

## Pollination services of *Apis cerana* and *Tetragonula laeviceps* (Hymenoptera: Apidae) on strawberry (*Fragaria x ananassa*)

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### Abstract

Strawberry (*Fragaria x ananassa*) is a fruit-producing plant with high economic value and essential horticultural commodities in Indonesia. Although strawberry plants have hermaphrodite flowers, this plant requires pollinating insects because the mature male and female organs are not often mature at the same time. Honey bees and stingless bees were reported as effective pollination agents of various plants. This study aims to measure the pollination services of *Apis cerana* and *Tetragonula laeviceps* on strawberry plants. Visiting activity of *A. cerana* and *T. laeviceps* were observed by focal sampling method from 8 am to 4 pm. The pollen load on insects were measured by using the acetolysis method. The results showed the highest duration visits of *A. cerana* ( $12.64 \pm 0.47$  seconds/flower) and the number of flowers visited ( $12.80 \pm 0.65$  flowers/three minutes) occurred at 8 to 9 am. Meanwhile, in *T. laeviceps*, the highest duration visits ( $89.15 \pm 9.03$  seconds/number) and the number of flowers visited ( $2.18 \pm 0.22$  flowers/three minutes) occurred at 9 to 10 am. The number of pollens carried by *A. cerana* and *T. laeviceps* were 303275 and 86281 pollen grains, respectively. Manual and bee pollinations increased the number of fruits formation, fruit size and weight, and vitamin C content compared with control plants.

**Keywords:** Pollination services, *Apis cerana*, *Tetragonula laeviceps*, Strawberry

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## Introduction

Insects provide services to natural and artificial ecosystems (Winfree et al., 2008). One of the important services of insects for human life is pollination. About 35% of food crops in the world need insects to ensure the success of pollination (Widhiono and Sudiana, 2016). Insect pollination provides a significant

contribution to agricultural production (Atmowidi et al., 2007; Rianti et al., 2010; Garibaldi et al., 2014). In tomato, pollination by insects increased by 189% the number of seeds per fruit and 355% the weight of seeds per fruit (Indraswari et al., 2016). Bees are the most effective pollinator (Pires et al., 2014).

Bees (honey bees and stingless bees) play an important role in pollination process and fruit production (Rader et al., 2015). *Apis cerana* was reported as an effective



pollinator on strawberry (MacInnis and Forrest, 2019), apple (Verma and Dulta, 2015), melon (Revanasidda and Belavadi, 2019), and cauliflower and cabbage (Verma and Partap, 2015). This species has a body length of 10-11 mm (Michener, 2000) and active foraging with a distance of 2-3 km (Amano et al., 2000). Stingless bees have high-frequency visits to flowers (Ruslan et al., 2015) and have a prospect as a crop pollinators because they are small, do not sting, have high adaptability to environmental stress, and are easy to handle (Jalil and Shuib, 2014). Previously was reported that *Heterotrigona itama* increased agricultural crop production, such as watermelon (Azmi et al., 2018) and chayote (A'yunin et al., 2019), while *Melipona fasciculata* increased eggplant production (Nunes et al., 2013). Pollination by *Tetragonula laeviceps* was reported to increase seed production, the number of pods, and seed germination of kale (Wulandari et al., 2017), and strawberries (Widhiono et al., 2012; Roselino et al., 2009).

Strawberries have hermaphrodite flowers, which are male and female reproductive organs found in one flower. Although strawberry have hermaphrodite flowers, female organs mature first compared to the male organ (Roselino et al., 2009) and need pollinating insects. Pollination by insects increased the number of fruits, fruit quality, and economic value of strawberries (Klatt et al., 2013). Herrmann et al. (2018) also reported bees were effective pollinators on strawberries and generally increased yields and quality of fruits. The current study aims to measure the pollination services of *A. cerana* and *T. laeviceps* on strawberries by observing visiting activity and measuring fruit production, i.e., the number of perfect fruits and abnormal fruits, fruits size, and sugar and vitamin C contents.

## Material and Methods

### Preparation of strawberry plants and flowers observations

The Variety of strawberry used in this study was sweet charlie owned by farmers at Sembalun Lawang, West Nusa Tenggara, Indonesia. Strawberries were planted in the soil media with the distance between plants was 50cm. The plants were fertilized once a month using NPK fertilizer fertilizer (16:16:16) and weed control was done manually once a month. Pest and disease controls were carried out by spraying insecticides, i.e., abamectin and cypermethrin, while fungi disease was controlled by difenoconazole fungicide every two

weeks. Strawberry flower properties were observed, such as morphology, colour, the number of sepals and petals, number of stamens and nectary position, and flower bloom time.

