



## EFFECT OF ENDOSULFAN ON ENZYME ACTIVITY IN SOME AQUATIC ORGANISMS

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### ABSTRACT:

The extensive and indiscriminate use of economic poison (pesticide) has resulted in their entrance into the ecosystem affecting different species in the aquatic environment. Proteins are important biomolecules involved in wide spectrum of cellular metabolic function. The indiscriminate use of organochlorine pesticide causes deleterious effect or even death to aquatic organisms. The physiological and biochemical alternations observed in animals with pesticide stress can correlate with structural and functional changes of cellular proteins. Majority of enzyme catalyzed reactions takes place within the living cells. Enzyme bioassays can provide diagnostic means to assess damage or injury caused to organism due to exposure to harmful substances. Effect of endosulfan on enzymes protease, Glutamate dehydrogenase (GDH), Aspartate aminotransferase (AAT) and Alanine aminotransferase (AIAT) were analyzed in this study. The crab *P. jacquemontii*, fish *C. orientalis*, *B. bengalensis* exposed to endosulfan showed variations in enzymes protease, glutamate dehydrogenase, aspartate amino transferase and alanine amino transferase activity levels at 24, 48, 72 and 96 h treatment period. The freshwater crab *p. jacquemontii*, fish *c. orientalis*, *B. bengalensis* showed variations in activity exposed to lethal and sub lethal concentration of endosulfan at different time intervals. Results of present study at lethal of concentrations of endosulfan showed general increase in enzyme activity (protease, glutamate dehydrogenase, aspartate amino transferase, alanine amino transferase) at 24, 48, 72 and 96 hours, except in fish, in which declined enzyme levels were observed at 24 h treatment period, which suggested synthesis of protein at early exposure period.

**Key words:** - Organochlorine, Enzyme activity, Lethal, Biomolecules

### INTRODUCTION:

Pesticides released into the environment has imbalanced the biology of ecosystem (Holden, 1973). Furthermore pesticides carried through food chains are very harmful to the aquatic animals and a potential danger to man when he consumes. The inhibitory effect was observed in *Lepomis macrochirus*, *Labeo rohita*, *Danio rerio*, and *Jenynsia multidentata* exposed to endosulfan (Ballesteros et al. 2009; Kumar et al. 2012); Entry of these pesticides into water bodies through industrial effluents, run off of direct applications affects many aquatic organisms (Kulkarni and Tankar, 1984 a.

Sherekar, 1986; Kabra, 1988; Malu, 1989). The pest controllers and weed-killers are moderately to highly toxic to aquatic invertebrates such as crab, shellfish and fish species (Narra et al., 2012).

Proteins as most important constituents of the animal tissue and amino acids as the building blocks of proteins have an important role to play in cellular metabolism. As enzymes, proteins participate in the intricately balanced subcellular functions. Since enhanced proteolysis causes major alteration in nitrogen metabolism, it was felt necessary to investigate comparative protease activity in tissues. The

enzyme aspartate aminotransferase catalyzes the interconversion of aspartic acid and keto glutamic acid to oxaloacetic acid and glutamic acid, whereas alanine and aminotransferase catalyzes the interconversion of alanine and ketoglutaric acid to pyruvic acid and glutamic acid. Aminotransferases functions at the junction between carbohydrate and protein metabolism by interconverting the strategic compound ketoglutarate, pyruvate and oxaloacetate. On one side alanine aspartic acid and glutamate on the other. The enzyme glutamate dehydrogenase catalyses the reversible oxidative deamination of glutamate to ketoglutarate and ammonia with pyridine nucleotide as a co-enzyme. This reaction serves as a link between protein metabolism and carbohydrate metabolism. MacKim et al (1970) reported an increase in blood AAT activity in Salvelinus fontinalis when exposed to short term copper toxicity. Many workers have observed inhibition of different enzymes due to exposure to metals (Satapathy et al., 1988; Shaikh and Bhagwat 1988; Katti and Sathyanesan, 1986; Choudhary, 1987). Bakthavathsalam and Reddy (1982), reported effect of Lindane on the enzyme activities of Anabas testudineus several workers have reported ALAT, AAT activities in different animals. Kabeer Ahmed (1981) studied in Tilapia mossambica. Ravinde Reddy (1985) reported ALAT, AAT, GDH activity in freshwater mussels Parresysia rugosa exposed to mercury and copper. Venkateswaralu (1988) observed GDH activity in fish Clarias batrachus exposed to endosulfan.

