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PESTICIDES' INDUCED TOXICITY ON SURVIVABILITY OF *EISENIA FETIDA*

RITU RANI*, DHARAMBIR SINGH, JYOTI YADAV AND NEETU
Department of Zoology,
CCS Haryana Agricultural University, Hisar - 125 004, INDIA
e-mail: riturani1713@gmail.com

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***Corresponding author**

ABSTRACT
The present study conducted for 90 days dealt with the study of effects of two pesticides *viz*. Carbendazim and Thiram on survivability and reproduction of E*isenia fetida*. As compared to control, the reduction upto 75.29% and 99.5% in adult population of earthworm and reduction in cocoon production has been observed respectively in earthworms treated with thiram along with carbendazim (1.25 + 1.25 mg/kg). Dose dependent reduction (69.5%) in body weight was recorded in earthworms exposed to thiram in combination with carbendazim (1.25 + 1.25 mg/kg) followed by Thiram (1.25mg/kg) in which 62.5% decrement has been recorded as compared to control. Reduction upto 44.7% in body length has been observed in earthworms exposed to thiram along with carbendazim (1.25 + 1.25 mg/kg) followed by earthworms exposed to thiram (1.25mg/kg) in which 39.4% decrement has been recorded as compared to control. Maximum decrease in survivability has been recorded in worms exposed to thiram along with carbendazim that marks their synergistic mode of action. The present study revealed that cocoon production was the most sensitive parameter as far as pesticide exposure is concerned. However, thiram was found to be more detrimental to earthworms as compared to carbendazim.

INTRODUCTION

Earthworms are believed to be the farmer's friends for their immense capabilities to transform the organic waste to nutrient rich manure (Yadav and Gupta, 2017). Recent developments in toxicology have revealed the presence of pesticides' residues in soil leading to soil pollution. Pesticides' pollution will inevitably become the major outcome of intensive agriculture practices. To analyse the extent of toxicology of pesticides, earthworms acts as suitable test animal as mentioned by OECD (Zhang *et al*., 2014). The remains of active ingredients of agrochemicals in plant residues have been reported and they are widely used for vermicomposting process (Sandeep *et al*., 2017).However, the earthworms still remain the most neglected part when it comes to pesticides' application. The survivability of earthworms in terms of growth and reproduction can be seen as major end points in ecotoxicology (Wu *et al*., 2011). The pesticide exposure induces detrimental effects on the physiology and metabolism of earthworm (Bansiwal and Rai, 2010). Growth and reproduction, despite being the vital factors for analysing the survivability of any organisms, remains the most neglected part of research. If the earthworms of any particular soil ecosystem is at threat, it will be difficult to develop that ecosystem again (Dutta and Dutta, 2016). *Eisenia fetida* is one of the most widely used species for vermicomposting (Yadav *et al*., 2017). Carbendazim and thiram are widely used as fungicides in agriculture. Hence, the present paper aims to compute the effects of thiram and carbendazim on growth and reproduction of *Eisenia fetida*.

MATERIALS AND METHODS

Collection of test animal

The species *Eisenia fetida* was collected from vermicomposting unit of Department of Zoology, CCS HAU, Hisar. The culture of worms was maintained and third generation of earthworms was used to avoid the pre exposure or residual effects of pesticides. Only, mature clitellated earthworms were used for assessing the worm reproduction test in presence of pesticides.

Collection of Substrate

The cow dung was used as substrate and was collected from Biogas Plant of Department of Microbiology, CCS HAU, Hisar. The cow dung was pre decomposed for 15 days prior to study to avoid the temperature increase during vermicomposting.

Experimental set up

The experiment was conducted for the duration of 90 days in tubs of 70 l capacity. Fungicides Thiram (75 WP) and Carbendazim (50 WP) were used in this study. Triplicates of each treatment were maintained. Thirty healthy and fully clitellated earthworm were put in each tub. Each tub wassprayed with different concentrations of fungicides as mentioned in Table 1.

Analysis of worm survivability

The total no. of adult earthworms, no. of cocoons, length and body weight of earthworms was counted on 30th, 60th and 90th day of the experiment as described by Lock *et al*. (2002). The earthworms were considered dead if they didn't respond to any stimulus.

Statistical analysis: The experimental design for screen house study was completely randomized block with three replicates.The data collected were subjected to ANOVA at 0.05% level of significance for computing the degree of variance among the different treatments given in the experiment.

