

ACTIVITIES OF DEHYDROGENASE, PHOSPHATASE AND PROTEASE AS INFLUENCED BY THE CYPERMETHRIN AND FENVALERATE TO COTTON SOIL

¹Swetha K, ²Srinivasulu M, ³Madhavi A and ⁴Rangaswamy V*.

Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu- 515003. Andhra Pradesh, India.

¹& ³Research scholars – Microbiology ;

²Academic consultant – Microbiology

⁴Professor of Microbiology

Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu – 515003.

Abstract: To understand the effect of two insecticide, cypermethin and fenvalerate on the activities, of dehydrogenase, phosphatase and protease, the experiments have been conducted at different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha⁻¹) of pesticides on under laboratory conditions in three cotton (*Gossypium arboreum L.*) soils, collected from Proddatur mandal, Kadapa district of Andhra Pradesh, India. The dehydrogenase activity in terms of formazan formed from triphenyl tetrazolium chloride was more pronounced in cotton soils treated with 2.5 kg ha⁻¹ of the cypermethrin and fenvalerate. But higher concentrations (5.0, 7.5 and 10 kg ha⁻¹) were toxic to dehydrogenase activity. The activity of phosphatase in terms of *p*-nitrophenol formed from *p*-nitrophenyl phosphate and protease activity in terms of tyrosin from formed 1% casein. Phosphatase and protease was higher in cotton soils, treated with cypermethrin and fenvalerate at 5.0 kg ha⁻¹, but higher levels (7.5 and 10 kg ha⁻¹) were toxic or innocuous to phosphatase and protease activity.

Keywords: Cypermethin, Fenvalerate, Dehydrogenase, Phosphatase, Protease, Cotton soil.

INTRODUCTION

Cotton is the most important fiber and as a cash crop of India, it plays a dominant role in the industrial and agricultural economy of the country. Cotton fiber as the basic raw material is very important to textile industry. In India 40 -50 million are employed in cotton trade followed by processing. Cotton crops provides direct livelihood to 6 million farmers in India. In order to control plant pathogens, pesticides are chemicals which play a major role in maintaining adequate quality of agricultural products. Qualitatively pesticides are used human and animal hygiene, in the protection of feed, food, natural raw materials lining products made of them (Chatterjee *et al.*, 2013). Microorganisms play vital role in soil biochemical processes can also affect the health of plants through interactions (Oliveira *et al.*, 2009). Due to the, fungicides and other substances microbial activity can be distorted by various types of contaminants dissipating into the terrestrial environment. Variations in microbial activity can be manifested by their lower count and biodiversity, which a strong impact on maintaining correct soil quality (Saha *et al.*, 2012). The occurrence of chemical reactions that existing in the nature is by the enzyme activities as the most important key consideration in the environment and changes. The pesticides application can be provide some adverse impact role, referring as enzymes are associated with microbial population in the soil, enzymatic activities and biological processes (Antonious, 2003). Here, it is cleared the pesticide contamination is one of the important aspects nowadays as they are polluting the environment as well. In order to step down the effect of chemical pesticides on environment, the use of bio - pesticides is gaining importance. The natural are safer and less damaging to the ecosystem, when compared these to chemical pesticides and quantitatively less affect the ecosystem adversely (Gopal *et al.*, 2007). This soil biochemical process driven by enzymatic reactions by pesticidal influences. The pesticides adversely microbial affect the microbial mineralization of organic compounds and associated biotransformation, such as nutrient dynamics and their bio availability (Demanou *et al.*, 2004; Kinney *et al.*, 2005; Mahia *et al.*, 2008 and Niewiadomska, 2004).

MATERIALS AND METHODS

Soils used in the present study

Black clay soil collected from cotton cultivating fields of Proddatur mandal, Kadapa district, of Andhra Pradesh, India, the soil sample collected from the depth of 12 cm by using a trowel. Soil samples are air dried and sieved through 2mm sieve prior to analysis. Before studying the effect of selected pesticides in the collected soil samples the physico-chemical characteristics were analyzed using standard methods. The results were listed in Table 1.

Analytical methods for characterization of soil samples for physicochemical properties

Mineral matter of soil samples such as sand, silt and clay contents were analysed with use of different sizes of sieves by following the method of Alexander (1961). The measurement of percentage of water holding capacity of soil samples by finding the amount of added distilled water to both the soil samples to get saturation point and then 60% water holding capacity of soil was calculated (Johnson and Ulrich, 1960). Soil pH was measured at 1:1.25 soils to the water ratio in Systronics digital pH meter with calomel glass electrode assembly. The estimation of organic carbon content in soil samples by the Walkley and Black method and the estimation of organic matter content in the soil samples was calculated by multiplying the values with 1.72 (Jackson, 19071). The estimation of electrical conductivity of the soil samples after addition of 100 ml distilled water to 1 gram soil samples measured by Conductivity Bridge. The estimation of total nitrogen content in soil samples determined by the method of Micro-kjeldhal method (Jackson, 1971). Content of inorganic ammonium-nitrogen in soil samples was measured after extraction with 1M KCl by Nesslerization method (Jackson, 1971). The contents of nitrite-nitrogen (Barnes F Folkard, 1972) and of nitrate-nitrogen by Brucin method (Rnney and Bartleet, 1972) after extraction with water was determined respectively.

