INT. J. BIOL. BIOTECH., 13 (4): 581-585, 2016.

TOXICITY OF NITENPYRAM AND NEEM LEAF EXTRACT AGAINST EARTHWORM

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ABSTRACT

The present study describes the impact of nitenpyram and neem leaf extract against *Pheretima posthuma* earthworm. The LD_{50} of nitenpyram was found to be 0.2908 ppm. Mean mortality against *Pheretima posthuma* after 48-hours exposure has been observed as 20, 30, 40, 60 and 80% in respect of 0.06, 0.12, 0.24, 0.48 and 0.96 ppm, in soil, respectively. The LD_{50} of neem leave extract was found to be 5.47 ppm. Mean mortality against *Pheretima posthuma* after 48-hours exposure has been observed as 10, 20, 30, 40 and 60% in respect of 0.50, 1.0, 2.0, 4.0 and 8.0 ppm, respectively.

Key words: Toxicity, Pesticides, Earthworm, leaf extract, nitenpyram, neem leaf extract.

INTRODUCTION

Earthworms perform several functions in the soil ecosystem by improving soil makeup and nutrient recycling, utilized as marker to determine the impactof insecticides against land organisms, significance for health of soil, enormous effect on the whole ecosystem and managed the level of influences (Blouin *et al.*, 2013, Utsumi and Yoshimura, 2011; Zhang *et al.* 2007).

Nitenpyram treated foliages get way into both the soil (or) nearest to water courses and drains, which leave transferred the nitenpyram to the decay cycling biota. Through the toxic residue of nitenpyram create the basis for troubling tothe degradation way that reason a uncertainty in the cycling of the necessary substances and improvement in the organic matter of the ecological unit (Dilly and Munch, 1996; Suberkropp 1998). The extensive uses of neonicotinoids have been shown by its traces established in soil tests (Raloff, 2005). Nitenpyram obstructs the neuronal passageway in insect nAChR (Nicotinic acetylcholine receptor) and killing them (Yasutaka *et al.*, 2012; Chatellier, 2001). Only small number of studies has been carried out for the evaluated effects on earthworms (Tu, 1995).

In the current study, neem leaf extract has been selected as entire crude extract compounds, still also being recommended and applied for agricultural organic farming; consequently, earthworms have been more probably exposed to neem extract, as compared with isolated azadiractin compounds. Wild plants extract have been believed to be naturally excellent resource of pesticides (Raguraman and Singh, 1997). Regardless of it, the localities of wild plant extracts are lacking in wide studies on the species of earthworms, all ingredient of *Azadirachta indica* have been some active biologically components which formed in animals, majority of researchers have been worked on unusual aspects of the *A. indica* including, (Naqvi et *al*., 1991; Xie *et al.*, 1995; Isman, 1997; Ahmad *et al*., 2001). Some studied on neem as element protector, before suggestion as a protector, conversely, it has important to estimate its determination on exposure surfaces of particles and also monitor its usefulness (Naqvi, 1996; Khan and Ahmed, 2000).The plant extracts have got significant position in integrated pest management (IPM). Believed as environment friendly products and not much unsafe to soil, non-target animals, trouble-free, cheap and could be applied effectively with usual techniques in the developing countries of world (Shoukry and Hussein, 1998; Mohamed *et al.*, 2003).

MATERIALS AND METHODS

For the present study earthworms *Pheretima posthuma* Kinberg were collected from the crop fields. After collection, earthworms were kept in plastic bags, having some amount of moist organic soil. Plant extract was prepared after Faheem and Khan (2010). Dilutions were prepared using Charle's formula $C_1V_1 = C_2V_2$. Concentrations of 0.50, 1.0, 2.0, 4.0 and 8.0 ppm doses were obtained after dilutions from original 100% extract. Each dilution was mixed in one kg soil for treatment of earthworm. Nitenpyram was purchased from pesticide market as trade name knockout 25%w/w, serial/Batch No FMU3FD0708. Dilutions of concentrations 0.06, 0.12, 0.24, 0.48 and 0.96 ppm doses were obtained after dilutions in conventional grade and then mixed in one kg soil for treatment of earthworm.

