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COMPARATIVE TOXIC STUDY OF TWO NOVEL INSECTICIDES AGAINST EARTHWORM (*PHERETIMA POSTHUMA*)

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ABSTRACT

The present study describes the impact of sulfoxaflor and spinetoram against *Pheretima posthuma* earthworm. The LD_{50} of sulfoxaflor was found to be 0.1841 *ppm* of soil. Mean mortality of *Pheretima posthuma* after 48-hours exposure has been observed as 20, 40, 60, 70 and 90% in respect of 0.06, 0.12, 0.24, 0.48 and 0.96 *ppm* of soil. The LD_{50} of spinetoram was found to be 1.4288 *ppm* of soil. Mean mortality of *Pheretima posthuma* after 48-hours exposure has been observed as 20, 40, 60, 80 and 90% in respect of 0.50, 1.0, 2.0, 4.0 and 8.0 *ppm* of soil, respectively.

Key words: Toxicity, Pesticides, Earthworm

INTRODUCTION

Earthworm carried out a lot of functions in ecosystem such as improving soil quality and recycling of nutrient, commonly used as gauge in the soil to determinate the effects of pesticides on terrestrial organisms, considerable important to health of fertile soil, several impacts on entire ecosystem and help in manage the different level of influences (Blouin *et al.*, 2013, Zhang *et al.*, 2007). Aristotle was the first person to pay consideration to the role of earthworm in airing the soil. Earthworm is the more important animal in the fauna of soil-dwelling organisms (Fouad *et al.*, 2023). Long time ago, several work were stated on their significant impacts the cultivated soils (Badawy *et al*., 2013, Fouad, 2021, Fouad *et al*., 2023).

Sulfoxaflor has a novel mode of action on insect populations, particularly those insects that developed resistance to formerly conventional neonicotinoids. Sulfoxaflor showed exceptional rapid action against sap feeding insects as green mirids and cotton aphids in either methods systemic and contact (Annetts and Thomas, 2012), successfully used in cereal and cotton fields to control the species of aphids in the worldwide (Annetts and Welsh, 2012 and Annetts and Elias, 2012). Sulfoxaflor (Neonicotinoid) sprayed foliages run into the ways either the soil and nearest to drains and water courses, which run off the neonicotinoid to the decomposed cycling of terrestrial biota. The residue of neonicotinoid proved gate way for disturbing to the degradation that caused a reason in the cycling of the essential substances and enhancement in the ecological units (Dilly and Munch, 1996, Suberkropp, 1998). The wide range uses of neonicotinoids showed its traces in soil trials (Raloff, 2005). Whereas only few studies carried out on pesticides effects against earthworms (Tu, 1995).

Spinetoram showed directly its effects on either neurotransmitters receptors as γ-aminobutyric acid receptors or nicotinic acetylecholine receptors that are present in insects nervous system at postsynaptic membranes, these serve for spinosyns as allosteric activators (Yasutaka *et al*., 2012). Spinetoram compound in respect of its toxicity and environmental fate were decreased threaten (Dow Agro Sciences, 2008). Spinetoram and its similar other compound such as spinosad and spinosyns were frequently used a successful insecticides against *Frnkliniella occidentalis* and *Orius insidiosus* at its recommended doses (Srivastava *et al.*, 2008, Reita *et al.*, 2003). The spinetoram could be controlling a broad range of insects in variety of agricultural crops specially Lepidoptera, Thysanoptera and Diptera. They showed least adverse impacts against valuable insects such as miniature pirate bugs, big eyed bugs, lacewings and ladybirds (El Kady *et al.*, 2007, Mahmoud *et al.*, 2009, Copping and Menn, 2001).

The toxicity of spinetoram investigated to control green peach aphid and onion thrips, its novel type of action create excitation in nervous system of insect pests by changing the functions of GABA-gated ion and nicotinic receptors (Laila and Hassan 2008, Mertz and Yao, 1990). For the first time, conclusively, present findings the differential action of conventional pesticide and neem leaf extract on the non-target organisms.

