

RESISTANCE STATUS OF BLOWFLY, *CHRYSOMYA MEGACEPHALA* AGAINST SELECTED INSECTICIDES FROM CENTRAL PUNJAB, PAKISTAN

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ABSTRACT

The role of *Chrysomya megacephala* in transmitting various pathogens highlights the urgent need to control this species to ensure public health safety. In the present study resistance status of blowfly populations was determined against five insecticides (deltamethrin, fipronil, chlorpyrifos, imidacloprid and pyriproxyfen). Blowfly populations were collected from different localities of Central Punjab, Pakistan i.e Sargodha, Bhalwal, Faisalabad and Chiniot. The adult flies of F1 generation were used for residual bioassay against five concentrations of each insecticide. Moderate level of resistance at LC₅₀ ranged from 8.57-15.84 folds was found against deltamethrin, 4.84-8.38 folds for fipronil, and 3.51-6.65 folds for chlorpyrifos. The RR values at LC₅₀ for imidacloprid and pyriproxyfen ranged from 3.98-5.63 folds and 3.34-4.66 folds, respectively. Faisalabad population of blowflies revealed very low resistance ratios against all tested insecticides whereas Bhalwal population of blowflies showed highest RR values as compared to populations of other locations. The present study showed varying level of resistance in blowfly populations from area to area and insecticide to insecticide. Therefore, for the control of resistance development in blowflies in near future to mitigate the risks associated with insecticide use, it is essential to establish a proper control mechanism, incorporating techniques like mosaic, rotational, and periodic application strategies.

Key words: *Chrysomya megacephala*, Insecticides, Resistance, Toxicity, Lethal concentration

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INTRODUCTION

The blowfly, *Chrysomya megacephala* (Fabricius, 1794), a notable pest species, is distinguished by its scavenging behavior (Mondal *et al.*, 2016). This necrophagous fly is typically the first insect to colonize cadaveric remains, arriving swiftly after death of organism, for oviposition (Shi *et al.*, 2009; Silahuddin *et al.*, 2015; Wang *et al.*, 2017). This fly species is widely distributed in tropical and temperate regions (Williams and Villet, 2006). Despite the significance of this species, the current understanding of resistance status of *C. megacephala* in Pakistan is limited. It highlights the requirement for systematic research to investigate resistance patterns and trends in blowflies.

The adult *C. megacephala* is a slightly large fly measuring 9.5mm, featuring bright metallic green body with large prominent red eyes (Siriwattananarungsee *et al.*, 2005). *C. megacephala* manifests holometabolous life cycle, consisting of four major phases of development, egg, larvae, pupa, and adult stage. The total life duration

is 8-9 days during summer at 25-30°C temperature (Goff, 2010; Siddiki and Zambare, 2017; Verma and Paul, 2016). The female *C. megacephala* exhibits moderate to high fecundity during summer (approximately 150-300 ova/batch) when temperature and humidity are most favorable (Gabre *et al.*, 2005; Sontigun *et al.*, 2018). *C. megacephala* prefers to stay in anthropogenic environments to breed near animal wastes and carcasses (Bansal, 2022; Kumarasinghe *et al.*, 2000).

Adult *C. megacephala* flies are diurnal insects that display an adaptable feeding behavior (Badenhorst and Villet, 2018). It serves as a mechanical vector of several other pathogens including helminthic parasites, *Taenia* spp., and protozoans (Sukontason *et al.*, 2007; Carneiro *et al.*, 2014; Schrottner *et al.*, 2017). The transmission of these pathogens can cause several health issues including gastrointestinal and urinary tract infections in humans and other animals (Chaiwong *et al.*, 2012). Research indicates that *C. megacephala* harbors 12 times more bacteria than *Musca domestica* L. due to

its contaminative feeding behavior (Chaiwong *et al.*, 2014).

Infestation caused by *C. megacephala* in sundried fish, resulted in contamination of stored fish with larvae that has substantial economic consequences for fishing industry in many parts of the world (Wall *et al.*, 2001). Blowfly larvae are known to induce human and veterinary myiasis, cause damage through infection by invading wounds and tissues (Ferraz *et al.*, 2010; Sinha, 2012). Therefore, to reduce disease transmission and loss caused by this vector species, it is essential to follow combined strategy of using targeted chemical insecticides and precise hygiene/sanitation practices (Maddheshiya *et al.*, 2021).

The most common practices to control this veterinary pest include the use of various insecticides, such as diazinon, malathion, imidacloprid, permethrin, and cypermethrin, chlorpyrifos, ivermectin, deltamethrin, and spinosad, few insect growth regulators (IGRs) such as cyromazine, dicyclanil, diflubenzuron, pyriproxyfen, and diofenolan, have been previously utilized to manage blowfly population throughout the world (Levot *et al.*, 2004; Rust, 2005).

The most commonly used insecticides in Pakistan are pyrethroids, organophosphates, organochlorines, and carbamates (Rashid *et al.*, 2022). Farmers rely heavily on chemical pesticides to control pests, with limited adoption of biological control measures in the Punjab (Luqman *et al.*, 2023). The improper use of pesticides can promote environmental disaster and several toxic health crises (Masson *et al.*, 2017). The misuse of synthetic insecticides can induce the development of resistant blowfly populations with the passage of time (Viana *et al.*, 2020). The development of metabolic resistance in different insects has been investigated globally.

