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EFFECT OF ENDOSULFAN ON FREE AMINO ACIDS OF CRAB,FISH AND SNAIL

Zoology

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ABSTRACT
Aquatic animals are often exposed to insecticides.Knowledge about the mode of action of toxicants is better understood from biochemical investigations . These effects can be studied by lethal and sublethal effects. Aminoacid and nitrogen metabolism are of the utmost importance in animals, since amino acids are essential intermediates in protein synthesis Furthermore amino acids are known to play an important part in several phenomenon occuring in the lifetime of animals during osmotic stress. The variations in the amino acids after exposure to endosulfan in crab P. Jacquemontii, Fish C. orientalis and snail B. bengalensis at 24, 48, 72 and 96 h were studied in present investigation. Free amino acid content o showed general increasing trend in all tissues at 24, 48, 78 and 96 h after endosulfan exposure. Except fish, in which the free amino acid level was slightly decreased at 24 h of treatment period.

KEYWORDS
insecticide,sub lethal,toxicants,osmotic pressure

Introduction

Aquatic animals are often exposed to insecticides as a result of treated areas as well as from direct application to the water. Knowledge about the mode of action of toxicants and causes of death in poisoned aquatic animals is better understood from biochemical investigations besides mortality studies. These effects can be studied by lethal and sublethal effects which can be shown by apparent external signs toxicity and by internal disturbances.

Environmental pollutants brings about the damage to different organs or disturb the physiological and biochemical processes with the organism following exposure to pollutant. Effect of different pesticides, heavy metals and other chemicals on biochemical constituent in aquatic animals is the current topic of interest because of threatening of the natural resources from industrial effluents (Everall, 1989; Giudici, 1981; Danota Szerow and Anna Milian, 1987).[1][2][3]

Endosulfan remains in the environment for longer periods and bio-accumulates in plants and animals which leads to contamination of food consumed by humans [4]. It affects mainly the central nervous system and was found to have higher acute inhalation toxicity than dermal toxicity. Gastrointestinal absorption of endosulfan is very high [5].Many workers attempted to evaluate these hazards on biota (Maria and Quasin, 1983; Tankar, 1985; Sherekar, 1986; Kabra, 1988; Malu, 1989;[6][7][8][9][10]

Biochemical investigations constitute an excellent evidence for the accumulation of pesticides in the tissues of animals which showed a wise degree of fluctuations in their organic components. Exposure to chemical pollutants elicit many molecular and biochemical changes in fish which precide cellular and systematic disfunction so that it appropriate parameters are early inconsistent signs of distress may be detected. To encounter stress, metabolic cycles involved in the interchange of organic constituents, that are responsible for production of energy, production. undergo change for energy production.

The organochlorine and organophosphate pesticide used in the present study occur in persistant nonpersistant state in the aquatic medium. Ramaswmy (1987) investigated effect of sevin on free amino acid in fish Sarotherodon mossambicus.[11] Gautam (1988), Bhale et al., (1988) also studied the effect of pesticide on physiological and biochemical aspects of fish and crab. [12][13] Patil et al., (1991) discussed some physiological and biochemical aspects of Paratelphusa jacquemontii exposed to organochlorine pesticides (BHC and DDT)[14].Since the stress conditions caused alterations in the metabolic cycles such as ionic composition, enzyme system etc. It is necessary to understand the significance of these variations in the organic compounds of the tissues. Under such conditions,these variations in turn can have an impact on food web relation and energy cycles and therefore is an important aspect for study. Hence present study was undertaken to account for the changes involved in protein nitrogen metabolism in the tissues like liver, muscle, gill, foot of crab, Paratelphusa jacquemontii; fish, Channa orientalis and snail, Bellamya bengalensis f. typica exposed to organochlorine pesticide Endosulfan .

Since the physiological characteristics and energy functions of the fish are known to alter during pesticide treatment (Holden, 1973). [15]These changes would certainly have an impact on the free amino acid level. In such altered physiological conditions, the free amino acid certainly play a vital role in maintaining the intracellular osmotic balance and also act as precursors for the synthesis of enzymes, hormones and other constituents of the metabolic machinery (Lenhinger , 1978).[16]

Anino acid and nitrogen metabolism are of the utmost importance in animals, since amino acids are essential intermediates in protein synthesis and the catabolise products of these prime constituents appear in the form of different nitrogen substances. Furthermore amino acids and certain other nitrogenous compounds are known to play an important part in several phenomenon occurring in the lifetime of animals and more specifically, during osmotic stress.