MATERIALS AND METHODS

Initial culture of earthworm *Pheretima posthuma* was procured from the curtsey of Dr. Abid, Govt. National Degree Science College Karachi. After collection they kept in plastic bag which contained fresh manure plus soils then they were brought to the laboratory for further reared and studied in laboratory conditions at Mohammad Afzal Hussain Qadri Biological Research Center, University of Karachi, and laboratory # 11.

Soil preparation for test

Soil was heated in an oven at 100 ^{o}C for its complete dryness, after that weighted 1 kg soil was added 100-120 ml sterile water for maintained of the soil moisture at above 80% humid level in which earthworms were treated. After that constant doses of different concentration were mixed to already prepared 1kg soil in each plastic basket for treatment of earthworms.

Preparation of solution under test compounds & their dilutions

Sulfoxaflor was obtained from pesticide market under trade name of Closer 50% w/w, sampling Schacht serial no, 1L102332C3 having total weight of ingredients 7.5 g, produced by Dow Agro Sciences Corporation, Indonesia and distributed by Dow Agro Sciences in Pakistan. Further dilutions were made by the use of Charl's formula $C_1V_1 = C_2 V_2$ for concentration of 0.06, 0.12, 0.24, 0.48 and 0.96 *ppm* doses were obtained after dilutions in conventional grades and then mixed in one kg soil for treatment of earthworm

Spinetoram was purchased from pesticide market as trade name Delegate 25%w/w, serial no, produced by Dow agro sciences corporation and distribution by Ali Akbar group in Pakistan. Further dilutions were made by the use of Charl's formula $C_1V_1 = C_2V_2$ for concentration of 0.50, 1.0, 2.0, 4.0 and 8.0 *ppm* doses were obtained after dilutions in conventional grades and then mixed in one kg soil for treatment of earthworm.

The earthworms were tested with the different concentrations of the sulfoxaflor and spinetoram. The desired concentrations of the sulfoxaflor and spinetoram were prepared in the soil used to expose them. LD_{50} of the sulfoxaflor and spinetoram were calculated based on (Finney, 1967).

RESULTS

In the present work toxicity of sulfoxaflor and spinetoram were determined against adult earthworm *Pheretima posthuma* Kinberg in respect of their impact on LD_{50}.

Toxicity of sulfoxaflor

The LD_{50} of sulfoxaflor was found to be 0.1841 *ppm* of soil. Mean mortality of *Pheretima posthuma* after 48-hours exposure has been observed as 20%, 40%, 60%, 70% and 90% in respect of 0.06 *ppm*, 0.12 *ppm*, 0.24 *ppm*, 0.48 *ppm* and 0.96 *ppm* of soil by plotting the values of average death for the doses of the sulfoxaflor through probit analysis, LD_{50} of sulfoxaflor was found to be 0.1841 *ppm* of soil. The lowest concentration of sulfoxaflor i.e. 0.06 *ppm* of soil showed 20% mortality at 48 hours post treatment, while 0.96 *ppm* of soil concentration of sulfoxaflor caused 90% mortality in imago earthworms at 48 hours at post treatment. For the determination of the ecological impacts the untreated worms have been placed as well (Table 1 & Fig. I).

Toxicity of spinetoram

The LD_{50} of spinetoram was found to be 1.4288 *ppm* of soil. Mean mortality of *Pheretima posthuma* after 48-hours exposure has been observed as 20%, 40%, 60%, 80% and 90% in respect of 0.50 *ppm*, 1.0 *ppm*, 2.0 *ppm*, 4.0 *ppm* and 8.0 *ppm* of soil by plotting the values of average death for the doses of the spinetoram through probit analysis, LD_{50} of spinetoram was found to be 1.4288 ppm of soil. The lowest concentration of spinetoram i.e. 0.50 *ppm* of soil showed 20% mortality at 48 hours post treatment, while 8.0 *ppm* of soil concentration of spinetoram caused 90% mortality in imago earthworms at 48 hours post-treatment. For the determination of the ecological impacts the untreated worms have been placed as well (Table 2 & Fig. 2).

DISCUSSION

Toxicity of sulfoxaflor (Neonicotinoids).

