Dynamic Soil, Dynamic Plant ©2011 Global Science Books

Influence of Selected Insecticides on Enzyme Activities in Groundnut (*Arachis hypogaea* L.) Soils

Gooty Jaffer Mohiddin[1] • Mandala Srinivasulu[1] • Mekapogu Madakka[2] • Kondeti Subramanyam[3] • Vengatampalli Rangaswamy[1*]

[1] Department of Microbiology, Sri Krishnadevaraya University, Anantapur -515055, Andhra Pradesh, India
[2] Department of Biotechnology and Bioinformatics, Yogivemana University, Kadapa -516 003, Andhra Pradesh, India
[3] Plant Molecular Biology Unit, Department of Biotechnolgy, Bharathidasan University, Tiruchirappalli– 620024, Tamil Nadu, India

Corresponding author: * **mekapogu@gmail.com**

ABSTRACT

The influence of acephate and imidacloprid on important soil enzyme activities, such as dehydrogenase and urease in two groundnut (*Arachis hypogaea* L.) soils, collected from Anantapur District of Andhra Pradesh, India, was studied under laboratory conditions. The activity of dehydrogenase, in terms of formazan formed from triphenyl tetrazolium chloride, was more pronounced in both soils treated with 2.5 kg ha^{-1} of the acephate and imidacloprid. But higher concentrations (5.0, 7.5 and 10 kg ha^{-1}) were toxic to dehydrogenase activity. The activity of urease in terms of ammonia formed from hydrolysis of urea was higher in both soils, treated with acephate and imidacloprid at 5.0 kg ha^{-1}, but higher levels (7.5 and 10 kg ha^{-1}) were toxic or innocuous to urease activity.

Keywords: acephate, dehydrogenase, imidacloprid, soil enzymes, urease

INTRODUCTION

The economy of India is largely dependent on the quality and quantity of agricultural produce. Better harvest requires intensive cultivation, irrigation, fertilizers and more importantly peticides to protect plants from pests and plant diseases. In India, about 15–20% of agricultural production is negatively influenced by pests (Bhalerao and Puranik 2007). Organophosphates, synthetic pyrethroids, carbamates, triazole and organochlorine pesticides either singly or in combination are routinely used to control major pests which affect economically important crops like groundnut, cotton, and tomato (Megharaj *et al*. 1989; Rangaswamy and Venkateswarlu 1992; Vijay Gundi *et al*. 2007; Jayashree and Vasudevan 2007; Romeh *et al*. 2009). In modern agriculture, pesticides are used in large quantities for controlling not only pests and weeds but also improving the crop yield. A study of the effect of pesticides on soil microflora and their beneficial activities forms an important part of the pesticides risk assessment. However, intensive use of common pesticides can lead to toxicity to soils, which may inhibit several biochemical reactions. Due to a high degree of toxicity, some pesticides, particularly those persistent in soil, constitute a very important group of contaminants. When pesticides are applied to soils, they may interact with non-target soil microorganisms and exhibit chronic diverse effects on soil microflora (Omar and Abdel Sater 2001; Moorman 1989; Tu 1995; Pimentol and Levitan 1986; Sarfraz *et al.* 2009). Further, they affect ecological balance in terms of soil fertility (Aramendia *et al.* 2007; Swaminathan *et al.* 2009). Although these pesticides have been restrictively used or even banned in some countries for several years, their persistence and bioaccumulation can still be found in many soils and plants (Vinas *et al.* 2002). It is well a known fact that a soil is an open but self regulating ecosystem with a large diversity of microbial populations (Kizilkaya *et al.* 2004). The living dynamic nature of living organisms is one of the important features of soil quality and often used as a bio indicator for soil health (Gianfreda *et al.* 2005; Sukul 2006).

Soil enzymes, that represent the major living organism activities, are involved in catalyzing various reactions necessary for organic matter metabolism, nutrient cycling, energy transfer, and crop productivity (Kizilkaya *et al.* 2004). Soil enzymes are potential indicators and act as biological catalysts of various important biochemical reactions to produce essential components besides playing an important role in soil fertility (Pascual *et al.* 2000; Garcia *et al.* 2000; Bending *et al.* 2006; Benedetti and Dilly 2006; Quian *et al.* 2009). The composition of the soil surroundings, insecticides, may be directly or indirectly influences the catalytic efficiency of soil enzymes (Bollag and Liu 1990; Min *et al.* 2001). Soil enzyme activities are used to assess the negative effects of pollutants such as pesticides, illicit drugs, petroleum hydrocarbons and heavy metals on soil ecosystem (Pandey and Singh 2006). In spite of the maximum potential of soil enzymes in maintaining soil biodynamics, only limited studies are available on influence of organochemicals on soil enzymes (Nannipieri and Landa 2000; Walker *et al*. 2001; Ramesh *et al.* 2003; Pessagno *et al.* 2008). Some of the microbial processes for assessing the effects of contaminants on soil health include dehydrogenase; an intracellular enzyme belonging to oxidoreductases present in all soil microorganisms used as a measure of total microbial activity in soil (Trevors 1984). Soil dehydrogenase is a specific kind of enzyme which plays significant role in the biological oxidation of soil organic matter by transferring protons and electrons from substrates to acceptors (Sebiomo *et al*. 2011). The objective of the present study is to evaluate the effect of imidacloprid and acephate applied at normal field and high concentrations in laboratory. Urease and dehdrogenase activities are very important for soil quality (Wang *et al*. 2010). Hence dehydrogenase and urease activities were selected because of their significance in soils.

