POLLINATION SERVICES OF *Tetragonula laeviceps* SMITH (APIDAE: MELIPONINAE) ON MELON PLANTS (*Cucumis melo* L.)

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ABSTRACT

Melon plants (*Cucumis melo* L.) have male, female, and hermaphrodite flowers. To optimize the pollination process, melon needs pollinating agents. *Tetragonula laeviceps* is a stingless bee species that play as an agent of pollination. This study aimed to measure the pollination services of *T. laeviceps* in melon plants in the greenhouse. Observation of visiting activity of bee using focal sampling method showed that the highest number of flowers visited by *T. laevceps* was 6.40 flowers/5 minutes. The longest visit duration of *T. laeviceps* was 28.04 seconds/flower. Visiting activity of *T. laeviceps* significantly correlated with environmental factors such as the air temperature and humidity ($P<0.001$). The pollen load on *T. laeviceps* was 26,200 pollen grains per individual. Pollination by *T. laeviceps* increased the fruit size, fruit weight, number of seeds per fruit, seed germination, and fruit sugar content compared with control treatment. Our result indicated that the pollination services of *T. laeviceps* can be applied in melon farms to improved production quality.

Key words: Stingless bees, crops, pollen, pollinating, *Tetragonula laeviceps*

ABSTRAK

Tanaman melon (*Cucumis melo* L.) mempunyai bunga jantan, betina dan hermafrodit. Spesies melon ini memerlukan agen pendebungaan untuk mengoptimumkan proses pendebungaan. *Tetragonula laeviceps* adalah spesies lebah yang tidak menyengat yang bertindak sebagai agen pendebungaan. Kajian ini bertujuan untuk mengukur perkhidmatan pendebungaan *T. laeviceps* pada tembikai di dalam rumah hijau. Pemerhatian aktiviti lawatan lebah menggunakan kaedah persampelan fokal menunjukkan bahawa jumlah bunga yang paling banyak dikunjungi oleh *T. laeviceps* ialah 6.40 bunga/minit. Tempoh lawatan terpanjang untuk *T. laeviceps* ialah 28.04 s/bunga. Aktiviti lawatan *T. laeviceps* mempunyai hubungan yang signifikan dengan faktor abiotik persekitaran, seperti suhu dan kelembapan ($P<0.001$). Beban debunga diukur menggunakan kaedah asetolisis, *T. laeviceps* membawa 26.200 butir debunga. Pendebungaan oleh *T. laeviceps* dapat meningkatkan ukuran buah, berat buah, jumlah biji per buah, percambahan biji dan kandungan gula buah jika dibandingkan dengan tanaman kawalan. Berdasarkan hasil kajian ini, perkhidmatan pendebungaan oleh *T. laeviceps* dapat diaplikasikan di ladang melon untuk meningkatkan kualiti hasil.

Kata kunci: Debunga, lebah, pendebungaan, tanaman, *Tetragonula laeviceps*

INTRODUCTION

Tetragonula laeviceps (Apidae: Meliponinae) is a stingless bee species that acts as a pollinating agent of plant (Gadhiya & Pastagia 2019). The species who lives in colonies has a wide distribution in Indonesia with various body size ranging from 3.42 to 4.88 mm (Azizi et al. 2020; Jalil & Shuib 2014; Purwanto & Trianto 2021). One colony of stingless bees has three castes, consist of about 3000 workers, hundreds of drones (males), and one or more individual queens. Each caste has its role in the colony, such as worker bees to protect the hive from predators, build and clean the hive and searching for nectar, pollen, and resin. The male bee fertilizes the queen, while the queen is responsible for reproduction (Michener 2007). The nests of stingless bees can be found in crevices walls of houses, logs, bamboo, and other hidden places (Roubik 2006). As a pollinating agent, stingless bee has several advantages, i.e., adaptive to environmental change, flower constancy, easy to manage, high colony resistance, generalist (polylectic), high food recruitment (Slaa et al. 2006) and increase the economic value of crops (Novais et al. 2016).

Tetragonula laeviceps has no sting, so it is easy to manage and suitable for pollinating plants cultivated in residential areas and greenhouses (Gadhiya & Pastagia 2019). As a generalist pollinator species, *T. laeviceps* actively collect nectar and pollen from various plant taxa (Cholis et al. 2019). Pollination by *T. laeviceps* makes a significant contribution to crop production (Atmowidi et al. 2007; Wulandari et al. 2017). Melon (*Cucumis melo* L.) is one of the most widely cultivated crops in Indonesia.

