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Effect of Pesticides on Microbial Diversity and Urease in Groundnut (*Arachis hypogaea* L.) Soil

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ABSTRACT

The influence of four pesticides viz., profenofos, deltamethrin, thiram and difenoconazole and two insecticide combinations viz., profenofos + cypermethrin and deltamethrin + endosulfan at 0.0, 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} were assessed for their effects on the activity of urease (measured in terms of hydrolysis of urea by sodium hypochlorite method) and microbial populations like bacteria, fungi and actinomycetes in two agricultural soils, collected from a fallow groundnut (*Arachis hypogaea* L.) fields of Anantapur district. The effects of selected pesticides profenofos, deltamethrin, thiram and difenoconazole and profenofos + cypermethrin and deltamethrin + endosulfan on microbial population and urease activity were dose dependent. Urease activity and microbial populations increased with increasing concentrations of the pesticides up to 5.0 kg ha^{-1}. Higher rates (7.5, 10.0 kg ha^{-1}) of these pesticides were either toxic or innocuous to the urease activity and microbial population. The significant stimulation in the activity of urease was associated with 2.5 kg ha^{-1} of pesticides in black soil, where as in red soil it was 5.0 kg ha^{-1} of profenofos, 2.5 kg ha^{-1} of deltamethrin, thiram, difeneconozole. In the insecticide combinations urease activity was 5.0 kg ha^{-1} profenofos + cypermethrin, 5.0 kg ha^{-1} of deltamethrin + endosulfan in black clay soils whereas in red sandy clay soils, population of microorganisms increased with 2.5 to 5.0 kg ha^{-1} of pesticides. With further incubation the activity of urease was significantly more at day 20 and enzyme activity decreased progressively with increasing incubation period.

Keywords: deltamethrin, difenoconazole, insecticide combinations, microbial populations, profenofos, thiram

INTRODUCTION

Pesticides in recent years are widely used in modern agriculture to control various insect pest populations and with the growing use of these pesticides in agricultural soils, there may be an interaction with soil organisms and their metabolic activities (Baxter and Cummings 2008). Therefore the behaviour of the total micro flora and their biological activities under continue pesticide input is an important aspect of research of the agricultural ecology (Li *et al*. 2008). The testing programs included soil enzymes as good indicators of soil biological fertility because of their participation in the decomposition of organic matter (Qian *et al*. 2007; Swaminathan *et al.* 2009). Microflora of soils are of major concern because of their role in sustaining agricultural productivity through various biochemical reactions mediated by soil enzymes (Mahía *et al*. 2008; Madakka *et al*. 2009). Additionally, soil microbes serve as principal agents of pesticide cleavage and modification of these compounds (White *et al*. 2010). In the majority of studies for testing side-effects of pesticides on non-target organisms and enzyme activities, the addition of a single insecticide was made to soil systems (Vijay Gundi *et al*. 2007). 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 (Surya Kalyani *et al.* 2010). Consequently, different pesticides may often exist together in the soil ecosystem at a given point of time. According to Schuster and Schroder (1990) and Srinivasulu *et al.* (2010), the effect of various combinations of pesticides may deviate from the behaviour of an individual pesticide because of the occurrence of synergistic, antagonistic or additive interactions between different pesticides. 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 (Gundi *et al*. 2007; Tejada 2009). Soil enzymes are remarkable biomolecules that show extraordinary specificity in catalyzing biological reactions, important for both soil microorganisms and plants (Kumar and Philip 2006; Hussain *et al*. 2007a, 2007b; Srinivasulu *et al*. 2010). Further, they act as important indices of soil fertility (Pascual *et al.* 2000). The extensive application of pesticides leads to interference with the normal enzymatic activity of proliferating soil microorganisms, and disturbing the delicate balance of soil ecosystem (Shushkova *et al*. 2010). Adverse impacts of pesticides on soil microbial diversity and activities have been described by many researchers (Littlefield-Wyer *et al*. 2008; Singh *et al*. 2008; Dutta *et al*. 2010). In spite of the maximum potential of soil enzymes in maintaining soil biodynamics, only limited studies were available (Mahía *et al*. 2008; Kalyani *et al*. 2009) on influence of soil enzymes with organochemicals. However, no literature was found regarding the interaction of the present selected pesticides viz., profenofos, deltamethrin, thiram, difenoconazole and insecticide combinations profenofos + cypermethrin, deltamethrin + endosulfan on enzymatic activities of soils. 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 common practice (Jayashree and Vasudevan 2007; Vijay Gundi *et al*. 2007; Romeh *et al*. 2009). Hence, an attempt was made in this study to find out the interaction effects of these pesticides on urease which is in-

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

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

* wettable powder ** emulsifying concentration

volved in the nitrogen cycle and microbial populations, in two soils of agricultural importance.

The present paper reveals that the effect of pesticides on urease activity and microflora in groundnut soils.

MATERIALS AND METHODS

Soils

Samples of black clay soil and red sandy clay soils, collected from groundnut cultivated fields of Anantapur district in a semi–arid zone of Andhrapradesh, India, to a depth of 12 cm, were air–dried and sieved through a 2 mm mesh screen before use. These soil samples were chosen because of agricultural importance as farmers are growing two crops per year in these fields.