Punjab, being the most populous province of Pakistan, plays a vital role in the country's economy, contributing 19% to the National GDP for agricultural production and food security. Investigating resistance patterns in this area is essential due to the high disease prevalence, intensive farming practices, and unique environmental factors that may contribute to the development of resistance.

This study aimed to investigate the resistance levels of *C. megacephala* populations collected from various localities (Sargodha, Bhalwal, Chiniot, and Faisalabad) in Central Punjab, Pakistan against five commonly used insecticides including deltamethrin, fipronil, chlorpyrifos, imidacloprid, and pyriproxyfen. The primary objectives were to assess the resistance status of *C. megacephala* to these insecticides and identify the most effective compounds for controlling this pest. Our findings indicate that implementation of proper control mechanism through mosaic, rotational, periodic

application strategies must be ensured to delay resistance development in blowflies.

MATERIALS AND METHODS

Insects: Adult blowflies, *C. megacephala* were collected from four locations of the Central Punjab i.e. Sargodha (32° N, 72° E), Bhalwal (32° N, 72° E), Faisalabad (31° N, 73° E), and Chiniot (31° N, 72° E), by using trap method. Trap method was used to employ a purposive sampling strategy to target areas with high blowfly activity. The blowfly sampling was conducted between March 2024 through May 2024 in which three traps were set up at each location.

Collected specimens were brought into the laboratory for rearing and bioassay. An attractive medium for fly collection and maggot rearing was created by using 100g chicken pieces sealed in airtight polythene bags for 48 hours to induce putrid odor. Three semi-transparent jars (10.5 × 6.5 × 7.5 inches) were used for developmental stages. In oviposition jar, chicken meat with 5 ml water was provided to facilitate adult fly collection. In rearing jar, larvae were fed chicken meat as a protein source, while dry leaves were provided in pupation jar to facilitate smooth pupation and completion of the development phase.

The rearing conditions were standardized by maintaining temperature (25-26°C), humidity (50-70%), along with 12:12 light/dark period (Claver and Yaqub, 2015). Chicken meat was provided until the third instar larvae attained the pre-pupal stage. Subsequently, prepupae were transferred to the pupation jar. After fly emergence, adult flies were fed chicken meat and cotton soaked in sugar-water paste under natural conditions (El-Ebiarie and Taha, 2012). For bioassay experiments, blowflies from F₁ generation (7-9 days old) and larvae (72-96 hours old) were used (Figure 1). The lab. strain, exhibiting relatively low LC₅₀ value, was selected as a baseline for assessing resistance levels in this study, despite not being completely susceptible.

Insecticides: This study employed technical-grade insecticides, specifically deltamethrin (98% purity), fipronil (95%), chlorpyrifos (97%), imidacloprid (95%), and pyriproxyfen (96%). These insecticides were obtained from NuChem Private Limited, Multan. The selection of these chemicals was based on a thorough literature review, which highlighted their widespread use in current field practices for insect pest control. Stock solutions of these insecticides were diluted to five concentrations following the methodology of Heong *et al.* (2011) i.e. 6.25 parts per million (ppm), 12.5 ppm, 25 ppm, 50 ppm, and 100 ppm by using serial dilution method. Acetone, a volatile solvent, was used to formulate these concentrations. The concentration range

was selected based on literature reviews of previous studies (Khater, 2018).

Adulticide bioassay technique: Following the methodology of Khater *et al.* (2021), a residual bioassay was conducted to assess the resistance status of blowflies. During the experiment, water served as the negative control, whereas acetone and various insecticide concentrations constituted the positive control. The resulting solutions (1.5ml/trial) were placed in transparent plastic jars (10 × 7 × 6 cm). To ensure

uniform distribution and solvent evaporation, the jars were rotated. Six experimental groups (five treated+one control) were created. During bioassay, each jar contained 10 flies of F₁ generation and three replicates per insecticide were conducted. After one-hour exposure to insecticidal treatment, flies were transferred to small boxes with water and sugar solution. Mortality was assessed after 12 and 24 hours. A 24-hour survival period post-insecticide exposure was used as the criterion for resistance.



a). Eggs



b). First instar larvae (maggots)



c). Third instar larvae



d). Pupa



e). Newly emerged blowflies



f). Adult male and female

Figure 1: Different stages of blowfly *Chrysomya megacephala* (a-f)

Larvicidal bioassay technique: The larvicidal bioassay followed the protocol outlined by Sukontason *et al.* (2004). Pyriproxyfen served as the positive control to determine its lethal concentration against third instar larvae of *C. megacephala*. For each trial, thirty third instar larvae from the same egg batch were used to estimate resistance level. Larvae were exposed to formulated solutions using dipping nets (pore size: 334×522 μm) for 30 seconds. Exposed larvae were placed in tightly sealed, transparent plastic jars (10 × 7 × 6 cm) containing 10g chicken meat. Three replicates per group were used during the bioassay. Mortality was assessed after 24 hours. A soft paint brush was used to stimulate each larva. Non-responsive larvae were recorded as dead.

