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RESEARCH ARTICLE

Enzymatic Detoxification of Insecticides in Blowfly (*Chrysomya megacephala*): A Threat to Ectoparasite Control in Livestock from Central Punjab, Pakistan

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ABSTRACT

A strong relationship exists between the livestock and blowfly, *Chrysomya megacephala* (Order: Diptera, Family: Calliphoridae), because it plays a major role in causing the veterinary condition known as myiasis, which involves the fly larvae invading the living animal tissues. Animal health and welfare gets severely impacted by this condition which also leads to major economic losses. The adult *C. megacephala* plays active role in transferring different types of pathogens, primarily bacteria, protozoans and helminthic parasites. The common practice followed for the management of insect population including blowflies in Central Punjab involves the use of insecticides. But their repeated exposure has resulted into emergence of resistances in various insect species against these insecticides. This study assessed the resistance levels of blowfly populations against several insecticides, including deltamethrin, fipronil, chlorpyrifos, imidacloprid, and pyriproxyfen. Insecticide bioassays involving both adult and larval stages of *C. megacephala* were followed to assess the resistance profiles. The studied populations displayed different levels of resistance at different concentrations. Overall results showed moderate resistance to deltamethrin (8.57-15.84) fold, whereas the resistance to fipronil, chlorpyrifos, imidacloprid and pyriproxyfen was found to be very low with resistance values ranging from (3.34-8.38) fold. The enzyme assays demonstrated significant upregulation of certain enzymes including monooxygenase, glutathione-*S*-transferase, and non-specific (α and β) esterase activities in response to insecticide exposure. This suggests a functional role for these enzymes in the development of resistance, potentially through the detoxification of insecticides or the modification of target site sensitivity. Our findings provide insights into the biochemical basis of insecticide resistance in this species, with implications for the development of novel control strategies in veterinary medicine. This research aims to inform evidence-based approaches for managing blowfly populations and mitigating their impact on animal health.

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INTRODUCTION

Chrysomya megacephala (Fabricius, 1794) (Order: Diptera, Family: Calliphoridae), is a cosmopolitan fly species found in tropical and subtropical regions (Verves, 2005; Williams and Villet, 2006). Blowflies pose a significant threat to animal health and productivity worldwide (Wall and Shearer, 2001). In most localities, these flies cause myiasis in livestock, invading the wounds and tissues and causing damage through infection (Chhabra *et al*., 2009; Sinha, 2012). The sheep industry is particularly affected, with blowfly-strike being a major concern, especially in Australia, New Zealand and England, where it has had an extensive economic and animal welfare impact (Sandeman *et al*., 2014; Kotze and James, 2022). Animal dung is an excellent breeding site for blowflies that also ties the link between livestock and blowflies. Understanding the mechanisms of insecticide

resistance in blowflies is essential for developing sustainable control strategies that minimize the risk of resistance development and promoting animal health and welfare.

The research on this necrophagous species shows that blowflies carry 12 times more bacterial contents than their closely related *Musca domestica* species (Chaiwong *et al*., 2014; Xu *et al*., 2022). The fly species *C. megacephala* strongly relates to human activities because it demonstrates the ability to inhabit diverse ecological areas (Chaiwong *et al*., 2012; Badenhorst and Villet, 2018). Insecticides hold extensive use for pest control globally because they destroy the target pest populations quickly (Sharma *et al*., 2019). The repeated contact with insecticides has led flies particularly *C. megacephala* to build resistance against these hazardous chemicals (Sukontason *et al*., 2005; Viana *et al*., 2020; Maddheshiya *et al*., 2021).

The occurrence of blowflies typically reveals inadequate animal health along with unsanitary and unhygienic practices in the environment. The situation leads to elevated pesticide application and simultaneous contamination of water supplies and food sources and ultimately violates the balance of natural environment (Massan *et al*., 2017; Bakhtawer and Afsheen, 2021). Several genetic and environmental factors promote the resistance development in insects. This phenomenon has been reported in global research studies. The rising concern about insecticide resistance requires a complete shift toward sustainable pest management practices (Smith *et al*., 2000; Meola *et al*., 2000; Levot *et al*., 2004; Rust, 2005; Singh *et al*., 2015).

Blowflies show resistance development to insecticides by intensifying various enzyme levels which metabolize chemical active substances thus reducing their effectiveness. Resistant insects utilize three enzyme groups namely esterases, monooxygenases and glutathione-*S*-transferases to defend themselves from these toxic chemicals (Siddiqui *et al*., 2023). The investigation of detoxifying enzymes that contribute to resistance development, holds great importance. Because it provides critical information necessary to develop effective insecticide resistance management solutions.

In Pakistan, Central Punjab is an agricultural region that largely depends on chemical-intensive practices for soil fertility management and pest control (Javaid *et al*., 2023). This over dependence on pesticides has accelerated the emergence of resistance in various pest species, including blowflies. The mechanisms driving resistance in *C. megacephala* populations in Pakistan remain poorly understood. This study aims to investigate the resistance mechanism in *C. megacephala*, in order to provide new insights into the evolution of resistance in this region.

MATERIALS AND METHODS

Sample collection: The experiment was conducted between March-July, 2024 considering the ideal environmental conditions for blowfly rearing. Adult *C. megacephala* were collected by using trap method from four locations in Central Punjab namely, Sargodha (32°N, 72°E), Bhalwal 32°N, 72°E), Faisalabad (31°N, 73°E) and Chiniot (31°N, 72°E). It is worth noting that insecticides were widely used at these locations. On the other hand, the reference population was obtained from areas with low insecticide exposure. This blowfly population was maintained in an insecticide-free environment. The F1 field-derived population was used for the bioassay. In contrast, the F1 field-derived reference population, which exhibited relatively low LC_{50} values, served as the standard for evaluating resistance levels. Flies were fed a diet of chicken meat and sugar solution in controlled laboratory conditions (El-Ebiarie and Taha, 2012).