Van der Sluijs *et al.* (2015) investigated that the neonicotinoid compounds kept highest solubility to water that determined the causes as an important element of pollution of surface water, soils, ground and tissues of plant from treated crops. Similarly, the most neonicotinoids could be caused pollution in soil and probably in plant tissues. The

use of neonicotinoids in ecological pollution that regularly go over threshold recognized to cause unfavorable impact for non-target organisms. Asrar *et al.* (2014) worked on toxicity to *Chrysoperla carnea* and *Coccinella septempunctata* (Predators) of seven pesticides including neonicotinoid, and results were related to present results of sulfoxaflor in respect of toxicity. Faheem and Khan (2010) also reported the toxicity of imidacloprid (Nicotinoid) against earthworm. In the present finding sulfoxaflor LD_{50} was found to be 0.1841 *ppm* which is highly toxic against earthworm by contact cum feeding method than spinetoram LD_{50} 1.4288 *ppm* as being a water soluble compound. The sulfoxaflor can spread deeper and could be cover plentiful area using water as medium in the soil and it could be absorbed by the earthworm integument. Zewain *et al.* (2013) evaluated sulfoxaflor 24% SC was effective against *Bemisia tabaci* Genn at fields recommended doses on cucumber crop. Wang *et al.* (2012) reported that the toxicities of five neonicotinoids against earthworm *Eisenia fetida* as >acetamiprid > imidacloprid > clothianidin > nitenpyram > thiacloprid. Generally, present findings are in agreement with Wang *et al.* (2012). As a result, the threat, in the honey bee might be universal on the uses of nearly all neonicotinoids (Tennekes and Sánchez-Bayo, 2012). Mostert *et al.* (2000) also investigated the effect of neonicotinoed against earthworm mortality. The previous findings of toxicity in respect of neonicotinoids are reported above, especially sulfoxaflor is usually in conformities with the reports of current work with least modifications in the LD_{50} of sulfoxaflor was observed 0.1841 *ppm* might be owing to dissimilarity in organism habitat and nature of under trial compounds.

Toxicity of Spinetoram

Legwaila *et al.* (2014) evaluated the spinosad against 2nd instar larvae of diamond back moth in 5 dilutions (1.08, 0.84, 0.60, 0.36 and 0.12 g/L). They observed that the spinosad was very toxic against eggs as compared to larvae but in present findings of spinosad related spinetoram biopesticide showed moderately toxicity to worms LD_{50} of spinetoram was found to be 1.4288 *ppm*.

Sabry *et al.* (2014) reported that the toxicity of modern three insecticides spinetoram, chlorantraniliprole and thiamethoxam were studies against the 1st instar larvae stages of *Pectinophora gossypiella* Saunders. The LC_{50} for spinetoram, chlorantraniliprole and thiamethoxam, were found as 19.0 *ppm*, 13.9 *ppm* and 8.9 *ppm* respectively. The evaluation of results were showed most effective was thiamethoxam>chlorantraniliprole>spinetoram, residual bioassays was carried out against trichogramma, *Trichogramma evanescens, Chrysoperla carnea* and *Coccinella septempunctata,* results showed that three examined pesticides were low toxic against to the 2nd instar larvae stages of *Chrysoperla carnea*. While mortality percent were found high against to the 2nd instar larval stages of *Coccinella septempunctata*. Amongst all spinetoram was very high toxic against e 2nd instar larval stage of *Trichogramma evanescens*. It was suggested that during the low peak of pink bollworm larvae population spinetoram can be recommended to use. Hardke *et al.* (2011) reported that the toxicities of spinetoram 0.066 µg /mL and chlorantraniliprole 0.068 µg/mL have been showed significantly high toxic impacts to fall armyworm. In current work the LD_{50} of spinetoram was found to be 1.4288 *ppm* showed moderate toxicity as compared to work of Legwaila *et al.* (2014), Sabry *et al.,* (2014) and Hardke *et al.* (2011) and less toxic than sulfoxaflor as reported in current carried out work.

For the assessment of data, probit analysis statistical method was used. In the present study, the LD_{50} of spinetoram was observed to be 1.4288 *ppm.* On the contrary, a little differences between the current carried out work and data on the record may be due to the difference in the test organism as earlier finding on different (vertebrate and invertebrate) but current results are on the earthworm (invertebrate).

Table 1. Toxicity of sulfoxaflor to *Pheretima posthuma*.

