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Influence of Pesticides, Alone and in Combination, on Phosphatase Activity in Soils of Groundnut (*Arachis hypogaea* L.) Fields

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ABSTRACT

Pesticides such as thiram and difenoconazole (fungicides), and deltamethrin and profenofos (insecticides), alone and in combination with insecticides viz., profenofos (organophosphate) + cypermethrin (synthetic pyrethroid) and deltamethrin (synthetic pyrethroid) + endosulfan (organochlorine) were applied at five concentrations (0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) to test their non-target effects towards the activity of phosphatase in black clay and red sandy clay soils of groundnut (*Arachis hypogaea* L.) fields under laboratory conditions. Phosphatase activity was more pronounced in soil samples treated with 2.5 kg ha^{-1} of the insecticide combinations and individual pesticides. The activity of phosphatase was increased with increasing concentration of insecticide combinations up to 2.5 kg ha^{-1}. Higher levels of these pesticides and insecticide combinations eliminated phosphatase activity. Soil samples receiving 2.5 kg ha^{-1} of insecticide combinations profenofos + cypermethrin and deltamethrin + endosulfan accumulated *para*-nitrophenol most after 20 days of incubation; enzyme activity decreased as the period of incubation increased. Results of this study suggest that enzyme activity is influenced by the concentration of profenofos + cypermethrin and deltamethrin + endosulfan combinations.

Keywords: groundnut soil, insecticide combinations, *para*-nitrophenol, pesticides, phosphatase

INTRODUCTION

In modern agriculture, it has become a common trend to apply different groups of pesticides, either simultaneously or in succession, for effective control of a variety of pests (Quazi *et al*. 2011). The intensive and extensive use of agricultural chemicals in crop production, ultimately influence the soil fertility which is primarily contributed by soil microflora (Hussain *et al*. 2009; Ahemad and Khan 2011a, 2011b). The extensive application of pesticides interferes with the normal enzymatic activity of proliferating soil microorganisms, and disturbs the delicate balance of the soil ecosystem. Different pesticides may often exist together in the soil ecosystem as residues at a given point of time and disturb ecological balance in longer run (Aramendia *et al*. 2007; Swaminathan *et al.* 2009; Defo *et al*. 2011). The effect of various combinations of pesticides may deviate from the behavior of an individual pesticide because of the occurrence of synergistic, antagonistic or additive interactions between different pesticides (Gundi *et al*. 2007). Information generated from studies with the application of single pesticide to soils cannot be extrapolated to soil systems containing more than one pesticide. Apparently, it has become necessary to determine the effects of agronomically needed combinations of pesticides, applied at recommended levels, in order to establish the ecological significance of the pesticide effects (Tejada 2009; Yan *et al*. 2011). Therefore, the behaviour of the total microflora and their biological activities under continue pesticide input is an important aspect of research of the ecology (Pandey and Singh 2004; Singh and Singh 2005; Fenlon *et al*. 2011).

Increasing use of pesticides in agricultural led to the development of soil microbial testing programmes for examination of the side effects (Swaminathan *et al.* 2009). The testing programmes included measurement of activities of soil enzymes, and physico-chemical properties of soil and degradation of pesticides. Soil enzymes are remarkable biomolecules that show extraordinary specificity in catalyzing biological reactions, important for both soil microorganisms and plants (Madakka *et al*. 2009). Soil enzymes are considered as good indicators of soil biological fertility because of their participation in the decomposition of organic matter (Pascual *et al.* 2000; Van Dyk and Pletschke 2011). Soil enzymes react to changes in soil ecosystem more quickly than other variables and therefore these soil enzymes are early indicators of various biological changes to the influence of pesticides (Masciandaro *et al.* 2004). Essential components produced through various important biochemical changes of soil plays an essential role in indicating the soil fertility (Bending *et al*. 2006; Quian *et al*. 2009). In spite of the maximum potential of soil enzymes in maintaining soil biodynamics, only limited studies were available on influence of soil enzymes on organochemicals (Walker *et al*. 2001; Pessagno *et al.* 2008; Ahemad and Khan 2011a). The majority of studies focused on testing side-effects of pesticides on non-target organisms and enzyme activities. However intensive use of common pesticides can lead to toxicity to soils, may inhibit biochemical reactions in soil which are important for its health. When pesticides are applied to soils, they may interact with non target soil micro organisms and have a chronic diverse effect on soil health (Hussain *et al.* 2009).

