



Activity of soil protease enzyme as influenced by dimethomorph and pyraclostrobin fungicides to groundnut (*Arachis hypogaea* L.) soil.

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ABSTRACT

Soil microbial diversity is indispensable to maintain functional diversity and enzyme-mediated critical soil processes that detoxify soil from environmental pollutants, like pesticides. Thus, the present study was carried out to assess the effect of different concentrations of the fungicides on the activity of protease, in clay and sandy loam groundnut soils of Anantapuramu District of Andhra Pradesh, India. The use of pesticides in agriculture has highly increased during the last 40 years to increase crop yields. However, today most pesticides are polluting water, soil, atmosphere and food. Pesticides also impact soil enzymes, which are essential catalysts ruling the quality of soil life. A laboratory experiment was conducted to study the effect of selected fungicides, dimethomorph and pyraclostrobin, at concentrations ranging from 0 to 10 kg ha⁻¹ on the activity of protease. The activities of protease were significantly more at pyraclostrobin and dimethomorph levels of 5.0 and 2.5 kg ha⁻¹, respectively. But at higher concentrations of 7.5 and 10 kg ha⁻¹ respectively, dimethomorph and pyraclostrobin were toxic to protease activities. In soil samples receiving 2.5-5.0 kg ha⁻¹ of the fungicides, the accumulation of reducing sugar was pronounced more at 20 days, and the activity of the protease was drastically decreased with increasing period of incubation up to 30 and 40 days. The results of the present study thus, clearly, indicate that application of the Fungicides in cultivation of groundnut, at field application rates improved the activity of protease in soils.

INTRODUCTION:

Agriculture happens to be the backbone of Indian economy. It comprises major share in gross domestic production (GDP) and this sector is the largest employer in economy. On the contrary, 15-20 % of all crops are destroyed by pests every year (Bhalerao and puranik., 2009). Modern day agriculture greatly relies on the use of different group of pesticides in order to manage the crop pests and to gain a better crop yield (Quazi, R. Datta, D Sarkar., 2011). Agricultural management practices (e.g. crop rotation, mulching, burning, tillage and application of fertilizers and pesticides) have diverse effects on the various soil enzyme activities (Ajwa *et al.*, 1999, Bandick and Dick 1999, Aon and Colaneri 2001, Xu *et al.*, 2002). Groundnut (*Arachis hypogaea* L.) is one of the vastly produced oilseed crop, cultivated in more than 100 countries in the world, referred as a universal crop. India is one of the largest producers and consumer of groundnut in the world. Groundnut crop has major threat of pathogen infection that affect the groundnut production process from planting to storage (Singh & Oswalt, 1992). The application of fungicides to soil to control plant diseases has become a common practice in crop production in many parts of the world. Pesticides can be classified in a number of ways: by target pest, their mode of action or chemical family. Several studies have shown that chemical products used in farming (e.g., fertilisers, pesticides, herbicides) have a marked influence on the soil enzyme activity (Alvear *et al.*, 2006). The main chemical groups are organochlorine, organophosphate, carbamate, pyrethroids, triazine and sulfonylurea (Afify *et al.*, 2010). In India,

Anantapuramu District, a semiarid region of Andhra Pradesh, ranks first in the area of groundnut (*Arachis hypogaea* L.) cultivation, but its productivity is low and fluctuates around 9q/ha⁻¹ on average (Anonymous 2014). Enzymes in soils mainly come from plants, soil animals and microorganisms and connected covalently, crosslinked, copolymerized, adsorbed and included in the microcapsules of soil particles (Girish S., Ajit V., 2011). Soil enzyme activities vary seasonally and have been related to soil physico-chemical characters, microbial community structure, vegetation, disturbance and succession (Caldwell B.A. 2005). The biological and chemical properties of soil are very important in the biogeochemical cycles of nutrients, enzymes produced by soil microorganisms being responsible with biochemical transformations. Like other pesticides, fungicides are bio toxicants which interfere not only with the biochemical and physiological reactions of the target plant pathogens but may also influence populations or activity of other non target organisms in soil (Tu, C.M., 1992). Due to soil enzymes considered sensitive to disturbances in ecosystem (Zwikel *et al.*, 2007), their activities were increased to different degrees by organic manure incorporation and gave a significant and positive relationship of enzyme activity with organic C and total N (Nayak *et al.*, 2007). The physicochemical nature of the soil is important for persistence, metabolism and binding of pesticides in the soil (Burns, R.G 1982). An ideal pesticide should be toxic to the only target organism, biodegradable and should not leach in to ground water. The presence of enzymes in soil depends on their continuous release by organisms inhabiting the soil ecosystem (Burns, 1982; Han *et al.*, 2010). The study of enzyme activity of soils may reflect the potential capacity of a soil to perform certain biological transformations of importance to soil fertility. The present work thus aimed at finding out the effect of very widely used fungicides in Indian agricultural system on activity of various soil enzymes at the recommended and higher dosages. The aim of the study was to determine the effects of the fungicides on the activity of soil enzyme protease.

