INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 7, p. 98-106, 2013

RESEARCH PAPER OPEN ACCESS

Acute toxicity of dimethoate on soil health : A study of its impact on earthworm

Anindita Bhattacharya[1*], Sanjat Kumar Sahu[2]

[1]*Department of Forestry, Wildlife & Environmental Sciences, Guru Ghasidas University, Bilaspur, Chhattisgarh, India*

[2]*P.G. Department of Environmental Sciences, Sambalpur University, Odisha, India*

Key words: Dimethoate, *Drawida willsi*, soil fertility, agrochemical toxicity.

doi: http://dx.doi.org/10.12692/ijb/3.7.98-106 Article published on July 25, 2013

Abstract

Several studies were conducted on the acute toxicity of agrochemicals. However, very little information is available on the toxic effect of dimethoate which is one of the most popular organophosphorous insecticide widely used in India. Therefore the present experiment was conducted to find out the eco-toxicity of dimethoate on soil health. For this, earthworm (*Drawida willsi*, Michaelsen) and soil was collected from such agricultural field where there had no record of input of agrochemicals. Different concentrations of dimethoate were prepared in dilution with acetone and sprayed on the soil surface. Five replicates for each concentration of the pesticides were prepared and ten numbers of juvenile, immature and adult earthworms were added separately into all the replicates of different concentration of the pesticides. All the samples were kept in the laboratory for 96 hours and number of earthworm death with respect to doses and replicates were recorded. The 96h LC_{50} values for juvenile, immature and adult earthworm were calculated by the Finney's Probit Method (Finney, 1971). The juvenile, immature and adult worms did not die in soil containing up to 0.5, 1 and 1 mg/kg of dimethoate respectively. When earthworms were exposed to concentration as high as 13 mg/kg of dimethoate, 100% of juveniles, 94% of immature and 81% of adults died. The 96 h LC_{50} values with their 95% confidence limits for juvenile, immature and adult worms were 5.5 (4.4-6.6), 6.7 (5.2-8.2) and 9.0 (7.3-10.7) mg/kg respectively. Although the recommended dose of dimethoate (0.4 mg a.i./ kg dry soil) was lower than the 96 h LC_{50} values of *D. willsi*, but still due to bioaccumulation, it may hamper the soil biota and also the soil health by triggering the domino effect.

* **Corresponding Author:** Anindita Bhattacharya ✉ anindita_bhattacharya1@rediffmail.com

Introduction

An excessive use of agrochemical (pesticides and fertilizers) in the agricultural field has caused an environmental concern since last few decades. When applied into the soil, they affect both the target as well as the non-target organisms like microorganisms, earthworm etc. and ultimately deteriorate the soil health.

Among all the biological factors, earthworm plays one of the major roles in maintaining soil health. They constitute a major fraction of the soil invertebrate biomass (> 80%), and they have ability to improve soil structure by breaking organic matter and release of plant nutrient (Edwards and Bohlen, 1996). They also maintain soil status through their burrowing and casting activities (Panda and Sahu, 1997). Since earthworm has direct contact with the ground, they were also found to absorb agro-chemical (Edwards and Bohlen, 1996). So any change in the soil due to agrochemical must have an effect on earthworm as well as on soil health. Therefore, they have been used as indicator species for ecotoxicological evaluation, risk assessment and monitoring of environment quality (Curry *et al*, 2008; Mahajan *et al*., 2007; Muthukaruppan *et al*., 2005; Hund-Rinke *et al*., 2003; Booth and O'Halloran, 2001; Hund-Rinke and Wiechering, 2001; Leland *et al*., 2001; Paoletti, 1999; Reinecke and Reinecke, 1998; Booth *et al*., 1998).

In India, dimethoate is one of the most popular insecticides that has been using in the agricultural field for crop protection. It is a kind of organophosphates pesticide which is used extensively due to low toxicity and less persistence (Jemec *et al*., 2007; Panda and Sahu, 2004). But its excessive use must have an effect on the non-target organisms like earthworm and soil microorganism and ultimately degrading the soil fertility.

Several studies were conducted on the toxic impact of different pesticides on earthworm (Rallmbke *et al*., 2007; Lagan and Shaw, 2006; Lydy and Linck, 2003; Kalka *et al.,* 2002; Ribera *et al.,* 2001; Morowati, 2000). Majority of the works were carried out to assess the potential risk of organophosphorus pesticides like malathion, phorate, monocrotophos, fenitrothion in tropical agro-ecosystem using earthworm as test species (Panda and Sahu, 1999, 2004; Patnaik and Dash, 1990). Few studies were also conducted on the toxic effect of imidacloprid, a common neonicotinoid insecticide, on earthworm (Capowiez *et al*., 2005, 2006), honey bee and predatory stinkbug (Torres and Ruberson, 2004; Suchail *et al*., 2001) in the tropical agro-ecosystem. By contrast, studies pertaining to dimethoate are scare (Patil *et al*., 1987). Hence, the present work was aimed to find out the lethal effect of dimethoate and its toxicity level at the recommended dose on earthworm which possesses a major role in maintaining soil health.

