Mortality, Biological, and Biochemical Response of *Musca domestica* (Diptera: Muscidae) to Selected Insecticides[1]

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Abstract The concentration–mortality response of *Musca domestica* L. (Diptera: Muscidae) to nine insecticides, and the impacts of these insecticides on selected biological and biochemical parameters of the insect, were determined in laboratory assays. Adults displayed a concentration-dependent response for each insecticide. Median lethal concentration (LC_{50}) values in baits were: acetamiprid (0.39 μg/ml), bifenthrin (0.22 μg/ml), chlorpyriphos (0.21 μg/ml), deltamethrin (0.41 μg/ml), emamectin benzoate (0.001 μg/ml), fipronil (0.002 μg/ml), imidacloprid (0.27 μg/ml), profenophos (0.63 μg/ml), and lufenuron (0.001 μg/ml). Based on 95% confidence intervals, fipronil proved to be the most lethal of the insecticides tested. LC_{10}, LC_{30}, and LC_{50} values of each of the insecticides were used to assess impacts on *M. domestica* longevity, fecundity, percentage eclosion, larval duration, percentage pupation, pupal weight, pupal duration, adult emergence, and sex ratio. In general, development parameters, with the exception of larval duration, were significantly ($P > 0.05$) altered in a concentration-dependent manner for each insecticide. Furthermore, enzymatic activity of total glutathione S-transferases, total esterases, acetylcholinesterase, and acid and alkaline phosphates was elevated at the LC_{10}, LC_{30}, and LC_{50} levels of the nine insecticides, which may contribute to development of resistance to these insecticides.

Key Words biological parameters, detoxification enzymes, insecticides, *Musca domestica*, progeny

The house fly, *Musca domestica* L. (Diptera: Muscidae), is a highly mobile insect pest that is often associated with decomposing matter and can vector disease-causing microbes (Fasanella et al. 2010; Ugbogu et al. 2006). Management relies mainly on insecticides (Ahmed et al. 2004; Shi et al. 2011); however, nonjudicial use of insecticides has resulted in development of resistance to the insecticide (Butler et al. 2007; Kozaki et al. 2009; Memmi 2010) and to environmental contamination (Yadav 2010). Insecticides with novel modes of action are now employed due to their effectiveness and low mammalian toxicity (Korrat et al. 2012; Shi et al. 2011) but should be used wisely to avoid development of resistance (Khan et al. 2013).

In addition to the direct lethal effects of insecticides on the target insect, which is indicated by lethal concentration or lethal dose values (Piri et al. 2014), sublethal effects of exposure to insecticides may affect physiological, behavioral, and

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developmental factors that will impact the next generation of the insect (Desneux et al. 2007; Miao et al. 2014). Longevity, fecundity, fertility, and changes in enzymatic activity reflect physiological impacts while behavioral changes may result in altering feeding and oviposition (Fujiwara et al. 2002; Liu and Trumble 2005; Zalizniak and Nugegoda 2006). Previous studies have demonstrated stimulated reproductive potential of target pests at low concentrations of insecticides (Tang et al. 2015; Zhang et al. 2015), decreased adult fecundity and survival at low concentrations (Han et al. 2012; Rehan and Freed 2015a), and alteration of the function of glutathione S-transferases (GSTs), esterases (ESTs), and other metabolic enzymes (Mouches et al. 1986; Piri et al. 2014).

In insects, GSTs are involved in the defense of target insects against insecticides (Yu 2004) and induce resistance against insecticides by coalescing reduced glutathione to the insecticide, as observed with organophosphate and pyrethroid resistance in insect species (Fragoso et al. 2003; Wei et al. 2001). Increased esterase levels also have been reported to illicit resistance to different insecticide groups such as organophosphates, carbamates, and pyrethroids (Mouches et al. 1986; Peiris and Hemingway 1993). Acetylcholinesterase (AChE) plays a vital role in neurotransmission and its function is targeted by organophosphate and carbamate insecticides. AChE found not to respond to those insecticides is an important detoxification mechanism against insecticides in many insect species (Walsh et al. 2001; Weill et al. 2003). For identification of underlying resistance mechanisms, enzyme assay therefore is an easy and insightful method for identifying underlying resistance mechanisms. The positive correlation of insecticide resistance with detoxification enzyme activity underlines the need for quantification of these enzymes in monitoring resistance development for improved management of insect pests (Yaqoob et al. 2013).

The importance of studying sublethal effects of insecticides on target insects is critical to developing and using new insecticides, delaying development of resistance, and decreasing pest resurgence risk (Xu et al. 2016). Our objectives were to define the toxicity of acetamiprid, bifenthrin, chlorpyriphos, deltamethrin, emamectin benzoate, fipronil, imidacloprid, profenophos, and lufenuron against *M. domestica* and to determine the sublethal effects of these insecticides on selected developmental and biological parameters of the insect as well as the activity of selected enzymes (i.e., total glutathione S-transferases, total esterases, acetylcholinesterase, acid and alkaline phosphatases) following exposure.

Materials and Methods

Insects. *Musca domestica* adults were reared in the Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan. The adults were maintained in rearing cages ($30 \times 30 \times 30$ cm) covered with mesh screen and equipped with a cloth sleeve at the front for handling rearing cage contents. The rearing conditions were 26 ± 2°C, 50 ± 5% relative humidity (RH), and a 12:12 (L:D) period (Farooq and Freed 2016). Adults were provided with sugar and powdered milk (3:1) and water *ad libitum* while wheat bran, rice meal, yeast, and dry milk powder (40:10:3:3:1, respectively) as a water-based paste was provided in cages as an egg-laying medium (Bell et al. 2010).

