INTERNATIONAL JOURNAL OF ADVANCE RESEARCH, IDEAS AND INNOVATIONS IN TECHNOLOGY

ISSN: 2454-132X
Impact factor: 4.295
(Volume3, Issue3)
Available online at www.ijariit.com

Effect of the fungicide dimethomorph at different application rates on enzyme activities in Groundnut (*arachis hypogaea* l.) Soils

P. Ramanamma
Sri Krishnadevaraya University, Anantapuramu-515001, Andhra Pradesh
n.nagashekar@gmail.com

D. Meghana
Sri Krishnadevaraya University, Anantapuramu-515001, Andhra Pradesh
meghanadhasetty@gmail.com

V. Rangaswamy
Sri Krishnadevaraya University, Anantapuramu-515001, Andhra Pradesh
rangamanjula@gmail.com

Abstract: ***Soil microbial diversity is indispensable to maintain functional diversity and enzyme-mediated critical soil processes that detoxify soil from environmental pollutants, like pesticides. Thus, the present study was carried out to assess the effect of different concentrations of the fungicide dimethomorph on the activities of phosphatase, and urease, of black and red soils of groundnut cultivated fields of Anantapuramu District, Andhra Pradesh, India. A laboratory experiment was conducted to determine the effect of selected fungicide, dimethomoph at different concentrations ranging from 1.0 to 10 kg ha^{-1} on the activity of phosphatase and urease in two groundnuts (Arachis hypogaea L.) soils. The activity of urease was significantly more at dimethomorph levels of 5.0 kg ha^{-1} in black and red soils respectively. The activity of phosphatase was significantly more at dimethomorph levels of 2.5 kg ha^{-1} in black and red soils respectively. But at higher concentrations of 7.5 and 10.0 kg ha^{-1} respectively, dimethomorph were toxic to urease and phosphatase activity. The activity of phosphatase and urease was drastically decreased with increasing period of incubation up to 30 and 40 days. The results of the present study clearly indicate that the field application rates show no effect on enzyme activity for longer period of incubation (40 days). But higher concentration (10 kg ha^{-1}) of fungicide leads to the inhibition of the enzymatic activity.***

Keywords: Phosphatase, Urease, Dimethomorph, Groundnut Soils.

INTRODUCTION

In India, it has become a common trend in modern agriculture to apply agrochemicals such as pesticides to increase agricultural productivity and has become an integral part of agriculture. About one-third of the world's food crop is destroyed by pests annually (Punitha *et al*., 2012). Pesticides enable to achieve higher crop yields (Ray *et al*., 2004, Pasquer *et a*l., 2005, Valenciano *et al*., 2006), but at the same time negatively affect the natural environment, including the soil environment, disturbing its homeostasis. Many synthetic fungicides were developed in the 1940–1950's based on 'old fungicide chemistry' (Wightwick *et al*., 2013). An analysis of soil enzymatic activity is one of the microbiological indicators of soil quality (Winding *et al*., 2005). Enzymes participate in numerous biochemical processes occurring in the soil, and as shown by the results of studies – they are sensitive to all environmental changes caused by natural and anthropogenic factors (Trasar-Capeda *et al*., 2000). Soil enzymes are recognized as sensitive indicators of soil health and quality (Bandick & Dick 1999; Caldwell 2005; Dick *et al.*, 1996). The transformation of natural ecosystems into agricultural ecosystems characterized by a low biodiversity, as well as the intensive development of farming systems, resulted in a large-scale application of crop protection chemicals. Like other pesticides, fungicides are toxicants which interfere not only with the biochemical and physiological reactions of the target plant pathogens but may also influence population or activity of other non-target organisms in soil (Tu, C.M., 1992). Soil enzymes serve as sensitive indicators of ecological change (Das and Varma, 2011). The monocropping of groundnut with high-yielding varieties resulted in an eruption of fungi, causing damage to groundnut crop in many areas of Andhra Pradesh; more than 90 insect pests and mites were found to be associated with the groundnut crop (Das *et al*., 1995). Currently, soils are becoming more and more polluted by pesticides molecules because of their wide use in agriculture practices.Groundnut is the most important oilseed crop in India. Especially in drought prone District of Anantapuramu, the farmers mainly depend on groundnut cultivation. Due to lack of irrigation facilities and poor alternative cropping pattern in rain-fed areas like Anantapuramu and in other Rayalaseema Districts the farmers have been cultivating groundnut crop

