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Impact of organophosphate pesticides, methyl parathion and chlorpyrifos on some tissue enzymes in fish (*Aphanius dispar)*

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In the present study acute effects of methyl parathion and chlorpyrifos on tissue enzyme activity (acetyl cholinesterase (AChE), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), acid phosphatase (ACP) and alkaline phosphatase (ALP) were assessed in fish juveniles using enzyme analysis kit. Results depict both elevation and inhibition of enzymes in the treated fish. The results revealed significant elevation in ALT activity of both pesticide i.e., methyl parathion and chlorpyrifos by (105%) and (118%) as compared to control and inhibition in AChE, LDH, AST and ALP enzymes. The AChE and ALP activity levels decreased significantly ($p< 0.05$) in both pesticide treated groups. Significant decrease levels of ACP activity in fish tissue were noticed in methyl parathion treated fish. But in case of chlorpyrifos treated fish, increase in ACP activity (101%) was observed, however the increase was not significant ($p< 0.05$).

[**Keywords:** Pesticides; methyl parathion; chlorpyrifos; alanine aminotransferase; acetyl cholinesterase; lactate dehydrogenase.]

Introduction

Agriculture is the main stay of Pakistan's economy and consequently pesticides are abundantly used on its croplands. The use of agro-chemicals has increased tremendously over the last two decades. Organophosphorus pesticides are widely used in agricultural crops to control pest. The organophosphorus pesticides replaced the organochlorine due to their nonpersistent nature[1]. Pesticides have benefited mankind by increasing the yield of crop in agriculture but at the same time their adverse effects on non-target organisms are significant[2,3]. Fish may be considered as good indicators of contamination by pollutants because their biochemical effects are similar to mammals[4] . The effect of pollution in fish is well documented[5,6,7,4,8,9]. Biochemical changes induced by pesticide stress lead to disturbances in metabolism, inhibition of enzymes, retardation of growth, reduce fecundity and biodiversity of the organism[10]. Bioaccumulation of pesticides in tissues of marine organisms may have drastic effect on human[11]. Organophosphorus pesticides produce toxicity by inhibiting cholinesterase enzymes in aquatic organisms. These enzymes remove the neurotransmitter acetylcholine (ACh) from the synaptic cleft through hydrolysis[12]. Acetyl cholinesterase (AChE) is an indicator of the effect of pollutants on aquatic organisms[13]. Monitoring of AChE inhibition has been widely used in marine ecosystems as an indicator of organophosphate pesticide exposure and effects in exposed animals[14,15,16]. The activities of ALT, AST and LDH enzymes are also used as a stress indicator[17]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate tissue damages in liver and kidney[18,19]. Increase in Lactate dehydrogenase (LDH), a cytoplasmic enzyme, indicates cell lysis[20]. Acid phosphatase (ACP), is associated with lysosomal activity and its elevation reflects proliferation of lysosomes[21]. An increase in alkaline phosphatase (ALP) levels is suggestive of increased osteoblastic activity and intra and extra hepatic obstructions of biliary passage[22].

The aims and objectives of the present study was to assess the toxicity of organophosphate pesticide, methyl parathion and chlorpyrifos on enzymes of fish *Aphanius dispar*. This work is expected to help in understanding biochemical basis of pesticide effect on fish and hence planning strategies for release of chemicals in the aquatic medium.

Material and Methods

Pesticides, methyl parathion 5% EC and chlorpyrifos 40% EC were procured from Pakistan Agricultural Research Center.

The 24h acute toxicity bioassay was carried out as described by Shoaib et al.[9] Juveniles of *Aphanius dispar* (1.7 ± 0.2cm in length; 112mg±1 in weight) were collected from Sandspit backwater. Organophosphate pesticide (methyl parathion and chlorpyrifos) was used in this experiment at a concentration equal to LC_{50} value i.e. 0.039 ppm and 0.0025 ppm (Shoaib *et al.)*[9] The fish were divided into two groups: Group A (Control, without exposure) and Group B (Experimental, with exposure). Both A and B group were in triplicate. At the end of exposure period (24h) fish were removed from jars and frozen for further tissue enzyme analysis.

