e-ISSN:2320-3528
p-ISSN:2347-2286

Research and Reviews: Journal of Microbiology and Biotechnology

# Impact of Pesticides Combination on Soil Microorganisms.

Maddela Naga Raju[a]* and Kadiyala Venkateswarlu[b]

[a]Universidad Estatal Amazonica, Puyo – 60150, Ecuador.
[b]Department of Microbiology, Sri Krishnadevaraya University, Anantapur - 515055, India.

Research Article

Received: 22/08/2013
Revised: 17/09/2013
Accepted: 25/09/2013

***For Correspondence**

Universidad Estatal Amazonica,
Puyo – 160150, Ecuador.
Cell: +593 968124116

**Keywords:** Buprofezin – acephate – nontarget effects – soil enzymes – NPK-amendments

## ABSTRACT

The nontarget effects of buprofezin and acephate, in combination at concentrations ranging from 2.5 to 10 μg $g^{-1}$, towards activities of cellulases, amylase and invertase in unamended and NPK-fertilizer-amended cotton soils were studied. Enzyme activities were determined spectrophotometrically by using standard *in vitro* soil incubation studies. The influence of the selected insecticides on enzyme activities was dose-dependent. Additive and synergistic stimulatory responses were noticed at lower concentrations; where as, at higher rates of insecticides, antagonistic inhibitory reactions were observed. It is apparent, therefore, that the soil application of buprofezin and acephate combinations, at higher concentrations, resulted in an interaction leading to significant antagonistic effect on activities of the soil enzymes tested. Thus, this study undoubtedly highlights the impact of insecticides on soil microflora, and also it aids in the deterrence of intensive and extensive usage of insecticides in agriculture.

## INTRODUCTION

A major problem that arises consistently in modern agriculture is that, it has become a common trend to apply different groups of pesticides, either simultaneously or in succession, for effective control of a variety of pests. It is quite apparent that the crop protection by pesticides results in pesticide residues in the soil, which ultimately the sink of all these xenobiotic compounds. It has long been known that pesticide applications at recommended rates have little or no effect on enzyme activity in soils [17, 5]. Numerous reports, however, have indicated that soil enzyme activities were significantly affected when pesticides were applied to soil at higher than recommended rates over long periods [11,34].

In recent decades, there is a serious concern about the vast economic damage caused to cotton by many insect pests. Although several insecticides are used on need basis for the effective control of these insect pests on cotton, two insecticides, particularly, buprofezin (Applaud®) and acephate (Hythene®) are widely used in the recent years to combat major insect pests of cotton. Although, more data exist for other pesticides of individual treatments, virtually no information is available in the literature on nontarget effects of combination of these two insecticides towards microbial activities in soil. In the present investigation, an attempt has, therefore, been made to assess the impact of combination of buprofezin and acephate on cellulases, amylase and invertase activities in unamended and NPK-fertilizer-amended soils.

## MATERIALS AND METHODS

### Soil collection

Soils with and with out known history of insecticide (buprofezin or acephate) use, were collected from fields (inherently very fertile) under cultivation of cotton at Nandyal, a semi-arid region of Andhra Pradesh, India, to a depth of 12 cm. The collected samples were mixed, air-dried and sieved through a 2-mm mesh prior to use. The physico-chemical properties (Table 1) and microbiological characteristics were determined following standard procedures. Soil pH was determined using an electrode and 1:1.25 of soil-water slurry [25]. Electrical conductivity was determined by the addition of 100 mL water to 1 g soil sample using Elico conductivity meter. The method described by Johnson and Ulrich [9] was employed for estimating 60% water-holding capacity. Organic carbon and total nitrogen content in soil were quantified using Walkley-Black method [14] and Micro-Kjeldahl's method [8], respectively. Populations of bacteria and fungi (Table 2), in both the soil samples, were isolated and enumerated following serial dilution and plating method.

