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Wahizatul Afzan Azmi
Universiti Malaysia Terengganu

Tse-Seng Chuah
Universiti Malaysia Terengganu

POLLINATION EFFICIENCY OF THE STINGLESS BEE, *Heterotrigona itama* (HYMENOPTERA: APIDAE) ON CHILI (*Capsicum annuum*) IN GREENHOUSE

Wahizatul Afzan Azmi[1], Chuah Tse Seng[2] and Nur Suhaili Solihin[2]
[1]School of Marine and Environmental Sciences, Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Terengganu
[2]School of Food Science and Technology, Universiti Malaysia Terengganu,
21030 Kuala Terengganu, Terengganu
Tel: 6(09)-6683751; Fax: 6(09)-6683193; Email: wahizatul@umt.edu.my

ABSTRACT

Chili (*Capsicum annuum*) is one of the important cultivated crops in Malaysia and is a source of income for thousands of Malaysian farmers. Even though chili flowers are self-pollinated, the anthers need to be shaken to allow effective pollen release. Many researchers found that pollination by stingless bees can increase fruit size and weight, pericarp volume, high percentage of seed per fruits and fastens harvesting time. This study aimed to investigate and determine the quality of chili produced by three different pollination treatments; (1) self-pollination, (2) hand-cross pollination and (3) stingless bees (*Heterotrigona itama*) pollination. Results showed that chilies produced from pollination by the stingless bees, *H. itama* and hand-cross pollination were significantly heavier, longer and containing greater number of seeds per fruit than self-pollinated chilies. Overall results showed that the effects of stingless bee pollination on chili production was similar to the chilies produced from hand-cross pollination. Thus, it can be concluded that besides manual pollination, *H. itama* can be considered as an effective pollinator for the chilies grown in greenhouse. This study provides important information on the potential use of *H. itama* as an alternative pollinator for crop pollination purpose.

Keywords: Stingless bees, *Heterotrigona itama, Capsicum annuum,* pollination, greenhouse

INTRODUCTION

Chili (*Capsicum annuum*) is one of the most widely grown vegetable crops in the world, and is commonly cultivated and consumed in Malaysia. Departments of Statistics Malaysia (2011) recorded that chili production in Malaysia was consistent from 2005 to 2009 with average production of 33.1 thousand tons. The imports of chili recorded fluctuating trends where 2005 was the highest chili imported with 39.1 thousand tons, and the lowest was in 2008 with 22.1 thousand tons (Departments of Statistics Malaysia 2011). Compared to imported chili, only small quantities of chilies were exported and they range about 4.9 to 5.8 thousand tons (Departments of Statistics Malaysia 2011). This shows that chili production in Malaysia still cannot meet the high demand by local consumer.

The major obstacle in achieving high chili production is the susceptibility of chili plants to diseases, pests and viral attacks (Touhidur et al. 2006). In Malaysia, available control measures developed for the control of viruses are the use of reflective plastic mulch, intercropping chili with maize and spraying insecticide against vectors (Touhidur et al. 2006). Currently, greenhouse cultivation is widely practiced by the local farmers to meet the urban demands on chilies. In addition, greenhouse cultivation also protects from direct sunlight, bad weather and pest attack. However, the major problem of cultivation in greenhouse is poor pollination due to the protected structure of the greenhouse which may result in low production and low quality of yield (Yong and Shafqat 2003). Thus, pollination agents are of absolute importance for a high quality fruit sets and seeds formation. Insufficient pollination causes low pollen supply which also affects the progeny vigor by reducing the selectivity among the gametes before and during fertilization (Bertin 1990).

At least 67% of flowering plant depends on insects for pollination while others depend on birds and mammals (Tepedino 1981). Insect pollinators include bees, flies, moths, butterflies and beetles (McGregor 1976). Bee species are considered the most important insect pollinators of crop worldwide (Shipp et al. 1994). Interestingly, good candidates for future alternatives in commercial pollination particularly in the tropical region can be found in the diverse group of stingless bees (Meliponini) (Sommeijer & de Ruijter 2000).

