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Research Paper

Effect of Triazophos and Pendimethalin on Avoidance Behavior of *Eisenia fetida* in Natural Soil of Kota (Rajasthan)

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Abstract: Global warming and biodiversity are always the matter of concern for the environmentalists and biologists. These issues have been raised on various platforms to aware masses. Vermicomposting is one of the important approaches towards its solution by using natural resources. Kota is situated in the south eastern part of Rajasthan state alongside Chambal River with very productive deep black/brown clayey soil. Maize, Wheat, Mustard and Soybean are the major crops of this region. In our research, it has been found that the main species of Earthworm in agricultural area around Kota city is *Eisenia fetida*. The main objective of this paper is to observe the effect of pesticides used for one Kharif season crop (Soybean). Triazophos and Pendimethalin are two popularly used insecticide and herbicide respectively for Soybean. For this, study has been conducted to observe the effect of Triazophos and Pendimethalin on avoidance behavior of *Eisenia fetida* in natural soil of Kota. 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 use these dosages and thus help to protect environment.

Keywords: Triazophos, Pendimethalin Avoidance, Global warming, Pesticides, *Eisenia fetida*

INTRODUCTION

In recent years, disposal of organic wastes from various sources like domestic, agriculture and industrial has caused serious environmental hazards and economic problems. Burning of organic wastes contributes tremendously to environmental pollution thus, leading to polluted air, water and land. This process also releases large amounts of carbon dioxide in the atmosphere, a main contributor to global warming. Burning also destroys the soil organic matter content, kills the microbial population and affects the physical properties of the soil. It has been demonstrated that earthworms

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can process household garbage, city refuse, sewage sludge and waste from paper, wood and food industries (Kale et al., 1982; Muyima et al., 1994; Edwards and Bohlen, 1996). The use of earthworms in composting process decreases the time of stabilisation of the waste and produces an efficient bio-product, i.e., vermicompost. Recycling of organic waste is feasible to produce useful organic manure for agricultural application. Vermicomposting offers a solution to tonnes of organic agro-wastes that are being burned by farmers and to recycle and reuse these refuse to promote our agricultural development in more efficient, economical and environmentally friendly manner. Therefore organic farming helps to provide many advantages such as; eliminate the use of chemicals in the form of fertilizers/pesticides, recycle and regenerate waste into wealth; improve soil, plant, animal and human health; and creating an ecofriendly, sustainable and economical bio-system models (Ansari and Ismail, 2001). Tripathi and Bhardwaj (2004) compared the composting potential, biomass growth and biology of a non-native (*Eisenia fetida*) and an endemic (*Lampito mauritii*) species of earthworm in the semiarid environment of Jodhpur district of Rajasthan in India. Results showed that percentage of organic carbon of the culture bedding material declined upto 105 days with *E. fetida* and 120 with *L. mauritii* and percentage of nitrogen, phosphorous and potassium increased as a function of the vermicomposting period. Both species were found to be effective for decomposition and mineralization of mixed bedding in the semiarid environment.

Although 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 behavior 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 behavior 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.,

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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). 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°C to 45°C through winter to summer season. It mainly contains deep black/brown clayey soil. *E. fetida* is used as the test organism to the avoidance behavior test for the two commonly used agro-chemicals (Triazophos and Pendimethalin) as per usual practice in Soybean 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° C (mean about 20° C). For all tests, only adult worms with clitellum with a fresh weight between 250 to 350 mg 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: Triazophos 40% EC is an Organophosphates and it is a Acetylcholinesterase (AChE) class of inhibitor. Triazophos was tested as Trizocel (250 ml, Excel Crop Care Ltd). It is a broad-spectrum insecticide which acts upon insects through contact and stomach action. It penetrates deeply into plant tissues. It is effective for control of stem borer, leaf folder, green leaf hopper, Aphids and Bollworms in Soybean.

Pendimethalin (3, 4-Dimethyl-2, 6-dinitro-*N*-pentan-3-yl-aniline) is an herbicide of the dinitroaniline class. Pendimethalin was tested as Panida 30EC (250 ml, Rallis India Ltd). It is used to control annual grasses and certain broadleaf weeds by inhibiting their cell division as well as cell elongation in wheat, barley, corn, soybeans, rice, potato, legumes, fruits, vegetables, nuts and other ornamental plants. 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 ° 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

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worms in both sections were counted. For each replicate, the avoidance response is calculated using

NR = [(C – T)/ N] x 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.

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 Pendimethalin (1000 mg a.i./kg of dry soil). Hence, this concentration was excluded for the statistical analysis of avoidance behavior.