Concentration–mortality response. Nine commercial-grade formulated insecticides belonging to different mode of action groups (Table 1) were tested in these bioassays; all were being applied at local poultry farms for control of *M. domestica*. Concentrations, ranging from 0.25 to 2.0 ppm for each insecticide, were prepared by serial dilution. Each suspension was mixed with sugar, which was used as a food bait for adult *M. domestica*. Two hundred flies including controls were employed for each insecticide, which was replicated three times. Insects and baits were placed in plastic containers ($15 \times 6 \times 6$ cm) and maintained at the aforementioned conditions. Mortality was recorded at 24, 48, and 72 h after initiation of the test.

Sublethal effects. The LC_{10}, LC_{30}, and LC_{50} concentrations of each insecticide were used to determine the sublethal effects on longevity, fecundity, eclosion, pupal weight, and sex ratio. The bait method was again used, with each concentration of insecticide being mixed with sugar, and with four replicates per treatment and 40 insects per replicate. Adults 4–5 days old at a sex ratio of 1:1 were placed in the plastic containers and provided baits and egg-laying medium as previously described. Baits with no insecticide served as controls. Adult longevity was recorded for each sex as per Fletcher et al. (1990). The egg-laying medium was examined daily for eggs and, if present, the eggs were counted with the aid of a hand lens. Eggs remained in the medium until eclosion. Fecundity was calculated by dividing the total number of eggs oviposited in the medium by the number of females in the containers (Crystal 1964). Percentage eclosion was calculated by dividing the number of larvae hatched by the total number of eggs oviposited (Sanil and Shetty 2012). Neonates remained in the rearing medium and were examined daily to estimate larval duration as being the interval between initiation of the 1st instar until pupation (Elkattan et al. 2011). To calculate percentage pupation, numbers of pupae were counted and divided by the total number of larvae. Pupae were also weighed and placed in separate containers until adult emergence, at which point pupal duration and percent emergence could be calculated as per Khazanie (1979) while number of males and females were counted to calculate sex ratio.

Enzyme and protein activity. *Musca domestica* adults were exposed to the LC_{10}, LC_{30}, and LC_{50} concentrations of each insecticide, and subsequent survivors at 24, 48, and 72 h after exposure were homogenized in PBG (100 mM phosphate buffer, pH = 7.5, 20% glycerol) and centrifuged at 13684.3 g for 10 min at 4°C. The supernatant was stored at −20°C for further testing. Total GST activity was determined with 1-chloro-2,4dinitrobenzene (CDNB) as substrate (Kristensen 2005). The reaction rate was determined for 5 min at 30°C at 340 nm using kinetic and lag period of 2 min. The incubation mixture for a 1-ml quartz cuvette contained 30 μl sample, 950 μl phosphate buffer (PB), 10 μl of CNDB (100 mM), and 10 μl of GSH (100 mM) for each sample. Total EST activity was determined by hydrolysis rate of p-nitrophenylacetate (PNPA) (Joffe et al. 2012). The reaction rate was determined for 5 min at 405 nm using a kinetic and lag period of 1 min. Each sample 1-ml quartz cuvette contained 10 μl sample, 980 μl PB, and 10 μl of PNPA (100 mM). AChE activity was determined by ATCI and DTNB solution at 30°C (Kristensen et al. 2006). The incubation mixture contained a 15-μl sample, 950 μl PB, and was incubated for 2 min at 30°C. Later, 30 μl of DTNB (10 mM) and 5 μl of ATCI (10 mM) were added for color development. The optical density was measured every 30 s for 5 min at 412 nm. Acid and alkaline

Table 1. List of tested insecticides, with concentrations, for evaluation of their effects on biological parameters of *M. domestica*. Number of adult flies used in the bioassay = 140.[A]

Insecticides	Trade Name, Manufacturer	Formulation	LC_{50} [μgmL^{-1}] [95% CI] (Limits)
Acetamiprid	Mospilan®, Arysta Life Sciences	20SP	0.39 (0.14–0.64)
Bifenthrin	Talstar®, FMC United	10EC	0.22 (0.049–0.396)
Chlorpyriphos	Lorsban®, Arysta Life Sciences	40EC	0.21 (0.036–0.404)
Deltamethrin	Decis®, Bayer Crop Science	10 EC	0.41 (0.03–0.67)
Emamectin benzoate	Proclaim®,Syngenta	19EC	0.001 (0.009–0.007)
Fipronil	Regent®, Bayer Crop Sciences	5EC	0.002 (0.001–0.009)
Imidacloprid	Confidor®, Bayer Crop Sciences	20SL	0.27 (0.06–0.43)
Profenophos	Curacuron®, Syngenta	50EC	0.63 (0.31–0.99)
Lufenuron	Match®, Syngenta	50EC	0.001 (0.0009–0.006)

[A] CI = confidence interval; χ^2 = Chi-squared; *df* = degrees of freedom.

phosphatase activity was determined by the hydrolysis rate of p-nitrophenyl phosphate (Serebrov et al. 2006). For acid phosphatase, a 25-µl sample and 535 µl citrate PB (pH = 5.0) were incubated for 2 h at 30°C while alkaline phosphatase employed a 25-µl sample and 535 µl Tris HCl buffer (pH = 8.8) and was incubated for 2 h at 30°C. Later, 425 µl NaOH (0.05N) was added to each well for color development. The optical density was measured at 410 nm. Protein concentration of the samples was determined by the Bradford (1976) assay with bovine serum albumin as the standard at 595 nm.

Statistical analysis. Morality data were corrected using Abbott's formula (Abbott 1925), and the concentration–mortality response was analyzed by probit analysis (Finney 1971) using POLO-PC software (Polo-PC 1987) to determine LC_{10}, LC_{30}, and LC_{50} values with associated slopes and 95% confidence intervals. Data from biological parameter testing and enzymatic activity assays were analyzed using analytical software Statistix version 8.1 (McGraw-Hill 2008), and treatment means were compared using the honest significant difference (HSD) test at $P = 0.05$.

Table 1. Extended.