#### MATERIAL & METHODS:

The test animals crab P. jacquemontii, fish. C. orientalis and the snail B. bengalensis were procured from Fish Market and local pond. Methods used for different enzyme analysis of endosulfan exposed selected aquatic organisms are

| S.No | Enzyme                           | Method                     |
|------|----------------------------------|----------------------------|
| 1    | Protease                         | Moor and Stein, (1957)     |
| 2    | Glutamate dehydrogenase (GDH)    | Loe and Landy, (1965)      |
| 3    | Aspartate aminotransferase (AAT) | Reitman and Frankel (1957) |
| 4    | Alanine aminotransferase (AIAT)  | Reitman and Frankel (1957) |

#### RESULT AND DISCUSSION:

The crab p. jacquemontii, fish c. orientalis, snail B. bengalensis exposed to endosulfan showed variations in enzymes protease, glutamate dehydrogenase, aspartate aminotransferase and alanine aminotransferase activity levels at 24, 48, 72 and 96 h treatment period. Hepatopancrease/Liver being an important organ is selected for protease, GDH, AAT, ALAT estimation. Crab, fish and snails enzyme activity was studied after endosulfan exposure.

##### Protease activity.

P. jacquemontii : Protease activity increased from 1.23, 1.41, 1.51 to 1.61 for lethal treatment and from 1.20, 1.38, 1.49 to 1.58 for sublethal treatment.

C. orientalis : Lower activity of protease was observed at 24 h, (-8.27) which further increased from 39.26 to 56.18 treatment period. and 69.33 percent at 48, 72 and 96 h treatment period. For sublethal treatment values changed from -6.89 to 37.30, 54.08 to 66.72.

B. bengalensis: Protease activity was seen to be increased throughout the experimental period. Values ranged from 1.12 to 1.47 for lethal and 1.08 to 1.46 for sublethal effect of endosulfan.

##### (Refer Table 1.1)

##### Glutamate dehydrogenase

P. jacquemontii : In crab treated to endosulfan glutamate dehydrogenase activity showed trend. Values were in increasing order

up to 96, 2.60, 2.86, 3.17, 3.36 and 2.57, 2.84, 3.13, 3.32 were corresponding values for lethal and sublethal concentrations.

C. orientalis : Glutamate dehydrogenase activity was decreased upto 24 h and thereafter increased upto 96 h. The changed percent activity for lethal was - 80.14, 11.57, 69.65, 33.2 and for sublethal -6.23, 39.22, 55.62, 63.29 at 24, 48, 72 and 96 h exposure period.

B. bengalensis : Enhanced activity was observed after endosulfan treatment, at 24, 48, 72 and 96 h. During lethal treatment values range between 0.71 and 0.90 and between 0.70 and 0.90 at sublethal treatment. **(Refer Table 1.2)**

#### **Aspartate aminotransferase**

P. jacquemontii: The AAT activity was beginning upto increasing from the experiment. At the end of seen the Changes in percent values were 21.69, 37.67, 46.78, 51.29 for lethal and 18.64, 39.61, 40.72, 49.04 for sublethal endosulfan exposure.

C. orientalis : The activity was less upto 24 h, then the activity was increased upto last exposure period i.e. 96 hour. Variations in values were 2.06, 3.13, 3.46, 3.69 for lethal and 2.09, 3.08, 3.36 and 3.62 for sublethal treatment.

B. bengalensis : The percent elevations in AAT activity were from 15.98 to 48.69 for lethal and from 14.92 to 46.63 for sublethal endosulfan treatments. **(Refer Table 1.3)**

#### **Alanine aminotransferase**

P. jacquemontii : At all tested exposure periods the activity was higher than the control. Activity for Lethal concentration ranged from 3.87 to 4.84 and from 3.83 to 4.79 for sublethal concentration.

