Impact of Selected Pesticides on Some Soil Enzymes Activity in Soil Cultivated with Wheat Crop

G. Abdel-Nasser*, M. A. Massoud, A. S. T. Barakat*** and A. M. Attia*****
*Soil and Agricultural Chemistry Dept., Faculty of Agriculture Saba Basha, Alexandria University
** Plant Protection Dept., Faculty of Agriculture Saba Basha, Alexandria University.
***Field Crop Pests Research Dept., Plant Protection Research Institute, Agricultural Research Center, Sabahia, Bacous, Alexandria, Egypt.

ABSTRACT: Wide areas of agricultural soil are grown with wheat crop in Egypt using different groups of pesticides especially; herbicides and fungicides, it is important to evaluate the potential risks of pesticides residue to non-target organisms in agro-ecosystems such as soil microorganisms which could be indicated by determining certain soil enzyme activities**.** Therefore, a field experiment was conducted at Research and Experimental Farm, Faculty of Agriculture, Abees region - Alexandria- Egypt to investigate the effects of some pesticides on some soil enzymes activity in soil cultivated with wheat crop. Three herbicides namely; Granstar® 75% (at rates of 0, 4 and 8 g/fed), Panther® 55% SC (at rates of 0, 300 and 600 ml/fed), Topik® 15% WP (at rates of 0, 70 and 140 g/fed) and one fungicide; Sumi-eight®12.5% WP (at rates of 0, 17.5 and 35 ml/100 l). The pesticides were applied during the growing season of wheat on Jan 19 and March 10, 2013. The soil samples were collected at the end of growing season of wheat crop May 2013 for three soil depths, i.e. 0-5, 5-10 and 10-20 cm. Enzymes activity were assayed in triplicate air-dried samples. Three soil enzymes were tested, i.e. catalase, urease, and phosphatase. Enzyme activities were assayed in triplicate air-dried samples. The results indicated that Panther has highest activity soil and Granstar has less activity. Also, the concentration of pesticides significantly ($p <= 0.05$) decreased the activity of soil enzymes in which increasing the application rate decreased the enzyme activity. The activity of soil enzymes was significantly ($p <= 0.05$) increased as soil depth increased. The surface layer (0–5 cm) has the least value of enzyme activity. These findings match the fact that the pesticide transport in soil is low and the pesticide did not reach more than 10 cm. In general, the pesticides decreased the activity of soil enzymes and this decreasing increased with increasing the pesticides application rate. Also, the decreasing of soil enzyme activity was less with increasing soil depth.

Keywords: pesticides, soil enzymes, catalase, urease, phosphatase, enzyme activity

INTRODUCTION

Pesticides are widely used in crop production and are known to cause major environmental problems. With increasing pesticide use, questions are rising on potential effect regarding public health and environment. Pesticides pollute air, soil, water resources and contaminate the food chain. The interaction between soil components and pesticides influences the biochemical processes driven by microorganisms. The effect of Pesticides on soil microorganisms could be determined by the study of functional parameters such as carbon and nitrogen mineralization that are governed by enzymatic activities. Those activities play an important role because all biochemical transformations in soil depend on or are related to the presence of enzymes (Raith *et al*., 2014).

The large-scale use of pesticides has created a biotic factor which affecting every ecosystem mostly. Application of pesticides to agricultural soils may affect soil biological activity in a variety of ways (Shetty and Magu, 1998). Pesticides may affect the beneficial microorganisms in the soil which play a good role on the fertility and productivity of the agricultural soils through their effect on the biological processes such as the mineralization of organic matter and soil enzymes (El-Shahaat, 1993). Soil enzyme activity is a key feature of plant nutrients and cycling processes, and therefore measurements of specific enzyme activities may be useful in determining soil biological activity, which might be used as an index of soil fertility (Reddy *et al*., 1995).

Several studies were conducted to find out the effects of pesticide on soil enzymes (Burrows and Edwards, 2004; Klose and Ajwa, 2004;Gundi *et al*., 2007; Menon *et al*., 2005; Stromberger *et al*., 2005;Pampulha and Oliveria, 2006; Bending *et al*., 2007; Qian *et al.,* 2007; Wang *et al*., 2007). Most of these studies conclude that pesticides at higher doses inhibit enzymatic activities.

Commercial fungicides namely dhanustin ,Dithane M-45 and Contaf are widely used for control of fungal pests in agricultural crops like groundnut. These agrochemicals may affect local metabolism or soil enzyme activities besides controlling pest problem. They may have both negative and positive effects on function of soil enzymes (Liu *et al*. 2008). So far little information is available on these fungicides behavior towards soil dehydrogenase, phosphatase, protease and urease.

Urease is an enzyme that catalysis the hydrolysis of urea into carbon dioxide and ammonia and is a key component in the nitrogen cycle in soils. Urease activity in soils has received a lot of attention since it was first reported by Rotini (1935), a process considered vital in the regulation of nitrogen supply to plants after urea fertilization (Makoi and Ndakidemi 2008). Most of the referenced studies reported that herbicides and fungicides appear to have no effect (Cycon*et al.*, 2010; Romero *et al*., 2010; Tejada *et al*., 2011; Yan *et al*., 2011; Bacmaga *et al*., 2012) or reduced effect on urease activity (Sukul, 2006; Caceres *et al*., 2009; Tejada, 2009). Decreased urease activity in soil due to the application of pesticides reduces urea hydrolysis, which is generally beneficial, because it helps to maintain nitrogen availability to plants (Antonious, 2003). On the contrary, the fungicides carbendazim and validamycin enhanced urease activity, respectively, up to 70 % and to 13–21 % (Qian *et al*., 2007; Yan *et al*., 2011). The urease activity appears to be either unaffected or inhibited by the addition of pesticides except carbendazim and validamycin, which tend to stimulate this enzyme activity. Thus, it is difficult to identify a clear response of this enzymatic activity to pesticides because this enzyme has received little attention during the last years.

Phosphatases are a broad group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid (Schmidt and Lawoski

1961). In soil ecosystems, these enzymes are believed to play critical roles in P cycles (Speir and Ross, 1978) as evidence shows that they are correlated to P stress and plant growth. Apart from being good indicators of soil fertility, phosphatase enzymes play key roles in the soil system (Dick and Tabatai, 1992; Dick *et al*., 2000).

