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Toxicity of Commonly Used Insecticides against *Apis dorsata* (Hymenoptera: Apidae) in South Punjab, Pakistan

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WA and AS conceived the research, conducted experiments and collected data. AS and MA designed the experiments. WA, AS, SUF, HAAK and SM collected and prepared the materials. AS supervised the experiments. HAAK, SM and MA analyzed the data. WA, AS, MA and SUF wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

Key words
***Apis dorsata*, Topical bioassay, Surface residual bioassay, Mortality, LT_{50}**

ABSTRACT

Honeybees are considered important pollinators of economically important crops that play a significant role in food security. The population of honeybees is declining due to the non-judicious and extensive use of pesticides. The current study was planned to evaluate the toxicity of five solely (abamectin, cypermethrin, imidacloprid, acetamiprid, and pyriproxyfen) and two mixtures of insecticides (carbosulfan+emamectin benzoate and pymetrozine+dinotefuran) formulations against workers of *Apis dorsata*. Five concentrations of each insecticide were prepared in distilled water and two types of contact bioassay were used i.e. topical bioassay and surface residual bioassay. In topical bioassay, pymetrozine + dinotefuran was found the most toxic insecticide with lower LD_{50} values (0.09 mg/L) followed by abamectin (0.30 mg/L), carbosulfan+emamectin benzoate (0.68 mg/L) and cypermethrin (0.94 mg/L) after 48 h of exposure. Whereas, in surface residual bioassay, pymetrozine+dinotefuran was found the most toxic insecticide with lower LD_{50} values (0.30 mg/L) followed by pyriproxyfen (0.48 mg/L), cypermethrin (0.93 mg/L) and carbosulfan+emamectin benzoate (0.96 mg/L) after 48 h of exposure. In topical bioassay, carbosulfan+emamectin benzoate showed faster mortality with a low LT_{50} value (4.98 h at 2 mg/L) followed by pymetrozine+dinotefuran (6 h at 2 mg/L), cypermethrin (9.01 h at 16 mg/L) and abamectin (9.72 h at 16 mg/L). Whereas, in surface residual bioassay, cypermethrin showed faster mortality with a low LT_{50} value (2.65 h at 16 mg/L) followed by carbosulfan+emamectin benzoate (4.57 h at 2 mg/L), pymetrozine+dinotefuran (8.76 h at 2 mg/L) and abamectin (10.51 h at 16 mg/L). The findings of the present study revealed that insecticide mixtures were the most toxic towards *A. dorsata* followed by cypermethrin and abamectin alone. Therefore, care should be taken during the selection of insecticides for the control of pests in field crops.

INTRODUCTION

Pollination is one of the most important ecosystem services that is provided by insects (Klein *et al*., 2007; Ahmad *et al*., 2021; Khan and Ghramh, 2021). Among insect pollinators, bees are considered important pollinators (Akram *et al*., 2019, 2022; Akram and Sajjad, 2022) especially honeybees as they contribute to more than 80 % of insect pollination (Hu *et al*., 2008; Suwannapong *et al*., 2011). Besides this, honeybees are also important because they provide many economically important products i.e., honey, royal jelly, bee pollen, propolis, bee venom, and wax (Nieh, 1998; Khan *et al*., 2016; Ghramh *et al*., 2019, 2020). The giant honeybee, *Apis dorsata* is larger than other honeybees therefore its foraging range is significantly higher (Crane, 1990). Moreover, *A. dorsata* is considered the efficient pollinator of various agronomic crops, fruits, and vegetables (Saeed and Masood, 2008; Saeed *et al*., 2008; Thangjam *et al*., 2016; Padamshali *et al*., 2018; Said *et al*., 2018; Abrol *et al*., 2019; Das *et al*.,

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2019; Akram *et al*., 2022; Trivedi *et al*., 2022).

There are many pivotal factors i.e., climate change, urbanization, deforestation, industrialization, loss of biodiversity and their habitat, and extensive application of broad-spectrum pesticides that cause a decline in the population of honeybees especially non-domesticated like *A. dorsata* and *A. florea* (Becher *et al*., 2013; Yang *et al*., 2018). In Pakistan, the commercial production of crops mostly depends on the application of pesticides like insecticides, fungicides, weedicides, and entomopathogenic fungi (Basit *et al*., 2013; Qasim *et al*., 2018, 2021). Among these pesticides, most of them are broad-spectrum and have widely been used since the 1940s (Coats, 2012; Panico *et al*., 2022). This extensive use of pesticides not only causes environmental pollution but also adversely affects the biodiversity of non-target organisms and human health (Desneux *et al*., 2007; Aktar *et al*., 2009; Khan *et al*., 2010; Sheikh *et al*., 2011; Khan and Damalas, 2015; Khan, 2020, 2021, 2022).

Pesticide application is usually considered a quick, easy, and inexpensive method for the control of insect pests, weeds, and diseases. Insecticides are the most widely used group of pesticides in Pakistan (Khan, 1998; Khooharo *et al*., 2008). Currently, various classes of insecticides are available in the market for the control of insect pests i.e., organophosphates, carbamates, pyrethroids, neonicotinoids, insect growth regulators (IGRs), botanicals, and some other insecticides derived from different origin that affect insect metabolism and nervous system (Kodandaram *et al*., 2010; Mustafa and Al-Baggou, 2020).

The major factor leading to bees decline when bees pollinate the crops, is the direct or indirect exposure to insecticides, weedicides, fungicides and some other groups of pesticides that are applied to the crops via seed treatments, soil applications and foliar applications (Hooven *et al*., 2013; OPP *et al*., 2014; Hopwood *et al*., 2016). After exposure, pesticides enter the foraging honeybees through two main routes such as ingestion of nectar and pollen and direct contact with sprayed parts of the plant (Hooven *et al*., 2013). In opened flowers, nectar and pollen directly acquire pesticides that are applied via foliar applications. Whereas, in closed flowers, nectar and pollen acquire those pesticides that translocate systemically through the plant vascular system (Kubik *et al*., 1999; Bonmatin *et al*., 2015; Simon-Delso *et al*., 2015). Some pesticides applied during bloom can lead to direct exposure to pollinators (Stanley and Preetha, 2016; Roubik, 2018).