Known weight of fish both test and control (six replicates each) was homogenized with distilled water using mortar and pestle. Content was centrifuged in Hitachi refrigerated centrifuge at 15000 rpm at 4° C for 15 min. Supernatant was immediately analyzed for enzymes using enzyme analysis kits. Samples were kept on ice during the preparation and analyses. Activities of six enzymes, namely acetyl cholinesterase, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, alkaline phosphatase and acid phosphatase, were determined in exposed and control sample of fish.

Samples of the fish tissue were subjected for calorimetrical analysis of alanine aminotransferase activity, method[23] (Clonital kit (EC 2.6.1.2.)), for determination of aspartate aminotransferase activity, method[23] (Clonital kit No. EC 2.6.1.1.) and for the quantitative determination of acetyl cholinesterase, method [24](Randox kit (CE 190)) was used. For the quantitative determination of lactate dehydrogenase, method[25] (Clonital kit (EC 1.1.1.27.)) was used. For the quantitative determination of alkaline phosphatase,[26] (Clonital kit (EC 3.1.3.1.)) and for quantitative determination of acid phosphatase, method [27](Randox kit (AC 1011) was used. For statistical analysis Student's't' test was applied using standard computer program SPSS to find significant differences between treated and control group.

Result

Variable effects of organophosphate pesticides on tissue enzyme activity levels were observed (Table 1, 2). The results depict both elevation and inhibition of enzyme activity in the treated fish. AChE activity levels decreased significantly ($p< 0.05$) in both pesticide treated groups. Maximum reduction of AChE activity level was observed in methyl parathion (49%) treated fish tissues as compare to chlorpyrifos treated fish tissues. AST activity was generally inhibited by both pesticides tested. Inhibitory effect of pesticide chlorpyrifos was noticeable and significantly different from control group values ($p< 0.05$) (Table 2). The ALT activity level was increased in tissues exposed to the pesticides, however, chlorpyrifos had more pronounced effect on ALT activity exhibiting 118% increase ($p<0.05$) over methyl parathion effect (105%)". LDH activity levels were decreased in pesticides (methyl parathion, chlorpyrifos) treated fish tissues. Effect of methyl parathion was more pronounced (31% decrease) as compare to chlorpyrifos. ALP activity was reduced significantly ($p< 0.05$) in tissues of methyl parathion and chlorpyrifos treated fish. Significant decrease levels of ACP activity in fish tissue were noticed in methyl parathion treated fish. But in case of chlorpyrifos treated fish, increase in ACP activity (101%) was observed, however the increase was not significant ($p< 0.05$).

Discussion

The present study suggests that when fish is exposed to methyl parathion and chlorpyrifos, the major biochemical response to the effect of pesticides is inhibition of acetylcholinesterase (AChE) activity.Our result is in agreement with [28,29,30,31,32,33,34,35,36,37]. Inhibition of AChE has adverse effects on the heart, as the cholinergic system has an important role in the enervation of the fish heart[38]. AChE inhibition can have drastic affect on the growth, social interactions, altered feeding, reduced swimming stamina, fecundity and biodiversity of fish[39,40,41,42,43,44,45,46,47]. Present study depicts that in pesticides treated fish the aspartate amino transferase (AST) was inhibited. Our result is in agreement with[48,49,50] alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are liver specific enzymes and can be measured within a short period of time[19]. The determination of AST and ALT, enzyme activities is being used as a stress factor in fish[51,52].

This study reveals that the activity of alanine amino transferase (ALT) was increased in

pesticides treated fish our result corresponds with previous results[48,19,53,54]. The increase in level of aminotransferases indicates the tissue damages in liver, kidney and gill[19,55,56]. Toxic action of pesticide combine with an enzyme to form an enzyme inhibition complex which reacts with functional groups of the enzymes inhibits the normal enzyme activity of major metabolic site [57].

