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The pyrethroid knockdown resistance (*kdr*) gene frequency and its impact on the enzyme activity in house fly, *Muscadomestica* L. from Faisalabad, Pakistan

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Abstract

House fly, *Muscadomestica*act as a vector of various pathogens *viz*. virus, bacteria, fungi, protozoa and nematodes; and is responsible for transmitting wide variety of human and veterinary diseases. It is one of the major concerns due to its high fecundity and hence, poses a serious concern to control. Although, different groups of insecticides are being used for its control; however, resistance has been reported against pyrethroids. The current study was conducted to monitor the frequency of pyrethroid resistance gene *kdr* in house fly populations of District Faisalabad. The molecular and biochemical assays were performed on fly samples from eleven different sites. DNA was amplified for knock down resistance genethrough PASA (PCR Amplification of Specific Alleles) method by using outer primers *kdr1* and *kdr4*, and the inner primers *kdr2* and *kdr3* which specifically amplify the domain-II of kdrgene. Two populations were found homozygous susceptible (+/+; 18%); whereas three populations were found genetically homozygous resistant (-/-; 27%) which are insensitive to pyrethroid insecticides. Similarly, six populations were found heterozygous (+/-; 55%) for kdr suggesting thereby that at least 1/4th homozygous resistant (-/-) house fly populations with insensitivity to pyrethroids would be produced in future keeping in view the Mendelian ratio. Biochemical assay showed that homozygous resistant and heterozygous populations had increased activity of Acetylcholinestarse (AChE), α-Carboxylesterase (α-Carboxyl), β-Carboxylesterase (β-Carboxyl), Alkaline Phosphatase (AKP) and Acidic Phosphatase (ACP) enzymes. The current results, strongly suggests that management program for pyrethoidsinsecticides resistance should be implementedin future countrywide.

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Introduction

Housefly is one of the major concerns of arthropods in agriculture field because of its high fecundity (Malik *et al.*, 2007). Houseflies act as a vector of many pathogens of above than 100 diseases of humans and animals (Forster *et al.*, 2009). The earliest record about housefly was as far back as 1577, when Mercurialis suggested that houseflies were the reason behind the prevalence of plague (Malik *et al.*, 2007). There is huge documentation on houseflies hazardous to human and animals (Denholm *et al.*, 1985; Hogsette, 1996). House flies mostly found in livestock and poultry farms all across the globe causing huge economic lose. According to revenue almost there was loss of about four million dollars per annum in the USA in1980, s (Axtell, 1986). The poor management of wastes by municipality and large manure heaps on dairy farms results in development of huge population of housefly (Moon *et al.*, 2001). There is need of effective management of pests in agriculture since the beginning of communities of insects. Therefore, different methods for housefly control are developed and manipulated.

Different practices were done to combat housefly population, chemical control were considered to be most reliable method for control while, *Muscadomestica* showed potential to resist against insecticidal groups. The resistance of house fly against chemicals had lead to great attention of researchers (Akiner and Caglar, 2012). Increase in metabolic detoxification of insects of agricultural importance is considered to be major factor of resistance against pyrethroids (McCaffery, 1998). Metabolic studies of radio labeled pyrethroids and biochemical studies about esterase provide evidence that activity of esterase play important role in resistance against pyrethroids (Soderlund and Bloomquist, 1990). For example elevated level of esterase has been known as the mechanism of pyrethroid resistance in household insects like mosquitoes and houseflies *Muscadomestica* (Naqqash *et al.*, 2016).

Non-metabolic resistance factor was first documented conferring as rapid paralytic knock down and severe action of Pyrethroids and DDT in house fly, *Muscadomestica* in 1951 (Busvine,1951).The mechanism is currently named *kdr* (knock down resistance).Different alleles having resistance factor including kdr and super *kdr* which have been mapped to a locus on autosome 3 in the housefly. Therefore the term *kdr* only refers to the resistance due to *kdr* allele in adult housefly (Sawicki, 1978). Now the term *kdr* or *kdr*like resistance is widely used in literature regarding to resistance against pyrethroids because of nerve insensitivity (Sawicki, 1985; Soderlund and Bloomquist, 1990). The *kdr* mechanism is expressed as an obvious reduction in susceptibility of insect nervous system towards pyrethroids (Sawicki, 1978).