SCOPE OF THE STUDY

The objective of the study is to determine the behavior of dimethomorph and pyraclostrobin 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 for continuous use and for understanding the potential adverse effects of intensive treatment with

dimethomorph and pyraclostrobin on soil protease activity.

II. MATERIALS AND METHODS

2.1 Soils used in the present study:

Agricultural soil samples such as samples of black clay soil and red sandy loam soil, collected from groundnut cultivated fields of Anantapuramu District of Andhra Pradesh, India in a semi arid zone from the depth of 12 cm and mixed thoroughly to prepare a homogenate composite sample. Soil samples air dried at room temperature, were cleaned by removing plant material and other debris and passed through a 2 mm sieve and stored at 4°C prior to analysis.

2.2 Analysis of Physico- chemical characteristics of soil samples:

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 (1961). Water holding capacity of the soil samples were 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 (1960). pH of soil samples was determined by mixing soil and water in the ratio of 1:1.25 using systronics digital pH meter with calomel glass electrode. Organic carbon content in soil samples was estimated by Walkey-Black method and the organic matter was calculated by multiplying the values with 1.72 (Jackson, 1971). Electrical conductivity of soil samples was measured by a conductivity bridge. Total nitrogen content in soil samples was determined by the method (Jackson, 1971). The inorganic ammonium nitrogen content in the soil samples after extraction of 1M KCl by Nesslerization method (Jackson, 1971) and contents of nitrite nitrogen (Barnes and Folkard, 1951), and the contents of nitrate-nitrogen by Brucine method (Ranney and Bartlett, 1972) after extraction with distilled water were determined respectively.

Physico-chemical characters of the two soil samples are listed in table 1.

2.3 Table1 Physico-chemical properties of the soils

Properties	Black clay soil	Red sandy loam soil
Sand (%)	66.4	52.4
Silt (%)	23.6	26.9
Clay (%)	9.3	19.4
pH ^a	7.9	7.1
Water holding capacity (ml g ⁻¹ soil)	0.45	0.32
Organic matter (%) b	1.44	0.74
Total nitrogen (%) ^c	0.089	0.048
NH ⁺ ₄ -N (µg g ⁻¹ soil) ^d	8.57	7.02
NO ⁻ ₂ -N (µg g ⁻¹ soil) ^e	0.45	0.66
NO ⁻ ₃ -N (µg g ⁻¹ soil) ^f	0.92	0.74

^a1:1.25 = soil: water slurry

^b Walkley- Black method (Johnson and Ulrich, 1960)

^c Micro-Kjeldahl method (Johnson and Ulrich, 1960)

^d Nesslerization method (Johnson and ulrich, 1960)

^e Diazotization method (Ranney and Bartlett, 1972)

^f Brucine method (Barnes and Folkard, 1951)

Fungicides used in the present study :

To determine the influence of selected fungicides on soil enzyme activities, Pyraclostrobin Chemical Name (IUPAC): methyl *N*-(2-{{[1-(4-chlorophenyl)-1H-pyrazol-3-yl]oxymethyl}phenyl)-*N*-methoxy carbamate. Pyraclostrobin is very effective against leaf spot diseases (18), but high rates (0.21 to 0.27 kg /ha) are also recommended for control of stem rot (Kemerait, B., Brenneman, T., and Culbreath, A. 2006). Dimethomorph (Chemical Name: 4-(3-(4-chlorophenyl)-3-(3,4-dimethoxy phenyl) acryloyl)morpholine). Therefore, this study aimed to induce systemic resistance in groundnut by foliar application of fungicides to protect the crop from opportunistic pathogen and yield losses. commercial fungicides were dissolved in distilled water.