The harmful effects of insecticides have been demonstrated for the honeybees (Laurino *et al*., 2011; Henry *et al*., 2012; Zhu *et al*., 2015; Feazel-Orr *et al*., 2016; Pashte and Patil, 2017) and few wild bee species (Laycock *et al*., 2014; Mallinger *et al*., 2015; Park *et al*., 2015). Insecticides are considered a major factor that has a detrimental effect on honeybee colony characteristics such as development of deformed larvae and pupae, greater risk of pest attack, death of foraging bees, disturbance of antioxidant activities, acetylcholinesterase activity, learning process, behavioral stress and other biological aspects (Decourtye *et al*., 2004; Aliouane *et al*., 2009; Fasasi, 2012; Gill *et al*., 2012; Boily *et al*., 2013; Husain *et al*., 2014; Hayat *et al*., 2018).

It is proven that insecticides are a major factor in the population decline of honeybees (Klein *et al*., 2007) because of the slower detoxification mechanism that leads to the death of honeybees (Husain *et al*., 2014; Jung *et al*., 2020). Besides this, residues of insecticides have also been reported in hive products i.e., honey, bee pollen, propolis, royal jelly and wax which may cause bio-magnification of insecticidal residues at higher trophic levels (Gómez-Ramos *et al*., 2016; Gonzalez-Martin *et al*., 2017; Giroud *et al*., 2019; Hou *et al*., 2019; Tomšič *et al*., 2020).

From Pakistan, many studies reported the effects of various insecticides on honey bees i.e., *Apis mellifera* Linnaeus, 1758 and *A. florea* Fabricius, 1787 by using residual and diet incorporation bioassay (Husain *et al*., 2014; Imran *et al*., 2018; Farooqi *et al*., 2016, 2020; Pervez and Manzoor, 2021; Anwar *et al*., 2022), but there is a scarce literature about the lethal effects of insecticides on *A. dorsata* Fabricius, 1793 (Husain *et al*., 2014). The current study aimed to evaluate the toxicity of seven insecticides among these, two are combinations of different insecticides against *A. dorsata*. The insecticides with different modes of action and usage history in the study area were used (Razaq *et al*., 2013; Iqbal *et al*., 2014; Ali, 2018). Abamectin and emamectin benzoate affect gamma-aminobutyric acid (GABA) receptors resulting in the disruption of nerve impulses (Jansson *et al*., 1997; Campbell, 2012; Casida and Durkin, 2015). Acetamiprid, imidacloprid, and dinotefuran affect the activity of nicotinic acetylcholine receptors (nAChRs) (Simon-Delso *et al*., 2015; Taillebois *et al*., 2018). Carbosulfan causes the inhibition of acetylcholinesterase (ACHE) (Fukuto, 1990). Cypermethrin causes dysfunction of mitochondrial dehydrogenase (Kaisarevic *et al*., 2019). Pyriproxyfen is an insect growth regulator that affects the embryogenesis, morphogenesis, and reproduction of insects (Invest and Lucas, 2008). Pymetrozine is an insect behavior regulator that causes rapid cessation of feeding (Ausborn *et al*., 2005).

The farmers have extensively used these insecticides on crops. Hence, the purpose of the study was to find out the most toxic and harmful insecticide for *A. dorsata* by topical

application and residual bioassay so that recommendations can be made on their proper and judicious use to conserve honeybees in the area.

MATERIALS AND METHODS

Insecticides

Commercially used five insecticides and two combinations of insecticides were purchased from their respective manufacturing companies to check their topical and residual contact toxicity against *Apis dorsata* under laboratory conditions (Table I).

Collection of Apis dorsata

For the collection of *A. dorsata*, three combs containing capped cells were directly collected from trees located at 3 different locations i.e., Cholistan Institute of Desert Studies (29.3784° N, 71.7696° E), Lal Suhanra National Park (29.4426° N, 71.9852° E), and the Agricultural Research Farm (29.3714° N, 71.7652° E). Smoke was continuously provided to calm down the *A. dorsata* and then removed from the comb with the help of a bee brush. Only the sealed brood portion was cut from the tree and placed in the plastic boxes. These combs were shifted to the laboratory of the Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur. Each comb was placed in a separate plastic cage provided with *ad libitum* 50% w/v sucrose solution. These cages were placed in the Incubator or Versatile Environmental Test Chamber MLR-352H (Panasonic Healthcare Co. Ltd.) at 35°C, 65% relative humidity, and without light for the emergence of bees (Williams *et al*., 2013).

Bioassay

The emerged bees were collected from the cages in plastic jars. Prior to conduct the bioassay, the jars were placed in a freezer to immobilize the worker bees by chilling for five min at -20 °C for easy handling (Tutun *et al*., 2020). Two types of contact bioassay were used to check the toxicity of insecticides i.e., topical bioassay and surface residual bioassay. Five doses of each formulated insecticide were tested. Range finding bioassay was used to determine the proper range of doses for each insecticide. Thus, the highest and lowest doses of each insecticide needed to cause 100% and 0% mortality, respectively were determined. Solutions of doses 1, 2, 4, 8, and 16 mg/L for abamectin, cypermethrin, imidacloprid, acetamiprid, and pyriproxyfen and 0.125, 0.25, 0.5, 1, and 2 mg/L for carbosulfan+emamectin benzoate and pymetrozine+dinotefuran were prepared. To prepare the solution of each dose, 10 ml of distilled water was used.

Topical bioassay

For topical bioassays, 2 µl solution was applied on the thorax of a worker bee using the Burkard handheld micro applicator (Burkard Manufacturing Co. Ltd.). Ten newly emerged worker bees were treated with each solution. Control bees were treated with just 2 µl distilled water. Treated worker bees were released in plastic jars, provided *ad libitum* with a 50% w/v sucrose solution, and kept in an Incubator or Versatile Environmental Test Chamber MLR-352H (Panasonic Healthcare Co. Ltd.) at 28±2 °C temperature and 65±5% relative humidity for the duration of the test period.