Chili flowers are very attractive to a wide array of insects including honeybees (*Apis mellifera*) and stingless bees (Heard 1999; Klein et al. 2007). Chili flowers, like those of most cultivated Solanaceae, are pendant from leaf axils, show a white corolla, five to seven stamens containing 1.0 to 1.5 mg of pollen, and one central style with a round sticky stigma on its top. The anthers are tubular, and dehiscence occurs through lateral opening. Both flower anthesis and anther dehiscence take place in the morning, between 7h and 9h (Dag and Kammer 2001). Although *C. annuum* is largely self-fertile, and a relatively large fruit set may be obtained without any pollinators, several studies indicate that stingless bee pollination increases chilli production (McGregor 1976; Jarlan et al. 1997; Cruz et al. 2005; Slaa et al. 2006). Even though stingless bees are common pollinators in Malaysian agricultural ecosystem, their successful role in pollination is still poorly understood. Certain species of stingless bees are regarded as candidates for commercial pollination because of their specialised foraging adaptations and frequent visitation to cultivated fields. There are several studies on chili pollination (Shipp et al. 1994; Jarlan et al. 1997; Dag and Kammer 2001), and the role of stingless bees in producing quality fruits of this crop is still unknown.

It has been suggested that stingless bees pollinate the flowers effectively and that their small foraging areas are important in keeping the cultivars of chilies genetically distinct when several cultivars are grown close together. However, to what extent the foraging behaviour, such as the number of visits necessary to achieve pollination and average time spent foraging on a flower is correlated with fruit characteristics (e.g. weight, size, seed number) remains unknown. In fact, there is a limited study carried out in Malaysia that uses stingless bees to enhance crop or horticultural productions. In Malaysia, most of the greenhouse farmers still use the hand pollination method which require more time, laborious and costly especially on large scale plantations. Therefore, pollination agents such as bees are important for crop pollination management.

Malaysia has 30 of the 150 *Trigona* bee species in the world and *Heterotrigona itama* is one of the potential candidates for commercial pollination (Salim et al. 2012). To date, there have been a few attempts to domesticate the species for pollination service in Malaysian agricultural ecosystem. However, their contributions to effective pollination have yet to be studied and the foraging behaviour of stingless bees is still unknown. This study is aimed to investigate the potential use of stingless bee, *H. itama* to pollinate flowers of chili grown in greenhouse. The outcome from this study will be used to develop suitable strategies for improving chili pollination.

MATERIALS AND METHODS

Plant materials and experimental location

The study was carried out from September 2013 to January 2014 in a 6.0 x 24.0 m^2 greenhouse at the School of Food Science and Technology, Universiti Malaysia Terengganu (N05^{0}24.541', E103^{0}05.347'). Chili seeds variety 630 were provided by Pejabat Ladang, Universiti Malaysia Terengganu. Seeds were initially soaked in a mixed solution of water and Thiram (seed treatment) for 15 to 25 minutes to prevent fungal diseases in seeds. Then, the seeds were rinsed for about 5 minutes under running water and were sown in shallow seed trays with moist compost. Generally, the swollen seeds started to sprout after 2 to 5 days. In about 3 to 4 weeks, the chili seedlings were transplanted into the polybags (60 x 40 cm) containing 5 kg of coco peat. Chili plants were treated with the same amount of fertilizers, i.e. 10 g of an equal mixture of urea and potassium chlorate and 7 g of superphosphate per polybag according to crop requirement. Plants were irrigated daily by drip irrigation.

