Original Article

EVALUATING TOXICITY OF COMMONLY USED INSECTICIDES ON RED DWARF HONEY BEE, *APIS FLOREA* F. (HYMENOPTERA: APIDAE)

Muhammad Sarmad[1]* ORCID: 0000-0002-5166-2982
Muhammad Shahid[2]
Asif Sajjad[2]* ORCID: 0000-0002-4246-1484
Muhammad Sajjad[2]
Pavel Ryšánek[1]

[1]Department of Plant Protection, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Prague 16500, Czech Republic
[2]Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

*corresponding author: sarmad@af.czu.cz; asif.sajjad@iub.edu.pk
Received: 05 November 2024; accepted: 03 June 2025

Abstract

The extensive use of insecticides has raised concern regarding their toxicological risk to honey bee species, *Apis florea* (Hymenoptera: Apidae). Bees may come into contact with pesticides while foraging in the field to gather nectar and pollen from agricultural blooming plants, which cause the death of their workers. In this respect, the current study was conducted to evaluate the toxicity of certain commonly used insecticides against the population of *A. florea*. The concentrations tested in the residual bioassays were 1, 2, 4, 8 and 16 ppm for abamectin, cypermethrin, imidacloprid, acetamiprid andpyriproxyfen, while for emamectin benzoate and carbosulfan, 0.125, 0.25, 0.5, 1 and 2 ppm. Each concentration was replicated three times. Our findings showed that the combination of emamectin benzoate and carbosulfan (LC_{50} 0.54) was more toxic, followed by imidacloprid (LC_{50} 0.70), acetamiprid (LC_{50} 1.05), cypermethrin (LC_{50} 1.57), pyriproxyfen (LC_{50} 1.83) and abamectin (LC_{50} 5.51). The present study shows the relative toxicity of various insecticides on *A. florea* besides the generalization that combinations of two insecticides (synergism) are more toxic to *A. florea* than a single insecticide. Therefore, combinations should be avoided for the management of insect pests.

Keywords: *Apis florea*, combination, honey bees, insecticides, toxicity

INTRODUCTION

Honey bees play a significant role as pollinators in agricultural ecosystems (Calderone 2012). In 146 countries worldwide, crops pollinated by bees contribute to 90% of the food supply (Klein et al., 2007). The production capacity of the crops is significantly mitigating due to the lack of honey bees (Klein et al., 2006). Among honey bee species, the red dwarf honey bee, *Apis florea* F. (Hymenoptera: Apidae), serves as a significant pollinator for agricultural plants and inhabits in plain areas at altitudes of 500 meters. However, seasonal migrations can be found as high as 1500 meters (Muttoo, 1956). It is mainly found in the Oman, Iran and Indian subcontinent (Hepburn & Hepburn, 2005). They build their nests in bushes and shades of trees. It successfully competes with other *Apis* species despite its small size (Koeniger, 1976).

Honey bees require a variety of plants to obtain essential nutrients (Branchiccela et al., 2019). Insect pollination is primarily stressed by agrochemicals (Kuldna et al., 2009). The global decline of honey bee colonies is due to the excessive and indiscriminate use of agrochemicals. Several agrochemicals can disrupt pollinating communities (Potts et al., 2010). Furthermore, their long-lasting toxic effects on honey bees impair their foraging abilities (Koch & Weisser, 1997). Moreover, insecticides harm the immune system of honey bees, making them more vulnerable to diseases (Desenex et al., 2007; Wu et al., 2011; Pettis et al., 2012).

Considering the intensified application of insecticides in district Bahawalpur to control cotton and maize pests, it is important to assess the comparative toxicity of frequently used insecticides on wild honey bees. This knowledge can be utilized to develop a more effective pest control approach in the area. The present study was conducted in the same context, with the goal of evaluating the harmful effects of widely used insecticides on *A. florea* in controlled conditions.

MATERIAL AND METODS

Collection of *Apis florea*

Honey bee, *A. florea* was collected from the trees located at the Bahawalpur district, placed in the plastic buckets and shifted to the laboratory at Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur, Punjab, Pakistan. The broods were transferred into wire mesh circular boxes (Fig. 1) and set inside in an incubator at 33°C with 65% relative humidity, and kept in the dark for the bees to emerge. The newly emerging bees were fed with 50% sucrose solution and water until used in the experiment (Williams et al., 2013).

