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Toxic effect of superphosphate on soil ecosystem using earthworm *Drawida willsi* as test specimen

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Abstract

In the name of "Green Revolution", enormous use of agrochemical had resulted threat to soil ecosystem by killing the beneficial soil organisms including earthworm. Several studies were conducted on the toxic impact of some fertilizers on earthworm. On contrary, few studies were conducted on the effect of superphosphate on earthworm and no concrete conclusion could be found out. So the present experiment was undertaken to find out the effect of superphosphate on soil ecosystem using earthworm (*Drawida willsi*) as test animal. For this different concentration of superphosphate were mixed with water and added to the replicates of soil containing different age group of *D.willsi*. In control, only water was added to soil. Earthworm deaths were recorded with respect to different dose of Superphosphate and Finney's probit method was followed to calculate 96h LC_{50} values. The experiment found that all the juvenile and immature worms thrived in soil containing up to 50 mg/kg while adult at 100 mg/kg of superphosphate. Worms were when exposed to concentration as high as 400 mg/kg of superphosphate, 100% of juveniles were killed. However at the same concentration 74% of immature and 53% of adults were killed. The 96 h LC_{50} values with their 95% confidence limits for juvenile, immature and adult worms were 222.8 (222.77-222.83), 262.4 (262.35-262.45) and 314.05 (313.98-314.12) mg/kg respectively. This indicates that the earthworms were affected by the use of Superphosphate. So it is suggested that Superphosphate should be used in their recommended doses to maintain the soil health.

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Introduction

The application of agrochemicals (i.e. fertilizers and pesticides) and use of heavy machineries, aptly described as "Green Revolution" has indeed brought about dramatic increases in crop yields (Ramakrishnan, 1992). Therefore, India has made an impressive progress in food production.

While agrochemicals usage has led to increased crop production and greater economic returns to farmers, their impact on soil and water environment and crop quality was not considered relevant until the last ten to twenty years. There is now increasing evidence to show that indiscriminate use of agrochemicals can have adverse effect on the physical, chemical and biological status of the soil (Lee and Pankhrust, 1992; Sebiomo *et al.,* 2011).

Soil harbors many beneficial organisms like earthworm. The soil organisms perform two major functions in soil subsystem: (i) The formation of soil organic matter and (ii) the mineralization of essential elements. In addition to influencing the carbon and nutrient fluxes, soil organisms play an important role in affecting soil structure through physical activities (Hendrix *et al.,* 1990). When fertilizers are 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. So any change in the soil due to agrochemical must have an effect on earthworm as well as on soil health. 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).

Several studies were conducted on the toxic impact of some pesticides on soil organisms especially the earthworms. On contrary, very few studies were conducted on the toxicity of fertilizers on earthworm. Among these, some of them reported a positive effect of fertilizers on earthworms and increases their populations (Edwards and Lofty, 1982; Lofs-Holmin, 1983; Syers and Springett, 1984; Scullion and Ramshaw, 1987; Estevez *et al.,* 1996; Curry *et al.,* 2008). Few workers also reported negative effects of fertilizers on earthworms (Syers and Springett, 1984; Estevez *et al.,* 1996; Edwards and Lofty, 1975; 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; Xiao *et al.,* 2004; Marhan and Scheu, 2005; Bunemann *et al.,* 2006; Mahajan *et al.,* 2007; Tindaon *et al.,* 2011; Bhattacharya and Sahu, 2014). However, no study was conducted till date on the effect of superphosphate using tropical 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 superphosphate on soil health.

Materials and methods

Soil and Earthworm

Since the experiment aimed at toxicological study, soil and earthworms (*D. 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 of soil, 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^{o}C with a diet of 10% organic matter (cow dung + leaf litter) (Senapati and Dash, 1984).

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.

Fertilizer

Different concentrations of superphosphate were prepared in dilution of water. These were added to the soil surface and then mixed thoroughly with enough water to ensure a homogeneous mixture. The same procedure with only water was applied to prepare the control.

