INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 3, No. 5, p. 78-86, 2013

RESEARCH PAPER OPEN ACCESS

Eco-toxicity of Neonicotinoid: a case study on the impact of imidacloprid using *Drawida willsi* earthworm as bioindicator

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

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

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

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

doi: http://dx.doi.org/10.12692/ijb/3.5.78-86 Article published on May 20, 2013

Abstract

Many pesticides are used in the world and in India to boost the crop productivity, among which imidacloprid; a neonicotinoid is widely used now a day due to its low toxicity. These pesticides no doubt have protected the crops from the dangerous pest but in turn are becoming dangerous for soil health. Therefore an experiment was conducted to find out the eco-toxicity of imidacloprid. 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 imidacloprid were prepared in dilution with acetone and sprayed on the soil surface. Five replicates for each concentration of the pesticides were prepared. Then ten numbers of juvenile, immature and adult earthworms were added separately into all the replicates of different concentrated samples. All the samples were kept in the laboratory under close vigil for 96 hours. Number of earthworm death with respect to doses and replicates were recorded and the 96h LC_{50} values for juvenile, immature and adult earthworm were calculated by Finney's Probit Method (Finney, 1971). It was found that the 96 h LC_{50} values with their 95% confidence limit of juvenile, immature and adult earthworm were 4.43, 7.96 and 12.45 mg a.i. imidacloprid/kg dry soil respectively. Although the recommended dose of imidacloprid was lower than the 96 h LC_{50} values of *D. willsi* for imidacloprid, but still it could affect the soil biota by altering its vital rates and metabolism due to bioaccumulation of the agrochemicals.

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

Introduction

Use of bioindicator in the study of eco-toxicity is a common practice throughout the world. Among the most bioindicators, the earthworms, snail and soil micro-organisms are widely used in toxicological study since they are the immediate victim of the agro-chemicals. Earthworms are used as bioindicator for assessing soil health due to easy availability, easy to handle and capability to improve the structure and fertility of the soil (Edwards and Lofty, 1982; Syers and Springett, 1984; Tiwari, 1993; Viswanathan, 1997; Booth *et al.*, 1998; Paoletti, 1999, Booth and O'Halloran, 2001; Hund-Rinke and Wiechering, 2001; Leland *et al.*, 2001; Hund-Rinke *et al.*, 2003; Muthukaruppan and Ganasekaran, 2010; Muthukaruppan *et al.*, 2005; Mahajan *et al.*, 2007; Curry *et al.*, 2008).

Pesticides are extensively used in the agricultural field to boost the crop production. However being an economic boon, they are proving themselves as ecological ban because they are deteriorating the soil fertility and health. Imidacloprid is a systemic insecticide used for control of insects on cereal crops, fruit trees etc. It is considered as the first generation of neonicotinoid compounds belonging to the chloronicotinyl subclass with both having gut and contact activities (Mullins, 1993).

The menace of agrochemical contaminated soil could be assessed through earthworm using acute toxicity test (Lukkari *et al.*, 2005). The acute toxicity test is an important tool until now for prediction of environmental contamination by different chemicals as it serves as a qualitative screen for the detection of other eco-toxicological effects (Neuhauser and Callahan, 1990; Panda and Sahu, 1999).

Imidacloprid is considered as the first generation of neonicotinoid compounds which is being used mostly in the Indian crop fields today due to its less toxicity. Several studies were conducted on toxic impact of imidacloprid on honeybees and predatory stinkbug (Suchail *et al.*, 2001; Torres and Ruberson, 2004). Few studies were conducted on toxic impact of imidacloprid using earthworms as a bioindicator on the soil health. Zang *et al.*, (2000) conducted a comparative genotoxicity study of imidacloprid on earthworm, *Eisenia felida*. Mosterrt *et al.*, (2002, 2000) conducted a comparative study of imidacloprid and other four insecticides on mortality and biomass by using earthworms of Pheretima group (Megascolecidae). Capowiez *et al.*, (2010; 2006; 2005; 2003a,b) and Capowiez and Berard (2006) conducted few studies on lethal and sub-lethal effects, acetylcholinesterase enzyme and behavioural response using two species of earthworms namely *Aporrectodea nocturna and Allolobophora iceterica* in response to imidacloprid. Faheem and Khan (2010) also conducted toxicity study of imidacloprid on protein contents using earthworm *Pheretima posthuma*. In contrary, no study so far has been conducted till date on earthworm *Drawida willsi* Michaelsen which constitutes the dominant earthworm in crop field of India. So the present study was carried out on the toxicity of imidacloprid in rice field soil using *Drawida willsi* Michaelsen, a dominant crop field earthworm under the following objectives .

