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Effect of pesticides on the population of *Azospirillum* sp. and on ammonification rate in two soils planted to groundnut (*Arachis hypogaea* L.)

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Abstract: Pesticides are usually applied simultaneously or serially for groundnut (*Arachis hypogaea* L.) crop protection. This type of pesticide application often leads to a combined contamination of pesticides and their residues in the soil. Pesticides used in groundnut cultivation – monocrotophos and chlorpyrifos, singly and in combination with mancozeb and carbendazim, respectively – were tested for their effects on the population of *Azospirillum* sp. and ammonification in two soils planted to groundnut in the Anantapur District of Andhra Pradesh, India. The size of the *Azospirillum* sp. population was initially low in both soils. Application of pesticides, singly and in combination up to 5.0 kg ha^{-1} , significantly increased the population of *Azospirillum* sp. after 7 and 14 days of incubation in vertisol soil, whereas in laterite soil, the application of pesticides in the same combination up to 2.5 kg ha^{-1} increased the population of *Azospirillum* sp. in similar conditions. Mineralization of peptone-nitrogen was profoundly increased when soil was treated with pesticides, singly or in combination, at 2.5 kg ha^{-1} in both soils. Vertisol soil that received monocrotophos and chlorpyrifos singly at 5.0 kg ha^{-1} showed a considerable increase in ammonification rate after 14 days of incubation. Due to the synergistic and additive interactions between pesticides and microorganisms, the population of *Azospirillum* sp. and the rate of ammonification increased at particular concentrations of pesticides (i.e 2.5 to 5.0 kg ha^{-1}), but at higher concentrations (7.5 and 10.0 kg ha^{-1}) the pesticides exerted antagonistic interactions on the population of *Azospirillum* sp. and ammonification.

Resumen: Comúnmente se aplican pesticidas de forma simultánea o escalonada para proteger el cultivo del cacahuate o maní (*Arachis hypogaea* L.). Con frecuencia la aplicación de este tipo de pesticidas causa una contaminación combinada de pesticidas y sus residuos en el suelo. Se probaron los efectos de los pesticidas usados en el cultivo de maní –monocrotofos y clorpirifos, solos y en combinación con mancozeb y carbendazim, respectivamente– sobre la población de *Azospirillum* sp. y la amonificación en dos suelos plantados con maní en el Distrito Anantapur de Andhra Pradesh, India. El tamaño de la población de *Azospirillum* sp. era inicialmente bajo en ambos suelos. La aplicación de pesticidas –solos y en combinación– de hasta 5.0 kg ha^{-1} incrementó significativamente la población de *Azospirillum* sp. después de 7 y 14 días de incubación en suelo de vertisol, mientras que en suelo de laterita, la aplicación de pesticidas en la misma combinación y hasta de 2.5 kg ha^{-1} incrementó la población de *Azospirillum* sp. en condiciones similares. La mineralización del nitrógeno de la peptona se incrementó sensiblemente cuando el suelo fue tratado con pesticidas, solos o en combinación, a una concentración de 2.5 kg ha^{-1} en ambos suelos. El vertisol que recibió monocrotofos y clorpirifos solos en una concentración de 5.0 kg ha^{-1} mostró un incremento considerable en la tasa de amonificación después de 14 días de incubación. Debido a las interacciones sinérgicas y aditivas entre pesticidas y microorganismos, la población de *Azospirillum* sp. y la tasa de

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amonificación aumentaron a ciertas concentraciones de pesticidas (i.e. 2.5 a 5.0 kg ha^{-1}), pero a concentraciones más altas (7.5 y 10.0 kg ha^{-1}) los pesticidas produjeron interacciones antagónicas sobre la población de *Azospirillum* sp. y la amonificación.

Resumo: Os pesticidas são normalmente aplicados simultaneamente ou serialmente para a protecção das culturas deamendoim (*Arachis hypogaea* L.). Este tipo de aplicação de pesticidas muitas vezes leva a uma contaminação combinado de pesticidas e dos seus resíduos no solo. Os pesticidas usados no cultivo de amendoim - monocrotofos e clorpirifos, isoladamente ou em combinação com "mancozeb" e "carbendazim", respectivamente - foram testados quanto aos seus efeitos sobre a população de *Azospirillum* sp. ea amonificação em dois solos plantados com amendoim no Distrito de Anantapur em Andhra Pradesh, na Índia. O tamanho da população de *Azospirillum* sp. foi inicialmente baixo em ambos os solos. A aplicação de pesticidas até 5,0 kg ha^{-1}- isoladamente ou em combinação - aumentou significativamente a população de *Azospirillum* sp. após 7 e 14 dias de incubação num Vertissolo, enquanto que num solo laterítico, a aplicação de pesticidas na mesma combinação de até 2,5 kg ha^{-1} aumentou a população de *Azospirillum* sp. em condições semelhantes. A mineralização do azoto-peptonaaumentou profundamente quando o solo foi tratado com pesticidas, isolados ou em combinação de 2,5 kg ha^{-1} em ambos os solos. Num Vertissolo que recebeu monocrotofos e clorpirifos, isoladamente numa dose de 5,0 kg ha^{-1}, apresentou um aumento considerável na taxa de amonificação após 14 dias de incubação. Devido às interacções sinérgicas e aditivas entre os pesticidas e microorganismos, a população de *Azospirillum* sp. ea taxa de amonificação aumentaram nas concentrações particularesde pesticidas (ou seja, 2,5 a 5,0 kg ha^{-1}), mas em concentrações mais elevadas (7,5 e 10,0 kg ha^{-1}), os pesticidas exerceram interacções antagonistas sobre a população de *Azospirillum* sp. ea amonificação.

