Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 4, No. 6, p. 64-72, 2014
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Lethal effect of urea on soil biota : a laboratory study on earthworm (*Drawida willsi*)

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Article published on June 05, 2014

Key words:

Abstract

The lethal effect of agrochemicals especially the pesticides on the soil ecosystem are well studied. Contrary to this, very few studies were conducted on the impact of fertilizer on the soil health. Urea among the fertilizers has been widely used in the agricultural field to boost up production. But only few studies were conducted to find out its toxic effect on soil ecosystem. However none of the study was undertaken on earthworm *Drawida willsi* Michaelsen as a test animal which constitutes the dominant earthworm in crop fields of India. Therefore an experiment was conducted to find out the eco-toxicity of urea on *D. willsi*. For this, soil and earthworm were collected from such agricultural field where there had no record of input of agrochemicals. Different concentrations of urea were prepared in dilution of water which then added to the experiment sets. Ten healthy gut evacuated earthworms of each age group (juvenile, immature, adult) were added to five replicates (polythene packets) for each concentration of fertilizer. On the other hand, the control group was given only water, and same numbers of earthworms were added with each age group. The experiment was maintained at 20±2g% soil moisture and 25±2°C soil temperature. Earthworm deaths were recorded and Finney's probit method was followed to calculate 96h LC_{50} values. Study found no mortality of juvenile, immature and adult *D. willsi* worms at the dose of 100, 200 and 200 mg urea /kg dry soil respectively. So, all the age groups of earthworm could tolerate up to 100 mg urea per kg of dry soil. At 800 mg urea /kg of dry soil 100%, 76% and 52% of mortality for juvenile, immature and adult earthworm was observed. Therefore, at this dose all the age groups of earthworms are in danger of extinction. The 96 h LC_{50} values with their 95% confidence limits for juvenile, immature and adult worms were 422.7 (422.66-422.74), 547.02 (546.96-547.08) and 827.9 (827.89-827.90) mg/kg respectively which is much more than the recommended agricultural doses of urea (79.04 mg/kg soil). Though the recommended dose of urea fertilizer is safe so far as the mortality is concerned, there must be some sub-lethal effect on earthworm like growth, reproduction and metabolism. Even the use of urea at higher dose may cause severe mortality of earthworm. So proper care should be taken on doses during use of urea in the agricultural field.

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Introduction

Population of humans is growing by leaps and bounce. To feed the mouth of millions, there is urgent need of more agricultural productivity that could be enhanced naturally (soil biota i.e. earthworms and microorganisms and litter) by enriching the soil fertility. But instead of this, the food productivity has been amplified by indiscriminate application of fertilizers like urea, superphosphate, potash and NPK along with pesticides. This must have been damaging the soil ecosystem by hampering soil dwelling micro and macro organisms (Haynes and Naidu, 1998; Reinecke and Reinecke, 2004) which play a major role in maintaining nutrient cycle of the soil. Since earthworms constitute more than 80% of the invertebrate biomass in most of the agro ecosystem of the world (Lee, 1985), it plays an important roles in improving the structure and fertility of the soil (Edward and Bohlen, 1996; Aira *et al*., 2006; Asawalam, 2006). So earthworm along with the microbial activity may serve as a useful soil quality indicator in sustained and short term field trials (Jordan *et al*., 2004).

Laboratory testing is often considered to be a better option for assessing the impact of agro-chemicals to living organisms (Christensen and Mather, 1994). Toxicity test in laboratory and field conditions offer reliable information on the effect of agrochemicals on earthworm. It evaluates acute, sub chronic and chronic exposures. Acute toxicity studies are conducted to ascertain the total adverse biological effects caused during a finite period of time, following the administration of single, normally large doses of a chemical (Reinecke and Reinecke, 1998). The end point of earthworm acute toxicity test is mortality and potency of the toxicant is expressed in terms of median lethal dose (LD_{50}) or median lethal concentration (LC_{50}).

