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EFFECTS OF SUBLETHAL DOSES OF MALATHION ON REGENERATION OF EARTHWORM *D. WILLSI*

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KEYWORDS

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Orientation
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Double agricultural dose of Malathion

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ABSTRACT

Malathion is an organophosphate which is indiscriminately used in agriculture, house and gardens and has lethal effects on many organisms. Earthworms are one of the non–target species which are adversely affected due to increased use of Malathion in modern agricultural practices. The main theme of the present study is to assess the effect of Malathion on the earthworm, *Drawida willsi*, were transected in different orientation like 25, 50 and 75% anterior portion and similarly 25, 50 and 75% posterior portion and thereafter inoculated into plastic pots containing soil mixed with single and double agricultural doses of Malathion. Habitat soil was taken as control. Measurements were carried after making earthworms straight on ice at an interval of four days for the length regenerated. The one way ANOVA showed that the regeneration abilities were significantly affected by different doses of Malathion ($p < 0.01$). With respect to the control no regeneration took place upto 8th day in single agricultural dose, however in maximum cases complete inhibition of regeneration was seen in double agricultural doses of Malathion. The double agricultural dose of Malathion seems to be more harmful in altering life functions of non target soil organisms and thus care should be exercise to select compounds that do not excessively damage earthworm population.

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INTRODUCTION

The nutrients recycled by the processes of decomposition of dead organic matter provides the major nutrients source for the growth of plants. Decomposition is brought about by the whole range of micro-organisms, fungi and soil animals that live in soil. Earthworms are important contributors to the decomposition process. In addition to helping in decomposition, earthworms along with termites are the main agents which bring about physical modification of soil profiles. By burrowing, cast formation and construction they move materials between and within soil horizons, mixing organic and inorganic soil constituents, disintegrating and reforming soil aggregation and modifying soil structure porosity, aeration and water infiltration. Both are very successful animal groups, about 3000 species of earthworms and 2000 species of termites are known (Lee, 1983). As earthworms play an important part in enhancement of soil structure and fertility, the benefit to plant growth as a result of earthworm activity is greatly recognized (Delahaut and Koval, 1989).

Earthworm have an unavoidable beneficial role, however, remain neglected during pest control practices (Faheem and Khan, 2010). The development of new biocides to control microbial, fungal, animal and plant pests in agricultural and pastoral industries has been a feature of modern agricultural chemistry. Their use has made it possible to greatly increase food production and it would not be possible to maintain or increase present levels of nutrition for the expending human population without these potential chemicals particularly in a country like India. However, it is important to understand that their effects are not necessarily confined to species that do harm to the crops or domestic animals whose protection is intended. Some of the most effective pesticides are infact broad – spectrum biocides, whose effects include the accidental destruction of beneficial species. Indiscriminate use of pesticides to suppress pests may results in the derangement of soil ecosystems leading to the destruction of many beneficial soil organisms (Anderson, 1978). Pesticides reach the soil directly, by their intentional application to the soil, to the foliage of crops and also through the seeds treated with pesticides during their storage (Bernhart and Vestal, 1983). Pesticide in soil may be accumulated by worms through two processes, either by injecting contaminated soil and by absorption from the soil water (Brunninger *et al*., 1995; Viswanathan, 1994) Organisms exposed to toxic chemicals, depending on the concentration and site of action may alter the physiological site of functions. In some cases, organisms may succumb to adapt to the change, whereas in other cases they may successfully adapt to the change, and recover from the exposure (Zachariassen, 1991).

Numerous reports have demonstrated the adverse effects of pesticides on earthworms (O'Brien, 1967; Randall *et al.,* 1972; Senapati *et al.,* 1992; Stenerson, 1979; Lofs-Holmin, 1982). The toxicity of pesticides to earthworms deals predominantly with recently some research has focused on measuring sublethal effect on the either of the animal physiology (Reproduction and weight loss) or behavior (avoidance) (Reinecke *et al*., 2002). Neuhauser and Callahan (1990) have observed the recovery of growth and reproduction in *E. foetida* following short term exposure to sublethal concentration of Dendrin and Carbaryl. Panda and Sahu (1999) have reported, recovery of growth and reproduction of *D. willsi*. The present paper deals with the study of effect of Malathion (a widely used organophosphorus insecticides) on regeneration of *D. willsi* (a cropland earthworm) in normal and under stressed laboratory conditions.

