J. Pest Cont. & Environ. Sci. 14 (2): 365 - 380 (2006).

The influence of certain pesticides on soil respiration and urease activity

Ashraf E. Mostafa, A. A. Komeil, A. F. El-Aswad and M. I. Aly
Pesticide Chemistry Dept., Faculty of Agriculture, Alexandria University

ABSTRACT

The side effect of pesticides on soil microflora could be investigated by studies of microbial respiration and soil enzymes. The influence of the insecticide, chlorpyrifos; the fungicide mancozeb and the herbicide, glyphosate on soil respiration and soil urease activity, kinetic and inhibition was evaluated. Two common Egyptian soil types, clay loam and sandy clay loam soil with two concentrations of tested pesticides, recommended and three fold of recommended field rate were tested. The results indicated that the soil type was found to be the most effective factor on soil respiration. In contrast, the tested concentrations of pesticides did not affect soil respiration. The rate of soil respiration was more in the case of clay loam soil (Kafre El-Zyate soil) under different treatment than that in sandy clay loam soil (Borg El-Arab soil). According to the statistical analysis, the effect of each tested pesticide on urease activity did not change either with soil type or with pesticide concentrations. The optimum substrate and protein concentrations are 83 mM and 75 μg in the standard assay. Also, the optimum pH value is 6.7. Indirect proportionality between the incubation time and the velocity of urease reaction was recorded. The Michaelis-Menten constant (K_m) was 53.1 mM and the V_{max} value was 434.78 μM hydrolyzed urea min^{-1}. The V_{max} values were 14.592, 18.727 and 10.10 μM min^{-1} for chlorpyrifos, mancozeb and glyphosate, respectively. Regarding the K_i values, all tested pesticides inhibited urease enzyme with the following ascending order; glyphosate < chlorpyrifos < mancozeb.

Keywords: Pesticides, soil respiration, soil urease, urease kinetic.

INTRODUCTION

The agricultural chemicals especially pesticides are perhaps the largest group of poisonous substances being disseminated throughout our environment (El-Aswad *et al.,* 2001). Pesticides may enter the soil either directly or indirectly (Burns, 1975). Once in the soil, pesticides may influence the growth and activity of microorganisms in soil. This kind of effect is significant localized of the essential role of these microorganisms in maintaining soil fertility (Wainwright, 1978 and Somerville and Greaves, 1987). Greaves and Malkones (1980) reported that the side effect of pesticides on soil microflora could be investigated by studies of microbial respiration and soil enzymes.

Several measurements have emerged as important parameters of the general biological activity in soils, especially respiration rates. Several pesticides had no effect on soil microorganisms or on the biochemical processes they mediated in soil. Other pesticides had adverse or beneficial effects. While other inhibited CO_2 evolution from soil. Imidacloprid and its metabolites have little effect on soil microorganism (Liu *et al.,* 2001). Sulfonylurea herbicide, cionsulfuron has a negative effect on a few aspects of the microbial community in soil ecosystems (Allievi and Gigliotti, 2001). A decrease in bacteria number was observed with thiram for two days and simulation with chlorpyrifos after seven days from treatment (Tu, 1981). Moreover, some pesticides increased enzyme activities in soil, whereas other decreased them (Heinonen–Tanski *et al.,* 1986; Walton and Anderson, 1990 and Boyle and Shann, 1995).

It is known that urea is used, as an organic nitrogen fertilizer requires an enzymatic hydrolysis to make its nitrogen content available to plants. Urease enzyme (urea amidohydrolase, EC 3.5.1.5) in the soil is the enzyme that catalysis hydrolysis of urea which could be absorbed by plant and roots causes a harmful effect on plant cells. Thus a serious problem may arise when urea fertilizer was added to soil having low urease activity (Lieoyd and Sheaffe, 1973 and Lethbridge *et al.,* 1981). The addition of glyphosate and paraquate activated urease activity in several soils (Sannino and Gianfreda, 2001). The effect of herbicide penoxalin and insecticide carbofuran at ranging from 2.5 to 10 kg / fed, on soil urease activity were a stimulatory effect and in contrast in the case of fluometuron (Aly and Nassef, 1985). It has been suggested that soil urease is trapped within organic matter, humus-enzyme complex behave similarity to synthetic organic polymer enzyme derivatives for these reasons measurement of stability and kinetic properties will effectively increase our knowledge of enzyme in soil (Nannipieri *et al.,* 1977). To characterize enzymatic activities in soil, several investigators have tried to determine the kinetics based on the Mechaelis-menten equation (Tabatabai, 1982).