Chemical control of several major pests of groundnut, cotton and tomato by spraying organophosphates, synthetic pyrethroid, carbamate, triazole and organochlorine groups of pesticides either singly or in combination, has been a common practice (Jayashree and Vasudevan 2007; Romeh *et al*. 2009). So far there have been no reports on the interaction of pesticides viz., profenofos, deltamethrin, thiram, difenoconazole and insecticide combinations profenofos +

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cypermethrin, deltamethrin + endosulfan) on enzymatic activities of soils. In the present study, for the first time an attempt has been made to study the interaction effects of these pesticides profenofos + cypermethrin, deltamethrin + endosulfan on the phosphatase enzyme which is involved in the phosphorous cycle, in black clay an red sandy clay soils from groundnut cultivated fields of Anantapur District of Andhra Pardesh, India.

MATERIALS AND METHODS

Soils

Agricultural soil samples such as black clay and red sandy clay were collected from groundnut cultivated fields of Anantapur District in a semi-arid zone of Andhra Pradesh, India. Samples to a depth of 12 cm, were air–dried and sieved through a 2 mm mesh screen before analysis. Samples were chosen because of their agricultural importance.

Pesticides

Two fungicides, thiram (carbamate), difenoconazole (triazole) and two insecticides, deltamethrin (synthetic pyrethroid) and profenofos (organophosphate) and insecticide combinations like profenofos (organophosphate) + cypermethrin (synthetic pyrethroid) and deltamethrin (synthetic pyrethroid) + endosulfan (organochlorine) were obtained from commercial market (**Table 1**).

Soil incubation

The soil ecosystem stimulating non-flooded conditions consisting of ten gram portions of soil samples were added in test tubes (25 × 150 mm) and moistened to a water potential of 0.090 MPa, in order to maintain at 60% water holding capacity. The same model was used previously to elucidate the effects of insecticides on microbial activities (Tu *et al.* 1996; Jaya Madhuri and Rangaswamy 2003).

Phosphatase activity

Two gram portions of each soil, in duplicates, were treated with the selected insecticides at five different concentrations i.e. 1, 2.5, 5.0, 7.5 and 10 kg ha^{-1}. Soil samples without insecticide treatment served as control. Soil samples in test tubes with and without insecticide treatment were incubated at room temperature in the lab (28 ± 4°C). After 10 days of incubation, soil extract was prepared in distilled water for assay of phosphatase (E.C. 3.1.3.1.) as per Rangaswamy and Venkateswarlu (1996).

Assay of phosphatase

Soil samples were transferred to 100 ml Erlenmeyer flasks and 0.2 ml of toluene, 6 ml of 0.1 M maleate buffer (pH 6.5), and 2 ml of *p*-nitrophenyl phosphate disodium salt were added. The flasks were swirled for a few seconds to mix the contents, stoppered and incubated at 37°C for 30 min. The reaction was stopped by adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH followed by swirling of the flask, for a few seconds and the soil suspension was filtered through a Whatmann No. 1 filter paper. The liberated *p*-nitrophenol in the filtrate was determined at 410 nm in a Spectronic-20D.

Statistical analysis

The concentration of phosphatase was calculated on a soil weight (oven dried) basis. The insecticide treatments were contrasted with untreated controls and the significant level $P \leq 0.05$ between values of each sampling and each insecticide were assessed using SYSTAT statistical software package to find the results of one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Jaffer *et al*. 2010).

RESULTS AND DISCUSSION

Phosphatases are a group of enzymes that catalyse the hydrolysis of both esters and anhydrides of phosphoric acid. The mineralisation of organic phosphorus by the activity of phosphatase in soils makes one of essential elements, phosphorus in soil for plant growth (Izaguirre-Mayoral *et al*. 2002; Li *et al*. 2004; Ndakidemi 2006). Hence, phosphatase activity was measured under the influence of profenofos, deltamethrin thiram and difenoconazole and the insecticide combinations profenofos + cypermethrin and deltamethrin + endosulfon, in two soils. The data obtained from these experiments are presented in **Tables 2-6**. Phosphatase activity increased in all individual tested pesticide-treated soils throughout the experiment. The stimulation in the activity of phosphatase increased up to 2.5 kg ha^{-1} and then turn down in both black and red soil after 10 days of incubation (**Tables 2, 3**). As increasing the concentration of pesticides, the stimulation of the enzyme activity was decreased after 2.5 kg ha^{-1}. This enzyme activity continues up to 20 days of incubation and then decline afterwards (**Table 4**). About 22-53, 15-63 and 02-45% enhancement in phosphatase activity over control was recorded in the black soil treated with profenofos, deltamethrin, thiram and difenoconozole alone at concentrations of 1.0 and 2.5 kg ha^{-1} by the end of 10 days incubation, respectively (**Table 2**). In respect of the red sandy clay soil, the corresponding figures of percent enhancement by four pesticides were 20-61, 12-85, 25-59 and 11-79% during the same period (**Table 3**). Of all four pesticides used in the present study, thiram in black clay soil deltamethrin in red sandy clay soil caused maximum stimulation in phosphatase activity. The phosphatase activity was enhanced in both soils with/without pesticides upon further incubation for another 10 days (20-day interval). At 20-day interval, slight increment in the phosphatase activity in both soils with or without insecticides occurred in comparison to the activity of the respective increment at 10-day interval (**Table 4**).

