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Research Article

COMPARATIVE IMPACT OF PHORATE AND CARTAP ON BIOMOLECULES OF *E. fetida*

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Abstract- The study of 60 days was carried out to analyze the effects of pesticides *viz.* cartap and phorate on the biomolecules i.e. carbohydrate, lipid and protein content of *Eisenia fetida*. The earthworms were exposed to cartap (1.0, 1.5 and 2.0 mg/Kg), phorate (1.0, 1.5 and 2.0 mg/kg) and cartap + phorate (0.5 + 0.5, 0.75 + 0.75 and 1.0 + 1.0 mg/kg). The samples of *E. fetida* were collected on first day and 60th day of pesticide exposure. Significant reduction in the carbohydrate, protein and lipid contents as compared to control has been recorded on the final day of study. Maximum reduction in biomolecules *i.e.* 6.949% protein, 27.632% lipid and 2.000% carbohydrates was observed with Cartap (2.0 mg/kg) followed by 6.237% protein, 25.981% lipid and 1.410% carbohydrates in Phorate + Cartap (1.0 + 1.0 mg/kg). Among the two insecticides (Phorate and Cartap) under investigation, Cartap was found to have more toxic effects when used alone as well as in combination with Phorate.

Keywords- Phorate, Cartap, Biomolecules, Pesticides.

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Introduction

Earthworms have capability to act upon soil mechanically, physically and biochemically to make it more fertile. They can transform organic waste into nutrient rich manure [1]. However, highly intensive agriculture in recent years has marked increased exposure of earthworms to pesticides. Being widely distributed in agroecosystems, earthworms are prone to the non-target effects of pesticides' exposure. The impact of insecticide on non-target organisms may be determined by assaying the changes at physiological, biochemical and molecular levels of the exposed organisms [2]. The deteriorating effects of pesticides on the physiology and metabolism of earthworm have previously been documented by Bansiwal and Rai [3]. The active ingredients remains of pesticides have also been reported in plant residues like husk etc. These plant residues are also being widely used as substrate for the process of vermicomposting. Thus, either by contact or by feeding they enter the body of earthworm so as to cause damage to earthworms [4]. Phorate and cartap are directly used for soil applications for controlling insect pests. *E. fetida* being an epigeic species thus is more prone to damaging effects of phorate and cartap. Keeping in view the above mentioned fact, the present study was carried out to analyze the impact of cartap and phorate on the biomolecule composition of *E. fetida*.

Material and Methods

Procurement of materials

The test earthworms were collected from Vermiculture unit of Department of Zoology, CCS HAU, Hissar. Phorate (10G) and Cartap (4G) were used for assessing the pesticides' induced toxicity on earthworms. Cowdung was procured from the Biogas plant, Department of Microbiology, CCS HAU, Hisar. The cow dung was predigested for 15 days before release of worms to avoid the overheating as it may cause damage to worms.

Experimental set up

20 selected fully clitellated worms were washed and weighed before inoculating them in tubs having cow dung as substrate. Three replicates were maintained for each treatment. Then the tubs were sprayed with the concentrations mentioned in [Table-1] along with the control. The factors like moisture, pH and aeration were maintained at optimum levels to avoid mortality of worms.

Table-1 *Different concentrations to which the earthworms were exposed to*

Sr. No.	Treatment	Concentration (mg/kg)
1.	Control	No treatment of Insecticides
2.	Phorate	1.0, 1.5 and 2.0
3.	Cartap	1.0, 1.5 and 2.0
4.	Phorate + Cartap (P+C)	0.5 + 0.5, 0.75 + 0.75 and 1.0 + 1.0

Chemical analysis

After 60 days of pesticide exposure, earthworms were dissected and various organs were removed rapidly for biochemical analysis. Body tissues were weighed and the whole tissue homogenate was prepared by macerating tissue in 20% cold trichloroacetic acid (TCA). 1.0 ml of TCA was used for each 100 mg of tissue weight. These contents were then incubated at 70^0C for 20 minutes and the supernatant was used for further chemical analysis.

Estimation of total tissue carbohydrates

Total tissue carbohydrates have been assayed by using Standard Phenol Sulphuric Method [5] taking glucose dilutions as standard. 1ml extract was added to 5% phenol solution was added and then 5.0 ml of concentrated sulphuric acid was added. The contents were shaken rapidly and allowed to stand for half an hour at room temperature. The optical density was read at 490 nm against a reagent blank.

Estimation of total lipids

The total lipid content has been determined by Soxhlet Extraction Method [6]. Tissue were dried in a hot air oven at 55^{0}C for 48 hours and powdered sample was taken in a pouch made of Whatman filter paper no. 40 and the same was placed in thimble connected with Soxhlet's apparatus. The initial weight of the Soxhlet's flask was recorded and gradually filled up with 100 ml petroleum ether (boiling point 60-80^{0}C). The total apparatus was then placed over a mantle and the petroleum ether was allowed to boil for 6-8 hours for circulation through the thimble by siphon process. After boiling, the petroleum ether was allowed to evaporate. The crude lipid was determined by difference between the initial weight of flask and final weight.

