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Response of the Microbial Activity to Fosetyl-Aluminium and Copper Oxychloride (Fungicides) in Groundnut (*Arachis hypogaea* L.) Soils

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ABSTRACT

The evaluation of the adverse effect of Fungicides on the microbial community and the soil enzyme activity were evidenced in soil amended with fosetyl-aluminium and copper oxychloride (Fungicides) in groundnut (*Arachis hypogaea* L.) soils. Variations in activity were independent upon the period of incubation. A fungicide disturbs the activities of soil enzymes and soil micro biota. So we investigated in laboratory conditions that the effect of two fungicides, fosetyl-aluminium and copper oxychloride on enzyme activities, such as protease in two soils collected from groundnut (*Arachis hypogaea* L.) cultivated fields of Anantapuram district of Andhra Pradesh, India, by conducting experiments at different concentrations (10, 25, 50, 75 and 100 ppm) which are equivalent to field application rates (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}). In our present study we observed, protease activities were significantly enhanced at 2.5 and 5.0 kg ha^{-1} in black and red soils after 10 days of incubation. Furthermore increase in concentration of fungicides and decreased the rate of enzyme activities. However, the stimulatory effect was continued up to 20 days of incubation in black and red soils. Whereas, the decline phase was started after 20 days and the minimum enzyme activities were noticed at the end of 40 days of incubation. But higher concentrations of fungicides at the level of 7.5 to 10.0 kg ha^{-1} were either toxic or innocuous to protease activity in black and red soils. An increasing trend in the activity of microbial population and soil enzyme activities were observed during 20 days of incubation in both control and treated soil with fungicides. These fluctuations in activities were in accordance to impact of selected fungicides in groundnut (*Arachis hypogaea* L.) soils.

Keywords: Microbial activity , Protease enzyme, fungicides (fosetyl-aluminium and copper oxychloride and groundnut (*Arachis hypogaea* L.) soils

I. INTRODUCTION

India is an agriculture based country, where more than 60% of population is depend on agriculture [1]. Agricultural soils harbour enormous microbial diversity. The soil environment is very complex and provides diverse microbial habitats. Microbes exist throughout the soil profile; however, they are most abundant in surface soils, the rhizosphere of plants, and around macropores [2 and 3]. Hence, soil microbial abundance and diversity are highest in the top 10 cm and decline with depth. In soil microbial communities, maintaining critical functions may ultimately be more important than maintaining taxonomic diversity. One essential microbial function in soils is the processing and recovery of key nutrients from detrital inputs and accumulated soil organic matter. The field of soil enzymology, including numerous methods and applications, has been extensively reviewed [4 and 5]. Soil enzyme activities have been related to soil physio-chemical characters [6], microbial community structure [7 and 8], vegetation [7 and 9], disturbance [10, 11, 12 and 13] and succession [14]. Protection of farm and agricultural lands means protection of all forms of life.

Groundnut (*Arachis hypogaea* L.) is the most important oilseed crop grown in India. Globally India ranks first in area and second in production. Groundnut plays a significant role in the livelihoods of smallholder farmers of rain fed area. Rain fed groundnut cultivation coupled with attack by a variety of insect, pests and diseases are the major reason for lower productivity. Pesticides are widely used against a range of pests infesting agricultural crops. Globally, about 3×10^9 kg of pesticides is applied annually with a purchase price of nearly $40 billions each year [15]. The term "pesticide" covers a wide range of compounds including insecticides, fungicides, herbicides, rodenticides, molluscacides, nematocides, plant growth regulators and others. The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the soil environment [17 and 16]. With the growing use of pesticides in contemporary agriculture, the issue of the impact of these chemicals on the composition of soil microorganisms and the processes they direct has received more attention [18 and 19]. The applied pesticides may harm the indigenous microorganisms, disturb soil ecosystem, and thus, may affect human health by entering in the food chain. Adverse impacts of pesticides on soil microbial diversity and activities have been described by many researchers [20, 21, 22 and 23]. Similarly, pesticides also influences soil biochemical processes driven by microbial and enzymatic reactions. The microbial mineralization of organic compounds and associated bio transformations such as nutrient dynamics and their bioavailability are also more or less adversely affected by the pesticides [24 and 25]. The applied pesticides also reduce soil enzymatic activities that act as a ‘‘biological index’’ of soil fertility and biological processes in the soil environment [26].

