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Population Growth and Insecticide Residues of Honey Bees in Tropical Agricultural Landscapes

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Abstract: Global decline of pollinators, especially bees, has been documented in many countries. Several causes such as land-use change and agricultural intensification are reported to be the main drivers of the decline. The objective of this study was to investigate the effect of land use on honey bee and stingless bee populations. Research was conducted in Bogor and Malang to compare between two different geographical areas. Managed bees such as honey bees (*Apis cerana* and *A. mellifera*) and stingless bees (*Tetragonula laeviceps*) were investigated to examine the effect of agricultural intensification. Field experiments were conducted by placing beehives in selected habitats (i.e., beekeeper gardens, forests areas, and agriculture areas). Population growth and neonicotinoid residue analysis of bees in different hive locations were measured to study the effect of habitat type. Population growth of bees represents the forager abundance and colony weight. Based on the analysis, we found that habitat type affected forager abundance and colony weight of honey bees ($p < 0.05$), although the patterns were different between species, region, as well as season. Forests could support the stingless bee colony better than agriculture and home garden habitats. Insecticide (neonicotinoid) was barely recorded in both honey bees and stingless bees.

Keywords: *Apis cerana*; *Apis mellifera*; agriculture; forests; home garden; neonicotinoid; *Tetragonula laeviceps*

1. Introduction

In the agroecosystem, pollinators are a pivotal component of biodiversity that provide an important ecosystem service through crop pollination [1] and increasing fruit set [2]. Pollinators also can be used as indicators of ecosystem health because of their sensitiveness to environmental stressors [3], for instance, the negative impacts of pesticide application [4]. There is growing concern relating to declines found in pollinators around the world [5]. In Europe and the US, a decline in wild bee species richness has been recorded, where the declining trends are in the abundance of honey bees (*Apis mellifera*) and a small number of wild pollinators [6]. Although high diversity of bees is found in the tropics (e.g., [7,8]), there is a lack of information about this phenomenon. Therefore, investigation needs to be undertaken into the scale, magnitude, and causes of the decline and the effects on pollination services.

Global declines in honey bees and wild bees have been associated to habitat loss and fragmentation, pesticide application, pathogens, invasive species, and climate change [9,10]. The potential threat of insecticides, such as neonicotinoids, for honey bees and wild bees has been reported, although it is still in debate [11]. Neonicotinoids have negative impacts such as increasing the mortality of honey bees by impairing their homing ability [12] and reducing the reproductive success of bumble bees and solitary bees [13], although other studies have reported no effects [13]. There is limited information from comprehensive studies on the impact of neonicotinoids toward long-term survival of honey bee colonies [14]. Landscape-scale experiments in different geographical regions are needed to investigate the impacts of neonicotinoids on bees [13,14].

The research was conducted in various land-use types both in Bogor (West Java) and Malang (East Java), Indonesia. Bogor has unique agricultural characteristics, as it is surrounded by mountain areas, and has a seminatural habitat dominated by agricultural fields cultivated with rotations of crop plants, which are mainly rice and vegetables [15]. Similar to Bogor, Malang is also surrounded by mountain areas and consists of tropical rainforests as well as cultivated and settlement areas. Agriculture is the primary land-use on the island of Java, so its management has profound consequences for the environment and for biodiversity. Agricultural intensification, especially pesticide application, is commonly used as a consequence of the green revolution [16] and has a negative effect on biodiversity, especially pollinators [17].

The objectives of this research were to investigate land-use effects and the indirect impact of insecticide application in agricultural areas on insect pollinators, particularly bees. Most evidence of the impact of pesticides on pollinators, especially honey bees, has come from laboratory-based toxicity tests. Negative effects of insecticide have been reported (e.g., [18]), but field research is still needed to understand how laboratory-derived toxicity levels effect pollinator communities in the agroecosystem, although some field- and semi-field-based studies have been conducted [19].

2. Materials and Methods

2.1. Research Site and Experiment Plot Selection

Research sites were located in West Java (Bogor and its surrounding area) and East Java (Malang and its surrounding area) to compare between two different geographical areas (Figure 1). Both areas are reported as honey producers in Indonesia [20]. Beekeepers in Bogor were characterized by breeding in a lower elevation (<300 m asl), while in Malang it was in a higher elevation (>500 m asl) (https://en.climate-data.org). To conduct the experiment, we selected two different habitat types, agriculture and forest, both in Bogor and Malang. As a comparison, we also observed the hives in selected beekeeper gardens (home garden) (Table 1). We used three different species of bees during the experiment that were commonly breed by beekeepers: *A. mellifera* and *A. cerana* in Malang and *A. cerana* and *T. laeviceps* in Bogor. In each habitat, we selected three experiment plots in different locations for replication, and the minimum distance between plots was 2 km (Figure 1).

