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ENZYMOLOGICAL ALTERATIONS PRODUCED BY CHRONIC MALATHION EXPOSURE IN FRESHWATER CRAB, *PARATELPHUSA (BARYTELPHUSA) JACQUEMONTII*

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Abstarct: Pesticides are concerned with problem of pollution especially of water. Increased level of these xenobiotic compounds in water is reason of damage to aquatic ecosystem. Aquatic biota is getting hampered due to load of insecticides, herbicides and other agrochemicals in agro intensives areas as well as around industries making production and use of these synthetic products. Freshwater crabs are one of the ecologically important species which are exposed to variety of organic and inorganic pollutants coming in their contact. Malathion, a widely used insecticide was used in present investigation to assess its impact on enzymes in fresh water crab, *Paratelphusa (Barytelphusa) jacquemontii*. Crabs were exposed to sub lethal concentration of 0.555 ppm for 30 days. Enzymes such as Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Glutamate Oxaloacetate Transaminase (GOT), Glutamate Pyruvate Transaminase (GPT) and Adenosine Tri-phosphatase (ATPase) showed marked changes in enzyme composition as compared to control. Significant increase was observed in phosphatases while decrease in ATPase was observed as compared to control. Present study gives details of these enzyme alterations in exposed group of animals. The results are discussed in the paper.
Keywords: Insecticide, Malathion, freshwater crab, enzymes.

Introduction

Present day intensive farming for food and heavy industrialization for production of goods to meet the need of growing population has led to problem of pollution. Environmental pollutants are becoming toxicants due to their adverse effects on living beings. Ecological impacts of waste from agro industries are inevitable due to their wide composition (Thakur, 2006). Now a days, toxicity studies of such pollutants in environment are gaining immense importance. Pollution of the ecosystem due to various anthropogenic substances known as xenobiotics is rising in recent years. Some of the examples of xenobiotics are toxic chemicals used in industries, substances from wastewater, heavy metals in different applications and these contaminants may occur in air, water and soil those are released by human activities but not present in natural condition. Coastal water resources are found to be contaminated with industrial and urban waste (Shanthi and Gajendran, 2009). These xenobiotics cause harm to

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organisms coming in their contact. They act indirectly by modifying essential bioconstituents, affecting nutrition and altering physiological mechanism. Some compounds are readily toxic in nature or sometime need to get change in chemical nature before they cause toxicity. They can get accumulated or distributed in target organism and affect specific cells, tissue or whole organ getting exposed to these compounds.

Pesticide is one such group which consists of clastogenic compounds leading to mutations in exposed organisms. Carcinogenicity checking is becoming important aspect as people are getting exposed to different chemicals directly and indirectly in day to day life. There has been lot of concern about the cellular and molecular changes leading to carcinogenesis. Studies regarding these chemicals need screening for their properties that do not cause mutation but still are having carcinogenic potential (Tweats *et al*., 2007). Indiscriminate use of pesticides and their untreated effluents affects fish and other aquatic animal (Wanee *et al.*, 2002) If concentration of pesticides reaches above certain limits in exposed organism, it leads to harms like change in metabolism, reproductive disorders, disruption of endocrine system. Death of the organisms occurs when pesticide is more toxic in nature. Several physiological and biochemical functions can get impaired due to exposure to pesticides. Antioxidant stress is another effect of their exposure which may involve change in levels of many enzymatic and non enzymatic factors. The effects of 2, 4-D were evaluated in fish, *Leporinus obtusidens* by Fonseca *et al*. (2008). It was observed that after 96 hrs. muscle protein levels were enhanced after exposure at 10 mg/L, but no significant changes were observed in muscle and liver glucose. Liver lactate and protein were significantly reduced after exposure in the same study. Profenofos, a broad spectrum Organophosphate insecticide is found to be inducing biochemical changes as a sign of hepatocellular injury and disturbed amino acid metabolism Gomes *et al.*, (1999).