The rearing conditions were standardized as follows: Temperature (25°C-26°C), humidity (50%-70%), and photoperiod (12:12 light/dark). Stock solutions of five insecticides i.e. deltamethrin, fipronil, chlorpyrifos, imidacloprid, and pyriproxyfen were diluted to five concentrations i.e. 6.25 parts per million (ppm), 12.5, 25, 50, and 100ppm by using serial dilution method (Heong *et al*., 2011). These chemicals were tested against *C. megacephala* populations and their third instar larvae by following residual method as described by Khater *et al*. (2021). While adults are the primary stage of veterinary concern, we expanded our investigation to include detoxifying enzyme activities in third instar larvae. This comprehensive approach allows us to better understand the mechanisms underlying resistance development in *C. megacephala*. Moreover, larval stages may play a significant role in driving resistance evolution, as many insecticides target both larval and adult life stages, thereby exerting selection pressure across the entire lifecycle.

Mortality rates were assessed for 24h post insecticidal treatment. These mortality levels served as the basis to determine the susceptibility status of blowfly populations. Resistant flies were identified through a mortality test at 100ppm that showed 80% or less mortality whereas susceptible flies demonstrated 95% or higher mortality at the same concentration according to World Health Organization (WHO) guidelines (2013). Prior to enzymatic analysis, resistant blowfly larvae and adult flies were preserved at -20°C to maintain tissue integrity. The biochemical systems causing insecticide resistance in *C. megacephala* were investigated through enzyme activity analysis. From preserved samples, crude extracts (supernatants) were prepared from adult blowflies with legs and wings removed and whole larvae. The enzyme assays aimed to reveal potential resistance mechanisms that occur in this particular blowfly population.

Enzyme preparation: Enzyme preparations were made using the method described by Naseem *et al*. (2013), involving homogenization and centrifugation of fly samples at 13,000rpm for 5 minutes at 4°C.

Total protein extraction: The Bradford dye-binding technique (Bradford, 1976) following few modifications was applied to estimate protein levels by mixing Bradford reagent with supernatant and sodium phosphate buffer. The absorbance reading was measured after incubation at 30°C for 15min by using a spectrophotometer at 595nm while using a BSA standard curve for results calibration.

Monooxygenases activity assays: The analysis of monooxygenase enzyme activities occurred through a modified Vulule *et al.* (1999) assay method. The measurement parameters included the reaction mixture

containing H_2O_2, tetramethylbenzidine and supernatant. Absorbance was analyzed at 620nm. The enzyme activity measurement utilized a calibration curve made with cytochrome c as a reference.

Esterase activity assay: Researchers assessed α and β esterase enzyme activities through a modified method of the Van Asperen (1962) assay. The assay mixture included alpha and beta naphthyl acetate as the substrate. A spectrophotometer measured optical densities at wavelengths of 620nm and 545nm in order to establish protein (mM/min/mg) enzyme activities through standardized curves.

Glutathione-*S*-transferase assay: A modified methodology of Habig *et al.* (1974), was followed to evaluate GST activity towards CDNB. The reaction mixture consisted of supernatant, reduced glutathione, 1-chloro-2,4-dinitrobenzene, and sodium phosphate buffer. The absorbance was measured at 340nm and GST activity was calculated by utilizing the molar extinction coefficient.

$$\text{CDNB-GSH conjugate formed in nM/ mg protein/ min} = \frac{\text{AB\S (increase in 5min) x 2.7 x 1000}}{\text{5.33 x 5 x protein in mg}}$$

Data analysis: Probit analysis (SPSS, version 23 for Windows) was used to determine the LC_{50} values. The resistance ratios (RR) were calculated by dividing the LC_{50} of the field strain by the LC_{50} of the F1 field-derived population. Graphpad prism (version 10 for windows) was used to apply one way ANOVA to determine the activity of different detoxifying enzymes (Table 1).

RESULTS

The results of toxicity bioassays revealed varying levels of resistance to tested insecticides in different populations. The field populations of *C. megacephala* exhibited 8.57-15.84, 4.84-8.38, 3.51-6.65, 3.98-5.63, and 3.34-4.66-fold RR to deltamethrin, fipronil, chlorpyrifos, imidacloprid, and pyriproxyfen, respectively (Data has been submitted somewhere else). The results showed significant variations in enzyme activity among different populations. α-esterase activity was significantly higher in Sargodha and Chiniot populations (P<0.0001) compared to the F1 field-derived reference population, while the Bhalwal population showed a significant increase (P=0.001). β-esterase activity was also significantly higher in Sargodha and Bhalwal populations (P<0.0001), with a significant increase in Faisalabad (P=0.0006), while the Chiniot population showed no significant difference (P>0.05). Monooxygenase activity was significantly increased in Bhalwal and Faisalabad populations (P<0.0001), with a significant increase in the Sargodha population (P=0.001). Bhalwal and Faisalabad populations exhibited a highly significant increase in protein concentration (P<0.0001), while the Sargodha and Chiniot populations showed no significant difference (P>0.19 and P>0.99, respectively) compared to the F1 field-derived reference population. GST activity was significantly higher in the Bhalwal population (P<0.0001), with a significant increase in Sargodha (P=0.003) (Table 1; Fig. 1).

Notably, in the case of fipronil exposed populations, significant inter-population variations in enzyme activity were observed. For instance, α-esterase activity was markedly elevated in Bhalwal and Chiniot populations (P<0.0001), with a notable increase in Sargodha (P=0.05). In contrast, β-esterase activity was substantially increased in Sargodha, Bhalwal, and Faisalabad populations (P<0.0001). Monooxygenase activity, on the other hand, was significantly enhanced in the Chiniot population (P=0.0008) relative to the F1 field-derived reference population. Furthermore, total protein content was significantly augmented in Bhalwal (P=0.0003) and Chiniot (P<0.0001), moderately increased in Sargodha (P=0.027), but remained unchanged in the Faisalabad population (P=0.30). Glutathione-*S*-transferase activity was uniformly elevated across all populations, with highly significant increases observed in Bhalwal, Faisalabad, and Chiniot (P<0.0001), and a moderately significant increase in Sargodha (P=0.005) (Table 1; Fig. 2).