from the last several decades. But of nine oilseed crops grown in India, the area under groundnut accounts for about 45 percent of the total cropped area and 55 percent of the total oilseeds area. India is the major groundnut producing country in the world. Fungicides contain one or several active substances, including, among others, benzimidazoles – mitosis inhibitors, azimino compounds and imidazoles inhibiting ergosterol biosynthesis, morpholines – inhibitors of the biosynthesis of nucleic acids and ergosterol, and strobilurins – fungal respiration inhibitors (Jańczak *et al*., 2004).**T**he indiscriminate use of various pesticides to reduce the impact of pests on agricultural crops has increased over the years, especially in developing countries (Santhakumar and Balaji, 2000). Natural and anthropogenic factors may affect the soil enzyme activities directly or indirectly (Gainfreda and Bollag, 1996).

2. MATERIALS AND METHODS

Soils used in the present study

Agricultural soil samples such as samples of black clay soil and red sandy loam soil, collected from groundnut cultivated fields of Anantapuramu District of Andhra Pradesh, India in a semi-arid zone from the depth of 12 cm and mixed thoroughly to prepare a homogenate composite sample, air dried at room temperature samples were cleaned by removing plant material and other debris and passed through a 2 mm sieve and stored at 4°c prior to analysis.

Analysis of Physico- chemical characteristics of soil samples

Mineral matter of soil samples such as sand, silt, and clay contents were analyzed with the use of different sizes of sieves by following the method of Alexander (1961). Water holding capacity of the soil samples was determined by adding distilled water up to the saturation point and then 60 % water-holding capacity of the soil samples was calculated by Johnson and Ulrich (1960). pH of soil samples was determined by mixing soil and water in the ratio of 1:1.25 using systolic digital pH meter with calomel glass electrode. Organic carbon content in soil samples was estimated by Walkey-Black method and the organic matter was calculated by multiplying the values with 1.72 (Jackson 1971). The electrical conductivity of soil samples was measured by a conductivity bridge. The total nitrogen content in soil samples was determined by the method (Jackson 1971). The inorganic ammonium nitrogen content in the soil samples after extraction of 1M KCL by Nesslerization method (Jackson 1971) and contents of nitrite nitrogen (Barnes and Folkard 1951) and the contents of nitrate-nitrogen by Brucine method (Ranney and Bartlett, 1972) after extraction with distilled water were determined respectively. Physico-chemical characters of the two soil samples are listed in Table 1.

1. Physico-chemical properties of the soils

Properties	**Black soil**	**Red soil**
Sand (%)	66.4	52.4
Silt (%)	23.6	26.9
Clay (%)	9.3	19.4
pH	7.9	6.8
Water holding capacity (ml g-1 soil) a	0.45	0.32
Organic matter (%) b	1.44	0.74
Total nitrogen (%) c	0.089	0.048
NH^{+}_{4} - N (µg g^{-1} soil) d	8.57	7.02
No^{-}_{2}-N (µg g^{-1} soil) e	0.45	0.66
No^{-}_{3}-N (µg g^{-1} soil) f	0.92	0.74

a 1:1.25= soil: water slurry

b Walkley- Black method (Johnson and Ulrich 1960)

c Micro-Kjeldahl method (Johnson and Ulrich 1960)

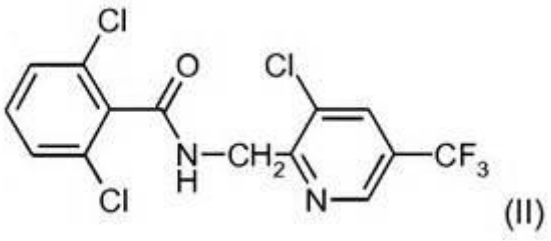
d Nesslerization method (Johnson and ulrich 1960)

c Diazotization method (Ranney and Bartlett 1972)

f Brucine method (Barnes and Folkard 1951)

FUNGICIDES USED IN THE PRESENT STUDY

To determine the effect of selected fungicide on soil enzyme activities dimethomorph (Chemical Name: 4-(3-(4-chlorophenyl)-3-(3,4-dimethoxy phenyl) acryloyl)morpholine). The used commercial grade fungicide was dissolved in water.