Table 1. Physico-chemical properties of cotton soil used in the present study

Properties	Cotton soil
Sand (%)	48
Silt (%)	13
Clay (%)	39
pH a	8.26
Water holding capacity (ml g ⁻¹ soil)	45.8
Electrical Conductivity (m. mhos)	0.18
Organic matter (%) b	0.41
Total nitrogen (%) c	0.62
NH ₄ ⁺ -N(μg ⁻¹ soil)d	5.42
NO ₂ ⁻ -N(μg ⁻¹ soil)e	7.32
NO ₃ ⁻ -N(μg ⁻¹ soil)f	0.48

Where

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 and Folkard, 1951),

f = Brucine method (Ranney and Bartlett, 1972).

Insecticides used in the present study

In order to determine the influence of selected insecticides on the microbial activities, cypermethrin and fenvalerate were selected in the present study. Commercial grades of used pesticides were Bayer science, India.

Dehydrogenase Activity

Five gram portions of each soil samples, in triplicates, in test tubes (25 x 150 mm) were treated with the selected pesticides to provide the final concentration of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha⁻¹. All the test tubes including controls were incubated at room temperature in the laboratory (28 ± 4°C). After particular time of incubation, the triplicate, soil samples were withdrawn for the assay of dehydrogenase.

Assay of dehydrogenase

Dehydrogenase activity was quantified according to the method of Casida et al., (1964) and Rangaswamy et al., (1994) and Srinivasulu and Rangaswamy (2013) in terms of triphenyl formazan accumulation from triphenyl tetrazolium chloride added. Five grams of soil samples were treated with 0.1 g CaCO₃ and 1 ml of 0.18 M aqueous triphenyl tetrazolium chloride and incubated for 24 hours at 37°C. Then the reaction mixture was treated with methanol for extraction of triphenyl formazan formed and assayed at 485 nm in a UV Visible Spectrophotometer (Thermo Scientific) Evolution 201. Rate of dehydrogenase activity was measured at 7, 14, 21, 28 and 35 days of soil incubation.

Phosphatase activity

Two gram portions of each soil samples, in triplicates, in test tubes (25 x 150 mm) treated with the selected pesticides individually to provide the final concentration of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha⁻¹. All the test tubes including the controls were incubated at room temperature in the laboratory (28 ± 4°C). After 10 days of incubation the triplicate soil samples were withdrawn for the assay of phosphatases (Tabatabai and Bremner, 1969) and also adopted by Rangaswamy and Venkateswarulu (1996). The experiment was done similarly and incubated for 10, 20, 30 and 40 days.

Assay of phosphatase

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*-nitrophenyl 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 minutes. The reaction was stopped by adding 1 ml of 0.5 M CaCl₂ and 4 ml of 0.5 M NaOH followed by swirling of the flask, for a few seconds and the soil suspension is filtered through a Whatman No. 1 filter paper. The liberated *p*-nitrophenol in the filtrate was determined at 410 nm in a UV Visible Spectrophotometer (Thermo Scientific) Evolution 201.

Protease activity

Soil protease enzyme activity is done by Speir and Ross (1975) and adopted by Ismail *et al.*, (1998). It is measured from tyrosine equivalents formed from casein. Two gram portions of soil samples were taken in the test tubes (25 × 150 mm) and treated with the selected pesticides individually to provide the final concentration of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha⁻¹. All the test tubes including the controls were incubated at room temperature in the laboratory (28 ± 4°C). After 10 days of incubation the triplicate soil samples can be withdrawn for the assay of protease.

Assay of protease

Two gram portion of soil samples incubated were treated with 10 ml of 0.1 M Tris (2-amino-2-(hydroxymethyl)-propane-1, 3-diol at pH 7.5) containing sodium caseinate (2% w/v) and incubated for 2 hrs at 30°C. To this, 4 ml of aqueous trichloro acetic acid (17.5% w/v) was added and the contents were centrifuged. Suitable aliquot of the supernatant further treated with 1.4 M Na₂CO₃, followed by 1 ml of Folin-Ciocalteu reagent (33.33% v/v) with rapid swirling. After 30 minutes blue colour was formed and read at 700 nm in a UV Visible Spectrophotometer (Thermo Scientific) Evolution 201.

Statistical analysis

All data were expressed on an air-dry soil basis and were averages of three replicates. The data were analyzed for significant differences ($P \leq 0.05$) between pesticide treated and untreated soil samples using Duncan's multiple range (DMR) test (Megharaj *et al.*, 1999 and Jaffer Mohiddin *et al.*, 2013).

Results and discussion

Dehydrogenase activity

Soil samples treated with different concentrations (1.0, 2.5, 5.0, 7.5 and 10.0 kg ha⁻¹) of cypermethrin and fenvalerate were incubated for 24 hrs after 7 days to determine the influence of two insecticides. Concentrations of cypermethrin and fenvalerate effected as significantly stimulation in enzyme activity during 7 day incubation in soil samples ranged from 1 to 7.5 kg ha⁻¹ level in case of dehydrogenase. The dehydrogenase activity ranged from 0-81% and 104-142% cotton soil due to stimulation of dehydrogenase by cypermethrin and fenvalerate (Table 2). The activity of dehydrogenase significantly more stated in soil samples received 2.5 kg ha⁻¹ of the two insecticides until 21 days of incubation (Table 3 and Fig. 1). Subjected to, incubation of insecticides-treated soil samples up to 35 days reported no stimulation of the enzyme activity. Based on the results of the present study revealed that the insecticides, widely used in cultivation of cotton, at field application rates improved the activity of dehydrogenase in cotton soils.