A large proportion of the dry matter of molluscan bodies is made up of amino acids, either free or combined to form macro molecules, among which proteins are constant. The pattern of the amino acid composition of the bulk of the proteins of molluscan tissues is similar to the pattern generally observed in animal tissues.

Increase or decrease in free amino acid content may be considered as the operation of the stress phenomenon at the tissue level. Very little information is available concerning the physiological or biochemical effects of pesticides on nucleic acid levels and protein content.

Amiared et al (1986), observed changes in amino acid content in haemolymph of crab after exposure to cobalt chlonde.[17] Krishaja et al (1987) mentioned the effects of heavy metals (mercury cadmium, lead, arsenic and selenium) on amino acid content of industrial crab Scylla serrata.[18] Hushanam (1987), studied alteration in free amino acids in freshwater prawn Macrobrachium kistnensis exposed to napthalene.[19] Srinivasulu Reddy et al (1989), reported alteration in amino acid content of peaneld praun Metapenaeus sonmceros exposed to phosphamidon.[20] Regulation of free amino acid in the tissues of teleost Tilapia mossambica exposed to sublethal concentration of malathion was reported by Kabeer Ahmand Sahib (1979)[21]. Seshagiri et al (1987) reported changes in amino acid level due to toxicity of benthiocarb in fresh water teleost Sarotherodon mossambicus [22].

Kabeer Ahmad et al(1978) reported changes in amino acid levels in the snail Pila globosa (Swainson) exposed to malathion [23]. Decrease in free amino acid level in body fluid of the snail Pila globosa was reported by Satyaprasad (1982)[24] when exposed to methyl parathion. Rajeshwara Rao (1983) studied elevation in amino acids in snail Pila globosa exposed to phenothoate [25]. Decrease in levels of free amino acid in fresh water mussel Lamellides marginalis under dichlorovas exposure was reported by Srinivas Moorthy (1983)[26]

Graney et al, (1986) studied effect of long ters exposure to pentachlorophenol on the free amino acid pool and energy reserves of the freshwater amphipod, Gasarus pseudol Linnaeus [27].

In present work, changes in free amino acid contents were studied in freshwater crab, P. jacquemontii, fish C. orientalis and snail, B. bengalensis after endosulfan exposure.

MATERIALAND METHOD

The test crab, P. Jacquemontii, the fish, C. orientalis and the snail B. bengalensis were procured from around Amravati fish market and local ponds. Maintenance, acclimation, selection of specimens size, weight, cleaning and treatment of pesticide was as per norms. Pesticide concentration was prepared as per procedure for experimentation .

Estimation of free amino acids (Danilson and Harold, 1958) Homogenated tissue in 10 ml folin fungstic acid. filter and take 5 ml of this filtrate, add a drop of phenolphthaline, add 0.1 M NaOH drop by drop till permanent pink colour was produced. Similarly for blank, add 5 al o water and treat as above (For blank test tube). 1 al o Borax solution and 1 ml freshly prepared Napthoquinon solution (0.5 %) was added. Keep in boiling water for 1 minute. After cooling, dilute it to 13 ml. To that add ml of farmaldehyde reagent and 1 ml of 0.05 N thiosulphat Make up to 15 ml by water. Read at 470 to 480 u

RESULTAND OBSERVATION

Animals were treated with organochlorine pesticide-endosulfan.The crab P. Jacquemontii, fish C. orientalis and snall B. bengalensis exposed to endosulfan showed variations in free amino acid content at 24, 48, 72 and 96 h treatment period in the tissues Hepatopancreas, Muscle and Gills. Hepatopancrease (Table 1.1) P. jacquemontii: Free amino acids were found be elevated at all treatment periods. Regarding values lethal and sublethal concentrations were 19.78, 23. 25.90, 27.27 and 19.24, 22.69, 24.46, 26.98 after endosulfan exposure.

C. orientalis: At 24 h exposure period free amino acid contents were observed less, which later on showed an ascending trend up to the end of experiment. Percent changes for lethal and sublethal concentrations were -6.28, 46.81, 63.28. 75.79 an -5.31, 44.29, 61.11, 72.86 after endosulfan effect.

