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RESEARCH ARTICLE

INTERACTION EFFECTS OF SELECTED PESTICIDES ON GROUNDNUT (*ARACHIS HYPOGAEA L.*) SOIL ENZYMES

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ABSTRACT

The impact of two selected insecticides i.e. phenthoate (Ethyl (dimethoxyphosphorothioyl) sulfanyl (phenyl) acetate) and λ-cyhalothrin (alpha-cyano-3-phenoxybenzyl-3-(2-chloro3,3,3-trifluoroprop-1-enyl)-2,2-dimethyl-cyclopropane-carboxylate) on selected soil enzymes phosphatase and urease were determined in two different soil samples (red sandy loam and black clay soils) of groundnut (*Arachis hypogaea* L.) cultivated fields in Anantapuramu district of Andhra Pradesh, India. A laboratory experiment was conducted to determine the effect of selected insecticides, Phenthoate (organo thio phosphorus) and λ-cyhalothrin (pyrethroid) at different concentrations ranging from 1.0 to 10 kg ha^{-1}. The soil samples receiving 5.0 kg ha^{-1} of selected insecticides were significantly more in both soil samples after 10 days of incubation. The activity of the phosphatase and urease was decreased progressively with the increasing period of incubation up to 30 and 40 days. Similarities were found between the results obtained with the selected enzymes in the soils but comparatively the stimulatory effect was more by phenthoate than λ-cyhalothrin.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is one of the important profitable oil seed crop grown all over the year in India (Guha and Chandrasekhar, 2001). Groundnut is the principal crop grown in Anantapuramu, a semi-arid district of Andhrapradesh, India. The application of pesticides in recent years is widely used in modern agriculture for control of various insect pest population. Pesticides are of primary importance due to their continuous entry into soil environment by direct applications (e.g. Agricultural practices) or indirect applications (e.g. Accidental spillage) leaks at pesticide dump sites, discharge of wastes from production facilities or urban pollution (Sannio and Gianfreda, 2001). Repeated application of the pesticide ultimately reaches the soil, which inturn may interact with soil organisms and their metabolic activities (Sharma and Roomiro, 2002). Soil is a dynamic living system containing many free enzymes, immobilized extracellular enzymes and some enzymes within microbial cells (Vineela Deborah *et al*, 2014). Soil enzymes play an important role in organic matter decomposition and nutrient cycling. Several investigations were performed to study the effect of various pesticides on the activity of soil enzymes from different origins (Sannio and Gianfreda, 2001). Now-a-days pesticides are widely used to improve the yield and quality of agricultural products and for controlling pests and diseases in crop products (Crum *et al*, 1999; Mc. Donald *et al*, 1999). The application of pesticides has increased in recent years and the potential negative effects on human health and environment. Pesticides are developed and applied to destroy or suppress only the target organisms in agricultural crops, but they also affect non-target organisms which are responsible for increasing the soil fertility. Organic phosphorus is abundant in soils that can contribute to the phosphorus nutrition of plants and microorganisms that results in the hydrolysis and releases free phosphate (Condron *et al*, 2005). Pesticides are generally categorized as insecticides, fungicides and herbicides based on the type of pest which show effective function (Milligi *et al*, 2006). Chemical pesticides are commonly used by farmers to protect the crops from various pests.

In the present study, we described the enzymatic degradation of phenthoate [O,O-dimethyl S-(α-(carboxy) benzyl) phosphoro dithioate], a common organophosphorus insecticide, in two soils. Because of the presence of one asymmetric carbon atom, phenthoate is chiral and has more insecticidal activity to most organisms (Ohkawa *et al*, 1976). λ- cyhalothrin is an insecticide that belongs to a group of chemicals called pyrethroids (organo phosphorus insecticide). Pyrethroids are man-made chemicals that are similar to the natural insecticide

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pyrethrin. On soil surfaces and in aqueous solutions at p^H -5, λ-cyhalothrin is degraded in sunlight with a half-life of approximately 30 days. The degradation in soil primarily occurs through hydroxylation followed by cleavage of the ester linkage to give two main degradation products that are further degraded to Co_2. Gianfreda *et al*, (1994, 1995) addressed this topic with a different methodological approach. Synthetic enzymatic systems which stimulate enzymes free in soil solution and enzyme-soil colloid associations were considered and the effects of some pesticides on the activity of these enzymes were studied. The activities of microorganisms in soil are crucial to the global cycling of carbon, nitrogen, sulphur, phosphorus and other elements, because of many substances cannot be degraded by organisms other than microbes. So, the biochemical activity of accumulated enzymes for certain reactions has been estimated to be more important than that of the microbial cells (Srinivasulu and Rangaswamy 2014).