(II)

SOIL INCUBATION STUDIES

Enzymes used in the present study

Phosphatase activity:

The activity of phosphatase under the influence of the fungicide at different concentrations was determined in black clay and red sandy loam soils. Two-gram portions of soil samples were transferred into test tubes (12 × 125 mm) were treated with fungicide to provide final concentrations of 10, 25, 50, 75 and 100 µg g^{-1} soil (equivalent to 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1} field application rates). The soil samples without fungicide treatment served as control. All the treatments including controls were incubated in the laboratory at 28 ± 4 °C by maintaining 60 % water-holding capacity. After 10 days of incubation period, triplicate soil samples were withdrawn for the assay of phosphatase (Tabatabai and Bremner 1969; Srinivasulu *et al*., 2012).

Assay of phosphatase

For the assay of phosphatase activity, 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*-nitrophenyl phosphate and the tubes were incubated at 37 °C for 30 min. After incubation, 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 were added and the yellow color developed was read at 405 nm in a Spectrophotometer.

Urease activity

For estimating the enzymatic activity of urease, 1 gm portions of soil samples (black clay and red sandy loam soils) placed in 15 × 150 mm test tubes were treated with 1 ml of aqueous solutions of fungicide to provide different concentrations of 1.0, 2.5, 5.0, 7.5 and 10.0 kg ha^{-1}. The soil samples without fungicide treatment served as control. All the treatments including control were maintained at 60 % WHC and the tubes were incubated at 28 ± 4 °C. After 10 days of incubation, triplicate soil samples were withdrawn for the assay of urease.

Assay of urease

Fawcett and Scott (1960) described the assay of urease at the desired intervals; 1 ml of 3 % urea and 2 ml of 0.1 M phosphate buffer (pH 7.1) were added to 1 g soil and the tubes were incubated at 37 °C for 30 min in a water bath. After incubation, the tubes were shaken and placed in ice until the ammonia was extracted with 10 ml of 2 M KCl. 5 ml of phenol–sodium nitroprusside solution and 3 ml of 0.02 M sodium hypochlorite were added to 4 ml of the filtrate. The mixture was shaken, incubated for 30 min in dark and the developed blue color was measured at 630 nm in a Spectrophotometer. After determining the effective concentration, the experiment was carried out further for 20, 30 and 40 days and assayed similarly.

Statistical analysis

The concentrations of the phosphatase and urease enzymes were calculated on soil weight (oven-dried) basis. The fungicide treatments with untreated controls and the significant levels $p \leq 0.05$ between the values of each sampling for each fungicide were obtained using SYSTAT statistical software package to find the results of Duncan's multiple range (DMR) test (Megharaj *et al.*,1999).

RESULTS AND DISCUSSION

Phosphatase activity

These two enzymes have been selected because they play an important role in the carbon–nitrogen, and phosphorus–sulfur cycles in soils. In soil, phosphatases have been the most studied enzymes contributing to P nutrition of plants and microbes. Urease is responsible for hydrolysis of urea fertilizers applied to the soil into ammonia and carbon dioxide. It may be degraded by soil proteolytic enzymes. The enzyme was very sensitive to toxic concentrations of xenobiotics (Yang *et al*., 2006). Urease has been studied more extensively relative to other soil enzymes because of its involvement in the breakdown of urea, a commonly used fertilizer (Martens & Bremner 1997). While the inhibition of urease activity could be due the presence of Mn and Zn ions in the pesticide, as reported by Tabatabai (1977). The influence on microbial processes and soil microorganisms of fungi used depends upon many factors. Some of the major factors include physical, biochemical properties (such as (pH organic matter, temperature, and moisture) of soil. Phosphatase, which is an extracellular enzyme produced by many soil microorganisms, is responsible for the

