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Effect of Malathion on Catalase Enzyme and Acetylcholinesterase Activity in Adult Flies and 3rd Instar Larvae of *Drosophila melanogaster*

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ABSTRACT: Farmers widely use insecticides and pesticides, in agriculture, which main ingredient is organophosphate, like, malathion. For prolong exposure of this organophosphate may affect neuronal behaviour and metabolic mechanism of non-target organisms. To study of these impacts, the objective of this paper was to evaluate the effect of Malathion on catalase enzyme and Acetylcholinesterase activity in adult flies and 3rd instar larvae of *Drosophila melanogaster* using appropriate standard methods. The study results have showed that both the activity level of Acetylcholinesterase and Catalase enzymes has been significantly dropped down in adults and 3rd instar larvae of *Drosophila*. Decrease level of Acetylcholinesterase indicates over accumulation of Acetylcholine neuro-transmitter, which can lead to hamper in their locomotion, behaviour, vision and other neuronal functions. Reducing level of Catalase indicates ROS generation, mainly H_2O_2 that can damage any metabolic activity. Thereby, we can suggest to control the usage of malathion organophosphate in agriculture purpose.

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In recent days, insecticides, mainly organophosphates are widely used in agriculture work to protect agriculture products from harmful insects, which can simultaneously contaminate crops and vegetables. For a long time, consuming these contaminated crops and vegetables, it causes an extensive exposure of several non-target organisms to these hazardous chemicals (Richmond, 2021). Non-target insects are also not manage themselves from the effect of organophosphate; their sense of smell and behaviour can hampered: examples from earlier studies like, chlorpyrifos diminished the learning ability (foraging and pollination) in bees (Urlacher *et al*., 2016) and disrupted the function of digestive enzymes in silkworms (Kalita *et al*., 2016); acephate, one of the highly used organophosphate, diminished reproductive capacity in *Drosophila melanogaster* (Mandi *et al*., 2020); dichlorvos, another organophosphate, which interfere in sensing of male silk moth from pheromone source (Chen *et al*., 2022).

Parathion and malathion emerging as the first organophosphate pesticides manufactured in the United States. Recent days, Malathion is one of most commonly used organophosphate for producing insecticides and pesticides as well as pest control in agriculture purpose. (Adeyinka *et al*., 2024) Organophosphates are also play a key role in formation

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of nerve gas. (Dressel *et al*., 1979). In 1920, Otto Loewi demonstrated that ACh (Acetylcholine) is a chemical intermediary that transmit nerve impulses across chemical synapses from one neuron to another neuron. (Borges *et al*., 2021). Acetylcholine (ACh) is a neurotransmitter synthesized from acetyl-CoA (acetyl-coenzyme A), which in turn is generated from glucose and choline via the catalytic action of choline acetyltransferase. In presynaptic membrane ACh is stored small vesicles, after receiving stimulation Ach are released and bind to several specific receptors to bring the stimulation forward. Through a hydrolytic process, AChE (Acetylcholinesterase) can degrade the neurotransmitter ACh into choline and acetate, for the termination of its effect on the muscarinic and nicotinic receptors (Rusyniak *et al*., 2004) (Adeyinka *et al*., 2024). During organophosphate metabolism, released nerve gas induces overstimulation of muscarinic and nicotinic receptors due to ACh accumulation, which can result in seizures, agitation, and centrally induced respiratory arrest at high doses. Peripheral overexpression of these receptors can generate cholinergic crisis, with excessive sweating, salivation, lacrimation, miosis-induced blurred vision, and respiratory distress due to bronchorrhea and bronchospasm (Abou *et al*., 2016). Chronic exposure to sarin nerve gas, may leads to neuroendocrine manifestations, (Faiz *et al*., 2011) such as delayed neurotoxicity, chronic neurotoxicity, and endocrine disruption. (Adeyinka *et al*., 2024). At present, several research works are processing on effect of organophosphates on insects (Kalita *et al*., 2016; Perveen and Ahmad, 2017). A very widely use model species for research work in genetics, developmental biology, biochemistry and biomedical sciences, is *Drosophila*, commonly known as fruit fly (Bindhani *et al*., 2022). Thereby, for the observation of neurological effect on exposure of malathion, we take *Drosophila* as a model of our study. Aerobic organisms (organisms surviving in presence of oxygen) generate reactive oxygen species (ROS) as a result of their metabolism. These ROS include hydrogen peroxide (H_2O_2), superoxide radical, hydroxyl radical and singlet oxygen. ROS can cause damage to DNA, protein, lipid and cell organelles. To maintain homeostasis, antioxidant enzymes balance ROS in living organisms. Catalase is an enzyme that decomposes hydrogen peroxide into water and oxygen. This enzyme prevents cells from oxidative damage by inhibiting hydrogen peroxide, thus protects from DNA damage. Catalase contains either heme or manganese (Mn) as a cofactor. It is an antioxidant enzyme found in all aerobic organisms. Deficiency of catalase enzyme (also called acatalesemia) could cause type 2 diabetes, loss of skin colour (called vitiligo), Alzheimer's disease and mouth ulcer and gangrene (Takahara disease) in human. Catalase activity in Drosophila peaks in third instar larvae stage and during metamorphosis (Bewley, Nahmias and Cook, 1983). Catalase level becomes normal after the adult fly emerges from the pupa. Organophosphate insecticides affect the antioxidant enzymes in various ways. Diazinon and dichlorvos increased superoxide dismutase (SOD) in rat erythrocyte (Sutcu *et al*., 2007, Cankayali *et al*., 2005); Chlorpyrifos inhibits the activity of antioxidant enzymes in mosquitofish, *Gambusia affinis* (Kavitha and Rao, 2008); Malathion increased the activity of catalase and superoxide dismutase in the root of *Allium cepa* (Srivastava and Singh, 2020).