The insecticides applied at the level of above 5.0 or 7.5 kg ha⁻¹ gradually decreased the activity of dehydrogenase (Table 2). Dehydrogenase, activity at highest concentration (41 mg kg⁻¹) and decreased effect at lower pentachlorophenol additions reported by Christina Diez *et al.*, (2006). The development of dehydrogenase activity in all pesticides treated soil up to 2.5 kg ha⁻¹ than the control at 21 days of incubation. Due to the application of glyphosate applied up to 200 mg kg⁻¹ soil on dehydrogenase activity has been reported by some authors (Accinelli *et al.*, 2002 and Araujo *et al.*, 2003). Due to atrazine application dehydrogenase activity has been found stimulated at various levels (Moreno *et al.*, 2007). A reference (Nada and Mitar, 2002).

The application of midacloprid (insecticide) and triadimefon (fungicide) to the soil samples stimulated the activity of dehydrogenase at all concentrations (0.2 µg g⁻¹, 0.5 µg g⁻¹ and 0.7 µg g⁻¹) (Deborah *et al.*, 2013). Stimulation of enzyme activity in response to soil amendment with diazinon and other insecticides have been observed (Gundi *et al.*, 2007 and Singh and Singh, 2005). Acetamiprid increased dehydrogenase activity up to 22% after first insecticide application (Singh and Kumar, 2008). 42.7% inhibition of dehydrogenase activity by the application of nicosulfuron herbicide at 3 mg concentration Radivojevic *et al.*, (2012). Application of Metalaxyl (fungicide) initially increased and then decreased the dehydrogenase activity in fungicide treated soil (Sukul, 2006).

Table 2. Activity of dehydrogenase* under the influence of different concentrations of selected pesticides in cotton soil for 24 hrs after 7days

Conc. of pesticides (kg ha ⁻¹)	Cypermethrin	Fenvaleraate
	24 hrs	24 hrs
0.0	124a (100)	124a (100)
1.0	98b (79)	82b (66)
2.5	118c (95)	101c (81)
5.0	84d (67)	92d (74)
7.5	70e (56)	88e (70)
10.0	59f (47)	68f (54)

*µg ammonia g⁻¹ soil formed after 24 hrs incubation with triphenyl tetrazolium chloride (TTC).

Figures, in parentheses, indicate relative production percentages.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

*Values in the table are means of triplicates.

Table 3. Influence of selected pesticides at 2.5 kg ha⁻¹ on dehydrogenase* activity in cotton soil after 24 hrs

Name of the pesticide	7 days	14 days	21 days	28 days	40 days
Control (0.0)	78a (100)	96a (100)	128a (100)	110a (100)	88a (100)
Cypermethrin (2.5 kg ha ⁻¹)	218b (278)	304b (316)	474b (370)	357b (274)	208b (176)
Fenvalerate (2.5 kg ha ⁻¹)	198c (253)	295c (236)	402c (314)	307c (236)	249c (211)

*µg of formazan g⁻¹ soil formed after 24 hrs incubation with triphenyl tetrazolium chloride (TTC).

Figures, in parentheses, indicate relative production percentages.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

* Values in the table are means of triplicates.

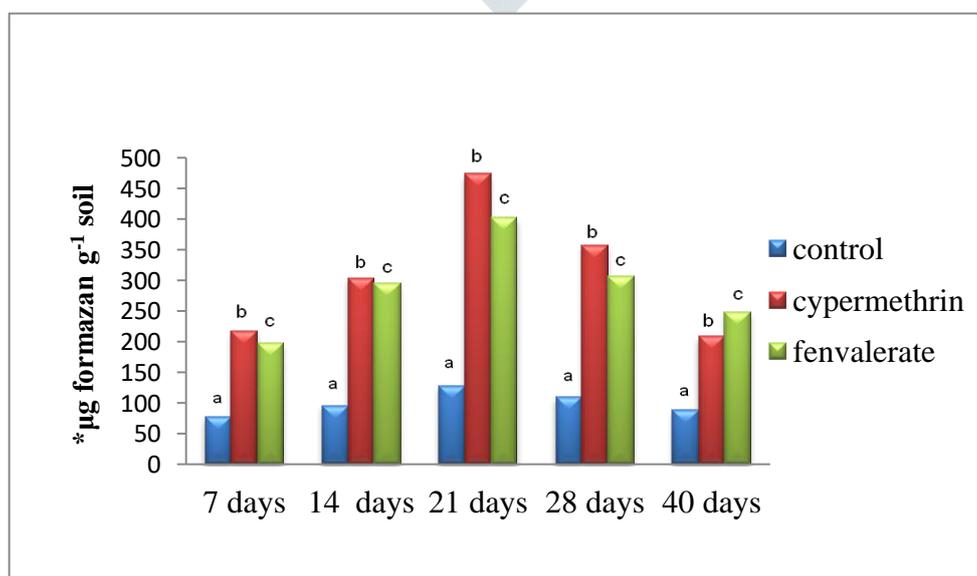


Figure 1. Influence of cypermethrin and fenvalerate at 2.5 kg ha⁻¹ on dehydrogenase* activity in cotton black soil.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

* Values in the table are means of triplicates.