**Table 1: Physicochemical properties of the soil used in the present study**

| Colour | Thick black |
|---|---|
| pH (1:1.25 soil–water slurry) | 8.2 |
| Texture: | |
| Clay (%) | 64 |
| Silt (%) | 15 |
| Sand (%) | 21 |
| Electrical conductivity (μmhos/cm) | 0.24 |
| 60% Water-holding capacity (mL 100 $g^{-1}$) | 45.6 |
| Organic matter (%) | 3.602 |
| Total nitrogen (g $kg^{-1}$ soil) | 0.14 |
| Available potassium ($K_2O$) in (g $kg^{-1}$) | 4.19 |
| Available phosphorus ($P_2O_5$) in (mg $g^{-1}$ soil) | 4.25 |
| Calcium | High |

**Table 2: Occurrence of bacteria and fungi (CFU $g^{-1}$ soil) in selected soil samples**

| Microflora | Soil with no history of insecticide use | Soil with history of insecticide use |
|---|---|---|
| Bacteria | $3 \times 10^8$ | $2 \times 10^9$ |
| Fungi | $6 \times 10^4$ | $2 \times 10^5$ |

**Insecticides and fertilizers selected in the present study**

Two insecticides, buprofezin (2-tert-butylimino-3-isopropyl-5-phenyl-1,3,5-thiadiazinan-4-one) and acephate (O,S-dimethyl acetyl phosphoramidothioate), were selected for the present investigation in view of their extensive and intensive usage in modern agriculture in India. The mineral fertilizer urea, calcium perphosphate and potassium were used at a specified rate (shown in section 1).

**Effect of selected insecticides on soil enzyme activities**

Aqueous solutions of commercial formulations of the two insecticides, buprofezin and acephate were prepared in water and added to 5 g portions of the soil in test tubes (25 × 200 mm) to get insecticide combinations, buprofezin + acephate with graded concentrations (2.5 to 10 μg $g^{-1}$ soil) as described earlier by Gundi *et al.* [7]. The final concentrations (on w/w basis) of each insecticide included 2.5, 5.0, 7.5 and 10.0 μg $g^{-1}$ soil, which correspond to 0.25, 0.5, 0.75 and 1.0 kg $ha^{-1}$, respectively [1]. These concentrations were chosen because of the fact that the field application dose of the selected insecticides range from ~ 0.3 to 0.6 kg $ha^{-1}$. Also, in one set, soil samples that received insecticide combinations were amended with fertilizers. All the tubes including control were maintained at 60% water-holding capacity, and incubated at 28 ± 4 °C, activities tested after 3 days. After 3 days of incubation, triplicate soil samples were withdrawn for the assay of cellulases, amylase, and invertase activities.

The per cent inhibition values were calculated relative to the activity in untreated controls. Interaction data for the combinations employed were analyzed by the multiplicative survival model as outlined by Stratton [24]. The expected interaction responses for the insecticide combinations were calculated using the formula, $E = X + [(100 - X)/100] \times Y$; where E = the expected additive effect of the mixture, X = the per cent inhibition due to component A alone, and Y = the per cent inhibition due to component B alone. The mean ratios between actual inhibition and expected inhibition significantly greater or less than 1.0 indicated synergism and antagonism, respectively, while an additive effect occurred when the actual and expected inhibitions did not differ significantly [23].

### Assay of Selected Soil Enzymes

### Cellulases

The activities of cellulases were assayed by the method described by Pancholy and Rice [16] expressed in terms of mg glucose $g^{-1}$ 30 $min^{-1}$ using glucose as a standard. Triplicate samples (5 g) of soil were withdrawn after desired intervals, placed in 50 mL Erlenmeyer flasks and 0.5 mL of toluene was added. Contents in the flasks were mixed thoroughly, 10 mL of 0.5 M acetate buffer (pH 5.9) was added after 15 min followed by the addition of 10 mL of 1% carboxy methyl cellulose (CMC). After 30 min of incubation, approximately 50 mL of distilled water was added. Then the suspension was filtered by Whatman No.1 filter paper and volume of the filtrate was made up to 100 mL with distilled water. A suitable aliquot of the supernatant was treated with 2 mL of alkaline copper reagent [13]; tubes were placed in boiling water bath for 10 min, cooled to room temperature. Then, mixture was treated with 1 mL of arsenomolybdate reagent followed by the addition of 5 mL of distilled water. The blue color developed was read at 620 nm in a spectrophotometer.