Data analysis: Median lethal concentrations (LC₅₀), slope and Chi-square was determined by using probit analysis (SPSS version 23). The resistance ratios (RR) among various blowfly populations were calculated by dividing the LC₅₀ of the field strain by the LC₅₀ of the laboratory-susceptible strain.

RESULTS

The study conducted a dose-response bioassay with five deltamethrin concentrations revealing decreased mortality in blowfly populations from four selected

localities. The mortality results indicate tolerance to deltamethrin in blowflies. At 6.25 ppm concentration, the highest mortality rate (20.0%) among *C. megacephala* populations was recorded in the Faisalabad population, followed by Bhalwal (16.6%), Chiniot and Sargodha, which exhibited equal mortality rates (13.3%). At 12.5 ppm, *C. megacephala* populations from Bhalwal and Chiniot showed the highest mortality (33.3%), followed by Sargodha (26.6%), and the lowest mortality was observed in Faisalabad (23.3%). A concentration of 25 ppm resulted in mortality rates of 43.3% in Bhalwal, 36.6% in Chiniot, 33.3% in Sargodha, and 30.0% in Faisalabad populations. The highest mortality rate (53.3%) at 50 ppm was observed in Faisalabad population, followed by Bhalwal (46.6%), whereas Sargodha and Chiniot (40.0%). The mortality rate at 100 ppm was highest in Faisalabad population (73.3%), followed by Sargodha (70.0%), and then Bhalwal and Chiniot, which both recorded a mortality rate of 53.3% (Figure 2). The laboratory strain's mortality response to deltamethrin increased significantly with concentration, ranging from 53.3% at the lowest concentration (6.25 ppm) to 96.6% at the highest concentration (100 ppm). Analysis of the resistance ratio (RR) for deltamethrin indicated a range of 8.57-15.84. The strain from Chiniot demonstrated the highest RR value, whereas the strain from Faisalabad had the lowest RR value (Table 1).

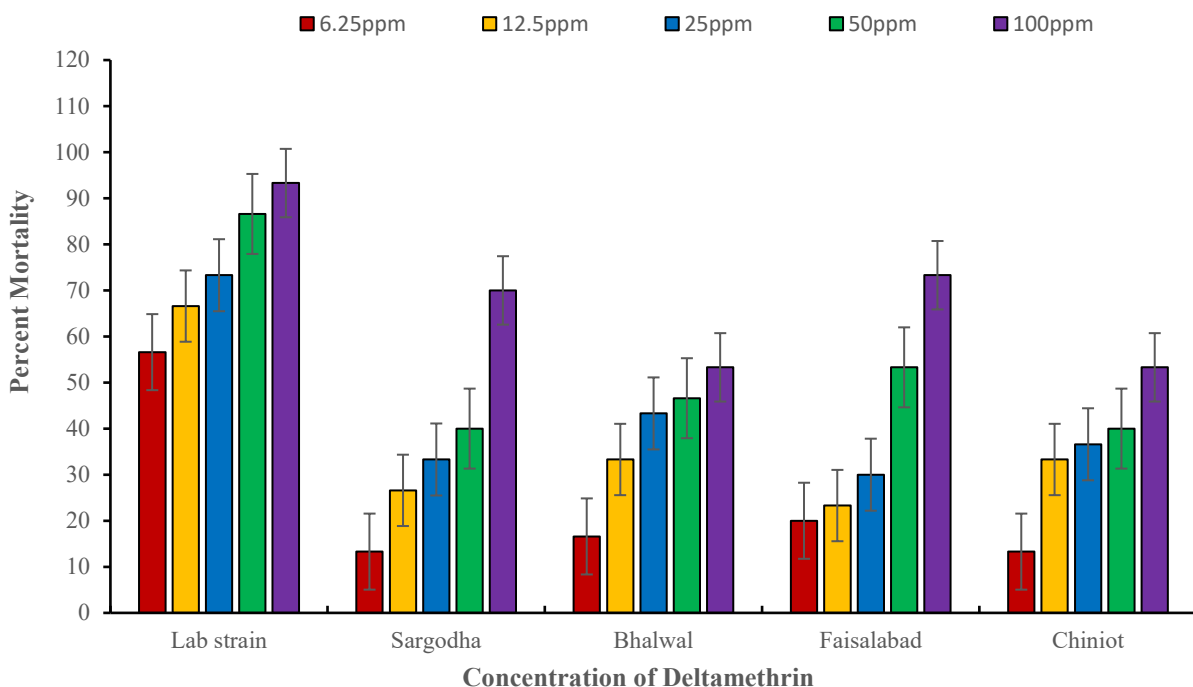


Figure 2: Percent mortality of *C. megacephala* exposed to deltamethrin across different locations and concentrations.