In the case of chlorpyrifos, significant inter-population variations in enzyme activity were observed. α-esterase activity was markedly elevated in the Sargodha population (P<0.0001), with a moderate increase in the Faisalabad population (P=0.01). β-Esterase activity, on the other hand, was highly significantly increased in the Chiniot population (P<0.0001), with a moderate increase in the Faisalabad population (P=0.001). Monooxygenase activity

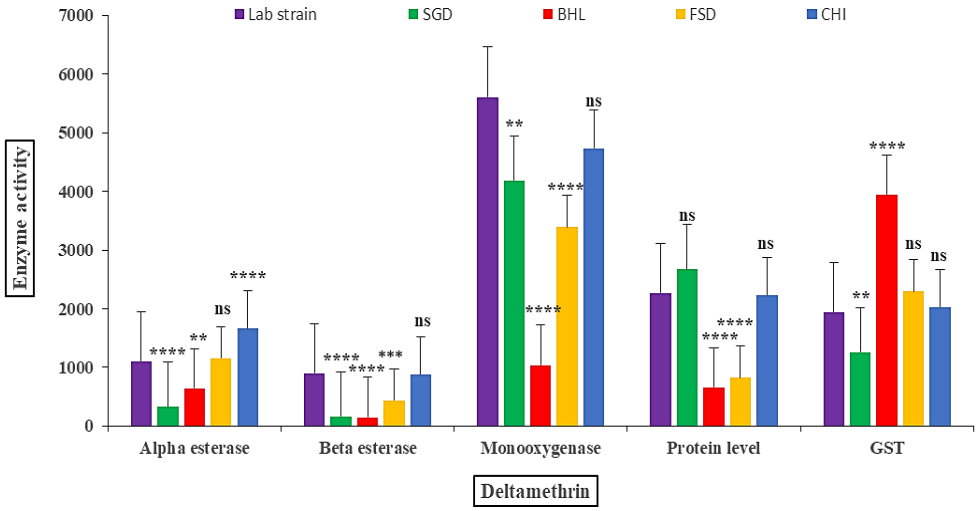

Fig. 1: Comparison of detoxifying enzyme activity in resistant and susceptible *C. megacephala* populations against deltamethrin from Central Punjab, Pakistan.

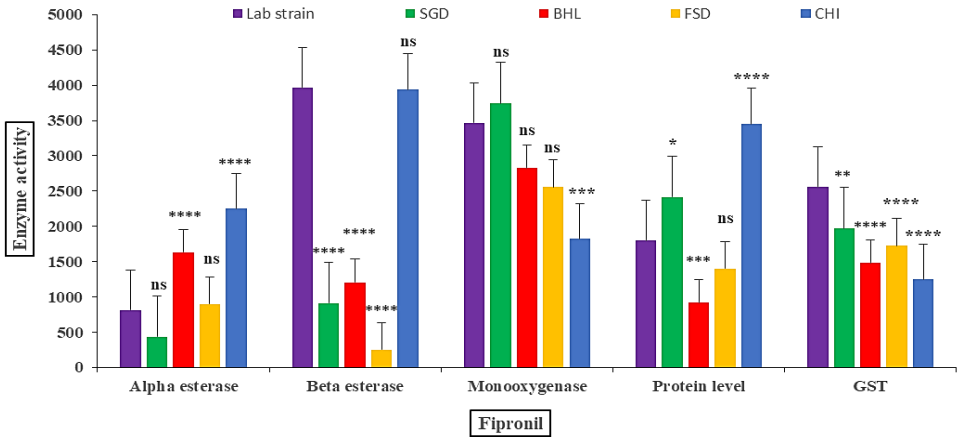

Fig. 2: Comparison of detoxifying enzyme activity in resistant and susceptible *C. megacephala* populations against fipronil from Central Punjab, Pakistan.

Table 1: Comparison of detoxifying enzyme activity in resistant and susceptible *C. megacephala* populations from Central Punjab, Pakistan

Deltamethrin																
Population	n	Alpha Esterase (mM/min/mg of protein)			Beta Esterase (mM/min/mg of protein)			Monooxygenase (µg/min/mg of protein)			Protein activity (microgram/ml			GST (nM/mg of protein/min)		
		Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value
FI field-derived reference population	180	1099	64.91		900.6	78.45		5610	253.9		2269	155.7		1937	114.4	
Sargodha	180	324.1	31.22	<.0001	160.9	17.49	<.0001	4184	279.0	0.001	2670	144.4	0.19	1257	101.2	0.003
Bhalwal	180	642.8	48.99	0.001	150.5	14.45	<.0001	1039	225.6	<0.0001	658.2	60.06	<0.0001	3939	168.7	<0.0001
Faisalabad	180	1155	71.92	0.99	435.3	52.51	0.0006	3390	328.9	<0.0001	820.1	79.94	<0.0001	2292	105.3	0.34
Chiniot	180	1659	155.2	<.0001	872.8	153.1	0.99	4736	215.5	0.13	2222	172.9	0.99	2023	167.9	0.99
Fipronil																
Population	n	Alpha Esterase			Beta Esterase			Monooxygenase			Protein activity			GST		
		Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value
FI field-derived reference population	180	811.8	60.5		3963	396.9		3468	251.8		1799	143.2		2558	143.0	
Sargodha	180	434.5	28.98	0.05	910.4	82.75	<0.0001	3743	340.8	0.96	2413	170.6	0.027	1969	100.2	0.005
Bhalwal	180	1632	154.4	<0.0001	1208	121.8	<0.0001	2827	326.0	0.53	916.3	60.46	0.0003	1482	129.3	<0.0001
Faisalabad	180	900.1	47.22	0.96	250.6	34.85	<0.0001	2554	262.8	0.17	1399	133.0	0.30	1725	96.73	<0.0001
Chiniot	180	2251	134.2	<0.0001	3939	168.7	>0.99	1820	269.3	0.0008	3450	192.2	<0.0001	1246	120.3	<0.0001
Chlorpyrifos																
Population	n	Alpha Esterase			Beta Esterase			Monooxygenase			Protein activity			GST		
		Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value
FI field-derived reference population	180	1170	65.93		250.6	34.85		3739	288.5		1311	156.9		1208	121.8	
Sargodha	180	2462	143.6	<0.0001	149.7	20.20	0.74	3911	362.9	0.99	1131	74.40	0.86	2041	109.8	<0.0001
Bhalwal	180	1141	106.8	0.99	459.8	72.57	0.09	2549	283.7	0.05	1018	73.80	0.51	1349	112.3	0.91
Faisalabad	180	704.8	58.95	0.01	569.9	48.08	0.001	3288	303.0	0.83	2462	143.6	<0.0001	1463	85.10	0.54
Chiniot	180	1467	112.0	0.24	824.4	91.28	<0.0001	2536	292.4	0.04	4929	174.1	<0.0001	2105	151.4	<0.0001
Imidacloprid																
Population	n	Alpha Esterase			Beta Esterase			Monooxygenase			Protein activity			GST		
		Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value
FI field-derived reference population	180	1026	80.71		766.2	74.22		3963	396.9		2273	122.0		1619	115.9	
Sargodha	180	773.1	37.16	0.19	424.7	31.64	0.001	3940	304.9	>0.99	1349	131.8	<0.0001	1954	102.9	0.44
Bhalwal	180	948.2	76.70	0.96	648.6	73.20	0.66	4643	261.2	0.54	718.0	60.77	<0.0001	1497	100.8	0.97
Faisalabad	180	1298	73.65	0.13	250.9	40.95	<0.0001	2279	278.6	0.001	1468	96.45	0.0001	1625	169.3	>0.99
Chiniot	180	1536	120.5	0.0002	444.1	74.67	0.002	3029	309.2	0.22	3223	188.6	<0.0001	2015	192.0	0.27
Pyriproxyfen																
Population	n	Alpha Esterase			Beta Esterase			Monooxygenase			Protein activity			GST		
		Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value	Mean	SE	P-value
FI field-derived reference population	180	2062	207.4		753.4	86.54		3501	350.6		2115	138.3		1937	204.3	
Sargodha	180	992.6	60.73	0.0002	795.1	72.80	0.99	3931	354.4	0.87	2188	151.8	0.99	3737	267.0	<0.0001
Bhalwal	180	1846	269.6	0.90	910.4	82.75	0.75	1027	183.7	<0.0001	1732	173.8	0.36	2716	158.9	0.032
Faisalabad	180	2636	149.1	0.14	1439	131.2	<0.0001	5753	353.9	<0.0001	1255	113.5	0.0006	2095	148.2	0.97
Chiniot	180	1171	103.7	0.003	841.1	81.70	0.96	2089	309.2	0.015	2321	164.2	0.86	2022	140.8	0.99