### Amylase

Amylase activity in untreated and insecticide- and/or fertilizer-treated soil samples was determined following the method developed by Cole [3] and modified by Tu [29,31]. Five grams of soil samples were placed in 25 mL of boiling test tubes, and 1 mL of toluene was then added. All the contents in the tubes were mixed thoroughly, and after 15 min, 6 mL of 2% starch in 0.2 M acetate buffer (pH 5.5) was added. The tubes were incubated for 48 h. The soil suspension was filtered through Whatman No.1 filter paper, and the amount of reducing sugar content in the filtrate was determined by Nelson-Somagyi method in an Elico digital spectrophotometer and activity was expressed in terms of mg glucose $g^{-1}$ 48 $h^{-1}$.

### Invertase

Invertase activity of the test soil samples was determined following the method of Tu [32]. Five grams of soil samples were placed in 25 mL of boiling test tubes, and 1 mL of toluene was added. The contents in the tubes were mixed thoroughly. After 15 min, 6 mL of 18 mM sucrose in 0.2 M acetate buffer (pH 5.5) was added, and the tubes were incubated for 6 h. Finally, the suspension was filtered through Whatman No.1 filter paper, and the amount of reducing sugar in the filtrate was determined by Nelson-Somagyi method in an Elico digital spectrophotometer. The enzyme activity was expressed as mg of glucose released per g of soil per 6 h (mg glucose $g^{-1}$ 6 $h^{-1}$).

## RESULTS

### Nontarget Effects of Buprofezin and Acephate on Soil Cellulases

Acephate treatment, at 2.5 µg $g^{-1}$ soil in combination with buprofezin up to 7.5 µg $g^{-1}$ soil caused significant synergistic effect on enzyme activity (Table 3a). Additive response was observed only with 5 µg $g^{-1}$ soil concentration of acephate and 2.5 µg $g^{-1}$ soil of buprofezin. Higher concentrations of buprofezin or acephate were significantly toxic, resulting in antagonistic interaction towards the enzyme activity. In actual fact, fertilizer amendments to the soil samples together with the insecticide combinations at graded levels had no measurable effect in altering the interaction effects noticed with the soil samples that received no NPK fertilizers (Table 4a).

### Nontarget Effects of Buprofezin and Acephate on Soil Amylase

In insecticide combination, buprofezin at a rate of 2.5 µg $g^{-1}$ exerted synergistic response on amylase with acephate up to 7.5 µg $g^{-1}$ soil (Table 3b). Where as, same combination at 5 µg $g^{-1}$ in the mixture has shown additive effect on enzyme activity. Nonetheless, higher concentrations of two insecticides combination have shown significant antagonistic effect on soil amylase. Furthermore, combination of two insecticides interacted more antagonistically with amylase in soil received with NPK-fertilizer (Table 4b).

Table 3. Interaction effects of insecticide combinations on (a) cellulase, (b) amylase, and (c) invertase activities in soil

Buprofezin (µg $g^{-1}$ soil)

Acephate (µg $g^{-1}$ soil)

| (a) | 0 | 2.5 | 5 | 7.5 | 10 |
|---|---|---|---|---|---|
| 0 | Control[x] | 40[a] | 140 | 100 | -60 |
| 2.5 | 1[a] | 220[a]/40.6[b] | 380/140 | 240/100 | 1/-58.4 |
| 5 | 240 | 180/184 | 80/44 | 1.0/100 | 240/324 |
| 7.5 | -20 | 220/28 | 240/148 | 40/100 | 1.0/92 |
| 10 | -60 | -20/4 | 140/164 | 60/100 | -20/156 |

