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Original article

Toxicity of different insecticides against the dwarf honey bee, *Apis florea* Fabricius (Hymenoptera: Apidae)

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ABSTRACT

Honey bees are considered as critical beneficial insects in the term of honey production and pollination of crops. One of the essential honey bee species in Pakistan is *Apis florea* Fabricius (Hymenoptera: Apidae). These make nests on trees near human dwellings and agriculture crops. During foraging in the field for nectar and pollen collection from agriculture flowering plants, honey bees may be exposed to pesticide sprays which may cause a change in their foraging behavior and the death of their workers. The current study evaluates the toxicity of six insecticides (emamectin benzoate, spinetoram, chlorantraniliprole, fipronil, flonicamid, and imidacloprid) against workers of *A. florea*. There were six concentrations of each insecticide (causing > 0% to < 100 % mortality) prepared in water, and each concentration was replicated four times. The experiment was conducted using the diet incorporation method in a plastic container. Emamectin benzoate was found the most toxic insecticide with lower LC_{50} values (1.02 µg/mL) followed by spinetoram (1.10 µg/mL), chlorantraniliprole (2.74 µg/mL), imidacloprid (3.09 µg/mL), flonicamid (3.94 µg/mL), fipronil (6.00 µg/mL) after 48 h of exposure. The results showed that insecticides are very toxic to *A. florea* and should be used on agriculture crops with great care and during less activity.

1. Introduction

Commercial production in Agriculture usually depends on pesticides such as herbicides, fungicides, insecticides, and other biological controls, including entomopathogenic fungi (Ngowi et al., 2007; Qasim et al., 2018; Qasim et al., 2021). Most of these synthetic pesticides are broad-spectrum and have been extensively used in agriculture since the 1940s (Coats, 2012). This over-reliance on pesticides had not only caused environmental contamination but also negatively affected biodiversity (Desneux et al., 2007; Khan, 2021).

Pollination is the essential ecosystem service that animals mainly provide. Among animals, insect arthropods occupy a significant place because they contribute more to farmlands in terms of pollination (Ahmad et al., 2021; Khan and Ghramh, 2021; Klein et al., 2007). Insect pollination is worth €153 billion a year globally (Gallai et al., 2009). When it comes to pollination, bees, especially honey bees, are the most important, as they account for more than 80% of the process (Hu et al., 2008; Suwannapong et al., 2011). Honey bees are also important because they provide many other valuable products such as bee wax and royal jelly and bee pollen and honey (Ghramh et al., 2020; Ghramh et al., 2019; Khan et al., 2016; Nieh, 1998).

Among honey bees, dwarf honey bee, *Apis florea* Fabricius (Hymenoptera: Apidae) is an essential pollinator of vegetables, fruits, and other flowering plants (Abrol, 2010) and is mainly found

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in the Indian subcontinent, Iran, and Oman (Hepburn and Hepburn, 2005). It also has a higher temperature tolerance than other honeybee species (Bhattacharyya et al., 2019), but its population decreases drastically near agriculture farms. According to Sihag (2021), the foraging populations of this species had declined in Raya (*Brassica juncea* Czern & Coss) and Carrot (*Daucus carota* L.) from 31.20 ± 3 bees/m^2 to 9.20 ± 2 bees/m^2 in crops. As a result of the colony and forager surveys, it appeared that *A. florea* was in danger of extermination, resulting in a pollination crisis (Sihag, 2021). It is believed that insecticides constitute a significant factor in the current decline of *A. florae* populations (Klein et al., 2007) because honeybees have a slower detoxification system that leads to bees' death (Husain et al., 2014; Jung et al., 2020). Moreover, insecticide residues have also been reported in hive products such as wax, honey, and pollen which may cause bio-magnification of residues at higher trophic levels (Gómez-Ramos et al., 2016).