Basing the reports Kucharski *et al.*, (2011) the changes in the enzymatic activity referring to sandy loam soil export to Zn pressure noticed that contamination of soil with zinc in doses from 70 to 10,000 mg kg⁻¹ cleared inhibition of dehydrogenase activity. The application of bromoxynil and a sulfonylurea (prosulfuron) at the three increasing doses inhibited dehydrogenase activity with no recovery over time (Pampulha and Oliveira 2006). Acetamiprid enhanced the activity of dehydrogenase (Singh and Kumar 2008). The decrease of dehydrogenase activity by the influence of flufenacet and isoxaflutole (boreal 58 WG herbicide) reported by Kucharski *et al.*, (2016).

Phosphatase activity

Phosphatase activity was measured under the influence of cypermethrin and fenvalerate at different concentrations of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha⁻¹. The data obtained from these experiments were presented in the Table 4. Cypermethrin and fenvalerate at concentrations ranging from 1.0 to 2.5 kg ha⁻¹ gradually increased the phosphatase activity and reached maximum at the concentration of 5.0 kg ha⁻¹ in cotton soil samples. About 30-148% and 26-129% enhancement in phosphatase activity over control was noticed in cotton black soil for 10-days incubation (Table 4) and significantly inhibited at higher concentration of 10.0 Kg ha⁻¹ in both pesticides treatments (Table 4). The activity of phosphatase was significantly more pronounced in soil samples that received 5.0 kg ha⁻¹ of the two pesticides until 20 days of soil incubation (Table 5 and Fig 2). However, incubation of pesticides-treated samples up to 40 days resulted in no stimulation of the enzyme activity. The results of the present study clearly indicates that the pesticides, widely used in the cultivation of cotton, at field application rates enhance the activities of phosphatase in soil.

The stimulation of phosphatases activity was observed in the presence of butachlor (Xia *et al.*, 2011). The phosphatase activity is increased up to 5.0 kg ha⁻¹ treated with monocrotophos, chlorpyrifos alone and in combination, monocrotophos + mancozeb, chlorpyrifos + carbendazim reported by Srinivasulu *et al.*, (2012b). The suppressive effect on phosphatase activity exerted at 10% of azadirachtin granules at all doses (Gopal *et al.*, 2007). The phosphatase activity with mancozeb, indicating the maximum phosphatase activity at zero ppm (121.8 U) and the average activity decreased significantly to 113.2 U at 100 ppm mancozeb concentration reported by Walia (2014). Mancozeb and dimethomorph the fungicidal mixture negatively affected the phosphatases in sandy soils by (Cycon *et al.*, 2010). Methyl isothiocyanate and dichloropropane at 300 and 80 mg kg⁻¹, inhibit the phosphatase activity (Tu, 1981). At the beginning of 2 to 4 weeks of incubation in stability of phosphatase activity along with decomposing of mixed pesticides deltamethrin and propineb in soil and decline to last part of 12 weeks incubation (Rahmansyah *et al.*, 2009). The development of phosphatase activity in sandy loam soil treated with barban at 200 ppm was reported by Quilt *et al.*, (1979). The development of acid phosphatase activity 1.8 times by the 14th day of incubation with 1 ppm endosulfan Suryakalyani *et al.*, (2010). Due to application acetamiprid decrease in the activity of alkaline phosphatase reported by Xiaohua *et al.*, (2005). At higher inhibitory effect of acetamiprid on phosphatase activity was reported by Yao *et al.*, (2006). However, stimulation in phosphatase activity was noticed while treated with dose of pesticides cocktail-falcon 460 EC fungicide (aspiroxamine, tebuconazole and triadimenol mixture (Bacmaga *et al.*, 2016). An increase in the phosphatase activity by the applied herbicides at the recommended field rate (2 ppm) and ten times more than field rate to a clay loam soil which resulted in an initial increase in the activity of enzyme (Perucci *et al.*, 1988).

Table 4. Activity of phosphatase* under the impact of different concentration of selected pesticides in cotton soil after 24 hrs.

Conc. of pesticides (Kg ha ⁻¹)	Cypermethrin	Fenvalerate
	24 hrs	24 hrs
0.0	132a (100)	132a (100)
1.0	144b (109)	184b (139)
2.5	248c (187)	352c (266)
5.0	372d (281)	448d (339)
7.5	218e (165)	364e (275)
10.0	128a (96)	144a (109)

*µg ammonia g⁻¹ soil formed after 24 hrs incubation with *p*-nitrophenyl phosphate (PNPP).

Figures, in parentheses, indicate relative production percentages.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

* Values in the table are means of triplicates.

Table 5. Influence of selected pesticides at 5.0 kg ha⁻¹ on phosphatase* activity in cotton soil after 10, 20, 30, and 40 days.

Name of the pesticides	10 days	20 days	30 days	40 days
Control (0.0)	132a (100)	150a (100)	124a (100)	112a (100)
Cypermethrin (5.0 kg ha ⁻¹)	378b (287)	448b (298)	366 b (295)	284b (253)
Fenvalerate (5.0 kg ha ⁻¹)	458c (346)	367c (244)	284c (229)	189c (168)

*µg ammonia g⁻¹ soil formed after 24 hrs incubation with p-nitrophenyl phosphate (PNPP)

Figures, in parentheses, indicate relative production percentages.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

* Values in the table are means of triplicates.