B. bengalensis: Enhancement in free amino acid was recorded at all exposure periods. Values for lethal concentration Increased from 13.50 to 17.68 and from 13.22 to 17.40 for sublethal concentration.

Table 1.1 a & 1.1 b Changes in hepatopancreas /liver free amino acid content of some aquatic organisms exposed to endosulfan lethal and sublethal concentrations at different exposure period. (mg /gm wet wt.tissue)

	Lethal (mg/l) (1.1 a)		
Concentration	0.121	0.13	0.048
Exposure period (h)	P.jacquemontii	C. ori-entalis	B. bengalensis f. typica
Control	16.13	30.51	0.97
	± 4.91	± 9.19	± 3.01
24	19.78***	28.59*	13.50**
	± 3.33	± 4.91	± 3.91
	-22.68	(-6.28)	-17.29
48	23.16*	44.81*	15.38**
	± 1.73	± 4.24	± 3.80
	-43.67	-46.89	-33.7
72	25.90NS	49.81**	16.72**
	± 2.01	± 3.30	± 2.71
	-60.62	-63.28	-45.29
96	27.27NS	53.63*	17.68**
	± 1.67	± 1.73	± 2.82
	69-09)	-75.79	-53.68

Parenthesis fig.( ) percent change *P=0.01, **P=0.001, ***P=0.05, NS = Not Significant

	sublethal(mg/l) (1.1. b)		
Concentration	0.04	0.043	0.162
Exposure period (h)	P.jacquemontii	C. ori-entalis	B. bengalensis f. typica
Control	9.81	16.28	7.89
	± 1.73	± 2.01	± 3.33
24	11.19NS	15.78***	8.68*
	± 4.91	± 1.81	± 1.73
	-14.09	(-5.31)	-10.11
48	13.00***	22.77NS	9.82**
	± 3.33	± 6.49	± 0.01
	-32.52	-39.87	-24.57
72	13.71*	25.46**	10.48NS
	± 1.73	± 4.91	± 2.30
	-39.79	-56.39	-32.89
96	14.61**	26.74***	11.401*
	± 0.01	±3.33	± 0.81
	-49.02	-64.27	-39.6

Parenthesis fig.( ) percent change *P=0.01, **P=0.001, ***P=0.05, NS = Not Significant Muscle (Table 1.2)

P. jacquemontii: Ever increasing trend was seen at 24, 48, 72 and 96 h treatment period. Amount of percent change was from 20.52 to 41.67, 58.19, 67.09 for lethal and from 17.39 to 38.60, 55.89, 64.76 for sublethal concentration of endosulfan.

C. orientalis: Free aminoacid level was found to be less at 24 h, which later on showed remarkable increase up to 96 doir exposure period. For lethal concentratie freeamino acid values were 19.87, 31.78, 35.05, 37.75 and 20.14, 31.03, 34.63, 37.01 for sublethal concentration after endosulfan exposure. In muscle slightly higher values were seen as compared to hepatopancrease.

B. bengalensis: Endosulfan influenced animals reflected increase in freeamino acid level through out the experimental course. The corresponding values for lethil and sublethal concentrations were 15.10, 31.69, 43.60, $1.63 and 13.29, 28.43, 40.09, 49.27.

Table 1.2 a & 1.1 b Changes in muscle free amino acid content of some aquatic organisms exposed to endosulfan lethal and sublethal concentrations at different exposure period. (mg /gm wet wt.tissue)

	Lethal (mg/l) (1.2 a)		
Concentration	0.121	0.13	0.048
Exposure period (h)	P.jacquemontii	C. ori-entalis	B. bengalensis f. typica
Control	10.56	21.23	8.21
	± 4.91	± 2.92	± 3.33
24	12.72***	19.87**	9.44*
	± 3.33	± 3.80	± 1.73
	-20.52	(-6.37)	-15.1
48	19.40*	31.78**	10.81*
	± 1.73	± 1.73	± 1.40
	-41.67	-47.72	-31.69
72	14.96**	35.05**	11.78*
	± 0.01	± 0.01	± 1.04
	-58.19	-65.13	-43.6
96	17.64NS	37.75NS	12.45**
	± 5.37	± 5.20	± 0.01
	(67-09)	-77.83	-51.65