LC_{30} [μgmL^{-1}] [95% CI] (Limits)	LC_{10} [μgmL^{-1}] [95% CI] (Limits)	Slope	$\chi 2$	*df*	*P*
0.140 (0.22–0.288)	0.032 (0.001–0.102)	1.19 ± 0.31	1.32	3	0.23
0.085 (0.008–0.199)	0.022 (0.000–0.78)	1.28 ± 0.34	0.32	3	0.27
0.07 (0.003–0.186)	0.015 (0.001–0.065)	1.11 ± 0.31	0.76	3	0.30
0.19 (0.06–0.21)	0.01 (0.001–0.05)	1.01 ± 0.23	2.6	5	0.67
0.0002 (0.0001–0.0006)	0.00002 (0.00001–0.001)	0.75 ± 0.25	1.3	3	0.53
0.0004 (0.0001–0.0008)	0.00003 (0.00001–0.0001)	0.67 ± 0.11	2.1	5	0.76
0.09 (0.009–0.21)	0.022 (0.0014–0.068)	1.18 ± 0.31	0.28	3	0.26
0.24 (0.06–0.43)	0.05 (0.004–0.13)	1.24 ± 0.29	1.62	3	0.54
0.0002 (0.0001–0.0007)	0.00002 (0.00001–0.00008)	1.23 ± 0.43	1.03	3	0.82

Results

Concentration–mortality responses. Lethal concentrations (LC) as determined by probit analysis with associated confidence intervals and regression line slopes for each insecticide are listed in Table 1. Based on comparison of LC_{50} values, fipronil proved be to the most toxic insecticide against *M. domestica* adults followed by emamectin benzoate and lufenuron. The LC_{10}, LC_{30}, and LC_{50} values were used in assessing the sublethal effects of the insecticides on *M. domestica* adults and their progeny.

Sublethal effects on longevity. Male longevity decreased as insecticide concentration increased (Table 2). In comparison to the controls, male longevity was significantly reduced by the LC_{50} concentration of acetamiprid (8.03 ± 0.09 d) and the LC_{50} concentration of emamectin benzoate (8.68 ± 0.32 d) ($F = 2.81$; $df = 9, 18$; $P = 0.0008$) (Table 2). A similar trend was observed with female longevity where the LC_{50} concentrations of fipronil and acetamiprid significantly reduced longevity to 8.25 ± 0.25 d and 8.66 ± 0.27 d, respectively ($F = 5.51$; $df = 9, 18$; $P < 0.0001$) (Table 2) .

Table 2. Sublethal effects of different insecticides on life history traits of *M. domestica*.

	Male Longevity (Days) (±SEM)			Female Longevity (Days) (±SEM)		
Insecticides	**LC_{50}**	**LC_{30}**	**LC_{10}**	**LC_{50}**	**LC_{30}**	**LC_{10}**
Acetamiprid	8.03 ± 0.10l	11.83 ± 0.28gh	14.90 ± 0.27de	8.66 ± 0.27mn	12.43 ± 0.58fg	15.96 ± 0.40b
Bifenthrin	11.55 ± 0.69gh	14.13 ± 0.37e	16.23 ± 0.10cd	10.85 ± 0.43jk	13.83 ± 0.37de	16.50 ± 0.29b
Chlorpyriphos	11.25 ± 0.25hi	13.90 ± 0.40ef	16.90 ± 0.35c	10.26 ± 0.70kl	13.01 ± 0.37de	16.39 ± 0.18b
Deltamethrin	12.38 ± 0.29gh	14.39 ± 0.44e	16.63 ± 0.31c	12.40 ± 0.56gh	13.45 ± 0.29de	15.63 ± 0.55bc
Emamectin	8.68 ± 0.32kl	12.05 ± 0.61gh	14.98 ± 0.34de	9.75 ± 0.85kl	12.90 ± 0.31efg	15.38 ± 0.55bc
Fipronil	9.20 ± 0.21jkl	11.95 ± 0.33gh	15.00 ± 0.41de	8.25 ± 0.25n	12.60 ± 0.54efg	15.88 ± 0.31b
Imidacloprid	9.78 ± 0.47jk	12.70 ± 0.45fg	14.73 ± 0.62e	9.25 ± 0.95lm	13.10 ± 0.42de	15.58 ± 0.37bc
Profenophos	11.75 ± 0.48gh	15.13 ± 0.31de	18.40 ± 0.47b	11.25 ± 0.25hij	14.34 ± 0.26cd	16.50 ± 0.37b
Lufenuron	10.03 ± 0.57ij	12.45 ± 0.21gh	15.23 ± 1.29de	10.25 ± 0.63jkl	11.85 ± 0.81gh	15.38 ± 0.55bc
Control	21.63 ± 0.24a	21.75 ± 0.48a	22.25 ± 0.48a	20.63 ± 0.55a	21.10 ± 0.06a	20.60 ± 0.44a
F-value	3.55			5.77		
P-value	0.0001			0.0000		
HSD-value	1.36			1.35		

* Means (±SEM) followed by same lowercase letters are not statistically different; HSD = honest significant difference, $P < 0.05$; LC_{50}, LC_{30}, and LC_{10} represent different levels of insecticides doses.

Sublethal effects on fecundity and eclosion. In general, fecundity decreased with the increased concentration of insecticide. The fewest number of eggs was observed in the treatment with an emamectin benzoate LC_{50} concentration (116.50 ± 5.18) followed by the LC_{50} concentrations of lufenuron (125.00 ± 5.93) and acetamiprid (128.00 ± 5.35) ($F = 5.09$; $df = 9, 18$; $P < 0.0001$) (Table 2). A similar trend was observed with egg eclosion. The lowest percentage of eclosion was observed with the LC_{50} concentrations of fipronil (69.25 ± 1.00), emamectin

Table 2. Extended.