### The use of *A. cerana* and *T. laeviceps* as strawberry pollination

Strawberry plants used in this study were divided into four treatments, i.e., (a) 50 plants were caged by screen without bee colonies as a control, (b) 50 plants were caged by screen for hand pollination with pollen from a different plant, (c) 50 plants were caged by the screen and added by one colony of *T. laeviceps*, and (d) 50 open plants added by one colony of *A. cerana*. The size of each cage was 1.50 m high, 4 m in length, and 2 m in width. No replication of each treatment was conducted.

### Observation of *A. cerana* and *T. laeviceps* visiting activity

Visiting activity of *A. cerana* were observed in open plants, while *T. laeviceps* was observed in caged plants using the focal sampling method (Martin and Bateson, 1993). Visiting activities observed were visit duration in a flower (flower handling time), visit duration in a plant (plant handling time), and the number of flowers visited per three minutes (foraging rate) (Dafni, 1992). During observation of bees visiting activities, environmental parameters were measured, i.e., relative humidity, air temperature, light intensity, and wind speed in every hour, starting at 8 am to 4 pm for 20 days.

### Pollen load measurements

*Pollen load* on *A. cerana* and *T. laeviceps* were measured by the acetolysis method (Dafni, 1992). Pollens were collected from *A. cerana* and *T. laeviceps* that returned to the hive. One individual of bee was captured and put in a microtube containing 70% ethanol. Then, the microtube was rotated for 24 hours and then centrifuged at 3500 rpm for 5 minutes. Then, bees were removed from the microtube and centrifuged at 2000 rpm for 3 minutes and the supernatant was removed. The precipitate pollens was added by 1 mL of solution acetolysis (acetic anhydride and sulfuric acid, 9:1). Samples were heated in a water bath at 80°C for 5 minutes and centrifuged at 2000 rpm for 10 minutes. One mL of distilled water was added to rinse the pollens until the solution was clear. As much as 0.1 mL of a solution containing pollen was dropped on the hemocytometer. Pollens found in four quadrants of the



hemocytometer were counted under a light microscope. Measurement of pollen load on each species of bee was conducted as much as 20 times. The number of pollen in one individual bee was calculated by the following formula.

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

Where V1= volume of four quadrants, V2= total volume of solution, N1= number of pollen counted, and N2= total of pollen load

**Measurement of fruit formation**

Measurements of fruit formation were carried out on 25 plants that were randomly selected in each treatment. The fruits formation measured were the number of fruits per plant, the number of perfect and abnormal fruits, the length, thickness, and the weight of fruits, the number of seeds per fruit, and the sugar content. The fruits vitamin C content was also measured by the HPLC method (Seal and Chaudhuri, 2017) in the Analytical Laboratory of the Department of Chemistry, Mataram University, Indonesia.

**Statistical analysis**

The relationship between environmental parameters and bees visiting activity was analysed using Pearson correlation in Paleontological Statistics (PAST) 3.20 software. Fruits formation production among treatments were analysed using One-way ANOVA and compared the means using the Tukey test (Hammer et al., 2001).

**Results and Discussion**

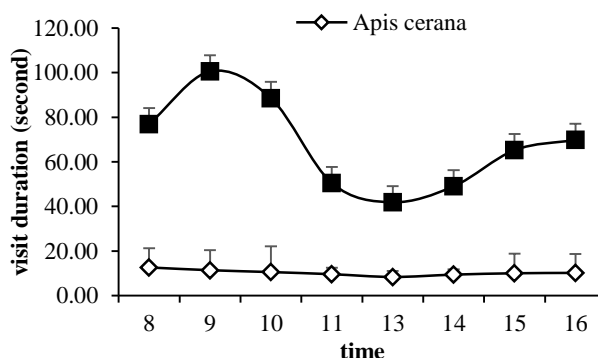
**Flowers morphology**

Strawberry has hermaphrodite flowers that the stamens and pistil are in a flower. The flower is white and has a cluster-like shape. Flower diameter ranges from 2-3.5 cm, 6-7 white petals with 10-14 green sepals. There are 25-40 stamens attached to the base of the receptacle. The nectaries are situated at the bottom of the stamens and close to the ovary. The blossoms bloom is in the morning (about 6 am) and flowers do not close until the pistil turns to brown.

