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TOXIC EFFECT OF UREA ON EARTH WORMS DETERMINED BY A SIMPLE PAPER CONTACT METHOD

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ABSTRACT

Objective: Nowadays, the utilization of chemical fertilizer is hiked tremendously to achieve a high yield. The aim of this experimental research work is to confirm and detect the lethal or toxic effect of those chemical fertilizers on the earthworms which are the natural friend of soil as well as the farmer and also maintains the soil health by porosity and aeration.

Methods: A simple paper contact method was adopted for the toxicity testing on the earthworms on the bed prepared using Petri plate and filter paper.

Results: The lethal dose value was determined and the toxic effect of the urea on earthworm was calculated and represented using statistical inferences, and a scope of future study was created for the biochemical profiling toward the body degradation of the earthworms.

Conclusion: From the above results, it may be concluded that the acute toxicity of urea on Eisenia *fetida* using a simple paper contact method was actually significant in confirming the toxic impending. The application of environmentally levelheaded doses of urea exposed the possible harmful effects on earthworms when comes in contact directly. Thus, in future, this method will be necessary to find an approach to establish the sensitivity of the earthworm's acute toxicity before going for the evaluation in soil (i.e., acute and chronic toxicity tests in artificial soil sample).

Keywords: Earthworms, Urea, Acute toxicity, LC50 value.

INTRODUCTION

Much consideration is paid to soil health and environmental safety. Earthworms are an important indicator of soil ecosystem health and safety issues. Ecological toxicity of excessive urea, in both their single and joint effects, on earthworm *Eisenia fetida* was thus studied using the soil culture methodology [1,2]. The post epoch of green revolution has

led to environmental pollution due to disproportionate use of agrochemicals and fertilizers and thus threatened the fragile ecosystem. Indian farmers have adopted the green revolution equipment for the past three decades, leading to the exploitation of agricultural and cultivation lands [3,4]. Fertilizers are deliberately utilized in agriculture in huge tonnages each year to supplement soils artificially to sustain food productivity [4,5]. The general recommendation for urea is 120 kg/hectare in agricultural fields as per the Indian soil testing manual declared in 2011 by the Department of Agriculture, Ministry of Agriculture, Government of India [6,7]. However, Indian farmers using excess urea to achieve more productivity and ignoring the negative effects on soil organisms, particularly the earthworms which are the best friend of soil. This condition actually prevails worldwide. Recently, much more attention is paid to soil health and environmental safety issues [8]. Earthworms play an important key role in soil fertility, and they constitute the 60% of the soil macrofauna biomass compositions. They are tremendously important in soil formation, principally by consuming organic matter, fragmenting it, and mixing it thoroughly with soil mineral particles to form water-stable aggregates [3,9]. Thus, it is important for the presence of earthworms in the soil to preserve its structure and function, particularly in agricultural fields. Due to their natural habitat, earthworms are exposed to a multiplicity of chemicals such as chemical fertilizers, pesticides, and insecticides in agricultural fields and therefore are suitable indicators for the assessment of toxic effects of chemicals [10,11]. Although effects of urea on plant growth and soil biota communities are studied; in fact, there are no data about its effects on earthworms [12,13]. The nitrogenous fertilizers usage on earthworms in soil has the toxic potential for them. Only single and joint effects of acetochlor and urea on earthworm *E. fetida* populations in phaiozem were premeditated by Xiao *et a*l., (2004). Urea is also an artificially manufactured chemical and could affect earthworms due to their general usage in the agriculture [14,15]. Since the data about its effects on earthworms are scarce, the endeavor of this study was to determine the toxic effects of urea on the earthworm *E. fetida*. Ecological toxic effects of urea on earthworm, *E. fetida*, were thus studied using a simple paper contact method procedure. Paper contact method was proposed by OECD (1984) as a screening test to prove the toxic potential of chemical as well as fertilizers [16,17]. This method has been consecutively adopted by many scientists (Karanjkar and Naik, 2010; Miyazaki *et al*., 2002; Roberts and Dorough, 1984; Wang *et al*., 2012; and Velki *et al*., 2013) to determine the toxicity of chemical to earthworms.