Received: 9 December, 2010. Accepted: 20 July, 2011.

Original Research Paper

Table 1 Physico-chemical characteristics of the soils.

Properties	Black soil	Red soil
Sand (%)	65.8	55.3
Silt (%)	25.2	27.2
Clay (%)	9.0	17.5
pH[a]	7.2	6.2
Water holding capacity (ml g^{-1} soil)	0.47	0.27
Electrical conductivity (m.mhos)	260	244
Organic matter [b] (%)	1.33	0.72
Total nitrogen [c] (%)	0.082	0.046
NH_4^+ - N (µg g^{-1} soil)[d]	7.93	7.02
NO_2^- - N (µg g^{-1} soil)[e]	0.54	0.43
NO_3^- - N (µg g^{-1} soil) [f]	0.86	0.62

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)

MATERIALS AND METHODS

Soils used in the present study

Two soils, a black clay soil and red sandy loam soil were collected from groundnut cultivating fields of Anantapur district, Andhra Pradesh, India. The soil samples were collected randomly from 5 places at a depth of 0-12 cm near the rhizosphere region. The samples were air dried at room temperature, mixed thoroughly to prepare a homogenate composite sample, and sieved through 2 mm sieve. Physico-chemical characteristics of the two soil samples were analyzed by standard methods and listed in **Table 1**.

Analysis of physicochemical characteristics of soil samples

Mineral matter of soil samples such as sand, silt, and clay contents were analyzed by means of different sizes of sieves by following the method described by Alexander (1961). The water holding capacity of soil samples was determined by adding distilled water up to the saturation point and then 60% water holding capacity of soil was calculated as described by Johnson and Ulrich (1960). Soil pH was measured by mixing soil and water in the ratio of 1: 1.25 using Systronics digital pH meter with calomel glass electrode assembly. Organic carbon content in soil samples was estimated by the Walkley and Black method and the organic matter was calculated by multiplying the values with 1.72 (Jackson 1971). Electrical conductivity of soil samples after the addition of 100 ml of distilled water to 1 gram of soil sample was measured by a conductivity bridge. The total nitrogen content in soil samples was determined by the micro-Kjehdal method (Jackson 1971). Inorganic ammonium-N content was estimated after extraction of soil samples with 1M KCl by the Nesslerization method (Jackson 1971). The soil samples were extracted with water to determine the concentration of nitrite to nitrogen (Barnes and Folkard 1951) and nitrate-N by the Brucine method (Ranney and Bartlett 1972).

Insecticides used in the present study

To determine the influence of selected insecticides on soil enzyme activities, imidacloprid a neonicotinoid (17.8% soluble liquid) and acephate an organophosphate (75% soluble powder) were obtained from Saraswati Agrochemicals Pvt. Ltd. and A.B. Chem. Jammu, India. The used commercial grade insecticides were dissolved in distilled water.

Soil incubation studies

1. Dehydrogenase activity **(E.C. 1.1.1.1)**

To study the effect of imidacloprid and acephate on dehydrogenase, 5 g of dried black and red soils were taken separately in test tubes (12 × 125 mm) containing different concentration of insecticides 10, 25, 50, 75, and 100 µg g^{-1} soil which are equal to 1.0, 2.5, 5.0, 7.5, and 10.0 kg ha^{-1} of field application rates. In order to maintain 60% water holding capacity (WHC), about 2 ml of deionized water was added to test tubes containing black soil and 1 ml into tubes containing red soil. Untreated soil samples served as controls. All the treatments, including controls were incubated in the dark at 28 ± 4°C for 7, 14, 28, and 35 days. During incubation period certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the enzyme assay.

Assay of dehydrogenase: The method employed for the assay of dehydrogenase was developed by Casida *et al.* (1964). This method is based on the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF). Each soil sample was treated with 0.1 g of $CaCO_3$ and 1 ml of 0.18 mM aqueous solution of TTC and incubated for 24 h at 30°C. The TPF formed was extracted with methanol from the reaction mixture and assayed at 485 nm in a Spectronic 20D spectrophotometer (Milton Roy Co.).