For Triazophos, earthworms showed strong attraction behavior at 0.316 mg/kg of dry soil. At 1 mg/kg concentration avoidance was found minimum (10%). At 3.16 mg/kg, avoidance was 70%. 85% avoidance was shown at 10 mg/kg. Avoidance was 95% at concentrations of 100 mg/kg and 316 mg/kg. At highest concentration of 1000 mg/kg, 100% avoidance was observed in earthworms. Result of this experiment is shown in figure 1.

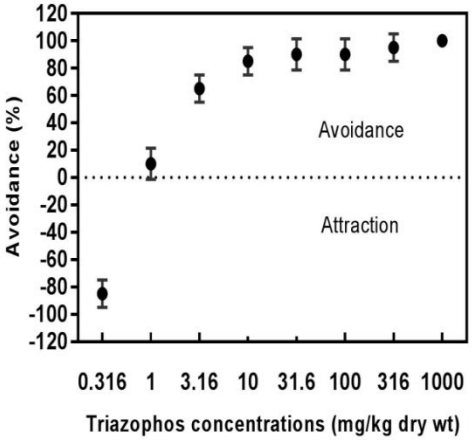

Figure 1: Avoidance behavior of *E. fetida* in Triazophos with varying concentrations.

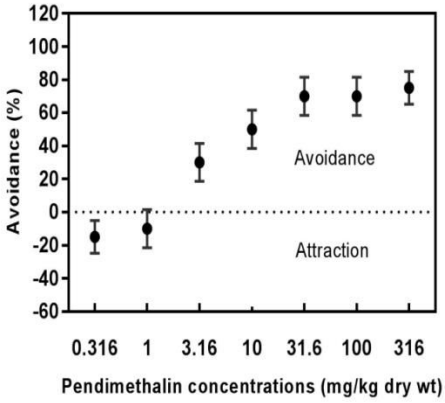

Figure 2: Avoidance behavior of *E. fetida* in Pendimethalin with varying concentrations.

When exposed to Pendimethalin, *E. fetida* showed average higher avoidance with higher concentrations. At the very low concentration of 0.316 mg/kg and 1 mg/kg, *E. fetida* showed attraction response. At 3.16 mg/kg concentration, 30% avoidance was observed and at 10 mg/kg, 50% avoidance was shown by earthworms. At concentrations of 31.6 and 100 mg/kg, earthworm showed 70% avoidance behavior. 80% avoidance was showed at concentration 316 mg/kg of dry soil. Result of this experiment is shown in figure 2.

DISCUSSION

Avoidance behavior of earthworms for two chemicals used for Soybean crops has been studied. Results show that the earthworms have a very strong avoidance at higher concentration of these chemicals. Attraction is shown at lowest concentration of these chemicals. Numerous research papers presented study on avoidance behavior of *E. fetida* for different chemicals. Study on avoidance behavior of *E. fetida* for Triazophos and

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Pendimethalin is not reported. This may be due to more use of these chemicals in India in comparison to other countries.

De Silva and Gestel (2009) found same type of attraction behavior with *P. excavatus* at the lowest three concentrations (1–10 mg/kg dry soil) by chlorpyrifos. Alves et al. (2013) also reported the same type of strong attraction by *E. Andrei* with fipronil, carboxin plus thiram, captan and thiametoxam chemicals. *E. Andrei* also showed same attraction behavior with carbofuran (Bucha et al. 2013). Li et al. (2015) reported that at concentration of 0.1 mg/kg of Enrofloxacin, *E. fetida* showed attraction behavior. Marques et al. (2009) found that *E. Andrei* showed attraction behavior at concentration of 31.7 mg/kg of formulated herbicide Mikado (a.i. is sulcotrione). M. Garcia et al. (2008) observed that tropical *E. fetida* in tropical artificial soil (TAS) soil and in LUFA soil indicate a significant avoidance behavior of earthworms at concentrations ≥1.0 mg a.i./kg for fungicide carbendazim. While in artificial OECD (1984) soil, a significant attraction was observed at the lowest concentration (1.0 mg a.i./kg). Alves et al. (2013) observed that more worms were found in the polluted compartments of thiametoxam, fungicides captan and carboxin plus thiram, at the lowest concentrations than in the control compartments. However, TAS treated with higher concentrations of these three pesticides was avoided by the worms.

In the present study, it has been found that the earthworms found attraction towards lower concentrations of Triazophos and Pendimethalin. However, attraction was found to be very high at lowest concentration (0.316 mg/kg) of Triazophos. Similarly, avoidance response was higher in Triazophos, in comparison to Pendimethalin, for other higher concentration values. In general, increased avoidance behavior was visible for higher values of concentration in both of these chemicals.

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