The aim of the present work was to: (1) evaluate the effect of some pesticides; chlorpyrifos, mancozeb, and glyphosate on soil respiration and soil urease activity (2) optimizing the factors affecting urease activity and (3) determine the urease inhibition and kinetic.

MATERIALS AND METHODS

1. Chemicals: The technical and formulated pesticides were supplied by the National Company for Agrochem. The insecticide, chlorpyrifos (O,O diethyl-O-(3,5,6-Trichloro-2-pyridinyl) phosphoro-thioate), the fungicide, mancozeb (coordinate product of zinc ion, manganese ethylene bisdithiocarbamate related to both maneb and zeneb) and the herbicide, glyphosate (isopropyl salt of N-(phosphonomethyl) glycien).

2. Soils: Two common Egyptian soil types were tested, clay loam (Kafr El-Zyate) and sandy clay loam soi (Borg El-Arab). Physical and chemical properties of the tested soils were shown in Table (1).

Table (1): Physical and chemical properties of the tested soils

Location		Kafr El-Zyate	Borg El-Arab
Particle size distribution (%)	Sand	16	52
	Silt	48	23
	Clay	36	25
Texture		Clay loam	Sandy clay loam
pH		7.9	4.8
EC		8.2	4.7
Soluble salt mg/l		105	36
Total carbonate %		4.5	44.5
O.M %		1.24	0.31

3. Soil treatment: Quantity of 150 g of each soil type was treated with two concentrations (recommended and three fold of recommended field rate) of the tested pesticides. Its equivalent to 0.48 and 1.44 ppm for clorpyrifos, 0.8 and 2.4 ppm for mancozeb and 1.29 and 5.76 ppm for glyphosate. Three replicates were made for each treatment.

4. Soil respiration determination: The CO_2 evolution from soil was determined according to the method described by Singh *et al.* (1969). The evolution of carbon dioxide during incubation period of 24 hrs at 25 ^oC from the pretreated soil samples was measured at the time intervals of 0, 7, 14, 21, 28, 35 and 42 days from the pesticide application. The carbon dioxide evolved was trapped in 0.02N sodium hydroxide solution, the alkali was quantitatively determined and the CO_2 was calculated as follow:

$$CO_2\ \mu g/100g = \frac{(B-A)\,x\,N\,x\,E}{Wt\ of\ sample}\,x\,100$$

Where, B = the blank titer, A = the sample titer, N= normality of hydrochloric acid, E = equivalent weight of CO_2. Wt = weight of sample

5. Soil urease activity: The urease activity was measured using the spectrophotometric method of Zantua and Bremner (1975). This method depends on determination of remaining urea after incubation of urea as a substrate with urease enzyme. The determination of urea depends on the color complex formed between urea and p-dimethyl aminobenzaldhyde.

6. Kinetic of urease enzyme:

6.1. Preparation of urease stock: The nutrient broth media 20 g / l was prepared in 1000 ml conical flask; the media was autoclaved at 121^oC for two hrs. The medium was enriched by the suspension of tested alluvial soil. The flask was shaking 24 hrs at 25 ^oC, and then centrifuged at 4000 rpm for 20 min. Few amounts of toluene (1 % v/v) was added and the urease stock was kept at 4 ^oC.

6.2. Optimization of urease activity assay:

6.2.1. Enzyme concentration: Different concentrations of the enzyme in the range of 0.5 – 5 ml of urease stock, equivalent with ranging from 12.5 to 125 µg/ml protein, determined according to the method of Lowry *et al.* (1951) using bovine serum albumin as a standard. The optimum concentration of enzyme could be indicating from the relation between O.D at 740 nm and protein concentration.

6.2.2. Substrate concentration: Five concentrations of urea, 8.3, 16, 30, 83 and 160 mM in 0.1M phosphate buffer (pH 7.1) were used. Incubation was carried out at 37^oC for 2 hrs. This experiment indicates the optimum concentration of substrate (Pettit *et al*., 1976).

6.2.3. Incubation time: Six incubation periods; 15, 30, 60, 90, 120 and 180 min. were evaluated to obtain the optimum incubation time of microbial urease activity.

6.2.4. pH value: Phosphate buffer 0.01M in the pH range of 6.5 - 8.8 was used to detect the optimum pH for microbial urease activity.