Many studies report the effects of various pesticides on different species of honey bees, but there are a few studies from Pakistan reporting lethal effects on *A. florea*. In the current study, we aimed to evaluate the toxicity of six insecticides against *A. florea*. The insecticides were selected based on their different mode of action. Spinetoram affects postsynaptic acetylcholine receptors and GABA receptors and is a broad-spectrum insecticide (Shimokawatoko et al., 2012). In insects, emamectin benzoate (avermectins) may bind to multiple sites in chloride channels such as glutamate and GABA, resulting in generalized cell dysfunction and nerve impulse disruption (Jansson et al., 1997). Activator of insect ryanodine receptors, chlorantraniliprole causes muscle dysfunction and paralysis (Hannig et al., 2009). The nAChR-binding to neonicotinoids has been studied, while fipronil has been reported as binding to GABA-receptors in the nervous systems (Simon-Delso et al., 2015).

The farmers have extensively used these insecticides on-field and fruit crops. Hence, the purpose of the study was to find out the most toxic and harmful insecticide for *A. florea* so that the recommendations can be made on their proper and judicious use to conserve *A. florea* in the area.

2. Materials and methods

2.1. Collection of Apis florea

The research was conducted at MNS University of Agriculture, Multan, Punjab, Pakistan. The experts collected three hives of *A. florea* from the trees located at Multan and Lodhran districts, placed them in the plastic bucket, and shifted them to the laboratory. They were placed in the laboratory for one day for acclimatization before use. During this time, they were fed a 20% sugar-water solution.

2.2. Insecticides

The insecticide used in the study include Emamectin benzoate 1.5%, Spinetoram 12% SC, Chlorantraniliprole 20% SC, Fipronil 5% SC, Flonicamid 50% DF and Imidacloprid 20% SL. These insecticides were purchased from the commercial market.

2.3. Lethal concentration and lethal time estimation

No-choice feeding bioassays were performed to determine the trends in mortality of *A. florea* by following the methodology of Laurino et al. (2011) with slight modification. Six concentrations (causing > 0% and < 100 % mortality) were prepared by serial dilution in 50% sugar solution for each of the six insecticides. Five healthy foraging workers were introduced into the box (0.5 L). Each concentration was repeated four times. There were four control boxes for each insecticide. The boxes were then placed at 25 ± 2 °C, and 70% ± 5 % R.H. A filter paper underneath the box's cover was wetted with distilled water daily during the treatment. Mortality was recorded after 12, 24, 36, and 48 h of exposure. Workers were considered dead when they showed no movement upon probing with a fine brush. From this data, lethal time was also calculated.

2.4. Data analysis

Abbott's formula (1925) was used for corrected mortality if the mortality in control was more than 5%. Data were analyzed by probit analysis using SPSS software (Version 23.0 for windows, SPSS Inc., Chicago, USA) to determine median lethal concentrations (LC_{50}) and lethal time (LT_{50}).

3. Results

3.1. Lethal concentration estimation (LC_{50})

Emamectin benzoate was found more toxic due to low value of LC_{50} followed by spinetoram, chlorantraniliprole, imidacloprid, flonicamid and fipronil. The LC_{50} value of emamectin benzoate was 2.01, 1.67, 1.02 and 0.81 μg/mL after 12, 24, and 36 h, respectively. The LC_{50} of imidacloprid ranged from 6.82 to 1.05 μg/mL during 24–48 h (Table 1).