A dose-response bioassay was conducted using five fipronil concentrations. The blowflies from all localities exhibited high mortality rates across all concentrations of fipronil, indicating high susceptibility. The Faisalabad population of *C. megacephala* exhibited the highest mortality rate (26.6%) at 6.25 ppm, surpassing Bhalwal (20.0%) Sargodha (16.6%) and Chiniot (13.3%). Among *C. megacephala* populations exposed to 12.5 ppm, mortality rates decreased in the following order: Faisalabad (36.6%), Chiniot (33.3%), Sargodha (30.0%), and Bhalwal (26.6%). Exposure to 25 ppm resulted in mortality rates of 50.0% (Faisalabad), 40.0% (Chiniot), 36.6% (Sargodha), and 33.3% (Bhalwal). At 50 ppm, the mortality rates of *C.*

megacephala populations were 53.3% (Faisalabad), 46.6% (Sargodha and Chiniot), and 43.3% (Bhalwal). The mortality trend among the four blowfly populations at 100 ppm fipronil indicates variable trend i.e. Chiniot (90.0%) > Faisalabad (83.3%) > Sargodha (80.0%) > Bhalwal (76.6%) (Figure 3). The laboratory strain exhibited a concentration-dependent mortality trend against fipronil i.e. at 6.25 ppm (56.6%), 12.5 ppm (66.6%), 25 ppm (73.3%), 50 ppm (86.6%), and 100 ppm (93.3%), respectively. A 4.84-8.38 fold resistance ratio was observed that indicates varying levels of susceptibility to fipronil among the blowfly populations (Table 1).

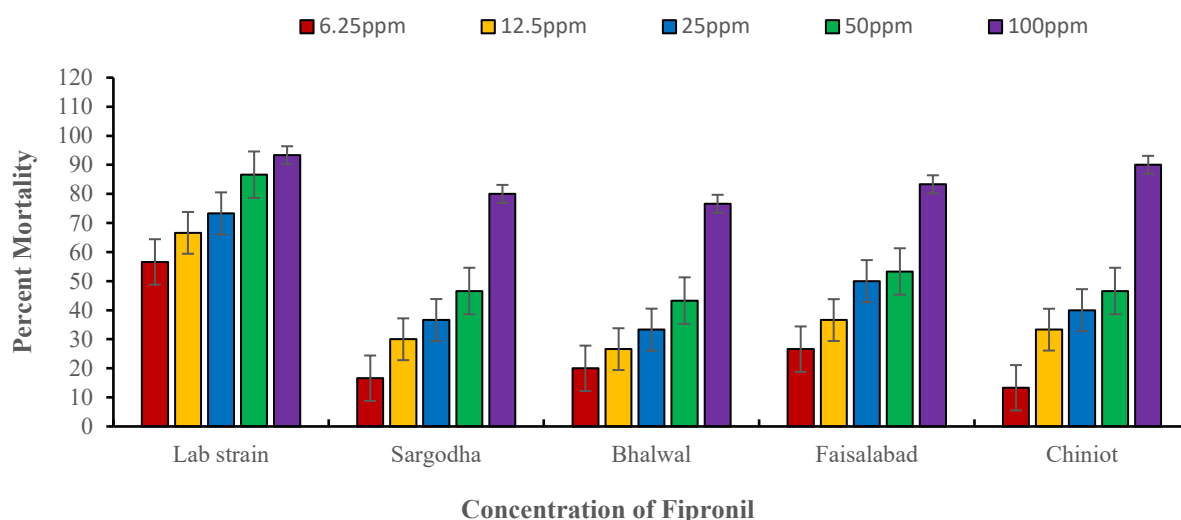


Figure 3: Percent mortality of *C. megacephala* exposed to fipronil across different locations and concentrations.

High mortality rates were observed across all concentrations of chlorpyrifos at all localities, indicating susceptibility to chlorpyrifos. At 6.25 ppm concentration of chlorpyrifos resulted in a mortality rate of 30.0% in Sargodha population of *C. megacephala*, which was the highest among the tested locations, followed by Bhalwal, Faisalabad, and Chiniot. The highest mortality rate (43.3%) at 12.5 ppm was observed in *C. megacephala* populations from Faisalabad and Chiniot, followed by Sargodha (36.6%), and the lowest mortality rate (30.0%) was found in Bhalwal. At 25 ppm, the mortality rates of *C. megacephala* populations were 66.6% in Faisalabad, 53.3% in Chiniot, 43.3% in Sargodha, and 36.6% in Bhalwal. The mortality pattern at 50 ppm concentration was similar, with mortality rates decreasing in the order of Faisalabad > Chiniot > Sargodha > Bhalwal. Comparative mortality at highest lethal dose (100 ppm) indicates Faisalabad (93.3%) > Bhalwal (90.0%) > Sargodha (86.6%) > Chiniot (83.3%) (Figure 4). A positive correlation was observed between concentration

and mortality in the lab strain. The resistance ratio for chlorpyrifos was found to range from 3.51 (Faisalabad) to 6.65 (Bhalwal), indicating significant variation in resistance levels among the tested populations (Table 1).

