2007).

Diagnosis foliar symptoms are rare, but may include stunting and chlorosis if the level of infection is extremely high. Root symptoms are the presence of distinct necrotic lesions, often elliptical in shape, ranging from a few millimetres to several centimetres in length. *P. Brachyurus* typically also cause distinct necrotic lesions on the pods. These lesions are characterized by diffuse rather than sharply delineated margins. Eggs are deposited singly in infected tissues and surroundings soil. The nematode may survive for 24 months in infected pods at room temperature. Diagnosis requires identification of the nematode in addition to observation of symptoms. Because of the endoparasitic nature of these nematodes, detection of the nematode is the best accomplished by extraction of root and pod samples.

Figure 2.2.2 Effects of *Pratylenchus sp.* on groundnuts (Source: www.sardi.sa.gov.au)

2.3 Effects and the Economical Consequences of Plant Parasitic Nematodes towards Groundnut Plantation and Production.

Plant parasitic nematodes cause damage resulting in an estimated $8 billion/year crop loss to US growers, and a $78 billion/year a global scale. Of these, root-knot nematodes (*Meloidogyne* spp.) cause the most serious damage to many crops worldwide. Three *Meloidogyne* spp. Parasitize groundnuts and each are capable of causing severe suppression of groundnuts yields and fruit quality.

Yield losses of more than 50% in heavily infested fields have been documented for *M. arenaria* and *M. javanica* (Bridge and Starr, 2007). Losses due to *M. hapla* are usually less and rarely exceed 25% of the yield potential. Pre-plant damage threshold population density for *M. arenaria* and *M. javanica* of 1-10 juveniles/500 cm^3 soil, and 16 juveniles/500 cm^3 soil for *M. hapla*. Because of their widespread distribution and high frequency of occurrence, the root knot nematodes are

considered to be very important pathogens of peanut. In some production areas of the USA, nearly 30% of the fields are infested with one or more of these nematodes.

P. brachyurus also effects marketable value of pod appearances (Bridge and Starr, 2007). Incidences of pod rot caused by soil-borne fungi, especially *Phytium* spp. and *Rhizoctonia solani*, are increased by concomitant infection by nematode. Aflatoxin contamination of pods due to colonization of nematode-infected pods by *Aspergillus flavus* is also increased. More than 90% of the total nematode populations are typically associated with the host tissues during the cropping season and, immediately after harvest, affecting the yield quantity.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

Two types of soil were used in this study. Sample soil was taken from kenaf plantation in Bris soil located in Telaga Papan, Terengganu. The soil was believed to have several species of plant parasitic nematodes based on the symptoms that shown by the kenaf plants including *Meloidogyne* sp. and *Pratylenchus* sp.. While for the control treatment, the soil used was from the John Innes Mixture (3 parts soil: 2 parts sand: 1 part organic matter). The groundnut seeds used is from variety menglambu. The planting area was carried out in two culverts cylinders.

3.2 Methods

3.2.1 Soil Sampling and Preparation

The soil first was sample off in nematodes infected soil in kenaf plantation in Telaga. During the sampling, the sample of kenaf plants was also taken to study the

symptom of nematode infected. The root of infected kenaf plants then was processed for Scanning Electron Microscope observation.

3.2.2 Bioassay Using Tomato and Kenaf Plants

Tomato and kenaf seeds were germinated in a petri dish for 7 days. The germinated seedlings then were transferred into 10 small cups of 100 ml^3 volume filled with sample soil. They were grown with consistent watering. After 28 days, all of the plants were taken out of the cup and the roots were washed gently. The roots were stained in Phloxine B to stain the egg mass by soaking the roots in 0.5% Phloxine for several minutes. The number of galls and egg masses on both tomato and kenaf hosts counted under the dissecting microscope.

3.2.3 Groundnuts Planting, Post Harvest Yield and Plant Analysis.

Seeds of groundnuts also were germinated first in petri dish and germinated seeds were transferred into 30 small cups with 250 ml^3 volume. 15 cups were filled with control soil and the other 15 with sample soil. Both cups were put into 2 separate culverts labelled as control treatment and sample soil.

Figure 3.2.3.1 Seedlings after transferred into cups

After 14 days, the cups were removed. Consistent and similar amount of watering and fertilization were assured to be applied to the seedlings in the culverts.

The growth patterns of both treatments were observed regularly. Some of the plants were harvested after 57 days. The roots were cut, tied in muslin cloth, and then dipped in boiling Fushin acid for 2 to 3 minutes. The stained roots were washed and cleaned in clear Lactophenol. The roots were observed under compound microscope.

After 75 days, all the groundnut plants were harvested. The pods then were detached, counted, and weight for each plants. The height of each plant also measured. All of the measurements were analysis and studied. The quality of groundnuts produced was observed whether any signs of symptoms of nematode damage. The aspects that were concern are appearance of the pods, size, and colour.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Soil Sampling

Figure 4.1.1 Galling in kenaf root caused by *Meloidogyne* sp. in Telaga Papan.

Root samples that had been taken, and had been proceeded for observing under scanning electron microscope showed that some female Meloidogyne sp. were still in the kenaf root cell.