In Pakistan, chemical pesticides were consistently used for the control of houseflies but there is no proper work done to detect alleles causing resistance against pyrethroid group of insecticides. Therefore, current study was designed to investigate the frequency of pyrethroid insecticide resistance kdr allele in house fly population from different locations of District Faisalabad, Punjab, Pakistan.

Materials and methods

Houseflies samples were collected from eleven sites from Faisalabad during 2018; *viz.* Jhang bazar, Gulfishan colony, Jinnah colony, Samundari, Shahkot, Ghulam Muhammad abad, Khalidabad, Dhobi ghat, Khurianwala, SamanabadandRehmat Ali Park.

Bioassay

The collected flies were reared in the Entomology Lab of Department of Zoology, Government College University Faisalabad, on artificial diet under optimum conditions following the protocol of Keiding (1964). The lab strain was considered as reference strain. Five Insecticides (Lambda-cyalothrin, Deltamethrin, Cypermethrin, Chlorpyrifos and Tetramethrin.) were used in bioassay against five concentrations (2.5, 5, 10, 20 and 40 ppm) of each insecticide. The experiment was replicated for three times. About 60 flies were used in each concentration causing > 0% and <100% mortality.

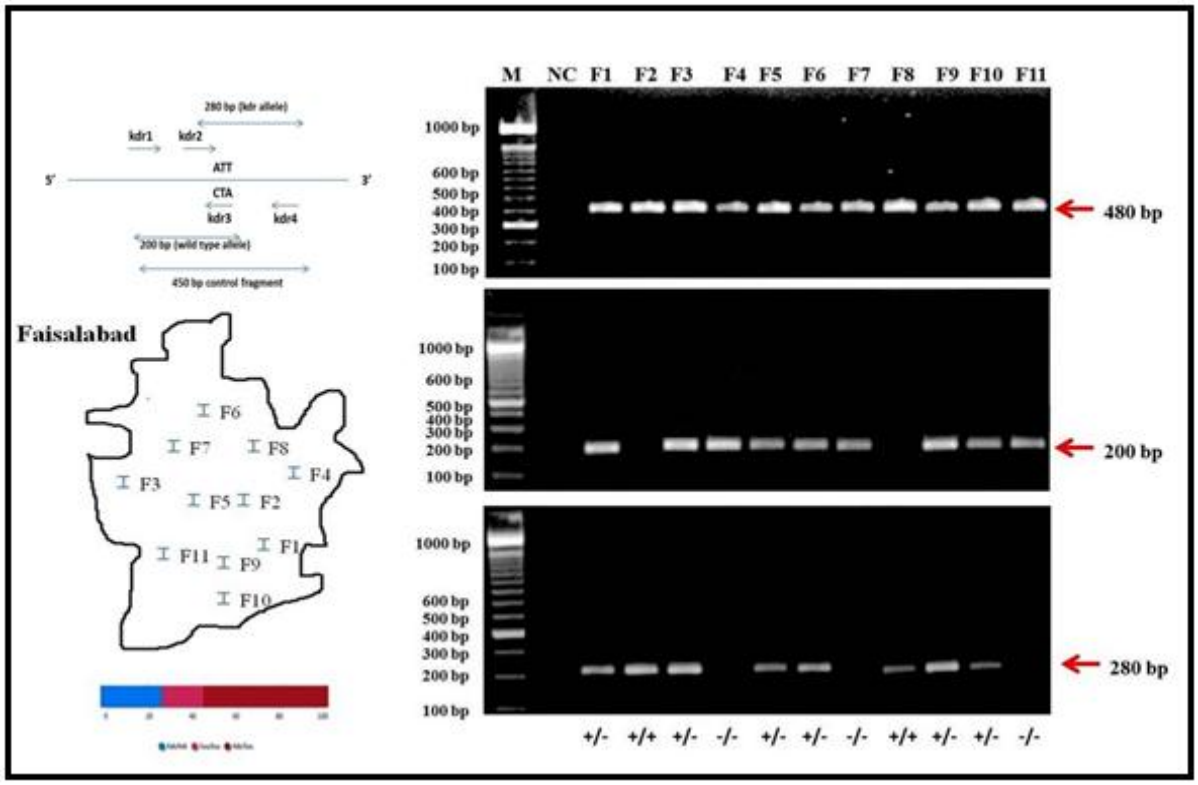

Fig. 1. Genotyping of kdr mutation through PASA (PCR Amplification of Specific Allele) from District Faisalabad
Upper left panel: kdr alleles.
Middle left panel: Sampling sites from district Faisalabad.
Lower left panel: Frequency of homozygous sus/sus, heterozygous kdr/sus and homozygous kdr/kdr*loci*
Upper right panel: A control fragment amplified using kdr1 and kdr4 primers.
Middle right panel: Susceptible allele fragment of 200-bp size amplified using kdr1 and kdr3 primers.
Lower right panel: kdr allele fragment of 280-bp size amplified by kdr2 and kdr4 primers.
F1: Jhang Bazar; F2: Jinnah Colony; F3: Shah Kot; F4: Gulfishan Colony;
F5: Samundari Road; F6: GM Abad; F7: Rehmat Ali Park; F8: Khalidabad;
F9: Dhobi Ghat; F10: Khurianwala; F11: Samanabad