3.2. Percentage mortality

Percent mortality of the *A. florea* population exposed to emamectin benzoate is shown in Fig. 1A. Mortality was ranged between 45.00 and 95.00% after 24 h of exposure. While, mortality range observed after 48 h of exposure was 65.00–100.00%. After 24 h of exposure, lower mortality i.e. 45.00% was observed in case of the lowest concentration viz. 1.25 μg/mL. It increased to the highest i.e. 95.00% after 24 h of exposure to emamectin benzoate. Similarly, after 48 h of exposure the mortality was low i.e. 65.00% which gradually increased to maximum (100.00%) in the highest concentration viz. 40 μg/mL. Fig. 1B shows the percent mortality of *A. florea* subjected to imidacloprid. After 24 h of exposure, the mortality rate ranged from 25.00 to 95.00%. After 48 h of exposure, percent mortality varied from 55.00 to 100.00%. The lowest concentration of 1.25 μg/mL resulted in 25.00 percent mortality after 24 h of exposure. After 24 h of exposure to the highest dose of imidacloprid (80 μg/mL), it reached to the maximum of 100.00%. Mortality was reduced to 55.00% after 48 h of exposure, but reached a maximum (100.00%) at the highest concentration of 80 μg/mL. In Fig. 1C, the chlorantraniliprole-induced mortality rate of *A. florea* is depicted. The mortality varied from 30.00 to 95.00% after 24 h of exposure. Similarly, mortality rates ranged from 45 to 100% after 48 h of exposure. Even at the lowest dosage of 1.25 μg/mL, the mortality rate after 24 h was 30.00%. Chlorantraniliprole (80 μg/mL) exposure for 24 h resulted in a maximum of 100.00%. Mortality was 45.00% at the lowest concentration after 48 h of exposure and 100.00% at the highest concentration of 80 μg/mL.

Percent mortality of the *A. florea* populations have been shown in Fig. 1D, after exposure to flonicamid. After 24 h of exposure, mortality rate ranged between 30.00 and 77.00%. While, mortality range was ranged between 50.00% and 100.00% after 48 h of treatment. After 24 h, the lowest percent mortality (30.00%) was found at the lowest concentration viz. 1.25 μg/mL. The highest i.e. 77.00% mortality rate was observed after 24 h of exposure to flonicamid. Similarly after 48 h, percent mortality was the lowest (50.00%)

Table 1
Toxicity (LC_{50}) of six insecticides against foraging workers of *A. florea* at different exposure times (h).

Insecticide	Time (hr)	LC_{50}[a]	(95%CL[b])	d.f	χ^{2} [c]	*P*	n[d]
Chlorantraniliprole	12	4.95	3.90–9.16	5	0.75	0.98	120
Emamectin benzoate		2.01	0.39–3.55	4	0.62	0.96	120
Fipronil		11.57	6.63–17.08	3	0.83	0.84	120
Flonicamid		12.67	4.56–47.99	5	0.36	0.99	120
Imidacloprid		16.61	11.26–26.23	5	4.11	0.53	120
Spinetoram		4.91	3.64–11.20	4	0.5	0.97	120
Chlorantraniliprole	24	3.3	1.92–4.90	5	0.94	0.96	120
Emamectin benzoate		1.67	0.52–2.76	4	1.41	0.84	120
Fipronil		8.41	4.22–12.58	3	0.09	0.99	120
Flonicamid		6.09	2.27–12.17	5	0.25	0.99	120
Imidacloprid		6.82	3.90–11.01	5	4.68	0.45	120
Spinetoram		4.75	3.54–10.35	4	0.21	0.99	120
Chlorantraniliprole	36	2.74	1.50–4.14	5	1.38	0.92	120
Emamectin benzoate		1.02	0.36–2.05	4	2.97	0.56	120
Fipronil		6	3.77–9.69	3	0.81	0.84	120
Flonicamid		3.94	1.84–6.57	5	1.28	0.93	120
Imidacloprid		3.09	1.81–4.56	5	0.58	0.9	120
Spinetoram		1.1	0.19–2.21	4	0.26	0.99	120
Chlorantraniliprole	48	1.47	0.53–2.51	5	0.77	0.98	120
Emamectin benzoate		0.81	0.14–1.53	4	1.13	0.89	120
Fipronil		3.52	0.99–6.14	3	0.24	0.97	120
Flonicamid		1.34	0.34–2.58	5	1.36	0.92	120
Imidacloprid		1.05	0.28–1.91	5	1.38	0.92	120
Spinetoram		0.97	0.26–1.62	4	0.44	0.97	120

[c] = Chi-square.
[a] LT_{50} = Lethal time to kill 50% population.
[b] CL = Confidence limits.
[d] = Numbers of workers exposed.