Malathion, S-1, 2-bis (ethoxycarbonyl) ethyl O, O-dimethylphosphorodithioate) is Organophosphorus class insecticide with worldwide use. It is also used in malaria eradication programmes since long back. Malathion has implication with mutagenic and carcinogenic properties and also was one of the insecticide with legacy problem, Brenner (1992). It is commonly used OP insecticide to control wide variety of pests on crops. It is also involved in Malaria eradication programs. Large scale use of this insecticide has raised concerned due to potential to cause genetic damage (Flessel *et al.* 1993). Organophosphorus insecticides are widely used and harmful to non target organisms due to run off (Joseph, 2011). Crustaceans such as crabs are exposed to variety of agrochemicals in their surroundings. Studies on

toxicity of pesticides to these non target organisms are essential in ecological exposure analysis investigations.

Present study was carried out to assess toxicity potential of Organophosphate pesticide (OP) Malathion in freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii.* Significant increase in ACP was observed while ALP, GOT, GPT showed insignificant increase. ATPase activity was found to be insignificantly inhibited in present investigation.

Material and Methods

The animals were collected from local market and brought to the laboratory. Animals were transported through plastic troughs of suitable size covered with wire mesh free from sharp points or projections. All the equipments were cleaned every time after their use for handling the animals. Commercial formulation of Organophosphate pesticide, Malathion was used in the study as commercial products are used mainly by farmers than that of analytical one.

LC_{50} value of the insecticide for 96 hrs was determined by exposing crabs to range of concentrations diluted with tap water. It was calculated by using Finney Probit analysis table. LC_{50} value for 96 hrs. was found to be 5.55 ppm. Chronic toxicity bioassay was carried out by exposing crabs to 0.555 ppm i.e. $1/10^{th}$ concentration of LC_{50}. Animals were exposed to this concentration for 30 days. Simultaneously control set of animals was maintained. Water from containers was changed twice a day and concentration of test compound was added every time. After completion of exposure period of 30 days animals were sacrificed and various tissues were excised to carry out enzyme studies. ACP and ALP were estimated using method given by Butterworth and Probert (1970) while Reitman and Frankel, (1957) method was used to determine GOT and GPT activities in the tissues. ATPase was analyzed by the method given by Du Boise and Potter, (1943).

The ANOVa test was used to determine the significance of difference between the mean value of control and experimental groups.

Results and Discussions

Pesticides are becoming environmental pollutants as these are used intensively agriculture for protection against diseases and pests. Pest control is crucial element of crop protection and is becoming associated with public health. The estimated annual application of pesticides worldwide is more than 4 million tons, but only 1% of this reaches the target pests (Gavrilescu, 2005). Studies of specific organ in terms of physiology and biochemistry are important tool in toxicity assessment. Different toxicants may have varying effects on particular organ at various levels.

Table-1. ACP activity in the different tissues of freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to Malathion for 30 days.

Sr. No.	Tissue	Control	Exposed
1	Gills	4.214 ±0.3547	4.7261 ±0.3527
2	Hepatopancreas	3.095 ±0.3198	5.4523** ±0.1104
3	Muscles	1.0238 ±0.4240	2.7142* ±0.4412
4	Testis	5.8690 ±0.4688	6.3095* ±0.6297
5	Ovary	4.1309 ±0.2645	6.1231** ±0.3120

Activity expressed as mg Pi liberated / h / mg Protein. Values are mean ± S.D. of 5 estimations. * Significantly different from control.
* P> 0.05 by ANOVA. ** P< 0.05 by ANOVA.