Phosphatase is an extracellular enzyme produced by many soil microorganisms and is responsible for the hydrolysis of organic phosphorus compounds to inorganic phosphorus (Monkiedje, Ilori, Spiteller, 2002). Phosphatases represent a broad range of intracellular as well as soil-accumulated activities that catalyse the hydrolysis of both the esters and anhydrides of phosphoric acid. Urease, in particular is a useful indicator to evaluate the soil pollution situation. Decreased urease activity in soil with the application of pesticides reduces urea hydrolysis which is beneficial. It helps to maintain N in a form (NH_4^+) less leachable (Antonious, 2003). Therefore, in the present study it has become necessary to determine the soil biological responses to the pesticides. To date, efforts have been made to understand the effect of pesticides on soil enzyme activities, phosphatase and urease but little is known about the effect of phenthoate and lambda-cyhalothrin.

MATERIALS AND METHODS

Soils used in the present study

Two soil samples, a black clay soil and red sandy loam soil, were collected from groundnut-cultivated fields of Anantapuramu district, Andhrapradesh, India. The soil samples were chosen with a known history of pesticide use, from a depth of 12cm and mixed thoroughly to prepare a homogenous composite sample, air-dried at room temperature and the soil samples were sieved through 2mm sieve and stored at 4^0c.

Analysis of physico-chemical characteristics of soil samples

Mineral matter of soil samples such as sand, silt and clay contents were analysed with the use of different sizes of sieves by following the method of Alexander (1961). Water-holding capacity(WHC) of the soil samples were determined by adding distilled water upto the saturation point and then 60% water holding capacity of the soil samples was calculated by Johnson and Ulrich (1960). P^H of soil samples was determined by mixing soil and water in the ratio of 1:1.25 using Systonics digital P^H meter with calomel glass electrode assembly. 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 after addition of 100ml distilled water to 1g 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 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.

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

Properties	Black clay soil	Red sandy loam soil
Sand (%)	72.6	53.4
Silt (%)	18.3	27.8
Clay (%)	9.1	18.8
pH[a]	7.8	6.5
Water holding capacity (ml g^{-1} soil)	0.43	0.35
Electric conductivity (m.mhos)	264	228
Organic matter[b](%)	1.206	0.67
Total nitrogen[c] (%)	0.078	0.045
NH_4^+- N (µg g^{-1} soil)[d]	7.09	6.01
NO_2^-- N (µg g^{-1} soil)[e]	0.62	0.48
NO_3^-- N (µg g^{-1} soil)[f]	0.98	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)

Insecticides used in the in the present study

In order to determine the influence of selected insecticides on the groundnut soil microbial activities, commercial grades of phenthoate and lambda- cyhalothrin were obtained from Bayer's Science India.

Enzymes Used In The Present Study

Phosphatase activity (E.C. 3.1.3.1.)

The activity of phosphatases under the influence of the insecticides, at different concentrations was determined in black clay and red sandy loam soils. Two gram portions of soil samples, transferred into test tubes (12x125 mm), was treated with two insecticides to provide final concentrations of 10, 25, 50, 75 and 100µg g^{-1} soil (equivalent to 1.0,2.5,5.0,7.5 and 10.0 kg ha^{-1} field application rates). The soil samples without insecticides treatment served as control. All the treatments, including controls were incubated in the laboratory at 28 ± 4^0c. After ten days of incubation, triplicate soil samples were withdrawn for the assay of phosphatase (Tabatabai and Bremner, 1969; Srinivasulu *et al*, 2012). Similarly the influence of the two insecticides at stimulatory concentration (5.0 kg ha^{-1}) on the rate of phosphatase activity in two different soils was also determined in triplicate soil samples at 10, 20, 30 and 40 days of incubation.

