Original Research

Evaluation of Resistance to Some Pyrethroid and Organophosphate Insecticides and Their Underlying Impact on the Activity of Esterases and Phosphatases in House Fly, *Musca domestica* (Diptera: Muscidae)

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Abstract

Resistance development against some frequently used insecticides, i.e., deltamethrin (1.5% EC), cypermethrin (10% SC), permethrin (0.5% WP) and DDVP (50% EC) was evaluated against *Musca domestica* L. Insecticide bioassays were carried out against susceptible and resistant strains at 2.5, 5, 10, 20 and 40 µg/µl concentrations. Mortality data was recorded after 24, 48 and 72 hours. Resistance was monitored up to three generations and the flies with higher LD₅₀ values than the F₁ generation were considered resistant. LD₅₀ values for Permethrin increased from 58.258 µg/µl to 85.1375 µg/µl with highest resistance ratio (RR) in F₁ to F₃. The lowest resistance ratio was observed with DDVP. Maximum inhibition in adult emergence was observed against DDVP. The inhibitory activity of Esterases; Acetylcholine and Phosphatases; ACP, AKP was recorded. Deltamethrin inhibited the maximum activity of AChE (50%), whereas, DDVP caused maximum inhibition of acid phosphatases. The results suggested that house fly populations are more resistant to pyrethroids compared to organophosphate insecticides.

Keywords: *Musca domestica*, organophosphates, pyrethroids, enzyme inhibition, resistance

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Introduction

The house fly, *Musca domestica* belongs to order Diptera, family Muscidae. It is considered an important pest of humans and livestock [1]. *Musca domestica* is a general nuisance and is responsible for food contamination by transferring various contagious pathogens of diseases including bacillary dysentery, cholera, shigellosis, avian influenza and salmonellosis [2,3]. House flies are an economic concern to poultry and animal farms resulting in low milk production because cows spend additional energy in fending off these flies (fly worry) [3, 4].

Chemical control has been the primary method of control strategy since 1950’s with organophosphates and pyrethroids frequently used [5]. Organophosphate pesticides are generally broad spectrum with esters of phosphoric acids, which affect the activity of neuromuscular enzyme acetylcholine-esterase. This enzyme is essential for normal body functioning of insects [6, 7]. Similarly, pyrethroid insecticides including Cypermethrin, Deltamethrin, and Permethrin are primary chemical insecticides used against many insect pests including house fly [8-10]. It is well established that the indiscriminate use of pesticides has resulted in the development of insect strains that are resistant to those insecticides [11]. The development of resistance is a potential threat to the future of insect control strategies [12]. *Musca domestica*, the common house fly, has been shown to develop resistance to insecticides [13, 14]. The monitoring of insecticide resistance through bioassays is a helpful tool to recognize resistant development to any insecticide or group of insecticides. Documentation of resistance, thus, helps in developing alternative solutions to devise better management strategies [15].

Metabolic degradation for many pyrethroids is reported through esterase-mediated metabolism and considered as one of the major resistance methods developed by insects [16-18]. Pyrethroid resistance in house flies is due to the insensitive acetylcholinesterase (AChE) as a defence mechanism to counter the effect of the insecticide [19, 20]. AChE is also involved in the detoxification of synthetic insecticides to form less toxic metabolites [21, 22]. Resistant house flies show changed AChE activity, rendering it less sensitive to the inhibitors [23]. Furthermore, phosphatases are reported to be involved in various physiological mechanisms [24-26] and used as indicator enzymes to evaluate the damaging effects of numerous toxicants related to the physiological status of insect pests. Similarly, Alkaline-phosphatases (AKP) are hydrolytic enzymes that detoxify many insecticides like the organophosphate group [27]. Insect growth regulators (IGRs) affect insects by regulating or inhibiting certain biochemical paths or routes necessary for growth and development. Insect pests exposed to IGRs possibly die due to irregular regulation of organ development or after an abnormal inhibition of a developmental stage [28].