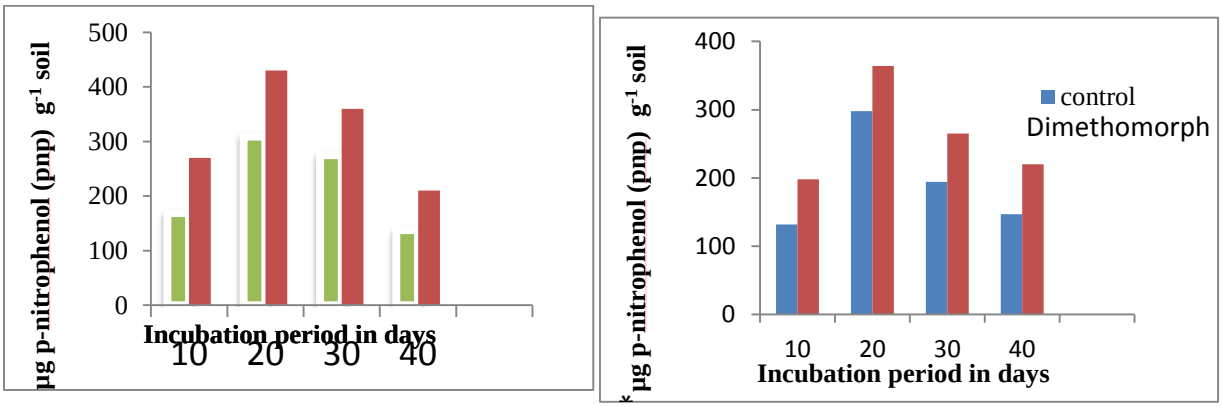
hydrolysis of organic phosphorus compounds to inorganic phosphorus (Monkiedje and Spiteller 2002). Hence, phosphatase activity was measured under the influence of dimethomorph at different concentrations (1.0, 2.5, 5.0, 7.5, 10.0 Kg ha^{-1}). Phosphatase activity was increased in treated soils up to 5.0 Kg ha^{-1} than the controls in 10 days incubated soil samples. The enhancement of enzyme activity continued up to 20 days and then gradually decreased after 30 and 40 days of incubation. Dimethomorph significantly enhanced in increasing of the phosphatase activity in 10 days incubated soil samples. Dimethomorph at concentrations ranging from 1.0 to 5.0 Kg ha^{-1}gradually increased the phosphatase activity and reached a maximum at the concentrations of 5.0 Kg ha^{-1} in both soil samples. Beyond 5.0 Kg ha^{-1} dimethomorph showed minimum phosphatase activity and also at 10.0 Kg ha^{-1}. But higher concentrations of pesticides at the levels 7.5-10.0 Kg ha^{-1} show inhibitory effect on the phosphatase activity and represents antagonistic interaction. Rangaswamy and Venkateswarulu (1996) reported that though exact mechanisms for the proliferation/non-proliferation of biological activities are not known, lowering of phosphatase activity at higher concentrations of pesticides alone in soils may affect the availability of phosphate for the growth of plants. Phosphatase carries out a broad range of intracellular as well as soil-accumulated activities that catalyze the hydrolysis of both the esters and anhydrides of phosphoric acid. The maximum enhancement in phosphatase activity over control was noticed in the black clay soil and red sandy loam soils at 20 days of incubation period. Further incubation periods, i.e., 30 and 40 days, the activity was decreased slowly in both the soil samples. Phosphatases, a group of enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid. The mineralization of organic phosphorous by the activity of phosphatase in soils makes one of the essential elements, phosphorous in the soil for plant growth. Phosphatase is a soil enzyme find widely in the soil environment which is responsible for hydrolytic cleavage of a variety of ester phosphate bonds of organophosphates and anhydrides of orthophosphoric acid (H3PO4) into inorganic phosphate (Rahmansyah *et al*., 2009). Phosphatase is concentrated in the surface layer and rhizosphere where most of the fresh and less humified organic matter is prevailing (Rojo *et al*., 1990 and Tarafdar *et al*., 2001). Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms and thus in the cycling of it within the soil (Schneider et al., 2001).