Plants have evolved many morphological and enzymatic adaptations to tolerate low phosphate availability. This includes transcription activity of acid phosphatases, which tend to increase with high P stress (Haran *et al*., 2000; Baldwin *et al*., 2001; Miller *et al*., 2001; Li *et al*., 2002). The amount of acid phosphatase exuded by plant roots has been shown to differ between crop species and varieties, (Ndakidemi, 2006; Izaguirre-Mayoral and Carballo, 2002) as well as crop management practices (Ndakidemi, 2006; Patra *et al*., 1990; Staddon *et al*., 1998;Wright and Reddy, 2001). For instance, research has shown that legumes secrete more phosphatase enzymes than cereal (Yadav and Tarafdar, 2001). This may probably be due to a higher requirement of P by legumes in the symbiotic nitrogen fixation process as compared to cereals. In their studies, Li *et al*. (2004) reported that chickpea roots were also able to secrete greater amounts of acid phosphatase than maize. The ability to solubilize soil mineral elements by these phosphomonoesteraces is expected to be a higher in biologically-managed systems because of a higher quantity of organic C found in those systems. In fact, the activity of acid and alkaline phosphatases was found to correlate with organic matter in various studies (Guan 1986; Jordan and Kremer, 1994;Aon and Colaneri, 2001). Another factor that influences the rate of synthesis, release and stability of this enzyme is the soil pH (Eivazi and Tabatabai, 1977; Juma and Tabatabai, 1977; Tabatabai, 1994). For example, phosphor monoesteracesinducibility and its intensity by plant roots and micro-organisms are determined by their orthophosphate need, which is in turn affected by soil pH. It is, therefore, anticipated that management practices that induce P stress in the rhizosphere may also affect the secretion of these enzymes in the ecosystem (Ndakidemi, 2006).

Catalase is an iron porphyrin enzyme which catalase very rapid decomposition of hydrogen peroxide to water and oxygen (Nelson and Cox, 2000). The enzyme is widely present in nature, which accounts for its diverse activities in soil. Catalase activity alongside with the dehydrogenase activity is used to give information on the microbial activities in soil. Both catalase and dehydrogenase activity is very sensitive to heavy metal pollution (Naplekova and Bulavko, 1983; Perez and Gonzalez, 1987; Wilkes, 1991). Their values have been suggested to be used as a simple toxicity test (Roger and Li, 1985).

Until present, there have been few studies examining the effect of pesticides application on the ecosystem of soil enzymes activity where major crops are grown. Understanding dynamics of enzyme activity in these systems is crucial for predicting nutrient uptake. Therefore, the present study was conducted to

investigate the effect of some pesticides on some soil enzymes activity in soil cultivated with wheat.

MATERIALS AND METHODS

Field experiment

The field experiment was conducted at Research and Experimental Farm, Faculty of Agriculture, Abees region - Alexandria-Egypt, during wheat growing season of 2012/2013. Wheat (*Triticumastivum*) cv. MISR 2 was cultivated on November 23, 2012. Each plot was 10.5 m^2 (3m X 3.5m) and separated from another one by an adjacent border (1m) to minimize the pesticides drift.

The field experiment was arranged as a randomized complete blocks design and each treatment was replicated three times and the treatments were three herbicides namely;Granstar® 75% DF, Panther® 55% SC, Topik® 15% EC and one fungicide; Sumi-eight®12.5% WP, two rates of each pesticide besides the check treatment were applied as described in Table (1)and Fig (1). The pesticides applications were applied during the growing season on Jan 19 and March 10, 2013, for fungicide. The agricultural practices were done according to the recommendations of the Egyptian Ministry of Agriculture including irrigation through surface irrigation system.

Table (1). The rates of pesticides applied during the growing season of wheat crop

Common name	Trade name	application rates
Tribenuron-methyl	Granstar® 75% DF	0, 4 and 8 g/fed
Diflufenican+Isoproturon	Panther® 55% SC	0, 300 and 600 ml /fed
Clodinafop-propargyl	Topik® 15% WP	0, 70 and140 g /fed
Diniconazole	Sumi-eight®12.5% WP	0, 17.5 and 35 ml/ 100 L water

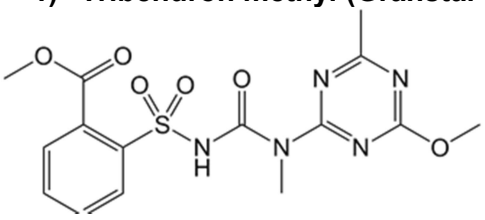
Soil sampling and analysis

Soil samples were collected at the end of growing season of wheat crop at May 2013 for three soil depths, i.e. 0-5, 5-10 and 10-20 cm. The samples were collected from four points randomly and were immediately stored in sealed plastic bags and transported to the laboratory for analysis. The samples were passed through a 2-mm sieve and a composite sample was made for each soil depth and replication. Each mixed soil sample was stored at 4 °C for enzyme analysis.

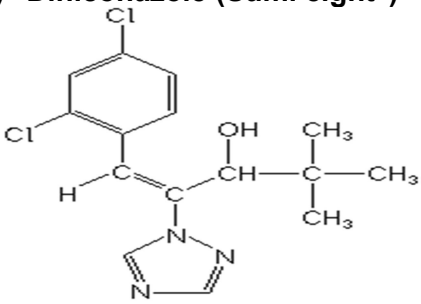
Soil physical and chemical properties were determined according to routine methods (Table 2). The particle-size distribution and saturation percentage (SP) of these soil samples were estimated as described by Jackson (1973). Soil pH was measured in the soil: water suspension (1:2) using a pH meter with a glass electrode. Soil organic matter (OM) was determined using Walkley and Black method (Jackson, 1973). The calcium carbonate ($CaCO_3$) content was estimated

using a volumetric calcium carbonate calcimeter (Nelson, 1982). The electrical conductivity of the soil water extract, 1:2 (EC_{sw}) was determined using an electrical conductivity meter according to Jackson (1973). Soluble calcium (Ca^{+2}) and magnesium (Mg^{+2}) in the saturated soil paste extract were estimated using the titration method by EDTA (Ethyline-diamine tetra acetic acid) solution. However, soluble sodium (Na^{+}) and potassium (K^{+}) in this extract were determined by flame photometry method **(**Hesse, 1998). Also, soluble anions such as bicarbonate (HCO_3^{-}) and chloride (Cl^{-}) were determined using the titration with HCl acid (Richards, 1969) and silver nitrate solution (Jackson, 1973), respectively.

