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Research Article

Evaluation of Native Stingless Bee Species (*Heterotrigona itama* and *Geniotrigona thoracica*) for Pollination Efficiency on Melon Manis Terengganu

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ABSTRACT

Melon Manis Terengganu (MMT) is a newly developed melon cultivar that is exclusively planted in Terengganu and has monoecious flowers where female and male parts are in different flowers of the same plant. Current practise for MMT pollination mainly depends on humans by hand-cross pollination treatment. However, until now little study on the potential of stingless bees as pollinator for greenhouse MMT has been documented in Malaysia. In this study, two species of stingless bees, *Heterotrigona itama* and *Geniotrigona thoracica* were placed with the MMT in the greenhouse. This study is aimed to investigate and compare the quality of MMT produced by four different pollination treatments; (1) self-pollination, (2) hand-cross pollination, (3) *H. itama* pollination and (4) *G. thoracica* pollination. Two hives of each stingless bee species were placed into the greenhouse at least two days before the MMT flowers bloomed. MMT produced from pollination by both stingless bee species and hand-cross pollination were significantly heavier in fresh weight, larger in diameter, higher in total soluble solid (TSS), and greater number of seeds per fruit compared to those produced from self-pollination. Pollination by stingless bees reached fruits with higher sweetness than hand-cross pollination and self-pollination. Results revealed that the stingless bee pollination on MMT production was similar to the MMT produced from hand-cross pollination. This study provides essential information on the potential of native stingless bees, *H.itama* and *G. thoracica* which can be effective pollinators for the MMT grown in the greenhouse besides manual pollination.

Key words: *Geniotrigona thoracica*, *Heterotrigona itama,* melon, pollination, stingless bee

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INTRODUCTION

Melon Manis Terengganu (MMT) (*Cucumis melo* var. *inodorous* cv. Manis Terengganu 1) is a newly developed variety of melon that was released exclusively for Terengganu in 2015. MMT belongs to the Cucurbitaceae family, which includes various squashes and melons. MMT is announced as the iconic fruit of Terengganu by the state government of Terengganu (Department of Agriculture Terengganu, 2019). It has become one of the most popular crops planted in Terengganu and has huge potential to become an important horticultural crop for the international market.

It has orange coloured flesh like other rockmelons and smooth yellow golden outer surface without netting of skin. To get high quality MMT, only one of six melons for each tendril is left and keep growing until it matures to reduce competition and get complete nutrition (Department of Agriculture Terengganu, 2019). Anthesis and pollination of MMT occur within 30 to 35 days of drilling, and around 75 days is needed for a complete life cycle of MMT plants (Mahmud *et al.,* 2009). MMT has monoecious flowers where the female (pistilate) and male (staminate) are both in the same plant (Tepedino, 1981). The male flower will present first, followed by the female flower, but they appear on different branches. Thus, insects of effective pollinators are needed to ensure pollination occurs as plants with monoecious flowers need the pollination agents (Azmi *et al.,* 2019).

In Terengganu, MMT is usually planted in greenhouses; however, the production and quality of the fruits are restricted by poor pollination because insect pollinators have no access to the greenhouse (Yong & Shafqat, 2003). As a solution, workers are hired to hand pollinate the flower of MMT, but this method requires a high cost and is ineffective compared to insect pollinators (Tengku Norulhuda T.M.M., personal communication).

Stingless bees are significant pollinators for some commercial crops and can efficiently forage in the greenhouse (Heard, 1999). Several farm crops in Malaysia such as star fruits, mango, watermelon, coconut and chilli are pollinated by stingless bees (Slaa *et al.,* 2006). In addition, several studies have shown that stingless bee pollination may also increase the production as well as the quality of fruits such as strawberry, tomato, eggplant, sweet pepper and cucumber (Del Sarto *et al.,* 2005; Santos *et al.,* 2008;Nunes-Silva *et al.,* 2013; Azmi *et al.,* 2017).

Heterotrigona itama and *Geniotrigona thoracica* are the native stingless bees in Malaysia and are commonly used for pollination services for crops in agricultural ecosystems (Mohd Norowi *et al.,* 2010). To date, very little attention has been made to investigating the pollination efficiency of these stingless bee species specifically on MMT flowers. Therefore, the current study was carried out to determine the efficiency of native stingless bees as a potential pollinator for MMT grown in the greenhouse.

MATERIALS AND METHODS

Study Site

This study was carried out in three greenhouses at Taman Kekal Pengeluaran Makanan (TKPM), Peradong, Manir, Terengganu (5°28'00.03"N 103°04'.50"E) from September 2019 until January 2020 (Figure 1). Experiments were conducted in three greenhouses (120 feet × 25 feet) where 1200 polybags of MMT were planted in each greenhouse.