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

RESULTS

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

Toxicity of nitenpyram

The LD_{50} of nitenpyram was found to be 0.2908ppm. Mean mortality of *Pheretima posthuma* after 48-hours exposure has been observed as 20, 30, 40, 60 and 80% in respect of 0.06, 0.12, 0.24, 0.48 and 0.96 ppm, by plotting the values of average death for the doses of the nitenpyram through probit analysis. The lowest concentration of nitenpyram i.e. 0.06 ppm showed 20% mortality at 48 hours post-treatment, while 0.96 ppm concentration of nitenpyram caused 80% mortality in adult earthworms at 48 hours post treatment. For the determination of the ecological impacts the untreated worms have been placed as well (Table 1 and Fig. 1).

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

No	Concentrations (ppm)	Mean % Mortality	S.E	% Mortality
I	0.06	2.2	0.22	17.60-26.30
II	0.12	3.4	0.27	28.70-39.29
III	0.24	4.2	0.22	37.60-46.30
IV	0.48	6.2	0.41	53.96-70.00
V	0.96	8.4	0.27	78.70-89.20

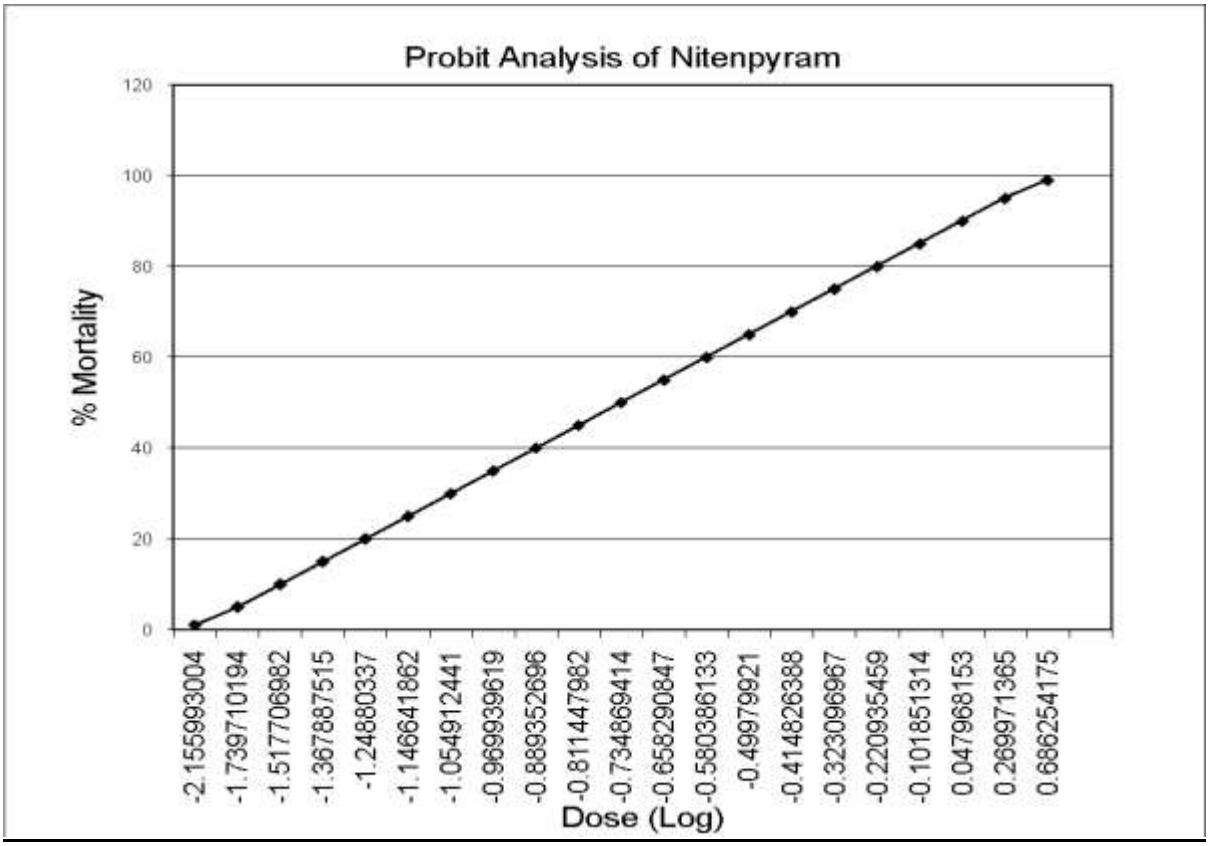

Fig. 1. Log dose & % mortality curve at post treatment of nitenpyram on earthworm *Pheretima posthuma*.