was significantly enhanced in the Bhalwal and Chiniot populations (P=0.05 and P=0.04, respectively). Furthermore, total protein levels were highly significantly elevated in the Faisalabad and Chiniot populations (P<0.0001), with no significant changes observed in the Sargodha (P=0.86) and Bhalwal (P=0.51) populations compared to the F1 field-derived reference population. GST activity was highly increased in the Sargodha and Chiniot populations

(P<0.0001) than the rest of the populations (Table 1; Fig. 3). In the case of imidacloprid, the results showed significant variations in enzyme activity among different populations of *C. megacephala*. α-esterase activity was moderately increased in the Chiniot population (P=0.002), while no significant changes were observed in the rest of the field populations. In contrast, β-esterase activity was significantly altered across populations, with a highly significant increase in the Faisalabad population (P<0.0001), moderate significance in the Sargodha and Chiniot populations (P=0.001 and P=0.002, respectively), and no significant difference in the Bhalwal population (P=0.66). Monooxygenase activity was significantly increased in the Faisalabad population (P=0.001). Imidacloprid exposure significantly altered protein expression across all populations, with highly significant increases observed in the Sargodha, Bhalwal, and Chiniot populations (P<0.0001). GST activity, on the other hand, showed no significant differences among the imidacloprid-treated populations from Sargodha, Bhalwal, Faisalabad, and Chiniot (P=0.44, P=0.97, P>0.99, and P=0.27, respectively) compared to the F1 field-derived reference population (Table 1; Fig. 4).

In the case of pyriproxyfen, α-esterase activity was significantly elevated in the Sargodha (P=0.0002) and Chiniot (P=0.003) populations, indicating a potential adaptive response to pyriproxyfen. In contrast, the Bhalwal (P=0.90) and Faisalabad (P=0.14) populations showed no significant change in α-esterase activity, suggesting a different enzymatic profile. Analysis indicates that Faisalabad population demonstrated an uncommon mechanism through β-esterase to respond to pyriproxyfen exposure. The analysis showed that Bhalwal and Faisalabad populations positively expressed monooxygenase activity (P<0.0001), but Chiniot population showed a moderate response (P=0.015). The flies from Sargodha avoided any significant alteration in monooxygenase activity levels (P=0.87) although the metabolic process remained different from other populations. Finally, the Faisalabad population showed a moderate elevation in total protein levels (P=0.0006), whereas the Sargodha, Bhalwal, and Chiniot populations remained unchanged (P=0.99, P=0.36, and P=0.86, respectively). This may indicate a stress response or adaptive mechanism in the Faisalabad population. The blowfly population from Sargodha displayed extensive GST increase (P<0.0001) while Bhalwal showed moderate GST elevation (P=0.032). Faisalabad (P=0.97) and Chiniot (P=0.99) populations exhibited no change in enzyme activity, compared to the F1 field-derived reference population (Table 1; Fig. 5).

DISCUSSION

This research assessed the resistance patterns along with enzymatic detoxification mechanisms of insecticides among *C. megacephala* populations from four different locations of Central Punjab including Sargodha, Bhalwal, Faisalabad and Chiniot. The findings of this study contribute towards the development of better insecticides and their proper usage for veterinary parasitology. Through various pathogen transmission to human beings and

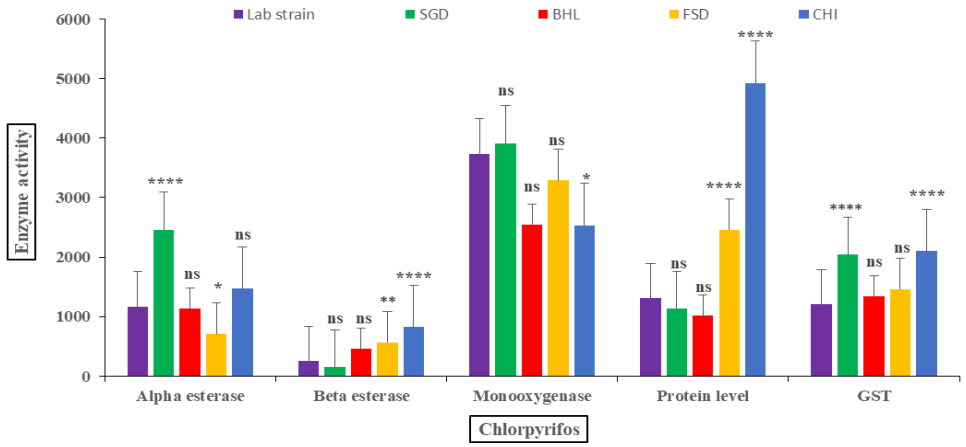

Fig. 3: Comparison of detoxifying enzyme activity in resistant and susceptible *C. megacephala* populations against chlorpyrifos from Central Punjab, Pakistan.