| (b) | 0 | 2.5 | 5 | 7.5 | 10 |
|---|---|---|---|---|---|
| 0 | Control[y] | 5[a] | 24 | 67 | -29 |
| 2.5 | 5[a] | 90[a]/9[b] | 248/27 | 81/68 | 19/-22 |
| 5 | 57 | 67/59 | 62/67 | 48/86 | 19/45 |
| 7.5 | 29 | 95/32 | 29/46 | 43/76 | 1/8 |
| 10 | 19 | 19/23 | 33/38 | 14/70 | 33/-4 |

| (c) | 0 | 2.5 | 5 | 7.5 | 10 |
|---|---|---|---|---|---|
| 0 | Control[z] | 145[a] | 482 | 173 | 82 |
| 2.5 | 127[a] | 518[a]/203[b] | 1664/967 | 118/265 | 100/105 |
| 5 | 209 | 236/240 | 1354/1280 | 1.0/325 | -82/120 |
| 7.5 | 73 | 164/112 | -9/203 | -45/120 | -55/95 |
| 10 | -36 | 45/161 | -54/619 | 1/235 | -27/75 |

All entries are means of per cent stimulation/inhibition values of enzyme activity relative to untreated control ([x]0.1, [y]0.004, and [z]0.185 mg glucose $g^{-1}$ $h^{-1}$)

[a]Experimental per cent values over control; [b]Expected per cent values over control

Antagonistic Synergistic Additive response

Table 4. Interaction effects of insecticide combinations on (a) cellulase, (b) amylase, and (c) invertase activities in NPK-amended soil

Buprofezin (μg $g^{-1}$ soil) (columns); Acephate (μg $g^{-1}$ soil) (rows)

(a)

| | 0 | 2.5 | 5 | 7.5 | 10 |
|---|---|---|---|---|---|
| 0 | Control[x] | 40[a] | 100 | 80 | 1.0 |
| 2.5 | 40[a] | 80[a]/64[b] | 200/100 | 100/88 | 1/41 |
| 5 | 200 | 160/160 | 340/100 | 40/120 | 140/199 |
| 7.5 | 40 | 140/64 | 80/100 | 40/80 | 1/41 |
| 10 | 1.0 | -20/41 | 40/100 | -40/80 | -20/1.99 |

(b)

| | 0 | 2.5 | 5 | 7.5 | 10 |
|---|---|---|---|---|---|
| 0 | Control[y] | 60[a] | 70 | 190 | 30 |
| 2.5 | 70[a] | 140[a]/88[b] | 140/91 | 190/127 | 40/79 |
| 5 | 160 | 320/118 | 27/124 | 210/46 | 120/142 |
| 7.5 | 240 | 120/156 | 110/142 | 210/-26 | 110/198 |
| 10 | 220 | 50/124 | 180/236 | 120/-8 | 140/184 |

(c)

| | 0 | 2.5 | 5 | 7.5 | 10 |
|---|---|---|---|---|---|
| 0 | Control[z] | 9[a] | 26 | 11 | -1 |
| 2.5 | 1[a] | 40[a]/10[b] | 54/27 | 37/12 | -18/0.01 |
| 5 | 40 | 151/45 | 63/56 | 21/47 | -20/39 |
| 7.5 | 6 | 20/14 | -17/36 | 11/16 | -14/5 |
| 10 | -17 | 6/-6 | -21/13 | 1/-4 | -14/-18 |

All entries are means of per cent stimulation/inhibition values of enzyme activity relative to untreated control ([x]0.05, [y]0.1, and [z]0.65 mg glucose $g^{-1}$ $h^{-1}$)

[a]Experimental per cent values over control; [b]Expected per cent values over control

Antagonistic   Synergistic   Additive response

### Nontarget Effects of Buprofezin and Acephate on Invertase

Buprofezin and acephate at equal concentrations of 2.5 μg g⁻¹ in combination caused synergistic stimulation on soil invertase activity (Table 3c). Additive response was observed only with 2.5 μg g⁻¹ soil of buprofezin and 5 μg g⁻¹ soil of acephate. However, combinations of 7.5 and 10 μg g⁻¹ buprofezin with graded concentrations of acephate elicited antagonistic response towards invertase activity in soil. Similar results were also recorded in NPK amended soil (Table 4c).