Table 2. Toxicity of neem leaf extract to *Pheretima posthuma*.

No	Concentrations (ppm)	Mean of Mortality	S.E	Rang % Mortality
I	0.50	1.2	0.22	07.0-16.0
II	1.0	1.8	0.27	18.70-29.20
III	2.0	3.8	0.27	27.60-36.30
IV	4.0	4.4	0.27	38.70-49.20
V	8.0	6.2	0.22	57.60-69.00

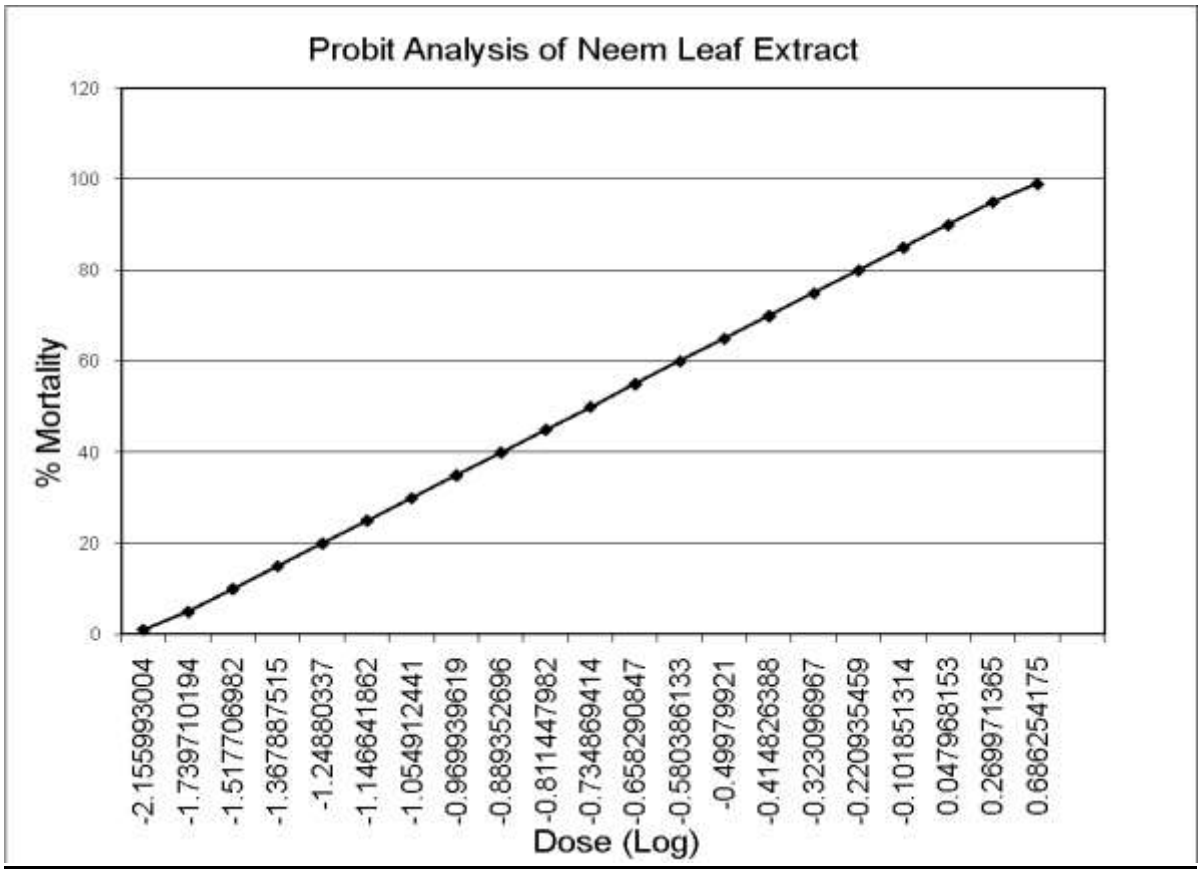

Fig. 2. Log dose & % mortality curve at post treatment of neem leaf extract on earthworm *Pheretima posthuma*.