Results

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

Table 1. 96 h toxicity test for *D. willsi* exposed to different concentrations of superphosphate.

	Concentration (mg a.i./kg dry soil)	Mortality	Emperical probit	Expected probit	96 h LC_{50} with 95% confidence limit
JUVENILE	50	0	--	--	
	100	7	3.52	2.20	
	150	15	3.96	3.55	
	200	35	4.61	4.45	222.8
	250	58	5.20	5.20	(222.77-222.83)
	300	77	5.74	5.80	
	350	93	6.48	6.25	
	400	100	8.09	6.70	
IMMATURE	50	0	--	--	
	100	3	3.12	1.95	
	150	11	3.77	3.10	
	200	25	4.33	3.93	
	250	41	4.77	4.55	262.4
	300	52	5.05	5.05	(262.35-262.45)
	350	63	5.33	5.45	
	400	74	5.64	5.77	
	450	81	5.88	6.15	
	500	92	6.41	6.47	
	550	100	8.09	6.70	
ADULT	100	0	--	--	
	150	5	3.36	1.50	
	200	12	3.82	2.55	
	250	22	4.23	3.40	
	300	34	4.59	4.05	
	350	41	4.77	4.55	314.05
	400	53	5.08	5.08	(313.98-314.12)
	450	67	5.44	5.50	
	500	79	5.81	5.95	
	550	85	6.04	6.30	
	600	93	7.46	6.60	
	650	100	8.09	7.02	

Effect on juvenile earthworm

There was no mortality of juvenile earthworm when exposed to superphosphate at the dose of 50 mg/kg of soil. Gradually the mortality increased with the increase of doses and 100% mortality was observed at the dose of 400 mg/ kg soil.

There was a distinct variation of juvenile earthworm mortality with respect to different doses of

superphosphate conducted in five replicates (Table-1). About 7% mortality of earthworms were recorded when they were exposed to 100 mg/kg of Superphosphate in all the five replicates. The mortality increased to 15% while they were exposed to 150 mg/ kg soil followed by 35% at 200 mg/kg, 58% at 250 mg/kg, 77% at 300 mg/kg, 93% at 350 mg/kg and all the juvenile earthworms died at 400 mg/kg.

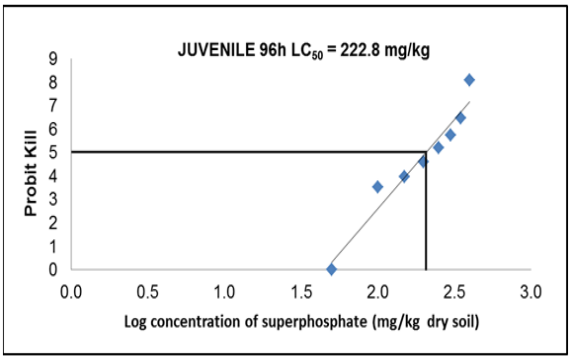

Fig. 1. 96 h toxicity test for juvenile *D. willsi* exposed to different concentrations of superphosphate.

The Finney's Probit analysis indicates that the 96 h LC_{50} values with 95% confidence limit for juvenile earthworm was 222.8 with a range of 222.77 to 222.83 (Fig-1, Table-1).

Effect on immature earthworm

There was no mortality of immature earthworm when exposed to superphosphate at the dose up to 50 mg/kg soil. But mortality started when they were exposed at 100 mg/kg soil and 100% mortality was observed at the dose of 550 mg/ kg soil.

A wide variation of toxicity of Superphosphate was recorded on immature earthworm mortality with respect to different doses conducted in five replicates (Table-1). About 3% mortality was recorded when earthworms were exposed at 100 mg/kg of superphosphate to soil followed by 11% at 150 mg/kg, 25% at 200 mg/kg, 41% at 250 mg/kg, 52% at 300 mg/kg, 63% at 350 mg/kg, 74% at 400 mg/kg and 81% at 450 mg/kg, 92% at 500 mg/kg soil. However, 100% mortality was observed at 550 mg/kg in all five replicates.