MATERIALS AND METHODS

Earthworms *D. willsi* for experiment were collected and stored from cropland soil. For gut evacuation, they were kept in glass jars for a day such that 3/4th part was immersed in water and 1/4th part was exposed. Individuals with any indication of damage by collecting were rigorously rejected and only those which were clitellate were used. The worms were left in moist soil, taken from the habitat of the worm kept in a plastic pot, for one week. Transections were made under a dissecting binocular microscope using sharp rajor blade exactly across the worm on an intersegmental furrow beginning at 28/29 in one series, at 56/57 in second series and at 84/85 in third series in different orientation. Anterior parts of three series were called as anterior 25%, anterior 50%, anterior 75% and posterior parts as posterior 75%, posterior 50%, posterior 25%. After cutting all fragments from the same level were kept together in one pot under oxygenated condition at room temperature (28±2°C). Habitat soil was used as control. Agricultural recommended dose 2.2 mg Malathion per kg soil as a single dose and 4.4 mg Malathion per kg soil as a double dose was used to study the toxic impact of Malathion with respect to control. These concentrations were prepared in dilutions of acetone and sprayed on to the soil surface. After evaporation of the solvent the treated soil was thoroughly mixed to distribute the insecticide evenly and enough water was added to maintain the moisture content.

RESULTS AND DISCUSSION

In anterior 25% segments of earthworm *D. willsi* initially both in treated and untreated soil showed no regeneration till 8th day. After 8th day 2.2 mg /kg Malathion treated soil has shown similar trend as untreated soil but in 4.4 mg/kg treated soil no regeneration took place after 8th day. The one way ANOVA analysis showed that the result is significant ($p<0.01$) (Table 1).

Anterior 50% showed totally different results in three types of soil. 4.4 mg/kg treated soil showed complete inhibition of regeneration however in 2.2 mg/kg treated soil regeneration could be seen till 8th day after 12th day no regeneration took place, which is not in accordance with the trend observed in control where regeneration continued till the end of the experiment (Table 2) and the results were found to be significant ($p<0.01$).Malathion , in single dose has shown its peak on 12th day before and after the day regeneration was inhibited in anterior 75% segment. Double dose of Malathion has shown inhibition of regeneration till 12th day after which it is enhanced. Regular regeneration could be seen in control soil (Table 3).

Regeneration in posterior 50% portion of earthworm in control soil was observed upto 20th day. One way analysis of Variance (ANOVA), however showed significant effect of Malathion on

Table 1: Regeneration of anterior 25% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

	Doses of malathion in soil				
Days	control	2.2mg/kg	4.4mg/kg	F(Anova)	p
o	1.6±0.18	1.7±0.24	2.4±0.08		
4th	1.6±0.18	1.7±0.24	2.4±0.08	22.80	0.003
8th	1.6±0.18	1.7±0.24	2.5±0.08	22.80	0.003
12th	1.7±0.18	1.8±0.24	2.5±0.07	22.80	0.003
16th	1.8±0.18	1.9±0.24	2.5±0.07	15.21	0.001
20th	1.9±0.12	2.0±0.24	2.5±0.08	13.32	0.002

Table 2: Regeneration of anterior 50% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

	Doses of malathion in soil				
Days	control	2.2mg/kg	4.4mg/kg	F	p
o	2.5±0.25	3.8+0.33	2.9+0.53		
4th	2.6±0.25	3.8+0.33	2.9+0.53	9.99	0.005
8th	2.8+0.28	3.8+0.33	2.9+0.53	7.68	0.01
12th	2.9+0.28	3.9+0.33	2.9+0.53	8.37	0.008
16th	3.2+0.36	3.9+0.33	2.9+0.53	5.78	0.02
20th	3.3+0.36	3.9+0.33	2.9+0.53	5.50	0.02

Table 3: Regeneration of anterior 75% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

