[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

Original Article

Interaction Effects of Selected Pesticides on Soil Enzymes

B. Vineela Deborah, M. Jaffer Mohiddin, R. Jaya Madhuri

Department of Applied Microbiology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati, Andhra Pradesh, India

ABSTRACT

The laboratory studies were conducted to resolute the effects of imidacloprid (insecticide) and triadimefon (fungicide) singly and in combination on enzymatic activities of soil microorganisms in tomato cultivated soils at different concentrations of 0.2, 0.5 and 0.7 kg/ha. The rate of amylase activity was stimulated by the application of pesticides at field rate. High dosage decreased the activity of amylase. Decline in the activity of cellulase was observed at all concentrations than control. Imidacloprid had an improved activity of cellulase at 0.5 µg/g than tridimefon and combination. At higher concentration (0.7 µg/g), the combination of insecticide and fungicide showed an antagonistic interaction toward cellulase. After 24 h, maximum inhibition was observed in invertase enzyme rate at all examined dosages. After 48 h, the activity was revived to some extent and imidacloprid showed enhanced activity at 0.5 µg/g (field rate). However at 0.7 µg/g, imidacloprid has a noticeable effect on the invertase. The pesticide application in single and in combination (0.2-0.7 µg/g soil) triggered the dehydrogenase activity. At field rate triadimefon significantly quickened the activity.

Key words: Imidacloprid, soil enzymes, tomato cultivated soils, triadimefon

INTRODUCTION

Modern agriculture depends upon a wide variety of synthetically produced chemicals including insecticides, fungicides, herbicides and other pesticides.[1] When a synthetic pesticide is released into the environment, about 0.1% is reaching the target organism, while the remaining 0.99% of interferes local metabolism or enzymatic activities, and also affects human health by entering into the food chain, which has raised considerable public concern.[2] Soil is a living dynamic system containing many free enzymes, immobilized extracellular enzymes and enzymes within microbial cells. Soil enzymes are the soil quality indicators which play an important role in organic matter decomposition and nutrient cycling.[3,4] Thus, it is required to estimate soil biological responses to the pesticides in terms of soil enzyme activities.

Access this article online

Quick Response Code:

Website: www.toxicologyinternational.com

DOI: 10.4103/0971-6580.121665

Tomato (*Lycopersicum esculentum*) is a major vegetable crop in Madanapalle, Chittoor district of Rayalaseema region, Andhra Pradesh, India. It is grown in abundance in the district with an average of 35,000 acres producing 3-4 lakh million tons per annum and extensively used in fruit processing industries. Pesticides like imidacloprid and triadimefon are commonly used for pest control in tomato crop nowadays. Imidacloprid is a systemic nicotinic compound with a potent insecticidal activity against a wide range of pests of vegetable crops.[5] and triadimefon is a systemic triazole foliar fungicide with a good fungicidal activity (Extension Technology Network, Cornell university). Despite of their potent role in pest control, there is no information available on the interaction effects of imidacloprid and triadimefon in tomato cultivated fields of Madanapalle.

Cellulose, the most abundant organic compound in the biosphere comprising almost 50% of the biomass

Address for correspondence: Ms. B. Vineela Deborah, Department of Applied Microbiology, Sri Padmavathi Mahila Visvavidyalayam, Tirupati - 517 502, Andhra Pradesh, India. E-mail: vinnideborah@gmail.com

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

synthesized by photosynthetic fixation of CO_2. Cellulases catalyse the degradation of cellulose and polysaccharide buildup of β-1, four linked glucose units.[6] Amylase hydrolyses α bond of polysaccharides or starch in soil yielding glucose and maltose.[7] Invertase is an enzyme that catalyses the hydrolysis of sucrose to fructose and glucose.[8] Dehydrogenases, as respiratory chain enzymes, play a major role in the energy production of organisms, which are responsible for the decomposition and conversion of organic matter.[9]

It is the farmer's practice in Madanapalle to use pesticides in combination to prevent the number of pests of tomato at the same time, which will enable sustainable management of pests. The rampant use of these pesticides exhibits a detrimental effect on non-target forms, which are beneficial to the agricultural fields. The present study will provide information on whether the selected pesticides are beneficial or harmful to the soil microbial activities. Inspite of the increased use of pesticides imidacloprid and triadimefon in the agricultural sector of Madanapalle, Chittor District of Andhra Pradesh, which occupied 19% of India's total tomato production, no research was performed in the direction of interaction of pesticides on soil microbial activities in this area. The present study has been aimed to determine the effects of pesticides on amylase, cellulase, invertase and dehydrogenase enzymes in tomato cultivated fields of Madanapalle.