The effect of malathion on *Drosophila* catalase is still unknown. Therefore, the objective of this paper was to evaluate effect of Malathion on catalase enzyme and Acetylcholinesterase activity in adult flies and 3rd instar larvae of *Drosophila melanogaster.*

MATERIALS AND METHODS

Drosophila culture medium: Drosophila larvae were reared in a standard culture medium. In India, the culture medium is prepared by using maize powder, agar agar, dried yeast, brown sugar, nipagin, propionic acid and water (Poddar, Mukhopadhyay, & Das, 2015). Both 3rd instar larva and adult flies were chosen for our experiment to assess the effect of malathion on AChE and Catalase enzymes.

Malathion treatment: LC_{50} dose of malathion was determined (Table 1). 0.80 µg of malathion insecticide was mixed with 10 ml of *Drosophila* culture medium in a clean vial. 3rd instar larvae and adult flies of *Drosophila* were fed the food containing the insecticide and observed for 24 hours after the treatment. Control samples were also prepared at the same time.

Sample Collection: For the study of Acetylcholinesterase, a total of 40 3rd instar larvae (20 from control sample and 20 from treated sample) and also 40 adult flies (20 from control sample and 20 from treated sample) were collected from the culture medium for study and were washed in few drops of Phosphate-buffered saline (PBS) solution separately. Both control and treated samples were homogenized in 1 ml of PBS solution and centrifuged at 10000 rpm for 5 minutes at 4^0C. Supernatant were collected.

For estimation of Catalase activity, 24 adult *Drosophila* (12 for control and 12 for treated) were homogenized in 1 ml of Phosphate-buffered saline (PBS) and centrifuged at 10000 rpm for 5 minutes.

Table 1: LC_{50} value determination, 20 *Drosophila*/vial / 10 ml food

Hours	Concentration (µl)	log_{10} (conc.)	% dead	Probit Value	LC_{50}	Final LC_{50} Value
24 hours LC_{50} value determination	2	0	15	3.96	LC_{50}= 1.28	LC_{50}=1.2µl
	1.7	0.113943352	25	4.33		
	1.5	0.176091259	85	6.04		
	1.3	0.230448921	85	6.04		
	1.1	0.301029996	100	7.33		
48 hours LC_{50} value determination	2	0.301029996	99	7.33	LC_{50}= 1.25	
	1.7	0.230448921	85	6.04		
	1.5	0.176091259	75	5.67		
	1.3	0.113943352	50	5		
	1.1	0.041392685	35	4.61		
72 hours LC_{50} value determination	2	0.301029996	99	7.33	LC_{50}= 1.2	
	1.7	0.230448921	90	6.28		
	1.5	0.176091259	85	6.04		
	1.3	0.113943352	60	5.25		
	1.1	0.041392685	40	4.75		
96 hours LC_{50} value determination	2	0.301029996	99	7.33	LC_{50}= 1.1	
	1.7	0.230448921	90	6.28		
	1.5	0.176091259	85	6.04		
	1.3	0.113943352	70	5.52		
	1.1	0.041392685	55	5.13		

Determination of AChE activity: Specific activity of AChE for each sample including both adult flies and 3rd instar larvae, was measured by Ellman's reagent. The enzyme kinetics was observed at 412 nm using a UV-VIS Spectrophotometer and a standard mixture (final volume is 3.12 ml) containing the supernatant, 0.1 M phosphate buffer (pH 8.0), 100 µl of Dithiobisnitrobenzoic acid (DTNB) and 20 µl of Acetylthiocholine iodide (Ellman, Courtney, Andrres, & Featherstone,1961).