MMT cultivation

MMT was used as the plant material in this study. MMT seeds were sowed into a sowing tray that contained peat moss as a nursery medium. Seed germination took about three to four days. The seedlings then were transferred into polybags containing coco peat into the greenhouse during the seventh to tenth days of seed germination. Supported ropes were used to support the plant's growth and help the plant climb properly. In the fertigation system, all plants were irrigated daily with the same amount of water and fertilizer by the drip irrigation system. The fertilizer concentration was provided based on the age of the plant, where the fertilizer solution was increased according to plant growth day by day. The stingless bee colonies were introduced during the MMT's anthesis period, which was 30 to 37 days. After that, the fruit set formation was recorded and the MMT fruits were harvested on day 75th for laboratory analysis.

Experimental design

The experimental design for this study was a Randomized Completely Blocked Design (RCBD). The MMT pollination treatments were classified into four treatment groups which were self-pollination (geitonogamy), hand-cross pollination, *H. itama* pollination and *G. thoracica* pollination. stingless bee pollination. The greenhouse was divided into four equal sections with a mist net (2 meters), where one-fourth was used for self-pollination (geitonogamy), the second section for hand cross pollination and the other two remaining sections were occupied for stingless bee pollination, respectively. Each treatment was replicated three times, and nine random MMT plants were chosen and used for each treatment.

In self-pollination (geitonogamy), the shaking technique was used. Only nine flower buds were chosen randomly in each treatment. The nine selected plants were shaken for the fertilization process to have occurred. The flower buds were bagged until anthesis and tagged (date) with a mesh net (mesh size: 1 mm × 1 mm). For hand-cross pollination, the other nine flower buds were bagged and tagged with a mesh net. After the anthesis, the blooming flower was unbagged and the flower was hand-cross pollinated with pollen from other male flowers that came from other plants. This process was done by touching the female flower stigma with stamen. Then, the flowers of hand-cross pollinated were bagged and tagged again. This was done to avoid other intruders and pollination agents. For stingless bee pollination, the hives of each species were placed two days before the plants started flowering. The hives were placed on each side of the blocks in the greenhouse. The pollen from flowers stuck onto the hind legs of the stingless bee workers as they moved from one flower to another. The pollination process happens as the bees transfer pollen from one flower to another. Stingless bee hives were removed from the greenhouse after one week of pollination.

Laboratory analysis

MMT fruits on the stalk in the range of 5-13 were harvested after 70-75 days. The fruit set for the MMT fruit was recorded. Only the best and high-

Fig. 1. The location of the greenhouses at Taman Kekal Pengeluaran Makanan (TKPM), Peradong, Manir, Terengganu

quality MMT fruits were selected. For post-harvest parameters, the fruit weight was measured using a weight balance and the fruit diameter was measured using a ruler positioned at the fruit's midpoint. For the fruit firmness, the fruit was measured using a texture analyzer, while the total soluble solid (TSS) was measured by a handheld refractometer and the fruit colour was tested by a chromameter. After the diameter of the fruit was measured, it was cut in half. The seeds were taken out from the fruit and manually counted. The total number of seeds per fruit was recorded.

Statistical analysis

A normality test was conducted to check the distribution of data before doing ANOVA. One-way ANOVA was used to determine if there were any differences between the mean groups of the tested variable. All the data were analyzed using Paleontological Statistics (PAST) software. The Post-hoc Test of Tukey at significance levels of 5 % (significant) was used if there were significant differences between the treatments ($p<0.05$).

RESULTS AND DISCUSSION

In this study, MMT fruits from pollination treatments by both stingless bees species (*H. itama* and *G. thoracica*) and hand-cross pollination were longer, heavier, wider, sweeter and more dried seeds than self-pollination treatment (Table 1). Interestingly, results of post-harvest parameters showed that MMT fruits produced from the stingless bee *G. thoracica* pollination were more presentable than fruits from *H. itama* pollination, hand cross pollination and self-pollination. However, there were no significant differences between MMT fruits produced from stingless bees pollination and hand cross pollination treatments. The fruits produced were slightly similar in weight, diameter, number of seeds per fruit and firmness.

MMT fruits produced from *H. itama* and *G. thoracica* pollination and hand-cross pollination were significantly heavier than self-pollination ($F = 62.77$, $df = 3$, $p < 0.05$). MMT produced from *G. thoracica* pollination was heavier (1.6 ±0.24 kg) than the weight of MMT produced from *H. itama* pollination, hand-cross pollination and self-pollination. Besides, MMT produced from *G. thoracica* pollination was longer in diameter (14.71 ± 0.56 cm) compared with MMT produced from other pollination treatments ($F = 100.1$, $df = 3$, $p < 0.05$), but no significant difference was observed between both stingless bee pollination treatments.