Fecundity (No. of Eggs) (±SEM)			Percent Hatching (% ± SEM)		
LC_{50}	LC_{30}	LC_{10}	LC_{50}	LC_{30}	LC_{10}
128.00 ± 5.35d	163.25 ± 6.31f	222.00 ± 7.31gh	78.23 ± 0.47ghi	82.00 ± 0.91bcd	83.44 ± 0.93bcd
145.25 ± 8.28c	184.25 ± 2.93e	232.88 ± 3.94g	79.55 ± 0.85fgh	82.25 ± 0.95bcd	84.35 ± 0.57bc
150.00 ± 4.23c	174.75 ± 3.39ef	236.75 ± 5.03fg	81.00 ± 0.43cde	80.00 ± 1.35efg	84.10 ± 1.23bc
185.50 ± 6.06bc	203.75 ± 8.25de	255.50 ± 7.60e	81.25 ± 1.21cde	81.53 ± 0.30def	84.98 ± 1.06b
116.50 ± 5.18c	149.33 ± 4.29g	247.00 ± 3.03h	72.15 ± 1.63m	78.19 ± 1.48hij	82.24 ± 0.61bcd
134.00 ± 6.19d	159.58 ± 7.19f	218.00 ± 7.17g	69.25 ± 1.00n	77.48 ± 1.83ijk	80.45 ± 1.68def
135.50 ± 5.85d	163.08 ± 5.59f	212.50 ± 3.69g	74.50 ± 0.65lm	75.25 ± 1.55klm	82.25 ± 1.70bcd
162.00 ± 6.81f	194.50 ± 4.30e	278.00 ± 5.19b	77.95 ± 0.67ijk	79.25 ± 1.03ghi	83.00 ± 1.15bcd
125.00 ± 5.93gh	172.25 ± 6.61f	227.50 ± 5.61cd	76.25 ± 0.95jkl	77.00 ± 0.71jkl	79.45 ± 1.21ghi
380.38 ± 5.63a	377.38 ± 7.88a	365.25 ± 8.49a	94.80 ± 0.58a	94.00 ± 1.23a	95.44 ± 1.17a
	5.57			5.62	
	0.0000			0.0000	
	26.35			3.18	

benzoate (72.15 ± 1.63), and imidacloprid (74.50 ± 0.65) ($F = 3.31$; $df = 9, 18$; $P = 0.0001$) (Table 2).

Sublethal effects on larvae and pupae. Larval duration was not significantly affected by insecticide or sublethal insecticide concentration. However, the pupation percentage decreased with increasing concentration of insecticide. Imidacloprid, emamectin benzoate, and fipronil at their LC_{50} concentrations caused significant reductions in percentage of larvae successfully pupating ($F = 2.27$; $df = 9, 18$; $P = 0.006$) (Table 2). Pupal weight was also significantly reduced in treatments of the LC_{50} concentrations of fipronil, imidacloprid, and profenophos ($F = 3.57$; $df = 9, 18$; $P < 0.0001$) (Table 2). Insecticidal treatments also prolonged the

Table 2. Extended.

Percent Pupation (% ± SEM)			Pupal Weight (mg) (±SEM)		
LC_{50}	LC_{30}	LC_{10}	LC_{50}	LC_{30}	LC_{10}
68.75 ± 2.78ijk	69.33 ± 1.44hij	76.88 ± 0.77bcd	12.13 ± 0.13l	14.55 ± 0.23hij	15.93 ± 0.42cde
75.00 ± 2.71def	75.35 ± 1.12efg	77.31 ± 0.76bcd	14.20 ± 0.40ijk	15.03 ± 0.52efg	16.03 ± 0.45cde
74.50 ± 2.63def	73.46 ± 1.44efg	81.84 ± 0.77b	14.00 ± 0.41jk	16.22 ± 0.21bcd	17.18 ± 0.29b
75.75 ± 2.93cde	72.85 ± 1.64efg	80.28 ± 0.48bc	13.81 ± 1.31k	15.40 ± 0.27cde	16.25 ± 0.48bcd
63.08 ± 1.42mn	71.90 ± 1.93fgh	78.14 ± 1.48bcd	11.25 ± 0.44lm	15.50 ± 0.20cde	16.15 ± 0.40bcd
64.74 ± 1.37lmn	69.61 ± 1.84ghi	75.12 ± 0.81def	10.30 ± 0.23m	14.83 ± 0.50ghi	15.23 ± 0.14def
61.15 ± 2.78n	68.11 ± 1.50jkl	75.94 ± 0.78def	11.13 ± 0.44lm	15.65 ± 0.28cde	15.00 ± 0.22fgh
72.53 ± 2.73efg	75.35 ± 1.09cde	77.90 ± 0.96cde	11.45 ± 0.52l	15.53 ± 0.34cde	16.40 ± 0.17bc
66.93 ± 1.10klm	67.80 ± 3.63jkl	77.11 ± 1.29bcd	11.88 ± 0.24l	15.80 ± 0.28cde	15.33 ± 0.24cde
93.63 ± 2.39a	92.63 ± 1.93a	94.50 ± 1.52a	18.75 ± 0.28a	19.43 ± 0.17a	19.35 ± 0.24a
	2.23			4.83	
	0.01			0.000	
	5.22			1.13	

duration of pupal period with the longest durations observed with the LC_{50} concentrations of lufenuron (8.91 ± 0.33 d), emamectin benzoate (8.63 ± 0.18 d), and profenophos (8.51 ± 0.19 d) ($F = 4.40$; $df = 9, 18$; $P < 0.0001$) (Table 2).

Sublethal effects on adult emergence and sex ratio. A significantly lower percentage of adults emerged in the LC_{50} (64.83 ± 1.77) and LC_{30} (67.58 ± 1.31) concentrations of fipronil ($F = 1.92$; $df = 9, 18$; $P < 0.03$) (Table 2). A significantly lower percentage of females emerged in the acetamiprid LC_{50} treatment (42.25 ± 2.29) ($F = 1.83$; $df = 9, 18$; $P = 0.03$) (Table 2).

Sublethal effects on enzymatic activity. GST activity was significantly increased after 72 h of exposure to the acetamiprid, bifenthrin, and imidacloprid

Table 2. Extended.