MATERIALS AND METHODS

Soil

Deep red loamy soil from tomato cultivated field at Madanapalle which is a semi-arid zone of Andhra Pradesh, India, was collected to a depth of 12 cm from the four corner parts and the center of the field. The samples were pooled, sieved through the 2 mm mesh, and brought to the laboratory in polyethylene bags and kept in the refrigerator at 5-6°C to maintain the biological activity of the soil microbes. The same was done during the entire studies. The soil physico chemical properties were studied and represented in Table 1.

Table 1: Soil physico-chemical properties

Properties	Untreated soil	Soil treated with imidacloprid	Soil treated with triadimefon
Sand %	70	72	72
Silt %	11	12	16
Clay %	5.0	4.8	4.8
pH	8.46	7.89	8.0
WHC ml/g soil	0.4	0.4	0.4
Organic matter[a] %	0.44	0.38	0.27
Total nitrogen content[b]	0.04	0.19	0.07
NH_4^+-N (µg/g soil)[c]	1.78	1.07	0.86
NO_2-N (µg/g soil)[d]	0.58	0.25	0.21
NO_3^--N(µg/g soil)[e]	0.72	0.23	0.21

WHC=Water holding capacity, [a]Walkley-Black method (Jackson, 1971), [b]Micro-Kjeldhal method (Jackson, 1971), [c]Nesslerization method (Jackson, 1971), [d]Diazotization method (Barnes and Folkyard, 1951), [e]Brucine method (Ranney and Bartlett, 1972)

Pesticides

Two pesticides, imidacloprid (insecticide) and triadimefon (fungicide) were selected for the present investigation in view of their usage in Madanapalle tomato cultivation for the control of insect pests and fungi. Imidacloprid and triadimefon were purchased from Saraswathi agrochemicals, Jammu and Kashmir and Bayer Crop sciences, Himatnagar, India. Imidacloprid: The chemical formula of imidacloprid is $C_9H_{10}ClN_5O_2$ and the international union of pure and applied chemistry (IUPAC) name is (E)-1-(6-chloro-3-pyridylmethyl)-N -nitroimidazolidin-2-ylideneamine. It is also known as nicotinamide and a common insecticide. The chemical formula of triadimefon (Triazole) is $C_{14}H_{16}ClN_3O_2$ and the IUPAC name of it is 1-(4-chlorophenoxy)-3, 3-dimethyl-1-(1H-1, 2, 4-triazol-1-yl) butan-2-one. It is a common fungicide.

Amylase activity

A total of 5 g soil samples in test tubes (15 mm × 150 mm) were incubated with selected pesticides singly and in combination. Duplicate soil samples were withdrawn after 10 days of incubation at room temperature (28 ± 4°C) to determine the amylase activity. The method employed for determining amylase activity is the method adapted by Tu.[10]

Assay of soil amylase

Soil samples were transferred to 100 ml erlenmeyer flasks and 1 ml of toluene was added. After 15 min, 6 ml of 0.2 M acetate-phosphate buffer (pH - 5.5) containing 2% of starch was added to the soil samples and the flasks were stoppered and held for 24 and 72 h at 30°C. Soil extracts were passed through Whatmann No. 1 filter paper and glucose content in the filtrate was assayed.

