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Soil biochemical changes induced by three applied insecticides/nematicides

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ABSTRACT

The effect of three granulated insecticides / nematicides; carbofuran, oxamyl and terbufos on the activities of three common soil enzymes (dehyrogenase, urease and catalase) and on soil respiration in clay loam soil was studied after prolonged time of applications under laboratory conditions. The relationships between the assayed enzymes and microbial respiration in treated and untreated soils were also investigated. The results indicated that carbofuran increased dehydrogenase activity at 15 and 50 days, while it decreased urease and catalase activities at 50 days from application compared with the control. Oxamyl treated soil, increased dehydrogenase and urease activities at 25 days and decreased the activities at 50 and 5 days, respectively. In case of catalase enzyme, oxamyl enhanced the activity at 7 days, but decreased its activity at the first day and at 35 days from application. Also, terbufos treatment appeared to cause an increase in dehydrogenase activity at 3, 25, and 50 days; urease at 25 days; and catalase at 7 days. Whereas the trend of increasing activity was reversed for urease at 7 and 50 days and for catalase at 35days from application compared with control treatment. Respiration, as indicated by CO_2-evolution form soil, showed significant differences was noticed with carbofuran or terbufos treatment over control treatment at 35 days. Oxamyl, on the other hand, has stimulatory effect in this respect at three level of times; 25, 35, and 50 days. Simple correlation analysis for the untreated soils, indicated that dehydrogenase activity was significantly ($p<0.05$) related to microbial respiration or to urease enzyme. As for the treated soils, only dehydrogenase activity showed a highly significant ($p<0.01$) negative correlation with urease

activity in soil treated with carbofuran, oxamyl or terbufos. Besides, oxamyl treated soil, exhibited significant positive correlation between dehydrogenase and catalase enzyme activities.

INTRODUCTION

Enzyme assays,in conjunction with other activity indices in soil such as microbial respiration, can provide a clearer picture of the microbial growth and their activities of significance in soil fertility (Frankenberger and Dick,1983 and Tu,1988). Some of the biochemical transformations in soil are catalyzed by enzymes found outside the living organisms (Skujins, 1967). The extracellular enzymes (present in soil as free enzymes and/or enzymes bound to organic and inorganic colloids) play an important role in nutrient cycling and hence soil fertility (Burns,1978) and even in pesticide decay (Burns and Edwards,1980 and Sikora *et al.*,1990).

The increasing use and heavy application of soil insecticides /nematicides in agriculture resulted in hazards to the environment and side effects on non-target soil microorganisms and their activities which are of significance in soil fertility and plant nutrition (Wainwright,1978 and Cheng,1990).

The interaction of insecticides/nematicides with the non-target soil microorganisms and their activities has been studied by many investigators (Tu,1972,1980,1988 & 1994; Radwan *et al.*,1990 and Radwan and El-Doksch 1991). Moreover,relatively little attention has been given to the interaction of these chemicals with soil enzymes (Zamorski *et al.*,1986; Ebieda,1987; Aly and Nassef,1988 and Basavaraj and Sidaramappa,1991). Since detrimental effects on soil fertility could outweigh the benefit of nematicide as nematode control measure, it is interesting to study their side effects on soil enzymatic activities and on soil respiration. Consequently, each negative or positive effect on the activity of these enzymatic systems or microbial respiration may be reflected on the overall microbial and biochemical soil activity.

The purpose of this study was to determine the effects of three soil applied insecticides/nematicides namely; carbofuran, oxamyl and terbufos on the activity of dehydrogenase, urease and catalase enzymes and on soil respiration after prolonged time of applications under laboratory conditions. The relationships between the activity of the tested enzymes and microbial respiration were also determined.

