EFFICIENCY OF SOME SOIL MICROORGANISMS IN DEGRADATION OF DIAZINON PESTICIDE

ABSTRACT

A pot experiment was carried out to study the efficiency of *Bacillus polymyxa*, *Pseudomonas fluorescens* and/or *Streptomyces aureofaciens* on biodegradation of the pesticide diazinon in sterilized and non-sterilized soil, either non-cultivated or cultivated. The obtained results showed that disappearance rate of diazinon was higher in the non-sterilized soil than in the sterilized one. Inoculation of soil with mixture of *Bacillus polymyxa + Streptomyces aureofaciens and Pseudomonas fluorescens + Streptomyces aureofaciens* exhibited higher dissipation rate of diazinon in either sterilized or non-sterilized soil, as compared with the soil inoculated with each microorganism individually. When the soil was cultivated with tomato plants and inoculation was made, biodegradation of diazinon increased, compared to the uncultivated and inoculated soil. The metabolites produced from biodegradation of diazinon by the tested microorganisms were 1,3-dimethy-2-nitrobenzene, diethylphosphate, 2-isopropyl-6-methyl-4-hydroxypyrimidine (IMHP) and 2-(1-hydroxy-1-methyl)-6-methyl-4 (IH)-pyrimidine (HMMP).

Key words: Diazinon, biodegradation , *B. polymyxa*, *P. fluorescens* and *S. aureofaciens*.

INTRODUCTION

Organophosphorus pesticides are more toxic to mammals than organochlorine ones. The persistence of pesticides has been of a considerable benefit to the user. Also, organophosphorus pesticide compounds introduce serious problems particularly with improper usage by farmers.

Although, there are many factors affect the persistence of pesticides in soil, such as temperature, pH, water content and others. But, soil microorganisms appeared to play a major role in the degradation of pesticides.

Primarily, biodegradation of pesticides under aerobic conditions is a major fate process for diazinon associated with water and soil. Also, diazinon can be biodegraded under anaerobic condition **(Environmental Protection Agency, 1990)**. Diazinon has a relatively short half-life in soil ranging from 70 hrs to 12 weeks depending on pH, temperature as well as on the presence of specific lytic microorganisms **(Ferrando *et al.* 1992** and **Scheunert *et al.* 1993).**

Also, **Aislabie & Jones (1997)** and **Megharaj *et al.* (2003)** reported that pesticides fate in the environment is mainly affected by microbial activity.

Li *et al.* (2002) reported that the degrading compounds from diazinon had identified as 2-iospropyl-6-methyl-4-hydroxypyrimidine (IMHP); IMHP is a major degradation product when low concentration of diazinon was studied in soil and water. Although (IMHP) is found to be potentially leacheable, it is less toxic than diazinon. Moreover, diazinon-O-analog (or diazoxon), 1,3-dimethyl-2-nitrobenzene, O,O,O,O-tetraethyl dithiopyrophosphate can also be produced as hydrolytic degradation. This research aimed at studying the efficiency of *Bacillus polymyxa*,

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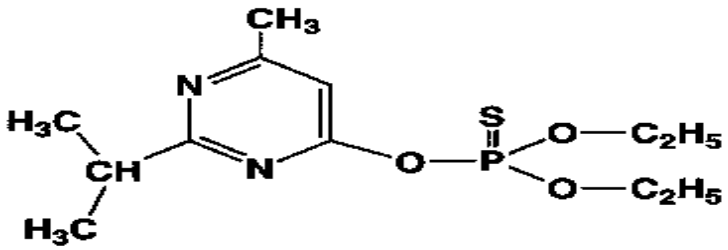
Pseudomonas fluorescens and *Streptomyces aureofaciens* on biodegradation of diazinon in sterilized and non-sterilized soils, both non-cultivated and cultivated.

MATERIALS AND METHODS

The pesticide used.

Organophosphorus pesticide, which is widely used in controlling many pests in Egyptian agricultural, was used, namely Diazinon (Dimpylate), "0,0-Diethyl-0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphorothioate".



Structural formula of diazinon

Efficiency of the tested bacterial strains on biodegradation of diazinon in soil.

A pot experiment was carried out to study the efficiency of *Bacillus polymyxa* , *Pseudomonas fluorescens* and/or *Streptomyces aureofaciens* on biodegradation of diazinon in non-cultivated or cultivated, sterilized or non-sterilized soils. Textural class of the investigated soil was clayey loam. The collected soil was sieved and divided into two parts, one of them was sterilized by autoclaving at 121°C for 3 hrs. Whereas, the second part of soil was left without sterilization. A 30 cm diameter pots were filled with the sterilized or the non-sterilized soils (3 kg/pot). Each soil part was divided into three portions. The first and second potted portions were left without cultivation. The third portion was amended with NPK fertilizers at recommended dose and immediately planted with tomato seedlings c.v. super strain B. After 15 days of planting, each portion was divided into two sub-portion. One of them was left as a control (without pesticide application), while, the other was mixed with diazinon to give a final concentration (50 ppm). After 15 days of pesticide application, the following treatments were carried out:

a) No bacterial inoculation.

b) Inoculation with *B. polymyxa* (20 ml/pot, 930×10^6 c.f.u. / ml) 48 hrs old culture.

c) Inoculation with *P. fluorescens* (20 ml/pot, 93×10^6 c.f.u./ ml) 48 hrs old culture.

d) Inoculation with *S. aureofaciens* (20 ml/pot, 7×10^6 spores/ml)7 days old culture.

e) Inoculation with *B. polymyxa* and *S. aureofaciens* (20 ml/pot) from each of them.

f) Inoculation with *P. fluorescens* and *S. aureofaciens* (20 ml/pot) from each of them.