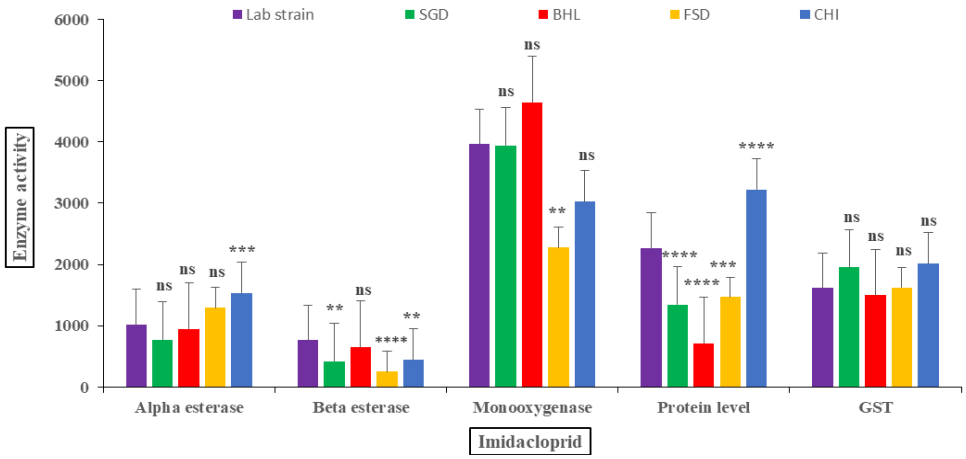

Fig. 4: Comparison of detoxifying enzyme activity in resistant and susceptible *C. megacephala* populations against imidacloprid from Central Punjab, Pakistan.

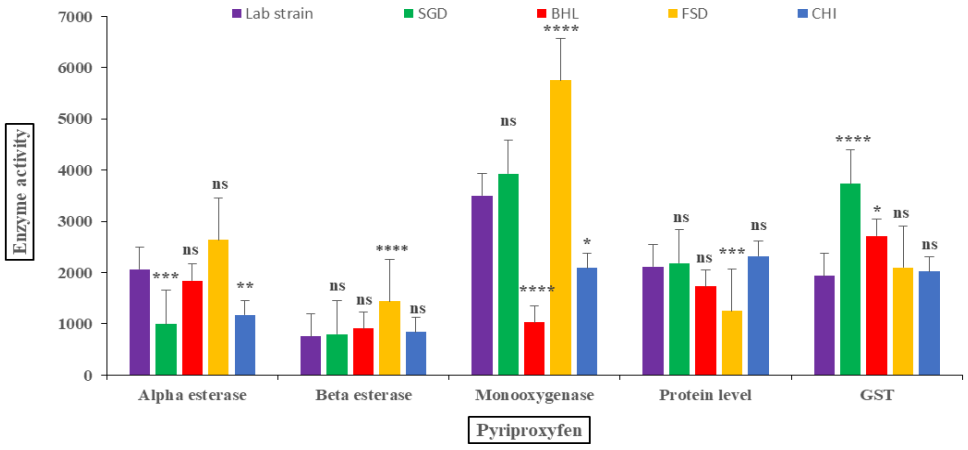

Fig. 5: Comparison of detoxifying enzyme activity in resistant and susceptible *C. megacephala* populations against pyriproxyfen from Central Punjab, Pakistan.

livestock, blowflies spread different diseases including bluetongue disease in sheep, and various infectious diseases in cattle leading to their elimination (Mullen and Durden, 2009; Carneiro *et al*., 2014; Brits *et al*., 2016; Hall *et al*., 2016; Schrottner *et al*., 2017), alongside gastrointestinal, respiratory and urinary infections in human beings (Chaiwong *et al*., 2012).

A combined strategy of using targeted chemical insecticides and precise hygiene/sanitation practice is essential for reducing disease transmission by these vector species (Maddheshiya *et al*., 2021). Our findings suggest that insecticide rotation/combination strategies should be followed to mitigate the development of insecticide resistance. Additionally, alternative control strategies, such as biological control using natural parasites, e.g. *Nasonia vitripennis* should be considered. Few cultural control tactics through proper waste management, or physical control using traps or barriers, may offer effective and sustainable solutions for managing *C. megacephala* populations. The blowfly, *C. megacephala*, uses advanced toxin-defense system through enzymes in order to develop resistance against insecticides. This situation creates substantial obstacles for controlling myiasis (Ferraz *et al*., 2010). Blowfly resistance levels demonstrated different degrees in populations across Central Punjab, Pakistan during the study through evaluation of specific insecticide resistance ratios.

Enzymes within insects control the rapid speed of chemical reactions that occur inside the cells. According to Zibaee (2011) insects develop resistance through these detoxifying enzymes. Exposure to insecticides resulted in disrupted enzyme activity in blowfly populations, causing them to target specific biochemical compounds aberrantly. Notably, the detoxifying enzymes e.g. monooxygenases, glutathione-S-transferase, and non-specific esterases exhibited enhanced activity against these insecticides. In the present study, blowflies have shown (8.57-15.84-fold) resistance to deltamethrin. Interestingly, our findings are consistent with those of Khan *et al*. (2015), who reported a similar level of resistance to deltamethrin (8.41-fold) in *M. domestica* (Diptera) due to increased esterase activity. Our study extends these findings by identifying increased monooxygenase enzyme activity as an additional contributing factor to deltamethrin resistance in blowflies. Jahan and Shahid (2013) documented very low resistance (1.95-fold) against deltamethrin in dengue mosquito, *Aedes aegypti* (Diptera) from Lahore, Pakistan. Khan *et al*. (2017) reported notable resistance in house flies of urban areas of Punjab. The researchers observed moderate level (7.22-19.31-fold) resistance in tested populations of *M. domestica*. This variable pattern of resistance ratio in various dipteran species suggests that deltamethrin resistance is a widespread issue in Punjab, Pakistan affecting multiple pest management strategies.