Insecticide resistance is a major challenge to the management of house fly. Hence, the current study was conducted to evaluate the resistance of most frequently used pyrethroids and organophosphates against *M. domestica*. Biochemical response of insecticide resistance was also addressed focusing on the modified activities of esterases and phosphatases.

Materials and Methods

*Musca domestica* was collected in Faisalabad, Pakistan by sweep-netting and reared in the Entomology Lab of the Department of Zoology, Government College University Faisalabad, on artificial diet under optimum conditions of 28°C±2° and 60-70% RH with 12L: 12D (h) photoperiod. The collected fly samples (Fig. 1) were
initially sorted out in two groups through PCR based on knockdown resistance (kdr allele) against pyrethroids in VGSC gene (voltage-gated sodium channels) as described by Gomes et al. [18], Scott [19], Al-Deeb [29] and Sing et al.[30]. Resistant populations were pooled and considered as a resistant strain used during the study. The susceptible population was referred to as the laboratory reference strain and was used as control.

Technical grade formulated pyrethroid insecticides: Cypermethrin (1.5%EC), Deltamethrin (1.5% w/v), Permethrin (0.5%WP) and the organophosphate DDVP (50%EC) were selected to evaluate the toxicity and further resistance through feeding bioassays. Twenty, 3-5-day-old flies were introduced into plastic containers (250 ml) and were provided with two pieces of cotton dental wick (2 cm length) moistened with a 20% sugar:water solution containing different concentrations (2.5, 5, 10, 20 and 40 µg/µl) of an insecticide. The control plastic jar was given cotton wicks soaked in a 20% sugar solution without any toxicant [31,32]. Experiments were replicated three times under Completely Randomized Design (CRD). The LD$_{50}$ values for 24 h, 48 h, and 72 h were calculated using Finney’s Probit analysis [14, 33].

Data regarding percentage mortality was recorded after 24 h, 48 h and 72 h of treatment. Mortality in control groups was also noted to obtain the corrected mortality according to Abbot’s formula [34]. The corrected mortality data were subjected to ANOVA using Statistica 13.0 for Windows. The means were separated using Tuckey’s HSD (Honest Significant Difference) test at a significance level of 0.05. A value of $p<0.05$ was considered statistically significant.

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

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

Evaluation of Resistance of Pyrethroid and Organophosphate Insecticides in _M. domestica_

For the detection of resistance, three generations of _M. domestica_ were reared. After the adult mortality had been recorded after 72 h, the flies that remained alive were allowed to complete their three generations. Twenty adult flies were used to evaluate the resistance after 48 hours feeding on the insecticide. Mortality data was recorded after 48 hours of insecticide treatment on the emerged flies of the F1 and F2 (parental) generations. The number of emerging flies was recorded after 2 weeks and meanwhile, the mortality was calculated [35]. The resistance level was measured in each successive generation in order to evaluate the increase in resistance level following the protocol of Singh & Prakash [36]. The resistance ratios (Resistant/Susceptible) were estimated by dividing the LD$_{50}$ for resistant strain to the LD$_{50}$ for the reference strain [32, 35, 37].

Bioassay for Growth Inhibitory Effects

To assess the toxicity of IGR insecticides, larval bioassays were performed by following the methodology of Cetin et al. [38]. Five concentrations (2.5, 5, 10, 20 and 40 µg/µl) of each insecticide was applied on 20 g culturing media. Twenty newly hatched 1st instar larvae were introduced onto the treated media and allowed to complete the development. Time interval data was collected for stage to stage development; larvae to pupae and then pupae to the adult emergence in the F1 generation up to 30 days and was compared with the control.