There are also reports documenting the ability of soil microorganisms to degrade pesticides in the soil environment [27, 28, 29 and 30]. The degradation products of these pesticides are assimilated by soil microorganisms [31] resulting in increased population sizes and activities of microorganisms [32 and 33]. Recently, molecular techniques have been used to elucidate the impact of pesticides on microbial community structure and functioning [34]. Here, we attempt to describe recent advances in the impact of pesticides on soil microorganisms and soil enzymes in groundnut soils.

II. METHODS AND MATERIALS

A. Soils used in the present study

Black clay soil and Red sandy loam soil samples, with a known history of Fungicides used were collected from fields of groundnut cultivated area of Anantapuramu District, Andhra Pradesh, India. The collected soil samples were chosen from a depth of 12 cm mixed, air-dried, and sieved through a 2-mm mesh prior to use. Two soil samples, a black clay soil and red sandy loam soil were used in the present study.

B. Analysis of Physico-chemical properties of soils

For soil sample characterization, selected physical and chemical properties were determined by using the well established laboratory procedures. Potential for hydrogen ion (pH), of the soil samples was determined by mixing soil and water in the ratio of 1:1.25 using Systronic digital pH meter with calomel glass electrode assembly. The electrical conductivity of soil samples after addition of 100 ml distilled water to 1 g soil samples was measured by a conductivity bridge. Water-holding capacity (WHC) of the soil samples was determined by adding distilled water up to the saturation point and then 60 % water-holding capacity of the soil samples was calculated by Johnson and Ulrich [35]. Mineral matter of soil samples such as sand, silt and clay contents were analyzed with the use of different sizes of sieves by following the method of Alexander [36]. Organic carbon content in soil samples was estimated by Walkley Black method and the organic matter was calculated by multiplying the values with 1.72 as per Johnson and Ulrich [37]. The total nitrogen content in soil samples was determined by the Micro-Kjeldhal method reported by Johnson and Ulrich [37]. The inorganic ammonium

nitrogen content in soil samples after extraction of 1 M KCl by the Nesslerization method [37] and the contents of nitrite nitrogen were determined by the method reported by Barnes and Folkard [38], and the contents of nitrate-nitrogen by Brucine method [39] after extraction with distilled water were determined. Physico- Chemical characters of the two soil samples are listed in Table1.

C. Fungicides used in the present study

To determine the influence of selected fungicides on the groundnut soil, the commercial grades of fosetyl-Al (95 %) were obtained from Bayer Crop Science (Germany) and copper oxychloride (50%) were obtained from Dhanuka Agritech Limited .

D. Soil Incubation Studies

Protease Activity:

For determination of protease activity, two grams of soil samples were distributed in test tubes (12 x 150 mm) and treated with the selected fungicides to provide a final concentration of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} level. All the treatments including control were incubated in the laboratory at room temperature (28 ± 4°C).Ten days after incubation, triplicate soil samples were withdrawn for the assay of protease as adopted by Speir and Ross [40] also similar method was followed by Jayamadhuri [41 and 42].

Assay of Protease:

Soil samples including controls were incubated with 10 ml of 0.1 M tris (2- amino-2 (hydroxyl methyl) propane-1:3-diol) (pH- 7.5) containing sodium caseinate (2%w/v) for 24 hrs at 30° C. An aqueous solution of tri-chloro acetic acid (4 ml, 17.5% w/w) was then added and the mixer was centrifuged. The supernatant liquid, in suitable aliquots, was treated with 3 ml of 1.4 M Na_2CO_3 and 1 ml of Folin-ciocalteau reagent (33.3% v/v) with rapid swirling. The blue colour, thus formed after 30 minutes, was read at 700 nm in a U V visible Spectrophotometer (Thermo scientific). Tyrosine equivalents in soil extracts were estimated by referring to a calibration curve prepared with known concentration of tyrosine.

E. Statistical Analysis

The concentrations of the protease enzyme were calculated on soil weight (over dried) basis. The fungicide treatments with untreated controls and the significant levels $P \leq 0.05$ between values of each sampling, each fungicide were performed using SYSTAT statistical software package to find the results of Duncan's Multiple Range (DMR) test Megharaj [43].

