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Interaction Effects of Insecticides on Enzyme Activities in Black Clay Soil from Groundnut (*Arachis hypogaea* L.) Fields

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In practice pesticides are extensively used in agriculture as a part of pest control strategies. Two insecticides, endosulfan (organochlorine) and profenophos (organophosphate), were assessed for their effects on the activities of protease (in terms of tyrosine formed from casein) and urease (as ammonia released from urea) in soil, collected from a fallow groundnut field by applications of insecticides at normal field rates and at higher concentrations (1.0, 2.5, 5.0, 7.5, 10.0 kg ha^{-1}), in a laboratory study. The results showed a strong positive influence on protease and urease enzyme activities in soil treated with 2.5 and 5.0 kg ha^{-1} dry soil and they were significantly ($P \leq 0.05$) higher than the control over the course of incubation. In soil treatment, there was a significant increase in protease and decrease in urease activities after 24h of incubation which continued up to 20 days. However, a significant decrease in both protease and urease enzyme activities was observed in 30 and 40 days of incubation.

Key words: *Endosulfan, profenophos, soil enzymes, groundnut (Arachis hypogaea L.) soil.*

1. Introduction

Since several decades, xenobiotic substances have been widely used in agriculture as a part of pest control strategies with the growing use of pesticides. The issue of an impact of these chemicals on the composition of soil microorganisms and the processes they direct have received more attention (Andrea et al. 2000, Baxter and Cummings 2008) and their background levels in the environment have increased greatly. As a consequence, these agrochemicals (biotoxicants) have been found in numerous natural systems and have a great impact on environment quality (Madhun and Freed 1990). Pesticides are developed and applied to destroy or suppress their only target organisms in agricultural crops; however, most often they also affect non-target organisms. Among the oil seeds and cash crops, the groundnut crop is frequently treated with pesticides. Groundnut (*Arachis hypogaea* L.) is one of the important major profitable crops grown in India throughout the year and is a world leader in groundnut farming, with eight million hectares of cultivated area in the year 2002-2003 (FAO 2004). The current productivity of groundnut in India is about quintal (50 kg) per ha (Talawar 2004). The present day agriculture involves abundant cultivation of the crop because of its vital role in edible oil seeds production (Kori et al. 2002). Groundnut ranks seventh among crops in terms of insecticide consumption in India (Giraddi et al. 1999). More than 120 pests affect economically important crops like groundnut, cotton, and tomato (Megharaj et al. 1999; Rangaswamy and Venkateswarlu 1992; Vijay Gundi et al. 2007; Jayashree and Vasudevan 2007; Romeh et al. 2009). When an anthropogenic factor is released deliberately or accidentally into the environment, about 0.1% of it reaches the target organism while the remaining 0.99% reaches the soil causing not only trouble to local metabolism or enzymatic activities (Carriger et al. 2006; Pimentel 1995 Engelen et al. 1998; Liu et al. 2008; Topp et al. 1997), but also disturbs the soil ecosystem and thus may affect human health by entering the food chain, and thus it has raised a considerable public concern.

Profenophos (0-4-bromo-2-chlorophenyl-O-ethyl-S propylphosphorothioate) is a non systemic

insecticide and acaricide with contact and stomach action used against mites, leafhoppers, thrips, aphids, mealy bugs and cotton stainers. 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 (Kwon et al. 2002). Endosulfan, due to its high degree of toxicity it persists in soils and water, has become an important group of contaminants. Although this pesticide has been restrictively used or even banned due to its persistence and bioaccumulation, it may be still found in soils. For this reason, soil biological responses to the pesticides are required to be estimated. To date, many efforts have been taken to understand the effect of pesticides on soil enzyme activities, protease urease, nevertheless little is known about the effect of endosulfan and profenophos. Proteases occur naturally in all organisms. Bacteria also secrete proteases to hydrolyse (digest) the peptide bonds in proteins and, therefore they break the proteins down into their constituent monomers. Urease (urea amidohydrolase, EC 3.5.1.5) is the enzyme that catalyses the hydrolysis of urea to Co_2 and $NH4^+$ ions by acting on C-N non – peptide bonds in linear amides. It is an important enzyme in soil that mediates the conversion of organic nitrogen to inorganic nitrogen by hydrolysis of urea to ammonia. The activities of these protease and urease enzymes are important in soil in releasing simple carbon and nitrogen sources for the growth and multiplication of soil microorganisms. Soil enzymes can be tracked as indicators of the soil quality following the addition of pesticide application. The objective of this work was to determine the impact of endosulfan, profenophos formulations on soil protease and urease activity.

