Available online at http://www.journalijdr.com

ISSN: 2230-9926

IJDR

International Journal of Development Research
Vol. 3, Issue, 05, pp.009-013, May, 2013

International Journal of
DEVELOPMENT RESEARCH

Full Length Research Article

INFLUENCE OF SELECTED INSECTICIDES ON PHOSPHATASE ACTIVITY IN TWO GROUNDNUT (*Arachis hypogaea* L.) SOILS

[1]Jaffer Mohiddin, G., [1,3]Srinivasulu, M., [2]Mdakka, M., [1]Vasundara, P., [1]Meghana, D. and [1]Rangaswamy, V.

[1]Department of Microbiology, Sri Krishnadevaraya University, Anantapur – 515003, Andhra Pradesh, India
[2]Department of Biotechnology and Bioinformatics, Yogi Vemana University, Kadappah – 516003, Andhra Pradesh, India
[3]Division of Advanced Nuclear Engineering, Pohang University of Science and Technology (POSTECH), San 31, Hyoja-Dong, Nam Gu, Pohang, Gyungbuk, 790-784, South Korea

ARTICLE INFO

Article History:
Received 16th March, 2012
Received in revised form
12th March, 2013
Accepted 28th April, 2013
Published online 13th May, 2013

Key words:
Imidacloprid,
Acephate,
Phosphatase,
Groundnut (Arachis hypogaea L.) soil

ABSTRACT

Organophosphorus, neonicotinoid insecticides are widely used in India for protection of agricultural yields from more than 120 pests. However, these pesticides pose various threats to organisms, including humans, and hamper soil microbial activity; thus, they are a cause for serious concern. Among them, peanut stem necrosis disease and sucking pests are the major threats to the groundnut (*Arachis hypogaea* L.) crop in Anantapur district of Andhra Pradesh. In the present study, we used imidacloprid, which is a neonicotinoid and acephate an organophosphorus insecticide against these diseases to check the soil health and fertility by knowing the activities of enzymes epecially phosphatase, under laboratory conditions in two groundnut (*Arachis hypogaea* L.) soils. The activity of phosphatase was more pronounced at 5.0 kg ha^{-1} in black and red sandy clay soils after incubation for 10 days. The enzyme activity was further enhanced up to 20 days of incubation, whereas, decreased the activity at 30 and 40 days of incubation. However higher concentrations of 7.5kg ha^{-1} and 10.0 kg ha^{-1} were toxic to phosphatase activity in both soils.

INTRODUCTION

Since from several decades, xenobiotic substances have been widely used in agriculture, either simultaneously or in succession, as a part of pest - control strategies (Quian *et al*., 2009). Better harvest requires intensive cultivation, irrigation, fertilizers and – what is more important – the use of agricultural chemicals to protect plants from pests and plant diseases. In India, 15 – 20% of all produce is destroyed by pests (Bhalerao and Puranik 2007). Organophosphorus, neonicotinoid insecticides are widely used in India for protection of agricultural yields from more than 120 pests. Chemical control of several major pests of groundnut, cotton and tomato by spraying organophosphates, synthetic pyrethroids, carbamates, triazole and organochlorine groups of pesticides either singly or in combination, has been a common practice (Romeh *et al*., 2009; Srinivasulu *et al*., 2012). However intensive use of common pesticides can lead to toxicity to soils, may inhibit biochemical reactions in soil, which are important for its health (Hussain *et al*., 2009; Ahemad and Khan 2011 a,b). The extensive application of pesticide interferes with the normal enzymatic activity of proliferating soil microorganisms, and disturbs the delicate balance of the soil residues at the given point of time and disturb ecological balance in longer-run (Swaminathan *et al*., 2009; Defo *et al*., 2011). As a result, the development of soil microbial testing programmes was developed for examination of the side effects of the pesticides (Swaminathan *et al*., 2009). The testing programmes include measurement of activities of soil enzymes and physico-chemical properties of soil. Soil enzymes are remarkable molecules that show extraordinary specificity in catalyzing biological reactions, important for both soil microorganisms and plants. Soil enzymes are considered as good indicators of soil biological fertility because of their participation in the decomposition of organic matter (Van Dyk and Pletschke 2011). Soil enzymes react to changes in the soil ecosystem more quickly than other variables and therefore, these soil enzymes are early indicators of various biological changes to the influence of pesticides (Masciandro 2004), essential components produced through

