POLLINATION EFFICIENCY OF THE STINGLESS BEE, *Heterotrigona itama* (HYMENOPTERA: APIDAE) ON SOLANACEAE AND CUCURBITACEAE CROPS IN GREENHOUSE

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Outline

• Introduction on Pollination
• The potential use of stingless bees as pollinator for agriculture crops
• Objective of Study
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• Results & Discussion
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Pollination is the transfer of pollen from the anther (the male part of the flower) to the stigma (the female part of the flower) of the plant.

Insect pollinators pollinate about 80% of flowering plants (FAO, 2007).

Insects are largely responsible for a major part of the angiosperm pollination process (Zanette et al., 2004).
Pollen grain grows a tube.

Sperm and egg join - a tiny plant forms.

Lupin seed:
- Cotyledon (food supply)
- Testa (outer covering) of seed
- Plumule (young shoot)
- Radicle (young root)

Together the plumule and radicle is called the embryo.
Pollination

- Self pollination and cross pollination can occur in the same plant or different plant of the same species.
- Pollination may be aided by the presence of the pollinators:
  - Abiotic (wind, water)
  - Biotic (bats, birds, insects, human)
Elaeidobius kamerunicus, the African oil palm weevil
Pollination by Bees

• Solitary Bees

• Social Bees
Solitary Bees

Xylocopa, Amegilla, Megachile & Halictus
Social Bees
Stingless bees

- Stingless bees can be found in most tropical or subtropical regions of the world.
- Stingless bees are known to be important pollinator in tropical rainforest (Eltz et al., 2003).
- They are also good candidates for providing pollination services in agricultural ecosystem such as star fruits, mango, durian, watermelon, guava and coconut (Slaa et al., 2006).
Heterotrigona itama

- Tribe to Meliponini, family Apidae
- Native to Malaysia
- Active throughout the year
- Harmless / safe
- Resistance to diseases and parasites
- Low cost in rearing them
- Modified hind leg called corbiculae or pollen basket
- One of the potential candidates in commercial pollination
- Largely domesticated stingless bee in Malaysia
Advantages of stingless bee:

- Polylecty
- Adaptability
- Floral fidelity
- Can be domesticated
- Perennial colonies
- Large food reserves
- Important and effective pollinators of many crop species
Several studies found that pollination by stingless bees can affect the fruits produced especially for major cultivated crops.

Santos et al., 2008 - cucumber pollination by the stingless bees helped in increasing fruit quantity and improving quality of the fruit produced.

Klatt et al. (2013) - bee-pollinated strawberries produced were heavier, had less malformed fruits and reached higher commercial grades.

Cruz et al. 2005; Slaa et al. 2006 - stingless bee pollination increases chilli production.

However, most of the studies on the effects of the stingless bee pollination on crops were conducted outside Malaysia.

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.
Rockmelon,
(Cucumis melo var. glamour).
- Family: Cucurbitaceae
- Commercial crop in Malaysia
- Monoecious flower
- Pistillate and staminate both in the same plant but different branches.
- The female flower has different shape of receptacle than male flower.
Cucumber (*Cucumis sativus*)

- Family: Cucurbitaceae
- Commonly cultivated and consumed in Malaysia.
- **Monoecious** plants - separate male and female in the same plant.
- Most Cucurbitacea family depends on **insect-pollination** (Richards, 2001).
- Native stingless bee role in pollination is still poorly understood.
Chili (*Capsicum annuum*)

- Family: Solanaceae
- One of the important cultivated crops in Malaysia & a source of income for thousands of Malaysian farmers.
- Chili flowers are self-pollinated, the anthers need to be shaken to allow effective pollen release.
- The role of stingless bees in producing quality fruits of this crop is still unknown.
Objective of Study

• To determine the effects of stingless bee, Heterotrigona itama pollination on greenhouse rock melon (Cucumis melo var. glamour), cucumber (Cucumis sativus) dan chili (Capsicum annum).
Materials & Methods

- Experimental location:
  Greenhouses at GM Peladang Sdn. Bhd. Kuala Ibai, Terengganu (5°20'19.8"N103°07'07.4"E)
Greenhouse at the School of Food Science and Technology, UMT (N05°24.541', E103°05.347').
The greenhouse was divided into three sections which have three different treatments; pollination without stingless bees (1), manual cross-pollination (2) & pollination with stingless bees (3).

