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Activity of Gut Enzymes in Three Tropical Grassland Earthworm Species Exposed to Sub-Lethal Malathion Suspension

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Earthworms account for 80% of the soil invertebrate biomass in agroecosystems of tropical and sub-tropical India (Senapati and Dash, 1981). They contribute a lot to the organic matter degradation and soil fertility (Senapati and Dash, 1984; Lee 1985). Numerous studies demonstrated the occurrence of gut enzyme activities in gut wall homogenate of various species of earthworms (Tracey, 1951; Devigne and Jeniaux, 1961; Nielsen, 1962; Marcuzzi and Turchetto Lafisca, 1980; Mishra and Dash, 1980 and Urbasek and Pizl, 1991). Reviews have been written by Bostrom and Holmin (1982), Ross (1983) and Lee (1985) on the methods for assessment of the toxicity of environmental chemicals to earthworms. Cook and Swait (1975), Wright (1977) and Lee (1985) reported significant reduction in the rate of metabolic processes like feeding, casting and cocoon production in earthworms due to sub-lethal pesticide exposure. Malathion (S-1,2-bis (ethoxycarbonyl) ethyl0, 0-dimethyl Phosphorodithioate) an organophosphorus insecticide is widely used in India for agriculture and public health purpoe and is reported to be less toxic to earthworms. The present work was undertaken to study the sub-lethal malathion toxicity on gut enzyme activities of three tropical grassland earthworms (Lampito mauritii, Kinberg, Octochaetona surensis (Michaelsen), and Drawida willsi (Michaelsen).

MATERIALS AND METHODS

Malathion (50% EC) was obtained from M/S Hindustan Agrochemical Industries, Titlagarh, Bolangir for the study purpose. Pot culture method was adopted for rearing adult worms in earthern pots of size 75cmx50cmx 75cm. Natural garden soils with organic manures (cow dungs + leaf litter) were used for preparation of the culture bed with adequate care to maintain proper pH and moisture percentage. Adult worms of the three

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Species were maintained in the laboratory pot culture. For experimentation worms were collected from culture pots and kept half immersed in petridishes containing 30mL of tap water at 25 ± 30°c for 24h to clean the gut content. Six worms of a species per petridish in five replicates were a set. Two such set for control and one for each concentration of malathion (2,5,7.5 and 10 ppm) for each species was taken as experimental (Patnaik and Dash, 1990). The experimental sets were exposed to 30mL suspensions of the sub-lethal concentrations of the pesticide mentioned above for two hours continuously. Control worms were kept in 30mL of deionised water.

Amylase, invertase, cellulase and urease activity of the gut homogenate was estimated after 48h of reintroducing the worms into fresh petridishes containing 30mL of deionised water. Carbohydrases activity were measured following the method of Burton et al (1977) with modification by Mishra and Dash (1980). The reaction mixture contained 1mL of enzyme preparation with 1mL of substrate (1% soluble starch, 6% sucrose and 3% carboxymethyl cellulose respectively for amylase, invertase and cellulase), 1mL of Sorenson's buffer (pH=6.0) and 0.2mL of toluene for 24h. The enzyme activities are expressed in ug of glucose per mg of protein per hour. Urease activity was measured by determining the amount of ammonia released (Kaplan, 1969) by incubating the enzyme extract with 1mL tris HCl buffer (pH=9.0), 1mL of urea solution (1% w/v) and 0.2mL toluene for 96h. Urease activity is expressed in ug ammonia per mg protein per hour. The protein content of the tissue homogenate was determined as per Lowry et al (1951) using bovine serum albumin as standard.