Invertase activity

The soil treatments are maintained at 60% of water holding capacity (WHC) and incubated at 28 ± 4°C. After 10 days, soil samples in duplicates were withdrawn for the assay of invertase activity. The method employed for determining invertase is developed by Cole.[11]

Assay of soil invertase

Soil samples were transferred to 100 ml erlenmeyer flasks and 1 ml of toluene was added. After 15 min, 6 ml of 0.2 M acetate phosphate buffer containing 18 Mm sucrose was added to the soil samples. The flasks were closed and held for 24 h and 48 h at 30°C. Soil

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

extracts in sterile distilled water were passed through Whatmann No. 1 filter paper and glucose in the filtrate was assayed (Nelson, 1944). Suitable aliquots of filtrate were taken in test tubes and 1 ml alkaline copper reagent was added and covered with marbles, placed in boiling water bath for 20 min. The tubes were cooled under running tap water and then 1 ml of arsenomolybdate reagent was added. The final volume was made up to 5 ml with distilled water and bluish green color developed was read at 500 nm in a spectronic 20D spectrometer. The amount of glucose was calculated by referring to a calibration curve.

Cellulase activity

A total of 5 g portions of soil placed in 15 mm × 150 mm test tubes were amended with imidacloprid and triadimefon singly and in combination. All the treatments including untreated controls were maintained at 60% WHC and incubated in the laboratory at 28 ± 4°C. Moisture levels were restored to their initial levels during incubation. Duplicate samples of soils were withdrawn after 10 days of incubation for determining cellulase activity following the method of Pancholy and Rice.[12]

Assay of soil cellulase

The soil samples were transferred into 100 ml of erlenmeyer flasks and 0.5 ml of toluene was added. The contents in the flasks were mixed thoroughly and after 15 min 10 ml of acetone buffer at PH 5.9 was added followed by 10 ml of 1% of carboxy methyl cellulose. The flasks were then incubated for 24 h at 30°C. At the end of this period, 50 ml of distilled water was added. The suspension was filtered through Whatmann No. 1 filter paper and the volume of the contents was made up to 100 ml with distilled water. The reducing sugar content in the filtrate was determined by the method of Nelson-Somagi (1944).

Suitable aliquots of filtrate were taken in test tubes and 1 ml of alkaline copper reagent was added, covered with marbles and placed in boiling water bath for 20 min. The tubes were then cooled under running tap water and then 1 ml of arsenomolybdate reagent was added. The final volume in tubes was made up to 5 ml with distilled water and bluish green color was read at 500 nm in a spectronic 20D spectrophotometer. The amount of glucose was calculated by referring to a calibration curve.

Dehydrogenase activity

To determine the dehydrogenase activity, 5 g portions of soil were incubated in test tubes with selected pesticides. Soil samples were withdrawn after 10 days of incubation to determine the dehydrogenase activity by the method of Casida *et al*.[13] adapted by Adam and Duncan (2001).

Assay of dehydrogenase

Assay of dehydrogenase activity was based on the reduction of 2, 3, 5-tri phenyl tetrazolium chloride (TTC). Soil samples were treated with 0.1 g of $CaCO_3$ and 1 ml of 0.18M aqueous TTC and incubated for 24 h at 30°C. The tri phenyl formazen formed was extracted in methanol from the reaction mixture and assayed at 485 nm in a spectronic 20D spectrophotometer.

Statistical analysis

Statistical analysis was performed by using Duncan's multiple range test.

RESULTS AND DISCUSSION

After 24 h of incubation with starch, the pesticide treatment individually and in combination at 0.5 μg/g (field rate) showed an increment in the amylase activity than that of control. The combination of pesticides at field rate augmented the rate of enzyme activity. The higher dosage drastically reduced the activity. After 72 h, there is continuous enhancement in the activity at field rate. However, the activity was restored to some extent at remaining application rates. The insecticide endosulfan and quinalphos are at normal residue to an elevated level (0-100) ppm which is equivalent to field application rates of 0-10 kg/ha was studied. Amylase enzyme activity was declined significantly after the application of pesticides at higher concentrations (7.5-10 kg/h), amylase activity showed individual increments of 53-171, 45-139 and 34-192, 69-183% of increase at 24 and 72 h of black paddy soil. The activity was decreased gradually on the prolonged period of incubation up to 30 and 40 days. Over all higher concentrations were toxic or innocuous to amylase activity.[14] This supported our observations. Triadimefon reduced the rate of enzyme activity at high dosage. The effect of two triazole compounds triadimefon and hexaconazole on carbohydrate metabolism was studied at the treatments 50 mg/L and 10 mg/L respectively. Both the triazoles resulted in a marginal increase in starch content and decreased the sugar contents. The α and β amylase activities were reduced under triadimefon and hexaconazole treatment.[15] Data pertaining to amylase activity was represented in Figures 1 and 2.