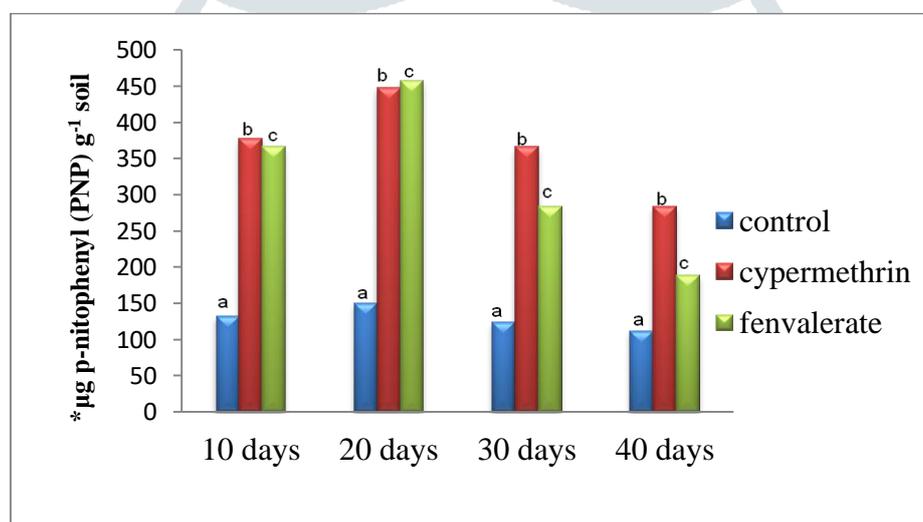


Figure 2. Influence of selected insecticides at 5.0 Kg ha⁻¹ phosphatase * activity in black soil after 10, 20, 30 and 40 days

*µg p-nitro phenol (PNP) g⁻¹ soil formed after 24 hrs incubation with P- nitrophenyl Phosphate (PNPP).

Means in each column, followed by the same letter are not significantly different ($P < 0.05$) from each other according to DMR test.

*Values in the table are means of triplicates.

Protease activity

Protease plays an important role in nitrogen cycle by hydrolyzing proteinaceous components of organic nitrogen to simpler in organic amino acid in soils. The data obtained from these experiments are presented in the Table 6. Interestingly, stimulatory effects were observed at 10-50 ppm concentrations tested at 10-day incubation period in black soils. The percentages of increasing in protease activity of the two pesticides treatments, over control are as follows 59-99% and 51-79% in cotton black soil at 10 days interval (pesticides treated at 10, 25, and 5.0 kg ha⁻¹ of cypermethrin and fenvalerate in cotton soils incubated for 10 days (Table 6). The activity of protease was significantly more pronounced in soil samples that received 5.0 Kg ha⁻¹ of the two pesticides until 30 days of soil incubation (Table 7 and Fig 3). However, incubation of insecticide-treated samples up to 40 days resulted in to stimulation of the enzyme activity.

Table 6. Activity of protease* under the impact of different concentrations of selected pesticides in cotton black soil for 24 hrs after 10 days.

Conc. of pesticides (kg ha ⁻¹)	Cypermethrin	Fenvalerate
	24 hrs	24 hrs
0.0	414a (100)	414a (100)
1.0	544b (131)	526b (127)
2.5	674c (162)	634c (153)
5.0	784d (189)	724d (174)
7.5	634e (153)	614e (140)
10.0	448f (108)	402f (97)

*µg ammonia g⁻¹ formed after 24 hrs incubation at 30°C with 1% casein.

Figures, in parentheses, indicate relative production percentages.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

*Values in the table are means of triplicates.

Table 7. Influence of selected pesticides at 5.0 kg ha⁻¹ on protease* activity in cotton black soil after 10, 20, 30, and 40 days

Name of the pesticides	10 days	20 days	30 days	40 days
Control (0.0)	424a (100)	568a (100)	342a (100)	216a (100)
Cypermethrin (5.0 kg ha ⁻¹)	782b (184)	824b (145)	752b (219)	590b (273)
Fenvalerate (5.0 kg ha ⁻¹)	738c (174)	808c (142)	712c (208)	614c (284)

*µg ammonia g⁻¹ soil formed after 24 hrs incubation 30°C with 1% casein.

Figures, in parentheses, indicate relative production percentages.

Means, in each row, obtained for each sampling, followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

*Values in the table are means of triplicates.

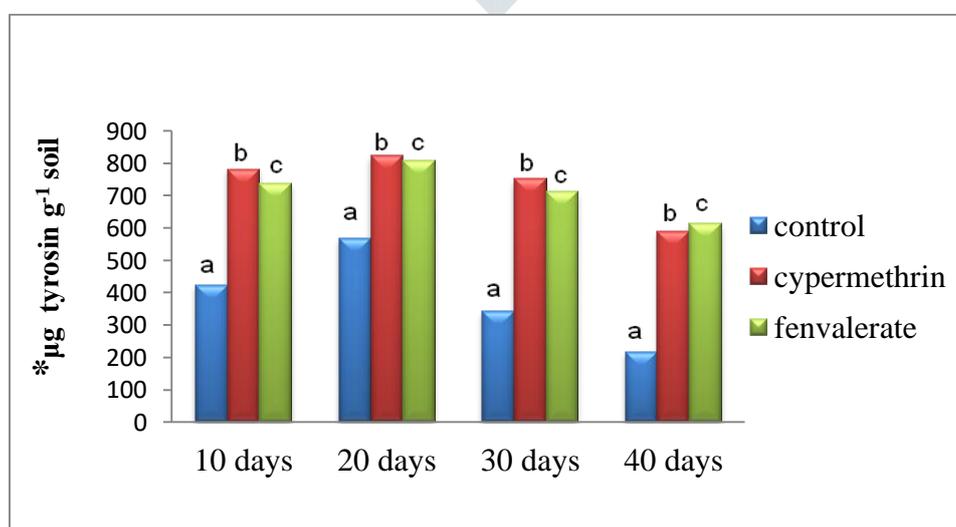


Figure 3. Influence of selected insecticides at 5.0 Kg ha⁻¹ on protease* activity in cotton black soil after 24 hrs.