Another research was conducted by Kojima *et al*. (2022) to test the susceptibility of synthetic pyrethrins including deltamethrin against blowfly, *Phormia regina*. The researchers reported the effectiveness of deltamethrin as feeding repellents against this species, highlighting its significance as control agent. In contrast, the tested populations of blowfly (*C. megacephala)* in our study have developed significant resistance against deltamethrin, limiting its effectiveness for blowfly control. Another study by Farahani *et al*. (2022) was conducted to evaluate the toxicity of deltamethrin at the larval stage of blowfly, *Lucilia sericata*. They reported lethal effects of deltamethrin exposure on larvae resulting into delayed adult formation. Comparing their results with our study highlights a significant change in impact of deltamethrin exposure on adult populations of *C. megacephala*, resulting into notable resistance due to involvement of detoxifying enzymes. Therefore, it is important to monitor emerging resistance at various stages of blowfly development in order to control this ongoing situation. The detrimental effects of deltamethrin on the developmental stages of blowfly, *C. megacephala* larvae were documented in a study conducted by de Oliveira *et al*. (2021). Surprisingly, the results of their research are consistent with our findings as both studies highlight the significance of esterase enzymes in developing insecticide-resistant blowflies.

In another study conducted by Khater *et al*. (2021), the lethal effects of deltamethrin exposure on blowfly, *Lucilia silvarum* were determined. They reported concentration-dependent effects of deltamethrin insecticide on developmental and reproductive systems of this blowfly species. A key finding shared by both research work is the crucial role of biochemical modifications leading to detoxification of insecticides in blowflies. The major shift in enzyme activity played a significant role to induce blowfly susceptibility towards tested toxic insecticide. Al-Jameeli *et al*. (2021) conducted research to determine resistance against deltamethrin in blowfly, *C. albiceps* and reported variable resistance levels in tested populations due to insecticide exposure. Comparing their results with our

findings, the present study identified a well-defined resistance mechanism in *C. megacephala* involving detoxifying enzymes. The findings of the current study lack the involvement of serine proteases in the development of *C. megacephala* as reported by El-Ebiarie and Taha (2012). However, similarity in both studies highlights the significant role of esterase and monooxygenase enzymes in inducing resistance to deltamethrin in blowflies. The effectiveness of permethrin and deltamethrin as control agents against blowflies was confirmed in early research by Sukontason *et al*. (2005). They reported the effectiveness of permethrin over deltamethrin as control agent against blowfly, *C. megacephala*. However, the findings of our research reveals that blowflies have developed major resistance over a period of time against deltamethrin in Central Punjab, Pakistan. Therefore, it is much needed to understand the complex interactions between enzyme systems and their role in insecticide resistance, in order to develop effective strategies for resistance management.

According to the findings of present research work, tested populations of *C. megacephala* from selected locations have expressed substantial resistance to fipronil (4.84-8.38-fold) due to overexpression of non-specific esterases (α and β), GSTs, and proteins levels. These detoxifying enzymes contributed to the ability of blowflies to minimize the lethal effects of fipronil. By comparing with the findings of Abbas *et al*. (2014) regarding fipronil resistance in *M. domestica* population from Punjab, our study demonstrates that blowfly (*C. megacephala*) populations have also evolved substantial resistance to fipronil due to increased enzyme activity. The findings of both these research work highlights the significance of thorough understanding of resistance mechanisms in various dipteran species to develop effective and long-lasting management strategies. In present study, notable (3.51-6.65-fold) resistance to chlorpyrifos was detected in blowfly populations. This observed resistance is correlated with enhanced activity of glutathione-*S*-transferase enzyme in tested flies, indicating an adaptive response to exposure of chlorpyrifos. In another research conducted by Yasmeen and Amir (2022) related to chlorpyrifos exposure highlights the harmful effects on the ultrastructural integrity of the midgut. This exposure leads to notable biochemical shifts in the larvae of *C. megacephala* and damaged the cellular components of larvae. Recent studies have explored the efficacy of citronella oil and chlorpyrifos against *C. megacephala*, documenting enhanced mortality and reduced oviposition (Denis *et al*., 2018). Al-Jameeli *et al*. (2021) worked on blowfly, *Chrysomya albiceps* and reported varying degrees of resistance to chlorpyrifos exposure in this species. In comparison to our results, both studies emphasize the significance of GSTs in the detoxification process leading to resistance development in different blowfly species.

Blowflies in the current study expressed significant (3.98-5.63-fold) resistance to imidacloprid, correlated with elevated protein activity and esterase levels as an adaptive response towards this chemical in tested populations. Kotze and Rolls (2022) in a related study revealed that blowfly, *Lucilia cuprina* developed notable resistance (3.1-3.8-fold) to imidacloprid. The researchers reported the role of cytochrome P450 and aminobenzotriazole (ABT) as inhibitor in inducing this resistance in the tested population of blowflies. Comparing these observations to our results demonstrate that imidacloprid resistance is a complex phenomenon in different blowfly species involving molecular mechanisms. The third instar larvae of *C. megacephala* in this study exhibited very low level (3.34-4.66-fold) resistance to pyriproxyfen (IGR) due to increased activity of monooxygenase enzymes. In related research conducted by Singh and Kumar (2015) highlights the lethal effects of pyriproxyfen exposure on larvae of blowfly, *C. megacephala*. They reported significant impact of pyriproxyfen on blowfly larvae including inhibited embryonic development and delayed adult emergence as consequence of this exposure. Their findings highlight the potential utility of pyriproxyfen as a control agent in managing *C. megacephala* population. However, the result of our study reveals that third instar larvae of *C. megacephala* has developed low level resistance against pyriproxyfen in tested populations. Therefore restricts the efficacy of pyriproxyfen as a control agent against blowflies. Qamar-ul-Haq *et al*. (2008) conducted research in Central Punjab, Pakistan to report the reasons behind resistance development. According to their observations, the excessive and unfair use of insecticides in Central Punjab is one of the main reasons that contributes to the emergence of insecticides-resistant blowflies.