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

various important biochemical changes of soil plays an important role in indicating soil fertility (Quian *et al.*, 2009). In spite of the maximum potential of soil enzymes in maintaining soil biodynamics, only limited studies were available (Pessagno Rosa *et al*., 2008; Ahemad and Khan 2011a) on influence of soil enzymes with organochemicals. Soil enzyme activities have been used to assess the negative effects of pollutants such as pesticides, illict drugs, petroleum hydrocarbons and heavy metals (Pandey and Singh 2006). Some of the widely used microbial processes for assessing the effects of contaminants on soil health include phosphatase. Phosphatase has been selected because of their importance in phosphorus soil cycles. Imidacloprid a neonicotinoid and acephate an organophosphorus insecticide used against peanut stem necrosis disease and sucking pests in groundnut fields. Imidacloprid and acephate are widely used pesticides at recommended and higher dosages because of an important agent in controlling the plant pathogens. There was very little information is available on the phosphatase activity with these pesticides. Therefore in the present study it has become necessary to determine the effects of imidacloprid and acephate at recommended and higher doses in order to establish the significance, in terms of biogeochemical reactions and nutrient cycling.

MATERIALS AND METHODS

Soils used in the present study

Agricultural soil samples such as black clay and red sandy clay soils were collected from groundnut cultivated fields of Anantapur District, a semi-arid zone of Andhra Pradesh, India. Samples were collected from the depth of 12 cm, air dried and sieved through a 2mm sieve and stored at 4°c prior to analysis. Mineral matter of soil samples was done by following the method (Jhonson and Ulrich 1960). Soil pH was determined by using 1:1.25 soils to water ratio in systronic digital pH meter. Organic matter in soil samples was estimated by walkley-black oxidation. Total nitrogen content in soil samples was determined by Micro-Kjeldhal method (Jackson 1971). Electrical conductivity was measured by conductivity bridge and contents of nitrite – nitrogen (Barnes and Folkard 1951). Contents of nitrate – nitrogen by Brucine method (Ranney and Bartlett 1972). The important Physico-chemical properties of the two soils are presented in Table 1.

Table 1. Physicochemical characteristics of the soils

Properties	Black clay soil	Red sandy clay soil
Sand (%)	65.8	55.3
Silt (%)	25.2	27.2
Clay (%)	09.0	17.5
pH[a]	8.6	8.2
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

Where a = 1:1.25 = Soil: Water slurry; b = Walkley-Black Method [16]; c = Micro-Kjeldhal Method [16]; d = Nesslerization method [16]; e =Diazotization Method [17]; f = Brucine Method [18]

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 from A.B.Chem. Jammu, India. The used commercial-grade insecticides were dissolved in distilled water.

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 and red sandy clay soils. Two gram portions of soil samples, transferred into test tubes (12 x 125 mm), was treated with two insecticides to provide final concentrations of 10, 25, 50, 75 and 100 µg g^{-1} soil (equivalent to 1, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} field application rates). The soil samples without insecticide's treatment served as control. All the treatments, including controls were maintained at 60% water-holding capacity (WHC) and the tubes were incubated in the laboratory at 28 ± 4°C. 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 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

Each soil sample was treated with 6 ml of 0.1 M Maleate buffer (pH 6.5) and 2 ml of 0.03 M *p*-nitro phenyl phosphate. After incubation for 30 minutes at 37°C, the tubes were placed on ice before the soil extracts were passed through Whatman No. 1 filter paper. To suitable aliquots of the extract, 1 ml of 5 M $CaCl_2$ and 4 ml of 0.05 M NaOH was added, and the yellow color developed was read at 405 nm in a Spectronic 20 D Spectrophotometer (Milton Roy Company).