which reached to the highest (100.00%) at the maximum concentration viz. 80 μg/mL. Fig. 1E is showed the percent mortality of *A. florea* subjected to fipronil. After 24 h of exposure, the percent mortality ranged from 35.00 to 95.00%. After 48 h of exposure, percent mortality varied from 65.00 to 100.00%. The lowest concentration of 5.00 μg/mL resulted in 35.00% mortality after 24 h of exposure. After 24 h of exposure to the highest concentration of fipronil (40 μg/mL), it reached a maximum of 100.00%. Mortality was 65.00% at the lowest concentration after 48 h of exposure, but reached to maximum (100.00%) at the highest concentration of 40 μg/mL. In Fig. 1F, the spinetoram-induced mortality rate of *A. florea* is depicted. The mortality varied from 0.00 to 85.00% after 24 h of exposure. Similarly, mortality rates ranged from 10 to 100% after 48 h of exposure. Even at the lowest dosage of 1.25 μg/mL, the mortality rate after 24 h was 25.00%. The highest concentration of spinetoram (40 μg/mL) showed 85.00% mortality rate after 24 h. The mortality was 60.00% at the lowest concentration after 48 h of exposure and 100.00% at the highest concentration of 40 μg/mL.

3.3. Lethal time estimation (LT_{50})

A decrease in LT_{50} values was observed as the concentration of insecticide increased. The minimum recorded LT_{50} values for Emamectin benzoate was 5.09 h at 10 μg/ml and 5.63 h at 40 μg/mL and followed by spinetoram with LT_{50} values of 8.99 h at 10 μg/mL and 6.46 h at 20 μg/mL. The LT_{50} value of spinetoram was 5.22 h at 40 μg/ml, and no LT_{50} value was calculated at 80 μg/mL because all individuals died. For Chlorantraniliprole, the LT_{50} values were 14.11 h at 10 μg/mL and 5.16 h at 80 μg/mL. The LT_{50} values for other insecticides are given in Table 2.

4. Discussion

Different insecticides pose varying risks to dwarf honey bees, as shown in the present study. The present study also identifies which insecticide has the most negligible impact on honey. Our research shows that the toxic effects of Emamectin benzoate are more significant on beneficial arthropods, especially on pollinators and honeybees, and are well-documented in the literature (Ioriatti et al., 2009). The emamectin benzoate LC_{50} was 2.01, 1.67, and 1.02 g/mL after 12 h, 24 h, and 48 h. These findings are comparable to the findings of Cang (2007), who have reported that emamectin benzoate was the most toxic against bees among different insecticides. Emamectin benzoate is more toxic because of its lower detoxification during metabolism, and it can penetrate more. The absorption coefficient of avermectins is high, and due to this reason, avermectin is considered highly toxic to bees (Abdu-Allah, 2011; Lumaret et al., 2012). However, field trials conducted on emamectin benzoate have depicted a low half-life in the sunlight. So, it can be added to the IPM program depending on the location (Lumaret et al., 2012). The LC_{50} values for fipronil were 8.41, 6.00, and 3.52 μg/mL after 24, 48, and 72 h respectively. The value is higher compared to other LC_{50} values of other insecticides. It is less toxic because of higher LC_{50} results; following the findings of Castro-Janer (2010), fipronil effectiveness against many organisms has decreased due to its overuse in the field. Many arthropods like houseflies, diamondback moths, cockroaches, etc., have developed resistance (Kristensen et al., 2004; Li et al., 2006; Khan, 2021). However, it has been proven that fipronil degradation varies with plant species. A single enantiomer of fipronil is unlikely to decrease the harm fipronil causes to honeybees. If researchers reduce the use of fipronil, they should improve the application methods based on the safest time and bloom circumstances (Li et al., 2009).