Five different concentrations of imidacloprid were evaluated to test the susceptibility in different populations of blowflies and the results showed high mortality rates across all concentrations and localities, indicating susceptibility of blowflies to imidacloprid. The mortality rate at 6.25 ppm was highest in the Bhalwal population (26.6%), followed closely by Sargodha and Chiniot (23.3% each), and then Faisalabad (20.0%). Comparative mortality analysis at 12.5 ppm revealed that Faisalabad had the highest mortality rate (46.6%), followed by Bhalwal and Chiniot (40.0% each), and then Sargodha (36.6%). A concentration of 25 ppm induced mortality rates of 63.3% in Bhalwal, 53.3% in both Faisalabad and Chiniot, and 46.6% in Sargodha. At 50 ppm, the mortality rates of *C. megacephala* populations were 76.6% (Chiniot), 73.3% (Bhalwal), 70.0%

(Faisalabad), and 63.3% (Sargodha). Comparative mortality trend at highest lethal dose (100 ppm) indicates Bhalwal and Chiniot (93.3%) > Faisalabad (90.0%) > Sargodha (86.6%) (Figure 5). Concentration-mortality profile of lab strain against imidacloprid shows positive

correlation. The resistance ratio (RR) for imidacloprid ranged from 3.98 to 5.63, with the Sargodha strain exhibiting the highest level of resistance (RR = 5.63) and the Bhalwal strain showing the lowest level of resistance (RR = 3.98) (Table 1).

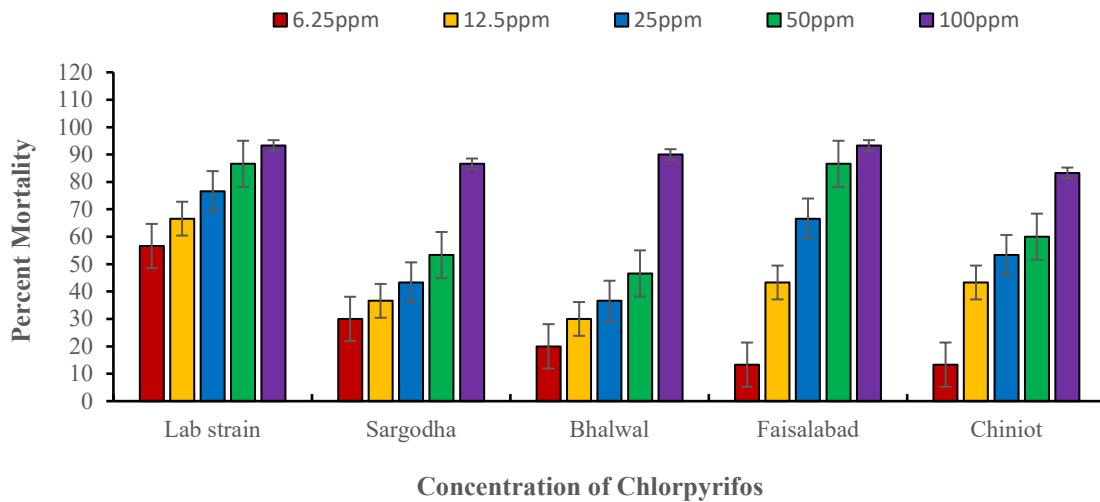


Figure 4: Percent mortality of *C. megacephala* exposed to chlorpyrifos across different locations and concentrations.

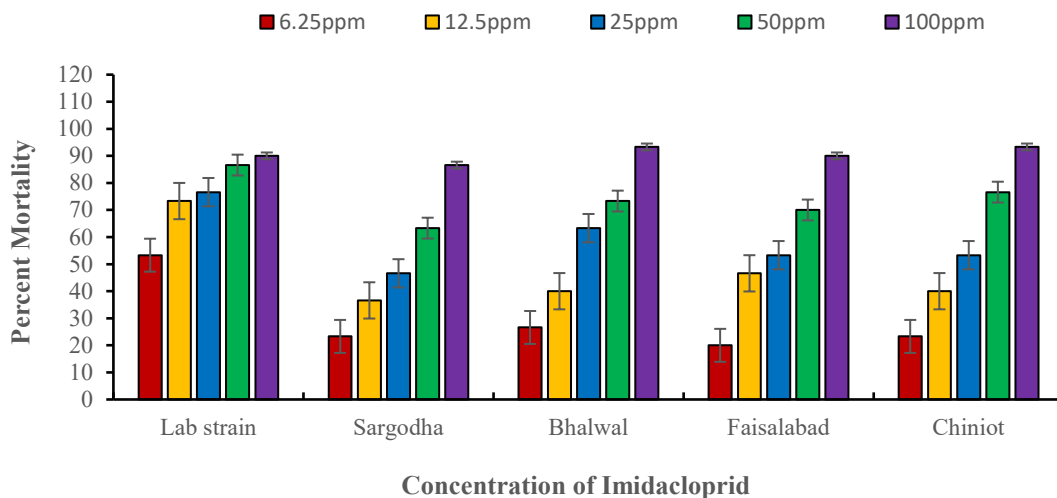


Figure 5: Percent mortality of *C. megacephala* exposed to imidacloprid across different locations and concentrations.