Statistical Analysis

The concentration of the phosphatase enzyme was calculated on a soil weight (oven dried) basis. The insecticide treatments were contrasted with untreated controls and the significant level $P \leq 0.05$ between values of each sampling, and each insecticide was performed using SYSTAT statistical software package to find the results of Duncan's Multiple Range (DMR) test (Megharaj *et al*., 1999; Gooty Jaffer Mohiddin *et al*., 2011).

RESULTS AND DISCUSSION

Phosphatase activity

Phosphatases, a group of enzymes that catalyse the hydrolysis of both esters and anhydrides of phosphoric acid. The mineralization of organic phosphorus by the activity of phosphatase in soils makes one of the essential elements, phosphorus in soil for plant growth. Hence, phosphatase activity was measured under the influence of imidacloprid and acephate at different concentrations of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}, on the soil phophatase activity has been determined in two soils. The data obtained from these experiments are presented in the Table 2. Hydrolysis of an exogenously added substrate, *p*-nitrophenyl disodium orthophosphate, by phosphatases was greater in both soils amended with two insecticides than in the control at 1.0, 2.5 and 5.0 kg ha^{-1} levels incubated for 10 days. About 22 – 55%

Table 2. Activity of phosphatase* under the impact of different concentrations of selected insecticides in soils (both black clay and red sandy clay soil) after 10 days.

Concentration of Inseticides (Kg ha^{-1})	Black clay soil		Red sandy clay soil	
	Imidacloprid	Acephate	Imidacloprid	Acephate
0.0	360 ± 4.618^{d} (100)	360 ± 1.732^{d} (100)	300 ± 0.577^{e} (100)	300 ± 0.577^{f} (100)
1.0	410 ± 1.154^{c} (114)	400 ± 5.773^{c} (111)	350 ± 1.154^{c} (117)	360 ± 4.618^{e} (120)
2.5	440 ± 2.886^{b} (122)	480 ± 5.773^{b} (133)	370 ± 2.886^{b} (123)	403 ± 1.732^{c} (134)
5.0	560 ± 2.886^{a} (155)	500 ± 5.773^{a} (139)	400 ± 3.464^{a} (133)	502 ± 1.154^{a} (167)
7.5	325 ± 1.452^{e} (90)	410 ± 1.154^{c} (114)	330 ± 5.773^{d} (110)	450 ± 0.577^{b} (150)
10.0	250 ± 1.154^{f} (69)	360 ± 1.732^{d} (100)	240 ± 2.886^{f} (80)	380 ± 0.577^{d} (127)

*µg *p*-nitrophenol (PNP) g^{-1} soil formed after 24 hours incubation with *p*- nitrophenyl phosphate (PNPP).
Figures, in parentheses, indicate relative production percentages.
Means ± S.E. in each column, followed by the same letter are not significantly different *(P < 0.05)* from each other according to DMR test.

and 33-39 % enhancement in phosphatase activity over control was noticed in the black clay soil for 10 days of incubation. In respect of the red sandy clay soil, the corresponding figures of percent enhancement by two insecticides at two levels were 3-33% and 34-67% during the same period (Figure 1a,b). Comparatively, imidacloprid at 5.0 kg ha^{-1} produced maximum stimulation in phosphatase activity in black clay soil than red sandy clay soil.

