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Early Colony Growth of the Stingless Bee *Heterotrigona itama* After Transfer to Hive Boxes in Three Types of Anthropogenic Landscapes

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Abstract: This study aimed to evaluate changes in colony weight during the first ten weeks after transfer to managed hive boxes and to assess nest-external activity (foraging) across mixed fruit orchard, suburban, and urban landscapes. A total of 27 colonies were observed over ten weeks. Colony growth was measured based on changes in colony weight, whereas worker activity was recorded using video observations. The data were analyzed using Linear Mixed Models (LMMs) and Generalized Linear Mixed Models (GLMMs). The results showed that time since placement in the hive boxes (LMM: $\chi^2 = 17.17$; $p < 0.001$) and its interaction with landscape type (LMM: $\chi^2 = 22.13$; $p < 0.001$) had significant effects on changes in colony weight. Colonies in mixed fruit orchard landscapes showed more progressive weight gain, reaching 654.82 ± 81.96 g in week 8 and 737.20 ± 108.37 g in week 10, whereas colonies in suburban and urban landscapes tended to remain stagnant. Landscape type had no significant effect on foraging activity ($p > 0.05$). In contrast, time had a significant effect on the number of workers carrying nectar (GLMM: $\chi^2 = 9.30$; $p = 0.0023$) and resin (GLMM: $\chi^2 = 10.23$; $p = 0.0014$). Nectar-carrying activity increased and peaked between weeks 8 and 9, whereas resin-carrying activity was higher during the early weeks and declined in subsequent weeks. Under the specific locations and observation period of this study, mixed fruit orchard landscapes provided more favorable conditions for the early growth of *H. itama* colonies than suburban and urban landscapes.
Keywords: Meliponini; orchard; urban; adaptation; Apidae

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INTRODUCTION

Stingless bees (Meliponini) are important pollinators in tropical regions, where they contribute to ecosystem sustainability and support agricultural productivity (Asmini et al., 2022; Azmi et al., 2022; Bueno et al., 2023). In Southeast Asia, *Heterotrigona itama* is among the most widely cultivated species because of its strong adaptability to box-based cultivation systems and its role in pollinating various horticultural crops and tropical fruits. With increasing land-use conversion and the intensification of human activities, stingless bee colonies are increasingly maintained in anthropogenic landscapes, including urban areas, suburban areas, and mixed orchards (Buchori et al., 2022).

Despite the rapid expansion of stingless bee cultivation, information on the early responses of colonies to different types of anthropogenic landscapes remains limited, particularly after colonies are transferred into hive boxes. Most studies on *H. itama* have focused on nest-transfer techniques and colony survival success (Jailani et al., 2019; Shilan et al., 2022), whereas the early growth of colonies across different landscape types has rarely been examined comparatively. In fact, the initial post-transfer phase is a critical period that determines the success of colony adaptation and subsequent development.

Differences in the characteristics of anthropogenic landscapes, such as vegetation composition and land-management intensity, may influence resource availability for stingless bee colonies. Mixed orchard landscapes generally support a higher diversity of flowering plants than urban areas, which are often dominated by ornamental vegetation, and therefore may provide more stable resources for colonies (Wayo et al., 2020, 2024). In addition, worker activity outside the nest, including the number of workers leaving the nest and those carrying nectar, pollen, and resin, can serve as an indicator of colony responses to environmental conditions and post-transfer adaptation processes (Azmi et al., 2022; Barbosa et al., 2020).

Unlike previous studies that have primarily emphasized nest-transfer success or foraging activity within a single habitat type, this study compares the early growth of *H. itama* colonies after transfer into hive boxes across three types of anthropogenic landscapes: mixed fruit orchads, suburban areas, and urban areas. The study employed repeated colony-level observations over a ten-week period. Specifically, this study aimed to evaluate early colony growth based on changes in colony weight and worker activity outside the nest following transfer into hive boxes. The findings are expected to provide a scientific basis for improving stingless bee cultivation practices and for managing landscapes that support the sustainability of pollinator populations in tropical regions.

