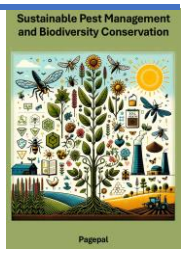
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Evaluating the sub-lethal effects of Cypermethrin and Lambda-cyhalothrin to quantify the behavior of domestic honey bee (*Apis mellifera*) under In-vitro conditions

***Muneeba Noor*[1*], *Fareesa Ameeer*[2], *Muhammad Hammad Akram*[1]**
[1] Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan
[2] Institute of Computing, Muhammad Nawaz Shareef University of Agriculture, Multan, Punjab, Pakistan
***Corresponding Author's email address:** muneebanoor7@gmail.com

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Abstract

The European honey bee, *Apis mellifera* (L.), is an economically important species that provides essential pollination services and is widely used for large-scale honey production. However, the extensive use of agricultural pesticides poses significant threats to the social behavior, foraging activity, and navigation of *A. mellifera*. This study was conducted to evaluate the comparative residual effects of two commonly used insecticides lambda-cyhalothrin and cypermethrin on the social and individual behaviors of *A. mellifera* workers under laboratory conditions. Worker bees were exposed to insecticide solutions at concentrations of 0, 250, 500, 750, and 1000 ppm by placing them in contact with filter papers soaked and dried in each dilution. Social behaviors (antennation and mutual interaction) and individual behaviors (flight ability, locomotion, buzzing, hiding tendency, and feeding) were systematically recorded. Bees exposed to either insecticide exhibited clear alterations in both social and individual behaviors compared with the control. Overall, cypermethrin induced more pronounced behavioral disruptions than lambda-cyhalothrin. Notably, even the lowest tested concentrations of both insecticides resulted in measurable behavioral deviations. Such disruptions may contribute to colony collapse disorders, as impaired behavior affects the efficiency and stability of honey bee colonies. Therefore, excessive or unmanaged use of these insecticides could severely compromise pollination services and, consequently, crop productivity.

1. Introduction

Honeybees are widely recognized as the most important pollinators globally because of their substantial contribution to crop pollination and the maintenance of diverse wild plant communities (Ollerton et al., 2011). Their pollination services support an estimated €153 billion worth of global food production each year (Gallali et al., 2009). Although many organisms participate in pollination, honeybees particularly *Apis mellifera* (L.) (Hymenoptera: Apidae) play a dominant role, pollinating more than 75% of flowering plant species worldwide (Buchmann and Nabhan, 1996; Kremen et al., 2002) and approximately 70% of the world's most economically important crops (Klein et al., 2007; Moritz et al., 2010).

Overall, pollinators, with honeybees as the primary contributors, are responsible for about 35% of global food production (Klein et al., 2007). *Apis mellifera* is particularly valuable in agriculture, pollinating roughly two-thirds of crop species and around one-third of all food crops worldwide (Crailsheim et al., 1992; Roubik, 2005; Klein et al., 2007). Insect pollination is a critical ecological service, benefiting 477 crop species and supporting 35% of the world's crop-derived food output (Pashte et al., 2017).

However, wild pollinator populations are declining at unprecedented rates due to anthropogenic pressures (Ghazoul, 2005). Improper pesticide use is a major contributor to these declines, as toxic compounds can contaminate nectar and pollen, reducing honeybee survival

Corresponding Author: Muneeba Noor Email: muneebanoor7@gmail.com

and potentially causing colony collapse (Chmiel et al., 2020). Although pesticides play an essential role in agricultural productivity reducing pest damage and improving the affordability and quality of food (Strassemeyer et al., 2017) their increased use has raised serious environmental and public-health concerns. The rapid expansion of pesticide production began during World War II (1939-1945) to combat insect-borne diseases, and chemical seed treatments introduced in the 1940s further boosted crop yields (Bernardes et al., 2015).