(i) To find out whether the earthworm mortality is there at the recommended dose or not.
(ii) To find out the lethal effect of imidacloprid on earthworm.

Materials and methods

Soil and earthworm

Since the experiment aimed at toxicological study, soil and earthworms (*Drawida willsi*) were collected from such agricultural field where there had no record of input of agrochemicals. These were collected from an upland non-irrigated paddy field which was of laterite type, with sandy loam texture, pH 6.8, organic matter (g%) 4.7, nitrogen (g%) 0.22 and a C/N ratio of 12.27. 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 clitellum. The gut of earthworms was cleaned by immersing them in glass petriplates having 30 ml of tap water in 25±2°C for 24h.

Test agrochemicals

For this experiment, Victor 17.8% SL (Insecticide (India) Limited, Jammu), a commercial solution of imidacloprid was used as a test solution. The chemical composition of imidacloprid is 1-(1-(6-chloro-2-pyridimyl) methyl)-N-nitro-2-imidazolidinimene.

Different concentrations of imidacloprid were prepared in dilution with acetone (Table-1, 2 & 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. Such five replicates for each concentration of imidacloprid was prepared. Then ten numbers of juvenile, immature and adult earthworms were added separately into all the replicates of different concentrated samples. All the samples were kept in the laboratory under close vigil for 96 hours. Number of earthworm death (juvenile, immature and adult) with respect to dose and replicate samples were recorded and 96 h LC_{50} values for juvenile, immature and adult earthworms were calculated by the Finney's Probit Method (Finney, 1971).

Results

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

Table 1. Lethal effect at different concentration of imidacloprid 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 (Total)		
01	1 mg	10	0	0	0	0	0	0	0	No lethal effect
02	2 mg	10	1	1	1	1	1	1 (5)	10%	
03	3 mg	10	3	3	3	3	3	3 (15)	30%	
04	4 mg	10	4	4	4	4	4	4 (20)	40%	
05	5 mg	10	5	5	5	5	6	5.2 (26)	52%	
06	6 mg	10	6	7	7	7	7	6.8 (34)	68%	
07	7 mg	10	7	7	7	7	8	7.2 (36)	72%	
08	8 mg	10	8	9	9	9	9	8.8 (44)	88%	
09	9 mg	10	9	9	9	10	10	9.4 (47)	94%	
10	10 mg	10	10	10	10	10	10	10 (50)	100%	100% mortality

Effect on juvenile earthworm

There was no mortality of juvenile earthworm when exposed to imidacloprid at the dose of 1 mg/kg of soil. But about 10% of mortality was recorded at the expose of 2 mg/ kg of soil. Gradually the mortality increased with the increase of doses and 100% mortality was observed at the dose of 10 mg/ kg soil.

There was a distinct variation of juvenile earthworm mortality with respect to different doses of imidacloprid conducted in five replicates (Table-1). When they were exposed of 2 mg/kg of the imidacloprid, one earthworm out of ten earthworms died in all the five replicates which constituted 10%

of the total mortality. At 3 mg/kg of the pesticide, an average of 30% juveniles (average 3 in each replicate) died. Gradually the mortality increased to 40% (average 4 in each replicate) at 4 mg/ kg, 52% (average 5.2; range 5-6) at 5 mg/kg, 68% (average 6.8; range 6-7) at 6 mg/kg, 72% (average 7.2; range 7-8) at 7 mg/kg, 88% (average 8.8; range 8-9) at 8 mg/kg, 94% (average 9.4; range 9-10) at 9 mg/kg, and all the juvenile earthworm died at 10 mg/kg. The Finney's Probit analysis indicates the 96 h LC_{50} values of 4.43 (95% confidence limit) for juvenile earthworm with a range of 4.39 to 4.47 (Fig. 1, Table 4).