RESULTS AND DISCUSSION

No. of adult earthworms

Significant reduction in the number of adult earthworms due to fungicide's exposure has been observed as compared to control on the final day of study.Maximum reduction (75.29%) in survivability was observedin worms exposed to Thiram alongwithcarbendazim (1.25 + 1.25mg/kg) followed worms exposed to thiram (1.25mg/kg)in which there is 72.06% decrease in survivability of earthworms was observed as compared to control. Whereas 65.6% decrement in survivability has been observed at carbendazim (1.25mg/kg). Thiram was observed to be more toxic as compared to carbendazim (Table 2). The reduction in total adult count may be the result of pesticides' induced mortality. However, it may also be noted that the decline in earthworms' population was gradual after their exposure to thiram and carbendazim. Dose dependent increased mortality of earthworms on pesticide exposure has also been previously stated by Jovana *et al*. (2014).

Number of cocoons

No. of cocoons found is the most suitable parameter for analysing the effect of fungicides on reproduction of earthworm. Decrease in the no. of cocoons drastically on exposure to pollutants attributes to the lesser survivability of earthworms. Significant decrease in cocoon production of the pesticide exposed earthworms as compared to control has been observed from the initial day of exposure to 90th day of study (Table 3). Maximum reduction (99.5%) in no. of cocoon production was observed inThiram + Carbendazim (1.25 + 1.25mg/kg) followed by Thiram (1.25mg/kg) in which there is 98.7% decrease in no. of cocoons occurs. Carbendazim had also adverse effect on the production of cocoons as there is 97.8% decrement occurs in case of Carbendazim (1.25mg/kg) as compared to control.The dose dependent reduction in the cocoon production of earthworms due to pesticide exposure has also been previously documented by Yasmin and D'Souza (2007). Decreased spermatic viability, alterations in cell proliferation and genotoxicity may be the most probable cause of low reproduction rates or less viable cocoon formation as stated by Navarro and Obregon (2005).

Body weight of Earthworm

Significantreduction in body weight in pesticide exposed worms as compared to control has been observed on 60th and 90th day of the present study (Table 4). It may also be noted that the reduction in body weight of earthworms was non- significant as compared to control on 30th day that marks the fact that reduction in body weight is induced due to prolonged exposure of earthworms towards pesticides. The normal gain in biomass may be attributed to the bioaccumulation of pollutant upto a certain limit (Aleagha and Ebadi, 2011). Maximum reduction (69.5%) was observed in earthworms exposed to Thiram + Carbendazim (1.25 + 1.25mg/kg) followed by Thiram (1.25mg/kg) in which 65.2% decrease in body weight has been observed as compared to control. Low feeing rate on the pesticide exposed

Table 1: Description of treatments (sprayed) given to earthworms along with control

Sr. No.	Treatment	Concentration (mg/kg)
1.	Thiram	0.75, 1.00 and 1.25
2.	Carbendazim	0.75, 1.00 and 1.25
3.	Thiram + Carbendazim	0.375 + 0.375, 0.50 + 0.50, 0.625 + 0.625, 0.75 + 0.75, 1.00 + 1.00 and 1.25 + 1.25

Table 2: Effect of pesticides on the no. of adult earthworm, *Eisenia fetida*

Treatments (mg/kg)	No. of adult earthworm			
	30 days	60 days	90 days	Mean
Control	30.00 ± 0.00^{d}	30.33 ± 0.33	31.00 ± 0.57	30.44
Thiram(0.75)	28.66 ± 0.33^{c}	27.66 ± 0.33^{c}	26.66 ± 0.33	27.66
Thiram(1.00)	27.33 ± 0.33^{a}	22.66 ± 0.66^{b}	17.33 ± 0.33^{b}	22.44
Thiram(1.25)	26.33 ± 0.33^{a}	17.33 ± 1.20	8.66 ± 0.33^{a}	17.44
Carbendazim(0.75)	29.66 ± 0.33^{c}	28.00 ± 0.57^{c}	26.66 ± 0.33	28.10
Carbendazim(1.00)	27.66 ± 0.66^{b}	26.33 ± 0.33^{c}	25.00 ± 0.57	26.33
Carbendazim(1.25)	27.00 ± 0.57^{ab}	20.00 ± 1.00	10.66 ± 0.33	19.22
Thiram + Carbendazim(0.75 + 0.75)	28.33 ± 0.66^{bc}	19.66 ± 0.88^{a}	16.66 ± 0.88^{b}	21.55
Thiram + Carbendazim(1.00 + 1.00)	26.00 ± 0.57^{a}	15.00 ± 0.57	13.00 ± 0.57	18
Thiram + Carbendazim(1.25 + 1.25)	22.00 ± 0.57	13.00 ± 0.57	7.66 ± 0.33^{a}	14.22
Thiram + Carbendazim(0.375 + 0.375)	29.66 ± 0.33^{cd}	27.00 ± 0.57^{c}	25.00 ± 0.57	27.22
Thiram + Carbendazim(0.50 + 0.50)	28.00 ± 0.57^{b}	22.33 ± 0.33^{b}	20.33 ± 0.33	23.55
Thiram + Carbendazim(0.625 + 0.625)	27.66 ± 0.66^{b}	19.66 ± 0.66^{a}	17.33 ± 0.33^{b}	21.55
Mean	27.56	22.22	18.91	

