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Research Paper

Avoidance behavior of *Eisenia fetida* to Chlorpyrifos and 2, 4-D Ethyl ester in natural soil from Kota (Rajasthan) in India

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Abstract: Kota is situated in the south eastern part of Rajasthan state alongside Chambal river with good agriculture produce. Maize, Wheat, Mustard and Soyabean are the major crops of this region. This humid south eastern plain of Rajasthan contains very productive deep black/brown clayey soil. In our research, it has been found that the main species of Earthworm in agricultural area around Kota city is *Eisenia fetida*. The use of pesticides to treat seeds and other agricultural applications for high yield of food production is a common practice. The main objective of this paper is to observe the effect of pesticides used for one Rabi season crop. Wheat crop has been selected for this study. Chlorpyrifos and 2, 4-D Ethyl ester (2,4-DE) are two popularly used insecticide and herbicide respectively for wheat. Study of avoidance behavior of earthworm to pesticides is quick and easy to perform, and it is known to observe the sensitivity of earthworms towards a wide range of chemicals. Avoidance test has been performed by two compartment method in a container. This way optimal concentration of a specific pesticide may be determined so that farmers are encouraged to follow the same. This way, organic farming can be protected together with usual practice of using pesticides with recommended dosage.

Keywords: Chlorpyrifos, 2,4-D Ethyl ester, Avoidance, Pesticides, Tropical, *Eisenia fetida*

INTRODUCTION

Soil pollution has important consequences to all forms of life and to the food, water and air quality because the soil is the source of water and nutrients to plants and animals, as well as the habitat for many species that are important to the nutrient cycling and availability (Wild, 1993; Ingham, 2014). Although the pesticides are used to treat seeds and other agricultural applications for high yield of food production, there are serious concerns about potential of pollution by these substances (Alves et al, 2013). Several studies have been conducted to observe the

effects on earthworms for various classes of chemicals (Frampton et al., 2006; Singh and Singh, 2015; Ahmed 2015; Singh and Singh, 2016). This way optimal toxicity of soil can assessed to balance production yield and its harm due to poisonous chemicals. Toxicity studies on soil fauna can be done by various established approaches.

The earthworm avoidance test, originally developed in USA (Yeardley et al., 1996), is selected because it is quick and easy to perform, and it is known to be sensitive towards a wide range of chemicals. The principle of this test is that the earthworms are simultaneously exposed to the soil sample spiked with the pesticide, and to the control soil. After a test period of two days the location of the animals is determined. The existing standardized protocol for the Earthworm avoidance test (ISO, 2008) was modified in terms of test species, substrate and conditions in order to make it suitable for tropical regions.

The tendency of a species to avoid a certain study soil in favour of the control soil (free of contaminants) is used as avoidance test to control soil quality and the effects of certain chemicals on the behaviour of earthworm species (ISO, 2011). These tests are based on the fact that chemicals in soil are in different factions depending on the contamination level and soil type and can be absorbed by earthworms. Earthworms can detect a wide range of contaminants due to their chemoreceptors on their anterior segments and sensory tubercles located on the surface body (Reinecke et al., 2002).

The importance to carry out the avoidance behaviour test in the tropics comes also from the fact that most of developing countries are high users of agrochemicals i.e. Brazil (Chrisman et al., 2009), Colombia (García-Santos et al., 2011), South-African countries (Williamson et al., 2008), India (Abhilash and Singh, 2009) or China (Wei et al., 2007). Field studies on soil toxicity are much harder to be found in developing countries except very few (Forster et al., 2006; De Silva et al. 2009). There is often a lack of monitoring of chemical residues in soil, surface water and groundwater with little information on effects in soil, water quality and aquatic toxicology (Lacher and Goldstein, 1997). Pelosi et al. (2013) presented a review paper on pesticides and earthworms. In most of the cases, damage to ecosystems is ignored.

Study area

Kota region is situated in the south eastern plain of Rajasthan having normal annual rainfall of 735 mm and temperature varies from 10^{o}C to 45^{o}C through winter to summer season. It mainly contains deep black/brown clayey soil. Soil of an agricultural area in the Kota region has been selected and assessed. *E. fetida* is used as the test organism to the avoidance behaviour test for the two commonly used agro-chemicals (Chlorpyrifos, 2,4-D Ethyl ester) as per usual practice in wheat growing in the region.