Soil samples were investigated five times throughout the experiment, i.e. initially and at 3, 7, 15, 35 days after inoculation with the tested microorganisms for assessment of the following:

a) Dehydrogenase activity. b) Nitrogenase activity.

c) Phosphatase activity.

d) Persistent amount of the pesticide and its metabolites.

Dehydrogenase activity was assayed according to **Thalmann (1967)**. Phosphatase activity was determined by the method recomended by **Drobnikova (1961)**. Nitrogenase activity was measured using the technique adopted by **Diloworth (1970).**

Extraction of diazinon and its degradation products.

The method of **Lichtenstein *et al* (1967)** for extraction of diazinon and its metabolites from soil was used with some modifications. Gas Liquid Chromatography and Gas Chromatography/Mass Spectrometer were employed for detection of diazinon and its metabolites.

RESULTS AND DISCUSSION

Periodical changes of dehydrogenase activity in non-sterilized soil non-cultivated and cultivated with tomato.

Data illustrated in Table (1) showed that the untreated soil with diazinon gave higher values of DHA activity as compared with the soil treated with the pesticide. This result was expected since pesticides application to the soil inhibits the activities of different soil microorganisms. This result is in agreement with that obtained by **Balinove *et al.* (1997) and Lan *et. al.* (2006)** who reported that pesticides application to the soil had adverse effect on microbial populations and consequently the microbial enzyme activities were decreased.

Data in Table (1) also emphasized that soil treated with diazinon and uninoculated with tested microorganisms (control) gave lower values of DHA activity, compared to soil treated with diazinon and inoculated.

Table (1): Periodical changes of dehydrogenase activity in non-sterilized soil non-cultivated and cultivated with tomato.

Treatments / Incubation periods(days)			Dehydrogenase activity (µg TPF / g dry soil / day)													
			Soil untreated with diazinon		Soil treated with diazinon*											
					Non-cultivated soil						Cultivated soil					
Planting	Pesticide addition	Inoculation	Non-cultivated soil	Cultivated soil	Control	A	B	C	A+C	B+C	Control	A	B	C	A+C	B+C
30	**15**	**0**	33.7	37.5	23.6	23.6	23.6	23.6	23.6	23.6	23.5	23.5	23.5	23.5	23.5	23.5
33	**18**	**3**	30.1	38.7	20.1	25.0	26.0	22.6	36.2	38.8	24.9	29.3	28.6	28.4	43.5	44.3
37	**22**	**7**	42.9	45.6	21.9	27.5	20.7	23.3	29.4	32.8	12.3	31.0	31.2	31.2	32.1	37.5
45	**30**	**15**	32.3	41.3	18.3	35.7	30.7	28.0	38.6	40.0	14.4	39.1	37.1	37.1	41.3	36.4
65	**50**	**35**	28.9	30.2	22.1	25.0	19.7	23.0	26.3	31.8	19.0	30.5	23.1	23.1	28.2	46.3

* A: *Bacillus polymyxa* B: *Pseudomonas fluorescens* C: *Streptomyces aureofaciens*

In addition, the soil treated with diazinon and inoculated with the mixture of the tested bacteria showed higher DHA activity than the soil inoculated with each one individually. Higher values of DHA activity in case of the soil inoculated with the mixture of the strains is likely due to the synergistic effect between the strains. Similar result was observed by **Aislabie & Jones (1997)** who mentioned that some pesticides are readily degraded by microorganisms including members of genera *Alcaligenes, Flavobacterium, Pseudomonas, Streptomyces and Rhodococcus.*

Moreover, data revealed that the soil treated with diazinon, inoculated with bacteria and cultivated with tomato plants gave higher values of DHA activity as compared with the uncultivated soil. Higher values of DHA activity in the cultivated soil may be due to the beneficial effect of root exudates and root debris which represent nutritional substances for soil microorganisms.

Periodical changes of N_2-ase activity in non-sterilized soil non-cultivated and cultivated with tomato.

Data illustrated in Table (2) showed that the untreated soil gave higher values of N_2-ase activity in rhizosphere soil compared to the soil treated with diazinon. This result is in harmony with that of **Trabue *et al.* (2001)** who mentioned that the microbial activity was adversely affected by the nematicides application.

Yueh & Hensley (1993) reported that diazinon significantly decreased C_2H_2 reduction. The soil amended with diazinon and uninoculated with the biodegrading strains showed lower values of N_2-ase activity, which can be attributed to the inhibitive effect of diazinon pesticide on free living N_2-fixers originally occurred in soil. This result is in accordance with **Doggette & Rhodes (1991)** who found that the growth rate of nitrogen fixers was suppressed by diazinon application at a concentration of 40 ppm.

