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Estimation of Amylase Enzyme Levels in *Drosophila melanogaster* Exposure to Malathion Organophosphate Poisoning

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ABSTRACT: Amylase, being a digestive enzyme breaks down polysaccharides, such as starch into simple sugars like glucose and maltose. Malathion is an organophosphate insecticide which is widely used in the agricultural field. Hence, the objective of this paper was to estimate amylase level in *Drosophila melanogaster* as a consequence of exposure to malathion organophosphate poisoning using appropriate standard methods. Data obtained show that mean amylase activity for control sample was 8.41±0.001 whereas mean Amylase activity for treated sample was 5.67±0.021 (mean ± standard error). The difference was statistically significant ($p<0.0001$). In malathion treated 3rd instar larvae, a significant reduction in amylase levels was observed in this study which indicates potential metabolic abnormalities and impaired energy production due to decreased starch digestion. Low amylase levels are associated with increased morbidity and delayed growth rate due to reduced glucose levels. This study represents the relationship between amylase levels and clinical outcomes in acute organophosphate poisoning. Long-term organophosphate exposure may also affect human health, particularly in relation to chronic diseases such as type II diabetes mellitus. Our finding underscores that exposure to malathion like insecticide disrupts amylase activity and plays a critical role in energy metabolism.

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Amylase is a digestive enzyme that breaks down polysaccharides like starch into short-chain sugars like glucose, maltose. In this study, the dipteran model insect *Drosophila melanogaster* was treated with malathion and used to investigate the effects of amylase. Malathion is an organophosphate insecticide that is commonly required to control a variety of insects that attack fruits, vegetables, and shrubs. In foragers of *Apis mellifera*, the effect of sub lethal concentrations of three organophosphates and one biopesticide on the activity of digestive enzyme Amylase was observed. The data came by treated groups of bees suggested that all the pesticides decreased the Amylase activity (Kumar, 2017). Organophosphates are important material for the common cause of acute poisonings in developing areas. This study determines the effect of serum amylase level for evaluation of prognosis in patients with acute organophosphate poisoning (Akdur *et al*.; 2010). It is observed that, the victims with higher serum amylase than normal were related with severe clinical issues and the risk for mortality increased (Zobeiri, 2021; Rao *et al*., 2017).

It already had been seen that sea cucumber *Holothuria parva* was accumulated and was then put through extraction. The results exhibited that compounds extracted from *Holothuria parva* had a stimulatory effect on enzyme activity. If the concentration of silver nanoparticles became increased, it inhibited the α-

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amylase activity and the nanoparticles also could reduce intestinal glucose uptake in diabetic individuals (Edrispour and Homaei; 2023) (Nasab *et al*.; 2020)

Another study was directed to understand the effects of organophosphate on activities of invertase, amylase and respiration of microorganisms in a sandy soil. The result determined that although pesticide tests unveiled some important effects on activities of soil enzymes and respiration, the soil microorganisms and enzymes can bear with the toxicities of most of the pesticides (Ernst *et al*.; 2008) (Gudbrandsen *et al*.; 2007).

Our previous study was undertaken to know the prognostic (Sumathi *et al.*; 2014) significance of various biochemical parameters in acute organophosphorus poisoning. However, the objective of this paper is to estimate amylase level in *Drosophila melanogaster* as a consequence of exposure to malathion organophosphate poisoning.

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 *et al.*; 2015). 60 (30 for control and 30 for treatment) 3rd instar larvae were chosen for our experiment to assess the effect of malathion on Amylase enzyme.

Malathion treatment: LC_{50} dose of malathion was determined by **Probit analysis method** (Table 1). 0.80 µg of malathion insecticide was mixed with 10 ml of *Drosophila* culture medium in a clean vial. 3rd instar larvae 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.

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		

Sample Collection: For the study of Amylase, a total of 60 3rd instar larvae (30 from control sample and 30 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. Prior to the experiment, weight of 30 larvae for control had taken and the value was 0.44gm and approximate same weighted 30 larvae were taken for treatment.

Estimation of Amylase activity: At first the Stock solution of 1% Maltose solution is prepared by dilution 100 mg of Maltose in 1000 ml of distilled water. Then 5 test samples are taken in separate test tubes marked A,B,C,D,E (representing 0.1% , 0.2%, 0.3%, 0.4%, 0.5% Maltose solution) (Poddar *et al.*; 2015).

Preparation of phosphate buffer:

A solution (Sol-A) is prepared by dissolving 1.42 g of Na_2HPO_4 in 100 ml of distilled water.A second solution (Sol-B) is prepared by dissolving 1.56 g of NaH_2PO_4 in 100 ml of distilled water. Sol-B is then added dropwise to Sol-A to adjust the pH to the desired value.

Preparation of substrate: To prepare the substrate, 2 g of starch is dissolved in 100 ml of distilled water.

Preparation of DNSA solution: A solution (Sol-C) is prepared by mixing 20 ml of 2N NaOH with 50 ml of distilled water. o Sol-C, 1 g of DNSA (3,5-dinitrosalicylic acid) is added slowly with continuous stirring. Then, 30 g of sodium potassium tartrate is added to the solution and wait until it is dissolved completely.