However, the chemicals hereby studied are pesticides and herbicides, and no work has been done in chemical fertilizers by today. This is the first toxicity study made on chemical fertilizer using this methodology and protocol. Roberts and Dorough (1984) tested the toxicity of 90 chemicals on *E. fetida* using the simple paper contact method and classified the chemicals as super toxic effect, extremely toxic, very toxic, moderately toxic, and relatively non-toxic circumstances [18]. This was the pioneer study made adopting this method, and here, the same protocol was followed.

METHODS

Earthworms

E. fetida, the common earthworm found in agricultural land, was adopted as the test species because it is the recommended and prescribed species in OECD (1984) guideline and regulation for testing of chemicals no. 207, earthworm, and acute toxicity tests and experiments. The earthworms were collected from the agricultural fields of Nuapadhi village, nearer to F.M University, Nuapadhi, Balasore, Odisha. They were all cultured under the same conditions and ambient environmental condition fed essentially the mixture of soil and manures (Fig. 1). This culture was judged to be free for contaminants

under controlled conditions. Adult and mature earthworms, which possessed clitellae and having an individual wet weight of 200±30 mg (after excreting the gut content) were selected for testing and further experiment [19].

Used chemicals and test solution preparation

The commonly used chemical fertilizer urea was used as testing chemical. It was purchased from the regular fertilizer distributor of Balasore district. Aqueous solutions of various concentrations were prepared by dissolving the urea in deionized water (distilled water) [20,21]. The concentrations were prepared in 5 mg/ml, and the toxicity was measured as µg/cm^2.

Acute toxicity testing

An acute toxicity testing of urea was performed on earthworms using a simple paper contact method projected by OECD testing guideline No. 207 [22,23]. This is a plain screening test to identify the toxic potential of the chemical fertilizer to earthworm. The protocol test vial was a plastic Petri dish of 14 cm in diameter and 2 cm in height. Round filter paper (Whatman No. 1 filter paper) of size 8.5 cm diameter was cut and resized to the suitable size and placed in such a way that sides are lined with the filter paper. Five regular concentrations were prepared by dissolving urea in 5 ml deionized (distilled) water in a general geometric series (5, 10, 20, 40, and

80 mg) and were pipette into each test vial to wet the filter paper bed. Blank control tests were performed with the 5 ml of deionized water only. For each treatment, 10 replicates of experimental series were used, each consisting of single earthworm per test vial. Adult and mature earthworms, which possessed clitellum and having an individual wet weight of 250–350 mg, were selected for testing and experiments. Earthworms were washed briefly with deionized water and were kept on moist filter paper for 3 h to get excrete the gut content, after which it was rinsed again with deionized water regularly, blotted on the filter paper, and placed in a testing vial (one earthworm per vial). After the introduction of earthworm, the vial was covered with plastic film that had been punched with small holes using needles for the respiration and air accumulation purposes. Tests were done in the darkroom at 28±20°C for 48 h. In a regular time interval of 1 h, the earthworm was monitored for mortality by a gentle mechanical stimulus to the front part of the body.

RESULTS AND DISCUSSION

The lethal toxic concentration of urea to *E. fetida* was evaluated as 28 µg/cm^2 by an average calculation. Thus, the comparative toxicity grade of urea was categorized as "very toxic" to *E. fetida*. With consistent area of contact exposure of urea to the earthworm in different concentrations showed various toxic effects in filter paper substrate medium on the Petri plate bed. A geometric concentration series of test solution (5, 10, 20, 40, and 80 mg/5 ml) was prepared and tested, in which mortality of earthworms was observed in 20, 40, and 80 mg concentrations after 18, 12, and 6 h, respectively (Table 1). Only the 5 mg/5 ml concentration was non-toxic to earthworm for 48 h. The lethal concentration for 48 h to *E. fetida* was 10 mg/5 ml concentration (Fig. 2a). The deleterious effects of urea on earthworm were lesions, inflammations, and disjointing of the posterior body parts (Fig. 2b). The earthworm was not able to move around after 40 h, neural retention and defoliation starts, and the body parts separate leading to the death.

As the neural degradation and the body part deformation are occurred,

the filter paper bed was absorbed with the body fluid and serum of the earthworms. Hence, the lethal effect of the regularly used chemical fertilizer urea is killing the friend of farmer in a drastic way.

