Effects of meliponiculture *Tetragonula laeviceps* on diversity and foraging behavior wild bee pollinators and *Citrus limon* Eureka pollination efficacy

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Abstract

The augmentation of pollination success in lemon (*Citrus limon* Eureka) flowers remains contingent on the involvement of bee pollinators. With wild bee pollinator populations declining in agroecosystems, meliponiculture has emerged as a potential option in Indonesia. This study aimed to investigate the effects of meliponiculture *Tetragonula laeviceps* on diversity, foraging behavior, and monthly population of wild bee pollinators as well as the pollination efficacy during two periods. Using scan and focal sampling methods in first and second periods, the study found that the diversity of lemon pollinators are 6 species of wild bees, 4 species of wild non-bees, and *T. laeviceps* when with meliponiculture. The relative abundance and daily foraging activity of wild bee pollinators were initially reduced in the first period and then maintained in the second period. The foraging behavior of *T. laeviceps* on lemon flowers involved specific sequences, with time spent of 72 seconds and the highest visitation rate of 0.25 flowers per hour observed at noon (10:00 to 13:00). Environmental factors influencing the number of bee pollinators visiting lemon flowers, with light intensity being the most influential factor. Pollination efficacy results showed that meliponiculture was greater compared to without meliponiculture across various parameters, including fruit sets, fruit weight, yield, and estimated productivity. The effects of meliponiculture *T. laeviceps* can enhance lemon pollination efficacy while preserving the diversity of wild insect pollinators. This suggests that meliponiculture stingless bees could be a beneficial practice in agroecosystems, especially in tropical regions where wild bee populations and diversity are declining.

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Do you mean that T. laeviceps meliponiculture will effect the diversity, foraging behavior of wild bee polinators and the pollination efficacy of Citrus limon?

Please correct the title!

Commented [H2]: The abstract only explains about T. laeviceps foraging behavior. It does not explain wild bees' foraging behavior after you cultivate T. laeviceps.

Keywords: Diversity, Foraging behavior, Meliponiculture, Pollination sequences, *Tetragonula laeviceps*

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Introduction

Lemon (*Citrus limon* Eureka) is a fruit agricultural commodity grown in open farming with monoecious flower types that bloom all year. Wind pollination is sufficient to pollinate lemon flowers, while bee pollinators can ensure increased pollination success (Aizen et al., 2019; Dymond et al., 2021; Vanlalhmangaiha et al., 2022). However, numerous studies have reported that the population of wild bee pollinators is declining by 20–57% in various regions (Potts et al., 2010; Koh et al., 2016; Rhodes, 2018; Panziera et al., 2022; MacInnis, Normandin & Ziter, 2023). This issue has prompted several studies on applied bee cultivation in agroecosystems to enhance productivity (Aslan & Yavuksuz, 2010; Nunes-Silva et al., 2013; Hall et al., 2020; Amon et al., 2023). The application of meliponiculture stingless bees has expanded in tropical regions (da Silva et al., 2017; Azmi et al., 2019; Layek, Das & Karmakar, 2022; Reddy, Chauhan & Singh, 2022; Balaji et al., 2023; Wongsa, Duangphakdee & Rattanawannee, 2023), particularly in Indonesia (Putra, Permana & Kinasih, 2014; Alpionita, Atmowidi & Kahono, 2021; Atmowidi et al., 2022; Nurdiansyah, Abduh & Permana, 2023).

Commented [H4]: You need to explain, why you choose Citrus limon? Is it important commodity in Indonesia? How about the production? Is it decrease, so you need insect to pollinate it?

You need to explain the effect of meliponiculture on agroecosystem for pollination efficacy. You can show the result of the pollination on crop in the world, also in Indonesia. So it is important to make this research.

Why foraging behavior is important?

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In Indonesia, a total of 19 stingless bees species have been cultivated (Buchori et al., 2022), and 7 species has been applied for meliponiculture, including *Heterotrigona itama*, *Lepidotrigona terminata*, *Trigona laeviceps*, *Trigona iridipennis*, *Tetragonula biroi*, *Tetragonula clypearis*, and *Tetragonula laeviceps* (Putra, Permana & Kinasih, 2014; Alpionita, Atmowidi & Kahono, 2021; Asmini, Atmowidi & Kahono, 2022; Djakaria, Atmowidi & Priawandiputra, 2022; Putra et al., 2022; Suhri et al., 2022). *T. laeviceps* was the most commonly used species in meliponiculture in agricultural commodities such us true shallot, strawberry, okra, pummelo, and orange, and it became main pollinator in orange orchards at 11:00 with 53.17% (Nurdiansyah, Abduh & Permana, 2023). The effects of meliponiculture stingless bees have been widely reported to improve quality and productivity of seeds and fruits, while there is unreported whether it affects wild insect pollinators in open farming.

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Maintaining the diversity of wild insect pollinators is critical for preserving ecological services, including the sustainability of natural habitats and population biodiversity (Garibaldi et al., 2011; Tschoeke et al., 2015). In agroecosystems, a rich diversity of wild insect pollinators contributed to enhanced pollination services (Katumo et al., 2022). However, the introduction of a new species into agroecosystems may lead to resource competition, potentially displacing existing wild bee pollinators from their role in pollination (Nielsen et al., 2017). A comprehensive study on meliponiculture stingless bees in open farming is needed to address this issue.

This study aimed to investigate the effects of meliponiculture stingless bee *T. laeviceps* on wild bee pollinators in a lemon orchard based on diversity, foraging behavior, and monthly populations. Additionally, the study evaluated the pollination efficacy by meliponiculture *T. laeviceps* on pollination efficacy and its consequences on lemon productivity. The findings of this study provide valuable insights into the application of meliponiculture to support sustainable agriculture.

