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RESEARCH

Foraging behavior and pollination efficiency of honey bees (*Apis mellifera* L.) and stingless bees (*Tetragonula laeviceps* species complex) on mango (*Mangifera indica* L., cv. Nam Dokmai) in Northern Thailand

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Background: The mango is one of the essential fruit trees for the economy of Thailand. Mango pollination relies primarily on insects. Other external forces, such as wind, are less efficient since pollen is sticky and aggregating. There is only one report from Thailand on the use of bees as mango pollinators. The study of the behavior and pollination efficiency of honey bees (*Apis mellifera*) and stingless bees (*Tetragonula laeviceps* species complex) was conducted in Nam Dokmai mango plantings in Phrao and Mae Taeng districts, Chiang Mai province, between February and March 2019.
Results: Our results reveal that the honey bees commenced foraging earlier than the stingless bee. The number of flowers visited within 1 minute by honey bees was higher than that visited by stingless bees. The average numbers of honey bees and stingless bees that flew out of the hive per minute from 7 a.m. and 6 p.m. in the Phrao district were 4.21 ± 1.62 and 9.88 ± 7.63 bees/min, respectively, i.e., higher than those observed in Mae Taeng, which were 3.46 ± 1.13 and 1.23 ± 1.20 bees/min, respectively. The numbers of fruits per tree were significantly higher in the honey bee and stingless bee treatments (T1 and T2) than in the open pollination treatment (T3). The number of fruits between T1 and T2 treatments was not different. In the pollinator exclusion treatment (T4), no fruit was produced. Fruit size factors were not significantly different among T1, T2, and T3 treatments.
Conclusions: Our results showed that insect pollination is crucial for mango production, especially with the Nam Dokmai variety in Northern Thailand. As pollinator exclusion treatment showed no fruit set, and pollinator treatment significantly increased the fruit sets compared to open access plots, a managed pollinator program would benefit the mango growers for better productivity. Both the honey bee and the stingless bee were shown to be effective as pollinators.

Keywords: *Apis mellifera*, foraging, mango, pollination, stingless bee

Introduction

The mango (*Mangifera indica* L.) is one of Thailand's most economically significant fruit trees. The mango plantation area in Thailand covers 3,152 km^2 and yields approximately 3.12 million tons of fruit per year. Mangoes are exported in fresh and other processed conditions, amounting to 117,472 tons, valued at 131 million USD. Phitsanulok province has the largest mango-growing area (132.68 km^2), followed by Loei and Chiang Mai, at 82.70 and 71.90 km^2, respectively (Department of Agricultural Extension 2018).

Pollen transmission from stamens to pistils, a vital stage in most flowering plants' seed reproduction, depends on the behavior of various animals, from insects to birds and mammals. Bee pollination is essential for the world's most important commercial crops, including mango (Tanda 2021). Studies in India and Israel examined the characteristics and biology of mango pollination and discovered that insects of the orders Diptera such as *Syrphus* sp., *Musca domestica* (L.) and Hymenoptera (*Melipona* sp.) played significant roles in mango fruiting, but house flies were not a very common pollinator (Singh 1989; Singh, 1997). Since mango pollen is sticky and aggregating, wind pollination is ineffective (Puangjik 2000; Kumar et al. 2016). Mango pollen sticks to the bees' legs and body hairs as they collect nectar and pollen from flowers, allowing pollen to be transferred from one flower to another (Thongkong 2018). Bee species have evolved significant adaptations to aid plant pollination due to the bees' sizes and foraging behaviors. Bees gather pollen and nectar from flowering plants to get carbohydrates and protein for themselves and the rest of their colony.