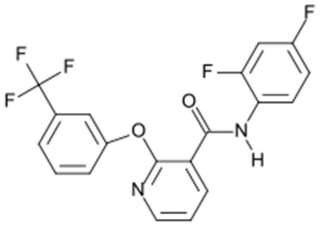
1) Tribenuron methyl (Granstar®)



2) Diniconazole (Sumi eight®)

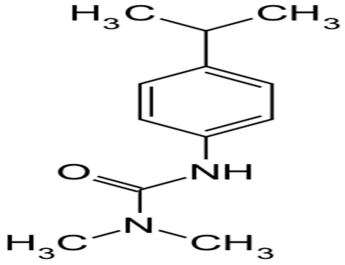


3) Diflufenican



Diflufenican+ Isoproturon = Panther®

1) Isoproturon



2) Clodinafop-propargyl (Topik®)

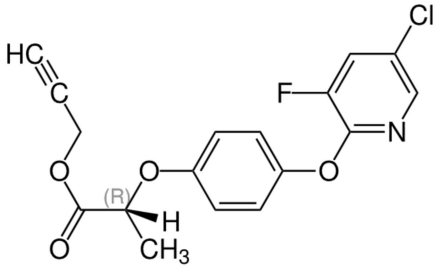


Fig. (1). Chemical structure of used pesticides in the present study

Table (2). Some physical and chemical properties of the experimental soil

Parameters	value	unit
Sand	26.72	%
Silt	24.00	%
Clay	49.28	%
Textural class	Clay	
pH(1:2 water suspension)	8.2	
EC(1:2 water extract)	2.0	dS/m
OM	1.6	%
$CaCO_3$	21.05	%
Soluble cations:		
Ca^{+2}	2.32	me/l
Mg^{+2}	3.48	me/l
Na^{+}	18.26	me/l
K^{+}	0.45	me/l
Soluble anions:		
HCO_3^{-}	5.67	me/l
CL^{-}	13.8	me/l
$SO_4^{=}$	3.0	me/l
Available nutrients:		
Nitrogen(N)	20.32	mg/kg
Phosphorus (P)	47.25	mg/kg
Potassium (K)	850	mg/kg

Measurement of soil enzyme activity

Activities were assayed in triplicate air-dried samples as described by Guan (1986). Briefly, urease activity was determined using urea as substrate, and the soil mixture was incubated at 37°C for 24 h, the produced NH_4-N was determined by a colorimetric method, and urease activity was expressed as µg NH_4-N/g soil/h, phosphatase activity was measured using sodium phenolphthalein phosphate as a substrate, incubation at 37°C for 24 h, and the liberated phenol was determined colorimetrically, acid phosphatase activity was expressed as µg phenol/g soil/h and catalase activity was measured by mixing 2 g of soil, 40 ml of distilled water and 5 ml of 0.3% H_2O_2 were added. The mixture was shaken at 25^{o}C for 20 min. Then, 5 ml of 1.5 M H_2SO_4 was added and the contents were titrated with 0.1 M K_2MnO_4. Catalase activity was expressed as mg/g soil/h.

Statistical Analysis

The results were expressed as mean values and compared with respect to the check treatment. Comparisons between the test and the check were made by using analysis of variance (ANOVA). Differences between treatment means at $p < 0.05$ were considered as significant using Statistix 8.0 software(Statistix, 2003).

RESULTS and DISCUSION

Several factors influenced enzymatic activity. These are both natural factors (e.g., seasonal changes, geographical conditions, depth, physicochemical properties of the soil) and anthropogenic factors (such as pollution with heavy metals, organic compounds, soil acidification, pesticides, etc.). With the development of civilization, anthropogenic factors started to play the main role in soil enzymatic activity changes (Gianfreda and Bollag, 1996).

Tables (3 and 4) show the activity of studied soil enzymes i.e. phosphatase, urease and catalase as affected by some pesticides application. Phosphatase activity ranged between 333.32 and 242.44 µg phenol/g soil/h. The potential of pesticides activity significantly ($p <= 0.05$) differ from each other. The concentration of pesticides significantly ($p <= 0.05$) decreased the activity of soil enzymes in witch increasing the application rate decreased the enzyme activity as 8.61 and 11.44% for half dose and full dose, respectively as compared to control treatment (no addition of herbicides). The activity of phosphatase was significantly ($p <= 0.05$) increased as soil depth increased. The surface layer (0–5 cm) has the least value of activity (327.76 µg phenol/g soil/h), then increased to 350.42 µg phenol/g soil/h as soil depth increased to 10 – 20 cm depth. This is true because of pesticides transport in soil is low and did not reach more the 10 cm.

The persistence of pesticides and their degradation products depend on how deeply they are mixed into the soil; even the most persistent compounds disappear relatively quickly when on the soil surface, yet when incorporated into the soil they are very persistent (Edwards, 1966). Generally, pesticide residues will occur in the top 15 cm of soil (Harris and Sans, 1967). This is also the region of greatest activity of soil flora and fauna in the soil ecosystem (Alexander, 1991).
Phosphatase activity, as indicated by the release of p-nitrophenol, is an index of the activity of microflora involved in soil organic phosphate decomposition. All herbicides suppressed phosphatase enzymes activities. It was demonstrated by some workers (Hoffmann and Seegerer, 1950) that phosphatase activity varies in some cases among soils. It correlates with the intensity of respiration and activity of the soil microflora.

Soil phosphatases because of their involvement in making phosphorus available to plants, need to be extensively studied in relation to pesticide use.

Results obtained from the experiment show stimulation of phosphatase activity followed by fungicide treatment at field application rate of 5 $kg.ha^{-1}$. PRasool and Reshi (2010) declared that the fungicide mancozeb initiated stimulation under normal recommended dosage and ten times the normal dosage. In the same manner, phosphatase increased significantly in agricultural soil amended with the fungicide probineb at field application rate (Rahmansyahet.al.,

2009). Enzyme activity was enhanced up to 7.5 kg.haP-1P than the controls in 10 days incubated soil samples (Srinivasulu*et.al*., 2012).