Rearing of stingless bees, *H. itama*

Two strong colonies of wild stingless bees, *H. itama* were obtained from the local suppliers of Tumpat district, Kelantan. A strong and active colony can contain up to 3000 *H. itama* adults. The stingless bees were transferred from their natural habitat into the man-made hives. The hives were built from wooden box of 60 cm x 25 cm x 10 cm. The stingless bee colonies were maintained and acclimatized for approximately 1 month in the field under the natural climatic conditions (± 22 to 31°C, 83% humidity). Environmental parameters such as minimum/maximum temperature (ºC) and relative humidity (%) were recorded using a Relative Humidity meter (Model EM-9000, Lutron Electronic Supplies, Taiwan). During acclimatisation, the entrance hole of the hives were covered with metal wire mesh (1.27 x 1.27 cm) to protect the hives from pests such as lizards, ants, black soldier flies and others.

Experimental design

The greenhouse was divided into three equal parts by using approximately 2 m of mist net with the opening size of 250 x 720 µm, where one third was occupied by plots using self-pollination and the other two parts were occupied by the remaining treatments which were hand-cross pollination and stingless bee polllination, respectively. The treatments in this study were (1) self-pollination as control, (2) hand-cross pollination and (3) stingless bees (*H. itama*) pollination with four replications each. There were 5 plants per replicate and overall, 60 chili plants were used in this study. In the self-pollination (control), 20 flower buds were chosen randomly and were bagged with fine muslin bags (mesh 1 x 1 mm) until fruit set to prevent the flowers from being pollinated by other pollination agents. The flowers were individually tagged. In the hand-cross pollination, 20 flower buds were selected, tagged and bagged. After the anthesis of chili flowers at 0700 to 1000 h, the flowers were unbagged and hand-pollinated with pollen taken from flowers of other chili plants by gently rubbing the flower stigma with a stamen containing fresh dehisced pollen grains. Then the flowers were rebagged to avoid other pollinators and properly tagged. In the pollination treatment by stingless bees, two hives of strong colonies of *H. itama* were placed at the centre of the greenhouse for at least two days before the anthesis period in order for the stingless bees to adapt with the new environment (Fig. 1). Foraging activity of *H. itama* was monitored and observed from 0700 to 1200 h (Fig. 2). Twenty flowers that received one *H. itama* visit were tagged and bagged until fruit set.

Sample collection and production-related parameters

After about 4 to 5 weeks, the matured chilies were harvested using a sharp knife or scissors after the chili fruits have reached its commercial standard, i.e. firm, smooth, thick-fleshed, reddish in colour and length of about 7 to 15 cm. In each treatment, total number of fruit malformation was counted. All the harvested chilies were kept in different trays and stored at room temperature. Eight parameters were evaluated in this experiment namely fruit weight, fruit size (diameter and length), average number of seeds per fruit, fruit colours, fruit firmness, Total Soluble Solid (TSS) and Titratable Acidity (TA). The fruit weight (g) was measured using an electronic weight balance, the diameter (mm) and length (cm) measured using vernier caliper and fruit colour was measured using Minolta Chroma meter (Model CR-400, Konica Minolta, US). The chili fruit firmness was measured using a Texture Analyzer (Model TA1, Lloyd, UK) with probe 2N. For firmness analysis, the force required to puncture the chili fruit was measured at three locations on the fruit, i.e. near the basal end, center, and tip of the pod and averaged to obtain a value for each fruit. The values were recorded in Newtons (N). Then the fruits were cut open and the number of seeds per fruit was counted. The Total Soluble Solid (TSS) content was measured using a hand-held Refractometer (Model MT-032, Reed Instruments, UK) where solid concentration of a sucrose containing solution was determined

(Tsegay et al. 2013). Juice of chili fruits was extracted using the method by Tsegay et al. (2013) and the filtrate was used for TSS determination using hand-held refractometer by placing a few drops of clear juice on the prism surface. Titratable Acidity (TA) was analysed using the method developed by Tsegay et al. (2013).

Data Analysis

Statistical analyses were performed using the SPSS version 21.0 software package. ANOVA was used to test whether or not there are significant differences in each parameter measured among the treatments. When there were significant differences, the Tukey post-hoc test was applied to determine which means were most alike (or different) and to test the equality of means for each pair of variables.