III. 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 fungicide to provide final concentration of 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 water holding capacity (WHC), about 2 MI of deionized water was added to test tubes containing black soil and 1 MI into tubes containing red soil. Untreated soil samples served as control. All the test tubes including controls were incubated in the laboratory at room temperature (28 ± 4°C) for 10, 20, 30 and 40 days. After incubation, triplicate soil samples were withdrawn for the assay of protease as adopted by Speir and Ross 1975, also similar method was followed by Rangaswamy *et al.* and Jayamadhuri 2004

Assay of soil 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 trichloro acetic acid (4ml, 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 Na₂CO₃ and 1 ml of Folin-Ciocalteu reagent (33.3% v/v) with rapid swirling. The blue colour, thus formed after 30 minutes, was read at 700 nm in a Spectrophotometer. Tyrosine equivalents in soil extracts were estimated by referring to a calibration curve prepared with known concentration of tyrosine.

Statistical analysis

The concentration of protease was calculated on the basis of soil weight (oven dried). Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test (DMRT) (Jaffer *et al.*, 2010). All statistical analysis was performed at using SYSTAT Statistical software package.

RESULTS AND DISCUSSION

Groundnut (*Arachis hypogaea L.*) is predominantly grown in both black and red clay soils of Anantapuramu district, Andhra Pradesh, India. Proteases in soil plays a significant role in nitrogen mineralization, more active in soils with a high water and humus content forest soils and landfills. Activity of this enzyme does not depend on microbial community of soils and expresses the biological activity of the soil. During last decades, pesticides were increasingly used in agriculture in order to limit crop diseases and increase food production. Over the years, increased crop productivity has lead into the use of many kinds of fungicides. Constant and indiscriminate use of these

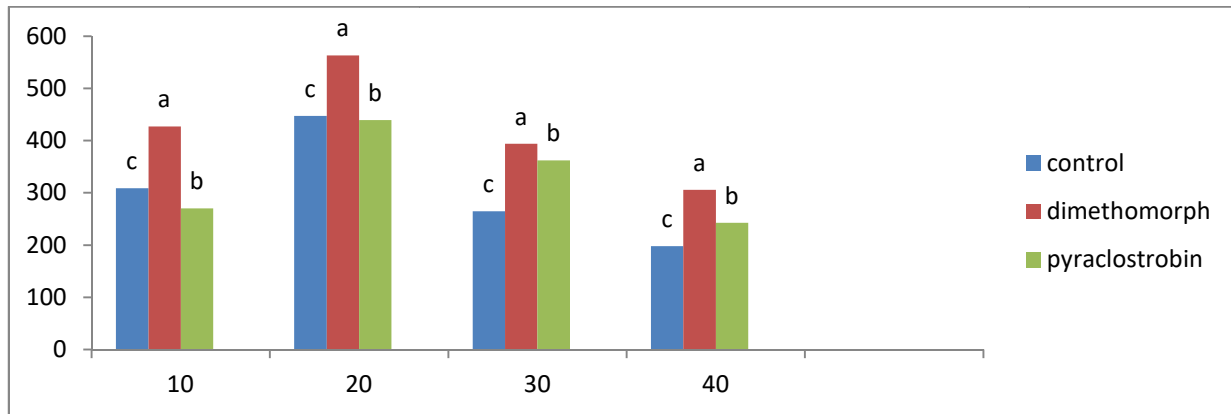
fungicides poses greater risk to soil health. Our analysis revealed that protease activity was depressed at higher concentrations (7.5 and 10.0 kg ha⁻¹) of dimethomorph and pyraclostrobin treated soils than untreated controls throughout the experiment (Table 2) suggesting that the enzyme is rather sensitive to dimethomorph and pyraclostrobin. The activities of protease were significantly more at pyraclostrobin and dimethomorph levels of 5.0 and 2.5 kg ha⁻¹, respectively. But at higher concentrations of 7.5 and 10 kg ha⁻¹ respectively, dimethomorph and pyraclostrobin were toxic to protease activities. In soil samples receiving 2.5-5.0 kg ha⁻¹ of the fungicides, the accumulation of reducing sugar was pronounced more at 20 days, and the activity of the protease was drastically decreased with increasing period of incubation up to 30 and 40 days. The results of the present study thus, clearly, indicate that application of the Fungicides in cultivation of groundnut, at field application rates improved the activity of protease in soils. The stimulatory concentration was noticed for dimethomorph and pyraclostrobin 2.5 and 5.0 kg ha⁻¹. Rasool and Zafar Reshi (2010) noticed the stimulatory effect on protease activity in comparison with the control up to 21 days of incubation. Similar observations on protease activity in soil treated with monocrototphos, quinalphos, cypermethrin and fenvelerate up to 25 ppm level was recorded by Rangaswamy *et al.*, (1994). Endo *et al.* (1982) observed that stimulation of protease activity in a native soil was reported after treatment with Linuron at 10 mg kg⁻¹, whereas Cartap-HCL at 100-1000 mg kg⁻¹ inhibited the enzyme activity without recovery during a period of 60 days. In accordance with the maximum increase in protease activity at 2.5 kg ha⁻¹ of dithane M-45 and 5.0 kg ha⁻¹ of dhanustin and contaf in black and red soils of groundnut, Mohiddin *et al.*, (2011) noticed that two insecticides acephate and imidacloprid at 25 and 50 ppm caused increment of 46-54% in the enzyme activity at 10 day interval respectively.

