

BIODEGRADATION OF SOME ORGANOPHOSPHORUS PESTICIDES BY SOIL MICROORGANISMS.

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ABSTRACT

This research was carried out to isolate some soil microorganisms capable of degrading organophosphorus pesticides (diazinon and nemacur). Three isolates which confirmed in their ability to degrade and utilize diazinon and nemacur as a sole source of carbon and nitrogen were identified as *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*. Obtained data showed that diazinon and nemacur amounts decreased with elapsed time. The rate of decrease in inoculated medium was faster than that in uninoculated one. Since, 22.71, 63.82, 62.60 and 47.51% of the added diazinon disappeared from the uninoculated, inoculated medium with *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively throughout the first 15 days of diazinon addition. Whereas, the disappearance rate of nemacur at 15 days of incubation period were 4.8, 28.04, 28.30 and 12.11% for uninoculated, inoculated medium with *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively. The compounds produced from the biodegradation of diazinon pesticide by various investigated microorganisms in this study are diazoxon, diethylphosphate, 2-isopropyl-4-methyl-6-hydroxypyrimidine, 2-hydroxy-1-methyl-6-methyl-4(1H)pyrimidine and 1,3-dimethyl-2-nitrobenzene. While, the compounds produced from the biodegradation of nemacur (fenamiphos) pesticide are fenamiphos phenol, fenamiphos sulfone and fenamiphos sulfoxide phenol.

INTRODUCTION

Organophosphorus pesticides are widely used in several countries in the world. They are relatively long-lived, their activity in soil lasting for several years (Franzmann *et al.*, 2000). Organophosphorus pesticides are more toxic to mammals than organochlorine one. In addition, organophosphorus pesticides such as diazinon and nemacur have been reported to be associated with chronic neurobehavioral effects. It is worthily to mention

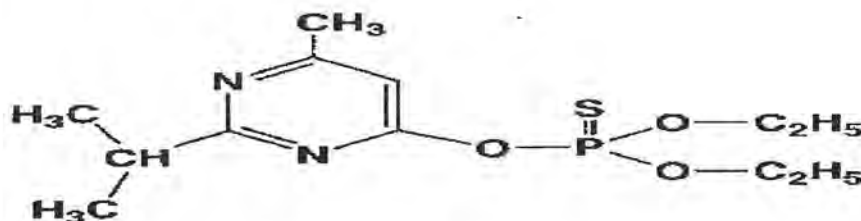
that after repeated application of pesticides, soil microorganisms become capable of detoxify the pesticides. Some soil microorganisms become adapted to use the pesticides as carbon and nitrogen sources . It is well known that the pesticides application to the soil affect on the activity of soil microorganisms. **Gerber *et al.* (1991)** reported that dehydrogenase activity is a useful indicator for overall microbial activity of soil. They also reported that dehydrogenase activity decreased with pesticides application. Also, **Hensley (1991)** and **Yueh & Hensley (1993)** found that diazinon application decreased the N₂-ase activity of soybean nodules. Microbial degradation appears to be the major pathway for the degradation of pesticides in soil. **Smelt *et al.* (1996)** and **Aislabie & Lloyed-Jones (1997)** reported that pesticides are readily degraded by diverse group of bacteria including species of *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium* and *Streptomyces* which are able to metabolize of pesticides. This research aimed to select an microbial strains from soil of Egypt capable of degrading organophosphorus pesticides to be used as an inoculants to remove the residues of pesticides from the pollutant soil and protect the environment from pollution.

MATERIALS AND METHODS

The pesticides used

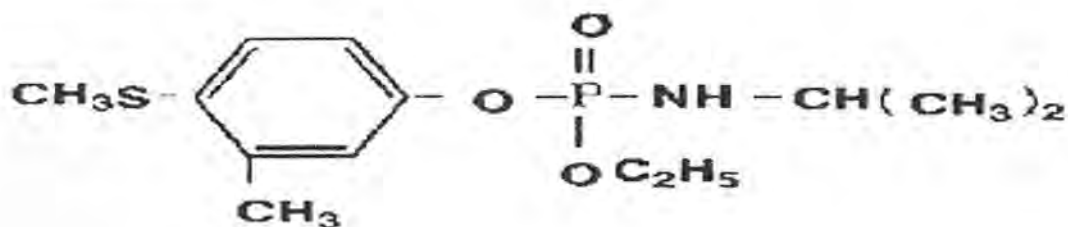
Two organophosphorus pesticides which are widely used in controlling many pests in Egyptian agricultural farms were chosen. These pesticides are:

Diazinon (Dimpylate). 0,0-Diethyl-0-(2-isopropyl-4-methyl-6-pyrimidiny) phosphorothioate.



