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Research Article

Toxicity and Resistance Status of *Musca domestica* against Bifenthrin and Dimethoate from Bhalwal, Punjab, Pakistan

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Authors' Contributions
MKM deigned and supervised the study and wrote the manuscript. SW and NA collected research material and conducted the experiments. SW helped in write up. SYK guided and assisted in execution of experiments and analysis of data.

Keywords
Toxicity, Resistance status, *Musca domestica,* Bifenthrin, Dimethoate, Bhalwal, Pakistan

Abstract | The house fly (*Musca domestica*) is a pest that acts as a vector of various diseases. Chemical insecticides are used on large scale for the effective and quick management of this pest but it has developed resistance against various insecticides in many countries of the world. This study was designed to check the resistance in *M. domestica* against selected insecticides from Bhalwal as no previous work has been reported from this area. Flies were collected from two different localities i.e., location 1 (L1) was Chak 07 SB Bhalwal (32°14'52.3"N 72°55'25.5"E) and location 2 (L2) was Chak 02 NB Bhalwal (32°19'20.1"N 72°54'56.5"E), Punjab, Pakistan. The flies were reared in mesh-cages and maintained on an artificial diet in the lab. Lab strain flies were collected from area where minimum insecticides were used. Flies from the F1 generation were used in the bioassays. The results showed that LC_{50} for the lab strain against bifenthrin and dimethoate was 11.068 µg/ml and 8.879 µg/ml, respectively. LC_{50} for field strains, Bhalwal L1 and L2, against bifenthrin was 96.184 µg/ml and 130.903 µg/ml, respectively whereas against dimethoate it was 74.340 µg/ml and 84.531 µg/ml, respectively. The resistance ratios for field strains of locations 1 and 2 against bifenthrin were 8.690 fold and 11.827 fold, respectively whereas these were 8.372 fold and 9.520 fold against dimethoate, respectively. In conclusion, the study revealed varying levels of resistance to bifenthrin and dimethoate that suggest cautious application of these pesticides for the control of *M. domestica*.

Novelty Statement | The resistance status of *Musca domestica* against selected insecticides has been first time studied from Bhalwal, Punjab, Pakistan

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Introduction

The house fly is a synanthropic pest with worldwide distribution. It prefers to lay eggs in moist organic matter; therefore, its population is more abundant in livestock, poultry ranches, dairy cattle sheds, horse corrals, and pig ranches, which provide favorable conditions to complete life cycle. The *Musca domestica* carries various disease-causing agents when it feeds and resides on filthy places such as the decomposing matter, humans and animals waste (Malik *et al.*, 2007; Deguenon *et al.*, 2019; Suwannayod *et al.*, 2019).

The house fly (*M. domestica*) secretes digestive juices, enzymes, and saliva on food and then sucks liquefy food using proboscis. As they may take food from unhygienic places, so the germs may stick to their mouth and other parts and when these flies land on the human food they transfer

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the disease-causing agents. It is a major public health pest and a vector of more than 100 pathogens of humans and domestic animals including protozoans, bacteria, viruses, and helminths (Forster *et al*., 2007; Malik *et al*., 2007; Scott *et al*., 2009; Attaullah *et al*., 2019; Zahoor *et al*., 2020).

Different methods are being used for the control of *M. domestica* but it is mainly controlled by the use of chemical insecticides viz. pyrethroids, carbamates, organophosphates, organochlorines neonicotinoids, and others (Deguenon *et al*., 2019). These insecticides have different modes of action, pyrethroids function by halting the closure of voltage gates of sodium channels present in the membrane of the axon (Scott *et al*., 2009); organophosphates obstruct the activity of cholinesterases and acetylcholinesterases, interrupt nerve impulses and result in killing or disabling the insect.

From the estimated 10000 arthropod pests, 553 species are reported to have insecticide resistance. The main problem in controlling pests is the resistance of pesticides (Sharififard and Safdari, 2013). Due to the extensive use of insecticides, the house fly has developed resistance to them (Azzam and Hussein, 2002; White et al., 2007; Jin and Feng, 2001) and it has successfully adapted to most insecticides (Ghosal, 2018) and it is one of 20 species that have shown the highest resistance to insecticides and is placed in the fifth row. This insect has become resistant to 44 different chemical insecticides, and its resistance is found to be due to a specific gene expression in the adult and larvae (Whalon *et al*., 2008).