Table (2): Periodical changes of N_2-ase activity in non-sterilized soil non-cultivated and cultivated with tomato.

Treatments / Incubation periods (days)			Nitrogenase activity (nmoles C_2H_4 / g dry soil / hr.)													
			Soil untreated with diazinon		Soil treated with diazinon*											
					Non-cultivated soil						Cultivated soil					
Planting	Pesticide addition	Inoculation	Non-cultivated soil	Cultivated soil	Control	A	B	C	A+C	B+C	Control	A	B	C	A+C	B+C
30	**15**	**0**	50.06	95.48	45.28	45.28	45.28	45.28	45.28	45.28	45.25	45.25	45.25	45.25	45.25	45.25
33	**18**	**3**	50.01	65.62	15.02	15.9	11.68	15.06	25.35	25.33	18.26	26.16	28.14	25.96	55.17	48.31
37	**22**	**7**	40.51	55.59	5.40	15.73	15.95	20.23	30.89	25.96	20.85	30.65	32.27	23.0	65.3	60.55
45	**30**	**15**	50.01	55.09	5.07	15.28	15.03	15.07	25.16	25.02	24.47	33.90	28.68	28.19	70.60	65.66
65	**50**	**35**	43.8	48.2	21.6	26.1	24.18	21.36	28.61	26.8	30.38	36.69	34.4	31.14	78.6	68.16

*A: *Bacillus polymyxa* B: *Pseudomonas fluorescens* C: *Streptomyces aureofaciens*

On the contrary, the soil inoculated with the investigated microorganisms and treated with diazinon showed higher values of N_2-ase activity than the uninoculated one. The soil amended with diazinon and inoculated with the mixture of strains gave higher values of N_2-ase activity as compared with soil inoculated with the strains individually.

The higher values of N_2-ase activity in rhizosphere soil of inoculation treatments can be attributed to the hydrolysis and biodegradation of diazinon either by normal habitate or inoculated microorganisms in soil. Microbial degradation appeared to be the major pathway for the degradation of diazinon in soil **(Aislabie and Jones, 1997)**.

Generally, the obtained data emphasized that the N_2-ase activity was higher in the cultivated soil treatments compared with the uncultivated ones. Such increase is likely due to the difference in multiplication rate of different N_2-fixers in the cultivated soil as a result of qualitative and quantitative changes in the nature of root exudates during different determination periods.

Periodical changes of phosphatase activity in non-sterilized soil non-cultivated and cultivated with tomato.

Data presented in Table (3) showed that the untreated soil gave higher values of phosphatase activity compared to the soil treated with diazinon during the first 15 days of the experimental period. This result was expected since pesticides application to the soil inhibits various microbial activities.

Whereas, results showed that phosphatase activity was enhanced later on the 35^{th} day sample in the soil treated with diazinon as compared with the untreated one. This result can be attributed to the hydrolysis and biodegradation of diazinon either by the tested strains or by the natural flora of the soil. Similar results were obtained by **Li *et al.* (2002)** who found that metabolites of diazinon were less toxic than the parent compound.

Data also emphasized that the soil treated with diazinon and uninoculated with any of the tested microorganisms (control) gave lowest values of phosphatase activity in the periods of 3, 7 days compared to the soil treated with diazinon and inoculated with each strain individually.

Table (3): Periodical changes of phosphatase activity in non-sterilized soil non-cultivated and cultivated with tomato.

Treatments / Incubation periods (days)			Phosphatase activity (µg inorganic phosphorus / g dry soil / 24 hrs.)													
			Soil untreated with diazinon		Soil treated with diazinon*											
					Non-cultivated soil						Cultivated soil					
Planting	Pesticide addition	Inoculation	Non-cultivated soil	Cultivated soil	Control	A	B	C	A+C	B+C	Control	A	B	C	A+C	B+C
30	**15**	**0**	16.6	19.2	12.8	12.8	12.8	12.8	12.8	12.8	11.9	11.9	11.9	11.9	11.9	11.9
33	**18**	**3**	21.3	23.1	11.0	16.0	13.4	14.8	17.5	19.8	20.0	18.1	26.7	16.1	34.3	31.7
37	**22**	**7**	23.4	28.2	9.2	19.7	14.9	16.8	23.9	36.4	16.0	25.2	22.5	18.2	33.1	37.0
45	**30**	**15**	19.4	21.3	14.7	15.0	14.7	11.9	22.9	22.6	18.9	18.6	16.4	19.69	23.4	22.7
65	**50**	**35**	14.6	27.9	24.7	23.7	26.1	24.4	24.9	25.1	26.7	26.2	28.2	29.8	32.4	30.2

*A: *Bacillus polymyxa* B: *Pseudomonas fluorescens* C: *Streptomyces aureofaciens*

In addition, the soil treated with diazinon and inoculated with a mixture of the tested strains showed higher phosphatase activity than the soil inoculated with each strain individually. Similar results were observed by **Aislabie & Jones (1997) and Lui *et. al.* (2005)**.