C. orientalis : In endosulfan exposed fishes. low activity was recorded at 24 h which thereafter was increased up to 96 h. Recorded values – 8.02, 41.27, 55.36, 67.09 and -7.89, 39.22, 53.16, 66.28 were percent changes after lethal and sublethal treatment.

B. bengalensis : Similar results were observed as that of crab. Activity ranged from 3.45 to 4.37 and 3.39 to 4.29 for lethal and sublethal exposure.

#### **(Refer Table 1.4)**

The freshwater crab p. jacquemontii, fish c. orientalis, snail B. bengalensis showed variations in activity exposed to lethal and sublethal concentration of endosulfan at different time intervals. Results of present study at lethal and sublethal concentrations of endosulfan showed general increase in enzyme activity (protease, glutamate dehydrogenase, aspartate amino transferase, alanine amino transferase) at 24, 48, 72 and 96 hours, except in fish, in which declined enzyme levels were observed at 24 h treatment period.

In crab P. jacquemontii all enzyme levels were be increased after lethal and sublethal concentrations of endosulfan. The protease enzyme functions in the breakdown of proteins leading to the formation of free amino acids at neutral pH. Glutamate dehydrogenase catalyzed oxidative deamination of glutamate to ammonia and ketoglutarate. It not only channels the nitrogen from glutamate to ammonia but also catalyzes the animation of keto glutamate to free ammonia. Sumati *et al* (1989) observed the increased GDH, ALAT and AAT activities in crab Oziotelphusa senex senex exposed to endosulfan. Present study results are in agreement with the above study: This increased GDH activity attributed to increased oxidation of glutamate, since GDH catalyzes the key reactions which provide substrates for protein synthesis. Increased AAT, ALAT content might suggest the increased oxidation of glutamate for sustained generation of ketoglutarate. Amino transferase activity acts as the intermediary point between carbohydrate and proteins by interconverting strategic crossover in metabolites like ketoglutarate, pyruvate and oxaloacetate, and

other side alanine aspartate and glutamate. The increased activity levels of AAT and ALAT may be due to tissue damage or disruption of mitochondrial integrity or may be the result of increased synthesis of enzyme. Similar reports were observed in crab *O. senex senex* exposed to endosulfan (Sumati *et al.*, 1989). Increased AAT and ALAT activities were noticed with pesticide stress (Swani *et al.* 1983; Srinivasulu Reddy *et al.*, 1985) on mussel and prawn.

In fish *C. orientalis* increase in the neutral protease activity was progressive with the progression directly proportional to the exposure period. The rise in activity was more at lethal as compared to sublethal concentration. The protease were known to breakdown proteins to small peptides and ultimately to amino acid. Protease activity and free amino acid levels were found to be maximum in liver tissue, least in gills and intermediary in muscle. It clearly indicated that tissue, pesticide favoured protein breakdown by inducing protease activity which lead more free amino acid synthesis or production. Thus pesticide creates metabolic stress as proteins have dynamic role to play the tissue metabolism. Results in present study are in consistent with Choudhary (1987) in fish *R. daniconius* exposed to BHC and malathion. Similar increase in protease activity was recorded by Kabeer *et al.* Dikshit *et al.* (1975). Decrease in protease activity at early hour exposure may be due to synthesis of protein. In fish *C. orientalis* aspartate amino transferase and alanine aminotransferase levels were more pronounced in liver. Increased AAT and ALAT activity may be due to more conversion of amino acid into ketoacids to be utilized for energy synthesis. Aminotransferase showed that the tissue seen to favour more pyruvate formation over oxaloacetate and this might be associated with the metabolic needs of respective tissue. Liver showed remarkable elevation in ALAT activity as compared to AAT activity. Since liver was the

seat of metabolism and contain fuel complement of all enzymes concerned with carbohydrate metabolism; pyruvate formed through ALAT activity may be converted to acetyl coenzyme to mobilized by the TCA cycle. Increased activity in liver may be, to compensate the low activity level in other tested tissue.