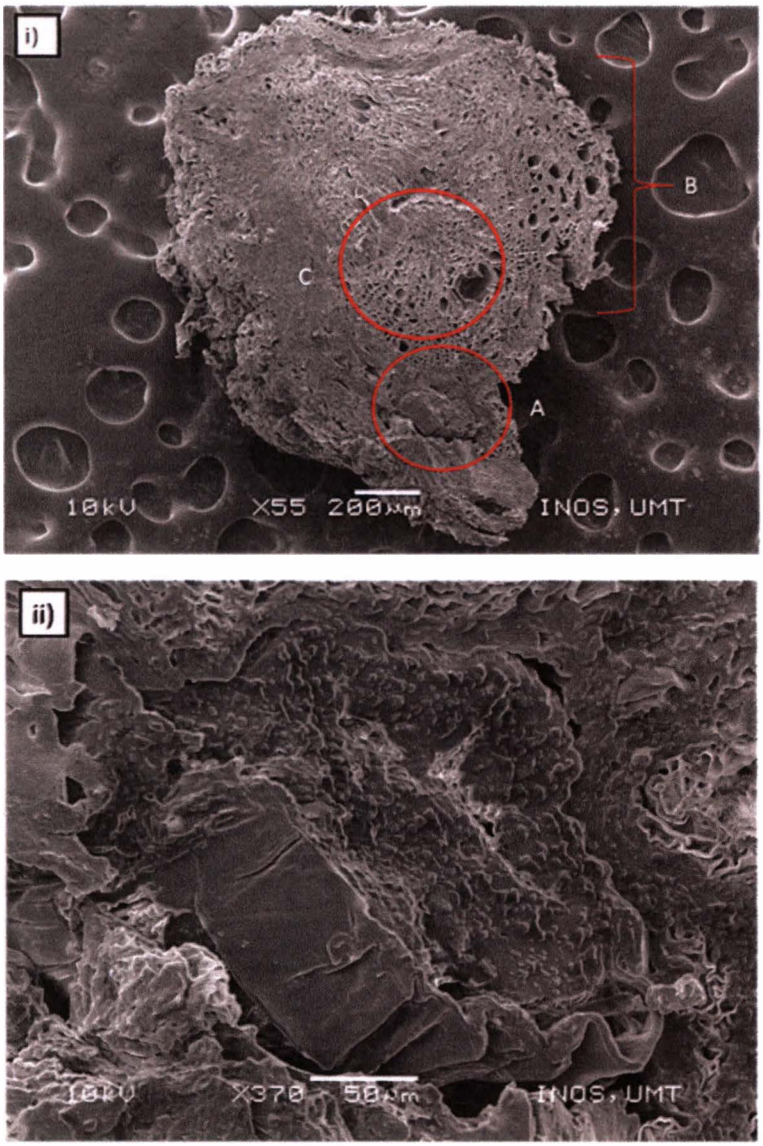

Figure 4.1.2 i) shows the phloem and xylem tissue of kenaf root had been disturbed (B), the giant cells (C) and the presence of Meloidogyne sp. in root cell (A) while ii) shows close up look of the nematode.

The observation had found out the existence of the female nematodes of *Meloidogyne* sp. in the plant root cells. Female *Meloidogyne* sp. tends to live in the root cell and produce egg mass instead of migrating to other plant root. The xylem and phloem tissue had been disturbed due to feeding activity of the nematodes and causing the forming of giant cells, where instigate galling on the root cells. A normal giant cell illustrates a dense cytoplasm, thick cell wall, and deformed xylem cells that surround them. In a susceptible host, the giant cells develop and provide the nematode with the

nourishment needed for it intense metabolic rate. This type of nematode probably in 3rd to 4th stage juveniles based from the maturity of the kenaf plants and egg mass were never existed. There is no male nematode which is in large vermiform shape since they migrate from root after matured.

4.2 Bioassay Techniques Using Tomato and Kenaf Plants

All 10 plants of kenaf survived until 28 days of planting but only 7 of tomato plants survived. It was found out that upper part of the plants seemed to be healthy. But both of the plants seemed to be susceptible to the suspected species of nematodes contained in the sample soil which is *Meloidogyne* spp. based on galls on the root with egg mass stained with Phloxine B (Figure 4.2.1). The number of gall occurred in the root of tomato plants were fewer than in kenaf root.

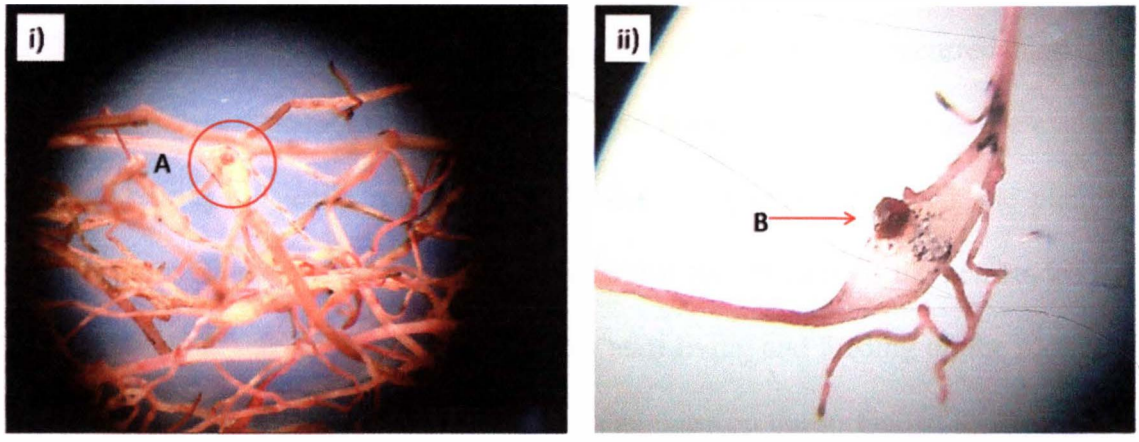

Figure 4.2.1. i) The stained egg mass on a gall on a root of kenaf (A) while in ii) shows the egg mass (B) on a gall in tomato root.

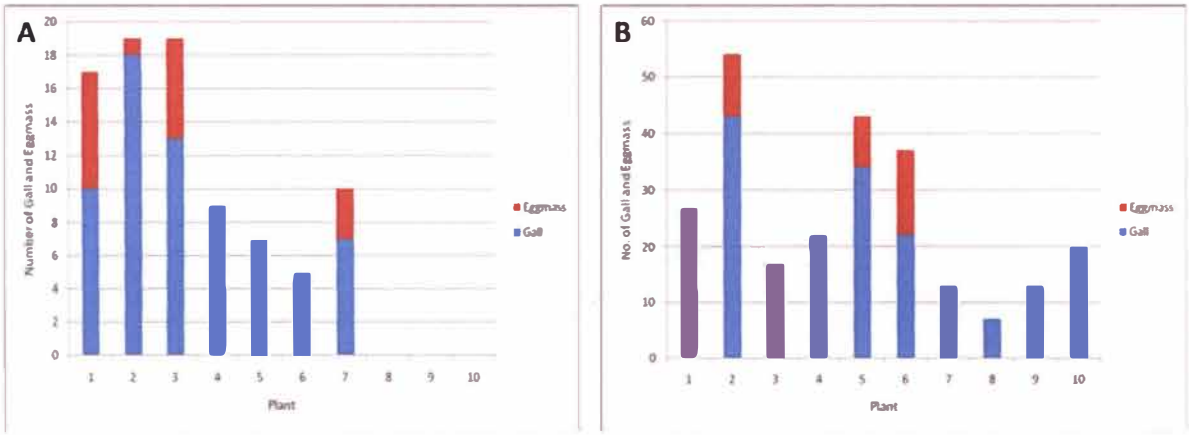

Figure 4.2.2 (A): Graph of number of galls and egg mass in tomato plant. (B): Graph of number of galls and egg mass in kenaf plants.

Both plants seemed to have galls and egg mass but the egg mass production did not seem to appear in every galls of the root (Figure 4.2.2). Fewer gall number found on tomato root is 5 to 18 with a mean of 6.9 galls per root. In contrast, kenaf roots a range of 7 to 43 galls with an average of 19.7. As for egg a mass, tomato has average number of 1.7 while kenaf is 5.6. The highest number of egg mass is 7 for tomato and 15 for kenaf while least number was 1 for both tomato and kenaf.

The variation of the number of galls and egg masses occurred since the maturities of the nematodes appear in every gall might be different. The life cycle of *Meloidogyne* sp. typically requires 2 to 50 days (Trigiano, 2008). As the planting period is only 28 days, there were possibilities that the maturity of the nematodes had only reached the second juvenile, where they start living and feeding on the roots and third juvenile where the female started to lay egg mass on the surface of the roots.

