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Responses of Soil Enzymes to Insecticides in Groundnut (*Arachis hypogaea* L.) Cultivated Black Soil

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Abstract: We investigated the impact of two insecticides, endosulfan and profenofos on enzyme activities, such as cellulase and amylase in black soil, collected from groundnut (*Arachis hypogaea*. L) cultivated fields of Kurnool district of Andhra Pradesh, India, by conducting experiments in laboratory at different concentrations (10, 25, 50, 75, 100ppm) which are equivalent to field application rates (1.0, 2.5, 5.0, 7.5, 10.0 kg ha^{-1}). In our study we observed, cellulase and amylase activities were significantly enhanced at 2.5 and 5.0 kg ha^{-1} in black soil after 10 days of incubation. Furthermore increase in concentration of insectcides decreased the rate of enzyme activity. However the stimulatory effect was continued up to 20 days of incubation in black soil. Whereas, the decline phase was started after 20 days and the minimum enzyme activities were noticed at the end of 40 days of incubation. But higher concentrations of insecticides at the level of 7.5 to 10.0 kg ha^{-1} were either toxic or innocuous to cellulase and amylase activities in black soil.

Key words: Endosulfan · Profenofos · Soil enzymes · Groundnut (*Arachis hypogeae* L.) soil

INTRODUCTION

In practice, different agrochemicals are used in modern agriculture as important tools that help the farmer to minimize economic losses caused by weeds, pathogens and insect pests. The economy of India is largely dependent on agriculture but about 15-20% agricultural production is negatively influenced by pests [1]. Application of individual pesticides particularly with many cash crops like groundnut, cotton and sugarcane to minimize the crop loss. Groundnut (*Arachis hypogaea*. L) is one of the important major profitable crop grown throughout the year in India and is a world leader in groundnut farming, with eight million hectare of cultivated area in the year 2002-2003 [2]. The current productivity of groundnut in India is about quintal per ha [3]. The present day agriculture involves abundant cultivation of the groundnut crop because of its vital role in edible oil seeds production [4]. Groundnut ranks seventh among crops in terms of insecticide consumption in India [5]. More than 120 pests affect economically important crops like groundnut, cotton and tomato [6-10]. Pesticides are recognized as a source of potential adverse environmental impacts and their persistence in soil and ground water has grown considerably [11,12]. When a pesticide is released deliberately or accidentally in to the environment about 0.1% reaching the target organism while the remaining 0.99% reaches the soil causing not only trouble local metabolism or enzymatic activities [13-17] but also disturb soil ecosystem and thus, may affect human health by entering in the food chain, have raised considerable public concern. Profenofos (*0*-4-bromo-2-chlorophenyl-*O*-ethyl-*S* propylphosphorothioate). It is a non systemic insecticide and acaricide with contact and stomach action used against mites, leafhoppers, thrips, aphids, mealy bugs and cotton stainer. Endosulfan is a chlorinated cyclodiene insecticide currently used throughout the world for the control of numerous insects in a wide variety of food and non food crops. Endosulfan has been ubiquitously detected in the atmosphere, soils, sediments, surface waters, rain waters and foodstuffs [18]. Endosulfan comprises two parent isomers alpha and beta endosulfan and the alpha to beta ratio of technical endosulfan is about 7:3 and both isomers are extremely toxic to aqueous organisms. Due to its high degree of toxicity it persists in soils, water and become an important group of contaminants.

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Although this pesticide have been restrictively used or even banned their persistence and bioaccumulation still be found in soils. Thus it is required to estimate soil biological responses to the pesticides. To date, many efforts have been made to understand the effect of pesticides on soil enzyme activities, amylase and cellulase but little is known about the effect of endosulfan and profenofos. So from past 10 decades more specific prominence has been given to soil enzymes because these are indicators of biological equilibrium [19], fertility [20], quality [21] and changes in the biological status of soil due to pollution [22, 23]. When compared with enzymes from different sources, soil enzymes commonly show particular and peculiar feature. Soil enzymes are involved in energy transfer, nutrient cycling, environmental quality and crop productivity. Negative impact of pesticides on soil enzyme activities has been widely reported in the literature [24, 25]. Hence the present study has been undertaken to investigate the effect of endosulfan and profenofos on cellulase and amylase activities in groundnut cultivated black soil.