A number of toxicity studies were conducted on the effect of agro-chemicals on earthworm. Most of these studies were conducted on the effect of pesticides (Rallmbke *et al*., 2007; Lagan and Shaw, 2006; Lydy and Linck, 2003; Kalka *et al.,* 2002; Ribera *et al.,* 2001; Morowati, 2000, Capowiez *et al*., 2005, 2006, Panda and Sahu, 1999, 2004; Patnaik and Dash, 1990). Very few studies were conducted on the toxicity of fertilizers on earthworm. Previous studies conducted by Edwards and Lofty (1982), Lofs-Holmin (1983), Syers and Springett (1984), Scullion and Ramshaw (1987), Estevez *et al*. (1996) and Curry *et al*. (2008) reported a positive effects of fertilizers on earthworms and increases their populations. On the other hand, studies conducted by Escritt and Arthur (1948), Edwards and Lofty (1975), Syers and Springett (1984), Mackay and Kladivko (1985), Potter *et al*. (1985), Ma *et al*. (1990), Marinissen (1992), Baker *et al*. (1993), Whalen *et al*. (1998), Bohlen *et al*. (1999), Hansen and Engelstad (1999), Whalen and Parmelee (2000), Emmerling and Paulsch (2001), Muldowney *et al*. (2003), Schmidt *et al*. (2003), Marhan and Scheu (2005), Bunemann *et al*. (2006), Mahajan *et al*. (2007) and Tindaon *et al*. (2011) reported a negative effects of fertilizers on earthworms. Most of these studies were conducted on bio-fertilizers and inorganic fertilizers excluding urea. Only few studies were conducted on the effect of urea on earthworm. Xu and Oldham (1997) have studied the lethal and sublethal effects of nitrogen fertilizer Ammonium nitrate on common toad while Xiao *et al*. (2004) used earthworm *Esisenia foelide* as the test animal. However, no study was conducted till date using earthworm *Drawida willsi*, a dominant crop field earthworm (>80% both in number and biomass) in India, as indicator species. Therefore, the present investigation was undertaken to assess the potential risk of urea on soil health.

Materials and methods

Fertilizers: Urea is used as a test chemical in the present investigation.

Soil and Earthworm: For the experiment, earthworm (*Drawida willsi*) and soil were collected from an upland non-irrigated paddy field which had no record of input of agrochemicals.

The soil was of laterite type with sandy loam texture. It had a pH of 6.8, organic matter (g %) 4.7, nitrogen g (%) 0.22 and a C/N ratio of 12.27. Prior to use, the soil was air dried and sieved. Then several experiment sets were prepared each with 500 gm soil in the polythene packet.

After collection, the earthworms were cultured for one month at their native soil in the laboratory condition at moisture (20±2g%) and temperature (25±2°C) (Senapati and Dash, 1979) with a diet of 10% organic matter (cow dung + leaf litter). Then earthworms were removed from culture pots and gut cleaned by immersing them in glass petriplates having 30 ml of tap water in 25±2°c for 24h. Then they were categorized into three age classes (juveniles: <2cm, immature : ≥2<4cm and adults : ≥4cm) on the basis of size and presence or absence of genital papilla and clitellium.

Toxicity Test: Different concentrations of urea were prepared in dilution of water. These were added to the experiment sets and then mixed thoroughly to ensure a homogeneous mixture with soil. Then ten healthy gut evacuated earthworms of each age group (juvenile, immature, adult) were added to five replicates (polythene packets) for each concentration of fertilizer. On the other hand, the control group was given only water and same numbers of earthworms were added for each age group. The experiment was maintained at 20±2g% soil moisture and 25±2°C soil temperature. Earthworm deaths were recorded and Finney's probit method was followed to calculate 96h LC_{50} values.

Result

Toxicity test of urea

Effect on juvenile earthworm

No mortality was reported when juvenile *D. willsi* earthworms were exposed to a concentration of 100 mg of urea fertilizers. But about 8% of mortality was recorded at the exposure of 200 mg/ kg of soil. Gradually the mortality increased with the increase of doses and 100% mortality was observed at the dose of 800 mg/ kg soil.

There was a distinct variation of juvenile earthworm mortality with respect to different doses of urea conducted in five replicates (Table-1). When they were exposed to 200 mg/kg of the urea, then one earthworm out of ten from each of the four replicates died which constituted 8% of the total mortality. At 300 mg/kg of the urea, an average of 16% juveniles (average 2 in each of three replicate and 2 each from other two replicates) died. Gradually the mortality increased to 32% (average 3 in each four replicate and 1 in one replicate) at 400 mg/ kg, 56% (average 5.6; range 5-6) at 500 mg/kg, 78% (average 7.8; range 7-8) at 600 mg/kg, 88% (average 8.8; range 8-9) at 700 mg/kg and all the juvenile earthworm died at 800 mg/kg.

Table 1. Lethal effect at different concentration of urea 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	100	10	0	0	0	0	0	0	0	No lethal effect
02	200	10	1	1	1	1	0	0.8(4)	8%	
03	300	10	1	1	2	2	2	1.6 (8)	16%	
04	400	10	3	3	3	3	4	3.2(16)	32%	
05	500	10	5	5	6	6	6	5.6(28)	56%	
06	600	10	7	8	8	8	8	7.8(39)	78%	
07	700	10	8	9	9	9	9	8.8(44)	88%	
08	800	10	10	10	10	10	10	10.0 (50)	100%	100% mortality

The Finney's Probit analysis indicates the 96 h LC_{50} values of 422.7 (95% confidence limit) for juvenile earthworm with a range of 422.66 to 422.74 (Fig-1).