Pupal Duration (Days) (±SEM)			Adult Emergence (% ± SEM)		
LC_{50}	LC_{30}	LC_{10}	LC_{50}	LC_{30}	LC_{10}
8.03 ± 0.09ab	7.33 ± 0.18bc	6.05 ± 0.10ef	68.80 ± 0.84ijk	72.16 ± 0.76def	73.85 ± 1.37bcd
7.01 ± 0.35cd	7.08 ± 0.10cd	6.11 ± 0.13ef	70.40 ± 0.32fgh	71.95 ± 0.64def	75.10 ± 1.38bcd
7.53 ± 0.19bc	6.43 ± 0.25e	6.25 ± 0.10e	69.45 ± 0.89ijk	70.45 ± 0.32efg	76.40 ± 0.80bc
7.77 ± 0.53b	7.33 ± 0.19bc	6.23 ± 0.11ef	68.25 ± 0.48jkl	69.74 ± 1.20ghi	75.44 ± 1.25bcd
8.63 ± 0.18a	7.75 ± 0.19b	6.36 ± 0.12ef	68.83 ± 0.97ijk	70.83 ± 0.28efg	74.29 ± 1.03bcd
8.15 ± 0.60a	7.50 ± 0.29ab	5.78 ± 0.23ef	64.83 ± 1.77l	67.58 ± 1.56kl	73.63 ± 1.12bcd
8.51 ± 0.19a	7.81 ± 0.18b	6.33 ± 0.11e	69.00 ± 1.00ijk	70.10 ± 3.06ghi	74.58 ± 1.50bcd
8.40 ± 0.10a	6.78 ± 0.46d	6.15 ± 0.10e	68.50 ± 1.85jkl	72.60 ± 1.67cde	76.83 ± 1.21b
8.91 ± 0.33a	7.11 ± 0.39cd	6.26 ± 0.60ef	69.48 ± 3.18hjk	71.73 ± 2.91def	75.48 ± 1.17bcd
5.78 ± 0.15fgh	5.68 ± 0.16fgh	5.56 ± 0.24fgh	94.58 ± 1.12a	94.25 ± 0.66a	94.08 ± 1.16a
	4.54			1.09	
	0.000			0.04	
	0.71			4.27	

LC_{50} concentrations as compared to other treatments ($F = 3.39$; $df = 9, 18$; $P < 0.001$) (Fig. 1A). Similar trends were observed for the LC_{30} and LC_{10} concentrations of acetamiprid and bifenthrin (Fig. 1B, C) while chlorpyriphos induced a much different GST activity after 24 h of exposure to the LC_{10} concentration ($F = 5.54$; $df = 9, 18$; $P < 0.001$) (Fig. 1C).

In comparison to other treatments, deltamethrin significantly increased EST activity in the LC_{50} concentration after 24 h ($F = 2.39$; $df = 9, 18$; $P = 0.01$) (Fig. 2A). Increased activity was also observed in treatments with the LC_{30} concentrations of deltamethrin at 24 h and bifenthrin and acetamiprid at 72 h ($F = 2.02$; $df = 9, 18$; $P = 0.03$) (Fig. 2B). At the LC_{10} concentration, bifenthrin, acetamiprid, and deltamethrin

Table 2. Extended.

Sex Ratio (♀/(♀+♂)) (% ± SEM)		
LC_{50}	LC_{30}	LC_{10}
42.25 ± 2.29h	47.25 ± 0.63fg	47.00 ± 0.70g
47.75 ± 0.48efg	47.75 ± 0.75efg	48.25 ± 0.48def
47.25 ± 1.44fg	50.75 ± 2.10abc	49.25 ± 0.85def
48.75 ± 0.78def	49.45 ± 0.32cde	49.75 ± 0.75bcd
52.75 ± 1.49a	50.10 ± 0.84abc	49.18 ± 0.55def
49.25 ± 0.48def	49.63 ± 0.85cde	48.75 ± 0.63def
52.50 ± 1.66ab	49.88 ± 0.72bcd	52.50 ± 1.70ab
52.25 ± 1.70abc	49.13 ± 0.43def	50.75 ± 1.32abc
50.50 ± 0.65abc	49.38 ± 0.24def	50.23 ± 1.02abc
50.25 ± 0.63abc	49.35 ± 0.22def	49.17 ± 0.44def
	1.98	
	0.03	
	2.89	

showed elevated EST activity after 72 h. Elevated EST activity was also observed with LC_{10} concentrations of fipronil and chlorpyriphos after 24 h of treatment ($F = 4.47$; $df = 9, 18$; $P < 0.001$) (Fig. 2C).

Acetamiprid, fipronil and bifenthrin at their LC_{50} concentrations significantly amplified AChE activity at 72 h ($F = 2.46$; $df = 9, 18$; $P = 0.01$) (Fig. 3A). Elevated enzymatic activities were also observed with fipronil and acetamiprid at 48 and 72 h ($F = 2.09$; $df = 9, 18$; $P = 0.03$) (Fig. 3B) while acetamiprid and bifenthrin at their LC_{10} concentrations elevated AChE activity at 72 h and deltamethrin, emamection benzoate, and fipronil elevated AChE activity at 48 and 72 h ($F = 2.23$; $df = 9, 18$; $P = 0.01$) (Fig. 3C).