In a study on mango pollinators in northern Australia, Anderson et al. (1982) reported that insects of the order of Diptera and stingless bees of the genus *Trigona* were most effective. Managing insects for pollination is widely adopted in field mango cultivation in many tropical countries (Ramírez and Davenport 2016), as well as in greenhouse cultivation in Japan (Alqarni et al. 2017). Honey bee foraging activity was also recorded in the study conducted in fruit orchards with avocado, mango, and litchi trees (Du Toit 1994). According to a study conducted in the Letsitele Valley's mango orchards, honey bees make frequent visits to the inflorescences (Du Toit and Swart 1993). The management of other pollinators has evolved during the past few decades, in which other insects such as flies and stingless bees have proven to be more effective than the honey bee. Heard (1999) reported the use of an Australian stingless bee (*Trigona* spp.) as a mango pollinator. Surprisingly, limited information is available in Thailand on selecting more suitably adapted pollinating insects and using a superior methodology (Srisuksai 2021). Here we compare the pollination behaviors of the honey bee, *Apis mellifera*, and the stingless bee, *Tetragonula laeviceps* species complex, both locally available in two districts, Phrao and Mae Teang, Chiang Mai province, northern Thailand. We also compare the rates of fruiting and the quality of the fruit to figure out how pollination affects fruit production in Northern Thailand.

Materials and Methods

Pollinators used in the experiment

The honey bee colonies had approximately 8,000 bees/hive. We used Nucleus hives (Nuc-hives), which contained four combs per hive. Each hive had about 80% of the adult bee population. We applied the Burgett and Burikam (1985) method to evaluate the adult honey bee population used in the experiment.

There is no standard or published method to estimate the population size of the stingless bee (*Tetragonula laeviceps* complex) colonies. The colonies used in the experiment had a similar age, being *ca*. 2.5 years old. We estimated the size of the colony by measuring the diameter of the brood area for each colony. All the stingless bee colonies used had an approximate brood cell area 10–13 cm in diameter. According to Chuttong and Burgett (2017), we estimated the size of the colonies used in the experiment at *ca*. 5,500 workers due to the brood cell area.

Study area

Two locations of the Nam Dokmai mango cultivar in Chiang Mai province were studied to examine the efficacy of insect pollination. The initial location was a Nam Dokmai mango cultivation plot in Pa Tum Subdistrict, Phrao District, Chiang Mai Province, with latitude and longitude coordinates of 19°22'32" N, 99°14'22" E and an elevation of 330 m above sea level. Another location was the Nam Dokmai mango cultivation plot in Khie Lek Subdistrict, Mae Taeng District, Chiang Mai Province, with 19°6'37" N, 98°52'34" E coordinates, at a height of 450 m above sea level.

The mango trees were randomly selected from each location. A total of four experimental plots were identified in each research location. Mango trees with a height of around 3.5 m, a similar age (three years old), and an identical growing cycle from flowering period to fruiting period were chosen for each plot. There were four different experimental treatments. Treatment 1 (T1) was caged with honey bees (*A. mellifera* as pollinators), treatment 2 (T2) was caged with a stingless bee colony (*T. laeviceps* species complex as pollinators), treatment 3 (T3) was an open plot (no cage and open pollinated trees), and treatment 4 (T4) was a closed cage (no pollinators). Mango trees were covered with nets for treatments 1, 2 and 4. Three replications were used for each treatment. Before the mango flowering period, nylon nets (cages) were installed to cover the mango trees in the experimental plots of treatments 1, 2, and 4. Each plot consists of four mango trees covered with a net measuring 10 × 10.5 × 4 m (wide × long × height).

Observation of foraging behavior

During the flowering period, colonies of honey bees (*A. mellifera*) and stingless bees (*T. laeviceps* species complex) were introduced into nylon net houses for four weeks. In 2019, mango flower blooming occurred in February in Phrao District and March in Mae Taeng District. The pollination efficacy of insect pollinators was recorded weekly in the field. The data recorded were as follows: 1) the time of the first bee departed the hive and the last bee returned to the colony. 2) the number of bees that depart the colony per minute, and 3) the number of mango blossoms that the bees visit in one minute, with the data recorded once every hour. In addition, humidity/temperature data loggers (EBI

20-TH1; Ebro Electronic GmbH & Co. KG, Ingolstadt, Germany) were used to record temperature and relative humidity every hour.