Melon flowers are bell-shaped, yellow in colour, have male, female and hermaphrodite flowers in one plant (Girek et al. 2013). The cross-pollination of melon flowers occurs naturally by insects (Kouonon et al. 2009). Some species of bees, such as *Apis mellifera*, *Halictus sp*., *Plebeia* sp., *Trigona spinipes* and *T. pallens* were reported as pollinators for melon (Tschoeke et al. 2015). Andromonoecious of melon plants require a pollinating agent to help the pollination process (Kouonon et al. 2009; Ribeiro et al. 2015; Tschoeke et al. 2015). Although some studies reported that bees are known a can bee pollinator, the utilization of *T. laeviceps* pollination services as native bees in Indonesia has not been applied to melon plants and the scientific evidences has not been reported yet. This study aims to measure the pollination services of *T. laeviceps* in melons with the measure of visiting activity related to abiotic environmental parameters, measuring the amount of pollen carried by bees and the number of fruit formation as a result of the pollination.

MATERIALS AND METHODS

Preparation of Melon Plants and Stingless Bee Colonies

Seeds of melon hybrid F1 Sisilia were obtained from an agricultural shop in Bogor, West Java, Indonesia. The seeds were planted in trays with a media consist of latosol soil, regosol soil, and cocopeat compost with a ratio of 1: 1: 1. After 2 weeks, the seedlings were transferred into the polybags. Melon plants were sprayed with fungicides to control fungi. One hundred melon plants in the greenhouse (12 m x 6 m) of IPB University were used. Fifty plants were caged by screen without *T. laeviceps* colony is used as a control. Other 50 plants were caged by screen with one colony of *T. laeviceps* for 23 days. After the plants were flowering (approximately 21 days), the number of male and hermaphrodite flowers in plants with and without bee were observed every day for 20 days.

Observation of Melon Flowers Characteristics

Characteristics of melon flowers were observed to determine the morphology of flowers by observing ovaries, number of petals, number of sepal, number of anther and number of stigma. The flowers were taken to laboratory and were photographed using Nikkon D5000. The observation of the number of male and hermaphrodite flowers were manually conducted based on Tschoeke et al. (2015).

Measurement of Visiting Activity of *T. laeviceps*

The focal sampling method was used to measure the activity of *T. laeviceps*. The activities of the bee were observed in 08.00-11.00 am and 02.00-05.00 pm during the flowering period. The focal sampling method observed the animal behavior by selecting one individual within a certain time period (Martin & Bateson 1993). Visiting activities observed were foraging rate by observed the number of flowers visited per 5 minutes, flower handling time by observed the duration of visits per male flower and hermaphrodite flower, and duration of visits in one plant. During the measurement of visiting activities of bee, environmental parameters were measured, i.e, air humidity, temperature, and light intensity using Lutron 4 in 1 environmental meter LM-8000A.

Measurement of Pollen Load

Pollen load measurements used the acetolysis method (Kearns & Inouye 1993). The measurements were carried out at the Laboratory of the Division of Animal Biosystematics and Ecology, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Bogor, Indonesia. The bee that returned to the hive was captured and put into a microtube contained 70% ethanol and then rotated for 24 hours. After that, a microtube was then centrifuged at 3500 rpm for 5 minutes and then, the bee was removed from the microtube. After that, it was centrifuged at 2000 rpm for 3 minutes, then the supernatant was removed, and remained the pellet of pollens. The pellet was added with 1 mL of acetolysis solution with composition of acetic anhydride and sulfuric acid in a ratio of 9:1. The sample was heated in water bath with temperature of 80^{o}C for 5 minutes and the sample was centrifuged at 2000 rpm for 5 minutes. The supernatant was discarded, and then 1 mL of distilled water was added to rinse the pollen until the solution was clear. One mL of the supernatant containing pollens was dropped on the hemocytometer. Pollens observed in four quadrants of hemocytometer were counted under a light microscope (Olympus CX31 embbeded with Indomicro camera). The number of pollens found in one individual of bee was calculated using the formula (Dafni 1992):

$$\frac{V_1}{N_1} = \frac{V_2}{N_2}$$

Where,
$V_{1=}$ volume of four quadrants
$V_{2=}$ total volume of solution
$N_{1=}$ number of pollens counted
$N_{2=}$ total of pollen load

Measurement of Fruit Set

Fruit sets of melon were harvested on the 120 days after planting and measured randomly in 23 control plants and 23 plants pollinated by stingless bees. Fruit measurements consist of number of seeds per fruit, fruit diameter and weight, and seed germination. The proximate analysis of fruits was carried out at the Calibration and Analytical Laboratory of the Agro-Industry Center using the method of AOAC (2005).