While in case of combinations all the tested insecticide combinations have shown stimulation in phosphatase activity at 2.5 kg ha^{-1} in both black and red soil (**Table 5**) after this concentration increasing in concentration decreases the stimulation of enzyme activity. With reference to individuals in insecticide combinations about 09-36 and 18-51% enhancement in phosphatase activity over control was recorded in the black soil treated with profenofos + cypermethrin and deltamethrin + endosulfan at concentrations of 1.0 and 2.5 kg ha^{-1} by the end of 10 days incubation, respectively (**Table 5**). In respect of the red clay soil, the corresponding figures of enhancement by two insecticide combinations were 12-84% and 15-76% during the same period (**Table 5**). Phosphatase activity was improved in both soils with/without insecticide combinations upon further incubation for another 10 days (20-day interval). At 20-day interval, irrelevant rise in the phosphatase activity in both soils with or without insecticide combinations occurred in comparison to the activity of the respective increment at 10-day interval (**Table 6**). Later, the phosphatase activity declined in both pesticide-amended and control. The release of *p*-nitrophenol from *p*-nitrophenyl disodium orthophosphate was significantly higher in the black clay soil when compared to red sandy clay soil. Similarly, mancozeb N_{10} {10 times the normal application (60 kg/ha)} brought about a 41% stimulation in activity after 14 days of incubation compared to control, but after 28 days of incubation a 30% decrease in enzyme activity was recorded (Rasool and Zafar 2010). In the pot study alakaline phosphatase activity was inhibited by swing at high doses and stimulated by unix (Jastrzebska and Kucharski 2007). Gopal *et al*. (2007) showed that 10% of azadirachtin granules at all doses exerted suppressive effect on phosphatase activity. Interestingly, Qian *et al*. (2007) reported that the validamycin stimulated its activity higher than that of control, only highest dose stimulated acid phosphatase activity by 29.7%. The Cd reduced the activity of phosphatase at

Table 1 Details of the pesticides used in the present study.

Technical name	Commercial name	Chemical class	Commercial formulation	Sources
Thiram	Spotrete	Carbamate	75% WP*	Rallis India Ltd., Mumbai, India
Difenoconazole	Score	Triazole	25% EC**	Syngenta India Ltd., Mumbai, India
Deltamethrin	Decis	Synthetic pyrethroid	2.8% EC**	Bayer Carpo Science India Ltd., Gujarath, India
Cypermethrin	Cyperkill	Synthetic pyrethroid	25% EC**	Bharat Pulversing Mills (Pvt) Ltd., Mumbai, India
Endosulfan	Thiodam	Organochlorine insecticide	35% EC**	Hoechst Schering Agro Evo (Ltd.), Gujarat, India
Profenofos	Prowess	Organophosphate	50% EC**	Sudharsha Industries Ltd, Pune, India

* Wettable powder, ** Emulsifying concentration

Table 2 Effect of different concentrations of selected pesticides on activity of phosphatase* in black soil after 10 days.

Concentration of insecticides (Kg ha^{-1})	Profenofos	Deltamethrin	Thiram	Difenoconazole
0.0	98 ± 2.309 d	98 ± 2.309 c	98 ± 2.309 d	98 ± 2.309 d
1.0	110 ± 2.886 c	112 ± 1.154 b	115 ± 2.886 c	120 ± 5.773 b
2.5	150 ± 2.886 a	148 ± 1.154 a	160 ± 11.547 a	145 ± 2.886 a
5.0	125 ± 2.886 b	110 ± 5.773 b	135 ± 2.886 b	102 ± 1.154 c
7.5	91 ± 0.577 d	80 ± 2.886 d	119 ± 0.577 c	90 ± 2.886 d
10.0	80 ± 2.886 e	75 ± 2.886 d	70 ± 5.773 e	64 ± 1.154 e

Each column is mean ± S.E. for 6 concentrations in each group; Columns with a different letter differ significantly with each other ($P \leq 0.05$; DMRT).