Key words: Ammonification, *Azospirillum* sp., groundnut soils, pesticides combination.

Introduction

Groundnut (*Arachis hypogaea* L.) is a major, significant, commercial oil seed crop in India (Guha & Chandrasekhar 2001). It is one of the major cash crops grown in dryland of India (Dharne *et al.* 2001), contributing 41.3 % of the country's oil seed production (Giraddi *et al.* 1999). Groundnut is also the single largest source of edible oils in India, accounting for about 50 % of the total oil seed production (Talwar 2004). Synthetic pesticides are purposely introduced into agricultural systems to protect crops, such as groundnut, against weeds, insects, fungi and other pests (Yang *et al.* 2007). The majority of pesticides applied to crops eventually reach the soil, even if sprayed on the foliage of crop plants and weeds, which may affect the growth and activity of soil microbial communities (Cope 1971; Omar & Abdel-Sater 2001; Singh & Singh 2005). Extensive and excessive use of pesticides has aroused concern on their fate in soil and possible effects on soil microbial communities. Although a number of studies have been conducted, most of these studies have focused on individual pesticides (Getenga *et al.* 2000; Gundi *et al.* 2005; Hill & Stratton 1991; Wainwright 1978; Xie *et al.* 2004), yet more than one pesticide is often applied to a single crop.

Agricultural management practices often involve application of different classes of pesticides – such as insecticides, fungicides and herbicides – simultaneously or sequentially for the purpose of crop protection, and these chemicals may interact with each other within the soil (Flieabach & Mader 2004). *Azospirillum* is a free-living, microaerophilic, heterotrophic, diazotrophic bacterium that is actively involved in heterotrophic nitrogen fixation in several grass-bacteria associations (Charyulu & Rao 1978). The occurrence of nitrogen-fixing *Azospirillum* sp. in rice roots and soils has also been reported (Nayak & Rao 1980,1982). The influence of several pesticides on the growth and nitrogen fixation of *Azospirillum* sp. has been investigated in pure culture systems by few workers

Table 1. Particulars of the pesticides.

Technical name	Commercial and chemical name	Chemical class	Commercial formulation	Sources
Monocrotophos	Monocron (dimethyl(*E*)-1-methyl-2-(methylcarbamoyl)vinyl phosphate)	Organophosphate	36 % EC*	Cheminova India Limited, 242/P& 27/28, GIDC, Industrial Estate, Panoli, Distt. Bharuch, Gujarat
Chlorpyrifos	Dursban (diethoxy-sulfanylidene-(3,5,6-trichloropyridin-2-yl) oxyphos-phorane)	Organophosphate	20 % EC*	Rallis India Limited Regd office: Apeejay House, 7th floor, 3 Dinshaw Vachha Road, Mumbai
Mancozeb	Dhanuka M-45 (manganese ethylenebis (dithocarbamate) (polymeric) complex with zinc salt)	Dithiocarbamate	75 % WP**	Northern Minerals Limited, Daultabad, Road, Gurgaon-122 001 (Haryana)
Carbendazim	Bavistin (methyl–[2–^{14}C]benzimidazol–2–yl– carbamate)	Benzimidazole	50 % WP**	BASF India Limited, Regd. Off: Mhindra Towers, Dr. Bhosale Marg, Worli, Mumbai 400 018

*EC : Emulsifying concentration, **WP : Wettable powder.

(Charyulu *et al.* 1980; Nayak & Rao 1980; Rangaswamy *et al.* 1989; Rangaswamy & Venkateswarlu 2000). Ammonification is the first step in nitrogen mineralization, during which NH_4^+ is liberated from organic nitrogen compounds, such as proteins, by the action of heterotrophic microorganisms in soil (Tu & Miles 1976). This process supplies readily available nitrogen to plants and microorganisms. Populations dynamics of ammonifiers are dependent on soil moisture level, being greatest at 60 % water-holding capacity (Naumann 1972).

Microbial populations and their metabolic processes, particularly those associated with the nitrogen cycle, are often chosen as test systems to know the ecological significance of nitrogen transformation in the agricultural ecosystems. The objectives of this study were to test the effect of monocrotophos and chlorpyrifos, singly and in combination with mancozeb and carbendazim, respectively, on the population of *Azospirillum* sp. and on ammonification rates in two soils planted to groundnut.

Materials and methods

Soils

Samples of vertisol and laterite soils were collected from cultivated groundnut fields of the Anantapur district of Andhra Pradesh, India on 20 May 2008 to a depth of 12 cm, because the groundnut crop is predominantly cultivated in these two soil types. The soil samples were air-dried and passed through a 2-mm sieve before use and the physico-chemical properties of soils were analyzed (Table 1).

Pesticides

In order to determine the impact of pesticides on *Azospirillum* sp. and ammonification activity, monocrotophos and chlorpyrifos, singly and in combination with mancozeb and carbendazim, respectively, were used in the present study. For incubation studies, commercial formulations of tested pesticides dissolved in distilled water were used. The details of the pesticides can be found in Table 2.

Population of Azospirillum sp. in soils

To determine the effect of monocrotophos and chlorpyrifos, singly (10, 25, 50, 75 and 100 μg g^{-1} soil) and in combination (i.e. monocrotophos + mancozeb and chlorpyrifos + carbendazim) at the same concentration (5+5, 12.5+12.5, 25+25, 37.5+37.5 and 50+50 μg g^{-1} soil) on the population of *Azospirillum* sp., 5 g (dry weight) portions of each soil were placed in 15 × 150 mm test tubes and treated with different concentrations of pesticides, which were equivalent to 1, 2.5, 5, 7.5 and 10 kg ha^{-1} (Jaya Madhuri & Rangaswamy 2003; Ranga-

Table 2. Physico-chemical properties of the soils.