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Materials & Methods

Study site

This study was carried out in Cibodas, West Bandung Regency, West Java, Indonesia, at coordinates latitude 6°49'20"S and longitude 107°40'35"E, with an altitude of 1,219 meters above sea level. The study area covered a total land area of 60 m^2 and included 200 plants of lemon (*Citrus limon* Eureka) aged three years, with an average height of 2 m and a canopy width of 2 m. The eastern, southern, and western parts of the lemon orchard are surrounded by horticultural farming including eggplants, tomatoes, and cabbage, while the northern part is bordered by teak forests. The colony carrying capacity in lemon has been calculated and the results require 4 colonies of stingless bee *Tetragonula laeviceps*, with approximately 400 to 600 adult worker bees each colony (Bareke et al., 2020). The colonies were obtained from cultivators in Banjaran, West Java, Indonesia and acclimatized for one week before the observations. The study was investigated in two periods, with the first period from March to June 2023 and from July to October 2023 for the second period, with cultivated 4 colonies *T. laeviceps* in Tetragonula hive (Abduh et al., 2020) at the middle of lemon orchard in late March and left until October 2023. The study employed 4 plots placed in accordance with the compass, each measuring 15 x 15 m^2, with 16 lemon plants per plot. Each plant receives 5 kg of organic fertilizer made from chicken manure in March and July 2023. Observations were conducted from 07:00 to 15:00, employing 15 min intervals for each plot. Observations for plots without meliponiculture were conducted weekly throughout March 2023, whereas those ~~with~~ meliponiculture were undertaken weekly from April to October 2023. Microclimate conditions at the study site were measured using the Data Logger HOBO U10-003.

Diversity, foraging behavior, and population of bee pollinators

Wild insect pollinators were collected by sweep net (mesh size 0.9×0.3 mm) using a dried preservation technique and subsequently pinned. Furthermore, lemon flowers and bee pollinators carrying pollen were captured and inserted into a 25 mL colonial tube containing 15 mL of 70% alcohol. These specimens were sent to the Laboratory of Entomology, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia for taxonomic identification (Gibb & Oseto, 2019; Mason et al., 2022) and number of lemon pollen and pollen load of bee pollinators analysis. After determining the taxonomic identification of pollinators, the diversity of pollinators was analyzed using the percentage of relative abundance calculated by dividing the total number of each pollinator species by total number of pollinators multiplied by 100%. Foraging behavior of bee pollinators was observed using scan sampling method across 4 plots, with each plot observed 15 min to determine the number of bees visiting blooming lemon flowers, while the time spent by bee pollinators per flower was recorded using focal sampling method (Putra, Permana & Kinasih, 2014; Nurdiansyah, Abduh & Permana, 2023). Nectar content from lemon flowers was collected utilizing micro hematocrit capillaries (length 75 mm and diameter 1.55 mm) and volume was measured with a micropipette, and the concentration was determined using a hand refractometer.

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Commented [H11]: Is it effect the result of your study?

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Commented [H13]: Why the period is short?

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1. When you get the nectar? Every day or hour?
2. How to collect the nectar from the flower?
3. Are you take the nectar each period?

For pollen analysis, flowers or bees were centrifuged for 5 min at 3,500 rpm, and then the flowers or bees were removed. Subsequently, another centrifugation was performed for 3 min at 2,000 rpm, and the supernatant was removed. A solution of acetolysis (0.9 mL acetic anhydride + 0.1 mL sulfuric acid) was added, and the samples were heated in water bath at 80 ºC for 5 min. Afterward, 1 mL of distilled water was added. The number of pollen was quantified using 0.1 mL of samples at hemocytometer in 4 quadrants under a light microscope (eyepiece lens 10x and objective lens 10x/0.24). The number of pollens from flowers and bees was calculated using the total volume of solution multiplied number of pollen counted divided volume of 4 quadrants (Alpionita, Atmowidi & Kahono, 2021).

The foraging behaviors of bee pollinators were assessed based on visitation rates, which were categorized into three intervals: morning (7:00 to 10:00), noon (10:00 to 13:00), and afternoon (13:00 to 16:00). The visitation rates of bee pollinators was calculated as the number of bee pollinators visiting flowers divided by the number of flowers available per observation (Gallagher & Campbell, 2020). The total number of bees visiting blooming flowers of each species and the total number of blooming flowers per month were utilized to analyze the monthly population of bee pollinators.

Pollination efficacy and productivity estimation of lemon

Pollination efficacy was investigated in two periods, with the first period from flower until harvest was observed from March to June 2023, and the second period observed from July to October 2023. Pollination efficacy was compared between without and with meliponiculture *T. laeviceps* based on various parameters, including number of fruit sets, pollination success, fruit weight, and yield per plant in the first period, as well as with meliponiculture in the first and second period. The comparison involves 15 flowers per plant from 64 plants were randomly tagged, and after 7 days of blooming stages, the number of fruits set was calculated. Percentage of fruit set was determined by calculating as the number of fruit sets divided by the number of flowers, multiplied by 100% (Nurdiansyah, Abduh & Permana, 2023). When harvesting, one lemon was selected at random from each plant and weighed using an analytical balance. The yield per lemon plant was determined by multiplying the average fruit weight by the number of harvested fruits. Furthermore, lemon productivity was estimated at 900 plants per hectare using a 3x3 m^2 spacing. The estimated lemon productivity was determined by multiplying the average fruit weight by the number of fruits harvested, the number of plants, and accounting for three harvest cycles within a single year (Nurdiansyah, Abduh & Permana, 2023).

Commented [H19]: How you can get the number of productivity while non meliponiculture only cultivated on March? Please explain clearly the procedure. Why the harvesting on meliponiculture used 2 periods while non meliponiculture only one period

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Statistical analysis

All data were analyzed for normality and homogeneity of variance, and no data transformations were applied. Effects of meliponiculture *T. laeviceps* were analyzed using two-sample t-test ($p < 0.05$) for the parameter’s relative abundance, daily foraging activity, fruit sets, fruit weight, and yield per plant. Analysis of variance (ANOVA) followed by Tukey’s multiple comparison test ($p < 0.05$) was performed to compare the pollen load, time spent, and visitation rates of bee pollinators. Pearson's correlation coefficient was employed to assess the significance of the correlation between the number of pollinators visiting lemon flower with environmental factors. Additionally, monthly correlation calculations were conducted to examine the relationship between the monthly population of bee pollinators and the number of flowers. To identify the primary components influencing the foraging behavior of bee pollinators on lemon flowers, a principal component analysis was performed. The statistical analyses were performed using the R program version 4.3.2. (R Core Development Team, 2023).