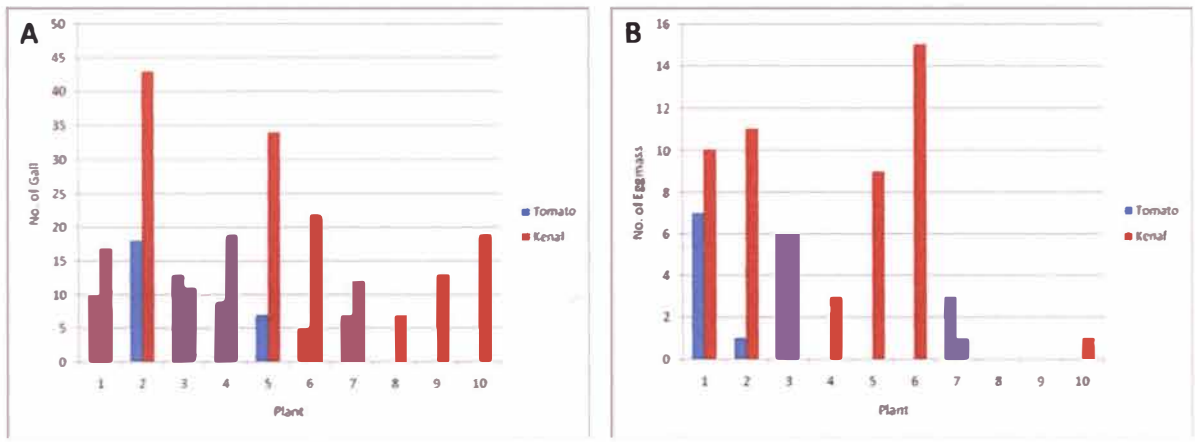

Figure 4.2.3 shows graph of gall number (A) and number of egg mass (B) in both tomato and kenaf plants.

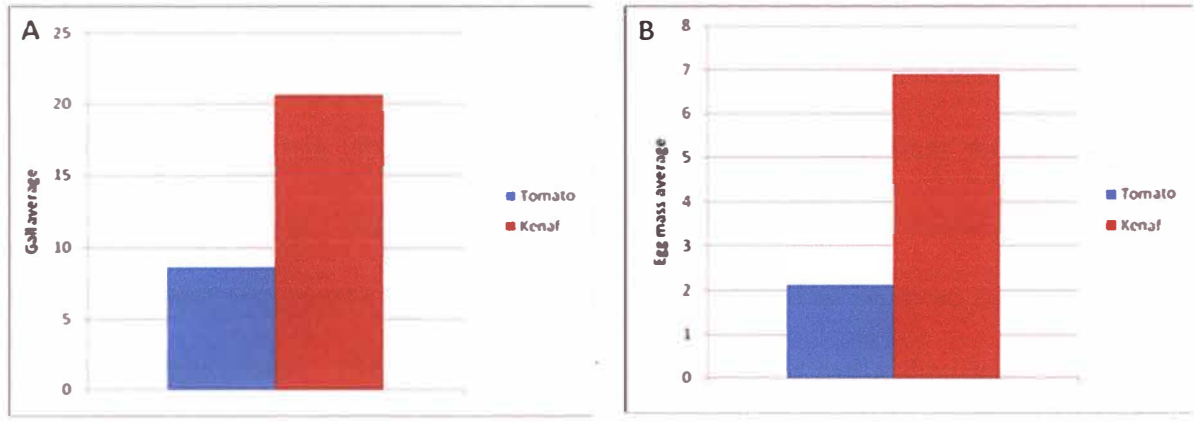

Figure 4.2.4 Average graph of (A) galls and (B) egg mass in both tomato and kenaf plant.

A major difference was obviously seen on number of galls and egg masses in both tomato and kenaf. Kenaf plants seemed to have higher number of galls and egg masses compared to tomato plants. As discussed earlier, average number of galls and egg masses produced by kenaf plants is significantly higher than tomato plants (Figure 4.2.4). Figure 4.2.3 explains the comparison of number of galls produced by each plant. It is clearly illustrated that each kenaf plant has higher number of galls and egg masses formed compared to each plant of tomato. The structure of kenaf root that is more fibrous and longer than its counterpart's made kenaf plants are more susceptible to this nematode.

4.3 Observation of Groundnut Plants and Analysis

Plants in the sampled soil showed growth and flower compared to plants in control soil. For the first few weeks, plants in sample soil started to produce flower on the 6th day of plantation (Figure 4.2.1 B) while plants in control soil flowered 4 days later. As the soil is taken from kenaf plantation, there might be nutrients that came from the plantation that induces the plant growth before the 1st and 2nd juvenile of *Meloidogyne* sp. had developed and show visible effect to the plants.

Above ground symptoms attributed to plant-parasitic nematodes includes generally vague growth problems related to root impairment, such as stunting, yellowing, and wilting (Trigiano, 2008). This can be seen after several weeks of plantation where the growth of plants in control soil seemed to be more rapid rather than the other treatment. The height of plants in both culverts had a significant higher than the plants in sample soil. The plants in sample soil also show symptoms of yellowing and chlorosis of leaves.

Figure 4.3.1 Yellowing of young leaves and stunted plants in sample soil.

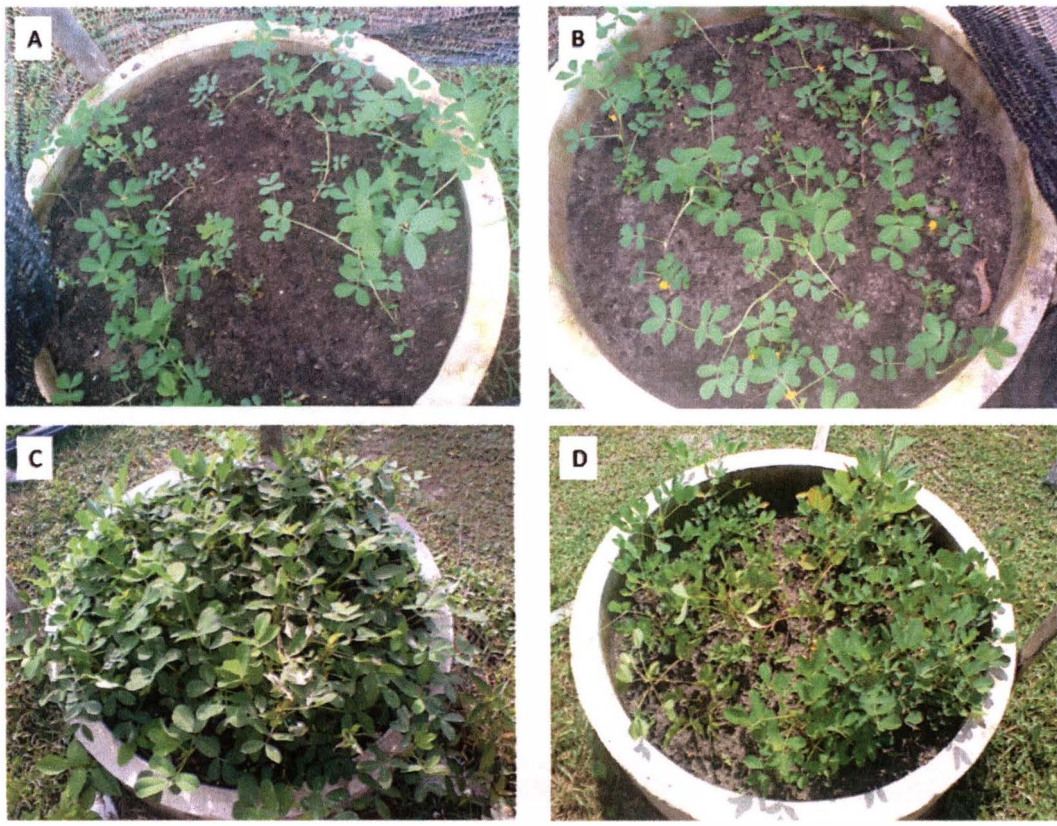

Figure 4.3.2 The plant growth (A) control treatment and (B) sample soil after 6 days plantation in calbad. (C) control treatment and (D) sample soil after 77 days of plantation.