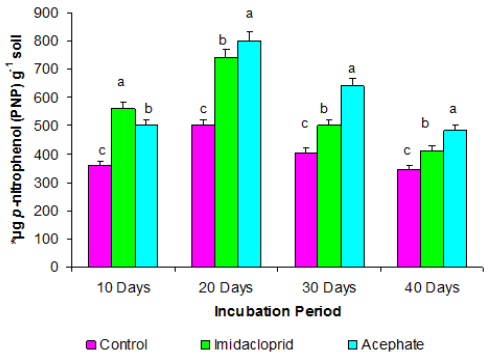

Figure 1a

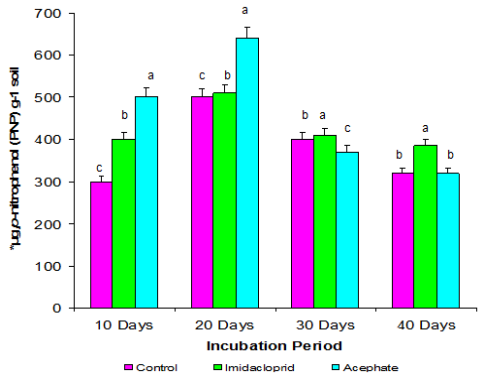

Figure 1b

Figure 1. Effect of selected insecticides at 5.0 kg ha^{-1} on phosphatase* activity in (a) black clay soil and (b) red sandy clay soil. *µg p-nitrophenol (PNP) g^{-1} soil formed after 24 hours incubation with *p*- nitrophenyl phosphate (PNPP). After 10, 20, 30, and 40 days. The values are the means ± S.E, for each incubation period, followed by the different letter which are significantly different ($P \leq 0.05$) from each other according to DMR test. Bars in the Figures represent means of three replicates.

In general, the organic matter content is high in black soil than red soil (Srinivasulu *et al*., 2012). Therefore, the biological activity was also pronounced more in black clay soil than in red sandy clay soil under the influence of insecticides. Phosphatase activity was significantly inhibited at higher concentrations of 7.5 kg ha^{-1} and 10.0 kg ha^{-1} in both insecticidal treatments. Approximating the present results, two organophosphorus insecticides, chlorpyrifos, increased activities of acid phosphatase in the groundnut field (Pandey and Singh 2006). Acetamiprid exhibits an inhibitory effect on phosphatase activity over the incubation time (Yao *et al*., 2006). Quinalphos, monocrotophos and quinalphos and two pyrethroids, cypermethrin and fenvalerate all at the concentrations of 1 to 5 kg ha^{-1}, significantly increased the 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.

Chlorpyrifos, terbufos and fonofos increased activities of acid phosphatase in loam soil sites in the field (Sikora *et al*., 1990). In a similar way, phorate and fenvalerate had a stimulatory effect towards phosphorus mobilization in soils under laboratory and field conditions (Das and Mukherjee 1994; Das and Mukherjee 1998a, b). On the contrary, tefluthrin, DOWCO 429X and DPX 43898, when applied at 10 mg kg^{-1}, induced a reduction of phosphatase activity in an organic soil, but stimulation in a sandy soil was reported by Tu (1990). Likewise, in an organic soil, parathion, triazophos, permethrin and fonofos at 5 mg kg^{-1} reduced the phosphatase activity by 2-fold (Tu 1981b). But malathion and parathion at the same level stimulated the activity in a clay soil (Tu 1989). On the other hand, the phosphatase activity was not affected by the application of pesticides to some other soils. In a clay loam soil, diazinon, chlorpyrifos, thionazin and trichloronate at 1 or 10 kg ha^{-1} were innocuous to phosphate mobilization (Tu 1981a). Tu (1970) reported that the addition of Bay-37289, diazinon, dursban and zinophos at 10 and 100 µg g^{-1} did not result in significant difference in phosphorus mineralisation. In the field study, fenamiphos at 18.6 kg ha^{-1} had no effect on phosphatase activity (Ross *et al*., 1984). In a similar study under laboratory conditions, fenamiphos at 37 and 930 mg kg^{-1} had no deleterious effect on the activity of phosphatase (Ross and Speir 1985). Srinivasulu *et al*., (2012) showed that the

phosphatase activity was enhanced up to 5.0 kg ha^{-1} treated with monocrotophos, chlorpyrifos alone and in combination monocrotophos + mancozeb, chlorpyrifos + carbendazim. Similarly, mancozeb N10 {10 times the normal application (60 kg/ha)} brought about a 41% stimulation in activity after 14 days of incubation compared to control, but after 28 days of incubation a 30% decrease in enzyme activity was recorded (Rasool and Zafar Reshi 2010). In the pot study alakaline phosphatase activity was inhibited by swing at high doses and stimulated by unix (Jastrzebska and Kucharski 2007). Gopal *et al*., (2007) showed that 10% of azadirachtin granules at all doses exerted a suppressive effect on phosphatase activity.

