Malays. Appl. Biol. (2017) 46(1): 51–55

EFFECTS OF STINGLESS BEE (*Heterotrigona itama*) POLLINATION ON GREENHOUSE CUCUMBER (*Cucumis sativus*)

WAHIZATUL AFZAN AZMI[1*], NURHIDAYAH SAMSURI[1], MUHAMMAD FIRDAUS MOHD. HATTA[1], ROZIAH GHAZI[1] and CHUAH TSE SENG[2]

[1]*School of Marine Science and Environmental Sciences, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu*
[2]*School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu*
[*]*Email: wahizatul@umt.edu.my*

Accepted 24 January 2017, Published online 31 March 2017

ABSTRACT

Stingless bee is one of the important pollinators in open field crops as well as in the greenhouse crops. However, the potential use of stingless bees as pollinator for greenhouse cucumber is less documented in Malaysia. In this study, the stingless bees, *Heterotrigona itama* were placed together with the greenhouse cucumbers, *Cucumis sativus* in order to investigate the effects of *H. itama* pollination on greenhouse cucumber. This experiment involved three pollination treatments; namely pollination without stingless bee, hand-cross pollination and pollination by stingless bee, *H. itama*. Two hives of stingless bees were introduced into the greenhouse at least two days before the cucumber flowers started to bloom. Results showed that the cucumbers pollinated by stingless bee and hand-cross pollination produced heavier, longer and larger cucumbers compared to those produced from pollination without stingless bees. However, in terms of dried weight seed, firmness and colour of cucumbers, no significant difference between the treatments were detected. Further study is required in order to determine the pollination efficiency of stingless bees in greenhouse crops.

Key words: Stingless bees, *Heterotrigona itama,* pollination, greenhouse, cucumber

INTRODUCTION

Pollination is the process of transferring the pollen grain from anther of male flower to the stigma of female flower to enable fertilization to take place (Abrol, 2013). There are two types of pollination; namely self-pollination and cross-pollination which can occur in the same plant or different plant of the same species. Pollination may be aided by the presence of the pollinators such as water, wind, human and animals (Tepedino, 1981).

According to Abrol (2013), there are about 25,000 to 30,000 bee species worldwide, which are considered as obligate flower visitor and are the efficient pollinators to the cultivated crops and wild nature. One of the efficient pollinators is the stingless bees. The stingless bees can forage effectively in glasshouses (Heard, 1999). Most of the species of bees such as honeybees and stingless bees can be managed for crop pollination.

Several studies found that pollination by stingless bees can affect the fruits produced, especially for major cultivated crops. For example, cucumber pollination by the stingless bees helped in increasing fruit quantity and improving quality of the fruit produced (Santos *et al.,* 2008). However, most of the studies on the effects of the stingless bee pollination on crops were conducted outside Malaysia. For instance, a study done by Klatt *et al* (2013) conducted at Gottingen, Germany showed that bee-pollinated strawberries produced were heavier, had less malformed fruits and reached higher commercial grades. Another study done by Koffi *et al* (2013) showed that *Citrullus lanatus* produced more fruits and seeds from cross pollination with multiple sources compared to natural pollination and self-pollination. This showed that bee species can be an effective pollinator for specific crops which can be beneficial for improving fruit quantity and quality.

In Malaysia, cucumbers are usually grown outdoors. However, due to better control of plant

* To whom correspondence should be addressed.

growth and environmental conditions, cucumbers are grown in the greenhouse where bees are excluded. To overcome the problems, most farmers use many workers to help in manual cross-pollination of the flowers which causes increase in hiring costs and time needed to pollinate the flowers. Therefore, the aim of this study was to investigate the potential of stingless bees as pollinator for greenhouse cucumber.