Moreover, the obtained data revealed that the soil treated with diazinon and cultivated with tomato plants gave higher values of phosphatase activity, compared to the uncultivated one. The phosphatase activity in the inoculated soil showed higher values than in the control; and the inoculation with the bacterial mixture apparently enhanced phosphatase activity than the individuall strains. This may be due to the beneficial effect of root exudates and root debris which represent nutritional substances for different soil microorganisms.

Persistence rate of diazinon in sterilized and non-sterilized soil.

Persistence rates of diazinon are presented in Table (4) as percentages of the initial concentrations. The obtained results showed that disappearance rate of diazinon was higher in the non-sterilized soil than in the sterilized one. Disappearance rate of diazinon in the non-sterilized and uninoculated soils was faster than in the sterilized uninoculated one, since 42.0 and 72.1 % of the added amounts were detected in the non-sterilized uninoculated and the sterilized uninoculated soil, respectively at the end of the experiment. This result revealed the importance of indigenous soil microorganisms in diazinon decomposition. This result is in agreement with that obtained by **Seyfried (1994)** who found that losses of diazinon from sterilized samples of soil were much slower than from non-sterilized one, indicating microbial participation in diazinon degradation in soils. **Talebi & Walker (1993)** and **Kale *et al.* (1996)** found a similar trend of results when they studied the degradation of pesticide (carbofuran) in sterilized soil compared to non-sterilized one.

Dissipation rate of diazinon was accelerated in soil inoculated with *Bacillus polymyxa, Pseudomonas fluorescens* and *Streptomyces aureofaciens* compared to the uninoculated soil. Soil inoculated with *Bacillus polymyxa* showed the highest dissipation rate of diazinon compared to other microorganisms when each strain was individually used. These results revealed that all tested microorganisms are capable to hydrolyze diazinon. These results are in harmony with those reported by **Kale *et al.* (1996), Trabue *et al.* (2001) and Singh *et. al.*(2005)** who revealed that there were many soil microorganisms capable of pesticides degradation when they were used as soil application.

Also, **Ferrando *et al.* (1992)** and **Scheunert *et al.* (1993)** found that diazinon was degraded by several genera of microorganisms and the microbial degradation appeared to be the major pathway for the degradation of diazinon in soils. Also, **Aislabie & Jones (1997), Miguel *et. al.*(2002), Wander *et. al.*(2003) and Vijay *et. al.*(2006)** reported that some pesticides are readily degraded by microorganisms, others have proven to be recalcitrant. A diverse group of bacteria including members of genera *Pseudomonas, Alcaligenes, Flavobacterium* and *Rhodococcus* were able to metabolize pesticides.

Table (4): Persistence rate of diazinon (%) in non-sterilized and sterilized soil.

Treatments / Days after		Uninoculated soil	Soil inoculated with *				
			A	B	C	A + C	B + C
Diazinon addition	**Inoculation**	**Non-sterilized soil**					
0	**-**	100	100	100	100	100	100
15	**0**	64.5	64.5	64.5	64.5	64.5	64.5
18	**3**	57.3	39.4	32.1	28.3	24.3	31.9
22	**7**	51.8	29.7	18.5	27.9	24.2	22.7
30	**15**	48.4	22.2	16.7	13.5	9.5	16.2
50	**35**	42.0	7.3	9.3	11.5	0.0	4.0
		Sterilized soil					
0	**-**	100	100	100	100	100	100
15	**0**	88.6	88.6	88.6	88.6	88.6	88.6
18	**3**	82.3	56.2	56.0	56.3	54.3	53.8
22	**7**	76.3	48.2	51.3	41.7	41.9	40.3
30	**15**	73.0	29.9	30.1	30.2	32.5	22.0
50	**35**	72.1	14.7	20.7	18.3	10.0	8.0

*A: *Bacillus polymyxa* B: *Pseudomonas fluorescens* C: *Streptomyces aureofaciens*

Inoculation of the soil with mixtures of *Bacillus polymyxa* + *Streptomyces aureofaciens* and *Pseudomonas fluorescens* + *Streptomyces aureofaciens* exhibited higher dissipation rate of diazinon either in sterilized or non-sterilized soils, as compared with the soil inoculated with each microorganism individually. This increase in dissipation rate in the case of soil inoculation with the mixture of strains was likely being due to their synergistic interaction to improve the degradation of diazinon.

Persistence rate of diazinon in non-sterilized soil uncultivated and cultivated with tomato.

Data in Table (5) showed that the uninoculated soil gave lower dissipation rate of diazinon, compared to the inoculated one. This result was observed in either the cultivated or uncultivated soils. When the soil was cultivated with tomato plants and bacterially inoculated, biodegradation of diazinon was increased as compared with the uncultivated and inoculated soil. Since, the persistence percentages of diazinon at the end of the experiment in the cultivated soil was lower than those recorded in the uncultivated one. This difference in disappearance rate of diazinon might be due to the root excretions of tomato plants which may synergist the proliferation of soil microorganisms either the indigenous or the introduced and therefore the decomposition rate of diazinon was increased in the cultivated soil. These results are in agreement with those obtained by **Balinove *et al.* (1997) Trabue *et al.* (2001) and Dimitrios *et. al.*(2005)** who found that the persistence rate of pesticides in soil cultivated with tomato was lower than in uncultivated one.