MATERIALS AND METHODS

Soil: A black soil from different sites of groundnut cultivated fields of Kurnool district of Andhra Pradesh, India collected randomly near the rhizosphere zone using trowel at a depth of 0-12 centimeters and mixed thoroughly to prepare a homogenate composite sample, air dried at room temperature samples were cleaned by removing plant material and other debris and passed through 2 millimeter sieve, stored at 4°C prior to analysis. Mineral matter of soil samples was done by following the method [26]. Soil pH was determined by using 1:1.25 soils to water ratio in systronic digital pH meter. Organic matter in soil samples was estimated by walkley and black oxidation, total nitrogen content in soil samples was determined by Micro-Kjeldhal method [27]. Electrical conductivity was measured by conductivity bridge and contents of nitrite-nitrogen [28] contents of nitrate-nitrogen by Brucine method [29]. The important Physico-chemical properties of the two soils are presented in Table 1.

Insecticides: To determine the influence of selected insecticides on soil enzyme activities, endosulfan a organochlorine insecticide (35% emulsifying concentration) was obtained from Hoechstschering agro ero (ltd). Gujarat and profenofos an organophosphate (50% emulsifying concentration) was obtained from Sudarsha industries ltd, Pune 411001, India.

Table 1: Physicochemical characteristics of the soil

Properties	Black soil
Sand (%)	61.7
Silt (%)	15.2
Clay (%)	23.8
pH[a]	7.4
Water holding capacity (ml g^{-1} soil)	0.31
Electrical conductivity (m.mhos)	260
Organic matter [b] (%)	1.078
Total nitrogen [c] (%)	0.046
NH_4^+-N (μg g^{-1} soil) [d]	8.97
NO_2^--N (μg g^{-1} soil) [e]	0.412
NO_3^--N (μg g^{-1} soil) [f]	1.340

Where a = 1:1.25 = Soil: Water slurry b = Walkley-Black Method (Jackson, 1971),
c = Micro-Kjeldhal Method (Jackson, 1971) d = Nesslerization method (Jackson, 1971)
e =Diazotization Method (Barnes and Folkard, 1951) f = Brucine Method (Ranney and Bartlett, 1972)

Cellulase and Amylase Activities in Soils: Five gram portion of the soil samples were weighed and dispersed into sterile test tubes (25 x 150 mm). Stock solutions from selected insecticides were added at the rate of 10, 25, 50, 75 and 100 μg g^{-1} soil equivalent to field application rates of 1.0, 2.5, 5.0, 7.5 and 100 kg ha^{-1} respectively. Soil samples without insecticide treatment served as controls. Soil samples were mixed thoroughly for uniform distribution of insecticide added. Triplicates were maintained for each treatment at room temperature (28 ± 4°C) with 60% water holding capacity throughout the incubation period. After desired intervals of incubation, soil samples were extracted in distilled water for estimation of enzyme activities.

Assay of Cellulase (EC 3.2.1.4.): In order to determine cellulase enzyme activity in soils, 10 ml of carboxy methyl cellulose (CMC) 1% was used as a substrate followed by10 ml of acetate buffer (pH 5.9) and incubated for 24 hours to determine the reducing sugar content in the filtrate [30] followed by Jaffermohiddin *et al.* [31].

Assay of Amylase (EC 3.2.1.1): The method employed for the assay of amylase was developed by Cole [32] and followed by Tu [33,34]. The soil samples were transferred to 100 ml Erlenmeyer flasks and were treated with 1 ml of toluene to arrest the enzyme activity. After 15 minutes, 6ml of 0.2M of acetate phosphate buffer (pH 5.5) containing 2% starch was added to each of the testing samples and closed with cotton plugs. After 24 hours and 72 hours of incubation the testing samples were made up to a volume of 50 ml with sterile distilled water and passed through Whatman No. 1 filter paper and the filtrate was assayed for amount of glucose by Nelson method [35] followed by Jaffer Mohiddin *et al.* [31] in a Spectronic 20D Spectrophotometer.

Statistical Analysis: The activities of the cellulase and amylase was calculated on the basis of soil weight (oven dried). Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range test (DMRT) [6, 36,]. All statistical analysis was performed at (P≤ 0.05) using SPSS statistical software package.