## DISCUSSION

Two insecticides employed in the present study for their nontarget effects towards the activities of enzymes in soil have shown apparent pessimistic impact. From the experimental data, it is clearly evident that soil cellulase is highly sensitive to anthropogenic substances in soil. Gundi *et al*. [6] reported that the soil treated with monocrotophos, up to 25 μg g⁻¹, was either nontoxic or stimulatory, but soil cellulase activities were adversely affected at higher rates of this insecticide. Several studies have noted to evidence that, though the activity of cellulases were stimulated at lower rates, but were affected badly when soil treated with higher concentrations of different pesticides include baythroid [10], diazinon [20], etc. In another work, brominal and selecron inhibited cellulase activity in soil after most incubation periods [15]. Surprisingly, Cycon and Piotrowska-Seget [4] reported that there was an initial drop in the population of heterotrophic bacteria and fungi, but was stimulated at higher doses of an organophosphate insecticide, diazinon, in soil. On the other hand, though lower rates of insecticide combination effected synergistic and additive responses in the activity of cellulases, but at higher rates, an antagonistic response was recorded in both soils. A study [6] has found evidence to support that the combination of monocrotophos and cypermethrin or quinalphos and cypermethrin, at different levels yielded synergistic and antagonistic responses towards cellulase activity in soil. Concluding, researchers warn that indiscriminate use of buprofezin and acephate, at higher rates but not at field applications rates, is deleterious to cellulases in soils.

As was emphasized previously, buprofezin and acephate at higher concentrations were also toxic to the amylase activity in soil and the same was reported in many studies [10, 21, 6]. For example, endosulfan at 32 and 48 μL L⁻¹ was reported to stimulate α-amylase activity in the supernatant of culture filtrate, but the enzyme activity was adversely affected at 64 and 80 μL L⁻¹ [26]. But in other studies, activity was stimulated at lower concentrations, but was negatively affected when soil was treated with higher rates of monocrotophos, quinalphos, cypermethrin, fenvalerate [18, 6], malathion and permethrin [30] and other organophosphate insecticides [32]. On the contrary, application of cyfluthrin and imidacloprid to soil initially inhibited amylase activity followed by the stimulatory effect at the end of 3 weeks of incubation [27]. Interestingly, at a concentration of 930 mg kg⁻¹, fenamiphos had a deleterious effect on amylase activity, which was reduced by 24%, after 62 days treatment under laboratory conditions [19]. On the other hand, combinations of buprofezin and acephate at higher concentrations yielded an antagonistic response for amylase activity in unamended and NPK-amended soils. There are very few published examples of pesticides combination effects on amylase activity in soil. In a recent study, Gundi *et al.* [6] reported that an increment of about 51% of amylase activity occurred with the combination of monocrotophos and cypermethrin at 5 μg g⁻¹ by the end of 10 days incubation; individually, the increments were 30% and 17%, respectively. Therefore, the present results indicate that higher application rates of the two insecticides, either singly or repeated applications or in combination greatly affect the soil amylase.

