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# Comparative Toxicity and Resistance to Insecticides in *Musca domestica* from Some Livestock Farms of Punjab, Pakistan

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**MKM and HAAK designed and supervised the study. NA and SW collected flies and conducted the experimental work. SYK assisted and guided in experimental work and analyzed the data. NA and MKM wrote the manuscript. HAAK proofread the manuscript.**

**Key words**
**Toxicity, Resistance, Insecticides, *Musca domestica*, Livestock**

## ABSTRACT

The *Musca domestica* is a serious hygienic pest of poultry, humans, and livestock facilities with the immense ability for resistance development against chemical insecticides. The current study aimed to evaluate the susceptibility and resistance status of *M. domestica* against different classes of insecticides. For this purpose, adult *M. domestica* populations were collected from five different localities of Sargodha division (Sargodha, Khushab, Jauharabad, Mianwali, and Bhakkar), Punjab, Pakistan, and tested against selected insecticides. The resistance ratios (RR) at $LC_{50}$ ranged from 10.32-35.37 folds for deltamethrin, 17.49-38.13 folds for fipronil, and 10.70-18.81 folds for chlorpyrifos. The RR values at $LC_{50}$ for imidacloprid and pyriproxyfen ranged from 4.35-28.0 and 10.56-21.45 folds, respectively. The study showed varying levels of resistance in *M. domestica* populations from area to area and from insecticide to insecticide. Therefore, to control resistance development in *M. domestica* from livestock facilities, inappropriate and excessive use of insecticides must be controlled through proper mechanisms and strategies.

## INTRODUCTION

*Musca domestica* is found in almost all habitats but is more common in warmer areas and is a pest of poultry and dairy (Scott *et al*., 2000; Kaufman and Rutz, 2002; Kaufman *et al*., 2005; Acevedo *et al*., 2009). It is a potential vector for various zoonotic diseases like typhoid, dysentery, cutaneous diphtheria, and trachoma and transmits more than 100 pathogens like viruses, helminth, protozoa, and bacteria etc. (Kumar *et al*., 2013). In Pakistan, climatic conditions are favorable for reproduction and development of *M. domestica* and warmer summer is optimal for its shorter life cycle. The average life span of a *M. domestica* is 21 days. It has a fast reproductive rate and a single female can lay up to 900 eggs (Abbas *et al*., 2014; Khan and Akram, 2014; Scott *et al*., 2014).

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Mostly chemical control methods are used to control flies. These methods play a vital role during the disease epidemics as they provide rapid and effective control. Different types of insecticides are used to overcome vector-borne diseases (World Health Organization, 2006). Four main groups of insecticides that are used to control these disease vectors are organophosphate, carbamates, neonicotinoids, and pyrethroids (World Health Organization, 2006; Ahmad *et al*., 2009; Tian *et al*., 2011; Khan *et al*., 2017). The insecticidal spray plays an integral role in sustainable livestock production and agriculture to control insect pests (Hemingway and Ranson, 2000). Unfortunately, insecticides are not used judiciously. Farming communities do not follow the labelled recommendations of insecticides and frequently apply overdose of the insecticide (Khan *et al*., 2013). Therefore, due to repetitive and sequential use of the same insecticide or an insecticide with the same mode of action, insect pests such as *M. domestica* develop resistance against these insecticides (White *et al*., 2007).

*Musca domestica* has various biological characteristics which help it in resistance development, for example, their ability to cope with different environmental conditions, increased fecundity, and a short development period (Khan *et al*., 2013; Kaufman *et al*., 2010). Insecticidal resistance turned into an alarming situation, as in the last 30 years the number of resistant insect pests has increased a lot and in 2009 the number of resistant insect species was 600 (Ambethgar, 2009). According to the pesticides resistance database, from the 20 top resistant arthropod pests, *M. domestica* is at number one (Whalon *et al*., 2012).

Considering the significance of *M. domestica* and its ability to develop resistance against various insecticides, the present study was designed to monitor the resistance level in *M. domestica* collected from different localities of Punjab Pakistan viz., Khushab, Jauharabad, Mainwali, and Bhakkar, against the insecticides deltamethrin, fipronil, chlorpyrifos, imidacloprid, and pyriproxyfen. The objectives of the study were to investigate the resistance status of *M. domestica* against these selected insecticides and to find out more effective insecticides in controlling this insect pest from study area.