MATERIALS AND METHODS

Test soil

The natural soil samples were collected from an abandoned area where no agricultural activity has done and which was an area with no known history of pesticides use. The physico–chemical characterization of soil was provided by

Nanta agricultural farm (office of Project Director, crop) in the Kota district.

Test organisms

The earthworms *Eisenia fetida* (Lumbricidae), were taken from Krishi Vigyan Kendra ,Borkhera, Kota. The animals were bred in cattle manure as food at temperature 15-30 ^{o}C (mean about 20 ^{o}C). For all tests, only adult worms with clitellum with a fresh weight between 250 to 350mg were used. All earthworms were fed according to demand, usually once a week, with finely ground cattle manure free of any chemical contamination. The worms selected for the test were acclimatized in the respective soil under test conditions for at least 24 hrs before starting the test.

Test chemicals

Chlorpyrifos (O,O-diethyl-O-3.5.6-trichloro-2-pyridyl phosphorothioate) is an organophosphate insecticide. Chlorpyrifos was tested as Radar 20 EC (100ml, Isagro (Asia) Agrochemical Pvt Ltd, Mumbai). It is used to kill insect pests by disrupting their nervous system. Chlorpyrifos has an advantage over other products in that it is effective against a wide range of plant-eating insect pests.

2, 4-D Ethyl ester 38 % EC (Based on 86% w/w 2-4 D acid) is a selective herbicide effective against broad leaves weeds in sorgham, maize, wheat, aquatic weeds etc. It was tested as Cut Off 38 (250 ml, Crystal Crop Protection Pvt Ltd). It is a selective, systematic, post emergent herbicides used mainly for control of annual/perennial broad leaved weeds in cereals, tea, wheat, rice, maize, millets, grasslands, established turf, grass seed crops, orchards, cranberries, asparagus, sugarcane and aquatic weeds etc in accordance with climatic conditions and approval of local authorities.

The concentrations tested were 0.316, 1, 3.162, 10, 31.62, 100, 316.22 and 1000 mg a. i /kg dry soil of all chemicals.

Test performance

The avoidance assays with the earthworms were made based on the ISO guideline 17512-2 (ISO 2011). Each plastic container (15.5 cm height and diameter 13 cm) was divided into two equal sections with a plastic card, one-half of the container received 250 g (dry weight) of control soil (section A) and the other half 250 g (dry weight) of soil contaminated with the pesticide (section B). All combinations of contaminated/ uncontaminated soil were tested, each one with four replicates. After placing the soils into each container, the card divider was removed and 10 worms were placed on the middle line. Afterwards, each container was covered with a transparent lid perforated in order to allow aeration. The organisms were incubated at 20 ± 2 ^{o}C with a photoperiod of 16 hrs: 8 hrs (light: dark) for 2 days. After the test period, the divider was put back to separate the control and test soils, and the number of worms in both sections were counted. For each replicate, the avoidance response is calculated using

$$NR = [(C – T)/ N] \times 100$$

where, NR = net avoidance response (%), C = number of worms in control soil, T = number of worms in pesticide-amended soil, and N = total number of worms exposed.

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RESULTS

In our experiment, no dead or missing worms were found in the test for all the different concentrations, except for 100% mortality at the highest concentration of chlorpyrifos (1000 mg a.i. /kg of dry soil). Hence, this concentration was excluded for the statistical analysis of avoidance behaviour. When exposed to chlorpyrifos, *E. fetida* showed on average higher avoidance with higher concentrations. At the very low concentration of 0.316 mg/ kg, *E. fetida* showed 40% avoidance. 55% avoidance ($p < 0.05$) was observed at 1 mg/kg. At 3.16 mg/kg concentration, 75% avoidance was observed and at 10 mg/kg 60% avoidance showed by earthworms. At highest three concentrations (31.6, 100 and 316 mg/kg), earthworm showed more than 90% avoidance behaviour. Result of this experiment is shown in figure 1.