Global pesticide production increased dramatically from 0.2 million tons in the 1950s to more than 5 million tons by 2000, reflecting an annual growth rate of approximately 11% (Carvalho et al., 2017). Despite their benefits, pesticides often harm non-target organisms, including fish, birds, beneficial insects, and native plant communities (Tuncel et al., 2009). Residues can disperse through air, soil, water, and food systems, posing risks to human health and contributing to environmental degradation (Kim et al., 2017; Singh et al., 2012). Cypermethrin, a widely used synthetic pyrethroid insecticide developed by Elliott and colleagues, is applied extensively in agricultural, commercial, and domestic settings. Pyrethroids are classified into Type I and Type II, with Type II compounds distinguished by the presence of an α-cyano group. While Type I pyrethroids primarily disrupt voltage-gated sodium channels, Type II pyrethroids additionally affect calcium and chloride channels, leading to more pronounced neurotoxic effects (Nasuti et al., 2003). Cypermethrin contamination has been detected in hive products, including up to 49 ng g^{-1} in pollen (Mullin et al., 2010), 20–30 ng g^{-1} in honey (Sanchez-Bayo and Goka, 2014), and similar concentrations in beeswax and adult bees (Chauzat et al., 2006; Wiest et al., 2011). In some regions, concentrations as high as 1290 ng g^{-1} have been reported (Tong et al., 2018). Synthetic pyrethroids are derived from natural pyrethrins but modified to enhance photostability and maintain strong insecticidal activity with relatively low acute toxicity to mammals (Soderlund et al., 2002). Their health impacts vary depending on exposure duration, frequency, and environmental conditions (Soderlund et al., 2002; Ye et al., 2015; Jain, 2016; Hu et al., 2019). These compounds exert their insecticidal action by altering sodium channel function, prolonging sodium ion influx, and ultimately causing paralysis and death in target insects (Ray and Fry, 2006; Martínez et al., 2019). The present study is designed to assess the effects of cypermethrin and lambda-cyhalothrin on the behavior of the domesticated honeybee (*Apis mellifera*) under controlled in-vitro conditions.

2. Materials and Methods

2.1 Insecticides formulation

Two locally available commercial-grade formulated insecticides viz. Arrivo and Karate were purchased from the spray market of Bahawalpur to evaluate their negative impact on social behavior of *A. mellifera* under laboratory conditions. The detailed description of the products is given in (Table 1).

Table 1. Detailed description of commercial-grade insecticides

Sr. #	Generic name	Trade name	Active ingredient	Manufacturer	Recommended dose/ acre
1.	Cypermethrin	Arrivo	2-dimethylcyclopropane carboxylate	Kalyani Industries Pvt. Ltd	250 ml
2.	Lambda-Cyhalothrin	Karate	2-dimethylcyclopropane carboxylic acid	Syngenta, Pakistan	330ml

2.2 Experimental site

This research was conducted in the Centralized Laboratory Department of Entomology, Faculty of Agriculture and Environment (FA&E), The Islamia University of Bahawalpur (IUB).

2.3 Collection of *A. mellifera*

The adults of *A. mellifera* foragers were collected from the apiary established at the farm area of FA&E, IUB, where honey bee colonies were maintained according to the standard commercial technique in the field. For this kind of risk assessment, foraging bees are considered the most ecologically relevant when they start performing external tasks (Picard-Nizou et al., 1995). Based on farming records, no obvious diseases were observed on units or colonies, and no hives were treated with pesticides. This was confirmed during the collection of bees. Foraging workers were collected as explained by Iwasa et al. (2004).

Before collection, safety measures were adopted to avoid the attack of honey bees which included, bee veils, gloves, overall and long boots, participants were clothed in full sleeved shirts to minimize the exposure to bees. By removing the lid of the bee box, the boxes were disclosed from the top. The bees were collected in petri dishes with vented lid that were slightly larger than usual. Honey bees were gently swiped into the petri dish using a bee brush. In each petri dish, ten honey bees were collected and a total of 50 honey bees were used for testing each pesticide. The bees were then transferred to the laboratory for experimentation.