Monitoring of *M. domestica* population`s susceptibility to insecticides is required for the effective use of insecticides. Early detection of an insect pest's resistance to chemical insecticides and selecting a more effective strategy to control them can efficiently reduce operational, financial, and social losses (Sharififard and Safdari, 2013).

Increased frequency and application rate is a major cause of resistance to pesticides and consequently, insecticides effectiveness decreased. Insecticides are applied by farmers to control grain crops from pests. In Pakistan, farmers do not use insecticides according to their specificity and these trends are very important in developing resistance against pests (Khan *et al*., 2013a, b; Abbas *et al*., 2015).

The resistance in house fly has been recorded in various countries, including Pakistan (Khan et al., 2013b, c; Kaufman *et al*., 2006; Deacutis *et al*., 2006; Acevedo *et al*., 2009). However, no data was available on the resistance status of *M. domestica* against different insecticides in the area of Bhalwal. The current study was conducted to check the level of resistance in *M. domestica* against bifenthrin (pyrethroid) and dimethoate (organophosphate), from two locations of Bhalwal, Punjab, Pakistan and to promote a constructive resistance management programme as well as to provide baseline details for upcoming tracking.

Materials and Methods

Collecting and rearing

With the help of sweep-netting, adults of *Musca domestica* were collected from two different locations of Bhalwal, Punjab, Pakistan. The first locality (L1) was Chak 07 SB Bhalwal, Punjab, Pakistan (32°14'52.3"N 72°55'25.5"E) while the second locality (L2) was Chak 02 NB Bhalwal, Punjab, Pakistan (32°19'20.1"N 72°54'56.5"E). The flies were taken to the laboratory for colonization. The adult flies were reared in mesh-cages with a dimension of 40 × 30 × 30 cm^3. Adult flies feed consisted of icing sugar and milk in powdered form in a 1:1 ratio and water. A mixture of sugar, powdered milk, yeast, and water was prepared for larvae to feed and these items were mixed in a ratio 0.3:0.3:1:4, respectively following Bell *et al*. (2010). The flies were kept in the lab following Khan *et al*. (2013a) and flies from F1 were used for bioassays. The lab strain flies were gathered from such areas in which there was minimum use of insecticides and then reared in a laboratory for three generations to be used as control. The Lab strain (3^{rd} generation) was not completely susceptible but values of LC_{50} of this particular strain were much less and, in the future, it provided us a baseline for resistance determination (Ahmed and Arif, 2009).

Insecticides and bioassay

Two insecticides in commercial-grade, bifenthrin (Eco Pest Solutions, 20.5 % SC) and dimethoate (Hi-Grade Chemicals, 40% EC) were used for residual bioassay. Four concentrations each for bifenthrin (0.8 ml/2ml, 0.6 ml/2ml, 0.4 ml/2ml and 0.2 ml/2ml) and dimethoate (8.4 ml/2ml, 4.53 ml/2ml, 0.67 ml/2ml and 0.33 ml/2ml) were prepared. Residual bioassays were performed using insecticide-treated filter papers (12×14 cm); set in bioassay tubes while tubes with only distilled-water-treated filter papers were served as control according to the World Health Organization (2006). Fifteen flies were introduced into each tube provided with adult diet to avoid starvation during the bioassays. Flies were given exposure for one hour after that they were transferred to clean bioassay susceptibility tubes for 24 hours observation. Mortality data was recorded at four-hour intervals till 24 hours. The flies that survived after 24 hours of insecticide exposure were considered resistant. Three replicates were performed for both insecticides.

Data analysis

The probit analysis was carried out with help of SPSS to assess the values of LC_{50}, slope and chi square. The data were corrected by the use of the Abbott formula (Abbott, 1925).

Table 1: Toxicity of bifenthrin and dimethoate to adults of *Musca domestica* from Bhalwal, Punjab, Pakistan.