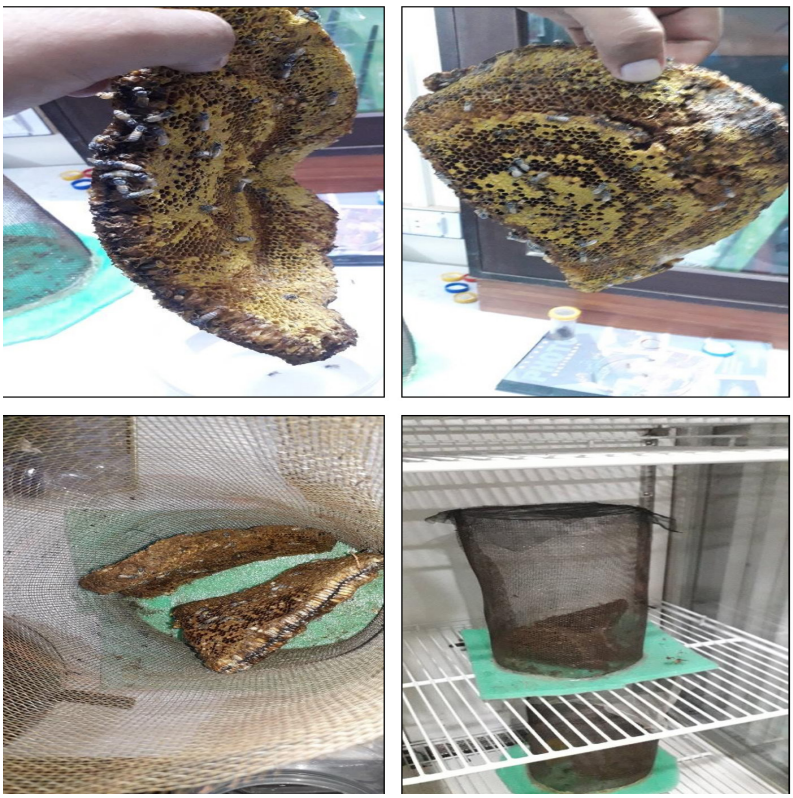

Fig. 1. Honey bee brood collected from the trees located at Bahawalpur district, Pakistan.

Insecticides

The insecticides abamectin 1.8% EC, cypermethrin 25% EW, imidacloprid 20% SL, acetamiprid 20% SL, pyriproxyfen 10.8% EC and emamectin benzoate and carbosulfan 25% EW were used in the experiment. These insecticides were purchased from licensed commercial insecticide retailers.

Residual toxicity bioassay

Residual bioassay was performed to determine the toxicity of six insecticides at different concentrations: 1, 2, 4, 8 and 16 ppm for abamectin, cypermethrin, imidacloprid, acetamiprid and pyriproxyfen, while 0.125, 0.25, 0.5, 1 and 2 ppm for emamectin benzoate and carbosulfan. The concentrations were prepared through a serial dilution in 50% sucrose solution, and each concentration was replicated three times (10 bees per replicate). The control bees were only fed a 50% sucrose solution. The appropriate range of doses for each insecticide was determined using a bioassay for range finding. The highest and lowest doses of each insecticide are needed to cause 0% and 100% mortality. Mortality was assessed after 24, 48 and 72 hours of exposure. The treated bees were considered dead if they were truly dead, rather than severely affected.

Lethal concentrated bioassay

For surface residual bioassay, a 5 ml solution of each concentration (from above prepared serial dilution) was poured into 1-liter glass beakers, shaken thoroughly for 2 minutes and air-dried for two hours (Radwan & Taha, 2012; Farooqi et al., 2020). Ten bees were released in each treated glass beaker with a 50% sucrose solution and kept in an incubator at 33±1°C, 65±5% relative humidity, with permanent darkness for 48 and 72 hours.

Statistical analysis

The statistical analysis was performed using SPSS to analyze the data (version 21 for Windows, SPSS Inc., Chicago, IL, USA). Probit analysis (Finny, 1971)

was used to determine the LC_{50}, chi-square and 95% confidence interval values. The ANOVA was performed for the means comparison of percent mortality using the Duncan multiple range test. The percent mortality was calculated using Abbott's formula (Abbott, 1925) as follows:

$$\text{Corrected percent mortality} = \frac{\text{Observed mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

RESULTS

Lethal concentration of *A. florea* against insecticides

The toxicity of different insecticides by residual application is presented in Tables 1 and 2. The combination of emamectin benzoate and carbosulfan was more toxic to *A. florea* than solely used insecticides. Emamectin benzoate and carbosulfan was found to be more toxic (0.54) followed by pyriproxyfen (1.83), acetamiprid (1.05), cypermethrin (1.57), imidacloprid (0.70) and abamectin (5.51) after 48 h. The insecticides were tested for their toxicity in the following order: emamectin benzoate and carbosulfan > imidacloprid > acetamiprid > cypermethrin > pyriproxyfen > abamectin.

