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TOXICITY OF AZOXYSTROBIN (FUNGICIDE) AGAINST EARTHWORM (*PHERETIMA POSTHUMA)*

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ABSTRACT

The present study describes the impact of azoxystrobin against *Pheretima posthuma* earthworm. LD_{50} of azoxystrobin (Fungicide) was found to be at 3.21 *ppm* in soil. Mortality has been found as 20%, 30%, 40%, 50% and 70% against doses of 0.50 *ppm*, 1.0 *ppm*, 2.0 *ppm*, 4.0 *ppm* and 8.0 *ppm* of soil at post exposure of 48-hours by plotting the doses against average death values through probit analysis. The concentrations of azoxystrobin 0.50 *ppm* and 8.0 *ppm* of soil showed 20% and 70% mortality respectively at post treatment of 48-hours in adult earthworms.

Key words: *Pheretima posthuma*, Toxicity, Fungicide.

INTRODUCTION

Earthworm plays significant role in agro ecosystem by developing makeup of soil, nutrient recycling, used as marker to monitor the effects of pesticides against land terrestrial organisms, enormous key effect on the whole ecosystem, significance for health of soil and deal with the intensity of its influences (Zhang *et al.*, 2007, Utsumi and Yoshimura, 2011; Blouin *et al.*, 2013). Earthworm's enhanced yield in domestic crops often decreased levels of wild associations (Turcotte and Poveda, 2017). It has been determined that diversity of earthworm communities has been considerably reduced by applying chemical and mechanical practices (Decaens and Jimenez, 2002, Smith *et al.*, 2008). Earthworms have been used in toxicological tests due to their highest biomass. Researchers associated the earthworm's sensitivity and soil arthropods to azoxystrobin, and the findings revealed that earthworms have been sensitive to azoxystrobin (Kohlschmid and Ruf, 2016). It found that the pesticides application in the cropping fields decrease the earthworm population. The replacement rate of earthworm is very much low (Lavelle and Spain, 2001), communities of earthworm living in polluted soils of pesticide survived constantly anxiety of chemical, furthermore via physiological struggles and avoidance activities (Posthuma and Van Straalen, 1993). They take part in different ways to fertile soil and improved adaptation of agricultural ecosystem to changes in atmosphere. The earthworms providing input soil functions by moving soil nutrients and substances from the subsoil to the topsoil, which helpful in many services and sustain the strength of the agricultural soil, thus, earthworms could be achieved further consideration in agricultural system. In a healthy agricultural soil per hector three million earthworms could be recognized, added up to 6 tons of organic substances decomposed and contribute as 1/2 centimeter of the coat in fields of cultivation annually. The vertical burrows of earthworm in the farming soil make sure as well exposure, improved macrospores, promote in the proliferations and propagations of fungi and beneficial bacteria, pests and pathogenic organism are biologically degraded, its tunnels also help in water penetration, storage and drainage of the agricultural soil and deliberately utilize of conventional pesticides have been in several places reduced earthworms in agricultural fields, mainly uses of those pesticides which have higher toxicity reported (Pfiffner *et al.*, 2014).

There are many significant investigation focused on earthworms (Han *et al.*, 2014, Kohlschmid and Ruf, 2016, Leitao *et al.*, 2014, Wang *et al.*, 2012), fish (Cao *et al.*, 2018, Jiang *et al.*, 2018; Liu *et al.*, 2013; Olsvik *et al.*, 2010), algae (Garanzini and Menone, 2015, Kunz *et al.*, 2017; Lu *et al.*, 2017) and soil microorganisms (Bacmaga *et al.*, 2015, Guo *et al.*, 2015; Howell *et al.*, 2014, Wang *et al.*, 2015, 2018). Above mentioned research revealed that azoxystrobin poses ecological hazards to non-target organisms. Fungicides are considered as either biological agent or chemical that controls the growth of fungi and their spores. Azoxystrobin is used worldwide for the control of fungal problems in cultivated fields (Battaglin *et al.*, 2011). The presence of fungicides sample in soil could be almost certainly increases of fungicides to manage the fungal diseases in various crops to increase in the yield (Battaglin *et al.*, 2011). Fungicides have been applied in agriculture and also in livestock against fungal diseases. For earthworms both fungicides and fumigants are the most lethal substances (Abdul Rida and Bouche, 1997 and Booth and Halloran, 2001). All fungicides showed contact action or systemic action protected those parts of plant wherever the fungicides treated, distributed in the whole plant where it applied and taken up to higher altitude parts