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2.4 Bioassay procedure

A residual toxicity bioassay method was used to determine the toxicities of insecticides on *A. mellifera* behavior. Stock solutions (1000 ml) were prepared for each insecticide and from these, four serial dilution (250, 500, 750 and 1000 ppm) were made for each insecticide. The experiment was performed in transparent plastic boxes measuring 10cm × 5cm × 5cm (L × W × H) having lid covered with mesh for aeration and avoid the insects to escape. 5ml of insecticide concentration from each insecticide was added in each jar with the help of a dropper and a 5 ml of distilled water served as a control. After air drying, 10 bees were introduced in each jar and numbered. After releasing various behaviors viz. buzzing, flight, locomotion, interaction, hiding, feeding and antennation were noted for half an hour. Each treatment was replicated three times under randomized complete block design.

2.5 Statistical analysis

The data collected for each behavior parameter was subjected to one way analysis of variance (ANOVA) in Minitab (Minitab 2003) using Tukey's Kramer test (HSD) (Sokal and Rohlf 1995) at 1 % significance level.

3. Results

3.1 Effect of cypermethrin and lambda-cyhalothrin on buzzing of *Apis mellifera*

The results suggested that both cypermethrin and lambda-cyhalothrin posed adverse effects on the buzzing behavior of *A. mellifera*. There was a significant difference between all the treatments for tested pesticides (cypermethrin: $F_{4,\ 14} = 70.9$, $P \leq 0.01$; lambda-cyhalothrin: $F_{4,\ 14} = 46.4$, $P \leq 0.01$).

Both tested pesticides significantly increased the buzzing time of bees as compared to the control (Fig 1). For Cypermethrin at 250 ppm and 1000 ppm, bees showed anxiety doing buzzing for (14±1.05 min) and (26±1.32 min) respectively which is significantly high as compared to control (11.33± 0.97min). For lambda-cyhalothrin at 250 ppm and 1000 ppm, bees showed anxiety doing buzzing for (11±0.57 min) and (20±1.23 min) respectively which is significantly high as compared to control (9.67±0.57 min). Comparing both insecticides, cypermethrin significantly increased the buzzing time compared to lambda-cyhalothrin, and also with the increase of pesticide concentration, the buzzing time of *A. mellifera* bees increased.

3.2 Effect of cypermethrin and lambda-cyhalothrin on flight of *Apis mellifera*

The results suggested that both cypermethrin and lambda-cyhalothrin posed adverse effects on the flight behavior of *A. mellifera*. There was a significant difference between all the treatments for tested pesticides (cypermethrin: $F_{4,\ 14} = 78.5$, $P \leq 0.01$; lambda-cyhalothrin: $F_{4,\ 14} = 41.3$, $P \leq 0.01$).

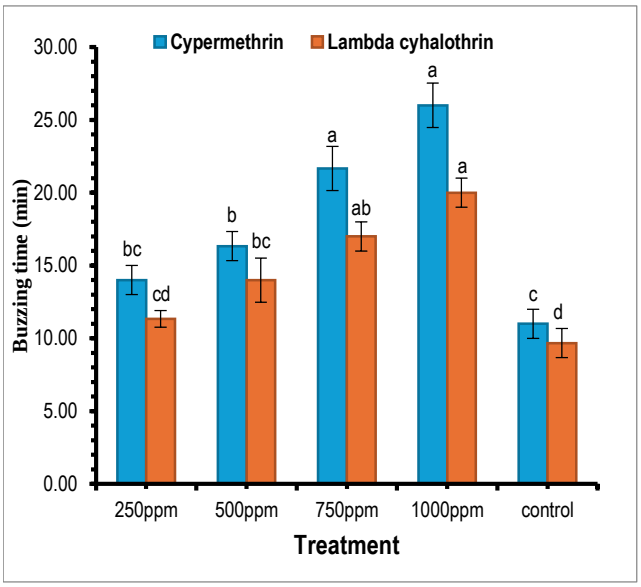


Fig 1. Mean buzzing time (± SE) of *Apis mellifera* treated with various doses of Cypermethrin and Lambda-cyhalothrin. Means followed by the same letter are not significantly different; HSD test $P \leq 0.01$.