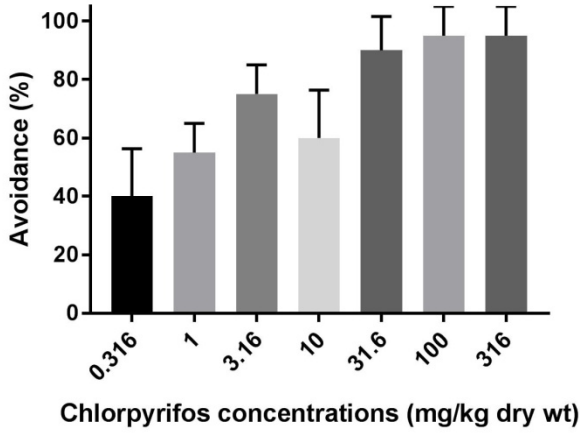

Figure 1: Avoidance behaviour of *E. fetida* in Chlorpyrifos with varying concentrations.

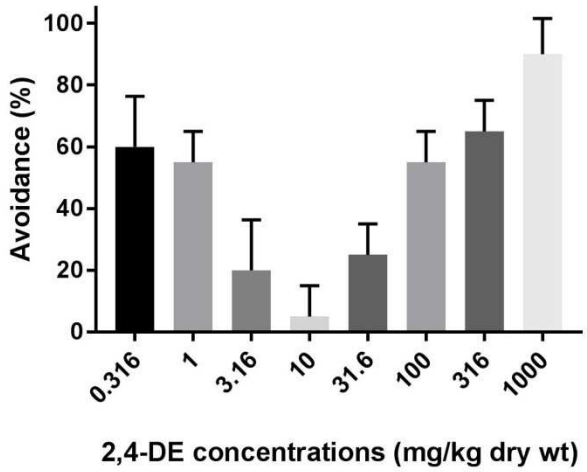

Figure 2: Avoidance behaviour of *E. fetida* in 2, 4-D Ethyl ester with varying concentrations.

For 2,4-D E chemical, avoidance at 0.316mg/kg and 1mg/kg were found to be around 60%. At 3.16 mg/kg, avoidance was 20%. Minimum avoidance of 5% was shown at 10 mg/kg. 25% avoidance was reflected at the concentration of 31.6mg/kg. The result of the experiment shown gradual increase for the remaining higher three concentrations and varied between 55% to 90% progressively. Result of this experiment is shown in figure 2.

Experiments for both of these chemicals represented common behaviour in terms of avoidance (with increase in concentration), which is evident. However, 2,4-DE shown little avoidance (5%) for 10 mg/kg.

DISCUSSION

Avoidance behavior of earthworms for two chemicals used for wheat crops has been studied. Results show that the earthworms have a very strong avoidance at higher concentration of these chemicals. Avoidance is low to medium for lower to

medium values of concentrations. Numerous research papers presented study on avoidance behavior of *E. fetida* for chlorpyrifos. Whereas, avoidance behavior of *E. fetida* for 2,4-DE chemical has not been attempted much by researchers.

For the organophosphate chlorpyrifos, *E. fetida* proved to be a potentially suitable species to assess the soil contamination. Chlorpyrifos showed increased avoidance response with higher contaminant levels.

Comparable trends of avoidance behaviour was reported at higher levels of concentrations of chlorpyrifos using *E. fetida* (Zhou et al., 2007) and by De Silva (2009) using *E. andrei* in a two compartment system for natural tropical soil from Sri Lanka. De Silva et al. (2009) observed that *P. excavatus* was significantly attracted by chlorpyrifos at the lowest three concentrations (1-10 mg a.i. kg_1 dry soil), such an effect was not seen for *E. andrei*. When neglecting this negative avoidance behaviour and focusing on the avoidance of chlorpyrifos in OECD (1984) artificial and natural soil, *E. andrei* was 2.5 and 2.0 times more sensitive, respectively, than *P. Excavates*. García-santos et al. (2011) also reported that *E. fetida* showed increased avoidance response with higher contaminant levels of Chlorpyrifos. The higher avoidance obtained by these researchers might be explained because of differences in the methodology. As reported in the literature, the avoidance response from the concentration tested under field conditions was comparable to the obtained avoidance rate under control conditions, which might potentially indicate that physical and chemical properties of the soil did not affect earthworm behaviour to chlorpyrifos and 2,4-DE. However, this result is not definitive and further research should consider testing more concentrations under field conditions.

Experimental results for Chlorpyrifos by and large shows an increased avoidance behaviour for higher values of concentration. In the present study the avoidance behaviour for 2,4-DE reflects decreased value around 10 mg/kg and thereafter increased avoidance behaviour for higher values of concentrations were observed.

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