Mortality levels were assessed at 24, 48 and 72 hours of exposure to insecticides. Corrected mortality was also obtained by noticing mortality in control group according to Abbot's formula (Abott, 1925). Ataxic individuals considered died (Khan *et al.*, 2014).

$$P = \frac{T - C}{100 - C} \times 100$$

Here P is the % corrected mortality, C is the % mortality in the non-treated group and T is the % mortality in the treated group.

The flies which remained alive were allowed to complete their three generations. The resistance level was measured in each succeeding generation to evaluate the increase in level of resistance following the protocol of Singh andParkash (2013). The resistance to susceptible ratios was estimated by dividing the LD50 for resistant strain to the LD50 for the Lab/reference strain.

DNA Extraction

An ideal DNA isolation method requires small amount of tissues, simple procedures and use of minimal amount of chemicals to extract good quality as well as quantity of DNA.

The standardized and modified TNE buffer method was preferred for DNA extraction as described by Zahoor *et al.* (2017) and Ashraf *et al.* (2016).

PCR Amplification of Specific Alleles (PASA) for the presence of kdr resistant gene in the housefly population

The amplification of template DNA was performed for *knock down resistance* gene using PASA method (PCR Amplification of Specific Alleles) following the protocol of Huang *et al*., (2004). The two outer allele specific primers *kdr1*,5'-AAGGATCGCTTCAAGG-3'and *kdr4,* 5'-TTCACCCAGTTCTTAAAACGAG-3'of 10pmol and two inner primers *kdr2,* 5'-TCGTGATCGGCAATT-3' *kdr3,* 5'-GTCAACTTACCACAAG-3' of 40pmol were used. The PCR reaction included; 2 µl of genomic DNA, 10pmol of each outer primer and 40pmol of each inner primer,*Taq*PCR Master Mixture 12.5 µl and filtered nuclease-free water 8.5 µl. PCR amplification was initiated by 95°c for 2 minutes, proceed by 40cycles, 94°c for 45 seconds, 54°c for 30 seconds and 72°c for 90 seconds and final extension step was at 72°c for10 minutes. Every PCR reaction includes negative control (no-Template DNA) to make sure that there was no contamination. The PCR amplified fragments were resolved by using electrophoresis on 1.5 % agarosegel stained with ethidium bromide and visualized under UV light documentation system.