Similarly, pollination by *G. thoracica* recorded a significantly higher number of seeds (614.44 ± 36.54) compared to the other pollination treatments ($F = 99.99$, $df = 3$, $p < 0.05$). In terms of the sweetness of fruit, stingless bees pollination resulted in higher fruit sweetness than hand-cross pollination and self-pollination (F = 128.4, df = 3, $p < 0.05$). There was a significant difference in fruit texture between the three pollination treatments (F= 10.16, df = 3, $p < 0.05$), where the MMT flesh firmness was softer in *G. thoracica* pollination compared with other treatments.

However, the effect of pollination treatments on outer colour and flesh colour varied according to different pollination methods (Table 2). The colour of fruit tends to be an indicator of flavour (sweetness) and freshness (quality). In this study, the L* value for MMT pollinated by hand-cross pollination showed higher lightness (skin: 74.62 ± 2.96, flesh: 60.13 ± 7.94) compared to the colour of MMT from stingless bee pollination and self-pollination. MMT produced from self- pollination showed higher green colour of a* value of MMT (skin: 5.80 ± 3.18) than other treatments. MMT produced from stingless bees pollination showed higher yellowness colour of b* value of MMT than hand-cross pollination and self- pollination. Generally, the flesh colour of MMT between four pollination treatments does not show any significant differences.

In this study, most of the post-harvest parameters of fruit produced from stingless bees and hand cross-pollination treatments were similar (Table 1). A study by Roselino *et al.* (2009) reported similar results, where they found that the quality of fruit produced from flowers pollinated by the stingless bees of *Scaptotrigona aff. depilis* and *Nannotrigona testaceicornis* were higher than in fruit produced from self-pollination. Santos *et al.* (2008) also found that *Melipona quadrifasciata* stingless bee-pollinated crops produced higher quality, heavier and larger fruit than those pollinated without the species. Similarly, Cruz *et al.* (2005) revealed that *M. subnitida* produced more seeds that developed within the fruits. Thus, it is clear that stingless bee pollination could produce larger and heavier fruits.

The stingless bees in the greenhouse had settled long enough to adapt to the conditions in the field, suggesting that once stingless bees become accustomed to conditions in the field, they show pollination effectiveness. It is suggested that the stingless bees' active foraging behavior be at its maximum. The bloom of experimental MMT flowers could positively influence the number of viable pollen grains deposited. The pollen load ability is also considered an indirect indicator of the relative foraging efficiency of bee foragers in various pollen types. Besides, Ramalho *et al.* (1994) found that the pollen load ability amongst stingless bees is likely not limited by weight as medium and large foragers can carry greater loads of pollen.

The sweetness of all MMT from stingless bees pollination showed a higher average sweetness compared with other treatments. During the maturing and ripening of fruits, it was found that the sweetness of sugar usually increases, which can be used as an indicator of maturity and the stage of ripeness (Syahidah *et al.*, 2015). Beaulieu and Gorny (2001) reported that the optimum sugar content is between 10 and 13° Brix in fresh-cut cantaloupe. We found that MMT fruits from the stingless bees pollination treatments were softer than in the self-pollination and hand-cross pollination treatments. According to Klatt *et al.* (2014), greater fruit firmness is associated with more stable cell walls, which was considered higher quality in stingless bee-pollinated fruit. The firmness decreases as the fruit matures and ripens entirely, where reduction of respiration and restricted metabolic processes may affect the sugar and acid content during storage. Consumers usually select their fruit choices based on the textured and external appearance of whole fruit which is used as an indicator of fruit maturity. This shows that the stingless bee pollination affects the quality and sweetness of MMT. Auxin and gibberellic acid prolong fruit softening and therefore improve firmness (Klatt *et al.*, 2014).

In terms of fruit colour, MMT resulting from stingless bee pollination is yellow with increased colour intensity compared to fruit from hand-cross pollination and self-pollination. Pollination by insects can influence the colour of fruits (Klatt *et al.,* 2014). Colour and appearance tend to be indicators of flavour (sweetness) and freshness quality. The cantaloupes are climacteric fruits that lose the color green of their fruit rinds upon ripening. Consumers easily recognize their yellow appearance as a sign of ripeness. In this study, we found that self-pollination and hand-cross pollination produced MMT fruit with higher colour intensity in green and yellow than those produced from *G. thoracica* and *H. itama* pollinations.