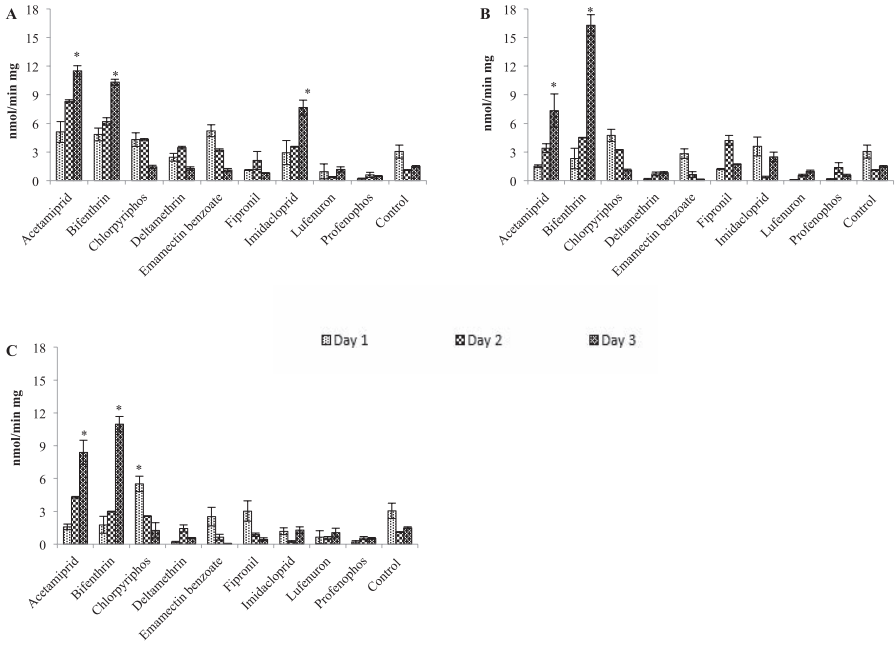

Fig. 1. Total glutathione S-transferases (GST) activity of *M. domestica* at (A) LC_{50}, (B) LC_{30}, and (C) LC_{10} of different insecticides. Asterisk (*) shows significant difference between the enzyme activity at 24, 48, and 72 h (honest significant difference test, $P \leq 0.05$).

The LC_{50} concentrations of acetamiprid and deltamethrin elevated acid phosphatase activity 48 and 24 h, respectively ($F = 2.88$; $df = 9, 18$; $P < 0.001$) (Fig. 4A) while the LC_{30} concentration of bifenthrin significantly increased enzymatic activity at 72 h ($F = 1.13$; $df = 9, 18$; $P = 0.01$) (Fig. 4B). Elevated activity was also observed with the LC_{10} concentration of acetamiprid at 72 and 48 h, bifenthrin at 72 h, deltamethrin and lufenuron at 48 h, and fipronil at 24 h ($F = 3.54$; $df = 9, 18$; $P < 0.001$) (Fig. 4C). Alkaline phosphatase activity was elevated by LC_{50} concentrations of fipronil at 48 h, profenophos at 24 and 48 h, and imidacloprid at 72 h ($F = 2.69$; $df = 9, 18$; $P < 0.001$) (Fig. 5A). Acetamiprid at the LC_{30} concentration significantly increased enzymatic activity ($F = 3.70$; $df = 9, 18$; $P < 0.001$) (Fig. 5B) while for LC_{10} the concentration of chlorpyriphos elevated activity at 24 h and acetamiprid at 72 h ($F = 3.98$; $df = 9, 18$; $P < 0.001$) (Fig. 5C).

Discussion

In the current study, LC_{10}, LC_{30}, and LC_{50} of nine different insecticides calculated by preliminary experimentation were assessed for their sublethal effects on biological and biochemical parameters of *M. domestica*. The sublethal effects of insecticides must be taken into account for their impact on the next generation of insect pests, as it explains the behavioral and physiological impacts which enable

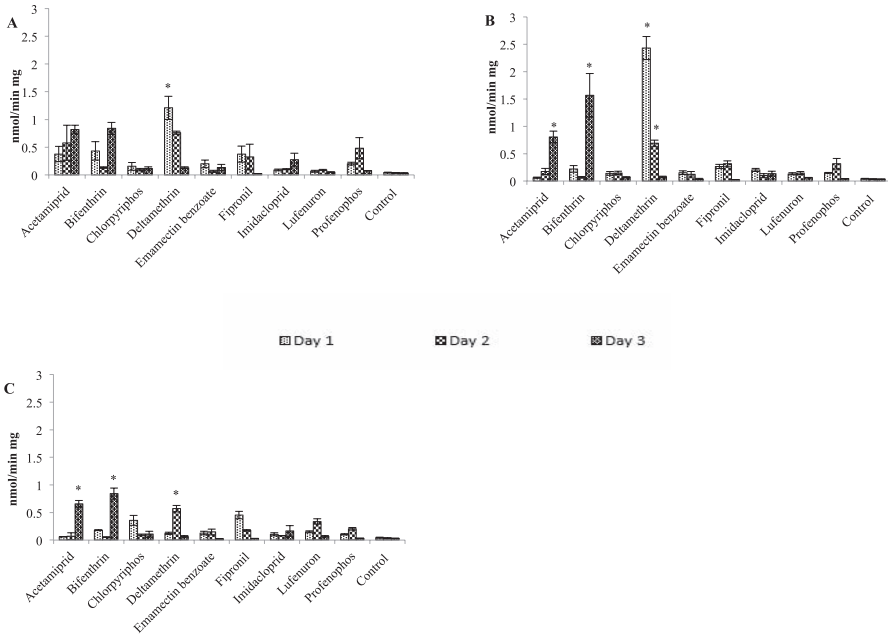

Fig. 2. Total esterase (EST) activity of *M. domestica* at (A) LC_{50}, (B) LC_{30}, and (C) LC_{10} of different insecticides. Asterisk (*) shows significant difference between the enzyme activity at 24, 48, and 72 h (honest significant difference test, $P \leq 0.05$).

insects to survive after pesticide exposure (Desneux et al. 2007). In addition, a life table study was deemed as an inclusive method to evaluate the insecticide for its total effect on an insect population (Tuan et al. 2016).