METHOD

Preparation of *Heterotrigona itama* Colonies

Heterotrigona itama is a stingless bee species commonly cultivated and was used in this study. This species has an effective flight range of approximately 500 m, with a maximum range of up to 1 km (Rusdimansyah et al., 2024). Between 23 July and 1 August 2024, a total of 27 colonies were transferred from wooden logs into wooden hive boxes measuring 20 × 19 × 20 cm (Figure 1). All boxes had the same design, with one entrance at the front and ventilation holes on the sides to maintain air circulation. Colony transfer was conducted at Universitas OSO.

The colonies selected for this study were healthy, as indicated by the presence of eggs, honey, pollen, and active worker activity. Before placement in the study landscapes, the initial weight of each colony was measured to ensure uniformity in colony size. A Kruskal–Wallis test showed that initial colony weight did not differ significantly among landscapes ($\chi^2 = 2.33$; df = 2; $p = 0.312$). Therefore, differences in initial colony size were not considered to influence the growth responses observed during the study.

Before colony transfer, each empty hive box was weighed. The natural nest was then carefully dismantled to transfer all essential nest components into the new box. Propolis was placed at the entrance of the hive box as a nest marker and to guide workers into the hive box. After the transfer process was completed, the box was covered with transparent plastic.

The colonies were acclimatized for 10 days at Universitas OSO before being moved to the study sites. During acclimatization, the hive boxes were placed in shaded areas, protected from direct sunlight, and equipped with ceramic roof covers to reduce exposure to rainfall. To minimize the influence of microclimatic variation during the study, all hive boxes at the study sites were also placed in shaded areas protected from direct sunlight.

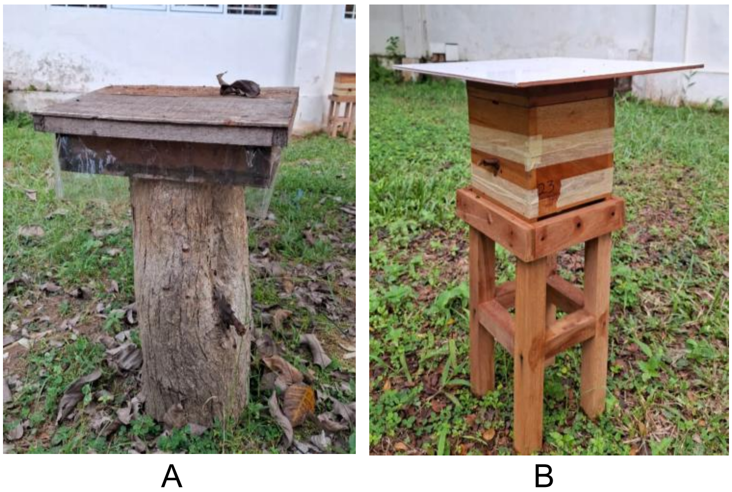

Figure 1. Stingless bee colonies in natural logs and hive boxes. (A) Nest appearance in a natural tree-trunk log; (B) stingless bee colony in a hive box after transfer from a natural tree log

Study Sites and Experimental Design

This study was conducted in Pontianak City and Kubu Raya Regency, West Kalimantan Province, Indonesia (Figure 2). Three landscape types were selected based on the dominant land-use characteristics surrounding the study sites: mixed fruit orchard, suburban, and urban landscapes. The mixed fruit orchard landscape was dominated by plantation vegetation and fruit trees, particularly langsat (*Lansium domesticum* Corr.), coconut (*Cocos nucifera* L.), and durian (*Durio zibethinus* L.). The urban landscape was characterized by a high density of buildings and residential areas, whereas the suburban landscape represented a transitional zone directly adjacent to plantation and residential areas. Landscape classification in this study was based on the dominant land-use characteristics at each study site and was used as the basis for treatment grouping.