Table 2 . Lethal effect at different concentration of imidacloprid 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 (Total)		
01	1 mg	10	0	0	0	0	0	0	0	No lethal effect
02	2 mg	10	0	0	0	0	0	0	0	No lethal effect
03	4 mg	10	1	1	1	1	1	1 (5)	11%	
04	6 mg	10	3	3	3	3	3	3 (15)	21%	
05	8 mg	10	4	5	5	5	5	4.8 (24)	48%	
06	10 mg	10	6	6	6	6	7	6.2 (31)	62%	
07	12 mg	10	8	8	8	9	9	8.4 (42)	84%	
08	14 mg	10	9	10	10	10	10	9.6 (49)	96%	
09	15 mg	10	10	10	10	10	10	10 (50)	100%	100% mortality

Table 3 . Lethal effect at different concentration of imidacloprid 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	4 mg	10	0	0	0	0	0	0	0	No lethal effect
02	6 mg	10	1	1	1	1	2	1.2 (6)	12%	
03	8 mg	10	2	2	2	3	4	2.6 (13)	26%	
04	10 mg	10	3	3	3	3	4	3.2 (16)	32%	
05	12 mg	10	4	4	4	4	5	4.6 (21)	46%	
06	14 mg	10	5	5	5	6	7	5.6 (28)	56%	
07	16 mg	10	6	7	7	7	7	6.8 (34)	68%	
08	18 mg	10	7	7	7	8	8	7.4 (37)	74%	
09	20 mg	10	8	8	9	9	9	8.6 (43)	86%	
10	22 mg	10	9	9	9	9	10	9.2 (46)	92%	
11	24 mg	10	10	10	10	10	10	10 (100)	100%	100% mortality

Effect on immature earthworm

There was no mortality of immature earthworm when exposed to imidacloprid at the dose up to 2 mg/kg soil. But mortality started when they were exposed at 4 mg/kg soil and 100% mortality was observed at the dose of 15 mg/ kg soil.

A wide variation of toxicity of imidacloprid was recorded on immature earthworm mortality with respect to different doses conducted in five replicates (Table-2). On an average of one immature earthworm died in five replicates at the exposure of 4 mg/ kg soil which constituted 11% of the total mortality. The mortality increased to 21% (average 3) when they were exposed to 6 mg/kg soil, 48% (average 4.8; range 4-5) to 8 mg/kg, 62% (average 6.2; range 6-7) at 10mg/kg, 84% (average 8.4; range 8-9) 12mg/ kg and 96% (average 9.6; range 9-10) at 14 mg/kg. However, 100% mortality was observed at 15 mg/kg in all five replicates.

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

	Concentration (mg a.i./kg dry soil)	Mortality	Emperical probit	Expected probit	96 h LC_{50} with 95% confidence limit
JUVENILE	1	0	-	-	4.43 (4.39-4.47)
	2	10	3.72	3.85	
	3	30	4.48	4.375	
	4	40	4.75	4.75	
	5	52	5.05	5.05	
	6	68	5.47	5.275	
	7	72	5.58	5.50	
	8	88	6.18	5.65	
	9	94	6.55	5.80	
	10	100	8.09	5.95	
IMMATURE	2	0	-	-	7.96 (7.92-7.99)
	4	11	3.77	3.77	
	6	21	4.19	4.52	
	8	48	4.95	4.95	
	10	62	5.31	5.40	
	12	84	5.99	5.65	
	14	96	6.75	5.98	
ADULT	4	0	-	-	12.45 (12.43-2.47)
	6	12	3.82	3.82	
	8	26	4.33	4.33	
	10	32	4.56	4.68	
	12	46	4.92	5.00	
	14	56	5.13	5.30	
	16	68	5.47	5.47	
	18	74	5.64	5.75	
	20	86	6.08	5.90	
	22	92	6.41	6.08	

The Finney's Probit analysis indicates the 96 h LC_{50} values of 7.96 (95% confidence limit) for juvenile earthworm with a range of 7.92 to 7.99 (Fig. 2, Table 4).