Data Analysis

The relationship between environmental parameters and visiting activity of the bees was analyzed by using Pearson's correlation test in the R 3.1.2 software (R Core Team 2014). Fruit set of plants pollinated by bee and control plants was analyzed by using t-test in Paleontological Statistics (PAST) 3.20 software.

RESULTS AND DISCUSSION

Morphology of Melon Flowers

Melon plants observed in this study produced several male flowers and hermaphrodite flowers. The hermaphrodite flower appeared solitary on a thick stalk in axillary and a few slightly larger than male flowers. The male flowers were produced either singly or sometimes in clusters of two or three on a thin stalk in between the leaf stalk and stem. As described by Kiill et al. (2016), the hermaphrodite flowers were produced along with a leaf on a thick and longer stalk raised from axillary points orienting themselves at higher position.

Male flowers have 5-6 yellow sepals, 5 green petals around the sepals, 5 anthers with fused filaments (Figure 1a &1b). This structure of sepals, petals, and anthers in hermaphrodite flowers are same as male flower, but hermaphrodite flowers have 3 stigmas located in the middle, and between the pistils (Figure 1c & 1d). As described by Revanasidda and Belavadi (2019) male flowers only have anthers while hermaphrodite flowers have pistils and anthers.

Based on the observations for 20 days during flowering in control plants, the number of male flowers was 4987 (average of 99.74±51.14 flowers/plant) and hermaphrodite flowers was 490 (average of 9.80±4.4 flowers/plant). While in plants with bees, the number of male flowers was 4668 (average of 93.36±29.19 flowers/plant) and hermaphrodite flowers were 431 (average of 8.62±2.85 flowers/plant). The number of male flowers was higher than hermaphrodite flowers with a ratio of 1:9. Results of the study were similar to Tschoeke et al. (2015) and Revanasidda & Belavadi (2019) that reported the number of male flowers melon was higher than hermaphrodite flowers. The determination of flower sex in plants is regulated by the andromonoecious and gynoecious genes that influence each other (Boualem et al. 2008). Brantley & Warren (1960) also stated that the number of male and hermaphrodite flowers in plants could be influenced by external factors, such as mineral nutrition, temperature, light intensity, and application of growth regulators.