RESULTS AND DISCUSSION

Enzyme activity of gut homogenate of adult earthworm species are represented in Fig.1. Average amylase, invertase, cellulase and urease activities in the gut homogenate of untreated *L. mauritii* were 142.4 ± 31.8 ug, 46.5 ± 21.6 ug, 56.5 ± 13.6 ug and 2.41 ± 1.9 ug/mg protein/h respectively. The corresponding values for *D. willsi* were 191.7 ± 17.6 ug, 114.7 ± 33.6 ug, 78.3 ± 14.5 ug and 1.61 ± 0.8 ug/mg protein/h. Urease activity was not detected in *O. surrensis* and the values for amylase, invertase and cellulase activity were 168.2 ± 25.7 ug, 189.8 ± 49.8 ug and 45.5 ± 9.5 ug/mg protein/h respectively.

Gut amylase activity was greater in *D. willsi* than *O. surensis* and *L. mauritii*. The activity decreased with an increase in malathion concentration except 2 ppm exposed worm which showed no significant effect. The

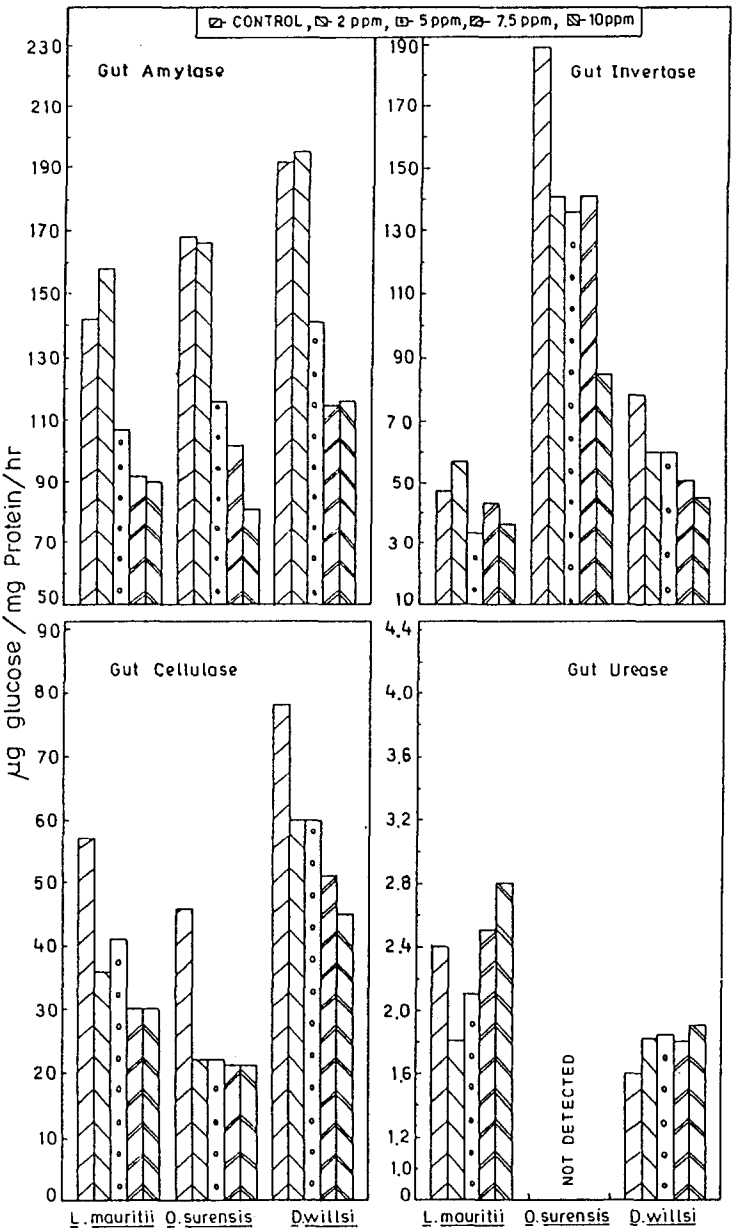

FIGURE - 1 Effect of Malathion on gut enzymatic activities of Lampito mauritii, Octochaetona surensis & Drawida willsi.

average percentage inhibition in amylase activity due to malathion exposure at 5, 7.5 and 10 ppm was 32.4 ± 6.8 for L. mauritii, 40.9 ± 10.5 for O. surensis and 35.5 ± 7.6 for D. willsi. However the statistical analysis shows significant differences only in 5, 7.5 and 10 ppm exposed D. willsi and O. surensis (Table-1).

