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Impact of chlorothalonil and propiconazole on enzyme activities in groundnut (*Arachis hypogaea* L.) soils

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ABSTRACT

This study was undertaken to determine the impact of fungicides, propiconazole and chlorothalonil on dehydrogenase and phosphatase activities in two groundnut (Arachis hypogaea L.) soils. The effect of these two fungicides was assessed over a period of five weeks for dehydrogenase and a period of 40 days for phosphatase. Soil samples collected from groundnut fields were treated with fungicides at different concentrations i.e 10, 25, 50, 75, 100 ppm, which are equivalent to field application rates (1.0, 2.5, 5.0, 7.5, 10.0 kg ha^{-1}) in the laboratory. The formation of triphenyl formazan (TPF) was significantly enhanced at 5.0 kg ha^{-1} up to 3 weeks in laterite and vertisol soils, individual increments in dehydrogenase activity ranged from a low increase +15% +93%, +6% to +71% and +17% to +118%, +12% to +100% in black and red soils. Furthermore increase in concentration of fungicides i.e., at 7.5 and 10.0 kg ha^{-1} decreased the rate of dehydrogenase activity after a week, and then decline phase was started gradually from 3 to 5 weeks of incubation. Where as in case of phosphatase the accumulation of p- nitrophenol was significantly increased in propiconazole and chlorothalonil treated soils at 5.0 and 2.5 kg ha^{-1}, respectively. Individual increments of phosphatase activity ranged from a low increase +7% +33%, +1% to +23% and +2% to +25%, +5% to +22% in black and red soils. Furthermore increase in concentration of fungicides (7.5 and 10.0 kg ha^{-1}) decreased the rate of phosphatase activity after 10 days, and then decline phase was started gradually after 30 and 40 days of incubation.

Key words: Chlorothalonil, Propiconazole, Dehydrogenase, Phosphatase, Groundnut soils.

INTRODUCTION

Since from past four decades, a large number of fungicides have been introduced to control plant pathogens in agricultural systems, to obtain high crop production in modern agriculture in India**.** The economy of India is largely dependent on the quality and quantity of agricultural produce. Better harvest requires intensive cultivation, irrigation, fertilizers and more importantly pesticides to protect plants from pests and plant diseases. In India, 15–20% of agricultural production is negatively influenced by pests [1]. Groundnut (*Arachis hypogaea* L.) is one of the important major, profitable oil seed crop grown throughout the year in India [2]. Groundnut ranks seventh among crops in terms of insecticide consumption in India. It contributes to 41.3% of countries oil seed production [3]. Fungicides are playing a very important role in the elimination of pathogenic fungi. However, they are increasingly polluting agricultural soils because of the wide and indiscriminate use of these artificial chemicals in agriculture. Like other pesticides (anthropogenic factors) fungicides may not only hamper with the biochemical and

physiological reactions of the target plant pathogens but also influence the activities of micro organisms in soil [4, 5]. Currently more specific prominence has been given to soil enzymes in relation to reclamation management and the enzyme process which play a significant role in bioremediation. Soil contains microorganisms (bacteria, fungi, protozoans, algae) and excretes a variety of enzymes (dehydrogenases and phosphatases). Soil enzymatic measurements can be used to provide a "biological index" of soil fertility and as an indicator for many soil biological processes. There is a need for more detailed information regarding soil enzymes and their functions in soil systems especially in relation to inhibitory materials like pesticides that may alter the natural microbiological processes in soil and there by influence soil fertility. Although many studies have reported on the effects of pesticides on soil microorganisms and soil enzymes [6, 7, 8, 9], inconsistent trends are often found [8, 9, 10]. Chlorothalonil (2, 4, 5, 6-tetrachloroisopthalonitrile), a non-systemic widely used foliar fungicide for the control of plant pathogens causing broad spectrum of plant diseases in agricultural systems. Propiconazole is a systemic foliar fungicide with a broad range of activity. It is used on grasses grown for seed, mushrooms, corn, wild rice, peanuts, almonds, sorghum, oats, pecans, apricots, peaches, nectarines, plums and prunes.