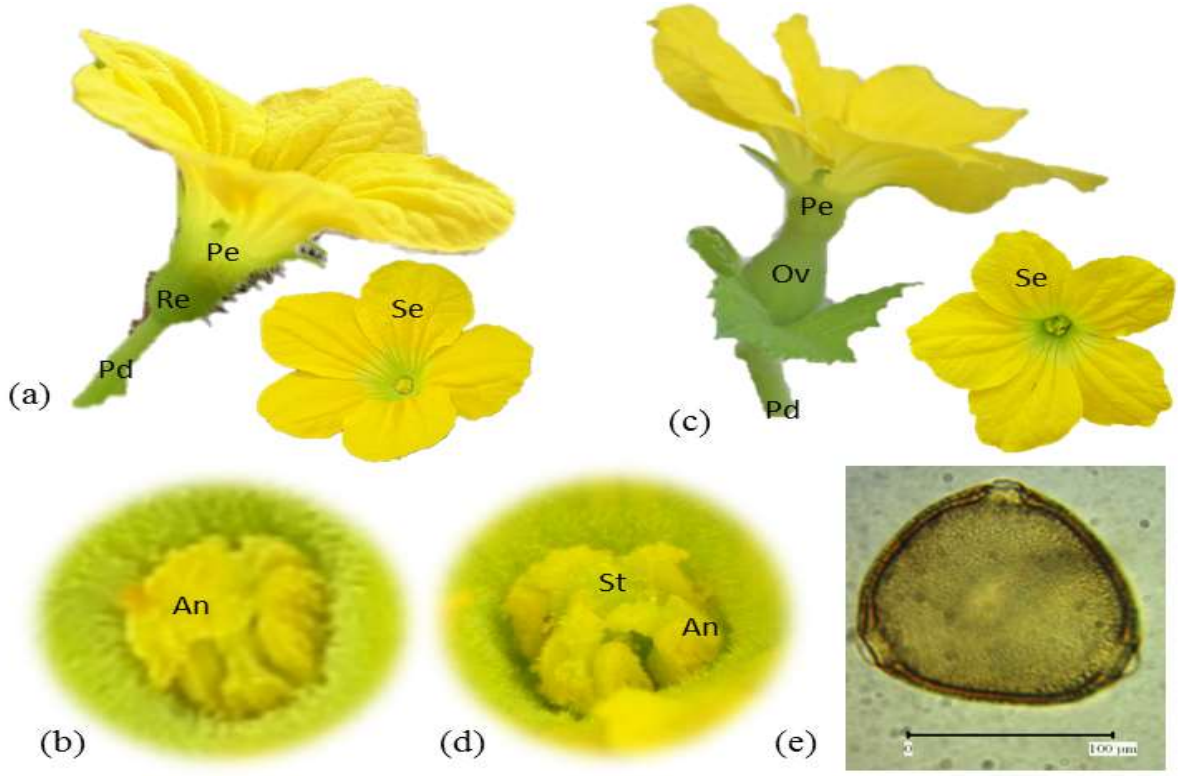

Figure 1. Flower of melon (*Cucumis melo* L.). (a) male flower, (b) male organ, (c) hermaphrodite flower, (d) hermaphrodite organ, and (e) morphology of melon pollen grain. Pe-petal; Se-sepal; Ov-ovary; St-stigma; An-anther; Re-receptacle; Pd-pedicel

Visiting Activities of *T. laeviceps*

Visiting activity of *T. laeviceps* on melon flowers varied on the period. In the morning (10.00-11.00 am), *T. laeviceps* used a lot of time to exploit one flower compared to the day and afternoon (Figure 2a). A similar trend also was found in duration of visits per plant (Figure 2b). The numbers of flowers visited per five minutes in the morning and afternoon were lower than in the noon (Figure 2c). These results supported data of Tschoeke et al. (2015) that the peak activity of the *Tetragonula pallens* visits to melon plants occurred in the morning (08.00-10.00 am and decreased in the afternoon (01.00 pm). The similar pattern of visit also was shown by *T. laeviceps* visited pummelo flowers (Cholis et al. 2020). Atmowidi et al. (2018) also reported *T. laeviceps* started to fly leaving the nest at around 07.00 am. In *Brassica oleracea*, activity of *T. laeviceps* also was high in the morning and decreases in the afternoon (Wulandari et al. 2017). Inoue et al. (1984) reported *T. laeviceps* was very active collecting pollen in the morning. Generally, the volume of nectar in flowers was high in the morning and decreases in the afternoon, that affecting the duration of insect visits to flowers (Dudareva & Pichersky 2006).

