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Research Article

Impact of pesticides alone and in combinations on bacterial and fungal populations in soils

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Abstract

The impact of selected pesticides, monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim, respectively, were assessed on bacterial and fungal populations in vertisol and laterite soils collected from fallow groundnut (*Arachis hypogaea* L) field of Anantapur district, Andhra Pradesh, India. The impact of pesticides on bacterial and fungal populations was dose dependent; monocrotophos alone and in combination with mancozeb showed the maximum stimulation in bacterial populations at 5.0 kg ha^{-1}, whereas chlorpyrifos with carbendazim showed maximum enhancement at 2.5 kg ha^{-1} level in both soils. Monocrotophos and chlorpyrifos alone at 5.0 and 2.5 kg ha^{-1} respectively, showed significant enhancement in fungal population in both vertisol and laterite soils. However, higher doses (7.5 and 10 kg ha^{-1}) of the pesticides were either innocuous or toxic to the bacterial and fungal populations. The highest stimulation in bacterial and fungal population was observed at 20-day incubation.

Keywords: Insecticides; Fungicides; Combinations; Bacterial and fungal populations.

Introduction

Soil bacteria occupy a key position in the global cycling of carbon and other elements because of their abundance in range of 10^6 and 10^9 bacteria per gram of soil and diverse metabolic capabilities to exploit many sources of energy and carbon [1]. The population of different groups of bacteria like autotrophs, heterotrophs, N_2-fixers, cellulose degraders, thermophiles, psychrophiles etc., found in soil are generally altered by pesticide application. Insecticides alone and combination with fungicides usually applied to control insects and pathogenic fungi in groundnut crop and maintain high crop production in Indian agriculture [2,3]. Most of the studies on fungicide or insecticide effects indicate that when they are applied at recommended rates, they usually have no significant effects or have transitory effects on soil microbial characteristics [4-6]. Measuring bacterial community structure revealed shifts due to fungicide and insecticide application [7]. Pesticides like fonofos, trichloronate, chlorfenvinphos, fensulfothion, parathion, chlorpyrifos and diazinon (organophosphorus insecticides) were innocuous to bacteria in an organic soil and a sandy loam soil [8]. Chlorpyrifos at 10-300 µg g^{-1} decreased the total number of bacteria in loamy soil whereas profenofos at the same level increased the total number of bacteria [9]. Fungi are usually abundant in the upper layer of the soil, where aerobic conditions prevail. Although numerically much less abundant (between 10^4 and 10^6 fungal propagules per gram soil) than bacteria, fungi are the major contributors to soil biomass and can account for as much as 70% by weight of the biomass [1].

Almost there are no reports available on the influence of insecticide, fungicide combinations on bacterial and fungal populations in soil. However, very recently, the effect of insecticide and fungicide combinations on *Azospirillum* sp. population and enzyme activities in soil was reported [2,10]. Therefore the present study was carried out to determine the influence of insecticides alone and in combination with fungicides on bacterial and fungal populations.

Materials and Methods

Soils

A vertisol soil (pH 8.6; organic matter 1.48 %; total nitrogen 0.091 %; EC 265 (m.mhos); sand 68.3 %; silt 22.7 %; clay 19.0 %) and a laterite soil (pH 8.0; organic matter content 0.76 %; total nitrogen 0.052 %; EC 247 (m.mhos); sand 53.3 %; silt 27.1 %; clay 19.6 %) were collected from groundnut cultivated fields, to a depth of 12 cm, air dried and sieved through a 2-mm sieve before use [2].

Pesticides

To determine the effect of pesticides on bacterial and fungal populations, monocrotophos (36 % EC), and chlorpyrifos (20 % EC) alone and in combination with mancozeb (75 % WP) and carbendazim (50 % WP), respectively, were selected in the present study. For the incubation studies, commercial formulations of tested pesticides dissolved in distilled water were used.