Percent mortality of *A. florea* against insecticides

The concentrations of abamectin showed sinificant differences after 24-hour and 48-hour (p<0.001) exposures. The mortality rate of the bees increased as the dosage and exposure time increased. After 24 and 48 hours of exposure to abamectin, the highest mortality was recorded at 16 ppm (73.33% and 100%) while the lowest at 1 ppm (36.67% and 46.66%) (Fig. 2). There was a significant difference among the concentrations of acetamiprid after 24 and 48 h (p<0.001) of exposure. The mortality rate was the maximum at 16 ppm (100%) and the minimum and comparable at 1 and 2 ppm (53.33±1.16% and 56.66±0.99%) after 24 h. Similarly, after 48 h

Table 1.

Lethal concentration (LC_{50}) and confidence interval (95% CI) of *A. florea* against insecticides after 48 hours

Insecticides	Time (Hours)	LC_{50}	95% CI	df	$X^{2,a}$	*p*-value
Abamectin	48	5.51	1.320-8.097	3	0.358	0.003
Acetamiprid		1.05	0.581-2.224	3	0.846	<0.001
Emamectin Benzoate + Carbosulfan		0.54	0.263-0.902	3	1.557	<0.001
Cypermethrin		1.57	0.263-4.925	3	1.580	0.034
Pyriproxyfen		1.83	0.582-3.402	3	2.330	0.022
Imidacloprid		0.70	0.237-1.850	3	1.150	<0.001

[a]Estimated parameter of Probit analysis

Table 2.

Lethal concentration (LC_{50}) and confidence interval (95% CI) of *A. florea* against insecticides after 72 hours

Insecticides	Time (Hours)	LC_{50}	95% CI	df	$X^{2,a}$	*p*-value
Abamectin	72	-	-	-	-	-
Acetamiprid		-	-	-	-	-
Emamectin Benzoate + Carbosulfan		0.017	0.010-0.105	3	0.730	<0.001
Cypermethrin		0.360	0.123-0.427	3	2.294	<0.001
Pyriproxyfen		1.124	0.579-2.390	3	0.530	<0.001
Imidacloprid		-	-	-	-	-

[a]Estimated parameter of Probit analysis

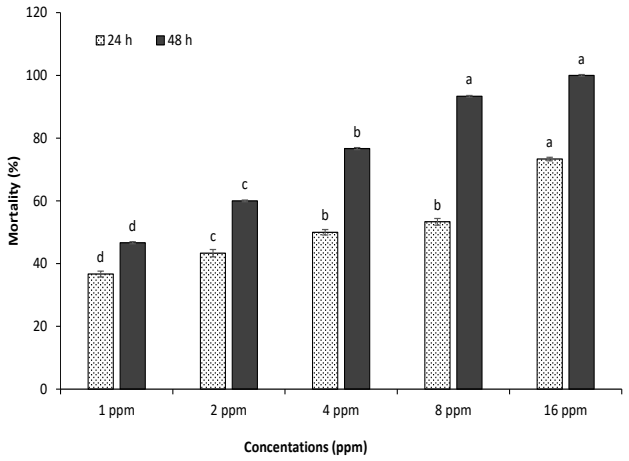

Fig. 2. Percentage mortality of *A. florea* after 24 and 48 hours of abamectin application.

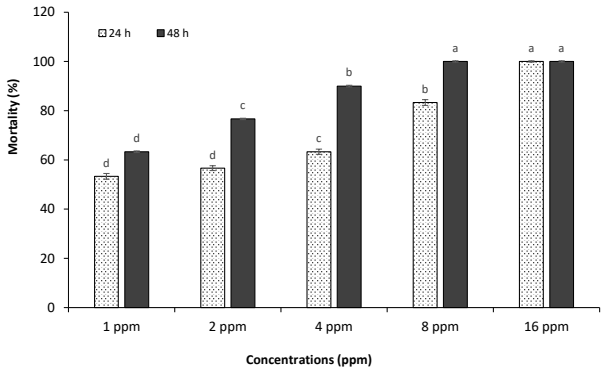

Fig. 3. Percentage mortality of *A. florea* after 24 and 48 hours of acetamiprid application.