Unlike other enzymes, dehydrogenase does not accumulate extracellularly in soil and are invariably linked to the variability of intact cells. Hence, its quantification has been recommended as a useful indicator for testing the side effects of agrochemicals [11]. The soil dehydrogenase system probably consists of different enzymes or enzyme systems, which have an important role in the initial stages of oxidation of soil organic matter**.** Dehydrogenase reduces 2, 3, 5 - triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). TTC is a water soluble and its redox potential is about -0.08v and function as an electron acceptor for several dehydrogenases [12]. Phosphatases find widely in bacteria's to mammals and indicate their importance in fundamental biochemical processes [13]. The term phosphatase in soil is used to describe a group of enzymes that are responsible for the hydrolytic cleavage of a variety of ester- phosphate bonds of organic phosphates and anhydrides of orthophosphoric acid (H_3PO_4) into inorganic phosphate. So far, little information is available on the behavior of chlorothalonil and propiconazole on soil enzymes. The objective of the study is to determine the behavior of chlorothalonil and propiconazole in both soils and to evaluate the responses of soil enzymes. This information will be useful for predicting the environmental fate of these widely used fungicides from continuous use and for understanding the potential adverse effects of intensive treatment with chlorothalonil and propiconazole on soil dehydrogenase and phosphatase activities.

EXPERIMENTAL SECTION

Soils

Two laterite and vertisol soils were collected randomly from different sites of groundnut cultivated fields of Anantapur district of Andhra Pradesh, India from a depth of 0-12 centimeters and mixed thoroughly to prepare a homogenate composite sample, air dried at room temperature samples were cleaned by removing plant material and other debris and passed through 2 millimeter sieve, stored at 4^{o}C prior to analysis. Mineral matter of soil samples was done by following the method [14]. Soil pH was determined by using 1:1.25 soil to water ratio in systronic digital pH meter. Organic matter in soil samples was estimated by Walkley-Black method, total nitrogen content in soil samples was determined by Micro-Kjeldhal method [15]. Electrical conductivity was measured by Conductivity Bridge method and contents of nitrite–nitrogen [16] by Brucine method [17]. The Physico-chemical properties of the two soils were furnished in Table 1.

Fungicides

To determine the influence of selected fungicides on soil enzyme activities, propiconazole (25% Emulsifying concentration*) and chlorothalonil (75% Wettable powder) was obtained from sergeant India Ltd 14, Mumbai-20.

Microbial assays

Dehydrogenase activity in soils (E.C. 1.1.1.1)

To determine the activity of dehydrogenase under the influence of two fungicides, at different concentrations in laterite and vertisol soils. A series of standard solutions of 2,3,5-triphenyltetrazolium chloride (TTC) covering the concentrations of 100–1000 µg per ml was prepared for calibration. To study the effect of two fungicides on dehydrogenase, 5 g of dried black and red soils were taken separately in test tubes (12 × 125 mm) containing different concentration of insecticides 10, 25, 50, 75, and 100 µg g^{-1} soil which are equal to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1} of field application rates. In order to maintain 60% water holding capacity (WHC), about 2 ml of deionized water was added to test tubes containing black soil and 1 ml into tubes containing red soil. Untreated soil samples served as controls. All the treatments, including controls were incubated in the dark at 28±4°C. After a

week triplicate soil samples were with drawn for the assay of dehydrogenase activity. The method employed for the assay of dehydrogenase was developed by Casida et al. [18]. This method is based on the reduction of 2, 3, 5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). Each soil sample was treated with 0.2 ml toluene, 0.1 g of $CaCO_3$ and 1 ml of 0.18 mM aqueous solution of TTC and incubated for 24 h at 30°C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a Spectronic-20D spectrophotometer. Further, the experiment was repeated with the stimulatory concentrations of pesticides (i.e. at 5.0 kg ha^{-1}) for 2, 3, 4 and 5 weeks to estimate the dehydrognase activity.