Fat contents mg/100 g of dried sample = W_i-W_f

WhereW_i = initial weight of the sample; W_f = final weight of the sample

Estimation of protein

The protein content has been estimated by Micro- Kjehdhal's Method [7]. About 100-500 mg of dried material was digested till the solution becomes clear. After cooling the digest, the solution was diluted to make the final volume up to 50 ml with distilled water. A blank was prepared by taking 10 ml of the acid (without sample) and digesting it in the same way as described above. 10 ml boric acid (4%) containing mixed indicator solution was taken and placed this receiving flask in such a way that the outlet of the condenser of the distillation apparatus dips into boric acid solution. Add about 10 ml of 40% NaOH to make it alkaline. Immediately closed the stop cork and passed the steam through steam chamber to distill ammonia and collected liberated NH_3 in boric acid. Then the solution was titrated against 0.01 NH_2SO_4 until the appearance of pinkish-violet color, the end point.

Statistical analysis

The experimental design for screen house study was completely randomized block with three replicates (tubs). The collected data were analyzed using ANOVA to find out the significant differences among various treatments.

Results and Discussion

Effect of insecticides on the carbohydrate content of the earthworms

Effect of different concentration of insecticides on carbohydrate content of the earthworms, *E. fetida* is given in [Table-2]. Maximum amount of carbohydrates content was seen in control *i.e.*, 22.114% on 0 day and 22.147% on 60th day whereas maximum decrease was observed in case of Cartap (2.0 mg/kg) and it was 21.989% on day zero later and on reduced to 21.543% by 60th day of the experiment. Phorate + Cartap (1.0 + 1.0 mg/kg) also have very harmful effect and carbohydrate content reduced from 21.982% on 0 day to 21.678% on 60th day. The earthworms were testes for the presence of total carbohydrate content after 24 hours of the exposure of pesticide on first day of experiment. The significant changes in the levels of carbohydrate have been observed as compared to control from the very initial day of exposure. However, among various treatments, non-significant decline in carbohydrate content has been observed. The decreased carbohydrate content can be attributed to the pesticide induced stress that increased the carbohydrate catabolism to generate energy to sustain. The f present findings may be advocated by the findings of Reddy *et al.* [8] who reported the above mentioned in *Lampito* mauritii. However, on final day of study comparatively more decline of carbohydrate level was recorded. Significant reduction in carbohydrate content has been observed when compared to control. However, among treatments non- significant values have also been recorded. As the prolonged pesticide exposure continues, it may cause hypoxia which in turn increase carbohydrate utilization and thus, the levels of carbohydrate decline to mitigate the individual energy demands [9]. Carbohydrate content of earthworms decreased gradually, as the concentration and duration of exposure to insecticides increased.

Effect of insecticides on the lipid content of the earthworms

Effects of different concentration of insecticides on the lipid content of the earthworms, *E. fetida* are presented in [Table-3]. Total lipid content decreased gradually with time and increase in concentration of insecticides. Maximum decrease was seen in case of earthworm reared in soil containing Cartap (2.0 mg/kg) which was 13.980% on day zero and then reduced to 10.117% on 60th day, followed by Phorate +Cartap (1.0+ 1.0 mg/kg) which was 13.879% on 0 day and 10.273% on 60th day after exposure to insecticides. As usual maximum increase in lipid content was observed in control *i.e.*, 14.280% on 0 day and 14.380% on 60th day. Significant reduction in the lipid content of *E. fetida* has been observed on first and final day of the study as compared to control. However, the level of lipid declination increased gradually with the prolonged exposure of *E. fetida* to cartap and phorate when used individually as well as in combination. The stress induced decline in lipid content may be stamped by the findings of Jatwani *et al.* [10]. Park *et al.* [11] observed that the stress induction may lead to the alteration in membrane lipid contents. The decreased level of lipids may be justified by the fact that lipids are used for energy production once the carbohydrates start depleting.

Table-2 *Effect of pesticides on carbohydrate content of Eisenia fetida*

Treatments	Carbohydrate on dry weight basis (%)	
	0th day	60th day
Control	22.114± 0.062	22.147± 0.071
Phorate (1.0 mg/kg)	22.031±0.034^a	21.964± 0.005^c
Phorate (1.5 mg/kg)	21.990±0.006^a	21.959± 0.004^c
Phorate (2.0 mg/kg)	21.980± 0.000^a	21.948± 0.007^c
Cartap (1.0 mg/kg)	21.985±0.005^a	21.809± 0.004^b
Cartap (1.5 mg/kg)	21.988±0.007^a	21.763±0.012^b
Cartap (2.0 mg/kg)	21.989±0.000^a	21.543± 0.009^a
P + C (0.5 + 0.5 mg/kg)	21.983±0.013^a	21.950± 0.006^c
P + C (0.75 + 0.75 mg/kg)	21.993±0.005^a	21.910± 0.008^c
P + C (1.0 + 1.0 mg/kg)	21.982±0.007^a	21.678± 0.033^a