Soil urease has attracted a great deal of attention due to the increasing use of urea as a fertilizer. In soil, urea is rapidly hydrolyzed to ammonium carbonate by urease activity resulting in the formation of nitrate and gaseous volatilization of ammonia.

All of the studied pesticides inhibited urease activity (Table 3). Sumi-eight herbicide was more inhibition of urease activity (100.36 µg NH4-N/g soil/h) and the least one was Granstar® (172.80 µg NH4-N/g soil/h). The differences between herbicides were significant ($p <= 0.05$). Also, the applied concentration of Granstar®, Topik®, Panther® and Sumi-eight® significantly ($p <= 0.05$) inhibited the urease activity by 37.06 and 49.62% for half and full doses, respectively as compared to control treatment (no addition of pesticides). Decreased urease activity in soil due to the application of pesticides reduces urea hydrolysis, which is generally beneficial because it helps to maintain nitrogen availability to plants (Antonious, 2003).

The activity of urease was significantly ($p <= 0.05$) increased as soil depth increased. The surface layer (0–5 cm) has the least value of activity (17.57 µg NH4-N/g soil/h), then to 154.26 µg NH4-N/g soil/h increased as soil depth increased to 10 – 20 cm depth.

This is true because of pesticides transport in soil is low and the transport of herbicides do not reach more the 10 cm. The inhibition of urease activity could be due to the presence of Mn and Zn ions in the pesticide, as reported by (Tabatabai, 1977).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 and Bremner, 1997).

Also, Yang *et al*.(2006) showed that furadan enhanced urease activity in four soils. Rahmansyah*et al*. (2009), also reported an increase in urease enzyme after two weeks incubation with fungicide probineb. Same findings were found by Maleeka Begum and Rajesh, 2015). But the majority of workers stated inhibition of the enzyme in fungicide amended soils, especially at higher concentration. According to Guo*et al.* (2008), urease activity was inhibited at all concentrations with napropamide. Similarly, Cycon et al. (2010) noticed that activity declined with dimethomorph and mancozeb in loamy and sandy loam soils at higher concentration. Likewise, 20% decrease in activity was observed in chlorpyrifos treated soil (Tejda*et al*., 2010; Wang *et al*., 2010).

Table (3).Average soil enzymes activity values as affected by certain pesticides (type and concentration) and soil depth

Pesticides	Concentration	Soil Depth (cm)	Phosphatase Activity µg phenol/ g soil/h	Urease Activity µg NH_4-N/g soil/h	Catalase Activity mg/g soil/h
Granstar® 75% DF	Check treatment	0 - 5.0	333.3	164.2	2.9
		5.0 - 10.0	343.6	288.2	2.9
		10.0 - 20.0	410.2	322.4	4.1
	Half dose	0 - 5.0	333.3	138.9	2.1
		5.0 - 10.0	328.2	138.9	2.1
		10.0 - 20.0	323.1	155.4	2.8
	Full dose	0 - 5.0	287.2	113.0	1.4
		5.0 - 10.0	317.9	116.8	1.9
		10.0 - 20.0	323.1	117.4	2.1
Topik® 15% WP	Check treatment	0 - 5.0	338.4	153.2	3.1
		5.0 - 10.0	374.3	171.4	3.7
		10.0 - 20.0	405.1	217.1	4.9
	Half dose	0 - 5.0	323.1	116.8	2.8
		5.0 - 10.0	328.2	138.9	3.0
		10.0 - 20.0	333.3	142.7	3.1
	Full dose	0 - 5.0	317.9	93.7	2.1
		5.0 - 10.0	317.9	97.5	2.1
		10.0 - 20.0	317.9	107.5	2.1
Panther® 55% SC	Check treatment	0 - 5.0	343.6	171.4	3.0
		5.0 - 10.0	353.8	197.3	3.4
		10.0 - 20.0	394.9	199.5	4.6
	Half dose	0 - 5.0	328.2	108	2.1
		5.0 - 10.0	338.4	118.5	2.2
		10.0 - 20.0	323.1	123.4	2.5
	Full dose	0 - 5.0	328.2	92.0	2.0
		5.0 - 10.0	328.2	98.1	2.1
		10.0 - 20.0	343.6	104.7	2.1
Sumi-eight® 12.5% WP	Check treatment	0 - 5.0	348.7	113.5	3.4
		5.0 - 10.0	348.7	117.9	3.5
		10.0 - 20.0	353.8	185.7	3.9
	Half dose	0 - 5.0	333.3	81.6	2.1
		5.0 - 10.0	338.4	90.9	3.3
		10.0 - 20.0	343.6	94.8	3.3
	Full dose	0 - 5.0	317.9	64.5	2.0
		5.0 - 10.0	317.9	73.8	2.1
		10.0 - 20.0	333.3	80.5	2.1

Table (4). Analysis of variance for soil enzymes activity as affected by cerain pesticides (type and concentration) and soil depth

	Phosphatase	Urease	Catalase
Main effect of Pesticides(A)			
Granstar® 75% DF	333.32	172.80	2.48
Topik ® 15% WP	339.57	137.64	2.99
Panther ® 55% SC	342.44	134.77	2.68
Sumi-eight®12.5% WP	337.29	100.36	2.86
LSD (0.05)	8.92*	5.10**	0.13**
Main effect of Concentrations (B)			
Check treatment	362.37	191.82	3.63
Half dose	331.18	120.73	2.62
Full dose	320.92	96.63	2.01
LSD (0.05)	4.18**	4.88**	0.05**
Main effect of soil depth(C)			
0 – 5 cm	327.76	117.57	2.42
5 – 10 cm	336.29	137.35	2.70
10 – 20 cm	350.42	154.26	3.13
LSD (0.05)	2.80**	4.39**	0.26**
Interactions (LSD 0.05)			
A X B	**	**	**
A X C	**	**	**
B XC	**	**	**
A X B X C	**	**	**

Concerning the catalase activity, all herbicides applied to soil significantly ($p <= 0.05$) inhibited the catalase activity. Granstar® herbicide was more inhibited the enzyme activity (2.48 mg/g soil/h) and the least one was Topik® (2.99 mg/g soil/h). The differences between herbicides were significant ($p <= 0.05$). Also, the applied concentration of Granstar®, Topik®, Panther® and Sumi-eight® significantly ($p <= 0.05$) inhibited the catalase activity by 27.82 and 44.63% for half and full doses, respectively as compared to control treatment (no addition of herbicides). The activity of catalase was significantly ($p <= 0.05$) increased as soil depth increased. The surface layer (0–5 cm) has the least value of activity (2.42 mg/g soil/h), then increased as soil depth increased to 3.13 mg/g soil/h at 10 – 20 cm depth. This is true because of pesticides transport in soil is low and the transport of pesticides do not reach more the 10 cm (Abdel-Nasser *et al*., 2011).