of sprayed plants by xylem vessels, then protected growth for a brief period of new leaf. Mostly fungicide deposits were found in foods that uses human being, from the postharvest treatments (Brooks and Roberts, 1999). Under uncertain conditions, precipitous of sulfur and dodine basically have been showed no effects on earthworm behavior used as fungicides (Edwards and Lofty, 1973), evaluated the key effects on worms physiology and behavior in farming units at post treatments of thiram, captan, formaldehyde, quintozene dicloran, and benomyl whereas benomyl found lethal effects (Stringer and Wright, 1973) and wide-range tests were carried out on the lethality of methyl benzimidazole-2-yl carbamate (MBC), benomyl, thiophanate methyl and thiabendazole against earthworm in different conditions (Stringer and Wright, 1973) Fungicides substituted groups like benzimidazole included carbendazim, benomyl, fuberidazole and thiabendazole showed extremely lethal impacts (Stringer and Lyons, 1974).

MATERIALS AND METHODS

During current work earthworms (*Pheretima posthuma)* Kinberg have been collected from laboratory. Afterward earthworms have been saved in plastic bags, taking particular amount of humid organic soil. Azoxystrobin (Generic 25%w/w, serial/Batch no, SPL1307AZ), purchased from the pesticide market produced by Sungro pesticide, private Limited in Pakistan. The concentrations of 0.50, 1.0, 2.0, 4.0 and 8.0 *ppm* doses have been obtained after diluted in conventional grade and after that assorted in 1 kg soil for earthworm exposure. The earthworms were tested with the different concentrations of the azoxystrobin. The desired concentrations of the azoxystrobin were prepared in the soil used to expose them. LD_{50} of the azoxystrobin was calculated after based on (Finney, 1967).

RESULTS

LD_{50} of azoxystrobin (Fungicide) was found to be at 3.21 *ppm* in soil. Mortality has been found as 20%, 30%, 40%, 50% and 70%, respectively against doses of 0.50 *ppm*, 1.0 *ppm*, 2.0 *ppm*, 4.0 *ppm* and 8.0 *ppm* of soil at post exposure of 48-hours by plotting the doses against average death values through probit analysis. The concentration of azoxystrobin 0.50 *ppm* and 8.0 *ppm* of soil showed 20% and 70% mortality respectively at post treatment of 48-hours in adult earthworms. For the environmental impacts the untreated earthworms have been kept as well (Table-1 and Fig. I).

DISCUSSION

LD_{50} of azoxystrobin (Fungicide) was observed as 3.21 *ppm* of soil, the lowest concentration 0.50 *ppm* showed 20% mortality and 8.0 *ppm* caused 70% mortality in adult earthworms at post treatments of 48-hours. Hou *et al., (*2016) measured the residue of azoxystrobin in soil and found that the initial residue of azoxystrobin in test field of Jilin Province, China was 9.54 mg kg^{-1}, and the residue of azoxystrobin in test field of Beijing, China was 8.57 mg kg^{-1}. Azoxystrobin (fungicide) was observed to have least solubility to the water; its distribution into porous water could be expected due to the least soluble product in the water. Previously, all tested animals to exposure of pesticides showed lethal responses due to the uptake of chemical into organisms and as well as different lethal activities of the tested pesticides (Frampton *et al.,* 2006). Presently observed LD_{50} of azoxystrobin to be 3.21 *ppm* showed conformity with azoxystrobin low solubility to the water as an active component proved.

Table 1. Mortality impact of *Pheretima posthuma* under the effects of azoxystrobin on earthworm.