Blowfly control program has become a massive challenge for the global insect management authorities due to the emergence of insecticide-resistant species. A deep understanding of biochemical mechanisms driving this situation is required in order to develop a novel, targeted and sustainable approach towards resistance management. Additionally, the misuse of pesticides to promote farming in Central Punjab as being the leading cause for resistance development in various insects should be monitored strictly. This present situation is threatening for both environmental sustainability and human well-being. The insufficient knowledge of integrated pest management (IPM) and insect resistance management (IRM) are also contributing to development of resistant insects (Aldosari *et al*., 2018). Further research and continuous efforts are required to understand various resistance mechanisms in blowflies and to discover novel, effective and environment friendly insecticides.

The significant role of different detoxifying enzymes in developing insecticide-resistant populations of *C. megacephala* was evaluated in present study. Whereas target site resistance in blowflies involving deltamethrin target gene mutation and the role of cytochrome P450 enzyme in inducing resistance were not discussed as avenues for future research. Further research would be a crucial step in understanding other complex mechanisms driving behavioral and cross resistance in blowfly, *C. megacephala*.

Conclusions: The present research work contributes significantly to our knowledge of mechanisms promoting resistance development in blowfly, *Chrysomya megacephala* and its larvae in Central Punjab, Pakistan. The findings of the study highlight the key role of detoxifying enzymes including non-specific esterase, glutathione-*S*-transferase, and monooxygenase. Exposure to deltamethrin, fipronil, chlorpyrifos, imidacloprid and pyriproxyfen enhanced the total protein activity of tested

populations of blowflies. These findings hold important steps towards adopting an integrated management strategy to address insecticide-resistance issues in Central Punjab. In order to elucidate the other complex mechanisms driving resistance in this veterinary pest, further investigation is required. This attempt will be helpful to develop more effective and targeted control methods to manage this pest species.

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Authors contribution: SJ: Conceptualization, Methodology, Investigation, Data curation, Writing-Original draft. MKM: Conceptualization, Supervision, Writing - Review & Editing. NA: Methodology, Data analysis, Writing - Review. SYK: Resources, Equipment provision. HAAK: Writing - Review & Editing.

REFERENCES

Abbas N, Khan HAA and Shad SA, 2014. Cross-resistance, genetics, and realized heritability of resistance to fipronil in house fly, *Musca domestica* (Diptera: Muscidae): a potential vector for disease transmission. Parasitol Res 113:1343-1352.

Aldosari F, Mubushar M and Baig MB, 2018. Assessment of farmers knowledge on pesticides and trainings on pesticide waste management in central Punjab–Pakistan. J Exp Biol Agric Sci 6(1):168-175.

Al-Jameeli MM, Mahyoub JA, Al-Ghamdi KM, *et al*., 2021. Susceptibility of screwworm fly, *Chrysomya albiceps* to commonly used pesticides in Jeddah Governorate. Life Sci J 18(7):1-8.

Badenhorst R and Villet MH, 2018. The uses of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) in forensic entomology. Forensic Sci Res 3(1):2-15.

Bakhtawer and Afsheen S, 2021. A cross sectional survey of knowledge, attitude and practices related to the use of insecticides among farmers in industrial triangle of Punjab, Pakistan. PloS one 16(8):e0255454.

Bradford MM, 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72(1-2):248-254.

Brits D, Brooks M and Villet MH, 2016. Diversity of bacteria isolated from the flies *Musca domestica* (Muscidae) and *Chrysomya megacephala* (Calliphoridae) with emphasis on vectored pathogens. Afr Entomol 24(2):365-375.

Carneiro JS, Pires EM, Nogueira RM, *et al*., 2014. Bacteria carried by *Chrysomya megacephala* (Fabricius, 1794)(Diptera: Calliphoridae) in Sinop, Mato Grosso, Brazil. Sci Electron Arch 6(1):18-22.

Chaiwong T, Srivoramas T, Sukontason KOM, *et al*., 2012. Bacterial fauna associated with the blow fly, *Chrysomya megacephala* (F.) in Ubon Ratchathani Province of Northeast Thailand. Int J Parasitol Res 4(1):71.

Chaiwong T, Srivoramas T, Sueabsamran P, *et al*., 2014. The blow fly, *Chrysomya megacephala*, and the house fly, *Musca domestica*, as mechanical vectors of pathogenic bacteria in Northeast Thailand. Trop Biomed 31(2):336-346.

Chhabra MB and Pathak KML, 2009. Myiasis of domestic animals and man in India. J Vet Parasitol 23(1):1-7.

de Oliveira, SR, Caleffe R R T, *et al*., 2021. Developmental changes in larvae of the oriental latrine fly, *Chrysomya megacephala*, exposed to deltamethrin. Parasitol Res 120(1):1-7.

Denis CI, Nordin NH, Azman AR, *et al*., 2018. Influence of citronella and chlorpyrifos on *Chrysomya megacephala* (Fabricius) and *Chrysomya rufifacies* (Macquart) (Diptera: Calliphoridae) infesting rabbit carcasses. Trop Biomed 35(3):755-768.

El-Ebiarie A and Taha N, 2012. Molecular characterization of serine proteases from both first and third larval instars of *Chrysomya megacephala*. Life Sci J 9(3):2086-93.

Farahani S, Zand AJ and Mahmoudi E, 2022. Lethal effects of insecticides deltamethrin, spinosad, pyridalyl and pirimiphos-methyl on third Instar larvae of blowfly (*Lucilia sericata*; Dip: Calliphoridae). J Vet Res 7(2):89-97.

Ferraz ACP, Proença B, Gadelha BQ, *et al*., 2010. First record of human myiasis caused by association of the species *Chrysomya megacephala* (Diptera: Calliphoridae), Sarcophaga (Liopygia) ruficornis (Diptera: Sarcophagidae), and *Musca domestica* (Diptera: Muscidae). J Med Entomol 47(3):487-490.

Habig WH, Pabst MJ and Jakoby WB, 1974. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem 249(22):7130-7139.

Hall MJ, Wall RL and Stevens JR, 2016. Traumatic myiasis: a neglected disease in a changing world. Annu Rev Entomol 61(1):159-176.

Heong KL, 2011. Research methods in toxicology and insecticide resistance monitoring of rice planthoppers. International Rice Research Institute, Los Baños, Philippines.