Effect on adult earthworm

There was no mortality of adult earthworm when exposed to imidacloprid up to the dose of 4 mg/kg soil. But mortality started at the exposure of 6 mg/ kg of soil. The mortality gradually increased with the increase of doses and 100% mortality was observed at the dose of 24 mg/ kg soil.

A distinct variation of adult earthworm mortality was also recorded with respect to different doses of imidacloprid conducted in five replicates (Table-3). When they were exposed of 6 mg/kg of the imidacloprid, an average of 1.2 adult earthworms out of ten died in all the five replicates which constituted 12% of the total mortality. The mortality of adult earthworm gradually increased to 26% (average 2.6, range 2 to 4) at 8 mg/kg of the pesticide, 32% (average 3.2; range 3-4) at 10 mg/kg, 46% (average 4.6; range 4-5) at 12 mg/kg, 56% (average 5.6; range 5-7) at 14 mg/kg, 68% (average 6.8; range 6-7) at 16 mg/kg, 74% (average 7.4; range 7-8) at 18 mg/kg, 86% (average 8.6; range 8-9) at 20 mg/kg, 92% (average 9.2; range 9-10) at 22 mg/kg and all the juvenile earthworm died at 24 mg/kg.

The Finney's Probit analysis indicates the 96 h LC_{50} values of 12.45 (95% confidence limit) for juvenile earthworm with a range of 12.43 to 12.47 (Fig-3, Table-4).

Discussion

The recommended dose for imidacloprid was 0.05 mg a.i. per kg dry soil. Our study found no mortality of earthworm *D. willsi* at this dose. The present study also showed that the 96 h LC_{50} value for all the age groups of *D. willsi* ranged from 4.43 and 12.45 mg a.i. imidacloprid/kg dry soil which is much higher than the recommended agricultural doses. Similar results were also found by a number of

earlier workers in other species of earthworm. Zang *et al.* (2000) found that LC_{50} value for *Eisenia fetida* earthworm was 2 and 4 ppm in soil following application of imidacloprid. Mostert *et al.* (2002) found that LC_{50} of imidacloprid was 5 mg/kg for 48-h to the earthworm *Pheritima*. Capowiez *et al.* (2005) also found that the LC_{50} of imidacloprid for the anecic species *Aporrectodea nocturna* and the endogeic species *Allobophora icterica* was between 2 and 4 mg/kg dry soil. Sardo and Soares (2010) found that at higher concentration of imidacloprid, more mortality of earthworm was seen. These clearly support the present findings that the recommended dose of imidacloprid is not toxic to the earthworm *D. willsi* so far as the toxicity test is concerned.

There is higher possibility of having sub-lethal effect of imidacloprid on earthworm. This is because; all the agro-chemical undergoes bioaccumulation in the animal and plants bodies. In such cases, the recommended dose may not have any practical meaning. Any small amount may triggers eco-toxicity due to bio-accumulation. In such case, the degree of eco-toxicity depends upon the degree of bioaccumulation. Therefore earthworm shows sub-lethal effects even at the recommended agricultural doses (Reddy and Goud, 1987; Neuhauser and Callahan, 1990; Panda and Sahu, 1999; Morowati, 2000; Capowiez *et al.*, 2005).

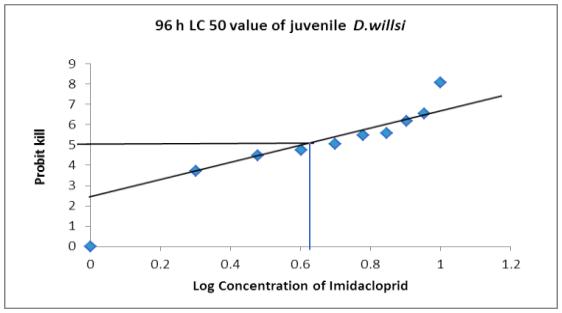

Fig. 1. Toxic impact of different concentrations of imidacloprid on juvenile *D. willsi*.