Table 1. Satellite images and description of habitat types for research experiments in Bogor and Malang. Satellite images were derived from Google Maps, accessed year 2019.

Area	Agriculture	Forest	Home Garden
Bogor			
Description	Dominated by rice, maize, and cucumber with frequent pesticide application	Tree plants, dominated by *Paraserienthes falcataria* or *Hevea braziliensis*	Habitat surrounding housing area, dominated by fruit trees and flowering plants

Table 1. *Cont.*

Area	Agriculture	Forest	Home Garden
Malang			
Description	Highland agroecosystem, dominated by vegetable crops with high insecticide application	Tree plants, dominated by pine (*Pinus merkusii*)	Vegetation surrounding housing area, dominated by fruit trees and flowering plants

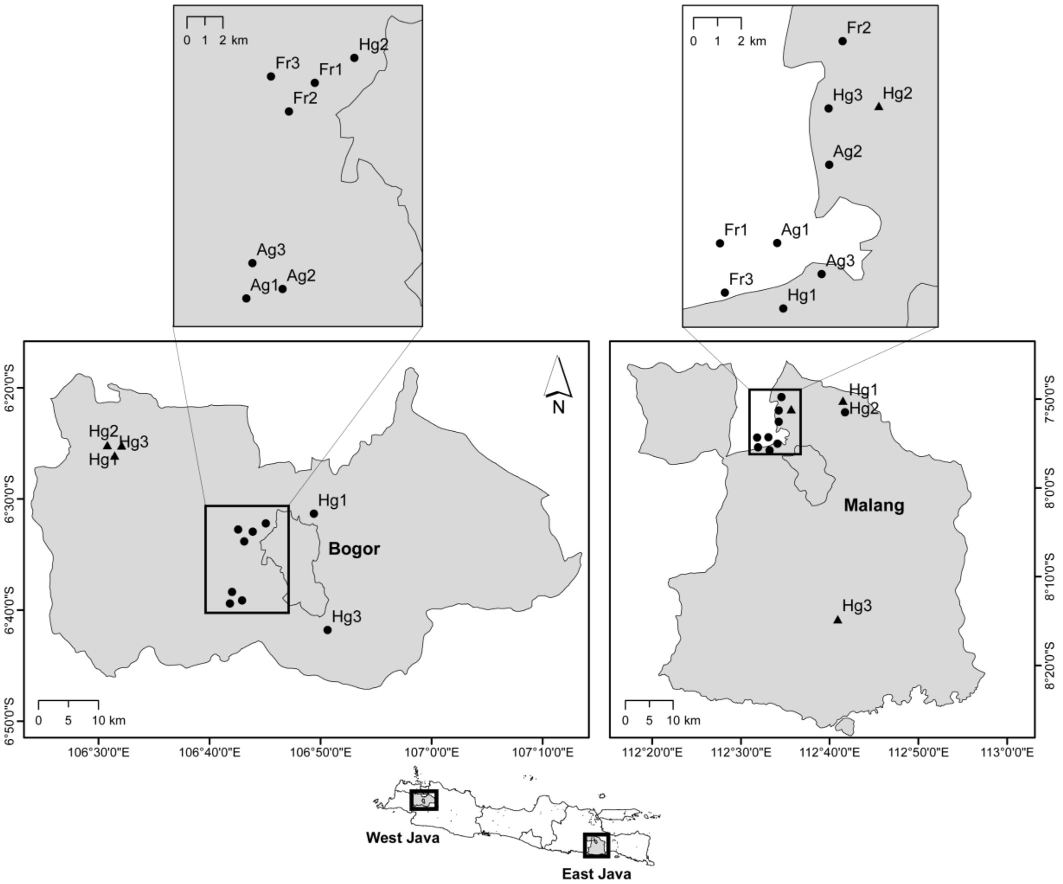

Figure 1. Map of study sites for research experiments in different habitat types (Ag: agriculture, Fr: forest, Hg: home garden (beekeepers place) in Bogor (West Java) and Malang (East Java)). The numbers after the habitat code indicates plot number. Home garden with triangle symbol indicates beekeepers of *Apis cerana*.