## MATERIALS AND METHODS

*Insects*

Adult *Musca domestica* populations were collected from five different areas of division Sargodha viz., Sargodha (32° 4' 56'' N, 72° 40' 8.8608'' E), Khushab (32°17'48 N, 72°21'9 E), Jauharabad (32.2899° N, 72.2719° E), Mainwali (32.5839° N, 71.5370° E), and Bhakkar (31.6082° N, 71.0854° E), and transferred to the laboratory for rearing. The adult flies were kept in mesh cages (40 × 30 × 30 $cm^3$) and fed with 1:1 ratio of powdered milk, icing sugar and water. The rearing medium for maggots was prepared in the laboratory in semi-transparent plastic jars by soaking cotton oilcake in water in order to develop a foul smell. Once the foul smell was developed, the collected flies were transferred to rearing media and the second instar larvae were used for larvicidal bioassay. The insects were maintained at 60 percent relative humidity, 25°C, and 12 h light/dark cycle. Bioassays were performed on the F1 generation flies collected from the field. The laboratory susceptible strain (lab) was acquired from places where insecticide use was low. The lab strain was not totally susceptible, but its $LC_{50}$ values were significantly lower, thus it was utilized to compare with field strain.

*Insecticides*

For residual bioassay, five insecticides of the technical grade deltamethrin, fipronil, chlorpyrifos, imidacloprid, and pyriproxyfen were used. For each insecticide, four different concentrations were prepared i.e. Deltamethrin: 0.125, 0.185, 0.25 and 0.375 ml/2ml; Fipronil: 0.52, 1.05, 2.1 and 3.15 ml/2ml; Chlorpyrifos, 3.35, 4.2, 5 and 7.5 ml/2ml; Imidacloprid: 2.52, 5.4, 10.2 and 21 ml/2ml: Pyriproxyfen: 0.84, 1.26, 1.68 and 2.5 ml/2ml. The control groups were treated with water and acetone separately.

*Adulticidal bioassay technique*

To check the resistance status of *M. domestica* a residual bioassay technique was used. For preparation of impregnated filter papers, World health organization recommended (WHO, 2006) procedure was used. Filter papers were cut according to the size (12×14cm) of WHO recommended bioassay cones. Concentrations (dilutions) of each insecticide were prepared by using acetone as solvent. Two ml of each dilution was necessary for impregnation of filter paper. Therefore, each filter paper was dipped in 2ml of dilution and then dried. For control groups filter papers were dipped in acetone and water separately.

Bioassays for field strains and lab strain were performed on the F1 generation. The lab strain (laboratory susceptible strain) originated from an area where the use of insecticides was low and the strain was maintained in the lab without exposure to any kind of insecticide. Lab strain was not completely a susceptible strain but had very low $LC_{50}$ values for insecticides. This particular strain was used as a baseline strain for resistance (Ahmed and Arif, 2009).

Insecticide-treated and control groups were separated and each susceptibility tube contained 15 flies. Sugar, condensed milk, and water paste were provided to both groups till satiation. In each treated group, flies were exposed to insecticides impregnated filter paper while in the control groups; flies were given exposure to acetone and distilled water impregnated filter papers separately. Flies were exposed for one hour before being transferred to clean bioassay susceptibility tubes for observation of next 48 h. Data on mortality were collected every 12 h interval till 48 h. The flies that survived 48 h after being exposed to an insecticide were classified as resistant. According to Kaufman *et al*. (2010b) water containing 10% sugar was provided to flies during 48 h observation period. Three replicated experiments were performed for insecticides and control groups.