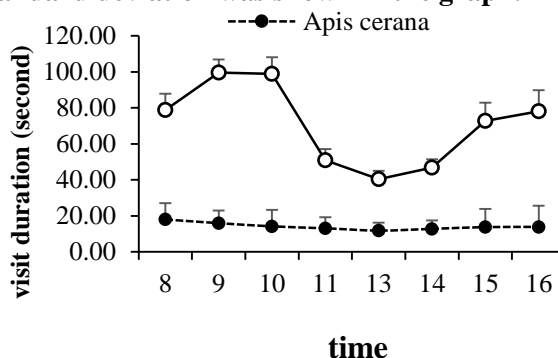
**Visiting activities of *A. cerana* and *T. laeviceps***

Results showed that the foraging activity of bees started in the morning until late afternoon. The longest visit duration in one flower of *A. cerana* (12.64±0.47 seconds/flower) occurred at 8 to 9 am and the shortest (8.36±0.48 seconds/flower) occurred at 1 to 2 pm.

While in *T. laeviceps*, the longest visit duration (89.15±9.03 seconds/flower) occurred at 9 to 10 am and the shortest (33.52±2.74 seconds/flower) occurred at 1 to 2 pm (Fig. 1). The longest visit duration of *A. cerana* was 18.02± 1.04 seconds/plant and the shortest was 11.76±0.57 seconds/plant. In *T. laeviceps*, the longest visit duration was 99.68± 7.12 seconds/plant and the shortest was 40.43± 4.55 seconds/plant (Fig. 2). The results of the current study were similar to Cholis et al. (2020) that peak of *A. cerana* and *T. laeviceps* visiting activity on pummelo flowers occurred at 8 am. During the day (at 1 pm) this activity decreased and increased again at 4 pm. Atmowidi et al. (2018) also reported that the highest activity of *H. itama* occurred at 9-10 am and decreased at 1-2 pm. Visiting activity of bees related to pollen availability. In the morning, the availability of pollens was high and reduced during the day (Tschoeke et al., 2015). The visiting activity of bees on flowers also related to flower colour, availability of nectar and pollen, and the suitability of flower and bee characters (Rianti et al., 2010). Generally, the volume of nectar is high in the morning and decreases in the afternoon that affected visiting duration of insects (Dudareva and Pichersky, 2006).



**Figure-1. Visit duration in a flower of *A. cerana* and *T. laeviceps* based on the time of observation. Standard deviation was shown in the graph.**



**Figure-2. Visit duration in one plant of *A. cerana* and *T. laeviceps* based on the time of observation.**



Standard deviation was shown in the graph.

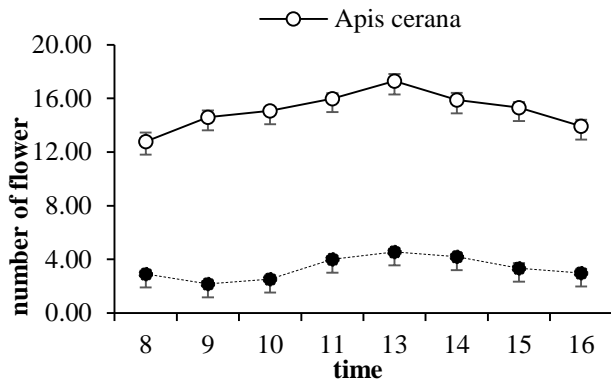


Figure-3. The number of flowers visited per three minutes by *A. cerana* and *T. laeviceps* based on the time of observation. The standard deviation was shown in the graph.

Visiting activity of *A. cerana* and *T. laeviceps* during foraging varied. The number of flowers visited by *A. cerana* at 8-9 am was lower (12.80±0.65 flowers/3 minutes) than the day at 11 am-1 pm (17.30 ± 0.52 flowers/3 minutes). Moreover, *T. laeviceps* visited 2.91±0.26 flowers/3 minutes at 8-9 am and at noon the species visited 4.55 ± 0.24 flowers/3 minutes (Fig. 3). Differences in behaviour and visit duration of *A. cerana* and *T. laeviceps* foraging activity possibly related to the body size. *Apis cerana* has a larger body size than *T. laeviceps*. These results supported Oranje et al. (2012) that larger bees visited more flowers with shorter visits in one flower. Masyitah et al. (2019) also reported bees (*A. cerana* and *Lasioglossum* sp.) visit flowers in shorter time (0.25-0.79 minutes/flower) than flies, *Episyrphus belteatus* and *Melanostoma* sp. (8-10 minutes/flower). The difference in the visit duration per flower among bee species affects the rate of visits, including the number of flowers visited per time unit. The behaviour of insect pollinators during visits the flowers was also related to environmental factors, flowers shape and colour, as well as the sugar content of nectar (Alao et al., 2016)