Materials and methods

Agrochemicals

Rogor 30% EC, the trade name for dimethoate, was used for carrying out the present work. The chemical composition of dimethoate is 0, 0-dimethyl-S-(N-methyl-carbamoylmethyl) di-thiophosphate.

Soil and earthworm

For conducting the toxicity test, soil and earthworms (*Drawida willsi*, Michaelsen) were collected from an upland non-irrigated paddy field which had no record of input of agrochemicals (fertilizers and pesticides). The soil had the following characteristics: laterite type, sandy loam texture, pH 6.8, organic matter (g%) 4.7, nitrogen (g%) 0.22 and a C/N ratio of 12.27.

Preparation of the experimental set

After collection, the soil was air dried and sieved. Simultaneously, the earthworms were cultured for one month at their native soil at moisture 20±2g% and temperature 25±2°C (Senapati and Dash, 1984) with a diet of 10% organic matter (cow dung + leaf litter).

At the time of toxicological study, earthworms were removed from culture pots and separated into three age-class on the basis of size (juveniles of <2cm,

immature of ≥2 to <4cm and adults of ≥4cm) and presence or absence of genital papilla and clitellium.

Various concentrations of dimethoate (mg a.i./ dry kg of soil equivalent) were first prepared in dilution of acetone (Table : 1-3) . After evaporation of the solvent, these were added to the soil surface maintaining moisture at 20±2g% and soil temperature 25±2°C and then mixed thoroughly to ensure a homogeneous mixture. Then 10 numbers of juvenile, immature and adult earthworms were released separately with respect to different dose. Five replicates were made for each dose and kept in the laboratory for 96 hours. Number of earthworm death in each sample with respect to different doses was recorded to find out the toxicity of dimethoate. The Finney's Probit Method (Finney, 1971) was also followed to calculate 96h LC_{50} values for juvenile, immature and adult earthworms.

Result

There was wide variation of toxicity of dimethoate on juvenile, immature and adult earthworms. Details of the toxicity with respect to different dose and replicates are given below.

Effect of dimethoate on juvenile earthworm

Mortality was not recorded when the worms were exposed to a concentration of 0.5 mg/kg of dimethoate in soil. But it resulted death of 12% earthworms at the exposure of 1 mg/kg of dimethoate in soil. However, 100% mortality was observed at the dose of 13 mg/ kg of dimethoate in soil.

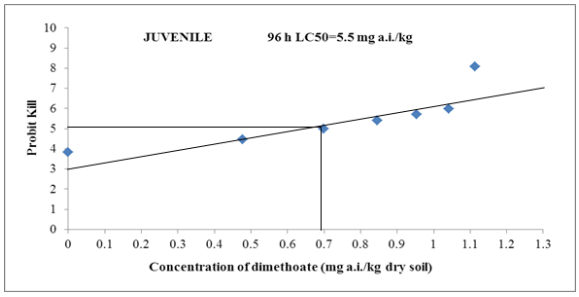

Fig. 1. Toxic impact of different concentrations of dimethoate on juvenile *D. willsi.*

There was variation of mortality with respect to different doses (Table-1). About 12% of juvenile died (average 1.2; range 1-2) at the exposure of 1 mg/kg of dimethoate in soil which gradually increased to 30% (average 3) at 3 mg/kg, 50% (average 5) at 5 mg/kg, 66% (average 6.6; range 6-7) at 7 mg/kg, 76% (average 7.6; range 7-8) at 9 mg/kg, 84% (average 8.4; range 8-9) at 11 mg/kg and no earthworm was alive at 13 mg/kg.

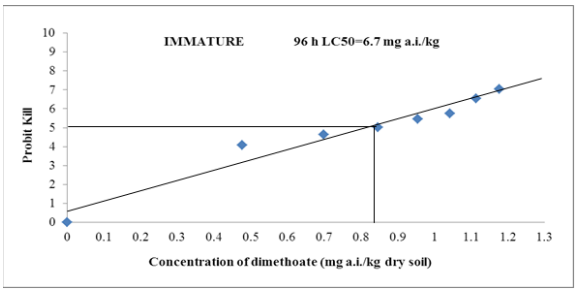

Fig. 2. Toxic impact of different concentrations of dimethoate on immature *D. willsi*.