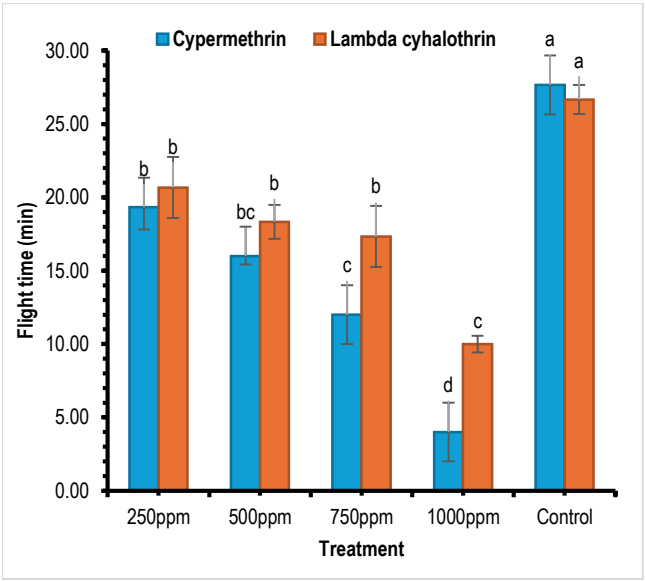


Fig 2. Mean flight time (± SE) of *Apis mellifera* treated with various doses of Cypermethrin and Lambda-cyhalothrin. Means followed by the same letter are not significantly different; HSD test $P < 0.01$.

Both tested pesticides significantly decreased the flight time of bees as compared to the control (Fig 2). For cypermethrin at 250 ppm and 1000 ppm, bees showed flight ability for (19.33±0.57 min) and (4.0±.34 min) respectively which is significantly slow down as compared to control (27.67±1.52 min). For lambda- cyhalothrin at 250 ppm and 1000 ppm,

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bees showed flight ability for the time (20.67±1.15 min) and (10±1.0 min) respectively which is significantly slow down as compared to control (26.67±2.08 min). Comparing both insecticides, cypermethrin significantly decreased the flight time compared to lambda-cyhalothrin, and also with the increase of pesticide concentration, the flight time of *A. mellifera* bees decreased.

3.3 Effect of cypermethrin and lambda-cyhalothrin on locomotion of *Apis mellifera*

The results suggested that both cypermethrin and lambda-cyhalothrin posed adverse effects on the locomotion behavior of *A. mellifera*. There was a significant difference between all the treatments for tested pesticides (cypermethrin: $F_{4,\ 14} = 98.1$, $P \leq 0.01$; lambda-cyhalothrin: $F_{4,\ 14} = 46.2$, $P \leq 0.01$). Both tested pesticides significantly decreased the locomotion time of bees as compared to the control (Fig 3). For Cypermethrin at 250 ppm and 1000 ppm, bees showed locomotion for (23.67±1.52 min) and (9.61±0.52 min) respectively which is significantly decreased as compared to control (29±1.0 min). For lambda-cyhalothrin at 250 ppm and 1000 ppm, bees showed locomotion for (21.33±0.57 min) and (13.56±1.52 min) respectively which is significantly decreased as compared to control (27.33±2.08 min). Comparing both insecticides, lambda-cyhalothrin significantly decreased the locomotion time compared to cypermethrin, and also with the increase of pesticide concentration, the locomotion time of *A. mellifera* bees decreased.

3.4 Effect of cypermethrin and lambda-cyhalothrin on interaction of *Apis mellifera*

The results suggested that both cypermethrin and lambda-cyhalothrin posed adverse effects on the interaction behavior of *A. mellifera*. There was a significant difference between all the treatments for tested pesticides (cypermethrin: $F_{4,\ 14} = 15.4$, $P \leq 0.01$; lambda-cyhalothrin: $F_{4,\ 14} = 17.1$, $P \leq 0.01$). Both tested pesticides significantly reduced the interaction time of bees as compared to the control (Fig 4).

