Research Paper :

Acute Toxicity : Novel Mode of Pesticides on Earthworm

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SUMMARY

Lab-experiments were carried out to asses the relative acute toxicity of eight novel modes of pesticides *viz.*, abamectin, diafenthiuron, fipronil, imidacloprid, spinosad, indoxacarb, novaluron and profenofos on earthworm (*Eisenia foetida*) comparing contact filter paper test and soil test wherein, the area of contact-exposure was kept uniform and the exposure-period was kept 48 h and 28 days, respectively. All the pesticides found to be toxic in former test were depicted comparatively less toxic in latter test, indicating that the estimation of toxicity differs significantly with the test method, type of chemical molecule and also physicochemical properties of the soil.

Key words : Acute- toxicity, Novel pesticides, Earthworm, *Eisenia foetida*

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Earthworms are the representative of soil fauna being largest invertebrate biomass of soil ecosystem and they are common in many soils and thus are vulnerable to various impacts on soil. The soil not only acts as a substrate for organisms but also as a recipient medium for chemicals. Agrochemicals irrespective of their place or mode of application exert certain unwanted influence on this non-target organism, which in turn becomes undesirable victim. It is used as an efficient biomonitor for eco-toxicological studies, both as an indicator and test species. It is an excellent tool to detect soil pollutants (Bouche, 1988). The earthworm, *Eisenia foetida* (Savigny) is reported to be a suitable test species (Heimbach, 1988).

The laboratory tests used for ecotoxicological assessment of pollutants to earthworms mainly constitute the acute and chronic exposure studies; majority are designed to measure acute toxicity *i.e.* mortality of earthworm, as the main end point (Reinecke and Reinecke, 1998). In general, nine distinct tests have been described. The European Economic Community (EEC) and the Organization for Economic Co-operation and Development (OECD) have standardized and advocated three tests for estimation of field hazards *viz*., contact filter paper test, artificial soil test and silica paste glass-ball test. Besides, United States Environmental Protection Agency (EPA) has also recommended ecological effects test guidelines. Amongst the nine tests, contact filter paper test is simpler, cheaper and quick method, which is designed in such a way that the earthworms are exposed to the toxicant both by contact and in the aquatic phase. This test is reported (Edwards and Bohlen, 1996) to be an excellent screening technique to assess the relative toxicity. Perusal of literature in respect of ecotoxicity studies reveals that most of the novel pesticides are relatively safe to birds, fishes, daphnia, bees, etc. however, there is a paucity of literature especially on the earthworms.

MATERIALS AND METHODS

Contact filter paper test and soil test in accordance with some modifications and on the similar lines advocated by EPA were adopted to assess relative acute toxicity of eight novel modes of pesticides on adult earthworm, *Eisenia foetida*. Laboratory experiments were carried out in CRD with three replicates for each of the pesticides to compare the acute toxicity. In both the tests, area of contact-exposure was kept uniform (56 cm^2/worm) while exposure-period was kept 48 h and 28 DAE for the former and latter tests, respectively. The toxicity was assessed on the basis of worm's mortality at recommended field concentration by following the former test on similar lines advocated by Goats and Edwards (1988) and Edwards and Bohlen (1996) and for the latter test by keeping views of EPA guidelines and also the studies carried out by Van-Gestel and Ma (1990). In soil test, clayey loam soil was provided as substrate medium. A block of thermocol (size 23.6 x 23.6 x 2 cm^3)

was prepared for each of the replicate to keep area of contact-exposure uniform. Sieved moist soil weighing about 150g was uniformly spread within each block by keeping 1 cm soil depth. Each of the concentration was uniformly sprayed with an atomizer on soil surface @ 2.8 ml/block, considering general field-spray coverage volume @ 500 l/ha. Ten worms were released in each block that was then covered with thin transparent plastic sheet having tiny holes. Uniform soil moisture content was maintained during the entire course of investigation. Relative humidity, maximum and minimum temperatures prevailing in laboratory were in the ranges of 50 to 65 per cent, 21.7 to 30.9°C and 16.1 to 21.4°C, respectively.

Progressive mortality was recorded during the respective exposure periods. If the worms did not respond even to gentle mechanical stimulus at front end, they were considered as dead (Srivastava *et al*., 1994). Per cent mortality for each of the pesticides was calculated and transformed into arcsin values. Data later on were subjected to statistical analysis. Relative toxicity of the pesticides was judged by comparing mean mortality recorded in both the tests.