RESULTS AND DISCUSSION

Results of post-harvest parameters clearly showed that chilies grown in the greenhouse benefit from pollination by *H. itama* (Table 1, Table 2, Fig. 3 and Fig. 4). Chilies produced from *H. itama* pollination and hand-cross pollination have significantly higher average number of seeds per fruit than self-pollination ($p<0.05$). In terms of fruit weight, chilies produced from *H. itama* pollination were significantly heavier compared to self-pollination ($p<0.05$). Interestingly, flowers pollinated by *H. itama* produced significantly longer chilies than self-pollination and hand-cross pollination ($p<0.05$). However, there was no significant difference in the average diameter of chili produced between self-pollination, hand-cross pollination and *H. itama* pollination ($p>0.05$) (Table 1).

Chili and other members of Solanaceae are noted for their low attractiveness to bees or other insect pollinators (Cruz et al. 2005). However, other researchers have proved that chili benefits from bee pollination by producing wider and longer chili with greater number of seeds per fruit and reduced malformation (improved fruit quality). For example, Shipp et al. (1994) reported that pollination of sweet pepper using bumble bee (*Bombus impatiens*) had shown significant differences for fruit weight, fruit width, fruit volume, pericarp volume, seed weight and days from fruit set to harvest between sweet peppers produced from bee pollination and without bee pollination.

In this study, flowers pollinated by *H. itama* produced chilies with higher number of seeds per fruit, heavier and longer fruit size than chilies produced by self-pollination treatment. Interestingly, average number of seeds and fruit weight produced from chilies pollinated by *H. itama* did not differ from hand-cross pollination. Therefore, it is clearly shown that the quality of chilies from stingless bee pollination was almost similar to the chilies produced from hand-cross pollination.

According to the previous research, Cruz et al. (2005) found that higher number of seed developing inside fruits produced from *Melipona subnitida* pollination would lead to bigger and heavier fruits in the greenhouse. Seeds play an important role in fruit setting process, since badly developed fruits are the result of an unequal seed distribution inside the fruit (McGregor 1976). Therefore, rapid development of ovary occurs in well pollinated flowers and cross seeds produce plant growth hormone that lead to good fruit development. Similar result was obtained by Shipp et al. (1994) where they obtained heavier and bigger sweet pepper fruits produced from the bumble bee pollination. In addition, a study by Kiatoko et al. (2013) found that flowers pollinated by *Hypotrigona gribodoi* produced heavier fruits. Jarlan et al. (1997) also worked with sweet pepper pollination and found similar results using the fly, *Eristalis tenax* as pollinator. Thus, it seems that bee visits to chili flowers are necessary to ensure desired fruit weight, length and diameter.

Figure 1. The experimental layout for pollination by the stingless bees (*H. itama*) in the greenhouse. (A) The red circle shows a colony of *H.itama* that was placed in the greenhouse for at least 2 days to acclimatize before the flower anthesis. (B) Self-pollination or control chili plants. (C) Stingless bee hive kept in the greenhouse after fruit set.