Experimental design

For determination of effect of monocrotophos, chlorpyrifos singly (10, 25, 50, 75, 100 µg g^{-1} soil) and in combinations i.e., monocrotophos + mancozeb and chlorpyrifos + carbendazim with equal concentration (5 + 5, 12.5 + 12.5, 25 + 25, 37.5 + 37.5, 50 + 50 µg g^{-1} soil) on the population of bacteria five gram portions of each soil were placed in 15 × 150 mm test tubes and were treated with different concentrations of pesticides, which were equivalent to 1, 2.5, 5, 7.5 and 10 kg ha^{-1}. The soil samples receiving only distilled water served as controls. Soil samples were then homogenized to distribute the pesticide, and sufficientdistilled water was added to maintain at 60% water holding capacity (WHC) and incubated at room temperature (28 ± 4^{o}C). After 10 days of incubation, triplicates of each treatment were withdrawn for the 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 (peptone 5g; beef extract 3g; agar 20g; distilled water 1litre and pH 7.2) and subsequently incubated for 48 hours in an incubator at 37^{o}C. 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 per gram of soil (dry weight basis) [11].

The impact of different concentrations of the selected pesticides alone and in combination on fungal population in two different groundnut soils, in triplicates, were determined, following the similar procedure as in the case of bacterial population. Soil plate method was used to assess fungal propagules developing on Rose-bengal agar medium (peptone 5g; $MgSO_4$ 0.5g; Rose-bengal 30mg; KH_2PO_4 500mg; glucose 10g; agar 20g; streptomycin 30mg; distilled water 1 liter and pH 4.6) and subsequently incubated for five days at 25^{o}C [12]. Once the stimulatory concentrations of pesticides were determined, the soil samples were further incubated for 20, 30 and 40 days for enumeration of bacterial and fungal populations. All data, expressed on an air dry soil basis, were averages of three replicates. Data were analysed for significant differences ($P \leq 0.05$) between pesticide treated and untreated soils using Duncan's multiple range (DMR) test [13,2].

Results and Discussion

Impact of pesticides on bacterial populations

The bacterial populations were significantly enhanced throughout the incubation period in vertisol and laterite soils treated with, insecticides alone and in combination with fungicides. The bacterial cell number was increased in both individual and binary mixtures of pesticides treated soils up to 7.5 kg ha^{-1} than the controls in 10-day incubated soil samples. The bacterial populations continued up to 20 days and then gradually decreased after 30 and 40 days of incubation (Fig. 1, 2). Organophosphorus insecticide, monocrotophos either singly or in combination significantly improved the bacterial populations in 10-day incubated soil samples.

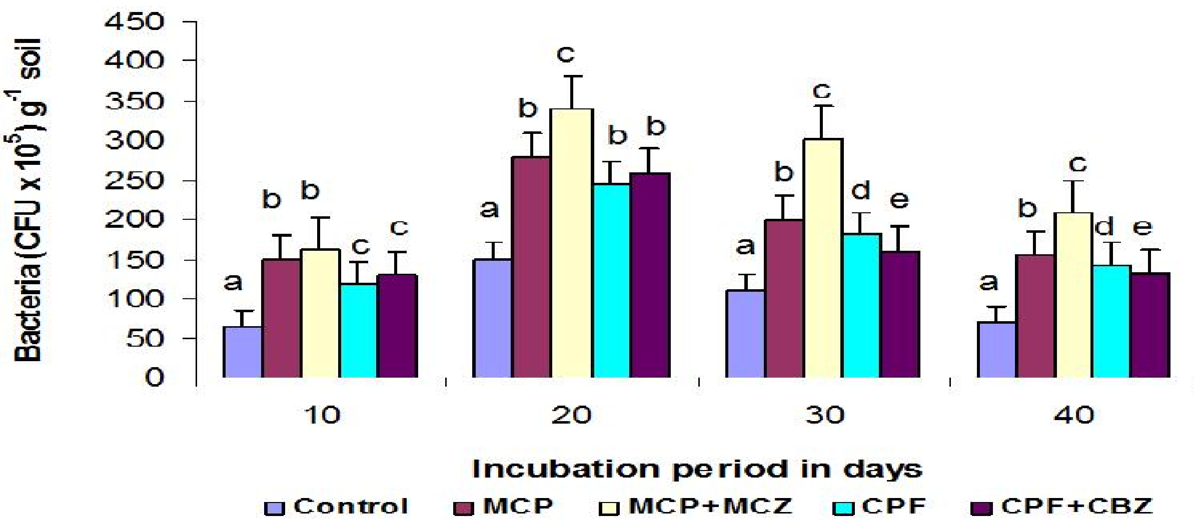

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Fig. 1 Influence of monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim respectively, at 2.5 or 5.0 kg ha^{-1} on the population of bacteria in vertisol soil. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figure are mean ± standard errors of three replicates.

