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Toxicity Assessment of Four Insecticides to Earthworm, *Pheretima posthuma*

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Pesticides are either directly applied to soil to control soil borne pests or are deposited on soil as run off from foliar applications. Whatever the source, earthworms are exposed to pesticides through (a) skin contact and (b) by feeding on contaminated litter in soil. The effect on earthworm may be either due to soil/foliar application at considerably higher concentrations or slow accumulation of low levels of persistant pesticide residues. In the former case heavy mortality is expected while in the latter it may partially impair physiological functions affecting arablity of soil or slowly impairing their growth and reproduction causing depletion in their production (Davey, 1963; Edwards and Thompson, 1973). Although many toxicity studies have been conducted (Bostrom and Lofs-Holmin, 1982; Dean Ross, 1983; Roberts and Dorough, 1984; Bharathi and Subbarao, 1985; Zoran et al, 1986; Gupta and Sunderaraman, 1987) the fact remains that only a few pesticides in use have been tested against relatively few earthworm species. Therefore, in this paper the toxicity of commonly used insecticides - aldrin, endosulfan, heptachlor and lindane to a native species of earthworm Pheretima posthuma has been determined.

MATERIALS AND METHODS

Technical grade aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4,5,8-dimethanonaphthalene); endosulfan (1,4,5,6,7,7-hexachloro-8,9,10-trinorborn-5-en-2,3-ylene dimethyl sulphite); heptachlor (1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene); lindane (γ-1,2,3,4,5,6-hexachlorocyclohexane); were used as test compounds obtained from Bharat Pulverising Mills Private Limited, Bombay; Hoechest India Limited, Bombay; Polyscience Corporation, Niles, U.S.A. and Swaroop Chemicals, Lucknow respectively. The stock solutions of insecticides were prepared in ethanol and diluted to

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required concentrations by adding an aliquot to water which was spread on filter paper or mixed with soil.

Healthy and sexually mature earthworms were collected, maintained and acclimatized at 25°C, R.H. 70±5 in a controlled environment chamber in the laboratory for three days before treatment either by contact filter paper method or soil pot method (European Economic community Directive, 79/831). The LC_{50} and confidence limit values were determined by probit analysis (Finney, 1978) statistical method.

RESULTS AND DISCUSSIONS

Exposure to different concentrations of insecticides revealed that the symptoms of toxicity were dose dependent (Table 1). The animals showed progressive signs and symptoms of toxicity ranging from visibly undetectable marks to coiling, curling, extrusion of coelomic fluid, segmental constriction and swelling. In several animals the swollen portion burst causing bloody lesions, limp and ultimately death. The symptoms produced were specific in nature, reproducible and so may help in recognizing the insecticide eg. coiling, curling (Aldrin); mucus secretion, sluggish movement (Endosulfan); lifting of body, extrusion of coelomic fluid (Heptachlor); globular swelling, segmental constriction, white band (Lindane) (Figure 1).

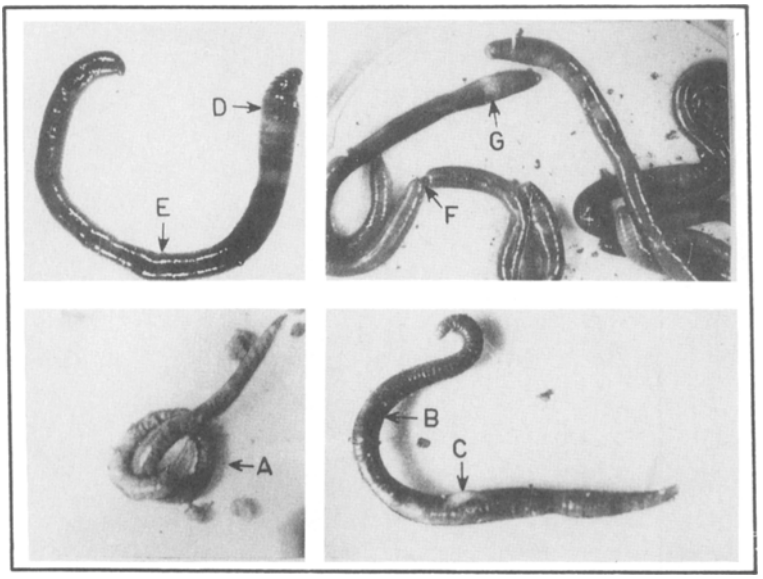

Figure 1. Specific toxicity symptoms in earthworms. A - Coiling, curling (Aldrin); B,C - Body lifting and extrusion of coelomic fluid (Heptachlor); D-G - Globular swelling, blister, segmental constriction and white band (Lindane).