Quantitative Analysis of Esterases and Phosphatases Activity

Flies that survived to the third generation were homogenized separately by adding 1.5 ml ice-cold sodium phosphate buffer (20 mM, pH up to 7.0). The homogenate was then centrifuged at 8000 revolution per minutes at 4°C for 20 minutes and the supernatant was separated for the estimation of esterases (Acetylcholinesterases) and phosphatases (Acid Phosphatases and Alkaline Phosphatases) by following the methodology of Younes et al. [39]. The percentage inhibition of the enzyme activity by the test extracts was calculated as follows

$$\%\text{ Enzyme inhibition} = \frac{\text{OD of Control} - \text{OD of treated}}{\text{OD of Control}} \times 100$$

Results and Discussion

Evaluation of Resistance of Commonly Used Insecticides Against _M. domestica_

The percentage mortality of _M. domestica_ was recorded against five different concentrations of Cypermethrin, Deltamethrin, Permethrin and DDVP. With a 40 µg/µl concentration and 72h of exposure period, DDVP showed the highest mean mortality (98.33%) and significant results against _M. domestica_. On the other hand, lowest mean mortality percentage was observed with Permethrin (73.80%) at the same exposure time. All tested insecticides caused maximum mortality at 72 h of exposure time period (Table 1). Overall, it was found that increased concentration caused increased mortality. In addition, prolonged exposure time also caused higher mortality. Moreover, Permethrin showed the highest LD$_{50}$ value of 20.10 µg/µl at 72 h exposure followed by Deltamethrin...
with an LD₅₀ of 9.53 µg/µl. Cypermethrin and DDVP showed LD₅₀ values of 4.36 µg/µl and 5.527 µg/µl, respectively at 72 h exposure time (Table 2).

The toxicity of four tested insecticides and resistance ratios of three generations of house flies was recorded based on their LD₅₀ values. Those insects with higher LD₅₀ values were considered resistant in successive generations. Very low level to no resistance was found against DDVP when compared with Cypermethrin, Deltamethrin and Permethrin (Table 3). Resistance ratios (RR) ranged between 0.976-0.975 in the case of DDVP. With Deltamethrin and Cypermethrin, moderate level of resistance (RRs) was found with a range between 1.034-1.182 and 1.1059-1.225, respectively. Maximum resistance was found in Permethrin with RRs ranging between 1.299-1.461. Nevertheless, a reduction in % age mortality was also observed in successive generations for Permethrin, Deltamethrin and Cypermethrin. The regression line slopes were found similar for all the generations against Deltamethrin, Cypermethrin, Permethrin and DDVP with p-value<0.05 (Fig. 2 and Table 3).

### Mean Adult Emergence and Percent Progeny Inhibition of *M. domestica*

Mean adult emergence and progeny inhibition were determined. DDVP caused maximum inhibition at 2.5 µg/µl concentration when compared to the other insecticides (59.33%), followed by Cypermethrin (58.33%), and Permethrin (53.33%), respectively. The lowest inhibitory activity was observed with Deltamethrin (48.33%). The lowest percentage of adult emergence (17.67%) was shown by DDVP at a 40 µg/µl concentration, which inhibited the F1 population by 82.33%. Similarly, Cypermethrin caused 80% adult inhibition and 20% adult emergence; Permethrin induced 80.67% inhibition and 19.33% adult emergence; while Deltamethrin caused 75% adult emergence and 25% inhibition (Table 4).

### Enzyme Bioassay

Cypermethrin, Deltamethrin, Permethrin and DDVP were also tested at three concentrations (2.5, 10, and 40 µg/µl) for their effect on the activity of Esterases
Evaluation of Resistance to Some Pyrethroid...