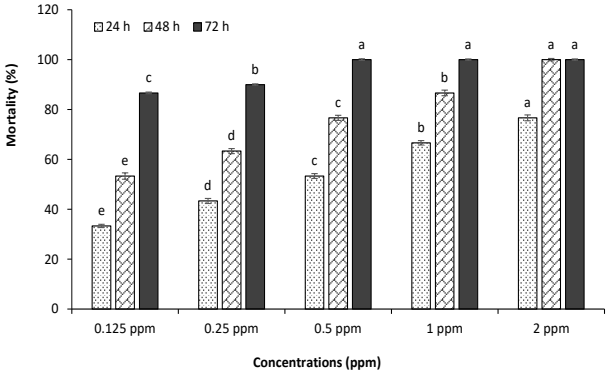

Fig. 4. Percentage mortality of *A. florea* after 24, 48 and 72 hours of emamectin benzoate and carbosulfan application.

exposure to acetamiprid, the highest percent mortality was noticed at 8 and 16 ppm (100%) while the lowest at 1 ppm (63.33±0.33%) (Fig. 3).

After 24, 48, and 72 hours of exposures, there was a notable difference among the concentrations of the emamectin benzoate and carbosulfan combination. The bees had the highest mortality rate at 2 ppm (100% and 100%) and the lowest at 0.125 ppm (33.33±0.65% and 53.33±1.27%) after 24 and 48 hours of exposure. The maximum mortality (100%) was found at 0.5, 1, and 2 ppm, while the minimum at 0.125 ppm (86.66±0.33%) after 72 hours of exposure to a combination of emamectin benzoate and carbosulfan (Fig. 4).

After 24, 48, and 72 hours of exposures, the concentrations of cypermethrin differed significantly (p<0.001). The highest percent mortality was exhibited at 8 (66.66±0.89% and 100%) and 16 ppm (70±1.18% and 100%) while the lowest at 1 ppm after 24 and 48 h (43.33±0.95% and 56.66±0.38%) of exposure. Similarly, mortality was the maximum at 4, 8 and 16 ppm (96.66±0.33%, 100% and 100%, respectively) while the minimum at 1 ppm (76.66±0.48%) (Fig. 5).

There was a statistical difference among the concentrations of pyriproxyfen after 24, 48 and 72 h of exposure. The highest mortality rate was revealed at 8 and 16 ppm (76.66±1.17% and 73.33±1.14%) while, the lowest at 1 ppm (40±1.07%) after 24 h of exposure. The percent mortality was the maximum at 16 ppm (93.33±0.29%) while the minimum at 1 ppm (46.66±1.01%) after 48 h of exposure. Likewise, the highest mortality rate was registered at 4, 8 and 16 ppm (83.33±0.33%, 96.66±0.27% and 100%) while the lowest at 1 ppm (60±0.33%) after 72 h exposure to pyriproxyfen (Fig. 6).

The concentration of imidacloprid showed significant (p<0.001) differences after 24 and 48 hour of exposure. The percentage mortality was increased with the increase of dose and exposure time. The maximum mortality (100%) was observed at 16 ppm in 24 and 48 hours. While, less than 50% mortality was showed at 1 ppm after 24 hour and gradually increased (63.33±2.45%) with the increase in exposure duration, 48 hours (Fig. 7). While, the mortality in the control groups was 0 to 8% in all experiment.

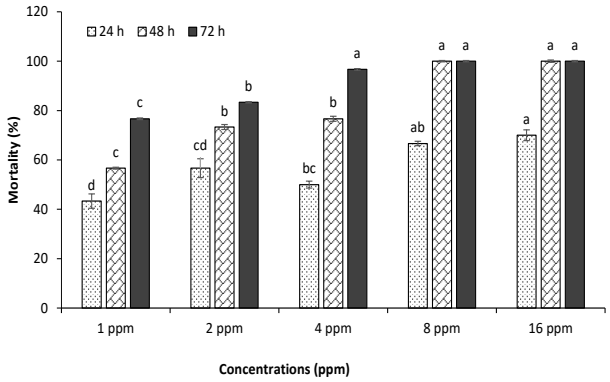

Fig. 5. Percentage mortality of *A. florea* after 24, 48 and 72 hours of cypermethrin application.