Table 1. Sign and symptoms of insecticide toxicity in earthworms during 6 hr exposure

Insecticide concentration* (ppm)	Aldrin	Endosulfan	Heptachlor	Lindane
10	Animal exhibit coiling and vigorous movement, faecal excretion	Body swollen, Excessive mucus secretion, sluggish movement.	Irritation, body swollen, fast locomotion.	Animals exhibit coiling and sluggish movement.
25	Irritation, tapering without movement, appearance of intersegmental white spot.	Body extended and weak muscle tone.	Irritation, lifting of body and oozing of coelomic fluid.	Severe irritation, vigorous movement, and exeessive mucus secretion.
50	Severe irritation with extrusion of coelomic fluid.	Above symptoms aggravated.	Clitellum developed patches on dorsal side, blister on ventral side and outgrowth of female genital pore.	Heavy mucus secretion, globular swelling in preclitellar region and deep segmental constriction in post clitellar region.
100	Death within 10 minutes.	Instant death.	Death within 15 minutes.	Frothing, decoloration, death within 2 hours.

* No visible change was observed upto 1 ppm.

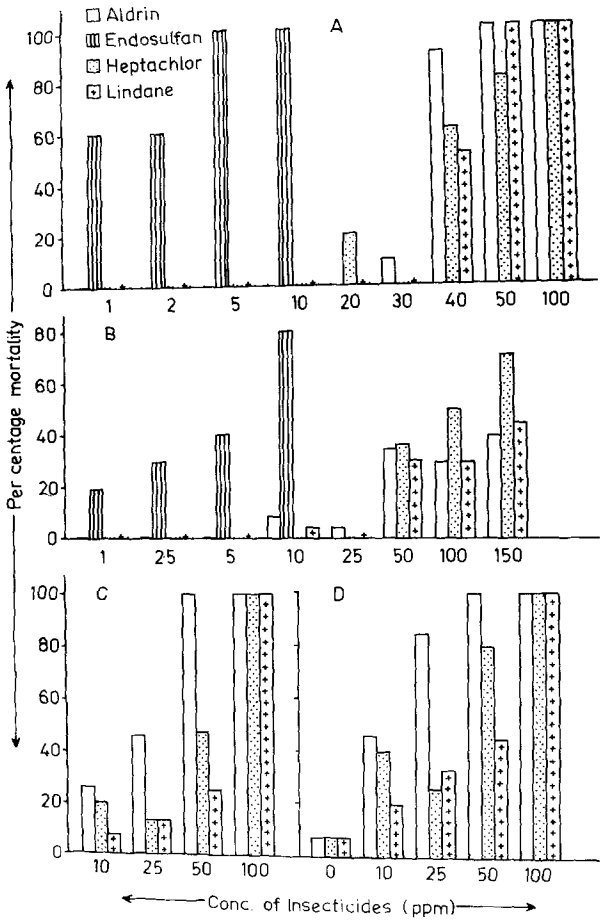

Figure 2. Percent mortality of earthworms exposed to insecticides. A - Filter Paper Contact Method (24 hr) B-D - Soil Pot Method (24 hr, 1 wk and 2 wk).

The mortality of animals at various exposure concentrations by contact filter paper method at 24 hr and soil pot method at 24 hr, 1 wk and 2 wk is shown in Figure 2. The pattern of lethality at 24 hr exposure was endosulfan > heptachlor > aldrin > lindane by contact filter paper method and endosulfan > heptachlor > lindane > aldrin by soil pot method.

The acute LC_{50} values for endosulfan, heptachlor, aldrin and lindane were 1.5, 32.4, 34.0 and 36.5 mgL^{-1} respectively, by contact filter paper method and 5,75,103 and 78 by soil pot method respectively (Table 2).

Table 2. LC_{50} values of four insecticides as determined by filter paper and soil pot method.