2. Materials and methods

Soil. Black clay soil, collected from a fallow groundnut cultivated field of Kurnool district of Andhra Pradesh, to a depth of 0-12 centimeters, air dried and sifted through 2 millimeter sieve was stored at 4^oC prior to analysis. Mineral matter of soil samples was obtained by following the method of Johnson and Ulrich 1960. Soil pH was determined by using 1:1.25 soils to the water ratio in a systronic digital pH meter. Organic matter in soil samples was estimated by Walkley and Black method, the total nitrogen content in soil samples was determined by Micro-Kjeldhal method (Jackson 1971). Electrical conductivity was measured by a conductivity bridge and the contents of nitrite – nitrogen (Barnes and Folkard 1951) the contents of nitrate – nitrogen - by Brucine method (Ranney and Bartlett 1972). Important physicochemical properties of the black clay soil are presented in Table 1.

Table.1. Physicochemical characteristics of the soil

Properties	Black clay 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)

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

Protease and urease activity. To determine protease and urease activities one gram and two gram portion soil samples were distributed in test tubes (12 x 150 mm) and treated with the selected insecticides to provide the final concentration of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} level. All the treatments including a control one were incubated in the laboratory at a room temperature (28 ± 4^oC). After ten days of incubation, triplicate soil samples were withdrawn for the assay of protease earlier studied by Speir and Ross (1975); Rangaswamy and Venkateswarlu (1994). For the assay of urease by a phenol hypochlorite method (Fawcett and Scott, 1960); Rangaswamy and Venkateswarlu (1992a) adopted by Jaffer Mohiddin et al. (2011).

In another experiment, black clay soil samples in triplicates were treated with two insecticides, endosulfan and profenophos at 2.5 and 5.0 kg ha^{-1}. After incubation for 10, 20, 30 and 40 days at 28 ± 4° C, triplicate soil samples were withdrawn to determine the rate of enzyme activity.

Assay of protease *(EC 3.4.21.19).* Untreated and insecticide- treated soil samples (2g) were incubated for 24 h at 30°C with 10 ml of 0.1 M tris (2-amino-2 (hydroxymethylmethyl)-propane-1:3-diol, pH, 7.5) containing sodium caseinate (2%w/v) of trichloro, acetic acid was added and the mixer was centrifuged. A suitable aliquot of the supernatant was treated with 3 ml of 1.4 M Na_2co_3 followed by the addition of 1.0 ml Folin-Ciocalteu reagent (33.3%v/v). The intensity of blue color was read after 30 min at 700 nm in a spectrophotometer. Tyrosine was used as a standard.

Assay of urease *(E.C. 3.5.1.5).* Urease activity in soil samples (1g) was determined following the method of phenol hypochlorite (Fawcett and Scott, 1960). Untreated and insecticide- treated soil samples (1g) were mixed with 4 ml of 0.1 M sodium phosphate buffer at pH-7.0 and 1 ml of 1 M urea solution and incubated for 30 minutes. After incubation, 10 ml of 2M KCl were added and the mixtures were kept at 4°C for 10 min, to stop the enzymatic reaction. Suspensions were centrifuged for 5 min. Two ml of supernatant was mixed with 5 ml of phenol sodium nitroprusside solution and 5ml of 0.02 M sodium hypochlorite, and the mixture was incubated for 30 minutes in the dark, and the blue color formed was read at 630 nm in a spectrophotometer.