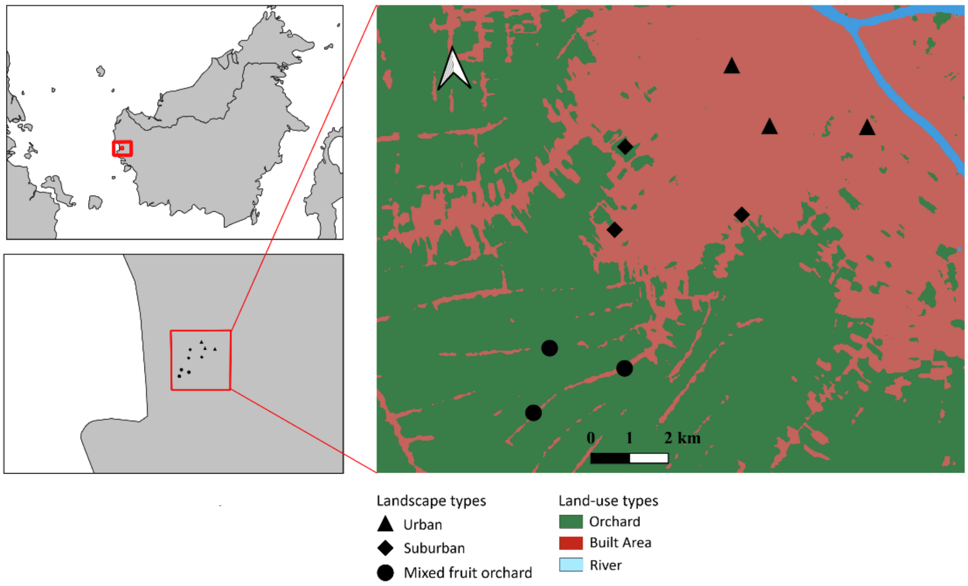

Figure 2. Map showing the locations of stingless bee hive boxes in Pontianak City and Kubu Raya Regency, West Kalimantan, across urban, suburban, and mixed fruit orchard landscapes. The land-use map was modified from the Sentinel-2 10 m Land Use/Land Cover basemap by Esri and Impact Observatory (https://livingatlas.arcgis.com/)

The study used a randomized complete block design with three landscape types—mixed fruit orchard, suburban, and urban—as treatments. Each landscape type consisted of three observation sites as replicates. Three *Heterotrigona itama* colonies were placed at each site, resulting in a total of 27 colonies used as experimental units (3 landscape types × 3 sites × 3 colonies). The distance between observation sites was maintained at more than 1 km to minimize potential interference in foraging activity among colonies (Rusdimansyah et al., 2024). All experimental units were placed in shaded locations protected from direct sunlight to support optimal colony development.

Data Collection on Colony Growth and Outside-Nest Activity (Foraging Activity)

Data were collected for 10 weeks after the colonies were placed at the study sites. However, data were not collected in weeks 6 and 7 because rainy weather prevented field observations. Routine observations were conducted weekly between 08:00 and 12:00 Western Indonesian Time. All observations were carried out under clear to cloudy conditions without rainfall, as stingless bee foraging activity is strongly influenced by weather conditions.

Colony growth was measured by weighing colonies periodically using a digital scale. Weighing was conducted by carefully lifting each hive box from its placement site to minimize disturbance to the colony. Colony weight was calculated by subtracting the empty hive box weight from the total weight of the hive box and colony. Changes in colony weight were used as an indicator of colony growth because they reflect overall colony development and represent a non-destructive method commonly used in stingless bee research (Vaidya et al., 2023; Wayo et al., 2024).

Foraging activity was observed by recording a 10-minute video for each colony and counting the number of workers leaving the nest, as well as the number of workers visibly carrying pollen, resin, or nectar. Observations were conducted during the designated observation period in each weekly sampling session. Specific visual characteristics were used to distinguish the materials carried by workers: rounded loads on the hind legs indicated pollen, irregularly shaped materials indicated resin, and an enlarged abdomen indicated nectar (Wayo et al., 2024).

Data Analysis

Data were analyzed using R software version 4.4.1. The effect of landscape type on changes in colony weight during the observation period was analyzed using a linear mixed model (LMM) with the lmer() function in the lme4 package. Colony weight data were square-root transformed to approximate a normal distribution. In the model, landscape type, observation week, and their interaction were treated as fixed effects, whereas colonies nested within observation sites were included as a hierarchical random effect. Observation week was treated as a continuous variable because the observations were conducted sequentially and represented progressive temporal changes.