*Larvicidal bioassay technique*

To check the resistance status of *M. domestica* larvae against Insect growth regulator, a residual bioassay technique was used with filter papers of the same size as mentioned in the adulticide bioassay. Third instar larvae were used for bioassays in order to get same age of maggots. Each filter paper was wet with 2ml of solution. The filter paper was then allowed to dry, cut with the help of scissors according to a diameter of the petri dish (9.2 mm). Each petri dish was labelled and was provided with cotton soaked in a solution containing caster sugar, powdered milk, and yeast. Fifteen maggots were used in each experiment. One h exposure of insecticide impregnated filter paper was given in each petri dish and after one h, the filter paper was removed and larvae were observed for 24 h. Those larvae that were unable to move and changed their color from skin to dark brown after 24 h of exposure to insect growth regulator (IGR) were considered dead, while those that survived were considered as resistant. All the bioassay experiments were repeated thrice.

*Data analysis*

To assess the values of $LC_{50}$, slope, and Chi-square, the probit analysis was done using SPSS software; data were corrected using the Abbott formula (Abbott, 1925). Resistance ratios were obtained by comparing $LC_{50}$ values of the field and lab strains.

## RESULTS

*Musca domestica* from all localities showed less mortality against different concentrations of deltamethrin. At 0.125 mg/2ml concentration, the highest mortality (31.1%) in *M. domestica* population was observed from Bhakkar followed by 26.6%, 6.6%, 4.6%, and 4.4%, from Mianwali, Jauhrabad, Khushab, and Sargodha, respectively. The almost similar trend with slight variation was also observed at 0.185 mg/2ml concentration, as *M. domestica* population from Bhakkar revealed highest mortality (42.2%) whereas lowest mortality (8.8%) was observed from Khushab. *Musca domestica* population from Mianwali, Jauhrabad and Sargodha showed following trend, 33.3%, 11.9%, and 11.1% mortality, respectively. At 0.25 mg/2ml concentration 55.5% mortality was observed from Bhakkar followed by Mianwali (42.2%), Jauhrabad (26.6%), Khushab (22.2%), and Sargodha (17.7%). At 0.375mg/2ml concentration the same mortality trend was observed. The lab strain showed slight increase in the mortality with increase in concentration of deltamethrin. The results showed that the resistance ratio (RR) for deltamethrin ranged 10.32-35.37 folds. The strain collected from Sargodha revealed the highest resistance ratio, whereas the strain collected from Bhakkar showed the lowest resistance ratio (Table I).

Flies from all localities showed moderate mortality against different concentrations of fipronil. At 0.52 mg/2ml concentration the *M. domestica* from Mianwali exhibited highest mortality (56.6%) followed by, Jauharabad, Bhakkar, Khushab and Sargodha, respectively. At 1.05 mg/2ml concentration the similar trend was also observed as *M. domestica* population from Mianwali revealed highest mortality (65.5%) followed by Khushab, Jauharabad, Bhakkar, and Sargodha. At 2.1 mg/2ml concentration, 69.9% was recorded from Mianwali whereas *M. domestica* from Jauharabad, Khushab, Bhakkar and Sargodha revealed 63.8%, 63.3%, 61.1% and 59.9% mortality, respectively. At 3.15mg/2ml trend of mortality was also similar with slight variation in Jauharabad, as highest mortality (73.3%) was recorded from Maianwali whereas *M. domestica* from Sargodha showed lowest mortality (64.4%). *Musca domestica* from Bhakkar, Jauharabad, and Khushab exhibited following trend 69.9%> 68.8%>67.7%. The lab strain showed slight increase in mortality with increase in concentration i.e., 0.52 mg/2ml (86.6%), 1.05 mg/2ml (88.8%), 2.1 mg/2ml (92.2%), and 3.15 mg/2ml (95.5%) (Supplementary Table II). Resistance ratio ranged 17.49-38.13 folds. Of five field strains tested against fipronil, four had a moderate level of resistance (23.28-38.17 folds) and one had a low level of resistance (17.49 fold) (Table I).