MATERIALS AND METHODS

Plant materials and experimental location

The study was carried out from November 2014 to January 2015 in a greenhouse of 70 feet × 5 feet (l × w) at GM Peladang, Kuala Ibai, Kuala Terengganu, Terengganu (5°20'19.8"N 103°07'07.4"E). *Cucumis sativus* (Cucumber) seeds were provided by Pejabat Ladang, Universiti Malaysia Terengganu. Cucumber seeds were soaked with Atonik (hormone growth) (Asahi Chemical MFG Co. Ltd.), Previcur (fungicide) (Bayer Crop Science) in warm water (40–50°C) for one hour. Seeds were then planted into moist peat moss or coco peat in the seed tray and closed with silver shine plastic. The seeds started to grow in two days and became young cucumber plantlets in a week. After 10 or 15 days, the young plants were transferred into polybags (size 16" × 16"), spaced 60 × 40 containing 5 kg of coco peat. Cucumber plants received the same amount of fertilizers with the combination of 10 g urea and potassium chlorate and 7 g of superphosphate per polybag according to crop growth. Plants were irrigated daily as needed.

Experimental design

The greenhouse was divided into three sections which have three different treatments namely pollination without stingless bees, manual cross-pollination and pollination with stingless bees, *Heterotrigona itama* (Figure 1). There were 120 polybags of *C. sativus* were planted in the greenhouse where each section have 40 replicates of *C. sativus* plants. Pollination without stingless bees was treated as control treatment. Only the treatment with manual cross-pollination and stingless bee pollination treatment were covered with mesh net with size of opening 250 × 720 micro thoroughly to prevent any other pollinators from pollinating the flowers. In the manual cross-pollination treatment, 40 buds of flowers were chosen randomly, tagged and bagged. After the anthesis of cucumber flowers at 0700 to 1000, the flowers were unbagged and manual cross-pollination was done. Two or three of male flowers were picked and their anthers were rubbed onto the stigma of female flowers for manual cross-pollination. All the 40 buds of female flowers were bagged again for a week until setting of fruit in order to avoid visits from other insect pollinators on the flowers. For pollination by stingless bees, two hives of strong colonies of *H. itama* were placed in the greenhouse for at least 2 days before the anthesis of flowers in order to initiate adaptation of the stingless bee with the new environment. Foraging activity of *H. itama* such as visiting the flowers had been observed from