Table 3 Effect of different concentrations of selected pesticides on activity of phosphatase* in red soil after 10 days.

Concentration of insecticides (Kg ha^{-1})	Profenofos	Deltamethrin	Thiram	Difenoconazole
0.0	62 ± 1.154 d	62 ± 1.154 e	62 ± 1.154 c	62 ± 1.154 d
1.0	75 ± 2.886 c	80 ± 5.773 c	78 ± 1.154 b	83 ± 1.732 c
2.5	100 ± 5.773 a	115 ± 8.660 a	99 ± 0.577 a	111 ± 0.577 a
5.0	88 ± 1.154 b	92 ± 1.154 b	80 ± 5.773 b	90 ± 0.577 b
7.5	60 ± 5.773 d	70 ± 5.773 d	60 ± 5.773 c	69 ± 0.577 d
10.0	55 ± 2.886 e	52 ± 1.154 f	43 ± 1.732 d	42 ± 1.154 e

Each column is mean ± S.E. for 6 concentrations in each group; Columns with a different letter differ significantly with each other ($P \leq 0.05$; DMRT).

Table 4 Influence of selected insecticide on phosphatase activity* in black and red soils. Soil incubation (in days).

	10 days	20 days	30 days	40 days
Black soil				
Control	98 ± 1.154 d	120 ± 5.773 d	110 ± 5.773 c	80 ± 5.773 d
Profenofos (2.5 kg ha^{-1})	150 ± 5.773 b	170 ± 5.773 b	155 ± 2.886 a	130 ± 5.773 a
Deltamethrin (2.5 kg ha^{-1})	148 ± 1.154 b	177 ± 1.732 a	140 ± 5.773 b	125 ± 5.773 b
Thiram (2.5 kg ha^{-1})	160 ± 5.773 a	175 ± 2.886 a	135 ± 2.886 b	110 ± 5.773 c
Difenoconazole (2.5 kg ha^{-1})	145 ± 2.886 c	165 ± 2.886 c	152 ± 1.154 a	121 ± 0.577 b
Red soil				
Control	62 ± 1.154 c	89 ± 0.577 c	50 ± 5.773 d	42 ± 1.154 d
Profenofos (2.5 kg ha^{-1})	100 ± 5.773 b	120 ± 5.773 b	90 ± 5.773 c	70 ± 5.773 c
Deltamethrin (2.5 kg ha^{-1})	115 ± 8.660 a	135 ± 2.886 a	120 ± 11.547 a	98 ± 1.154 a
Thiram (2.5 kg ha^{-1})	99 ± 0.577 b	125 ± 2.886 b	105 ± 2.886 b	72 ± 1.154 c
Difenoconazole (2.5 kg ha^{-1})	111 ± 0.577 a	131 ± 0.577 a	100 ± 5.773 b	81 ± 0.577 b

Each column is mean ± S.E. for 6 concentrations in each group; Columns with a different letter differ significantly with each other ($P \leq 0.05$; DMRT).

Table 5 Effect of different concentrations of selected insecticide combinations on activity of phosphatase* in black and red soil after 10 days.

Insecticide Concentration (Kg ha^{-1})	Profenofos + Cypermethrin	Deltamethrin + Endosulfan	Profenofos + Cypermethrin	Deltamethrin + Endosulfan
	Black soil		Red soil	
0.0	110 ± 5.773 c	110 ± 5.773 d	65 ± 2.886 e	65 ± 2.886 d
1.0	120 ± 11.547 b	130 ± 5.773 c	90 ± 5.773 c	88 ± 1.154 b
2.5	150 ± 5.773 a	167 ± 1.732 a	120 ± 5.773 a	115 ± 2.886a
5.0	125 ± 2.886 b	140 ± 5.773 b	110 ± 5.773 b	75 ± 2.886 c
7.5	90 ± 5.773 d	108 ± 1.154 d	73 ± 1.732 d	55 ± 2.886 e
10.0	85 ± 2.886 e	93 ± 1.732 e	55 ± 2.886 f	40 ± 5.773 f

Each column is mean ± S.E. for 6 concentrations in each group; Columns with a different letter differ significantly with each other ($P \leq 0.05$; DMRT).