Prior to the enzyme activity measurement, the protein concentration was determined by the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951) with the help of the Spectrophotometer, taking bovine serum albumin as the protein standard. The mean or average enzyme activity was measured for each sample. The whole experiment was repeated for seven times and the AChE activity was determined for four times in each sample.

Determination of Catalase activity: The assay of catalase activity was based on the conversion of cobalt (II) to cobalt (III) by hydrogen peroxide in presence of bicarbonate solution (Hadwan, 2018).

We prepared a working solution by mixing cobalt solution, Graham salt solution and sodium bicarbonate solution. 500 µl of *Drosophila* sample were mixed with 1 ml of hydrogen peroxide. After incubating for 2 minutes, working solution (6000 µl) was added to it. Changes in absorbance were recorded at 440 nm against blank.

Catalase activity = $2.303/t \log S^0/S$.

Statistical analysis: Mean density and standard error of the mean (SEM) were determined from three measurements. Statistical analysis was performed in MS Excel. To test the significance of difference between control and treated samples, Student's t test followed by one way analysis of variance (ANOVA) was applied.

RESULTS AND DISCUSSION

AChE activity in Adult Drosophila: The activity of Acetylcholinesterase decreases in treated adult *Drosophila*. Mean AChE activity for control sample was 4.56±0.042 whereas mean AChE activity for treated sample was 3.75±0.044 (mean ± standard error) (Figure 1). The difference was statistically significant ($p<0.0001$).

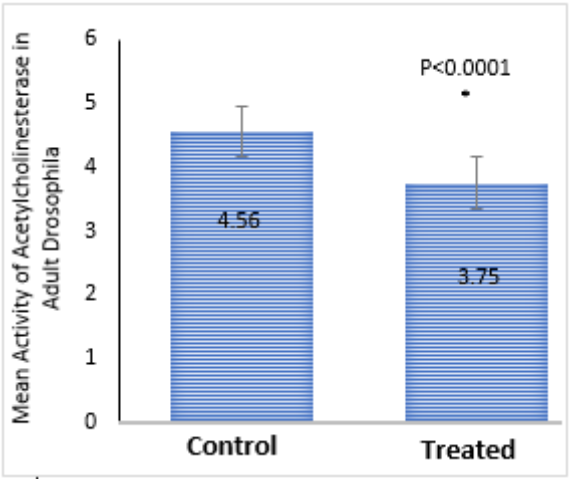

Fig 1: Mean Activity of Acetylcholinesterase decrease in treated Adult *Drosophila*

AChE activity in 3rd instar larvae of Drosophila: The activity of Acetylcholinesterase also drops in treated 3rd instar larvae of *Drosophila*. Mean AChE activity

for control sample was 3.72±0.0091 whereas mean AChE activity for treated sample was 2.5±0.0219 (mean ± standard error) (Figure 2). The difference was statistically significant (p<0.0001).

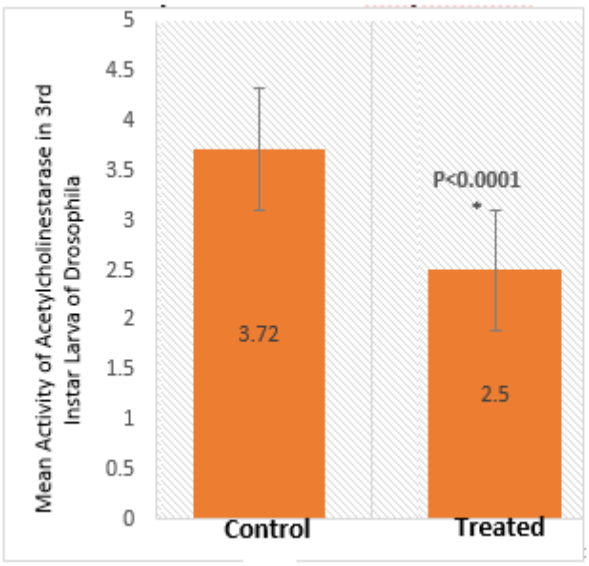

Fig 2: Mean Activity of Acetylcholinesterase decrease in treated 3rd instar larvae of *Drosophila*