6.3. Activity studies: The determination of urease activity was carried out under the optimum conditions. Activity was expressed as the different between sample and control (without enzyme). Net urease activity was obtained after correction for two controls, the first without enzyme and the second without substrate. The lineweaver-burk plot was drawn by plotting 1/V versus 1/S where S is the molar concentration of substrate at time 120 min., while the K_m (Michael's constant) and V_{max} (maximum velocity) were estimated.

6.4. Inhibition studies: The inhibition of urease enzyme was carried out by the tested pesticides ranged from 50 to1000 ppm. The Dixon plot was drown by plotting 1/V versus concentration of pesticides. The V_{max} and Ki (inhibition constant) were calculated.

RESULTS AND DISCUSSION

1. Effect of the tested pesticides on soil respiration: The amount of CO_2 evolved from the pretreated two tested soils at the recommended rate and three fold of recommended rate of the tested pesticides, were determined at different time intervals and presented in Table (2). An increase in clay loam soil respiration was observed in all pesticidal treatments at zero time and day 28, except of three fold glyphosate at zero time. Chlorpyrifos and mancozeb decreased carbon dioxide evolution after one week. Regarding to the mean values, no significant differences were recorded among the different treatments as well as control.

The obtained data show that all tested pesticides decreased respiration in case of sandy clay loam soil, at the interval from day 28 to day 42. While exhibited an increase at zero time and day 14. Chlorpyrifos caused highly increase in the soil respiration within the first two weeks after treatment. The mean of carbon dioxide evolution reached to equilibrium throughout the experiment. Almost the same trend was obtained from treatment of three fold of recommended rate.

Table (2): Effect of tested pesticides on soil respiration (mg CO_2 / 100g soil)

Treatment	Time (week)							Mean
	0	1	2	3	4	5	6	
				Sandy clay loam soil				
Control	55.40	36.73	25.78	18.38	18.02	17.14	23.23	27.81
				1 fold of recommended rate				-
Chlorpyrifos	76.83	14.09	24.86	18.37	19.94	19.99	25.21	28.47
Mancozeb	66.31	28.23	33.42	18.39	20.30	14.27	26.00	29.56
Glyphosate	59.71	37.18	27.74	19.39	23.83	15.56	22.46	29.41
$LSD_{0.05}$								5.13
				3 fold of recommended rate				-
Chlorpyrifos	77.97	30.65	31.56	19.07	20.28	18.40	22.48	31.49
Mancozeb	74.26	26.48	44.95	17.02	19.31	15.86	23.19	31.58
Glyphosate	54.75	19.91	38.56	19.41	22.55	15.59	23.98	27.82
$LSD_{0.05}$								5.75
				Loamy sand soil				
Control	39.55	20.25	7.91	9.83	17.14	20.08	20.51	19.32
				1 fold of recommended rate				-
Chlorpyrifos	41.37	26.97	15.66	9.43	19.99	22.27	17.96	21.95
Mancozeb	29.23	11.95	21.86	11.09	14.27	16.47	21.16	18.00
Glyphosate	45.42	14.98	21.10	10.88	15.56	14.20	17.02	19.88
$LSD_{0.05}$								4.65
				3 fold of recommended rate				-
Chlorpyrifos	63.62	8.20	18.23	10.12	19.00	14.21	15.70	21.30
Mancozeb	42.38	31.51	19.33	9.14	15.86	17.43	18.62	22.04
Glyphosate	48.45	30.01	38.06	13.16	15.56	17.10	18.62	25.85
$LSD_{0.05}$								4.23

The soil type was the most effective factor on soil respiration which was caused differences in CO_2 evolved in the same pesticide treatment. In contrast, the tested concentrations of pesticides did not affect soil respiration. No significant differences were recorded between both concentrations of each tested pesticide in the same soil type. Clearly, the soil respiration rate was more in the case of clay loam soil under different treatment than that in sandy clay loam soil. These results might be due to the high evolution of microorganisms in clay loam soil even after affecting by pesticides. Also, this might be due to the pesticide molecules in sandy clay loam soil were more available to microorganisms, therefore the affecting of microorganisms by pesticides is more in sandy clay loam soil than that in clay loam soil. These comment agreed with Chandra et al. (1960) and Lewis *et al.* (1978). In general, the effect of pesticides on soil respiration in the present study was soil type dependant, but the effect is unclear and fluctuated. This unclear trend was obtained by several investigators such as Kondratenko *et al.* (1981) and Sabra (1988). They found that neapropamide and pendimethalin increased soil respiration in the first period then followed by significant decrease of CO_2 evolution. Lewis *et al.* (1978) showed that trifluralin caused significant increase of soil respiration. While, Rankov and Velve (1976) found that metrobuzin decreased soil respiration.