Phosphatase activity in soils (E.C. 3.1.6.1)

To assay the activity of phosphatase under the influence of two fungicides, at different concentrations was determined in latterite and vertisol soils. A series of standard solutions of *p*-nitro phenyl phosphate (PNPP) covering the concentrations of 100–1000 μg μl^{-1} was prepared for calibration. Two-gram portions of each soil, in triplicates, were treated with the selected pesticides at 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} concentrations. Soil samples without insecticide treatment were served as controls. Soil samples in test tubes with and without insecticide treatment were incubated at room temperature (28±4°C). After 10 days of incubation, soil extract was prepared in distilled water for the assay of phosphatases according to the method as described by Tabatabai [19] and Srinivasulu et al. [20]. Soil samples were transferred to 100 ml Erlenmeyer flasks, and 0.2 ml of toluene, 6 ml of 0.1 M maleate buffer (pH 6.5) and 2 ml of *p*-nitro- phenyl phosphate disodium salt were added. The flasks were swirled for a few seconds to mix the contents, stoppered and incubated at 37°C for 30 min. The reaction was stopped by adding 1 ml of 0.5 M $CaCl_2$ and 4 ml of 0.5 M NaOH followed by swirling the flask, for a few seconds, and the soil suspension was filtered through a Whatman No.1 filter paper. The liberated *p*-nitrophenol in the filtrate was determined at 410 nm in a Spectronic-20D spectrophotometer. Further, the experiment was repeated with the stimulatory concentrations of pesticides (i.e. at 2.5 or 5.0 kg ha^{-1}) for 20, 30 and 40 days to estimate the phosphatase activity.

Statistical analysis

The concentration of the dehydrogenase and phosphatase was calculated on the basis of soil weight (oven dried). Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range test (DMRT) Megharaj et al. [21] and Jaffer et al. [22]. The statistical analysis was performed at $P \leq 0.05$ using SYSTAT statistical software package.

RESULTS

Dehydrogenase activity

The effect of different concentrations of propiconazole and chlorothalonil on dehydrogenase activity is presented in Table 2. After a week days of incubation enzyme activity increased in all the treatments (1.0, 2.5, 5.0, 7.5, kg ha^{-1}) except at 10.0 kg ha^{-1} level. The maximum dehydrogenase activity was observed at 5.0 kg ha^{-1} (stimulatory) and lowest activity was noticed at 10.0 kg ha^{-1} level. The dehydrogenase activity was significantly enhanced at 5.0 kg ha^{-1} level in both soils for propiconazole and chlorothalonil showed individual increments of dehydrogenase activity ranged from a low increase 15 – 93%, 6–71% and 17 – 118%, 12 – 100% in comparison to control (Table 2). The stimulatory concentration (5.0 kg ha^{-1}) induces the highest enzymatic activity after 2, 3, 4, 5 weeks of incubation in both soils (Figure 1a, b). Further increase in the period of incubation decreased the rate of dehydrogenase activity after 3 weeks and then decline phase was started from 4 to 5 weeks of incubation (Figure 1a, b).

Phosphatase activity

Phosphatase activity (Table 3) showed a variable pattern in response to different fungicide concentration after 10 days of incubation. The activity of enzyme was increased under all the treatments (1.0, 2.5, 5.0, 7.5, kg ha^{-1}) except 10 kg ha^{-1} level compared to the controls in both soils. The maximum activity was observed at 5.0 kg ha^{-1} (stimulatory) and lowest activity was noticed at 10.0 kg ha^{-1} level (Table 3). The activity of phosphatase was significantly increased at 2.5 and 5.0 kg ha^{-1} of chlorothalonil and propiconazole. In the case of both fungicides (at 2.5 and 5.0 kg ha^{-1}) the phosphatase activity significantly enhanced and caused individual increments ranged from 2 – 25%, 5 – 22% and 7 -33%, 1 -23% in comparison to control after 10 days of incubation of both soils (Table 3). The stimulatory concentration induces the highest enzymatic activity after 20, 30 and 40 days of incubation in comparison with control in both soils (Figure 2a, b). Further increase in the incubation period (up to 40 days) the stimulatory concentration of fungicides decreased the rate of phosphatase activity after 30 days and then decline phase was started from 30 to 40 days of incubation (Figure 2a, b).