*µg tyrosine g⁻¹ soil formed after 24 days incubation at 30°C with 1% Casein.

Means, in each column, followed by the same letter are not significantly Different ($P \leq 0.05$) from each other according to DMR test.

*Values in the table are means of triplicates.

The results of the present study clearly indicate that the insecticides, widely used in the cultivation of cotton, at field applications rates enhance the activity of protease in soil. Practically, little information is available on interaction effects of different agrochemicals in combination towards soil protease enzyme, involved in nutritional recycling.

The study was done on protease activity in soils when treated with pesticides and their incubation periods are compared with results by Rangaswamy *et al.*, (1994). Increase in protease activity by cypermethrin, monocrotophos and quinolphos up to 25 ppm noted in soil. The lower doses of endosulfan (0.05 and 0.1 w/v) stimulated protease activity, methyl parathion markedly stimulated enzyme activity at less concentration reported by Sabale and Misal (2000). It is observed that protease activity melathoin against 21 days of incubation (Satpathy and Behera 1993). The insecticides namely, kelthane and fenvalerate caused inhibition in enzyme activity was reported by Omar and Abd-Alla (2000). More enzyme activity due to ditera a nematicide applied at 2.5 and 5.0 kg ha⁻¹ for 1 day was recorded by Fernandez *et al.*, (2002).

Conclusion

Based on the results obtained in the present study from above, the dehydrogenase activity in cotton soils was profoundly increased in two pesticide concentrations (1.0 to 2.5 kg ha⁻¹ at 24 hrs 7 days) the selective influence of the two pesticides was determined, at higher concentrations (5.0, 7.5-10.0 kg ha⁻¹) a suppressed activity in the dehydrogenase enzyme with individual treatments of pesticides are compared to control, phosphatase activity is profoundly increased up to 5.0 kg ha⁻¹ where as at higher concentrations (7.5–10.0 kg ha⁻¹) of pesticide concentration the enzyme activities was dramatically decreased in cotton soils. Protease enzyme activity showed a stimulatory activity up to 5.0 kg ha⁻¹ further increase in the pesticide concentration repression in the enzyme activity was noticed in cotton soil. The pesticides cypermethrin and fenvalerate are as an important agent for the control of plant pathogens, cypermethrin and fenvalerate is often not used at much higher than the recommended dosage in order to maintain soil health.

Acknowledgement

We are grateful to the Sri Krishnadevaraya University authorities for providing necessary facilities throughout my research work.

References

- [1]. Accinelli, C., C. Screpanti, G. Dinelli and A. Vicari. **2002**. Short-time effects of pure and formulated herbicides on soil microbial activity and biomass. *International Journal of Environmental Analytical Chemistry*. **82(8)**: 519-527.
- [2]. Alexander, M., **1961**. Introduction to soil microbiology. 2nd edn. Wiley, New York.
- [3]. Alexander, M. **1965**. Most Probable Number Method for microbial populations. *In: Methods of Soil Analysis*. (Ed. C. A. Black). Part 2. pp. 1467-1472. *Am. Soc. Agr. Madison. Wisconsin, U.S.A.*
- [4]. Anonymous **2017**. Agriculture production plan for ProddaturMandalKadapa District of Andhra Pradesh, India. Department of Agriculture ProddaturMandalKadapa District of Andhra Pradesh, India.
- [5]. Antonious, G. F. **2003**. Impact of soil management and two botanical insecticides on urease and invertase activity. *J. Environ. Sci. Health B*. **38**: 479-488.
- [6]. Araujo, A. S. F., R. T. R. Monteiro and R. B. Abarkeli. **2003**. Effect of glyphosate on the microbial activity of two Brazilian soils. *Chemosphere*. doi:10.1016/S0045-6535(03)00266-2.
- [7]. Barnes, H. and B. R. Folkard. **1951**. The determination of nitrite. *Analyst* **76**: 599-603.
- [8]. Baćmaga, M., J. Wyszowska and J. Kucharski. **2016**. The effect of the Falcon 460 EC fungicide on soil microbial communities, enzyme activities and plant growth. *Ecotoxicology* **25**: 1575-1587.
- [9]. Casida, L. E., D. H. Klein and T. Santoro. **1964**. Soil dehydrogenase activity. *Soil Sci.* **98**: 371-376.
- [10]. Chatterjee N.S., Gupta S., Varghese E. **2013**. Degradation of metaflumizone in soil: impact of varying moisture, light, temperature, atmospheric CO₂ level, soil type and soil sterilization. *Chemosphere*. DOI: 10.1016.
- [11]. Christina Diez, J. M., A. F. Gallardo, G. Saavedra, L. Mara Cea, L. Gianfreda and Z. M. Alvear. **2006**. Effect of pentachlorophenol on selected soil enzyme activities in a Chilean Andisol. *Journal of Soil Science and Plant Nutrition* **6**: 40-51.
- [12]. Cycon M., Z. Piotrowska-Seget and J. Kozdroj. **2010**. Response of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *Int Biodeterior Biodegrad.* **64**: 316-323.
- [13]. Cycon, M., Z. Piotrowska-Seget and J. Kozdroj. **2010d**. Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *Int Biodeterior Biodegrad.* **64**: 316-323.
- [14]. Deborah, B. V., J. A. Mohiddin and R. J. Madhuri, **2013**. Interaction effect of selected pesticides on soil enzymes. *Toxicology International* **20**: 195-200.
- [15]. Demanou, J., A. Monkiedje, T. Njine, S. M. Foto, M. Nola, H. Serges, Z. Togouet and N. Kemka. **2004**. Changes in soil chemical properties and microbial activities in response to the fungicide Ridomil gold plus copper. *Int. J. Environ. Res. Public Health*. **1**: 26-34.
- [16]. Fernandez, A. A. R., D. Hernado, A. Aguer, J. Caceres and S. Malato. **2002**. Toxicity assays. A way for evaluating AOPs efficiency. *Water Research*. **36(17)**: 4255-4262.

