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Original Research Paper

Toxicity Assessment of *Annona squamosa* Oil Cake Using Earthworm Bioassay

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ABSTRACT

An earthworm bioassay was conducted to assess the ecotoxicity of *Annona squamosa* oil cake amended soil using the species *Eudrilus eugeniae*. Mortality and abnormal morphology of earthworms exposed to different concentrations of the oil cake, i.e., 10, 100, 112.5, 125 and 150g/kg were examined. Mortality was measured as the ecological end point in the tests. *Annona squamosa* oil cake was toxic to the earthworm and the severity of the response increased with increasing concentration of the oil cake. The LC_{50} value showed that the mortality observed was significantly different from the negative control, suggesting that it may be attributed to the effect of addition of *Annona squamosa* oil cake.

INTRODUCTION

Earthworms have been considered as an indicator of soil health and recommended as a critical representative of soil organisms reflecting the nature of the soil in which they inhabit (Culy & Berry 1995). The special emphasis in involving earthworm species in toxicological studies is based on the fact that earthworm skin is a significant route for contaminant uptake (Briggs et al. 1980). Earthworm bioassays are widely accepted tool for assessing toxicity of the contaminated soils (Callahan et al. 1985, Edwards 1984, Van Gestel & Van Dis 1988). This is largely due to the fact that they ingest large quantities of the soil and are in full contact with the substrate they consume (Sandoval et al. 2001). Mortality is the major parameter that has been mostly used to test the toxicity in earthworm (Robidoux et al. 1999).

This study is an attempt to determine the toxicity of *Annona squamosa* oil cake to earthworms as a predictive assessment tool in ecotoxicological studies.

MATERIALS AND METHODS

Earthworm collection: The test species *Eudrilus eugeniae* (Annelida; Oligochaeta; Eudrilidae) were collected from the stock culture and maintained in the laboratory conditions. Acclimatization to laboratory conditions was done for seven days. Earthworms were selected on sexual maturity as evidenced by presence of a clitellum and active in behaviour.

Methodology: The soil samples were prepared by mixing cow dung, red soil and oil cake in definitive proportion and made upto 1 kg in five different containers. The concentrations of oil cake taken in the present study were 10 g/kg, 100 g/kg, 112.5 g/kg, 125 g/kg and 150 g/kg. The remaining portion of the soil mixture was constituted by cow dung and red soil in the ratio 2:1.

The acclimatized earthworms in the laboratory conditions were kept on moist filter paper to void the contents of the stomach and intestinal tract before being introduced into the test containers. Thereafter, ten voided earthworms were cleaned, weighed and introduced into test samples of varying concentrations of oil cake-soil mixture. Three replicates per treatment were prepared for five exposure concentrations of oil cake. The containers were covered with perforated, transparent cover to prevent the medium from drying and kept under test conditions for 14 /28 days at a temperature of 25±2°C. A control was also prepared along with the test samples and the results were recorded at the end of 7 and 14 days.

Earthworm mortality was evaluated at the end of day 7 and 14 of the experiment in all the sets of the test samples. Direct contact was avoided to prevent stress on earthworm. Percentage mortality rate and morphological changes were observed (Spurgeon & Hopkin 1996).

RESULTS AND DISCUSSION

Mortality rate of earthworms exposed to different concentrations of *Annona squamosa* oil cake are shown in Fig. 1. Earthworms exposed to varying *Annona squamosa* oil cake concentration at day 7 recorded no mortality in concentration 10 and 100 g/kg. However, mortality rate of 30%, 43% and 67% was observed in the concentration of 112.5g, 125g, and 150 g oil cake/kg of soil mixture respectively, indicating that mortality increased with increasing concentrations of oil cake (Table 1). In day 14 of earthworm exposure to 10, 100, 112.5, 125, 150g/kg concentrations, 0%, 10%, 50%

Table 1: Mortality rate of earthworms exposed to different concentrations of *Annona squamosa* oil cake-Day 7.