Paranthesis fig.( ) percent change *P=0.01, **P=0.001, ***P=0.05, NS = Not Significant

	sublethal(mg/l) (1.2 b)		
Concentration	0.04	0.043	0.162
Exposure period (h)	P.jacquemontii	C. ori-entalis	B. bengalensis f. typica
Control	10.56	21.23	8.21
	± 4.94	± 2.62	± 3.24
24	12.39***	20.14**	9.30**
	± 3.33	± 2.71	± 1.37
	-17.39	(-5.11)	-13.29

48	14.63*	31.05**	10.54**
	± 1.73	± 2.23	± 2.28
	-38.6	-46.29	-28.43
72	16.46**	34.63**	11.50**
	± 0.01	± 3.20	± 3.04
	-55.89	-31.13	-40.09
96	17.39NS	37.01**	12.25**
	± 5.28	± 2.51	± 4.62
	-64.76	-74.37	-49.27

Parenthesis fig.( ) percent change *P=0.01, **P=0.001, ***P=0.05, NS = Not Significant

Gills (Table 1.3)

P. jacquemontii: Ever increasing trend was obtained in endosulfan exposed crab. Exposure in lethal concentration showed increase from 11.43 to 14.83 and from 11.19 to 14.61 for sublethal concentration at 24, 48, 72 and 96 h treatment period.

C. orientalis Enhanced free amino acid level was marked at 24 h which further went on decreasing up to 96 h treatment, Variations in percent values were -4.12, 43-29, 57.37, 69.37 and -3.07, 39.87, 56.39), 64.27 for lethal and sublethal concentrations respectively.

B. bengalensis: Free amino acid was seen to be Increased from 24 to 96 h exposure period. Increased for lethal concentration, was from 8.88 to 11.21 and from 8.68 to 11 al for sublethal concentration after endosulfan effect

Table 1.3 a& b Changes in gill free amino acid content of some aquatic organisms exposed to endosulfan lethal and sublethal concentrations at different exposure period. (mg /gm wet wt.tissue) Paranthesis fig.( ) percent change *P=0.01, **P=0.001, ***P=0.05, NS = Not Significant

	sublethal(mg/l) (1.3 b)		
Concentration	0.04	0.043	0.162
Exposure period (h)	P.jacquemontii	C. ori-entalis	B. bengalensis f. typica
Control	9.81	16.28	7.89
	± 1.73	± 2.01	± 3.33
24	11.19NS	15.78***	8.68*
	± 4.91	± 1.81	± 1.73
	-14.09	(-5.31)	-10.11
48	13.00***	22.77NS	9.82**
	± 3.33	± 6.49	± 0.01
	-32.52	-39.87	-24.57
72	13.71*	25.46**	10.48NS
	± 1.73	± 4.91	± 2.30
	-39.79	-56.39	-32.89
96	14.61**	26.74***	11.401*
	± 0.01	±3.33	± 0.81
	-49.02	-64.27	-39.6

Parenthesis fig.( ) percent change *P=0.01, **P=0.001, ***P=0.05, NS = Not Significant

	Lethal (mg/l) (1.3 a)		
Concentration	0.121	0.13	0.048
Exposure period (h)	P.jacquemontii	C. ori-entalis	B. bengalensis f. typica
Control	9.81	16.28	7.89
	± 2.62	± 4.91	± 0.56
24	11.43***	15.60**	8.88**
	± 2.71	± 3.33	± 0.38
	-16.59	(-4.12)	-12.58
48	13.21**	23.32*	9.97**
	± 2.23	± 1.73	± 0.22
	-34.67	-43.29	-26.37
72	13.99**	25.61**	10.70**
	± 3.20	± 0.01	± 0.92
	-42.69	-57.37	-35.62
96	14.83**	27.57NS	11.21**
	± 2.51	± 5.23	± 0.86
	69-09)	-69.37	-42.19

Discussion

Amino acid is utilized for protein synthesis and to which the products of protein degradation are returned. The variations in the amino acids after exposure to endosulfan in crab P. Jacquemontii, Fish C. orientalis and snail B. bengalensis at 24, 48, 72 and 96 h were studied in present investigation. values of freeamino acid and the comparative analysis of the acid recorded following facts.

Free amino acid content of above selected species showed general increasing trend in all tissues at 24, 48, 78 and 96 h after endosulfan exposure. Except fish, in which the free amino acid level was slightly decreased at 24 h of treatment period.