It was found out that the plants in sample soil gradually show signs of unhealthy condition throughout the plantation period. The plants appeared to be stunted, and more yellowing seemed to appear on the leaves (Figure 4.3.2). The results appeared to concur with repeat of Luc et al., (2005) findings that these are the major consequences of development of root knot nematode and giant cell formation that lead to malformation of the xylem and phloem tissues. As an outcome, infected roots do poorly in taking up nutrients and water resulting unhealthy growth of plants.Total chlorophyll content of leaves and leaf lamina is also reduced by 76.72 and 33.83 per cent respectively over normal healthy plants. As a result, infected plants looks stunted with smaller chlorotic leaves.

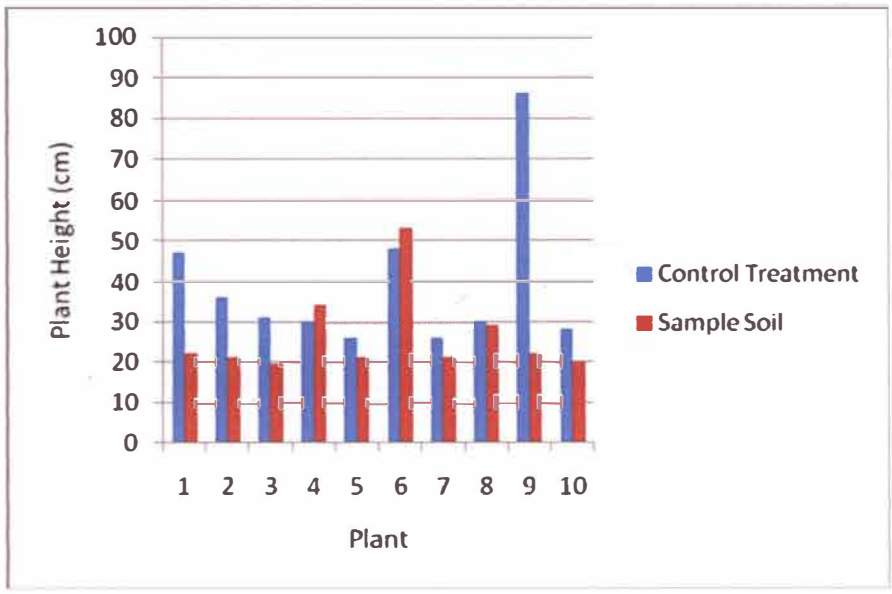

Figure 4.3.3 shows height of plants in both control treatment and sample soil at the harvesting period.

The differentiation of plants height between control soil and sample soil is obviously seen in Figure 4.3.3 where almost all of groundnut plants planted in control soil were higher compared to sample soil.

4.4 Observation of Plant Parasitic Nematodes in Groundnut Roots.

The root from both plants from control and sample soil is evaluated to find out any significant difference after 57 days after planting. It seemed that the roots from both plants had a few symptoms of nematodes infected. Lesions were observed on the roots in plant from sample soil compared to the plant from control soil. There were more on root of plant grown in control soil than sample soil. But there are obvious feature that can be seen by the roots where roots from sample soil is slightly shorter than the roots from control soil. The pods that still attach to the roots also fewer than pods attached to the roots in control soil.

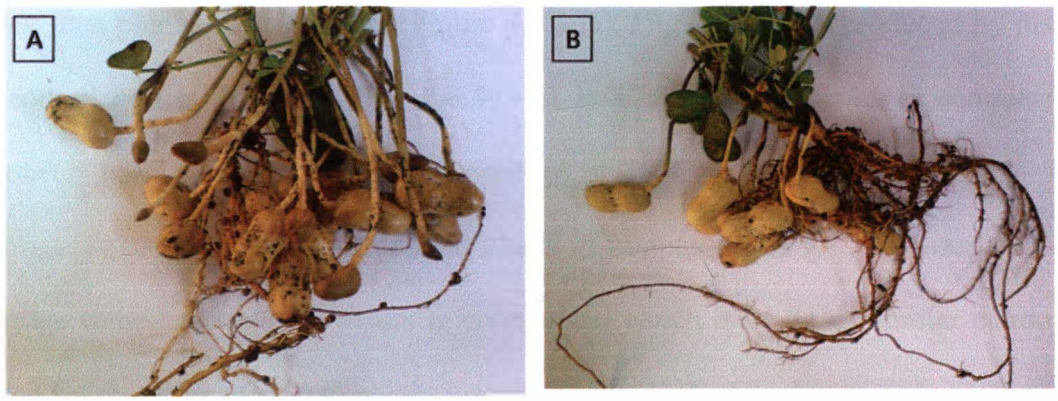

Figure 4.4.1 The differentiation of roots (A) from control soil, and (B) in sample soil after 57 days of plantation.

The observation under compound microscope had obtained the image of several nematodes in young roots. Figure 4.4.2 (A) shows a male Meloidogyne sp. based on its shaped that is in vermiform or sausage-shaped. While the other figure (Figure 4.4.2 B) is showing female Meloidogyne sp. is penetrating the root tissue.

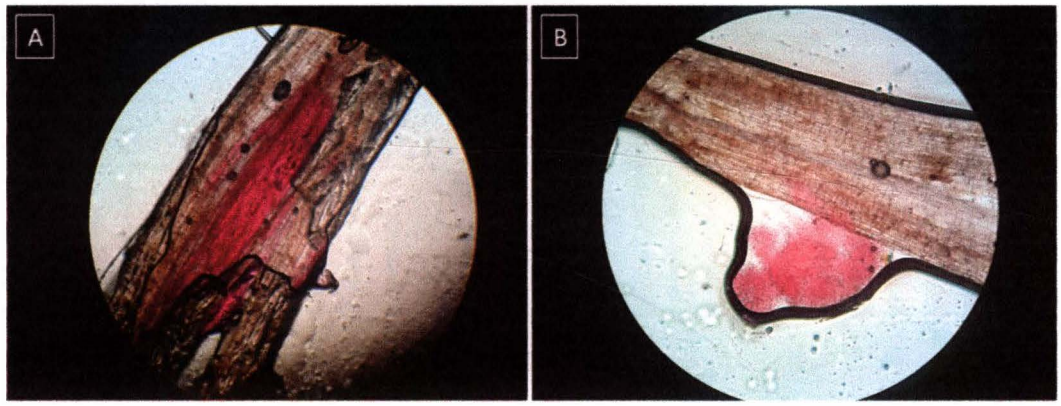

Figure 4.4.2 The observation of stained plant parasitic nematodes in young roots. (A) The nematode is already inside the root tissue and (B) probably a nematode penetrating the root tissue.