In the current study, the insecticide concentrations significantly reduced the longevity of adults, especially in the case of emamectin benzoate and fipronil. Lee (2000) reviewed the sublethal effects of insecticides on longevity and fecundity of insect pests including *Aedes aegypti* (L.) (Diptera: Culicidae), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), and *M. domestica*. The results of the current research are in accordance to Hamilton and Schal (1990), who reported a shorter life span of *Blattella germanica* L. (Dictyoptera: Blattellidae) as a result of application of chlorpyrifos-methyl at LC_{10}, LC_{20}, and LC_{60} levels. In the current study, the shorter life span of female flies affected the fecundity in all treatments, which is in accordance with Ahmed and Wilkins (2001), who reported the reduction in the fecundity of insecticide-resistant strains of *M. domestica*. Furthermore, it may also be speculated that the insecticides affected ovaries of female flies, resulting in reduced egg laying (Perveen and Miyata 2000).

The hatching percentage was reduced at higher levels of insecticide in comparison to lower concentrations and the control, favoring the prior studies where the LC_{25} concentration of methoxyfenozide reduced the hatching percentage of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Enríquez et al. 2010),

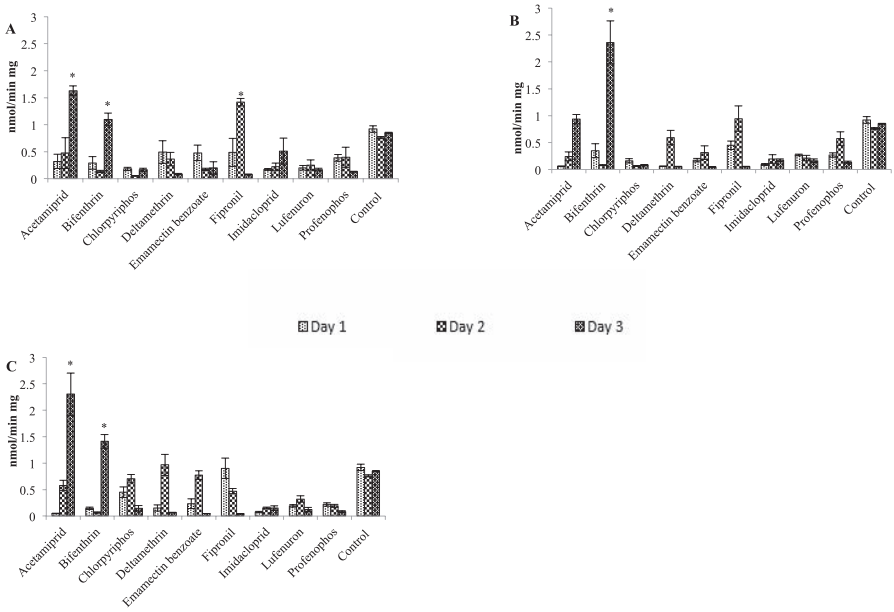

Fig. 3. Acetylcholinesterase (AChE) activity of *M. domestica* at (A) LC_{50}, (B) LC_{30}, and (C) LC_{10} of different insecticides. Asterisk (*) shows significant difference between the enzyme activity at 24, 48, and 72 h (honest significant difference test, $P \leq 0.05$).

while the larval duration was not significantly reduced for all levels of insecticides. In addition, pupation percentage of *M. domestica* was reduced and significantly differed in comparison to the control. Similar results comparable to the current study were observed by Abouelghar et al. (2013) when sublethal concentrations of spinosad reduced the pupal percentage of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). The results regarding pupal weight are in accordance to Rehan and Freed (2015b), where spinosad significantly affected the pupal weight of the *Spodoptera litura* (F.) (Lepidoptera: Noctuidae). Pupal duration was prolonged, in accordance with Xu et al. (2016), who found that doses of cyantraniliprole resulted in prolonged pupal duration of *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae). In our study, maximum reduction in adult emergence from 94.25–62.83**%** was observed in comparison to the control. Similar results were observed by Miao et al. (2016), who showed a significant reduction in adult emergence of *Megacopta cribraria* (F.) (Hemiptera: Plataspidae) at below-lethal concentrations of imidacloprid. In addition, our results showed sex ratio to significantly differ among the treatments, which is in agreement with Sanil and Shetty (2012), who observed sex ratio changes with *Anopheles stephensi* (Liston) (Diptera: Culicidae) following treatments with temephos and propoxur at LC_{10}, LC_{30}, and LC_{50} concentrations.

In general, increased activity of detoxification enzymes indicates existence of a resistance mechanism in the insects in which the activity occurs. The important

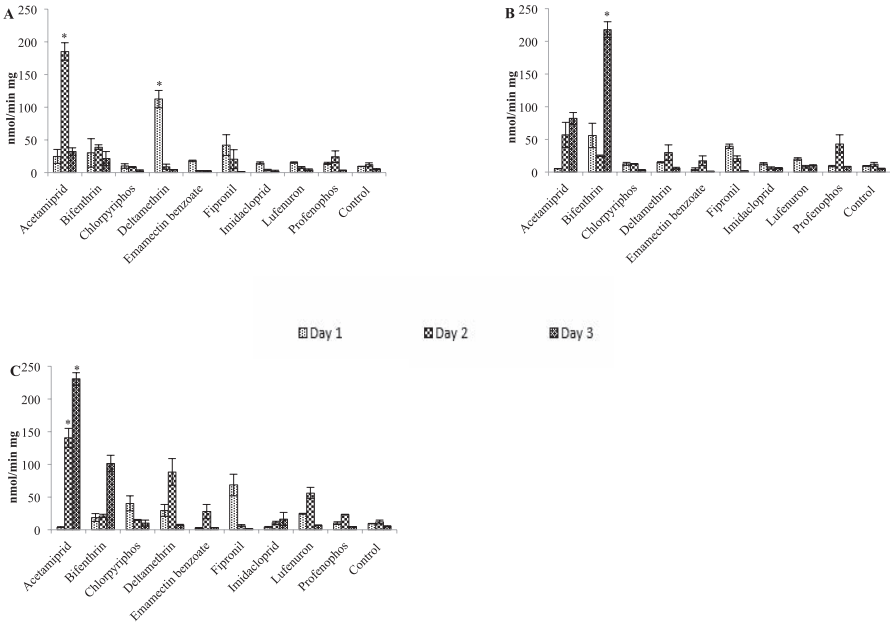

Fig. 4. Acid phosphatase activity of *M. domestica* at (A) LC_{50}, (B) LC_{30}, and (C) LC_{10} of different insecticides. Asterisk (*) shows significant difference between the enzyme activity at 24, 48, and 72 h (honest significant difference test, $P \leq 0.05$).