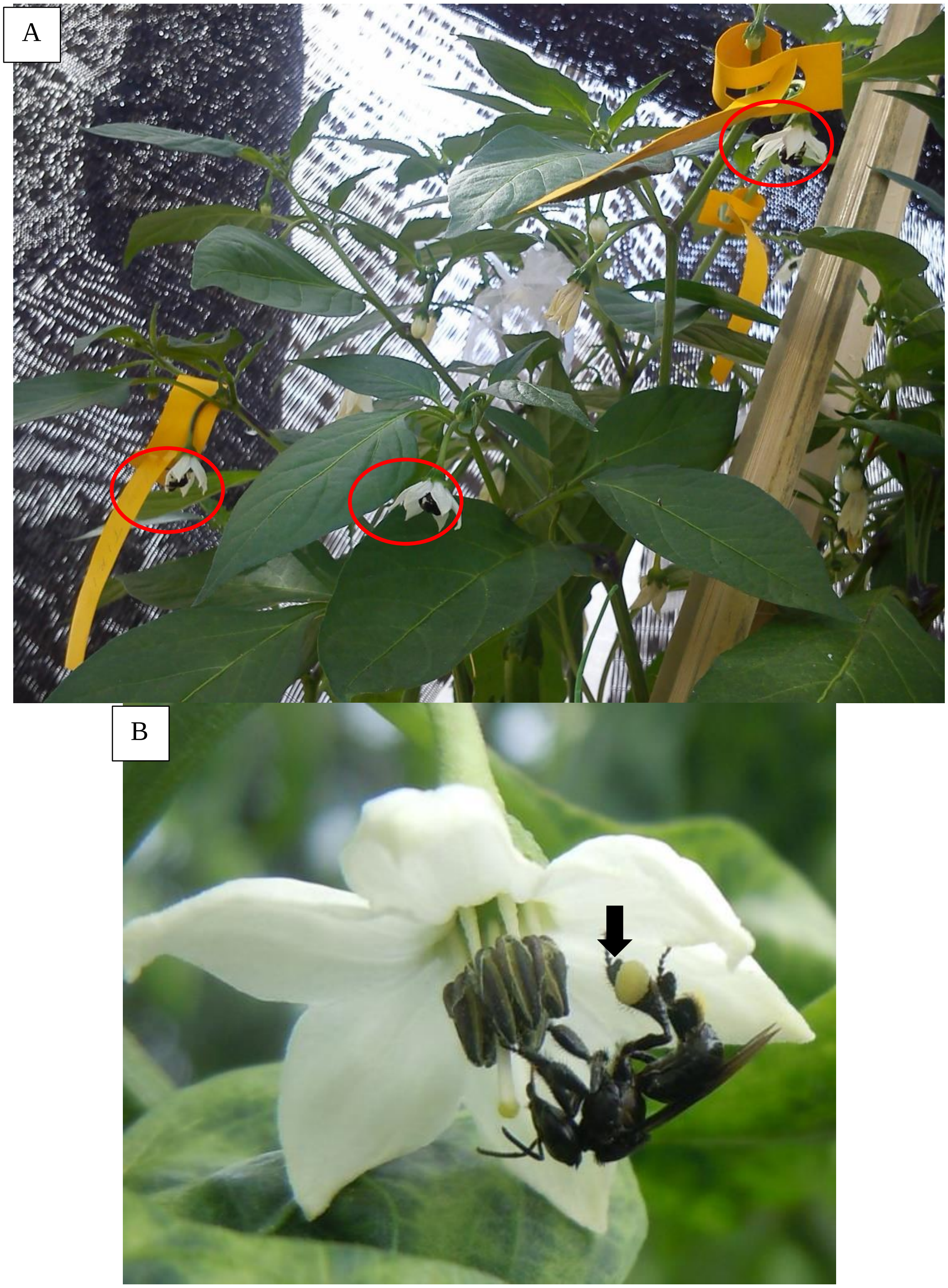

Figure 2. Foraging activity of *H. itama* was observed from 0700 to 1200 h during the chili flower anthesis period. (A) Red circles are the flowers that received *H. itama* visit. (B) Arrow shows a stingless bee with its corbicula (pollen collecting apparatus) full of pollens.

Table 1. Number of seeds, fruit weight and size of chilli (*Capsicum annuum*) fruits produced from self-pollination, hand-cross pollination and *Heterotrigona itama* pollination.

Treatment	No. of seeds (no/fruit)	Weight (g)	Length (cm)	Diameter (mm)
Self-pollination	48.54 ±15.28a	8.63 ±1.45a	9.02 ±0.77a	11.38 ±1.28a
Hand-cross pollination	102.92 ±24.25b	9.77 ±1.54a,b	9.23 ±1.89a	11.58 ±1.05a
H. itama pollination	112.54 ±21.15b	11.61 ±0.86b	13.00 ±0.59b	12.40 ±0.94a

*Means ± SD in column with same letter are not significantly different (p>0.05) according to Tukey Post Hoc test (n = 20).