For Cypermethrin at 250 ppm and 1000 ppm, bees showed interaction for (16.30±1.07 min), and (8.0±1.0 min), respectively which is significantly reduced as compared to control (18±2.08a). For lambda- cyhalothrin at 250 ppm and 1000 ppm, bees showed interaction for (19±1.15 min) and (8±0.51 min) respectively which is significantly reduced as compared to control (19.67±1.06 min). Comparing both insecticides, cypermethrin significantly reduced the interaction time compared to lambda-cyhalothrin, and also with the increase of pesticide concentration, the interaction time of *A. mellifera* bees reduced.

3.5 Effect of cypermethrin and lambda-cyhalothrin on hiding behavior of *Apis mellifera*

The results suggested that both cypermethrin and lambda-cyhalothrin posed adverse effects on the hiding behavior of

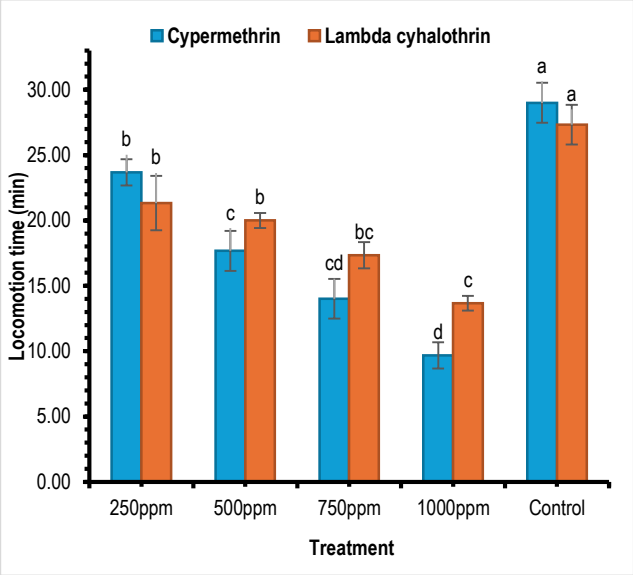


Fig 3. Mean locomotion time (± SE) of *Apis mellifera* treated with various doses of Cypermethrin and Lambda-cyhalothrin. Means followed by the same letter are not significantly different; HSD test $P \leq 0.01$

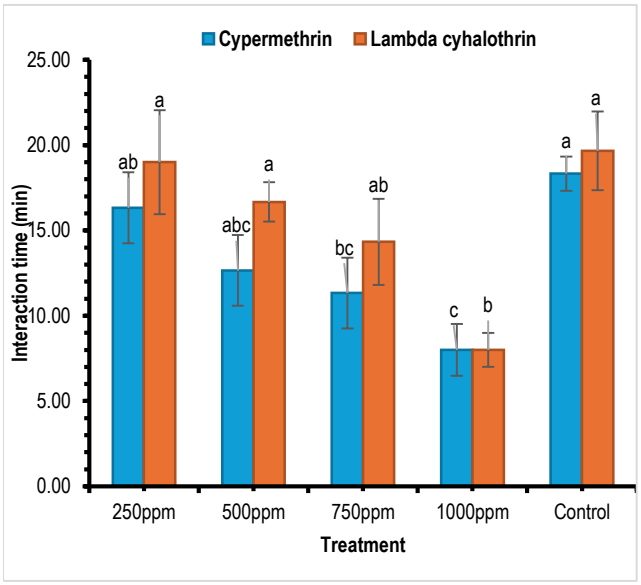


Fig 4. Mean interaction time (± SE) of *Apis mellifera* treated with various doses of Cypermethrin and Lambda-cyhalothrin. Means followed by the same letter are not significantly different; HSD test $P \leq 0.02$

A. mellifera. There was a significant difference between all the treatments for cypermethrin: $F_{4,\ 14} = 8.02$, $P \leq 0.01$, while no significant difference was recorded for lambda-cyhalothrin: $F_{4,\ 14} = 2.31$, $P < 0.12$. Cypermethrin significantly increased the hiding time of bees as compared

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to the control (Fig 5). For cypermethrin at 250 ppm and 1000 ppm, bees showed hiding tendency for (19.33±1.15 min) and (28.0±1.0 min) respectively which is significantly increased as compared to control (19.0±1.09 min). For lambda-cyhalothrin at 250 ppm and 1000 ppm, bees showed hiding tendency for (20.67±1.52 min) and (23.45±0.57 min) respectively which was not significantly increased as compared to control (21.33±1.52 min).