detoxification enzymes involved during degradation of toxic compounds include GSTs, ESTs, cytochrome P450 monooxygenases, acid phosphatases, and alkaline phosphatases (Yang et al. 2001). In our study, higher activities of detoxification enzymes were recorded with the assumption that increased levels of activity would result in resistance development. GST activity increased with exposure to acetamiprid, bifenthrin, and imidacloprid at the higher concentrations tested. The increase in GST activity may indicate its involvement in the detoxification process of acetamiprid, bifenthrin, and imidacloprid. Earlier studies recognized the GST system as a major mechanism involved in insecticide resistance in metabolizing several endogenous compounds (Flores et al. 2006; Gunasekaran et al. 2011; Yu 2004). In addition, noticeably increased levels of EST activities were recorded with deltamethrin, bifenthrin, and acetamiprid as compared to other treatments. However, no fixed trend was found for the increase in EST activity over time. In earlier studies, esterase-based resistance was reported for organophosphorus, carbamate, and pyrethroid insecticides (El-Latif and Subrahmanyam 2010; Field et al. 1988).

Our study demonstrates that AChE activity in *M. domestica* significantly increased after exposure to bifenthrin, acetamiprid, and fipronil. Regardless of the fact that AChE is not a target for bifenthrin, acetamiprid, and fipronil, the current study corroborates similar findings where AChE activity can be utilized as a biomarker for insecticide sensitivity (Jemec et al. 2007). Moreover, significantly

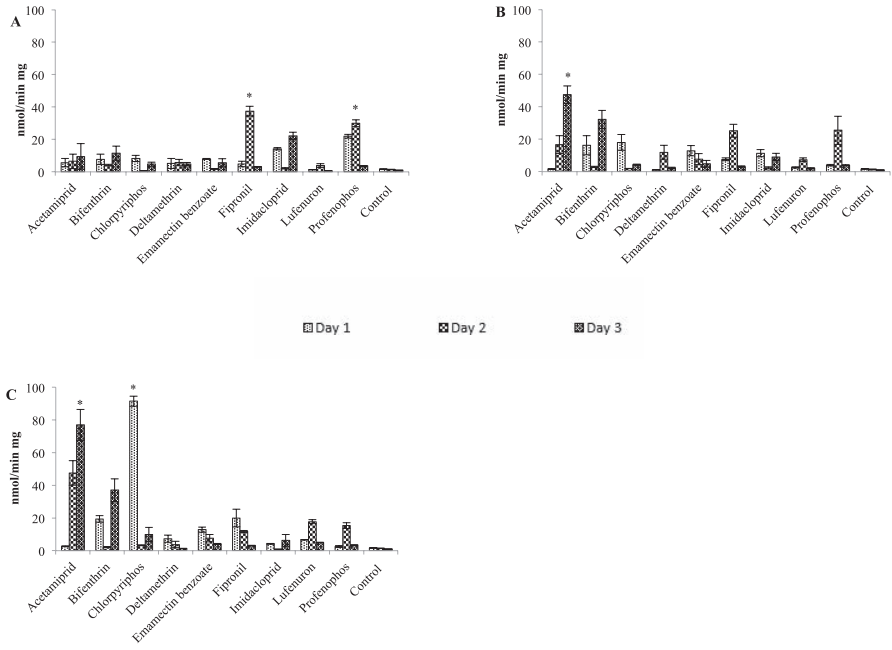

Fig. 5. Alkaline phosphatase activity of *M. domestica* at (A) LC_{50}, (B) LC_{30}, and (C) LC_{10} of different insecticides. Asterisk (*) shows significant difference between the enzyme activity at 24, 48 and 72 h (honest significant difference test, $P \leq 0.05$).

increased AChE activity in *M. cribraria* by imidacloprid (LC_{40}) (Miao et al. 2016) further supports the validity of our findings.

In addition, acid and alkaline phosphatases hydrolyze phospho-monoesters under acid or alkaline conditions. For acid phosphatases, the acetamiprid, bifenthrin, and deltamethrin showed significantly higher activities. Elevated levels of acid phosphatases were reported in *Schistocerca gregaria* (Forskal) (Orthoptera: Acrididae) by application of *Ammi visnaga* L. extracts (Ghoneim et al. 2014). Furthermore, chlorpyriphos, acetamiprid, bifenthrin, fipronil, and profenophos showed increased activities of alkaline phosphatases in comparison to other treatments. Similar results were reported by Emtithal and Thanaa (2012), where alkaline phosphatases may be the possible cause of detoxification of chlorpyriphos in *Culex pipiens* (L.) (Diptera: Culicidae).

Insect survival to sublethal levels of an insecticide results from increased selection pressure in favor of insecticide resistance based on physiological changes (i.e., increased gene copy number) in coding of a supplementary protective enzyme to aid breakdown of toxins into less toxic compounds (Daly et al. 1978). Moreover, pesticide adaption usually results in decreased relative fitness, and resistant insects have reduced reproductive potential and longevity (Stenersen 2004). The sublethal effects of LC_{10}, LC_{30}, and LC_{50} of insecticides in our study affected the normal developmental stages and longevity of *M. domestica*. In addition, increased activity

of detoxification enzymes may aid in resistance development. Insecticide application may result in population decline by not only killing susceptible individuals but also by reducing reproductive potential in the surviving insects (Rao and Shetty 1992). Moreover, elevated enzyme activity at LC_{10}, LC_{30}, and LC_{50} of insecticides provides information for underlying resistance development in the *M. domestica* population. However, further research is needed for exploring the association of these findings in field conditions as involves insecticide selection and resistance management.

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