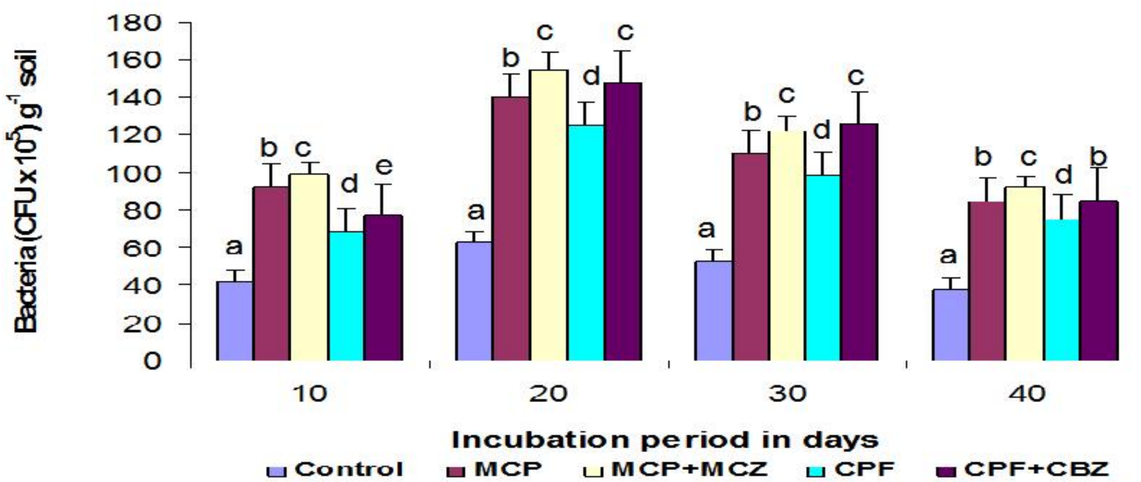

Fig. 2 Influence of monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim respectively, at 2.5 or 5.0 kg ha^{-1} on the population of bacteria in laterite soil. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figure are mean ± standard errors of three replicates.

Monocrotophos at concentrations ranging from 1.0 to 5.0 kg ha^{-1} gradually increased the population of bacteria and reached maximum at the concentration of 5.0 kg ha^{-1} indicates synergistic interaction, in both the soil samples. Similarly a stimulatory effect by an organophosphate insecticide, fenamiphos was observed towards the population of bacteria in soil [14]. Individual stimulatory effect of monocrotophos, quinalphos and cypermethrin has also been confirmed on nitrifiers, nitrogen-fixing organisms and the population of *Azospirillum* sp. in soils [15]. Both monocrotophos and quinalphos up to 20 µg ml^{-1} were either stimulatory or innocuous to culture yield, and photosynthetic pigments such as chlorophyll *a*, phycocyanin and carotenoids of *Anabaena torulosa* in pure culture studies [16]. Beyond 5.0 kg ha^{-1} monocrotophos showed negative effect on bacterial populations and showed minimum increase in cell number at 10.0 kg ha^{-1} (Tables 1, 2). At the end of 10-day incubation about 23-130% increase in the population of bacteria was observed in vertisol soil and 31-119% increase was observed in laterite soil treated with monocrotophos when compared to controls (Tables 1, 2).

Table 1. Impact of insecticides alone and in combination with fungicides on population of bacteria* (CFU × 10^5 g^{-1} soil) in vertisol soil after 10 days.

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + Mancozeb	Chlorpyrifos	Chlorpyrifos + Carbendazim
0.0	65a (100)	65a (100)	65a (100)	65a (100)
1.0	80b (123)	90b (138)	70a (107)	82b (126)
2.5	110c (169)	120b (184)	120b (184)	130c (200)
5.0	150d (230)	162c (249)	92c (141)	104d (160)
7.5	90e (138)	102d (157)	65a (100)	92e (141)
10.0	60a (92)	63fa (97)	52a (80)	58a (89)

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. Values in table represents means of three replicates.

Table 2. Impact of insecticides alone and in combination with fungicides on population of bacteria* (CFU × 10^5 g^{-1} soil) in laterite soil after 10 days.