Effect of dimethomorph on phosphatase* activity:

Concentration of fungicide (Kg ha -1)	*µg p- nitrophenol (pnp) g¯1soil*	*µg p- nitrophenol (pnp) g¯1soil*
0.0	78±2.443	64±1.662
1.0	197±1.212	167±3.221
2.5	360±4.550	310±6.471
5.0	260±5.343	223±2.884
7.5	218±2.113	179±1.457
10.0	128±2.774	108±2.127

Effect of dimethomorph on phosphatase* activity

Activity of Phosphatase under the impact of different concentrations of selected fungicide in black and red soil



Urease activity

The activity of urease implicated in the hydrolysis of urea was significantly enhanced by the fungicide dimethomorph up to 5.0 kg ha^{-1} in both soil samples, in comparison to the controls. However, increasing concentrations (7.5&10 kg ha^{-1}) were toxic to urease activity after 10 days incubation. Urease catalyzes the hydrolysis of urea to CO_2 and NH_4^+ ions by acting on C–N non-peptide bonds in linear amides (Ramudu *et al*., 2012). On hydrolysis, the substrate *p*-nitrophenyl disodium orthophosphate was added, phosphatase was increased in both the selected soil samples with the selected fungicide than in the control at 1.0, 2.5 and 5.0 kg ha^{-1} levels, incubated for 20 days. Our investigation revealed that phosphatase and urease activities were drastically decreased at higher concentrations (7.5 and 10.0 kg ha^{-1}) of dimethomorph treated soils than the untreated controls throughout the experiment. Urease, in general, helps to maintain nitrogen in the form of ammonia (NH_4^+) and is less leachable (Srinivasulu and Rangaswamy 2014). Omar and Abd-Alla [Omar SA, Abd Alla MH, 2000] observed that urease activity was promoted on the application of two fungicides pyrazofos and propiconazole (as tilt) at 50 ppm.The fungicide, pyroxyfur applied to a sandy loam soil at 10 µg/gm of soil had no inhibitory effect on the activity of urease [Tu CM, 1992]. Depending upon the type of soil, few fungicides remained innocuous initially and then inhibited or stimulated urease activity. For instance, triazophos, captan, maneb and thiram at 5 and 10 mg/kg in a clay loam soil resulted in no inhibition of urease activity within seven days of incubation. According to Shukla and Mishra [Shukla

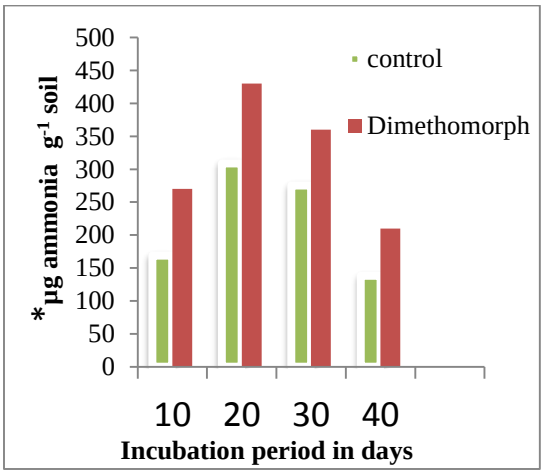
PK, Mishra RR 1996], urease activity was reduced in potato field soil, by the application of benomyl (0.37 kg ha^{-1}), copper oxychloride (7.4 kg $ha^{-1)}$ and ethane M-45 (2.0 kg ha^{-1}). Copper oxychloride and dithane M-45 are more effective than benomyl.Cycon et al 2010, noticed that urease activity declined in sandy loam and loamy sand soils with a combination (mancozeb+dimethomorph) at increasing concentration compared to the control.

Activity of Urease under the impact of different concentrations of selected fungicide in red & black soil