Comparing both insecticides, lambda-cyhalothrin significantly increased the hiding time compared to cypermethrin, and also with the increase of pesticide concentration, the hiding time of *A. mellifera* bees increased.

3.6 Effect of cypermethrin and lambda-cyhalothrin on feeding behavior of *Apis mellifera*

The results suggested that both cypermethrin and lambda-cyhalothrin posed adverse effects on the feeding behavior of *A. mellifera*. There was a significant difference between all the treatments for tested pesticides (cypermethrin: $F_{4,\ 14}$ = 21.3, $P \leq 0.01$; lambda-cyhalothrin: $F_{4,\ 14}$ = 29.9, $P \leq 0.01$). Both tested pesticides significantly reduced the feeding time of bees as compared to the control (Fig 6). For Cypermethrin at 250 ppm and 1000 ppm, bees showed feeding (16.0±1.03 min) and (8.0±0.56 min) respectively which is significantly reduced as compared to control (18.0±1.09 min). For lambda- cyhalothrin at 250 ppm and 1000 ppm, bees showed feeding for (21.67±1.15 min) and (13.33±1.52 min) respectively which is significantly reduced as compared to control (23.33±1.52 min). Comparing both insecticides, cypermethrin significantly reduced the feeding time compared to lambda-cyhalothrin, and also with the increase of pesticide concentration, the feeding time of *A. mellifera* bees reduced.

3.7 Effect of cypermethrin and lambda-cyhalothrin on antennation of *Apis mellifera*

The results suggested that both cypermethrin and lambda-cyhalothrin posed adverse effects on the antennation behavior of *A. mellifera*. There was a significant difference between all the treatments for tested pesticides (cypermethrin: $F_{4,\ 14}$ = 38.0, $P \leq 0.01$; lambda-cyhalothrin: $F_{4,\ 14}$ = 24.4, $P \leq 0.01$). Both tested pesticides significantly decreased the antennation time of bees as compared to the control (Fig 7). For Cypermethrin at 250 ppm and 1000 ppm, bees showed antennation for (22.0±1.73 min) and (11.33±1.0 min) respectively which is significantly decreased as compared to control (24.61±1.52 min). For lambda- cyhalothrin at 250 ppm and 1000 ppm, bees showed antennation for (22.98±1.15 min) and (17.0±1.23 min) respectively which is significantly decreased as compared to control (24.0±1.12 min). Comparing both insecticides, cypermethrin significantly decreased the antennation time compared to lambda-cyhalothrin, and also with the increase of pesticide concentration, the antennation time of *A. mellifera* bees decreased.

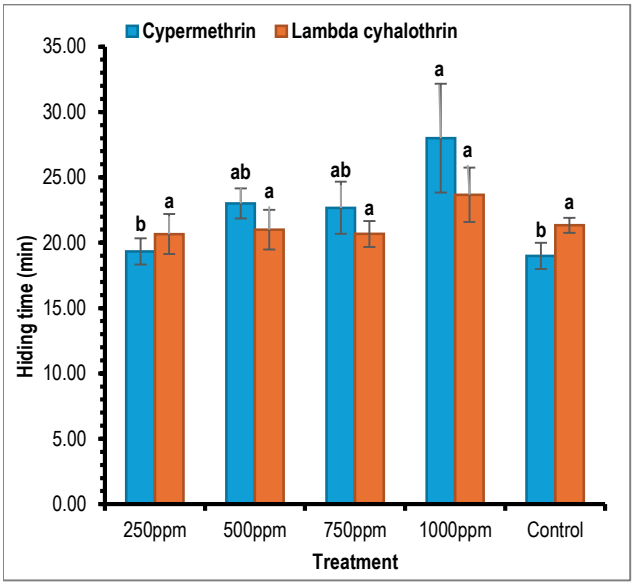


Fig 5. Mean hiding time (± SE) of *Apis mellifera* treated with various doses of Cypermethrin and Lambda-cyhalothrin. Means followed by the same letter are not significantly different; HSD test $P \leq 0.03$