The modeling process began with a full model that included the interaction between landscape type and observation time. Candidate models were then compared using the Akaike Information Criterion (AIC) and likelihood ratio tests (LRTs) through the anova() function to determine whether the addition of predictors or interactions significantly improved model fit. When significant main effects or interactions were detected, post hoc analyses were conducted using the emmeans package with Tukey adjustment to compare estimated marginal means (EMMs). For significant interactions between landscape type and observation time, comparisons among landscapes were conducted for each observation week, and differences in temporal trends were analyzed using the emtrends function.

Foraging activity was analyzed separately using generalized linear mixed models (GLMMs) to evaluate the effects of landscape type and observation time on foraging activity. The models included landscape type, observation week, and their interaction as fixed effects, while colonies nested within observation sites were included as a hierarchical random effect. Total worker foraging activity was analyzed using the glmer.nb function in the lme4 package with a negative binomial distribution. In contrast, the activity of workers carrying nectar, pollen, and resin was analyzed using the glmmTMB package with a zero-inflated negative binomial distribution. When significant main effects or interactions were found, Tukey post hoc tests were conducted using the emmeans package for pairwise comparisons.

RESULTS AND DISCUSSION

Colony Growth

The results showed that the full model best explained the effects of landscape type and time after hive-box placement on the colony weight of the stingless bee *Heterotrigona itama* (Table 1). Observation time, expressed as week, had a significant effect on colony weight (LMM: $\chi^2 = 17.17$; df = 1; $p < 0.001$). In addition, there was a significant interaction between landscape type and observation week (LMM: $\chi^2 = 22.13$; df = 2; $p < 0.001$). In contrast, the main effect of landscape type was not significant (LMM: $\chi^2 = 0.58$; df = 2; $p = 0.754$).

Further analysis using emtrends indicated that the rate of change in colony weight differed among landscapes. The mixed fruit orchard landscape showed the highest rate of increase (0.221 ± 0.035), followed by the urban landscape (0.066 ± 0.035), whereas the suburban landscape showed a rate close to zero (-0.020 ± 0.037). The rate of increase differed significantly between the mixed fruit orchard and suburban landscapes ($p < 0.001$) and between the mixed fruit orchard and urban landscapes ($p = 0.0063$), but not between the suburban and urban landscapes ($p = 0.211$). Simple-effects analysis using emmeans showed that differences in colony weight among landscapes within each observation week were not significant (all $p > 0.05$).

Overall, colonies placed in the mixed fruit orchard landscape exhibited a more progressive early growth pattern during the observation period than those in suburban and urban landscapes. The increase in colony weight in the mixed fruit orchard landscape was most evident from week 8 (654.82 ± 81.96 g, mean ± SE) to week 10 (737.20 ± 108.37 g) after hive-box placement. Colonies in urban and suburban landscapes showed relatively slower weight gain, while colonies in the suburban landscape exhibited a more stagnant growth pattern than those in the mixed fruit orchard landscape (Figure 3). The faster colony growth observed in the mixed fruit orchard landscape is consistent with previous studies reporting that plantation-based landscapes tend to support greater bee colony growth than urban landscapes (Buchori et al., 2020; Wayo et al., 2024).

This study is the first to specifically compare the early post-transfer growth of *H. itama* colonies placed in hive boxes across different anthropogenic landscape types. Previous studies have generally focused on the success of transferring nests from tree logs into hive boxes and have shown that colonies can survive well under this system (Shilan et al., 2022). Successful adaptation is strongly influenced by surrounding abiotic and biotic conditions, particularly the availability of food resources such as nectar, pollen, and resin near the nest. The presence of flowering plants that provide nectar and pollen is a key factor enabling *H. itama* to survive in anthropogenic areas, including orchards and urban environments (Benedick et al., 2021; Jailani et al., 2019; Samsudin et al., 2024).