Table-2. ALP activity in the different tissues of freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to Malathion for 30 days

Sr. No.	Tissue	Control	Exposed
1	Gills	2.2380 ±0.5137	4.7852* . ±0.4582
2	Hepatopancreas	6.6309 ±0.3379	7.5952* 0.3669
3	Muscles	2.1071 ±1.0105	4.7261* ±1.2073
4	Testis	4.6071 ±0.7004	5.2261* ±0.5418
5	Ovary	0.5595 ±0.1024	0.9112 ±0.0107

Activity expressed as mg Pi liberated / h / mg Protein. Values are mean ± S.D. of 5 estimations. * Significantly different from control.
* P> 0.05 by ANOVA. ** P< 0.05 by ANOVA.

Table-3. GOT activity in the different tissues of freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to Malathion for 30 days.

Sr. No.	Tissue	Control	Exposed
1	Gills	0.2566 ±0.0501	0.2972 ±0.0381
2	Hepatopancreas	0.0450 ±0.02360	0.2600 ±0.0075
3	Muscles	0.2077 ±0.01565	0.8055 ±0.06714

4	Testis	0.1922 ±0.01982	0.2627 ±0.02377
5	Ovary	1.8955 ±0.03261	2.508 ±0.0411

Activity expressed as units / mg Protein.
Values are mean ± S.D. of 5 estimations.
* Significantly different from control.
* P> 0.05 by ANOVA.
** P< 0.05 by ANOVA.

Table-4. GPT activity in the different tissues of freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to Malathion for 30 days.

Sr. No.	Tissue	Control	Exposed
1	Gills	0.2438 ±0.0068	0.2644 ±0.0191
2	Hepatopancreas	0.0683 ±0.0425	0.2394 ±0.0167
3	Muscles	0.1833 ±0.0108	0.3683 ±0.0294
4	Testis	0.3872 ±0.8105	0.3927 ±0.31534
5	Ovary	0.0055 ±0.0034	0.0612 ±0.0149

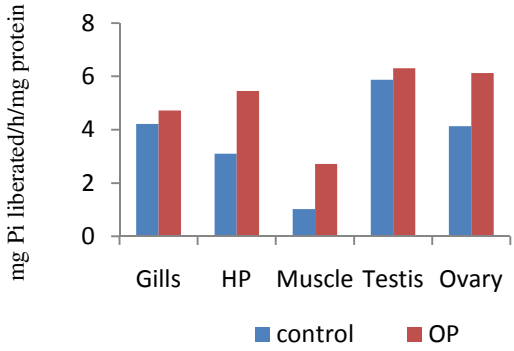
Activity expressed as units / mg Protein.
Values are mean ± S.D. of 5 estimations.
* Significantly different from control.
* P> 0.05 by ANOVA.
** P< 0.05 by ANOVA**.**

Table-5. ATPase activity in the different tissues of freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to Malathion for 30 days

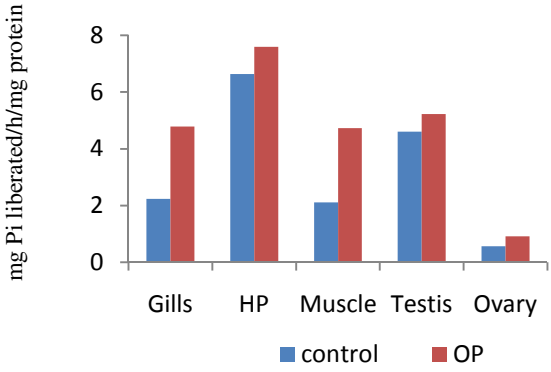
Sr. No.	Tissue	Control	Exposed
1	Gills	0.1602 ±0.115	0.1127 ±0.0083
2	Hepatopancreas	0.1637 ±0.0310	0.1296 ±0.0090
3	Muscles	0.1410 ±0.0070	0.06195** ±0.0094
4	Testis	0.577 ±0.0068	0.371 ±0.0071
5	Ovary	0.0904 ±0.0013	0.0207** ±0.0071

Activity expressed as mg Pi liberated / h / mg Protein. Values are mean ± S.D. of 5 estimations. * Significantly different from control.
* P> 0.05 by ANOVA. ** P< 0.05 by ANOVA.