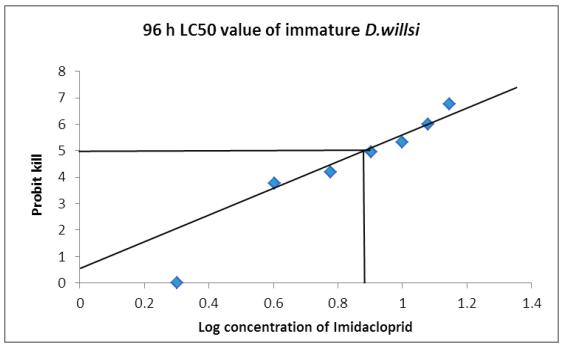

Fig. 2. Toxic impact of different concentrations of imidacloprid on immature *D. willsi*.

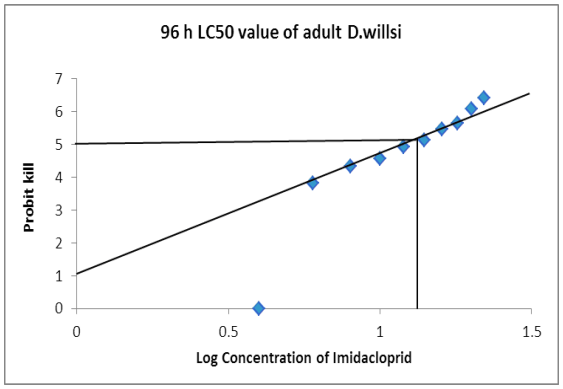

Fig. 3. Toxic impact of different concentrations of imidacloprid on adult *D. willsi*

So it is suggested that the application of imidacloprid must be restricted to recommended doses only for the sustenance of soil health. However it does not mean that the recommended dose is completely safe. Definitely it has some sub-lethal effect due to bioaccumulation of agro-chemicals in the plants and animals body that ultimately effect on 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.

References

Booth LH O, Halloran K. 2001. A comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus insecticides diazinon and chlropyrifos. Environmental Toxicology and Chemistry **20(11)** 2494-2502. http://dx.doi.org/10.1002/etc.5620201115

Booth LH, Heppelthwaite VJ, Eason CT. 1998. Choline esterase and glutathione s-transfer in the earthworm *Aporrectodea caliginosa* as biomarkers of organophosphate exposure. Proceedings of the 51st New Zealand Plant Protection Conference 138-142.

Capowiez Y, Dittbrenner N, Rault M,Triebskorn R, Hedde M, Mazzia C. 2010. Earthworm cast production as a new behavioural biomarker for toxicity testing. Environmental Pollution **158**, 388–393. http://dx.doi.org/10.1016/j.envpol.2009.09.003

Capowiez Y, Bastardie F, Costagliolia G. 2006. Sublethal effects of Imidacloprid on burrowing behaviour of two earthworm species L: Modification of the 3D burrow systems in artificial cores and consequences on gas diffusion in soil. Soil Biology and Biochemistry **38(2)**, 285-293. http://dx.doi.org/10.1016/j.soilbio.2005.05.014

Capowiez Y, Bérard A. 2006. Assessment of the effects of imidacloprid on the behavior of two earthworm species (Aporrectodea nocturna and Allolobophora icterica) using 2D terrraria. Ecotoxicology and Environmental Safety **64**, 198–206. http://dx.doi.org/10.1016/j.ecoenv.2005.02.013

Capowiez Y, Rault M, Costagliolia G, Mazzia, C. 2005. Lethal and sublethal effects of imidacloprid on two earthworm species (*Aporrectodea nocturna and Allolobophera icterica*). Biology and Fertility of Soils **41(3)**, 135-143. http://dx.doi.org/10.1007/s00374-004-0829-0