Concentration g/kg	Number tested	Number dead Trial 1	Trial 2	Trial 3	Mean mortality %	Mean probit value
Control	10	0	0	0	0	0
10	10	0	0	0	0	0
100	10	0	0	0	0	0
112.5	10	2	4	3	30	4.48
125	10	5	4	4	43	4.82
150	10	6	7	6	67	5.44

77% and 97% mean mortalities were recorded respectively indicating that exposed duration also had an influence on mortality rate of the test species (Table 2). The results observed in the control showed that no death or morphological changes were present in the negative controls on the day 7 and day 14 of the test.

Earthworm species vary in their tolerance to organic pollutants and reports have shown that a decline in earthworm population in response to large amounts of organic chemical deposition (Bayer & Foy 1982). The present study observed the vulnerability of earthworm to *Annona squamosa* oil cake. The mean lethal concentration of *Annona squamosa* oil cake was 112.5g/kg falling in the practically non-toxic category as per earthworm toxicity rating as presented in Table 3. Though, rated as non-toxic, the oil cake concentration above 125 g/kg of soil mixture proved to be highly toxic to earthworms. The earthworm mortality increased considerably above 125g/kg and 100 percent mortality was recorded for oil cake concentration above 150 g/kg with morphological distortions. The toxicity levels of *Annona squamosa* oil cake as shown in LC_{50} value may be attributed to the toxic constituents in oil cake and corresponding regulatory response by earthworm.

Certain morphological changes and behavioural alterations were observed in the earthworm exposed to *Annona squamosa* oil cake. The distinct morphological change observed in the dead worms was its discoloration from natural purplish red colour to black. Moreover, the worms exposed to the higher concentration showed high rigidity with immediate effect. The morphological changes in the surviving earthworm were discolouration, reduced elongation and contraction with slight rigidity. The behavioural alteration of the surviving worms included slow and sluggish movement, reduced burrowing ability, lack of active feeding and reduction in the formation of pellets (vermicast). The abnormalities and physical inability may be attributed to alteration in muscular function as reported by Bustos & Goicochea (2001). It appears that *Annona squamosa* oil cake induced physiological stress resulting in functional and metabolic damage to *Eudrilus eugeniae.*

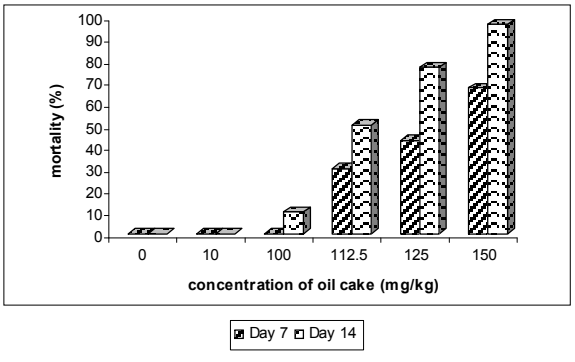

Fig. 1: Mortality rate of Earthworm exposed to different concentrations of *Annona squamosa* oil cake.

Table 2: Mortality rate of earthworms exposed to different concentrations of *Annona squamosa* oil cake-Day 14

Concentration g/kg	Number tested	Number dead Trial 1	Trial 2	Trial 3	Mean mortality %	Mean probit value
Control	10	0	0	0	0	0
10	10	0	0	0	0	0
100	10	1	2	0	10	3.72
112.5	10	5	6	4	50	5.0
125	10	8	7	8	76.6	5.74
150	10	10	9	10	96.6	6.88

Table 3: Earthworm toxicity rating (Data from OECD 2003).

Rating	Designation	LC_{50} (mg/kg)
1.	Super toxic	< 1.0
2.	Extremely toxic	1.0-10
3.	Very toxic	10-100
4.	Slightly toxic	100-1000
5.	Practically non-toxic	> 1000

LC_{50} – median lethal concentration.

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