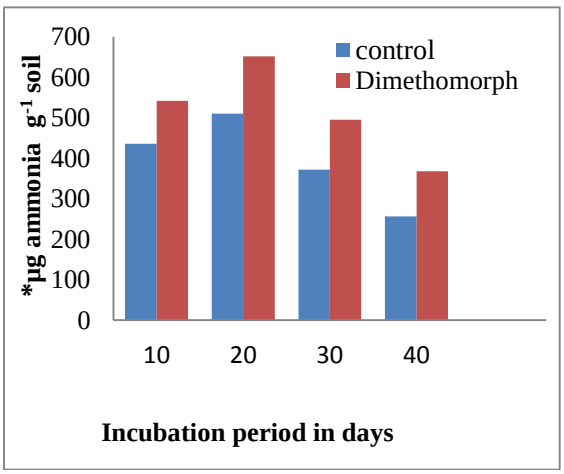
Concentration of fungicide (Kg ha $^{-1}$)	**µg ammonia g^{-}1soil*	*Concentration of fungicide (Kg ha $^{-1}$)*	**µg ammonia) g^{-}1soil*
0.0	141±8.651	0.0	300±0.777
1.0	192±5.236	1.0	420±5.379
2.5	290±3.932	2.5	496±1.273
5.0	465±7.567	5.0	574±5.868
7.5	230±4.681	7.5	414±0.757
10.0	170±8.449	10.0	370±2.775

Black

Red soil



Black soil



Influence of selected fungicide at 5.0 kg ha^{-1} on urease activity in the red soil after 10, 20, 30&40days*µg ammonia g^{-1} soil formed after 3Incubation 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.

Influence of selected fungicide at 5.0 kg ha^{-1} on urease activity in the black soil after 10,20,30&40days*µg ammonia g^{-1} soil formed after 3Incubation 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.

CONCLUSION

The results of the present study clearly indicated that the two selected fungicides, dime tho morph, profoundly enhanced both the phosphatase and urease activities at 1.0–5.0 kg ha^{-1}. Based on the above results, it is concluded that the microbial activities (i.e., enzyme activities) were not affected by the fungicides applied at recommended levels in the agricultural system to control the pests.

ACKNOWLEDGMENT

We are grateful to the University Grants Commission-BSR (RFSMS), New Delhi, India, for providing financial assistance. We are also thankful to the Department of Microbiology, Sri Krishnadevaraya University, Anantapuramu, Andhra Pradesh, for providing all the necessary facilities throughout my research work.

REFERENCES

[1] Alexander M. Introduction to soil microbiology. 2. New Delhi: Wiley, Eastern Ltd; 1961. Bandick, A.K. & R.P. Dick. 1999. Field management effects on soil enzyme activities. *Soil Biology and Biochemistry* **31**: 1471-1479.

[2] Barnes H, Folkard BR. The determination of nitrite. Analyst. 1951; 76:599–603. doi: 10.1039/an9517600599.