Contact filter paper testing is an original screening technique protocol to evaluate the comparative toxicity of chemicals and fertilizers to the earthworms. In this preliminary screening experiment, the chemicals are shocked or absorbed into the earthworm body mainly through the outer dermal layer of skin when it is moving around the filter paper [24-26]. Although it fails to signify the condition in soil, it is more significant to know the toxic grade of a particular chemical, whether it is toxic or non-toxic. If the chemical proved to be toxic, further extensive study on synthetic soil can be carried out and performed. If the chemical

proved to be non-toxic, there is no need for the comprehensive study [27]. It has been demonstrated for many decades that most of the inorganic mineral fertilizers are non-toxic to earthworms; however, it has been disproved in this systematic study.

Statistical analysis

For the filter paper contact method, the toxicity is expressed basically in µg/cm^2. Based on the resulting 48-h LC50 values lethal dose [28,29], the urea fertilizer will

be classified as super toxic element (<1.0 µg/cm^2), extremely toxic at 1–10 µg/cm^2, very toxic at 10–100 µg/cm^2, moderately toxic at 100–1000 µg/cm^2, or relatively non-toxic at >1000 µg/cm^2. Moreover, the lethal time duration for the individual earthworms per the 5 ml solution testing is determined and is graphically represented (Graph 1).

Fig. 1: The accumulated and adopted to the artificial soil bed

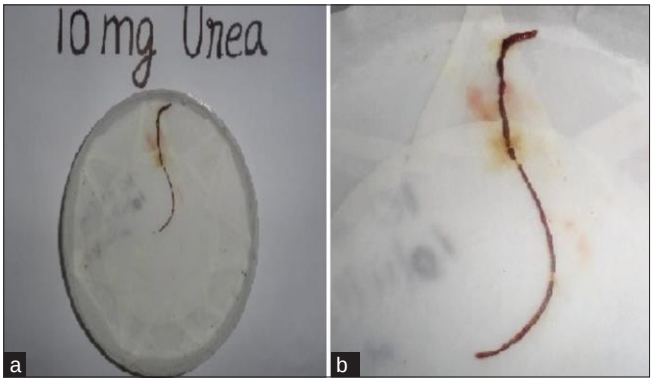
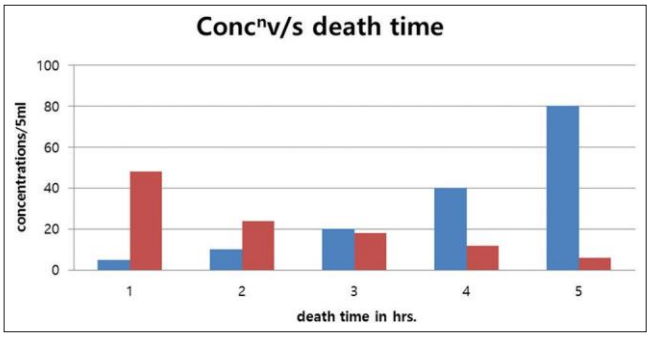


Fig. 2: (a) Mortality of *Eisenia fetida* at 10 mg/5 ml treatment of urea. (b): Degradation effects such as lesions, Inflammations, and separation of body parts observed on *E. fetida*



Graph 1: The death time and concentration ratio

Table 1: The death time period per different concentrations

Concentrations	5 mg/5 ml	10 mg/5 ml	20 mg/5 ml	40 mg/5 ml	80 mg/5 ml
Death hours	48	24	18	12	6

CONCLUSION

From the above results, it may be concluded that the acute toxicity of urea on *E. fetida* using a simple paper contact method was actually significant in confirming the toxic impending. The application of environmentally levelheaded doses of urea exposed the possible harmful effects on earthworms when comes in contact directly. Thus, in future, this method will be necessary to find an approach to establish the sensitivity

of the earthworm's acute toxicity before going for the evaluation in soil (i.e., acute and chronic toxicity tests in artificial soil sample). Soil being a very complex system, it is often difficult to compare toxicity information directly. Although the paper contact method ignores the contribution of soil compositions, it has a high advantage of reproducibility and the probable of direct comparison of results. Using the data obtained from this method, the assortment of test concentration for acute and chronic toxicity tests can also be indomitable. Thus, this work fulfills the objective that the soil health and its predator's effect. The necessary precautions and regulations should be implemented for the usage of the chemical fertilizers like urea on the agricultural lands. The social awareness is most needed for this serious issue about the soil health.

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