We observed that there is a suppressed activity in the cellulase enzyme with individual and combined treatments of pesticides at low field rate (0.2 μg/g), at field rate (0.5 μg/g) and high field rate (0.7 μg/g) compared to control. However, imidacloprid had an improved activity of cellulase enzyme. At high field rate, the combination of insecticides and fungicide showed an antagonistic interaction toward the activity, i.e., the percentage of glucose released from cellulose was only 43 compared with control. Contradictory to the present results, Mohiddin *et al*.[16] reported that the activity of cellulase in terms of glucose released from

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

cellulose was more pronounced at 0.5 kg/ha soil, under the influence of two insecticides imidacloprid and acephate. However at higher concentrations of 7.5 and 10 kg/ha, both the insecticides were either stimulatory or innocuous to cellulase activity. Cellulase activity in soil treated with two fungicides, brominal and selecron was inhibited at field application rate and fivefold field rates after most incubation periods.[17] According to Moharram *et al*.,[18] Kocide and Rodomil Plus (systemic fungicides) were incorporated in the liquid culture medium specified for enzyme production; there was a significant reduction in cellulase production particularly at higher doses (200-400 ppm). Exceptions were observed with lower doses (50 and 100 ppm). The data pertaining to cellulase was represented in Figure 3.

After 24 h of incubation with sucrose, we observed a maximum decrease in invertase activity in both individual and combined treatments pesticides at all dosages. Mixture of pesticides at a lower dose showed much decline in activity. The depressed activity might be due to the toxicity of the pesticide to soil microbes. At the prolonged incubation with sucrose (72 h), the activity was revived to extent than before. Imidacloprid showed an intensified activity of the invertase enzyme as that in control at 0.5 µg/g. But at higher dose, there was a marked effect of imidacloprid on the activity. Soils were treated with napropamide insecticide at 0, 2, 10, 20, 40 and 80 mg/kg soil and sampled at intervals of 1, 3, 7, 14, and 56 days. Application of napropamide at 2-80 mg/kg soil had an inhibitory effect on invertase activity. The depressed enzyme might be due to the toxicity of pesticides to soil microbes.[19] The activity of invertase in the rhizosphere of potato plants was determined under field conditions with the application of pyrethrins and neemix-4E. The effects were neither drastic nor prolonged enough to be considered deleterious to the invertase activity which is important to soil fertility.[20] These studies correlated with our observations. Data of invertase activity represented in Figures 4 and 5.

The insecticide (imidacloprid) and fungicide (triadimefon) applied to soil stimulated the dehydrogenase activity at all concentration (0.2 µg/g, 0.5 µg/g and 0.7 µg/g. Triadimefon showed its supremacy with a significant raise of dehydrogenase activity at field rate. In contrast to this, the insecticide diazinon dosage at three different dosages of 7, 35 and 700 mg/kg soil were studied in sandy soils on dehydrogenase activity. The activity decreased in the soil treated with the higher dosage of insecticide.[21] The impact of fungicides benomyl, kitazine, mancozeb and tridimorph on dehydrogenase activity was studied at 0.37 kg/ha, 0.98 L/ha, 2.0 kg/ha and 0.62 L/ha respectively. The enzyme activity was disrupted with the application. However, stimulation in dehydrogenase activity was observed with benomyl and tridimorph treated soils.[22] Data pertaining to dehydrogenase represented in Figure 6.