Values with the same superscript in same column do not differ significantly

Table 3: Effect of pesticides on the number of cocoons produced by earthworm, *Eisenia fetida*

Treatment(mg/kg)	No. of cocoons			
	30 days	60 days	90 days	Mean
Control	130.66 ± 5.20	134.33 ± 2.96	136.66 ± 3.28	133.88
Thiram (0.75)	95.00 ± 3.21	51.00 ± 2.88^{d}	48.00 ± 3.21^{d}	64.66
Thiram (1.00)	26.00 ± 2.08^{bc}	10.00 ± 1.15^{bc}	8.00 ± 1.15^{b}	14.66
Thiram (1.25)	13.00 ± 1.00^{a}	2.33 ± 0.88^{a}	1.66 ± 0.66^{a}	5.66
Carbendazim (0.75)	120.00 ± 2.88	63.66 ± 2.96	58.00 ± 1.52	80.55
Carbendazim (1.00)	28.66 ± 1.76^{c}	13.00 ± 2.08^{c}	10.33 ± 1.76^{b}	17.33
Carbendazim (1.25)	15.33 ± 1.45^{a}	4.00 ± 1.00^{ab}	3.00 ± 0.57^{a}	7.44
Thiram + Carbendazim (0.75 + 0.75)	51.00 ± 2.88^{d}	13.00 ± 2.08^{c}	10.00 ± 2.00^{b}	24.66
Thiram + Carbendazim (1.00 + 1.00)	26.00 ± 2.08^{bc}	8.66 ± 2.33^{b}	4.66 ± 2.02^{ab}	13.10
Thiram + Carbendazim (1.25 + 1.25)	11.66 ± 0.88^{a}	2.33 ± 0.88^{a}	0.66 ± 0.33^{a}	4.88
Thiram + Carbendazim (0.375 + 0.375)	77.00 ± 6.24	51.00 ± 2.88^{d}	48.33 ± 3.18^{d}	58.77
Thiram + Carbendazim (0.50 + 0.50)	62.66 ± 3.93	31.00 ± 2.08	27.33 ± 2.40	40.33
Thiram + Carbendazim (0.625 + 0.625)	49.00 ± 1.73^{d}	12.66 ± 1.45^{c}	10.00 ± 1.15^{b}	23.88
Mean	54.30	30.53	28.20	