Insecticide	Population	LC_{50}[µg/ml] (95%Cl)	Slope (± SE)	χ2	df	RR (95%)
Bifenthrin	Lab Strain	11.068 (5.456-18.567)	1.322±0.71	1.713	2	
	Field Strain L1	96.184 (64.276-136.654)	2.136 ±0.48	9.196	2	8.690
	Field Strain L2	130.903 (94.215-167.295)	2.604±0.47	10.119	2	11.827
Dimethoate	Lab Strain	8.879 (4.162-15.792)	1.18±0.66	0.491	2	
	Field Strain L1	74.340 (53.112-105.467)	1.68±0.45	1.256	2	8.372
	Field Strain L2	84.531(53.220-136.09)	1.65±0.4	2.197	2	9.520

Resistance ratio (RR)= LC_{50} of field population/ LC_{50} of Lab susceptible population.

Results and Discussions

The residual bioassays results showed that the LC_{50} for lab strain against bifenthrin and dimethoate was 11.068 µg/ml and 8.879 µg/ml, respectively. LC_{50} for field strains, Bhalwal location 1 and 2, against bifenthrin was 96.184 µg/ml and 130.903 µg/ml, respectively whereas against dimethoate it was 74.340 µg/ml and 84.531 µgm/ml, respectively. The resistance ratio against bifenthrin was 8.690 fold for field strain of location 1 and 11.827 fold for field strain of location 2. Slopes of both tested populations computed from the Probit analysis (Table 1) against bifenthrin were steeper as compared to the Lab strain. For dimethoate, value of resistance ratio was 8.372 fold for field strain of location 1 and 9.520 fold for field strain of location 2. Slopes of both tested populations against dimethoate were steeper as compared to lab strain. In tested *Musca domestica*, very low to low resistance level to bifenthrin was observed and its resistance ratio was ranging from 8.690-11.827 fold, whereas with dimethoate very low resistance level was observed and its resistance ratios were ranging from 8.372-9.520 fold (Table 1).

In this study, a very low to low resistance level was observed to bifenthrin with resistance ratios ranging from 8.690-11.827. Various pyrethroid insecticides, such as bifenthrin, lambda-cyhalothrin, cypermethrin, and deltamethrin, have been used in Pakistan to combat various pests of crops, dairy animals, and poultry (Saleem *et al*., 2008; Muhammad *et al*., 2008; Khan *et al*., 2013c; Ranian *et al*., 2021). Resistance against pyrethroids have been identified in a number of different insect pests, including tobacco cutworm, spotted bollworm, diamondback moth, house mosquito, coding moth, and American bollworm (Sauphanor *et al*., 2000; Kranthi *et al*., 2001; Sayyed *et al*., 2005; Daaboub *et al*., 2008; Saleem *et al*., 2008; Ahmad and Arif 2009; Faheem *et al*., 2013; Abbas *et al*., 2014).

Pyrethroid insecticides are being used to control the different types of pests and this wide range of use might be the cause of pyrethroid resistance in Pakistan. The results of the present study also revealed low resistance level against bifenthrin. There was < tenfold resistance ratio in house fly against dimethoate. The results are consistent with the fact that the selected localities had the usage of bifenthrin and dimethoate against different insect pests, which might be reflected in the development of insecticide resistance in *M. domestica*. Various organophosphate insecticides are utilized to control various pests in different cities of Punjab and high resistance level in *M. domestica* was recorded by various researchers from different cities of Punjab (Saleem *et al*., 2008; Khan *et al*., 2011, 2013c; Shad *et al*., 2012) and also globally (Cheikh *et al*., 2009; Wang *et al*., 2010). However, resistance to insecticides is a spatio-temporal phenomenon that changes with time and space. Therefore, resistance expression in *M. domestica* from Bhalwal is different from the results of the above studies. The process of resistance development can be slowed down by environment sanitation and proper disposal of waste and by doing this feeding and breeding sites of flies can be eliminated (Learmount *et al*., 2002; Malik *et al*., 2007; Khan *et al*., 2012).

Conclusions and Recommendations

The results indicated that housefly did not show high resistance against bifenthrin and dimethoate from both selected areas but it showed higher resistance against bifenthrin as compared to dimethoate. In future resistance against other insecticides should also be checked to view the boarder spectrum of insecticide resistance in housefly from the selected area. The use of insecticides should be monitored and insecticides having different modes of actions should be used in rotation to avoid the development of resistance.

Conflict of interest

The authors have declared no conflict of interest.

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