Pesticides

The influence of two fungicides, thiram (75% WP, wettable powder)(carbamate), difenoconazole (triazole) (25% EC, emulsifying concentration), two insecticides, deltamethrin (2.8% EC) (synthetic pyrethroid) and profenofos (50% EC)(organophosphate), and two insecticide combinations deltamethrin (2.8% EC) + endosulfan (35% EC), profenofos (50% EC) + cypermethrin (25% EC) was determined in the present study. Commercial formulations dissolved in distilled water of the tested pesticides were used for determining the microbial activities like soil enzymes. Details of the pesticides used in the investigation are furnished in **Table 1**.

Soil incubation

The soil ecosystem stimulating non-flooded conditions consisting of 10-g 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 Jaya Madhuri and Rangaswamy (2003).

Assay of urease

Soil urease (E.C. 3.5.1.5) activity was based on the hydrolysis of urea. The assay of urease enzyme activity was determined using the method of Fawcett and Scott (1960) and adopted by Malkomes (2001).

At desired intervals (i.e., 10, 20, 30 and 40 days), 1 ml of 3% urea (urease substrate) and 2 ml of 0.1 M phosphate buffer (pH 7.1) were added to one gram portion of soil samples and incubated for 30 min at 37°C in a water bath shaker. Then the tubes were placed in ice until ammonia was extracted with 10 ml of 2 M KCl and filtered through Whatman filter paper No.1. To 4 ml of the filtrate, 5 ml of phenol sodium nitroprusside solution and 3 ml of 0.03 M sodium hypochlorite solution were added. Mixture was shaken, and kept aside for 30 min in the dark, and the developed blue colour was measured at 630 nm. Rate of urease enzyme activity was assayed at 10, 20, 30 and 40 days of soil incubation with the respective stimulatory concentrations of pesticides.

Enumeration of microbial population

The effect of different concentrations of selected pesticides, Profenofos, deltamethrin, thiram and difenoconazole and the insecticide combinations profenofos + cypermethrin and deltamethrin + Endosulfon on bacterial population in two different agricultural soil samples, in duplicate, were determined. Aliquots (0.05 ml) from stock solutions of the pesticides were applied to 5-g portions of soil contained in test tubes (15 × 150 mm). The final concentrations (w/w) of each pesticide included 10, 25, 50, 75 and 100 µg g^{-1} soil, which are equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} (Anderson 1978). The soil samples receiving only distilled water served as controls.

Soil samples were then homogenized to distribute the fungicide, and enough distilled water was added to maintain at 60% water holding capacity (WHC) and incubated at room temperature (28 ± 4°C). Seven days after incubation, duplicates of each treatment were withdrawn for estimation of bacterial population. Aliquots were prepared from 10^{-1} to 10^{-7} from treated and untreated soil samples by serial dilution plate method on nutrient agar medium and subsequently incubated for 24 h in an incubator at 30°C (Shukla and Mishra 1997).

After incubation, bacterial colonies grown on nutrient agar medium were counted by Quebec colony counter. Bacterial populations were enumerated and expressed as number of colonies formed g^{-1} of soil (dry weight basis) (Shetty and Magu 2000). Soil plate method was used to assess fungal propagules developing on rose Bengal agar medium and subsequently incubated for five days at 25°C (Shukla and Mishra 1997). The population of actinomycetes was estimated by using Ken Knight's agar medium and subsequently incubated for 3 days in the dark at 30°C (Balasubramanian and Sankaran 2001).

Statistical analysis

All data were expressed on an air-dry soil basis and were averages of two or three replicate determinations. The data of pesticidal effects on microbial populations and urease activity was analyzed for significant differences ($P \leq 0.05$) between pesticide treated and untreated soils using Duncan's multiple range test (Megharaj *et al.* 1999).

RESULTS AND DISCUSSION

Urease activity

Urea is an organic chemical used as a nitrogenous fertilizer in agriculture. Conversion of organic nitrogen to inorganic nitrogen through hydrolysis of urea to ammonia and carbon dioxide is due to activity of urease enzyme secreted by certain microorganisms and plants. This enzyme is responsible for supply of nitrogen demand to growing crop and also in the evaluation of changes in soil quality (Pascual *et al.* 1999; Chakrabarti *et al.* 2000), in the present study the impact of selected pesticides on the activity of urease in two soils was assessed. The data are presented in **Tables 2-5**. All tested individual pesticides in all concentrations appeared to be innocuous to urease activity in black and red soil up to 2.5 kg ha^{-1} except in case of profenofos in red soil, which was shown maximum activity at 5 kg ha^{-1} (**Tables 2, 3**). This enzyme activity was continued up to 20 days of incubation and then decline in urease activity was observed (**Table 4**) in all individual pesticides in both black and red soils at all concentrations. This observation was in similar with results reported earlier by Rangaswamy and Venkateswarlu (1992b). According to this study, monocrotofos, Quinalphos and cypermethrin also stimulated urease activity up to 25 ppm but were inhibitory to urease at concentrations > 25 ppm. According to Gianfreda *et al*. (1994), glyphosate enhanced urease activity of soils by 1.1-1.4-fold