Increased protease activity in snail *B. bengalensis* suggested possible degradation of proteins (Sivaprasad Rao *et al.*, 1980; Rajeshwara Rao *et al.*, 1983). Alterations in the fatty acid profile, antioxidant enzymes and protein pattern of *Biomphalaria alexandrina* snails exposed to the pesticides diazinon and profenofos. (Bakry *et al.* 2013) GDH activity which is regulatory link between energy and nitrogen metabolism was and increased significantly. This results in increased formation of keto glutamate and its utilization in transamination. Enhanced AAT and ALAT activities might be due to imposition of stress on the animal during pesticide exposure. It has been suggested that the stress condition in general induced an elevation in the activities of transaminase (Knox *et al.*, 1965; Kulkarni *et al.*, 1973). It is likely that the toxic stress could be responsible for their elevation. Increase may be due to more feeding of amino acids into TCA cycle. It might be a compensatory phenomenon for the decreased mitochondrial oxidation. Resembling results with that of current study were observed in freshwater mussel *Lamellidens marginalis* exposed to dichlorvos (Srinivas Moorthy *et al.*, 1986). Similar reports were recorded by Reddy *et al.* (1985) in fresh water mussel exposed to mercury and copper. Kabeer *et al.* (1977), reported enhanced activity of above enzymes in snail *Pila globosa* exposed to malathion.

#### CONCLUSION

Increase enzyme levels might be indicated the more conversion of amino acid into ketoacid to be utilized for energy synthesis. Increase

activity clearly indicates that the amino acids were spread initially and when the sustained demands diminished the reverse glycogen store and amino acids were drawn into energy metabolism.

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**Table 1.1** Changes in hepatopancreas protease activity of some aquatic organisms exposed to endosulfan lethal and sublethal concentrations at different exposure period. (u mol of tyrosine/gm equivalent/mg protein/h)

| Concentration       | Lethal (mg/l)                |                               |                              | sublethal(mg/l)               |                              |                               |
|---------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|------------------------------|-------------------------------|
|                     | 0.121                        | 0.13                          | 0.048                        | 0.04                          | 0.043                        | 0.162                         |
| Exposure period (h) | p.jacquemontii               | c. orientalis                 | B. bengalensis f. typica     | p.jacquemontii                | c. orientalis                | B. bengalensis f. typica      |
| Control             | 1.03<br>+ -0.68              | 1.99<br>+ -0.88               | 0.97<br>+ -0.32              | 1.03<br>+ -0.49               | 1.19<br>+ -0.37              | 0.97<br>+ -0.22               |
| 24                  | 1.23*<br>+ -0.33<br>(18.29)  | 1.09***<br>+ -0.49<br>(-8.27) | 1.12**<br>+ -0.13<br>(15.28) | 1.20***<br>+ -0.33<br>(16.11) | 1.10**<br>+ -0.23<br>(-6.89) | 1.08*<br>+ -0.92<br>(13.27)   |
| 48                  | 1.41*<br>+ -0.17<br>(35.71)  | 1.66**<br>+ -0.33<br>(39.26)  | 1.29**<br>+ -0.22<br>(32.79) | 1.38**<br>+ -0.17<br>(33.28)  | 1.64**<br>+ -0.63<br>(37.30) | 1.26**<br>+ -0.86<br>(29.62)  |
| 72                  | 1.51<br>+ -0.01<br>(46.39)   | 1.86*<br>+ -0.17<br>(56.18)   | 1.40**<br>+ -0.30<br>(44.69) | 1.49**<br>+ -0.01<br>(44.86)  | 1.84**<br>+ -0.86<br>(54.08) | 1.37**<br>+ - 0.52<br>(41.59) |
| 96                  | 1.61ns<br>+ -0.28<br>(56.39) | 2.01**<br>+ -0.01<br>(69.33)  | 1.47**<br>+ -0.46<br>(51.27) | 1.58<br>+ -0.27<br>(53.61)    | 1.99**<br>+ -0.56<br>(66.72) | 1.46**<br>+ -0.68<br>(49.21)  |