In methyl parathion and chlorpyrifos treated fish LDH activity was inhibited similar reports were observe by[58,19,59,60]. The decreased muscle LDH activity may be due to its inhibition, or decreased synthesis. LDH activity is decreased due to tissue damage[18].

The activity of alkaline phosphatase was inhibited in chlorpyrifos and methyl parathion treated fish in the present study, our result is in agreement with Sastry and Sharma[61] who reported decrease of activities of alkaline phosphatases in the brain of fish following the effect of diazinon. This study shows that in chlorpyrifos treated fish, the activity of acid phosphatase was increased. Gill *et al.,*[21] speculate that acid phosphatase elevation reflects proliferation of lysosomes in attempt to sequester the toxic xenobiotic. While in methyl parathion, treated fish the acid phosphatase was inhibited. Our result corresponds with the result of Sastry and Sharma[61], who reported decrease of activities in acid phosphatases in the brain of fish following

the effect of diazinon.

This is evident from the forgoing that different kinds of pesticides may lead to physiological disturbances which in turn may affect fish health and biodiversity[50,62,63,64]. In conclusion, methyl parathion and chlorpyrifos pesticides induce its toxic effects by inhibiting and activating enzymes. Changes in the biochemical tissue profile reflect changes in metabolism of the fish, resulting from the toxic effect of methyl parathion and chlorpyrifos.

Table 1. Effect of methyl parathion on enzyme activity in tissue of *Aphanius dispar.* Values are mean percent activity of six replicate analysis, asterisk sign denotes significantly different from control ($p<0.05$).

METHYL PARATHION	TIME (Sec)	MEAN OF ENZYMES U/L	±S.D.	PERCENT ACTIVITY	95% CONFIDENCE LIMIT
AST activity Control	180	382±	48	100	339-424
AST activity Treated	180	387±	110	98.67	279-495
ALT activity Control	180	386 ±	108	100	280-492
ALT activity Treated	180	414 ±	36	105.19	379-450
LDH activity Control	180	5110 ±	917	100	4211-6009
LDH activity Treated	180	3485 ±	503	68.22*	2992-3979
AChE activity Control	90	28675 ±	11275	100	15917-41434
AChE activity Treated	90	14395 ±	4201	50.2*	12315 -1878
ACP activity Control	180	143 ±	23	100	117-170
ACP activity Treated	180	121 ±	34	84.17*	87-154
ALP activity Control	180	1542 ±	111	100	1432-1651
ALP activity Treated	180	1345 ±	198	87.24*	1150-1540

Tables 2. Effect of chlorpyrifos on enzyme activity in tissue of *Aphanius dispar.* Values are mean percent activity of six replicate analysis, asterisk sign denotes significantly different from control ($p<0.05$).

CHLORPYRIFOS	TIME (Sec)	MEAN OF ENZYMES U/L	±S.D.	PERCENT ACTIVITY	95% CONFIDENCE LIMIT
AST activity Control	180	369±	130	100	242-497
AST activity Treated	180	334±	86	90.56*	250-419
ALT activity Control	180	336 ±	39	100	297-374
ALT activity Treated	180	397 ±	107	118.33*	303-491
LDH activity Control	180	5168 ±	811	100	4457-5880
LDH activity Treated	180	4910 ±	384	95.02*	4603-5218
AChE activity Control	90	19033 ±	341	100	18647-19420
AChE activity Treated	90	17413 ±	2212	90.7*	15244 -19581
ACP activity Control	180	148 ±	13	100	135-160
ACP activity Treated	180	149 ±	48	101.29	111-188
ALP activity Control	180	1460 ±	252	100	1213-1708
ALP activity Treated	180	1399 ±	404	95.79*	1075-1722

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