We placed three beehives in each plot for observation. The sizes of beehives were different depending on the beekeeper's practice in rearing bees. In Malang, the hive size of *A. cerana* was $40 \times 25 \times 25$ cm with four combs and *A. mellifera* was $50 \times 40 \times 25$ cm with four combs. While in Bogor, the hive size of *A. cerana* was $35 \times 30 \times 25$ cm with six combs and *T. laeviceps* was $30 \times 10 \times 10$ cm without combs.

2.2. Observation of Bees in the Hives and Residue Analysis

To study the effect of habitat type on bees, we observed the hives on each experimental plot, measured the population growth of bees, and performed a residue analysis. Population growth of bees was measured by counting the forager abundance (foraging activity) and colony weight (weighing full hive). The method of foraging activity monitoring was based on [21] by counting the foragers departing or returning to the colony for thirty minutes per hive. In each plot, the observation was conducted from 7 a.m. until 11 a.m. Weighing full hives was conducted to calculate the colony weight that included the summed weight of the box, combs with food stores, and the bees [22]. Both forager abundance and colony weight were observed every two weeks during two months. Observations were conducted in different seasons (i.e., rainy and dry seasons). Observations in the dry season were done from March to May 2019, while for the rainy season, observations were from July to September 2019.

In addition, insecticide residue analysis was conducted by collecting honey and bee (foragers) samples in three habitat types. We collected 5 mg of bees and 5 mg of honey per plot and initially froze it before being analyzed using the QuEChERS protocol [23]. Insecticide residue analysis was conducted in the medical laboratory of Jakarta (https://labkesda.jakarta.go.id) and was focused on imidacloprid content as the representation of neonicotinoid insecticide.

2.3. Data Analysis

The difference of forager abundance and colony weight of bees between habitat types was analyzed using a Kruskal–Wallis test. If we found significant differences, a post-hoc test was done using Fisher's least-significant difference with $\alpha = 0.05$. To analyze the relationship between forager abundance as well as colony weight of bees and observation time, we analyzed using fitting median-based linear models based on the Theil–Sen single median. All analyses were performed using R statistical software [24] and package "mblm" for fitting median-based linear models [25].

3. Results

3.1. Effect of Different Habitat Types and Season on Honey Bees

Based on the analysis, we found that habitat type and season affected the forager abundance and colony weight of bees ($p < 0.05$), although the patterns were different between species and region. In general, habitat types showed to be the most important factor that affected both forager abundance and colony weight of bees compared to season and observation time (Table 2). Effect of habitat type on forager abundance and colony weight of *A. cerana* differed between Malang and Bogor. In Malang, the difference of habitat type significantly influenced the forager abundance and colony weight of *A. cerana,* which were found higher in home gardens and forests compared to the agricultural areas (Figures 2a and 3a). In Bogor, forager abundance of *A. cerana* was not significantly different among habitat type (Figure 2b), while the colony weight of *A. cerana* in forests was significantly higher than in home gardens (Figure 3b). The same pattern was observed with *A. cerana* in Malang for forager abundance of *A. mellifera* in Malang and *T. laeviceps* in Bogor, which were also found higher in home gardens and forests compared to agricultural areas (Figure 2c,d and Figure 3c,d).

Table 2. The results of Kruskal–Wallis tests of forager abundance and colony weight of bees in different seasons, habitat types, and observation times.

Variable	Bee	Region	Season (df = 1)		Habitat Type (df = 2)		Observation Time (df = 3)	
			χ^2	*p*-Value	χ^2	*p*-Value	χ^2	*p*-Value
Forager abundance	*Apis cerana*	Malang	0.960	0.327	22.448	<0.001	4.430	0.219
		Bogor	0.006	0.937	3.804	0.149	0.239	0.971
	Apis mellifera	Malang	0.323	0.570	18.543	<0.001	0.106	0.991
	Tetragonula laeviceps	Bogor	0.051	0.822	17.106	<0.001	1.159	0.763
Colony weight	*A. cerana*	Malang	2.144	0.143	8.737	0.013	0.581	0.901
		Bogor	4.202	0.040	4.425	0.109	3.383	0.336
	A. mellifera	Malang	18.365	<0.001	9.092	0.011	0.056	0.997
	T. laeviceps	Bogor	0.011	0.915	45.094	<0.001	1.615	0.656

