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Environmental impact of Imidacloprid on soil fertility : a case study on *Drawida willsi* earthworm

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Abstract

Imidacloprid one of the major components of many widely used insecticides and is relatively persistent in soils. Earthworms are used as indicator species for ecotoxicological evaluation and risk assessment. The effect of Imidacloprid on mortality of a dominant crop field earthworm (*Drawida willsi,* Michaelsen) was studied under ideal laboratory conditions to rice field soil. Soil and earthworm were collected from a non-irrigated paddy field by hand sorting method. Different concentration of Imidacloprid are prepared in dilution with acetone and sprayed on the soil surface. Five replicate for each concentration of the pesticides were prepared. Earthworm, *D. willsi* was added to each replicates and 96 h LC_{50} value of the earthworm were calculated by probit analysis. It was found that the 96 h LC_{50} value for with their 95% confidence limit of juvenile, immature and adult earthworm was 4.43, 7.96 and 12.45 mg a.i. Imidacloprid/kg dry soil respectively. Although the recommended dose of Imidacloprid was lower 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.

Keywords : Imidaclopid toxicity, *Drawida willsi,* Soil fertility, Agrochemical toxicity.

1. Introduction

Environmental contamination by agro-chemicals is of growing concern throughout the world including India. But these agrochemicals are posing toxicity to soil biota where the earthworms are severely affected. Chemical substances harm on reaching a susceptible site on or in an organism (Kannan, 1997). Toxicity test is either done in the laboratory or in field condition by either observing the acute toxic effects or chronic toxic effects. In the former, the test is done for short duration and the end point is usually mortality (Karnak and Hamelink, 1982; Dean Ross, 1983). Chronic exposures are those that result from 90 or more daily, often continuous doses over lifetime and related to changes in growth, metabolism, reproduction or even death (Lofs-Holmin, 1982; Reinecke, 1992; Dalby *et al.,* 1995; Panda and Sahu, 1997, 1999).

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).

There are some biotas including plants, animals, and honey bees etc. that are used as indicators for chemical contamination. Earthworms are used as bioindicator for assessing soil health due to easy availability, easy to handle and

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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 *et al.,* 2005; Mahajan *et al.,* 2007; Curry et al, 2008).

Acute toxicity of earthworm can be efficient tool in assessing ecological risks of contaminated soils (Lukkari *et al.,* 2005). Many studies were done on the toxic effect of pesticide on earthworms (Morowati, 2000; Ribera *et al.,* 2001; Kalka *et al.,* 2002; Lydy and Linck, 2003 : Lagan and Shaw, 2006; Rallmbke *et al.,* 2007). Few studies have been made using imidacloprid pesticide for determination of acute and chronic toxicity in honeybees and predatory stinkbug (Suchail *et al.,* 2001; Torres and Ruberson, 2004) and earthworm (Capowiez *et al.,* 2005, 2006). Review of literature indicates that study to assess the potential risk of neonicotinoid pesticide in tropical agro ecosystem using earthworm as test species is less. Therefore, the present investigation was aimed to assess the toxicity of Imidacloprid in rice field using Drawida willsi Michaelsen, a dominant crop field earthworm.

2. Materials and Methods

Soil and Earthworm : Earthworm *(Drawida willsi)* and soil were collected from an upland non-irrigated paddy field. The soil was of laterite type, with sandy loam texture having pH 6.8, organic matter (g%) 4.7, nitrogen (g%) 0.22 and a C/N ratio of 12.27. This soil had no record of input of agrochemicals. Prior to use, the soil was air dried and sieved. The earthworm were cultured for one month at their native soil at moisture (20±2g%) and temperature (25±2ºC) (Senapati and Dash, 1979) with a diet of 10% organic matter (cow dung + leaf litter). Then earthworms were removed from culture pots and categorized into tree age class on the basis of size and presence or absence of genital papilla and clitellium: juveniles (<2cm), immature (=2<4cm) and adults (=4cm). Gut cleaning was done by immersing them in glass petriplates having 30 ml of tap water in 25±2°c for 24h.

Test Agrochemicals : A commercial solution of Imidacloprid (Victor 17.8% SL, Insecticide (India) Limited, Jammu) was used as a test solution for carrying out the present work. The chemical composition of Imidacloprid is 1-(1-(6-chloro-2-pyridimyl) methyl)-N-nitro-2-imidazolidinimene.