A concentration-dependent mortality response was observed in third instar larvae of blowflies from all localities when exposed to pyriproxyfen. The mortality rate at 6.25 ppm was significantly higher in the Sargodha population (33.3%) compared to Faisalabad (30.0%), Bhalwal (23.3%), and Chiniot (23.3%). Faisalabad and Chiniot populations showed the highest mortality rate (56.6%) among *C. megacephala* populations at 12.5 ppm, followed by Sargodha and Bhalwal, which had mortality rates of 43.3% and 36.6%, respectively. The mortality

rate at 25 ppm was consistently high in Bhalwal, Faisalabad, and Chiniot (66.6%), while Sargodha recorded a relatively lower mortality rate of 50.0%. The mortality rate at 50 ppm was highest in Bhalwal (83.3%), with Faisalabad, Chiniot, and Sargodha exhibiting mortality rates of 80.0%, 76.6%, and 70.0%, respectively. At the highest lethal dose (100 ppm), the mortality trend indicates Bhalwal and Chiniot populations (93.3%) > Sargodha and Faisalabad populations (90.0%) (Figure 6). The lab strain exhibited a gradual, concentration-

dependent increase in mortality. At low concentration (6.25 ppm) 60.0% mortality, moderate concentration (12.5 - 25 ppm) 70.0 - 80.0% mortality, and high concentration (50 - 100 ppm) 86.6 -93.3%, mortality was

observed. Pyriproxyfen resistance ratios ranged from 3.34 (Faisalabad) to 4.66 (Sargodha), revealing significant differences in resistance levels among the tested strains (Table 1).

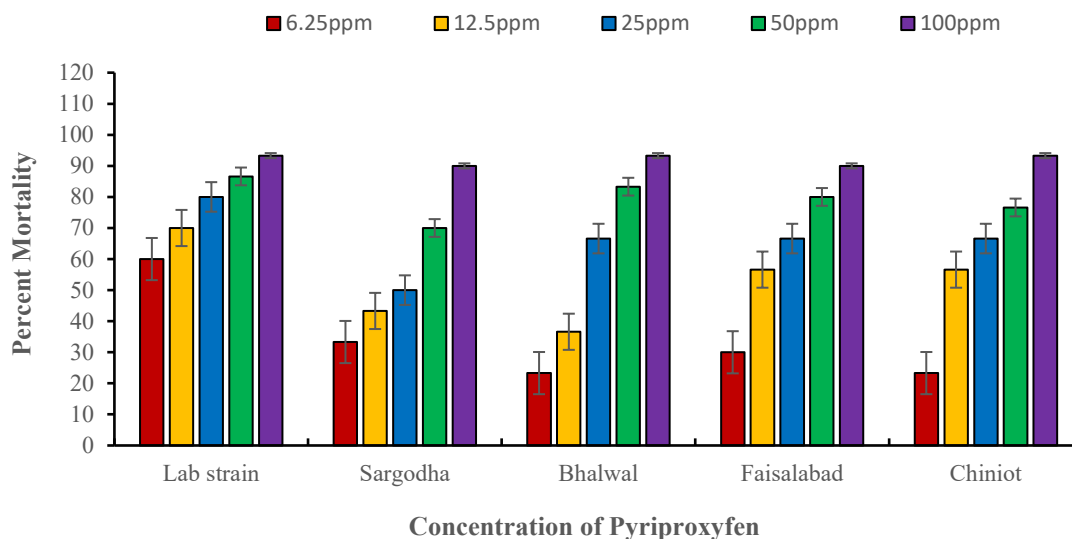


Figure 6: Percent mortality of *C. megacephala* exposed to pyriproxyfen across different locations and concentrations.

The present research demonstrates that all adult *C. megacephala* populations and third instar larvae from four locations revealed low to moderate level of resistance to five tested insecticides. A comparative analysis of insecticide resistance among *C. megacephala* populations indicated a moderate level of resistance against deltamethrin, whereas pyriproxyfen, fipronil, chlorpyrifos and imidacloprid exhibited very low to low level of resistance. In Sargodha, the insecticides induced mortality in the following order: pyriproxyfen > imidacloprid and chlorpyrifos > fipronil > deltamethrin. The mortality trend in Bhalwal revealed that

pyriproxyfen and imidacloprid were equally effective, followed by chlorpyrifos, fipronil, and then deltamethrin. The mortality trend in Faisalabad revealed chlorpyrifos as the most effective insecticide, followed closely by pyriproxyfen and imidacloprid, with fipronil and deltamethrin being the least effective. The insecticides induced mortality in Chiniot in the order of pyriproxyfen and imidacloprid > fipronil > chlorpyrifos > deltamethrin. The overall results showed that pyriproxyfen and imidacloprid were the most effective insecticides, inducing higher mortalities compared to other tested insecticides across all localities (Table 1).

Table 1: Comparative toxicity of insecticides against blowfly, *Chrysomya megacephala* in Central Punjab, Pakistan.