**Visiting activity of *A. cerana* and *T. laeviceps* related to environmental parameters**

During measuring the activity of *A. cerana*, the highest temperature and light intensity (35.3 °C and 18662 lux, respectively) occurred at 1-2 pm, while highest air relative humidity (67.8%) occurred at 4-5 pm and wind speed occurred at 3-5 pm (1.11 m/s). Meanwhile, during measuring activity of *T. laeviceps*, the highest

temperature and light intensity occurred at 1-2 pm (34.7 °C and 18249 lux, respectively) and highest relative humidity (70.1%) occurred at 8-9 pm (Table 1). High relative humidity affected sugar concentration in the nectar secreted by flowers and wet pollen make it difficult for bees to pick up a large number of pollens (Ruslan et al., 2015).

Table-1. The average of environmental parameters measured during the observation of *A. cerana* and *T. laeviceps*

| Time  | <i>A. cerana</i> |              |                       |                  | <i>T. laeviceps</i> |              |                       |
|-------|------------------|--------------|-----------------------|------------------|---------------------|--------------|-----------------------|
|       | Temperature (°C) | Humidity (%) | Light intensity (lux) | Wind speed (m/s) | Temperature (°C)    | Humidity (%) | Light intensity (lux) |
| 8-9   | 23.1             | 63.1         | 8164                  | 0.0              | 20.4                | 70.1         | 4555                  |
| 9-10  | 26.5             | 59.8         | 10423                 | 0.0              | 23.2                | 63.7         | 7087                  |
| 10-11 | 29.1             | 55.6         | 13174                 | 0.18             | 26                  | 58.4         | 11014                 |
| 11-12 | 32.2             | 50.3         | 16284                 | 0.43             | 30.4                | 51.8         | 15768                 |
| 13-14 | 35.3             | 41.5         | 18662                 | 1.11             | 34.7                | 46           | 18249                 |
| 14-15 | 32.0             | 50.6         | 16005                 | 0.52             | 30.5                | 52.6         | 15358                 |
| 15-16 | 27.3             | 56.2         | 15050                 | 0.2              | 26.9                | 60.2         | 10429                 |
| 16-17 | 24.2             | 67.8         | 7181                  | 0.0              | 23.6                | 65.2         | 5618                  |

Pearson correlation analysis showed that visiting activity of *A. cerana* had a positive correlation with air temperature (r=0.96; p=0.00), light intensity (r=0.94; p=0.00), and wind speed (r=0.89; p=0.00), and negatively correlated with humidity (r=-0.92; p=0.00). While in *T. laeviceps*, visiting activity had a positive correlation with air temperature (r=0.86; p = 0.01) and light intensity (r=0.83; p=0.01) (Table 2).

Table-2. Pearson correlation between visiting activities of *A. cerana* and *T. laeviceps* and environmental parameters.

| Environment parameters | Visiting activity |      | Visiting activity   |      |
|------------------------|-------------------|------|---------------------|------|
|                        | <i>A. cerana</i>  |      | <i>T. laeviceps</i> |      |
|                        | r                 | p    | r                   | p    |
| Temperature (°C)       | 0.96              | 0.00 | 0.86                | 0.01 |
| Humidity (%)           | -0.92             | 0.00 | -0.81               | 0.02 |
| Light intensity (lux)  | 0.94              | 0.00 | 0.83                | 0.01 |
| Wind speed (m/s)       | 0.89              | 0.00 | -                   | -    |

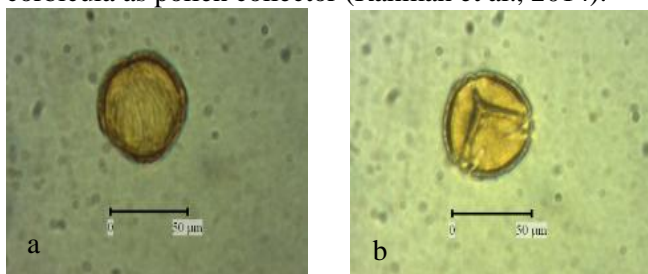




The visiting activity of bees on a strawberry is influenced by temperature, relative humidity, light intensity, and wind speed. Wulandari et al. (2017) reported the optimal temperature (26-34°C) supported the activity of pollinating insects on kale (*Brassica oleraceae*). High humidity and lower temperature caused decrease activity of bees on flowers (Ruslan et al., 2015). To maintain body temperature during flight, bees use a thermoregulation mechanism (Tan et al., 2014).