RESULTS AND DISCUSSION

Data (Table 1) in respect of filter paper test revealed that all the worms from untreated control were observed to be alive and active till the end (48 h) of exposure period. At 12 h, diafenthiuron, profenophos, fipronil and novaluron were found at par with untreated control. Indoxacarb and spinosad were at par with each other. Abamectin recorded 53.3 per cent mortality. Imidacloprid observed to be the most toxic (73.3%). At 24 h, no mortality was recorded in novaluron and profenofos. Diafenthiuron and fipronil were found almost in similar range (3.3%). Indoxacarb and spinosad recorded 23.3 and 33.3 per cent mortality, respectively. Imidacloprid recorded cent per cent mortality followed by abamectin (93.3%). At 36 h, mortality reached to hundred per cent in abamectin. Spinosad (86.7%) and indoxacarb (80%) were found to be statistically equal. Fipronil (23.3%), profenofos (16.7%), diafenthiuron (13.3%) and novaluron (13.3%) were observed to be at par with each other. At 48 h, cent per cent mortality was recorded in spinosad. Profenofos and indoxacarb recorded 96.7 per cent mortality and were at par with fipronil (93.3%), diafenthiuron (90%) and novaluron (90%).

Data (Table 2) in respect of soil test reveals that worms from untreated control were observed to be unaffected even after exposure period of 28DAE. At 7 DAE, all the pesticides except imidacloprid (33.3%) were at par with each other recording no mortality. At 14 DAE, novaluron and spinosad were at par with untreated control. Diafenthiuron (10%), abamectin (23.3%), indoxacarb (30%) and profenofos (30%) were at par with each other. At 21 DAE, diafenthiuron, fipronil, novaluron and spinosad recorded 10 per cent mortality. Remaining pesticides were found to be at par; amongst imidacloprid and abamectin recorded 63.3 per cent mortality. At 28 DAE, least (10%) mortality was recorded in diafenthiuron, fipronil, novaluron and spinosad as against highest (63.3%) mortality in abamectin and imidacloprid which were at par with profenofos and indoxacarb.

Pesticide-wise assessment of relative acute toxicity is summarized below:

Imidacloprid:

Cent per cent mortality was observed at 24 h with filter paper test as against 63.3 per cent in soil test at 21 DAE.

Table 1: Progressive mean earthworm's mortality in contact filter paper test

Sr. No.	Pesticide	Mortality observed hours after exposure			
		12 h	24 h	36 h	48 h
1.	Abamectin, 1.9 EC (0.0019 %)	53.333 (46.923)	93.333 (77.710)	100.000 (90.000)	100.000 (90.000)
2.	Diafenthiuron, 50 WP (0.0600 %)	0.000 (0.000)	3.333 (6.145)	13.333 (17.710)	90.000 (75.000)
3.	Fipronil, 5 SC (0.0100 %)	0.000 (0.000)	3.333 (6.145)	23.333 (28.780)	93.333 (81.145)
4.	Imidacloprid, 17.8 SL (0.0035 %)	73.333 (59.004)	100.000 (90.000)	100.000 (90.000)	100.000 (90.000)
5.	Indoxacarb, 14.5 EC (0.0120 %)	3.333 (6.145)	23.333 (28.780)	80.000 (63.930)	96.667 (83.855)
6.	Novaluron, 10 EC (0.0200 %)	0.000 (0.000)	0.000 (0.000)	13.333 (21.145)	90.000 (71.565)
7.	Profenofos, 50 EC (0.1500 %)	0.000 (0.000)	0.000 (0.000)	16.667 (23.855)	96.667 (83.855)
8.	Spinosad, 2.5 SC (0.0008 %)	10.000 (15.000)	33.333 (35.218)	86.667 (68.855)	100.000 (90.000)
9.	Untreated-control	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
	S.E.±	4.517	2.277	3.705	4.895
	C. D. (P=0.05)	13.421	6.767	11.011	14.545