Study on pollination efficiency

Ten inflorescences were randomly selected and tagged from each mango tree at the early inflorescence stage, for a total of 160 bunches per tree. During the harvesting phase, data on the amount and quality of mango fruit were obtained. In addition, the results from the tagged branches were examined for: 1) the average number of mango fruit per tree, 2) the average weight of fruit per tree, and 3) the average fruit size per tree (length, width and thickness).

Data analysis

The data regarding four different treatments (open plot, honey bee cage, stingless bee cage, and without pollinators/closed cage) were compared. The number of fruits per tree, the weight of fruit and fruit size were subjected to statistical analysis using analysis of variance. At $p = 0.05$, means were compared using the Least Significant Difference. SPSS version 26.0 (IBM Co., Armonk, NY, USA) was used to analyze the data.

Results

Foraging behavior of honey bees and stingless bees

At the Phrao location (Fig. 1), the foraging activity of *A. mellifera* usually began at 7 a.m., which was shortly after sunlight appeared. Although only 0.75 ± 0.95 bees flew out of their hives at 7 a.m., numbers increased at 9 a.m. and continued up to 5 a.m. The peak number of foragers flying out was at 5 p.m. (6.50 ± 4.43 bees). *T. laeviceps* species complex flew out of their hive after 8 a.m., with the number increased rapidly, with a maximum peak occurring at 9 a.m. (26.50 ± 18.69 bees). After this time, the number of foragers flying out of their nests decreased slowly until 5 p.m. (Table 1).

At the Mae Taeng location (Fig. 2), the foraging activity of *A. mellifera* frequently began at 7 a.m. (2.00 ± 2.16 bees exiting the hives). The number of bees that flew out increased at 8 a.m. and continued up to 5 p.m. The highest numbers of foragers flying out, occurring from 3–5 p.m., were 6.25 ± 2.98, 6.5 ± 3.69, and 6.75 ± 6.18 bees, respectively. The number of *T. laeviceps* species complex exiting their hives after 9 a.m. increased slowly and the maximum number of bees flying out was 3.50 ± 1.91 bees at 11 a.m. (Table 1).

Visitation frequency

The number of flowers visited by bees in a 1-minute period was counted at the Phrao location. *A. mellifera* visiting mango flowers varied between 3.19 ± 4.46 (at 6 p.m.) and 10.88 ± 1.85 (at 11 a.m.) flowers, with an average of 7.93 ± 3.54 flowers. *T. laeviceps* species complex visiting, mango flowers varied between 0.69 ± 1.24 (8 a.m.) and 6.13 ± 1.43 (11 a.m.) flowers, with an average of 3.86 ± 1.04 flowers. The numbers of flowers visited by *A. mellifera* and *T. laeviceps* species complex in a 1-minute period from 7 a.m. to 6 p.m. were compared only at 4 p.m., when the number of flowers visited by bees was not different ($p < 0.05$).

At the Mae Taeng location, the number of flowers visited by bees in a 1-minute period was observed. *A. mellifera* visiting mango flowers varied between 6.87 ± 4.60 (at 6 p.m.) and 11.39 ± 1.24 (at 8 a.m.) flowers, with an average of 10.04 ± 2.27 flowers. *T. laeviceps* species complex visiting mango flowers varied between 1.83 ± 2.59 (5 p.m.) to 5.07 ± 1.72 (1 p.m.) flowers, with an average of 2.72 ± 1.69 flowers. The numbers of flowers visited by *A. mellifera* and *T. laeviceps* species complex in a 1-minute period from 7 a.m. to 6 p.m. were compared. At 3 p.m. and 6 p.m., the numbers of flowers visited by bees were not different ($p < 0.05$).