Biochemical assays

For biochemical assay, adult house flies were washed properly with distilled water and dried with bloating paper. Adult houseflies were homogenized with ice cold 20 mM Sodium phosphate buffer having pH 7.0 with help of Teflon hand homogenizer and then homogenate was centrifuged at 8000 rpm at 4˚c for 20 minutes(Younes*et al*., 2011; Sultana *et al*., 2016; Riaz*et al*., 2018; Sultana *et al*., 2019).

Acetylcholinesterase assay

For preparation of 50 µl of solution, 50 µl of acetylcholine chloride (2.6mM) was added as a substrate and 1ml of 20Mm sodium phosphate buffer (pH7.0) were added and was incubated at 25°c for 5mins. 400 µl of 0.3% fast blue B salt was added to stop reaction. Blank and sample were run through spectrophotometer and Optical density (OD) was recorded at 405nm (Younes *et al*., 2011; Sultana *et al*., 2016; Riaz*et al*., 2018; Sultana *et al*., 2019).

Carboxylesterase assay

For preparation of 50 µl solution, 1ml of 20Mm Sodium phosphate buffer (pH 7.0) and 50 µl of each α-naphthyl acetate and β-naphthylacetate (substrate) were added separately to determine the activities of α-carboxyl esterase and β-carboxyl esterase, respectively. The prepared solutions were incubated for 20 minutes at 30˚c for 20mins. After incubation, 400 µl of 0.3% freshly prepared fast blue B salt in 3.3% SDS (Sodium dodecyl sulphate) was added to stop the reaction and allowed the color of solution to develop for 15 min at 2°c. Blank and sample were run on spectrophotometer. Optical density (OD) was recorded at 430 and 590 nm for α-carboxyl esterase and β-carboxyl esterase, respectively (Younes*et al*., 2011; Sultana *et al*., 2016; Riaz*et al*., 2018; Sultana *et al*., 2019).

Acid and Alkaline Phosphatases assay

The acid phosphatases assay was performed by mixing 50 µl of adult homogenate with 50 µl of 50mM Sodium phosphate buffer (pH 7.0) and 100 µl of 20mM p-nitro phenyl phosphate .For the estimation of alkaline phosphatases activity, 50 µl homogenate was mixed with 50 µl of 50mM TrisHCl buffer (pH9.0) and 100 µl of 20mM p-nitrophenylphosphate (substrate). After that, both acid phosphatases and alkaline phosphatases solutions were incubated at 37˚c for 15 minutes and the enzymatic reaction was stopped by adding 0.5M Naoh solution. Blank and sample were run on spectohotometer and the optical density (OD) was recorded at 440nm (Riaz *et al*., 2018; Sultana *et al*., 2016 & 2019; Younes *et al*., 2011)

$$\text{Percent Enzyme inhibition} = \frac{\text{OD of control flies - OD of tretaed flies}}{\text{OD of control flies}} \times 100$$

Statistical analysis

The recorded data was corrected by using Abbott's formula (Abbott, 1925) and subjected to evaluation of variance (ANOVA) using Statistica 13.0 for Windows. Post hoc testing was also carried out using the Tukey's

HSD test. A significant level of 5% was taken into consideration for all statistical tests.

Results

Bioassay

To evaluate the resistance against insecticides; Lab strain of *Muscadomestica* were treated with five insecticides Lambda cyhalothrin, deltamethrin, chlorpyrifos, cypermethrin and tetramethrin, having using different concentrations *viz*. 2.5%, 5%, 10%, 20% and 40% at exposure time of 24, 48 and 72 hr, respectively. Maximum percentage mortality percentage of Lambda cyhalothrin was found at concentration of 40 ppm (51.6%) after 48 hrs and 20 ppm (46.15%) after 72 hr followed by 20 ppm concentration. It was found that the mortality was increased with increase in concentrations but not decreased with exposure time; moreover, low mortality was found at lower concentrations. Similar results were found with Deltamethrin; maximum mortality percentage was found at 40 ppm (51.6%) after 48 hrs and 49.01% after 24 hr, respectively.