This study also tried to investigate the effects of buprofezin and acephate on invertase activity in soil. The most important impact of these findings is that the invertase is known to be affected badly by the repeated applications and in combination of two selected insecticides at higher rates. There is good evidence that invertase activity increased by 110.9% at 1.6 μg baythroid g⁻¹ soil and was decreased by 40.3% at the highest level studied [10]. In another study, Sreenivasulu and Rangaswamy [22] reported that invertase activity in soil was increased with increasing concentrations of fungicides; the enzyme activities were adversely affected at higher rates of each test chemical. On the other hand, carbaryl insecticide applied at a normal agricultural dose did not have any inhibitory effect on invertase activity in soil [12]. In contrast, a study by Tu [28] revealed that captafol and chlorothalonil suppressed invertase activity for one day temporarily in a sandy loam soil and later on, after 2 days, the inhibitory effect was alleviated. A report by Voets *et al*. [33] demonstrated for the fist time that long-term atrazine applications significantly reduced the activity of invertase in soil. Perhaps, more alarmingly, amendment of the soil with NPK fertilizers seems to lessen the toxicity of the insecticides towards invertase. A Study by Bielinska and Pranagal [2], however, showed that the application of high level of mineral fertilization simultaneously with chemical weed control appears to be detrimental particularly to biological activity of the soil. This means that the accompanying decrease in $pH_{KCl}$ was an additional cause of the strong lowering in soil enzymatic activity for that site. Therefore, from the above data, it can be concluded that invertase is highly sensitive to the selected insecticides when applied alone or in combinations. But, amendment of the soil with NPK fertilizers seems to alleviate the toxicity of the insecticides towards invertase. Although these results suggest that the studied enzymes are known to be affected by the selected insecticides *in vitro*, it remains unclear how these are actually influenced at field level. In conclusion, insecticide combinations, at higher concentrations, resulted in an interaction leading to significant antagonistic effect on activities of all the soil enzymes tested. This clearly warrants the judicious use of insecticides at recommended doses only.

## ACKNOWLEDGEMENTS

We wish to thank Dr. S. Imthiyaz Ahamed, Principal, National P.G. College, Nandyal, India for providing lab facilities and encouraging us through out this study.

## REFERENCES

1. Anderson JR Pesticide effects on non-target soil microorganisms. In: I.R. Hill and S.J.L. Write (Eds.), Pesticide Microbiology. Academic Press, London, pp. 1978, 313-533.
2. Bielinska EJ, Pranagal J. Enzymatic Activity of Soil Contaminated with Triazine Herbicides. Pol J Environ Stud. 2007;16:295-300.
3. Cole MA. Lead inhibition of enzyme synthesis in soil. Appl Environ Microbiol. 1977;33:262-268.
4. Cycon M, Piotrowska-Seget Z. Effect of selected pesticides on soil microflora involved in organic matter and nitrogen transformations: pot experiment. Pol J Ecol. 2007;55:207-220.
5. Dick RP. Soil enzyme activities as integrative indicators of soil health. In: Pankhurst, C.E., Doube, B.M. and Gupta, V.V.S.R. (Eds.). Biological Indicators of Soil Health. CAB International, Wallingford, UK. pp. 1997, 121-156.
6. Gundi VA, Viswanath B, Chandra MS, Kumar VN, Reddy BR. Activities of cellulase and amylase in soils as influenced by insecticide interactions. Ecotoxicol Environ Safe. 2007;68:278-285.
7. Gundi VAKB, Narasimha G, Reddy BR. Interaction effects of insecticides on microbial populations and dehydrogenase activity in black clay soil. J Sci Environ Heal B. 2005;40:269-283.
8. Jackson ML. The Text Book of Soil Chemical Analysis. Prentice-Hall Inc, Engle Wood Cliffs, Jersy. 1973, p187.
9. Johnson CM, Ulrich A. Determination of moisture in plant tissues. California Agricultural Bulletin No. 766. In: Wilde, S.A., (ed.), Soil and Plant Analysis for Tree Culture. Obrtage publishing Co, Oxford and Bombay. 1960, p112-115.
10. Lodhi A, Malik NN, Mahmood T, Azam F. Response of Soil Microflora, Microbial biomass and some Soil Enzymes to Baythroid (An insecticide). Pak J Biol Sci. 2000;3:868-871.
11. Megharaj M. Heavy pesticide use lowers soil health. Kondinin Landcare. http://www.clw.csiro.au/publications/farming_ahead/2002/p3738_FA121_JAN02.pdf, 2000.
12. Mishra PC, Pradhan SC. Seasonal variation in amylase, invertase, cellulase activity and carbon dioxide evolution in tropical protected grassland of Orrisa, India, sprayed with carbaryl insecticide. Environ Pol. 1987;43:291-300.
13. Nelson N. A photometric adaptation of Somagyi method for determination of glucose. The J Biol Chem. 1944;153:375-380.
14. Nelson DW, Sommers LE. Total carbon, organic and organic matter. In: Sparks, D.L. (Ed.), Methods of Soil Analysis, Part 3. Soil Science Society of America Book Series, vol. 5. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin. 1996, pp. 961–1010.
15. Omar SA, Abdel-Sater MA. Microbial populations and enzyme activities in soil treated with pesticides. Water, Air Soil Pol. 2001;127:49-63.
16. Pancholy SK, Rice EL. Soil enzymes in relation to old field succession: Amylase, cellulase, invertase, dehydrogenase and urease. Soil Sci. Soc. American Proc. 1973;37:47-50.
17. Rangaswamy V, Reddy BR, Venkateswarlu K. Activities of dehydrogenase and protease in soil as influenced by monocrotophos, quinalphos, cypermethrin and fenvalerate. Agric Eco Environ. 1994;47;319-326.
18. Rangaswamy V, Venkateswarlu K. Activities of amylase and invertase as influenced by the applications of monocrotophos, quinalphos, cypermethrin and fenvalerate to groundnut soil. Chemosphere. 1992;25:525-530.
19. Ross DJ, Speir TW. Changes in biochemical activities of soil incubated with the nematicides and fenamiphos. Soil Biol Biochem. 1985;17:123-125.
20. Shittu OB, Akintokun AK, Akintokun PO, Gbadebo MO. Effect of diazinon application on soil properties and soil Micro flora. Proceedings of International Conference and National Development, 25th – 28th October. COLNAS Proc. 2004, pp. 68-74.
21. Singh BK, Allan W, Denus JW. Degradation of chloropyrifos, fenamiphos and chlorothalonil alone and in combination and their effects on soil microbial activity. Environ Toxicol Chem. 2002;21:2600-2605.
22. Srinivasulu M, Rangaswamy V. Activities of invertase and cellulase as influenced by the application of tridemorph and captan to ground nut (*Araachis hypogeae*) soil. Afr J Biotech. 2006;5:175-180.