For chlorpyrifos at 3.35mg/2ml concentration *M. domestica* from Bhakkar showed highest mortality (81.1%), followed by Mianwali Jauharabad, Khushab and Sargodha. At 4.2mg/2ml concentration the trend of mortality as also same as highest mortality (83.3%) was observed from Bhakkar whereas lowest mortality (77.7%) was recorded from Sargodha. *Musca domestica* from Mianwali, Jauharabad, and Khushab revealed 81.1%, 80.0% and 79.9% mortality, respectively. At 5mg/2ml concentration 85.5% mortality was observed from Bhakkar followed by 83.3%, 82.2%, 81.1%, and 79.9% from Mianwali, Jauharabad, Khushab, and Sargodha respectively. At 7.5mg/2ml concentration the trend of mortality was also same, Bhakar > Mianwali > Jauharabad> Khushab >Sargodha (80%). The lab strain showed slight increase in mortality with increase in concentration (Supplementary Table III). For chlorpyrifos, resistance ratio ranged 10.70-18.81. The highest value of RR was found in the flies from Sargodha and lowest value of RR was found in the flies collected from Khushab (Table I).

For imidacloprid at 2.52mg/2ml concentration *M. domestica* from Bhakkar revealed highest mortality (81.1%) followed by Mianwali, Khauhab, Sargodha and Jauharabad (69.9%). At 5.2mg/2ml concentration the trend of mortality was also same with slight variations, as highest mortality (85.5%) was observed from Bhakkar, whereas *M. domestica* from Sargodha revealed lowest mortality, (74.4%). Housefly from Mianwali, Khushab and Jauharabad showed 77.7%, 76.6% and 75.5% mortality, respectively. At 10.2mg/2ml concentration 88.8% mortality was observed from Bhakkar, followed by 80%, 79.9%, 79.1%, and 78.8%, from Khushab, Sargodha, Mianwali, and Jauharabad, respectively. At 21mg/2ml concentration, the highest mortality was observed from Bhakkar (91.1%), whereas lowest mortality was observed from Jauharabad (81.2%).

**Table I. Toxicity of insecticides against *M. domestica* from livestock farms in Sargodha, Punjab, Pakistan.**

| Insecticides | Populations | N | $LC_{50}$[µgm/ml] (95%Cl) | Slope (± SE) | $\chi^2$ | df | RR |
|---|---|---|---|---|---|---|---|
| Deltamethrin | Lab Strain | 225 | 11.11 (8.67-16.93) | 0.66(±0.39) | 0.10 | 2 | |
| | Sargodha | 225 | 393.04(378.37-418.92) | 2.02(±0.72) | 0.27 | 2 | 35.37 |
| | Khushab | 225 | 278.51(201.32-318.52) | 2.62(±0.72) | 0.69 | 2 | 25.06 |
| | Jauharabad | 225 | 250.94(186.34-298.56) | 2.54(±0.61) | 0.84 | 2 | 22.58 |
| | Mianwali | 225 | 209.44(167.58-245.62) | 1.17(±0.55) | 0.15 | 2 | 18.85 |
| | Bhakkar | 225 | 114.72 (88.59-156.23) | 1.88(±0.55) | 0.16 | 2 | 10.32 |
| Fipronil | Lab Strain | 225 | 6.96 (4.78-8.48) | 0.67(±0.29) | 0.49 | 2 | |
| | Sargodha | 225 | 265.43(209.82-298.71) | 0.44(±0.21) | 0.26 | 2 | 38.13 |
| | Khushab | 225 | 187.98(165.52-218.62) | 0.49(±0.21) | 0.12 | 2 | 27.00 |
| | Jauharabad | 225 | 162.06(139.24-197.38) | 0.46(±0.21) | 0.16 | 2 | 23.28 |
| | Mianwali | 225 | 121.79 (89.67-143.72) | 0.56(±0.21) | 0.12 | 2 | 17.49 |
| | Bhakkar | 225 | 212.08(187.62-243.73) | 0.51(±0.210 | 0.83 | 2 | 30.45 |
| Chlorpyrifos | Lab Strain | 225 | 12.11 (8.23-14.92) | 0.53(±0.65) | 0.15 | 2 | |
| | Sargodha | 225 | 227.90(196.63-256.79) | 0.79(±0.56) | 0.007 | 2 | 18.81 |
| | Khushab | 225 | 129.60(103.02-155.78) | 0.68(±0.5)6 | 0.20 | 2 | 10.70 |
| | Jauharabad | 225 | 140.81(121.38-168.47) | 0.71(±57) | 0.04 | 2 | 11.62 |
| | Mianwali | 225 | 152.78(137.58-178.82) | 0.79(±59) | 0.03 | 2 | 12.61 |
| | Bhakkar | 225 | 132.15(115.29-162.95) | 0.80(±60) | 0.04 | 2 | 10.91 |
| Imidacloprid | Lab Strain | 225 | 6.44 (13.82-18.62) | 0.38(±0.24) | 0.09 | 2 | |
| | Sargodha | 225 | 169.76(137.62-198.68) | 0.46(±0.20) | 0.10 | 2 | 26.36 |
| | Khushab | 225 | 180.81(167.27-209.97) | 0.48(±0.20) | 0.01 | 2 | 28.07 |
| | Jauharabad | 225 | 109.07 (89.37-135.63) | 0.39(±0.20) | 0.08 | 2 | 16.93 |
| | Mianwali | 225 | 28.06 (21.82-47.37) | 0.32(±0.20) | 0.03 | 2 | 4.35 |
| | Bhakkar | 225 | 74.01 (58.62-89.06) | 0.41(±0.21) | 0.04 | 2 | 11.49 |
| Pyriproxyfen | Lab Strain | 225 | 3.21 (1.98-5.11) | 0.49(±0.46) | 0.04 | 2 | |
| | Sargodha | 225 | 43.80 (32.79-63.65) | 0.63(±0.40) | 0.21 | 2 | 13.64 |
| | Khushab | 225 | 33.90 (49.04-75.86) | 0.62(±0.41) | 0.18 | 2 | 10.56 |
| | Jauharabad | 225 | 68.87 (49.04-75.86) | 0.85(±0.42) | 0.18 | 2 | 21.45 |
| | Mianwali | 225 | 59.23 (41.06-76.83) | 0.86(±0.43) | 0.17 | 2 | 18.45 |
| | Bhakkar | 225 | 63.40 (47.82-79.62) | 0.72(±0.40) | 0.14 | 2 | 19.75 |