Table 2. Toxicity of tested pyrethroid and organophosphate insecticides against *M. domestica* after 24, 48 and 72 hours of exposure time.

| Pesticide         | Exop. Time | N  | LD₅₀              | Slope ±SE               | X²     | Df | SE  | p Value |
|-------------------|------------|----|-------------------|-------------------------|--------|----|-----|---------|
| Permethrin        | 24         | 20 | 85.1375           | (61.0016±165.704)       | 0.0196074 +0.0054831 | 9.3705 | 3   | 18.8184 | 0.025   |
|                   | 48         | 20 | 33.0004           | (29.4130±37.8084)       | 0.0449377 +0.0046953 | 14.6386 | 3   | 2.07405 | 0.002   |
|                   | 72         | 20 | 20.1069           | (16.9851±23.7781)       | 0.0373187 +0.0044735 | 11.2009 | 3   | 1.67891 | 0.011   |
| Cypermethrin      | 24         | 20 | 36.2375           | (31.0452±44.3310)       | 0.0322237 +0.0044740 | 41.4469 | 3   | 3.18306 | 0.000   |
|                   | 48         | 20 | 18.9100           | (15.3059±23.0703)       | 0.0315766 +0.0043692 | 24.4804 | 3   | 1.90163 | 0.000   |
|                   | 72         | 20 | 4.36554           | (0.421237±7.34286)      | 0.0437169 +0.0053817 | 29.4914 | 3   | 1.69692 | 0.000   |
| DDVP              | 24         | 20 | 47.1516           | (42.4189±54.3306)       | 0.0574333 +0.0073487 | 9.9447  | 3   | 2.87894 | 0.019   |
|                   | 48         | 20 | 26.9337           | (23.4027±31.6132)       | 0.0372045 +0.0044175 | 25.4148 | 3   | 2.01704 | 0.000   |
|                   | 72         | 20 | 5.52724           | (2.55867±7.89061)       | 0.0540393 +0.0064286 | 4.44034 | 3   | 1.31418 | 0.018   |
| Deltamethrin      | 24         | 20 | 43.1706           | (35.0771±58.8665)       | 0.0239581 +0.0043813 | 7.86628 | 3   | 5.37054 | 0.049   |
|                   | 48         | 20 | 9.53950           | (0.705754±15.5652)      | 0.0184913 +0.0042492 | 8.81434 | 3   | 3.32352 | 0.032   |
|                   | 72         | 20 | 6.89081           | (23.1837±0.718409)      | 0.0219155 +0.0046546 | 3.18390 | 3   | 5.16534 | 0.364   |

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

and Phosphatases: Acetylcholine-esterases (AChE), alkaline phosphatases (AKP) and Acid phosphatases (ACP) (Table 5). Maximum inhibitory effect was found at 40 µg/µl concentrations on AChE enzyme activity. Maximum inhibition in AChE was induced by Deltamethrin (50%; p-value 0.002) followed by Permethrin (42%; p-value 0.04) and then Cypermethrin (39%; p-value 0.01), respectively. DDVP showed minimum inhibition (38.33%; p-value 0.02) as shown in Table 4. Overall, a negative pattern of inhibition of
acid phosphatases was observed with Cypermethrin, Deltamethrin, and Permethrin, respectively. All the tested pyrethroid insecticides showed similar levels of reduction in AKP activity (21.167%) at 40 µg/µl concentrations. Moreover, DDVP caused 13% of AKP inhibitory activity. Although, increased concentration caused decreases in inhibitory activity against Deltamethrin, Cypermethrin, and Permethrin. However,

Table 4. Mean adult emergence and percent progeny inhibition of *M. domestica* against commonly used pyrethroid and organophosphate insecticides.