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

Concentration of pesticide (Kg ha^{-1})	Profenofos	Deltamethrin	Thiram	Difenoconazole
0.0	134 ± 2.309 c (100)	134 ± 2.309 c (100)	134 ± 2.309 b (100)	134 ± 2.309 b (100)
1.0	136 ± 2.309 c (102)	138 ± 1.155 c (102)	136 ± 2.309 b (102)	134 ± 2.309 b (111)
2.5	156 ± 2.309 a (116)	160 ± 2.887 a (119)	146 ± 1.154 a (108)	141 ± 0.577 a (105)
5.0	140 ± 1.154 b (104)	150 ± 2.887 b (111)	141 ± 0.577 a (105)	138 ± 1.155 b (102)
7.5	124 ± 1.154 d (92)	126 ± 2.309 d (94)	130 ± 2.886 b (97)	124 ± 1.154 c (92)
10.0	108 ± 1.154 e (80)	112 ± 1.155 e (83)	104 ± 1.154 c (77)	108 ± 1.154 d (80)

*µg ammonia g^{-1} soil formed after 30 min incubation at 37°C with urea. 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 DMRT.

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

Concentration of pesticide (kg ha^{-1})	Profenofos	Deltamethrin	Thiram	Difenoconazole
0.0	84 ± 2.309 c (100)	84 ± 2.309 c (100)	84 ± 2.309 c (100)	84 ± 1.154 c (100)
1.0	94 ± 1.154 b (111)	88 ± 1.154 c (104)	88 ± 1.154 c (104)	86 ± 1.154 c (102)
2.5	98 ± 1.154 b (116)	114 ± 1.154 a (135)	110 ± 1.154 a (130)	120 ± 1.154 a (142)
5.0	104 ± 1.154 a (123)	102 ± 1.154 b (120)	100 ± 2.886 b (119)	100 ± 2.886 b (119)
7.5	82 ± 1.154 c (97)	78 ± 1.154 d (92)	84 ± 1.154 c (100)	80 ± 2.886 c (95)
10.0	64 ± 1.154 d (76)	62 ± 1.154 e (76)	62 ± 1.154 d (73)	60 ± 2.886 d (71)

*µg ammonia g^{-1} soil formed after 30 min incubation at 37°C with urea. 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 DMRT.

Table 4 Influence of selected pesticides on urease activity* in black and red soil.

Pesticide	Soil incubation (days)			
	10 days	20 days	30 days	40 days
Black soil				
Control	134 ± 1.154 d	144 ± 2.309 c	136 ± 1.732 d	120 ± 2.886 c
Profenofos (2.5 kg ha^{-1})	156 ± 1.154 b	164 ± 1.154 b	158 ± 5.733 a	140 ± 2.886 a
Deltamethrin (2.5 kg ha^{-1})	160 ± 1.154 a	170 ± 2.886 a	160 ± 5.733 a	140 ± 2.886 a
Thiram (2.5 kg ha^{-1})	146 ± 1.154 c	160 ± 2.886 b	150 ± 5.733 b	122 ± 1.154 c
Difenoconazole (2.5 kg ha^{-1})	142 ± 1.154 c	162 ± 1.154 b	146 ± 1.732 c	130 ± 2.886 b
Red soil				
Control	84 ± 1.154 d	100 ± 1.154 c	88 ± 1.154 d	62 ± 1.154 c
Profenofos (2.5 kg ha^{-1})	104 ± 2.309 c	124 ± 1.154 b	108 ± 1.154 c	90 ± 1.154 a
Deltamethrin (2.5 kg ha^{-1})	114 ± 1.154 b	128 ± 1.154 b	116 ± 1.154 b	82 ± 1.154 b
Thiram (2.5 kg ha^{-1})	110 ± 1.154 b	122 ± 1.154 b	108 ± 1.154 c	80 ± 1.154 b
Difenoconazole (2.5 kg ha^{-1})	120 ± 1.154 a	136 ± 1.154 a	122 ± 1.154 a	90 ± 1.154 a

*µg ammonia g^{-1} soil formed after 30 min incubation at 37°C with urea. 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 DMRT.

Table 5 Effect of different concentrations of selected insecticide combinations on urease activity* 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	45 ± 2.886 d (100)	45 ± 2.886 e (100)	31 ± 0.577 c (100)	31 ± 0.577 d (100)
1.0	55 ± 2.886 c (122)	58 ± 1.154 c (128)	45 ± 2.886 b (145)	46 ± 1.154 b (148)
2.5	61 ± 0.577 b (135)	62 ± 1.154 b (137)	50 ± 2.886 b (161)	52 ± 1.154 a (167)
5.0	82 ± 0.577 a (182)	75 ± 2.886 a (166)	64 ± 2.309 a (206)	42 ± 1.154 c (135)
7.5	51 ± 0.577 c (113)	58 ± 1.154 c (128)	36 ± 2.886 c (116)	30 ± 2.886 d (96)
10.0	39 ± 0.577 e (86)	50 ± 1.154 d (111)	25 ± 2.309 d (80)	24 ± 2.309 e (77)

*µg ammonia g^{-1} soil formed after 30 min incubation at 37°C with urea. 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 DMRT.