2. Urease activity (E.C. 3.5.1.5)

To study the effect of imidacloprid and acephate on urease, the soil samples were prepared according to the method described in the assay of dehydrogenase. Untreated soil samples were considered as controls. All the treatments, including controls were incubated in the dark at 28 ± 4°C for 10, 20, 30, and 40 days. During the incubation period, a certain amount of distilled water was added to maintain the soil WHC. Triplicate soil samples were withdrawn for the assay of urease by following the phenol hypochlorite method (Fawcett and Scott 1960). The influence of two insecticides at 2.5 and 5.0 kg ha^{-1} on the rate of urease activity was also determined in two soil samples. After 10, 20, 30, and 40 days of incubation at room temperature (28 ± 4°C), triplicate soil samples of each treatment were withdrawn for the enzyme assay.

Assay of urease: For the assay of soil urease, the soil samples were mixed with 4 ml of 0.1 M sodium phosphate buffer (pH 7.0) and 1 ml of 1 M urea solution, and incubated for 30 min. After incubation, the enzymatic reaction was stopped by adding 10 ml of 2 M KCl at 4°C for 10 min. Suspensions were centrifuged at 5000 rpm for 5 min and the NH_4^+ ions content in supernatant was determined by the phenol hypochlorite method (Fawcett and Scott 1960). Two ml of supernatant was mixed with 5 ml of phenol sodium nitroprusside and 5 ml of 0.02 M sodium hypochlorite and incubated for 30 min in the dark. The absorbance of the blue color so formed was read at 630 nm in a Spectronic-20 D Spectrophotometer (Milton Roy).

Statistical analysis

The concentration of the dehydrogenase and urease was calculated on the basis of soil weight (oven dried). Data were analyzed using one-way ANOVA and the differences contrasted using Duncan's multiple range test (DMRT) (Jaffer *et al*. 2010). All statistical analysis was performed at $P \leq 0.05$ using SYSTAT statistical software package.

RESULTS AND DISCUSSION

Dehyrogenase activity

To determine the selective influence of the two pesticides (imidacloprid and acephate) on dehydrogenase activity, the soil samples were treated with different concentrations (1.0, 2.5, 5.0, 7.5, and 10 kg ha^{-1}) of the pesticides for 7 days and the treated samples were exposed to TTC, which is a water-soluble and its redox potential is about -0.08mV and functions as an electron acceptor for several dehydrogenases (Thalman 1968). Nearly all microorganisms reduce TTC into TPF. Dehydrogenase activity was enhanced in both the soil samples following the application of imidacloprid and acephate at the concentration of 2.5 and 1.0 kg ha^{-1}, whereas, higher levels (5.0 to 10.0 kg ha^{-1}) were either toxic or innocuous to the enzyme activity. The two soil samples (black and red soil) treated with imidacloprid and acephate at 10 and 25 µg g^{-1} levels for 7 days and exposed to TTC for 24 h showed individual increments of 38-43, 30-48, 43-46 and 65-76% in dehydrogenase activity over the control. A

Table 2 Activity of dehydrogenase* under the impact of different concentrations of selected insectides in soils (both black and red) for 24 h after 7days.

Concentration of insecticides (Kg ha^{-1})	Black soil		Red soil	
	Imidacloprid	Acephate	Imidacloprid	Acephate
0.0	1850 ± 15.773 e	1850 ± 15.773 e	1020 ± 11.547 f	1020 ± 11.547 f
1.0	2560 ± 5.774 b	2405 ± 2.886 b	1460 ± 11.547 b	1680 ± 11.547 c
2.5	2650 ± 5.773 a	2749 ± 0.577 a	1490 ± 5.773 a	1800 ± 11.547 a
5.0	2215 ± 8.660 c	2000 ± 5.773 c	1440 ± 23.094 c	1730 ± 17.320 b
7.5	1950 ± 11.547 d	1670 ± 5.773 d	1300 ± 11.547 d	1600 ± 23.094 d
10.0	1450 ± 5.773 f	1020 ± 11.547 f	1220 ± 11.547 e	1580 ± 11.547 e

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

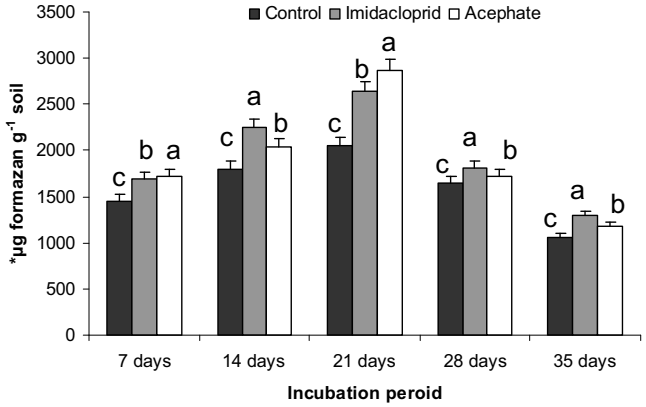

Fig. 1 Influence of selected insecticides at 2.5 kg ha^{-1} on dehydrogenase* activity in black soil after 24 h. *µg formazan g^{-1} soil formed after 24 h incubation with triphenyl tetrazolium chloride (TTC). Means, in each time period, followed by the same letter are significantly different ($P \leq 0.05$) from each other according to DMR test.