MATERIALS AND METHODS

Soil treatment:

The soil used in this study was previously cultivated with Alfa alfa and collected from Alexandria Agricultural College research station at Abis region. There is no history of pesticides application in the area and the soil properties are: pH,7.90,organic matter,1.5%; total nitrogen, o.14%; sand, 28.6%, silt,36.4%; clay 35%.and 63.5meq/l. total soluble salts. Its texture class was clay loam soil. The soil was air-dried and sieved using 2mm sieve, one hundred grams from soil was mixed with a nematicidal treatment. The treated soil in addition to the control was replicated three times and maintained at 60% of water holding capacity (WHC) through the incubation period at temperature of 30±1c.

Insecticides/nematicides used :

Carbofuran (Furadan 10%G) "2,3-dihydro-2,2-dimethyl-7-benzofuranyl methyl carbamate", oxamyl (Vydate 10%) Methyl N,N-dimethyl-N-[(methyl carbamoyl)oxy]-1-thiooxamindate, and terbufos (Counter 10%) S-(tert.butylthio) methyl O,O-diethyl phosphorodithioate. Each of these nematicides used at the rate of 15 kg/feddan (equal to 0.15 mg/100 gm soil), which is the field recommended rate.

Microbiological determination:

The activities of the various soil enzymes were based on the release and quantitative determination of the product in the reaction mixture when soil samples were incubated with substrate and buffer solution. The following enzyme assays were performed as described elsewhere: Dehydrogenase (Casida *et al.*, 1964 and Pancholy and Rice, 1973); Urease (Watt and Chrisp, 1954) and Catalase (Johnson and Temple,1964). Soil respiration as indicated by CO_2-evolution was estimated according to Singh *et al.* (1969) as quantity of CO_2 evolved per 100 gram soil. All treatments were assayed for enzyme activity and soil respiration after time intervals of 1,3,5,7,15,25,35,50,75,and 100 days from application. Data were expressed on air-dry basis and were analyzed by standard statistical procedures (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

It is well known that the activity of soil microorganisms may strictly depend upon the efficiency of extracellular enzymes and microbial respiration. So, any undesirable effect on either these enzymatic systems or microbial respiration may be reflected on the overall microbial and biochemical soil activity (Frankenberger and Dick,1983 and Gianfreda *et al.*,1993).

Table (1): Dehydrogenase enzyme activity among both control and treated soils with three nematicides after different time intervals.

Time intervals (days)	Nematicide treatments			
	Control	Carbfuran	Oxamyl	Terbufos
	Specific activity of dehydrogenase*			
1	33.78	26.06	33.78	20.27
3	17.45	50.67	21.42	66.99
5	19.08	36.59	54.04	34.91
7	23.65	37.16	36.59	33.78
15	49.94	118.70	78.17	52.84
25	42.78	48.98	131.73	127.22
35	83.64	112.59	113.23	99.72
50	235.21	386.14	194.17	592.14
75	64.98	75.63	84.28	65.62
100	70.13	93.93	87.50	86.21

* Dehdrogenase activity is expressed as Ug formazan/gm soil.
$LSD_{0.05}$ = 36.06 (for every two treatments at the same level of time).

Effect of the three tested nematicides on the activity of soil dehydrogenase, urease and catalase:

Enzyme activities in soil indicate the potential of soil to affect biochemical transformations necessary for maintenance of soil fertility (Skujins,1967 and Burns,1978). The effects of the nematicide; carbofuran, oxamyl or terbufos on the activity of dehydrogenase, urease and catalase enzymes after prolonged time of application are reported in Tables 1,2, and 3 , respectively.

The results shown in Table (1) demonstrate the effect of the three tested nematicides on soil dehydrogenase activity. Terbufos increased the activity of dehydrogenase enzyme at 3 days from application compared with control treatment. At 15 days after application, the formazan content obtained with carbofuran only was significantly higher than those obtained with the untreated treatment.

Table (2): Urease enzyme activity among both control and treated soils with three nematicides after different time intervals.