Table 6 Influence of selected insecticide combinations on phosphatase activity* in black and red soils after 30 min of incubation.

	10 days	20 days	30 days	40 days
Black soil				
Control	110 ± 5.773 c	135 ± 2.886 c	120 ± 5.773 b	91 ± 0.577 b
Profenofos + cypermethrin (2.5 kg ha^{-1})	150 ± 5.773 b	170 ± 5.773 b	127 ± 1.732 a	98 ± 1.154 a
Deltamethrin + endosulfan (2.5 kg ha^{-1})	167 ± 1.732 a	187 ± 1.732 a	130 ± 5.773 a	100 ± 5.773 a
Red soil				
Control	65 ± 2.886 c	85 ± 2.886 c	50 ± 5.773 c	42 ± 1.154 c
Profenofos + cypermethrin (2.5 kg ha^{-1})	120 ± 11.547 a	145 ± 2.886 b	125 ± 2.886 b	97 ± 1.732 b
Deltamethrin + endosulfan (2.5 kg ha^{-1})	115 ± 8.660 b	152 ± 1.154 a	131 ± 0.577 a	105 ± 2.886 a

Each column is mean ± S.E. for 6 concentrations in each group; Columns with a different letter differ significantly with each other ($P \leq 0.05$; DMRT).

early incubation time (1-7 days), while the reduction almost disappeared at the end of the incubation (Wang *et al*. 2007). When Cd (10 mg kg^{-1}) was combined with butachlor (50 and 100 mg kg^{-1}), the activity of phosphatase became lower than without combination at early incubation time, which indicated that the toxicity of Cd significantly increased ($P < 0.05$ or < 0.01). However, when Cd (10 mg kg^{-1}) was combined with butachlor (10 mg kg^{-1}), the activity of phosphatase became higher than those without combination at the end of the incubation, which indicated that the toxicity of Cd decreased. Piotrowska-Seget *et al*. (2008) noticed that the acid and alkaline phosphatase activities were significantly reduced by soil treatment with captan. Obviously, phosphatase activity is instability in the beginning of 2 to 4 weeks incubation along with decomposing of mixed pesticide of deltramethrin and probineb in soil, and decline to last part of 12 weeks incubation (Rahmansyah *et al*. 2009). Wang *et al*. (2009) showed that the phosphatase activity in copper concentrations of orchard soils significantly increased with increasing orchard ages ranging from 21.8 to 141 mg kg^{-1}, and the $CaCl_2$-extractable soil Cu concentrations varied from 0.00 to 4.26 mg kg^{-1}. The soil mean C_{mic} values varied from 43.6 to 116 mg kg^{-1} in the orchard soils, and were lower than the value of the reference soil (144 mg kg^{-1}). The ratio of soil C_{mic} to total organic C (Corg) increased from 8.10 to 18.3 mg C_{mic} g^{-1} Corg with decreasing orchard ages, and was 26.1 mg C_{mic} g^{-1} Corg for the reference soil. A significant correlation was observed between total- or $CaCl_2$-extractable soil Cu and soil C_{mic} or C_{mic} /Corg, suggesting that the soil Cu was responsible for the significant reductions in C_{mic} and C_{mic} /Corg. On the other hand Mariusz Cycoń *et al*. (2010) reported that the acid and alkaline phosphatase was more sensitive to mancozeb + dimethomorph at 1500 $mgkg^{-1}$ and its activity declined in both loamy soils and sandy loam soils. However, mancozeb, endosulfan and chloripyifos at 100 mg/kg inhibited 50% activity of phosphatase (Sharma *et al.* (2010). Similarly, Suryakalyani *et al*. (2010) observed that acid phosphatase activity 1.8 times, respectively by the 14th day of incubation with 1 ppm endosulfan.

CONCLUSION

It can be concluded that the application of the pesticides in combinations profenofos + cypermethrin, deltamethrin + endosulfan or in individual applications of profenofos, deltamethrin, difenoconazole, thiram to soils increased the enzyme activity up to 5.0 kg ha^{-1} and decreased the activity when increased pesticide concentration in both soils. Stimulation and pronounced activity of phosphatase by selective pesticides was at 20 day period of incubation. Prolonged incubation up to (40 days) of pesticides treated soils on the enzyme activity showed no effect. These results of the present study clearly indicate that these pesticides in combinations and their individuals widely used in the cultivation of groundnut, at field application rates, enhance the activity of phosphatase.

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