	Doses of Malathion in soil				
Days	control	2.2mg/kg	4.4mg/kg	F	p
o	4.8±0.08	2.6±0.24	4.1±0.21		
4th	5.1±0.08	2.6±0.24	4.1±0.21	167.64	0.001
8th	5.3±0.11	2.6±0.24	4.1±0.21	183.00	0.001
12th	5.4±0.09	2.7±0.24	4.1±0.21	185.46	0.001
16th	5.6±0.09	2.7±0.24	4.4±0.09	214.12	0.001
20th	5.8±0.09	2.7±0.24	4.6±0.12	249.43	0.001

Table 4: Regeneration of posterior 50% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

	Doses of Malathion in soil				
Days	control	2.2mg/kg	4.4mg/kg	F	p
o	1.9±0.08	3.8±0.24	2.9±0.53		
4th	2.0±0.51	3.8±0.24	2.9±0.53	27.84	0.0001
8th	2.2±0.08	3.8±0.24	2.9±0.53	22.05	0.0003
12th	2.4±0.09	3.8±0.24	2.9±0.53	18.77	0.0006
16th	2.6±0.13	3.8±0.24	2.9±0.53	13.74	0.001
20th	2.7±0.17	3.8±0.24	2.9±0.53	11.90	0.002

Table 5: Regeneration of posterior 75% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

	Doses of Malathion in soil				
Days	control	2.2mg/kg	4.4mg/kg	F	p
o	5.8±0.33	3.2±0.08	3.0±0.16		
4th	6.0±0.37	3.4±0.12	3.0±0.14	182.77	0.001
8th	6.1±0.36	3.5±0.08	3.0±0.16	207.75	0.001
12th	6.1±0.36	3.7±0.05	3.1±0.16	195.38	0.001
16th	6.1±0.36	3.7±0.05	3.1±0.16	195.38	0.001
20th	6.1±0.36	3.7±0.05	3.1±0.16	195.38	0.001

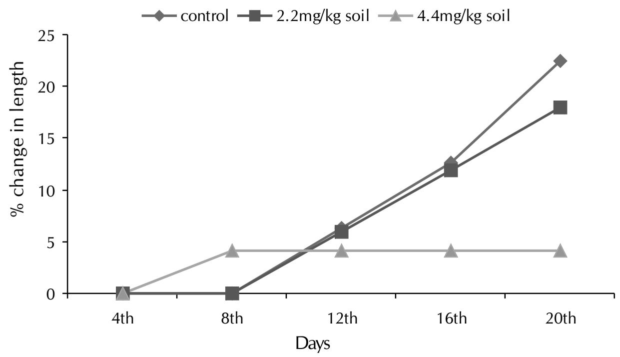

Figure 1: Percentage change in length of anterior 25% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

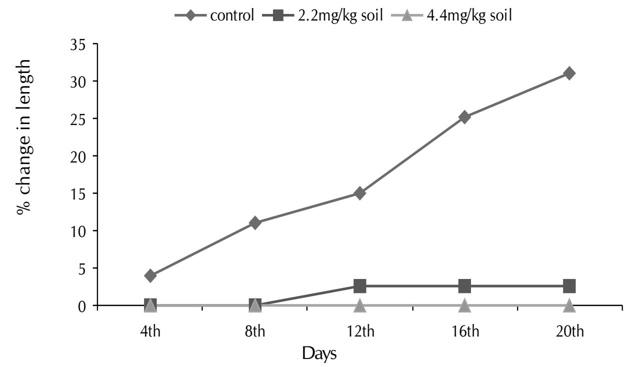

Figure 2: Percentage change in length of anterior 50% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

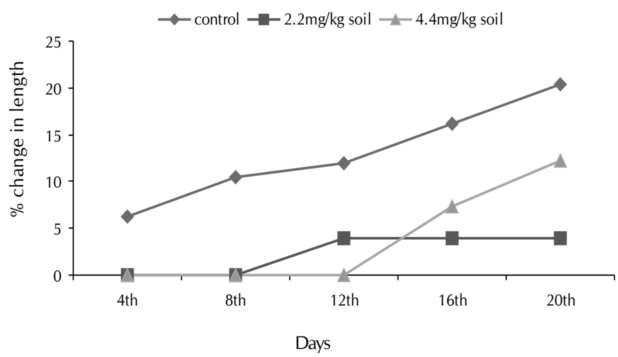

Figure 3: Percentage change in length of anterior 75% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