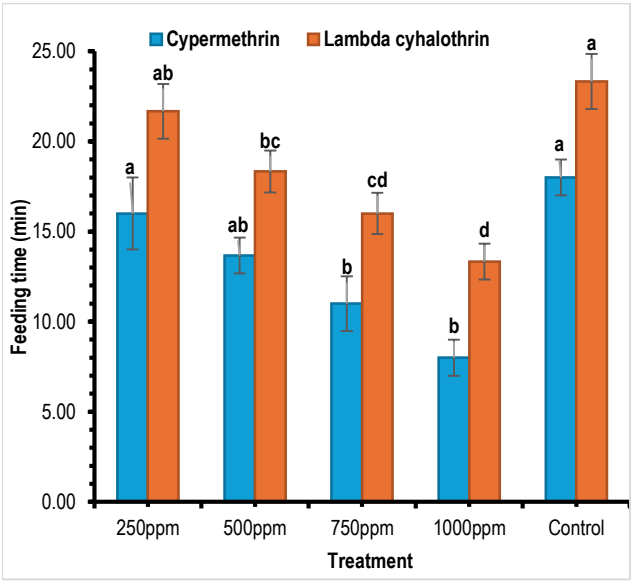


Fig 6. Mean feeding time (± SE) of *Apis mellifera* treated with various doses of Cypermethrin and Lambda-cyhalothrin. Means followed by the same letter are not significantly different; HSD test $P \leq 0.01$

4. Discussion

Synthetic pyrethroids are commonly used worldwide for the control of household and agricultural pests for 40 years

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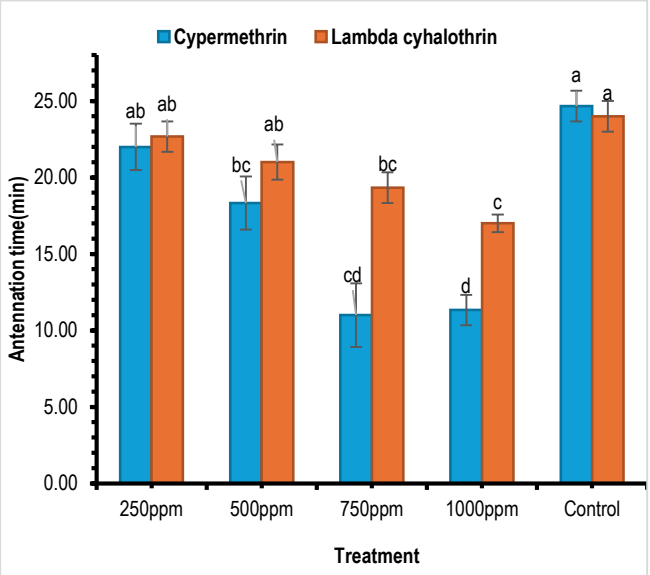


Fig 7. Mean antennation time (± SE) of *Apis mellifera* treated with various doses of Cypermethrin and Lambda-cyhalothrin. Means followed by the same letter are not significantly different; HSD test $P \leq 0.01$

(Kaisarevic et al., 2019). They have been found exerting detrimental effects on the environment, human health, aquatic life and non-target organisms. In current study, result revealed the detrimental effects of two commonly used synthetic pyrethroids, lambda-cyhalothrin, and cypermethrin on social and individual behaviors of worker *A. mellifera.* Individual behavior observed included was the flight ability, locomotion, buzzing, hiding, and feeding while social behaviors included antennation and mutual interactions.