Time intervals (days)	Nematicide treatments			
	Control	Carbfuran	Oxamyl	Terbufos
	Specific activity of urease*			
1	68.89	68.88	68.88	68.16
3	68.60	67.04	68.83	68.10
5	73.87	73.93	61.54	73.59
7	74.15	74.32	73.36	61.82
15	68.27	68.55	67.87	67.76
25	55.05	55.50	72.13	71.69
35	64.12	65.69	63.45	63.00
50	61.27	49.63	52.98	47.19
75	65.69	65.75	65.36	65.35
100	70.28	70.28	73.79	70.51

* Urease activity is expressed as Umole urea/gm soil.

$LSD_{0.05}$ = 8.34 (for every two treatments at the same level of time).

Also, the data showed that the significant increase in this respect was recorded with both oxamyl and terbufos after 25 days of incubation. Both carbofuran and terbufos treatments significantly enhanced dehydrogenase activity after 50 days. The stimulatory effect was pronounced in terbufos treated soil. On the contrary, after this period ,the inhibitory effect was noted in oxamyl treated soil. At the end of the experiment (100 days), all nematicide treatments appeared to cause an in dehydrogenase activity but without significant differences between treatments.

The results presented in Table (2) clearly indicated that significant increases and decreases of urease activity was observed with oxamyl treated soil after 25 and 5 days of application, respectively. Also, terbufos enhanced urease activity after 25 days and reduced its activity at the time of 7 and 50 days. On the other hand, carbofuran significantly inhibited urease activity only after 50 days of application compared with the control.

Data in Table (3) showed that the two nematicides, oxamyl and terbufos increased the activity of catalase enzyme at 7 days and decreased the activity at 35 days from applications compared with the control treatment. Also, oxamyl treated soil decreased catalase activity at 1st day after application. The effect of carbofuran on the catalase activity is similar to the untreated soil, along all the experiment except at 50 days when it significantly decreased.

It could be concluded that the following points : -

1- Carbofuran significantly increased dehydrogenase activity at 15 and 50 days after application, while it decreased urease and catalase activity at 50 days after treatment compared with the untreated soil.
2- Oxamyl significantly increased dehydrogenase and urease activities at 25 days and decreased the activities at 50 and 5 days from applications, respectively, compared with the control. In case of catalase enzyme, oxamyl increased the activity at 7 days, but decreased its activity at the first day and at 35 days after application.
3- Terbufos significantly increased dehydrogenase activity at time intervals of 3,25, and 50 days, uraese activity at 25 days, and catalase activity at 7 days. Whereas the trend of increasing activity was reversed for urease at 7 and 50 days and for catalase at 35 days from applications compared with control treatment.

Table (3): Catalase enzyme activity among both control and treated soils with three nematicides after different time intervals.

Time intervals (days)	Nematicide treatments			
	Control	Carbfuran	Oxamyl	Terbufos
	Specific activity of catalase*			
1	91.75	93.08	87.75	89.08
3	96.75	96.00	93.75	95.17
5	108.83	107.08	109.17	108.58
7	107.25	105.33	111.17	112.92
15	113.42	112.17	113.00	116.00
25	112.08	111.50	112.17	111.75
35	109.76	108.92	103.33	99.00
50	111.33	107.75	110.00	109.50
75	112.92	110.08	110.58	109.58
100	122.10	121.33	120.83	120.83

* Catalase activity is expressed as Umole H_2O_2 decomposed /gm soil/15 min.
$LSD_{0.05}$ = 3.35 (for every two treatments at the same level of time).

Carbofuran was not inhibitory to the activity of soil urease (Sahrawat,1981). Also, changes in catalase and urease activities in soil treated with carbofuran, oxamyl and phenamiphos were insignificant (Zamorski *et al.*,1986). On the other hand, Ebieda (1987) reported that aldicarb, carbofuran and terbufos stimulated both dehydrogenase and urease activities in uncultivated soils. Aly and Nassef (1988) found that aldicarb and carbofuran decreased urease activity at 1,3 and 56 days, while they increased dehydrogenase activity at 5 days. Carbofuran significantly inhibited dehydrogenase activity, while it had both stimulatory and inhibitory effect on soil urease when applied to the soil at 10 and 100 ppm levels, respectively (Basavaraj and Siddaramappa,1991).