Continuous and indiscriminate use of these pesticides causes a major risk to soil health. There have been many reports of the effects of pesticides on soil enzyme activities (Anonymous, 2011; and Loganathan *et al*., 2002) and it has been observed that the responses of soil enzymes on different pesticides are not the same. Soil enzyme activities are more sensitive to the environment. They reflect the soil quality more quickly and directly (Srinivasulu *et al*., 2012). Since enzyme

activity has been considered as a very sensitive indicator, any disturbance due to biotic or environmental stresses in the soil ecosystem may affect soil biological properties.

Finally, the measurement of specific enzymatic activities may contribute to understanding the metabolic processes involved in the biogeochemical cycles of nutrients. Pesticides reaching the soil may disturb local metabolism or enzymatic activities (Liu *et al*., 2008; Hussain *et al*., 2009). Negative impacts of pesticides on soil enzymes such as hydrolases, oxidoreductases, and dehydrogenase activities have been widely reported in the literature (Monkiedje and Spiteller 2002; Monkiedje *et al.*, 2002; Menon *et al*., 2005; Caceres *et al*., 2009). There is also evidence that soil enzymes may provide valuable general information on the transformation of pesticides in soils (Gianfreda and Bollag 1994; Kalam *et al*., 2004; Gil-Sotres *et al*., 2005; Hussain *et al*., 2009).

Contrarily, phosphatase activity in paddy cultivated soil showed a variable pattern in response to various concentrations of triazophos after 1st, 7th, 14th, 21st and 28th days of incubation periods. Enzyme activity significantly decreased at all the concentrations of triazophos and there was much difference in the activity compared to untreated control (Lakshmi Kalyani and Suvarnalatha Devi, 2015). Coinciding with the above, activities of two phosphatases were also inhibited (22%) in a sandy loam soil incorporated with captan fungicide at all incubations reported by Piotrowska*et.al*. (2008). the decrease in enzyme activity may be ascribed to suppression of a sensitive fraction of soil biota.

It is difficult to understand the role of pesticides in disturbing the microbial communities and their enzymatic activities in soil due to divergent research findings reported in the literature. A number of factors could be responsible for those debatable results such as soil properties, chemical nature, and concentration of pesticides, biological function observed. Even if pesticides applied at recommended rates may cause slight and transient changes to populations or activities of soil microorganisms (Johnsen *et al.,* 2001), it is obvious that long-term frequent applications of pesticides are known to interfere with the biochemical balance, which can reduce soil fertility and productivity by affecting local metabolism and enzymatic activities.

Finally, in this study, we attempt to look for general trends of enzymatic responses to pesticides, which could be useful for researchers and thus for policy decision makers. Thus, this work has allowed to future investigation for (i) identify patterns of enzymatic activity response to pesticides application, (ii) link them with the pesticides mechanisms of action, and (iii) classify the pesticides according to their stimulating, inhibiting or neutral effects on enzymatic activities. As general, application of pesticides decreased the activity of soil enzymes and this decreasing increased with increasing the pesticides application rate. Also, the decreasing of soil enzyme activity was less with increasing soil depth.

REFERENCES

Abdel-Nasser, G., A. M. Al-Turki, M. H. El-Saeid and M. I. Al-Wabel (2011). Behavior of Atrazine and Malathion Pesticides in Soil: Simulation of transport process using numerical and analytical models. Research Journal of Environmental Sciences, 5(3):221-235.

Alexander, M. (1991). Introduction to Soil Microbiology. 467pp. Krieger Pub Co; 2 edition

Anonymous (2011). Agriculture production plan for Anantapur District, Andhra Pradesh, India. Department of Agriculture, Anantapur, Andhra Pradesh, India

Antonious, G.F. (2003). Impact of soil management and two botanical insecticides on urease and invertase activity. J. Environ. Sci. Heal. B, 38:479–488.

Aon, M. A. and A. C. Colaneri (2001). Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. Appl. Soil Ecol., 18: 255–270

Bacmaga, M., E. Boros, J. Kucharski and J. Wyszkowska (2012). Enzymaticactivity in soil contaminated with the Aurora 40 Wg herbicide.Environ. Prot. Eng., 38:91–102

Baldwin, J.C., A. S. Karthikeyan and K. G. Raghothama (2001). LEPS2, a Phosphorus Starvation- Induced Novel Acid Phosphatase from TomatoLEPS2, Plant Physiol., 125: 728-737

Bending, G. D., M. S. Rodrıguez-Cruz and S. D. Lincoln (2007). Fungicide impacts on microbial communities in soils with contrasting management histories. Chemosphere, 69: 82-88.

Burrows LA, Edwards CA (2004). The use of integrated soilmicrocosms to assess the impact of carbendazim on soilecosystems. Ecotoxicology 13(1):143–161

Caceres, T.P., W.X. He, M. Megharaj and R. Naidu (2009). Effect of insecticide fenamiphos on soil microbial activities in Australian and Ecuadorean soils. J. Environ. Sci. Heal. B., 44:13–17.

