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Assessment of Neonicotinoid Insecticide Acetamiprid LC_{50} against Earthworm (*Eisenia fetida* L.)

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ABSTRACT

Earthworms are prominent invertebrates belonging to family lumbricidae and are dominant in the temperate and tropical soils and they are account for 60–80 % of the entire soil biomass and play a critical role in the ecosystem. *Eisenia fetida* is most suitable class of earthworms which is used for vermicomposting but now the number of earthworms decreasing day by day because of uncontrolled use of pesticides. The pesticides enhanced the yield of crop.Acetamiprid is a second-generation of neonicotinoid insecticide which is more effective against pests if it used as foliar sprays as compared with applied directly to the soil. The technical grades of acetamiprid (97%) were obtained from the l pesticide market of Hisar. Exposure of earthworms of *Eisenia fetida* to different concentrations of acetamiprid is usually done for determination of lethal concentration 50 value by using standard paper contact toxicity method and the following concentration of acetamiprid (0.100 µg/cm^2, 0.150 µg/cm^2, 0.170 µg/cm^2, 0.190 µg/cm^2, 0.210 µg/cm^2, 0.220 µg/cm^2, 0.230 µg/cm^2 and 0.240 µg/cm^2) were prepared with water for determination of lethal concentration of actetamiprid against *Eiseniafetida* and a concentration of 0.165µg/cm^2 caused fifty percent mortality of earthworms knwon as LC_{50} of acetamiprid. The mortality per cent has been calculated twenty four hours after treatment with relevant pesticide.

Keywords Actetamiprid, *Eisenia fetida,* Earthworms, Neonicotinoid, LC_{50}.

INTRODUCTION

Acetamiprid is a second-generation neonicotinoid insecticide which was initially commercialized in Japan in 1995 by Nippon Soda mainly for foliar applications while direct soil uses are restricted (Elbert *et al.* 2008). It has been proved that acetamiprid is more effective against pests if it used as foliar sprays as compared with applied directly to the soil (Palumbo *et al*. 2001). It is still relatively unclear that ecological risks posed by acetamiprid to non-target aquatic and terrestrial organisms. It's highly soluble (4250 mg L^{-1} at 25^{o}C) and stable in water (Jeschke and Nauen 2010). Like

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other pesticides, non-target aquatic organisms are usually exposed through off-target spray drift, surface water runoffs (Racke 2003).

Contribution of soil decomposing micro and macro organisms are affecting soil structural and physical properties in various agro-ecosystems and their effect in cropping patterns have been extensively studied (Fragoso *et al.* 1997, Beare *et al*. 1997). There were accumulated evidences suggests that the biodiversity of soil organisms impart resistance to biotic and abiotic stresses (Altieri and Miguel 1999). Many factors were responsible for green revolution in India, out of which one of the major factor that effected is the usage of pesticides at appropriate doses. These pesticides usage had grown up especially in case of high yielding varieties. These newly introduced varieties became major targets for ranges of pests and insects. Cultivation of high yielding varieties caused soil exhaustion of nutrients. The nutrient depletion was replenished by high doses of fertilizers which caused luxurious growth of crops which are made prime targets of pests. Hence there has indiscriminate usage of pesticides been noticed (Ecobichon 2000).

Neonicotinoids are now the most prominent class of insecticides and are being used globally as selective agonists of insect nicotinic acetylcholine receptors (Jeschke *et al.* 2011,Szczepaniec *et al.* 2013). Besides their use in agriculture in various formulations such as granules or foliar sprays, they even found their way to control house hold insects likes termites, cockroaches. They are also been used in veterinary medicine to control ectoparasites (Jeschke *et al*. 2011,Goulson 2013).

Vojoudi and Saber (2013), Miao *et al.* (2013) have studied the lethal and sub-lethal toxicity of neonicotinoids on target pests. Several researchers have also been carried out studies on the effect of neonicotinoids on bees and other non-target invertibrates both in aquatic and terrestrial ecosystems. Werner and Hitzfeld (2012) reported their role in pollinator crisis (Colony collapse disorder) which is a major environmental concern.

MATERIALS AND METHODS

The experiment was conducted during July to September 2020 at Chaudhary Charan Singh Haryana Agricultural University, Hisar and fully clitellated earthworms (*E.fetida*) were exposed to neonicotinoid insecticides viz., acetamiprid to determine toxicity.

Selection of test earthworm

Fully developed clitellated healthy earthworms of *E. fetida* with average weight of 700 mg were collected from the vermiculture unit of Department of Zoology, Chaudhary Charan Singh Haryana Agricultural University, Hisar. The earthworms were brought to the vermiculture labo ratory and their culture was maintained in plastic tubs having cow dung mixed with organic manure (60:40) with adequate moisture (60-65%) maintained by sprinkling of water on the substrate as and when required and they are covered with gunny bags to protect them from pests and to maintain moisture level.

Procurement of test neonicotinoid insecticides

The technical grade of acetamiprid (97%) was obtained from the pesticide market of Hisar, Haryana. Description of treatments (sprayed) given in Table 1.

Experimental set up for assessing the LC_{50} of acetamiprid

The selected earthworms were washed with distilled water and kept in moist filter paper for two h for cleaning of gut contents. The size of flat bottomed glass vials of 8 cm in length, 3 cm in diameter with the medium grade 0.2 mm thick were taken and whatmann filter papers were kept to fit properly to

Table 1. Description of treatments (sprayed) given to the test earthworm species along with control.