Table 2. Percentage of fruit malformation, firmness, Total Soluble Solid and Titratable Acidity of chilli (*Capsicum annuum*) fruits produced from self-pollination, hand-cross pollination and *Heterotrigona itama* pollination.

Treatment	Percentage of malformation (%)	Firmness (N)	Total Soluble Solid (°)	Titratable Acidity (%)
Self-pollination	0a	1.702 ±0.30a	9.64±1.07a	0.369±0.03a
Hand-cross pollination	10.00 ±2.00a	1.789 ±0.31a	9.48±2.18a	0.325±0.05a
H. itama pollination	0a	1.839 ±0.37a	7.35±0.51a	0.313±0.06a

*Means ± SD in column with same letter are not significantly different (p>0.05) according to Tukey Post Hoc test (n = 20).

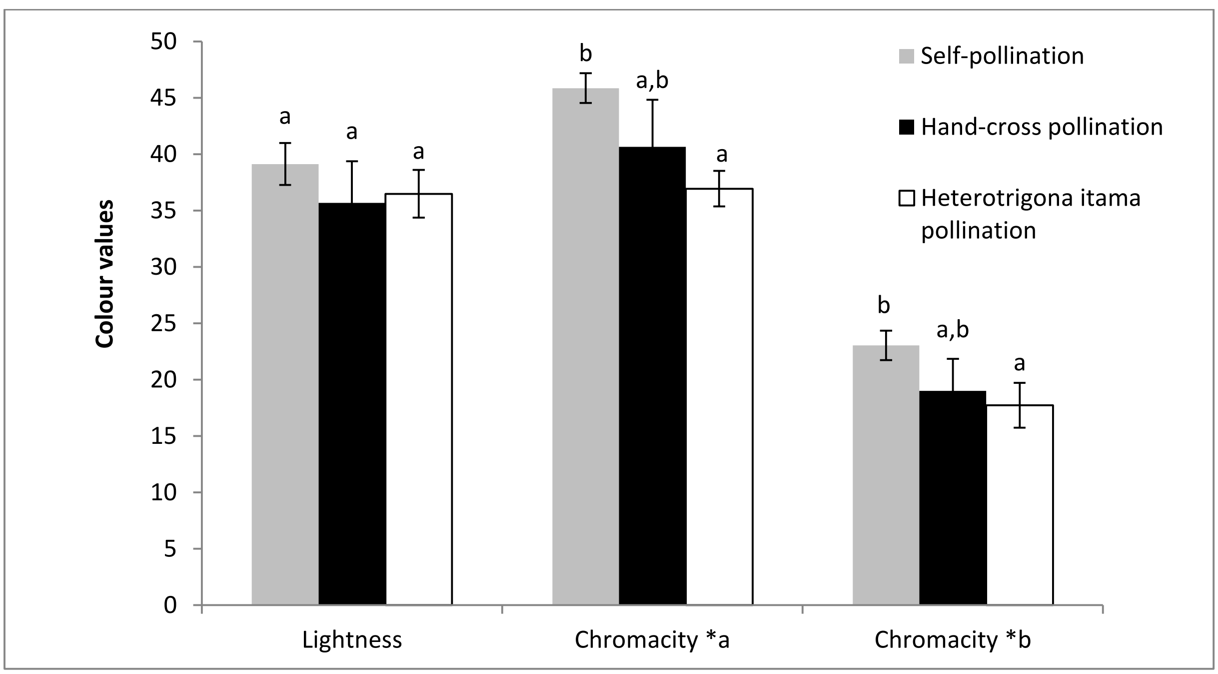

Figure 3. Average colour values of chilli (*Capsicum annuum*) fruits produced from self-pollination, hand-cross pollination and *Heterotrigona itama* pollination. *Bars with same letter are not significant difference (p>0.05) according to Tukey Post Hoc test.