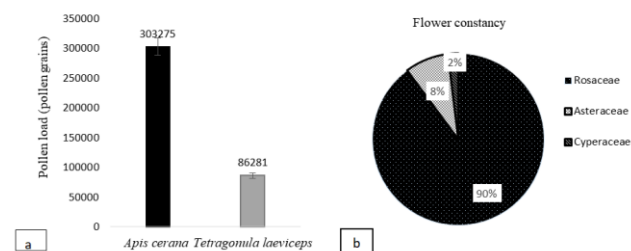
**Pollen load**

Pollen of strawberry is a tricolpate with prolate shape in equatorial view (Fig. 4a) and circular-lobate in polar view (Fig. 4b). Stingless bee is hairy and pollens easily attach to the body when bees visit the flowers. Two species of bees, *A. cerana* and *T. laeviceps* have corbicula as pollen collector (Rahman et al., 2014).



**Figure-4. Morphology of strawberry pollen: (a) prolate (equatorial view), (b) circular-lobate (polar view).**

Based on the number of pollens attached to the hind legs showed that bee actively visited the flowers. *Apis cerana* carried more pollens (303275 pollen grains) than *T. laeviceps* (8628 pollen grains) (Fig. 5 a). Current study showed that the body size effect the pollen carried. Pangestika et al. (2017) stated the higher pollen loads were observed in *H. itama* (31392 pollen grains) and *T. laeviceps* (8015 pollen grains). In stingless bees, pollens were commonly found in the thorax, tarsus, and corbicula (Chan and Saw, 2011).



**Figure-5. Pollen load on *A. cerana* and *T. laeviceps* (a) and type of pollens attached to *A. cerana* (b).**

In open plants, *A. cerana* prefer to visit strawberry flowers compared to other plants because the location of the bee colony is very close. Khairiah et al. (2012) reported bees tend to visit flowers of the same plant species that are close to their nests. In honey bees, information of food source location is informed to other bees using a round-dance (Schoonhoven et al., 1998). Results showed that the dominant pollen type attached on *A. cerana* was the pollen of Rosaceae (90%) followed by Asteraceae (8%), and Cyperaceae (2%) (Fig. 5 b). The percentage of flowers visited by bees depends on the plants around the nest and the availability of food and pollinators prefer to choose flowers that are easily accessible (Widhiono and Sudiana, 2016)

**Fruit formation**

Results showed that *A. cerana* pollination in strawberries yielded 4.76±1.05 fruits/plant with a percentage of perfect fruit was 77.31% and abnormal fruit was 22.69%. While, pollination by *T. laeviceps* yielded 4.24±0.92 fruits/plant with a percentage of perfect fruit was 71.70% and abnormal fruit was 28.30%. Human-assisted pollination using pollen from different plants yielded 5.04±1.04 fruits/plant with a percentage of perfect fruits was 76.98%. In control plants, the low yielded low number of fruits (2.08±0.70 fruits/plant), low perfect fruits (40.38%), and high abnormal fruits (59.62%) (Table 3).

Pollinating insects affected on the strawberry fruits. The Current study supported Adhikari and Miyanaga (2016) that reported pollination by *A. plumipes* increased strawberry fruit formation (59.9%) than that in control plants (31.0%). Abrol et al. (2017) also reported a lower percentage of abnormal fruit formation (11.20%) in plants pollinated by bees compared to control plants (17.44%). Hasan et al. (2017) also stated insects affected the cucumber production. Crop yielded also is influenced by genetic and environmental factors, as well as the status of soil nutrients and insect-plant interaction (Susilawati, 2016).

Pollination by *A. cerana* increased strawberries fruit size (3.90±0.45 cm in length, 3.61±0.36 cm in thickness, and 18.94 ± 4.00g in weight) and sugar content (8.07%). Likewise, pollination by *T. laeviceps* increased fruit size (3.57±0.48 cm in length, 3.43±0.40 cm in thickness, and 15.11±5.33g in weight). Pollination by insects especially bees produced heavier fruits and contains high nutrients (Neto et al., 2013). Control plants had a smaller fruit size (2.69±0.62 cm in

length,  $2.73 \pm 0.66$  cm in thickness, and  $10.27 \pm 2.41$  g in weight). Based on ANOVA analysis, fruit weight of hand pollination, pollination by *A. cerana*, and pollination by *T. laeviceps* was significantly different with control plants ( $p < 0.001$ ). Adhikari and Miyana (2016) also stated a higher fruit set of strawberry in hand- and *A. plumipes* pollination (95%) compared to control plants (40%). This may be related to visiting activities of *A. cerana* and *T. laeviceps* from morning to evening and allows cross-pollination occurred. Klatt et al. (2013) reported that increasing fruits quantity and size in strawberry pollination by bees. Absence of insects in control plants caused cross-pollination does not work well and transfer of pollen to the stigma does not occur.