Highest mortality rate was observed in case of Chlorpyrifos. Flies showed lowest resistance against Chlorpyrifos. At 20ppm concentration mortality rate was found 57.1%, 42.8% and 50% after 24, 48 and 72 hrs, respectively. Highest mortality percent (57.1%) was observed at 40ppm concentration after 24 hr. With Cypermethrin, mortality rate was found decreased with increase in exposure time (30%, 28% and 9.8 %) at 40ppm after 24, 48 and 72 hr, respectively. Tetramethrin showed least mortality among all tested insecticides. Overall, highest mortality rate (57.1429%) was observed at 40% concentration of Chlorpyrifos, and least mortality (14.7368%) was observed in case of Tetrametrhin at same concentrations (Table 1).

Table 1. Mean mortality of House fly (*Muscadomestica* L.) treated with different insecticides.

Code.	Conc.	F Value	df	P Value	Mean mortality with different time intervals		
					24 Hours	48 Hours	72 Hours
1	2.5ppm	0.095	2	0.910	23.889±5.77350^{a}	21.6667±1.6667^{a}	22.8205±1.6667^{a}
	5ppm	1.546	2	0.287	28.889±5.0000^{a}	28.333±1.6667^{a}	21.1538±2.8867^{a}
	10ppm	0.082	2	0.923	33.889±7.63763^{a}	31.667±3.333^{a}	31.1538±2.8867^{a}
	20ppm	1.762	2	0.507	38.889±2.88675^{a}	43.333±1.6667^{a}	41.1538±2.8876^{a}
	40ppm	4.321	2	0.069	43.889±2.88675^{a}	51.667±1.6667^{a}	46.1538±.0002^{a}
2	2.5ppm	0.322	2	0.737	2.6471±2.88675^{a}	8.333±10.1379^{a}	16.667±15.2752
	5ppm	0.410	2	0.681	7.3529±2.88675^{a}	18.333±10.379^{a}	6.6667±14.2401^{a}
	10ppm	0.873	2	0.465	14.0196±3.333^{a}	26.667±11.5470	8.333±12.58306^{a}
	20ppm	2.671	2	0.148	29.0196±1.6666^{a}	43.333±7.2648^{a}	28.33±5.000^{a}
	40ppm	1.061	2	0.403	49.0196±14.813	51.6667±7.6376	33.333±.0002^{a}
3	2.5ppm	180.4	2	0.001	38.8095±4.4095^{a}	37.8205±4.409^{a}	56.667±3.333^{b}
	5ppm	1.061	2	0.403	49.0196±14.813^{a}	51.6667±7.637^{a}	33.333±0.02^{a}
	10ppm	125.7	2	0.000	55.4762±1.6666^{a}	44.4872±1.666^{b}	51.6667±1.6667^{c}
	20ppm	913.7	2	0.000	57.1429±.0002^{a}	42.8205±3.333^{b}	50.000±0.000^{c}
	40ppm	372.1	2	0.000	57.1429±0.0000^{a}	44.4872±1.666^{b}	50.000±0.002^{c}
4	2.5ppm	200.3	2	0.000	1.66667±1.6667^{a}	16.6667±1.666^{b}	45.1515±1.6668^{c}
	5ppm	209.4	2	0.001	10.000±.0000^{a}	33.33±2.88675^{b}	36.8182±.0002^{c}
	10ppm	42.89	2	0.002	16.667±1.6666^{a}	6.6667±2.8867^{a}	23.4848±4.4095^{b}
	20ppm	48.06	2	0.000	15.000±.0000^{a}	13.333±1.6667^{a}	18.4848±4.4095^{b}
	40ppm	27.02	2	0.001	30.000±2.88675^{a}	28.333±1.6666^{a}	9.8485±1.6667^{b}
5	2.5ppm	5.018	2	0.052	4.7368±.000^{a}	5.4684±1.4787^{ab}	13.5714±6.9375^{c}
	5ppm	47.78	2	0.000	8.0702±1.6667^{a}	0.6863±1.6667^{b}	14.5238±1.6667^{c}
	10ppm	55.47	2	0.002	6.4035±1.6667^{a}	6.4035±1.6667^{a}	16.1905±1.6666^{b}
	20ppm	16.397	2	0.004	9.7368±2.88675^{a}	9.7368±2.88675^{a}	7.8571±2.88675^{b}
	40ppm	8.699	2	0.017	14.7368±2.8867	9.0196±2.8867^{ab}	2.8571±2.88675^{b}