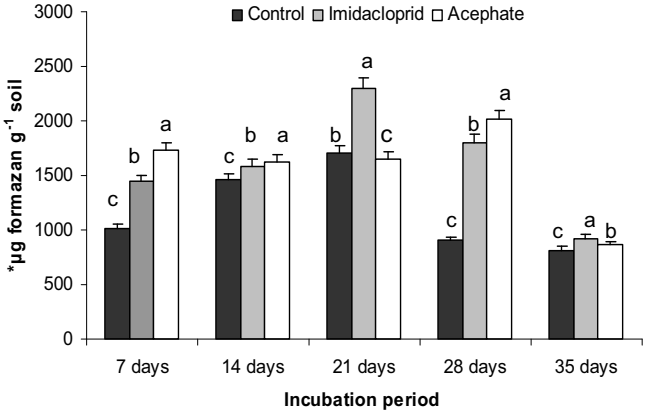

Fig. 2 Influence of selected insecticides at 2.5 kg ha^{-1} on dehydrogenase* activity red soil after 24 h. *µg formazan g^{-1} soil formed after 24 h incubation with triphenyl tetrazolium chloride (TTC). Means in each time period followed by the same letter are significantly different ($P \leq 0.05$) from each other according to DMR test.

significant increase in the activity of dehydrogenase was noticed after the application of acephate at 2.5 kg ha^{-1} in red soil (**Table 2**) and also in the accumulation of formazan when the incubation period was raised to 21 days (**Figs. 1, 2**). Hence, the results in the present study clearly indicated that the activity of dehydrogenase in black soil was comparatively higher than in red soil. In contrast, Cycon *et al.* (2010) reported that dehydrogenase activity decreased in sandy loam soils in combination of mancozeb and dimethomorph at higher concentrations. Similar observations were made by Monkiedje *et al.* (2002) with mefenoxam and metalaxyl and as well as azoxysstrobin, tebuconazole and chlorothalonil by Bending *et al.* (2007). In the same manner dehydrogenase activity was decreased to 39.3% in unamended polluted soils (with MCPA) (Tejda *et al*. 2010). Rasool and Reshi (2010) reported a significant increase in dehydrogenase activity with mancozeb at different application rates relative to the control. In another study, dehydrogenase activity was stimulated by fenamiphos (Caceres *et al.* 2009) in Australian and Ecuadorean soils. Akmal and Xu (2008) noticed a significant decrease in dehydrogenase (50%) enzyme activity with different concentrations (0, 200, 400, 600, 800, and 1000 mg kg^{-1}) of Pb-contaminated soil. Rangaswamy *et al.* (1994) reported a significant enhancement of dehydrogenase activity by monocrotophos, quinalphos, cypermethrin, and fenvalerate up to 2.5 kg ha^{-1}. However, at higher levels (5-12.5 kg ha^{-1}), these insecticides were either innocuous or toxic to dehydrogenase activity following 7 days of incubation. At 2.5 kg ha^{-1}, these insecticides stimulated dehydrogenase activity up to 21 days which then gradually declined (Rangaswamy *et al.* 1994). In the present investigation, pesticides at lower concentration stimulated dehydrogenase activity which is in agreement with findings of Rangaswamy *et al.* (1994). A significant increase in dehydrogenase activity was noticed with permethrin (FMC 33297), FMC 45498, Shell WL41706, Shell WL43467, and Shell WL43775 at 0.5 and 5 µg g^{-1} after 3 weeks of incubation (Tu 1980). Similarly, a significant increase in dehydrogenase activity up to 21 days was noticed with glyphosate, atrazine, primeextra and paraquat (Sebiomo *et al*. 2011). Tefluthrin, DOWCO 429X and DPX 43898 at 10 mg kg^{-1} increased dehydrogenase activity in a sandy loam soil after 2 weeks of incubation whereas dehydrogenase activity was initially reduced by tefluthrin and unaffected by other pesticides in an organic soil after 2 weeks (Tu 1990). In some cases, dehydrogenase activity was unaffected by several pesticides (Chendrayan *et al* 1980; Tu 1981).

The data presented in **Table 2** reveals that significant inhibition of dehydrogenase activity occurred at higher concentrations (10 kg ha^{-1}) of imidacloprid and acephate in both black and red soils collected from groundnut-cultivated fields. Similarly, Gowda (1973) also reported the inhibition of dehydrogenase activity in peptone-amended soil by benomyl at 100 to 10000 µg g^{-1} soil. The extent of dehydrogenase activity of soil samples under the impact of selected insecticides at 2.5 kg ha^{-1} was also determined by incubating the insecticide-treated samples for 7, 14, 21, 28 and 35 days (**Figs. 1, 2**). In general, dehydrogenase activity was relatively lower in the soil maintained under non-flooded conditions as reported by Chendrayan *et al.* (1980). This is expected because dehydrogenase activity is significantly more pronounced in flooded soils, as most dehydrogenases are anaerobic in origin (Chendrayan *et al* 1980). There was a progressive increase in the accumulation of formazan with increasing incubation period up to 21 days, which gradually decreased further. Hence, dehydrogenase activity was significantly enhanced with 2.5 kg ha^{-1} of the two insecticides up to 21 days of incubation. In fact, application of insecticides to soils led to an initial striking increase in dehydrogenase activity.