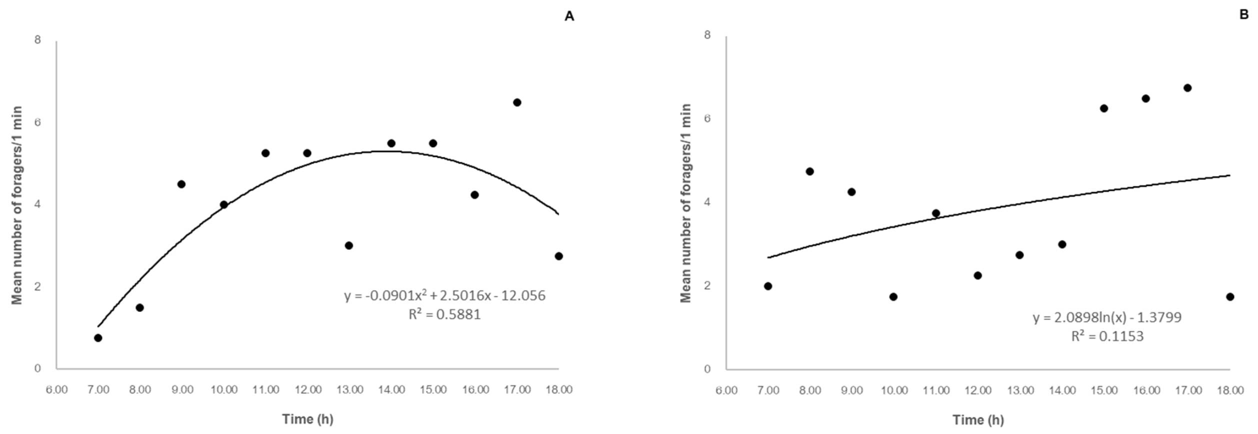

Fig. 1 Outgoing foragers during mango flower blooming period in Phrao mango plantation, Chiang Mai Province. (A) Mean hourly number of *Apis mellifera* workers leaving their hives within a 1-minute period. (B) Mean hourly number of *Tetragonula laeviceps* species complex workers leaving their hives within a 1-minute period.

Table 1 Average number of honey bees and stingless bees leaving the hives per minute, observed at every hour interval from 7 a.m. to 6 p.m. in mango plantations in Phrao and Mae Taeng, Chiang Mai Province, northern Thailand, during the 2019 season

Site	Bee species	Time (h)											
		7 a.m.	8 a.m.	9 a.m.	10 a.m.	11 a.m.	12 p.m.	1 p.m.	2 p.m.	3 p.m.	4 p.m.	5 p.m.	6 p.m.
Phrao	*Apis mellifera* L.	$0.75 \pm 0.95^{a,b}$	1.50 ± 1.29^{a}	4.50 ± 1.91^{b}	4.00 ± 1.82^{b}	5.25 ± 0.95^{b}	5.25 ± 2.21^{b}	3.00 ± 4.08^{b}	5.50 ± 4.20^{b}	5.50 ± 3.31^{a}	4.25 ± 3.77^{a}	6.50 ± 4.43^{a}	2.75 ± 2.21^{a}
	Tetragonula laeviceps species complex	0.00 ± 0.00^{b}	3.00 ± 6.00^{a}	26.50 ± 18.69^{a}	12.50 ± 5.19a	15.75 ± 4.57^{a}	14.25 ± 0.50^{a}	13.25 ± 2.98^{a}	13.50 ± 6.24^{a}	8.25 ± 2.06^{a}	6.25 ± 5.73^{a}	5.25 ± 3.50^{a}	0.00 ± 0.00^{b}
Mae Taeng	*Apis mellifera* L.	2.00 ± 2.16^{a}	4.75 ± 4.27^{a}	4.25 ± 4.03^{b}	1.75 ± 1.25^{b}	3.75 ± 2.06^{b}	2.25 ± 1.89^{b}	2.75 ± 2.21^{b}	3.00 ± 0.81^{b}	6.25 ± 2.98^{a}	6.50 ± 3.69^{a}	6.75 ± 6.18^{a}	1.75 ± 1.25^{a}
	Tetragonula laeviceps species complex	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}	0.25 ± 0.50^{b}	1.50 ± 1.00^{b}	3.50 ± 1.91^{b}	3.25 ± 1.25^{ab}	2.00 ± 0.81^{b}	1.50 ± 0.57^{b}	1.00 ± 0.81^{b}	0.25 ± 0.50^{a}	0.25 ± 0.50^{a}	0.00 ± 0.00^{b}

Values are presented as mean ± standard error.
The values with different superscript letters in a column are significantly different ($p < 0.05$).