The Finney's Probit analysis indicates the 96 h LC_{50} values with 95% confidence limit for immature earthworm was 262.4 with a range of 262.35 to 262.45 (Fig-2, Table-1).

Effect on adult earthworm

There was no mortality of adult earthworm when they were exposed to superphosphate up to the dose of 100 mg/kg soil. But mortality started at the exposure of 150 mg/ kg of soil. The mortality gradually increased with the increase of doses and 100% mortality was observed at the dose of 650 mg/ kg soil.

A distinct variation of adult earthworm mortality was also recorded with respect to different doses of superphosphate conducted in five replicates (Table-3). About 5% mortality was recorded when they were exposed at 150 mg/kg of superphosphate to soil followed by 12% at 200 mg/kg, 22% at 250 mg/kg, 34% at 300 mg/kg, 41% at 350 mg/kg, 53% at 400 mg/kg, 67% at 450 mg/kg, 79% at 500 mg/kg, 85% at 550 mg/kg, 93% at 600 mg/kg and 100% at 650 mg/kg of superphosphate to soil.

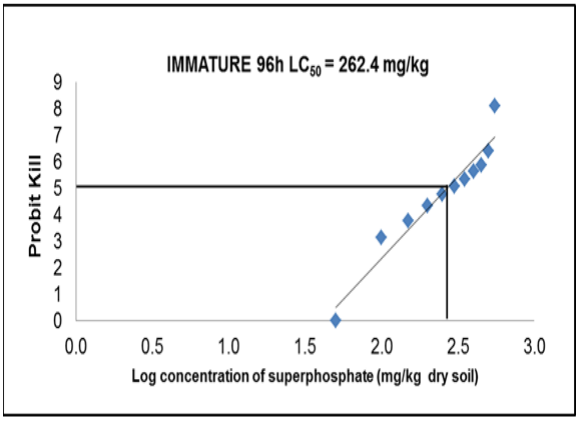

Fig. 2. 96 h toxicity test for immature *D. willsi* exposed to different concentrations of superphosphate.

The Finney's Probit analysis indicates the 96 h LC_{50} values with 95% confidence limit for adult earthworm was 314.05 with a range of 313.98 to 314.12 (Fig-3, Table-1).

Discussion

In agricultural field, superphosphate is applied at a

rate of 16 kg/acre which is equivalent to 39.52 mg/kg soil (Anon, 1987). This figure is much less than the LC_{50} value of the present experiment of 222.77 - 314.12 mg/kg for superphosphate. Previous experiment conducted on earthworm (*Eisenia foetida*) by filter contact method also found mortality at *210mg/5ml (i.e. 300µg/cm2)* (Abbiramy *et al.,* 2013). This clearly indicates that the use of superphosphate at the recommended dose is found to be safe for earthworm so far as the toxicity test is concerned. However, there is higher possibility of having sub-lethal effect of Superphosphate 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. Hence, the degree of eco-toxicity depends upon the degree of bioaccumulation.

It is also evident that the effects of various inorganic fertilizers on earthworms apparently vary from site to site. 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).

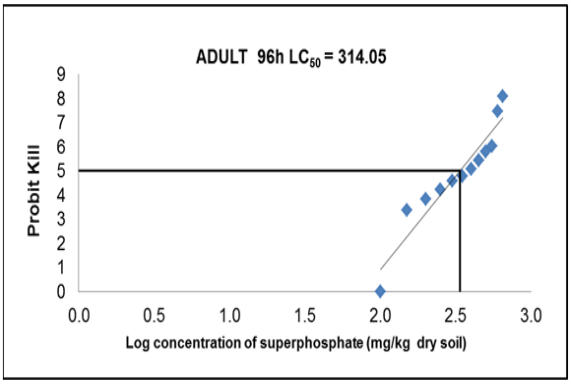

Fig. 3. 96 h toxicity test for adult *D. willsi* exposed to different concentrations of superphosphate.

So it is suggested that the application of Superphosphate 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 affect the soil health.

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