Preparation of reaction mixtures: In separate test tubes (control, treated, and labelled A, B, C, D, E), 0.5 ml of phosphate buffer, 0.5 ml of substrate and 0.5 ml of enzyme solution are added. For the blank solution 0.5 ml of phosphate buffer, 0.5 ml of substrate and 0.5 ml of distilled water are added to the test tube. All samples are incubated at 37°C temperature.

DNSA reaction and final steps: After incubation 0.5 ml of DNSA solution is added to each test tube. The samples are then placed in a water bath and boiled for 8 minutes. Following the boiling, the test tubes are cooled by holding them under running tap water. After that 10 ml of distilled water is added to each sample containing test tubes. Finally, the optical density (OD) of each sample is measured at 540 nm using a green filter using Colorimeter.

The amylase activity was calculated using equation 1 and 2.

$$Activity = \frac{A}{0.5\ ml\ x\ 10\ minutes} \quad (1)$$

$$\text{Activity} = \frac{B}{342.3\ g/mol} x1000\ \mu\text{mol/mg} \quad (2)$$

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

RESULTS AND DISCUSSION

Amylase activity in 3rd instar larvae of Drosophila: The activity of Amylase decreases in treated adult *Drosophila*. Mean Amylase activity for control sample was 8.41±0.001 whereas mean Amylase activity for treated sample was 5.67±0.021 (mean ± standard error) (Table 2). The difference was statistically significant ($p<0.0001$) (Fig 1).

Table 2: Value of Amylase activity in 3rd instar larvae of *Drosophila*

Group	Control	Treated
Number	**30**	**30**
Mean	**8.41**	**5.67**
SD	**0.006**	**0.12**
SEM	**0.001**	**0.021**

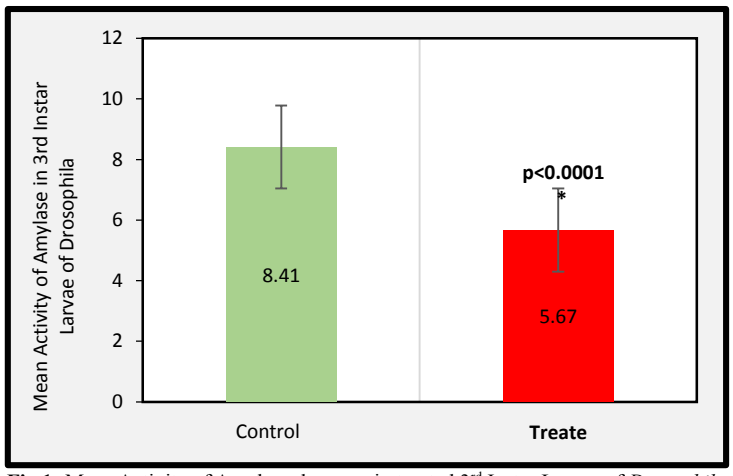

Fig 1: Mean Activity of Amylase decrease in treated 3rd Instar Larvae of *Drosophila*

The study results have suggested that the amylase activity was significantly reduced in treated 3rd instar larvae of *Drosophila melanogaster.* Decrease level of Amylase indicates increased risk of metabolic abnormalities. Amylase helps to break down starches into sugar, which can use for energy (Hickey and Bernhard; 1982) . Low amylase level retains the starch from undigested carbohydrate within the gut which leads to higher morbidity and mortality. Amylase acts as an indispensable hydrolase in insect growth and development. But in this study due to decreased level of amylase, the growth rate of the insect is also delayed. In an experiment, the toxicity of *Rubus fruticosus* and *Valeriana jatamansi* was compared against granary weevil, *Sitophilus granarius* followed by changes in enzyme activity which was responsible for grain damage. Here, the enzyme activity of α-amylase was reduced and increased in *R. fruticosus* and *V. jatamansi*, treated insects, respectively (Ahmed *et al.*; 2021). Another study is observed to determine the antidiabetic activity of glycosidic compound - saponin - derived from the Egyptian sea cucumber, *Holothuria thomasi*. The outcome suggested that saponin extract significantly reduced serum glucose, α-amylase activity (Barky *et al.*; 2016). Our experimental result shows that the consumption of malathion affects metabolic activities in *Drosophila melanogaster* which is reflected by the decrease in amylase enzyme level as well as dropping off glucose levels. Based on this study we can assume that Humans, are also affected by several chronic diseases related to aging, including type II diabetes mellitus due to the decrease of amylase enzyme through chronic exposure to Malathion (Kurt *et al.*; 2015) (Lando *et al.*; 2012)

Conclusion: The observation of the present study concluded that the amylase levels are considered a marker of malathion intoxication. Amylase enzyme has a significant role in digestive function both in insects and mammals as it can break down complex carbohydrates to ultimately form monosaccharides. So, a reduction in the Amylase level creates trouble in carbohydrate metabolism. From this study, it could be suggested that malathion poisoning hampers the metabolic pathway in insects as well as in Humans leading to serious conditions like Type 2 Diabetes, etc. that need rapid diagnosis and treatment. Hence, the use of this hazardous organophosphate chemical in agriculture should be controlled and we should look for other alternate methods.