2. Kinetic of urease enzyme:

Optimization of urease activity assay:

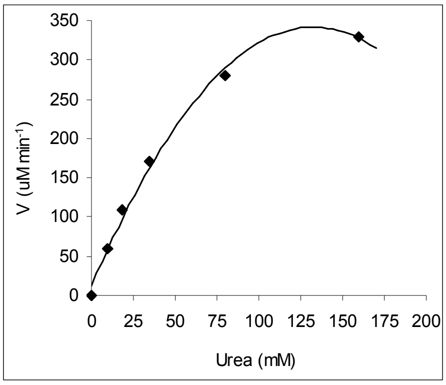
2.1. Substrate concentration: The urease activity (as a μM of hydrolysed urea per one minute) was measured at various concentrations of urea as a substrate (Fig. 1 A). No saturation of urea was observed. Based on this result, the concentration of urea was chosen to be 83 mM in the standard assay

2.2. Enzyme concentration: The protein concentration on urease stock was determined and it found to be 25 μg / ml. The increasing of protein concentrations to 75 μg increased the urease activity, while increasing to 120 μg the urease activity decreased. Therefore, the optimum protein concentration for this reaction was 75 μg (Fig. 1 B). This finding differs the finding with Dixon and Webb (1964) they found that the velocity of any enzymatic reaction is directly proportional to the enzyme concentration.

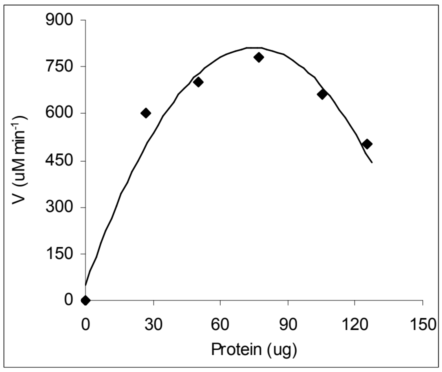
2.3. pH value: The effect of pH on the urease activities is shown in Fig. (1C). Three peaks of activity were observed with various pH ranged from 6.5 to 8.5. The higher enzyme activities were determined at pH 6.7 followed by 7.2 then 8.0. Almost the activities at other pH values were nil. According to this result, the optimum pH value is 6.7. Reithel (1971) found that urease extracted from various sources other than soil showed a pH optimum about 7.0. Nannipieri *et al.* (1977) observed that the optimum pH of urease prepared from soil was slightly alkaline.

2.4. Incubation time: The effect of various reaction time 15, 30, 60, 90, 120 and 180 min. on urease activity was investigated. The percentage of hydrolysed urea from the total amount of urea was 15.9, 17.6, 18.1, 21.1, 21.6 and 23.3 % during the reaction times 15, 30, 60, 90, 120 and 180 min., respectively. Indirect proportionality between the incubation time and the velocity of urease reaction under standard conditions was recorded (Fig. 1 D).

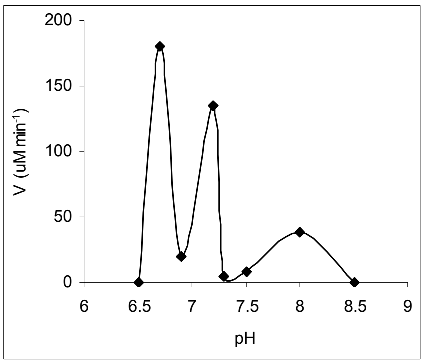
(A)



(B)



(C)



(D)