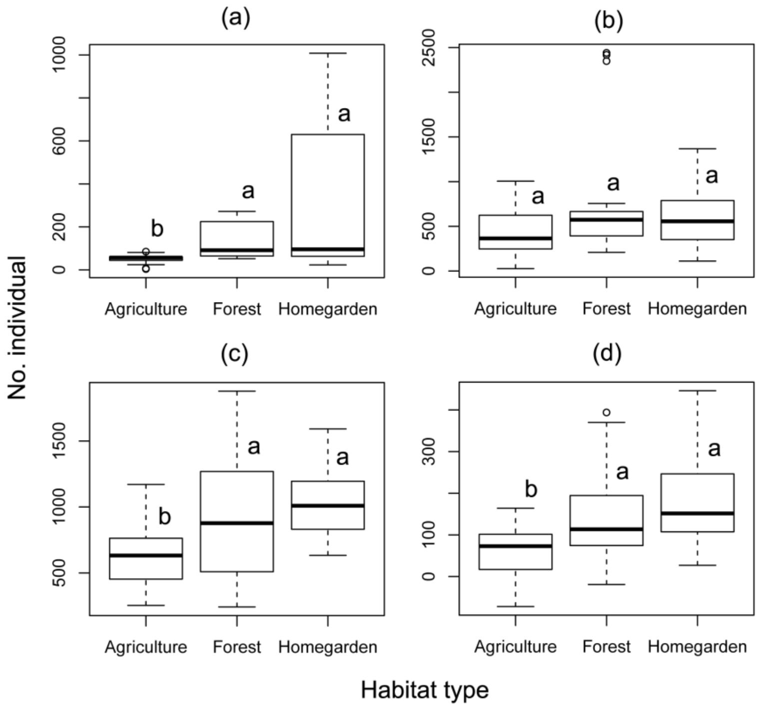

Figure 2. Forager abundance of honey bees and stingless bees in different habitats. (**a**) *A. cerana* in Malang, (**b**) *A. cerana* in Bogor, (**c**) *A. mellifera* in Malang, and (**d**) *T. laeviceps* in Bogor. Boxes with different letters are significantly different at $p < 0.05$ according to Fisher's least-significant difference.

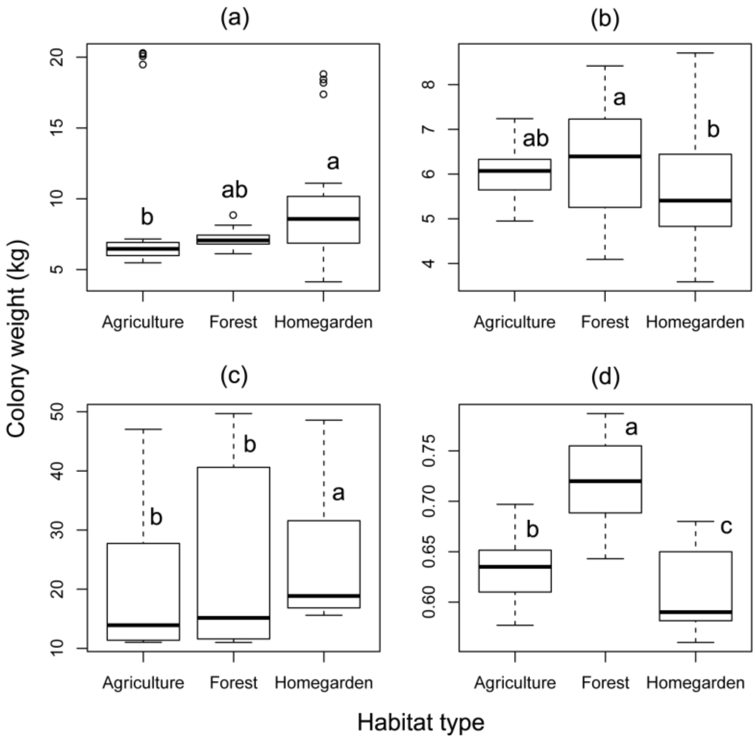

Figure 3. Colony weight of honey bees and stingless bees in different habitats. (**a**) *A. cerana* in Malang, (**b**) *A. cerana* in Bogor, (**c**) *A. mellifera* in Malang, and (**d**) *T. laeviceps* in Bogor. Box with different letters are significantly different at $p < 0.05$ according to Fisher's least significant difference.

Based on non-parametric regression, we found that the forager abundance of *A. cerana* in Malang was prone to decrease with increasing observation time ($p = 0.001$) (Table 3). In addition, the colony weights of *A. cerana* and *T. laeviceps* in Bogor as well as *A. mellifera* in Malang tended to increase with increasing observation time ($p < 0.05$).