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + Mancozeb	Chlorpyrifos	Chlorpyrifos + Carbendazim
0.0	42a (100)	42a (100)	42a (100)	42a (100)
1.0	55b (131)	58b (138)	44ab (104)	49b (116)
2.5	66c (157)	70c (166)	68c (161)	77c (183)
5.0	92d (219)	99d (235)	55d (130)	58d (138)
7.5	50b (119)	51b (121)	45ab (107)	36e (86)
10.0	40a (95)	38a (90)	32e (76)	29f (69)

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. Values in table represents means of three replicates.

Chlorpyrifos at the concentrations of 1.0 and 2.5 kg ha^{-1} showed marked increase in bacterial populations and beyond this concentration the bacterial populations reduced gradually and reached minimum at 10.0 kg ha^{-1} in both vertisol and laterite soils. Conversely, chlorpyrifos at 10-300 µg g^{-1} decreased the population of bacteria in loamy soil [17] whereas profenofos at the same levels increased the bacterial populations [9]. At the end of 10-day incubation about 7-84% increase in bacterial populations were observed in vertisol soil and 4-61% increase was observed in laterite soil treated with chlorpyrifos in comparison to control soil samples (Tables 1, 2).

The combination of monocrotophos with mancozeb and chlorpyrifos with carbendazim showed increase in bacterial populations at 1.0 and 2.5 or 5.0 kg ha^{-1} of each pesticide in both soils. But higher concentrations of pesticides at the level of 7.5 to 10 kg ha^{-1} shows inhibitory effect on the bacterial populations represents antagonistic interaction (Tables 1, 2). Similarly, the population of *Azosprilillum* sp. (nitrogen fixing bacteria) was significantly increased by the application of monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim respectively, at 2.5 or 5.0 kg ha^{-1} and the population was lowerd at higher dose (7.5 or 10.0 kg ha^{-1}) in ground nut soils [10]. In vertisol soil monocrotophos with mancozeb showed 38-149% increase and same combination in laterite soil showed 38-135% in bacterial populations at the end of 10 days incubation. The combination of chlorpyrifos and carbendazim showed 26-100% increase of bacterial populations in vertisol soil and in laterite soil the population of bacteria increased about 16-83% over controls (Tables 1 and 2). The biomass or population of bacteria increases because bacteria use the fungicide (during degradation) and fungal necromass (i.e., dead fungi) as carbon and nutrient sources for energy and growth. Insecticides seem to have similar effects to those of fungicides on soil fungi and bacteria [18-20].

Impact of pesticides on fungal populations

The fungal populations were increased in all individual and combinations of pesticides treated soils up to 5.0 kg ha^{-1} than the controls in 10-day incubated soil samples. The fungal populations continued up to 20 days and then gradually decreased after 30 and 40 days of incubation (Fig. 3, 4). Monocrotophos either singly or in combination significantly improved the fungal populations in 10-day incubated soil samples. Monocrotophos at concentrations ranging from 1.0 to 5.0 kg ha^{-1} gradually increased the population of fungi and reached maximum at the concentration of 5.0 kg ha^{-1} shows synergistic interaction in both soil samples. Beyond 5.0 kg ha^{-1} monocrotophos showed negative effect on fungal populations and showed minimum increase in fungal flora at 10.0 kg ha^{-1} (Tables 3, 4). At the end of the 10 days incubation about 13-118% increase in the fungal population was observed in vertisol soil and 6-50% increase was observed in laterite soil treated with monocrotophos when compared to controls (Tables 3, 4).