*Means sharing the same letter within each treatment is not statistically different

1: Lambda-cyalothrin; 2: Deltamethrin; 3: Chlorpyrifos; 4: Cypermethrin; 5: Tetramethrin.

Evaluation of Resistance of Pyrethroid Insecticides in Muscadomesticapopulations

The toxicity of four insecticides and resistance ratio of three generation of houseflies was recorded on basis of their LD50 values. Those flies with higher LD50 values were considered resistant in successive generations. Very low to no level of resistance was found against Lambda cyalothrin when compared with Cypermethrin, Deltamethrin and Tetramethrin. Resistance ratio (RR) ranged between 0.973-0.947 in the case of Lambda cyalothrin. With Deltamethrin and Cypermethrin moderate level of resistance was found with a range between 1.133-1.193 and 1.106-1.234 respectively. Maximum resistance was found in Tetramethrin with RR ranging between1.299-1.472 (Table 2).

Table 2. Evaluation of Resistance of Pyrethroid Insecticides in *Muscadomestica* L. from District Faisalabad.

Insecticide	Fn	LD50µg/µL	95%CL	Slope±S.E	χ^2 (df)	RR
Cypermethrin (1.5% EC)	F1	30.458	26.3617±33.406	0.0418±0.0041	35.964(3)	1.1066
	F2	31.702	27.068±37.6412	0.0329±0.0043	38.221(3)	1.1784
	F3	34.148	32.1452±42.3312	0.0342±0.0045	42.436(3)	1.234
Deltamethrin (1.5% EC)	F1	40.812	31.8007±61.2913	0.02153±0.0043	3.3601(3)	1.133
	F2	42.282	33.219±62.4278	0.02158±0.0042	3.4128(3)	1.174
	F3	47.628	38.7502±73.879	0.2128±0.0040	7.1535(3)	1.193
Tetramethrin (0.5 % WP)	F1	57.258	44.697±90.8273	0.215±0.0033	5.8721(3)	1.299
	F2	74.264	54.9657±149.531	0.0180±0.0047	2.3946(3)	1.287
	F3	84.137	61.001±164.702	0.1950±0.00647	2.3967(3)	1.472
LambdaCyalothrin (50 % EC)	F1	48.736	42.8485±61.4573	0.3272±0.00481	1.2787(3)	0.973
	F2	46.539	43.1142±57.7611	0.3892±0.0062	1.5689(3)	0.976
	F3	45.152	43.318±58.330	0.0584±0.0073	8.9336(3)	0.947

Molecular Assay

The four *kdr* primers designed by macrogen company were used for PASA (PCR Amplification of Specific Alleles) following the protocol of Huang *et al* (2004). *Kdr1* and *kdr4* amplified fragment of 480 bp, *kdr1* and *kdr3* amplified 200bp susceptible allelic fragments while *kdr2* and *kdr4* amplified 280bp *kdr*type allelic fragments in the domain-II of kdr gene. Two populations were found homozygous susceptible (+/+; 18%); whereas three populations were found genetically homozygous resistant (-/-; 27%) which are insensitive to pyrethroid insecticides. Similarly, six populations were found heterozygous (+/-; 55%) for kdr. Hence, following the Mendelian ratio in future generation, at least 1/4th homozygous resistant (-/-) house fly populations would be produced which would increase the insensivity to pyrethroid insecticides (Fig. 1).