Study on pollination efficiency

The average number of mango fruit set was recorded in both research locations. There was no statistically significant difference between treatment 1 (T1), which employed honey bees as pollinators, and treatment 2 (T2), which used stingless bees as pollinators. However, there was a significant difference between treatment 3 (T3) (open plot) and treatment 4 (T4) (closed plot) (p = 0.05) (Table 2). In T1 and T2, the average number of fruits was at 13.25 ± 0.96 and 12.50 ± 0.58, respectively, in the area of mango planting in the Phrao District, which was higher than in T3, which had an average number of fruits set at 10.25 ± 0.50 (Table 2). At the same time, the mango plantation area in the Mae Taeng District had comparable fruiting characteristics to the Phrao District area. Table 2 showed that the average number of fruit-bearing at (T1) 11.50 ± 1.29, (T2) 11.50 ± 0.58, and T3 was 9.50 ± 0.58, respectively (Table 2). There were no pollinators (closed plot) in the T4 trial. The average number of fruit was 0, indicating no fruit set.

Discussion

Our results indicated the peak foraging times of honey bees and stingless bees were different. In both locations we studied, *A. mellifera* active foraging time was in the afternoon from 3 to 5 p.m. However, *T. laeviceps* species complex highest foraging time was between 9 and 11 a.m., which is relevant to the maximum flowering time of mango between 9:30–10:30 a.m. and completed at 11 a.m. The dehiscence of anthers takes place at 11:30 a.m. and continues up to 3:45 p.m. (Srivastava et al. 2017).

Bees with larger wings are expected to be able to fly for a longer period than those with smaller wings (Mostajeran et al. 2006). Furthermore, the adaptation of honey bee subspecies to various climatic circumstances may influence foraging behavior (Alqarni et al. 2006). This study indicates that the honey bee had a more extended foraging period than the stingless bee. The results revealed that the average number of bees that flew out of the hive within one minute in Phrao was greater than in Mae Taeng. This difference could be due to microclimatic factors such as temperature and relative humidity (Amin et al. 2015). Honey bee foraging rates tended to be positively correlated with air temperature, while relative humidity had less effect on flight activity (Gebremedhn et al. 2014; Joshi and Joshi 2010). This is not correlated to our findings on the foraging behavior of the honey bee and stingless bee. The number of food plants, the degree of nectar and pollen demands inside the colony, and the interior environment of the plot all influence bee foraging activities (Abou-Shaara 2014). Maia-Silva et al. (2014) stated the stingless bee (*Melipona subnitida*) did not forage at very low temperatures, even when valuable pollen sources were in abundance.