Table 2: Activity of protease* under the different concentrations of selected fungicides in black and Red soil for 24 hours after 10 days

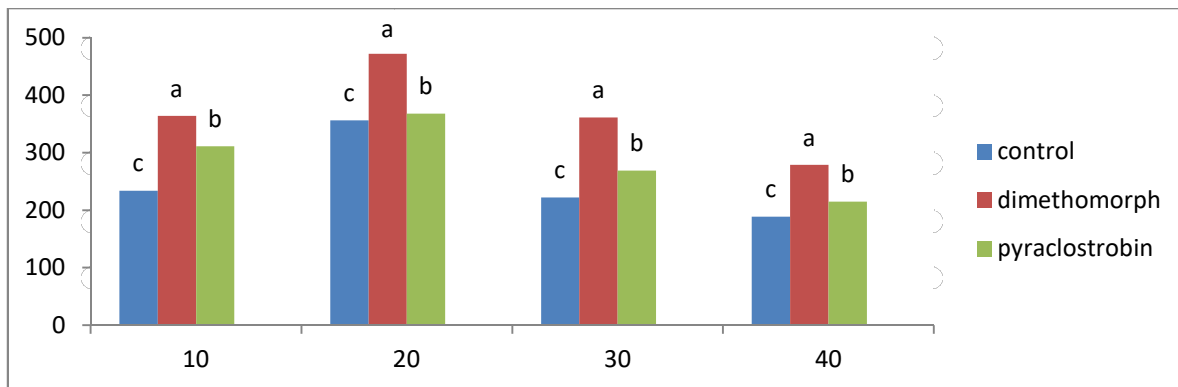
Pesticide concentration (kg ha ⁻¹)	Dimethomorph Black Soil	Pyraclostrobin Black soil	Dimethomorph Red Soil	Pyraclostrobin Red Soil
0.0	327±3.356d	287±3.637c	257±1.687e	245±2.964d
1.0	422±5.362b	238±4.237d	362±2.351b	284±5.791c
2.5	561±2.518 a	358±4.372b	463±9.651a	307±4.733b

5.0	390±4.681c	432±7.165a	356±5.863c	381±7.132a
7.5	303±3.932 e	263± 7.331e	270±9.169d	236±3.468e
10.0	270±8.449 f	228±4.632f	208±2.357f	172±2.357f

* μg tyrosine g^{-1} soil formed after 24 hours incubation at 30°C with 1% casein. 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 DMR test.



Influence of selected fungicides at 2.5 and 5.0 kg ha^{-1} on protease* activity in black soil after 24 hours. * μg tyrosine g^{-1} soil formed after 24 hours incubation at 30°C with 1% casein. The values are the means \pm S.E. for each incubation periods, the means with different letters are significantly different ($P \leq 0.05$) from each other according to DMR test.



Influence of selected fungicides at 2.5 and 5.0 kg ha^{-1} on protease* activity in red soil after 24 hours. * μg tyrosine g^{-1} soil formed after 24 hours incubation at 30°C with 1% casein. The values are the means \pm S.E. for each incubation periods, the means with different letters are significantly different ($P \leq 0.05$) from each other according to DMR test.

CONCLUSIONS

The results of the present study thus, clearly indicated that the fungicides, dimethomorph and pyraclostrobin were profoundly enhanced the activity protease at 2.5 and 5.0 kg ha^{-1} . Based on the above results, it is concluded that the protease were not affected, by the fungicides applied at recommended levels in agricultural systems. No reports are available on the influence of dimethomorph and pyraclostrobin on

protease activity in groundnut soils. Hence, further research is needed to evaluate the effect of these fungicides on soil enzyme activities.

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