Assay of Phosphatase

For assay of Phosphatase activity, each soil sample was treated with 6ml of 0.1M Maleate buffer (P^H 6.5) and 2ml of 0.03M p-nitro phenyl phosphate. After incubation for 30 minutes at 37^0c, the tubes were placed on ice before the soil extracts were passed through Whatmann No.1 filter paper. To suitable aliquots of the extract, 1ml of 5M $CaCl_2$ and 4ml of 0.05M NaOH was added, and the yellow colour developed read at 405nm in a Spectrophotometer (Milton Roy Company).

Urease activity (EC. 3.5.1.5)

For estimating the enzymatic activity of Urease, portions of 1gm Soil samples (Black clay and Red sandy loam soils) placed in 15x150 Mm test tubes were treated with 1ml of aqueous solutions of two insecticides to provide different concentrations of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}. The soil samples without insecticide treatment served as control. All the treatments including control were maintained at 60% WHC and the tubes were incubated at 28 ± 4^0c. After 10 days of incubation, triplicate soil samples were withdrawn for the assay of urease.

Assay of Urease

For the assay of urease (Fawcett and Scott, 1960), at desired intervals, 1ml of 3% urea and 2ml of 0.1M phosphate buffer (pH 7.1) were added to 1g soil. After incubation for 30 minutes at 37^0c in a water-bath, shaker the tubes and were placed in ice until the ammonia was extracted with 10ml 2M KCl. 5ml of phenol-sodium nitroprusside solution and 3ml of 0.02M Sodium hypochlorite were added to 4ml of the filtrate. The mixture was shaken, incubated for 30 minutes in the dark and the developed blue colour was measured at 630nm in a spectronic 20D spectrophotometer. After determining the effective concentration experiment further for 20, 30 and 40 days and assayed similarly.

Statistical Analysis

The concentrations of the phosphatase and urease enzymes were calculated on soil weight (over dried) basis. The insecticide treatments with untreated controls and the significant levels $P \leq 0.05$ between values of each sampling, each insecticide were performed using SYSTAT statistical software package to find the results of Duncan's Multiple Range (DMR) test (Megharaj *et al*, 1999; Jaffer Mohiddin *et al*, 2013).

RESULTS AND DISCUSSION

Realized laboratory experiments showed that, there exists a relationship between soil enzymatic activity and the selected insecticides concentrations in the selected soil samples. Hydrolysis of an exogenously added substrate *p*-nitrophenyl disodium orthophosphate, by phosphatases was increased in both the selected soil samples with the selected insecticides phenthoate and λ- cyhalothrin, than in the control at 1.0, 2.5 and 5.0 kg ha^{-1} levels incubated for 10 days. Our investigation has revealed that phosphatase activity was drastically decreased at higher concentrations (7.5 and 10.0 kg ha^{-1}) of phenthoate and λ- cyhalothrin treated soils than the untreated controls throughout the experiment. The data obtained from these experiments are represented in the tables 2 and 3. Hydrolysis of the added substrate, *p*-nitrophenyl disodium orthophosphate, by phosphatases was greater in both the soil samples with the selected two insecticides than in the control at 1.0, 2.5 and 5.0 kg ha^{-1} levels incubated for 10 days. The enhancement in phosphatase activity over control was noticed in the black clay soil and red sandy loam soil and for 10 days of incubation period. Further incubation periods i.e., 20, 30 and 40 days, the activity was decreased slowly in both the soil samples.

In case of urease activity, implicated in the hydrolysis of urea was significantly enhanced by the selected insecticides phenthoate and λ- cyhalothrin upto 5.0 kg ha^{-1}, in both the soil samples when compared to the controls. However, the higher concentrations (5.0 and 10.0 kg ha^{-1}) were toxic to the urease activity after 10 days of incubation period. The amount of ammonia formed from urea was more in soil samples treated with 2.5 and 5.0 kg ha^{-1} of phenthoate and λ- cyhalothrin, higher in red soil with 5.0 kg ha^{-1} phenthoate. In the present experiment, higher levels of 7.5 and 10.0 kg ha^{-1} , the selected insecticides inhibited urease activity in two soils at 10 day interval.