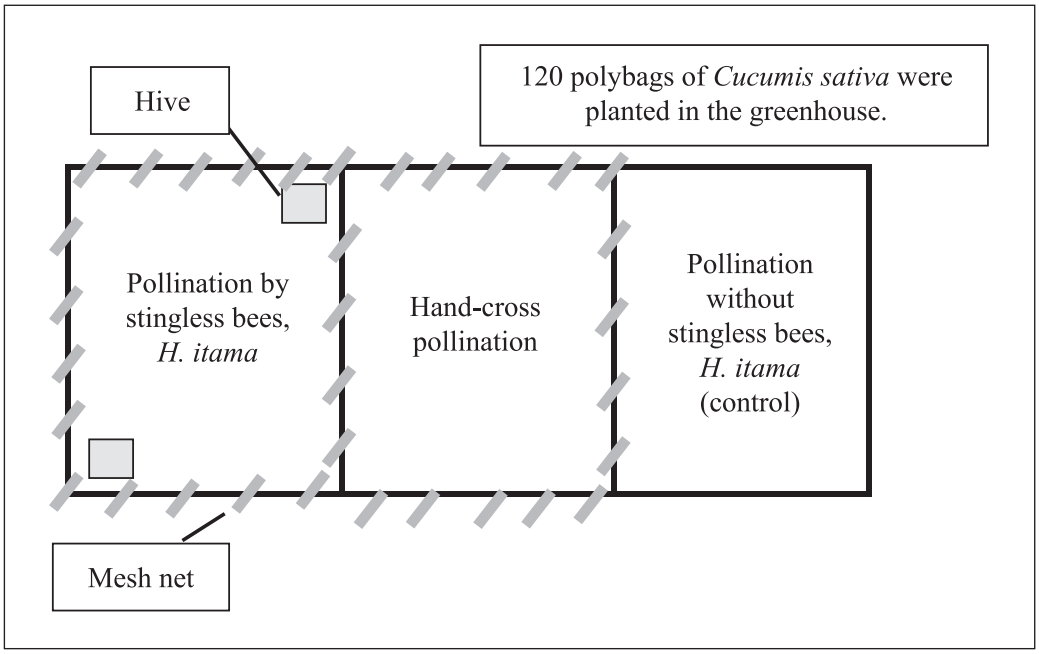

Fig. 1. The experimental layout for pollination by the stingless bees (*H. itama*) in the greenhouse.

Fig. 2. Foraging activity of *H. itama* was observed from 0700 to 1200 h during the cucumber flower anthesis period.

0700 to 1200 hr (Figure 2). Forty buds of female flowers visited by *H. itama* were tagged and bagged using the mist net (size opening: 0.5 mm).

Production-related parameters

After four to five weeks, the cucumbers became mature and were harvested. The cucumbers were harvested using sharp knife or scissors after the cucumber had reached the commercial standards which were firm, bright, green in colour, and with length of about 15 to 30 cm. For each treatment, total number of fruits produced and fruit malformation were counted. All the harvested cucumbers were kept in different trays and stored at room temperature. Six parameters were evaluated in this experiment: fruit weight, fruit size (diameter and length), average of dried seeds weight per fruit, fruit firmness and fruit colours. The fruit weights (g) were measured using weight balance, length (cm) using ruler or measuring tape and diameter (cm) was measured using the caliper or measuring tape which was positioned at the midpoint of cucumber. Fruit firmness was measured using a Stable Macro System, TA. XTplus texture analyzer. Fruit colour was measured using a CR-400/410 Chroma meter (Kinoca Minolta Photo Imaging USA Inc., Mahwah, NJ). External colour readings were taken and averaged on 10 cucumbers. For the average of dried cucumber seeds weight (g), 10 cucumbers from each treatment were chosen randomly. Fifty seeds were taken from the selected cucumbers, dried naturally and then weighed using weight balance.

Data Analysis

Statistical analyses were performed using the software package of SPSS version 21.0. ANOVA was used to test for the differences in the production-related parameters among the treatments. Where 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

In general, cucumbers produced from pollination by stingless bee and manual cross-pollination treatments were similar in weight, length, diameter and firmness, lower in green and yellow colour compared with cucumbers from treatment without stingless bee (Table 1).

Table 1. Results of fruit weight, length, diameter, firmness and dried seed weight of cucumber fruits produced from pollination without stingless bee, hand-cross pollination and *Heterotrigona itama* pollination

Treatment	Weight (g)	Length (cm)	Diameter (cm)	Firmness (Brixmeter)	Dried seed weight (g)
Without stingless bee	0.30±0.09a	19.61±1.62a	15.75±2.07a	26.49±4.51a	0.46±0.23a
Hand-cross pollination	0.42±0.07b	17.58±1.90b	17.58±1.21b	25.42±3.16b	0.82±0.32b
H. itama pollination	0.43±0.08b	22.20±1.12b	17.84±1.01b	23.85±2.12b	0.79±0.25b

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

The highest total number of cucumber produced was from treatment of pollination with stingless bee (n = 29), followed by manual cross-pollination (n = 26) and pollination without stingless bee (n = 24). Pollination without stingless bee showed higher number of fruit malformation (n = 14) compared with manual cross-pollination (n = 11) and stingless bee pollination treatment (n = 9). However, no significant differences in total number of fruit sets and in the total number of malformed fruits among treatments were detected (ANOVA, $p > 0.05$).