Urease activity

The activity of urease, implicated in the hydrolysis of urea, was significantly enhanced by the insecticides acephate and imidacloprid up to 5.0 kg ha^{-1}, in both soils, in comparison to the controls. However, higher concentrations (7.5 and 10 kg ha^{-1}) were toxic to urease activity after 10 days' incubation (**Table 3**). The activity of urease in terms of am-

Table 3 Activity of urease* under the impact of different concentrations of selected insecticides in soils (black and red) after 10 days.

Concentration of insecticides (Kg ha^{-1})	Black soil		Red soil	
	Imidacloprid	Acephate	Imidacloprid	Acephate
0.0	260 ± 5.774 d	260 ± 5.774 d	200 ± 11.542 e	200 ± 11.542 f
1.0	310 ± 57.735 c	300 ± 11.547 c	250 ± 5.774 c	260 ± 17.320 e
2.5	340 ± 23.094 b	380 ± 11.547 b	270 ± 5.773 b	303 ± 4.582 c
5.0	460 ± 17.320 a	400 ± 11.547 a	300 ± 11.547 a	402 ± 1.154 a
7.5	225 ± 14.433 e	310 ± 11.547 e	230 ± 17.320 d	350 ± 5.773 b
10.0	150 ± 5.774 f	160 ± 17.320 f	140 ± 23.094 f	280 ± 5.773 d

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

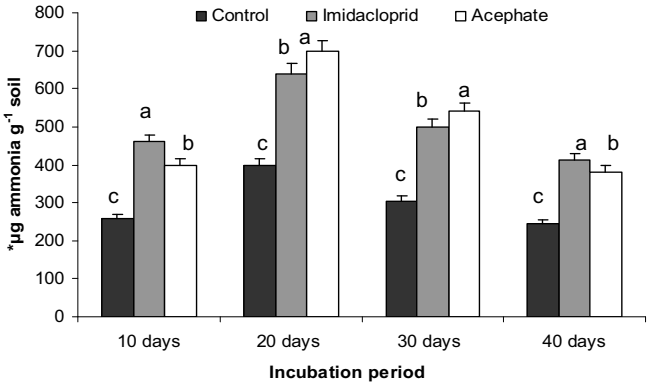

Fig. 3 Influence of selected insecticides at 5.0 kg ha^{-1} on urease* activity in black soil after 10, 20, 30 and 40 days. *µg ammonia g^{-1} soil formed after 3 h incubation at 37°C with 1 M urea. Means in each time period followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

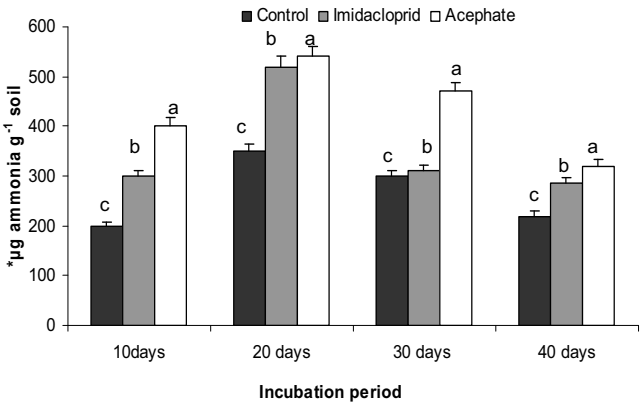

Fig. 4 Influence of selected insecticides at 5.0 kg ha^{-1} on urease* activity in red soil after 10, 20, 30 and 40 days. *µg ammonia g^{-1} soil formed after 3 h incubation at 37°C with 1 M urea. Means in each time period followed by the same letter are not significantly different ($P \leq 0.05$) from each other according to DMR test.

monia formed from urea was more pronounced in soil samples treated with 2.5 kg ha^{-1} and 5.0 kg ha^{-1} of acephate and imidacloprid, highest in red soil receiving 5.0 kg ha^{-1} acephate (**Table 3**). In fact, acephate and imidacloprid at 10, 25 and 50 µg g^{-1} individually caused a 30-77 and 46-54% increase in urease activity over the control, respectively in black soil after a 10-day interval. The corresponding values of urease activity in red soil for both insecticides at the same interval are 35-50 and 51-101% (**Table 3**). Urease activity decreased significantly after a longer period of incubation up to 30 or 40 days (**Figs. 3, 4**).