and of soil extracts by 2.59- to 6.73-fold at 0.3 and 1.5 mM but had no influence on free or immobilized jackbean urease. In contrast, thiram at 10 ppm decreased urease activity in both sandy and organic soils after 7 days (Tu 1990). Many researchers have revealed either unchanged, an increase or decrease in urease activity following the application of pesticides (Chen *et al*. 2001; Antonious 2003; Nowak *et al*. 2004; Ingram *et al*. 2005). Decreased urease activity in soil with the application of pesticides reduces urea hydrolysis which is generally beneficial, because it helps to maintain N in a form (NH_4^+) less leachable Antonious (2003). Yang *et al*. (2006) showed that chlorimuron ethyl and furadan activated urease in the four different soils. Both enhanced urease activity up to 14–18 and 13–21%, respectively. Contrarily, acetamiprid reduced up to 35% urease activity in soil at 43 days after sowing cotton (Singh and Kumar 2008). Srinivasulu *et al*. (2010) observed monocrotofos, chloropyrifos alone and in combination with mancozeb and carbendazim on urease activity in two agricultural soils of groundnut. Ingram *et al*. (2005) revealed that *Bacillus pasteurii* was unaffected by the insecticides diazinon and imidacloprid. Only diazinon significantly reduced urease activity in washed cells as well as in Maury soils (fine, mixed, semiactive, mesic Typic Paleudalf). The pyrethrins, neemix-4E inhibited urease activity (Antonious 2003). Increased soil urease was observed in sand and loam soils by the use of isoproturon (Nowak *et al*. 2004). Increased soil urease activity was observed with fungicide application, namely benomyl and captan (Chen *et al*. 2001). According to Srinivasulu *et al*. (2010), the rate of urea hydrolysis is more rapid in two different soils in which groundnut was grown and treated with the fungicides tridemorph and captan at 5.0 kg ha^{-1}.

Effect of pesticides and insecticides in combinations on soil microflora

1. Bacterial populations

The effect of profenofos, deltamethrin, thiram, difenoconazole and insecticide combinations, profenofos + cypermethrin and deltamethrin + endosulfan on bacterial popu-

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

Treatment	Soil incubation in days			
	10	20	30	40
Black soil				
Control	45 ± 2.886 c	64 ± 1.763 c	54 ± 1.154 b	32 ± 1.154 b
Profenofos + cypermethrin (5.0 kg ha^{-1})	82 ± 1.154 a	100 ± 1.154 a	57 ± 1.154 b	43 ± 1.732 a
deltamethrin + endosulfan (5.0 kg ha^{-1})	75 ± 2.886 b	82 ± 1.154 b	60 ± 1.154 a	40 ± 1.154 a
Red soil				
Control	31 ± 0.577 c	51 ± 0.577 c	37 ± 1.154 c	22 ± 1.154 c
Profenofos + cypermethrin (5.0 kg ha^{-1})	64 ± 2.309 a	80 ± 2.886 a	55 ± 2.886 a	43 ± 1.732 a
deltamethrin + endosulfan (5.0 kg ha^{-1})	52 ± 1.154 b	65 ± 2.886 b	45 ± 2.886 b	33 ± 1.732 b

*µg ammonia g^{-1} soil formed after 30 min incubation at 37°C with urea. 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 DMRT.

Table 7 Effect of selected pesticides, at varying concentrations, on population of bacteria (CFU × 10^6 g^{-1} dry soil). *

Pesticide concentration (kg ha^{-1})	Profenofos	Deltamethrin	Thiram	Difenoconazole
Black soil				
0.0	155 ± 2.886 e (100)	155 ± 2.886 e (100)	155 ± 2.886 d (100)	155 ± 2.886 d (100)
1.0	185 ± 2.886 d (119)	178 ± 1.154 d (114)	169 ± 0.577 c (109)	190 ± 2.886 c (122)
2.5	217 ± 1.15 4 b (140)	250 ± 2.886 a (161)	189 ± 0.557 b (121)	212 ± 1.155 b (136)
5.0	263 ± 1.732 a (169)	207 ± 1.155 b (133)	215 ± 8.660 a (138)	245 ± 8.660 a (158)
7.5	202 ± 1.154 c (130)	191 ± 0.577 c (123)	187 ± 1.154 b (120)	197 ± 1.154 c (127)
10.0	150 ± 2.886 e (96)	134 ± 1.155 f (86)	132 ± 1.154 e (85)	147 ± 1.154 e (94)
Red soil				
0.0	135 ± 2.886 d (100)	135 ± 2.886 e (100)	135 ± 2.886 d (100)	135 ± 2.886 d (100)
1.0	152 ± 1.154 c (112)	152 ± 1.154 d (105)	150 ± 2.886 c (107)	141 ± 0.577 c (104)
2.5	167 ± 1.154 b (123)	172 ± 1.154 b (127)	168 ± 1.154 b (124)	162 ± 1.154 b (120)
5.0	207 ± 1.154 a (153)	195 ± 2.886 a (144)	200 ± 2.886 a (148)	194 ± 1.154 a (143)
7.5	137 ± 1.154 d (101)	164 ± 1.154 c (121)	159 ± 2.886 c (117)	139 ± 0.577 c (102)
10.0	117 ± 1.154 e (86)	120 ± 2.886 f (88)	121 ± 0.577e (89)	103 ± 0.577 e (76)

* Number of colonies per gram soil = $\frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Dry weight of soil}}$

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 DMRT. CFU: Colony forming units.

lation was studied. Data on the effects of test compounds, profenofos, deltamethrin, thiram, difenoconazole and insecticide combinations such as profenofos + cypermethrin and deltamethrin + endosulfan on bacterial populations are summarized in **Table 7** and **Fig. 1**.