Diazinon

Nemacur (Fenamiphos). (1-methylethyl)-ethyl-3-methyl-4-(methylthio)phenyl phosphoramidate.



NEMACUR

Soil treatment with diazinon

Soil samples were put into a 500 ml glass beaker and treated with freshly prepared solution of diazinon to give a final concentration of 50 ppm. The soil was again treated with 50, 100, 200 and 400 ppm diazinon after 15, 30, 60 and 75 days respectively. During incubation period, changes of microbial populations and CO₂ evolution as a result of diazinon treatment have been studied.

Enrichment technique for isolation of diazinon degrading microorganisms

Enrichment technique was carried out to isolate microorganisms which were highly efficient in the degradation of diazinon insecticide (Cappuccino & Sherman, 1992).

After 75 days of diazinon addition, sterile basal mineral salts medium amended with 50 mg a.i. (active ingredient) diazinon as a sole source of carbon was inoculated with 5 g of diazinon treated soil. After 10 days of incubation, 1 ml from liquid culture was plated in mineral salt agar medium, incubated at 30°C for 7 days. Single colonies were picked up and purified by streaking plate method.

Screening and tolerance of isolates to pesticides

All bacterial and actinomycetes purified isolates were tested for their ability to grow in presence of diazinon or nemacur pesticides on nutrient broth and starch nitrate media, respectively. Five concentrations namely (0, 100, 500, 1000 and 5000 ppm) were applied.

Inoculated flasks were incubated at 30°C for 4 and 10 days for bacterial and actinomycetes isolates, respectively. The biomass of isolates which tolerated the toxicity

of pesticides was recorded as mg biomass / 50 ml medium. The most tolerant isolates were used for further experiments.

Identification of isolates

Three isolates which confirmed their abilities to degrade and/or utilize diazinon and nemacur as a sole source of carbon and nitrogen were identified . The purified isolates were subjected to detail morphological and physiological studies according to **Bergey's Manual of Determinative Bacteriology (2001)**.

Biodegradation of diazinon and nemacur pesticides by efficient strains in liquid culture

Streptomyces aureofaciens, *Bacillus polymyxa* and *Pseudomonas fluorescens* strains which showed higher tolerance to pesticides were tested for their ability to degrade of pesticides in vitro. Three treatments were used namely untreated medium with pesticides, medium supplemented with pesticides (500 ppm) without inoculation and medium supplemented with pesticides (500 ppm) and inoculation . Samples were periodically investigated at intervals of 0, 3, 7, 15, 21 and 30 days for the following assessments:

- 1- Medium pH, phosphatase activity and microbial biomass.
- 2- Gas liquid chromatography analysis to determine diazinon and nemacur residues.
- 3-GC/Mass to identify diazinon and nemacur metabolites.

Microbiological procedures

Total bacterial counts, counts of Actinomycetes and counts of fungi were determined according to **Labeda (1990)**, **Waksman & Lechevolier (1961)** and **Martin (1950)** respectively. CO₂ evolution was estimated using the method of **Maswadeh (1976)**. While , phosphatase activity was determined according to the method described by **Drobnikova (1961)** .

Extraction of diazinon, nemacur and their metabolites

Diazinon and its metabolites were extracted from liquid culture according to the method described by **Lichtenstein et al (1967)**. Whereas, nemacur and its metabolites

were extracted from liquid culture according to the method described by **Atmakuru & Muthukrishnan (1999)**.

Identification of diazinon, nematicur and their metabolites.

Diazinon, nematicur and their metabolites were determined by GLC and Gas/Mass Spectrometer.