Table 1. Comparison of linear mixed models (LMMs)

Model	AIC	χ^2	df	p-value
sqrt(colony weight) ~ week × landscape + (1 | site/colony)	723.57	28.60	2	<0.001
sqrt(colony weight) ~ week + landscape + (1 | site/colony)	741.69			
sqrt(colony weight) ~ week × landscape + (1 | site/colony)	723.57	40.24	5	<0.001
sqrt(colony weight) ~ 1 + (1 | site/colony)	753.81			

Note: Comparison of linear mixed models (LMMs) explaining the effects of habitat type and time after hive-box placement on stingless bee colony weight. The best model was selected based on the lowest AIC value and a significant likelihood ratio test result ($p < 0.05$).

The relatively high vegetation diversity in the mixed fruit orchard landscape, dominated by langsat and coconut trees as well as various fruit crops such as durian, mango, mangosteen, and areca palm, together with other surrounding vegetation, may provide more diverse and continuous sources of nectar, pollen, and resin. These conditions are likely to support increased nectar and pollen foraging activity and faster colony growth in stingless bees (Wayo et al., 2020; 2024). However, not all studies have reported the same pattern. Some studies have found that colony growth can be faster in urban areas than in plantation landscapes (Kaluza et al., 2016, 2018). This suggests that colony performance is determined not only by landscape type but also by landscape composition and management quality (Wayo et al., 2024). Urban areas with high flowering-plant diversity, such as city parks and home gardens, can provide sufficient nectar and pollen resources for bee colonies. Conversely, some plantation areas may experience resource limitations due to intensive management practices, such as monoculture systems or the use of pesticides and agrochemicals, which may affect food availability for bees (Buchori et al., 2020; Vaidya et al., 2023).

At the study sites, urban areas tended to be dominated by buildings and ornamental plants that likely provided fewer nectar and pollen resources than the vegetation present in mixed fruit orchard landscapes. This condition may have contributed to the lower colony growth rates observed in urban and suburban landscapes compared with the mixed fruit orchard landscape. This finding is consistent with Wayo et al. (2024), who reported that urban areas dominated by buildings generally have more limited nectar and pollen resources than other landscape types. Such resource limitation may restrict bee foraging activity and consequently reduce colony growth rates in urban landscapes.

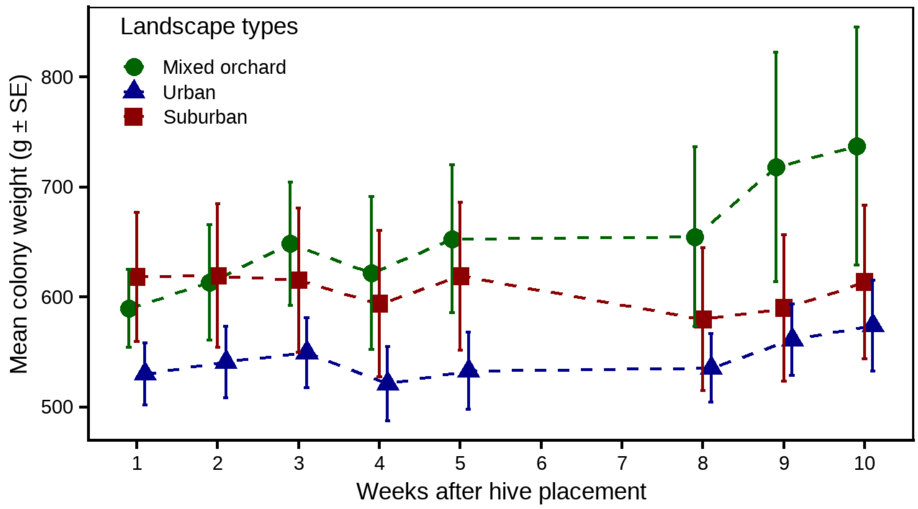

Figure 3. Mean colony weight (g ± SE) over the observation period in mixed fruit orchard, suburban, and urban landscapes

Foraging Activity

Habitat type did not significantly affect the number of workers leaving the hive or the number of workers carrying nectar, pollen, and resin ($P > 0.05$ for all variables). In contrast, time after hive-box placement had a significant effect on the number of workers carrying nectar (GLMM: $\chi^2 = 9.30$, df = 1, $p = 0.0023$) and resin (GLMM: $\chi^2 = 10.23$, df = 1, $p = 0.0014$), whereas the number of workers leaving the hive and carrying pollen was not significantly affected ($P > 0.05$ for both variables) (Figure 4).