Results

Diversity of insect pollinators

Wild insect pollinators during without meliponiculture on visiting lemon flowers are six species of bees, including *Apis cerana* (Hymenoptera: Apidae), *Lasioglossum albescens* (Hymenoptera: Halictidae), *Megachile laticeps* (Hymenoptera: Megachilidae), *Xylocopa confusa* (Hymenoptera: Apidae), *Xylocopa latipes* (Hymenoptera: Apidae), and *Xylocopa caerulea* (Hymenoptera: Apidae), along with 4 species of non-bees, including *Dolichoderus thoracicus* (Hymenoptera: Formicidae), *Papilio demoleus* (Lepidoptera: Papilionidae), *Delias belisama* (Lepidoptera: Pieridae), and *Hypolimnas misippus* (Lepidoptera: Nymphalidae). However, the diversity of wild insect pollinators with meliponiculture was maintained and the stingless bee *Tetragonula laeviceps* (Hymenoptera: Apidae) emerged as a new pollinator for lemon flowers became the most relative abundance ($t_{(3)} = 60.00$, $p = 1.02E^{-5}$). The relative abundance of wild bee pollinators visiting lemon flowers decreased significantly in the first period, including *A. cerana* ($t_{(6)} = 31.84$; $p = 6.38E^{-8}$), *L. albescens* ($t_{(6)} = 28.54$; $p = 1.23E^{-7}$), *M. laticeps* ($t_{(6)} = 8.69$; $p = 1.28E^{-4}$), *X. confusa* ($t_{(6)} = 31.72$; $p = 6.52E^{-8}$), *X. confusa* ($t_{(6)} = 31.72$; $p = 6.52E^{-8}$), *X. caerulea* ($t_{(6)} = 18.04$; $p = 1.87E^{-6}$), while the non-bee pollinators remained constant (Table 1). There was no change in the relative abundance of wild insect pollinators between with meliponiculture in the first and second periods ($p > 0.05$).

Foraging behavior of bee pollinators

The daily foraging activity of bee pollinators visiting lemon flowers starts from 7:00 to 16:00 (Fig. 1). The highest number of bee pollinators visiting lemon flowers occurred at 11:00 in both the first and second periods. However, the highest number of bee pollinators visiting lemon flowers in the first period significantly decreased between without and with meliponiculture, including *A. cerana* ($t_{(6)} = 85.90$; $p = 1.56E^{-10}$), *L. albescens* ($t_{(6)} = 48.18$; $p = 5.36E^{-9}$), *M. laticeps* ($t_{(6)} = 51.30$; $p = 3.68E^{-9}$), *X. confusa* ($t_{(6)} = 47.82$; $p = 6.60E^{-9}$), *X. latipes* ($t_{(6)} = 18.59$; $p = 1.56E^{-6}$), and *X. caerulea* ($t_{(6)} = 18.64$, $p = 1.54E^{-6}$). There were no significant different in daily foraging activity between with meliponiculture in the first and second period ($p > 0.05$). Each visit to lemon flowers by *T. laeviceps* follows a distinct pollination sequence (Fig. 2). It starts with approach (Fig. 2A) the flower at a position parallel to 45 degrees from its original position, followed by perching on a petal (Fig. 2B1) or an anther (Fig. 2B2). Subsequently, it enters the nectary flower to collect nectar (Fig. 2C), then climbs to the anther to collect pollen (Fig. 2D). When solely interested in pollen, *T. laeviceps* directly perching goes to the anther. Upon completing its activities, it leaves the lemon flower from the anther (Fig. 2E) without buzzing and moves searches for the nearest flower to resume collecting nectar and pollen (Fig. 2F). *T. laeviceps* exhibiting the longest time spent visiting lemon flower ($F_{(6, 105)}$ = 12.22; *p* = 0.000), while *M. laticeps* ($F_{(6, 105)}$ = 26.01; *p* = 0.000) spends the fastest time (Table 2). Lemon flowers produce 12,539 ± 376 pollen grains per flower. Each bee pollinator carries pollen on its body after visiting a lemon flower and the pollen load of each bee pollinator differs ($p < 0.05$). Based on pollen load on their bodies, bee pollinators seem to visit not only lemon flowers but also other flowers in the lemon orchard, such as tomatoes and eggplants. The honey bee *A. cerana* has the highest pollen load in lemon flowers, with 84,875 pollen grains ($F_{(6, 105)}$ = 71,728.25; *p* = 0.000), while the stingless bee *T. laeviceps* carries only 6,124 pollen grains ($F_{(6, 105)}$ = 5,239.94; *p* = 0.000). Furthermore, *M. laticeps* ($F_{(6, 105)}$ = 12,372.51; *p* = 0.000) carries the highest pollen grains from various plant flowers. The volume of nectar on lemon flowers continues to increase from 7:00 to 11:00 and decreases until 16:00 when the last bee pollinators visited lemon flowers (Fig 3A). However, the nectar concentration continued to increase from 7:00 to 16:00. The highest pollinator visitation rates occur in the noon (10:00 to 13:00), with *T. laeviceps* being the most significant pollinator visiting lemon flowers ($F_{(12, 483)}$ = 7.42; *p* = 0.000). However, the lowest pollinator visitation rate is observed *X. caerulea* ($F_{(12, 483)}$ = 0.01; *p* = 0.000) during the afternoon (13:00 to 16:00).