Cucumbers are monoecious plants which have separate female and male flowers in the same plant. The cucumber pollen is sticky and cannot be dispersed by wind or air. Thus, it needs the bees as pollination agent. As stated by Nicodemo *et al* (2013), the production of cucumber increased by 26% when the stingless bees were placed in the greenhouse. A study by Amano (2005) in Japan showed that stingless bee pollination produced higher number of fruit sets than honey bees. Honeybees is one of the important pollinators in crop yields but they are hard to manage and cannot be easily transported to new area (Slaa *et al.,* 2006). The costs for hiring beekeeper will be reduced because the stingless bees are harmless to beekeeper and greenhouse workers. Therefore, the stingless bee can act as efficient greenhouse crop pollinator.

The malformation of cucumber from stingless bee and manual cross-pollination treatment were lower than cucumber formed without pollination by stingless bee. The higher number of malformed cucumber formed in control treatment could be caused by the rain where infection can occur easily during bad weather. Meanwhile, lower number of malformed cucumber from stingless bee pollination could probably occur because *H. itama* deposited a great number of viable, compatible pollen as placed on the flower stigmas.

In this study, cucumbers from treatments with pollination by stingless bee and manual cross-pollination produced heavier, longer, wider and heavier in dried seed weight than treatment without stingless bee pollination. The cucumbers from stingless bee pollination were significantly heavier (0.43 kg ± 0.08) and longer (22.20 cm ± 1.12) than the weight and length of cucumbers produced from pollination treatment without stingless bee. However, there was no significant difference with manual cross treatment ($F = 0.98$, $df = 2$, $p<0.05$). Cucumbers produced from stingless bee pollination (17.84 cm ± 1.01) and manual cross-pollination (17.58 cm ± 1.21) were larger in diameter compared with cucumbers from pollination without stingless bee (15.75 cm ± 2.07). There were significant differences between diameter of cucumber from pollination without stingless bee and the other two treatments ($F = 5.75$, $df = 2$, $p < 0.05$).

According to Delaplane & Mayer (2000), when more seeds developed into fruit, the fruit size became larger and contributed to the increase in fruit weight than fruits with less seed. With stingless bee as pollinator, the cucumber will have more seeds. Thus, it shows that stingless bee can be the pollinator for greenhouse cucumber. Similar results were obtained with previous study by Nicodemo *et al* (2013) who found that the pollination by Brazilian native stingless bee contributed to a significant increase in cucumber diameter and length compared to the cucumbers pollinated by honeybees. This shows that cucumber flowers pollinated by the pollination agents such as bees have better qualities in terms of weight, length and diameter. In fact, cucumber flowers pollinated by the stingless bee may increase the quality of fruit production (Heard, 1999).

In terms of cucumber firmness, the three treatments showed no significant differences between them (ANOVA, $p > 0.05$). Similarly, no significant difference for dried seed weight were detected between the three treatments (ANOVA, $p > 0.05$). For brightness of cucumber, L* value for cucumbers pollinated by stingless bee showed low lightness (45.24 ± 5.24) compared to colour of cucumbers from the other two treatments but there was no significant difference in the lightness of cucumbers among the three treatments ($F = 1.09$, $df = 2$, $p < 0.05$) (Table 2). In terms of greenness of cucumber, a* value from

Table 2. Comparison of colour (L, a, b) between pollination without stingless bee, hand cross-pollination and pollination by stingless bee (*Heterotrigona itama*)

Treatment	Colour (mean)		
	Brightness (L*)	Greenness (a*)	Yellowness (b*)
Without stingless bee	48.21±6.43a	-9.99±0.95a	23.82±7.77a
Hand-cross pollination	48.66±5.08b	-8.80±1.01b	18.15±3.39b
H. itama pollination	45.24±5.24b	-9.07±0.99b	16.66±3.89b

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

cucumber pollinated by manual cross and stingless bee showed lower green colour (-8.80 ± 1.01; -9.07 ± 0.99) than cucumber pollinated without stingless bee (-9.99 ± 0.95). Cucumbers of pollination by stingless bee did not show highly significant difference for b* value (yellowness colour) with cucumbers from pollination without stingless bee, and no significant difference between manual cross pollination ($F = 4.92$, $df = 2$, $p < 0.05$). Thus, cucumbers from stingless bee treatment had lower lightness, green colour and lower in yellow colour compared to the other treatments.