Apart from variation in external fruit color, variation in melon flesh (mesocarp) colour can be noted that includes green, white, cream, and orange (colour intensity is determined by beta-carotene content) (Burger *et al.* 2006). Chlorophyll degradation, fruit softening, and activation of the abscission zones are ethylene-dependent (Nishiyama *et al.,* 2007). Whereas the accumulation of soluble sugars and beta-carotene in the flesh of ripening fruits is ethylene-independent. However, the effects of bee pollination on ethylene content and its relation to MMT fruit colour can be further evaluated.

Putra *et al.* (2014) reported that higher flower handling and flower constancy of stingless bees improve the chance of pollen deposited in the stigma. The current findings are consistent with other previous studies that showed higher fruit set rates in plants from stingless bee pollination

Table 1. Results of fruit weight, diameter, number of seeds per fruit, firmness, and sweetness of MMT fruit produced from self-pollination, hand-cross pollination, and stingless bees pollination. Data are means ± SD. Different letters indicate treatment differences at $p<0.05$

Pollination Treatments	Weight (kg)	Diameter (cm)	Number of seeds per fruit	Sweetness (°)	Fruit texture (N)
Self-Pollination	0.78 ± 0.22a	11.1 ± 1.01a	357.57 ± 40.19a	9.09 ± 2.05a	7.75 ± 2.72a
Hand-cross Pollination	1.36 ± 0 .24b	13.86 ± 0.93b	543.56 ± 30.57b	11.33 ± 2.10a	5.22 ± 2.37ab
H.itama Pollination	1.38 ± 0.18b	13.66 ± 0.93b	590.00 ± 38.77b	14.60 ± 0.85b	4.38 ± 0.44b
G.thoracica Pollination	1.6 ± 0.24bc	14.71 ± 0.56bc	614.44 ± 36.54bc	14.16 ± 0.95b	3.97 ± 0.46b

Table 2. The average value of fruit color of MMT between pollination treatments. Data are means ± SD. Different letters indicate treatment differences at $p<0.05$

Pollination Methods	Skin color			Flesh color		
	Luminosity (L*)	Greenness (a*)	Yellowness (b*)	Luminosity (L*)	Greenness (a*)	Yellowness (b*)
Self-Pollination	70.96 ± 3.18a	5.80 ± 3.18a	51.91 ± 5.77a	55.67 ± 6.37ab	3.16 ± 1.11a	23.27 ± 5.63a
Hand-cross Pollination	74.62 ± 2.96b	-2.20 ± 2.35a	62.85 ± 8.99ab	60.13 ± 7.94ab	5.06 ± 1.02b	25.12 ± 5.09a
H.itama Pollination	73.2 ± 1.64b	-4.06± 1.80b	66.21 ± 3.06b	54.67 ± 4.23ab	3.45 ±0.71b	23.89 ± 3.78a
G.thoracica Pollination	75.24 ± 2.78b	-3.06 ± 1.45b	71.70 ± 4.62b	63.88 ± 4.55ab	5.49 ± 0.97b	30.01 ± 3.62b

compared to the other pollination treatments. The presence of a huge amount of pollinators could increase the fruit set of a plant. In terms of seed production, the seed production in MMT from stingless bee pollination was more significant than in the other two pollination treatments. According to Michener (2007), the pollination method influences the seed number of fruit size.

The stingless bees could enhance seed production by raising the number of pollen grains by transferring them to other plants' stigmas (Cauich *et al.,* 2006). A study by Richards (1997) found that the mature pollen of the stingless bee produced and released from the anther usually has a short lifespan of about 30 minutes. Even though the pollen released in the species that is pollinated by insects, the viable pollen is hardly reaches one day. Thus, the efficient stingless bee foraging behavior throughout the maximum bloom of MMT flower may affect the number of viable pollen grains deposited (Azmi *et al.,* 2019). The efficiency of native stingless bees particularly *G. thoracica*, however, has not been tested and needs to be proven. This current study has revealed that both native species mainly collected pollen which resulted in intimate contact with the stigma. This foraging behaviour has proved that native stingless bees yielded better MMT quality and function as efficient pollinators for this crop.

CONCLUSION

In a conclusion, this study showed that stingless bees *Geniotrigona thoracica* and *Heterotrigona itama* are proven to be efficient pollinators in producing better MMT quality compared to hand-cross pollination and self-pollination. The fruits produced by stingless bee pollination were heavier in weight, longer in diameter, higher in firmness, higher intensity of colour, a greater number of seeds and higher sweetness. This study shows the potential of *G. thoracica* and *H. itama* as efficient pollinators in Malaysian agricultural ecosystems. It is also recommended to further investigate the floral constancy and foraging behaviour of native stingless bees in open field experiments on other important tropical crops.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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