The number of bee pollinators visiting lemon flowers was then analyzed in correlation to environmental factors such as microclimate conditions and nectar contents. Microclimate conditions including temperature, light intensity, and relative humidity during observations from March to October 2023 are ranging from 20.27–24.29 ºC, 589.3–5,442.5 lux, and 71.95–87.43% respectively. The temperature followed the same pattern as the number of pollinators and showed a very high positive correlation (*r* = 0.83; $p = 2.22E^{-16}$), as performed light intensity (*r* = 0.93; $p = 2.22E^{-16}$) (Fig. 4). Whereas relative humidity follows the opposite pattern and showed a high negative correlation (*r* = -0.68; $p = 3.26E^{-11}$). In addition, the number of bee pollinators visiting lemon flowers also correlates with nectar content, with a high positive correlation with volume (*r* = 0.65; $p = 6.20E^{-10}$) and a low positive correlation with concentration (*r* = 0.24; *p* = 0.04). Furthermore, a principal component analysis was performed to explore the relationship between microclimate conditions, nectar content, and the number of pollinators visiting lemon flowers (Fig. 5). The results indicated that light intensity (Dim1 = 2.29; Dim2 = -0.36) is most significant component influenced on the number of pollinators (Dim1 = 2.33; Dim2 = -0.02), followed by temperature (Dim1 = 2.08; Dim2 = 0.34), relative humidity (Dim1 = -1.89; Dim2 = -0.87), volume (Dim1 = 1.71; Dim2 = -1.62) and concentration of nectar (Dim1 = 0.74; Dim2 = 2.26).

Population of bee pollinators

The monthly population of bee pollinators visiting lemon flowers show fluctuations (Fig. 6). The population of bee pollinators and lemon flowers increased from March to April in the first period, but then decreased until June. In the second period, a similar pattern was observed. This indicates that the lemon production cycle lasts for 4 months, with phenological stages lasting 115-120 days in the first period and 121-125 days in the second period from full bloom to

Commented [H22]: How about other wild bee? Are they did not make the pattern like T. laeviceps?

Commented [H23]: Is it the same between non meliponiculture and meliponiculture?

Commented [H24]: Is it only for meliponiculture? How about non meliponiculture harvest from 20 flowers observed. The total number of bee pollinators exhibited a very high positive correlation ($r = 0.98$; $p = 2.25E^{-5}$) with the total number of blooming lemon flowers.

Pollination efficacy and productivity estimation of lemon

Commented [H25]: How you can get the result on non meliponiculture while it was conducted only for a month?

The pollination efficacy of 64 lemon plants was evaluated using 15 flowers per plant (Table 3). The results of the first period showed a significant difference in the fruit set ($t_{(126)} = 26.47$; $p = 1.29E^{-52}$), fruit weight ($t_{(126)} = 118.49$; $p = 4.44E^{-131}$), and yield per plant ($t_{(126)} = 108.63$; $p = 2.27E^{-126}$) between without meliponiculture and with meliponiculture *T. laeviceps*. There were no significant differences in the parameters of fruit sets, fruit weight, and yield per plant in the with meliponiculture results of both periods ($p > 0.05$). Estimated lemon productivity without meliponiculture is 11.62 ± 0.24 tons per hectare per year, while with meliponiculture is 15.19 ± 0.12 tons per hectare per year in the first period and 14.92 ± 0.16 tons per hectare per year in the second period.

Discussion

This study showed that meliponiculture *Tetragonula laeviceps* in lemon orchards does not affect the diversity of wild pollinators such as meliponiculture in orange orchards (Nurdiansyah, Abduh & Permana, 2023). However, the relative abundance of wild bee pollinators has decreased (42%) while the relative abundance of wild non-bee pollinators has maintained in the first period. This demonstrates the existence of competition between wild bee pollinators and *T. laeviceps*, leading to *T. laeviceps* being the most abundant pollinator of lemon flowers. Similar patterns have been observed with managed honeybees, which can reduce the density of wild bumble bees in homogeneous plant landscapes and raspberry farming (Herbertsson et al., 2016; Nielsen et al., 2017). This suggests that stingless bees *T. laeviceps* show high fidelity to lemon flowers followed by wild honeybees *A. cerana*. The consistent relative abundance between the first and second periods suggests the structure of insect pollinator community is stable, and the meliponiculture *T. laeviceps* has no potential to disrupt the community structure in the short-term. A study was required to evaluate the potential long-term disruptiveness of meliponiculture *T. laeviceps*, because honeybees disrupted the structure of plant-pollinator interactions (Valido, Rodríguez-Rodríguez & Jordano, 2019).

The daily foraging activity of wild bee pollinators visiting lemon flowers was reduced in the first period with meliponiculture *T. laeviceps*. The decline in wild bee pollinators can be attributed to flower constancy, which causes bee species to avoid previously visited flowers by colony bees (Grüter & Ratnieks, 2011; Nielsen et al., 2017). This is supported by the behavior of colony bees such as stingless bees, which leave trail pheromone on flowers to indicate that they have been visited (Jarau et al., 2010, 2011; Grüter, 2020). The daily foraging activity of *T. laeviceps* on lemon flowers peaked at 11:00 each day in with meliponiculture periods. This finding is supported by the highest number of *T. laeviceps* entering and exiting the hive (Abduh et al., 2023). However, the highest foraging activity in *Tetragonula pagdeni* was at 10:00 in greenhouse conditions (Wongsa, Duangphakdee & Rattanawannee, 2023). Furthermore, several studies was reported that at 11:00, *T. laeviceps* was the most visiting strawberry, mango, and orange flowers (Atmowidi et al., 2022; Chuttong et al., 2022; Nurdiansyah, Abduh & Permana, 2023).

The pollination sequences of *T. laeviceps* commence with the worker bees positioned parallel to or 45 degrees above the flower. This positioning is thought to be related to the flight method of *T. laeviceps*, which avoids flying over its resource's plants. Following that, worker bees approach lemon flowers directly, most likely guided by scouting bees that had marked the locations of flowers containing nectar and pollen (Grüter, 2020). When collecting nectar, *T. laeviceps* land on the petals and then enter the nectary of the flower, while when collecting pollen, they land directly on the anthers. *T. laeviceps* spends more time collecting resources in lemon flowers than honey bees, and the same occurs to other flower plants (Putra, Permana & Kinasih, 2014; Alpionita, Atmowidi & Kahono, 2021; Nurdiansyah, Abduh & Permana, 2023). This is due to its small size (± 0.5 cm) and its opportunistic approach to carrying as much as possible on its body, which can contain approximately 6,200 lemon pollen grains. This pollen load in lemon flowers was lower compared to other plants, such as strawberries with 8,600 pollen grains and melons with 26,200 pollen grains (Alpionita, Atmowidi & Kahono, 2021; Bahlis, Atmowidi & Priawandiputra, 2021). Subsequently, *T. laeviceps* departs from the flower via the anthers, facilitating pollen transfer to the stigma and enhancing pollination success, thus reaping the benefits of the plant-pollinator interaction.