*Musca domestica* population from Khushab, Sargodha and Mianwali showed following trend 84%>83.3%>82.2%. The lab strain showed slight increase in mortality with increase in concentration i.e., 2.52mg/2ml (84.4%), 5.2mg/2ml (86.6%), 10.2mg/2ml (90%) and 21mg/2ml (91%) (Supplementary Table IV). Resistance ratio ranged 4.35-28.07 folds. Of five field strains tested against imidacloprid, two had a moderate level of resistance (26.36-28.07 folds), two had low level of resistance (11.43-16.93 folds) and one had a very low level of resistance (4.35 fold) (Table I).

For pyriproxyfen at 0.84mg/2ml, concentration *M. domestica* from Mianwali revealed highest mortality (77.7%) followed by Jauharabad, Khushab, Bhakkar, and Sargodha. At 1.26mg/2ml concentration the highest mortality was observed from Khushab (79.9%) whereas house flies from Bhakkar exhibited lowest mortality (75.5%). *Musca domestica* from remaining cities showed following trend i.e., Mianwali>Jauharabad >Sargodha. At 1.68mg/2ml concentration *M. domestica* from Mianwali showed highest mortality (84.4%), followed by 81.1%, 80.9%, 80.0%, and 78.8% from Jauharabad, Khushab, Sargodha, and Bhakkar, respectively. At 2.5 mg/2ml concentration *M. domestica* from Mianwali exhibited

87.7% mortality followed by Juaharabad (86.6%), Bhakkar (83.9%), Khushab (83.3%) and Sargodha (81.1%). whereas lab strain exhibited 87.7% mortality (Supplementary Table V). Resistance ratio ranged from 10.56-21.45 folds. The highest value of RR was recorded in the house flies collected from Jauharabad and lowest value of RR was found in flies collected from Khushab (Table I).