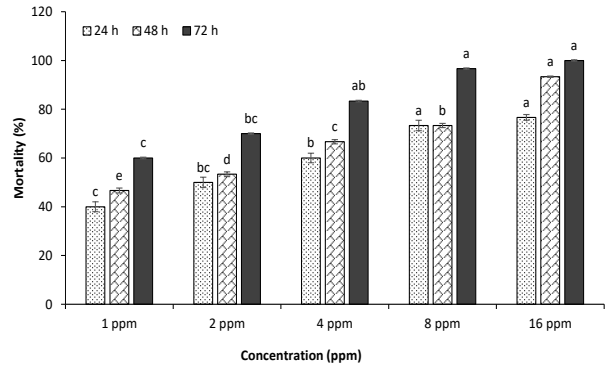

Fig. 6. Percentage mortality of *A. florea* after 24, 48 and 72 hours of pyriproxyfen application.

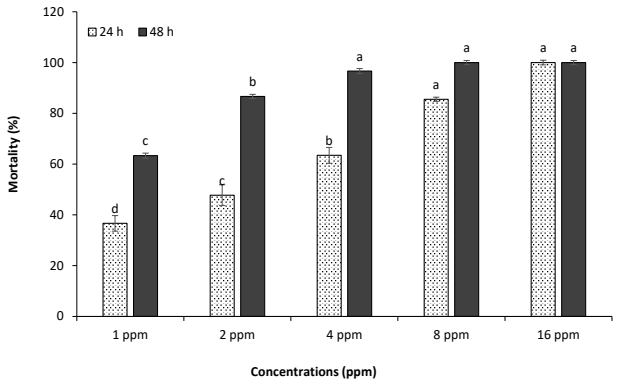

Fig. 7. Percentage mortality of *A. florea* after 24 and 48 hours of imidacloprid application.

DISCUSSION

The present study demonstrates that *A. florea* face different levels of risk from various insecticides. The most negligible impact on honey bees is also identified in the current study, concerning which insecticide has the most notable toxic effect on bees. In our findings, a combination of two insecticides was found to be more toxic than solely used insecticides. A useful approach for managing various pests is using a combination of insecticides (Dittrich et al., 1990). The mixtures of pesticides could prevent pests from becoming resistant to them (Bielza et al., 2009). Farmers could gain advantages from employing a strategy against resistance by utilizing mixtures of different insecticides, resulting in reduced insecticide usage in the field. The population of *Helicoverpa armigera* experienced an inhibition of esterases due to organophosphates, leading to an increase an the toxicity of pyrethroids because of their synergistic actions (Saddiq et al., 2017). The most extensive resistance mechanism in insets is enzyme detoxification (Wang et al., 2006). Byrne and Devonshire (1991) demonstrated that when organophosphates and pyrethroids are combined, they can block the metabolic detoxification enzymes in *Bimissia tabacii*. The populations and categories of insecticides had an effect on the synergistic or antagonistic interactions (Abbas et al., 2015; Khan et al., 2013). In comparison to individual exposure, a combination of imidacloprid, dichlorvos, and atrazine had synergistic effects on *Danio rerio* (Shukla et al., 2017). Thiamethoxam, nitenpyram and thiacloprid combined effectively targeted *Anopheles gambiae* and showed synergistic effects against pyrethroid-resistant *Aedes aegypti* and *Anopheles gambiae* strains (Darriet et al., 2013). The widespread use of synthetic chemicals has the potential to directly or indirectly extinguish non-target organisms. Nasreen et al. (2000) tested the field recommended doses of seven different insecticides. The insecticidal effects on non-target organisms are classified as harmless (50% mortality), mildly harmful (50-79% mortality),

moderately harmful (80-89% mortality), and harmful (> 90% mortality).

Although emamectin benzoate and carbosulfan showed the lowest LC_{50} at 48 hours of exposure, it did not give 100% mortality at 48 hours as did abamectin, acetamiprid and imidacloprid. On the other hand, at 48 hours of exposure emamectin benzoate and carbosulfan exhibited the lowest LC_{50} as compared to pyriproxyfen and cypermethrin. The lethal concentration values of insecticide mixture are usually affected by the duration of exposure as Akinwandea et al. (2021) showed high toxicity of cypermethrin at one hour of exposure in *Culex quinquefasciatus* but toxicity decreased after 1.5 hour of exposure.

Insecticides in the avermectin group include emamectin benzoate. Convulsions and excessive excitability are caused by them because they block chloride channels. The activation of glutamate (inhibitory) chloride channels is the most susceptible target site for avermectins in insects (Arena et al., 1995). Carbosulfan belongs to the carbamate insecticide group. Carbamates have an effect on insects similar to acetyl cholinesterase (AChE) inhibitors, which block the function of the neurotransmitter acetylcholine in the insect nervous system under normal circumstances (Hardstone et al., 2010). In our study, the combination of emamectin benzoate and carbosulfan showed a synergistic effect. The combination of thiamethoxam + lamba-cyhalothrin and bifenthrin + zeta-cypermethrin causes mortality >99% against honey bees (Zhu et al., 2015).