The highest pollinator visitation rate for lemon flowers occurred at noon (10:00 to 13:00), with *T. laeviceps* being the most frequent visitor at 0.25 flowers per hour, followed by *A. cerana* visiting at 0.18 flowers per hour, which was consistent with previous study on meliponiculture *T. laeviceps* in orange orchards (Nurdiansyah, Abduh & Permana, 2023). This can be attributed to the first full bloom of lemon flowers around 10:00, followed by an increase in the volume of nectar secretion. In the afternoon (13:00 to 16:00), the volume of nectar decreased, followed by a decline in the pollinator visitation rate. However, the nectar concentration increased during this period. The nectar secretion pattern of lemon flowers is similar to that of *Croton macrostachyus* flowers (Bareke et al., 2020). The flowers were rarely visited by bee pollinators on the second day they bloom, indicating that the rewards offered by lemon flowers had decreased because the volume and sugar content of nectar decreased over time (Chauhan, Chauhan & Galetto, 2017). This is supported by the Pearson's correlation analysis, indicating that the nectar contents including volume ($r = 0.65$) and concentration ($r = 0.24$) had a positive correlation with the number of bee pollinators visiting lemon flowers.

Following that, the microclimate conditions were investigated, and it was showed that temperature ($r = 0.83$) and light intensity ($r = 0.93$) had a positive correlation with the number of pollinators, while the relative humidity ($r = -0.68$) showed a negative correlation. These findings are consistent with previous studies indicating that temperature, light intensity, and relative humidity are factors influencing the foraging behavior of bee pollinators (Polatto, Chaud-Netto & Alves-Junior, 2014), honeybees (Taha, Al-Abdulsalam & Al-Kahtani, 2016), and *T. laeviceps* (Abduh et al., 2023; Nurdiansyah, Abduh & Permana, 2023). The principal component analysis revealed that light intensity is the most influential environmental factor affecting the number of pollinators visiting lemon flowers. This finding contradicts prior study, which emphasized temperature was the predominant factor in pollinator activity visits to flowers (Taha, Al-Abdulsalam & Al-Kahtani, 2016; Gallagher & Campbell, 2020; Layek, Kundu & Karmakar, 2020). While temperature significantly influences the activities of stingless bee *Plebeia* aff. *flavocincta* outside the hive (Barbosa et al., 2020). However, observations on stingless bee *Tetragonula pagdeni* suggest that foraging activity in collecting tomatoes pollen increases under stable temperature conditions (Wongsa, Duangphakdee & Rattanawannee, 2023). These findings suggest that when temperature conditions are relatively stable, other microclimate conditions such as light intensity can play an important role in augmenting bee pollinator activity, especially stingless bees during resource collection. The foraging behavior of bee pollinators is shaped by the intricate interaction of environmental factors in lemon orchards.

The monthly population of bee pollinators in lemon orchards showed a positive correlation (r = 0.98) with the number of blooming flowers. Bee pollinators visit a single lemon flower 1.41–1.95 times per day, indicating that lemon flowers provide a resource-rich environment for pollinators including pollen and nectar. Comparing the pollen load of *T. laeviceps* (6,124 pollen grains) to the amount of pollen of lemon flowers (12,539 pollen grains) suggests that each lemon flower could potentially be visited by at least 2 stingless bees. However, the nectar load of *T. laeviceps* and other stingless bee species remain unknown. In contrast, honey bees are reported to carry 22 µL of sugar syrup (50%) in one day (Huang, 2018), and the nectar from a lemon flower (58.54 µL) can potentially sustain at least 2 honey bees. Based on the number of visits of *T. laeviceps* visited lemon flowers 3.39 times per flower in one day. This shows that foraging behavior of *T. laeviceps* requires more flower visits to fill its body with pollen compared to the available amount of pollen, potentially influencing the pollination success of lemon flowers.

Pollination efficacy of meliponiculture *T. laeviceps* produces more fruit sets (15%) compared without meliponiculture, and these findings are consistent with previous studies in open farming (Layek et al., 2021; Chuttong et al., 2022; Chauhan & Singh, 2022; Balaji et al., 2023; Nurdiansyah, Abduh & Permana, 2023) and closed farming (Azmi et al., 2019; Moura-Moraes et al., 2021; Layek, Das & Karmakar, 2022; Reddy, Chauhan & Singh, 2022). According to Gallagher & Campbell (2020), there is a positive correlation between higher pollinator diversity and larger pollinator populations, leading to increased pollinator visitation rates in agricultural landscapes, with potential implications for enhancing pollination success. The consequences of increasing fruit set on fruit production were investigated, including fruit weight, yield per plant, and estimated productivity. Meliponiculture *T. laeviceps* contributes to producing larger fruits. However, it's essential to note that specific impact on seed formation in lemon was not quantified in this study. Numerous study suggest that variations in fruit weight attributed to different pollination method are often associated with the number of seeds formed during pollination process, influenced by the pollinator and the frequency of visits (Gallagher & Campbell, 2020; Azmi et al., 2022; Wongsa, Duangphakdee & Rattanawannee, 2023). A notable 28–34% of fruits drop from the initial fruit set to the harvested stage in both treatments.

Incorporating meliponiculture results in notable distinctions during the initial phases of fruit set and an augmented fruit weight, leading to an increased yield of lemon plants. Consequently, a higher fruit set corresponds to a greater overall yield. The estimated lemon productivity in this study is below the world average productivity in 2021, which was reported as 15.56 tons per hectare (FAO, 2023). Nonetheless, the estimated lemon productivity with meliponiculture closely aligns with world productivity, reaching 15 tons per hectare per year, representing a 23% increase compared to cultivation without meliponiculture. Meliponiculture emerges as an excellent option for enhancing the fruit set of lemon with consequences on lemon productivity in tropical regions.