Toxicity tests of juvenile, immature and adult *D. willsi* earthworms were conducted for 96 hours with various concentrations of Imidacloprid applied to the soil. Five replicates for each concentration of Imidacloprid was made. Different concentrations of Imidacloprid respectively were prepared in dilution of acetone. 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. Number of earthworm death with respect to dose and replicate samples were recorded. The Finney's Probit Method (Finney, 1971) was also followed to calculate 96h LC50 values for juvenile, immature and adult earthworms.

3. Results

3.1 Effect on juvenile earthworm

No mortality was recorded when earthworms were exposed to 1 mg/kg of Imidacloprid in soil. When there was an application of 2 mg/kg of the pesticide, one earthworm out of ten earthworms is found to be dead in all the five replicates. So ten percent mortality was observed at 2 mg/kg of pesticide. At 3 mg/kg of the pesticide, three earthworms out of ten earthworms had died. Therefore at 3 mg/kg of pesticide, thirty percent of death of earthworm was recorded When 4 mg/kg of the pesticide was used, four out of ten earthworms in each of the replicates had faced extinction. Worm faced forty percent of mortality at a concentration of 4 mg/kg of pesticide. Five earthworms in four replicates and six earthworms in the fifth replicate out of total ten earthworm in each of all the replicates has declined with 5 mg/

kg of pesticide in soil. On 5 mg/kg, fifty two percent of earthworms were demised. In one replicate six earthworm and in the rest four replicates seven earthworms each was decreased by giving 6 mg/kg of the pesticide to the soil. By 6 mg/kg, sixty eight percent of mortality was noted. On providing 7 mg/kg of the pesticide to the soil, seven earthworm in each of all the four replicates and eight earthworms in the fifth replicate was diminished. Seventy two percent of earthworms were declined when they were exposed to concentration of 7 mg/kg. Out of ten earthworms in each of the five replicates, eight earthworms in one replicate and in rest nine earthworms had died by application of 8 mg/kg of pesticide to the soil. In a concentration of 8 mg/kg, eighty eight percent of earthworms were demised. In 9 mg/kg of the pesticide in soil, nine earthworm each in the three replicates and ten earthworms each in rest two replicates out of ten earthworms each in all replicates was lessen. Earthworm faces ninety four percent of mortality when a concentration 9 mg/kg of pesticide was applied. All earthworms had faced mortality by applying 10 mg/kg of the pesticide to the soil (Table-1).

Table-1 : Lethal effect at different concentration of Imidaclopid 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	1 mg	10	0	0	0	0	0	0	0	No lethal effect at 1 mg/kg soil dose
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 at 10 mg/kg soil dose

The concentration at which fifty percent of mortality had occurred was found from the graph. It is found that the 96 h LC_{50} values with their 95% confidence limit of juvenile earthworm was 5.5 with a range of 4.4 to 6.6 (Fig. - 1).