Catalase activity in adult flies: In case of, Catalase activity shows significantly drop down in treated *Drosophila*. Mean catalase activity for control sample was 0.056±0.008 whereas mean catalase activity for treated sample was 0.034±0.007 (mean ± standard error) (Figure 3). The difference was statistically significant (p<0.5)

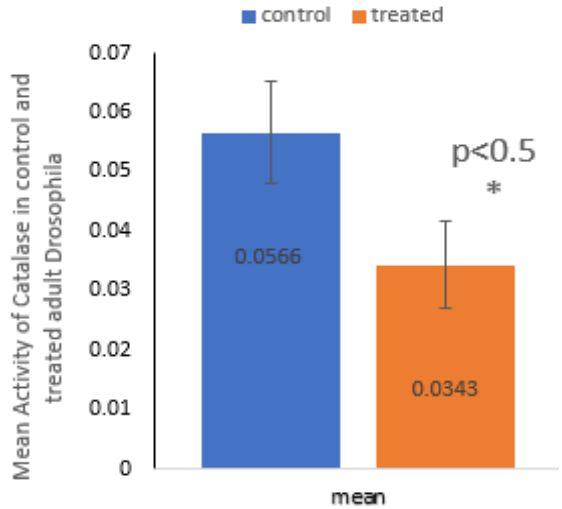

Fig 3: Mean Activity of Catalase decrease in treated Adult *Drosophila*

In our present experiments, we have found that Acetylcholinesterase drastically falling off in both adult and 3rd instar larvae of *Drosophila*. In case of adult flies, control sample showed mean value 4.56, whereas, treated sample (treated with malathion) showed mean value 3.75, and for 3rd instar larvae, control sample showed mean value 3.72, whereas, treated sample (treated with malathion) showed mean value 2.5. In both cases, Acetylcholinesterase activity significantly decreased (p<0.0001).

The results illustrated that malathion has a remarkable effect on Acetylcholinesterase activity. In different previous study, it is already demonstrated that reduction of Acetylcholinesterase level can increase the storage of Acetylcholine, which acts as a crucial neuro transmitter. Acetylcholine binds to muscarinic acetylcholine receptor leads to modify rhythm-generating networks, which are distributed in the central nervous system (CNS) of soft bodied *Drosophila* larvae (Jonaitis *et al*., 2022). Acetylcholine action also crucial for synaptic transmission and dendrite development as well as development of visual system and the ventral lateral neuron (Rosenthal *et al*., 2021). Over accumulation of Acetylcholine leads to sensory overload and bring on exceedingly strike on the visual, locomotion, behaviour and others neural function of *Drosophila*, both in adult flies and 3rd instar larvae.

Another experiment showed prominent decrease in catalase level in treated adult *Drosophila*. Result showed control sample of adult flies showed mean value 0.056, whereas, treated sample (treated with malathion) showed mean value 0.034, catalase activity significantly decreased (p<0.5). Lowering the level of catalase activity is responsible elevation the ROS level, which can generate critical adaptive stress that includes disfunction of midgut epithelial cell attachment to the extracellular matrix (ECM)-derived basement membrane and others metabolic activity (Mlih and Karpac, 2022).

Conclusion: Based on the above data, it can hypothesize that sublethal exposure to malathion leads to suppress the Acetylcholinesterase level in 3rd instar larvae as well as in adults of *Drosophila*; it directly reflects over expression of Acetylcholine neuro-transmitter that give rise to various neural functions distortion. This malathion exposure also reduces the Catalase enzyme level in adult *Drosophila*, brings on ROS generation which deliberately effect on various metabolic mechanism. On this statement, we can presume that malathion could have remarkable worst impact on Neurological and several metabolic functions of Human including other non-target organisms like pollinators; it causes disturbance in our ecosystem. Hence, use of this hazardous organophosphate chemical in agriculture should be

control and we should look for other alternate methods.

List of Abbreviations
AChE- Acetylcholinesterase
ACh- Acetylcholine
ECM- Extracellular matrix
PBS- Phosphate buffered saline
ROS- Reactive oxygen species

Declarations
Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: Datasets are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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