Table 1. Statistical table showing "t" values for difference in enzymatic activities between control and different ppm exposed earthworms. (Values in parenthesis are significant at $p < 0.05$ and df = 6).

Exposure Concentration / Enzyme activities	worm species	2 ppm	5 ppm	7.5 ppm	10 ppm
Amylase	L.mauritii	0.89	1.77	2.07	(2.45
	D.willsi	0.29	(2.67)	(3.63)	(6.28)
	O.surensis	0.12	(2.51)	(3.77)	(5.04)
Invertase	L.mauritii	0.67	1.02	0.27	0.72
	D.willsi	2.10	2.07	1.92	2.05
	O.surensis	1.62	1.74	1.51	(3.53)
Cellulase	L.mauritii	1.82	1.49	2.21	1.50
	D.willsi	1.51	1.39	(2.98)	(3.66)
	O.surensis	1.86	1.97	(3.65)	(2.47)
Urease	L.mauritii	0.49	0.24	0.07	0.77
	D.willsi	0.29	0.33	0.31	0.42
	O.surensis	x	x	x	x

Invertase activity of gut homogenates of O. surensis is significantly higher than the other species. The ensyme activity showed remarkable reduction even at 2 ppm (except L. mauritii) due to malathion exposure. The reduction was higher at 5 ppm exposed L. mauritii and D. willsi than 2, 7.5 and 10 ppm exposure. O. surensis, however, showed significant reduction at 10 ppm exposed worms ($t=3.53$, $p < 0.05$, $d.f=6$). The average percentage reduction in invertase activity for all concentrations of malathion exposure were 20.5 ± 11.5 in L. mauritii, 33.7 ± 14.3 in O. surensis an 37.9 ± 2.6 in D. willsi.

Cellulase activity was greater in D. willsi than the other two species. The activity showed significant reduction in malathion exposed worms and the percentage reduction increased with an increase in malathion concentration. The average percentage reduction in cellulase activity due to malathion exposure were 39.5 ±

8.8, 53.3 ± 1.3 and 31.2 ± 9.6 for L. mauritii, O. surensis and D. willsi respectively. Statistical analysis (Table-1) showed significant reduction only in 7.5 and 10 ppm exposed D. willsi and O. surensis.

Urease activity was not found in the gut homogenate of O. surensis at pH=9.0 and temperature = 25 ± 3°c. L. mauritii showed greater urease activity than D. willsi. Urease activity increased in the gut homogenates of worms exposed to all concentrations of pesticide suspension except 2 and 5 ppm for L. mauritii worm species. The average percentage increase for 7.5 and 10 ppm exposed worms were 9.97 ± 8.8 and 15.22 ± 4.8 for L. mauritii and D. willsi respectively.

Malathion was previously reported to be non-toxic at normal dose rates when applied in field conditions (Hopkins and Kirk, 1957). However, the question of whether sub-lethal concentrations of pesticides have any deleterious effects on non-target soil organisms like earthworms in agroecosystems have not received sufficient attntion.

Occurence of number of enzymes, particularly cellulase in the gut of earthworms indicate their role in the decomposition of plant litter and other cellulosic materials (Dash, 1987). This investigation clearly shows that higher sub-lethal malathion exposure reduced the gut carbohydrases activity indicating harmful effect of the pesticides on earthworms. Lampito mauritii was found to be more resistant to malathion exposure as minimum reduction in enzyme activity occurred in them. Patnaik and Dash (1991) reported that earthworms exposed to organophosphorus insecticide show higher rate of excretion than the untreated worms. In this study the urease activity was found to be greater in worms exposed to malathion and the worms produced higher amount of mucus and other excretory materials. Hence increased urease activity due to insecticide exposure is also not good for the earthworms.

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