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Correlation between Burrowing Capability and AChE Activity in the Earthworm, *Pheretima posthuma*, on Exposure to Carbaryl

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Earthworms are important biocomponents of ecosystem, contributing substantially to maintain soil structure and helps plant growth. Poisoning and other disturbances in the natural habitat of earthworms can lead to ecological imbalance (Laird and Kroger 1981). Soil is the main sink for most toxicants which are released in the environment through industrial effluents, application of pesticides for agriculture and by dumping of civil refuge. Toxic contaminants to the soil can adversely affect the non-target organisms including earthworm. The sensitivity of earthworms to relatively low concentrations of environmental pollutants in soil has been amply demonstrated thus making them a choice species for biomonitoring (Kaiser 1980).

Carbamate insecticides are increasingly used in agriculture as a substitute for organochlorine insecticides because of their high efficiency and lower persistance in the environment. Toxicity of carbamates in non-target organism like earthworms is poorly understood despite reports of mortality and segmental swelling on exposure to carbofuran (Sileo and Gilman 1975; Roberts and Dorough 1983) and tonic tremors and end to end reduction in size on exposure to carbaryl (Stenersen 1979; Gupta and Sundararaman 1988). Carbamates in general exhibit a prolonged effect on the ability of earthworms to work the soil (Gilman and Vardanis 1974) and sublethal concentrations of carbaryl retarded the growth and reproduction in E.fetida (Neuhauser and Clarence 1990).

Carbaryl, a N-methyl carbamate insecticide was commercially used in India for more than a decade particularly to control foliar insects on cotton, apple and vegetable crops. The recommended quantities of carbaryl spray is 0.25 to 2.0 Kg active ingredient per hectare. Contamination of soil with carbaryl results from its soil application and its runoff to the ground. The soil residues depend upon the presence of organic substances with affinity towards carbaryl as well as the rate of microbial and hydrolytic conversion into 1-naphthol

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and other water soluble metabolites (Fukuto 1972; Rodriguez and Dorough 1977). The soil residues of 1-naphthol in India have been recently reported to range between 0.153 to 0.656 ppm (Dikshith et al 1990) and using this criteria substantial concentrations of carbaryl (1 ppm and more) are expected in the soil. The potential toxicity of carbaryl and its equally toxic metabolite 1-naphthol residues in soil will tend to deplete earthworm population, however, its actual magnitude is not known.

The present communication demonstrates a dose and time of exposure dependent loss of burrowing capability, inhibition of acetylcholinesterase (AChE, EC, 3.1.1.7) activity and a correlation between these manifestations in the earthworm Pheretima posthuma on carbaryl exposure.

MATERIALS AND METHODS

Earthworms P.posthuma were collected locally from the bank of a water reservoir and kept in earthen pots with their native soil at laboratory temperature. They were acclimatized for 7 days before commencing the experiment. Healthy and clitellate earthworms (average weight - 3.24 gm, average length - 16.25 cm) were sorted out and kept for 24 hr in a glass trough with little water for moisture to evacuate their gut prior to experimentation.

Carbaryl (1-naphthyl methylcarbamate; sevin, technical grade) was supplied by Union Carbide Corporation, India. Stock solution was prepared in acetone and diluted with distilled water to yield final concentrations of 1,2,4 and 8 ppm. Five earthworms were individually exposed for 5, 10, 20, 40 and 80 min intervals in a 50ml beaker containing 2.0ml of various concentrations of test solution or distilled water (control). The test concentrations used in the present study are close to that of expected residues in the soil. Higher concentrations were used to exert significant inhibition of AChE activity and loss of burrowing in earthworms for establishing a dose effect correlationship.

The earthworms were quickly rinsed in tap water and kept on moist soil immediately after the completion of their exposure. The burrowing time from first segment to the last segment was noted with the help of a stop watch. The worms were recovered from the soil, rinsed with tap water and returned to the same beaker for further exposure. Likewise the burrowing time of control earthworms was also noted for comparison.

In another set of experiment, 80 worms each were collectively exposed to 1,2,4 and 8 ppm carbaryl for 5,10,20,40 and 80 min. All earthworms were rinsed with water after exposure time and incisioned quickly to take out nerve ring along with total nerve cord. The neuronal tissue was collected in a petridish on ice, pooled for 20 eathworms each, homogenized in 0.25M sucrose and used for determination of AChE activity (Hestrin 1949). Recovery from the adverse behavioural effects of carbaryl was observed by rinsing exposed earthworms in tap water and keeping in normal soil for a period of 24 hr.

All experiments were repeated ten times. The group means and standard errors for each observation were calculated and compared by Student's t-test (Fischer 1948) to evaluate statistical significance of results. P-values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Exposure of earthworms to carbaryl (1-8 ppm) significantly increased the burrowing time which was directly proportional to the dose and time of exposure. The reduction in the ability of worms to burrow in soil was clearly evident at the lowest test concentration (1 ppm) and the earliest period of exposure (5 min). This response was gradually increased to complete loss of burrowing at 40 min of exposure to 4 and 8 ppm and at 80 min of exposure to 1 and 2 ppm carbaryl (Table 1). Additionally, the time for onset of tonic tremors was shortened with the increased concentrations of carbaryl.