Conclusions

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Meliponiculture *Tetragonula laeviceps* did not affect pollinator diversity in both periods, while the relative abundance and daily foraging activity of wild bee pollinators were reduced in the first period and then maintained in the second period. Pollination sequences of *T. laeviceps* involve approaching the lemon flower from a parallel position or 45^{o} angle above the flower position by perching on the petal or anther with a time spent of 71.32 ± 5.64 seconds in collecting nectar and pollen, next consistently leaves the lemon flower specifically from the anther. *T. laeviceps* exhibits the highest pollinator visitation rate of 0.25 flowers per hour in the noon (10:00 to 13:00). The number of bee pollinators visiting lemon flowers influenced by environmental factors with light intensity being the most influencing factor. Pollination efficacy with meliponiculture *T. laeviceps* produces 15% more fruit sets and 23% more estimated productivity than without meliponiculture. This study suggests that meliponiculture stingless bees could be beneficial as pollinators in agricultural farming while maintaining pollinator diversity, which is critical for sustainable agriculture and enhanced pollination efficacy and productivity.

Commented [H27]: How about the oher species (Foraging behaviour)?

Commented [H28]: Which species is the lowest visitation rate

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References

Abduh MY, Adam A, Fadhlullah M, Putra RE, Manurung R. 2020. Production of propolis and honey from *Tetragonula laeviceps* cultivated in Modular Tetragonula Hives. *Heliyon* 6:e05405. DOI: 10.1016/j.heliyon.2020.e05405.

Abduh MY, Ramdhani F, Setiawan A, Rifqialdi G, Rahmawati A, Zainudin IM. 2023. Determination of productivity, yield and bioactivity of propolis extract produced by Tetragonula spp. Cultivated in Modular tetragonula hives. *Heliyon* 9:e17304. DOI: 10.1016/j.heliyon.2023.e17304.

Aizen MA, Aguiar S, Biesmeijer JC, Garibaldi LA, Inouye DW, Jung C, Martins DJ, Medel R, Morales CL, Ngo H, Pauw A, Paxton RJ, Sáez A, Seymour CL. 2019. Global agricultural productivity is threatened by increasing pollinator dependence without a parallel increase in crop diversification. *Global Change Biology* 25:3516–3527. DOI: 10.1111/gcb.14736.

Alpionita R, Atmowidi T, Kahono S. 2021. Pollination services of *Apis cerana* and *Tetragonula* *laeviceps* (Hymenoptera: Apidae) on strawberry (*Fragaria x ananassa*). *Asian Journal of Agriculture and Biology* 2021:1–8. DOI: 10.35495/ajab.2021.01.057.

Amon ND, Quezada M, Labarre D, Guédot C. 2023. Pollination practices and grower perceptions of managed bumble bees (Bombus spp.) as pollinators of cranberry in Quebec and Wisconsin. *Renewable Agriculture and Food Systems* 38:1–11. DOI: 10.1017/s1742170523000352.

Aslan MM, Yavuksuz C. 2010. Effect of honey bee (*Apis mellifera* L.) and Bumblebee (*Bombus terrestris* L.) pollinators on yield and yield factors in sunflower (*Helianthus annuus* L.) production areas. *Journal of Animal and Veterinary Advances* 9:332–335. DOI: 10.3923/javaa.2010.332.335.

Asmini A, Atmowidi T, Kahono S. 2022. Pollination by Three Species of Stingless Bees (Hymenoptera: Meliponini) Increase Seed Set of Mustard (*Brassica rapa* L.: Brassicaceae). *HAYATI Journal of Biosciences* 29:712–719. DOI: 10.4308/hjb.29.5.712-719.

Atmowidi T, Prawasti TS, Rianti P, Prasojo FA, Pradipta NB. 2022. Stingless Bees Pollination Increases Fruit Formation of Strawberry (*Fragaria x annanassa* Duch) and Melon (*Cucumis melo* L .). *Tropical Life Sciences Research* 33:43–54. DOI : https://doi.org/10.21315/tlsr2022.33.1.3.

Azmi WA, Sembok WZW, Nasaruddin SNM, Azli NS, Hatta MFM, Muhammad TNT. 2022. Evaluation of Native Stingless Bee Species (*Heterotrigona itama* and *Geniotrigona thoracica*) for Pollination Efficiency on Melon Manis Terengganu. *Malaysian Applied Biology* 51:229–235. DOI: 10.55230/mabjournal.v51i5.2360.

Azmi WA, Wan Sembok WZ, Yusuf N, Mohd Hatta MF, Salleh AF, Hamzah MAH, Ramli SN. 2019. Effects of Pollination by the Indo-Malaya Stingless Bee (Hymenoptera: Apidae) on the Quality of Greenhouse-Produced Rockmelon. *Journal of Economic Entomology* 112:20–24. DOI: 10.1093/jee/toy290.

Bahlis W, Atmowidi T, Priawandiputra W. 2021. Pollination Services of *Tetragonula laeviceps* Smith (Apidae: Meliponinae) on Melon Plants (*Cucumis melo* L.). *Serangga* 26:124–136.

Balaji K, Jayaraj J, Shanthi M, Vellaikumar S, Rajamanickam C, Chitra N, Suresh K. 2023. Pollinator diversity and pollination efficiency of stingless bee *Tetragonula iridipennis* smith in bitter gourd. *Indian Journal of Entomology* e23344:1–4. DOI: 10.55446/IJE.2023.1650.

Barbosa AB, Meneses HM, Rosa FL, Freitas BM. 2020. Flight Activity of the Stingless Bee Plebeia aff. flavocincta in Tropical Conditions as an Indicator of the General Health of the Colony. *Sociobiology* 67:545–553. DOI: 10.13102/sociobiology.v67i4.4926.

Bareke T, Kumsa T, Roba K, Addi A. 2020. Nectar Secretion Dynamics and Honey Production Potential of *Croton macrostachyus* L., Euphorbiaceae. *Bee World* 97:123–127. DOI: 10.1080/0005772x.2020.1763086.