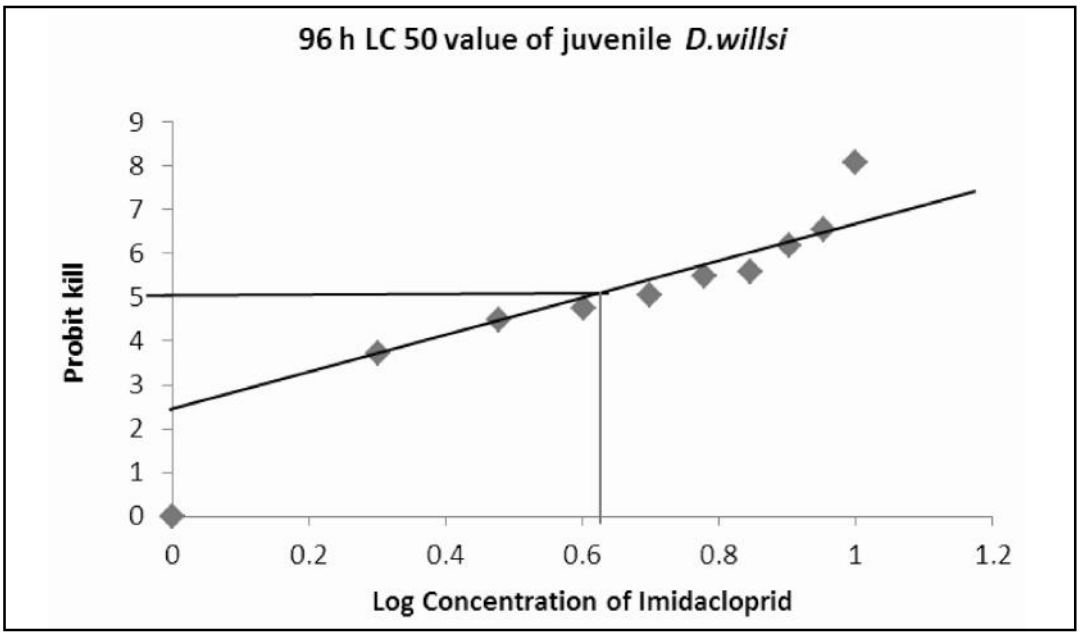

Fig. - 1 : Toxic impact of different concentrations of Imidacloprid on juvenile *D. willsi.*

3.2 Effect on immature earthworm

In our present investigation, we found that ninety six percent of the earthworm died when they were exposed to a concentration of 14 mg/kg of pesticide. Nine earthworms in one and ten earthworm in each of the rest four replicates died, out of total ten earthworms each in all the five replicates. By 12 mg/kg of pesticide concentration, eighty four percent of mortality was seen. In replicate first, second and third, eight earthworms in each and in fourth and fifth replicate nine earthworms in each were declined. Sixty two percent of earthworm death was recorded with 10 mg/kg of pesticide. Out of ten earthworms each in all the five replicates, six earthworms in four replicates and seven earthworms in the fifth replicate had declined. Forty eight percent of worm died when they were exposed to a concentration of 8 mg/kg of pesticide. Four earthworm in one replicate and five earthworms each in the rest of the five replicate were death. With 6 mg/kg of pesticide twenty one percent of earthworms were demised. On an average two to three earthworms faced mortality in all the replicates. By 4 and 2 mg/kg of pesticide concentration eleven and zero percent of earthworm were killed (Table-2).

Table-2 : Lethal effect at different concentration of Imidaclopid 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	2 mg	10	0	0	0	0	0	0	0	No lethal effect at 2 mg/kg soil dose
02	4 mg	10	1	1	1	1	1	1.2 (5)	11%	
03	6 mg	10	3	3	3	3	3	2.3 (15)	21%	
04	8 mg	10	4	5	5	5	5	4.8 (24)	48%	
05	10 mg	10	6	6	6	6	7	6.2 (31)	62%	
06	12 mg	10	8	8	8	9	9	8.4 (42)	84%	
07	14 mg	10	9	10	10	10	10	9.6 (49)	96%	96% mortality at 14 mg/kg soil dose

With the help of graph the 96 h LC50 values with their 95% confidence limit of immature earthworm was 7.96 with a range of 7.92 to 7.99 following the same method as above (Fig-2).