Cycon, M., Z. Piotrowska-Seget and J. Kozdroj (2010). Responses of indigenous microorganisms to a fungicidal mixture of mancozeb and dimethomorph added to sandy soils. Int. BiodetBiodegr, 64:316–323

Dick, W.A., L. Cheng and P. Wang (2000). Soil acid and alkaline phosphatase activity as pH adjustment indicators. Soil Biol Biochem 32:1915–1919

Dick, W.A. and M. A. Tabatai (1992). Potential uses of soil enzymes. In: Metting FB Jr (ed) Soil microbialecology: applications in agricultural and environmental management. Marcel Dekker, NewYork, pp 95–127

Edwards, C. A. (1966). Insecticide residues in soils. Res. Reviews, 13, 82 - 132

Eivazi, F. and M. A.Tabatabai (1977). Phosphatases in soils. Soil Biol Biochem 9:167–172

El-Shahaat, M. S. A. (1993). The impact of certain pesticides with cotton plants and soil: Effect of several pesticides on cotton leaves and soil biological processes during leaf worm control. Alex. J. Agric. Res., 38(2): 377-398.

Gianfreda, L. and J. M. Bollag (1994). Effect of soils on the behavior of immobilized enzymes. Soil Sci. Soc. Am. J., 58:1672–1681

Gianfreda, L. and J. M. Bollag (1996). Influence of natural and anthropogenic factors on enzyme activity in soil. In: Stotzky, G., Bollag, J.M. (Eds.), Soil Biochemistry. Marcel Dekker, New York, p. 427.

Gil-Sotres, F., C. Trasar-Cepeda, M. C. LeirAs and S. Seoane (2005). Different approaches to evaluating soil quality using biochemical properties. Soil Biol.Biochem., 37:877

Guan, S., D. Zhang and Z. Zhang (1986). Soil Enzyme and its Research Methods. Agriculture Press, Beijing.

Gundi, V., B. Viswanath, M. S. Chandra, V. N. Kumar and B. R. Reddy (2007). Activities of cellulase and amylase in soils as influenced byinsecticide interactions.Ecotox Environ Safe 68:278–285

Guo, H., G. F. Chen, Z. P. Lu, H. Zhao, and H. Yang.(2008). Alteration in microbial proerites in soils exposed to napopramide. J. Environ. Sci., 21: 494-502.

Haran, S., S. Logendra, M. Seskar, M. Bratanova and I. Raskin (2000). Characterization of Arabidopsis acid phosphatase promoter and regulation of acid phoaphatase expression. Plant Physiol., 124: 615- 626

Harris, C. R. and W. W. Sans (1967). Absorption of organochlorine insecticide residues from agricultural soils by root crops. Journal of Agricultural and Food Chemistry, 15: 861-863.

Hesse, P.R. (1998). A textbook of soil chemical analysis.CBS Publishers & Distributors. Delhi, India.

Hoffmann, E. and A. C. Seegerer (1950). Soil enzymes as measure of biological activity.Biochem. Z., 321: 97-99

Hussain, S., T. Siddique, M. Saleem, M. Arshad and A. Khalid (2009). Impact of pesticides on soil microbial diversity, enzymes, and biochemical reactions. Adv. Agron., 102:159–200

Izaguirre-Mayoral, M.L., S. Flores and O. Carballo (2002). Determination of acid phosphatases and dehydrogenase activities in the rhizosphere of nodulated legume species native to two contrasting savannah sites in Venezuela. Biol. Fert. Soils, 35: 470-472.

Jackson, M.L. (1973). Soil chemical analysis. Prentice-Hall, Inc., Englewood Cliffs. NJ, USA

Johnsen, K., C. S. Jacobsen, V. Torsvik V. and J. Sorensen J (2001). Pesticideeffects on bacterial diversity in agricultural soils: a review. Biol. Fert. Soils, 33:443–453

Jordan, D. and R. J. Kremer (1994). Potential use of microbial activity as an indicator of soil quality. In: Pankhurst CE, Double BM, Gupta VVSR, Grace PR (Eds.): Soil biota. Management in sustainable farming systems, CSIRO Australia pp. 245-249.

Juma, N.G. and M. A. Tabatabai (1977). Effects of trace elements on phosphatase activity in soils. SSSAJ, 41: 343–346.

Kalam, A., J. Tah and A. K.Mukherjee (2004). Pesticide effects on microbial population and soil enzyme activities during vermicomposting of agricultural waste. J. Environ.Biol., 25:201–208

Klose, S. and H. A. Ajwa (2004). Enzyme activities in agricultural soils fumigated with methyl bromide alternatives.36(10):1625-1635

Klose, S., V. Acosta-Martinez and H. A.Ajwa (2006). Microbial communitycomposition and enzyme activities in a sandy loam soil afterfumigation with methyl bromide or alternative biocides. Soil

Lakshmi Kalyani, B and P. Suvarnalatha Devi (2015). Soil phosphatase and protease activity in response to application of triazophos an organophosphorus insecticide.World J. Pharma. Res., 4(6): 1283-1291.

Li, D., H. Zhu, K. Liu, X. Liu, G. Leggewie, M. Udvardi and D. Wang (2002). Purple Acid Phosphatases of Arabidopsis thaliana comparative analysis and differential regulation by phosphate deprivation. J. Biol. Chem., 277: 27772-27781

Li, Y., M. Guohua, C Fanjun, Z. Jianhua and Z. Fusuo (2004). Rhizosphere effect and root growth of two maize (Zea mays L.) genotypes with contrasting P efficiency at low P availability. Plant Sci. 167: 217-223.

Liu, X. M., Q. Li, W. J. Liang and Y. Jiang (2008). Distribution of soil enzyme activities and microbial biomass along a latitudinal gradient in farmlands of songliao plain, Northeast China. Pedosphere, 18:431

Loganathan, M., P. C. Sundarababu and G. Balasubramanyam (2002). Efficacy of biopesitcides against *Spdeopteralitura*(Fab.) on groundnut (*Arachishypogaea*L.). Mad Agric J., 89(7–9): 521–524.

Makoi, J. and P. A. Ndakidemi (2008). Selected soil enzymes: examples of their potential roles in the ecosystem. Afr. J. Biotechnol., 7:181–191.

Martens, D. A. and J. M. Bremner (1997). Inhibitory effects of fungicides on hydrolysis of urea and nitrification of urea nitrogen in soil.Pest.Sci., 49: 344-452.