Bacterial populations were significantly higher in black soil treated with profenofos, deltamethrin, thiram and difenoconazole and the identical was happened in case of two insecticide combinations, profenofos + cypermethrin and deltamethrin + endosulfan at 1.0, 2.5, 5.0 and 7.5 kg ha^{-1} than in the untreated control, after 7 days of incubation (**Table 7; Fig. 1**). Bacterial population in black soil was enhanced with increasing concentrations (up to 7.5 kg ha^{-1}) of four pesticides and in the same was observed in case of insecticide combinations used in the present study. The escalating level of cell number in pesticides and insecticide combinations amended black soil at 1.0, 2.5, 5.0 and 7.5 kg ha^{-1} were 9-61% and 10-64% over control by the end of 7 days of incubation. As seen in the case of black soil the four pesticides and two insecticide combinations were individually stimulatory to the bacterial populations in the red soil too, at all tested concentrations (up to 7.5 kg ha^{-1}) as reflected by higher count of bacterial pollution in pesticide-treated soil than in soil without pesticides (**Table 7; Fig. 1**). Individual stimulatory effect of monocrotofos, quinalfos and cypermethrin at 5.0 kg ha^{-1} has been confirmed on nitrifiers, nitrogen-fixing organisms and the population of *Azospirillum* sp. in soils of groundnut fields (Rangaswamy 1990) and similar observations were observed when pesticides profenofos, deltamethrin, difenoconazole, thiram and their combinations viz., profenofos + cypermethrin and deltamethrin + endosulfan on the populations of *Azospirillum* sp. in groundnut soils (Madakka and Rangaswamy 2009). In Chinese loamy soils, methamidophos at 0.5, 2.5, 5 and 10 µg g^{-1} inhibited the population of bacteria strongly throughout the incubation period (Xu *et al*. 1997). Chlorpyrifos at 10-300 µg g^{-1} decreased the population of bacteria in loamy soil (Martínez-Toledo *et al*. 1992b) whereas profenofos at the same levels increased the populations of

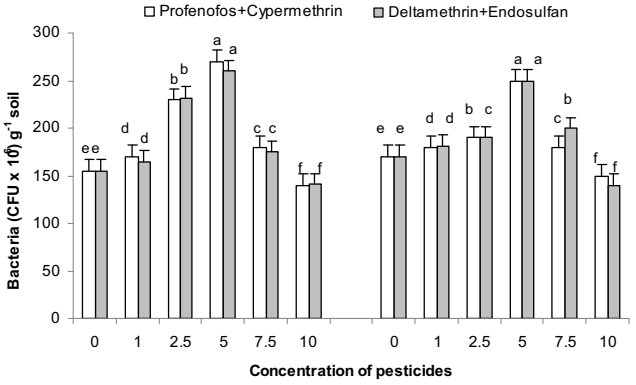

Fig. 1 Effect of insecticide combinations at varying concentrations, on population of bacteria (colony-forming units (CFU) × 10^{-6} g^{-1} dry soil) in black and red soils.

bacteria (Martínez-Toledo *et al*. 1992a). No significant change in total viable count of bacteria was observed when treated with phorate, carbofuran, carbosulfan, thiamethoxam, imidacloprid, chlorpyrifos, monocrotofos both at high and low concentrations (Sarnaik *et al*. 2006). Adversely-affected *Rhizobium* sp. populations were found after the application of any concentration of the herbicides atrazine, isoproturon, metribuzin, and sulfosulfuron in soils in which chickpea was grown (Khan *et al*. 2006). Wang *et al*. (2006) concluded that the effect of methamidophos and urea reduced microbial biomass and enhanced functional diversities of soil microbial communities, i.e., some species of bacteria might be enriched in soils under methamidophos stress. Similar observations were found by Demanou *et al*. (2006), who investigated the effect of a combined application of copper and mefenoxam on the functional diversity of soil and found that microbial populations increased. In another study, benzene and heavy metals reduced the number or diversity of bands in bacterial DGGE gels, indi-

cating toxicity responses (Girvan *et al*. 2005). Sáez *et al*. (2006) observed the effect of some pesticides on growth and denitrifying activity of *Xanthobacter autotrophicus* CECT 7064. Chen *et al*. (2007) and Lin *et al*. (2007) who investigated the associated impact of inorganic fertilizers, heavy metals, and pesticides on microbial com-munities in soils. Similarly, Madhaiyan *et al*. (2006) who studied the effect of various pesticides on the growth and survival of *Gluconacetobacter diazotrophicus* strain PAL5. The monocrotofos, lindane and dichlorvos proved lethal to *Gluconacetobacter*, while in case of endosulfan, chlorpyrifos, and malathion effects were intermediate. The influence of profenofos, deltamethrin, thiram, difenoconazole and insecticide combinations such as profenofos + cypermethrin and deltamethrin + endosulfan at different levels on the number of bacteria in both soils was assessed (**Table 7; Fig. 1**). The interaction responses are usually illustrious on the basis of the percent stimulation values (over control) regarding any parameter in soil treated with combination of insecticides or a single pesticide at specified dose in soil. In case, the per cent stimulation in parameter of interest by combination of two different insecticides at a particular dose is comparable to the sum of per cent stimulation in the same parameter shown by respective single insecticides at corresponding dose, an interaction between two insecticides is considered as an additive type. Percent stimulation observed for a combination of insecticides is significantly greater or less than the sum of individual effects of insecticides observed at respective concentrations, indicating synergistic or antagonistic interactions, respectively.