Saddiq et al. (2017) used different combinations of insecticides, i.e., chlorpyrifos and spinosad and deltamethrin and emmamectin benzoate, which showed synergistic effects against cotton mealybug (*Phenococus solenopsis*). Additionally, they claimed that insecticide synergism and combinations could help in the efficient suppression of *P. solenopsis* populations that were less susceptible. This might help manage this pest's resistance to insecticides in a way that has less negative effects on the ecosystem. Abamectin belongs to the avermectin group of insecticides. Avermectins cause convulsions and hyper excitability because they are chloride channel blockers. In insects, the activation of glutamate (inhibitory) chloride channels is the most sensitive target site for Avermectins (Arena et al., 1995).

Cypermethrin was toxic to *A. florea* which caused 100% mortality in 8 and 16 ppm concentrations after 48 hours, but it caused 100% mortality in all concentrations after 72 hours of exposure. It belongs to the pyrethroid group of insecticides, which is similar to pyrethrin. Despite having a natural origin, pyrethrin is an extremely deadly toxin for bees (Pettis et al., 2013). Pyrethroids cause the voltage-gated Na+ channel in the axons of nerve cells to take longer to close, extending the time it takes for the nerve cells to recover after transmitting an action potential (Haarmann et al., 2002). Synthetic pyrethroids have toxic side effects that include bees regurgitating food after eating, moving erratically, paralyzing and dying in large numbers between foraging areas and colonies (Kumar et al., 2020).

Acetamiprid and imidacloprid were found to be toxic to *A. florea.* Both insecticides belong to the neonicotinoids group of insecticides. These synthetic insecticides have a stronger affinity for nAChR in insect nervous systems, including that of bees (Kumar et al., 2020). A neonicotinoid insecticide named imidacloprid acts to kill insects by inhibiting their nicotinic acetylcholine receptors.

Pyriproxyfen was found to be less toxic against *A. florea* because 100% mortality was achieved in 72 hours of time which was high as compared to all other insecticides. Insect growth regulator (IGR), pyriproxyfen, has been extensively used since the early 1990s to protect crops from pests (Dennehy et al., 1996). Despite the fact that many pesticides (such as insect growth regulators) are not poisonous to adult bees, there is still a considerable risk to the development of larvae. It was reportedly not poisonous to adult honey bees and other non-target insects (Sullivan & Goh, 2008). High pyriproxyfen exposure levels may result in abnormalities and death, particularly during development. These investigations also demonstrated that pupae would become melanized when exposed to low amounts of pyriproxyfen. This could be as a result of

enhanced phenoloxidase activity controlling pupation and melanization (Bitondi et al., 1998; Zufelato et al., 2000).

In this study, all the tested insecticides were found to be highly toxic against *A. florea* except pyriproxyfen. Based on the results of our study, it is concluded that combinations of insecticides should be avoided as much as possible, while pyriproxyfen can be recommended for field application due to its low toxicity against *A. florea*. If the use of insecticides other than pyriproxyfen is necessary, then some precautionary measures should be adopted to prevent the loss of honey bees. These measures include applying less harmful and quickly biodegradable pesticides, applying pesticides in the evening, selecting the right formulations, changing the application method and setting up apiaries in secure areas.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Department of Entomology, for coordinating in the present research work.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Abbas, N., Crickmore, N., Shad, S. A. (2015). Efficacy of insecticide mixtures against a resistant strain of house fly (Diptera: Muscidae) collected from a poultry farm. *International Journal of Tropical Insect Science, 35*(1), 48-53.

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology, 18,* 265-267.

Akinwande, A. A., Dada, A. A., Umar, I. O. (2021). Acute toxicity of mesocarp of *Azadirachta indica* (L.) (*Neem plant*) on fingerlings of *Heterobranchus bidorsalis. African Scientist, 7*, 29-33.