- [17]. Gopal, M., A. Gupta, V. Arunachalam. and S. P. Magu. **2007**. Impact of azadirachtin, an insecticidal allelochemical from neem on soil microflora, enzyme and respiratory activities. *Bioresource Technology* (**98**): 3154-3158.
- [18]. Gundi, V. A. K. B., G. Narasimha and B. R. Reddy. **2005**. Interaction effects of insecticides on microbial populations and dehydrogenase activity in a black clay soil. *J. Environ. Sci. Health, Part B* **40(2)**: 269.
- [19]. Ismail, B. S., K. F. Yapp and U. Omar. **1998**. Effects of metasulfuron methyl on amylase, urease and protease activities in two soils. *Aust. J. Soil. Res.* **36**: 449-456.
- [20]. Jackson, M.L. (1971). Soil chemical analysis. Prentice Hall, New Delhi, India.
- [21]. Jaffer Mohiddin, G., M. Srinivasulu, M. Madakka and V. Rangaswamy. **2013**. Impact of selected insecticides on protease and invertase activities in groundnut soils. *Discovery Biotechnology*. **4(11)**: 24-31.
- [22]. Jackson ML **1971** Soil chemical analysis. Prentice Hall India, New Delhi.
- [23]. Johnson, C.M., Ulrich, A. (1960). Determination of moisture in plant tissues. Calif. Agric. Bull., 766:112-115.
- [24]. Kinney, C. A., K. W. Mandernack and A. R. Mosier. **2005**. Laboratory investigations in to the effects of the pesticides mancozeb, chlorothalonil and prosulfuron on nitrous oxide and nitric oxide production in fertilized soil. *Soil Biology and Biochemistry*. **3**: 837-850.
- [25]. Kucharski, J., M. Tomkiel, M. Baćmaga, A. Borowik and J. Wyszowska. **2016**. Enzyme activity and microorganisms diversity in soil contaminated with the Boreal 58 WG herbicide. *J Environ Sci Health B*. **51(7)**: 446-454.
- [26]. Kucharski, J. and J. Wyszowska. **2008**. Biological properties of soil contaminated with the herbicide Apyros 75 WG. *J Elem.* **13(3)**: 357-371.
- [27]. Mahia, J., A. Cabaneiro, T. Carbellar, and M. Diaz-Ravina. **2008**. Microbial biomass and C mineralization in agricultural soils as affected by atrazine addition. *Bio. Fertil. Soils*. **45:99-105**.
- [28]. Megharaj, M., I. Singleton and N. C. McClure. **1998**. Effect of penta-chlorophenol pollution towards microalgae and microbial activities in soil from a former timber processing facility. *Bulletin of Environmental Contamination and Toxicology*. **61**: 108-115.
- [29]. Moreno, J. L., A. Aliaga, S. Navarro, T. Hernandez and C. Garcia. **2007**. Effect of atrazine on microbial activity in semiarid soil. *Appl. Soil. Ecol.* **35**: 120-127.
- [30]. Nada, A. and M. Mitar. **2002**. Effect of herbicides on microbiological properties of soil. Proceedings for Natural Sciences, Matica Srpska Novi Sad. 102: 5-21.
- [31]. Nelson, E. E. and C. Y. Li. **1985**. Persistence of benomyl and captan and their effects on microbial activity in field soils. *Bulletin of Environmental Contamination and Toxicology* **34(4)**: 533-540.
- [32]. Niewiadomska, A. **2004**. Effect of carbendazim, imazetapir and thiram on nitrogenase activity, the number of microorganisms nsoil and yield of red clover (*Trifolium pretense* L.) *Pol. J. Environ. Stud.* **13**: 403-410.
- [33]. Oliveira C.A., Alves V.M.C., Marriel I.E., Gomes E.A., Scotti M.R., Carneiro N.P., Guimaraes C.T, Schaffert R.E, Sa N.M.H. **2009** Phosphate solubilizing microorganisms isolated from rhizosphere of maize cultivated in an oxisol of the Brazilian Cerrado biome. *Soil Biology and Biochemistry*. **41**:1782-1787
- [34]. Pampulha, M. E. and A. Oliveira. **2006**. Impact of an herbicide combination of bromoxynil and prosulfuron on soil microorganisms. *Curr. Microbiol.* (**53**): 238-243.
- [35]. Perucci, P., L. Scarpenui and M. Monotti. **1988**. Interference with soil phosphatase activity by maize herbicide treatment and incorporation of maize residues. *Biology and Fertility of Soils* **6**: 286-291.
- [36]. Quilt P., E. Grossbard and S. J. L. Wright. **1979**. Effects of the herbicide barban and its commercial formulation carbyne on soil microorganisms. *Appl. Bacteriol.* **46**: 431-444.
- [37]. Radivojevic, L., L. Santric and J. G. Umiljendic. **2012**. Rimsulfuron in soil: effects Microbiological properties under Varying Soil Conditions. *Pestic. Phytomed.* (Belgrade) **26**: 134-140.
- [38]. Ranney, T. A. and R. J. Bartlett. **1972**. Rapid field determination of nitrate in natural water. *Communications in Soil Science and Plant Analysis*. **3**: 183-186.
- [39]. Rangaswamy, V., B. R. Reddy and K. Venkateswarlu. **1994**. Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin and fenvelerate. *Agriculture Ecosystem Environment*. **47**: 319-326.
- [40]. Rangaswamy, V. and K. Venkateswarlu. **1996**. Phosphatase activity in groundnut soils as influenced by selected insecticides. *J. Environ. Biol.* **17 (2)**: 115-119.
- [41]. Rahmansyah, M., S. Antonius and N. Sulistinah. **2009**. Phosphatase and Urease instability caused by pesticides present in soil improved by grounded rice straw. *ARPN Journal of Agricultural and Biological Science*. **4**: 56-62.
- [42]. Sabale, A. and B. N. Misal. **2000**. Effect of endosulfan and methyl parathion on hydrolytic enzymes of germinating seeds of jowar. *J. Environ. Biol.* **21(1)**: 29-35. Singh, J. and K. S. Dileep. **2005**. Bacterial, azotobacter, actinomycetes and fungal population in soil after diazinon, imidacloprid and lindane treatments in groundnut (*Arachis hypogaea* L.) fields. *J. Environ. Sci. Heal.* Part B. **40**: 785 -800.
- [43]. Saha S., Dutta D., Karmakar R., Prasad R.D. **2012**. Structure–toxicity relationship of chloroacetanilide herbicides. Relative impact on soil microorganism. *Environmental Toxicology and Pharmacology*. **34(2)**: 307-314
- [44]. Singh, D. K. and S. Kumar. **2008**. Nitrate reductase, arginine deaminase, urease and dehydrogenase activities in natural soil (ridges with forest) and in cotton soil after acetamiprid treatments. *Chemosphere*. **71**: 412-418.
- [45]. Speir, T. W. and D. J. Ross. **1975**. Effects of storage on the activities of protease, urease, phosphatase and sulphatase in three soils under pasture. *New Zealand Journal Science*. **18**: 231-237.
- [46]. Srinivasulu, M. and V. Rangaswamy. **2013**. Influence of insecticides alone and in combination with fungicides on enzyme activities in soils. *Int. J. Environ. Sci. Technol.* **10(2)**: 340-341.