Table I. List of insecticides used to check their topical and residual toxicity.

Chemical name with formulation	Trade name	Group	Mode of action	Recommended dose/acre	Manufac-turer
Abamectin 1.8% EC	Flight	Avermectin	Stomach	400 ml	ICI
Cypermethrin 25% EW	Cypermethrin	Pyrethroid	Contact and stomach	200 ml	Kanzo AG
Imidacloprid 20% SL	Nexus	Neonicotinoid	Contact and stomach	250 ml	Swat Agro
Acetamiprid 20% SL	Acetamiprid	Neonicotinoid	Contact, stomach, and systemic	100-125 ml	Leader AG
Pyriproxyfen 10.8% EC	Pyriproxyfen	Insect growth regulator	Contact, stomach, and translaminar	400 ml	Swat Agro
Carbosulfan+Emamectin benzoate 25% EW	Locater	Carbamate+ Avermectin	Contact, stomach, and systemic	250 ml	Leader AG
Pymetrozine+Dinotefuran 60% WG	Veyong Jinteng	Pyridine+ Neonicotinoid	Contact, stomach, systemic and translaminar	100 grams	Jaffer Agro

*WG, Wettable granules; EC, Emulsifiable concentrate; SL, Soluble liquid; EW, Emulsions in water.

Residual bioassay

For surface residual bioassay, a 5 ml solution of each dose was poured into 2-L plastic jars, shaken thoroughly for 2 min, and air dried (Radwan and Taha, 2012; Farooqi *et al*., 2016, 2020). Ten newly emerged worker bees were released in each treated jar, provided *ad libitum* with a 50% w/v sucrose solution and kept in an incubator at 28±2 °C temperature and 65±5% relative humidity for the duration of the test period.

Assessment of bees mortality

Mortality for both topical and surface residual bioassays was assessed 6, 12, 24 and 48 h after the application of insecticides. The treated bees mortality was recorded during the bioassay.

Statistical analysis

To determine the LD_{50}, LT_{50}, chi-square and 95% confidence interval, Probit analysis (Finny, 1971) was performed by using IBM SPSS Statistics 26. The percent mortality was calculated by 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

Estimation of LD_{50} in topical bioassay

The toxicity of different insecticides by using topical application is presented in Table II. The combinations of two insecticides were more toxic to *Apis dorsata* than solely used insecticides. Carbosulfan + Emamectin benzoate was found more toxic after 6h with low LD_{50} value (7.66 mg/L) whereas, Pymetrozine+dinotefuran was found more toxic after 12, 24 and 48 h with LD_{50} of 0.48, 0.10 and 0.09 mg/L, respectively. Among solely used insecticides, topical application of cypermethrin was highly toxic after 6 and 12 h whereas after 24 and 48 h abamectin was more toxic (Table II).

Table II. Topical median lethal dose (LD_{50}) of different insecticides against *Apis dorsata*.

Insecticides	Time (h)	LD_{50} (mg/L)	95% CI	df	χ^2	p value
Abamectin	6	20.59	0.014-0.194	4	1.337	0.023
Cypermethrin		17.31	0.011-0.156	4	1.851	0.025
Imidacloprid		19.86	0.014-0.184	4	1.763	0.022
Acetamiprid		22.21	0.001-0.239	4	1.060	0.048
Pyriproxyfen		20.59	0.014-0.194	4	1.337	0.023
Carbosulfan+Emamectin benzoate		7.66	0.002-0.136	4	1.243	0.042
Pymetrozine+Dinotefuran		14.34	0.001-0.136	4	0.930	0.046
Abamectin	12	10.82	0.003-0.136	4	2.247	0.039
Cypermethrin		10.60	0.007-0.141	4	1.859	0.030
Imidacloprid		18.10	0.018-0.178	4	0.870	0.016
Acetamiprid		19.86	0.014-0.184	4	1.763	0.022
Pyriproxyfen		14.34	0.001-0.136	4	0.930	0.046
Carbosulfan+Emamectin benzoate		1.13	0.032-0.298	4	1.580	0.015
Pymetrozine+Dinotefuran		0.48	0.281-1.574	4	0.637	0.005
Abamectin	24	2.43	0.047-0.298	4	0.207	0.007
Cypermethrin		2.44	0.018-0.176	4	0.161	0.016
Imidacloprid		14.34	0.001-0.136	4	0.930	0.046
Acetamiprid		9.31	0.018-0.154	4	0.264	0.013
Pyriproxyfen		6.85	0.005-0.140	4	1.457	0.034
Carbosulfan+Emamectin benzoate		0.68	0.011-0.858	4	0.566	0.045
Pymetrozine+Dinotefuran		0.10	0.202-2.122	4	0.420	0.018
Abamectin	48	0.30	0.008-0.557	4	0.397	0.043
Cypermethrin		0.94	0.030-0.343	4	0.061	0.02
Imidacloprid		2.97	0.064-0.352	4	0.889	0.005
Acetamiprid		2.05	0.044-0.305	4	0.498	0.009
Pyriproxyfen		1.18	0.038-0.380	4	0.584	0.017
Carbosulfan+Emamectin benzoate		0.68	0.011-0.858	4	0.566	0.045
Pymetrozine+Dinotefuran		0.09	0.002-0.356	4	2.192	0.048