Properties	Vertisol soil	Laterite soil
Sand (%)	68.3	53.3
Silt (%)	22.7	27.1
Clay (%)	09.0	19.6
pH [a]	8.8	8.0
Water holding capacity (ml g^{-1} soil)	0.37	0.17
Electrical conductivity (mmhos)	265	247
Organic matter [b] (%)	1.48	0.76
Total nitrogen [c] (%)	0.091	0.052
NH_4^+ - N (µg g^{-1} soil) [d]	8.05	7.51
NO_2^- - N (µg g^{-1} soil) [e]	0.50	0.36
NO_3^- - N (µg g^{-1} soil) [f]	0.92	0.79

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 & Folkard 1951), f = Brucine Method (Ranney & Bartlett 1972).

swamy & Venkateswarlu 1999). Soil samples without pesticides served as controls. The soil samples were incubated at 28 ± 4°C in the laboratory. The soil moisture content was maintained at 60 % throughout the experimental period by adding appropriate amounts of water.

After 7 and 14 days of incubation, triplicate soil samples were used to estimate the population size of *Azospirillum* sp. using the MPN method described by Alexander (1965), with MPN values calculated using probability tables (Alexander 1965). The growth medium (sterile, nitrogen-free, semi-solid malate medium, pH=6.8 (Dobereiner *et al.* 1976) contained (per L): malic acid, 5 g; KOH, 4 g; K_2HPO_4, 0.5 g; $MgSO_4$, 0.2 g; NaCl, 0.1 g; $CaCl_2$, 0.02 g; $FeSO_4$, 0.5 g; Na_2MoO_4, 0.02 g; $MnSO_4$, 0.01 g; 5 % alcoholic solution of bromothymol blue, 2 ml; agar, 1.75 g). Five ml aliquots of medium were added to five MPN tubes and inoculated with 0.5 ml of a soil suspension from 10^{-1} to 10^{-5} soil dilutions, and incubated at 37° C. MPN tubes in which a typical white pellicle developed a few mm below the surface of the medium after incubation for 36 h were scored positive for *Azospirillum* sp.. Microscopic examination of the cultures revealed the characteristic rods adhered to the flat droplets of oil.

Ammonification

To investigate the effects of monocrotophos and chlorpyrifos, singly (10, 25, 50, 75 and 100 µg g^{-1} soil) and in combination (i.e. monocrotophos+ mancozeb and chlorpyrifos + carbendazim) at the same concentrations (5 + 5, 12.5 + 12.5, 25 + 25, 37.5 + 37.5, 50 + 50 µg g^{-1} soil) on mineralization of peptone-nitrogen, 0.05 ml aliquots from stock solutions of the insecticides were applied with 0.1 ml pipette to the surface of 5 g (dry weight) portions of vertisol and laterite soils contained in 25 × 200 mm test tubes, as described by Gundi *et al.* (2005). Soil samples receiving 0.05 ml distilled water served as controls. All the treatments were supplemented with AR-grade peptone at the concentration of 1,000 µg g^{-1}, as described by Rangaswamy & Venkateswarlu (1990), and the soil samples homogenized to distribute the pesticides and N source. Soil samples were maintained at 60 % of water holding capacity throughout the incubation period. After 7 days of incubation at room temperature (28 ± 4° C), triplicate samples of each treatment were withdrawn and extracted with 12.5 ml of 2 M KCl per 5 g soil for analysis of ammonia using the Nesslerisation method (Jackson 1971). To suitable aliquots of the soil extract, 0.5 ml of Nessler's reagent was added and the volume was made up to 7 ml. The yellow color developed was read at 495 nm in a Spectronic-20D spectrophotometer (Milton Roy).

In the case of ammonification, initially the soil samples were treated with different concentrations of pesticides (1.0, 2.5, 5.0, 7.5 and 10 kg ha^{-1}), and after 7 days of incubation the stimulatory concentrations of pesticides for ammonification were determined as 2.5 or 5.0 kg ha^{-1}, and these treatments were further incubated for 14, 21 and 28 days.

Statistical analysis

All data were expressed on an air dry soil basis and were averages of three replicates. Data were analysed by significant difference ($P \leq 0.05$) between pesticide-treated and untreated soils using Duncan multiple range (DMR) test (Jayamadhuri & Rangaswamy 2009; Megharaj *et al.* 1999). If A + B < AB, the response can be considered as synergistic interaction. If A + B > AB, the response can be considered as antagonistic interaction; if A + B = AB, the response can be considered as additive interaction (where, A = the percent stimulation in population of *Azospirillum* sp./ammonification caused by pesticide X alone over the control; B = the percent stimulation in population *Azospirillum* sp./ammonification caused by pesticide Y alone over the control; and AB = the percent stimulation in population of *Azospirillum* sp./ammonification caused by the combination of X + Y over the control). The percent stimulation values were calcu-