Insecticide	Populations	N	LC ₅₀ [ppm](95%CI)	Slope (± SE)	χ ²	df	RR
Deltamethrin	Lab Strain	180	4.91 (0.89 – 9.23)	1.07 (±0.28)	0.45	3	
	Sargodha	180	52.01 (34.22 – 105.25)	1.20 (±0.26)	1.87	3	10.59
	Bhalwal	180	61.31 (32.87 – 348.88)	0.78 (±0.25)	1.19	3	12.48
	Faisalabad	180	42.11 (28.51 – 74.23)	1.26 (±0.26)	2.04	3	8.57
	Chiniot	180	77.79 (40.79 – 561.44)	0.81 (±0.25)	1.65	3	15.84
Fipronil	Lab Strain	180	5.13 (1.81 – 8.31)	1.51 (±0.34)	0.19	3	
	Sargodha	180	37.24 (25.71 – 60.89)	1.33 (±0.26)	2.76	3	7.25
	Bhalwal	180	43.02 (28.55 – 80.24)	1.19 (±0.26)	3.22	3	8.38
	Faisalabad	180	24.86 (15.68 – 39.52)	1.16 (±0.26)	2.42	3	4.84
	Chiniot	180	31.42 (11.29 – 186.05)	1.59 (±0.28)	6.46	3	6.12
Chlorpyrifos	Lab Strain	180	4.76 (0.86 – 8.97)	1.08 (±0.29)	0.12	3	
	Sargodha	180	24.74 (15.63 – 39.38)	1.16 (±0.26)	4.41	3	5.19

Imidacloprid	Bhalwal	180	31.70 (8.56 – 808.01)	1.46 (± 0.27)	6.99	3	6.65
	Faisalabad	180	16.71 (12.57 – 21.40)	2.20 (± 0.32)	1.03	3	3.51
	Chiniot	180	24.31 (16.81 – 34.85)	1.45 (± 0.27)	2.91	3	5.10
	Lab Strain	180	4.13 (0.45 – 8.51)	0.98 (± 0.28)	0.65	3	
	Sargodha	180	23.28 (15.88 – 33.68)	1.41 (± 0.27)	1.28	3	5.63
	Bhalwal	180	16.45 (11.21 – 22.44)	1.64 (± 0.28)	1.01	3	3.98
Pyriproxyfen	Faisalabad	180	19.00 (13.01 – 26.28)	1.56 (± 0.28)	1.63	3	4.60
	Chiniot	180	18.15 (12.91 – 24.38)	1.74 (± 0.29)	0.96	3	4.39
	Lab Strain	180	3.64 (0.34 – 7.70)	1.00 (± 0.29)	0.04	3	
	Sargodha	180	16.98 (10.39 – 24.81)	1.30 (± 0.26)	2.14	3	4.66
	Bhalwal	180	16.16 (11.68 – 21.23)	1.93 (± 0.30)	0.44	3	4.43
	Faisalabad	180	12.19 (7.02 – 17.51)	1.42 (± 0.28)	0.67	3	3.34
Chiniot	180	13.76 (8.91 – 18.92)	1.62 (± 0.29)	2.13	3	3.78	

Note: RR 0-10 = Low resistance, RR 10-20 = Moderate resistance, RR 21-50 = High resistance, RR 51-100 = Extremely high resistance

DISCUSSION

This study evaluated few commonly used insecticides (deltamethrin, fipronil, chlorpyrifos, and imidacloprid) and larvicide (pyriproxyfen) against adult *C. megacephala*, and its third instar larvae, revealing moderate resistance to deltamethrin (8.57-15.84), low-level resistance to fipronil, chlorpyrifos, and imidacloprid (3.51-8.38), and minimal resistance to pyriproxyfen (3.34-4.66), highlighting the need for resistance management. Environmental adaptability, high reproductive capacity, rapid development cycle, and cross-resistance between insecticides are the key factors that drive resistance development in insects (Kaufman *et al.*, 2010). These factors make pest control more challenging and reduces the effectiveness of insecticide.

Blowfly resistance to pyrethroid insecticides with unique mechanisms of action has been documented in different blowfly species. Research by Farahani *et al.* (2022) assessed the insecticidal activity of deltamethrin, and few other insecticides against third-instar larvae of blowfly, *Lucilia sericata* (Meigen). The findings demonstrated significant lethal effects and dose-dependent mortality rates for these tested compounds. Research conducted by Kojima *et al.* (2022) examined the efficacy of synthetic pyrethrins (deltamethrin and permethrin) as feeding deterrents against blowfly, *Phormia regina* (Meigen). These chemicals targeted the voltage-gated sodium channels of insect and showed stronger deterrent effect.

de Oliveira *et al.* (2021) investigated the detrimental effects of deltamethrin on *C. megacephala* larval development. This exposure resulted in delayed larval development, reduced body size and weight, altered pupation and adult emergence, increased mortality rates, and histopathological changes in larval tissues. Khater *et al.* (2021) conducted a research to investigate the detrimental effects of deltamethrin on biology and physiology of blowfly, *Lucilia silvarum* (Meigen). The researchers reported concentration-dependent mortality

and reproductive impairment in adult flies. Al-Jameeli *et al.* (2021) documented the susceptibility of blowfly, *Chrysomya albiceps* (Wiedemann), to deltamethrin and reported moderate to high toxicity in blowflies against this chemical.