Table 1: Physico-chemical characteristics of the soil

Properties	Black Soil	Red Soil
Sand (%)	68.3	53.3
Silt (%)	22.7	27.1
Clay (%)	09.0	19.6
pH [a]	7.7	6.6
Water holding capacity (ml g^{-1} soil)	0.47	0.27
Electrical conductivity (m.mhos)	254	238
Organic matter [b] (%)	1.44	0.72
Total nitrogen [c] (%)	0.084	0.042
NH_4^+ - N (µg g^{-1} soil)[d]	7.96	7.01
NO_2^- - N (µg g^{-1} soil)[e]	0.48	0.32
NO_3^- - N (µg g^{-1} soil)[f]	0.98	0.76

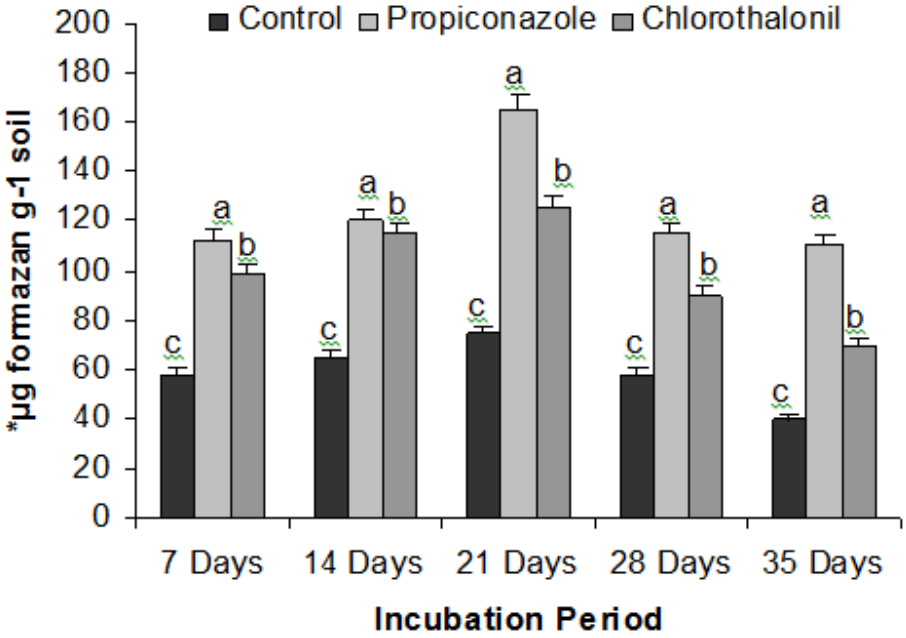
Where, a = 1:1.25 = Soil: Water slurry, b = Walkley-Black method (Jackson 1971)
c = Micro-Kjeldhal method (Jackson 1971), d = Nesslerization method (Jackson1971), e = Diazotization method (Barnes and Folkard 1951), f = Brucine method (Ranney and Bartlett 1972

Table 2: Activity of dehydrogease* under the impact of different concentrations of selected fungicides in black and red soils after 7 days

Concentration of fungicides (kg ha^{-1})	Black soil		Red soil	
	Propiconazole (Tilt)	Chlorothalonil (Kavach)	Propiconazole (Tilt)	Chlorothalonil (Kavach)
0.0	58 ± 8.165[e]	58 ± 8.165[e]	48 ± 1.633[e]	48 ± 1.633[e]
1.0	67 ± 1.633[d]	62 ± 1.633[c]	56 ± 1.633[d]	54 ± 3.266[c]
2.5	87 ± 1.633[b]	77 ± 1.633[b]	73 ± 2.449[b]	71 ± 0.816[b]
5.0	112 ± 3.266[a]	99 ± 4.899[a]	105 ± 4.082[a]	96 ± 3.266[a]
7.5	79 ± 2.867[c]	72 ± 1.633[b]	63 ± 2.449[c]	59 ± 2.449[c]
10.0	56 ± 3.266[e]	57 ± 1.633[d]	44 ± 3.266[e]	43 ± 2.449[d]

Each column is mean ±S.E. for six concentrations in each group; Columns not sharing a common letter (a, b, and c) differ significantly with each other (P ≤ 0.05; DMR test).