Interestingly, Qian *et al*., (2007) reported that the validamycin stimulated its activity higher than that of control, only highest dose stimulated acid phosphatase activity by 29.7%. The Cd reduced the activity of phosphatase at early incubation time (1-7 days), while the reduction almost disappeared at the end of the incubation (Wang *et al*., 2007). When Cd (10 mg kg^{-1}) was combined with butachlor (50 and 100 mg kg^{-1}), the activity of phosphatase became lower than without combination at early incubation time, which indicated that the toxicity of Cd significantly increased ($P < 0.05$ or < 0.01). However, when Cd (10 mg kg^{-1}) was combined with butachlor (10 mg kg^{-1}), the activity of phosphatase became higher than those without combination at the end of the incubation, which indicated that the toxicity of Cd decreased. Piotrowska-Seget *et al*., (2008) noticed that the acid and alkaline phosphatase activities were significantly reduced by soil treatment with captan. Obviously, phosphatase activity is instability in the beginning of 2 to four week incubation along with decomposing of mixed pesticide of deltramethrin and probineb in soil, and decline to last part of 12-week incubation (Rahmansyah *et al*., 2009). Wang *et al*., (2009) showed that the phosphatase activity in copper concentrations of orchard soils significantly increased with increasing orchard ages ranging from 21.8 to 141 mg kg^{-1}, and the $CaCl_2$ extractable soil Cu concentrations varied from 0.00 to 4.26 mg kg^{-1}.

The soil mean Cmic values varied from 43.6 to 116 mg kg^{-1} in the orchard soils, and were lower than the value of the reference soil (144 mg kg^{-1}). The ratio of soil Cmic to total organic C (Corg) increased from 8.10 to 18.3 mg Cmic g^{-1} Corg with decreasing orchard ages, and was 26.1 mg Cmic g^{-1} Corg for the reference soil. A significant correlation was observed between total or $CaCl_2$ extractable soil Cu and soil C mic or Cmic /Corg, suggesting that the soil Cu was responsible for the significant reductions in Cmic and Cmic /Corg. On the otherhand Cycoń *et al*., (2010) reported that the acid and alkaline phosphatase was more sensitive to mancozeb + dimethomorph at 1500 $mgkg^{-1}$ and its activity declined in both loamy soils and sandy loam soils. However, mancozeb, endosulfan and chloripyifos at 100 mg/kg inhibited 50% activity of phosphatase (2010). Similarly, Suryakalyani *et al*., (2010) observed that acid phosphatase activity 1.8 times, respectively by the 14 th day of incubation with 1 ppm endosulfan.

Conclusions

Results from this study indicated that soil phosphatase was affected by the application of imidacloprid and acephate at higher concentrations. Overall, imidacloprid and acephate at a normal field dose would not pose a threat to the phosphatase activity whenever the two pesticides concentration was increased, however, the threat to phosphatase increased. Based on the above results, it is concluded that the biological (phosphatase) activitiies were not affected, by the insecticides applied at recommended levels in the agricultural system to control insect pests.

Acknoledgements

We are grateful to the University Grants Commission-SAP, DST-FIST 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.

REFERENCES

Ahemad, M., and Khan, M.S. 2011b. Effects of insecticides on plant-growth-promoting activites of phosphatase-solubilizing Rhizobacterium Klebsiella sp. Strain PS19. *Pesticide Biochemistry and Physiology*, vol 100, pp 51-56.

Ahemad, M., and Khan, M.S.2011a. Assessment of plant growth promoting activities of Rhizobacterium putida under insecticide-stress. *Microbiology Journal*, vol 1, pp 54-56.

Barnes, H., and Folkard, A.R. 1951. The determination of nitrites, The Analyst, vol. 76, no. 907, pp. 599–603.

Bhalerao, T.S., and Puranik, P.R.2007. Biodegradation of organochlorine pesticide, endosulfan, by a fungal soil isolate, *Aspergillus niger*, *International Biodeterioration and Biodegradation*, vol. 59, no. 4, pp. 315–321.

Cycon, Z., Piotrowska-Seget, and Kozdrój, J. 2010. Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. *International Biodeterioration and Biodegradation* vol 64, pp 316-323.

Das, A.C., and Mukherjee, D. 1998a. Insecticidal effects on soil microorganisms and their biochemical processes related to soil fertility. *World Journal Microbiol. Biotechnoogy*, vol 14, pp 903-909.