Enhanced toxicity of deltamethrin was observed against blowflies on mixing with plant essential oils by Suwannayod *et al.* (2019). Susceptibility of *C. megacephala* to permethrin and deltamethrin was analyzed by Sukontason *et al.* (2005). The results of bioassay revealed LC₅₀ values of *C. megacephala* was 0.022% against permethrin, and 0.009% against deltamethrin. Deltamethrin resistance has emerged in various Diptera species in Punjab including the housefly, *M. domestica* and yellow fever mosquito, *Aedes aegypti* (L.). Research indicates that the driving force behind this resistance is the excessive use of insecticides in Punjab (Qamar-ul-Haq *et al.*, 2008).

The research findings indicates low-level resistance to fipronil among different blowfly populations of Central Punjab. Abbas *et al.* (2014) reported high resistance ratios to fipronil in *M. domestica* population of the Punjab. Smith *et al.* (2000) assessed the insecticidal effects of fipronil and β -cyfluthrin on *L. sericata* larvae and reported the increased larval mortality and disrupted development by combined application of these two insecticides. This scenario poses significant concerns regarding cross-resistance to various insecticide classes such as deltamethrin, permethrin, imidacloprid, malathion, and chlorpyrifos.

Resistance to chlorpyrifos in *C. megacephala* was remarkably low across selected locations. Agricultural pest control in Punjab frequently involves the application of organophosphate pesticides including chlorpyrifos. Yasmeen and Amir (2022) determined the detrimental effects of chlorpyrifos on the midgut of third instar larvae of *C. megacephala*. Their findings indicate that chlorpyrifos induced ultrastructural damage, including disruption of microvilli, mitochondrial swelling, compromising midgut integrity. Similar results

were observed by Khater (2018), while investigating the ultrastructural effects of chlorpyrifos on third instar larvae of *C. albiceps*. Recent studies have explored the efficacy of citronella oil and chlorpyrifos against *C. megacephala*, documenting enhanced mortality and reduced oviposition (Denis *et al.*, 2018). Chlorpyrifos resistance can facilitate cross-resistance to imidacloprid and pyriproxyfen due to overlapping molecular and biochemical mechanisms, impacting pest control strategies in Pakistan.

A negligible level of resistance to a neonicotinoid insecticide, imidacloprid was detected in *C. megacephala* populations from multiple cities in Punjab. Kotze and Rolls (2022) investigated the resistance status of sheep blowfly, *Lucilia cuprina* (Wiedemann), to imidacloprid in Australia and found 3.1-3.8-fold higher resistance ratios in treated larvae. The relatively low resistance levels observed in the present study against imidacloprid may be attributed to its efficacy and regional application patterns. The global rise in the utilization of neonicotinoids to control insect pests led researchers in Pakistan to investigate imidacloprid resistance and the possible causes of resistance development (Khan *et al.*, 2013).

The results of present study revealed an exceptionally low level resistance in third instar larvae of all tested populations of blowflies to pyriproxyfen. IGR (pyriproxyfen) work as a juvenile hormone analog and disrupts the normal hormone regulation in insects. It disturbs some crucial physiological processes such as development, reproduction, and metamorphosis. Singh and Kumar (2015) investigated the effects of juvenoids pyriproxyfen and diufenolan on embryogenesis and post embryonic development of *C. megacephala*. This exposure caused dose-dependent inhibition of embryonic development and delayed adult emergence. IGRs have been commercially available for use in Punjab, Pakistan since the early 2010s. They are considered as a valuable tool for managing pest populations.

The agricultural techniques and misuse of pesticides in Central Punjab increases the risk of resistance development. Farmers in the cotton belt of Punjab, Pakistan, apply large quantities of pesticides (4875 kg/year), mostly classified as moderately or highly hazardous (Khan *et al.*, 2015). The widespread use of highly toxic pesticides in agricultural sector of Punjab poses significant environmental and health risks, contaminating air, water, and land. This careless use of pesticides can lead to the accumulation of toxic residues in the food chain, causing various health issues in humans (Khan *et al.*, 2020).

The resistance observed in blowfly populations is attributed to the insufficient knowledge about IPM and improper use of various pesticides in order to promote farming (Aldosari *et al.*, 2018). To control this ongoing alarming situation, regular monitoring of pesticide use is

required. Basic farmer education and awareness of pesticide toxicity and promotion of IPM and insecticide resistance management (IRM) techniques for effective pest control, are essential (Mehmood *et al.*, 2021).

Conclusion: This research assesses the insecticide resistance profile of *Chrysomya megacephala* populations in Central Punjab, Pakistan. The present research work demonstrates that all adult blowfly, *C. megacephala* populations from four locations of Central Punjab, revealed low to moderate level of resistance to five tested insecticides. Therefore, the suitable mechanism and IPM techniques must be implemented to manage the improper and excessive use of insecticides in Punjab to prevent the development of resistance in blowflies. Farmers need to receive training on the proper usage of insecticides. Further research is needed to elucidate various resistance patterns in *C. megacephala* to devise effective management strategies.

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Supplementary Materials: The supplementary materials accompanying this article provide further details and supporting information.

Data Availability: The datasets generated and/or analyzed during the current study are not currently publicly available. However, we are committed to making the data available upon publication. Until then, the data can be obtained from the corresponding author upon reasonable request.

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

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