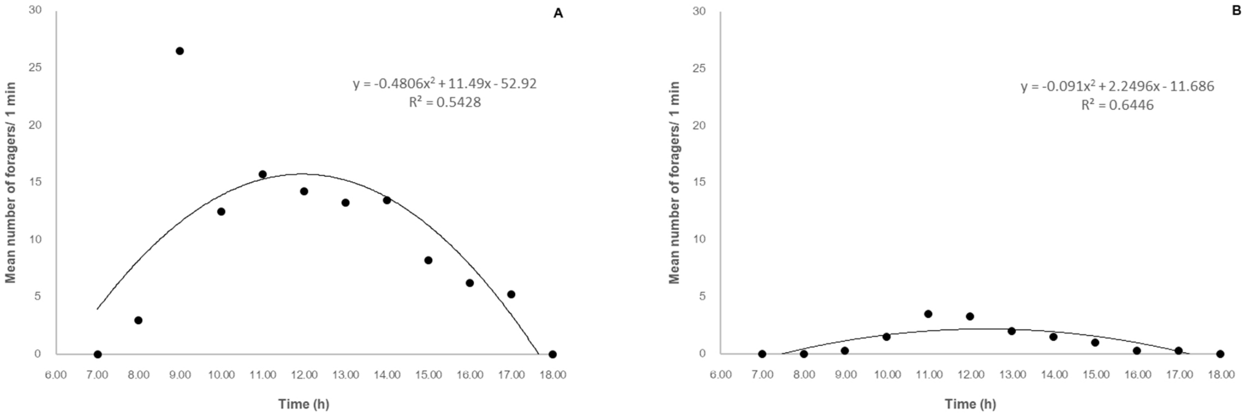

Fig. 2 Outgoing foragers during mango flower blooming period in Mae Taeng mango plantation, Chiang Mai Province. (A) Mean hourly number of *Apis mellifera* workers leaving their hives within a 1-minute period. (B) Mean hourly number of *Tetragonula laeviceps* species complex workers leaving their hives within a 1-minute period.

Table 2 Mean number of fruit per plant, weight per fruit, and size factors of fruit by treatments in two mango plantations located in Phrao and Mae Taeng, Chiang Mai Province, northern Thailand during 2019 season

Site	Treatment	No. fruit/tree	Weight/fruit (g)	Size of fruit (cm)		
				Length	Width	Height
Phrao	T1	13.25 ± 0.96^{a}	201.36 ± 36.36^{a}	12.30 ± 0.75^{a}	5.29 ± 0.31^{a}	5.95 ± 0.49^{a}
	T2	12.50 ± 0.58^{a}	213.55 ± 12.39^{a}	12.60 ± 0.18^{a}	5.40 ± 0.19^{a}	6.14 ± 0.14^{a}
	T3	10.25 ± 0.50^{b}	209.97 ± 40.39^{a}	12.60 ± 1.10^{a}	5.34 ± 0.40^{a}	6.12 ± 0.42^{a}
	T4	0.00	0.00	0.00	0.00	0.00
Mae Taeng	T1	11.50 ± 1.29^{a}	385.84 ± 25.35^{a}	15.46 ± 0.46^{a}	6.92 ± 0.21^{a}	7.36 ± 0.15^{a}
	T2	11.50 ± 0.58^{a}	410.92 ± 31.99^{a,b}	16.10 ± 0.78^{a,b}	7.09 ± 0.09^{a,b}	7.64 ± 0.06^{b}
	T3	9.50 ± 0.58^{b}	452.41 ± 23.06^{b}	16.91 ± 0.82^{b}	7.31 ± 0.13^{b}	7.83 ± 0.21^{b}
	T4	0.00	0.00	0.00	0.00	0.00

Values are presented as mean ± standard error.
T1 was honey bee cage (*A. mellifera* as pollinator), T2 was stingless bee cage (*T. laeviceps* species complex as pollinator), T3 was open plot (no cage and open pollinated trees) and T4 was closed cage (no pollinators).
The values with different superscript letters in a column are significantly different ($p < 0.05$)

There is a relationship between the amount of food stored and resource availability, demonstrating that when food is available, colonies collect more food (rainy season) (Aleixo et al. 2017). During the dry season, this helps stingless bee colonies stay alive even though there is less food and the temperature is lower.