Inoculation of soil with *B. polymyxa* individually showed the highest disappearance rate of diazinon compared to soil inoculated with either *P. fluorescens* or *Streptomyces aureofaciens*. From data presented in Table (5) it was clear that soil inoculation with the mixtures of strains exhibited highly disappearance rate of diazinon as compared with the soil inoculated with each microorganism apart.

Increasing of disappearance rate of diazinon from the soil inoculated with the mixtures of strains is likely due to the synergistic effect that occurred between the bacterial inocula and subsequently the biodegradation rate of diazinon was increased.

Table (5): Persistence rate of diazinon in non-sterilized soil non-cultivated and cultivated with tomato.

Treatments / Days after			Uninoculated soil	Soil inoculated with*				
				A	B	C	A + C	B + C
Planting	**Diazinon addition**	**Inoculation**	**Non-cultivated soil**					
15	**0**	**-**	100	100	100	100	100	100
30	**15**	**0**	64.5	64.5	64.5	64.5	64.5	64.5
33	**18**	**3**	57.3	39.4	32.1	28.3	24.3	31.9
37	**22**	**7**	51.8	29.7	18.5	27.9	24.2	22.7
45	**30**	**15**	48.4	22.2	16.7	13.5	9.5	16.2
65	**50**	**35**	42.0	7.3	9.3	11.5	0.0	4.0
			Cultivated soil					
15	**0**	**-**	100	100	100	100	100	100
30	**15**	**0**	52.0	52.0	52.0	52.0	52.0	52.0
33	**18**	**3**	50.2	30.8	21.8	20.9	19.5	18.9
37	**22**	**7**	47.8	22.4	15.7	19.9	13.0	16.0
45	**30**	**15**	34.4	13.0	9.6	19.2	12.4	11.1
65	**50**	**35**	35.5	1.4	1.9	2.9	1.2	0.0

*A: *Bacillus polymyxa* B: *Pseudomonas fluorescens* C: *Streptomyces aureofaciens*

Analysis of diazinon biodegradable products by the tested bacterial strains in sterilized and non-sterilized soil using GLC.

Analysis of soil samples was carried out by GLC after 30 days of diazinon treatment (15 days after inoculation). The obtained results are given in Table (6) and illustrated by Fig (1 a, b).

From data in Table (6) it is obvious that the non-sterilized soil treatments showed more metabolites from the biodegradation of diazinon by the investigated bacteria, compared to those produced from sterilized one. These results reveal the importance of indigenous (native) soil microorganisms in diazinon decomposition in the soil. This result is in harmony with that obtained by **Getzin & Shanks (1990)** who found that the degradation of pesticides was slower in sterilized soil and the half-life of pesticides was exceeded. **Arunachalam & Lakshamanan (1990)** and **Zaghloul *et al.* (2003)** who found that in sterilized soil about 75 % of the added carbofuran was recorded as residue after 60 days, whereas in non-sterilized soil about 75 % of the added carbofuran was metabolized.

Extract of the non-sterilized soil inoculated with either *Bacillus polymyxa* or *Pseudomonas fluorescens* cultures showed five metabolites having Rt 0.91, 1.7, 1.8, 1.9 and 2.3 minutes. Whereas, the extract of the abovementioned cultures in the sterilized soil showed four metabolites having Rt 0.91, 1.7, 1.8 and 1.9. Extract of the soil inoculated with *Streptomyces aureofaciens* showed lower metabolites than the soil inoculated with either *Bacillus polymyxa* or *Pseudomonas fluorescens*. Similar trend of results was observed in both the sterilized and non-sterilized soil.

Table (6): Analysis of diazinon biodegradable products by the tested bacterial strains in non-sterilized and sterilized soil using GLC.

Compounds	Uninoculated soil	Retention time (Rt min.)*				
		A	B	C	A + C	B + C
	Non-sterilized soil					
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
metabolites 1	0.91	0.91	0.91	0.91	-	0.91
2	-	1.7	1.7	1.7	-	-
3	1.8	1.8	1.8	-	-	1.8
4	-	1.9	1.9	1.9	1.9	-
5	-	2.3	2.3	-	-	-
	Sterilized soil					
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
metabolites 1	0.91	0.91	0.91	0.91	0.91	0.91
2	-	1.7	1.7	1.7	-	-
3	1.8	1.8	1.8	-	-	-
4	-	1.9	1.9	1.9	1.9	-
5	-	-	-	-	-	-

A: *Bacillus polymyxa* B*: Pseudomonas fluorescens* C*: Streptomyces aureofaciens*

Metabolites produced from diazinon decomposition can't be identified by GLC analysis because their authentic materials were not available.

Gas mass spectrometer was used to determine the values of M/e for each compound to be as reference to use these values for identification of the compounds produced from the biodegradation of diazinon by the investigated microorganisms. The limited data are presented in Table (7) and shown in Fig. (2).