Similarly, 0.5 and 5 µg g^{-1} of pyrethroids, permethrin (FMC 33297), FMC 45498, Shell WL41706, Shell WL43467 and Shell WL43775 had no effect on urease activity in a sandy loam soil (Tu 1980). According to Gianfreda *et al*. (1994), glyphosate enhanced urease activity of soils 1.1-1.4-fold and of soil extracts 2.59 to 6.73-fold at 0.3 and 1.5 mM but had no influence on free or immobilized jackbean (*Canavalia ensiformis*) urease. Rasool and Reshi (2010) reported a significant decrease in urease activity with mancozeb at different application rates over the control. In another study, urease activity was not inhibited by fenamiphos (Caceres *et al.* 2009) in Australian and Ecuadorean soils. Similarly, urease activity was inhibited by napropamide at all concentrations relative to the control with long periods of application (Guo *et al.* 2008). Similarly, Cycon *et al.* (2010) noticed that urease activity declined in sandy loam and loamy sand soils with a combination (mancozeb + dimethomorph) at higher concentrations compared to the control. Similarly, urease activity decreased by 20% in unamended polluted soils (with MCPA) (Tejda *et al*. 2010) and following exposure to chlorpyrifos (CPF) and its oxon derivative (CPO) at higher concentrations (Wang *et al.* 2010). In another study, a 55.6% decrease in urease enzyme activity was noticed with different concentrations (0, 200, 400,600, 800, and 1000 mg kg^{-1}) of Pb-contaminated soil (Akmal and Xu 2008). Similarly, Rhamansyah *et al*. (2009) reported an increase in urease enzyme activity after 2 weeks of incubation which declined to 12 weeks of incubation with the insecticide deltamethrin and the fungicide probineb. In contrast, thiram at 10 ppm decreased urease activity in both sandy and organic soils after 7 days (Tu 1990). In another study, urease activity was not affected by the presence of glyphosate at 5.4 kg ha^{-1} in soil (Davies and Greaves 1981). Pesticides, including organophosphorus insecticides, could disrupt urea hydrolysis in soils at higher doses ranging from 100-1000 ppm (Lethbridge *et al*. 1981). Fenamiphos at 18.6 kg ha^{-1} reduced the activity of urease under field conditions but after 5 months' activity was the same as in the control while no effect was observed under laboratory conditions (Ross *et al.* 1984; Ross and Speir 1985).

CONCLUSION

The results obtained in the present study clearly indicate that the insecticides imidacloprid and acephate profoundly enhanced the activities of both dehydrogenase and urease at field application rates. Based on these 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 insect pests.

ACKNOWLEDGEMENTS

We are grateful to the University Grants Commission-SAP New Delhi for providing the financial assistance. We are also thankful to the Sri Krishnadevaraya University authorities for providing necessary facilities throughout my research work. Finally, we thank Dr. Jaime A. Teixeira da Silva for significant improvements in language and grammar.

REFERENCES

Akmal M, Xu J-M (2008) Dehydrogenase, urease and phosphatase activities as affected by Pb contamination in the soil. *Soil and Environment* **27 (2)**, 139-142

Alexander M (1961) *Introduction to Soil Microbiology*, Wiley Estern Ltd., New Delhi, 472 pp

Aramendia MA, Borau V, Lafont F, Martinas A, Marians JM, Moreno JM, Urbano FJ (2007) Determination of herbicide residues in olive oil by gas chromatography-tandem mass spectrometry. *Food Chemistry* **105**, 855-861

Bending GD, Lincon SD, Edmondson RN (2006) Spatial variation in the degradation rate of the pesticides isoproturon, azoxystrobin and diflufenican in soil and its relationship with chemical and microbial properties. *Environmental Pollution* **139**, 279-287

Bending GD, Rodríguez-Cruz SD, Lincoln SD (2007) Fungicide impacts on microbial communities in soils with contrasting management histories. *Chemosphere* **69**, 82-88

Bhalerao TS, Puranik PR (2007) Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*. *International Biodeteri oration and Biodegradation* **59**, 315-319

Benedetti A, Dilly O (2006) In: Bloem J, Hopkins DH, Benedetti A (Eds) *Microbiological Methods for Assessing Soil Quality*, CABI Publishing, Wallingford, UK, pp 3-14

Barnes H, Folkard BR (1951) The determination of nitrite. *Analyst* **76**, 599-603

Bollag JM, Liu SY (1990) Biological transformation processes of pesticides. In: Cheng HH (Ed) *Pesticides in the Soil Environment: Processes, Impacts, ND Modeling*, Soil Science Society of America, Madison, WI, USA, pp 169-211

Caceres TP, He W-X, Megharaj M, Naidu R (2009) Effect of insecticide fenamiphos on soil microbial activities in Australian and Ecuadorean soils. *Journal of Environmental Science and Health Part B* **44**, 13-17

Casida LE Jr., Klein DA, Santoro T (1964) Soil dehydrogenase activity. *Soil Science* **98**, 371-376