(a)



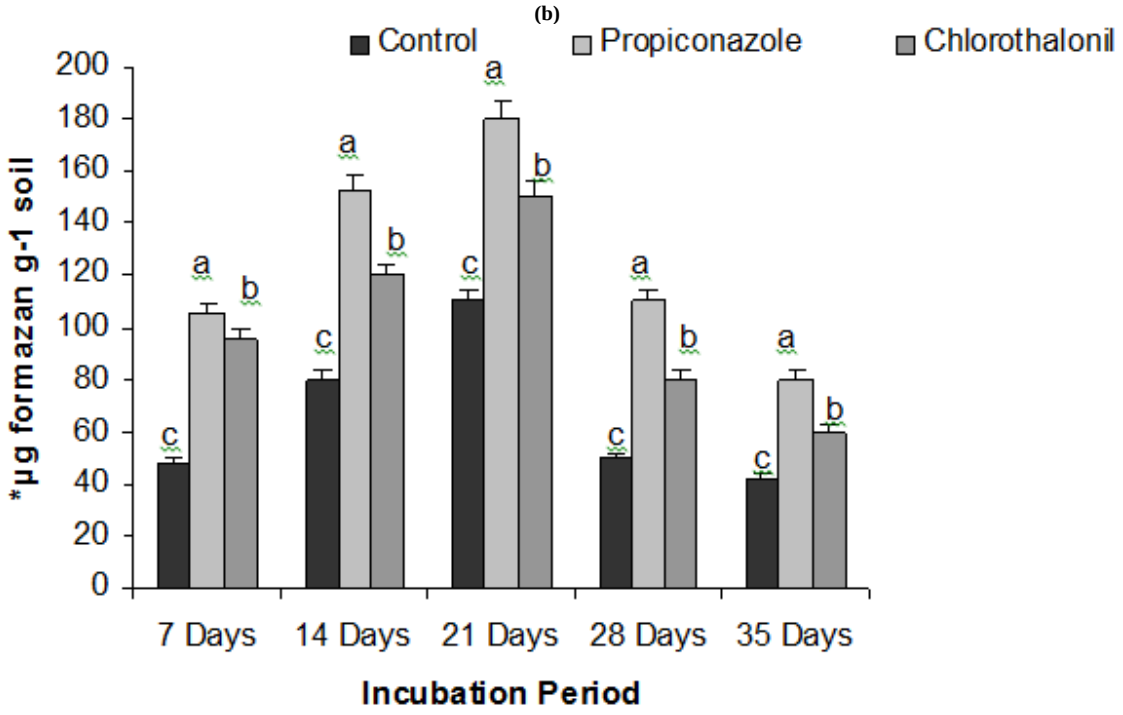
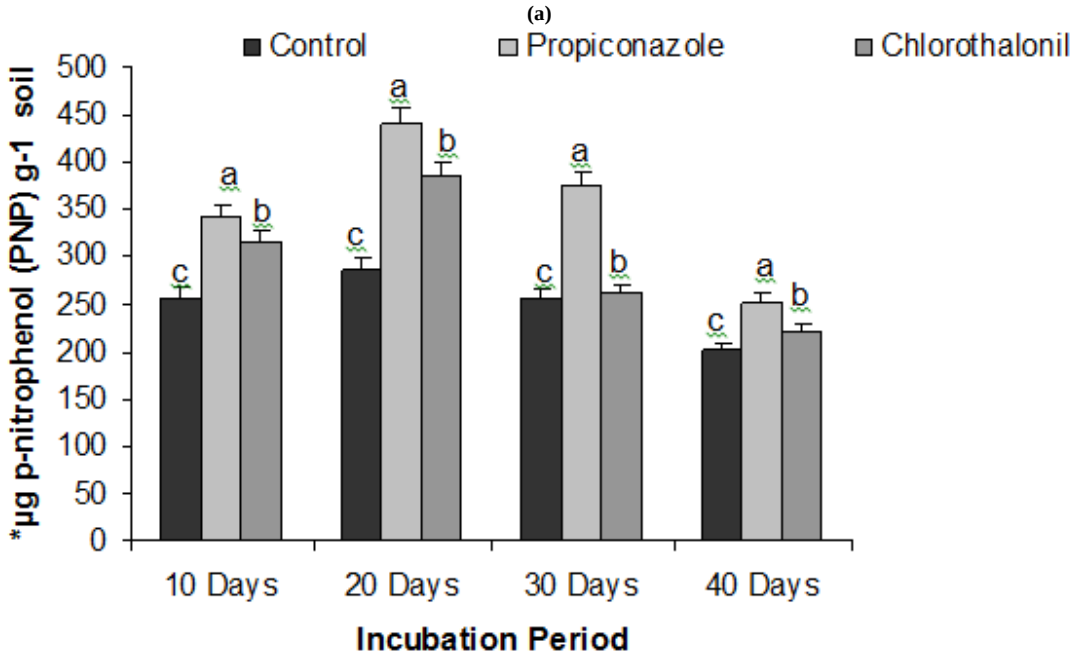