Arena, J. P., Liu, K. K., Paress, P. S., Frazier, E. G., Cully, D. F., Mrozik, H., Schaeffer, J. M. (1995). The mechanism of action of avermectins in *Caenorhabditis elegans*: correlation between activation of glutamate-sensitive chloride current, membrane binding, and biological activity. *The Journal of Parasitology, 81*(2), 286-294. https://doi.org/10.2307/3283936

Bielza, P., Fernández, E., Grávalos, C., Abellán, J. (2009). Carbamates synergize the toxicity of acrinathrin in resistant western flower thrips (Thysanoptera: Thripidae). *Journal of Economic Entomology, 102*(1), 393-397. https://doi.org/10.1603/029.102.0151

Bitondi, M. M., Mora, I. M., Simoes, Z. L., Figueiredo, V. L. (1998). The *Apis mellifera* pupal melanization program is affected by treatment with a juvenile hormone analogue. *Journal of Insect Physiology, 44*(6), 499-507. https://doi.org/10.1016/S0022-1910(97)00113-3

Branchiccela, B., Castelli, L., Corona, M., Díaz-Cetti, S., Invernizzi, C., Martínez de la Escalera, G., ... Antúnez, K. (2019). Impact of nutritional stress on the honeybee colony health. *Scientific Reports, 9,* 10156.

Byrne, F. J., & Devonshire, A. L. (1991). In vivo inhibition of esterase and acetylcholinesterase activities by profenofos treatments in the tobacco whitefly *Bemisia tabaci* (Genn.): implications for routine biochemical monitoring of these enzymes. *Pesticide Biochemistry and Physiology, 40*(3), 198-204. https://doi.org/10.1016/0048-3575(91)90090-9

Calderone, N. W. (2012). Insect pollinated crops, insect pollinators and US agriculture: trend analysis of aggregate data for the period 1992-2009. *PloS One, 7*, e37235. https://doi.org/10.1371/journal.pone.0037235

Darriet, F., & Chandre, F. (2013). Efficacy of six neonicotinoid insecticides alone and in combination with deltamethrin and piperonyl butoxide against pyrethroid-resistant *Aedes aegypti* and *Anopheles gambiae* (diptera: culicidae). *Pest Management Science, 69*(8), 905-910. https://doi.org/10.1002/ps.3446

Dennehy, T.J., Ellsworth, P.C., Nichols, R.L. (1996). The 1996 whitefly resistance management program for Arizona cotton. University of Arizona Press.

Desneux, N., Decourtye, A., Delpuech, J.M. (2007). The sub-lethal effects of pesticides on beneficial arthropods. *Annual Review of Entomology, 52*, 81-106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Dittrich, V., Uk, S., Ernst, G.H. (1990). Chemical control and insecticide resistance of whiteflies. In: Gerling DE (ed) Whiteflies: their Bionomics, pest status and management, Athenaeum Press, New Castle, UK.

Farooqi, M.A., Irsa, B., Ali, S., Sajjad, A., Muhammad, W.H., Akhtar, S. (2020). Impact of selected insecticides on *Apis mellifera* L. (Hymenoptera: Apidae) under controlled conditions. *Pakistan Journal of Zoology, 52*(1), 193-198. https://dx.doi.org/10.17582/journal.pjz/2020.52.1.193.198

Finney Probit analysis. (1971). Cambridge: Cambridge University Press, 333.

Hardstone, M.C., & Scott, J.G. (2010). Is *Apis mellifera* more sensitive to insecticides than other insects. *Pest Management Science, 66*, 1171-1180. https://doi.org/10.1002/ps.2001

Haarmann, T., Spivak, M., Weaver, D., Weaver, B., Glenn, T. (2002). Effects of fluvalinate and coumaphos on queen honey bees (Hymenoptera: Apidae) in two commercial queen rearing operations. *Journal of Economic Entomology, 95*(1), 28-35. https://doi.org/10.1603/0022-0493-95.1.28

Hepburn, R., & Hepburn, C. (2005). Bibiliography of *Apis florea. Apidologie 36*(3), 377-378. https://doi.org/10.1051/apido:2005024

Khan, H.A., Akram, W., Shad, S.A. (2013). Resistance to conventional insecticides in Pakistani populations of *Musca domestica* L. (Diptera: Muscidae): a potential ectoparasite of dairy animals. *Ecotoxicology, 22*, 522-527.