Das, A.C., and Mukherjee, D. 1994. Effect of insecticides on the availability of nutrients, nitrogen fixation, and phosphate solubility in the rhizosphere soil of rice. *Biology Fertile Soils*, vol 18, pp 37-41.

Das, A.C., and Mukherjee, D. 1998b. Insecticidal effects on the activity and numbers of non-symbiotic N_2-fixing bacteria and phosphate solubilizing microorganisms and on some chemical properties of rice soil. *Microbiological Research* vol 153, pp 355-361.3-14.

Defo, M.A., Njine, T., Nola, M., and Beboua, F.S. 2011. Microcosm study of the long term effect of endosulfan on enzyme and microbial activities on two groundnut soils of Yaounde-Cameroon. *African Journal of Agricultural Research*, vol 6, pp 2039-2050.

Gooty Jaffer Mohiddin, Mandala Srinivasulu, Mekapogu Madakka, and Venkatampalli Rangaswamy.2011. Influence of Selected Insecticides on Enzyme Activities in Groundnut (Arachis hypogaea L.) Soils. *Dynamic Soil Dynamic Plant* vol 5, no1, pp 65-69.

Gopal, M., Gupta, A., Arunachalam, V., and Magu, S.P. 2007. Impact of azadirachtin, an insecticidal allelochemical from neem on soil microflora, enzyme and respiratory activities. *Bioresource Technology* vol 98, pp 3154-3158.

Hussain, S., Siddique, T., Saleem, M., Arshad, and Khalid, A. 2009. Impactof pesticides on soil microbial diversity, enzymes and biochemical reactions. *Advances in Agronomy*, vol 102, pp 159-200.

Ismail, B.S., Fugon, D., and Omar, O. 1996. Effect of metolachlor on soil enzymes in Malaysian soil. *Journal of Environmental science*, vol 31, no 6, pp 1267-1278.

Jackson, M.L. 1971. Soil Chemical Analysis, Prentice Hall, New Delhi, India.

Jastrzebska, E., and Kucharski, J. 2007. Dehydrogenases, urease and phosphatases activities of soil contaminated with fungicides. *Plant Soil Environment* vol 53, pp 51-57.

Johnson, C.M., and Ulrich, A.1960. Determination of moisture in plant tissues, *California Agriculture Bulletin*, vol. 766, pp. 112–115.

Masciandro, G., Ceccanti, B., Benedicto, S., Lee, H.C., and Cook, F. 2004. Enzymes activity and C and N pools in soil following of mulches. *Canadian Journal of Soil Science*, vol 84, pp 19-30.

Megharaj, M., Singleton, I., Kookana, r., and Naidu, R.1999. Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil, *Soil Biology and Biochemistry*, vol. 31, no. 11, pp. 1549–1553.

Pandey, S., and Singh, D.K. 2006. Soil dehydrogenase, phosphomonoesterase and arginine deamidase activities in insecticide treated groundnut (Arachis hypogea L) field. Chemosphere vol 63, pp 869-880.

Pessagno Rosa, R.C., Torres, M., Sanchez, R.M., and Doss Santos Afonso, M. 2008. Glyphosate behavior at soil and mineral – water interfaces. *Environmental Pollution* vol153, pp 53-59.

Piotrowska, S.Z., Engel, R., Nowak, E., and Jacek, K.J. 2008. Successive soil treatment with captan or oxytetracycline affects non-target microorganisms. *World Journal of Microbiology and Biotechnology* vol 24, pp 2843-2848.

Qian, H., Hu, B., Wang, Z., Xu, X., and Hong, T. 2007. Effects of validamycin on some enzymatic activities in soil. *Environmental Monitoring and Assessment* vol 125, pp 1-8.

Quian, k., Tang, T., Shi, T., Wang, F., Li, J., and 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 Chimica Acta* vol 635, pp 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* vol 4, pp 56-62.

Rangaswamy, V., and Venkateswarlu, K. 1996. Phosphatase activity in groundnut soils as influenced by selected insecticides. *Journal Environmental Biology*, vol 17, pp 115-119.