RESULTS

Cellulase Activity: The activity of cellulase was increased in all the soil samples treated with endosulfan and profenofos throughout the incubation period (Table 2). The significant stimulation in cellulase activity was noticed by the application of insecticides at 5.0 kg ha^{-1} and the activity was dramatically decreased at higher concentrations of 7.5 and 10.0 kg ha^{-1} after 10 days of incubation. The endosulfan and profenofos showed individual increments in cellulase activity ranged from a low increase 10-66% and 5-61% in comparison to controls in black soil (Table 2). The stimulatory concentration (5.0 kg ha^{-1}) of both insecticides, endosulfan and profenofos have shown the highest enzymatic activity after 20 days and then decline phase was started and showed minimum activity after 30 and 40 days of incubation in black soil (Fig 1).

Amylase Activity: Amylase activity showed a variable pattern in response to different insecticide concentration after 10 days of incubation (Table 3)

Table 2: Activity of cellulase* under the impact of different concentrations of selected pesticides endosulfan and profenofos in black soil for 24 hours after 10 days

Conc., of insecticides (Kg ha^{-1})	Black soil	
	Endosulfan	Profenofos
0.0	450 ± 2.886^{d}(100)	450 ± 2.886^{d}(100)
1.0	495 ± 2.886^{c}(110)	474 ± 3.464^{c}(105)
2.5	615 ± 8.660^{b}(137)	576 ± 2.309^{b}(128)
5.0	749 ± 0.577^{a}(166)	725 ± 2.886^{a}(161)
7.5	617 ± 1.732^{b}(137)	553 ± 1.732^{b}(123)
10.0	445 ± 2.886^{d}(99)	437 ± 1.732^{d}(97)

*µg glucose per gram soil formed after 24 and 72 hrs incubation with 2% starch.

Figures, in parentheses, indicate relative production percentages.

Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan's Multiple Range (DMR) test.

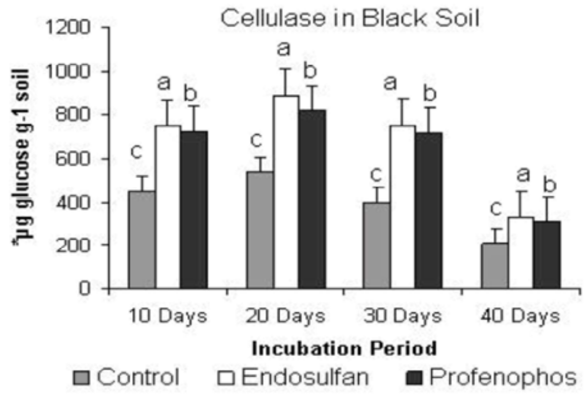

Fig. 1: Influence of insecticdes at (5 kg ha^{-1}) on cellulase*activity in black soil incubated for 24 hours with 1% carboxy methyl cellulose (CMC) after 10, 20, 30 and 40 days.
Means, in each column, followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan's Multiple Range (DMR) test

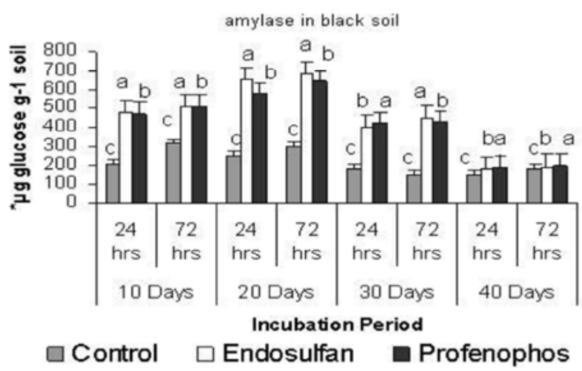

Fig. 2: Influence of insecticides at 2.5 and 5.0 kg ha^{-1} on amylase* activity in black soil after 24 and 72 hours.
*µg glucose per gram soil formed after 24 and 72 hrs incubation with starch. Means, in each column, followed by the same letter are not significantly Different (P ≤ 0.05) from each other according to Duncan's Multiple Range (DMR) test.