**Table-3. Fruits formation of strawberry pollinated by *A. cerana*, *T. laeviceps*, and manual pollination.**

| Yield Components              | Fruits Formation (Average $\pm$ st. deviation)* |                      |                        |                    |
|-------------------------------|---|----------------------|------------------------|--------------------|
|                               | Hand pollination                                | <i>A. cerana</i>     | <i>T. laeviceps</i>    | Control plants     |
| Number of perfect fruits (%)  | $5.04 \pm 1.04^a$                               | $4.76 \pm 1.05^{ab}$ | $4.24 \pm 0.92^b$      | $2.08 \pm 0.70^c$  |
|                               | 76.98 <sup>a</sup>                              | 77.31 <sup>a</sup>   | 71.70 <sup>b</sup>     | 40.38 <sup>c</sup> |
| Number of abnormal fruits (%) | 23.02 <sup>a</sup>                              | 22.69 <sup>a</sup>   | 28.30 <sup>b</sup>     | 59.62 <sup>c</sup> |
| Fruit length (cm)             | $3.77 \pm 0.44^a$                               | $3.90 \pm 0.45^a$    | $3.57 \pm 0.48^a$      | $2.69 \pm 0.62^b$  |
| Fruit thickness (cm)          | $3.53 \pm 0.40^a$                               | $3.61 \pm 0.36^a$    | $3.43 \pm 0.40^a$      | $2.73 \pm 0.66^b$  |
| Fruit weight (g)              | $17.07 \pm 3.08^{ab}$                           | $18.94 \pm 4.00^a$   | $15.11 \pm 5.33^b$     | $10.27 \pm 2.41^c$ |
| Number of fruit achenes       | $309.5 \pm 53.39^{ab}$                          | $344.8 \pm 83.92^a$  | $284.5 \pm 69.60^{ab}$ | $255.6 \pm 49.5^b$ |
| Sugar content (% Brix)        | 6.68 <sup>a</sup>                               | 8.07 <sup>ab</sup>   | 7.10 <sup>ab</sup>     | 4.48 <sup>c</sup>  |
| Vitamin C content (g/100g)    | -   | 60.75 <sup>a</sup>   | 50.85 <sup>b</sup>     | 38.5 <sup>c</sup>  |

\*Values on the same row followed by the same letters do not showed a significant difference (Tukey test 95%).

The insect pollination of strawberry results reddish and heavier fruits weight and also higher storage resistance compared to non-insect-pollination (Klatt et al., 2013). Successfully pollination trigger hormones in flowers, such as gibberellin, cytokines, auxin, and abscisic acid (Iglesias et al., 2007) to promote fruits growth. Also, fruit yielded by strawberry pollinated by *A. cerana* and *T. laeviceps* had a higher vitamin C content

(60.7%/100g sample) than control plants (38.5%/100g sample). Pollinators also increased fruits micronutrients, such as vitamins A and C, calcium, fluoride, and folic acid (Smith at al., 2015). Therefore, bees can be used as an alternative pollinator for strawberry plants (Adhikari and Miyana, 2016).

## Conclusion

*Apis cerana* and *T. laeviceps* contributed to increase of fruit production of strawberry plants. The high visiting activity of *A. cerana* and *T. laeviceps* on strawberry flowers occurred in the morning and decreased during the day. Visiting activity of bees correlated with environmental factors. The average, *A. cerana* carried more pollens (303275 pollen grains) than *T. laeviceps* (8628 pollen grains). Pollination by *A. cerana* and *T. laeviceps*, as well as hand-pollination significantly increased the formation of fruits, i.e., with the number of normal shapes, fruits size and weight, and vitamin C content.

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### Contribution of Authors

Alpionita R: Designed research methodology, collected and analysed data and wrote manuscript  
Atmowidi T & Kahono S: Helped in literature review, editing and proof reading of manuscript.