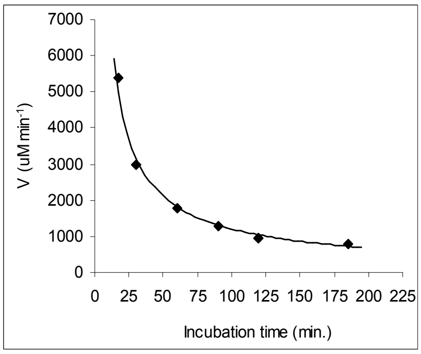


Fig. (1): Effect of different parameters on the urease activity assay

3. Kinetic studies of urease enzyme: Kinetic studies of urease activity were conducting by measuring the initial velocity of the enzyme activity at different substrate concentrations under optimum enzyme activity conditions. Fig. (2) illustrate the regression of duple reciprocal plots (1/V versus 1/S) of the points (Line-weaver and Burk, 1934). The Michaelis-Menten constant (K_m) was 53.1 mM and the V_{max} value was 434.78 µM hydrolyzed urea/min. The kinetic parameters were calculated by Y = 0.1221X + 0.0023, R_2 = 0.9986. The constant K_m is related to a number of rate constant and is a measure of the enzyme affinity for the substrate. The lower K_m the higher the enzyme affinity for the substrate (White *et al.,* 1968). In the absence of charge interactions and enzyme is immobilized sometimes exhibit K_m values higher than those of the free enzymes (Kay and Lilly, 1970). Accordingly, the source of urease; soil or pure media may by affect the K_m value.

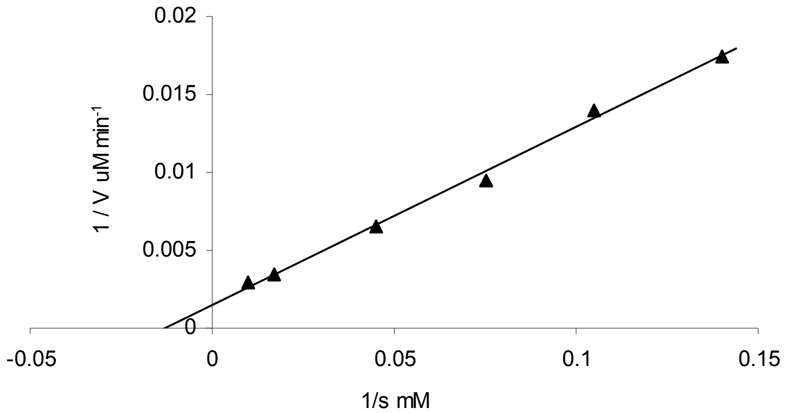


Fig. (2): Lineweaver-burk plot of urease enzyme

4. Effect of the tested pesticides on soil urease activity: The effect of chlorpyrifos, mancozeb and glyphosate with one and three fold of the recommended field rate on urease activity (% of control) in clay loam and sandy clay loam soils is presented in Table (3). Data of urease activity in clay loam soil were generally ranged ± 10 % compared to the control except the urease activity at day 21 with chlorpyrifos, 85.33 % and mancozeb, 191.19 %. For the effect of tested pesticides with three fold of recommended rate, the urease activity was >10 % as control in the case of mancozeb after the 1st week to the 3rd weeks from treatment. No significant differences in the mean values of urease activity in clay loam soil were observed among all tested pesticides.

Although the urease activity in sandy clay loam soil was ranged ± 5 % compared to the control, the significant differences were detected among the means of urease activity. The statistical analysis indicated that the lowest activity obtained with glyphosate. The effect of chlorpyrifos on urease activity was higher in the case of three fold than in the case of recommended rate. According to the statistical analysis, the effect of each tested pesticide on the urease activity did not change with either soil type or pesticides concentrations. These observations are in agreement with that reported by Emara (1986). In general, soil properties variation had a slight influence on pesticide actions. In the present study, there are no significant differences between both soil types on the behavior of the tested pesticides either with the recommended rate or three of the recommended rate. This statement fit with Lewis *et al.* (1978).

Table (3): Effect of tested pesticides on soil urease activity (% of control)

Treatment	Time (week)							Mean
	0	1	2	3	4	5	6	
				Sandy clay loam soil				
				1 fold of recommended rate				-
Chlorpyrifos	103.48	102.63	104.72	85.33	94.91	91.41	100.91	97.63
Mancozeb	107.43	93.52	102.05	191.19	98.15	107.52	98.04	113.99
Glyphosate	107.75	102.18	99.58	100.42	107.77	99.50	100.17	102.48
LSD $_{0.05}$								17.43
				3 fold of recommended rate				-
Chlorpyrifos	97.18	102.16	107.44	92.01	104.40	99.30	100.02	100.36
Mancozeb	96.52	111.73	114.82	116.66	103.23	103.12	101.02	106.73
Glyphosate	89.92	96.91	93.76	122.32	92.14	96.38	100.98	98.92
LSD $_{0.05}$								13.71
				Loamy sand soil				
				1 fold of recommended rate				-
Chlorpyrifos	99.15	99.29	99.67	100.34	97.37	100.21	101.49	99.65
Mancozeb	99.68	98.57	99.94	100.57	101.67	96.30	102.64	99.91
Glyphosate	98.39	97.62	99.24	100.57	96.70	95.96	102.90	98.77
LSD $_{0.05}$								0.40
				3 fold of recommended rate				-
Chlorpyrifos	99.50	98.51	87.47	101.05	96.44	99.68	102.23	97.84
Mancozeb	99.32	99.01	99.09	102.26	97.86	95.27	103.28	99.44
Glyphosate	100.33	97.50	98.21	100.42	96.43	96.89	102.90	98.95
LSD $_{0.05}$								0.35