Enzyme activity increased under all the treatments (1.0, 2.5, 5.0, 7.5, kg ha^{-1}) except 10 kg ha^{-1} level compared to controls in black soil. The endosulfan and prfenofos have shown maximum enhancement in cullulase activity at 2.5 and 5.0 kg ha^{-1} respectively and the activity was significantly decreased at higher concentration of 10.0 kg ha^{-1} in black soil (Table 3). The insecticides, endosulfan and profenofos showed individual increments in amylase activity were 142-134%, 19-61% and 25-131%, 8-61% in

Table 3: Activity of amylase* under the impact of different concentrations of selected pesticides endosulfan and profenofos in black soil for 24 and 72 hours after 10 days

Conc., of insecticides ($Kg\ ha^{-1}$)	Black soil			
	Endosulfan		Profenofos	
	24 hrs	72 hrs	24 hrs	72 hrs
0.0	204 ± 2.645^{d}(100)	317 ± 1.732^{d}(100)	204 ± 2.645^{e}(100)	317 ± 1.732^{d}(100)
1.0	290 ± 5.773^{c}(142)	378 ± 1.154^{c}(119)	256 ± 2.309^{d}(125)	342 ± 1.154^{c}(108)
2.5	477 ± 5.773^{a}(234)	510 ± 5.77^{a}(161)	369 ± 0.577^{b}(181)	419 ± 0.577^{b}(132)
5.0	374 ± 2.309^{b}(183)	451 ± 0.577^{b}(142)	472 ± 1.154^{a}(231)	512 ± 1.154^{a}(161)
7.5	243 ± 1.732^{c}(119)	301 ± 0.577^{d}(95)	305 ± 2.886^{c}(149)	403 ± 1.732^{b}(127)
10.0	189 ± 0.577^{e}(93)	250 ± 5.773^{e}(79)	156 ± 1.154^{f}(76)	260 ± 5.773^{e}(82)

*µg glucose per gram soil formed after 24 and 72 hrs incubation with 2% starch.

Figures, in parentheses, indicate relative production percentages.

Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test.

comparison to control after 24 and 72 hrs at 2.5 and 5.0 kg ha^{-1} respectively (Table 3). The stimulatory concentrations (2.5 to 5.0 kg ha^{-1}) of both endosulfon and profenofos have shown the highest enzymatic activity after 20 days and then decline phase was started and exhibited lowest activity after 30 and 40 days of incubation in black soil (Fig 2).

DISCUSSION

The black and red soils were predominantly used for the cultivation of groundnut (*Arachis hypogaea* L.) in the Kurnool district of Andhra Pradesh, India. Comparatively black soil has highest organic matter content than red soil [37]. For this reason black soil has been using predominantly for the cultivation of crops. Hence black soil was selected for our investigation under the influence of pesticides. Persistence of pesticide residues in the soil may have a significant impact on soil microbial communities and their functions such as the activity of enzymes, which are directly related to soil health and fertility and also to the removal of contaminants [38,39]. Our analysis revealed that cellulase activity was low at higher concentrations (7.5 and 10.0 kg ha^{-1}) of endosulfan and profenofos treated soil than untreated controls throughout the experiment (Table.2) suggesting that the enzyme is rather sensitive to endosulfan and profenofos. Interestingly, stimulatory effect was observed at 10 to 50 ppm concentrations. Analogous report was obtained by Ismail *et al*. and Gigliotti *et al*., [40, 41] on application of metolachor to Malaysian soil. Gherbawy and Abdel Zaher [42] also reported that bensulfurn methyl at 16 and 160 mug/g inhibited cellulase activity in soil samples. In a diverse study made by Arinze and Yubedee [43], revealed that alteration in the activity of cellulase by metalaxyl was marked in pure fungal cultures. Similar results were obtained by Katayama and Kuwatsuka [44], the kelthane and fenvalerate caused inhibition to enzyme activity. Similar observation was made by Jayamadhuri and Rangaswamy, Srinivasulu and Rangaswamy, Tu and Cole [31, 45-47] on the cellulase activity. On the other hand, for amylase the stimulatory concentrations for endosulfan is 2.5 kg ha^{-1} and for profenofos 5.0 kg ha^{-1}. Similar type of results were obtained by Tu, Cole [47,31]. Tu, Nelson, Prasad and Mathur [33,34,48], triazophos, a phosphorothioate triazole is stimulate for amylase at 5 and 10 mg/kg incubated for three days in an organic soil. As per the observation made by the Megharaj *et al*., [49] the amylase activity increased during germination in both control and Cuman treated seeds at 0.25, 0.5, 0.75 and 1% respectively. Interaction effects on soil enzyme activities including amylase activity received least attention.

CONCLUSION

The results obtained in the present study, clearly indicated that the insecticides endosulfan and profenofos were profoundly enhanced the activities of both amylase and cellulase, at the level of 1.0 to 5.0 kg ha^{-1}. Based on the above results, it is concluded that the microbial activities (enzyme activities) were not affected, by the insecticides applied at recommended levels in agricultural system to control insect pests.

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