Buchori D, Rizali A, Priawandiputra W, Raffiudin R, Sartiami D, Pujiastuti Y, Jauharlina, Pradana MG, Meilin A, Leatemia JA, Sudiarta IP, Rustam R, Nelly N, Lestari P, Syahputra E, Hasriyanti, Watung JF, Daud IDA, Hariani N, Jihadi A, Johannis M. 2022. Beekeeping and Managed Bee Diversity in Indonesia: Perspective and Preference of Beekeepers. *Diversity* 14:1–14. DOI: 10.3390/d14010052.

Chauhan S, Chauhan SVS, Galetto L. 2017. Floral and pollination biology, breeding system and nectar traits of *Callistemon citrinus* (Myrtaceae) cultivated in India. *South African Journal of Botany* 111:319–325. DOI: 10.1016/j.sajb.2017.04.010.

Chauhan A, Singh HK. 2022. Stingless Bee *Tetragonula iridipennis* and Honey Bee *Apis cerana* Pollination in Cucumber. *Indian Journal of Entomology* 84:1–5. DOI:

10.55446/IJE.2021.282.
Chuttong B, Panyaraksa L, Tiyayon C, Kumpoun W, Chantrasri P, Lertlakkanawat P, Jung C, Burgett M. 2022. 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. *Journal of Ecology and Environment* 46. DOI: 10.5141/jee.22.012.
Djakaria KM, Atmowidi T, Priawandiputra W. 2022. the Foraging Activity and Pollination Services of Three Stingless Bee Species To Enhance Fruit Quality and Quantity of Okra (Abelmoschus Esculentus L.). *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis* 70:215–226. DOI: 10.11118/actaun.2022.016.
Dymond K, Celis-Diez JL, Potts SG, Howlett BG, Willcox BK, Garratt MPD. 2021. The role of insect pollinators in avocado production: A global review. *Journal of Applied Entomology* 145:369–383. DOI: 10.1111/jen.12869.
Food and Agriculture Organization. 2023.Crops and livestock products. *Available at https://www.fao.org/faostat/en/#data/QCL* (accessed December 14, 2023).
Gallagher MK, Campbell DR. 2020. P. *American Journal of Botany* 107:445–455. DOI: 10.1002/ajb2.1439.
Garibaldi LA, Steffan-Dewenter I, Kremen C, Morales JM, Bommarco R, Cunningham SA, Carvalheiro LG, Chacoff NP, Dudenhöffer JH, Greenleaf SS, Holzschuh A, Isaacs R, Krewenka K, Mandelik Y, Mayfield MM, Morandin LA, Potts SG, Ricketts TH, Szentgyörgyi H, Viana BF, Westphal C, Winfree R, Klein AM. 2011. Stability of pollination services decreases with isolation from natural areas despite honey bee visits. *Ecology Letters* 14:1062–1072. DOI: 10.1111/j.1461-0248.2011.01669.x.
Gibb TJ, Oseto C. 2019. *Insect Collection and Identification Techniques for the Field and Laboratory*. Cambridge: Elsevier.
Grüter C. 2020. *Stingless Bees*. Cham:Springer International Publishing. DOI: 10.1007/978-3-030-60090-7.
Grüter C, Ratnieks F. 2011. Flower constancy in insect pollinators Adaptive foraging behavior or cognitive limitation? 4:633–436. DOI: 10.4161/cib.4.6.16972.
Hall MA, Jones J, Rocchetti M, Wright D, Rader R. 2020. Bee Visitation and Fruit Quality in Berries under Protected Cropping Vary along the Length of Polytunnels. *Journal of Economic Entomology* 113:1337–1346. DOI: 10.1093/jee/toaa037.
Herbertsson L, Lindström SAM, Rundlöf M, Bommarco R, Smith HG. 2016. Competition between managed honeybees and wild bumblebees depends on landscape context. *Basic and Applied Ecology* 17:609–616. DOI: 10.1016/j.baae.2016.05.001.
Huang Z. 2018. *Feeding honey bees*. Michigan: Michigan State University.
Jarau S, Dambacher J, Twele R, Aguilar I, Francke W, Ayasse M. 2010. The trail pheromone of a stingless bee, *Trigona corvina* (Hymenoptera, Apidae, Meliponini), varies between populations. *Chemical Senses* 35:593–601. DOI: 10.1093/chemse/bjq057.
Jarau S, Hemmeter K, Aguilar I, Ayasse M. 2011. A scientific note on trail pheromone communication in a stingless bee, *Scaptotrigona pectoralis* (Hymenoptera, Apidae, Meliponini). *Apidologie* 42:708–710. DOI: 10.1007/s13592-011-0070-4.
Katumo DM, Liang H, Ochola AC, Lv Min, Wang QF, Yang CF. 2022. Pollinator diversity benefits natural and agricultural ecosystems, environmental health, and human welfare. *Plant Diversity* 44:429–435. DOI: 10.1016/j.pld.2022.01.005.
Koh I, Lonsdorf E V., Williams NM, Brittain C, Isaacs R, Gibbs J, Ricketts TH. 2016. Modeling

the status, trends, and impacts of wild bee abundance in the United States. *Proceedings of the National Academy of Sciences of the United States of America* 113:140–145. DOI: 10.1073/pnas.1517685113.

Layek U, Das A, Karmakar P. 2022. Supplemental Stingless Bee Pollination in Fennel (*Foeniculum vulgare* Mill.): An Assessment of Impacts on Native Pollinators and Crop Yield. *Frontiers in Sustainable Food Systems* 6:1–13. DOI: 10.3389/fsufs.2022.820264.

Layek U, Kundu A, Bisui S, Karmakar P. 2021. Impact of managed stingless bee and western honey bee colonies on native pollinators and yield of watermelon: A comparative study. *Annals of Agricultural Sciences* 66:38–45. DOI: 10.1016/j.aoas.2021.02.004.

Layek U, Kundu A, Karmakar P. 2020. Floral ecology, floral visitors and breeding system of Gandharaj lemon (*Citrus* × *limon* L. Osbeck). *Botanica Pacifica* 9:1–7. DOI: 10.17581/bp.2020.09208.