Several workers have suggested that the urease enzyme in soil is protected by humus or clay colloids and this protection arises through immobilization of the enzyme within organic colloids during humus formation (Mclarin, 1963 and Burns *et al.,* 1972). Also, Burns (1982) suggested that the soil urease is immobilized on the organic mater portion of mineral complexes in soil. In addition, the urease activity in different Egyptian soils tended to be positively associated with organic mater and clay content, but negatively with total carbonate content (Fawaz *et al.,* 1981). On the other hand, the used pesticides at or near field rate may be adsorded on the soil particals. This adsorption behavior mask the pesticides at the adsorption sits, these means less free concentration available in the soil media to interact with the microorganisms which are responsible for urease release (Chandra *et al.,* 1960). Sannion and Gianfreda (2001) showed that glyphosate activated urease activity in several soils. Similary, Aly and Nassef (1985) found that the effect of penoxalin and fluometuron (herbicides) and aldicarb and carbofuran (insecticides) on soil urease activity were a stimulatory effect in general.

5. Inhibition studies of urease: Fig. (3) shows the Dixon plots for the inhibition of urease by three tested pesticides. Chlorpyrifos, mancozeb and glyphosate had K_i values 3115, 0.23 and 7611 mM, respectively. Moreover, the V_{max} values were 14.592, 18.727 and 10.10 μM min^{-1} (calculated by $Y = 0.000022X + 0.06853$, $R^2 = 0.97135$; $Y = 0.0004X + 0.00534$, $R^2 = 0.9917$ and $Y = 0.000013X + 0.098953$, $R^2 = 0.944283$) for chlorpyrifos, mancozeb and glyphosate, respectively. According to the K_i values, all tested pesticides inhibited urease enzyme with the following ascending order; glyphosate < chlorpyrifos < mancozeb.

In conclusion, the soil type was found to be the most effective factor on soil respiration. However, the tested concentrations of pesticides did not affect soil respiration. The respiration rate was more in clay loam soil under different treatments than that in sandy clay loam soil. The effect of each tested pesticide on urease activity was not changed either by soil type or by pesticide concentrations. The optimum substrate and protein concentrations are 83 mM and 75 μg in the standard assay. Also, the optimum pH value is 6.7. Indirect proportionality between the incubation time and the velocity of urease reaction was recorded. The calculation of kinetic parameters indicated that the Michaelis-Menten constant (K_m) was 53.1 mM and the V_{max} value was 434.78 μM hydrolyzed urea min^{-1}. Dixon plots for the inhibition presented that the V_{max} values were 14.592, 18.727 and 10.10 μM min^{-1} for chlorpyrifos, mancozeb and glyphosate, respectively. According to the K_i values, all tested pesticides inhibited urease enzyme with the following ascending order; glyphosate < chlorpyrifos < mancozeb.