There were 120 polybags of the studied crop were planted in the greenhouse where each section have 40 replicates.
N = 120 plants / experiment
Pollination without stingless bee

- No mesh net.
- 40 buds of female flowers- tagged.
- Bagged when fruit started to form.

Hand-cross pollination

- 40 buds of female flowers- tagged & bagged before anthesis (7.00 am – 10.00 am).
- After anthesis, the flower was unbagged and the pollen (male) were rubbed into stigma (female) - bagged again (1 week).

Pollination with stingless bee, H. itama

- Placed two hives of H. itama (2 days before first anthesis).
- Observed activity of stingless bee visited the flower.
- Tagged and bagged (1 week).
GREENHOUSE PROCEDURE

1) Germination process
2) Seeds took 5 days to germinate
3) Plants were transferred into polybags
4) The development and growth in a good condition
5) 2 days before pollination day - introduce stingless bees
6) Pollination day – hand cross
   - 2 male flowers from another cultivar - touched the flower stigma with stamen
7) Pollination day – Stingless bees pollination - activity of stingless bees visiting flowers were observed.

8) The female flowers that already pollinate were bagged and tagged. (Fruit set formation)

9) Bigger fruits – unbagged the mesh net

10) Growth perfectly

11) Harvest day!

12) Samples collection – brought to the lab
• **Self pollination**: Flower was bagged during anthesis until setting of fruits.
• Stingless bees pollination

Two hives were placed in greenhouse when flowering start.

Stingless bees were monitored.

Flower visited by stingless bee was bagged.
Hand cross pollination:

Flower was bagged during anthesis.

Flower from another plant was picked and rubbed gently to the bagged flower.

Flower was bagged again until setting of fruit.
## Production-related parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Apparatus/ machine/ way</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>Weighing balance</td>
</tr>
<tr>
<td>Diameter (cm)</td>
<td>Measuring tape</td>
</tr>
<tr>
<td>Thickness of the fleshes (cm)</td>
<td>Caliper</td>
</tr>
<tr>
<td>Sweetness (%)</td>
<td>Refractometer</td>
</tr>
<tr>
<td>Firmness (N)</td>
<td>Texture analyzer</td>
</tr>
<tr>
<td>Number of Seed</td>
<td>Dried, count</td>
</tr>
</tbody>
</table>
Harvested cucumber

Cutting process

Measuring diameter of cucumber

Measuring length of cucumber
Stable macro system, TA. XTplus texture analyzer

Dried cucumber seed at room temperature

Cucumis sativus
Data analysis

- One-way ANOVA to determine differences between the treatments (weight, length, diameter, color, firmness, dried seed weight, etc.).

- Post Hoc tests of Tukey to determine which treatment is significantly different from others.
Results – Rock Melon

Fruit weight, diameter, sweetness, flesh thickness and number of seeds of rock melon fruits produced from self-pollination, hand-cross pollination and *Heterotrigona itama* pollination.

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

<table>
<thead>
<tr>
<th>Type of pollinations</th>
<th>Weight (kg)</th>
<th>Diameter of fruits (cm)</th>
<th>Percentage of sweetness (%)</th>
<th>Average of firmness (N)</th>
<th>Thickness of flesh (cm)</th>
<th>Number of seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without stingless bee</td>
<td>1.2 ±0.14a</td>
<td>42.86±1.37a</td>
<td>8.6±1.39a</td>
<td>9.64±5.60a</td>
<td>3.30±0.23a</td>
<td>521±93.3a</td>
</tr>
<tr>
<td>Hand cross</td>
<td>1.5±0.24b</td>
<td>46.24±3.04b</td>
<td>9.95±1.66a</td>
<td>8.93±4.49a</td>
<td>3.46±0.30b</td>
<td>656±98.4b</td>
</tr>
<tr>
<td>By stingless bees, <em>H. itama</em></td>
<td>1.6±0.18b</td>
<td>48.02±1.86b</td>
<td>11.01±1.35b</td>
<td>11.85±3.95a</td>
<td>3.62±0.11b</td>
<td>625±131.6b</td>
</tr>
</tbody>
</table>