MacInnis G, Normandin E, Ziter CD. 2023. Decline in wild bee species richness associated with honey bee (*Apis mellifera* L.) abundance in an urban ecosystem . *PeerJ* 11:e14699. DOI: 10.7717/peerj.14699.

Mason L, Sayre-chavez B, Brien CO, Seshadri A, Murgel J, Miller A. 2022. *Family : Apidae A Beginner's Field Guide to Identifying Bees Acknowledgments*. Colorado: Colorado State University.

Moura-Moraes MC, Frantine-Silva W, Gaglianone MC, Campos LAO. 2021. The use of different stingless bee species to pollinate cherry tomatoes under protected cultivation. *Sociobiology* 68:1–9. DOI: 10.13102/SOCIOBIOLOGY.V68I1.5227.

Nielsen A, Reitan T, Rinvoll AW, Brysting AK. 2017. Effects of competition and climate on a crop pollinator community. *Agriculture, Ecosystems and Environment* 246:253–260. DOI: 10.1016/j.agee.2017.06.006.

Nunes-Silva P, Hnrcir M, Shipp L, Imperatriz-fonseca VL, Kevan PG. 2013. The *Bombus impatiens* (Apidae, Bombini) on tomato (*Lycopersicon esculentum* Mill*., Solanaceae*) flowers: pollination and reward perception. *Journal of Pollination Ecology* 1:33–40.

Nurdiansyah MA, Abduh MY, Permana AD. 2023. Effects of meliponiculture *Tetragonula laeviceps* on pollinator diversity and visitation rate and citrus productivity in West Java , Indonesia. *Biodiversitas* 24:5757–5763. DOI: 10.13057/biodiv/d241058.

Panziera D, Requier F, Chantawannakul P, Pirk CWW, Blacquière T. 2022. The Diversity Decline in Wild and Managed Honey Bee Populations Urges for an Integrated Conservation Approach. *Frontiers in Ecology and Evolution* 10:1–7. DOI: 10.3389/fevo.2022.767950.

Polatto LP, Chaud-Netto J, Alves-Junior V V. 2014. Influence of Abiotic Factors and Floral Resource Availability on Daily Foraging Activity of Bees: Influence of Abiotic and Biotic Factors on Bees. *Journal of Insect Behavior* 27:593–612. DOI: 10.1007/s10905-014-9452-6.

Potts SG, Roberts SPM, Dean R, Marris G, Brown MA, Jones R, Neumann P, Settele J. 2010. Declines of managed honey bees and beekeepers in Europe. *Journal of Apicultural Research* 49:15–22. DOI: 10.3896/IBRA.1.49.1.02.

Putra RE, Permana AD, Kinasih I. 2014. Application of Asiatic honey bees (Apis cerana) and stingless bees (Trigona laeviceps) as pollinator agents of hot pepper (Capsicum annuum L.) at local Indonesia farm system. *Psyche* 1-5. DOI: 10.1155/2014/687979.

Putra RE, Sulistia WN, Kinasih I, Raffiudin R, Purnobasuki H, Fajrina N, Juansa A. 2022. Comparison of common green bottle flies (*Lucilia sericata* Meigen) and stingless bees (*Tetragonula laeviceps* Smith) as pollinating agents for imported true shallot (*Allium cepa*

L.) seed production. *Agriculture and Natural Resources* 56:409–416. DOI: 10.34044/j.anres.2022.56.2.18.
R Core Development Team. 2023. The R Project for Statistical Computing. *Available at https://www.r-project.org/*
Reddy P, Chauhan A, Singh HK. 2022. Impact of Bee Pollination in Brinjal. *Indian Journal of Entomology* 84:687–689. DOI: 10.55446/IJE.2021.15.
Rhodes CJ. 2018. Pollinator decline – An ecological calamity in the making? *Science Progress* 101:121–160. DOI: 10.3184/003685018x15202512854527.
da Silva MA, Ferreira N da S, Teixeira-Souza VH da S, Maia-Silva C, de Oliveira F de A, Hrncir M. 2017. On the thermal limits for the use of stingless bees as pollinators in commercial greenhouses. *Journal of Apicultural Research* 56:81–90. DOI: 10.1080/00218839.2016.1260380.
Suhri AGMI, Soesilohadi RCH, Putra RE, Raffiudin R, Purnobasuki H, Agus A, Kahono S. 2022. The Effectiveness of Stingless Bees on Pollination of Bitter Melon Plants *Momordica charantia* L. (Cucurbitaceae). *Journal of Tropical Biodiversity and Biotechnology* 7:1–9. DOI: 10.22146/jtbb.69124.
Taha EKA, Al-Abdulsalam M, Al-Kahtani S. 2016. Insect Pollinators and Foraging Behavior of Honey Bees on Alfalfa (*Medicago sativa* L.) in Saudi Arabia. *Journal of the Kansas Entomological Society* 89:92–99. DOI: 10.2317/150402.1.
Tschoeke PH, Oliveira EE, Dalcin MS, Silveira-Tschoeke MCAC, Santos GR. 2015. Diversity and flower-visiting rates of bee species as potential pollinators of melon (*Cucumis melo* L.) in the Brazilian Cerrado. *Scientia Horticulturae* 186:207–216. DOI: 10.1016/j.scienta.2015.02.027.
Valido A, Rodríguez-Rodríguez MC, Jordano P. 2019. Honeybees disrupt the structure and functionality of plant-pollinator networks. *Scientific Reports* 9:1–11. DOI: 10.1038/s41598-019-41271-5.
Vanlalhmangaiha R, Singh HK, Boopathi T, Lalhruaitluangi S. 2022. Impact of insect pollination on the quantitative and qualitative characteristics of sweet orange, *Citrus sinensis* (L .) Osbeck. *Journal of Apicultural Research* 0:1–10. DOI: 10.1080/00218839.2021.2013401.
Wongsa K, Duangphakdee O, Rattanawannee A. 2023. Pollination efficacy of stingless bees, *Tetragonula pagdeni* Schwarz (Apidae: Meliponini), on greenhouse tomatoes (*Solanum lycopersicum* Linnaeus). *PeerJ* 11:1–14. DOI: 10.7717/PEERJ.15367.