- [47]. Srinivasulu, M., G. Jaffer Mohiddin and V. Rangaswamy. **2012b**. Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in two groundnut (*Arachis hypogaeae* L.) soils. *Environmental Geochemistry and Health*. **3(34)**: 365-374.
- [48]. Srinivasulu, M., G. Jaffer Mohiddin and V. Rangaswamy. **2012b**. Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in two groundnut (*Arachis hypogaeae* L.) soils. *Environmental Geochemistry and Health*. **3(34)**: 365-374.
- [49]. Surya Kalyani, S., Jitender Sharma, Prem Dureja, Surender Singh and Lata. **2010**. Influence of Endosulfan on Microbial Biomass and Soil Enzymatic Activities of a Tropical Alfisol. *Bull Environ Contam Toxicol*. **84**: 351-356.
- [50]. Sukul, P. **2006**. "Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues," *Soil Biology and Biochemistry*. **38(2)**: 320-326.
- [51]. Tabatabai, M. A. and J. M. Bremner. **1969**. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem*. **1**: 301-307.
- [52]. Tu, C. M. **1981**. Effects of pesticides on activities of enzymes and microorganism in a clay soil. *J. Environ. Sci*. **B16**: 179-191.
- [53]. Walia, A. **2014**. Impact of fungicide mancozeb at different application rates on soil microbial populations, soil biological processes, and enzyme activities in soil. *Sci World J*. Article ID 702909.
- [54]. Xia, X. M., M. Zhao, H. Y. Wang and H. Ma. **2011**. Influence of butachlor on soil enzymes and Microbial growth. *Journal of Food Agriculture and Environment*. **9**: 753-756.
- [55]. Xiaohua, Y., Min Hang and Yuan Haiping. **2005**. Effects of acetamiprid on enzymatic activities and respiration of upland soil. *Appl. Pesticide Sustainability*. **12**: 124-128.
- [56]. Yao, X. H., H. Min, Z. H. Lu and H.P. Yuan. **2006**. Influence of acetamiprid on soil enzymatic activities and respiration. *European J. Soil Biol*. **42(2)**: 120-126.