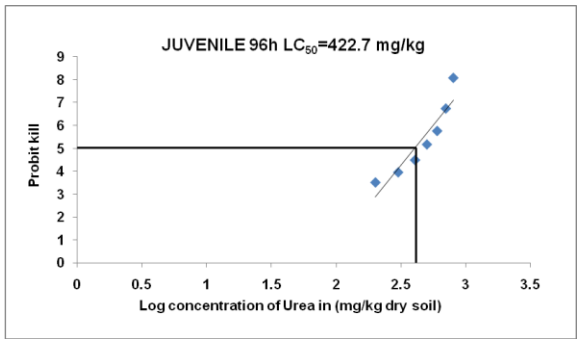

Fig. 1. Lethal effect at different concentrations of urea on juvenile *D. willsi*

Effect on immature earthworm

There was no mortality of immature earthworm when exposed to urea at the dose up to 200 mg/kg soil. But mortality started when they were exposed at 300 mg/kg soil and 100% mortality was observed at the dose of 1200 mg/ kg soil.

A wide variation of toxicity of urea was recorded on immature earthworm mortality with respect to different doses conducted in five replicates (Table 2). On an average, one immature earthworm died in five replicates at the exposure of 300 mg/ kg soil which constituted 10% of the total mortality. The mortality increased to 28% (average 2.8) when they were exposed to 400 mg/kg soil, 54% (average 5.4; range 5-6) to 600 mg/kg, 76% (average 7.6; range 7-8) at 800 mg/kg and 86% (average 8.6; range 8-9) 1000 mg/ kg. However, 100% mortality was observed at 1200 mg/kg in all five replicates.

Table 2. Lethal effect at different concentration of urea 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	100	10	0	0	0	0	0	0	0	No lethal effect
02	200	10	0	0	0	0	0	0	0	No lethal effect
03	300	10	1	1	1	1	1	1 (5)	10%	
04	400	10	2	3	3	3	3	2.8 (14)	28%	
05	600	10	5	5	5	6	6	5.4 (27)	54%	
06	800	10	7	7	8	8	8	7.6 (38)	76%	
07	1000	10	8	8	9	9	9	8.6 (43)	86%	
08	1200	10	10	10	10	10	10	10 (50)	100%	100% mortality

The Finney's Probit analysis indicates the 96 h LC_{50} values of 547.02 (95% confidence limit) for immature earthworm with a range of 546.96 to 547.08 (Fig-2).

Effect on adult earthworm

There was no mortality of adult earthworm when exposed to urea up to the dose of 200 mg/kg soil. But mortality started at the exposure of 300 mg/kg of soil. The mortality gradually increased with the increase of doses and 100% mortality was observed at the dose of 1300 mg/ kg soil.

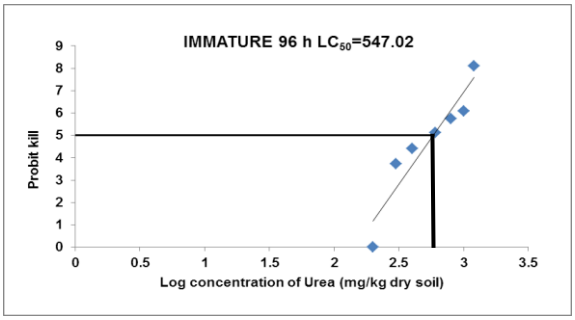

Fig. 2. Lethal effect at different concentrations of urea on immature *D. willsi*

A distinct variation of adult earthworm mortality was also recorded with respect to different doses of urea conducted in five replicates (Table-3). When they were exposed to 300 mg/kg of the urea, an average of 0.8 adult earthworms out of ten died in all the five

replicates which constituted 8% of the total mortality. The mortality of adult earthworm gradually increased to 18% (average 1.8, range 1 to 2) at 400 mg/kg of the urea, 22% (average 2.2; range 2-3) at 500 mg/kg, 38% (average 3.8; range 3-4) at 600 mg/kg, 42% (average 4.2; range 4-5) at 700 mg/kg, 52% (average 5.2; range 5-6) at 800 mg/kg, 68% (average 6.8; range 6-7) at 900 mg/kg, 76% (average 7.6; range 7-8) at 1000 mg/kg, 80% (average 8.0; range 8) at 1100 mg/kg, 94% (average 9.4: range 9-10) at 1200 mg/kg and all the adult earthworm died at 1300 mg/kg.