The interaction between different agrochemicals in combination on bacterial populations in soils received least attention in comparison to effects of a single agrochemical. There were no differences in the degree of diversity in bacterial populations by application of combination of 5 pesticides including chlorfenvinfos and glyphosate to a field plot for 20 years (Nicholson and Hirsch 1998). In this study, dominant strains in bacterial populations of treated and untreated plots were not same. Furthermore, higher count of cultured bacteria in treated plots was recorded. In all these studies, a variety of interaction effects such as synergistic, additive and antagonistic observed were dependent on the concentration of the interacting chemical. For instance, the combination of different pesticides, monocrotofos and cypermethrin or fenvalerate, at a lower level (5 µg ml^{-1}) yielded an additive or synergistic interaction response to inhibition of growth of *Scenedesmus bijugatus* in pure culture studies whereas the same combination at a higher level (25 µg ml^{-1}) exerted an antagonistic interaction with same test system (Megharaj *et al*. 1989). Antagonistic interactions were predominant in interaction studies with the same pesticides on another photosynthetic organism, *Synechococcus elongatus*. However, results from interaction studies with another combination, quinalphos and cypermethrin/fenvalerate were not clear because of high toxicity of quinalphos alone at an even lower level (5 µg ml^{-1}) to the growth of *S. bijugatus*. In another study, using a two-component mixture on a cyanobacterium, *Anabaena inaequalis*, all combinations containing permethrin or its degradation product, 3-phenoxybenzaldehyde interacted in an additive manner on toxicity towards photosynthesis whereas antagonistic interactions were predominant with any combination containing 3-phenoxybenzoic acid on inhibition of growth (Stratton and Corke 1982a). Correspondingly, consistent interactions took place between individual pesticides and insecticide combinations on bacterial populations in both soils in the present study. Gundi *et al*. (2007) investigated that such diverse effects of insecticides on two enzyme activities was in concomitant to populations of cellulolytic and amylolytic microbes in soils treated with insecticides and their combinations. Niewiadomska (2004) and Niewiadomska and Klama (2005) reported the adverse effects of carbendazime, thiram (fungicides), and imazetapir (herbicide) on nitrogenase activity of *Rhizobium leguminosarum*, *Sinorhizobium meliloti* and *Bradyrhizobium* sp. Wang *et al*. (2007) demonstrated that the addition of a high concentration of butachlor applied in combination with Cd significantly affected the diversity of microbial community.

2. Fungal populations

Profenofos, deltamethrin, thiram and difenoconazole and two insecticide combinations such as profenofos + cypermethrin and deltamethrin + endosulfan were tested for their effects on fungal populations in two soils. The data obtained from these experiments were furnished in **Tables 8** and **9**.

Fungal populations in both soils increased with increasing concentrations (up to 5 kg ha^{-1}) of all the tested pesticides and insecticide combinations. Stimulation in fungal populations in the range of 12-62% by profenofos, deltamethrin, thiram, difenaconazole and 11-55% by profeno-fos + cypermethrin and deltamethrin + endosulfon at all three levels i.e., 10, 25 and 50 ppm for 5 days incubation in black soil occurred. About 16-75% by individual pesticides and 13-66% by insecticide combinations increase in fungal flora was observed in red soil (**Tables 8, 9**). In contrast, phorate considerably stimulated a population of fungi in soil than fenvalerate under laboratory conditions (Das and Mukherjee 1998a). In their study, both insecticides affected fungal composition and diversity in soil by stimulating relative proportion of *Penicillium* and reducing *Rhizophus*. In a similar study, even under field conditions, fenvalerate exerted stimulatory effect on fungal populations (Das *et al*. 1995). Sigler and Turco (2002) revealed that chlorothalonil removed a number of bands from the fungus community DGGE profile of agricultural and turfgrass soils 2 weeks

Table 8 Effect of selected pesticides, at varying concentrations, on population of fungi (CFU × 10^5 g^{-1} dry soil).*

Pesticide concentration (kg ha^{-1})	Profenofos	Deltamethrin	Thiram	Difenoconazole
Black soil				
0.0	16 ± 1.154 b (100)	16 ± 1.154 b (100)	16 ± 1.154 c (100)	16 ± 1.154 c (100)
1.0	18 ± 1.154 b (112)	19 ± 0.577 a (118)	19 ± 0.577 b (118)	18 ± 1.154 b (112)
2.5	24 ± 1.154 a (150)	26 ± 1.154 a (162)	25 ± 2.886 a (156)	22 ± 1.154 a (137)
5.0	20 ± 1.154 a (125)	20 ± 1.154 a (125)	18 ± 1.154 b (112)	19 ± 0.577 b (118)
7.5	15 ± 2.886 b (93)	15 ± 0.577 b (93)	13 ± 1.732 c (81)	15 ± 0.577 c (93)
10.0	10 ± 1.154 c (62)	11 ± 0.577 c (68)	10 ± 1.154 d (62)	9 ± 0.577 d (56)
Red soil				
0.0	12 ± 1.154 b (100)	12 ± 1.154 c (100)	12 ± 1.154 c (100)	12 ± 1.154 c (100)
1.0	15 ± 1.154 b (125)	14 ± 1.154 b (116)	15 ± 0.577 b (125)	15 ± 0.577 b (125)
2.5	21 ± 0.577 a (175)	19 ± 0.577 a (158)	20 ± 1.154 a (166)	21 ± 0.577 a (175)
5.0	18 ± 1.154 a (150)	16 ± 1.154 b (133)	16 ± 1.154 b (133)	17 ± 0.577 b (141)
7.5	10 ± 1.154 c (63)	12 ± 1.154 c (100)	11 ± 0.577 c (91)	14 ± 0.577 b (116)
10.0	7 ± 0.577 d (58)	6 ± 1.154 d (50)	8 ± 0.577 d (66)	7 ± 0.577 d (58)