Figure 1. Effect of selected fungicides on dehydrogenase* in (a) laterite soil at 2.5 kg ha^{-1} and (b) vertisol soil at 2.5 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test. Bars in the figures represent means of three replicates



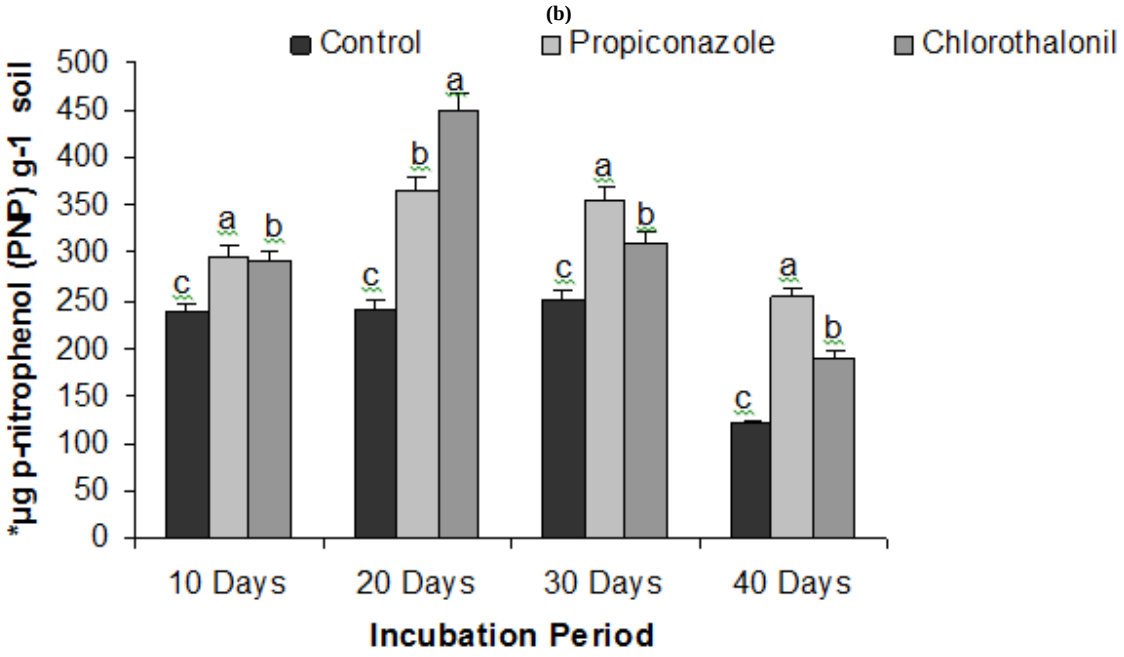


Figure 2. Effect of selected fungicides on phosphatase* in (a) laterite soil at 2.5 kg ha^{-1} and (b) vertisol soil at 2.5 kg ha^{-1}. Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test. Bars in the figures represent means of three replicates

Table 3. Activity of phosphatase* under the impact of different concentrations of selected fungicides in black and red soils incubated for 24 hours after 10 days