AChE activity in the neuronal tissue of earthworms was significantly inhibited in strict stoichiometric relationship to the increasing dose and the time of exposure to carbaryl (Table 2).

Post-exposure recovery was not observed in 4 and 8 ppm exposure groups and all worms died with in 18 hr. Recovery in 2 ppm exposure group was only 30%, the other 70% died between 18-24 hr. However all worms were alive and fully recovered to normal behaviour (no tremors, efficient burrowing) 18 hrs post-exposure to 1 ppm carbaryl.

Burrowing is an inherent behavioural mode of defence in earthworms to protect themselves from predators and to escape from undesirable exposure to toxicants. Presence of environmental pollutants in soil have been reported to affect the burrowing and cause extensive mortality of earthworms as seen here with carbaryl (Stenersen 1979).

Burrowing is a direct result of coordinated muscle contraction in earthworms. The physiological significance of this behaviour is in locomotion required for feeding, reproduction, defence etc. Muscle contraction in earthworms, as in most other cases, is an energy dependent process triggered by the release of Ca^{++} ions from sarcolemma in response to acetylcholine mediated nerve impulse. The concentration of

Table 1. Burrowing time (seconds) in the earthworm *Pheretima posthuma*

Control value	Carbaryl treatment	Exposure time in minutes				
		5	10	20	40	80
108.78 ± 1.10	1 ppm	128.1±1.44	148.70±3.73	218.92±5.22	286.72±4.41	NB
	2 ppm	162.43±3.72	224.01±2.45	298.32±5.44	487.40±4.39	NB
	4 ppm	223.83±3.36	291.00±7.16	402.78±6.33	NB	NB
	8 ppm	277.98±5.07	385.83±4.38	579.53±7.05	NB	NB

All values are mean ± S.E.
$P<0.05$ for all treatment groups as compared to control.
NB - Not burrowing

Table 2. Acetylcholinesterase activity (µmole/gm/min) in the nervous tissue of the earthworm *Pheretima posthuma*

Control value	Carbaryl treatment	Exposure time in minutes				
		5	10	20	40	80
12.54 ± 0.17	1 ppm	12.14±0.04	11.98±0.09	11.23±0.10*	10.88±0.15*	9.42±0.13*
	2 ppm	12.11±0.09	11.43±0.11*	11.17±0.13*	10.48±0.16*	9.12±0.16*
	4 ppm	12.11±0.27	11.13±0.14*	10.97±0.19*	9.09±0.24*	8.04±0.10*
	8 ppm	11.77±0.23*	10.82±0.14*	9.66±0.22*	8.10±0.10*	5.53±0.02*

All values are mean ± S.E., * $p < 0.05$

acetylcholine in the nerve synaptic terminals are regulated by AChE activity. A variety of chemicals commonly grouped as anticholinergic substances (organophosphates, carbamates, alkaloids etc.) are known to inhibit AChE activity and affect acetylcholine concentration to alter the nerve conduction and muscle contraction. The severity of AChE inhibition is characterized by tonic tremors, convulsions and eventual death as observed in this study. The observed inhibition of AChE activity is consistant with the previous reports on anticholinergic effect of carbamates (Stenersen 1979; Aspock and Ander Lan 1963).

This study, therefore, demonstrates a direct correlationship between the increased burrowing time and the inhibition of AChE activity. Burrowing is the primary response since carbaryl can be readily absorbed through the moist and highly permeable body wall of earthworms. The irritant effect of toxicants would be normally expected to stimulate the process of burrowing in order to avoid toxic exposure however, when combined with the neurotoxic effect (AChE inhibition) and muscular discoordination, the efficiency to burrow has decreased on exposure to carbaryl. Our observations indicate that approximately 30% inhibition of AChE activity can produce virtually complete muscular paralysis (no burrowing) and severe tonic tremors in earthworms. One of the reasons why burrowing was significantly affected at 5 min of exposure without substantial inhibition to AChE activity could be the peripheral effects of carbaryl which could not be measured in the neuronal tissue extracted from the interior of earthworm. Significant but delayed inhibition of AChE activity in the present study can be accounted for the relative rate of absorption and requisite concentrations of carbaryl to reach the interior tissues of earthworms.

The anticholinergic response of carbaryl is known to be prolonged but reversible in earthworms (Stenersen 1979). The observations of our recovery experiments confirm to this phenomenon, though evaluated through the indirect means of burrowing time. The prolonged effect of carbaryl led to the extensive mortality in high dose groups, whereas low dose group exhibited a complete recovery.

The moist and mucilagenous skin of earthworms facilitates efficient absorption of carbaryl and a variety of organicals from the soil. The present observations of a transient and reversible effect on earthworms at carbaryl concentrations close to soil residues (approx 1 ppm) is anticipated to produce little effect on earthworm population and soil fertility. On the other hand, insect infestations in absence of insecticide spray and soil treatment can cause major crop losses thus the benefits outweight the ecological effects from the commercial usage of carbaryl.

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