Sl.No.	Treatment	Description
1	Control	No dose
2.	T1	Acetamiprid 0.100 µg/cm^2
3.	T2	Acetamiprid 0.150 µg/cm^2
4.	T3	Acetamiprid 0.170 µg/cm^2
5.	T4	Acetamiprid 0.190 µg/cm^2
6.	T5	Acetamiprid 0.210 µg/cm^2
7.	T6	Acetamiprid 0.220 µg/cm^2
8.	T7	Acetamiprid 0.230 µg/cm^2
9.	T8	Acetamiprid 0.240 µg/cm^2

Table 2. LC_{50} value of acetamiprid against fully developed clitellum earthworm *(E. fetida).*

Insecticide	Expose time	Number	Slope (b)	χ^2	LC_{50}	LC_{95}
Acetamiprid	24h	8	5.293	0.98	0.165 µg/cm^2	0.331 µg/cm^2

the size of vials to avoid overlapping of sides. Eight concentrations of acetamiprid (0.100 µg/cm^2, 0.150 µg/cm^2, 0.170 µg/cm^2, 0.190 µg/cm^2, 0.210 µg/cm^2, 0.220 µg/cm^2, 0.230 µg/cm^2 and 0.240 µg/cm^2) were prepared with water (Table 1). Every vial with filter paper were poured with one ml concentration of insecticide and then rotated horizontally to ensure the homogenous distribution of insecticide over filter paper along with the control (having 1 ml deionized water only). Thereafter, inoculated one earthworm per vial to different concentrations of acetamiprid along with the control and all vials were covered with muslin cloth to avoid the escaping of earthworms from the vials. During the whole experimental process proper aeration, temperature range between 20±5^0 C and adequate moisture levels were maintained to minimize the mortality. All treated vials were laid horizontally and protected from the exposure of light to increase the efficiency of experiment. Eight replicates for each concentration along with the control were maintained.

Determination of LC_{50} of insecticides against adult

Eisenia fetida

After assessing the percent mortality, the corrected mortality was computed (Abbott 1925) and the data recorded during experiment was subjected to Probit analysis (Finney 1971) as recommended by standard paper contact toxicity method.

$$\text{Percent mortality} = \frac{\text{Total number of earthworms died}}{\text{Total number of earthworms released initially}} \times 100$$

$$\text{Corrected mortality} = \frac{\text{Mortality in treatment} - \text{Mortality in control}}{100 - \text{Mortality in control}} \times 100$$

RESULTS

Earthworms of *Eisenia fetida* were exposed to different concentrations of acetamiprid for determination of LC_{50} by using standard paper contact toxicity method. The obtained results are given in Table 2.

LC_{50} values have also been calculated and shown in Table 2. It is the concentration of pesticide at which it causes fifty percent death of the organisms under study. Acetamiprid at a concentration of 0.165µg/cm^2 caused fifty percent mortality of earthworms.

DISCUSSION

Earthworms have a key role in agriculture because of its excreta providing quickly available plant nutrients like Nitrogen, Phosphorus and Potassium. It burrows in the soil and increase its porosity but they are directly or indirectly affected by pollutants as a result of indiscriminate use of pesticides for better crop yield. Earthworms are important bio-indicators for ecological factor, key part of food web and are non-target species affected by insecticides. The chemical analysis of mortality of neonicotinoid insecticides exposure is expressed in terms of LC_{50}. Organization for Economic Cooperation and Development (OECD 2004) recommended that *E. fetida* is most appropriate earthworm species for ecotoxicological investigation due to their easy ability and better reproduction rates (Gestel 2012). There are several testing methods that were developed to estimate the toxicological effects of pesticides on earthworms (Wang *et al.* 2012b).

No mortality was observed at lowest dose of acetamiprid (0.100 µg/cm^2) and this dose is considered as minimal and dose of 0.240 µg/cm^2 resulted 100% mortality. The calculated LC_{50} by Probit analysis of insecticide were 0.165 µg/cm^2 it caused 50% mortality of *E. fetida* and the mortality percent has been calculated after 24 h exposure. Similar results were also reported by Enrico *et al*. (2019) in which LC_{50} of acetamiprid was (0.16 µg/cm^2) with 50% earthworm *E. andrei* mortality existed after 24 h of exposure. As par results were also reported by of Wang *et al*. (2012) where 24 pesticides were tested by filter paper contact test and artificial soil test and

demonstrated that different insecticides varied widely in their contact toxicities and the different insecticides within the same chemical classes had different toxicities to *Eisenia fetida.* In the experiment they have tested with five neonicotinoid insecticides by using contact filter paper toxicity and reported LC_{50} values for acetamiprid (97%) was 0.0088 µg/cm^2 , clothianidin (96.5%) was 0.28 µg/cm^2 , imidacloprid (95.3%) was 0.027 µg/cm^2, nitenpyram (95%) was 0.22 µg/cm^2 and thiacloprid (97.75%) was 0.45 µg/cm^2 and the LC_{50} values for imidacloprid, acetamiprid, nitenpyram, clothianidin and thiacloprid to *E. fetida* were 3.05 mg kg^{-1}, 2.69 mg kg^{-1}, 4.34 mg kg^{-1}, 0.93 mg kg^{-1} and 2.68 mg kg^{-1}.

According to the study of Saha *et al.* (2017), Wang *et al.* (2015b) the neonicotinoids insecticides have been observed as cholinesterase inhibitor insecticide substitutes (carbamate and organophosphate) as they show reduced special effects on environment because of competitively inhibit acetylcholine receptors in the central nervous system of insect.

CONCLUSION

It is concluded that acetamiprid is considered as very sensitive for the earthworm's *Eisenia fetida* even at lowest concentration and calculated LC_{50} of acetamiprid is 0.165µg/cm^2.

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