It could be noticed that the metabolites produced from the biodegradation of diazinon by the investigated bacteria showed molecular weights equivalent to the molecular weights of diazinon and its dominant metabolites which were observed and identified by **Robert *et al.* (2000)** in their study. They mentioned that diazinon pesticide was metabolized by microorganisms to diazoxone, diethylphosphate, 2-isopropyl-6-methyl-4-hydroxypyrimidine (IMHP), 2-(1-hydroxy-1-methyl)-6-methyl-4 (IH)-pyrimidine (HMMP) and 1,3-dimethyl-2-nitrobenzene. These compounds are recorded in Table (7).

Therefore, the metabolites produced from the biodegradation of diazinon by the tested bacterial strains were 1,3-dimethyl-2-nitrobenzene, diethylphosphate, HMMP and IMHP.

The identification deduction of the abovementioned metabolites was based on the relation between retention time obtained from GLC analysis and molecular weight obtained from Gas/Mass analysis which was achieved to confirm the obtained results. These results are in agreement with those obtained by **Li *et al.* (2002)** in their studies on diazinon decomposition. They reported that 2-isopropyl-6-methyl-4-hydroxypyrimidine was a major degradation product.

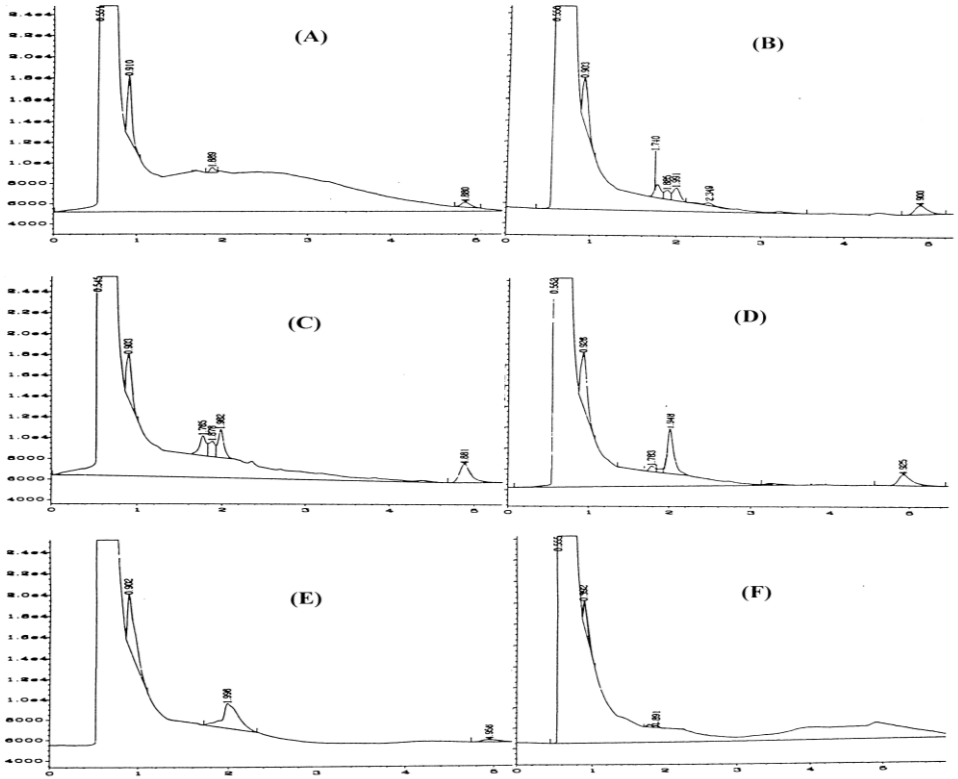


Fig 1 a. Gas liquid chromatography spectra of the non-sterilized soil extracts at 15 days of:

A) Soil treated with diazinon.
B) Soil treated with diazinon and inoculated with *Bacillus polymyxa* (B).
C) Soil treated with diazinon and inoculated with *Pseudomonas fluorescens* (P).
D) Soil treated with diazinon and inoculated with *Streptomyces aureofaciens* (S).
E) Soil treated with diazinon and inoculated with (B + S).
F) Soil treated with diazinon and inoculated with (P + S).