4.5 The Crop Yield

The crops yield produced by both plants in control soil and sample soil had significantly large difference. The weight of produce by each plant in sample soil is

lower than plants in control soil (Figure 4.5.1.A). Highest weight produced by plants in control soil is 40.36 g while the lowest is 17.83 g. The plants in sample soil however, had the highest weight of produce of 18.83 g and lowest is 2.71 g. The average weight produce by groundnut plants in sample soil is 9.48 g, lesser than the average weight produce by plants in control soil which is 26.54 g. Similar pattern is found for the analysis of pod number produced by plants. Number of pods produce by each plants in sample soil had enormous contrast towards the number of pods produced by each plants in control soil (Figure 4.5.1 B). The uppermost pod number produced by plants in sample soil is 14 much less than control soil which is 40 pods. Number of pods produced by plants in sample soil is 6 compared to control soil which is 20. Average number of pods produced by each plant in sample soil is 8.8 and 28.6 for control soil.

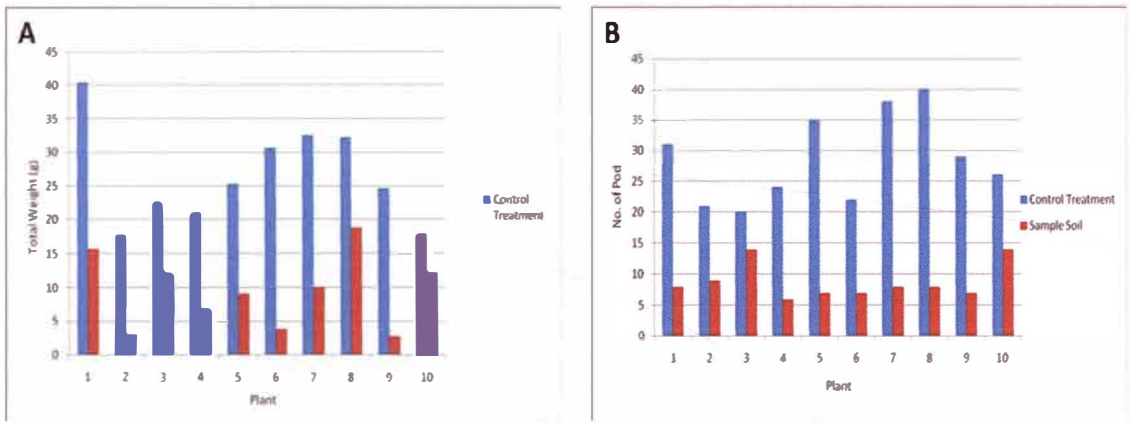

Figure 4.5.1 The weight of produce (A) Number of pods produced (B) from each plant.

The percentage of yield production (weight) also showed significant value between produce from plants in sample soil and plants in control soil (figure 4.5.2). Percentage of weight produced by groundnuts in sample soil is 26% which is less significant to the control treatment where the percentage is 74%.

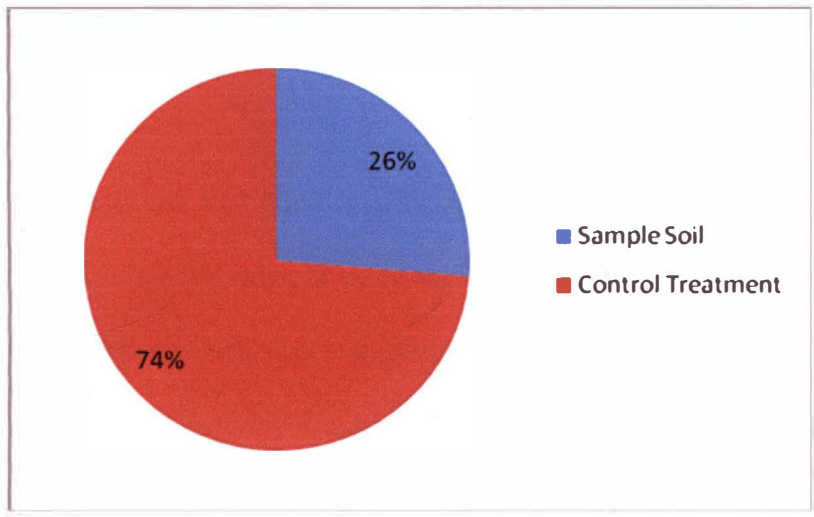

Figure 4.5.2 The percentage of yield produce by both from control treatment and sample soil.

4.6 Symptoms and Evaluation on Groundnut Pods

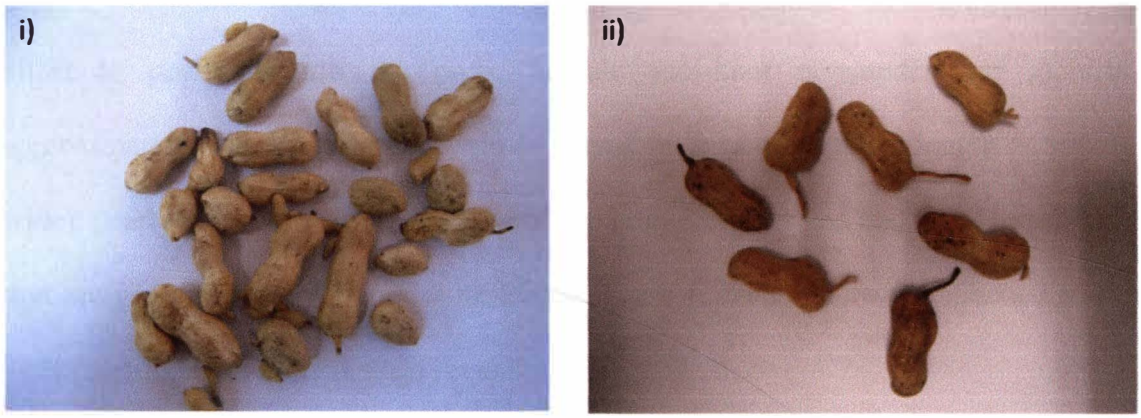

Figure 4.6.1 The differences of pods numbers between control soil (i) and sample soil (ii) in appearance, size, and colour.

There were significant difference of groundnut pods that can be seen clearly is the pods number and also the sizes. As shown in Figure 4.6.1, the quality of the pods of one of the groundnut plant planted in control soil (i) is clean compared to some lesions observed on pods that produce in sample soil (ii). The appearance of both pods also differs in terms of colours. Pods of control soil have slightly bright yellowish-brown colour compared to pods of sample soil that was darker in colour and had some brownish spots. The quality appearance also differed in terms of sizes. Based on

figure 4.6.2, the bigger size pods were obtained from control soil, as compared to pot from sample soil.