In crab P. jacquemontii increase in free amino acid may be due to degradation of protein synthesis or/and may be due to diminished utilization of amino acids In the tissue or their enhanced synthesis From other sources like glucose and fatty acids. Such Increase was also noticed in different animals like crab (Bhale et al., 1988)[28]and in army worm (Lomte and Patil, 1987 [29]).

These free amino acids might be fed in to TCA cycle as keto acids by way of transsination. Since in present study transaminase activity is known to elevate during pesticide exposure (Chapter IV). The Increased level of amino acid might also be due to increase synthetic potentiality. The Increased free amino acid may be partly utilized for the protein synthesis and partly for glyconeogenesis, through the transamination and transdeamination reactions to supply the necessary keto acids to act as a precursors for the maintenance of carbohydrate metabolism, to meet the energy demand during stress condition (Natarajan 1983)[30]Decreased free amino acid levels in fisl C. orientalis at early exposure period may be due to protein synthesis. This result was in agreement with Choudhary (1987)[31]. The free amino acid increased with increasing exposure time. The increased after 24 hour, may be due to enhanced proteolysis or synthesis of free amino acid, when there was deficit of glycogen content, these amino acids might be source of energy. The level of free amino acid say indicate the condition of the tissue. Increase or decrease in free amino acid content may be considered as the operation of the stress phenomenon at the tissue level. Mehrle et al. (1974)[32], Shkoori et al, (1976) [33]observed similar results in fishes exposed to toxicants.

The increased free amino acid pool suggested the increased protein brakedown, the related protein degradation and thus free amino acid pool was found to be enhanced, during pesticide exposure. The Increased free amino acid content might be due to the higher synthesis of free amino acid to full till the demand. However the increased synthesis of free amino acid can not be attributed to all types of amino acids, but to those which can be synthesized in fish tissues. Thus, amino acid pool might be contributed through two ways- viz. by the degration of proteins and by the enhanced synthesis of amino acids in the tissues and this might have resulted in consistent rise in free amino acid pool.

The increased free amino acid pool besides contributing to proteinsynthesis and energetic requirements may also be.useful to the fish in the maintenance of the osmotic and acid base balance as reported for other animals during altered physiological states of the fish consequence to pesticide exposure. Analogus results were observed in teleost Sartherodon mossambicus exposed to benthicarb by Seshagiri Rao (1987)[20], Kabeer et al. (1981)[34] and Malla Reddy (1987)[35] observed in free amino acid level in fish Tilapia mossambicus to sublethal malathion exposure. Higher free amino acid content may also be attributed to decreased utilization of amino acids in glyconeogenesis as consequence of pesticiee exposure (Sreenivasulu Moorthy, 1983[24]; Sherekar, 1986[6]).

Increase in free amino acid content of snail B. bengalensis might be because of different turn over between amino acid synthesis and amino acid degradation to TCA cycle, as the proteolytic activity increased (as -Chapter IV).

Proteins are degraded in to amino acids showed their increased level of free amino acid (Chapter 111). This increased free amino acid level later on was comparatively less, because these amino acids might have been used for energy purposes through TCA cycle, which wasclear from the ammonia formation as This might be because of the short span of experiments selected rather than longer duration as the gastoapod animals respond to the stress of pesticide slowly by projecting foot or opening operculum.

Conclusion

But in case of crab P. jacquemonti and snail B. bengalensis, pesticide can not be easily entered directly as small have effective dosing of operculum and crab can reduce the pestiedal entry by behavioral changes and so have less effect than fish C. orientalis.

Amino acids are representative of proteolysis. Amino acids are utilized for protein synthesis, to which the products of protein degradation are returned.There was increased free amino acid content upto end of the experimental period in the hepatopancreas /liver, muscle and gill in crab, fish and snail after endosulfan and cythion exposure. Only in fish free amino acid decrease was observed at 24 h of treatment period.

Increased proteolysis leads to increased free amino acid levels. Increased free amino acid may be partly utilized for glyconeogensis the protein synthesis and partly for through the transamination and transdeamination reactions to supply the necessary keto acids to act as precursors for the maintenance of carbohydrate metabolism to meet energy demand during stress conditions.Increased proteolysis may be due to breakdown of proteins leading to the formation of free amino acids.

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