No	Concentrations (*ppm*)	Mean mortality	S.E	Range % Mortality
I	0.06	2.4	0.27	18.70-29.20
II	0.12	4.2	0.22	37.60-46.30
III	0.24	6.0	0.35	53.10-66.80
IV	0.48	8.2	0.41	74.00-90.00
V	0.96	9.0	0.35	83.14-96.80

Table 2. Toxicity of spinetoram to *Pheretima posthuma*.

No	Concentrations (*ppm*)	Mean Mortality	S.E	Range % Mortality
I	0.50	2.2	0.22	17.60-27.30
II	1.0	4.2	0.27	38.70-49.20
III	2.0	6.2	0.41	54.00-70.00
IV	4.0	7.2	0.22	67.60-79.20
V	8.0	9.4	0.27	88.70-99.00

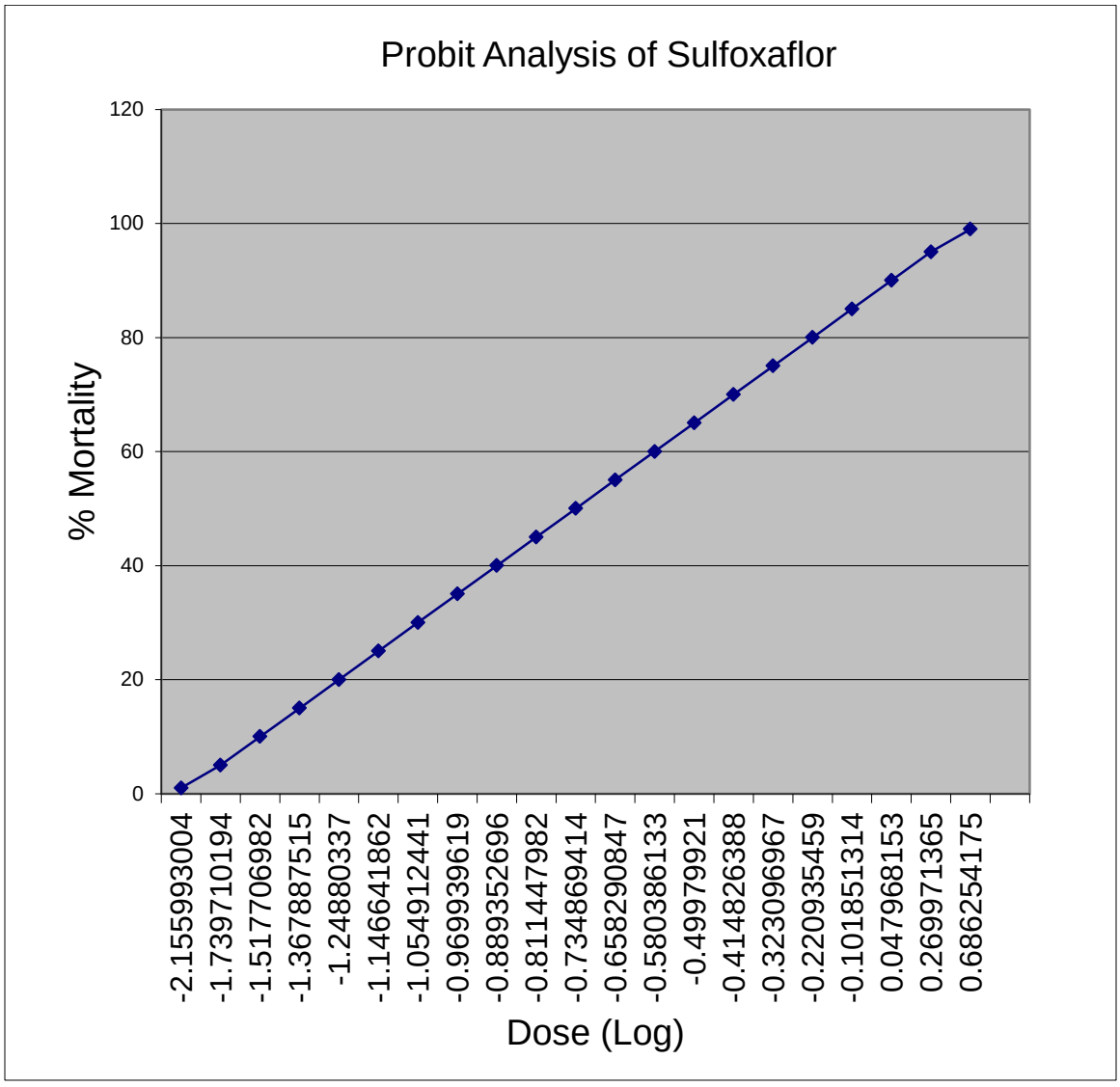

Fig. I. Log dose & % Mortality curve at post treatment of sulfoxaflor on earthworm.

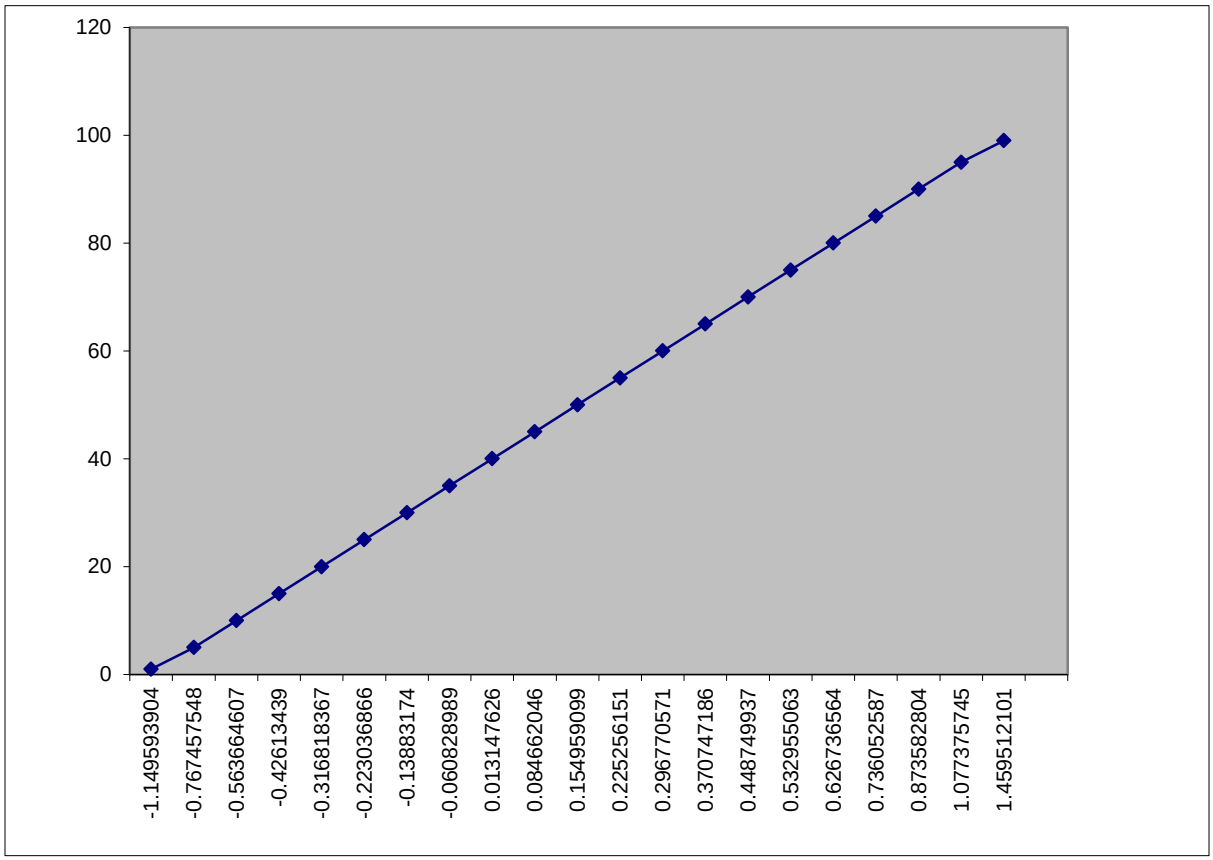

Fig. 2. Log dose & % Mortality curve at post treatment of spinetoram on earthworm *Pheretima posthuma*.

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