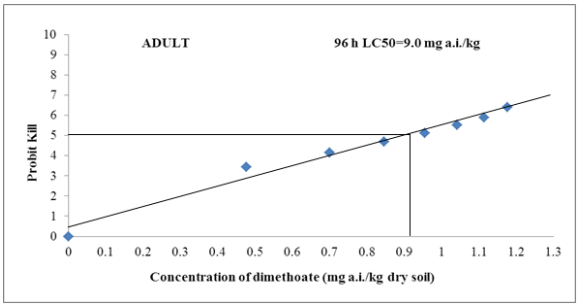

Fig. 3. Toxic impact of different concentrations of dimethoate on adult *D. willsi*.

The Finney's Probit analysis indicates the 96 h LC_{50} values of 5.5 (95% confidence limit) for juvenile earthworm with a range of 4.4 to 6.64 (Fig-1, Table-4).

Effect of dimethoate on immature earthworm

On exposure to 1 mg/kg of dimethoate in soil, the entire earthworm survived. When immature earthworms were exposed to 3 mg/kg of dimethoate in the soil, mortality initiated and 100% mortality was recorded at the exposure of 16 mg/kg.

The degree of toxicity was different with respect to different doses (Table-2). About 18% of immature died (average 1.8; range 1-2) at the exposure of 3 mg/kg of dimethoate in soil which gradually

increased to 36% (average 3.6; range 2-5) at 5 mg/kg, 52% (average 5.2; range 5-6) at 7 mg/kg, 68% (average 6.8; range 6-7) at 9 mg/kg, 76% (average 7.6; range 7-8) at 11 mg/kg, 94% (average 9.4; range 9-10) at 13 mg/kg and all the immature died at 16 mg/kg.

Table 1. Lethal effect at different concentration of dimethoate on juvenile *Drawida willsi*.

SL No.	Dose (mg/ kg soil)	No. of EW used in each replicate	Mortality in different replicates						Mortality (%)	Remark
			01	02	03	04	05	Average		
01	0.5 mg	10	0	0	0	0	0	0	0	No lethal effect
02	1 mg	10	1	1	1	1	2	1.2 (6)	12%	
03	3 mg	10	3	3	3	3	3	3 (15)	30%	
04	5 mg	10	5	5	5	5	5	5 (25)	50%	
05	7 mg	10	6	6	7	7	7	6.6 (33)	66%	
06	9 mg	10	7	7	8	8	8	7.6 (38)	76%	
07	11 mg	10	8	8	8	9	9	8.4 (42)	84%	
08	13 mg	10	10	10	10	10	10	10.0 (50)	100%	100% mortality

Table 2. Lethal effect at different concentration of dimethoate on immature *Drawida willsi*

SL No.	Dose (mg/ kg soil)	No. of EW used in each replicate	Mortality in different replicates						Mortality (%)	Remark
			01	02	03	04	05	Average		
01	1 mg	10	0	0	0	0	0	0	0	No lethal effect
02	3 mg	10	1	2	2	2	2	1.8 (9)	18%	
03	5 mg	10	2	3	4	4	5	3.6 (18)	36%	
04	7 mg	10	5	5	5	5	6	5.2 (26)	52%	
05	9 mg	10	6	7	7	7	7	6.8(34)	68%	
06	11 mg	10	7	7	8	8	8	7.6 (38)	76%	
07	13 mg	10	9	9	9	10	10	9.4 (47)	94%	
08	15 mg	10	9	10	10	10	10	9.8(49)	98%	
09	16 mg	10	10	10	10	10	10	10 (50)	100%	100% mortality

Table 3. Lethal effect at different concentration of dimethoate on adult *Drawida willsi*

SL No.	Dose (mg/ kg soil)	No. of EW used in each replicate	Mortality in different replicates						Mortality (%)	Remark
			01	02	03	04	05	Average		
01	1 mg	10	0	0	0	0	0	0	0	No lethal effect
02	2 mg									No lethal effect
03	3 mg	10	1	1	1	0	0	0.6 (3)	06%	
04	5 mg	10	2	2	2	2	2	2.0 (10)	20%	
05	7 mg	10	3	4	4	4	4	3.8 (19)	38%	
06	9 mg	10	5	5	5	6	6	5.4 (27)	54%	
07	11 mg	10	7	7	7	7	7	7.0 (35)	70%	
08	13 mg	10	8	8	8	8	9	8.2(41)	82%	
09	15 mg	10	9	9	9	9	10	9.2(46)	92%	
10	17 mg	10	10	10	10	10	10	10 (50)	100%	100% mortality

The Finney's Probit analysis indicates the 96 h LC_{50} values of 6.7 (95% confidence limit) for immature earthworm with a range of 5.2 to 8.2 (Fig-2, Table-4).

Effect of dimethoate on adult earthworm

There was no mortality of adult earthworm when exposed to dimethoate up to the dose of 2 mg/kg soil. But mortality started at the exposure of 3 mg/ kg of soil. The mortality gradually increased with the increase of doses and 100% mortality was observed at the dose of 17 mg/ kg soil.