Capowiez Y, Pierret A, Moran CJ. 2003a. Characterisation of the threedimensional structure of earthworm burrow systems using image analysis and mathematical morphology. Biology Fertility of Soils **38**, 301–310. http://dx.doi.org/10.1007/s00374-003-0647-9

Capowiez Y, Rault M, Mazzia C, Belzunces, L. 2003b. Earthworm behaviour as a biomarker – a case study using imidacloprid. Pedobiologia **47**, 542–547. http://dx.doi.org/10.1078/0031-4056-00226

Curry JP, Doherty P, Purvis G, Schmidt O. 2008. Relationship between earthworm populations and management intensity in cattle-grazed pastures in Ireland. Applied Soil Ecology **39(1)**, 58-64. http://dx.doi.org/10.1016/j.apsoil.2007.11.005

Edwards CA, Lofty JR. 1982. Nitrogenous fertilizers and earthworm populations in agricultural soils. Soil Biology and Biochemistry, **14(5)**, 515-521. http://dx.doi.org/10.1016/0038-0717(82)90112-2

Faheem M, Khan MF. 2010. Toxicity of imidacloprid (Nicotinoid) against earthworm, *Pheretima posthuma* with special reference to its effects on protein. Journal of Basic and Applied Sciences, **6(1)**, 55-62.

Finney DJ. 1971. Probit analysis, Cambridge Uni. Press, Cambridge.

Hund-Rinke K, Wiechering H. 2001. Earthworm avoidance test for soil assessments : An alternative for acute and reproduction tests. Journal of Soils and Sediments **1**, 15-20. http://dx.doi.org/10.1007/BF02986464

Hund-Rinke K, Achazi R, Römbke J, Warnecke D. 2003. Avoidance test with *Eisenia fetida* as indicators for the habitat function of soils: Results of a laboratory comparison test. Journal of Soils and Sediments **3(1),** 7-12. http://dx.doi.org/10.1007/BF02989462

Leland JE, Mullis DE, Berry DF, 2001. Evaluating environmental hazards of land applying composted diazinon using earthworm bioassay. Journal of Environmental Science and Health **36(6)**, 821-834. http://dx.doi.org/10.1081/PFC-100107415

Lukkari T, Aatsinki M, Vaisanen A, Haimi J. 2005. Toxicity of copper and zinc assessed with three different earthworm tests. Applied. Soil Ecology **30(2),** 133-146.
http://dx.doi.org/10.1016/j.apsoil.2005.02.001

Mahajan S, Kanwar SS, Sharma SP. 2007. Long-term effect of mineral fertilizers and amendements on microbial dynamics in an alfisol of western Himalayas. Indian Journal of Microbiology, **47(1)**, 86-89.
http://dx.doi.org/10.1007/s12088-007-0016-8

Morowati M. 2000. Histochemical and histopathological study of the intestine of the earthworm (*Pheretima elongate*) exposed to a field dose of the herbicide glyphosate. The Environmentalist **20(2)**, 105-111.
http://dx.doi.org/10.1023/A:1006704009184

Mostert MA, Schoeman AtS, Merwe Mac, van der. 2002. The relative toxicities of insecticides to earthworm of *Pheretima* (Oligochaeta). Pest Management Science **58(5)**,
446-450.
http://dx.doi.org/10.1002/ps.473

Mostert MA, Schoeman AS, Van der Merwe, M. 2000. The toxicity of five insecticides to earthworms of the Pheretima group, using an artificial soil test. Pest Management Science **58**, 1093–1097.
http://dx.doi.org/10.1002/1526-4998(200012)56:12<1093::AID-PS259>3.0.CO;2-6

Mullins JW, 1993. Imidacloprid. A new nitroguanidine insecticide. (In: Pest control with enhanced environmental safety). ACS Symposium Series, American Chemical Society **524**, 183-198.