However, firmness and colour did not show any difference among the three treatments. One of the reasons that may have caused these results was the monsoon season which can reduce the visitation rate of pollinators (Polatto *et al.,* 2014).

CONCLUSION

In general, cucumbers which resulted from pollination by stingless bee *H. itama,* and manual cross-pollination have improved in terms of crop quantity and fruit quality where heavier, longer and wider cucumbers were produced. However, firmness and colour of cucumbers produced did not have differences among the treatments. Thus, this study concluded that stingless bee, *H. itama* contributed to higher fruit quality compared to that of the pollination without stingless bee. This reveals the potential of *H. itama* to be utilized in the greenhouse for cucumber pollination.

ACKNOWLEDGEMENTS

We would like to thank the Ministry of Education (MOE) for the FRGS Fund (VOT: 59339), School of Food Science and Technology and School of Marine and Environmental Sciences at UMT for the laboratory facilities.

REFERENCES

Abrol, D.P. 2013. Pollination biology. In Asiatic Honeybee, *Apis cerana*: In *Biodiversity Conservation and Agricultural Production* (eds). Springer Publication No. 34, New York, pp: 509-574.

Amano, K. 2005. Attempts to introduce stingless bees for the pollination of crops under greenhouse conditions in Japan. Food and Fertilizer Technology Center, Taipei. pp: 1-9. http://www.fftc.agnet.org/library/article/-tb167.html.

Delaplane, K.S. & Mayer, D.F. 2000. *Crop pollination by bees*. USA. 33-36pp.

Heard, T.A. 1999. The role of stingless bees in crop pollination. *Annual Review of Entomology*, **44**: 183-206.

Klatt, B.K., Holzschuh, A., Westphal, C., Clough, Y., Smit, I., Pawelzik, E. & Tscharntke, T. 2013. Bee pollination improves crop quality, shelf life and commercial value. *Proceedings of the Royal Society B.* 1-8. doi.org/10.1098/rspb.2013.2440.

Koffi, A., Séverin, B.B., Guillaume, K.K., Bertin, Y.K., Clémence, K.L., Sylvère, Y.K. & Yao, D. 2013. Effect of pollen load, source and mixture on reproduction success of four cultivars of *Citrullus lanatus* (Thunb), Matsumara and Nakai (Cucurbitaceae). *Scienticia Horticulturae.* **164**: 521-531.

Nicodemo, D., Malheiros, E.B., Jong, D.D. & Couto, R.H.N. 2013. Enhanced production of parthenocarpic cucumbers pollinated with stingless bees and Africanized honey bees in greenhouses. *Ciências Agrárias,* **34(6)**: 3625-3634.

Polatto, L., Chaud-Netto, J. & Alves-Junior, V. 2014. Influence of abiotic factors and floral resource availability on daily foraging activity of bees. *Journal Insect Behaviour*, **27(5)**: 1-20.

Santos, S.A., Roselino, A.C. & Bego, L.R. 2008. Pollination of cucumber, *Cucumis sativus* L. (Cucurbitales: Cucurbitaceae) by the stingless bees *Scaptotrigona aff.* depilis Moure and *Nannotrigona testaceicornis* Lepeletier (Hymenoptera: Meliponini) in greenhouses. *Neotropical Entomology*, **37(5)**: 506-512.

Slaa, E.J., Sánchez Chaves, L.A., Malagodi-Braga, K.S. & Hofstede, F.E. 2006. Stingless bees in applied pollination: practice and perspectives. *Apidologie*, **37**: 293-315.

Tepedino V.J. 1981. The pollination efficiency of the squash bee (*Peponapis pruinosa*) and the honey bee (*Apis mellifera*) on summer squash (*Cucurbita pepo*). *Journal of the Kansas Entomological Society,* **54(2)**: 359-377.