Menon P, Gopal M, Parsad R (2005). Effects of chlorpyrifos andquinalphos on dehydrogenase activities and reduction of Fe3? Inthe soils of two semi-arid fields of tropical India. AgricEcosystEnviron 108:73–83

Menon, P., M. Gopal and R. Parsad (2005). Effects of chlorpyrifos and quinalphos on dehydrogenase activities and reduction of Fe_3? In the soils of two semi-arid fields of tropical India. Agric. Ecosys. Environ., 108:73–83.

Miller, S. S., J. Liu, D. L. Allan, C.J. Menzhuber, M. Fedorova and C. P. Vance (2001). Molecular Control of Acid Phosphatase Secretion into the Rhizosphere of Proteoid Roots from PhosphorusStressed White Lupin. Plant Physiol., 127: 594-606

Monkiedje A, Spiteller M (2002). Effects of the phenylamide fungicides, mefenoxam and metalaxyl, on the microbiological properties of a sandy loam and a sandy clay soil. Biol.Fertil. Soils 35:393–398

Monkiedje, A, Ilori MO, Spiteller M (2002). Soil quality changes resulting from the application of the fungicides mefenoxam and metalaxyl to a sandy loam soil. Soil Biol.Biochem. 34:1939–1948

Naplekova, N.N. and G. L. Bulavko(1983). Enzyme Activity of Soils Polluted by Lead Compounds. Soviet Soil Science,**15**: 33-38.

Ndakidemi, PA (2006). Manipulating legume/cereal mixtures to optimize the above and below ground interactions in the traditional African cropping systems. Afr. J. Biotechnol., 5 (25): 2526-2533.

Nelson, R.E. (1982). Carbonate and gypsum. p. 181-197. In A.L. Page (ed.) Methods of soil analysis. Part 2.2nd ed. Agron.Monogr. 9. ASA and SSSA, Madison, WI.

Nelson, D..L. and M. M. Cox (2000). Principles of Biochemistry. Macmillan Press, London, UK

Pampulha, M.E. and A. Oliveira (2006). Impact of an herbicide combination of bromoxynil and prosulfuron on soil microorganisms.Curr. Microb 53:238–243

Patra, D.D., P. C. Brookes, K. Coleman and D. S. Jenkinson (1990). Seasonal changes of soil microbial biomass in an arable and a grassland soil which have been under uniform management for many years. Soil Biol. Biochem., 22: 739-742.

Perez, M.M. and C. S. Gonzalez (1987). Effect of Cadmium and Lead on Soil Enzyme Activity. Review of Ecology and Biology Solutions, **1**: 11-18.

Piotrowska, S. Z., R. Engel, E. Nowak and J. Kozdroj (2008). Successive soil treatment with captan or oxytetracycline affects non target microorganisms. World. J. Microbio. Biotech. 24: 2843-2848.

Qian, H. F., B. L. Hu, Z. Y. Wang, X. Xu and T. Hong (2007). Effects of validamycin on some enzymatic activities in soil. Environ Monit Assess, 125:1–8.

Rahmansyah, M., S. Antonius, and N. Sulistinah (2009). Phosphatase and urease instability caused by pesticides in soil improved by groundnut straw. ARPN. J. Agric. And Biol. Sci., 4(2): 56-62.

Raith, W., K. Laval, E. Laroche-Ajzenberg, C. Mougin, X. Latour, I. Trinsoutrot-Gattin (2014). Effects of pesticides on soil enzymes: a review**.** Environ Chem Lett

Rasool, N. and Z. A. Reshi (2010). Effect of the fungicide mancozeb at different application rates on enzyme activities in a silt loam soil of the Kashmir Himalaya, India. Trop. Ecol., 51(2): 199-205.

Reddy, CA (1995). The potential for white-rot fungi in the treatment of pollutants.Curr.Opin Biotechnol., 6:320–328

Rogers, J.C. and S. Li (1985). Effect of metals and otherinorganic ions on soil microbial activity.Soil dehydrogenaseassay as a simple toxicity test. Bull. Environ.Contam.Toxicol., 34, 858-865.

Romero, E., J. Fernandez-Bayo, J. M. C. Diaz and R. Nogales (2010). Enzyme activities and diuron persistence in soil amended with vermicompost derived from spent grape marc and treated with urea. Appl. Soil Ecol., 44:198–204

Rotini, O. T. (1935). La trasformazioneenzimaticadell'ureanelterreno. Ann Labor RicFermSpallanrani, 3:143–154.

Richards, L. A. (1969). Diagnosis and Improvement of Saline and Alkali Soils.United States Salinity Laboratory Staff. Agriculture Handbook No. 60.United States Department of Agriculture. Reviewed Edition.

Schmidt G, Bartsch G, Liss M, Herman T, Levine M (1962). Intracellular distribution of acid phosphatase of fresh andp-starved baker's yeast. Fed Proc., 21(2):237

Schmidt, G. and M. Sr.Laskowski (1961). Phosphate ester cleavage (Survey). In: Boyer PD, Lardy H, Myrback K (eds) The enzymes, 2nd edn. Academic, New York, pp 3–35

Shetty, P. K. and S. P. Magu (1998). In *vitro* effect of pesticide on carbon dioxide evolution and dehydrogenase activities in soil. J. Environ. Biol., 19(2): 41-144.

Speir, T.W. and D. J. Ross (1978). Soil phosphatase and sulphatase. In: Burns RG (ed) Soil enzymes. Academic, London, UK, pp 197–250

Srinivasulu, M., G. Jaffer Mohiddin, K. Subramanyam, V. Rangaswamy (2012). Effect of insecticides alone and in combination withfungicides on nitrification and phosphatase activity in two groundnut (Arachishypogeae L.) soils. Environ Geochem Health34:365–374

Staddon, W.J., L. C. Duchesne and J. T. Trevors (1998). Impact of clear-cutting and prescribed burning on microbial diversity and community structure in a Jack pine (*Pinusbanksiana* Lamb.) clear-cut using BiOLOG gram-negative microplates. World J. Microbiol. Technol., 14: 119-123

Statistix (2003). Statistix 8.2, Analytical Software for Window. Tallahassee, FL.