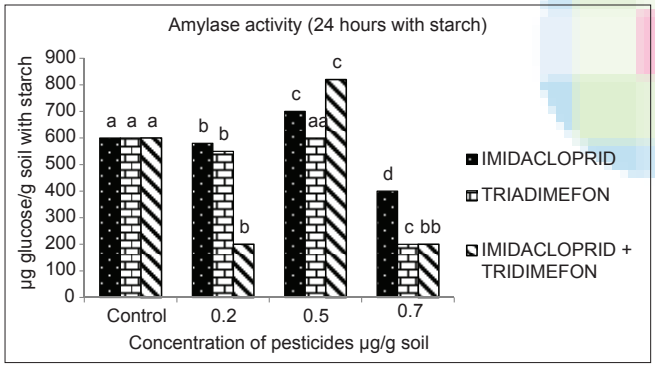


Figure 1: Effect of pesticides on amylase activity after 10 days of incubation. Bars marked by the same letter are not significantly different ($P < 0.05$) from each other according to Duncan's multiple range test

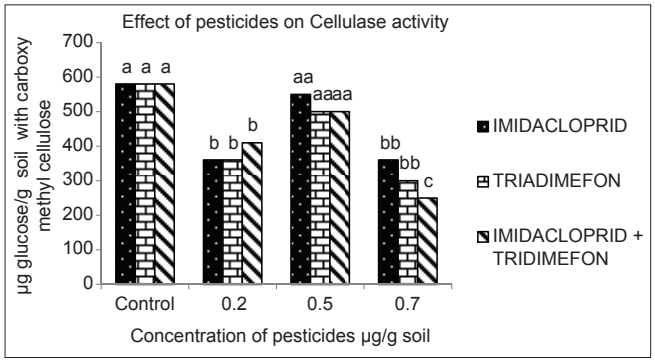


Figure 3: Effect of pesticides on cellulase activity after 10 days of incubation. Bars marked by the same letter are not significantly different ($P < 0.05$) from each other according to Duncan's multiple range test

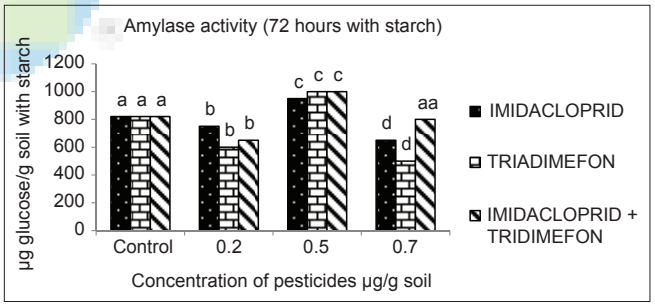


Figure 2: Effect of pesticides on amylase activity after 10 days of incubation. Bars marked by the same letter are not significantly different ($P < 0.05$) from each other according to Duncan's multiple range test

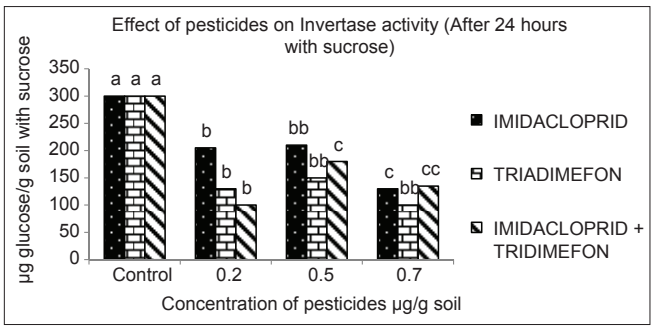


Figure 4: Effect of pesticides on invertase activity after 10 days of incubation. Bars marked by the same letter are not significantly different ($P < 0.05$) from each other according to Duncan's multiple range test

