Applied Biological Research 22(2): 207-209; (2019) **SHORT NOTE**
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In vitro EFFECT OF INSECTICIDE EMAMECTIN BENZOATE ON EARTHWORM, *Metaphire posthuma,* USING CONTACT FILTER PAPER METHOD

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Pesticides are extensively used across the globe to combat pests for higher crop production (Kohler and Triebskorn, 2013). In modern farming the use of intensive agriculture practices is mostly criticized as it adversely affects the environmental health, contaminate soil and reduces biodiversity (Datta *et al.*, 2016). More than 99.7% pesticides used in agriculture are persistent and accumulate in surrounding environment (Tejada *et al.*, 2017). Pesticides adversely affect soil organisms including earthworm (Maboeta *et al.*, 2004). Earthworms, the major biomass of soil macrofauna, are highly susceptible to chemicals so are considered as pollution-indicator soil organism (Jouquet *et al.*, 2010). The ingestion of soil minerals and organic material by earthworm facilitates humification and mineralization of soil nutrients. The pesticide residues in soil cease the physiological function and ecological structure of earthworms and cause their mortality (Maboeta *et al.*, 2004) as well as damage higher trophic levels (Van Gestel *et al.*, 2011). Since earthworm is a significant route for the uptake of soil contaminants, therefore toxicity study on earthworms may help in understanding the mechanism by which these pesticides affect the soil quality, especially soil biota. Emamectin benzoate is a semisynthetic bioinsecticide composed of naturally occurring compound, avermectin, extracted from *Streptomyces avermitilis*. Emamectin benzoate is used to control lepidopteron insects in vegetables, cotton and tobacco. Emamectin benzoate is a chloride channel activator that prevents muscle contraction, induces cessation of feeding and finally causes the death of pests. Earlier the toxic effects of antibiotics on earthworm have been demonstrated in many studies (Gunn and Sadd, 1994; Diao *et al.*, 2007). However, the chemical used in present study has been studied for the first time under Kumaun (Uttarakhand) agroclimatic conditions.

Earthworms were collected from grasslands free from agrochemicals; and cultured in the laboratory for 2 weeks at room temperature (20-25^{o}C) on cow manure. Adult earthworms (*Metaphire posthuma),* belonged to phylum Annelida and class Oligochaeta, with well-developed clitellum were selected for present study (Iordache and Borza, 2010). The acute toxicity test was performed as per OECD Guideline 207 (OECD, 1984). The earthworms were washed with distilled water and kept on moist filter paper for 3 h to allow voiding of their guts. Then they were re-washed, wiped and weighed before use. Emamectin benzoate (Avermectin group), selected as test insecticide, was procured from pesticide market of Kichha, U.S Nagar, Uttarakhand (India) in 2018. The aqueous solutions of variable concentrations of test chemical were prepared by dissolving emamectin benzoate in acetone. Filter paper contact test is a simple method but gives high reproducibility and possible direct comparison of results (Zhang *et al.*, 2009). The test chemical was dissolved in distilled water and six concentration prepared and transferred to filter papers (Whatman No. 1) in 9 cm petri-dish. Six replicates were maintained for each treatment (concentration) with one control using distilled water alone. Tests were done in dark at 28±2.0°C for 48 h. Earthworms were monitored for mortality by a gentle mechanical stimulus to front part. Probit analysis was conducted to assess acute toxicity of insecticide to earthworm using a program developed by Chi (1997). Based on the results after 48 h LC_{50} values, emamectin benzoate was classified as super toxic

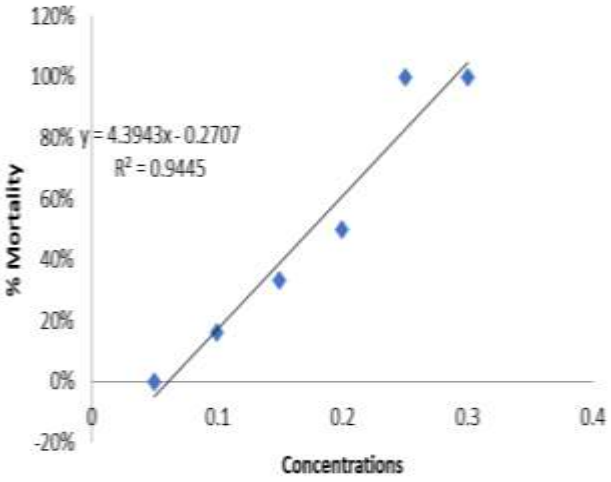

Fig. 1: Probit analysis curve showing LC_{50} of emamectin benzoate

Table 2: Morphological characteristics of emamectin benzoate-treated earthworms

Conc. (ppm)	Discoloration	Coelomic secretion	Muscle contraction	Swelling of clitellum	Body lesion	Bleeding
0.05	-	+	-	-	-	-
0.10	-	+	-	-	-	+
0.15	-	+	+	-	+	++
0.20	+	++	+	++	+	++
0.25	++	+++	++	++	+	++
0.30	+++	+++	+++	+++	+	++
Control	-	-	-	-	-	-