The number of flowers visited by bees per one minute is consistent with the Gadhiya and Pastagia (2019) study of the time *T. laeviceps* spent on musk melon flowers. The average pollen collection was 10.74 ± 0.38 seconds per flower and the average nectar collection was 17.2 ± 0.65 seconds per flower. According to Sung et al. (2006), *Apis cerana* visits individual mango flowers for 4.5 ± 2.9 seconds, while *A. mellifera* spends 3.9 ± 2.2 seconds on a flower. Honey bees are faster than stingless bees in collecting nectar and pollen from flowers. Pollinator movement between flowers improves pollination efficiency; therefore, a higher visitation rate should result in better pollination (Couvillon et al. 2015). Our findings at the Mae Taeng location show that *A. mellifera* and *T. laeviceps* species complex spend 5.97 and 22.05 seconds on a flower, respectively. At the Pharo location, *A. mellifera* spends 7.56 seconds and *T. laeviceps* species complex spends 15.54 seconds on a flower. In addition to landing and walking on the flower's reproductive parts, stingless bee foragers stay at a flower for a long time. This seems to help spread pollen grains as much as possible, which is important for the development of fruit without deformation (Bomfim et al. 2014). On the other hand, the stingless bee had a lower average visit rate than the honey bee. According to Biesmeijer and Tóth (1998), the forager groups of stingless bees (*Melipona beecheii*) in Costa Rica showed notably diverse activity patterns and lifespans. Nectar foragers were active throughout the day and died after an average of three days of foraging. Pollen foragers performed for 1–3 hours every day but lived only 12 days. In contrast, pollen and nectar foragers flew at the exact same times during their lifetimes.

Puangjik (2000) stated that using pollinators, such as honey bees and stingless bees, to support the pollination of Nam Dokmai mango flowers and enable natural insect pollination increased the fruiting efficiency of mango trees. However, pollinator-free treatments (closed plots) produced no fruit set. Our results demonstrated the number of fruits per tree with a pollinator trial (*A. mellifera* and *T. laeviceps* species complex) and an open plot (natural pollinators) was much higher when compared to no pollinator and no yield. These explain the need for pollinators for mango. And the number of fruits per tree of mango pollinated by *A. mellifera* and *T. laeviceps* species complex was higher than the natural pollinators at a significant level. Dag and Gazit (2000) conducted a preliminary pollinator trial on mango, The production was low without pollinators. The yield increased dramatically after pollinators such as honey bees, bumble bees, and house flies were introduced into the cages, yet the significant increase was not determined to be relevant due to high variability. The honey bee appears to be less effective than the bumble bee. This could be due to the fact that the bumble bee works better in small places. Cross pollination of mango is facilitated by insects, wind, and possibly vertebrate species (Ramírez and Davenport 2016). Even though our results showed no fruit set in a closed plot without a pollinator, this was due to the nylon net used in the experiment, which limited air ventilation in the cage. Huda et al. (2015) reported that Chok Anan mango flowers failed to produce fruit set in the covered treatment and hence were strongly pollinator dependent. However, the pollinator had no effect on mango fruit quality in terms of weight and size. The result showed no difference between insect pollinators and those with no pollinators.

Conclusions

According to our results on bees' foraging activity, stingless bees spend more time on flowers than honey bees. This behavior can assist in pollen transfer, which is necessary for fruit development. The most active time of the stingless bee foraging is comparable to the maximum flowering time of mango between 9:30 and 10:30 a.m., while the peak number of forager honey bees flying was in the afternoon. By combining our results, pollinators significantly affect the number of fruit sets where the yield can be significantly increased by insect pollinators. This highlights the significance of bees in mango pollination. However, pollinator species of either honey bee or stingless bee did not differ in pollination services, resulting in similar quality of mango, i.e., fruit weight and size, but showed superior service over both open pollination and exclusion treatments.

The management of insect pollination services in mango is significant, and the utilization of insect pollinators and conservation of natural pollinators is also very important to increase the mango yield.

Abbreviations

Not applicable.

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Author's contributions

BC and LP conducted the laboratory and field research, analyzed the data, and wrote the manuscript. The field investigation was conducted by CT and PL, who also edited the manuscript. The manuscript was reviewed and edited by WP, PC, CJ, and MB. The authors read and approved the final version.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interest

The authors declare that they have no competing interests.

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