III. RESULTS AND DISCUSSION

The black and red sandy loam soils are used for the cultivation of groundnut (Arachis hypogaea L.) in Anantapuramu district of Andhra Pradesh, India. The major limitations in the groundnut crop production are insects and fungi pests. Because of this reason pesticides are frequently used for crop protection. Continuous and indiscriminate use of these fungicides causes a major risk of soil health. Hence, these soils were selected to study the effect of fungicides on enzyme activities. In general, the organic matter content is high in black soil it leads to pronounced more activity in black soil than in red soil under the influence of fungicides. There have been many reports of the effects of fungicides on soil enzyme activities [44 and 45] and it has been observed that the responses of soil enzymes on different fungicides are not the same. Soil enzyme activities are more sensitive to the environment. They reflect the soil quality more quickly and directly [46]. Since enzyme activity has been considered as a very sensitive indicator, any disturbance due to biotic or environmental stresses in the soil ecosystem may affect soil biological properties. Our analysis revealed that protease activity was significantly increased in both soils by both fungicides from 1.0 to 5.0 kg ha-1

whereas the activity was decreased at higher concentrations (7.5–10.0 kg ha-1) of fungicides at 24 hrs. as shown in Table 2 and 3 respectively when compared to control in both black and red soils.

Table 1: Physico-chemical properties of soils used in the present study

Properties	Black soil	Red soil
Sand (%)	**80.2**	**63.6**
Silt (%)	**13.4**	**23.3**
Clay (%)	**6.4**	**13.1**
pH[a]	**8.0**	**7.5**
Water holding capacity (mlg^{-1} soil)	**0.45**	**0.33**
Electric conductivity (m.mhos)	**264**	**228**
Organic matter[b](%)	**1.85**	**0.054**
Total nitrogen[c](%)	**0.087**	**0.045**
NH_4^+- N (µg g^{-1} soil)[d]	**8.42**	**6.69**
NO_2^-- N (µg g^{-1} soil)[e]	**0.56**	**0.41**
NO_3^-- N (µg g^{-1} soil)[f]	**0.92**	**0.81**

[a]1:1.25 (Soil:Water)
[b]Walkley-Black method (Jackson, 1971)
[c]Micro-Kjeldhal method (Jackson, 1971)
[d]Nesslerization method (Jackson, 1971)
[e]Diazotization method (Barnes and Folkard, 1951)
[f]Brucine method (Ranney and Bartler, 1972)

Table 2. Influence of fosetyl-Al and copper oxychloride (fungicides) on soil enzyme of protease activity* in black clay soil after 10 days incubation.

Fungicide concentration (kg ha^{-1})	fosetyl-Al	copper oxychloride
0.0	200±1.94 a (100)	200±2.42 a (100)
1.0	300±1.72 b (150)	300±2.62 b (150)
2.5	370±1.29 c (185)	350±0.85 c (175)
5.0	400±1.93 d (200)	390±2.48 d (195)
7.5	350±2.56 c (175)	305±1.91 b (153)
10.0	180±3.62 a (90)	190±0.74 a (95)