In view of the fact that good quality of produce depends on the quality of the plants where the plants itself must have enough nutrients, and needs to complete their plant activity such photosynthesis, respiration, anthesis and many more. Other than that, the plant must be in healthy condition where the structure of the plant must not be disturbed or damaged by pests. It is undoubtedly that the root-knot nematode or *Meloidogyne* sp. effect on the quality of groundnut productions as this pest feeds on the nutrients of the plants and slowly interrupt the plant activity. As a result, the plants grow unhealthy and poor production of crops occurs.

The observation under dissecting microscope had had found that there are several female *Meloidogyne* sp. attached to the pods of the ground nuts, probably in effort to penetrate into the pods. Female root-knot nematodes are globose, approximately the size of a typewritten period on a page (800μm length × 500μm wide), pearly white in colour, and have sharp pointed necks and heads off to one side that are generally visible (Luc et al, 2005). These females that were attached to the pods most likely in second stage juvenile.

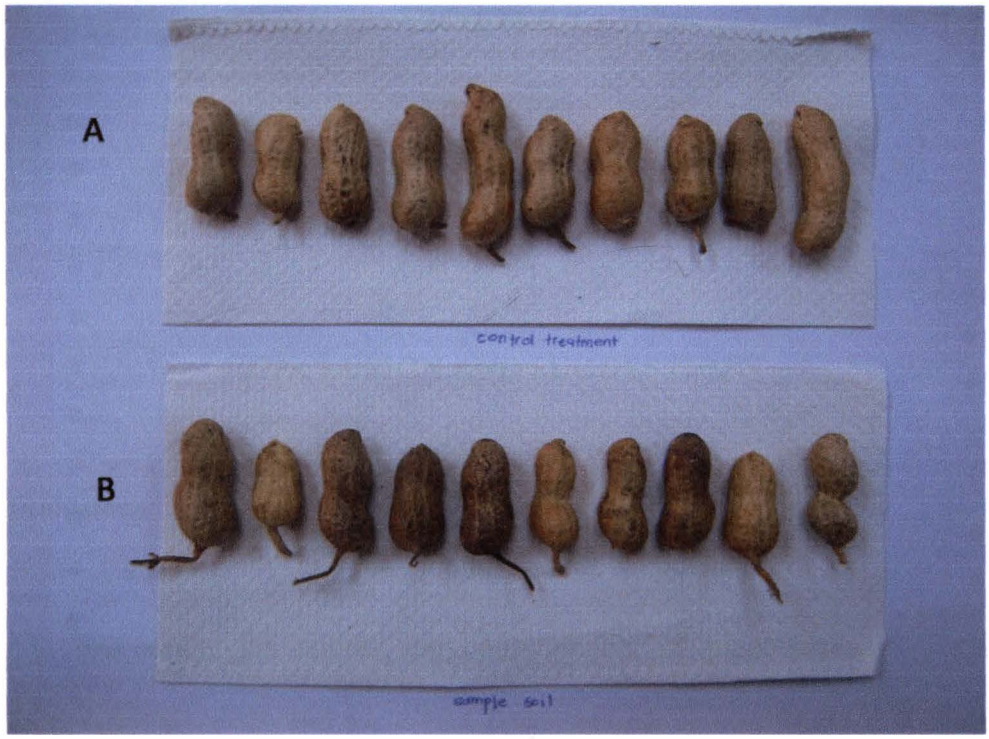

Figure 4.6.2 The difference quality appearance between produces by control treatment (A) and sample soil (B). The pods had been arranged by the plant number from left 1 to 10.

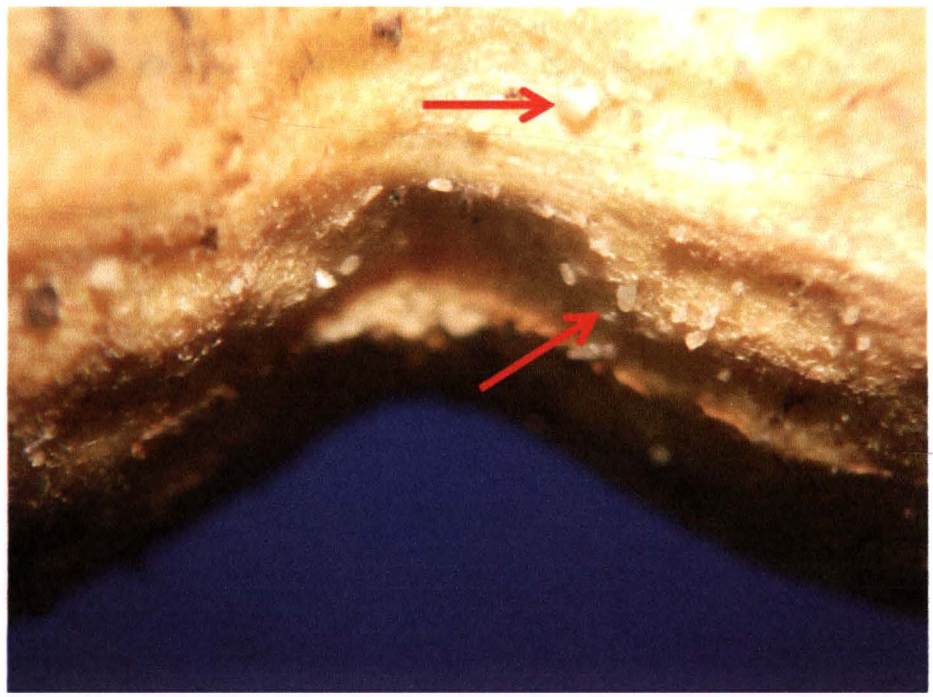

Figure 4.6.3: The female of *Meloidogyne* sp. penetrating the pods of groundnuts (red arrow).

CHAPTER 5

CONCLUSION AND SUGGESTIONS

5.1 Conclusions

In this study, the effects and consequences of plant parasitic nematodes towards groundnut plants and yield had been studied. It was found that the major symptoms of *Meloidogyne* sp. is not obviously seen in but several aspects of plants illustrate the effects. Observation of *Meloidogyne* sp. under Scanning Electron Microscope (SEM) shows that the mechanisms of this species of destructing root tissue of a plant which is formation of giant cells as feeder and lead to formation of galls in roots. The bioassay of tomato and kenaf also indicated that both of this plants is susceptible to *Meloidogyne* sp. and results shows the kenaf is more susceptible due to results that prove the number of galls and egg masses is more than the tomato. The negative effects also found by the plants where the plants in sample soil show stunted, yellowing, and unhealthy condition compared to control soil. This then leads to poor quality of pods produced during harvesting where the number of pods and weight of produce is lesser than the produce from control soil. The total weight produce by sample soil is less 48% from the produce by control soil. The quality evaluation like colour, appearance, and sizes of pods produce from sample soil resulting in poor characteristics on those three aspects. Even though there were no galls found, there are several female *Meloidogyne* sp. were penetrating into the pods as the pods observed

under microscope. In the early plantation of groundnuts, it is concluded that plant parasitic nematode, which is root knot nematode (*Meloidogyne* sp.), affect negatively on the yield of groundnuts especially in aspects of weight and quality appearance that is colour and sizes.