Effect of the three tested nematicides on soil respiration:

Soil respiration, as indicated by uptake and/or the release of carbon dioxide (CO_2-evolution) by living, metabolizing entities in the soil, is a good index of microbial activity (Tu,1980 and Tu and Miles,1976). The rate of CO_2-evolution from treated and untreated soils was determined and presented in Table (4).

The results illustrated in Table (4) revealed that the microbial respiration rate was significantly increased in soil treated with carbofuran oxamyl or terbufos after 35 days from application, where the values of CO_2 were 40.1, 37.1 and 35.2 mg CO_2/100gm soil. Also, oxamyl markedly increased CO_2-production at 25 and 50 days from applications compared with the control.

Table (4) : Microbial respiration among both control and treated soils with three nematicides after different time intervals.

Time intervals (days)	Nematicide treatments			
	Control	Carbfuran	Oxamyl	Terbufos
	Soil respiration*			
1	6.60	8.90	10.90	12 00
3	43.20	46.10	48.30	42.00
5	10.70	12.30	13.30	13.10
7	17.20	15.10	13.50	15.50
15	22.50	18.20	20.50	15.10
25	34.40	33.30	49.90	41.00
35	25.40	40.10	37.10	35.20
50	2.20	3.70	13.30	5.10
75	22.50	16.60	26.30	24.40
100	12.30	18.20	11.60	20.50

* Soil respiration is expressed as mg CO_2/100 gm soil.
$LSD_{0.05}$ = 9.08 (for every two treatments at the same level of time).

Oxygen consumption of soil microbes was not affected by carbofuran or terbufos treated sandy loam soil (Tu,1978). Also, oxamyl and terbufos stimulated the uptake of oxygen by loamy sand soil (Tu,1980). On the other hand, carbofuran and oxamyl when used at the recommended field rate did not alter CO_2-production in sandy clay loam soil cultivated with sweet potato (Radwan *et al.*,1990). Moreover, Radwan and El-Doksch (1991) found that aldicarb and carbofuran enhanced soil respiration when used at the rate of 5 ppm.

The present findings, as discussed above, indicate that the fluctuation of the inhibitory effect after some incubation intervals may be due to the toxic action of these nematicides and/or their metabolites on microorganisms, while the stimulatory effect in other occasions may be either due to the increased activity of few resistant species or to rapid degradation of these chemicals via existing enzymes and may serve as a source of nutrient (Bartha *et al.*, 1967 and Tu,1980).

Relationships between the three tested enzyme activities and microbial respiration:

Quantitative information about the relationships between soil enzymes and microbial respiration accurately reflected microbial growth and their activity (Frankenberger and Dick,1983). Relationships between the activities of the enzymes tested and microbial respiration were determined in untreated and treated soils with carbofuran, oxamyl and terbufos; and are presented in Table (5).

Simple correlation analysis for the untreated soils,showed that dehydrogenase activity was significantly related urease enzyme or to microbial respiration. Catalase and urease activities were not significantly correlated to microbial respiration, as measured by CO_2-evolution in soil. These results agree with those of Casida (1977) and Frankenberger and Dick (1983). They reported close correlations of dehydrogenase activity with CO_2 release or O_2 uptake, respectively. The latter authors stated the fact that urease enzyme was not significantly correlated with respiration. Also,they indicated that much of its activity may not be associated with the active microbial population.

Table (5) : Simple Correlation between some microbial activities in treated and untreated soils.