The absence of a significant habitat effect indicates that the foraging activity of *Heterotrigona itama* was relatively similar across urban, suburban, and mixed fruit orchard habitats. This finding is consistent with several studies reporting that stingless bees have a high capacity to adapt to different landscape types and can continue to exploit available food resources despite variation in habitat conditions (Kaluza et al., 2018; Wayo et al., 2024).

Nevertheless, this pattern differed from the colony growth results, which showed variation in the rate of colony weight increase among landscapes, with colonies in mixed fruit orchard gaining weight more rapidly than those in urban and suburban landscapes. This mismatch between foraging activity and colony growth suggests that the number of foraging workers does not necessarily reflect the amount or quality of resources successfully obtained by the colony. Food-resource quality, including nectar sugar concentration and the continuity of resource availability, can strongly influence colony development (Trinkl et al., 2020). Therefore, the higher colony growth observed in mixed fruit orchard landscapes may be associated with better resource quality or greater resource diversity, even though the observed number of foraging workers did not differ significantly among habitats.

In addition to colony growth, foraging activity can serve as an early indicator of colony health and development (Barbosa et al., 2020; Samsudin et al., 2024). Overall, the number of workers carrying nectar was relatively similar among habitats during most observation weeks. However, a significant increase in nectar-carrying activity was observed in colonies located in the mixed fruit orchard landscape during weeks 8 and 9 after hive-box placement (Figure 4B). This pattern corresponds with the increase in colony weight during the same period and suggests that mixed fruit orchard landscapes provided sufficient nectar resources to support colony growth.

The number of workers carrying resin was also influenced by time after hive-box placement. Resin-carrying activity was relatively high during the early weeks after colonies were transferred from tree logs into hive boxes and then gradually declined (Figure 4D). This pattern indicates that resin plays an important role in the early adaptation of colonies to a new nest environment. In stingless bees, resin is used as a major material for nest construction and repair, including the formation of the involucrum, sealing of gaps, and protection of the nest from environmental disturbances and microorganisms (Roubik, 2006; Shanahan & Spivak, 2021; Newis et al., 2023). Thus, the high resin-collecting activity observed at the beginning of the study likely reflected the colony's need to stabilize and strengthen nest structures after relocation. Once the nest became more stable, the demand for resin decreased, leading to a reduction in resin-carrying activity.