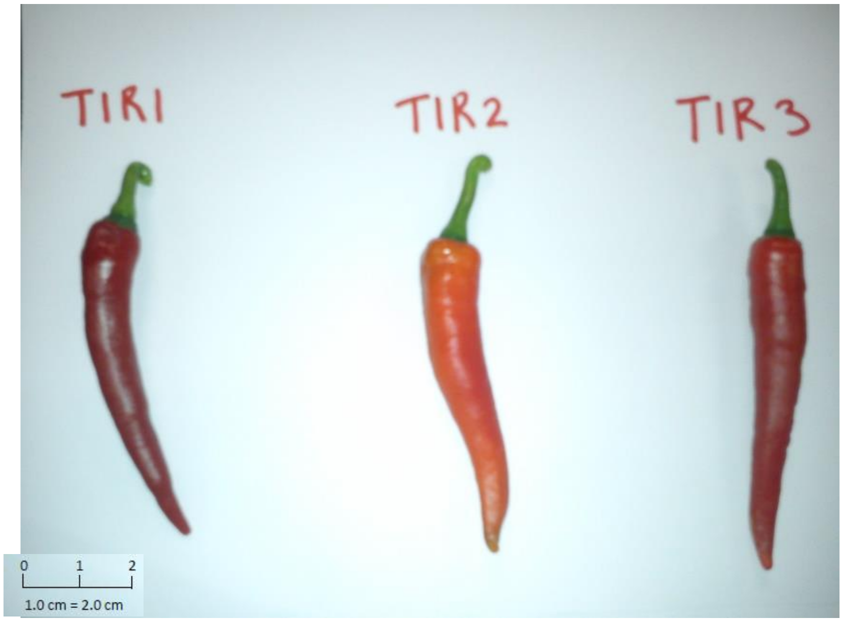

Figure 4. Chilli (*Capsicum annuum*) fruits produced from self-pollination (TIR1), hand-cross pollination (TIR2) and *Heterotrigona itama* pollination (TIR3).

Chilies produced from the self-pollination and pollination by stingless bee treatments had no malformed fruits compared to hand-cross pollination (Table 2). However, there was no significant difference in the percentage of malformed fruits among the three treatments. The pollination by stingless bees produced the highest number of seed set and no percentage of malformed fruits. Hand-cross pollination also set a high number of seeds, but produced a high percentage of deformed fruits. This probably occurs because *H. itama* deposited a great number of viable, compatible pollen as placed on the flower stigmas. In contrast, hand-cross pollination needs a lot of precision and requires skilled workers in order to produce high quality progeny. Failure to do so may probably lead to high number of malformed fruits. Thus, it can be suggested that there might be some effects from the number of pollen grains deposited by the stingless bee and pollen compatibility in seed set and fruit quality from the pollination by stingless bee which require further studied.

Even though there was no difference in sugar content in chilies among all treatments, the TSS content did not differ among treatments ($p>0.05$) (Table 2). Similarly, there were no significant differences of average TA ($p>0.05$) and fruit firmness ($p>0.05$) produced between self-pollination, hand-cross pollination and *H. itama* pollination. Determination of TSS in fruits indicated the sugar content while acidity represents the organic acid such as malic acid, citric acid and oxalic acid content in fruits. Chili is one of the fruit vegetables containing malic acid. Sugar content increases as the fruit ripen. It has been reported that bee pollination may increase auxin and gibberellic acid production which may delay fruit-softening and thereby enhance firmness (Klatt et al. 2013). Therefore, higher firmness of fruits is associated with more stable cell walls which might reduce respiration, which is known to limit metabolic processes affecting sugar and acid contents during storage (Klatt et al. 2013).

In this study, fruits produced from *H. itama* pollination had lower sugar content and acidity but higher in terms of firmness compared with self-pollination and hand-cross pollination. The current results were in accordance to a study by Klatt et al. (2013). They observed that bee pollination was able to reduce sugar and acid content and were firmer, thus improving the commercially important fruit shelf life. They also suggested that the difference between wind and self pollination remained variety-dependent, whereas the sugar–acid–ratio of fruits resulting from bee pollination improves the fruit quality and differed from both treatments across varieties (Klatt et al. 2013). Thus, it shows that stingless bee pollination not only improve the chili quality, but also retain the fruit firmness.