Emamectin benzoate depicted lower LT_{50} value than any other insecticides used in the study. At 10, 20, and 40 μg/mL, the LT_{50} values of emamectin benzoate were 5.09, 5.87, and 5.63 h, respectively. The LT_{50} could not be ascertained for 80 μg/mL because all 20 exposed individuals died. According to Jansson (1997), emamectin benzoate has shown different results under different conditions. Different conditions, such as in the labaoratory and field, produced different results during his experiment. For example, emamectin benzoate is less toxic during field trials than in laboratory experiments. When applied topically, emamectin benzoate killed insects at a rate of 100 percent (Reynolds, 2017). Imidaclo-

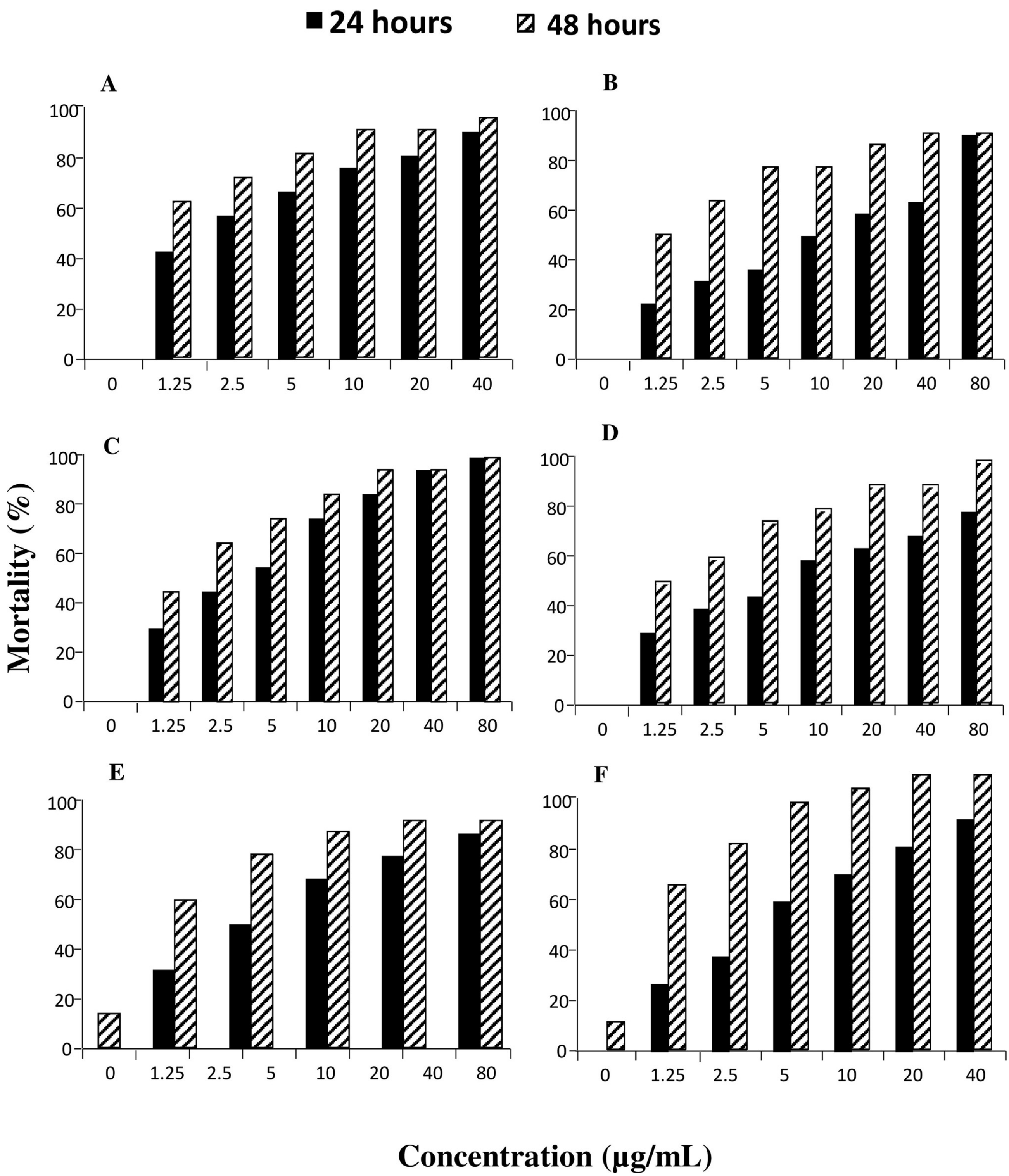

Fig. 1. Mortality (%) of *A. florea* after 24 and 48 hours of exposure to emamectin benzoate (A), imidacloprid (B), chlorantraniliprole (C), flonicamid (D), fipronil (E) and spinetoram (F).