* Number of colonies per gram soil = $\frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Dry weight of soil}}$

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 DMRT. CFU: Colony forming units.

Table 9 Effect of insecticide combinations, at varying concentrations, on population of fungi (CFU × 10^5 g^{-1} dry soil).*

Concentration of insecticide combinations (kg ha^{-1})	Profenofos + cypermethrin	Deltamehtrin + endosulfan
Black soil		
0.0	18 ± 1.154 d (100)	18 ± 1.154 c (100)
1.0	20 ± 1.154 c (111)	21 ± 0.577 b (116)
2.5	24 ± 1.154 b (133)	24 ± 1.154 b (133)
5.0	27 ± 0.577 a (150)	28 ± 1.154 a (155)
7.5	16 ± 1.154 d (88)	16 ± 1.154 c (88)
10.0	11 ± 0.577 e (61)	12 ± 1.154 d (66)
Red soil		
0.0	15 ± 0.577 c (100)	15 ± 0.577 d (100)
1.0	17 ± 0.577 b (113)	18 ± 1.154 c (120)
2.5	19 ± 0.577 b (126)	20 ± 1.154 b (133)
5.0	23 ± 1.732 a (153)	25 ± 0.577 a (166)
7.5	22 ± 1.154 a (146)	14 ± 0.577 d (93)
10.0	10 ± 1.154 d (66)	9 ± 0.577 e (60)

* Number of colonies per gram soil = $\frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Dry weight of soil}}$

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 DMRT. CFU: Colony forming units.

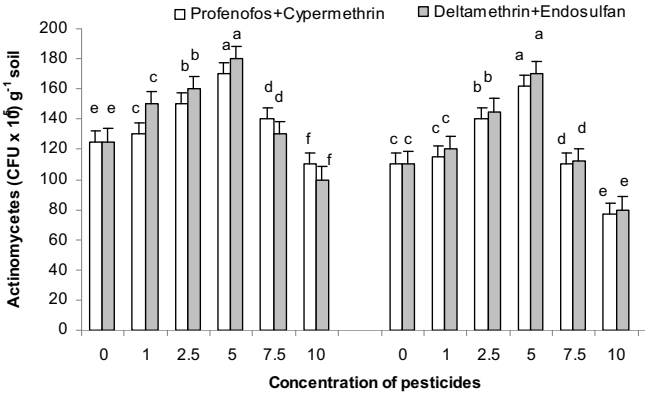

Fig. 2 Effect of insecticide concentration on population of actinomycetes (colony-forming units (CFU) × 10^{-5} g^{-1}dry soil) in black and red soil.

after application.

Like in bacterial populations, profenofos, deltamethrin, thiram and difenoconazole and the tested two insecticide combinations such as profenofos + cypermethrin and deltamethrin + endosulfan interacted differently and yielded synergistic, additive and antagonistic interactions towards fungal populations in both soils (**Table 8, 9**). According to studies of Houseworth and Tweedy (1973), additive responses occurred when atrazine was applied to the soil in combination with the fungicide, captan or thiram at 10 ppm towards the population of fungi. On the other hand, application of another fungicide, benomyl in combination with the thiram to a raw mulch soil as a dust significantly decreased the population of total fungi and *Fusarium* sp. over control (Ferris and Mitchell 1981). Permethrin and its degradation products in combination interacted to yield antagonistic, additive and synergistic interaction towards the growth of fungi in pure culture studies (Stratton and Corke 1982a). Similarly, a combination of DDT + parathion + zineb enhanced fungal population in soils when applied at field rates (Hubbel *et al*. 1973). According to Nowak *et al.* (2004), isoproturon decreased population of actinomycetes and fungi. Gundi *et al*. (2005) studied the effect of three insecticides (monochrotophos, quinalphos, and cypermethrin) on microbial populations in a black clay soil. They observed synergistic effects at the lower level and adverse effects at the highest level of the insecticides.

In the present study, considerable information was obtained regarding interaction effects between pesticides towards fungal populations in soils. Synergistic/additive interactions between insecticides in combinations at lowest concentration were predominant towards fungal population in soils whereas antagonistic response mostly figured in soils with combination of insecticides at the highest level in the present study.