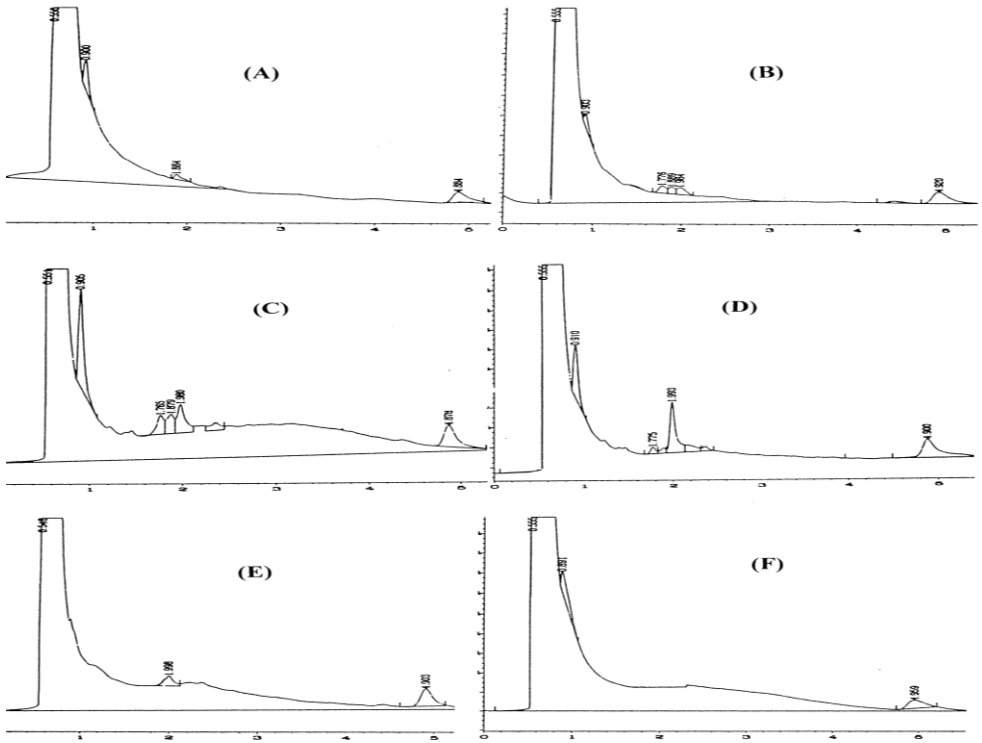


Fig 1 b. Gas liquid chromatography spectra of the sterilized soil extracts at 15 days of:

A) Soil treated with diazinon.
B) Soil treated with diazinon and inoculated with *Bacillus polymyxa* (B).
C) Soil treated with diazinon and inoculated with *Pseudomonas fluorescens* (P).
D) Soil treated with diazinon and inoculated with *Streptomyces aureofaciens* (S).
E) Soil treated with diazinon and inoculated with (B + S).
F) Soil treated with diazinon and inoculated with (P + S).

Dumas *et al.* (1990) stated that the organophosphorus hydrolase (OPH) is an organophosphotriester hydrolyzing enzyme that first discovered in the soil microorganisms *Pseudomonas diminuta* MG and *Flavobacterium* spp. The enzyme has broad substrate specificity and is able to hydrolyze a number of OPH pesticides such as paraoxon, parathion, coumaphos and diazinon. The reaction products, from hydrolysis by organophosphorus hydrolase (OPH) had generally reduced toxicity relative to the parent compound **(Lai *et al.*, 1994** and **Yakovlevsky *et al*, 1997).**

Summing up, from the obtained results it could be concluded that soil microorganisms play an important role in pesticides degradation.

Table (7): Gas/Mass analysis of diazinon and related compounds in soil.

Compounds	Molecular weight	M/e	Chemical formula
Diazinon	304	304	$C_{12}H_{21}O_3N_2PS$
Diethyl phosphate	154	154	$C_4H_{11}O_4P$
2-(1-hydroxy-1-methyl)-6-methyl-4(1H)-pyrimidinone (HMMP)	168	167	$C_8H_{12}O_2N_2$
2-isopropyl-6-methyl-4-hydroxypyrimidine (IMHP)	152	152	$C_8H_{12}ON_2$
1,3-dimethyl-2-nitrobenzene	151	151	$C_8H_9O_2N$

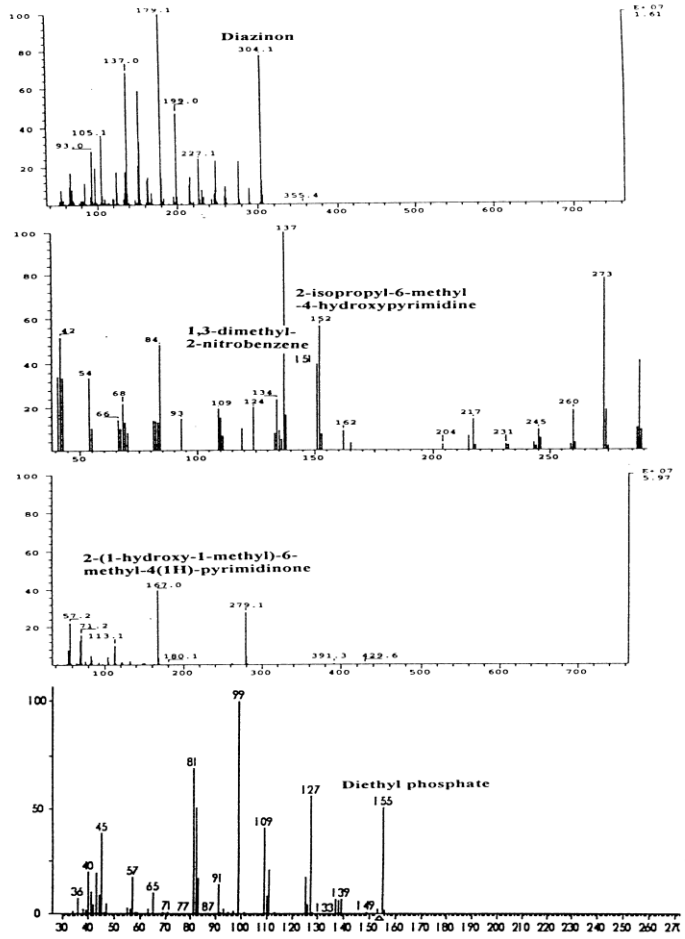


Fig (2): Mass spectrum of diazinon and its metabolites in soil.