Chendrayan K, Adhya TK, Sethunathan N (1980) Dehydrogenase and invertase activities of flooded soils. *Soil Biology and Biochemistry* **12 (3)**, 271-273

Cycon M, Piotrowska-Seget Z, Kozdroj J (2010) Response of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *International Biodeterioration and Biodegradation* **64**, 316-323

Davies HA, Greaves MP (1981) Effects of some herbicides on soil enzyme activities. *Weed Research* **21**, 205-209

Fawcett JK, Scott JE (1960) A rapid and precise method for the determination of urea. *Journal of Clinical Pathology* **13**, 156-159

Gianfreda L, Rao MA, Piotrowska A, Palumbo G, Colombo C (2005) Soil enzyme activities as affected by anthropogenic alterations: Intensive agricultural practices and organic pollution. *Science of the Total Environment* **341**, 265-279

Gianfreda L, Sannino F, Ortega N, Nannipieri P (1994) Activity of free and immobilized urease in soil. Effects of pesticides. *Soil Biology and Biochemistry* **26**, 777-784

Gowda TKS (1973) Studies on the effect of benlate on microflora and other biological properties of soils of Mysore State. MSc thesis, University of Agricultural Science, Banglore, India, 160 pp

Garcia C, Hernández T, Pascual JA, Moreno JL, Ros M (2000) Microbial processes and strategies for their rehabilitation In: Garcia C, Hernández MT (Eds) *Research and Perspectivies of Soil Enzymology in Spain*, CEBAS-CSIC (Centro de Edalfologia y Biologia Aplicada del Segura – Consejo Superior De Investigaciones Cientificas), Murcia, pp 93-143

Guo H, Chen G-F, Lu Z-P, Zhao H, Yang H (2008) Alteration of microbial properties and community structure in soils exposed to napropamide. *Journal of Environmental Science* **21**, 494-502

Jaffer Mohiddin G, Srinivasulu M, Madakka M, Rangaswamy V (2010) Influence of insecticides on the activity of amylase and cellulase in groundnut (*Arachis hypogea* L.) soil. *Ecology, Environment and Conservation* **16 (3)**, 383-388

Jayashree R, Vasudevan N (2007) Persistence and distribution of endosulfan under field condition. *Environmetal Monitoring and Assessment* **131**, 475-87

Jackson ML (1971) *Soil Chemical Analysis*, Prentice Hall, New Delhi, India, pp 535-538

Johnson CM, Ulrich A (1960) Determination of moisture in plant tissues. *California Agriculture Bulletin* **766**, 112-115

Kizilkaya R, Askin T, Bayrakli B, Salgam M (2004) Microbiological characteristics of soils contaminated with heavy metals. *European Journal of Soil Biology* **40**, 95-102

Lethbridge G, Bull AT, Burns RG (1981) Effects of pesticides on 1,3-α-glucanase and urease activities in soil in the presence and absence of fertilizers, lime and organic materials. *Pesticide Science* **12**, 147-155

Min H, Ye Y-F, Chen Z-Y, Wu W-X, Du Y-F (2001) Effects of butachlor on microbial populations and enzyme activities in paddy soil. *Journal of Environmental and Science Health B* **36 (5)**, 581-595

Monkiedje A, Ilori MO, Spiteller M (2002) Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to sandy loam soil. *Soil Biology and Biochemistry* **34**, 1939-1948

Moorman TB (1989) A review of pesticide effects on microorganisms and microbial processes related to soil fertility. *Journal of Production Agriculture* **2**, 14-23

Megharaj M, Singleton I, Kookana R, Naidu R (1999) Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. *Soil Biology and Biochemistry* **31**, 1549-1553

Nannipieri P, Landa L (2000) Soil enzymes. In: Sumner HE (Ed), *Handbook of Soil Science*, CRC Press, New York, pp C129-C137

Omar SA, Abdel Sater MA (2001) Microbial populations and enzyme activities in soil treated with pesticides. *Water, Air, and Soil Pollution* **127 (1-4)**, 49-63

Pandey S, Singh DK (2006) Soil dehydrogenase, phosphomonoesterase and ar ginine deamidase activities in insecticide treated groundnut (*Arachis hypogea* L) field. *Chemosphere* **63**, 869-880

Pimentol D, Levitan L (1986) Pesticides: Amounts applied and amounts reaching pests. *Bio-Science* **36**, 89-91

Pascual JA, Garcia C, Hernández T, Moreno JL, Ross M (2000) Soil microbial activity as a biomarker of degradation and remediation processes *Soil Biology and Biochemistry* **32**, 1877-1883

Pessago RC, Rosa M, Torres Sánchez RM, dos Santos Afonso M (2008) Glyphosate behavior at soil and mineral – water interfaces. *Environmental Pollution* **153**, 53-59