\*P=0.01, \*\*P=0.001, \*\*\*P=0.05, NS = Not Significant

**Table 1.2** Changes in hepatopancreas Glutamate dehydrogenase activity of some aquatic organisms exposed to endosulfan lethal and sublethal concentrations at different exposure period. (u mol of tyrosine/gm equivalent/mg protein/h)

| Concentration       | Lethal (mg/l)                 |                               |                                 | sublethal(mg/l)               |                               |                                 |
|---------------------|-------------------------------|-------------------------------|---------------------------------|-------------------------------|-------------------------------|---------------------------------|
|                     | 0.121                         | 0.13                          | 0.048                           | 0.04                          | 0.043                         | 0.162                           |
| Exposure period (h) | <i>p.jacquemontii</i>         | <i>c. orientalis</i>          | <i>B. bengalensis f. typica</i> | <i>p.jacquemontii</i>         | <i>c. orientalis</i>          | <i>B. bengalensis f. typica</i> |
| Control             | 2.18<br>+ -0.19               | 2.23<br>+ -0.64               | 0.61<br>+ -0.26                 | 2.18<br>+ -0.17               | 2.23<br>+ -0.49               | 0.61<br>+ -0.56                 |
| 24                  | 2.60*<br>+ -0.33<br>(19.27)   | 2.05***<br>+ -0.49<br>(-8.01) | 0.71**<br>+ -0.27<br>(17.68)    | 2.57 ns<br>+ -0.91<br>(18.03) | 2.09***<br>+ -0.33<br>(-6.23) | 0.70**<br>+ -0.38<br>(15.59)    |
| 48                  | 2.86*<br>+ -0.17<br>(31.57)   | 3.14***<br>+ -0.33<br>(41.11) | 0.78**<br>+ -0.22<br>(28.20)    | 2.84*<br>+ -0.49<br>(30.12)   | 3.1*<br>+ - 0.17<br>(39.22)   | 0.76**<br>+ -0.22<br>(26.18)    |
| 72                  | 3.17 ns<br>+ -0.88<br>(45.62) | 3.51*<br>+ -0.17<br>(57.69)   | 0.86**<br>+ - 0.32<br>(42.60)   | 3.13***<br>+ -0.33<br>(43.51) | 3.47**<br>+ -0.01<br>(55.62)  | 0.85**<br>+ -0.92<br>(40.10)    |
| 96                  | 3.36**<br>+ -0.17<br>(54.29)  | 3.68ns<br>+ -0.01<br>(65.32)  | 0.92**<br>+ -0.25<br>(51.62)    | 3.32*<br>+ - 0.17<br>(52.37)  | 3.64<br>+ -0.28<br>(63.29)    | 0.90**<br>+ -0.86<br>(48.37)    |

\*P=0.01, \*\*P=0.001, \*\*\*P=0.05, NS = Not Significant



**Table 1.3** Changes in Liver/Hp Aspartate aminotransferase activity of some aquatic organisms exposed to endosulfan lethal and sublethal concentrations at different exposure period. (u mol of tyrosine/gm equivalent/mg protein/h)