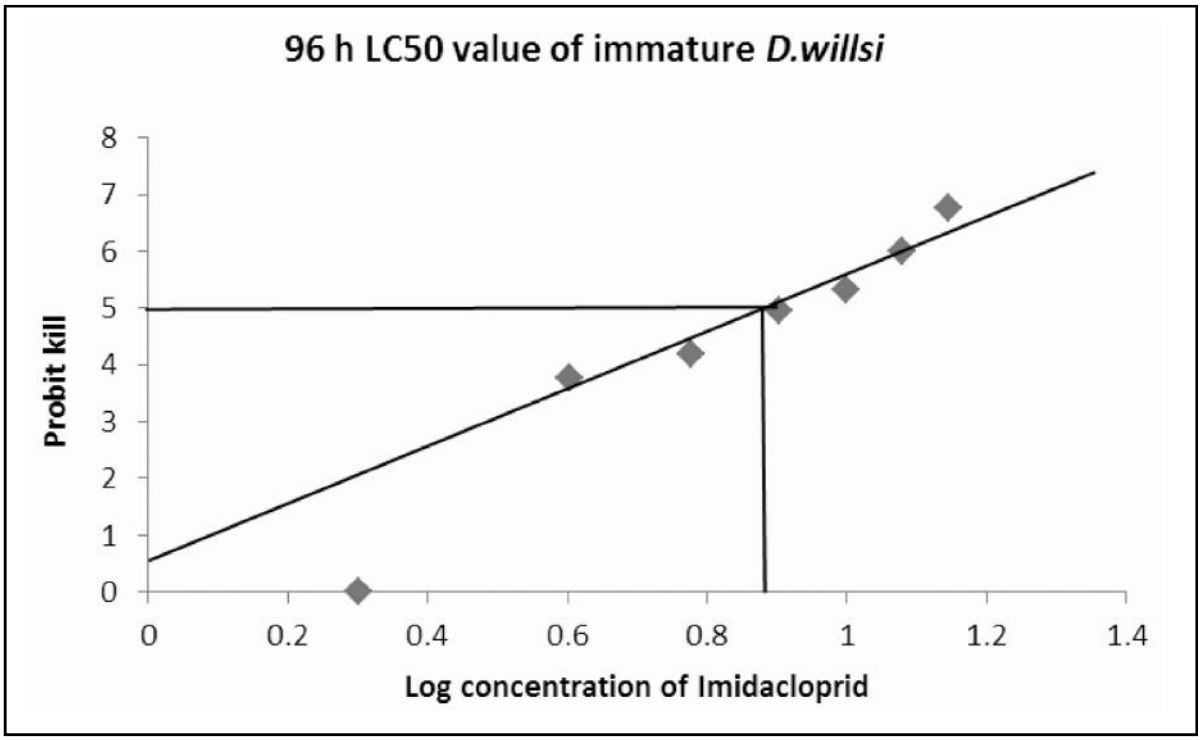

Fig. - 2 : Toxic impact of different concentrations of Imidacloprid on immature *D. willsi.*

3.3 Effect on adult earthworm

No mortality was observed with 4 mg/kg of pesticide concentration. Twelve percent of death was observed with 6 mg/kg of pesticide. One earthworm in each of the four replicate and two earthworms in the fifth replicate were found to dead out of ten earthworms each in all the five replicates by using 6 mg/kg of the pesticide. By 8 mg/kg, twenty six percent of earthworm kill was noticed. Out of ten earthworms each in all the five replicate, two earthworms in three sets, three and four earthworms in rest of the two replicates were found dead in 8 mg/kg of soil applied with the pesticide. With a concentration of 10 mg/kg of pesticide, thirty two percent of earthworm death was recorded. Three earthworm in four replicates and four earthworms in the last replicate were declined at a concentration of 10 mg/kg in soil. Forty six percent of mortality was observed with 12 mg/kg of pesticide application. Four earthworms in the first four replicate and five earthworms in the last replicate were found to be decreased. Earthworms were reduced by fifty six percent with 14 mg/kg of pesticide exposure. Out of ten earthworms each in five replicates, five earthworms in each of the three replicate and six and seven number of earthworms were found to reduce in the rest replicates. By application of 16 mg/kg of pesticide, sixty eight percent of worms were decreased. This time it was found that six earthworm in one replicate and seven earthworms in each of the four replicate was declined. Seven four percent of earthworm demised with 18 mg/kg of pesticide. Seven earthworms in each of the three replicates and in rest two replicates each, eight earthworms were reduced. Eighty six percent of earthworm faces death with 20 mg/kg of pesticide exposure. In each of the two replicates it was found that eight earthworms were no more whereas each of the rest three replicates

nine earthworms had died. By use of 22 mg/kg of pesticide, ninety two percent of earthworms were declined. In this time it was seen that nine earthworms in each of the four replicates were dead whereas no earthworm survived in the last replicate (Table-3).

Table-3 : Lethal effect at different concentration of Imidaclopid 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 at 4 mg/kg soil dose
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	5	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%	100% mortality at 22 mg/kg soil dose in 10th replicate

From the graph, the 96 h LC50 values with their 95% confidence limit of adult earthworm was 12.45 with a range of 12.43 to 12.47 (Fig. - 3).