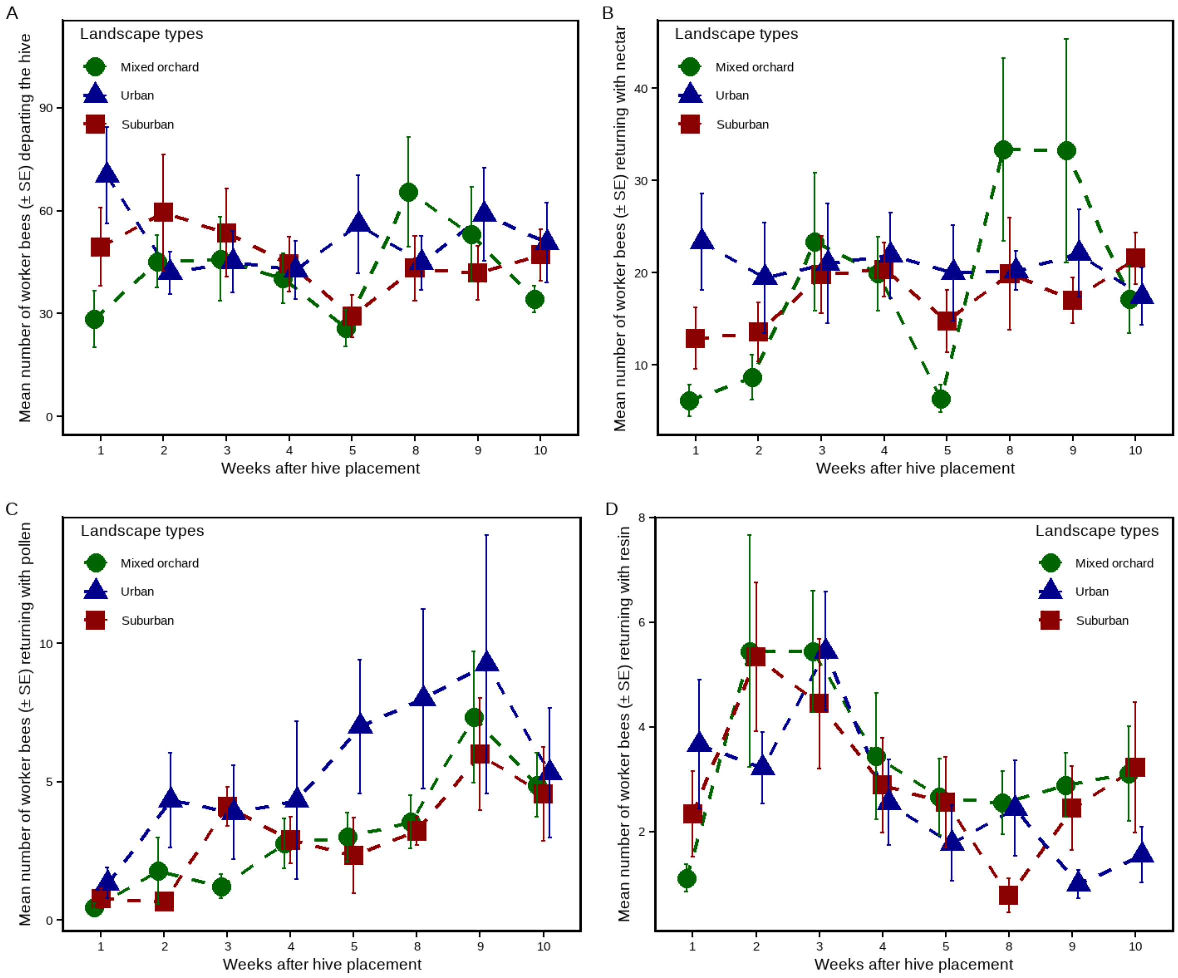

Figure 4. Mean number (±SE) of workers leaving the hive box (A), workers carrying nectar (B), pollen (C), and resin (D) in mixed fruit orchard, suburban, and urban landscapes

From a practical perspective, these findings indicate that mixed fruit orchard landscapes may be more suitable for the early cultivation stage of stingless bee colonies because they supported better colony growth during the observation period. However, landscape type did not significantly affect foraging activity, suggesting that the mechanisms underlying differences in colony growth require further investigation. These findings should also be validated through long-term observations to determine whether colony responses remain consistent across varying landscape conditions.

CONCLUSION

Mixed fruit orchard landscapes supported greater increases in the colony weight of *Heterotrigona itama* than urban and suburban landscapes during the observation period. Foraging activity did not differ significantly among landscape types, although nectar and resin collection changed over time. These findings indicate that mixed fruit orchards may be prioritized as suitable locations for colony placement during the early stage of stingless bee cultivation in anthropogenic landscapes. However, this study was limited to a 10-week observation period and to study sites in Pontianak City and Kubu Raya Regency. Further research across longer observation periods and broader geographic areas is therefore needed to test the consistency of the observed patterns.

RECOMMENDATION

This study did not examine long-term colony growth dynamics or a broader range of environmental conditions. Further research is needed to evaluate the growth of *H. itama* colonies over extended periods and to investigate the relationships among the diversity of forage plants, pollen type and quality, plant morphology, and the effects of landscape management practices on colony health and productivity. Such information is essential as a scientific basis for developing sustainable stingless bee cultivation, both for beekeeping purposes and for supporting agricultural systems in tropical regions.

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