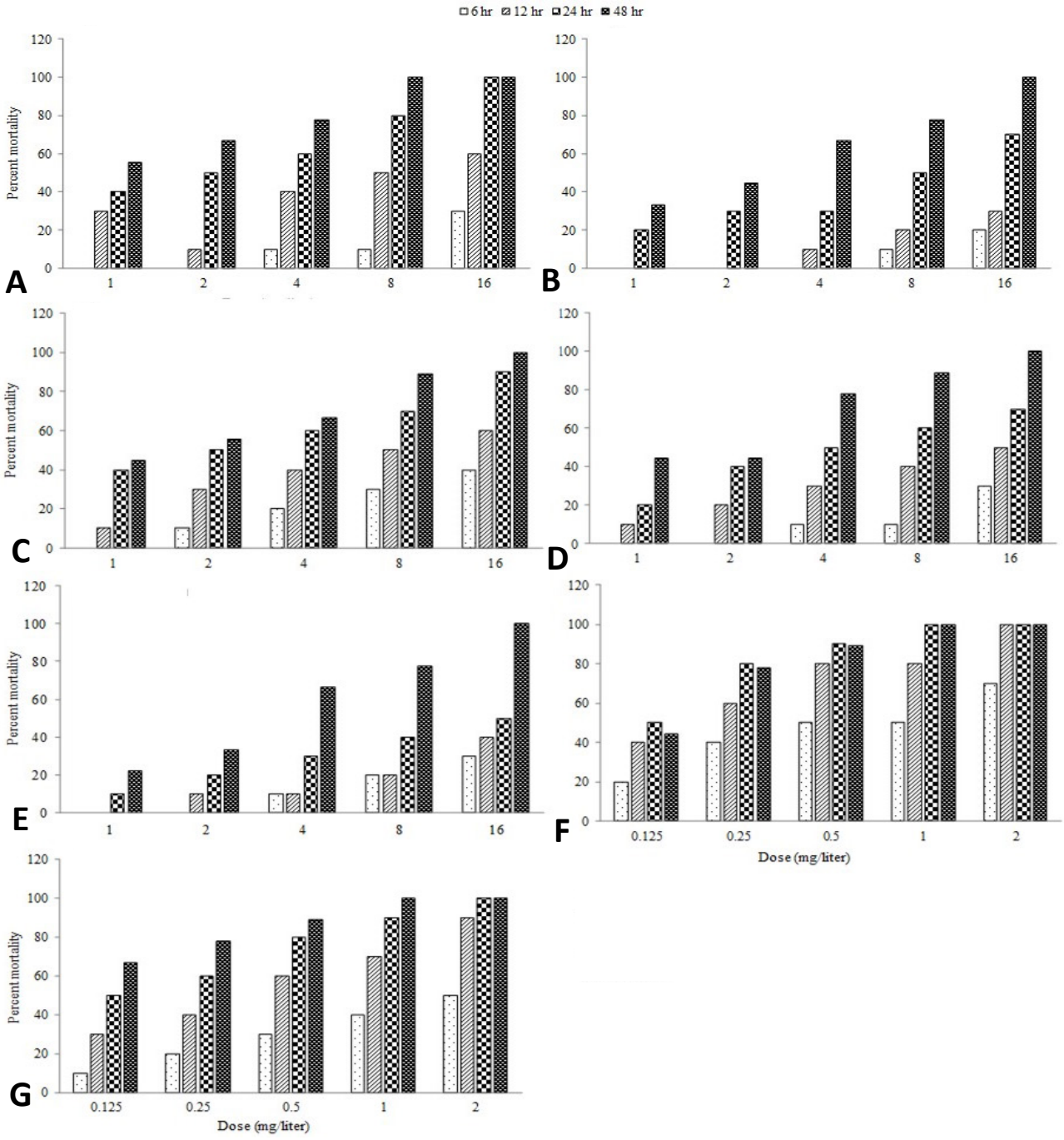

Fig. 1. Corrected percentage mortality of *A. dorsata* after 6, 12, 24 and 48 h of exposure to different insecticides in topical bioassay. (A) Abamectin, (B) Cypermethrin, (C) Imidacloprid, (D) Acetamiprid, (E) Pyriproxyfen, (F) Carbosulfan+Emamectin, (G) Pymetrozine+Dinotefuran.

Percent mortality by topical bioassay

The mortality increased with the increase in dose and exposure time. The lowest mortality was recorded at 1 mg/L ranging from 0% to 56% after 6 and 48 h of exposure to abamectin whereas 100% mortality was recorded at 8 and 16 mg/L after 24 and 48 h of exposure (Fig. 1A). The lowest mortality was recorded at 1 mg/L ranging from 0% to 44% after 6 and 48 h of exposure to cypermethrin

whereas 100% mortality was recorded at 16 mg/L after 48 h of exposure (Fig. 1B). The lowest mortality was recorded at 1 and 2 mg/L ranging from 0% to 33% after 6 and 48 h of exposure to imidacloprid whereas the highest was at 16 mg/L from 30 to 100% after 6 and 48 h of exposure (Fig. 1C). The lowest mortality was recorded at 1 mg/L ranging from 0% to 33% after 6 and 48 h of exposure to acetamiprid whereas the highest was at 16 mg/L from 20 to 100% after 6 and 48 h of exposure (Fig. 1D). The lowest mortality was recorded at 1 mg/L ranging from 0% to 44% after 6 and 48 h of exposure to pyriproxyfen whereas the highest was at 16 mg/L from 30% to 100% after 6 and 48 h of exposure (Fig. 1E). The lowest mortality was recorded at 0.125 mg/L ranging from 20% to 44% after 6 and 48 h of exposure to carbosulfan + emamectin benzoate whereas 100% mortality was recorded at 1 and 2 mg/L after 12, 24 and 48 h of exposure (Fig. 1F). The lowest mortality was recorded at 0.125 mg/L ranging from 10% to 67% after 6 and 48 h of exposure to pymetrozine+dinotefuran whereas 100% mortality was recorded at 1 and 2 mg/L after 24 and 48 h of exposure (Fig. 1G).

Estimation of LD_{50} in residual bioassay

The toxicity of different insecticides by using surface residual bioassay is presented in Table III. The combinations of two insecticides were also more toxic to *Apis dorsata* than solely used insecticides in terms of surface residual bioassay. Pymetrozine+dinotefuran was found more toxic after 6 and 48 h with low LD_{50} values of 2.26 and 0.30 mg/L, respectively. Carbosulfan + Emamectin benzoate was found more toxic after 12 and 24 h with LD_{50} of 0.39 and 0.21 mg/L, respectively. Among solely used insecticides, surface treatment of cypermethrin was highly toxic after 6, 12, and 24 h whereas after 48 h pyriproxyfen was more toxic (Table III).

