Toxic effect of insecticides on earthworms reducing soil fertility in Pakistan.

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Abstract- This research has been attempted to test acute toxicity of five commonly used insecticides in Pakistan. Two different methods, contact filter paper and artificial soil assessment were used for measuring toxicity effect on earthworm. Different concentrations of insecticides for 48 hours were used in contact filter paper test and found Sulfosulfuron super toxic to earthworms. In artificial soil test different concentrations of five insecticides were used for seven and fourteen days. Neonicotinoids however was found to be more toxic to earthworms after seven and even 14 days treatment. This research is limited to earthworms only, how much other beneficial organisms and nutrients are affected is not known. In order to save earthworms from such acute toxicity these insecticides should be emended.

Index Terms: *Earthworm,Insecticide, Neonicotinoids, Sulfosulfuron, Toxicity.*

I. INTRODUCTION

Annelida is large phylum containing earthworms, ragworms, leech etc. There are more than 5000 species of earthworms (Abdollahi *et al.,* 2004; Baker *et al.,* 2006). They are present in moist and dark places of mud; they live near the surface of soil (Heimbach, 1984). They indicate soil health and also amend it by horizontal and vertical movements through the soil. Due to these movements they carry the nutrients from bottom to top and organic matter from top to bottom during this process channels are made which help in gaseous exchange in tunnels. They are known as soil engineers and are studied most in scientific field (Baker *et al.,* 2006).

Earthworms are assailable to many toxic chemicals and act as model organisms in order to study effect of insecticides. These are an indispensable component of soil biomass and act as an important indicator of total soil metabolism as well as soil pollution and toxicity (Izadi *et al.,* 2012). In fact, several earthworm species have been used as bio-indicators and hence these are used in the evaluation of chemical environmental pollution (Fründ *et al.,* 2011). For environmental toxicological analysis, inspecting quality of environment and risk evaluation earthworms are best indicators. However, while using different insecticides the importance of earthworm has always been neglected, their role in asseverating soil health and

fertility is ignored (Lanno *et al.*, 2004; Lo, 2010). Earthworm's activity could be halted if the issue of toxicity of insecticides be ignored. This can also diminish fertility of soil and vegetation (Nasr and Badawy, 2015). Therefore, the present research has been attempted to investigate the unfavorable effect of five insecticides which are commonly used in Pakistan, on earthworm.

II. MATERIALS AND METHODS

Earthworm Samples

Samples containing different earthworms were collected. Various areas like Multan, Muzaffargarh and The Women University Multan premises were considered for collection of samples. Fine sand, ground sphagnum peat and kaolinite clay were mixed together to make artificial soil and after that the mixture was reserved at 25-260C. For the adjustment capability of maximum potential extent of water the method of Wang *et al* (2012) was applied for wetness of soil that was adjusted to 35% by adding sanitized water.

Insecticides

Insecticides like Pyriproxyfen, Pelegan, Counter, Juel and Uniform were selected on the basis of two reasons, as they are the most common and widely used in Pakistan (Table 01). The commercial formation was used alternatively than the active ingredients.

Toxicity Test Methods

Contact Filter Paper Test:

Insecticides (with concentrations 0, 0.5, 1, 2, 5 and 10µl) were used with 2ml of acetone each for contact filter paper test. The insecticides and acetone were mixed in five different 9cm petri dishes and that mixture was used to moist the filter papers. All petri dishes were marked with different insecticides name. When the solution evaporated, a single earthworm

was kept in each petri dish after remoistening the filter papers by using 2ml distilled water. Petri dishes were then incubated at 20ºC for 48hr considering a maximum interval of time and then the earthworm mortality was noted.

Class	Insecticide name	Common Name in Pakistan
Pyridine based pesticide	Pyriproxyfen	Pyriproxyfen
Organophosphate	Triazophos	Pelegan
Pyrethroid	Deltamethrin	Counter
Neonicotinoid	Nitenpyram	Juel
Sulfonylurea	Sulfosulfuron	Uniform

TABLE 01: Five commonly used insecticides in Pakistan.