Qian K, Tang T, Shi T, Wang F, Li J, Cao Y (2009) Residue determination of glyphosate in environmental water samples with high-performance liquid chromatography and UV detection after deviation with 4 chloro-3,5- dinitrobenzotrifluride. *Analytica et Chimica Acta* **635**, 222-226

Rahmansyah M, Antonius S, Sulistinah N (2009) phosphatase and urease instability caused by pesticides present in soil improved by grounded rice straw. *ARPN Journal of Agricultural and Biological Science* **4 (2)**, 56-62

Rasool N, Reshi ZA (2010) Effect of the fungicide Mancozeb at different application rates on enzyme activities in a silt loam soil of the Kashmir Himalaya, India. *Tropical Ecology* **51 (2)**, 199-205

Romeh AA, Mekky TM, Ramadan RA, Hendawi MY (2009) dissipation of profenofos, imidacloprid and penconazole in tomato fruits and products. *Bulletin of Environmental Contamination and Toxicology* **83 (6)**, 812-817

Ramesh PR, Kotur SC, Sudhir K (2003) Relationship of activities of urease and dehydrogenase with physico-chemical properties of some coffee-growing soils of Karnataka. *Journal of the Indian Society of Soil Science* **51**, 78-80

Rangaswamy V, Venkateswarlu K (1992) Activities of amylase and invertase as influenced by the application of monocrotophos, quinalphos, cypermethrin and fenvalerate to groundnut soil. *Chemosphere* **25 (4)**, 525-530

Rangaswamy V, Reddy BR, Venkateswarlu K (1994) Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin fenvalerate. *Agriculture, Ecosystems and Environment* **47**, 319-326

Ranney TA, Bartlett RJ (1972) Rapid field determination of nitrate in natural waters. *Communications in Soil Science and Plant Analysis* **3**, 183-186

Ross DJ, Speir TW (1985) Changes in biochemical activities of soil incubated with the nematicides oxamyl and fenamiphos. *Soil Biology and Biochemistry* **17**, 123-125

Ross DJ, Speir TW, Cowling JC, Whale KN (1984) Influence of field applications of oxamyl and fenamiphos on biochemical activities of soil under pasture. *New Zealand Journal of Science* **27**, 247-254

Sarfraz H, Siddique T, Saleem M, Arshad M, Khalid A (2009) Impact of pesticides on soil microbial diversity, enzymes and biochemical reactions. *Advances in Agronomy* **102**, 159-200

Sebiomo A, Ogundero VW, Bankole SA (2011) Effect of four herbicides on microbial population, soil organic matter and dehydrogenase activity. *African Journal of Biotechnology* **10 (5)**, 770-778

Swaminathan P, Prabaharan D, Uma L (2009) Fate of few pesticides-metabolizing enzymes in the marine cyanobacterium *Phormidium valderianum* BDU 20041 in perspective with chlorpyrifos exposure. *Pesticide Biochemistry and Physiology* **94**, 68-72

Sukul P (2006) Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues. *Soil Biology and Biochemistry* **38**, 320-326

Tejada M, Garcia-Martinez AM, Gomez I, Parrado J (2010) Application of MCPA herbicide on soils amended with biostimulants: Short-time effects on soil biological properties. *Chemosphere* **80**, 1088-1094

Trevors JT (1984) Dehydrogenase activity in soil: A comparison between the INT and TTC assay. *Soil Biology and Biochemistry* **16**, 673-674

Tu CM (1980) Influence of pesticides and some of the oxidised analogues on microbial population, nitrification and respiration activities in soil. *Bulletin of Environmental Contamination and Toxicology* **24**, 13-19

Tu CM (1981) Effect of pesticides on activity of enzymes and microorganisms in a clay loam soil. *Journal of Environmental Science and Health* **16**, 179-180

Tu CM (1990) Effect of four experimental insecticides on enzyme activities and levels of adenosine in mineral and organic soils. *Journal of Environmental Science and Health* **25**, 787-800

Tu CM (1995) Effect of five insecticides on microbial and enzymatic activities in sandy soil. *Journal of Environmental Science and Health B* **30**, 289-306

Vinas P, Campillo N, Lopez-Garcia I, Aguinaga N, Hernández-Cordoba M (2002) Determination of pesticides in waters by capillary gas chromatography with atomic emission detection. *Journal of Chromatography A* **978 (1-2)**, 249-256

Vijay Gundi AKB, Viswanath B, Subhosh Chandra M, Narahari Kumar V, Rajasekhar Reddy B (2007) Activities of cellulose and amylase in soils as influenced by insecticide interactions. *Ecotoxicology and Environmental Safety* **68**, 278-285

Walker A, Jurado-Exposito M, Bending GD, Smith VJR (2001) Spatial variability in the degradation rate of isoproturon in soil. *Environmental Pollution* **111**, 417-427

Wang F, Yao J, Chen H-L, Chen K, Trebse P, Zaray G (2010) Comparative toxicity of chlorpyrifos and its oxon derivatives to soil microbial activity by combined methods. *Chemosphere* **78**, 319-326