Percent mortality by residual bioassay

In case of surface residual bioassay, the lowest mortality was recorded at 1 mg/L ranging from 0% to 33% after 6 and 48 h of exposure to abamectin whereas 100% mortality was recorded at 8 and 16 mg/L after 48 h of exposure (Fig. 2A). The lowest mortality was recorded at 1 mg/L ranging from 20% to 44% after 6 and 48 h of exposure to cypermethrin whereas 100% mortality was recorded at 8 and 16 mg/L after 48 h of exposure (Fig. 2B). The lowest mortality was recorded at 1 and 2 mg/L ranging from 0% to 30% after 6 and 48 h of exposure to imidacloprid whereas the highest was at 16 mg/L from 20% to 100% after 6 and 48 h of exposure (Fig. 2C). The lowest mortality was recorded at 1 mg/L ranging from 0% to 33% after 6 and 48 h of exposure to acetamiprid whereas the highest was at 16 mg/L from 20 to 100% after 6 and 48 h

Table III. Residual median lethal dose (LD_{50}) of different insecticides against *Apis dorsata*.

Insecticides	Time (h)	LD_{50} (mg/L)	95% CI	df	χ^2	p value
Abamectin	6	19.05	0.015-0.178	4	3.334	0.021
Cypermethrin		10.93	0.002-0.134	4	0.496	0.045
Imidacloprid		26.20	0.013-0.188	4	3.044	0.090
Acetamiprid		22.21	0.001-0.239	4	1.060	0.048
Pyriproxyfen		19.86	0.014-0.184	4	1.763	0.022
Carbosulfan + Emamectin benzoate		10.60	0.007-0.141	4	1.859	0.030
Pymetrozine + Dinotefuran		2.26	0.142-1.372	4	0.87	0.016
Abamectin	12	10.87	0.015-0.150	4	1.958	0.016
Cypermethrin		5.33	0.000-0.134	4	1.264	0.051
Imidacloprid		15.37	0.014-0.154	4	0.238	0.018
Acetamiprid		15.02	0.037-0.188	4	1.245	0.004
Pyriproxyfen		15.69	0.004-0.141	4	0.279	0.037
Carbosulfan + Emamectin benzoate		0.39	0.172-1.424	4	0.148	0.012
Pymetrozine + Dinotefuran		1.41	0.162-1.251	4	1.255	0.011
Abamectin	24	2.94	0.037-0.207	4	2.098	0.005
Cypermethrin		1.42	0.020-0.185	4	0.982	0.015
Imidacloprid		6.56	0.031-0.175	4	0.967	0.005
Acetamiprid		6.59	0.027-0.170	4	0.770	0.007
Pyriproxyfen		7.46	0.013-0.149	4	1.469	0.020
Carbosulfan + Emamectin benzoate		0.21	0.020-0.366	4	2.472	0.029
Pymetrozine + Dinotefuran		0.54	0.586-2.523	4	0.790	0.002
Abamectin	48	1.70	0.110-0.933	4	0.263	0.013
Cypermethrin		0.934	0.030-0.844	4	0.047	0.035
Imidacloprid		3.96	0.078-0.356	4	0.190	0.002
Acetamiprid		1.99	0.055-0.395	4	0.061	0.010
Pyriproxyfen		0.48	0.012-0.176	4	1.063	0.024
Carbosulfan + Emamectin benzoate		0.96	0.002-0.356	4	2.192	0.048
Pymetrozine + Dinotefuran		0.30	0.590-3.459	4	0.379	0.006