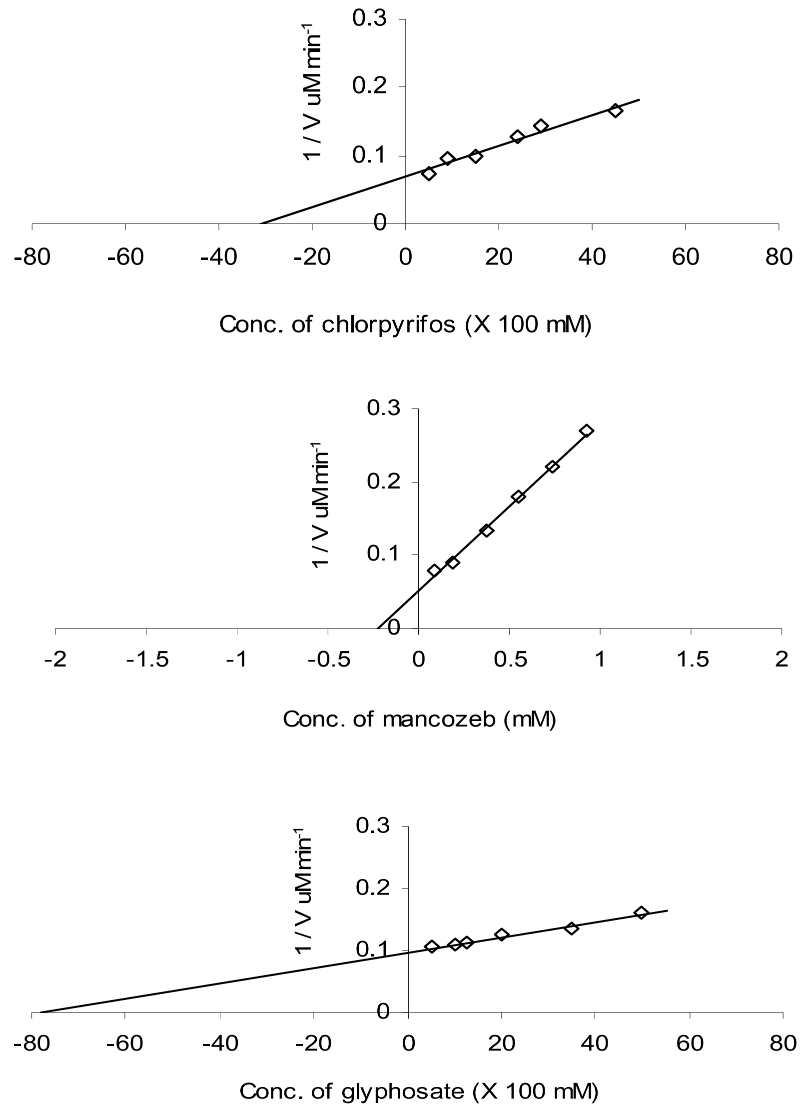


Fig. (3): Dixon plot: Effect of tested pesticides on urease activity

ACKNOWLEDGEMENT

Allah please Mr. Ashraf soul.

REFERENCES

Allievi, L. and C. Gigliotti (2001). Response of the bacteria and fungi of two soils to the sulfonylurea herbicide cinosulfuron. J. Environ. Sci. Health (B), 36 (2): 161-175.

Aly M. I. and O. B. Nassef (1985). Interaction effect of fluometuron, penoxalin herbicides and aldicarb, carbofuran insecticides alone and in combination on soil dehydrogenase and soil urease activity. Comm. In Sci. & Devel. Rese., 9 (80): 71-84.

Boyle J. and J. Shann (1995). Biodegradation of phenol, 2,4-DCP and 2,4,5-T in field-collected rhizosphere and non rhizosphere soils. J. Environ. Qual., 24: 782-785.

Burns R. G. (1982). Enzyme activity in soil: location and possible role in microbial ecology. Soil Biol. Biochem., 12: 423-427.

Burns, R. G.; M. H. Elsayed and A. D. Malaren (1972). Extraction of an urease active organocomplex from soil. Soil Biol. Biochem. 4: 107-108.

Burns R. G. (1975). Factors affecting pesticide loss from soil. In: Soil Biochemistry. E. A. Paul and A. D. Mclaren (eds). V (4) Marcel Dekker, Inc., NewYork, 103-141.

Chandra, P.; W. R. Furtic and W. B. Bollen (1960). The effect of four herbicides on microorganisms in nine Oregon soils. Weeds (8): 589-598.

Dixon, M. and E. C. Webb (1964). Enzymes, Longmans and London, p.950.

El-Aswad, A. F.; A. M. Attia and A. I. Khalil (2001). Influence of malathion and metribuzin on microbial populations and their cellulolytic activities during the composition of vegetable residues. Alex. J. Agric. Res., 46 (1): 253-268.

Emara, M. M. (1986). Interaction between pesticides and Egyptian soils. Ph.D. Thesis, Univ. of Alex. Fac. of Agric. Egypt.

Fawaz, K.; M. H. El-halfawi and M. El-W. Ekhlas (1981). Studies on soil urease enzyme presence and distribution in Egyptian soils. Alex. J. of Agric. Res., 29 (1): 345-350.

Greaves, M. P. and H. P. Malkones (1980). Effect on soil microflora. In: Interaction Between Herbicides and the Soil. R. J. Hance (ed.). Academic Press, Inc London, 223-253.