Ranney, T.A., and Bartlett, R.J. 1972. Rapid field determination of nitrate in natural waters, *Communications in Soil Science and Plant Analysis*, vol. 3, pp. 183–186.

Rasool, N., and Zafar Reshi, A. 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* vol 51, pp 199-205.

Romeh, A.A., Mekky, T.T., Ramadan, R.A. and Hendawi M.Y.2009. Dissipation of Profenofos, Imidacloprid and Penconazole in Tomato Fruits and Products. *Bull Environment Contam Toxicology*, vol 83, no 6, pp 812-7.

Ross, D.J., Speir, T.W., Cowling, J.C., and Whale, K.N. 1984. Influence of field applications of oxamyl and fenamiphos on biochemical activities of soil under pasture. *New Zealand Journal. Science*, vol 27, pp 247-254.

Ross, D.J., and Speir, T.W. 1985. Changes in biochemical activities of soil incubated with the nematicides oxamyl and fenamiphos. *Soil Biology Biochemistry*, vol 17, pp 123–125.

Sikora, L.J., Kaufman, D.D., and Horng, L.C. 1990. Enzyme activity in soils showing enhanced degradation of organophosphate insecticides. *Biology Fertile Soils*, vol 9, pp 14-18.

Srinivasulu, M., Jaffer Mohiddin, G. and Rangaswamy, V. 2012. Effect of insecticides alone and in combination with fungicides on nitrification and phosphatase activity in two groundnut (Arachis hypogeae L.) soils. *Environmental Geochemistry and Health*, vol 34, no 3, pp 365-374.

Surya Kalyani, S., Sharma, J., Dureja, P., Singh, S., and Lata, X.Y.Z. 2010. Influence of endosulfan on microbial biomass and soil enzymatic activities of a tropical alfisol. *Bulletin of Environmental Contamination and Toxicology*, vol 84, pp 351-6.

Swami Nathan, P., Prabharan, D., and 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.

Tabatabai, M.A., and Bremner, J.M. 1969. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biology Biochemistry*, vol 1, pp 301-307.

Tu, C.M. 1970. Effect of four organophosphorus insecticides on microbial activities in soil. Applied. *Microbiology*, vol 19 pp 479-484.

Tu, C.M. 1981a. Effects of pesticides on activities of enzymes and microorganisms in a clay soil, *Journal of Environmental Science and Health* - Part B, vol. 16, no. 2, pp. 179–191.

Tu, C.M. 1981b. Effects of some pesticides on enzyme activities in an organic soil, *Bulletin of Environmental Contamination and Toxicology*, vol. 27, no. 1, pp. 109–114, 1981b.

Tu, C.M. 1989. Effects of some experimental insecticides on Microbial activities on mineral and organic soils. *Journal of Environmental. Science and Health*, B vol 24, pp 57-64.

Tu, C.M. 1995. Effect of five insecticides on microbial and enzymatic activities in sandy soil. *Journal Environmental. Science. Health*, B vol 30, pp 289-306.

Tu, C.M.1990. Effect of four experimental insecticides on enzyme activities and levels of adenosine in mineral and organic soils. *Journal of Environmental Science*. Health B.. vol 25, pp787-800.

Van Dyk, J.S., and Pletschke, B. 2011. Review on the use of enzymes for the detection of organochlorine, organophosphate and carbamate pesticides in the environment. Chemosphere, vol 82, pp 291-307.

Wang, J., Lu, Y., Ding, H., Shen, G. 2007. Effect of cadmium alone and in combination with butachlor on soil enzymes. *Environmental Geochemistry and Health*, vol 29, pp 395-403.

Wang, Q.L., Zhou, D.M., and Cang, L. 2009. Microbial and enzyme properties of apple orchard soil as affected by long-term application of copper fungicide. *Soil Biology and Biochemistry*, vol 41, pp 1504-1509.

Yao. Xiao- hua Min. Hang Lu. Zhen-hua, and Yuan, Hai-ping. Influence of acetamiprid on soil enzymatic activities and respiration". *European journal of soil biology*, vol 42: 120-126, 2006.

****