[3] Chen, S.K., S.Subler and C.A.Edwards, 2001). Effects of the fungicides benomyl, captan, and chlorothalonil on soil microbial activity and nitrogen dynamics. Soil Bio.Biochem., 33:1971-80.
[4] Das AC, Chakravarty Sukul P, Mukherjee D. Insecticides: their effect on microorganisms and persistence in rice soil. Microbiol Res. 1995; 150:187–194. doi: 10.1016/S0944-5013(11)80055-8
[5] Das, S.K, and A.Verma. 2011. The role of soil enzymes in maintaining soil health. In: Soil Enzymology, Soil Biology (Ed. G.Shukla and A.Verma) Springer Verlag Publishers, Berlin Heidelberg, Vol 22, 25-42.
[6] Fawcett JK, Scott JE. A rapid and precise method for the determination of urea. J Clin Pathol. 1960; 13:156–159. doi: 10.1136/jcp.13.2.156.
[7] L. Gianfreda, J.M. Bollang, Influence of natural and anthropogenic factors on enzyme activity in soil. In Stotzky, G., Bollang, J.M. ed., Soil Biochem. Marcel Dekker, New York, 1996.
[8] Jackson ML. Soil chem. anal. New Delhi: Prentice Hall India; 1971.
[9] Johnson CM, Ulrich A, et al. Determination of moisture in plant tissues. In: Wilde SA, et al., editors. Soil and Plant analysis for tree culture. Oxford: Portage publishing Co.; 1960. pp. 112–115.
[10] Jańczak C., Filoda G., Pawlak A. (2004): Progress in the protection of cereals, fungicides of old and new generation. Program. Plant Prot., *44*: 747–749.
[11] Omar SA, Abd Alla MH, *Microbiol. Res*, **2000**, 154 (4), 339-347.
[12] Martens, D.A. & J.M. Bremner. 1997. Inhibitory effects of fungicides on hydrolysis of urea and nitrification of urea nitrogen in the soil. *Pesticide Science* **49**: 433-352.
[13] Megharaj M, Singleton I, Kookana R, Naidu R. Persistence and effects of fenamiphos on native algal populations and enzymatic activities in soil. Soil Biol Biochem. 1999; 31:1549–1553. doi: 10.1016/S0038-0717(99)00078-4.
[14] Monkiedje A, Spiteller M. Effects of the phenylamine fungicides, mefenoxam, and metalaxyl, on the biological properties of sandy loam and sandy clay soils. Biol Fertil Soils. 2002; 35:393–398. doi: 10.1007/s00374-002-0485-1.
[15] Punitha BC, Hanumantharaju TH, Jayaprakash R, Shilpashree V. Acetamiprid impact on urease and phosphates activity in selected soils of southern Karnataka. Int J Basic Appl Chem Sci. 2012;2(1):1–6.
[16] Ramudu AC, Srinivasulu M, Jaffer Mohiddin G, Rangaswamy V. Effect of Fungicides on Urease and Protease activity in two groundnuts (*Arachis hypogaea* L.) soils. Int J Environ Sci Technol. 2012;2(1):10–16
[17] M.J. Rojo, S. G. Carcedo, M. P. Mateos, Distribution and characterization of phosphatase and organic phosphorous in soil fraction. Soil Boil biochem 22(1990): 169-174.
[18] Rangaswamy V, Venkateswarlu K. Phosphatase activity in groundnut soils as influenced by selected insecticides. J Environ Biol. 1996; 17(2):115–119.
[19] Ranney TA, Bartlett RJ. Rapid field determination of nitrate in natural waters. Commun Soil Sci Plant Anal. 1972; 3:183–186. doi: 10.1080/00103627209366366
[20] Ray R.V., Jenkinson P., Edwards S.G. (2004): Effects of fungicides on eyespot, caused predominantly by *Oculimacula acuformis*, and yield of early-drilled winter wheat. Crop Prot., *23*: 1199–1207.
[21] SANTHAKUMAR, M. AND BALAJI, M., 2000. Acute toxicity of an organ op hours insecticide monocrotophas and its effects on the behaviour of an air-breathing fish, Anabas *testudineus* (Bloch). *J. environ. Biol*., **21**: 121-123. K. Schneider, M. B. Turrion, B.F. Grierson, J.F. Gallardo, Phosphatase activity, microbial phosphorous and fine root growth in forest soil in the sierra degata, western central Spain, Biio Fertil soils (2001).
[22] Shukla PK, Mishra RR, *Proc. Ind. Natl. Sci. Acad*, **1996**, B62 (5), 435-438.
[23] Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol Biochem. 1969; 1:301–307. doi: 10.1016/0038-0717(69)90012-1
[24] Tabatabai, M.A. 1977. Effect of trace elements on urease activity in soils. *Soil Biology and Biochemistry* **9**: 9-13.
[25] Trasar-Capeda C., Lieros M.C., Seoane S., Gil-Sotres F.(2000): Limitations of soil enzymes as indicators of soil pollution. Soil Biol. Biochem., *32*: 1867–1875.
[26] Tu, C.M., 1992. Effect of some herbicides on activities of microorganisms and enzymes in soil. J.Environ.Sci..Health Part B, 27:695-709.
[27] Vyas, S.C., 1988. Non-target effects of Agricultural Fungicides.
[28] Wightwick AM, et al. The effects of copper hydroxide, captan and trifloxystrobin fungicides on soil phosphomonoesterase and urease activity. Water Air Soil Pollut. 2013; 224:1703. doi: 10.1007/s11270-013-1703-1.
[29] Winding A., Hund-Rinke K., and Rutgers M. (2005): The use of microorganisms in ecological soil classification and assessment concept. Ecotoxicol. Environ. Saf., *62*: 230–248.
[30] Yang, Z., Liu, S., Zheng, D and Feng, S. 2006. Effect of heavy metals on soil enzyme acivities.J.Environ. Sci. Vol 18: 1135-1141.