Heinonen–Tanski, H.; H. Siltannen; S. Klipi; P. Simojoki; C. Rosenberg and S. Makinen (1986). The effect of the annualuse of some pesticides on soil microorganisms, pesticide residues in soil and carrot yields. Pestic. Sci. (17): 135-142.

Kay, G. and M. D. Lilly (1970). The chemical attachment of chimotrypsin to water insoluble polymers using 2-amino-4,6-dichloro-S-triazine. Biochem. Biophys Acta, 198: 276-285.

Kondratenko, V. L.; A. V. Voevodin and S. S. Istamov (1981). Changes in soil microflora under the influence of herbicides. Dokady vessoyuznoi Akademii sel' Skohyozyaistrennykh Nauk imeni.VI. Lenia, No.9, 25-26 (c.f., W.A.31 (4): 1246, 1982).

Lethbridge, G.; A. T. Bull and R. G. Burns (1981). Effect of pesticides on 1,3-B-blucanaseand urease activities in soil presence and absence of fertilizers, lime and organic materials. Pestic. Sci., 12: 147-155.

Lewis, J. A.; G. C. Papavizas and T. S. Hora (1978). Effect of some herbicides on microbial activity in soil. Soil Biochem., 10: 137-141.

Lieoyd, A. B. and M. J. Sheaffe (1973). Urease activity in soils. Plant and Soil (39): 71-80.

Line-weaver, J. and D. Burk (1934). The determination of enzyme dissociation constant. J. Am. Chem. Soc., 56: 657-666.

Liu, H.; W. Zheng and W. Liu (2001). Effects of pesticide imidacloprid and its metabolites on soil respiration. Huan Jing Ke Xue 22 (4): 73-76.

Lowry, O. H.; N. H. Rosenbrough; A. L. Farr and R. L. Andall (1951). Protein measurements with folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mclarin, A. D. (1963). Enzyme activity in soils sterilized by ionizing radiation and some comments on microenvironments in nature. In: Recent progress in microbiology, 8, N. E. Gibbons (Ed.) 221-229.University of Toronto Press.

Nannipieri, P.; B. Ceccanti; S. Cervelli and P. Sequi (1977). Stability and kinetic properties of humus urease complexes. Soil Biol. Biochem., 10: 143-147.

Pettit, N. M.; R. Smith; B. Freedman and R. G. Burns (1976). Soil urease: activity, stability and kinetic properties. Soil Biol. Biochem., 8: 479-484.

Rankov, V. and B. Velve (1976). Influence of temperature propachlor and soil microorganisms. Pochvozanzie I Agrokimia. 11 (5): 100-105. (c.f., W.A. 27 (1): 588, 1978).

Reithel, F. J. (1971). Urease. In the Enzymes. V. ll, 3rd, P. Boyer (ed.), 1-21. Academic Press, New York.

Sabra, F. (1988). Side effect of certain herbicides on fruit quality, yield of tomato plants and soil microorganisms. M.Sc. Thesis. Fac. of Agric., Alexandria University.

Sannino, F. and L. Gianfreda (2001). Pesticide influence on soil enzymatic activities. Chemosphere, 45 (4-5): 417-25.

Singh, B. R.; A. S. Agarwal and Y. Kamehiro (1969). Effect of chloride salts on ammonium nitrogen release in tow Hawaiian soils. Soil Sci. Am. Proc., 33: 257-266.

Somerville, L. and M. P. Greaves (1987). Pesticide effects on soil microflora. Taylor and Francis, London.

Tabatabai, M. A. (1982). Soil enzymes In: A. L. page (ed.) Methods of soil Analysis. Part 2. Chemical and microbiological properties. Am. Soc. of Agronomy, INC. Madison.WI.

Tu, C. M. (1981). Effects of some pesticides on enzyme activities in an organic soils. Bull. Environ. Contam. Toxicol., 27: 109-114.

Wainwright, M. (1978). A review of the effect of pesticides on microbial activity in soils. J. Soil Sci., 29: 287-289.

Walton, B. T. and T. A. Anderson (1990). Microbial degradation of trichloroethylene in the rhizophere: potential application to biological remediation of waste sites. Applied Environ. Microb., 56: 1012-1016.

White, A.; P. Hanler and E. L. Smith (1968). Principles of biochemistry. Mc. Graw-Hill, New York.

Zantua, M. I. and J. M. Bremner (1975). Comparison of methods of assaying urease activity in soils. Soil Biol. Biochem., 7: 291-295.