Biochemical assays

The effect of insecticides on the activity of Acetylcholine Esterase (AChE), Carboxylesterase (α-Carboxylesterases and β-Carboxylesterases), Acidic Phosphatase (AcP), Alkaline Phosphatases (AkP) is shown in Table 3. Maximum percent inhibition of AChE was observed in Samanabad (42.298%) followed by Gulfishan colony (20.7667 %). Low level of inhibition of AChE was shown by Khurianwala (11.201 %). Maximum percent inhibition of AkP was observed in Gulfishan colony (189.764%) followed by Khrianwala (175.634 %); whereas, very low percent inhibition was found in Jinnah colony (25.833%). Similarly, maximum percent inhibition of AcP was found in Gulfishan colony (175.633%) followed by Woda town (160.990 %). Low level of inhibition of AcP was found in samples from Khalidabad (20.578%). Maximum percent inhibition of α-Carboxylesterases was shown by Gulfishan colony (41.80%) followed by Samanabad (39.412%), whereas, low level of inhibition of α- Carboxyl was found in Khurianwala (10.786%). Similarly, maximum percent inhibition of β-Carboxylesterases

was observed in Samanabad (39.412%) followed by Gulfishan colony (38.919%); whereas, very low level of inhibition was found in Khurianwala (10.786%). Overall, the percentage inhibition of Alkaline Phosphatases (AkP) and Acidic Phosphatase (AcP) was found high as compared to Acetylcholineesterase (AChE) and Carboxylesterase (α- Carboxyl and β-Carboxyl) activity (Table 3).

Discussion

In the present study housefly (*Muscadomestica* L.) was collected from eleven (11) different sites of Faisalabad, Pakistan. A Polymerase chain reaction (PCR) based diagnosis assay was developed to detect resistance in field and lab strains. *Kdr* mutation was genotyped by allele specific PCR (PASA) which revealed that this allele was present in the tested populations. The findings showed three types of genotypes with amplification of 280 bp homozygous resistant allelic fragment (*kdr/kdr*), heterozygous genotype with amplification of two allelic fragments 280 bp and 200 bp (*kdr/sus*) or homozygous susceptible allelic fragment of 200 bp (sus/sus).

Table 3. Effect of insecticides on the percent enzyme inhibition in *Muscadomestica*L. From District Faisalabad.

Sr#	Location	AchE	AkP	AcP	α-Carboxyl	β-Carboxyl
		df=13,F=1.569, P= 0.05	df=13,F=16.28, P=0.00	df=13,F=10.699, P=0.000	df=6,F=5.826, P=0.00	df=6,F=13.82, P=0.00
	Control	11.13±1.192^{a}	33.113±17.35^{i}	31.101±2.165^{j}	10.0221±1.132^{a}	9.301±1.151^{a}
1	Jhang bazaar	15.343±1.229^{c}	22.199±3.339^{h}	20.120 ±3.41^{i}	80.921±3.121^{e}	82.333±2.241^{f}
2	Jinnahcolony	15.567±1.231^{e}	14.57±1.1219^{d}	11.68± 1.218^{b}	25.833±1.2983^{b}	21.4567±1.94^{c}
3	Shahkot	18.733±1.751^{e}	15.4667±1.25^{d}	13.412± 1.267^{e}	95.634±9.4200^{g}	109.900±19.6^{i}
4	Gulfishan	20.7667±3.82^{g}	41.800±8.362^{k}	38.919± 6.372^{l}	189.7667±6.11^{k}	175.633±8.79^{k}
5	Samundri	18.543±3.219^{e}	17.242±2.43^{g}	16.414± 1.43^{h}	109.9±3.842^{h}	100.241±6.24^{h}
6	G. M Abad	16.919±2.275^{d}	13.143±1.492^{b}	12.141± 2.491^{c}	78.125±2.421^{d}	60.912±3.212^{d}
7	Rehmat Ali	19.939±1.09^{f}	12.212±2.012^{a}	11.121± 1.012^{a}	149.95±12.291^{j}	154.21±6.792^{j}
8	Khalidabad	15.495±2.234^{c}	16.212±1.454^{e}	14.322 ±2.456^{f}	69.832±49.832^{c}	20.578±1.310^{b}
9	Dhobi ghat	15.4667±1.01^{c}	14.393±1.231^{c}	13.392 ±2.132^{d}	109.783±2.506^{h}	98.959±2.894^{g}
10	Khurianwala	13.201±1.228^{b}	16.786±1.108^{f}	15.787 ±2.208^{g}	90.783±18.506^{f}	71.959±1.895^{e}
11	Samanabad	42.298±4.115^{k}	39.412±2.209^{j}	36.413±2.219^{k}	145.22±12.004^{i}	119.1±3.414^{i}