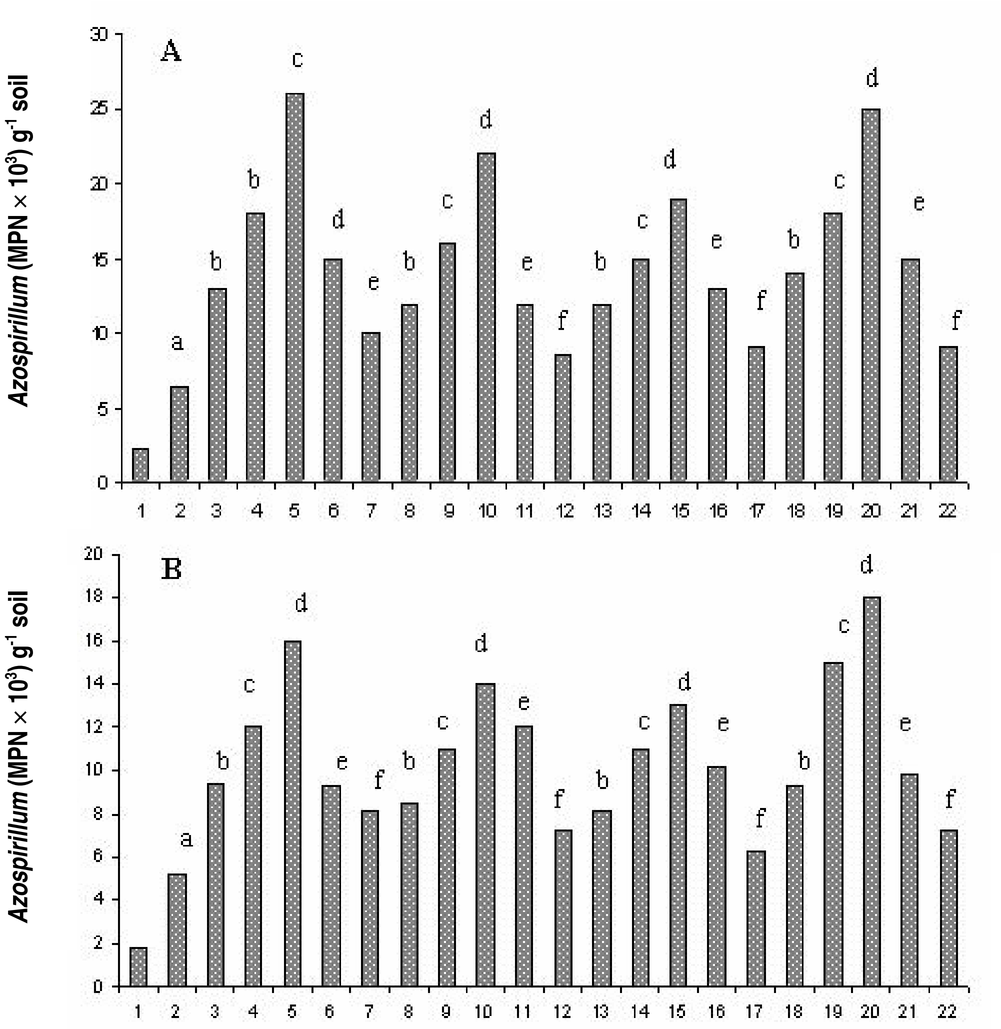

Fig. 1. Interaction effect of pesticides on the population of *Azospirillum* sp. in vertisol soil; (A) 7 days of incubation, (B) 14 days of incubation. Values plotted in figures are means of three replicates. Means, in each bar, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test. X-axis: 1 = 0 day, 2 = control, 3 = MCP 10 ppm, 4 = MCP 25 ppm, 5 = MCP 50 ppm, 6 = MCP 75 ppm, 7 = MCP 100 ppm, 8 = MCP 5 ppm + MCZ 5 ppm, 9 = MCP 12.5 ppm + MCZ 12.5 ppm, 10 = MCP 25 ppm + MCZ 25 ppm, 11 = MCP 37.5 ppm + MCZ 37.5 ppm, 12 = MCP 50 ppm + MCZ 50 ppm, 13 = CPF 10 ppm, 14 = CPF 25 ppm, 15 = CPF 50 ppm, 16 = CPF 75 ppm, 17 = CPF 100 ppm, 18 = CPF 5 ppm + CBZ 5 ppm, 19 =CPF 12.5 ppm + CBZ 12.5 ppm, 20 = CPF 25 ppm + CBZ 25 ppm, 21 = CPF 37.5 ppm + CBZ 37.5 ppm, 22 = CPF 50 ppm + CBZ 50 ppm.

MCP = Monocrotophos; MCZ = Mancozeb; CPF = Chlorpyrifos; CBZ = Carbendazim.

lated relative to population of *Azospirillum* sp. /the rate of ammonification in untreated control.

Results

Effect of pesticides on population of Azospirillum sp. in soils

The initial size of the population of *Azospirillum* sp. was low in both soils (Figs. 1 & 2). The population of *Azospirillum* sp. was significantly higher in soils treated with monocrotophos and chlorpyrifos, singly and in combination with mancozeb and carbendazim, respectively, than in untreated control soils during the course of experiment. The population of *Azospirillum* sp. in soils increased when pesticides were applied at 2.5 - 5.0 kg ha^{-1}; by contrast, as the concentration of pesticides increased to 7.5 - 10.0 kg ha^{-1}, the population of *Azospirillum* sp. gradually decreased in both soils. Application of pesticides, singly and