Table 3. Lethal effect at different concentration of urea 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	200	10	0	0	0	0	0	0	0	No lethal effect
02	300	10	0	1	1	1	1	0.8 (4)	8%	
03	400	10	1	2	2	2	2	1.8 (9)	18%	
04	500	10	2	2	2	2	3	2.2 (11)	22%	
05	600	10	3	4	4	4	4	3.8 (19)	38%	
06	700	10	4	4	4	4	5	4.2 (21)	42%	
07	800	10	5	5	5	5	6	5.2 (26)	52%	
08	900	10	6	7	7	7	7	6.8 (34)	68%	
09	1000	10	7	7	8	8	8	7.6 (38)	76%	
10	1100	10	8	8	8	8	8	8.0 (40)	80%	
11	1200	10	9	9	9	10	10	9.4 (47)	94%	
12	1300	10	10	10	10	10	10	10 (100)	100%	100% mortality

The Finney's Probit analysis indicates the 96 h LC_{50} values of 827.9 (95% confidence limit) for adult earthworm with a range of 827.89 to 827.90 (Fig-3).

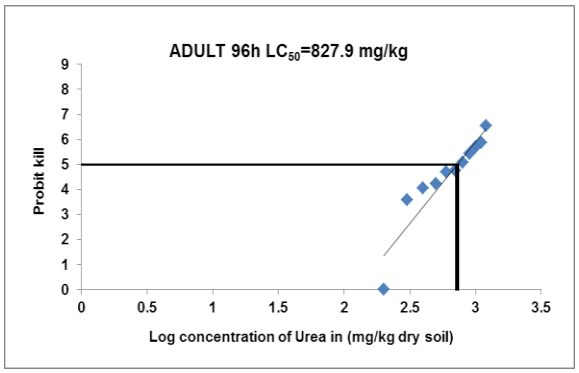

Fig. 3. Lethal effect at different concentrations of urea on adult *D. willsi*

From the present experiment, it was also found that the juvenile, immature and adult *D. willsi* worms did not die with application of urea upto 100, 200 and 200 mg/kg dry soil respectively. This indicates that all the age groups of earthworm could tolerate upto 100 mg urea per kg of soil. Similarly, 100%, 76% and 52% mortality of juvenile, immature and adult earthworm was observed at concentration of 800 mg urea / kg dry soil (Fig-4). This further indicates that all the age groups of earthworm are in danger of extinction at this dose.

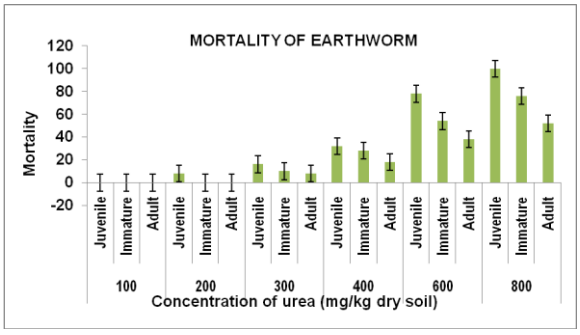

Fig. 4. Comparative lethal effect at different concentrations of urea on different age group of earthworm *D. willsi*.

Discussion

The present investigation found that there was a variation in mortality in different age class of *D. willsi* with response to toxicity of urea. There was 100% mortality at 800 mg urea/ kg dry soil for juvenile and immature while in case of adult, it was 1000 mg urea/ kg dry soil. The 96h LC_{50} value was found to be 422.7 for juvenile, 547.02 for immature and 547.02 for adult *D. willsi*. This clearly indicates that the recommended dose for urea (79.04 mg/kg) is safe so far point of mortality is concern.

Similar result also reported by Xiao *et al.* (2004). They recorded that the mortality rate of earthworm reached 100% when the concentration of urea was more than 1500 mg/kg. However there was positive and beneficial effect of urea at a lower concentration of 500 mg/kg soil. Xu and Oldham (1997) have also studied the lethal and sub-lethal effects of nitrogen fertilizer Ammonium nitrate on common toad and found that the 96h and 168h LC_{50} value were 1704mg/l and 1637 mg/l respectively.

Although there was no report of mortality of earthworm at the recommended dose, the use of urea fertilizer cannot be safe. It must have some sub-lethal effects even at the recommended agricultural doses (Reddy and Goud, 1987). Therefore further test on sub lethal effects of urea, on parameters like growth, reproduction, metabolism etc. are required to draw a safe conclusion.

The wide variations in the result of experiment may be explained in terms of the mode of action. The effect of fertilizers on earthworms may be direct by changing the acidity of the soil or indirect by changing the form and quantity of the vegetation that ultimately provides food for worms (Edward and Lofty, 1977). There is now increasing evidence to show that the use of inorganic fertilizers can be beneficial or adverse effect on both the crop productivity and earthworm populations (Edwards and Lofty, 1982; Syers and Springett, 1984, Tiwari, 1993; Mahajan *et al.*, 2007; Curry *et al.*, 2008).

In conclusion, the present experiment suggests that although the fertilizer urea is not very toxic to earthworm at the recommended doses still some sub-lethal effect may cause toxicity to the agro-ecosystem.

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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