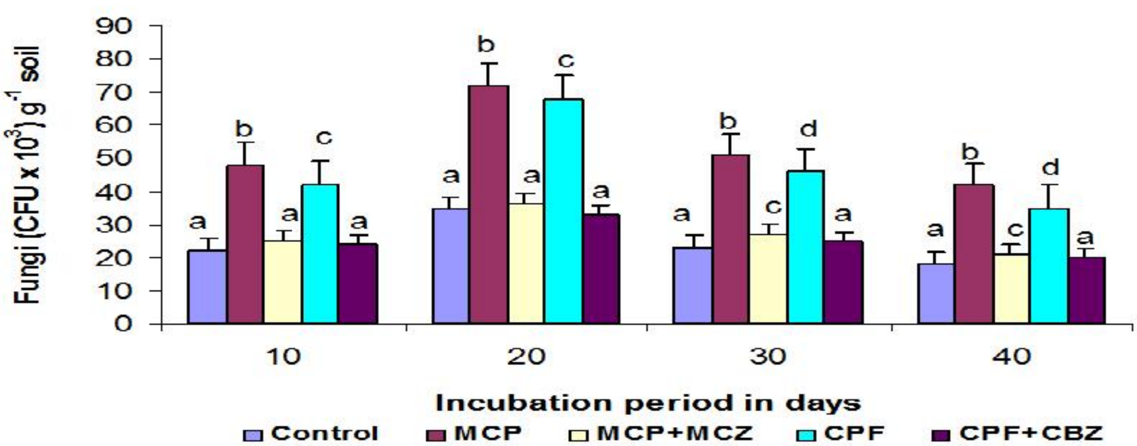

Fig. 3 Influence of monocrotophos (5.0 kg ha^{-1}), chlorpyrifos alone and in combination with mancozeb and carbendazim respectively, at 2.5 kg ha^{-1} on the population of fungi in vertisol soil. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figure are mean ± standard errors of three replicates.

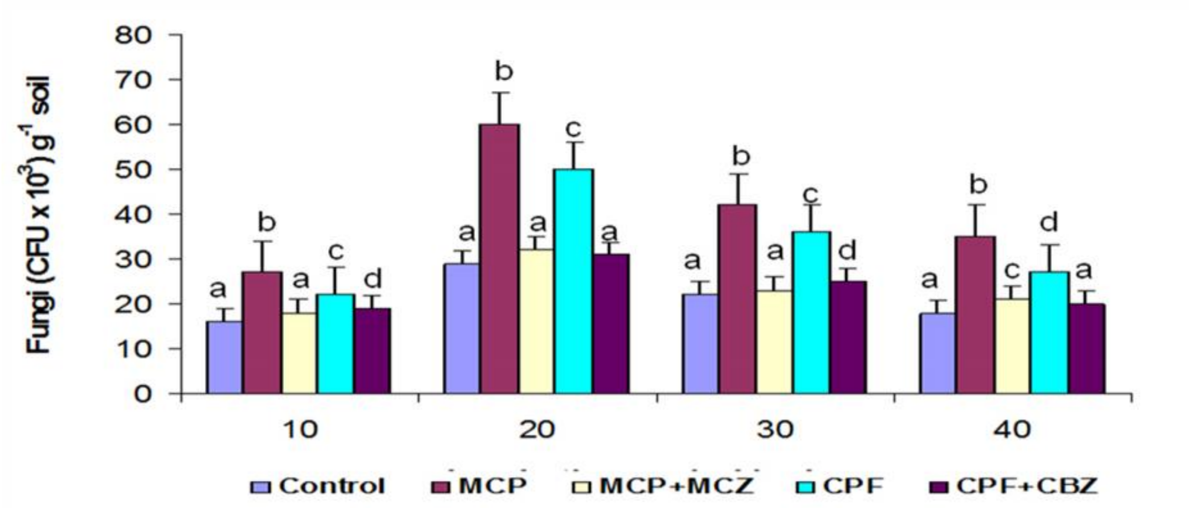

Fig. 4 Influence of monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim respectively, at 2.5 or 5.0 kg ha^{-1} on the population of fungi in laterite soil. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test. Values plotted in figure are mean ± standard errors of three replicates.

Table 3. Impact of insecticides alone and in combination with fungicides on population of fungi* (CFU × 10^3 g^{-1} dry soil) in vertisol soil after 10 days.

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + Mancozeb	Chlorpyrifos	Chlorpyrifos + Carbendazim
0.0	22a (100)	22a (100)	22a (100)	22a (100)
1.0	25a (113)	22b (100)	23a(104)	23a (104)
2.5	32b (145)	23b (104)	42b (190)	24b (109)
5.0	48c (218)	25c (113)	30c (136)	22b (100)
7.5	30b (136)	19b (86)	20a (90)	16a (72)
10.0	21a (95)	11d (50)	16d (72)	9c (40)

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. Values in table represents means of three replicates.

Table 4. Impact of insecticides alone and in combination with fungicides on population of fungi* (CFU × 10^3 g^{-1} dry soil) in laterite soil after 10 days. Figures, in parentheses indicate relative production percentages.