Toxicity of neem leaf extract

The LD_{50} of neem leave extract was found to be 5.47 ppm. Mean mortality of *Pheretima posthuma* after 48-hours exposure has been observed as 10, 20, 30, 40 and 60% in respect of 0.50, 1.0, 2.0, 4.0 and 8.0 *ppm*, by plotting the values of average death for the doses of the neem leave extract through probit analysis. The lowest concentration of neem leave extract i.e. 0.50 ppm showed 10% mortality at 48 hours post-treatment, while 8.0 *ppm* concentration of neem extract caused 60% mortality in adult 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 nitenpyram (Neonicotinoids).

Asrar *et al.* (2014) reported that the bioassay toxicities of nitenpyram pesticides with trade Pyramid® against both predators *Chrysoperla carnea* and *Coccinella septempunctata* in laboratory trials. Dobson *et al.* (2012) reported that the clinical trials of nitenpyram on flea invasion. Wang *et al.* (2012) reported that the toxicities of

tested five neonicotinoids remained asacetamiprid > imidacloprid > clothianidin > nitenpyram > thiacloprid to *E. fetida*. In the present finding nitenpyram LD_{50} was found to be 0.2908 ppm which is highly toxic against earthworm by contact cum feeding method than neem leaf extract LD_{50} values 5.472 ppm as being a water soluble compound.

Consequently, the risk, in honey bee could be common on the uses of most neonicotinoids, e.g., thiacloprid, imidacloprid and nitenpyram (Tennekes and Sánchez-Bayo, 2012). Mostert *et al.* (2000) also investigated the effect of neonicotinoid against earthworm mortality. The previous findings of toxicity in respect of neonicotinoids, especially nitenpyram is in generally agreement with the finding of present work with least changes in the LD_{50} values as LD_{50} of nitenpyram was observed to be 0.2908 ppm, could be due to difference in organism ecology and nature of tested compounds.

Toxicity of neem leaf extract

Kumar *et al*. (2015) evaluated that the effects of bio pesticide azadirachtin /quercetin concentrations of 4.7, 7.1 and 9.3 ml/ kg soil against species of earthworms *Eutyphoeus orientalis* and evaluated their reproductive behavior, survivability and casting activity. Sinha *et al.,* (2004) were evaluated that the effects of neem compound against *C. capsici* by using food method. They observed the neem compound application decrease the growth of *C. capsici* significantly. The previously findings disclose that the higher concentrations to current work however, the present work is in the agreement to the previous findings.

Singh and Gour (1993) investigated that the values of neem compounds on juveniles of *Mythimna separata* and matures of *Callosobruchus chinensis* (Linn). Relevant application results showed that cured petroleum ether extraction was observation highly toxic and LC_{50} = 0.0165% remain than that of ethanolic extraction LC_{50} = 0.1421%. Tanzubil and Mc Caffery (1990) reported the impacts on *Spodoptera exampta* by compound of azadirachtin. The toxic impact has been dependent on concentration of *Azadirachtin* compound at 10 µg/larvae were showed 100% mortality. Zebitz (1987) reported the effects of *Azadirachtin* on the mosquitos 4th instar of larvae. In the case of present study it observed that the neem leaf extract has been evaluated highly toxic compound to adult *Pheretima posthuma* than other non targeted insects. Currently, crude neem leaf extract was showed 50% mortalities at 5.472 ppm, against species of earthworm by contact cum feeding method.

For the assessment of data, the statistical method of analysis probit to be used and data was assessed through probit analysis statistical method. In the present study, the LD_{50} of neem leaf extract was observed to be 5.472 ppm. Conversely, a few differences between the present work and previous results may be owing to difference in the organism as previous finding on different (vertebrate and invertebrate) but current results are on the earthworm (invertebrate).

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(*Accepted for publication July 2016*)