All the behaviors of honey bees were significantly affected by the residues of various concentrations of both insecticides. Synthetic pyrethroids affect the nervous system of the insect and cause convulsion and paralysis in worker bees and ultimately cause death (He et al., 2008; Wang et al., 2020). This is generally assumed that insecticides affect the nervous system of bees and caused the flying bees to be still or diminish their ability to fly. Usually, bees perform buzzing when feel anxiety (Burkart et al., 2012). Both insecticides caused disturbance in the bees which resulted significantly more buzzing consecutively during the experiment as compare to the control. Comparing both insecticides, cypermethrin exerted more hazardous effects on individual and social behavior of workers bees compared to the lambda-cyhalothrin. Various studies have revealed such toxic effects of pyrethroids on various honey bee species and other beneficial insects (Liao et al., 2018; Arthidoro de Castro et al., 2020; Reegan et al., 2020). Hence, we hypothesized that honeybee exposure to insecticides causes social and individual behavior disorders.

As for as the mode of action of synthetic pyrethroids is concerned, they damage the nerve impulses of the insect which stop the insects from feeding and moving (Murray, 1985; He et al., 2008). They target the central nervous system in both target and non-target organisms. Their main mechanism of action is interacting with voltage-gated sodium channels in neurons. This interaction results in depolarization caused by the prolonged influx of sodium ions during excitation. The extended depolarization is what leads to repetitive nerve activity that can result in hyperexcitation and death. Some pyrethroids have also been found to inhibit ATPase activity. Studies have also found that some pyrethroids interact with the aminobutyric acid receptor; however, this is normally more common only with type II pyrethroids (He et al., 2008). Our study showed the lack of movement in the initially flying honeybees after their exposure to the insecticide residual area. So, we concluded that even the residues of these two insecticides can cause behavior disorders in the honeybees. It might be attributed to the fact that the pesticide damage some part of worker bees, resultantly may cause behavioral disorders. Arthidoro de Castro et al. (2020), narrated that sub-lethal dose of lambda-cyhalothrin damaged some parts of the brain which caused behavioral changes in bees.

In our case, the residual effects of different sub-lethal doses of the above mentioned insecticides did not cause sudden death but deviation in behavior from normal conditions can cause many disorders. As these insecticides affected the flight ability of honeybees, it shows that this shall damage the foraging capability of honeybees in the field. Nervous disorders will disturb the bee's duties inside the hive. Negative deviation in the social behavior of bees as antennation causes the lack of communication which will affect the trophallaxis and other messages given through waggle dance, round dance, and other movements by honeybees (Liao et al., 2018). We concluded that all this damage caused by the insecticide lead to many colony-related problems. Under natural conditions, bees remain in contact with the flora for longer times which has insecticide residues is a great threat to the reducing population of apiary as well as wild bees. We suggest that further studies should be done to observe the residual effects of these commonly used insecticides on *A. mellifera* in the field. These insecticides reduce the population of honeybees which ultimately affects the yield of crops and orchards (Klein et al., 2007).

5. Conclusions

Chemical pesticides pose serious threat to the life of pollinators, particularly forger honey bees. Here we tested various concentrations of two commonly used chemical

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insecticides including cypermethrin and Lambda-cyhalothrin on social and individual behavior of worker bees of *A. mellifera*, under in vitro conditions. We found that lambda cyhalothrin had more detrimental effects on all dosages on individual and social behavior of worker bees. Hence, we recommend to use cypermethrin must be preferred over lambda cyhalothrin when pest control decisions are made on economically important crops. Further study must be conducted to evaluate the effect of other commonly used insecticides and create awareness among the farming community about the hazardous effects of pesticides on environment and pollinators.

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7. Conflict of Interest
The authors declare no conflict of interest.

8. Authors' Contribution
MN designed the study and conducted bioassay
MA helped in collecting data and provided guidelines on bioassay setting and proof read the MS
HSB performed statistical analysis and prepared data tables
FA completed write up and finalized the MS
SJ Proof read the document
MHA Prepared tables and figures and help in collecting and rearing population

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