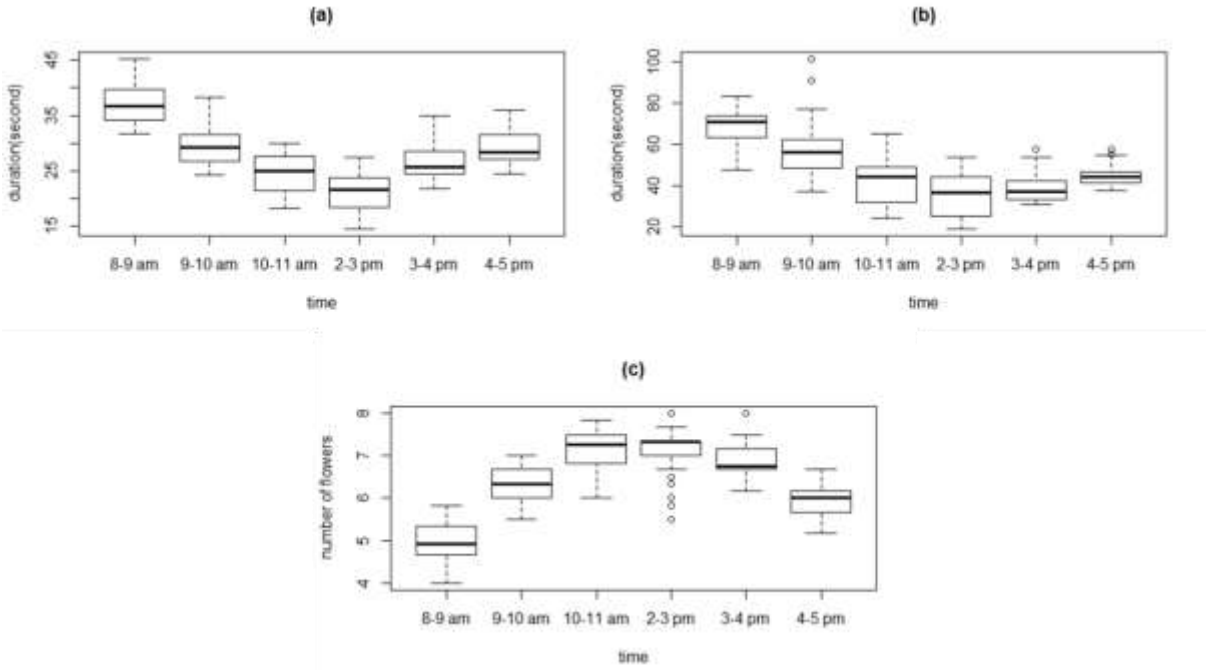

Figure 2. Visiting activity of *T. laeviceps* in melon flowers: (a) duration of visited per flower, (b) duration of visited per plant, and (c) the number of flowers visited per 5 minutes

The number of flowers visited and duration of visits *T. laeviceps* on male and hermaphrodite flowers varied. The highest number of male flowers visited (6.14 flowers/5 minutes) occurred at 02.00 pm, while in hermaphrodite flowers (1.56 flowers/5 minutes) occurred at 08.00 am (Figure 3a & 3b). The highest duration of visit of male and hermaphrodite flowers occurred at 08.00 am (28.69 & 47.14 seconds/flower) (Figure 3c & 3d). The duration of visits in one hermaphrodite flower was longer than in male flowers. This result supported of Ribeiro et al. (2015) that hermaphrodite flower received longer visits than male flower. Bees preferred hermaphrodite flowers with a high volume of nectar. The nectar volume of hermaphrodite flowers was 2µl higher than in male flowers (Revanasidda & Belavadi 2019). The higher nectar volume in hermaphrodite flowers caused bees prefer to visit than visits male flowers (Ribeiro et al. 2015). Flower morphology is related to the activity of pollinating insects on flowers (Hasan & Atmowidi 2017; Kiill et al. 2016). The number of flowering plants increased the number of individual pollinating insects (Atmowidi et al. 2007).