Analysis of diazinon biodegradable products by the tested bacterial strains in cultivated and non-cultivated soil using GLC.

Data presented in Table (8) clearly show that extracts of the non-cultivated soil inoculated with either *Bacillus polymyxa* or *Pseudomonas fluorescens* showed five compounds having Rt 0.91, 1.7, 1.8, 1.9 and 2.3 minute. Whereas, inoculation of the cultivated soil showed in chromatographic analysis four compounds in case of *Bacillus polymyxa* having Rt 0.91, 1.7, 1.8 and 1.9 minutes and two compounds in case of *Pseudomonas fluorescens* having Rt 0.91 and 1.9 minutes.

Extract of the soil inoculated with *Streptomyces aureofaciens* showed lower metabolites than the soil inoculated with *Bacillus polymyxa.* Similar trend of results was observed in both the cultivated and uncultivated soil.

From the obtained results, it could be concluded that diazinon in soil decomposed or metabolized to five metabolites having Rt 0.91, 1.3, 1.7, 1.8 and 1.9 minutes.

Metabolites produced from diazinon decomposition was identified by the same method mentioned before in the sterilized and non-sterilized soil **(Robert *et al.*, 2000)**.

Therefore, the metabolites products from the biodegradation of diazinon by various microorganisms used in this research were 1,3-dimethyl-2-nitrobenzene, diethylphosphate, HMMP and IMHP.

Table (8): Analysis of diazinon biodegradable products by the tested bacterial strains in non-cultivated and cultivated soil using GLC.

Compounds	Uninoculated soil	Soil inoculated with*				
		A	B	C	A + C	B + C
	Non-cultivated soil					
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
metabolites 1	0.91	0.91	0.91	0.91	0.91	0.91
2	-	-	-	-	-	-
3	-	1.7	1.7	1.7	-	-
4	1.8	1.8	1.8	-	-	1.8
5	-	1.9	1.9	1.9	1.9	-
6	-	2.3	2.3	-	-	-
	Cultivated soil					
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
metabolites 1	0.91	0.91	0.91	0.91	0.91	0.91
2	1.3	-	-	-	-	-
3	-	1.7	-	-	-	-
4	1.8	1.8	-	-	-	-
5	-	1.9	1.9	1.9	1.9	1.9
6	-	-	-	-	-	2.3

A: Bacillus polymyxa* B*: Pseudomonas fluorescens* C*: Streptomyces aureofaciens*

Similar result was observed by **Seyfried (1994)** who reported that the main metabolite produced from biodegradation of diazinon was 2-isopropyl-6-methyl-4-hydroxypyrimidine (IMHP). Also, **Michel *et al.* (1997)** found that IMHP and diazoxon were the major degradation products. Whereas, **Li *et al.* (2002)** found that 1,3-dimethyl-2-nitrobenzene, compound was produced from the biodegradation of diazinon.

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كفاءة بعض ميكروبات التربة فى تحليل مبيد الديازينون

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أقيمت تجربة بهدف دراسة كفاءة بعض ميكروبات التربة وهى

Pseudomonas fluorescens , Bacillus polymyxa , Streptomyces aureofaciens فى تحليل مبيد الديازينون فى التربة المعقمة وغير المعقمة وكذلك فى التربة المنزرعة بنباتات الطماطم . ولقد أوضحت نتائج هذة الدراسة ما يلى:–

كان معدل إختفاء مبيد الديازينون من التربة أعلى فى حالة التربة الغير معقمة مقارنة بمعدل إختفاؤه من التربة المعقمة . و كذلك أوضحت النتائج أن تلقيح التربة بمخلوط السلالات الميكروبية قد أدى إلى حدوث تحلل أسرع للمبيد مقارنة بالتلقيح الفردى بأى من السلالات. وعندما زرعت التربة بنباتات الطماطم وتم تلقيحها بأحد الميكروبات سالفة الذكر وجد أن مبيد الديازينون قد تحلل بمعدل أسرع مقارنة بالتربة غير المنزرعة.

و من أهم المركبات التى نتجت من تحلل مبيد الديازينون مركب ٣،١ داى ميثيل –٢– ريتروبنزين و داى إيثيل فوسفات و ٢– أيزوبروبيل– ٦– ميثيل–٤– هيدروكسى بيريميدين و ٢–(١– هيدروكسى –١– ميثيل)٦– ميثيل بيريميدين.

و إتضح من نتائج هذه الدراسة أن الأراضى المصرية غنية بالميكروبات ذات القدرة على تحليل مثل هذه المبيدات ولذلك يمكن إعداد لقاحات من هذه الميكروبات لتلقيح التربة بعد إضافة المبيدات و القيام بدورها في القضاء علي الافات و ذلك للتخلص من آثارها المتبقية وللحفاظ على التربة الزراعية من التلوث.