5.2 Suggestion for Further Study

More research should be carried out on the study of the effects of the plant parasitic nematodes towards groundnut plants and production. Among the suggestions for further study are as follows:

1. The study of plant parasitic nematodes should be done in Malaysia as this country is also one of the countries that produce groundnuts and it has economical importance into its people and export purposes.
2. Determination of which cultivar of groundnuts in Malaysia that resistant and susceptible to the infection of plant parasitic nematodes thus assist on the nematodes management in this plantations.

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Appendix A

Table of Gall and Egg mass Produced by Tomato and Kenaf on Bioassay

	Tomato		Kenaf	
	Gall	Egg mass	Gall	Egg mass
Plant 1	10	7	17	10
Plant 2	18	1	43	11
Plant 3	13	6	11	6
Plant 4	9	0	19	3
Plant 5	7	0	34	9
Plant 6	5	0	22	15
Plant 7	7	3	12	1
Plant 8	0	0	7	0
Plant 9	0	0	13	0
Plant 10	0	0	19	1

Appendix B

Table of weigh and Pod Number of Harvested Groundnuts of Both Control and Sample Soil

	Control Soil		Sample Soil	
	Weight (g)	Pod Number	Weight (g)	Pod Number
Plant 1	40.361	31	15.701	8
Plant 2	17.831	21	3.091	9
Plant 3	22.731	20	12.241	14
Plant 4	21.041	24	6.991	6
Plant 5	25.341	35	9.071	7
Plant 6	30.621	22	3.861	7
Plant 7	32.521	38	10.081	8
Plant 8	32.261	40	18.831	8
Plant 9	24.661	29	2.711	7
Plant 10	18.041	26	12.291	14

Appendix C

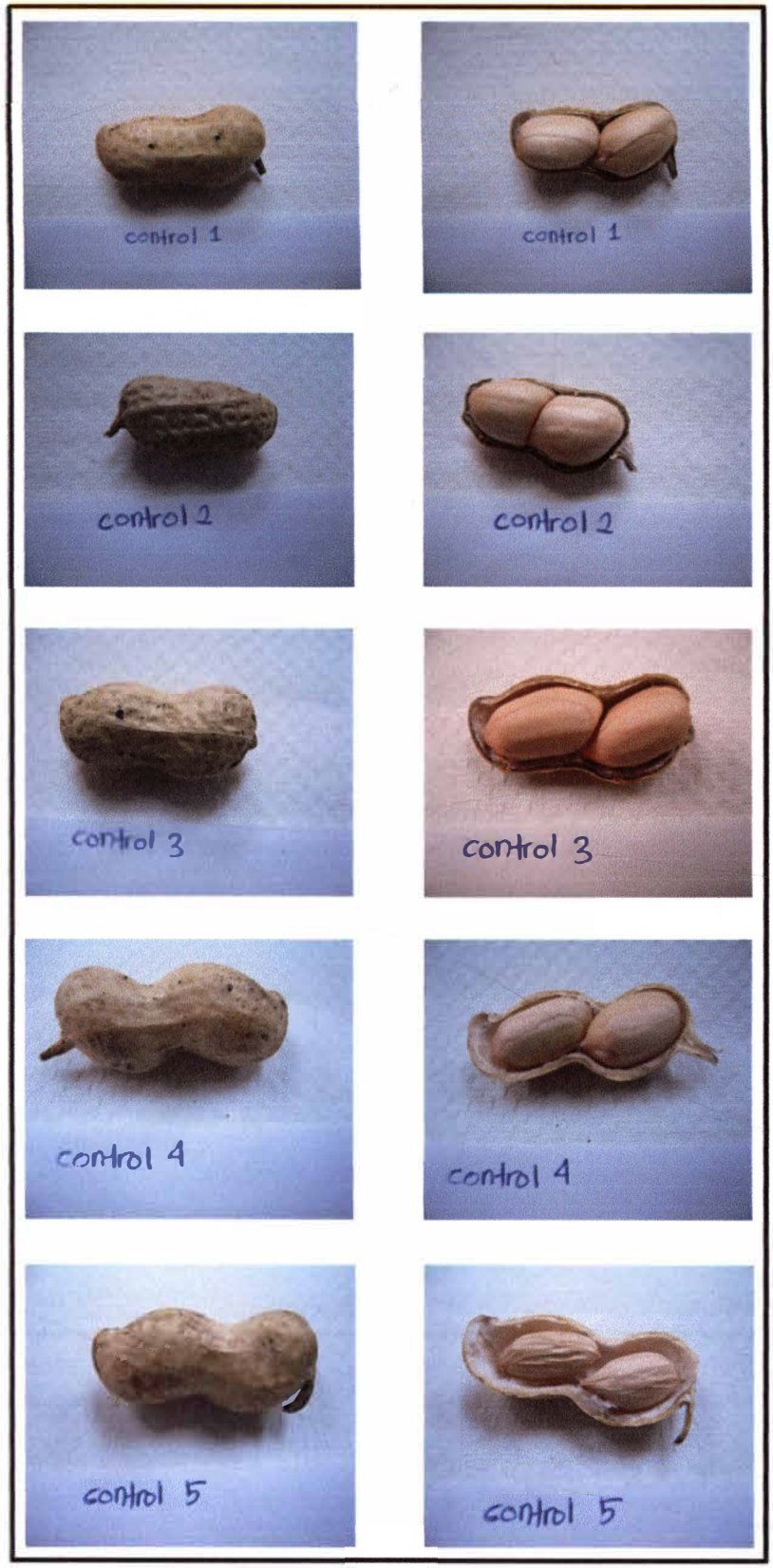
Table of Plants Height on During Harvesting

	Control Soil	Sample Soil
	Height (cm)	Height (cm)
Plant 1	47	22
Plant 2	36	21
Plant 3	31	19.5
Plant 4	30	34
Plant 5	26	21
Plant 6	48	53
Plant 7	26	21
Plant 8	30	29
Plant 9	86	22
Plant 10	28	20