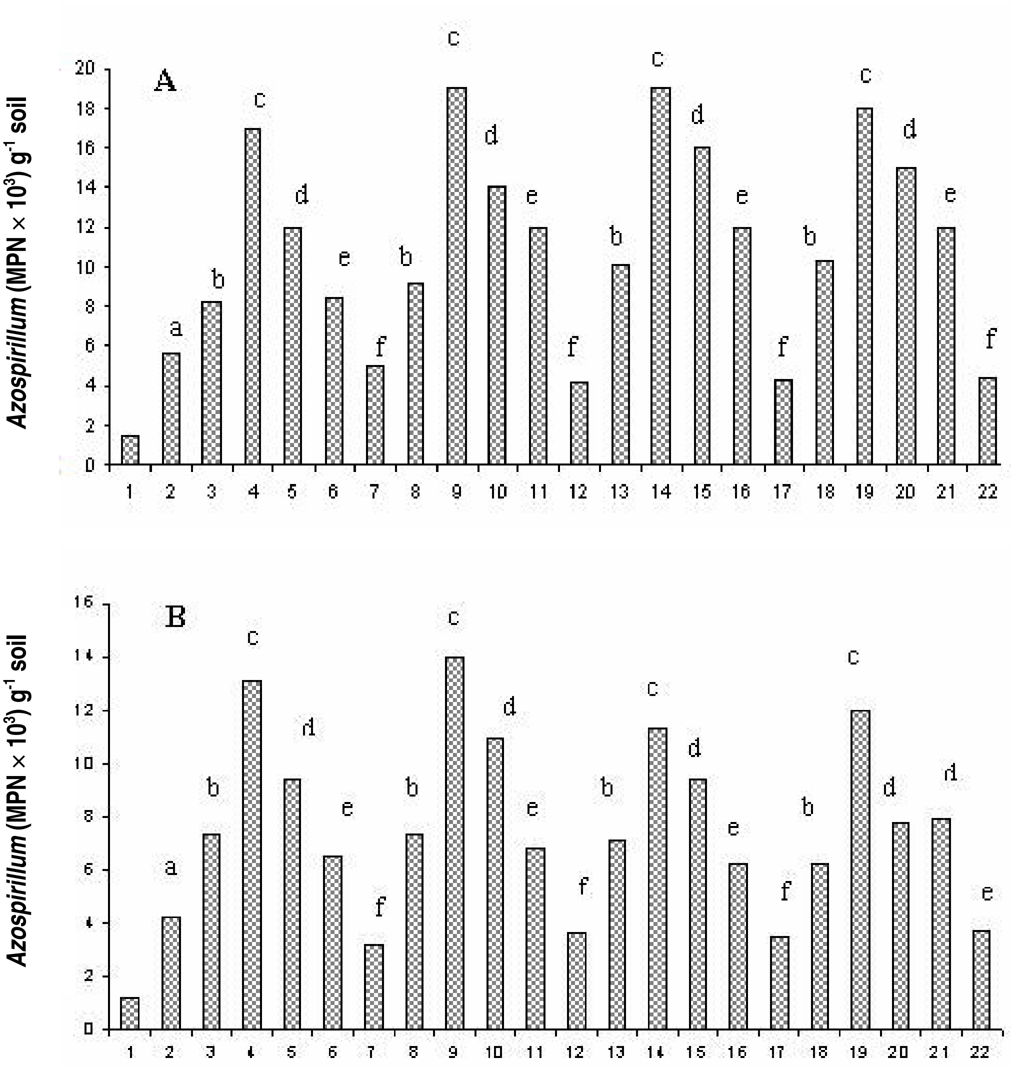

Fig. 2. Interaction effect of pesticides on the population of *Azospirillum* sp. in laterite soil; (A) 7 days of incubation (B) 14 days of incubation. Values plotted in figures are means of three replicates. Means, in each bar, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test. See Fig. 1 for explanation of x-axes.

in combination up to 5.0 kg ha^{-1}, profoundly enhanced the population of *Azospirillum* sp. in vertisol soil (Fig. 1). For the laterite soil, pesticide concentrations up to 2.5 kg ha^{-1} increased the population of *Azospirillum* sp. after 7 and 14 days of incubation (Fig. 2).

The increase in population of *Azospirillum* sp. in vertisol soil amended with monocrotophos and chlorpyrifos, singly and in combination (i.e. monocrotophos + mancozeb and chlorpyrifos + carbendazim at 1.0, 2.5 and 5.0 kg ha^{-1}) was 100 - 300, 85 -238, 82 - 192 and 115 - 284 %, respectively, over the control treatment after incubation for 7 days (Fig. 1a). The population of *Azospirillum* sp. in vertisol soil with or without pesticides decreased gradually after 14 days (Fig. 1b) compared to that after 7 days. The corresponding increases in population of *Azospirillum* sp. in laterite soil amended with four pesticides at 1.0 and 2.5 kg ha^{-1} were 46 - 203, 64 - 239, 80 - 239 and 84 - 221 %, respectively, over the control treatment by the end of 7 day interval (Fig. 2a). The population of *Azospirillum* sp. also decreased gradually under similar conditions after a 14 day incubation in laterite soil (Fig. 2b). The influence of monocrotophos, chlorpyrifos alone and in combination with mancozeb and carbendazim, respectively, at different levels on the population of *Azospirillum* sp. in the two soils was assessed to examine interaction between pesticides. Interaction responses are generally distinguished on the basis of percent stimulation values (over control) regarding any parameter in soil treated with