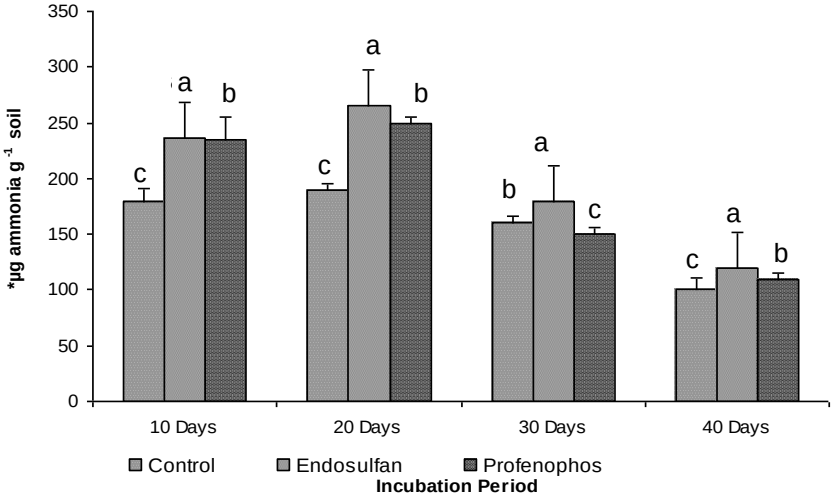

*Fig.1. Influence of insecticdes at (2.5 kg ha^{-1}) on protease*activity in black clay soil incubated for 24 hours with 1% casein after 10, 20, 30 and 40 days. The values are the means ± S.E.., for each incubation periods, in each column followed by the same letter are significantly Different (P ≤ 0.05) from each other according to Duncan's Multiple Range (DMR) test*

3. Statistical analysis

Table 2. Activity of protease under the impact of different concentrations of selected pesticides endosulfan and profenophos in black clay soil for 24 hours after 10 days

Conc., of insecticides (kg ha^{-1})	Black clay soil	
	Endosulfan	Profenophos
0.0	602 ± 1.154^{d} (100)	602 ± 1.154^{d} (100)
1.0	680 ± 5.773^{c} (113)	613 ± 1.732^{c} (102)
2.5	883 ± 5.773^{a} (147)	692 ± 5.773^{a} (115)
5.0	750 ± 5.773^{b} (124)	630 ± 17.320^{b} (105)
7.5	748 ± 1.154^{b} (124)	612 ± 1.154^{c} (102)
10.0	542 ± 1.154^{e} (90)	544 ± 2.309^{e} (90)

**µg glucose per gram soil formed after 24 hrs with 1% casein.. Figures in parentheses indicate relative production percentages. In each column means followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan's Multiple Range (DMR) test.*

The activity of the protease and urease 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) (Megharaj et al. 1999; Jaffer et al. 2011). All statistical analysis was performed at (P ≤ 0.05) using the SPSS statistical software package.

Table 3. Activity of urease under the impact of different concentrations of selected pesticides endosulfan and profenophos in black clay soil for 24 hours after 10 days*

Conc., of insecticides (kg ha^{-1})	Black clay soil	
	Endosulfan	Profenophos
0.0	180 ± 5.773^{d} (100)	180 ± 5.773^{d} (100)
1.0	192 ± 5.773^{c} (107)	180 ± 5.773^{d} (100)
2.5	223 ± 1.732^{b} (124)	202 ± 1.154^{b} (112)
5.0	236 ± 17.320^{a} (131)	235 ± 20.207^{a} (130)
7.5	196 ± 2.309^{c} (109)	192 ± 1.154^{c} (107)
10.0	154 ± 2.309^{e} (85)	152 ± 1.154^{e} (84)

**µg glucose per gram soil formed after 24 and 72 hrs incubation with 2% starch.*
Figures in parentheses indicate relative production percentages.
I n each column means followed by the same letter are not significantly different (P ≤ 0.05) from each other according to Duncan's Multiple Range (DMR) test.

4. Results

Our investigation has revealed that protease activity has more drastically decreased at higher concentrations (5.0, 7.5 10.0 kg ha^{-1}) of endosulfan and profenophos treated soils than the untreated controls throughout the experiment (Table 2), suggesting that the enzyme is rather sensitive to endosulfan and profenophos. Interestingly, a stimulatory effect was observed at 10 - 25 ppm concentrations with individual increments of two insecticidal treatments, on the control, they are as follows: 13-47% and 2-15 % in black clay soil after 10 days of incubation. This trend follows up to 20 days of incubation, when further prolonged in the period of incubation up to 40 days, a decline in enzyme activity was observed. Similar to protease, urease also follows the same trend, a stimulatory effect of endosulfan and profenophos was observed at 10-50 ppm concentrations with individual increments of two insecticidal treatments on the control, they were 7-31% and 12-30 % in black clay soil after 10 days of incubation (Table 3). Urease activity decreased significantly after a longer period of incubation up to 30 or 40 days (Fig. 2).