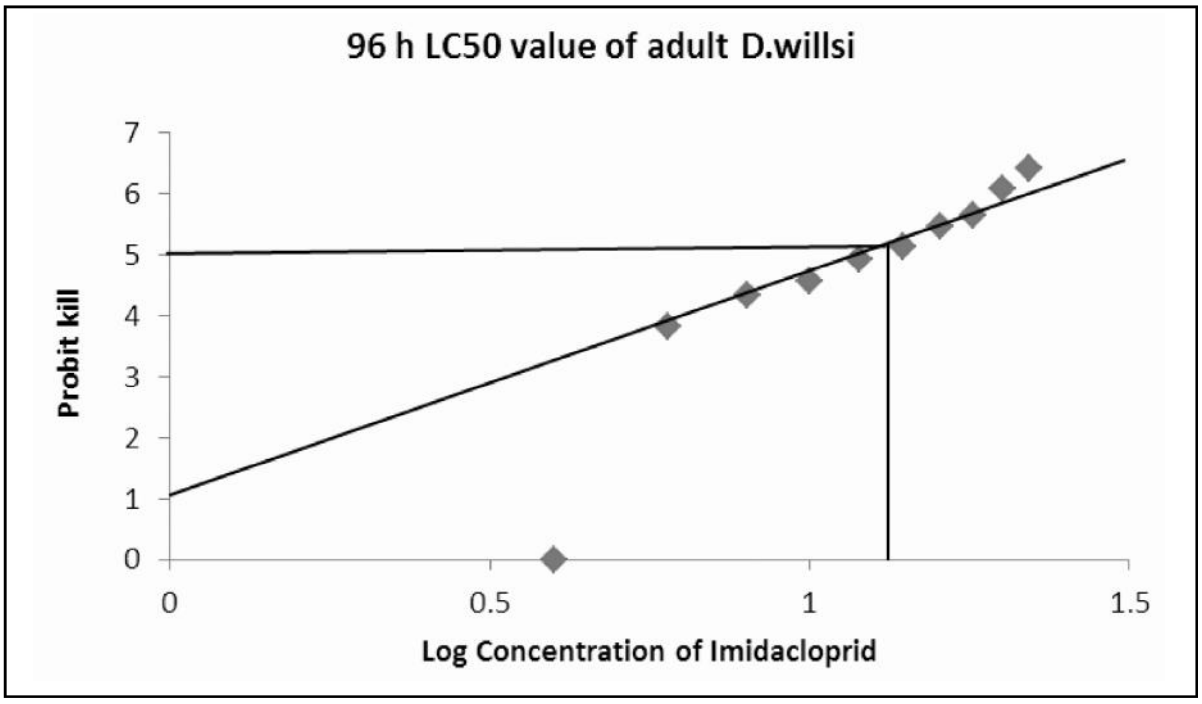

Fig - 3 : Toxic impact of different concentrations of Imidacloprid on adult *D. willsi.*

4. Discussion

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 ecotoxicological effects (Neuhauser and Callahan, 1990; Panda and Sahu, 1999).

Our results showed that the 96 h LC50 value for all the age groups of D. willsi ranged from 4.43 and 12.45 mg a.i. Imidacloprid/kg dry soil. The LC50 values of D. willsi for Imidacloprid was found to be much higher than the recommended agricultural doses (0.055 mg a.i./kg dry soil respectively).

Zang *et al.,* (2000) found that LC50 value of Eisenia fetida earthworm was 2 and 4 ppm in soil following application of Imidacloprid. Mostert et al. (2002) found that LC50 of Imidacloprid was 5 mg/kg for 48-h to the earthworm Pheritima. Capowiez *et al.,* (2005) have found that the LC50 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. Whatever study on Imidacloprid was made regarding toxicity on earthworms are consistent with our findings till date although no work had been done on effect of Imidacloprid on mortality of the earthworm Drawida willsi.

Toxicity data of all the agrochemicals in this present investigation indicate that these agrochemicals are not very toxic to the earthworm at recommended agricultural dose but it is reported that earthworm may show sublethal effects even at these recommended agricultural doses (Reddy and Goud, 1987; Neuhauser and Callahan, 1990; Panda and Sahu, 1999; Morowati, 2000; Capowiez *et al.,* 2005). Therefore further test on sub lethal effects of all the test chemicals used in the present investigation on parameters like growth, reproduction, metabolism etc. are required to draw a safe conclusion.

So it is suggested that the application of this pesticide should be restricted to recommended doses only for sustenance of soil health. Additional research is suggested as result from laboratory studies may differ from field experiment, in order to know the possible impact of this pesticide.

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