Appendix D

Groundnuts Harvested in Both Control and Sample Soil

Control Soil




control 6


control 6


control 7


control 7


control 8


control 8

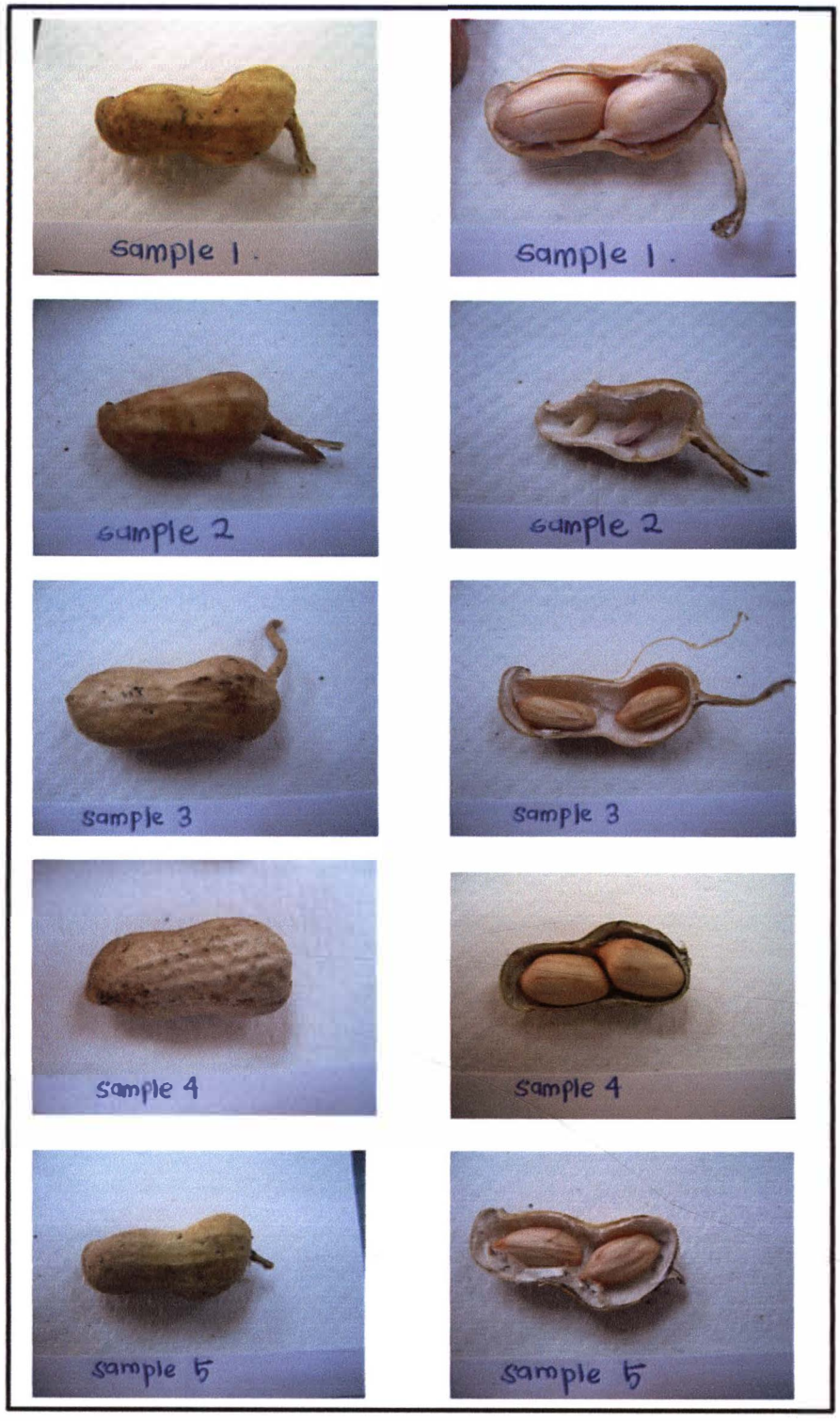

control 9


control 9


control 10


control 10

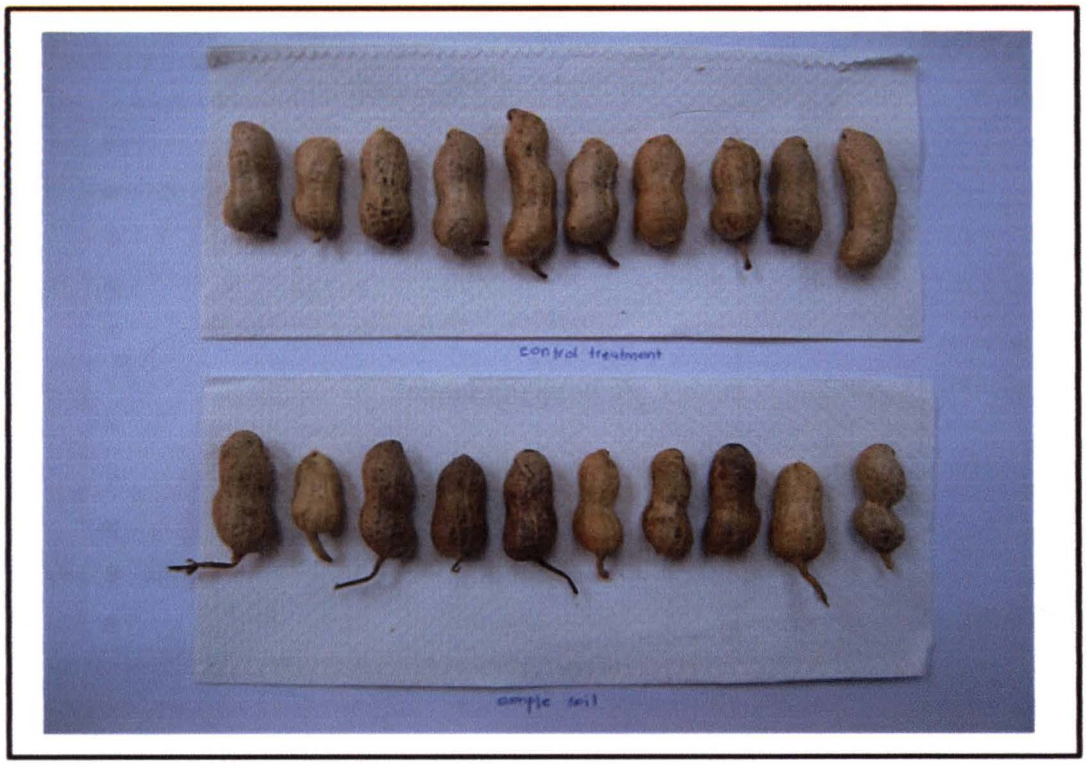
Sample Soil


sample 1.
sample 1.
sample 2
sample 2
sample 3
sample 3
sample 4
sample 4
sample 5
sample 5


Sample 6
Sample 6
Sample 7
Sample 7
Sample 8
Sample 8
Sample 9
Sample 9
Sample 10

Appendix E

Comparison of Groundnuts Planted on Both Soil



CURRICULUM VITAE

Name	: Marahaini binti M.Markam
Matric Number	: UK 15413
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Educational Background

2007-2010 Universiti Malaysia Terengganu

2006-2007 Kolej Matrikulasi Johor

2004-2005 Maktab Rendah Sains Mara Serting, Negri Sembilan

2001-2003 Maktab Rendah Sains Mara Batu Pahat, Johor

Skills and Experiences

- Fluent on Bahasa Malaysia and English
- Fast learner and love to try new things
- Experiences as a trainer in Deparment of Agriculture in Kota Bharu during internship
- Can be a good team player and like to work in groups.
- Microscopic handling
- Skillful in managing Microsoft Power Point, Microsoft Word, Microsoft Excel and Adobe Photoshop.
- Love to work in the field

Co-curricular Activities

- Attending pre-intership workshop of Faculty of Agrotechnology and Food Science 2009.
- Participant in Darul Falah Orphanage Visiting Programme Under Kelab Penyayang UMT 2009.
- Member of Sea Scout UMT 2008.

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By

Marahaini binti M. Markam

Research Report submitted in partial fulfillment of

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DEPARTMENT OF AGROTECHNOLOGY

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