Declaration of Conflict of Interest: The authors declare no conflict of interest.

Data Availability Statement: Data are available upon request from the corresponding author.

REFERENCES

Ahmed S, Zia A; Mehmood, SA; Panhwar, WA; Khan, W; Shah, M; Ullah, I; (2021). Change in malate dehydrogenase and alpha amylase activities in Rubus fruticosus and Valeriana jatamansi treated granary weevil, Sitophilus granarius. *Brazilian. J. Biol.* 81(2):387-391. doi: 10.1590/1519-6984.226952

Akdur, O; Durukan, P; Ozkan, S; (2010). Poisoning severity score, Glasgow coma scale, corrected QT interval in acute organophosphate poisoning. *Hum. Exp Toxicol*. 29(05):419–425. DOI: 10.1177/0960327110364640.

Barky, EI AR; Hussein, SA; Alm-Eldeen, AA; Hafez, YA; Mohamed, TM; (2016). Anti-diabetic activity of *Holothuria thomasi* saponin. *Biomed Pharmacother.* Dec; 84:1472-1487. doi: 10.1016/j.biopha.2016.10.002.

Edrispour, Z; Homaei, A; (2023). Exploring in vitro effect of silver nanoparticles and Holothuria parva extracts on kinetics and stability of α-amylase. *Biotechnol Appl. Biochem*. Apr; 70(2):885-894. doi: 10.1002/bab.2407.

Ernst, G; Zimmermann, S; Christie, P; Frey, B; (2008). Mercury, cadmium and lead concentrations in different ecophysiological groups of earthworms in forest soils. *Environ. Pollute.* 156 (3): 1304-1313;

Gudbrandsen, M; Sverdrup, LE; Aamodt, S; Stenersen, J; (2007). Short-term exposure increases earthworm tolerance to mercury European Journal of Soil Biology. *European J. Soil Biol.* 43, Supplementary 1, S261-S267; doi: https://doi.org/10.1016/j.ejsobi.2007.08.025.

Hickey, DA; Bernhard, B; (1982). Regulation of amylase activity in *Drosophila melanogaster*: effects of dietary carbohydrate. *Biochem. Genet.* 20, 1117–1129 10.1007/BF00498936, DOI: 10.1007/BF00498936

Kumar, S; (2017). Effect of pesticides on amylase activity in foragers of Apis mellifera L. under laboratory conditions. *Pollut. Res*. 36(3):619-622; doi: https://jstr.org.in/downloads/pub/v1/i1/3.pdf.

Kumar, S; (2017) Study of acetyl cholinesterase activity in forager worker bees of Apis mellifera under stress of Pesticides. *Flora and Fauna*, 23 (1): 237-244; doi: 10.33451/florafauna.v23i1pp237-234.

Kurt, O; Demirci, H; Ozturk, K; Kantarcioglu, M; Uygun, A; (2015). Severe serum amylase elevation, with only chronic kidney disease. *Renal Failure* 37 (5): doi: https://doi.org/10.3109/0886022X.2015.1022852.

Lando, HM; Alattar, M; Dua, AP; (2012). Elevated amylase and lipase levels in patients using glucagonlike peptide-1 receptor agonists or dipeptidyl-peptidase-4 inhibitors in the outpatient setting. *Endocr Pract*. Jul-Aug; 18(4):472-7. doi: 10.4158/EP11290.OR.

Nasab, SB; Homaei, A; Karami, L; (2020). Kinetic of α-amylase inhibition by Gracilaria corticata and Sargassum angustifolium extracts and zinc oxide nanoparticles. *Biocatal. Agric. Biotechnol.* 23: 101478. Doi: https://doi.org/10.1016/j.bcab.2019.101478.

Poddar, T; Mukhopadhyay, S; Das, SK; (2015). An advanced laboratory manual of Zoology. Trinity Press (An imprint of Laxmi Publications Pvt. Ltd.). India.

Rao, AN; Pati, A; Brodnik, ZD; Liang, Q; Rodrigo, AE; Kimberly, AS; (2017). Pharmacologically increasing microtubule acetylation corrects stress exacerbated effects of organophosphates on neurons. *Traffic.* 18(7):433–441. DOI: 10.1111/tra.12489.

Sumathi, ME; Kumar, SH; Shashidhar, KN; Takkalaki, N; (2014). Prognostic significance of various biochemical parameters in acute organophosphorus poisoning. *Toxicol. Int.* 21(02):167–171., DOI: 10.4103/0971-6580.139800.

Zobeiri, M; (2021). Serum amylase as a prognostic marker of organophosphate poisoning. *J Inj Violence Res.* 13(2):117-120. doi: 10.5249/jivr.v13i2.1632.