The present study showed varying level of resistance in house flies populations from area to area and insecticide to insecticide. In Sargodha, chlorpyrifos exhibited highest mortality followed by imidacloprid, pyriproxyfen, fipronil and deltamethrin. In Khushab, the following trend of mortalities was observed, chlorpyrifos > pyriproxyfen > imidacloprid > fipronil > deltamethrin. In Jauharabad, again chlorpyrifos was found to be more effective as it showed highest mortality rate followed by pyriproxyfen, Imidacloprid, fipronil, and deltamethrin. The following of mortalities were observed from Mianwali, chlorpyrifos > pyriproxyfen)> imidacloprid > fipronil > deltamethrin. In Bhakkar, imidacloprid exhibited highest mortality followed by chlorpyrifos, pyriproxyfen, fiproniland deltamethrin. Overall deltamethrin, chlorpyrifos and Imidacloprid revealed highest mortalities from Bhakkar as compared to other localities whereas fipronil and pyriproxyfen are found to be more effective in Mianwali.

## DISCUSSION

Resistance against insecticides is a serious issue to control the pests of health and agriculture (Scott *et al*., 2000) and as a result, their application rates have increased. Other biological features that enhance resistance development include adaptation to varied settings, shorter developing period, higher fecundity and cross-resistance (Kaufman *et al*., 2010a). Many scientific publications on the development of pesticide resistance in the *M. domestica* may be found all over the world (Scott *et al*., 2000; Tang *et al*., 2002; Kristensen and Jespersen, 2003; Kristensen *et al*., 2004; Deacutis *et al*., 2006; Acevedo *et al*., 2009; Bell *et al*., 2010; Memmi, 2010; Kaufman *et al*., 2010b; Khan *et al*., 2013).

The *M. domestica* populations were collected from five different localities of Punjab, Pakistan, and tested for resistance against insecticides belonging to pyrethroid, organophosphate, neonicotinoid, phenylpyrazoles, and Insect growth regulator groups. Varied resistance level was noted in *M. domestica* populations collected from different livestock facilities.

The current bioassay results showed a low to moderate resistance level to deltamethrin. *M. domestica* resistance against deltamethrin insecticide has been evaluated from different countries by different scientists such as Cao *et al*. (2006) from Northern China, Akinar and Coglar (2012) from Turkey, Sarifard and Safdari (2013) from Iran, Khan *et al*. (2017) from Punjab, Pakistan, and Wang *et al*. (2019) from Zheijang, China. The widespread use of pyrethroid insecticides to control livestock pests is due to their possible low mammalian toxicity and rapid mode of action. However, resistance can drive through several mechanisms such as modification of target site. Target site resistance is mainly linked with interference of electronic signaling in the nervous system which can lead to paralysis and ultimately death of insect (Brito *et al*., 2013). Single and multiple genes mutation may result in target site resistance which is usually referred as knockdown resistance (*kdr*). The *VGSC* (Voltage gate sodium channals) gene mutations also lead to *kdr*. The *kdr* resistance mechanism is well studied in insect pests e.g., *M. domestica* and *Aedes aegypti* (Hemingway and Ranson, 2000; Karunaratne *et al*., 2018) and could be a good predictor of efficiency of pyretheroids by genotyping of mutant *kdr* allele.

*Musca domestica* in Punjab, Pakistan is exposed to a number of chemicals that varied in chemical nature, and most dairies in Punjab, Pakistan have an open or semi-open design. Pour-on and dipping approaches have also been used to control certain dairy pests. Pyrethroids, particularly cypermethrin or deltamethrin, have been considered as viable pesticides in Pakistan for the dairy and poultry pest management are employed through pour-on, dipping, and spraying methods (Muhammad *et al*., 2008). When insecticides are sprayed directly on animals, *M. domestica* may be exposed to chemical residues during the day. Pest management was mostly done with leftover pesticides from crop farming, and the volume of pesticide applications was determined by chemical availability. These techniques lead to an abuse of dosages, which may be a contributing factor in the resistance development in dairy pests to various pesticide classes.

The current bioassay results demonstrated a low to moderate level of resistance against fipronil. *M. domestica* resistance to pesticides with unique mechanisms of action has already been documented from various regions (Kaufman *et al*., 2006; Acevedo *et al*., 2009; Memmi, 2010). Resistance against fipronil has been documented in various insect pests, including *Sogatella furcifera, Spodoptera litura* and *Plutella xylostella* (Sayyed and Wright, 2004; Ahmad *et al*., 2009; Tang *et al*., 2010) as well as *M. domestica* (Wen and Scott, 1999; Liu and Yue, 2000; Kristensen *et al*., 2004). The main cause of resistance against fipronil may be due to their unsuitable and injudicious use, as well as poor application tactics, and probable cross-resistance mechanisms.