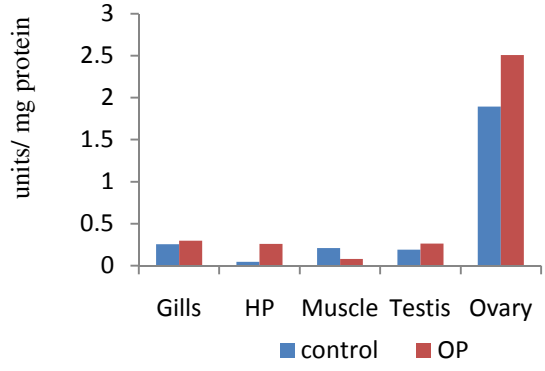
Graph 1
ACP activity in the different tissues of the crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to OP insecticide for 30 days



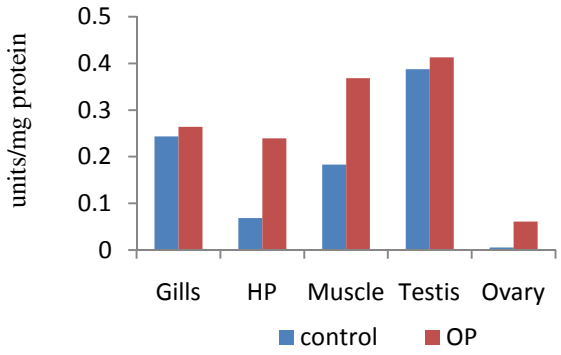
Graph 2
ALP activity in the different tissues of the crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to OP insecticide for 30 days



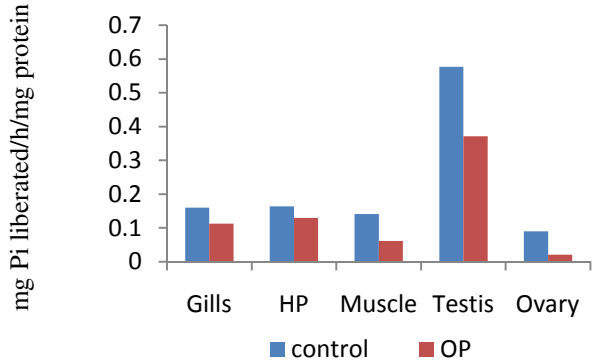
Graph 3
GOT activityin the different tissues of the crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to OP insecticide for 30 days



Graph 4
GPT activity in the different tissues of the crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to OP insecticide for 30 days



Graph 5
ATPase activity in the different tissues of the crab, *Paratelphusa (Barytelphusa) jacquemontii* exposed to OP insecticide for 30 days



This mechanism imparts scope for assessment of risk of xenobiotics in organ system. Benthic animals are frequently exposed to various pollutants out of which agrochemical products are one of them. Aquatic animals come in contact with many pollutants in their habitats. Water soluble crude oil like aromatic hydrocarbon of naphthalene got accumulated in brain tissue of the crab, *Uca pugilator* (Deecarman and Fingerman, 1985). Sub lethal exposure in aquatic organisms can develop tolerance to the toxic effect or physiological adaptation (Sprague, 1970). Qualitative and quantitative estimations of biochemicals and enzymes is important in toxicity assessment which throws light on insight of responses of physiological changes occurring in an organism exposed to it. Hence, toxicity studies of these pollutants are gaining importance now a days. Lipid content in hepatopancreas of freshwater crayfish, *Cherax quadricarinatus* following exposure to glyphosate was found to be declined, Frontera *et al.,* (2011) similarly Fahmy, (2012) has noted decrease in carbohydrate content in the teleost fish, *Oreochromis niloticus* exposed to Malathion.