Klein, A.M., Vaissiere, B.E., Cane, J.H., Dewenter, I.S., Cunningham, S.A., Kremen, C., Tscharntke, T. (2006). Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Socirty B: Biological Sciences, 274*, 303-313. https://doi.org/10.1098/rspb.2006.3721

Koch, H., & Weisser, P. (1997). Exposure of honey bee during pesticide application under field conditions. *Apidologie, 28*(6), 439-447. https://doi.org/10.1051/apido:19970610

Koeniger, N. (1976). Interspecific competition between *Apis florea* and *Apis mellifera* in the tropics. *Bee World, 57,* 110-112. https://doi.org/10.1080/0005772X.1976.11097604

Kuldna, P., Peterson, K., Poltimae, H., Luig, J. (2009). An application of DPSIR framework to identify issues of pollinator loss. *Ecological Economics, 69*(1), 32-42. https://doi.org/10.1016/j.ecolecon.2009.01.005

Kumar, G., Singh, S., Nagarajaiah, R.P.K. (2020). Detailed review on pesticidal toxicity to honey bees and its management. Modern Beekeeping-Bases for Sustainable Production. pp. 13-33.

Muttoo, R.N. (1956). Facts about beekeeping in India. *Bee World, 37,* 125-133. https://doi.org/10.1080/0005772X.1956.11094935

Nasreen, A., Ashfaq, M., Mustafa, G. (2000). Intrinsic toxicity of some insecticides to egg parasitoid, *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *Bulletin of the Institute of Tropical Agriculture Kyushu University, 23*, 41-44. https://doi.org/10.11189/bita.23.41

Pettis, J.S., Van Engeldsorp, D., Johnson, J., Dively, G. (2012). Pesticides exposure in honeybee results in increased levels of the gut pathogen *Nosema*. *The Science of Nature, 99*(12), 153-158. DOI: 10.1007/s00114-011-0881-1

Pettis, J.S., Lichtenberg, E.M., Andree, M., Stitzinger, J., Rose, R. (2013). Crop pollination exposes honey bees to pesticides which alters their susceptibility

to the gut pathogen *Nosema ceranae*. *PLoS One, 8*, 70182. https://doi.org/10.1371/journal.pone.0070182

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution, 25*(6), 345-353.

Radwan, E. M. M., & Taha, H. S. (2012). Toxic and biochemical effects of different insecticides on the tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae). *Egyptian Academic Journal of Biological Sciences, 4*(1), 1-10. https://doi.org/10.21608/eajbsf.2012.17272

Saddiq, B., Ejaz, M., Shad, S. A., Aslam, M. (2017). Assessing the combined toxicity of conventional and newer insecticides on the cotton mealybug *Phenacoccus solenopsis*. *Ecotoxicology, 26*, 1240-1249.

Shukla, S., Jhamtani, R. C., Dahiya, M. S., Agarwal, R. (2017). Oxidative injury caused by individual and combined exposure of neonicotinoid, organophosphate and herbicide in zebrafish. *Toxicology Reports, 4*, 240-244. https://doi.org/10.1016/j.toxrep.2017.05.002

Sullivan, J. J., & Goh, K. S. (2008). Environmental fate and properties of pyriproxyfen. *Journal of Pesticide Science, 33*, 339-350. https://doi.org/10.1584/jpestics.R08-02

Wang, W., Mo, J., Cheng, J., Zhuang, P., Tang, Z. (2006). Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology, 84*(3), 180-187. https://doi.org/10.1016/j.pestbp.2005.07.002

Williams, G. R., Alaux, C., Costa, C., Csaki, T., Doublet, V., Eisenhardt, D., Brodschneider, R. (2013). Standard methods for maintaining adult *Apis mellifera* in cages under in vitro laboratory conditions. *Journal of Apicultural Research, 52*(1), 1-36. https://doi.org/10.3896/IBRA.1.52.1.04

Wu, J. Y., Anelli, C. M., Sheppared, W. S. (2011). Sublethal effects of pesticides residues in brood comb on worker honeybees (*Apis mellifera* L.) development and longativity. *PLoS One, 6*, 11-17.

Zhu, Y. C., Adamczyk, J., Rinderer, T., Yao, J., Danka, R., Luttrell, R., Gore, J. (2015). Spray toxicity and risk potential of 42 commonly used formulations of row crop pesticides to adult honey bees (Hymenoptera: Apidae). *Journal of Economic Entomology, 108*(6), 2640-2647. https://doi.org/10.1093/jee/tov269

Zufelato, M. S., Bitondi, M. M., Simoes, Z. L., Hartfelder, K. (2000). The juvenile hormone analog pyriproxyfen affects ecdysteroid-dependent cuticle melanization and shifts the pupal ecdysteroid peak in the honey bee (*Apis mellifera*). *Arthropod Structure and Development, 29*(2), 111-119. https://doi.org/10.1016/S1467-8039(00)00023-2