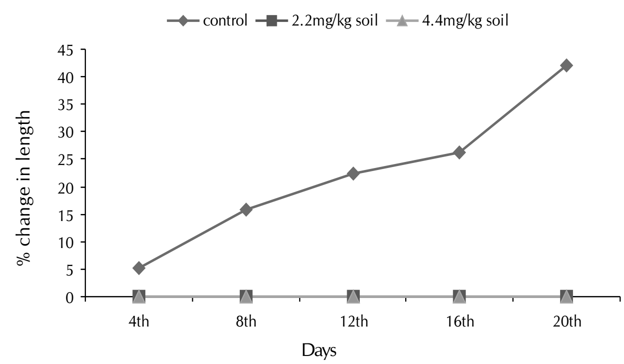

Figure 4: Percentage change in length of posterior 50% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

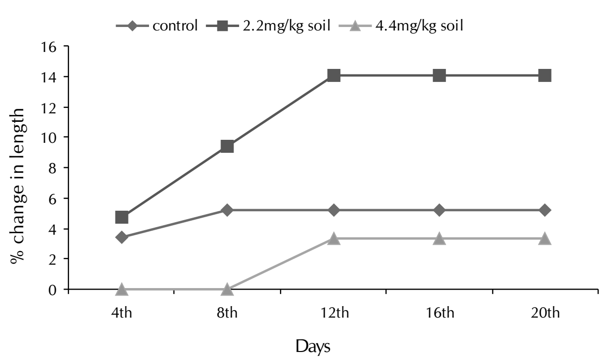

Figure 5: Percentage change in length of posterior 75% segment of earthworm *D. willsi* following application of different agricultural doses of Malathion

2.2 mg/kg and 4.4 mg/kg treated soil segments upto 20th day ($p<0.01$) (Table 4).

Segment having posterior 75% portion, treated in single agricultural dose soil got inhibited after 12th day. Portion in double agricultural dose soil was inhibited till 8th day. On 12th day it showed its peak and thereafter again remained inhibited, however, segment in control soil showed regeneration upto 8th day (Table 5). Different doses of Malathion on all the segments at different intervals significantly ($p<0.01$) affected the process of regeneration. Posterior 25% was unable to survive in both single and double agricultural dose of Malathion.

In the present experiment anterior segments treated with single agricultural dose showed no regeneration till 8th day after which regeneration took place. The effect of organophosphorous compounds are short lived (Verstraete and Voets, 1977). Malathion degrades rapidly in soil, with reported half-life ranging from hour to approximately 1 week (Konrad *et al.*, 1969; Gibson and Burns, 1977; Howard, 1991). Half life values in soil of 3-7 days (Bradman *et al.*, 1994). The toxic effects of Malathion are attributed to the acetyl cholinesterase inactivation that occurs after sufficient parent compound is oxidized to Malaoxan (Talcott, 1979). Posterior segments in single agricultural dose Malathion soil showed different result as 50% was completely inhibited. Kumari *et al.* (2010) reported that anterior segments of earthworm showed maximum regeneration towards posterior direction, however, ability to regenerate in anterior direction by posterior segments is less.This difference of regeneration shown by anterior and posterior segments may be due to absence of brain and supraesophaogeal ganglion in posterior segments (Bedate and Sequers, 1984). A stimulatory factor or growth factor from the brain is probably required for release of a regeneration factor from subesophageal ganglia or nerve cord neurosecretor cell (Bedate and Sequers, 1984). Both single and double agricultural doses of Malathion have decreased the percentage change in length of different portions of earthworm (Figs. 1 to 5).In double agricultural dose majority segments has shown complete inhibition of regeneration. It seems double agricultural dose of Malathion is more harmful in altering the life functions of non target soil organisms and thus should be avoided. Posterior 25% segment of earthworm in both doeses of Malathion has not survived. This is in accordance with the Drewes and Vining (1984) who reported that recovery may not occur in some cases after exposure to Benomyl pesticide. In respect with earthworms it is significant that it kills organisms through intake or contact by distressing the nervous system (Bacey, 2003).

From the preceding account of the importance of earthworms in promoting soil fertility and plant growth it is obvious that care should be exercised to select compounds that do not excessively damage earthworm population.

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