Low level of resistance to chlorpyrifos was found in *M. domestica* from different cities of Sargodha, in current study. In Punjab, Pakistan, organophosphate pesticides have been used to manage a variety of agricultural pests. Various workers from Punjab, Pakistan have discovered higher levels of resistance against these insecticides in *M. domestica*, *Laodelphax striatellus*, *Aedes albopictus*, *Culex pipiens* and *Spodoptera litura* (Saleem *et al*., 2008; Khan *et al*., 2011; Shad *et al*., 2012; Khan *et al*., 2013) and also worldwide resistance has been reported against them (Cheikh *et al*., 2009; Wang *et al*., 2010). The presence of pesticide resistance in *M. domestica* populations was discovered in this study, which could be attributable to a lack of a structured management for livestock pests, as well as farmers' habits of applying self-experiences on an irregular basis. In Pakistan, mostly dairy farms are surrounded by different crops. The widespread use of organophosphate pesticides on crops and dairy facilities is another likely source of resistance.

Resistance against imidacloprid was noted in *M. domestica* populations from different cities in Pakistan in the current study, ranging from very low to moderate. Neonicotinoids have been widely utilized for pest management in field crops, dairies, and poultry facilities around the world, but they were just introduced in the late 1990s (Kaufman *et al*., 2010; Basit *et al*., 2011; Khan *et al*., 2013). Resistance to neonicotinoid has been reported in a variety of pests, including *Bemisia. tabaci, Colorado potato beetle, and Leptinotarsa decemlineata, P. xylostella, S. litura, planthopper, Nilaparvata lugens,* and *M. domestica* (Elbert and Nauen, 2000; Mota-Sanchez *et al*., 2006; Sayyed and Crickmore, 2007; Wang *et al*., 2008; Kaufman *et al*., 2010; Basit *et al*., 2011; Abbas *et al*., 2012). The current study's findings demonstrate the occurrence of pesticide resistance in *M. domestica* populations, which could be related to a lack of a comprehensive dairy pest management plan. The resistance against imidacloprid may be due to its excessive use in dairy farms to control *M. domestica*.

Resistance to pyriproxyfen was found low to a moderate level in *M. domestica* populations from different cities of Pakistan in this study. For more than a decade, Insect growth regulator insecticides have been on the market in Punjab, Pakistan. The insecticide exposure to *M. domestica* may be different due to the architecture of poultry farms (semi-open or closed) or dairy farms (open or semi-open) in Pakistan (Khan *et al*., 2013). Therefore, it might be possible that they have been pre-exposed to the Insect growth regulators during feeding, flight, or breeding activities around open farms which might be the reason to develop resistance against Insect growth regulators (Khan *et al*., 2013). Improper monitoring, planning or lack of management plan might be the reason behind resistance development (Khan *et al*., 2013) and thus supported the present study results. The results found by Shah et al. (2015) were also similar to the present study results as they reported that the life history traits of *M. domestica* inherited from the previous generations exposed to larvicides; the *M. domestica* developed resistance against Insect growth regulator methoxyfenozide. Ishaaya *et al*. (2003) reported that the colony of whitefly showed 1200-2000 folds resistance against pyriproxyfen as compared to the susceptible colony.

To control resistance development in *M. domestica,* unsuitable and excessive use of insecticides must be controlled. Mosaic, rotational, periodic application strategies must be used to delay resistance development. Insecticide and its dose must be decided after consulting with an entomologist. WHO recommended dose should be used. Training on insecticide usage must be given to dairy farmers. Systematic and comprehensive strategies must be developed to control pest and biological method must be preferred over chemical methods.

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*IRB approval and ethical statement*

The study project was approved by Biosafety and Ethical Review Committee of University of Sargodha, Sargodha on November 30, 2022 for biosafety and ethical conduct.

*Supplementary material*

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20221020081040

*Statement of conflict of interest*

The authors have declared no conflict of interest.

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