Dose *(ppm)*	Log Dose	Earthworm exposed	Earthworm killed	Empirical probit	Chi- square
0.50	-0.301	10	02	1.9104	0.0042
1.0	0.	10	03	2.9175	0.0023
2.0	0.301	10	04	4.1197	0.0035
4.0	0.6021	10	05	5.4114	0.0313
8.0	0.9031	10	07	6.6607	0.0173

Chi-square = 0.0586, Degrees of Freedom = 3, p-level = 0.9963

Probit Analysis - Finney Method [Lognormal Distribution]
In all cases zero mortality data was consider for the analysis

	Dose *(ppm)*	**Standard Error**	**Log Dose**	**Standard Error**	**% killed**	**Probit (Y)**
Dose (Stimulus) Percentile	0.0227	3.103	-1.6434	2.4362	1	2.6732
	0.0969	2.2828	-1.0136	1.6733	5	3.3548
	0.21	1.9521	-0.6777	1.2705	10	3.7183
	0.387	1.7325	-0.4123	0.9573	16	4.0056
	0.5359	1.6259	-0.2709	0.7944	20	4.1585
	0.765	1.5128	-0.1164	0.6226	25	4.3258
	1.053	1.4143	0.0224	0.4797	30	4.476
	1.8745	1.4176	0.2729	0.3032	40	4.7471
	3.2107	3.0199	0.5066	0.3643	50	5.
	5.4994	9.7491	0.7403	0.5807	60	5.2529
	9.79	34.7212	0.9908	0.8592	70	5.524
	13.4759	69.997	1.1296	1.0205	75	5.6742
	19.236	152.8744	1.2841	1.203	80	5.8415
	26.6352	312.6475	1.4255	1.3714	84	5.9944
	49.0828	1,202.4261	1.6909	1.6903	90	6.2817
	106.3629	6,641.4251	2.0268	2.0965	95	6.6452
	453.4916	165,053.5864	2.6566	2.8621	99	7.3268
Regression Statistics	LD50	LD50 (S.E.)	Log LD50	LD50 (S.E.)	LD50 LCL	Y intercept
	3.2107	3.0199	0.5066	0.3643	1.3621	4.4517