Artificial Soil Test:

35% of water content was found by dry weight of soil after a soil toxicity test. The concentration range of insecticide 2, 5 and 10µl in 250g of artificial soil was mixed in 10ml acetone and 10g of fine quartz sand for every concentration that was examined. The sand is left for minimum period of one hour to evaporate acetone from it and then mix completely with artificial soil. All arrangement was made in glass jars. 10 adult earthworms and artificial soil were placed in 500ml glass jar for 7-14 days. The jars were kept within stable and permanent light source at 25-260C and enclosed with fine net for exchange of air with 80-85% comparative moisture.

Statistical Analysis

For statistical analysis of high toxicity of insecticides in earthworms LC50 values of insecticides were classified (Roberts and Wyman, 1984) into 5 groups named relatively nontoxic having >1000µl cm-2 value, moderately toxic ranging 100-1000µg cm-2, very toxic ranging 10-100µg cm-2, extremely toxic ranging 1-10µg cm-2 and super toxic having <1.0µg cm-2 value.

TABLE 02: Summarizing different parameters showing acute toxicity of insecticides in earthworm

Insecticide	Slope (se)	x2 (d.f)	LC50µg cm-2	Toxicity grade
Nitenpyram (Juel)	5.75 (0.85)	1.22 (3)	0.2	Super toxic
Deltamethrin (Counter)	4.00 (0.55)	1.37 (3)	10.53	Very toxic
Triazophos (Pelegan)	3.39 (0.41)	4.16 (3)	14.19	Very toxic
Pyriproxyfen	3.50 (0.51)	2.55 (3)	8.5	Extremely toxic
Sulfosulfuron (Uniform)	6.55 (0.95)	1.06 (3)	0.5	Super toxic

through contact filter paper test.

III. Results

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Contact Toxicity

Among all five insecticides, Sulfosulfuron and Nitenpyram conferred to be the most prominent toxicity followed by Pyriproxyfen, Deltamethrin and Triazophos. According to the insecticides common names in Pakistan their toxicity level can be described as uniform, most toxic followed by Juel, Pyriproxyfen, Counter and the least toxic Pelegan (Table 02).

At 48hrs, Sulfosulfuron was 28.38 fold more toxic than Triazophos. Nitenpyram was 3 folds less toxic

than Sulfosulfuron. Sulfosulfuron was super toxic to earthworms.

Soil Toxicity Test

Direct proportion between concentration and mortality rate was found through soil toxicity test of all insecticides, increase in length of exposure caused an increase in mortality rate. Nitenpyram was found to be highly toxic after seven days. It was 344.59 folds more toxic than Pyriproxyfen. Both Sulfosulfuron and Triazophos were found to be equally toxic with a minor difference and more toxic than Deltamethrin. Average acute toxicity in descending order is presented as:

Nitenpyram > Sulfosulfuron > Triazophos > Deltamethrin > Pyriproxyfen

At fourteen days interval, average acute toxicity of insecticides in descending order was same as exhibited at previous interval of seven days (Table 03).

On the base of on LC50 value, the toxicity of insecticides was ordered in following manner:

Nitenpyram > Sulfosulfuron > Triazophos > Deltamethrin > Pyriproxyfen.

The toxicity of Nitenpyram is 321.82 fold higher than Deltamethrin at 14 day interval. In 14 day time period (Table 04). Nitenpyram however demonstrate the most toxicant properties as alike as the 7-day time period.

TABLE 03. Summarizing different parameters showing acute toxicity of insecticides (7-days treatment) in earthworm through contact filter paper test.