* Figures in parentheses are transformed arcsin values

Table 2 : Progressive mean earthworm's mortality in soil test

Sr. No.	Pesticide	Mortality observed days after exposure			
		7 DAE	14 DAE	21 DAE	28 DAE
1.	Abamectin, 1.9 EC (0.0019 %)	0.000 (0.000)	23.333 (28.860)	63.333 (52.85)	63.333 (52.85)
2.	Diafenthiuron, 50 WP (0.0600 %)	0.000 (0.000)	10.000 (18.44)	10.000 (18.44)	10.000 (18.44)
3.	Fipronil, 5 SC (0.0100 %)	0.000 (0.000)	0.000 (0.000)	10.000 (18.44)	10.000 (18.44)
4.	Imidacloprid, 17.8 SL (0.0035 %)	33.333 (35.41)	53.333 (46.89)	63.333 (52.85)	63.333 (52.85)
5.	Indoxacarb, 14.5 EC (0.0120 %)	0.000 (0.000)	30.000 (33.21)	30.000 (33.21)	30.000 (33.21)
6.	Novaluron, 10 EC (0.0200 %)	0.000 (0.000)	0.000 (0.000)	10.000 (18.44)	10.000 (18.44)
7.	Profenofos, 50 EC (0.1500 %)	0.000 (0.000)	30.000 (33.21)	43.333 (41.13)	50.000 (45.000)
8.	Spinosad, 2.5 SC (0.0008 %)	0.000 (0.000)	0.000 (0.000)	10.000 (18.44)	10.000 (18.44)
9.	Untreated-Control	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)	0.000 (0.000)
	S.E.±	5.339	7.993	11.095	11.096
	C. D. (P=0.05)	15.865	23.750	32.967	32.967

* Figures in parentheses are transformed arcsin values

Abamectin:

At 36 h, cent per cent mortality was found in filter paper test while it was found to be 63.3 per cent in soil test at 21 DAE.

Profenofos:

In filter paper test at 48 h, mortality was tending towards cent per cent however in soil test, it was observered to be 30 and 50 per cent at 14 DAE and at 28 DAE, respectively.

Indoxacarb:

At 48 h, maximum mortality (96.7 %) was recorded in filter paper test while in soil test it was 30 per cent at 14 DAE.

Diafenthiuron:

In filter paper test, 90 per cent mortality was observed at 48 h as against in soil test it was maximum up to 10 per cent till 28 DAE.

Fipronil:

In filter paper test at 48 h, 96.7 per cent mortality was recorded while in soil test it was maximum up to 10 per cent even after 4 weeks exposure.

Spinosad:

At 48 h, cent per cent mortality was recorded in filter paper test as against in soil test it was only 10 per cent at 21 DAE and 28 DAE.

Novaluron:

In filter paper test, at 48 h, 90 per cent mortality was recorded and in soil test even at 28 DAE, it was recorded as 10 per cent.

In general context, the pesticide found to be more toxic in filter paper test was depicted comparatively less toxic in soil test. Toxicity of pesticides in soil is mainly determined by pore-water concentration, which can be derived from total concentration by means of sorption data (Van-Gestel and Ma, 1990). Under the present studies, imidacloprid and abamectin were appeared to be slightly toxic. Ishaaya and Degheeley (1998) pointed out that imidacloprid being apropetally systemic compound, may potentially endanger soil organisms, especially the endogeic earthworm, *Pheretima postural*. Imidacloprid in comparison with fipronil had a larger negative effect on individual earthworms (Moster *et. al.*, 2001). Soil treatment with imidacloprid was found to be harmful to anecic/endogeic species of earthworm (Lal *et al.*, 2001) however, its brief influence was observed on earthworm, *Lumbricus terrestris* (Pfluger and Schmuck, 1991). Yu-Luo *et al.* (1999) reported that imidacloprid exhibits different acute toxicity effects on *E. foetida* under different exposure systems. Relative toxicity of the remaining pesticides under the studies has not been discussed herein due to paucity of literature.

Comparison of acute toxicity to earthworm, *E. foetida* depicted in two distinct tests by keeping uniform area of contact exposure except the exposure period reveals that in filter paper substrate medium, all the test pesticides were found to be almost toxic. In soil substrate medium on the contrary, they were found to be comparatively less toxic. It indicates that toxicity estimation of a pesticide differs significantly with the test methods used therein and probably also with the type of chemical molecule(s); the findings are in confirmation with that of Heimbach (1984), Bouche (1992), Van-Gaestel (1992) and Neuhauster *et al.* (1985) wherein, the former test does not accurately represent the situation in soil system.

Present findings are however in contradiction with that reported by Delahaut and Koval (1990) wherein, similar acute toxicity was recorded in both the test. Lofs-Holmin (1981) reported that toxicity of pesticides was dependent on soil texture however; Harris (1967) found that it was dependent upon soil type.

Maurya and Chattoraj (1994) pointed out that soil temperature, moisture content, nature of soil, porosity of soil, soil organic matter, soil microbes and quantum of pesticides prevailing within soil play key role in causing acute toxicity to earthworms. It feels that the said attributes needs to be considered during the evaluation of pesticides by conducting field trials. Moreover, there is a felt need of collecting information on sub lethal chronic and sub-chronic toxicity effects and the ecological significance thereby, which can only be assessed under field conditions. It is also to point out that until no pesticide is reported to be cent per cent selective to target organism.

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