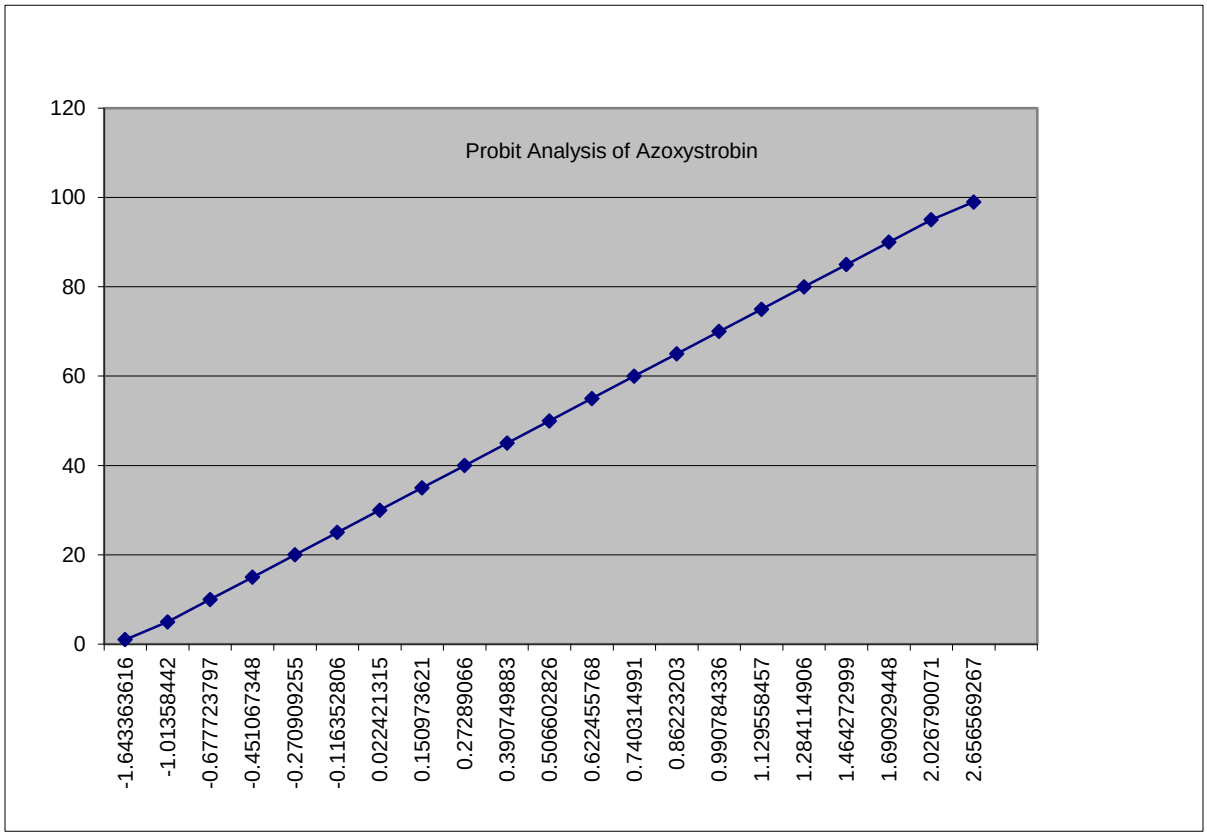

Fig. I. Log dose and % Mortality curve at post treatment of azoxystrobin on earthworm *Pheretima posthuma*.

The pollutants taken by surface dweller animals are directly linked with main pathway of soil as pores water (Styrishave *et al.,* 2008; EFSA, 2010a), uptake of pesticides through the pores water influenced by soil fertility. Organic matters and clay substances which communicate with the pesticide molecules in the original composition that are uptake by surface dweller organism (Kuperman *et al*., 2006; EFSA, 2009; Van-Gestel, 2012). Worms obtained pesticides from soil either by ingestion or skin media and passive diffusion (De Silva *et al.,* 2009). Similar details have been postulated by other researchers in respect of soil-dweller e.g. earthworms, few compounds of different pesticides belong to various groups as polychlorinated, benzimidazole fungicides, organochlorine and carbamate insecticides (Amorim *et al.,* 2002, Patakioutas and Albanis, 2002, Lanno *et al.,* 2004, De Silva *et al.,* 2009, EFSA 2010b). The fungicide azoxystrobin is approximated with least lethal effects to earthworms due to the belong strobilurin group of fungicide, which is quickly degraded in the environment causing a very little hazard for determined in the environment (Bartlett *et al.,* 2002). Leitao *et al.,* (2014) the studies of fungicide as azoxystrobin in cropping fields against two species of earthworm *Eisenia andre* and *Enchytraeus crypticus* evaluated by using reproduction as ecotoxicological standard, azoxystrobin showed highly toxic to either species of earthworm and the EC_{50} was found as 42.0 *ppm* in soil caused as 77% mortality, but in the current work, The azoxystrobin 0.50 *ppm* in soil caused 20% and 8.0 *ppm* in soil caused 70% mortality in adult earthworms. Current results of azoxystrobin in respect of mortality in earthworms agreed with the previous postulations class strobilurin (Potter *et al.,* 1994, Bartlett *et al.,* 2002, Kuperman *et al*., 2006, De Silva *et al.,* 2009, EFSA, 2009, Rouabhi *et al.,* 2009, Van-Gestel 2012 and Leitao *et al.,* 2014).

About fungicide low toxicities against earthworms and other untargeted organism with cropping soil Envirofacts (2005) postulations disagreed from the present results in respect of earthworms, while the results of azoxystrobin toxicity with the LD_{50} of 3.21 *ppm* are comparable to the results reported by OECD against untargeted soil tests values found of LC_{50} as 283.0 *ppm* of soil (EFSA 2010b) and values of LC_{50} as 327.40 *ppm* of soil (Wang *et al.,* 2012). Nevertheless, the assurance that the NOEC reported for earthworm assessment has not been achieved with the low concentration estimation 50.0 *ppm* of soil that was in conformity with the NOEC statement of 20 *ppm* of soil to earthworm Footprint (2012).

Currently, azoxystrobin was showed 50% mortalities at 3.210 *ppm*., against earthworm by contact cum feeding method. The data is assessed by statistical method of probit analysis. In the present work, LD_{50} of azoxystrobin was observed as 3.210 *ppm*. On the contrary, a few differences were found between the present work and earlier results could be due to variation of organism as previous finding on different organisms, but current results are observe on the earthworm *Pheretima posthuma.*

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