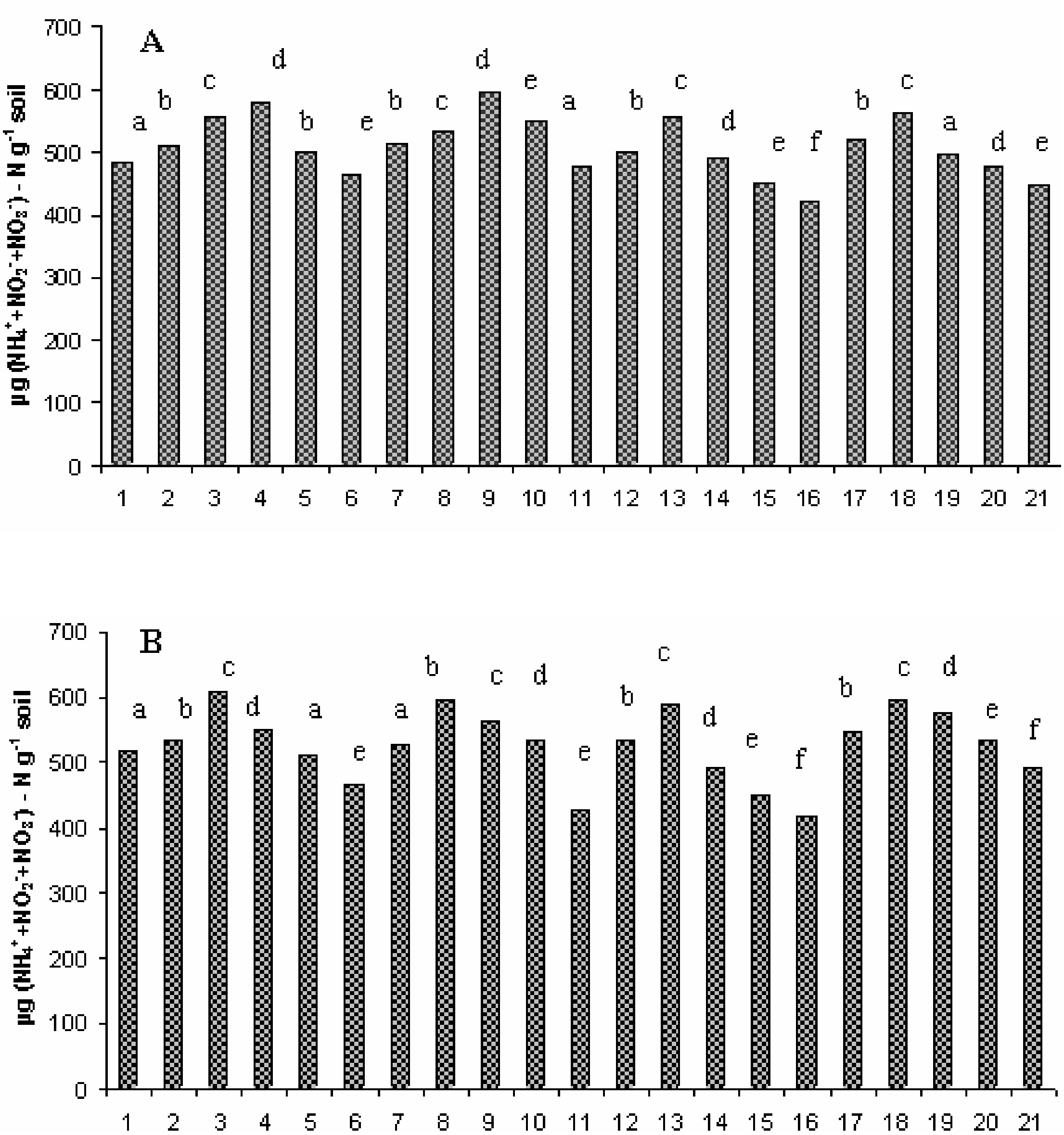

Fig. 3. Effect of pesticides on ammonification in soils: (a) 7 days of incubation in vertisol soil; (b) 7 days of incubation in laterite soil. Values plotted in figures are means of three replicates. Means, in each bar, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test. X-axis: 1 = control, 2 = MCP 10 ppm, 3 = MCP 25 ppm, 4 = MCP 50 ppm, 5 = MCP 75 ppm, 6 = MCP 100 ppm, 7 = MCP 5 ppm + MCZ 5 ppm, 8 = MCP 12.5 ppm + MCZ 12.5 ppm, 9 = MCP 25 ppm + MCZ 25 ppm, 10 = MCP 37.5 ppm + MCZ 37.5 ppm, 11 = MCP 50 ppm + MCZ 50 ppm, 12 = CPF 10 ppm, 13 = CPF 25 ppm, 14 = CPF 50 ppm, 15 = CPF 75 ppm, 16 = CPF 100 ppm, 17 = CPF 5 ppm + CBZ 5 ppm, 18 = CPF 12.5 ppm + CBZ 12.5 ppm, 19 = CPF 25 ppm + CBZ 25 ppm, 20 = CPF 37.5 ppm + CBZ 37.5 ppm, 21 = CPF 50 ppm + CBZ 50 ppm. Note: ppm = Parts per million, 1 ppm = 1 $\mu g\ g^{-1}$ soil.

single pesticide or in combination with another pesticide at a specified dose in soil. In this study monocrotophos, chlorpyrifos singly and in combination (i.e., monocrotophos + mancozeb, chlorpyrifos + carbendazim at 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) interacted synergistically, additively and antagonistically, respectively (Figs. 1 & 2). It is clear from these results that the occurrence of interactions between insecticides and fungicides was dose-dependent, and these interactions were