A distinct variation of adult earthworm mortality was also recorded with respect to different doses of

dimethoate conducted in five replicates (Table-3). There was 6% mortality (average 0.6; range 0-1) of adult earthworm with exposure of 3 mg/kg of dimethoate which gradually increased to 20% (average 2.0) at 5 mg/kg of the pesticide, 38% (average 3.8; range 3-4) at 7 mg/kg, 54% (average 5.4; range 5-6) at 9 mg/kg, 70% (average 7.0) at 11 mg/kg, 82% (average 8.2; range 8-9) at 13 mg/kg, 92% (average 9.2; range 9-10) at 15 mg/kg and 100% mortality was recorded at 17 mg/kg.

Table 4. 96 h toxicity test for *D. willsi* exposed to different concentrations of dimethoate.

Concentration (mg a.i./kg dry soil)	Mortality	Emperical probit	Expected probit	96 h LC_{50} with 95% confidence limit
JUVENILE				
13	100	8.09	7.00	
11	84	5.99	6.48	
9	76	5.71	5.95	5.5 (range : 4.4-6.6)
7	66	5.41	5.41	
5	50	5.00	4.87	
3	30	4.48	4.35	
1	12	3.82	3.82	
0.5	--	--	--	
IMMATURE				
15	98	7.05	7.05	
13	94	6.55	6.55	
11	76	5.74	6.05	6.7 (range : 5.2-8.2)
9	68	5.47	5.55	
7	52	5.03	5.05	
5	36	4.64	4.57	
3	18	4.08	4.08	
1	--	----	----	
ADULT				
15	92	6.41	6.83	
13	82	5.88	6.05	
11	70	5.52	5.52	9.0 (range : 7.3-10.7)
9	54	5.13	5.00	
7	38	4.69	4.50	
5	20	4.16	4.00	
3	06	3.45	3.45	
1	--	----	---	

The Finney's Probit analysis indicates the 96 h LC_{50} values of 9.0 (95% confidence limit) for adult earthworm with a range of 7.3 to 10.7 (Fig-3, Table-4).

Discussion

Acute toxicity of earthworm is an efficient tool in assessing ecological risks of contaminated soils (Lukkari *et al.,* 2005; Hemibach, 1985) and the end point is mortality (Karnak and Hamelink, 1982; Dean-Ross, 1983; Ellis *et al.,* 2007). In this present experiment, dimethoate showed no mortality at the recommended agricultural dose (0.4 mg a.i./kg dry soil). Our results also showed that the 96 h LC_{50} value for all the age groups of *D. willsi* ranged from 5.5 to 9.0 mg a.i. dimethoate/kg dry soil. This is found to be much higher than the recommended agricultural dose. Similar trend is also reported in other organophosphosphates pesticide by a number of previous workers. Bharthi and Subha Rao (1984) reported 96-h LC_{50} of phosphamidon, monocrotophos and dichlorvos as 10.35 ppm, 4.87 ppm and 0.22 ppm respectively for *Lampito mauritii* in artificial soil. Patnaik and Dash (1990) exposed earthworms to different concentrations of pesticides for two hours, recorded 48-h LC_{50} of monocrotophos for *Drawida calebi* and *Octochactona surensis* to be 14.79 ppm and 14.13 ppm and fenitrothion to be 15.67 ppm and

15.14 ppm respectively. Reddy and Rao (2008) observed 24 and 48h LC_{50} for profenofos, an organophosphate to be 4.56 and 3.55 µg/cm² respectively for *Eisenia foetida*. Gupta *et al.* (2010) found that cypermethrin was more toxic to *Perionyx excavates* (LC_{50}-0.008 mg/kg) followed by endosulfan (LC_{50}-0.03 mg/kg), carbaryl (LC_{50}-6.07 mg/kg), chlorpyrifos (LC_{50}-7.3 mg/kg), aldicarb (LC_{50}-10.63 mg/kg) and monocrotophos (LC_{50}-13.04 mg/kg).

Though no mortality of earthworm was observed at the recommended agricultural dose, still dimethoate must have sub-lethal effects. Study found some sub-lethal effects on growth, reproduction and metabolism of earthworm even at the recommended dose (Dalby *et al.,* 1995; Neuhauser and Callahan, 1990). There is always chance of having potential risk of dimethoate on soil health. So it is proposed that the application of dimethoate should be restricted to recommended doses only for protecting the soil biota and safeguarding the soil health.

Acknowledgement

The authors are thankful to the DST, Govt. of India, New Delhi for providing infrastructural facilities through FIST Grant. Anindita Bhattacharya is thankful to Sambalpur University for a fellowship.

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