Table 3. Relationship between forager abundance and colony weight of bees and observation time (day) based on non-parametric regression.

Parameter	*A. cerana*				*A. mellifera*		*T. laeviceps*	
	Malang		Bogor		Malang		Bogor	
	Estimate	*p*-Value	Estimate	*p*-Value	Estimate	*p*-Value	Estimate	*p*-Value
Forager abundance								
(Intercept)	89.000	<0.001	530.500	<0.001	783.750	<0.001	105.500	<0.001
Observation	−0.639	0.001	−0.342	0.567	1.700	0.637	0.339	0.160
Colony weight								
(Intercept)	7.095	<0.001	5.738	<0.001	15.915	<0.001	0.646	<0.001
Observation	−0.005	0.167	0.013	0.001	0.014	0.966	0.000	0.033

3.2. Detection Results of Insecticide Residue in Honey and Body of Honey Bees

Based on residue analysis, we barely detected insecticide residue (imidacloprid content) both in the honey and the body of bees (Table 4). Two of 18 honey samples (11.11%) contained a small amount of insecticide residue (<5 μg/kg). Insecticide residues were also detected in three bee samples (16.66%). Surprisingly, we did not detect the insecticide residue in the agriculture habitat, yet it was detected in forest and home garden habitats.

Table 4. Insecticide residue (imidacloprid content) detected from honey and bee bodies.

Species	Product	Habitat	Residue (μg/kg)	
			Bogor	Malang
A. mellifera	Honey	Agriculture	-	-
		Forest	4.4	-
		Home garden	-	-
	Bee body	Agriculture	-	-
		Forest	-	-
		Home garden	-	-
A. cerana	Honey	Agriculture	-	-
		Forest	-	0.5
		Home garden	-	-
	Bee body	Agriculture	-	-
		Forest	3.1	-
		Home garden	11.2	-
T. laeviceps	Honey	Agriculture	-	-
		Forest	-	-
		Home garden	-	-
	Bee body	Agriculture	-	-
		Forest	-	-
		Home garden	-	2.9

4. Discussion

Our research provided the evidence that habitat type significantly affects both forager abundance and colony weight of honey bees as well as stingless bees in Indonesia. The lowest forager abundance and colony weight was shown in agricultural areas, which indicated that agricultural areas had a negative effect on bees. Research by [26] showed that bee species have distinct preferences for different plant communities, and their abundance is related to the abundance of their host plants. Agricultural areas that are dominated by certain crop plants might affect the fitness and population growth of bees. This is due to plant diversity and is a key driver of bee fitness. Bees were found to be fitter and their populations grew faster in more florally diverse environments because of a continuous supply of food resources [27].

Landscape diversity also influences the growth and reproduction of honey bees, besides the availability of pollen in agricultural landscapes. For instance, *A. mellifera* compensated for lower landscape diversity by increasing their pollen foraging range in order to maintain pollen amounts and diversity [28]. This indicates the importance of agri-environmental schemes to support pollinators and not just the plant diversity and pollen availability. In Malang, *A. mellifera* is handled by beekeepers following "migratory management", which causes bees undue stress. In order to ease access to food source, beekeepers move the hives to flowering areas. However, this management may affect the population growth of bees. Research by [29] showed that the lifespan of migratory adult bees tends to decrease compared to stationary bees.

In this research, we only focused on environmental stressors (e.g., habitat condition) and did not investigate other potential drivers that affect population growth of bees, such as pests and pathogens, and genetic diversity as well as vitality of bees [9]. However, habitat type did not guarantee that bees were unhampered from insecticide. This might be related to the foraging range of bees as well as the food source, which is not only pollen but also honeydew. Honey bee presence was positively affected by the presence of honeydew and source of insecticide residue that affected honey quality [30]. Neonicotinoid (imidacloprid), which was found in our study, was also detected in 11% samples of honey from apiaries located in Poland [31]. However, the death of honey bees from Bologna were reported from different active ingredients of insecticide [32]. Thus, in this research, neonicotinoid was detected in the body of bees and honey, although it was in a small amount of residue.

5. Conclusions

Our research results showed the factor that affected forager abundance and colony weight of bees the most was habitat type. The agricultural habitat had lower bee forager abundance and colony weight compared to forest and home garden habitats. This indicates that the hypothesis from the beekeepers' perspective is accepted in relation to the negative effect of agriculture (especially pesticide application) on their honey production. However, our experiment revealed that habitat type, especially forest and home garden, did not guarantee that honey bees were unhampered from pesticide.

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