Jahan N and Shahid A, 2013. Evaluation of resistance against deltamethrin and cypermethrin in dengue vector from Lahore, Pakistan. J Anim Plant Sci 23(5):1321-1326.

Javaid Z, Ghazala, Ibrahim, *et al*., 2023. Pesticide contamination of potable water and its correlation with water quality in different regions of Punjab, Pakistan. Water 15(3):543.

Khan HAA, Akram W and Haider MS, 2015. Genetics and mechanism of resistance to deltamethrin in the house fly, *Musca domestica* L., from Pakistan. Ecotoxicol 24:1213-1220.

Khan HAA, Akram W and Fatima A, 2017. Resistance to pyrethroid insecticides in house flies, *Musca domestica* L.,(Diptera: Muscidae) collected from urban areas in Punjab, Pakistan. Parasitol Res 116:3381-3385.

Khater K, Elsobki AE, Ramadan M, *et al*., 2021. Biological and Physiological Disturbances of *Lucilia silvarum* Meigen (Diptera: Calliphoridae) Treated with Certain Insecticides. Egypt Acad J Biol Sci E Med Entomol Parasitol 13(1):63-83.

Kojima T, Yamato S and Kawamura S, 2022. Natural and Synthetic pyrethrins act as feeding deterrents against the black blowfly, *Phormia regina* (Meigen). Insects 13(8):678.

Kotze AC and James PJ, 2022. Control of sheep flystrike: what's been tried in the past and where to from here. Aust Vet J 100(1-2):1-19.

Levot G and Sales N, 2004. Insect growth regulator cross-resistance studies in field-and laboratory-selected strains of the Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) (Diptera: Calliphoridae). Aust J Entomol 43(4):374-377.

Maddheshiya R, 2021. Effect of a novel juvenoid fenoxycarb on the pupal-adult transformation in the blowfly, *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae). Parasitol Res 120(7): 2351-2356.

Meola R, Meier K, Dean S, *et al*., 2000. Effect of pyriproxyfen in the blood diet of cat fleas on adult survival, egg viability, and larval development. J Med Entomol 37(4):503-506.

Mullen G R and Durden L A, 2009. Medical and veterinary entomology (3rd Ed), Academic press, 125 London Wall, London EC2Y 5AS, United Kingdom.

Naseem S, Tahir HM and Yaqoob R, 2013. Effect of Chlorpyrifos on the Activities of Non-specific Esterases in *Musca domestica* L. Pak J Zool 45(6):1771-1772.

Qamar-ul-Haq TA, Ahmad M and Nosheen F, 2008. An analysis of pesticide usage by cotton growers: a case study of district Multan, Punjab-Pakistan. Pak J Agri Sci 45(1):133-137.

Rust MK, 2005. Advances in the control of *Ctenocephalides felis* (cat flea) on cats and dogs. Trends Parasitol 21(5):232-236.

Sandeman RM, Levot GW, Heath ACG, *et al*., 2014. Control of the sheep blowfly in Australia and New Zealand–are we there yet? Int J Parasitol 44(12):879-891.

Siddiqui JA, Fan R, Naz H, *et al*., 2023. Insights into insecticide-resistance mechanisms in invasive species: Challenges and control strategies. Front Physiol 13:1112278.

Singh S and Kumar K, 2015. Effect of juvenoids pyriproxyfen and diofenolan on embryogenesis and postembryonic development of blow fly *Chrysomya megacephala* (Diptera: Calliphoridae) following egg treatment. Parasitol Res 114(9):3213-3222.

Sinha S, 2012. Myiasis in domestic animals: new records of calyptrate Diptera. J Parasit Dis 36(2):277-279.

Sharma, A, Kumar V, Shahzad B, *et al*., 2019. Worldwide pesticide usage and its impacts on ecosystem. SN Applied Sciences 1:1-16.

Schrottner P, Rudolph WW, Damme U, *et al*., 2017. *Wohlfahrtiimonas chitiniclastica*: current insights into an emerging human pathogen. Epidemiol Infect 145(7):1292-1303.

Smith KE, Wall R, Howard JJ, *et al*., 2000. In vitro insecticidal effects of fipronil and β-cyfluthrin on larvae of the blowfly *Lucilia sericata*. Vet Parasitol 88(3-4):261-268.

Sukontason K, Chaiwong T, Tayutivutikul J, *et al*., 2005. Susceptibility of *Musca domestica* and *Chrysomya megacephala* to permethrin and deltamethrin in Thailand. J Med Entomol 42(5):812-814.

Van Asperen K, 1962. A study of housefly esterases by means of a sensitive colorimetric method. J Insect Physiol 8(4):401-416.

Verves YG, 2005. A catalogue of oriental Calliphoridae (Diptera). Int J Dipterol Res 16(4):233-310.

Viana TDS, Dias RF, Vianna ACDS, *et al*., 2020. Evaluation of Chilean Boldo essential oil as a natural insecticide Against *Chrysomya megacephala* (Diptera: Calliphoridae). J Med Entomol 57:1364-72.

Vulule JM, Beach RF, Atieli FK, *et al*., 1999. Elevated oxidase and esterase levels associated with permethrin tolerance in *Anopheles gambiae* from Kenyan villages using permethrin-impregnated nets. Med Vet Entomol 13(3):239-244.

Wall R and Shearer D, 2001. Veterinary ectoparasites: biology, pathology and control.

Williams KA and Villet MH, 2006. A history of southern African research relevant to forensic entomology. S Afr J Sci 102(1):59-65.

Xu W, Wang Y, Wang YH, *et al*., 2022. Diversity and dynamics of bacteria at the *Chrysomya megacephala* pupal stage revealed by third-generation sequencing. Sci Rep 12(1):2006.

Yasmeen S and Amir M, 2022. Ultrastructural, histochemical, and biochemical damage induced by chlorpyrifos in the midgut of *Chrysomya megacephala* larvae after acute exposure. Int J Trop Insect Sci 42(6):3793-3806.

Zibaee A, 2011. Botanical insecticides and their effects on insect biochemistry and immunity. In: Pesticides in the modern world: (Stoytcheva M, ed): InTech, Janeza Trdine 9, 5100, Rijeka, Croatia pp:55-68.