Acephate, an OP insecticide is tested for its toxicity by Venkateswara *et al.,* (2007) in brine shrimp, *A. salina* found LC_{50} higher than value reported by Osterberg *et al.* (2012) for blue crab megalopae and juveniles. Methamidophos, a metabolite of acephate has showed severe toxicity in shrimp, *Penaeus stylirostris* in a study carried out by Lin *et al.,* (2006). Crustaceans are exposed to insecticides in their proximate surrounding. Studies on toxic potential of such xenobiotics proves important tool for ecotoxicity rating of insecticides. Keeping these points in view present study was carried out to throw light on enzymological alterations in fresh water crab, *Paratelphusa* (*Barytelphusa*) *jacquemontii.*

In the present investigation it is found that, Malathion with concentration of 5.5 ppm is found to be LC_{50} concentration for fresh water crab, *Paratelphusa* (*Barytelphusa*) *jacquemontii.* Significant alterations in the enzyme contents in all the tissues as compared to control were observed. These alterations may be attributed to increased autolysis in tissues due to cytotoxicity. It indicates environmental stress on biological system (Verma *et al*., 1984, Murti and Shukla, 1984). Malathion and other pesticides led to induce severe physiological and biochemical disturbances in experimental animals as goats (Kaur *et al.,* 2000). ACP is a lysosomal enzyme. Several workers have reported that toxicant induced alterations release results in more production and release of ACP. Results of present investigation are in complimentary with this. One of the reasons of increased enzyme activity suggests proliferation of smooth endoplasmic reticulum (sER). Being hydrolytic enzyme taking part in dissolution of dead cells it is stress indicator on biological system. Rise in ALP is also response to hepatotoxicity. Increase in ALP level has been reported to be indicator of damage of the cells of liver, kidney, small intestine, and bone resulting in the liberation of this enzyme in the blood systems in many organisms. Present study concludes elevated level of ACP and ALP in all the tissues of exposed animals.

Transaminases are a group of enzymes that elevate activity of enzyme phosphorylase which plays an important role in glycogenolysis and also are major link between protein and carbohydrate metabolism. These are also key enzymes of nitrogen metabolism and are important in energy mobilization. These are precursors in studying alterations in protein and carbohydrate metabolism. GOT and GPT levels were found to be increased after pesticide exposure. The increase in GOT and GPT activity suggests that proteins are channeled into the metabolic pathway. Chambers *et al.,* (1979) has reported that this may be also related to possible failure of the tissues to initiate the required compensatory reactions under conditions of a prolonged stress.

Adenosine Tri-Phosphatase (ATPase) is a mitochondrial enzyme and it carries out oxidative phosphorylation i.e. it catalyses the hydrolysis of Adenosine triphosphate (ATP) to Adenosine Di-Phosphate (ADP) and Phosphoric acid. This brings about the release of enormous energy. It is also involved in osmoregulation. The bulk of cellular energy in normal cell is derived from ATP. The observed inhibition might be related to the activity of the compound to alter cellular configuration. ATPase is membrane enzyme and inhibition of ATPase in the present study is in accordance with the study carried out by Singh *et al*., (2006) for anti oxidant level assessment followed by Organophosphate toxicity. Inhibited ATPase may also be cause of altered cationic transport leading membrane disturbances.

The foregoing discussion suggests that, the exposure of the freshwater crab, *Paratelphusa (Barytelphusa) jacquemontii* to the sub lethal concentration of Organophosphorus insecticide, Malathion leads to altered enzyme activities in the organs like gills, hepatopancreas, ovaries and muscle.

Conclusion

In the present investigation stress exerted by exposure of crabs to insecticide altered activity of enzyme constituents. This indicates that there is significant influence of toxic nature of this insecticide to crab as an important species of aquatic ecosystem. The foregoing discussion suggests that the exposure of the crab to sub lethal concentrations of 5.5 ppm altered the metabolism of the organisms which has clearly shown by enzyme studies. This study gives scope to biochemical and histological studies for the assessment of effects of insecticide on the organism under study.

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