AChE=acetylcholineesterase, AcP= acidic phosphatase, AkP=alkaline phosphatase, α-Carboxyl= α-Carboxylesterases and β-Carboxyl= β-Carboxylesterases.

*Means sharing the same letter within each treatment is not statistically different.

Three locations *viz*. Gulfishan colony, Samanabad and Rehmat Ali park were found homozygous resistant (*kdr/kdr)* for house fly populations; whereas, Jinnah colony and Khalidabad were found homozygous susceptible (*sus/sus*). Six sampling sites viz. Jhang bazaar, Shahkot, Samundari, Ghulam Muhammad abad, Khurianwala and Dhobi Ghat were found heterozygous for kdr (*kdr/sus*). All the three genotypes were present with different frequency levels.The percentage of heterozygous genotype (*kdr/sus*) was higher as compared to homozygous genotypes. The results were in agreement with findings of (Al Deeb*et al*., 2014). Insecticidal bioassays and biochemical assays were also carried out to relate resistance level.

The percentage mortality of *Muscadomestica* L. was recorded using five different concentrations of five insecticides with time exposure of 24 hours, 48 hours and 72 hours. The highest mean mortality was observed in case of Lambda cyalothrin and Chlorpyrifos and the lowest mean mortality was observed in case of Tetramethrin. Data indicates a progressive increase in resistance level to Cypermethrin, Deltamethrin and Tetramethrin (Singh and Parkash, 2013).

In present study, the metabolic biochemical activity ratio of homozygous resistant (*kdr/kdr*) to homozygous susceptible (*sus/sus*) and heterozygous (*kdr/sus*) ratios varied among different populations.The current findings of enzyme assay of field strains while comparing with the molecular assay showed that percent inhibition of Acetyl choline E (AChE),Carboxyl Esterase (α-Carboxylesterases and β-Carboxylesterases), Alkaline phosphatae (AkP) and Acidic phosphatases (AcP) increase with increase in resistance and decrease with increase in susceptibility. In addition, the alkaline phosphatases (AkP) and Acidic phosphatases (AcP) activity increased more as compared to Acetylcholine Esterase (AChE) and Carboxylesterase (α-Carboxylesterases and β-Carboxylesterases).A decrease rate of detoxifying enzyme was responsible for imparting susceptibility of *Muscadomestica* L. (Wheelock *et al.*, 1992; Smirle*et al.*, 2010).

The results of present study could be helpful in the strategic development of management plans of *Muscadomestica* L. in Faisalabad, Pakistan. Moreover, enzyme level change could be served as a marker for the analysis of resistance which would also be helpful in future to devise a targeted control strategy against population of house fly.

Conclusion

The results showed homozygosity for both susceptible and resistant strains. Thus, keeping in view the simple Mendelian genetics; heterozygous for mutation ultimately lead towards $1/4^{th}$ of homozygous *kdr* mutants. Hence, increasing thereby; the frequency of resistant strains in a given area. The study also showed low resistance level in Chorpyrifos which indicates that combination of chemicals could be better choice rather to use pyrethroid insecticide individually. This study also showed that Acetylcholisnestrase (AChE), Acid Phophatase (ACP), Alkaline Phosphatase (AKP) and Carboxylesterase (α-Carboxylesterases and β-Carboxylesterases) had increased in resistant house fly samples. Thus, despite of having molecular approaches, being rather costly; these enzyme assays would be used as marker to check the susceptibility level of house fly samples from any area.

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