Table 2 Influence of selected insecticides on activity of phosphatase* in black clay soil after 10 days

Insecticide concentration (kg ha^{-1})	Phenthoate	Lambda-cyhalothrin
0	250±4.35f (100)	250±2.57f (100)
1.0	480±2.43d (192)	500±3.82d (200)
2.5	750±2.12c (300)	600±1.06c (264)
5.0	1000±2.83a (400)	900±3.13a (360)
7.5	860±2.22b (344)	750±0.72b (300)
10.0	400±1.19e (160)	480±0.66e (192)

*µg of *p*-nitrophenol (PNP) g^{-1} soil formed after 24 hours incubation with *p*-nitrophenyl phosphate (PNPP).
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.

Table 3 Influence of selected insecticides on activity of phosphatase* in red sandy loam soil after 10 days

Insecticide concentration (kg ha^{-1})	Phenthoate	Lambda-cyhalothrin
0.0	170±3.2f (100)	170±1.25f (100)
1.0	370±2.13d (217)	340±1.13d (200)
2.5	580±3.13b (341)	580±3.2c (341)
5.0	930±2.06a (547)	860±0.53a (505)
7.5	690±1.98c (405)	750±0.61b (441)
10.0	270±1.56e (158)	280±0.72e (164)

*µg of *p*-nitrophenol (PNP) g^{-1} soil formed after 24 hours incubation with *p*-nitrophenyl phosphate (PNPP).
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.

Phosphatase activity

About 92% to 300 % and 100% to 260% enhancement in phosphatase activity over control was noticed in the black clay soil for 10 days of incubation, whereas in case of the red sandy loam soil the corresponding figures of percentage enhancement by the two insecticides at two levels were 117% to 447% and 100% to 405% during the same period when compared to controls. The inhibition in the phosphatase activity was recorded in the 10^{th} day of incubation with the phenthoate and λ- cyhalothrin application. However, the inhibitory effect was reduced upon further incubation due to the degradation of applied insecticides (Yao xiao hua *et al*, 2005). Similarly, the soils without phenthoate and λ- cyhalothrin showed initial phosphatase activity. Yao xiao hua *et al*, (2005) studied the effect of acetamaprid at higher concentration showed inhibitory effect on phosphatase activity. Initial reduction in the phosphatase activity may be due to the lethal action of

acetamaprid on P- solubilizers population which alter the membrane permeability of the microorganisms releasing phosphatase enzymes. This statement is supported by Voets *et al*, (1974) in their study on the effect of Atrazine on phosphatase activityin a forest soil, who concluded that the inhibition of the phosphatase activity was upto 61.8%. Similar results were also noticed by Krishnamurthy *et al*, (1999) with Carbofuran. Stimulation in phosphatase activity under the influence of paraquat, trifluralin, glyphosate and atrazine has been reported by Hazel and Greaves (1981). Approximating the results, two organophosphorus insecticides, increased activities of phosphatase in the groundnut field (Pandey and Singh 2006), Phenthoate exhibits an inhibitory effect on phosphatase activity over the incubation period (Yao *et al*, 2006). Quinalphos, monocrotophos and two pyrethroids, cypermethrin and fenvalrate at the concentrations of phosphatase activity, but above this concentration i.e., 7.5 kg ha^{-1}, these insecticides were inhibitory to the activity (Rangaswamy and Venkateswarlu 1996). Similar inhibition in phosphatase activity was also reported by Tu (1995) and Ismail *et al*, (1996) with imidacloprid and Cyfluthrin. Srinivasulu *et al*., (2012) showed that the phosphatase activity was enhanced upto 5.0 kg ha^{-1} treated with monocrotophos, chlorpyriphos alone and in combination monocrotophos + mancozeb, chlorpyriphos + carbendazim. Similarly, mancozeb N 10 {10 times the normal application (60 kg/ha)} brought about by a 41% stimulation in activity after 14 days of incubation period compared to control, but after 28 days of incubation a 30% decrease in enzyme activity was recorded (Rasool and Zafar Reshi 2010).