Simple Correlation	Nematicide treatments			
	Control	Carbfuran	Oxamyl	Terbufos
r_{12}	- 0.419*	- 0.720**	-0.479**	-0.892**
r_{13}	0.290	0.224	0.434*	0.128
r_{14}	- 0.437*	- 0.312	0.025	-0.316
r_{23}	- 0.163	- 0.101	0.084	0.112
r_{24}	- 0.233	0.016	0.115	0.278
r_{34}	- 0.099	0.114	-0.207	-0.199

1- Dehydrogenase 2- Urease
3. Catalse 4- Microbial respiration
*,** Significant at $p<0.05$ and $p<0.01$, respectively

It is clear from Table (5) that there was no significant correlation between the assayed enzymes and CO_2-evolution in soil treated with any tested nematicides. Only dehydrogenase activity was high negatively correlated ($p<0.01$) to urease avtivity obtained in soil treated with carbofuran (r= -0.729), oxamyl (r= -0.479), and terbufos (r= -0.892). Oxamyl treated soil,showed a positive correlation between dehydrogenase and catalase enzymes in soil.

This study provides information about the impact of the three tested insecticides/nematicides on the biochemical activity of soil under laboratory conditions. However, the influence of these chemicals on soil biochemical activity in the presence of vegetation requries close attention.

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الملخص العربى

التغيرات البيوكيماوية فى التربة والناجمة عن تطبيق ثلاثة من المبيدات الحشرية / النيماتودية

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درس تأثير ثلاثة من المبيدات الحشرية / النيماتودية والمطبقة فى التربة (كاربوفيوران، أوكسامايل ، تربيوفوس) على نشاط ثلاثة إنزيمات من إنزيمات التربة الشائعة (الديهيدروجينيز ، اليوريز ، الكتاليز) وأيضا على تنفس التربة ولمدة ١٠٠ يوم تحت الظروف المعملية – وكذلك درست علاقة الارتباط بين هذه الانزيمات وتنفس التربة فى كل من التربة المعاملة والغير معاملة

وأوضحت النتائج أن الكاربوفيوران قد زاد من نشاط الديهيدروجينيز عند اليوم الخامس عشر ، واليوم الخمسون، بينما خفض من نشاط اليوريز والكتاليز عند خمسون يوما من المعاملة مقارنة بالكنترول – وفى التربة المعاملة بالأوكسامايل قد ازداد نشاط الديهيدروجينيز واليوريز عند خمسة وعشرون يوما وخفض عند خمسون يوما وخمسة أيام على الترتيب، وفى حالة إنزيم الكتاليز وجد ان النشاط قد إزداد عند سبعة أيام وأنخفض عند اليوم الاول وكذلك عند اليوم الخامس والثلاثون من المعاملة – والمعاملة بالتربيوفوس أظهرت زيادة فى نشاط الديهيدروجينيز عند ثلاثة ، خمسة وعشرون ، خمسون يوم وزيادة فى اليوريز عند خمسة وعشرون يوم وزيادة فى الكتاليز عند سبعة يوم فى حين إتجاه الزيادة فى النشاط قد انعكس لإنزيم اليوريز عند سبعة يوم وخمسون يوم ولانزيم الكتاليز عند خمسة وثلاثون يوم من المعاملة مقارنة بالكنترول.

كذلك وجد ان المعاملة بالكاربوفيوران أو التربيوفوس قد احدثت تنشيطا جوهريا لانطلاق ك أ٢ (كدالة لتنفس التربة) عند خمسة وثلاثون يوما من المعاملة مقارنة بالكنترول ومن جهة اخرى وجد ان الاوكسامايل ايضا قد أحدث تنشيط على ثلاثة فترات هى خمسة وعشرون ، خمسة وثلاثون ، وخمسون يوما من المعاملة.

وأظهرت نتائج تحليل الارتباط البسيط فى الترب الغير معاملة ان هناك علاقة ارتباط عند مستوى معنوية ٥% بين نشاط الديهيدروجينيز وتنفس التربة أو نشاط اليوريز – اما فى حالة الترب المعاملة فقد وجد علاقة ارتباط سالبة بين نشاط اليوريز فى التربة المعاملة بالكاربوفيوران او الاوكسامايل او التربيوفوس عند مستوى معنوية ١% بالاضافة الى ان التربة المعاملة بالاوكسامايل قد اظهرت علاقة ارتباط موجبة بين نشاط الديهيدروجينيز والكتاليز.