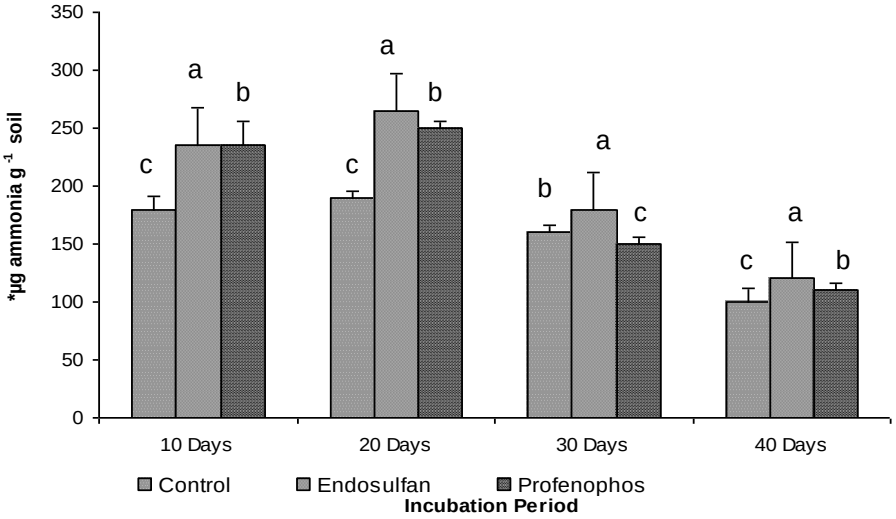

Fig. 2. Influence of insecticides at 5.0 kg ha^{-1} on urease activity in black soil after 30 min incubation at 37°c with urea. *µg ammonia per gram soil formed after 30 min incubation at 37°c with urea. The values are the means* ± S.E.., *for each incubation periods, in each column followed by the same letter they are significantly different ($P \leq 0.05$) from each other according to Duncan's Multiple Range (DMR) test.*

5. Discussion

The laterite and vertisol soils were predominantly used for the cultivation of groundnut (*Arachis hypogeae* L.) in Kurnool district of Andhra Pradesh, India. The major constraint in groundnut crop is insects and pathogenic fungi. Hence, pesticides were frequently used for crop protection. Due to continuous use of these 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 (Ingham et al. 1991, Beare et al. 1992). In general, the organic matter content is higher in black soil than in red soil. Hence, black soil is selected to study the effect of insecticides on the protease and urease activities (Srinivasulu et al. 2011). Our reports are in agreement with the previous studies carried out by Srinivasulu et al. (2010b) whose observed activity of protease was higher in black soil and received the monocrotophos and chlorpyrifos in combination with mancozeb and carbendazim, respectively, at 5.0 kg ha^{-1} incubated for 20 days. This increase in enzyme activity continued up to 20 days of incubation and afterwards there was a turn down in enzyme activity. Rasool and Reshi (2010) noticed the stimulatory effect on protease activity in comparison with the control up to 21 days of incubation. Similar observations on protease activity in soil treated with monocrototphos, quinolphos, cypermethrin and fenvalerate up to 25 ppm level was recorded by Rangaswamy et al. (1994). The same results were observed by Endo et al. (1982). The stimulation of protease activity in a native soil was reported after its treatment with Linuron at 10 mg kg^{-1}, where as Cartap-HCL at 100-1000 mg kg^{-1} inhibited the enzyme activity without recovery during the period of 60 days. On the other hand, several reports indicate that protease activity in soils was individually inhibited by the application of urea at 200-N kg ha^{-1} and of herbicides dalapon and paraquat at 10 kg ha^{-1} by 13 and 18%, respectively. The combination of urea and herbicides at the same concentrations reduced the protease activity in soils by 41-56% indicating the synergistic interaction at the end of the 10-day incubation (Namdeo and Dube, 1973), In contrast, thiram at 10 ppm decreased urease activity in both sandy and organic soils after 7 days (Tu 1990). Gooty Jaffer Mohiddin et al. (2011) noticed that two