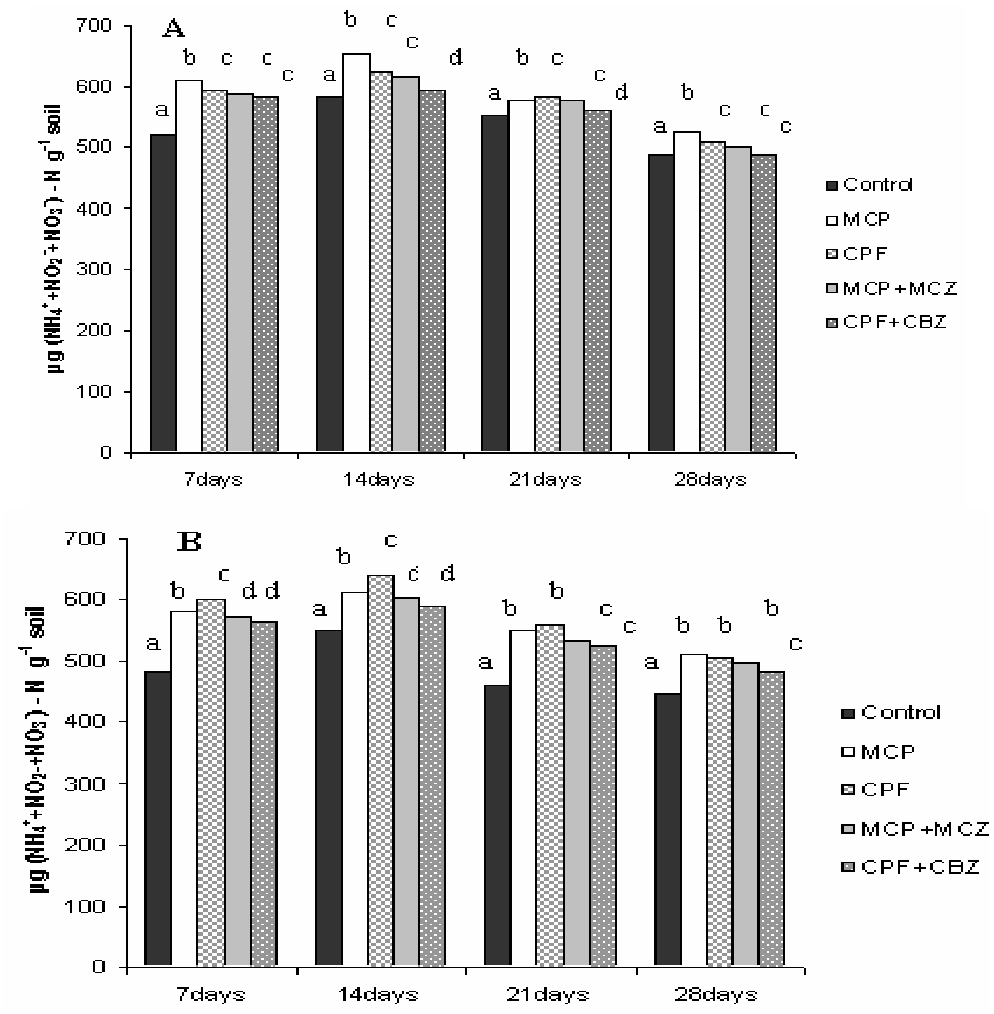

Fig. 4. Effect of pesticides on ammonification in soils: (A) vertisol soil; (B) laterite soil. Values plotted in figures are means of three replicates. Means, in each bar, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range test.

A) MCP 25 ppm, CPF 25 ppm, MCP 12.5 ppm + MCZ 12.5 ppm, CPF 12.5 ppm + CBZ 12.5 ppm B) MCP 50 ppm, CPF 50 ppm, MCP 12.5 ppm + MCZ 12.5 ppm, CPF 12.5 ppm + CBZ 12.5 ppm.

prevailed in soil even after incubation for 14 days.

Effect of pesticides on ammonification in soils

Soil application of pesticides, singly and in combinations, significantly enhanced the rate of ammonification when applied at 2.5 or 5.0 kg ha^{-1}, whereas an increase in the concentration of pesticides up to 10.0 kg ha^{-1} resulted in a slight decrease in the mineralization of peptone nitrogen in both soil types (Fig. 3). Significant stimulation in ammonification occurred after incubation for 14 days in both soils. Treatments receiving monocrotophos, chlorpyrifos, monocrotophos + mancozeb, and chlorpyrifos + carbendazim, showed a high nitrogen mineralization rate at 2.5 kg ha^{-1} relative to the control after incubation for 14 days in the laterite soil (Fig. 4a). In the vertisol soil that received monocrotophos and chlorpyrifos alone there was significant stimulation in mineralization of organic nitrogen at 5.0 kg ha^{-1}, and in treatments receiving monocrotophos + mancozeb and chlorpyrifos + carbendazim, a high mineralization rate was observed at 2.5 kg ha^{-1} relative to the control after incubation for 14 days (Fig. 4b). The highest rate of mineralization of peptone nitrogen occurred after incubation for 14 days for all tested pesticides in both soils.

Soil ammonification activity is considered a valuable parameter for assessing the side effects of pesticide treatments on the soil microbial community. In the present study ammonification activity was higher in the pesticide-amended soils than in the control soils at all incubation intervals (7, 14, 21 and 28 days after application), suggesting that the microbial metabolic activity increased due to the incorporation of pesticides in soil. Similar to the effects on the population of *Azospirillum* sp., monocrotophos, chlorpyrifos singly, and in combination, i.e, monocrotophos + mancozeb and chlorpyrifos + carbendazim, at 1.0, 2.5 and 5.0 kg ha^{-1} resulted in enhancement of ammonification activity by 15 - 20, 10 - 23, 3 - 15 and 8 - 17 %, respectively over the control treatment after 7 days in vertisol soil (Fig. 3a). The corresponding increase in laterite soil were 3 - 17, 1 - 14, 3 - 13, and 5 -15 %, respectively, at same concentrations of pesticides under similar conditions (Fig. 3b).