Pesticide concentration (kg ha^{-1})	Monocrotophos	Monocrotophos + Mancozeb	Chlorpyrifos	Chlorpyrifos + Carbendazim
0.0	16a (100)	16a (100)	16a (100)	16a (100)
1.0	17a (106)	16a (100)	16a (100)	17a (106)
2.5	19a (118)	18a (112)	22b (137)	19b (118)
5.0	27b (168)	20b (125)	17a (106)	18a (112)
7.5	16c (100)	12c (75)	13a (81)	9c (56)
10.0	11a (68)	5a (50)	10c (62)	4d (50)

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. Values in table represents means of three replicates.

Chlorpyrifos (Organophosphorus insecticide) at the concentrations of 1.0 and 2.5 kg ha^{-1} showed marked increase in fungal populations and beyond this concentration the fungal populations reduced gradually and reached minimum at 10.0 kg ha^{-1} in both vertisol and laterite soils. At the end of the 10 days incubation about 4-90% increase in fungal populations were observed in vertisol soil and 0-37% increase was observed in laterite soil treated with chlorpyrifos in comparison to control soil samples (Tables 3, 4). Likewise, eight organophosphorus insecticides, fonofos, trichloronate, chlorfenvinphos, fensulfothion, parathion, chlorpyrifos and diazinon were not toxic to fungal populations in an organic soil and a sandy loam soil [8,14] proved in his studies that individual application of an organophosphate, fenamiphos significantly stimulated the proliferation of fungal populations in soil. The combination of monocrotophos with mancozeb and chlorpyrifos with carbendazim showed no measurable effect on fungal populations at 1.0 and 2.5 or 5.0 kg ha^{-1} in both soils when compared to control. But higher concentrations of the fungicides in combination with insecticides at the level of 7.5 to 10 kg ha^{-1} showed significant inhibitory effect on the fungal population in comparison to bacterial population represents antagonistic interaction (Tables 3, 4). Similarly there was no significant difference in population of cellulolytic fungi with the use of fungicides (Carbendazim and mancozeb) at the recommended level; the cellulolytic bacteria were significantly lowered by the high fungicide rate than the recommended dose [21]. On the other hand individual application of carbendazim significantly reduced the fungal:bacterial ratio throughout the experiment compared to the control [22].

Application of pesticides significantly increased the bacterial and fungal populations in vertisol soil at 2.5-5.0 kg ha^{-1} than in laterite soil, may be due to the presence of high organic matter content in vertisol than in laterite soil. Conversely Srinivasulu et al. [3] reported that usually, the organic matter content is high in black (vertisol) soil. The biological activity was also pronounced more in black (vertisol) soil than in red (laterite) soil under the influence of insecticides alone and combination with fungicides at 2.5 or 5.0 kg ha^{-1}.

Conclusion

The results of the present study clearly indicate that application of the insecticides alone and in combination with fungicides, in cultivation of groundnut, at field application rates (2.5–5.0 kg ha^{-1}) significantly enhanced the bacterial populations, in vertisol and laterite soils. However, higher concentrations (7.5 and 10 kg ha^{-1}) of the pesticides were either innocuous or toxic to the bacterial and fungal populations in soils. The most efficient combination for the enhancement of bacterial population was monocrotophos + mancozeb, in both soils. Monocrotophos alone in vertisol soil and laterite soil, was more effective on the proliferation of fungal population. Little is known on the influence of insecticide and fungicide combinations on the bacterial and fungal populations in groundnut-cultivating vertisol and laterite soils. Therefore, further study is needed to evaluate the influence of insecticide and fungicide combinations on the bacterial and fungal populations in agricultural soils which are important and affect organic matter decomposition and nutrient cycling of soils.

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References

1.Alexander M. *Introduction to Soil Microbiology*. 2nd edn. Wiley, New York. pp. 113-330Anderson JR (1978) Pesticide effects on non-target soil microorganisms. In *Pesticide Microbiology*' (Edited by I.R. Hill and S.J.L. Wright). Academic Press. London. 1977; pp. 313-533.

2. Srinivasulu M, Rangaswamy V. Influence of insecticides alone and in combination with fungicides on microflora of groundnut soils. Int J Environ Sci Tech 2013; 10: 341–350.

3. Srinivasulu M, Jaffer Mohiddin G, Subramanyam K, Rangaswamy V. Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in groundnut (*Arachis hypogaea* L.) soil. Environ Geochem Health 2012b; 34: 365-374.

4. Ahtiainen JH, Vanhala P, Myllymaki A. Effects of different plant protection programs on soil microbes. Ecotoxicol Environ Saf 2003; 54: 56-64.