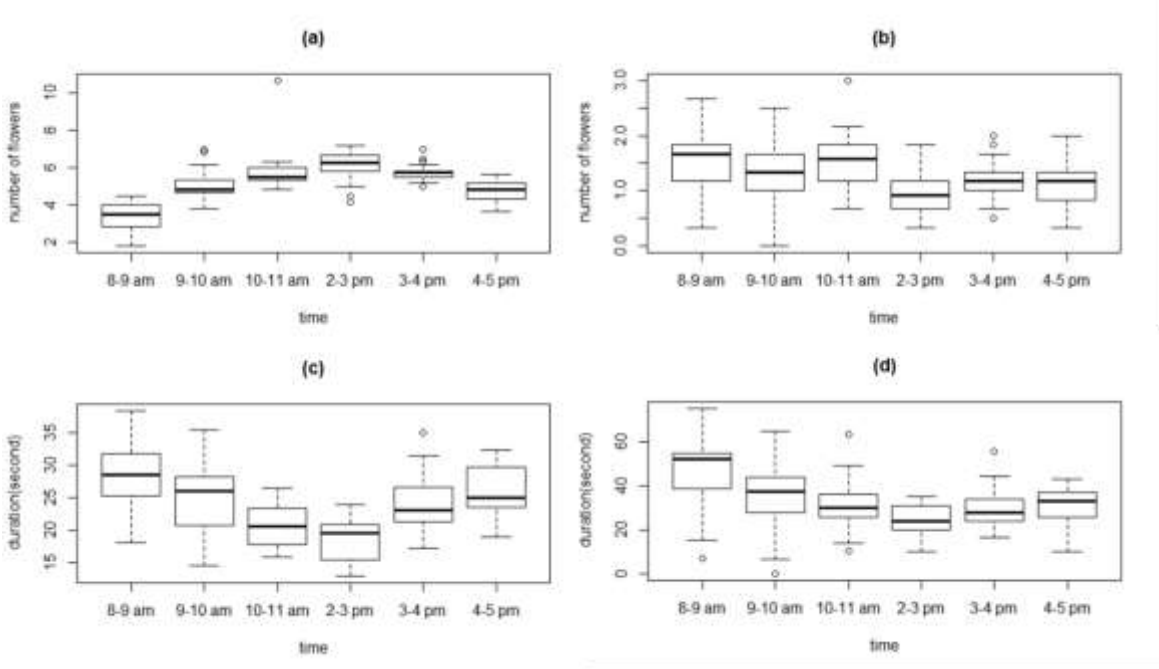

Figure 3. Visiting activity of *T. laeviceps* on melon flowers: (a) the number of male flowers visited per 5 minutes, (b) the number of hermaphrodite flowers visited per 5 minutes, (c) the duration of visited on one male flower, and (d) the duration of visited on a hermaphrodite flower

The observations showed that *T. laeviceps* was actively visits at 08.00 am (average temperature 27°C, humidity 66.3%, light intensity 11205.7 lux), decreased activity at 10.00 am (temperature 35.5 oC, humidity 41.1%, light intensity 22706.4 lux) and the activity increased again at 03:00 pm (mean temperature were 32.4°C and humidity 50.7%, light intensity 9055.8) (Table 1; Figure 2). Based on the Pearson correlation analysis, the air temperature was positively correlated with the number of flowers visited, while humidity was negatively correlated (r = -0.97; *P<0.0001*). These results similar with Wulandari et al. (2017) that stated visiting activity of *T. laeviceps* on *Brassica oleracea* was correlated with environmental factors. Wicaksono et al. (2020) also reported that air temperature and light intensity have a positive correlation with the flight activity of *Lepidotrigona terminata* carrying nectar and resin. In addition to abiotic factors, the visiting activity of pollinating insects on melon flowers also was influenced by the availability of flower resources, such as nectar and pollen (Tschoeke et al. 2015).

Table 1. Mean±SD air temperature, air humidity, and light intensity during the observation of *T. laeviceps* activity

Time	Temperature (ºC)	Humidity (%)	Light intensity (lux)
08.00-09.00 am	27.0±0.7	66.3±5.9	11205.7±11811
09.00-10.00 am	30.5±1.6	54.8±5.1	15911±1978
10.00-11.00 am	35.5±1.5	41.1±2.2	22706.4±2721
02.00-03.00 pm	33.8±1.2	46.2±4.4	17284.5±2712

03.00-04.00 pm	32.4±0.8	50.7±4.3	9055.8±3385
04.00-05.00 pm	30.7±1.1	57.0±4.3	5483.3±2417

Tetragonula laeviceps visit from one flower to other flower. The highest visitation percentage of *T. laeviceps* occurred from male to male flower (65%) and the lowest was from hermaphrodite to hermaphrodite flowers on the same plant or different plants (4%) (Figure 4). The number of male flowers were higher than hermaphrodite flowers so the flower visitation pattern of *T. laeviceps* was high from male to male flowers compared to others.

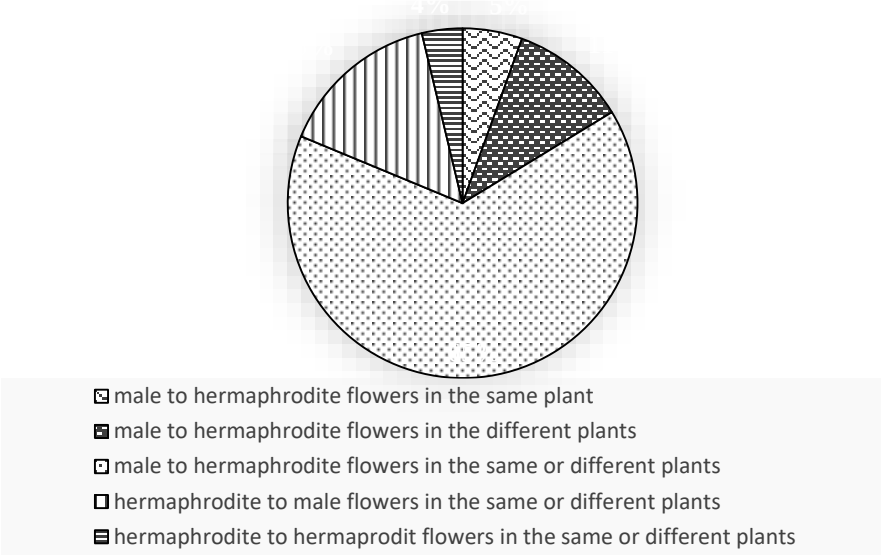

Figure 4. Percentage of *T. laeviceps* visits on male and hermaphrodite flowers in the same or different melon plants (*Cucumis melo* L.)