Values with the same superscript in same column do not differ significantly

Table 4: Effect of pesticides on the body weight of earthworm, *Eisenia fetida*

Treatment(mg/kg)	Body weight of earthworm (in grams)			
	30 days	60 days	90 days	Mean
Control	0.86 ± 0.03^{c}	0.88 ± 0.03	0.92 ± 0.02	0.88
Thiram (0.75)	0.53 ± 0.03^{b}	0.59 ± 0.04^{b}	0.52 ± 0.04^{b}	0.54
Thiram (1.00)	0.48 ± 0.03^{a}	0.50 ± 0.02^{b}	0.46 ± 0.03^{b}	0.48
Thiram (1.25)	0.38 ± 0.03^{a}	0.35 ± 0.02^{a}	0.32 ± 0.02^{a}	0.35
Carbendazim (0.75)	0.54 ± 0.07^{b}	0.71 ± 0.06^{c}	0.61 ± 0.02^{c}	0.62
Carbendazim (1.00)	0.52 ± 0.01^{b}	0.53 ± 0.07^{b}	0.49 ± 0.06^{b}	0.51
Carbendazim (1.25)	0.44 ± 0.01^{a}	0.50 ± 0.04^{b}	0.47 ± 0.03^{b}	0.47
Thiram + Carbendazim (0.75 + 0.75)	0.49 ± 0.03^{a}	0.65 ± 0.02^{c}	0.40 ± 0.03^{ab}	0.51
Thiram + Carbendazim (1.00 + 1.00)	0.49 ± 0.03^{a}	0.48 ± 0.02^{b}	0.46 ± 0.01^{b}	0.47
Thiram + Carbendazim (1.25 + 1.25)	0.37 ± 0.04^{a}	0.32 ± 0.00^{a}	0.28 ± 0.01^{a}	0.32
Thiram + Carbendazim (0.375 + 0.375)	0.56 ± 0.06^{bc}	0.60 ± 0.02^{bc}	0.57 ± 0.10^{c}	0.57
Thiram + Carbendazim (0.50 + 0.50)	0.50 ± 0.02^{ab}	0.56 ± 0.01^{b}	0.53 ± 0.01^{bc}	0.53
Thiram + Carbendazim (0.625 + 0.625)	0.44 ± 0.09^{a}	0.50 ± 0.08^{b}	0.47 ± 0.07^{b}	0.47
Mean	0.50	0.55	0.5	

Values with the same superscript in same column do not differ significantly

Table 5: Effect of pesticides on the length of earthworm, *Eisenia fetida*

Treatment(mg/kg)	Length of earthworm(in cm)			
	30 days	60 days	90 days	Mean
Control	8.00 ± 0.01^{f}	8.59 ± 0.16^{d}	8.83 ± 0.15	8.47
Thiram (0.75)	7.73 ± 0.06^{ef}	7.90 ± 0.11^{c}	7.69 ± 0.11^{de}	7.77
Thiram (1.00)	6.46 ± 0.03^{c}	6.50 ± 0.34^{ab}	6.06 ± 0.36^{b}	6.34
Thiram (1.25)	5.56 ± 0.34^{a}	6.03 ± 0.46^{a}	5.35 ± 0.23^{a}	5.64
Carbendazim (0.75)	8.00 ± 0.11^{f}	8.10 ± 0.03^{cd}	7.92 ± 0.05^{e}	8.00
Carbendazim (1.00)	7.13 ± 0.06^{d}	7.24 ± 0.09^{b}	6.28 ± 0.20^{b}	6.88
Carbendazim (1.25)	6.30 ± 0.20^{bc}	6.44 ± 0.11^{a}	6.16 ± 0.03^{b}	6.3
Thiram + Carbendazim (0.75 + 0.75)	7.26 ± 0.26^{de}	7.28 ± 0.20^{bc}	6.80 ± 0.24^{c}	7.11
Thiram + Carbendazim (1.00 + 1.00)	6.80 ± 0.36^{cd}	6.93 ± 0.47^{b}	6.00 ± 0.06^{b}	6.57
Thiram + Carbendazim (1.25 + 1.25)	5.24 ± 0.18^{a}	5.88 ± 0.18^{a}	4.88 ± 0.13^{a}	5.33
Thiram + Carbendazim (0.375 + 0.375)	7.73 ± 0.12^{ef}	8.27 ± 0.33^{d}	7.61 ± 0.32^{d}	7.87
Thiram + Carbendazim (0.50 + 0.50)	6.76 ± 0.50^{c}	6.84 ± 0.49^{b}	6.17 ± 0.24^{b}	6.59
Thiram + Carbendazim (0.625 + 0.625)	5.73 ± 0.12^{ab}	5.75 ± 0.12^{a}	5.11 ± 0.06^{a}	5.53
Mean	6.82	7.05	6.52	

Values with the same superscript in same column do not differ significantly

substrate alongwith the avoidance behaviour of earthworm may result in low biomass gain as advocated by Sivakumar, 2015.

Length of earthworm

Significant changes in the length of earthworms have been observed in pesticides exposed worms as compared to control

on the 90th day of study (Table 5).Reductionupto(44.7%) was observed in worms treated with Thiram + Carbendazim (1.25 + 1.25mg/kg) followed by Thiram (1.25mg/kg) in which 39.4%decrement in length occurred. The reduced growth of pesticide exposed earthworms has previously been stated by Jovana *et al*. (2014) and the reduced growth may be the most probable cause for low lengthening rates in pesticides exposed earthworms.

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