| Concentration       | Lethal (mg/l)                 |                               |                                 | sublethal(mg/l)               |                              |                                 |
|---------------------|-------------------------------|-------------------------------|---------------------------------|-------------------------------|------------------------------|---------------------------------|
|                     | 0.121                         | 0.13                          | 0.048                           | 0.04                          | 0.043                        | 0.162                           |
| Exposure period (h) | <i>p.jacquemontii</i>         | <i>c. orientalis</i>          | <i>B. bengalensis f. typica</i> | <i>p.jacquemontii</i>         | <i>c. orientalis</i>         | <i>B. bengalensis f. typica</i> |
| Control             | 2.04<br>+ -0.99               | 2.24<br>+ -0.88               | 1.87<br>+ -0.26                 | 2.04<br>+ -0.49               | 2.24<br>+ -0.56              | 1.87<br>+ -0.32                 |
| 24                  | 2.48<br>+ -0.88<br>(21.69)    | 2.06***<br>+ -0.64<br>(-7.69) | 2.17**<br>+ -0.27<br>(15.98)    | 2.42***<br>+ -0.33<br>(18.64) | 2.09**<br>+ -0.38<br>(-6.87) | 2.15**<br>+ -0.13<br>(14.92)    |
| 48                  | 2.81***<br>+ -0.64<br>(37.67) | 3.13***<br>+ -0.49<br>(39.69) | 2.42**<br>+ -0.22<br>(30.65)    | 2.75*<br>+ -0.17<br>(34.61)   | 3.08**<br>+ -0.22<br>(37.16) | 2.41**<br>+ -0.22<br>(28.62)    |
| 72                  | 2.97***<br>+ -0.49<br>(46.78) | 3.46*<br>+ -0.33<br>(54.29)   | 2.63**<br>+ -0.32<br>(41.62)    | 2.89**<br>+ -0.01<br>(43.72)  | 3.36**<br>+ -0.92<br>(50.21) | 2.59**<br>+ -0.30<br>(39.61)    |
| 96                  | 3.08***<br>+ -0.33<br>(51.09) | 3.69**<br>+ -0.17<br>(64.83)  | 2.78**<br>+ -0.25<br>(48.69)    | 3.04ns<br>+ -0.68<br>(49.04)  | 3.62**<br>+ -0.86<br>(61.80) | 2.74**<br>+ -0.46<br>(46.63)    |

\*P=0.01, \*\*P=0.001, \*\*\*P=0.05, NS = Not Significant



**Table 1.4** Changes in hepatopancreas Alanine aminotransferase activity of some aquatic organisms exposed to endosulfan lethal and sublethal concentrations at different exposure period. (u mol of tyrosine/gm equivalent/mg protein/h)

|                     | Lethal (mg/l)                |                               |                               | sublethal(mg/l)              |                               |                                |
|---------------------|------------------------------|-------------------------------|-------------------------------|------------------------------|-------------------------------|--------------------------------|
| Concentration       | 0.121                        | 0.13                          | 0.048                         | 0.04                         | 0.043                         | 0.162                          |
| Exposure period (h) | p.jacquemontii               | c. orientalis                 | B. bengalensis f. typica      | p.jacquemontii               | c. orientalis                 | B. bengalensis f. typica       |
| Control             | 3.15<br>+ -2.62              | 3.47<br>+ -0.99               | 3.02<br>+ -0.88               | 3.15<br>+ -0.56              | 3.47<br>+ -0.49               | 3.02<br>+ -0.64                |
| 24                  | 3.87**<br>+ -2.71<br>(23.37) | 8.19*<br>+ -0.49<br>(-8.02)   | 3.45***<br>+ -0.64<br>(14.37) | 3.83**<br>+ -0.38<br>(21.52) | 3.21***<br>+ -0.33<br>(-7.89) | 3.39***<br>+ -0.49<br>(12.09)  |
| 48                  | 4.40**<br>+ -2.23<br>(39.62) | 4.90***<br>+ -3.01<br>(41.27) | 3.92***<br>+ -0.49<br>(29.91) | 4.33**<br>+ -0.22<br>(37.53) | 4.83ns<br>+ -0.42<br>(39.22)  | 3.84***<br>+ - 0.33<br>(27.21) |
| 72                  | 4.56**<br>+ -3.20<br>(46.29) | 5.39**<br>+ -3.91<br>(55.36)  | 4.18***<br>+ -0.33<br>(38.61) | 4.47**<br>+ -0.92<br>(44.27) | 5.31**<br>+ -0.33<br>(53.16)  | 4.13ns<br>+ -0.56<br>(36.82)   |
| 96                  | 4.84**<br>+ -2.51<br>(53.66) | 5.80**<br>+ -3.80<br>(67.09)  | 4.37*<br>+ -0.17<br>(44.69)   | 4.79**<br>+ -0.52<br>(52.10) | 5.77*<br>+ -0.17<br>(66.28)   | 4.29**<br>+ -0.38<br>(42.11)   |

. \*P=0.01, \*\*P=0.001, \*\*\*P=0.05, NS = Not Significant