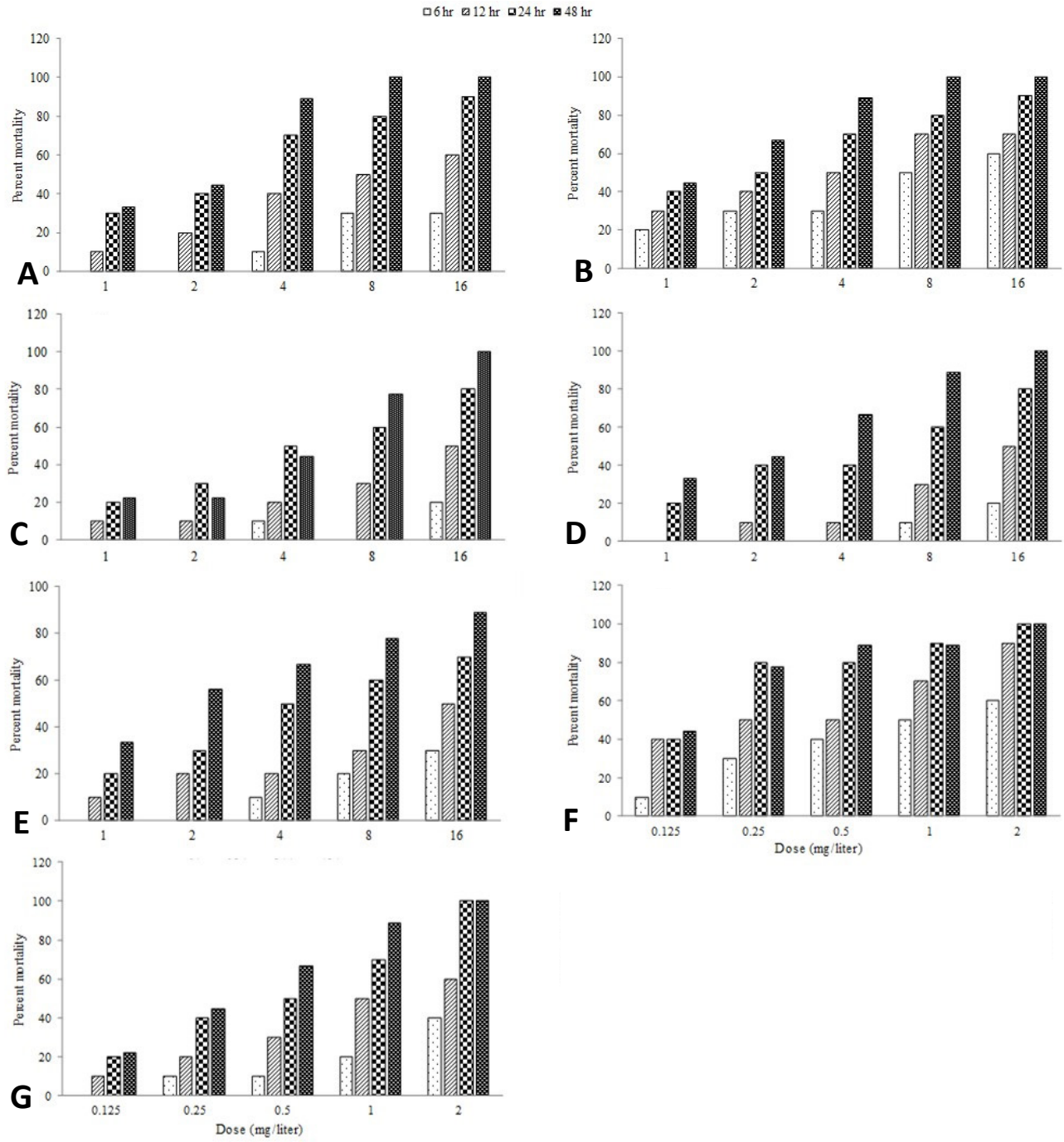

Fig. 2. Corrected percentage mortality of *A. dorsata* after 6, 12, 24 and 48 h of exposure to different insecticides in residual bioassay. (A) Abamectin, (B) Cypermethrin, (C) Imidacloprid, (D) Acetamiprid, (E) Pyriproxyfen, (F) Carbosulfan+Emamectin, (G) Pymetrozine+Dinotefuran.

of exposure (Fig. 2D). The lowest mortality was recorded at 1 mg/L ranging from 0% to 33% after 6 and 48 h of exposure to pyriproxyfen whereas the highest was at 16 mg/L from 30% to 89% after 6 and 48 h of exposure (Fig. 2E). The lowest mortality was recorded at 0.125 mg/L ranging from 10 to 44% after 6 and 48 h of exposure to carbosulfan+emamectin benzoate whereas 100% mortality was recorded at 2 mg/L after 24 and 48 h of exposure (Fig.

2F). The lowest mortality was recorded at 0.125 mg/L ranging from 0% to 22% after 6 and 48 h of exposure to pymetrozine + dinotefuran whereas 100% mortality was recorded at 2 mg/L after 24 and 48 h of exposure (Fig. 2G).

Estimation of LT_{50}

The LT_{50} of different insecticides by using a topical application is presented in Tables IV and V. The results showed that LT_{50} values decreased with an increase in the concentration of insecticides. The minimum LT_{50} values were recorded for abamectin 37.05 h at 1 mg/L, 33.67 h at 2 mg/L and 23.77 h at 4 followed by cypermethrin 43.04 h at 1 mg/L, 34.30 h at 2 mg/L and 24.68 h at 4 mg/L and vice versa at 8 and 16 mg/L. Whereas, the highest LT_{50} values were recorded for imidacloprid (Table IV). The minimum LT_{50} values were recorded for Carbosulfan+Emamectin benzoate 39.74 h at 0.125 mg/L, 9.62 h at 0.25 mg/L, 6.39 h at 0.5 mg/L, and 4.98 h at 2 mg/L whereas at 1 mg/L (3.25 h) for Pymetrozine+Dinotefuran (Table V).

The LT_{50} of different insecticides by using surface residual bioassay is presented in Tables VI and VII. The results showed that LT_{50} values decreased with an increase in the concentration of insecticides. The minimum LT_{50} values were recorded for cypermethrin 44.77 h at 1 mg/L, 25.09 h at 2 mg/L, 14.49 h at 4 mg/L, 5.25 h at 8 mg/L, and 2.65 h at 16 mg/L followed by acetamiprid 50.98 h at 1 mg/L, pyriproxyfen 39.84 at 2 mg/L and abamectin 19.92 h at 4 mg/L, 12.53 h at 8 mg/L and 10.51 h at 16 mg/L. Whereas the highest LT_{50} values were recorded for imidacloprid (Table VI). The minimum LT_{50} values were recorded for Carbosulfan+Emamectin benzoate 42.99 h at 0.125 mg/L, 13.24 h at 0.25 mg/L, 10.27 h at 0.5 mg/L, 5.67 h at 1 mg/L and 4.47 h at 2 mg/L (Table VII).

Table IV. Topical median lethal time (LT_{50}) of different insecticides against *Apis dorsata*.

Insecticides	Dose (mg/L)	LT_{50} (h)	95% CI	df	χ^2	p value
Abamectin	1	37.05	0.009-0.063	3	2.824	0.009
Cypermethrin		43.04	0.011-0.068	3	2.737	0.007
Imidacloprid		57.86	0.005-0.089	3	0.629	0.028
Acetamiprid		50.98	0.012-0.082	3	1.645	0.009
Pyriproxyfen		46.69	0.012-0.073	3	0.684	0.006
Abamectin	2	33.67	0.023-0.083	3	2.981	0.001
Cypermethrin		34.30	0.004-0.055	3	1.501	0.024
Imidacloprid		52.96	0.006-0.066	3	0.840	0.021
Acetamiprid		44.78	0.017-0.083	3	2.748	0.003
Pyriproxyfen		42.80	0.006-0.061	3	2.555	0.016
Abamectin	4	23.77	0.014-0.070	3	1.838	0.003
Cypermethrin		24.68	0.003-0.055	3	1.109	0.028
Imidacloprid		36.44	0.017-0.074	3	0.144	0.002
Acetamiprid		36.94	0.025-0.087	3	0.766	0.001
Pyriproxyfen		27.06	0.017-0.073	3	0.675	0.002
Abamectin	8	14.85	0.037-0.177	3	1.070	0.003
Cypermethrin		14.49	0.011-0.073	3	0.375	0.007
Imidacloprid		29.06	0.015-0.070	3	0.178	0.003
Acetamiprid		28.44	0.020-0.078	3	0.491	0.001
Pyriproxyfen		21.42	0.022-0.086	3	1.244	0.001
Abamectin	16	9.72	0.036-0.294	3	0.234	0.012
Cypermethrin		9.01	0.016-0.156	3	0.004	0.017
Imidacloprid		17.77	0.020-0.095	3	1.461	0.003
Acetamiprid		17.32	0.030-0.138	3	0.150	0.002
Pyriproxyfen		13.67	0.019-0.114	3	0.352	0.006

Table V. Topical median lethal time (LT_{50}) of insecticide mixtures against *Apis dorsata*.