Insecticide	**Slope (se)**	**x2 (d.f)**	**LC50 µg cm-2**
Nitenpyram	9.62 (1.34)	2.72 (3)	4.44
Deltamethrin	8.90 (1.55)	0.17(3)	1353
Triazophos	8.89 (1.41)	1.76 (3)	374.19
Pyriproxyfen	7.45 (1.31)	1.45 (3)	1530

Sulfosulfuron	8.55 (1.65)	1.26 (3)	361.07

TABLE 04. Summarizing different parameters showing acute toxicity of insecticides (14-days treatment) in earthworm through contact filter paper test.

Insecticide	**Slope**	**x2 (d.f)**	**LC50 µgcm-2**
Nitenpyram	9.15 (1.20)	1.60 (3)	3.94
Deltamethrin	10.60 (1.55)	1.97 (3)	1268
Triazophos	7.61 (1.21)	1.12 (3)	344.09
Pyriproxyfen	8.85 (1.41)	1.25 (3)	1250
Sulfosulfuron	7.85 (1.65)	2.46 (3)	255.37

IV. DISCUSSION

Insecticides are toxic chemicals that have the potential to sternly damage beneficial non targeted soil fauna. This could bring down the population of soil fauna specially earthworms that are beneficial for soil fertility. Earthworms are extremely assailable to the adverse effects of insecticides (Nurhidayati *et al.*, 2012). This study involves toxic effect of commonly used insecticides on earthworms in Pakistan.

In filter paper test, insecticides were ingested directly as there was no food intake involved so it was considered as more toxic procedure. Although same insecticides were found to be less toxic in soil method except for Nitenpyram.

The contact filter paper test was first method for analyzing the toxicity of insecticides to earthworm. This test is fast, effective and relatively simple to perform because the chemicals are absorbed via epidermis but its drawback is that it is unable to find the soil's original form (Cang *et al.*, 2017).

Nitenpyram's toxicity was statistically greater than other insecticides after 14 days (Nauen and Denholm, 2005; Wang *et al.*, 2015). Some previous studies reveal that juvenile earthworms are more sensitive to insecticides as compare to the adults. The studies demonstrate that the toxic effect of chemicals reduces their sexual development along with their growth (Shiping *et al.*, 2008). Nitenpyram is being widely use because of their highest quality insecticidal effect (Narahashi, 2000).

On the base of LC50 values, all the insecticides were low in toxicity except Nitenpyram which was found to be highly toxic to earthworms and it was termed as super toxic. Earthworms' death rate was directly proportional to the concentration of insecticide and time of exposure of insecticide. Triazophos was found to be less toxic. Nitenpyram was found to be more toxic after 14 days interval.

In both tests, Deltamethrin was found to be much toxic on the earthworm. In the contact filter paper test, the LC50 value of Deltamethrin was 327.8µg cm-2

within 48hrs. However, the LC50 value of Deltamethrin in 7 days and 14 days were found to be 92.8mgkg^{-1} and 68.1mgkg^{-1} respectively, in artificial soil test (Velki and Hackenberger, 2013; Song et al., 2015). This research revealed that Deltamethrin is fatal for earthworms and under severe contact in 14 days treatment, Deltamethrin was toxic or even lethal to earthworms. Deltamethrin is toxic in dose dependent manner.

Many insect pests are killed by Triazophos. In contact and soil methods, Triazophos is found to be very toxic against insects (Reddy and Rao, 2008; Wang *et al.*, 2012). In artificial soil, Pyriproxyfen acts as insect growth regulator in the dose dependent manner so by increasing concentration of this insecticide causes decrease in number of earthworms.

V. CONCLUSION

This research reveals that in contact filter paper test insecticides were proved to be more toxic on earthworm as compared to artificial soil test. Different insecticides showed varied amount of toxicity due to their chemical nature. In contact filter paper test, Sulfosulfuron was highly damaging. In artificial soil test, Nitenpyram was found to be super toxic for earthworms. There is still need to work more on chemical nature of diverse group of insecticides and their mode of action so that soil fauna could be saved, which are beneficial for plant growth.

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