| Sr No. | Insecticide       | Concentrations µg/µl | Percent Emergence | Percent Inhibition |
|--------|-------------------|----------------------|-------------------|--------------------|
|        |                   | 2.5 µg/µl            | 10 µg/µl          | 40 µg/µl           |
|        |                   | Percent | %          | Percent | %          | Percent | %          |
| 1      | Cypermethrin      | 41.67±2.89 a         | 58.33±2.89 b      | 28.67±2.89 b      | 73.33±2.89 a | 20.00±0.00 b | 80.00±0.00 a |
|        | (1.5% EC)         |                     |                   |                    |            |            |            |
| 2      | Deltamethrin      | 51.67±2.89 a         | 48.33±2.89 c      | 36.67±2.89 b      | 63.33±2.89 b | 25.00±5.00 c | 75.00±5.00 a |
|        | (1.5% w/v)        |                     |                   |                    |            |            |            |
| 3      | Permethrin        | 46.67±2.89 a         | 53.33±2.89 b      | 38.33±2.89 b      | 61.67±2.89 b | 19.33±5.77 c | 80.67±5.77 a |
|        | (0.5% WP)         |                     |                   |                    |            |            |            |
| 4      | DDVP              | 41.67±2.89 a         | 59.33±2.89        | 26.67±2.89 a      | 73.33±2.89 b | 17.67±2.52 b | 82.33±2.52 a |
|        | (50% EC)          |                     |                   |                    |            |            |            |

EC = emulsified concentration, WP = Wettable Powder, W/V = weight by Volume
P<0.001 = highly significant, P<0.01 = highly significant, P<0.05 = significant, P>0.05 = NS
Means sharing the same letter within each treatment is not statistically different.
DDVP showed a positive correlation as increasing the concentration also increased inhibitory activity (Table 5).

House fly has a great ability to develop resistance to insecticides [10, 32]. The intensive use of pyrethroids for its control has led to many instances of pyrethroid resistance worldwide (19, 22). Monitoring of insecticide resistance through bioassays helps recognize a particular resistant insecticide in order to devise a management strategy [10, 13, 14, 15, 32] that lessens resistance development. The present work was focussed on evaluating the resistance level in house fly against frequently used insecticides in Faisalabad, Pakistan and to add to our knowledge of resistance in general. Five different concentrations of insecticides were used following the protocol of Khan [32] and it was observed that increased concentration caused increased mortality, which is in agreement with the findings of Farooq & Fareed [40]. Farooq & Fareed [40] employed nine commonly used different insecticides and found concentration -dependent response for each insecticide. Similarly, increased exposure time also caused increased mortality [41]. DDVP showed highest mean mortality (100%), whereas, lowest mean mortality was observed against Permethrin (73.80%).

Monitoring the susceptibility status of insecticides is an important tool to improve the use of existing insecticides and otherwise to induce the delay in the development of insecticide resistance. It has been reported that resistance towards pyrethroid group of insecticides mainly Permethrin, Cypermethrin, and Deltamethrin was found to be comparatively high [31, 37]. In our study, the resistance bioassay was performed to the third generation of house fly, *M. domestica* at 48 hours (F1, F2, and F3). The data show a progressive increase in resistance to Cypermethrin, Deltamethrin and Permethrin, but very low or no resistance was observed against DDVP. This group of insecticides, is being used separately or in combination with pyrethroids for the control of *M. domestica* [31, 37, 42, 43]. In addition, high resistance was found in Permethrin with a RR of 1.461 in the third generation followed by Cypermethrin and Deltamethrin, respectively (RR = 1.22 and 1.1820). Subsequently, an increase in the resistance ratio was observed in successive generations of *M. domestica* [32]. Interestingly, DDVP showed a slight decrease in the resistance ratio in successive generations.

The objectives of an insect pest management strategy can be immediate control of insect pest or the inhibition of its progeny production. Previously, it was reported by Tunaz et al. [28] and Castro et al. [44] that insecticide affects the population emergence either by suppressing oviposition or induced inhibition in adult emergence though developmental delay from larvae to pupae or pupae to adult in *M. domestica*. The toxic component present in the insecticide subsequently affects the biochemical process that ultimately influences the embryonic development and inhibition of adult emergence in F1 progeny [45].