insecticides, acephate and imidacloprid at 10, 25, 50 μg g^{-1} levels, individually caused increments of 30-77 and 46-54% increase in urease activity on the control in black soil at a 10-day interval, respectively. The enzyme activity decreased significantly in a longer period of incubation up to 30 and 40 days. A similar trend was observed by Srinivasulu et al. (2010a) with tridemorph and captan which showed individual increments of 28.37-93.70 and 18.32-90.37% increase in urease activity over the control, respectively, in black soil at 10-day interval. According to Gianfreda et al. (1994), glyphosate enhanced urease activity of soils 1.1-1.4-fold and of soil extracts 2.59 to 6.73-fold at 0.3 and 1.5 mM, but had no influence on free or immobilized jackbean (*Canavalia ensiformis*) urease. In contrast, Rasool and Reshi (2010) reported a significant decrease in urease activity with mancozeb at different application rates over the control. In another study, urease activity was not inhibited by fenamiphos (Caceres et al. 2009) in Australian and Ecuadorean soils. On the other hand, urease activity was inhibited by napropamide at all concentrations relative to the control with long periods of application (Guo et al. 2008). Similarly, Cycon et al. (2010) noticed that urease activity declined in sandy loam and loamy sand soils with a combination (mancozeb + dimethomorph) at higher concentrations compared to the control. Similarly, urease activity decreased by 20% in unamended polluted soils (with MCPA) (Tejda et al. 2010) following the exposure to chlorpyrifos (CPF) and its oxon derivative (CPO) at higher concentrations (Wang et al. 2010). In another study a 55.6% decrease in urease enzyme activity was noticed with different concentrations (0, 200, 400,600, 800, and 1000 mg kg^{-1}) of Pb-contaminated soil (Akmal and Xu 2008). In contrast, Rahmansyah et al. (2009) reported an increase in urease enzyme activity after 2 weeks of incubation which declined after 12 weeks of incubation with insecticide deltamethrin and fungicide probineb. In another study, urease activity was not affected by the presence of glyphosate at 5.4 kg ha^{-1} in soil (Davies and Greaves 1981).

6. Conclusions

The results of the present study have clearly indicated that the insecticides endosulfan and profenophos profoundly enhance the activities of both protease and urease when used 2.0-5.0 kg ha^{-1}. Thereby, proteases promote a breakdown of proteinaceous substance and urease promotes hydrolysis of urea to $NH4^{+}$ in soil to simpler nitrogen compounds that are available for plant nutrition, and in turn, the plant yield will be good which is beneficial to the farmer and the society. Based on the above results, it is concluded that the protease and urease are not affected by the insecticides applied at the recommended levels to the agricultural system. Very little information is available on the influence of endosulfan and profenophos on protease and urease activities in groundnut soils. Hence, further research is needed to evaluate the effect of these insecticides on soil enzyme activities.

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Insekticidų poveikis juodojo molio fermento aktyvumui laukuose, kuriuose auginami žemės riešutai (*Arachis Hypogaea* L.)

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Pesticidai plačiai naudojami žemės ūkyje kaip viena iš kenkėjų naikinimo priemonių. Straipsnyje aprašomas tyrimas, kurį atliekant buvo vertinama dviejų insekticidų – endosulfano (organinio chloro) ir profenofoso (organinio fosfato) – poveikis peptidazei (tirozinas susiformavęs iš kazeino) ir ureazei (iš šlapalo išsiskyręs amoniakas) dirvoje. Mėginiai buvo imti iš pūdymui palikto žemės riešutų lauko, kuriame buvo naudojami įprasti insekticidų kiekiai. Laboratoriškai buvo tirta didesnė insekticidų koncentracija (1,0; 2,5; 5,0; 7,5; 10,0 kg ha^{-1}). Rezultatai parodė stiprų proteazės ir ureazės fermentų aktyvumą dirvoje, kur insekticidų koncentracija sausoje dirvoje buvo lygi 2,5 ir 5,0 kg ha^{-1}. Išdirbus žemę, po 24 valandų inkubacinio periodo proteazės aktyvumas reikšmingai padidėjo, ureazės aktyvumas sumažėjo. Tačiau abiejų fermentų aktyvumas gerokai sumažėjo tik po 30 ir 40 dienų inkubacinio periodo.