23. Stratton GW. Effects of the herbicide atrazine and its degradation products, alone and in combination on phototrophic microorganisms. Arch. Environ. Cont Toxicol. 1984;13:35-42.
24. Stratton GW. Interaction effects of permethrin and atrazine combinations towards several non target microorganisms. Bull Enviro Con Toxicol. 1983;31:297-303.
25. Thomas GW. Soil pH and soil acidity, In: Sparks, D.L. (ed.), Methods of Soil Analysis, Part 3. Soil Science Society of America Book Series. American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin. 1996, 5: 475-490.
26. Tolan V, Ensari Y. Effect of endosulfan on growth, α-amylase activity and plasmid amplification in *Bacillus subtylis*. Indian J Biochem Biophy. 2006;43:123-126.
27. Tu CM. Effect of five insecticides on microbial and enzymatic activities in sandy soil. J Environ Sci Heal Part B. Pesticides, Food Contaminants, and Agricultural Wastes. 1995;30:289-306.
28. Tu CM. Effect of fungicides, captafol and chlorothalonil, on microbial and enzymatic activities in mineral soil. J Environ Sci Heal. 1993;28:67-80.
29. Tu CM. Effect of pesticides on activity of enzymes and microorganisms in a clay loam soil. J Environ Sci Heal. 1981a;16:179-191.
30. Tu CM. Effect of selected pesticides on activities of invertase, amylase and microbial respiration in sandy soil. Chemosphere. 1988;17:159-163.
31. Tu CM. Effect of some pesticides on enzyme activities in an organic soil. Bull. Environ. Cont Toxicol. 1981b;27:109-114.
32. Tu CM. Influence of pesticides on activities of amylase, invertase and level of adenosine triphosphate in organic soil. Chemosphere. 1982;2:909-914.
33. Voets JP, Meerschman P, Verstraete W. Soil microbiological and biochemical effects of long term atrazine applications. Soil Biol Biochem. 1974;6:149-152.
34. Wyszkowska J, Kucharski J. Biochemical and physicochemical properties of soil contaminated with herbicide Triflurotex 250 EC. Pol J Environ Stud. 2004;3:223-231.