Pollen Load

During foraging, *T. laeviceps* collect pollen and stored in the pollen basket. The average pollens collected by *T. laeviceps* after visiting the melon flower was 26.200 pollen grains. Melon has a triporate pollen, from the polar view is semi-angular (Morley 1990) (Figure 1e). Chan & Saw (2011) stated that *T. laeviceps* is a potential pollinator in *Johannesteijsmannia* spp. because of the higher pollen load in thorax, tarsus, and corbicula. The species of bee carried 8015 pollen grains (Pangestika et al. 2017) and 122,594 pollen grains (Cholis et al. 2019) from plants around the nest. Wulandari et al. (2017) also reported *T. laeviceps* were effective pollinators of *Brassica oleracea* with high pollen load (8125 pollen grains). Ramalho et al. (2009) reported the number of pollens carried by bees is an adaptive foraging activity to supply the colony's needs.

Fruit Formation

Melon plants with *T. laeviceps* colonies produced a higher percentage of fruit formation (13.68%) compared to control plants (4.69%). Fruit set of melon with *T. laeviceps* was significantly different with control plants (t-test, *P<0.001*). Fruit produced by melon plants with *T. laeviceps* colonies increased 41% of fruit diameter, 39% of fruit length, 173% of fruit weight, 133% of number of seeds/fruit, 97% of seed germination, and 47% Brix of sugar content (Table 2). The foraging activity of *T. laeviceps* affected the yield of melon. Flowers

frequently visited by wild and managed pollinating bees accelerate the fertilization process and fruits were successfully formed (Husby et al. 2015; Priawandiputra et al. 2018). Tschoeke et al. (2015) reported that the intensity of bee visits was significantly correlated with melon fruit weight. These results were similar with Wulandari et al. (2017) that stated the pollination by *T. laeviceps* on *B. oleracea* plants increased the number of pods per plant, number of seeds per pod, seed weight per plant, and seed germination. The results also supported data of Indraswari et al. (2016) on *Solanum lycopersicum* fruit pollinated by bees increased the fruit length, fruit diameter, fruit weight, number of seeds per fruit, and seed weight.

Table 2. Fruit and seed properties of melon pollinated by *T. laeviceps*

Components	**n**	**Control plants**	**Plants with *T. laeviceps*)**	**Increasing (%)**
Fruit diameter (cm)	23	5.73	8.1*	41
Fruit length (cm)	23	5.82	8.11*	39
Fruit weight (g)	23	94.7	259.35*	173
Number of seeds/fruit	23	84.91	198.57*	133
Seed germination (%)	100	47	93*	97
Sugar content (% Brix)	23	8.13	12*	47

*Indicate significantly different in the same row (control plant) of the t-test level 95%

Table 3. Proximate analysis of melon fruits pollinated by *T. laeviceps*

Fruit Components	**n**	**Control plants**	**Plant pollinated by T. *laeviceps***
Moisture (%)	1	95.9	91
Ash (%)	1	0.32	0.86
Protein (%)	1	0.87	1.67
Fat (%)	1	0.12	0
Carbohydrate (%)	1	2.79	6.47

Based on proximate analysis, melon fruit yielded by *T. laeviceps* pollination contain high protein and carbohydrate (1.67% and 6.47%) compared to control plants (0.87% and 2.79% Table 3). Previous study also reported that fruit pollinated by insects had a high amount of nutrients, such as vitamin A, vitamin C, vitamin E, lipids, protein, and minerals (Eilers et al. 2011).

CONCLUSION

Stingless bee, *Tetragonula laeviceps* contribute to the increase of fruit set of melon plants. The high visiting activity of *T. laeviceps* on melon flowers occurred in the morning and decreased

during the day. The number of flowers visited by *T. laeviceps* was positively correlated with air temperature, while humidity was negatively correlated correlated. The average number of pollen loads on the body of *T. laeviceps* after visiting the melon flowers was 26,200 pollen grants. Pollination by *T. laeviceps* increased the fruit size, fruit weight, number of seeds per fruit, seed germination, and fruit sugar content. The melon farming industry can utilize the pollination of *T. laeviceps* to improve the fruit production quality.

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