Muthukaruppan G, Ganasekaran P. 2010. Effect of Butachlor herbicide on earthworm *Eisenia fetida* – its histological perspicuity. Applied and Environmental Soil Science **2010**, 1-5.
http://dx.doi.org/10.1155/2010/850758

Muthukaruppan G, Janardhanan S. Vijayalakshmi G. 2005. Sublethal toxicity of the herbicide butachlor on the earthworm *Perionyx sansibaricus* and its histogical changes (5pp). Journal of Soils and Sediments **5(2)**, 82-86.
http://dx.doi.org/10.1065/jss2004.09.111

Neuhauser EF, Callahan CA. 1990. Growth and reproduction of the earthworm *Eisenia fetida* exposed to sub-lethal concentrations of organic chemicals. Soil Biology and Biochemistry **22(2)**,175-179.
http://dx.doi.org/10.1016/0038-0717(90)90083-C

Panda S, Sahu SK. 1999. Effects of malathion on the growth and reproduction of *Drawida willsi* (Oligochaeta) under laboratory conditions. Soil Biology and Biochemistry **31**, 363-366.
http://dx.doi.org/10.1016/S0038-0717(98)00135-7

Paoletti MG. 1999. The role of earthworms for assessment of sustainability and as bioindicators. Agriculture Ecosystems and Environment **74(1-3)**, 137-155.
http://dx.doi.org/10.1016/S0167-8809(99)00034-1

Rallmbke J, Jaonsch S, Junker T, Pohl B, Scheffezyk A, Schallnaay HJ. 2007. The effect of tributyltin-oxide on earthworm, springtails and plants in artificial and natural soils. Archives of Environmental Contamination and Toxicology, **52(4)**, 525-534.
http://dx.doi.org/10.1007/s00244-006-0099-y

Reddy MV, Goud AN, 1987. Impact of inorganic fertilizers on the earthworm population density of wetland rice (*Oryza sativa*) agroecosystems. Proceedings of the National Symposium on Ecotoxicology 154-158.

Ribera D, Narbonne JF, Arnaud C, Saint-Denis M. 2001. Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil, effect of carbaryl. Soil Biology and Biochemistry **33(7-8)**, 1123-1130.

http://dx.doi.org/10.1016/S0038-0717(01)00035-9

Sardo AM, Soares AM VM. 2010. Assessment of the effects of the pesticide imidacloprid on the behavior of the aquatic oligochaeta *Lumbricus variegatus*. Archives of Environmental Contamination and Toxicology **58(3)**, 648-656. http://dx.doi.org/10.1007/s00244-010-9470-0

Senapati BK, Dash MC. 1984. Functional role of earthworms in decomposer subsystem. Tropical Ecology **25(2)**, 54-73.

Suchail S, Guez D. Belzunces LP. 2001. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis melifera*. Environmental Toxicology and Chemistry **20(11)**, 2482-2486. http://dx.doi.org/10.1002/etc.5620201113

Syers JK, Springett JA, 1984. Earthworms and soil fertility. Plant and Soil **76 (1-3)**, 93-104. http://dx.doi.org/10.1007/BF02205570

Tiwari SC. 1993. Effects of organic manure and NPK fertilization on earthworm activity in an Oxisol. Biology and Fertility of Soils **16(4)**, 293-295. http://dx.doi.org/10.1007/BF00369307

Torres JB, Ruberson JR. 2004. Toxicity of thiamethoxam and imidacloprid to *Podisus nigrispinus* (Dallas) (Heteroptera: Pentatomidae) nymphs associated to aphid and whitefly control in cotton. Neotropical Entomology **33(1),** 99-106. http://dx.doi.org/10.1590/S1519-566X2004000100017

Viswanathan, R. 1997. Physiological basis in assessment of Ecotoxicity of pesticides to soil organisms. Chemosphere, **35(1-2)**, 323-334. http://dx.doi.org/10.1016/S0045-6535(97)00158-6

Zang Y, Zhong Y, Luo Y, Kong ZM. 2000. Genotoxicity of two novel pesticides for the earthworm *Eisenia fetida*. Environmental Pollution **108(2)**, 271-278. http://dx.doi.org/10.1016/S0269-7491(99)00191-8