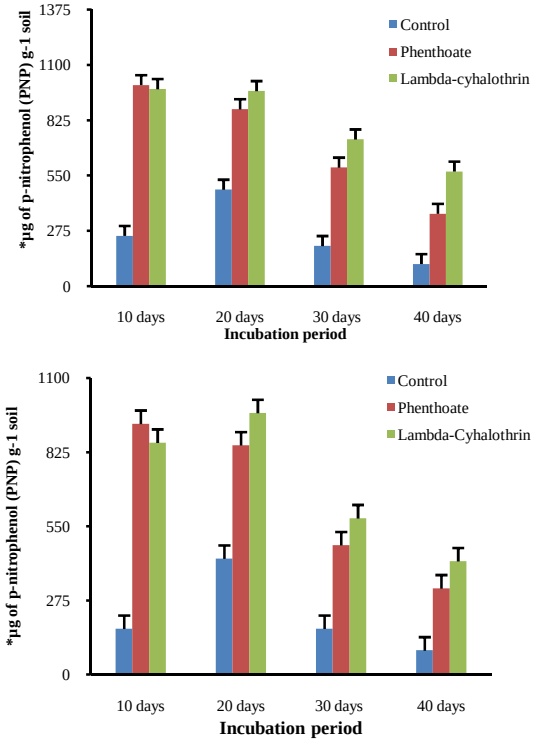

Fig. 1a,b. Influence of Phenthoate and Lambda-Cyhalothrin on Phosphatase*activity in a black clay and red sandy loam soil at 5.0 kg $^{ha-1}$. *µg of p-nitrophenol (PNP) g-1 soil formed after 24 hours of incubation with p-nitrophenyl phosphate (PNPP). after 10, 20, 30 and 40 days of incubation. The values are the mean ± SE for each incubation period, are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test

The activity of phosphatase was decreased significantly after a longer period of incubation upto 20, 30 and 40 days (figures 1a,b).

Table 4 Influence of selected insecticides on urease activity* in black clay soil after 10 days

Insecticide concentration (kg ha^{-1})	Phenthoate	Lambda-cyhalothrin
0.0	390±2.13f (100)	390±1.61f (100)
1.0	600±0.53d (352)	500±2.4e (294)
2.5	750±2.57c (441)	660±2.13c (388)
5.0	1000±2.43a (588)	900±0.77a (529)
7.5	830±2.12b (488)	750±0.72b (441)
10.0	550±1.36e (323)	480±0.52d (282)

*µg ammonia g^{-1} soil formed after 30 minutes incubation at 37°C with urea.
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.

Table 5 Influence of selected insecticides on urease activity* in red sandy loam soil after 10 days

Insecticide concentration (kg ha^{-1})	Phenthoate	Lambda-cyhalothrin
0.0	300±1.79 f (100)	300±2.13f (100)
1.0	490±1.13d (163)	430±2.06d (143)
2.5	580±1.56c (193)	570±0.72c (190)
5.0	910±1.98a (303)	860±2.13a (286)
7.5	690±2.06b (230)	750±1.98b (250)
10.0	420±3.2e (140)	360±0.61e (120)

*µg ammonia g^{-1} soil formed after 30 minutes incubation at 37°C with urea.
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.

Urease activity

The absence of urease enzyme in many groundnut soils and its low activity in other soils are not easily explainable. It is well accepted that groundnut soils exhibit appreciable urease activity. Measurable urease activities were detected even in stored and geologically preserved soils (Bremner and Mulvaney, 1978). These findings led to the conclusion that native soil ureases are mainly extracellular and are persistant because of their association with organic and inorganic colloids (Burns *et al*, 1972 a, b; Gianfreda *et al*,1992, Gianfreda *et al*, 1995. The data on the effect of phenthoate and λ- cyhalothrin on urease activity is shown in the tables 3 and 4, and the variation has been observed in all the enzyme activity with respect to the selected pesticides for the present study. The initial urease activity in treated black clay soil were 252% to 488%µg urea g^{-1} soil ha^{-1}. The inhibition in the urease activity was recorded in the 10^{th} day of incubation period with phenthoate and λ- cyhalothrin application.The inhibitory effect was reduced upon further incubation due to the reduction in the concentration and degradation of applied insecticides. The urease activity, in the hydrolysis of urea urea was significantly enhanced by the application of insecticides phenthoate and λ-cyhalothrin upto 5.0 kg ha^{-1} in both soils when compared to the

controls. However, the higher concentrations (7.5 & 10.0 kg ha^{-1}) were toxic to urease activity of the soils after 10 days incubation period. The ammonia formed from urea in the activity of urease was pronounced in soil samples treated with 2.5 & 5.0 kg ha^{-1}. In case of acetamaprid & imidacloprid at 10, 25 & 50 µg g^{-1} individually caused a 30 –77% and 46 –54% increase in urease activity over the control, respectively in black clay soil after 10 day interval. Corresponding values of urease activity in red sandy loam soil for both the selected insecticides at the same incubation are 63% to 203%and %. The activity of urease was decreased significantly after a longer period of incubation upto 20, 30 & 40 days (figures 2a, b). Similarly, 0.5 & 5.0 µg g^{-1} of pyrethroids, permethrin (FMC 33297), of FM 4598, Shell WL 41706, Shell WL 43467 & Shell WL 43775 had no effect on urease activity in a sandy loam soil (Tu 1980). According to Rasool & Reshi (2010) reported that, there is a significant decrease in urease activity with mancozeb at different application rates over the control.