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

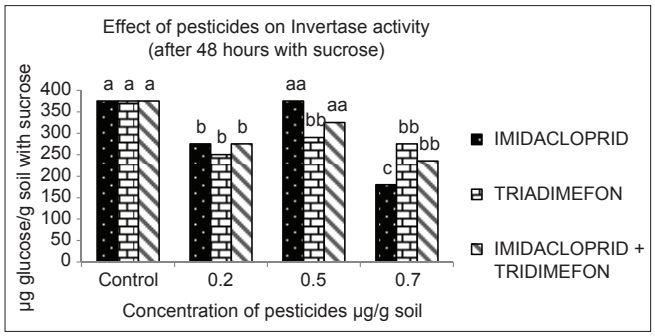


Figure 5: Effect of pesticides on invertase activity after 10 days of incubation. Bars marked by the same letter are not significantly different ($P < 0.05$) from each other according to Duncan's multiple range test

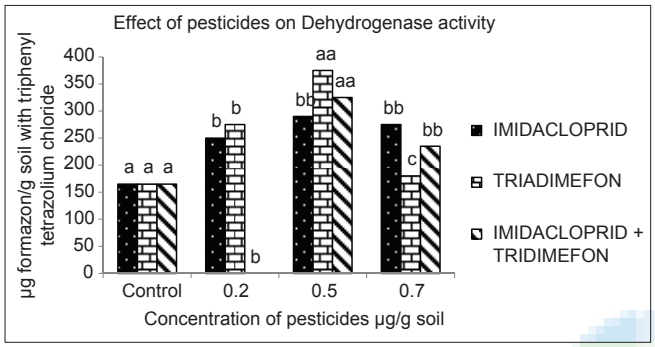


Figure 6: Effect of pesticides on dehydrogenase activity after 10 days. Bars marked by the same letter are not significantly different ($P < 0.05$) from each other according to Duncan's multiple range test

CONCLUSIONS

The effect of pesticides generally decreased with the increase in incubation period. Increase in the concentration of pesticides decreased the rate of enzyme activities. The rate of amylase was restored after 72 h. The higher concentration was toxic or innocuous to the amylase activity. Imidacloprid increased the cellulase activity much. However, the combination at high dosage was detrimental towards cellulase activity. There was a deleterious effect of pesticide on invertase enzyme earlier, but later the activity was restored. Imidacloprid had a great effect in the raise of invertase activity rate. Dehydrogenase activity was stimulated with individual and mixture of pesticide concentrations at all levels. Triadimefon ameliorated the dehydrogenase activity. It was concluded that the enzyme activities were not harmed at the recommended field rates. Future work is to clarify whether the changes in enzymatic activities is due to the effect of the pesticides rather than the heterogenecity of soil itself.