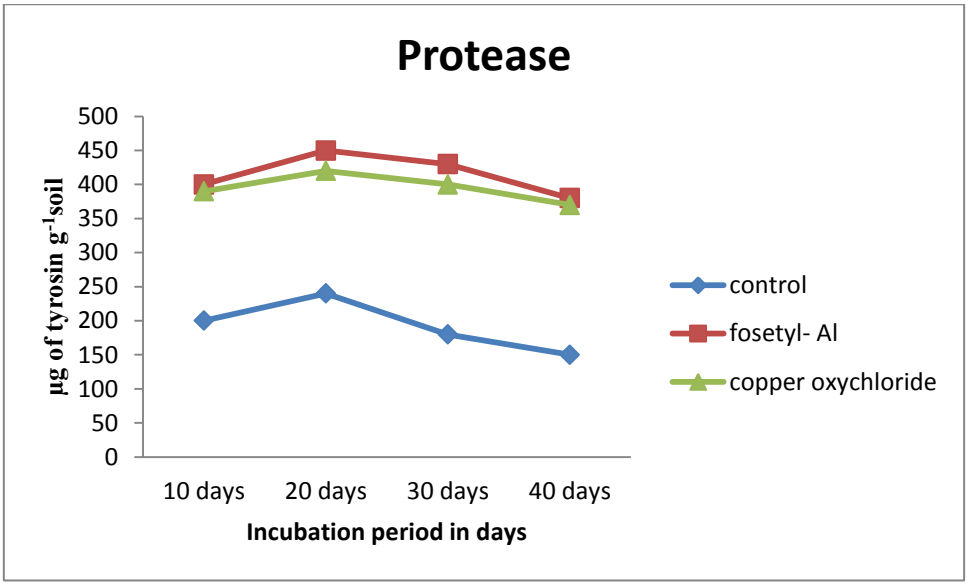
*µg of tyrosine g^{-1} soil formed after 24 hours incubation with sodium casinate.
Figures, in parentheses indicate relative production percentages.
Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test.

Table3: Influence of fosetyl-Al and copper oxychloride (fungicides) on soil enzyme of protease activity* in red sandy loam soil after 10 days incubation.

Fungicide concentration (kg h^{-1})	fosetyl-Al	copper oxychloride
0.0	180±1.94 a (100)	180±2.42 a (100)
1.0	240±1.72 b (133)	220±2.62 c (122)
2.5	280±1.29 c (156)	260±0.85 b (144)
5.0	300±1.93 d (167)	290±2.48 a (161)
7.5	260±2.56 c (144)	250±1.91 e (139)
10.0	160±3.62 a (89)	165±0.74 f (92)

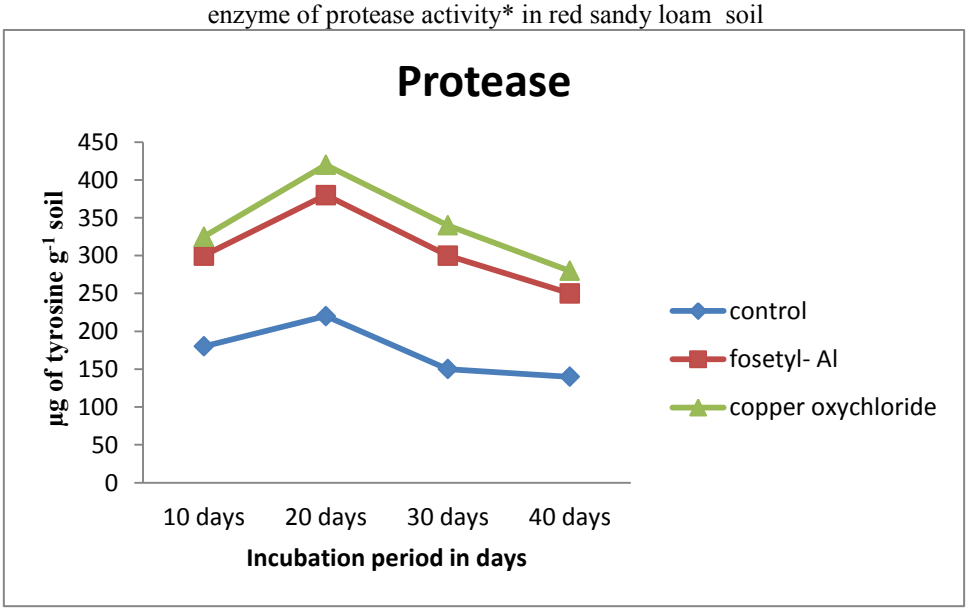
*µg of tyrosine g^{-1} soil formed after 24 hours incubation with sodium casinate.
Figures, in parentheses indicate relative production percentages.
Means, in each column, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test

Figure 1. Influence of fosetyl-Al and copper oxychloride (fungicides) at 5.0 Kg ha^{-1} on soil enzyme of protease activity* in black clay soil



*µg of tyrosine g^{-1} soil formed after 24 hours incubation with sodium casinate.
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

Figure 2. Influence of fosetyl-Al and copper oxychloride (fungicides) at 5.0 Kg ha^{-1} on soil enzyme of protease activity* in red sandy loam soil