Mean ± S.D. Values with the same superscript in same column do not differ significantly

Table-3 Effect of pesticides on the lipid content of *Eisenia fetida*

Treatments	Lipid on dry weight basis (%)	
	0th day	60th day
Control	14.280±0.009	14.380±0.270
Phorate (1.0 mg/kg)	14.000±0.009	12.850±0.180^e
Phorate (1.5 mg/kg)	13.950±0.010	11.867±0.186^{cd}
Phorate (2.0 mg/kg)	13.920±0.009	11.450±0.229^c
Cartap (1.0 mg/kg)	13.890±0.010^a	10.800±0.115^b
Cartap (1.5 mg/kg)	14.040±0.007	10.483±0.044^{ab}
Cartap (2.0 mg/kg)	13.980±0.007	10.117±0.235^a
P + C (0.5 + 0.5 mg/kg)	13.840±0.007	12.416±0.017^{de}
P + C (0.75 + 0.75 mg/kg)	13.810±0.007	11.800±0.305^c
P + C (1.0 + 1.0 mg/kg)	13.879±0.009^a	10.273±0.300^a

Mean ± S.D. Values with the same superscript in same column do not differ significantly

Effect of insecticides on the protein content of the earthworms

The effects of insecticides on the protein content of the earthworm, *E. fetida* and as the concentration of insecticides increased, protein content of earthworms decreased gradually as indicated in [Table-4], which shows that total protein content decreased gradually and the maximum decrease was seen in case of earthworm reared in soil containing Cartap @ 2.0 mg/kg. Total protein was 54.647% on day zero and then reduced to 51.150% on day 60th. Similar results were obtained in earthworm treated with Phorate + Cartap @ 1.0 + 1.0 mg/kg. The protein contents which were 54.720% on day zero reduced to 51.564% at day 60$^{th.}$ Cartap @ 2.0 mg/kg exhibited maximum toxicity followed by Phorate + Cartap. Maximum protein content were observed in control *i.e.*, 55.020% and 55.183% on 0 and 60th day, respectively. At the initial phase non- significant changes in protein content as compared to control has been observed. At initial levels, proteins are not used as source of energy. However, on 60th day, the significant decline in protein content as compared to control has been recorded. The findings are similar to those of Mosleh [12] and Jeyanthi *et al.* [13] in *P. excavatus*. Carbohydrates are the major and immediate energy precursors for the earthworm exposed to stress conditions while protein is spared during chronic period of pollutant stress [14]. As stated by Vaidya [15], the declined level of proteins may

be due to catalysis of proteins to amino acids to mitigate the stress conditions as proteins act as alternate source of energy especially during stress conditions.

Table-4 *Effect of pesticides on the protein content of Eisenia fetida*

Treatments	Protein on dry weight basis (%)	
	0th day	60th day
Control	55.020± 0.043a	55.183± 0.116
Phorate (1.0 mg/kg)	54.990±0.000a	54.783± 0.100e
Phorate (1.5 mg/kg)	54.983±0.000a	54.483± 0.116de
Phorate (2.0 mg/kg)	54.993±0.000a	54.386± 0.165d
Cartap (1.0 mg/kg)	54.973±0.011a	52.683± 0.092c
Cartap (1.5 mg/kg)	54.996±0.003a	52.117± 0.413bc
Cartap (2.0 mg/kg)	54.970±0.000a	51.150± 0.104a
P + C (0.5 + 0.5 mg/kg)	55.027±0.036a	54.483± 0.116d
P + C (0.75 + 0.75 mg/kg)	54.983±0.000a	53.900± 0.160d
P + C (1.0 + 1.0 mg/kg)	54.990±0.000a	51.564± 0.467ab

Mean ± S.D. Values with the same superscript in same column do not differ significantly

Table-5 *Percent changes in biomolecules of Eisenia fetida*

Treatments	Percent changes in biomolecules		
	Protein (%)	Lipid (%)	Carbohydrate (%)
Control	-	-	-
Phorate (1.0 mg/kg)	0.39	8.21	0.32
Phorate (1.5 mg/kg)	0.91	14.93	0.18
Phorate (2.0 mg/kg)	1.11	17.74	0.18
Cartap (1.0 mg/kg)	4.17	22.25	0.82
Cartap (1.5 mg/kg)	5.24	25.33	1.00
Cartap (2.0 mg/kg)	6.95	27.63	2.00
P + C (0.5 + 0.5 mg/kg)	0.98	10.29	0.14
P + C (0.75 + 0.75 mg/kg)	1.96	14.55	0.36
P + C (1.0 + 1.0 mg/kg)	6.24	25.98	1.41
Mean	3.11	18.55	0.71

Conclusion

It may be concluded that the pesticides induce stress and alters the biochemical composition of the earthworms. However, cartap if found to be more detrimental as compared to phorate. The present study leaves an avenue to further analyze the pathway by which the pesticides effects biochemical composition in earthworms.

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Abbreviations: mg/kg= milligram/ kilogram

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of Interest: None declared

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