### Table 5: Effect of commonly used pyrethroid and organophosphate insecticides on percent inhibition of Acetylcholinesterase, Acid-phosphatases, and Alkaline-phosphatases in *M. domestica*.

| Insecticide | AChE | AcP | AkP |
|-------------|------|-----|-----|
| **Concentrations (µg /µl)** | 2.5 | 10 | 40 |
| **Cypermethrin** | 35.66±0.57B | 39.33±0.57A | 37.33±0.57A |
| **Deltamethrin** | 31.66±0.57C | 40.33±0.5B | 32.33±0.5A |
| **Permethrin** | 31.66±0.57C | 40.33±0.5B | 32.33±0.5A |
| **DDVP** | 27.33±2.08C | 33.33±2.89B | 38.33±7.6A |

Means sharing the same letter within each treatment is not statistically different.
application of some insecticides to last instar larvae causes disturbance in larval/pupal transformation, which leads to prolongation of last larval instar [40, 46-49]. Farooq & Fareed [40] studied different biological parameters against nine insecticides in *M. domestica* and found that with the exception of larval duration, developmental parameters were significantly (*P* > 0.05) altered in a concentration-dependent manner for each insecticide. Similarly, in the present findings, DDVP was found to cause a maximum developmental delay in F1 progeny, which is also in agreement to studies by Schneider et al. [49, 50].

Insecticide resistance in house fly is reported to be linked with the differential expression of certain genes encoding metabolic detoxification enzymes [51]. Thereby, the enzymatic profiles are modulated in response to various insecticides. Acetylcholinesterase (AChE) serves as a target site for the major group of insecticides, organophosphate (OP) and carbamate compound [23, 52]. Resistance to organophosphates and carbamate insecticides is due to mutations in the Acetylcholinesterase gene (Ace) [51, 53]. Moreover, binary combinations of organophosphate and synthetic pyrethroids are reported as more potent acetylcholinesterase inhibitors than organophosphate and carbamate mixtures [54]. Organophosphate resistance is mainly mediated by enhanced metabolic detoxification through quantitative changes in esterases [55]. Farooq & Fareed [40] studied biochemical parameters in *M. domestica* and found enzymic modulation in the activity of glutathione S-transferases, total esterases, acetylcholinesterase, and acid and alkaline phosphates for each insecticide, which ultimately contributes to the development of resistance. Resistant larvae exhibited higher activities of esterases and phosphatases compared to susceptible larvae [11]. The current findings showed that Deltamethrin inhibited maximum percent activity of AChE (50%) followed by Permethrin (42.33%), Cypermethrin and DDVP, respectively. Similarly, Deltamethrin, Cypermethrin and Permethrin also caused inhibitory effects on Alkaline-Phosphatases activity (AKP). Interestingly, DDVP showed low AKP inhibitory activity (13%). Here we showed a negative effect of insecticides on Acid phosphatases inhibitory activity. Increase in concentration caused decreased enzyme inhibition activity. Maximum inhibition of acid phosphatases activity was obtained by DDVP (37%). In the case of other three tested insecticides, with an increase in concentration the inhibitory activity was reduced [22]. Deltamethrin induced reduction of enzyme activity followed by Cypermethrin and Permethrin. The current results, thus, supports the notion that decreased rate of detoxifying enzymes was responsible for imparting the susceptibility to *M. domestica* against applied insecticides which is consistent to the previous studies described by Farooq & Freed et al. [40], Smirle et al. [23] and Walsh et al. [24].

Thus, in order to manage the resistance level in house fly, it is suggested that pyrethroid insecticides should be used carefully and preferably in combination with organophosphate groups of insecticides.

**Conclusions**

Highest mean mortality, lowest resistance ratio (RR) and maximum inhibition in adult emergence was found with DDVP. The inhibitory activity of the esterases, Acetylcholine and Phosphatases; ACP and AKP showed that Deltamethrin inhibited maximum activity of AChE (50%), whereas, DDVP caused maximum inhibition of acid phosphatases. In conclusion, house fly populations are more resistant to pyrethroids compared to organophosphate insecticides.

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