*µg of tyrosine g^{-1} soil formed after 24 hours incubation with sodium casinate.
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

Protease enzymes contribute to the breakdown of proteinaceous substances in soil to simpler nitrogen compounds that are available for plant nutrition. It has been shown that proteases in soil can hydrolyse not only added [47] but also native soil proteins and peptides [48]. Proteases, which are widely distributed

among soils, show a wide range of activities [49] and properties [50]. Discharge of effluents from sugar industry, enhanced the soil protease activity but it declined with the time. In a study of Rangaswamy et al. [51], insecticides, monocrotophos and quinalphos of organophosphates and cypermethrin and fenvalerate of pyrethroid within a range of 2.5 kg ha-1 signifcantly stimulated the protease activity in a soil but these insecticides at higher concentration were toxic to the protease activity. Protease activity drastically decreased at higher concentrations (5.0, 7.5 and 10.0 kg ha-1) of endosulfan and profenophos treated soils than the untreated controls throughout the experiment, suggesting that the enzyme is rather sensitive to endosulfan and profenophos. Interestingly, a stimulatory effect was observed at 10-25 ppm concentrations with individual increments of two insecticidal treatments, than the control, they are as follows: 13-47% and 2-15% in black clay soil afer10 days of incubation. This trend follows up to 20 days of incubation, when further prolonged in the period of incubation up to 40 days; a decline in enzyme activity was observed [52]. The impact of different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}) of two selective fungicides, propiconazole and chlorothalonil on protease activity has been studied in two groundnut soils (laterite and vertisol) supplemented with 1% casein. Interestingly, stimulatory effect was observed with all concentrations tested at 10-day incubation period in both soils. The percentages of increasing in protease activity of the two fungicidal treatments, over control are as follows: 14-47% and 2-15% in laterite soil and 7-65%, 17-48% in vertisol soil respectively at 10-day interval over control (fungicides treated at 10, 25, 50 ppm level). However, stimulatory effect was more pronounced at 5.0 kg ha-1 of propiconazole and chlorothalonil in both soils incubated for 10-days. This trend follows up to 20 days of incubation further prolong in period of incubation up to 40 days decline in enzyme activity was observed [53]. From the above results, about 10–50% and 5-50% increase in protease activity over the control was noticed in the black soil with 20 days of incubation, whereas in the case of red sandy loam soil, the corresponding table (3) of the percentage enhancement by the two selected fungicides at two levels were 11–33% and 8–22% during the same period of incubation (10 days). In comparison, fosetyl-aluminium and copper oxychloride, at 5.0 kg ha-1 produced maximum stimulation in protease activity in black clay soil than red sandy loam soil. At higher concentrations, i.e., 7.5 and 10.0 kg ha-1, protease activity was significantly inhibited by treating the selected soil samples with both the selected fungicides. Among the two fungicides treatments fosetyl-aluminium and copper oxychloride, produced a different stimulation over the control. In the present study, comparatively, black soil showed higher enzyme activity than red soil throughout the experiment (table 2 and 3).

IV. CONCLUSIONS

The results obtained in the present study clearly indicate that the fungicides fosetyl-aluminium and copper oxychloride profoundly enhanced the activitiy of protease at field application rates. On the basis of these results, it is concluded that the microbial activities (i.e., enzyme activities) were increased by the fungicides applied at recommended levels in agricultural system, which are used for control of insect pests. Therefore, we are concluded that the observed stimulation or inhibition of this enzyme at low or high concentrations of the fungicides could be attributed to number of populations of proteolytic organisms present in both soils, the protease activity in both soils is profoundly increased in both fungicide concentrations 5.0 kg ha-1 at 24 hrs, at higher contractions (7.5–10.0 kg ha-1) a suppressed activity in the protease enzyme with individual treatments of fungicides compared to control, the fungicides fosetyl-aluminium and copper oxychloride are as an important agents for the control of plant pathogens.

Fosetyl-aluminium and copper oxychloride is often not used at much higher than the recommended dosage in order to maintain soil health, A very few reports are available on the influence of selected fungicides on protease enzyme. The results of the present study shows that the enzyme activity gradually decreased as incubation period increases (Figure 1 and 2).

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