Insecticides	Dose (mg/L)	LT_{50}(h)	95% CI	df	χ^2	p value
Carbosulfan+Emamectin benzoate	0.125	39.74	0.010-0.040	3	1.161	0.230
Pymetrozine+Dinotefuran		40.32	0.002-0.048	3	2.016	0.075
Carbosulfan+Emamectin benzoate	0.25	9.62	0.002-0.052	3	1.417	0.075
Pymetrozine+Dinotefuran		22.01	0.010-0.064	3	0.655	0.008
Carbosulfan+Emamectin benzoate	0.5	6.39	0.003-0.061	3	2.157	0.077
Pymetrozine+Dinotefuran		10.76	0.010-0.073	3	1.530	0.010
Carbosulfan+Emamectin benzoate	1	6.18	0.002-0.311	3	0.051	0.053
Pymetrozine+Dinotefuran		3.25	0.004-0.069	3	2.419	0.027
Carbosulfan+Emamectin benzoate	2	4.98	1.845-2.883	3	0.001	0.667
Pymetrozine+Dinotefuran		6.00	0.002-0.430	3	0.001	0.520

Table VI. Residual median lethal time (LT_{50}) of different insecticides against *Apis dorsata*.

Insecticides	Dose (mg/L)	LT_{50} (h)	95% CI	df	χ^2	p value
Abamectin	1	51.09	0.005-0.063	3	1.801	0.023
Cypermethrin		44.77	0.007-0.043	3	0.237	0.159
Imidacloprid		62.87	0.002-0.059	3	1.081	0.067
Acetamiprid		50.98	0.012-0.082	3	1.645	0.009
Pyriproxyfen		52.96	0.006-0.066	3	0.840	0.021
Abamectin	2	42.80	0.006-0.061	3	2.555	0.016
Cypermethrin		25.09	0.001-0.049	3	0.056	0.065
Imidacloprid		60.84	0.003-0.056	3	2.340	0.074
Acetamiprid		43.04	0.011-0.068	3	2.737	0.007
Pyriproxyfen		39.84	0.013-0.069	3	1.540	0.004
Abamectin	4	19.92	0.023-0.089	3	1.877	0.001
Cypermethrin		14.49	0.011-0.073	3	0.375	0.007
Imidacloprid		41.03	0.001-0.052	3	2.032	0.043
Acetamiprid		35.30	0.024-0.085	3	1.613	0.001
Pyriproxyfen		31.57	0.013-0.068	3	0.943	0.003
Abamectin	8	12.53	0.020-0.137	3	0.056	0.008
Cypermethrin		5.25	0.006-0.104	3	0.424	0.029
Imidacloprid		26.68	0.023-0.082	3	3.363	0.001
Acetamiprid		22.81	0.025-0.091	3	0.695	0.001
Pyriproxyfen		23.81	0.013-0.068	3	0.552	0.004
Abamectin	16	10.51	0.026-0.174	3	0.115	0.008
Cypermethrin		2.65	0.001-0.123	3	0.058	0.052
Imidacloprid		13.80	0.027-0.155	3	0.303	0.005
Acetamiprid		13.80	0.027-0.155	3	0.303	0.005
Pyriproxyfen		14.49	0.011-0.073	3	0.375	0.007

DISCUSSION

The study of pesticide effects on honeybees is vital because of the need to control a wide variety of agricultural pests without deleterious impact on bees. This toxicity study provides valuable information about the harmful effects of insecticides on wild honeybees. In both topical and residual methods, cypermethrin was highly toxic for *Apis dorsata* workers after 6 h (topical: 17.31 mg/L and residual: 10.93 mg/L) and 12 h (topical: 10.60 mg/L and residual: 5.33 mg/L). Pyrethroids are highly toxic insecticides even in small doses for both beneficial and harmful insects (Andreescu *et al*., 2008). Cypermethrin is a highly toxic insecticide to honeybees because it shows its effect within two days (Delabie *et al*., 1985). The age of the bees could be the major factor in the susceptibility of bees. Delabie *et al*. (1985) found that the susceptibility of *A. mellifera* to cypermethrin increases with increasing the age of the bee. Contrarily to our findings, few studies found that cypermethrin was less toxic to honeybees than other insecticides i.e., imidacloprid, fipronil, indoxacarb, malathion, clothianidin and thiamethoxam (Sharma and Abrol, 2005; Jeyalakshmi *et al*., 2011; Pashte and Patil, 2018). These differences could be due to several factors i.e., the origin of the population, age of bees, the effect of post-treatment temperature, and application methods which can influence the toxicity of insecticides.

In the present study, by using the topical application, abamectin was highly toxic after 24 and 48 h with LD_{50} of 2.43 mg/L and 0.30 mg/L, respectively. Baolan *et al*. (2017) concluded that oral abamectin was highly toxic to honeybees, and acute poisoning resulting from high-dose exposure normally led to instant death. Many studies reported that abamectin showed its insecticidal activity after 7 days of application against stored grain insect pests (Kavallieratos *et al*., 2009; Perišić *et al*., 2020). Few factors i.e., exposure interval and increase in dose rate enhanced the efficacy of abamectin (Kavallieratos *et al*., 2009). In the present study, by using the residual application, pyriproxyfen was highly toxic after 48 h with an LD_{50} of 0.48 mg/L. Pyriproxyfen is considered to have low acute toxicity against adult honey bees.