- = No effect, + = Minor effect, ++ = Major effect, +++ = Lethal

at <1 μg cm^{-2}, extremely toxic at 1-10 μg cm^{-2}, very toxic at 10-100 μg cm^{-2}, moderately toxic at 100-1000 μg cm^{-2} or relatively non-toxic at >1000 μg cm^{-2} (Roberts and Dorough, 1984).

Earthworms are widely used to assess the toxic effect of pesticides. In present study the concentrations of emamectin benzoate ranged from 0.05 to 0.30 ppm along with a control (acetone). High mortality was found in 0.25 ppm concentration and onwards; and no mortality in control and 0.05 ppm (Fig. 1). Probit analysis showed that LC_{50} mortality in emamectin benzoate was at 22.9 μg cm^{-2} in contact filter paper method using acetone as reference. The LC_{50} of dimethoate, pirimphos methyl and deltamethrin to *L. rubellus* using filter paper bioassay for 48 h was 2.24, 0.41 and 0.11 μg cm^{-2} (Velki and Hackenberger, 2013). Abamectin, emamectin and ivermectin exhibit LC_{50} values of 23.08, 30.2 and 4.4 μg cm^{-2}, respectively (Wang *et al.*, 2012). In present study, the exposure of earthworms to emamectin benzoate induced morphological changes (Fig. 2) such as excessive secretion of coelomic fluid, blood extrusion, longitudinal muscle contraction and loss of pigmentation. Other morphological changes such as constriction and swelling appeared within 48 h in anterior regions of worms exposed to 0.25 and 0.30 mg cm^{-2} and degenerative changes appeared at posterior end of exposed earthworms after 3 h exposure. The study indicated that the insecticides before use in crops require to be evaluated in laboratory and then under control field conditions.

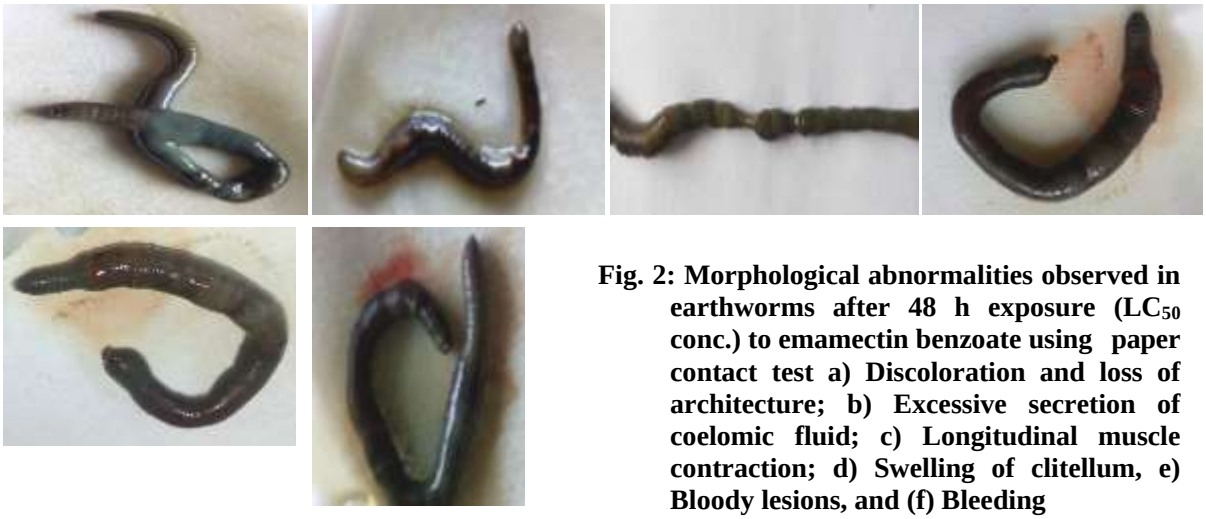

Fig. 2: Morphological abnormalities observed in earthworms after 48 h exposure (LC_{50} conc.) to emamectin benzoate using paper contact test a) Discoloration and loss of architecture; b) Excessive secretion of coelomic fluid; c) Longitudinal muscle contraction; d) Swelling of clitellum, e) Bloody lesions, and (f) Bleeding

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