prid had a long half-life (LT_{50}) when compared to other insecticides. LT_{50} values were found for imidacloprid at 10, 20, and 40 ug/mL: 18.70, 13.28, and 11.21 h. Honey bees' acute response to imidacloprid has been documented in the past (Suchail, 2000). As Roy et al. (2016) found, the LT_{50} value in our study was about 10 h and 100 ug/mL, and the fiducial limit was around 9.30–12.20. A low concentration of 40 µg/mL provided the LT_{50} value of 11.22 h. These values showed that honey bees are susceptible to insecticidal exposures. In the present study, fipronil and chlorantraniliprole were the least toxic insecticides, while emamectin benzoate was the most toxic insecticide against A. florea. The low toxicity of chlorantraniliprole can be attributed to the fact that it is unlikely to reach and affect the target sites, ryanodine receptors, in honey bees when dissolved in watery solutions (Dinter

Table 2
Toxicity (LT_{50}) of six insecticides against foraging workers of *A. florea* at different exposure times (h).

Insecticide	Concentration (µg/mL)	LT_{50}[a]	(95%CL[b])	d.f	χ^2 [c]	*P*
Chlorantraniliprole	10	14.11	0.00–23.20	2	0.54	0.76
Emamectin benzoate		5.09	0.00–12.41	2	0.29	0.86
Fipronil		15.89	4.74–22.89	2	0.86	0.65
Flonicamid		14.97	1.96–22.68	2	0.13	0.93
Imidacloprid		18.71	10.52–25.02	2	0.31	0.86
Spinetoram		8.99	0.15–15.45	2	2.58	0.28
Chlorantraniliprole	20	8.17	0.01–17.02	2	1.81	0.4
Emamectin benzoate		5.87	0.00–12.74	2	0.61	0.74
Fipronil		8.05	0.07–14.38	2	0.79	0.67
Flonicamid		11.46	0.97–18.06	2	0.75	0.68
Imidacloprid		13.29	6.16–18.09	2	1.52	0.46
Spinetoram		6.46	0.00–12.48	2	3.23	0.19
Chlorantraniliprole	40	5.87	1.62–12.74	2	0.61	0.73
Emamectin benzoate		5.63	1.27–10.83	2	0.54	0.76
Fipronil		6.76	0.17–11.85	2	1.15	0.56
Flonicamid		9.07	0.01–16.23	2	0.49	0.78
Imidacloprid		11.29	4.58–15.53	2	3.58	0.17
Spinetoram		5.22	0.00–10.88	2	1.49	0.48
Chlorantraniliprole	80	5.16	0.00–11.50	2	0.68	0.71
Emamectin benzoate		–	–	–	–	–
Fipronil		5.19	0.00–10.38	2	0.77	0.68
Flonicamid		9.52	2.65–13.91	2	1.49	0.47
Imidacloprid		–	–	–	–	–
Spinetoram		–	–	–	–	–

[c] = Chi-square.
[d] = Numbers of workers exposed.
[a] LT_{50} = Lethal time to kill 50% population.
[b] CL = Confidence limits.

et al. 2010). Hence, care should be taken while using pesticides in agriculture area by taking into account the foraging time of bees.

5. Conclusion

The *A. florea* is one of the essential pollinators in Southeast Asia and the most common dwarf honeybee. Its importance is due to its services like pollination and honey production. But insecticides are toxic to *A. florea,* which in turn significantly reducing their numbers. Insecticides residues in pollen grains result in physiological changes, behavioral changes, and also mortality; as a result, decreased colony performance is observed. Honeybees have minor adaptation and are essential as well, so care should be taken during pesticide application. And steps should be taken to conserve this vital creature.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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