Table VII. Residual median lethal time (LT_{50}) of insecticide mixtures against *Apis dorsata*.

Insecticides	Dose (mg/L)	LT_{50} (h)	95% CI	df	χ^2	p value
Carbosulfan+Emamectin benzoate	0.125	42.99	0.005-0.045	3	1.990	0.125
Pymetrozine+Dinotefuran		62.87	0.002-0.059	3	1.081	0.067
Carbosulfan+Emamectin benzoate	0.25	13.24	0.004-0.059	3	2.055	0.024
Pymetrozine+Dinotefuran		43.72	0.001-0.053	3	0.815	0.043
Carbosulfan+Emamectin benzoate	0.5	10.27	0.008-0.070	3	0.653	0.014
Pymetrozine+Dinotefuran		30.20	0.010-0.063	3	1.056	0.007
Carbosulfan+Emamectin benzoate	1	5.67	0.000-0.064	3	1.462	0.053
Pymetrozine+Dinotefuran		16.62	0.016-0.079	3	1.178	0.003
Carbosulfan+Emamectin benzoate	2	4.57	0.032-0.380	3	0.004	0.098
Pymetrozine+Dinotefuran		8.76	0.027-0.254	3	0.537	0.015

Machado Baptista *et al*. (2009) concluded that the direct application of pyriproxyfen on *A. mellifera* workers led to an LT_{50} value of 466 h. Costa *et al*. (2013) found the LT_{50} value of more than 100 h by direct spraying 0.1 g a.i./L of pyriproxyfen on groups of 10 honeybees. The absorption of pyriproxyfen and subsequently its toxicity mostly depended on the solvent used. For example, acetone might cause an underestimation of the adverse effects due to restricted absorption. Whereas, dimethyl sulfoxide (DMSO) significantly enhances the absorption of pyriproxyfen (Phillips, 2013).

In the present study, the combinations of two insecticides were more toxic to *A. dorsata* than solely used insecticides. Most of the studies reported that the binary mixtures of pesticides are more toxic to honeybees (Iwasa *et al*., 2004; Rinkevich *et al*., 2015; Guseman *et al*., 2016; Zhu *et al*., 2017; Raimets *et al*., 2018; Wang *et al*., 2020a, 2020b, 2020c). In both topical and residual methods, Carbosulfan + Emamectin benzoate showed a knockdown effect and was found more toxic than Pymetrozine+dinotefuran. Carbosulfan and emamectin benzoate showed synergistic effects however having different modes of action. Carbosulfan causes the inhibition of acetylcholinesterase (ACHE) (Fukuto, 1990) whereas, emamectin benzoate affects gamma-aminobutyric acid (GABA) receptors resulting in the disruption of nerve impulse (Jansson *et al*., 1997; Campbell, 2012; Casida and Durkin, 2015). Several studies have reported that carbosulfan and emamectin benzoate are lethal to honey bees (Cang *et al*., 2007; Akca *et al*., 2009; Anwar *et al*., 2022; Deepika *et al*., 2022). The absorption coefficient of avermectins is high and due to this reason, avermectins are considered highly toxic to bees. Emamectin benzoate is more toxic due to its lower detoxification during metabolism and it can penetrate more through insect cuticle (Abdu-Allah, 2011; Lumaret *et al*., 2012). Anwar *et al*. (2022) reported that emamectin benzoate caused high mortality in *A. florea* at 12h, 24h and 48h with LC_{50} values of 2.01, 1.67, and 1.02 g/mL after 12 h, 24 h, and 48 h when incorporated with diet. However, field trials conducted on emamectin benzoate have shown a low lifespan in the sunlight. Thus, it can be added to the integrated pest management program depending on the location (Lumaret *et al*., 2012).

By using the topical application, the minimum LT_{50} values were recorded for abamectin whereas, by residual method, the minimum LT_{50} values were recorded for cypermethrin. The main factors that affect the mortality in bioassays are the choice of insecticide bioassay response, the stage of the insects, health of the organism, bioassay environment, method of application, diet, sample size, sampling, and operator skill (Ball, 1981). Aljedani (2017) found that abamectin has an adverse effect on *A. mellifera* that causes faster mortality with a minimum LT_{50} value of 21.026 h as compared to deltamethrin which has an LT_{50} value of 72.011 h. Anwar *et al*. (2022) also recorded the minimum LT_{50} values i.e., 5.09 h at 10 µg/ml and 5.63 h at 40 µg/ml for emamectin benzoate against *A. florea*. In the present study, the minimum LT_{50} values were recorded for carbosulfan + emamectin benzoate. Carbosulfan and emamectin benzoate are considered lethal insecticides to beneficial insects because of their lower detoxification during metabolism (Abdu-Allah, 2011; Lumaret *et al*., 2012; Deepika *et al*., 2022).

CONCLUSION

Apis dorsata, the giant honeybee is considered the efficient pollinator in the studied locality of the Punjab province, Pakistan. It is crucial not only for providing ecological services but also for honey production. However, the extensive use of broad-spectrum pesticides significantly reduces their population. Cypermethrin and abamectin are highly toxic to *A. dorsata* workers due to low LD_{50} values. Nowadays, insecticide mixtures are commonly used for the efficient control of pests that have a lethal effect on honeybees. It is concluded that all insecticide combinations or certain classes of insecticides when combined yielded a toxic effect on bees. Carbosulfan + Emamectin benzoate was found more toxic to *A. dorsata*. Due to field application of pesticides during bloom, their residues persist in pollen grains resulting in behavioral changes, physiological changes, and mortality. Honeybees have minor adaptations so care should be taken during pesticide application to conserve their population and associated environmental benefits.

DECLARATIONS

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Ethical statement and IRB approval

The study was approved by the Departmental Research Committee of the Department of Entomology, Faculty of Agriculture and Environment, The Islamia University of Bahawalpur.

Statement of conflict of interest

The authors have declared no conflict of interest.

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