Compound	Method of exposure	Exposure period	Acute Toxicity Range (98% confidence Limit) Upper (ppm)	Lower (ppm)	Median LC_{50} (ppm)	Fold difference in toxicity against filter paper
Aldrin	Filter Paper	24 hr	36.70	31.45	33.98	
	Soil Pot	24 hr	121.51	92.66	103.16	3.04
	Soil Pot	1 wk	35.76	14.38	22.68	0.67
	Soil Pot	2 wk	18.56	6.78	10.79	0.30
Endosulfan	Filter Paper	24 hr	1.98	1.02	1.46	
	Soil Pot	24 hr	8.69	2.88	5.01	3.43
	Soil Pot	1 wk	ND	ND	ND	ND
	Soil Pot	2 wk	ND	ND	ND	ND
Heptachlor	Filter Paper	24 hr	41.77	25.15	32.41	
	Soil Pot	24 hr	87.42	60.73	75.05	2.31
	Soil Pot	1 wk	64.12	37.09	48.77	1.50
	Soil Pot	2 wk	41.40	24.77	32.02	0.79
Lindane	Filter Paper	24 hr	42.15	29.39	36.52	
	Soil Pot	24 hr	91.53	63.92	78.26	2.14
	Soil Pot	1 wk	71.24	42.90	55.29	1.31
	Soil Pot	2 wk	63.79	25.62	40.43	1.10

The results suggested that animals can tolerate higher insecticide concentration in soil than on moist filter paper and the concentration ratio is three fold for aldrin and endosulfan and two fold for heptachlor and lindane.

This difference in the behaviour of these insecticides in both the media may probably be due to the rate of diffusion of insecticides from the medium into the body of earthworm. However, prolong exposure to the soil medium reduced the LC_{50} values for each compound compared to 24 hr values. It was interesting to note that 2 wk LC_{50} values in soil were close to 24 hr values determined by filter paper method. It is well documented that insecticides are adsorbed on soil and also tightly bound with its organic matter (Edwards, 1966; Thompson, 1973). The availability, therefore, of insecticide for diffusion will presumbly be less from the soil than impregnated filter paper. However, in long term exposure the passage of soil through gut might release the residue in the system to enhance toxicity.

Roberts and Dorough (1984) tested ninety chemicals against Eisenia foetida through contact filter paper method and on the basis of their LC_{50} values, classified them into 5 categories; Of the chemicals tested - Carbofuran, eserine salicylate were super toxic; aldicarb, carbaryl, benomyl, phenolic derivatives of 2,4-D and 2,4,5-T were extremely toxic; endosulfan, parathion, malathion very toxic; kepone, acephate, paraquat moderately toxic and DDT, premethrin, diuron relatively non-toxic. Several chemicals eg. carbaryl, malathion, cypermethrin which were moderately toxic or relatively non-toxic to mammals were very toxic to earthworms. This demonstrates the unpredictability of chemicals toxicity to different animal species.

The present study indicates that among the insecticides tested endosulfan is most toxic to earthworm Pheretima posthuma causing severe mortality while aldrin the least. Lindane and heptachlor were toxic at about 75 percent concentration of the aldrin and therefore, they may be categorized as moderately toxic.

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REFERENCES

Bharathi Ch, Subbarao BVSSR (1985) Toxic effects of two organophosphate insecticide monocrotophos and dichlorovos to common earthworm Lampito mauritii. Water Air Soil Pollut 28:127-130.

Bostrom U, Lofs-Holmin A (1982) Testing side effects of pesticides on soil fauna. A critical literature review. Reports 12 Swedish University of Agricultural Sciences and Department of Ecology and Environmental Research Uppsala Sweden.

Davey S P (1963) Effects of chemicals on earthworms - A review of the literature U.S. Fish and Wildlife Service Special Scientific Report Wildlife no. 74.

Dean Ross D (1983) Methods for assessment of the toxicity of environmental chemicals to earthworms. Ecotoxicol Environ Safety 6:216-222.

Edwards C A (1966) Insecticide residues in soil. Residue Rev 13:83-132.

Edwards C A, Thompson A R (1973) Pesticides in the soil fauna. Residue Rev 45:1-181.

Finney D J (1978) Statistical methods in biological assay. Charles Griffin & Company Limited, London.

Gupta S K, Sunderaraman V (1987) Carbaryl induced light microscopic changes in the earthworm Pheretima posthuma. J Res Adv Appl Sci 2:350-356.

Pimental D, Edwards C A (1982) Pesticides and Ecosystem Biosciences 32:595-600.

Roberts B L, Dorough H W (1984) Relative toxicities of chemicals to earthworms Eisenia foetida. Environ Toxicol Chem 3:67-78

Thompson A R (1973) Pesticide residue in soil invertebrates In: Edwards C A (ed) Environmental Pollution by Pesticides. Plenum pp. 87-133.

Zoran M J, Heppner T J, Drewes C D (1986) Teratogenic effects of the fungicide benomyl on posterior segmental regeneration in the earthworm Eisenia foetida. Pestic Sci 17:641-652.

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