Ammonification activity increased in pesticide treated and untreated soils during incubation for 14 days, decreasing after incubation for 21 and 28 days. Application of insecticides in combination with fungicides to soils resulted in mixed effects on ammonification activity in comparison to the single insecticide. Three types of interactions - synergistic, additive and antagonistic – were observed toward ammonification activity when the two soils received insecticides alone and in combination with fungicides (Figs. 3 & 4).

Discussion

In the present study, four pesticides applied to soil, singly or in combination, at concentrations ranging from 1.0 to 5.0 kg ha^{-1}, had no deleterious effect on *Azospirillum* sp. and ammonification activity; rather both the bacterial population and activity increased. A similar individual stimulatory effect of monocrotophos and chlorpyrifos was previously demonstrated on the population of *Azospirillum* sp. (Rangaswamy *et al.* 1989). Similar observations with other organophosphorus and pyrethroid insecticides and fungicides have also been reported (Jayamadhuri & Rangaswamy 2003; Rangaswamy & Venkateswarlu 2000).

Interactions between different agrochemicals applied in combination on microorganisms and their activities in soils have received little attention in comparison to effects of a single agrochemical. There were no differences in degree of diversity in bacterial populations from the application of a combination of five pesticides, including chlorfenviphos and glyphosate, to field plot of 20 years (Nicholson & Hirsch 1998). In the present study the application of pesticides to the soils at certain concentrations was not harmful the population of *Azospirillum* sp. A few reports have been published on interactions between pesticides and their solvents, pesticides and their degradation products, and two different pesticides on growth of organisms in pure culture studies of fungi, algae and cyanobacteria (EI-Jay 1996; Megharaj *et al.* 1989, 1990; Stratton 1984; Stratton & Corke 1982a, 1982b; Stratton & Smith 1988). In all these studies, a variety of interaction effects such as synergistic, additive and antagonistic were observed, depending on concentration of the interacting chemicals. For example, the combination of permethrin and its degradation product interact to yield antagonistic, additive and synergistic interactions toward the growth of fungi in pure culture (Stratton & Corke 1982b), because the degradation rate of an individual pesticide may be changed due to the combinations of pesticides, ultimately leading to different types of interactions. In the present study, similar types of interactions occurred between selected pesticide combinations and population of *Azospirillum* sp. in two soils. Increases in the population of *Azospirillum* sp. at high concentrations (100 ppm) of benomyl or 2-aminobenzimidazole (a hydrolysis product of benomyl) have been reported in paddy soil (Charyulu & Rao 1978; Charyulu *et al.* 1980). Nayak & Rao (1980) have observed stimulation in population of *Azospirillum* sp. when treated with benomyl at lower concentration (5 ppm) in alluvial, laterite and saline soils, and carbofuran in alluvial soil only.

The mineralization of peptone-nitrogen was greater in soils amended with pesticides at the concentrations of 2.5 - 5.0 kg ha^{-1} - values similar to field application rates - but higher concentrations of these pesticides were innocuous or toxic to the mineralization process in both soils. Similarly, monocrotophos and quinolphos at levels of 2.5 kg ha^{-1}, and cypermethrin fenvalerate at 5 kg ha^{-1}, considerably stimulated amonification, but at higher concentrations these pesticides were toxic to the N mineralization process in two agricultural soils (Rangaswamy & Venkateswalru 1990, 1993). Ammonification of peptone nitrogen was also enhanced by diazinon, chlorpyrifos and thionazin added at concentrations of 1 or 10 kg ha^{-1} in sandy loam soil (Tu 1970, 1972). Higher concentrations of monocrotophos and methidathion were toxic to ammonification (Idris 1973). These observations are in agreement with the results of the present study. The overall effects of combinations of pesticides on microbial activities in soil may be subject to interactions between pesticides (i.e. additive, synergistic and antagonistic) and may deviate from the behaviour of the individual pesticide components (Auspurg 1985). In the present study similar types of interactions occurred between selected insecticide and fungicide combinations on ammonification in two soils.

Although the mechanisms of interactions are not known, interaction patterns may have a profound influence on soil microflora and their activities, thereby affecting soil fertility. Pesticides added to soil undergo degradation to metabolites in the course of time. For instance, monocrotophos is hydrolysed to N-methyl acetoacetamide (Lee *et al*. 1990). Pesticides are generally applied simultaneously or serially for crop protection, hence the degradation behavior of a pesticide may be changed after it interacts with other pesticides (or their degradation products) already present in the soil; such changes in pesticide degradation may have different side effects on biological processes, such as ammonification, and on microbial populations.

The presence of chlorothalonil has been suggested as altering the degradation behavior of chlorpyrifos-degrading microbes (Chu *et al*. 2008). The persistent interaction responses recorded in the present study cannot be attributed exclusively to parent pesticides, since metabolites may also have biological effects. Generally pesticides are recalcitrant (not easily degradable) substances, hence they persist for long periods in the soils. This may be one of the main reasons for persistent interactive effects in soil. The present study further accentuates the need for a systemic study on the interactive effects of pesticides used extensively, as well as their metabolites.

The results of the present investigation clearly indicate that the selected pesticides - monocrotophos and chlorpyrifos, singly and in combination with mancozeb and carbendazim, respectively - at levels ranging from 1.0 to 5.0 kg ha^{-1} - significantly increased the population of *Azospirillum* sp. and the rate of ammonification in a vertisol and a laterite soil from a groundnut plantation. Furthermore, these pesticides, singly and in combination, at levels of 1.0 to 10.0 kg ha^{-1} exerted synergistic, additive or antagonistic interactions toward population of *Azospirillum* sp. and ammonification in these soils.

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