5. Griffiths BS, Caul S, Thompson J, Birch ANE, Scrimgeour C, Cortet J, Foggo A, Hackett CA, Krogh PH. Soil microbial and faunal community responses to Bt maize and insecticide in two soils. J Environ Qual 2006; 35: 734-741.

6. Vig K, Singh DK, Agarwal HC, Dhawan AK, Dureja P. Soil microorganisms in cotton fields sequentially treated with insecticides. Ecotoxicol Environ Saf 2008; 69: 263-276.

7. Lupwayi NZ, Hanson KG, Harker KN, Clayton GW, Blackshaw RE, Donovan JTO, Johnson E N, Gan Y, Irvine RB, Monreal MA. Soil microbial biomass, functional diversity and enzyme activity in glyphosate-resistant wheat-canola rotations under low-disturbance direct seeding and conventional tillage. Soil Biol Biochem 2000; 39: 1418–1427.

8. Miles JRW, Tu CM, Harris CR. Persistence of eight organophosphorus insecticides in sterile and non-sterile mineral and organic soils. Bull Environ Contam Toxicol 1979; 22: 312-318.

9. Martinez-Toledo MV, Salmeron V, Gonzalez-Lopez J. Effect of an organophosphorus insecticide, profenofos, on agricultural soil microflora. Chemosphere 1992a. 24: 71-80.

10. Srinivasulu M, Jaffer Mohiddin G, Madakka M, Rangaswamy V. Influence of pesticides on population of *Azospirillum* sp. in groundnut (*Arachis hypogaea* L.) soil. Trop Eco 2012a; 53: 93-104.

11. Shetty PK, Magu SP. Effect of metalaxyl on soil microbial population. J Trop Agric 2000; 38: 63-65.

12. Shukla PK. Mishra RR. Influence of herbicides on microbial population and enzyme activities in potato (*Solanum tuberosum*) field soil. J Agric Sci 1997; 67: 610-611.

13. Megharaj M, Singleton I, Kookana R, Naidu R. Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. Soil Biol Biochem 1999; 31: 1549-1553.

14. Tu CM, Marks CF, Johnson PW, Elliot JM. Effects of nematicides on populations of soil microflora and *Pratylenchus penetrans*, soil enzymes and flue-cured tobacco. J Environ Sci Health B 1995; 30: 95-111.

15. Rangaswamy V, Charyulu PBBN, Venkateswarlu K. Effect of monocrotophos and quinalphos on soil population and nitrogen-fixing activity of *Azospirillum* sp. Biomed Environ Sci 1989; 2: 305-314.

16. Bhaskar M, Sreenivasulu C, Venkateswarlu K. Non-target effects of monocrotophos and quinalphos towards *Anabaena torulosa* isolated from rice soil. Microbiol Res 1994; 149: 395-400.

17. Martinez-Toledo MV, Salmeron V, Gonzalez-Lopez J. Effect of the insecticides pyrimifos-methyl and chlorpyrifos on soil microflora in an agricultural loam. Plant Soil 1992b;147: 25-30.
18. Cycon M, Piotrowska-Seget Z, Kaczynska A, Kozdroj J. Microbiological characteristics of a loamy sand soil exposed to tebuconazole and λ-cyhalothrin under laboratory conditions. Ecotoxicology 2000; 15: 639-646.
19. Adebayo TA, Ojo OA, Olaniran OA. Effects of two insecticides Karate1 and Thiodan1 on population dynamics of four different soil microorganisms. Res J Biol Sci 2007; 2: 557–560.
20. Wang M, Liu Y, Wang Q, Gong M, Hua X, Pang Y, Shuijin H, Yong H Y. Impacts of methamidophos on the biochemical, catabolic and genetic characteristics of soil microbial communities. Soil Biol Biochem 2008; 40: 778–788
21. Fawole OB, Aluko M, Olownithi TE. Effects of a carbendazim-mancozeb fungicidal mixture on microbial populations and some enzyme activities in soil. Agrosearch 2009; 10: 65-74.
22. Yan H, Wang D, Dong B, Tang F, Wang B, Fang H, Yunlong Y. Dissipation of carbendazim and chloramphenicol alone and in combination and their effects on soil fungal: bacterial ratios and soil enzyme activities. Chemosphere 2011; 84: 634-641.