3. Actinomycete populations

Profenofos, deltamethrin, thiram and difenoconazole and two insecticide combinations such as profenofos + cypermethrin and deltamethrin + endosulfan were tested for their effects on actinomycetes populations in two soils as described. The data obtained from these experiments, furnished in **Table 10** and **Fig. 2**, reveal the impact of different concentrations of pesticides profenofos, deltamethrin, thiram, difenoconazole and the tested insecticide combinations profenofos + cypermethrin and deltamethrin + endosulphan on actinomycetes population in black and red soil after 7 days of soil incubation. Concentrations of the four pesticides and the tested insecticide combinations up to 7.5 kg ha^{-1} significantly increased the population of actinomycetes. The increase in cell number of actinomycetes was 4-47% in black soil, whereas in red soil, it was 14-59%. However, in the case of insecticide combination applications to the soil samples, the increase cell number of actinomycetes was 3-40% in black soil and 9-54% in red soil. Even though the actinomycetes population was increased up to 7.5 kg ha^{-1}, the stimulation was more pronounced at the 5.0 kg ha^{-1} of profenofos, deltamethrin, thiram, difenoconazole and insecticide combinations profenofos + cypermethrin and delta-

Table 10 Effect of selected pesticides, at varying concentrations, on population of actinomycetes (CFU × 10^5 g^{-1} dry soil).*

Pesticide concentration (kg ha^{-1})	Profenofos	Deltamethrin	Thiram	Difenoconazole
Black soil				
0.0	115 ± 2.886 d (100)	115 ± 2.886 d (100)	115 ± 2.886 d (100)	115 ± 2.886 d (100)
1.0	129 ± 0.577 c (112)	120 ± 2.886 c (104)	127 ± 1.154 c (110)	124 ± 2.309 c (107)
2.5	144 ± 1.154 b (125)	140 ± 2.886 b (121)	146 ± 1.154 b (126)	137 ± 1.154 b (119)
5.0	169 ± 0.577 a (146)	164 ± 2.309 a (142)	165 ± 2.886 a (143)	170 ± 2.886 a (147)
7.5	127 ± 1.154 c (110)	120 ± 2.886 c (104)	115 ± 2.886 d (100)	118 ± 2.886 d (156)
10.0	105 ± 2.886 e (91)	95 ± 2.886 e (82)	87 ± 1.154 e (75)	96 ± 2.309 e (83)
Red soil				
0.0	96 ± 1.154 e (100)	96 ± 1.154 e (100)	96 ± 1.154 e (100)	96 ± 1.154 e (100)
1.0	112 ± 1.154 d (116)	121 ± 0.577 c (126)	112 ± 1.154 c (116)	110 ± 2.886 c (114)
2.5	127 ± 1.154 b (132)	135 ± 2.886 b (140)	123 ± 1.732 b (128)	127 ± 1.154 b (132)
5.0	142 ± 1.154 a (147)	151 ± 0.577 a (157)	143 ± 1.732 a (148)	153 ± 1.732 a (159)
7.5	119 ± 2.309 c (123)	110 ± 2.886 d (114)	107 ± 1.154 d (111)	104 ± 2.309 d (108)
10.0	87 ± 1.154 f (90)	86 ± 1.154 f (89)	89 ± 0.577 f (92)	92 ± 1.154 e (95)

* Number of colonies per gram soil = $\frac{\text{No. of colonies} \times \text{Dilution factor}}{\text{Dry weight of soil}}$

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 DMRT. CFU: Colony forming units.

methrin + endosulfan in black and red soil. Significant stimulation of actinomycetes population was more in black soil than the red soil (**Table 10; Fig. 2**).

Shetty and Magu (2000) reported that metalaxyl at 0.5 ppm, incubated for 4 and 8 weeks significantly stimulated the actinomycete population in a sandy loam soil. Current findings revealed that actinomycete population was inhibited at 10 kg ha^{-1} of the selected pesticides and the used insecticide combination application in both soils. The inhibitory effect was more distinct in red soil compared to black soil (**Table 10; Fig. 2**). Gundi *et al*. (2005) studied the effect of three insecticides (monochrotophos, quinalphos, and cypermethrin) on microbial populations in a black clay soil. They observed synergistic effects at the lower level and adverse effects at the highest level of the insecticides. In contrary, toxic effects of pesticides (captan, deltameth-rin, isoproturon, and pirimicarb) were observed on freshwater sediment microbial communities even at concentrations predicted to be environmentally safe (Widenfalk *et al.* 2004). Wang *et al*. (2007) investigated the combined effect of cadmium (Cd) and butachlor on microbial activity. They demonstrated that the addition of high concentration of butachlor applied in combination with Cd significantly affected the diversity of microbial community. Almost similar results were reported by Chen *et al*. (2007) and Lin *et al*. (2007) who investigated the associated impact of inorganic fertilizers, heavy metals, and pesticides on microbial communities in soils.

CONCLUSION

This study has shown that the variation in soil microbial activity reflects the capacity of microorganisms to respond to inputs of pesticides in combinations profenofos + cypermethrin, deltamethrin + endosulfan or in individual applications of profenofos, deltamethrin, difenoconazole, thiram to soils increased the enzyme activity and microbial populations up to 5.0 kg ha^{-1} and decreased the activity when increased pesticide concentration in both soils. Stimulation and pronounced activity of urease 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. Individual application of pesticides the maximum observed increment of urease activity in two soils was up to 42%, where as in combinations of pesticides it was up to 82%. The results of the present study clearly indicate that these pesticides in combinations and their individuals at field application rates, enhance the activity of urease and microbial populations in soils.

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