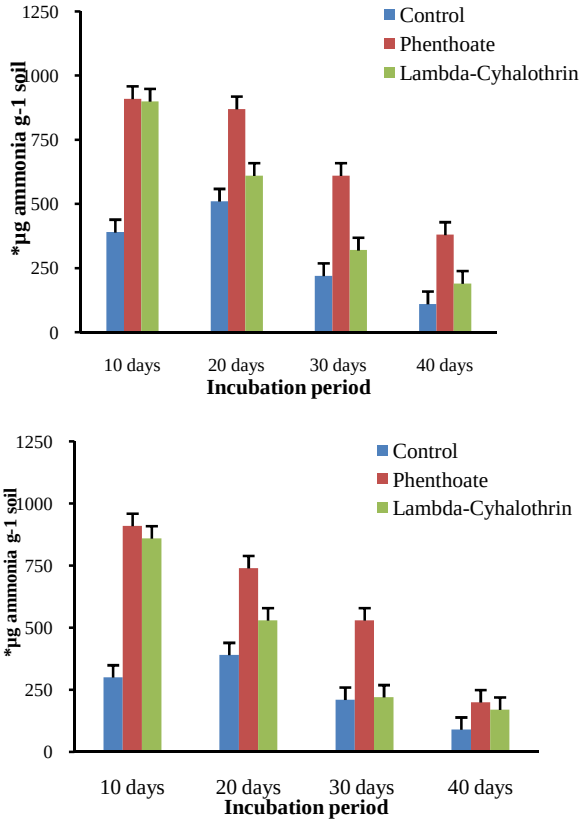

Fig. 2a,b. Influence of Phenthoate and Lambda-Cyhalothrin on Urease*activity in a black clay and red sandy loam soil at 5.0 kg $^{ha-1}$. *µg ammonia g-1 soil formed after 30 minutes incubation at 37oC with urea. after 10, 20, 30 and 40 days of incubation. The values are the mean ± SE for each incubation period, are not significantly different ($P \leq 0.05$) from each other according to Duncan's multiple range (DMR) test

In another studies, urease activity was inhibited by ŧenamiphos (Caceres *et al*, 2009) in Australian and Ecuodorean soils. Urease activity was inhibited by napropamide at all concencentrations relative to the control with longer periods of insecticide application (Guo *et al*, 2008). The data and the reports obtained from the present studies are in agreement with the previous studies carried out by Srinivasulu *et al*, (2010 b) observed that the urease activity in combination with mancozeb & carbendazim at 5.0 kg ha^{-1} incubated for 20 days. Further increase in enzyme activity continued up to 20 days of incubation and afterwards there was a decrease in enzyme activity. Urease activity was decreased by 20% in unamended polluted soils (with MCPA) (Tejda *et al*.,2010) and exposure to chlorpyriphos and its oxon derivative (CPO) at higher concentrations (Wang *et al*, 2010) similarly. The other studies stated that a 55.6% decrease in urease enzyme was observed different concentrations (0, 200, 400,600, 800 & 1000 mg kg^{-1}) of Pb- contaminated soil (Akmal & Xu 2008) . However, pesticides including organophosphorus insecticides, could disrupt urea hydrolysis in soils at higher doses ranging from 100 –1000 ppm. (Lethbridge *et al*, 1981).

CONCLUSION

The results obtained in the present study thus, clearly indicated that the insecticides, Phenthoate and λ- cyhalothrin were profoundly enhanced both the phosphatase and urease activities, at 1.0 –5.0 kg ha^{-1}. Based on the above results, it is concluded that the microbial activities (i.e., enzyme activities) were not affected by the insecticides applied at recommended levels in agricultural system to control the insect pests.

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