Concentration of fungicides (kg ha^{-1})	Black soil		Red soil	
	Propiconazole (Tilt)	Chlorothalonil (Kavach)	Propiconazole (Tilt)	Chlorothalonil (Kavach)
0.0	257.4 ± 8.164^{d}	257.4 ± 8.164^{c}	237.6 ± 8.164^{c}	237.6 ± 8.164^{c}
1.0	277.2 ± 8.164^{c}	259.8 ± 8.165^{c}	244.4 ± 16.330^{c}	250.8 ± 8.165^{c}
2.5	307.2 ± 4.967^{b}	316.8 ± 8.164^{a}	261.3 ± 8.165^{b}	290.4 ± 8.165^{a}
5.0	343.2 ± 6.329^{a}	270.6 ± 8.165^{b}	297.0 ± 8.165^{a}	260.0 ± 8.165^{b}
7.5	270.6 ± 8.165^{c}	261.5 ± 8.165^{c}	261.5 ± 8.165^{b}	241.6 ± 8.165^{d}
10.0	231.0 ± 8.165^{e}	217.8 ± 8.164^{d}	211.2 ± 8.576^{d}	204.6 ± 8.165^{f}

Each column is mean ± S.E. for six concentrations in each group; Columns not sharing a common letter (a, b, and c) differ significantly with each other ($P \leq 0.05$; DMR test).

DISCUSSION

The laterite and vertisol soils were predominantly used for the cultivation of groundnut (*Arachis hypogaea* L.) in the Anatapur district of Andhra Pradesh, India. Persistence of pesticide residues in the soil may have a significant impact on soil microbial communities and their functions such as the activity of enzymes, which are directly related to soil health and fertility and also to the removal of contaminants [23, 24]. Hence these soils were selected to study the effect of fungicides. The microbial characteristics were adversely affected by chlorothalonil treatment, these findings are in agreement with the results of previous studies [25, 26] in which dehydrogenase and phosphatase activity were reduced following application of chlorothalonil. The dehydrogenase activity was not affected significantly by benomyl at 20-200 ppm in soil [27]. In contrast Gowda [28] and Vyas [29], reported inhibition of dehydrogenase activity of peptone-amended soil by benomyl at 100 and 1000 µg g^{-1} and 20-200 ppm respectively. Similarly carbaryl at 10, 50, and 100 ppm, quinalphos at 25 ppm, carbofuran and quintazene at 100 ppm was known to inhibit dehydrogenase activity [30, 31]. Tu [32] found inhibition in the activity of dehydrogenase after application of captan, mancozeb and thiram fungicides in soil. However, our results contrast with recent reports [33] that chlorothalonil stimulate dehydrogenase activity. The phosphatase activity was stimulated at 50 ppm by fungicides in soils was noticed by several researchers [31, 6, 32, 34]. These observations were well in agreement with the results of the present study. Unlike the above results , the application of fungicides, benomyl (a systemic fungicide) and two

contact fungicides (copperoxy chloride and dithane M-45) even at low concentrations (0.37 kg ha^{-1} and 7.4 kg ha^{-1}) inhibited the phosphatase activity in potato field soils, throughout the incubation period (0, 15, 30, 45, and 60 days) [35]. Similarly, application of quintozene at 10 ppm in red soil incubated fro 1 and 15 days, inhibited enzyme activity in soil [31]. In our present investigation, phosphatase activity was significantly inhibited at higher concentrations of 7.5 kg ha^{-1} and 10.0 kg ha^{-1} in both fungicidal treatments (Table 2 and 3). Similarly, few workers [31, 36, 37] observed inhibition of phosphatase activity at higher concentrations of the fungicides.

CONCLUSION

The results obtained in the present study clearly indicate that the fungicides chlorothalonil and propiconazole profoundly enhanced the activities of both dehydrogenase and phosphatase at 2.5 to 5.0 kg ha^{-1}. Based on these results, it is concluded that the microbial activities (i.e., enzyme activities) were not affected by the fungicides applied at recommended levels in agricultural system to control pathogenic fungi.

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