Source: Wahizatul et al. (unpublished)
Pollination by *H. itama* produced rock melon fruits with higher number of seeds per fruit, larger, heavier and sweeter than rock melons produced by other pollination treatments.
The stingless bee pollinated treatment had added about RM 12.00 to the net profit of rock melon sales price per plant set (per polybag). Whereas, hand-cross had proved to have about at least RM 5.00 per plant set for the net profit of sale.
## Results – Cucumber

### 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

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

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

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Brightness (L*)</th>
<th>Greenness (a*)</th>
<th>Yellowness (b*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without stingless bee</td>
<td>48.21±6.43a</td>
<td>-9.99±0.95a</td>
<td>23.82±7.77a</td>
</tr>
<tr>
<td>Hand-cross pollination</td>
<td>48.66±5.08b</td>
<td>-8.80±1.01b</td>
<td>18.15±3.39b</td>
</tr>
<tr>
<td><em>H. itama</em> pollination</td>
<td>45.24±5.24b</td>
<td>-9.07±0.99b</td>
<td>16.66±3.89b</td>
</tr>
</tbody>
</table>

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

Source: Wahizatul et al. (2017)
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.
## Results – Chili

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

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

*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 *Heterotrigoa itama* pollination.

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

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

Source: Wahizatul et al. (2016)
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.
• Rock melons, cucumbers & chillies produced from pollination by stingless bee were higher in fruit set and less malformed fruits than other treatments.

• Nicodemo et al. (2013) - the production of cucumber increased by 26% when the stingless bees placed in the greenhouse.

• Amano (2005) - stingless bee pollination produced higher number of fruit sets than honey bees.

• The rock melon, cucumber & chili pollens are sticky and cannot be dispersed by wind or air. Thus, they need the bees as pollination agent.

• Lower number of malformed cucumber from stingless bee pollination - *H. itama* deposited a great number of viable, compatible pollen as placed on the flower stigmas.
• Nicodemo et al. (2013);
- Done in the greenhouses at Ribeirao Preto, Brazil.
- Brazilian native stingless bee, Irai (*Nannotrigona testaceicornis*) contributed to a significant increase in cucumber diameter & length.

• Santos et al. (2008) reported that stingless bees (*Scaptotrigona depilis* & *Nannotrigona testaceicornis*) effectively pollinated the greenhouse cucumber (located at Universidade de Sao Paolo, Brazil); higher fruit production and higher fruit weight.

• Cruz et al. (2005) found that higher number of seed developing inside fruits produced from *Melipona subnitida* pollination would lead to bigger and heavier sweet peppers in the greenhouse.
• Stingless bees were active during morning and evening to collect nectar and pollen due to anthesis of the flowers (in the morning), energy requirements and better condition in the greenhouse (Cruz et al., 2005).

• More pollens are being transferred, more seeds will developed and the fruit size become larger and also contribute to increase of fruit weight than fruits with less seed (Delaplane & Mayer, 2000).


Our study revealed that pollination by stingless bees can increase fruit size and weight, seed number, pericarp volume, high percentage of fruitsets and fastens harvesting time.

This study provides important information on the potential use of *H. itama* as an alternative pollinator for crop pollination purpose based on scientific findings.

Outcome from this study is hoped will be used to develop application strategies suitable for future use in crop pollination success.

It is hoped that this study will enhance the awareness of the importance of stingless bee as an alternative pollinator in agricultural ecosystem in Malaysia.
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• School of Food Science & Technology, UMT
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• Department of Agriculture, Terengganu
• GM Peladang Sdn. Bhd.
• Beloved students…

*Do not over-hunting of wild stingless bees...
* Protect wild habitat and host plants of stingless bees..
Thank you....

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* Protect wild habitat and host plants of stingless bees..