Stromberger, M. E., S. Klose, H. Ajwa, T. Trout, and S. Fennimore (2005). Microbial Populations and Enzyme Activities in Soils Fumigated with Methyl Bromide Alternatives. Soil Sci. Soc. Am. J., 69:1987–1999

Sukul, P. (2006). Enzymatic activities and microbial biomass in soil as influenced by metalaxyl residues. Soil Biol. Biochem., 38:320–326.

Tabatabai, M. A. (1977). Effects of trace elements on urease activity in soils.Soil.Bio.Soil.Biochem., 9(1): 9- 13.

Tabatabai, M.A. (1994). Soil enzymes. In: WA Dick (ed) Methods of soil analysis, 2. Microbiological and biochemical properties. Soil Science Society of America, Madison, pp 775–833

Tabatabai, M.A. and W. A. Dick (2002). Enzymes in soil: research and developments in measuring activities. In: Burns, R.G., Dick, R.P. (Eds.), Enzymes in the Environment: Activity, Ecology, and Applications. Marcel Dekker, New York, pp. 567- 596.

Tejada, M. (2009). Evolution of soil biological properties after addition of glyphosate, diflufenican and glyphosate plus diflufenican herbicides. Chemosphere, 76:365–373.

Tejada, M., A. M. Garcinia Martinez, I. Gomez and J. Parrado (2010). Applications of MCPA herbicide on soil amended with biostimulants. Short term effects on soil biological properties. Chemosphere, 80: 1088-1094.

Tejada, M., I. Gomez, A. M. Garcia-Martinez,P. Osta and J.Parrado (2011). Effects of Prochloraz fungicide on soil enzymatic activities and bacterial communities. Ecotox Environ Safe 74:1708–1714.

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

Wang, J., Y. Lu and G. Shen (2007). Combined effects of cadmium and butachlor on soil enzyme activities and microbial community structure.Environmental Geology , 51:1093-1284.

Wilkes, B.M. (1991). Effects of Single and Successive Addition of Cd., Ni and Zn on Carbon Dioxide Evolution and Dehydrogenase Activity in Sandy Soil. Biology and Fertility of Soils, **11**: 34-37.

Wright, A. L. and K. R. Reddy (2001). Phosphorus Loading Effects on Extracellular Enzyme Activity in Everglades Wetland Soil. SSSAJ, 65: 588-595.

Yadav, R.S. and J. C. Tarafdar (2001). Influence of organic and inorganic phosphorous supply on the maximum secretion of acid phosphatase by plants. Biol. Fert. Soils, 34: 140-143.

Yan, H., D.D. Wang, B. Dong, F. F. Tang, B. C. Wang, H. Fang and Y. L. Yu (2011). Dissipation of carbendazim and chloramphenicol alone and in combination and their effects on soil fungal: bacterial ratios and soil enzyme activities. Chemosphere, 84:634–641

Yang, Z., S. Liu, D. Zheng and S. Feng (2006). Effect of heavy metals on soil enzyme acivities. J. Environ. Sci., 18: 1135-1141.

الملخص العربي

تأثير بعض المبيدات على نشاط بعض إنزيمات التربة فى أرض منزرعة بالقمح

جمال عبد الناصر خليل * مجدى عبد الظاهر مسعود أحمد صبحى بركات *** أحمد محمد عطية*****

* قسم الأراضى والكيمياء الزراعية – كلية الزراعة سابا باشا – جامعة الاسكندرية

** قسم وقاية النبات – كلية الزراعة سابا باشا – جامعة الاسكندرية

*** قسم بحوث آفات المحاصيل الحقلية – معهد بحوث وقاية النبات مركز البحوث الزراعية

يتم زراعة مساحات شاسعة من الارض الزراعية بمحصول القمح فى مصر. ومع استخدام انواع مختلفة من المبيدات على نظاق واسع خاصة مبيدات الحشائش ومبيدات الفطريات فمن المهم تقييم الاخطار الشديدة لبقايا المبيدات على الكائنات غيرالمستهدفة فى البيئة الزراعية مثل الكائنات الحية الدقيقة بالتربة والتى يمكن تقديرها بقياس نشاط بعض انزيمات التربة.

لهذا فقد اجريت تجربة حقلية بمزرعة كلية الزراعة سابا باشا بمنطقة ابيس ١٠ – الاسكندرية لدراسة تأثيرات بعض المبيدات على نشاط بعض انزيمات التربة فى ارض مزروعة بمحصول القمح. تم استخدام ثلاثة مبيدات حشائش هى جرانستار ٧٥% بمعدلات صفر, ٤ و ٨ جم/فدان , بانثر ٥٥% بمعدلات صفر, ٣٠٠ و ٦٠٠ مل/فدان , توبك ١٥% بمعدلات صفر , ٧٠ و ١٤٠ جم/فدان ومبيد واحد من مبيدات الفطريات هو سومى ايت بمعدلات صفر, ١٧,٥ و ٣٥ مل/١٠٠ لتر. تم اضافة المبيدات خلال موسم نمو القمح . تم جمع عينات التربة فى نهاية موسم النمو خلال مايو ٢٠١٣ لثلاثة اعماق للتربة هى من صفر :٥سم , ٥ : ١٠ سم و ١٠ : ٢٠ سم . تم تقدير نشاط الانزيمات فى ثلاث مكررات . تم قياس نشاط ثلاث من انزيمات التربة وهى اليوريز , الفوسفاتيز و الكاتاليز. أوضحت النتائج الحالية ان مبيد بانثر كان له أعلى نشاط فى التربة بينما مبيد جرانستار كان له اقل نشاط. كما أن تركيزات المبيدات كان لها اثر معنوى فى تقليل نشاط انزيمات التربة حيث ان زيادة تركيز المبيد المضاف قلل من نشاط الانزيمات. كما وجد ان نشاط انزيمات التربة يزداد مع زيادة عمق التربة . وقد وجد ان الطبقة السطحية للتربة كان لها اقل نشاط لانزيمات التربة حيث ان انتقال المبيدات فى التربة قليل ولا يتعدى عمق ١٠ سم . بصفة عامة إضافة المبيدات للتربة قلل من نشاط انزيمات التربة وهذا النقص إزداد مع زيادة تركيز المبيد المضاف كما ان انخفاض نشاط انزيمات التربة قل مع زيادة عمق التربة .