In terms of fruit colours, there was no significant difference (p>0.05) of average lightness of chili colour between pollination treatments (Fig. 1 and Fig. 2). However, there were significant differences in average chromacity *a (p<0.05) and chromacity *b (p<0.05) of chilies produced between self-pollination, hand-cross pollination and *H. itama* pollination. Chromacity *a indicates the redness of chili, while chromacity *b indicates the yellowness of chili. In this study, self-pollination produced chili with high intensity of red and yellow colours than chili produced from *H. itama* pollination that had the lowest redness and yellowness of chilies (Fig. 5). This result is different from Klatt et al. (2013) who found that bee pollination may influence the fruit colour. Klatt et al. (2013) showed that bee pollination resulted in bright strawberries with a more intense red color than wind pollination fruits, whereas self pollinated strawberries were darker and less red. In this study, chilies produced from self pollination have better intensity of red and yellow colour compared to chilies from *H. itama* pollination. However, there is little information available about the effects of bee pollination on the fruit colour. Thus, more study is needed to confirm whether pollination by *H. itama* has effects on the colour of the chilies.

CONCLUSION

This study shows that chili produced from *H. itama* pollination and hand-cross pollination were significantly heavier, longer and contained greater number of seeds per fruit than self-pollination. However, fruit diameter, sugar and organic acid content, firmness and colour of chili were not significantly different among treatments. Thus, this study has found that *H. itama* contributed to higher fruit quality compared to that of the self-pollination. This reveals the potential of *H. itama* to be utilised in greenhouse for chili pollination. It is hoped that this study will enhance the awareness of the importance of stingless bee particularly *H. itama* as an alternative pollinator for *C. annuum* in agricultural ecosystem in Malaysia.

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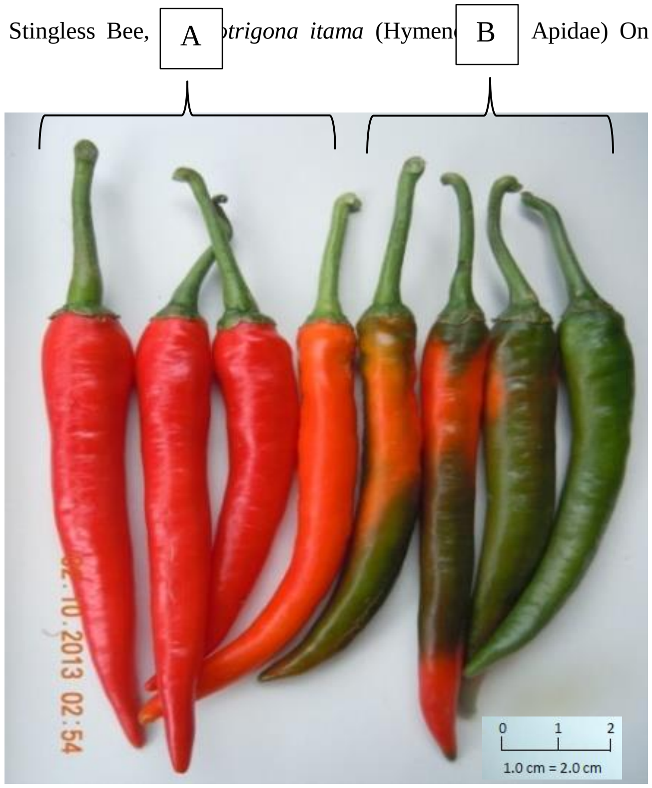

Figure 5. Chilies produced from self pollination have better intensity of red and yellow colour (A) compared to chilies from *H. itama* pollination (B). However, no significant difference ($p<0.05$) was detected between pollination treatments for the colour value of chilli fruit.