REFERENCES

1. Zhang WJ, Jiang FB, Ou JF. Global pesticides consumption and pollution: With China as a focus. Proc Int Acad Ecol Environ Sci 2011;1:125-44.
2. Ramudu AC, Mohiddin GJ, Srinivasulu M, Madakka M, Rangaswamy V. Impact of fungicides chlorothalonil and propiconazole on microbial activities in groundnut (*Arachis hypogaea* L.) Soils. ISRN Microbiol 2011;2011:623404.
3. Garcı'a-Ruiz R, Ochoaa MV, Hinojosa BB, Carreiraa JA. Suitability of enzyme activities for the monitoring of soil quality improvement in organic agricultural systems. Soil Biol Biochem 2008;40:2137-45.
4. Mishra S, Behera N. Amylase activity of starch degrading bacteria is isolated from soil receiving kitchen wastes. Afr J Biotechnol 2008;7:3326-31.
5. Nasreen C, Jaffer Mohiddin G, Srinivasulu M, Rekhapadmini A, Ramanamma P, Rangaswamy V. Interaction effects of insecticides on enzyme activities in black clay soil from groundnut (*Arachis hypogeal* L) fields. Environ Res Eng Manage 2012;60:21-2.
6. Gilad R, Rabinovich L, Yaron S, Bayer EA, Lamed R, Gilbert HJ, *et al*. CelI, a noncellulosomal family 9 enzyme from Clostridium thermocellum, is a processive endoglucanase that degrades crystalline cellulose. J Bacteriol 2003;185:391-8.
7. Makoi, Joachim HJ, Ndakidemi AP. Selected soil enzymes: Examples of their potential roles in the ecosystem. Afr J Biotechnol 2008;7:181-91, 5.
8. Hubert S, Clarke M, Pollach G. Sugar. In: Ullmann's Encyclopedia of Industrial Chemistry. Weinheim: Wiley-VCH; 2007.
9. Consuelo Q, Marañón T. Dehydrogenase activity in mediterranean forest soils. Biol Fertil Soils 2002;35:102-7.
10. Tu CM. Chemosphere 1982;2:909-14.
11. Cole MA. Lead inhibition of enzyme synthesis in soil. Appl Environ Microbiol 1977;33:262-8.
12. Pancholy SK, Rice EL. Soil enzymes in relation to old field succession: Amylase, cellulase, invertase, dehydrogenase and urease. Soil Sci Soc Am Proc 1973;37:47-50.
13. Casida L, Klein D, Santoro T. Soil dehydrogenase activity. Soil Sci 1964;98:371-6.
14. Vijaya KV, Srinivasulu M, Jaffer Mohiddin G, Padmini AR, Ramanamma P, Naik JG, *et al*. Effect of endosulfan and quinolphos on enzyme activities in paddy soil. Int J Res Environ Sci Technol 2012;2:10-6.
15. Gundi VA, Viswanath B, Chandra MS, Kumar VN, Reddy BR. Activities of cellulase and amylase in soils as influenced by insecticide interactions. Ecotoxicol Environ Saf 2007;68:278-85.
16. Mohiddin Jaffer G, Srinivasulu M, Madakka M, Rangaswamy V. Influence of insecticides on the activity of amylase and cellulase in groundnut soils. Ecol Environ Conserv 2010;16:383-8.
17. Omar SA, Abdel-Sater MA. Microbial populations and enzyme activities in soil treated with pesticides. Water Air Soil Pollut 2001;127:49-63.
18. Moharram AM, Abdel-Hafez SI, El-Said AH, Saleem A. Effect of two systemic fungicides on cellulose decomposing fungi of tomato plants and on some enzymatic activities. Acta Microbiol Immunol Hung 2004;51:403-30.
19. Guo H, Chen G, Lv Z, Zhao H, Yang H. Alteration of microbial properties and community structure in soils exposed to napropamide. J Environ Sci (China) 2009;21:494-502.
20. Antonious GF. Impact of soil management and two botanical insecticides on urease and invertase activity. J Environ Sci Health B 2003;38:479-88.

[Downloaded free from http://www.toxicologyinternational.com on Tuesday, April 26, 2016, IP: 14.139.224.243]

21. Cycoń M, Piotrowska-Seget Z, Kozdrój J. Microbial characteristics of sandy soils exposed to diazinon under laboratory conditions. World J Microbiol Biotechnol 2010;26:409-18.
22. Shukla AK. Impact of fungicides on soil microbial population and enzyme activities. Acta Bot Indica 2000;28:85-7.
23. Kishorekumar A, Jaleel CA, Manivannan P, Sankar B, Sridharan R, Panneerselvam R. Comparative effects of different triazole compounds on growth, photosynthetic pigments and carbohydrate metabolism of *Solenostemon rotundifolius*. Colloids Surf B Biointerfaces 2007;60:207-12.

How to cite this article: Deborah BV, Mohiddin MJ, Madhuri RJ. Interaction effects of selected pesticides on soil enzymes. Toxicol Int 2013;20:195-200.
Source of Support: Nil. **Conflict of Interest:** None declared.

Announcement

"QUICK RESPONSE CODE" LINK FOR FULL TEXT ARTICLES

The journal issue has a unique new feature for reaching to the journal's website without typing a single letter. Each article on its first page has a "Quick Response Code". Using any mobile or other hand-held device with camera and GPRS/other internet source, one can reach to the full text of that particular article on the journal's website. Start a QR-code reading software (see list of free applications from http://tinyurl.com/yzlh2tc) and point the camera to the QR-code printed in the journal. It will automatically take you to the HTML full text of that article. One can also use a desktop or laptop with web camera for similar functionality. See http://tinyurl.com/2bw7fn3 or http://tinyurl.com/3ysr3me for the free applications.