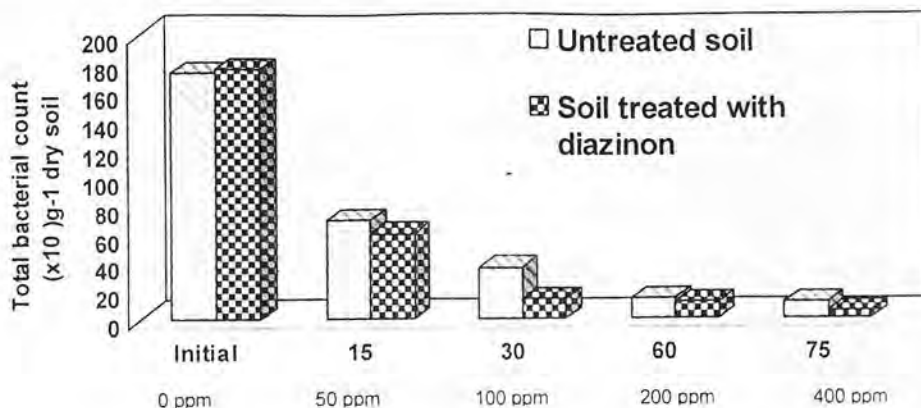
RESULTS AND DISCUSSION

Isolation of degrading diazinon microorganisms from soil

Data illustrated by (**Figs 1&2**) indicated that the densities of different microbial groups showed lower counts in diazinon treated soil than untreated one. Data clearly showed that the increasing of diazinon additions enhanced observed selection of resistant isolates since, the counts sharply decreased all over the experimental periods and specially after 75 days of incubation and 400 ppm diazinon concentration. On the contrary, CO₂ evolution slightly increased after 15 days in the untreated soil samples and then decreased thereafter. While, the treated soil showed slight decrease at first then the decline was sharp thereafter.

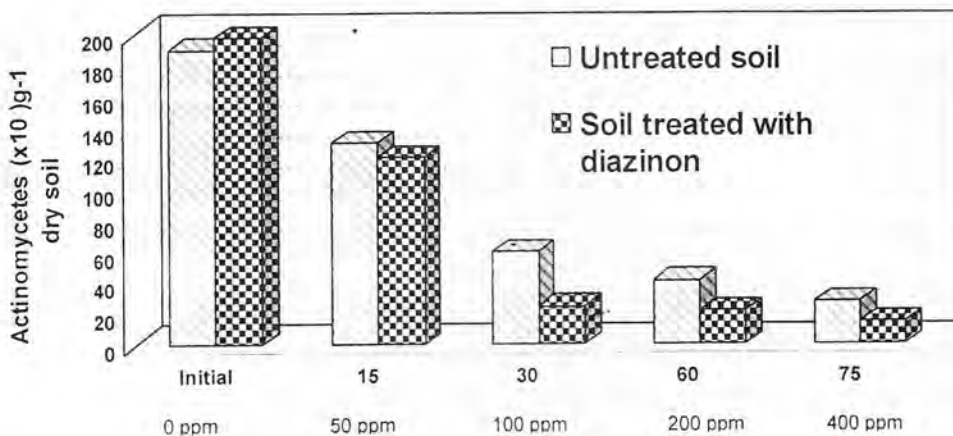
The soil treated with increasingly concentrations of diazinon for 75 days was used to obtain resistant isolates for approaching experiment.

Only five isolates were able to grow and resist the toxicity of diazinon in soil. Three of them were belonging to bacteria and the two others were belonging to actinomycetes. These isolates were purified on their specific media and kept for screening process.



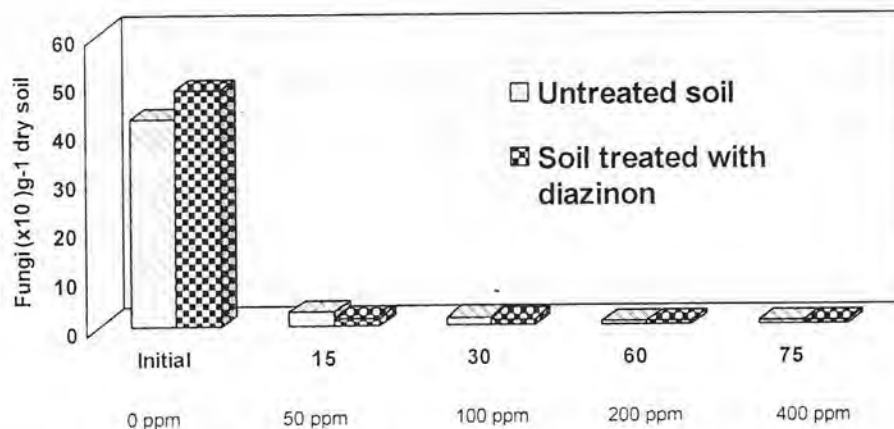
(A)

Incubation period (days) and concentrations of diazinon addition.



(B)

Incubation period (days) and concentrations of diazinon addition.



(C)

Incubation period (days) and concentrations of diazinon addition.

Fig.1. Microbial changes in diazinon treated soil during incubation period.
 A: Total bacterial count B: Total actinomycetes count C: Total fungal count

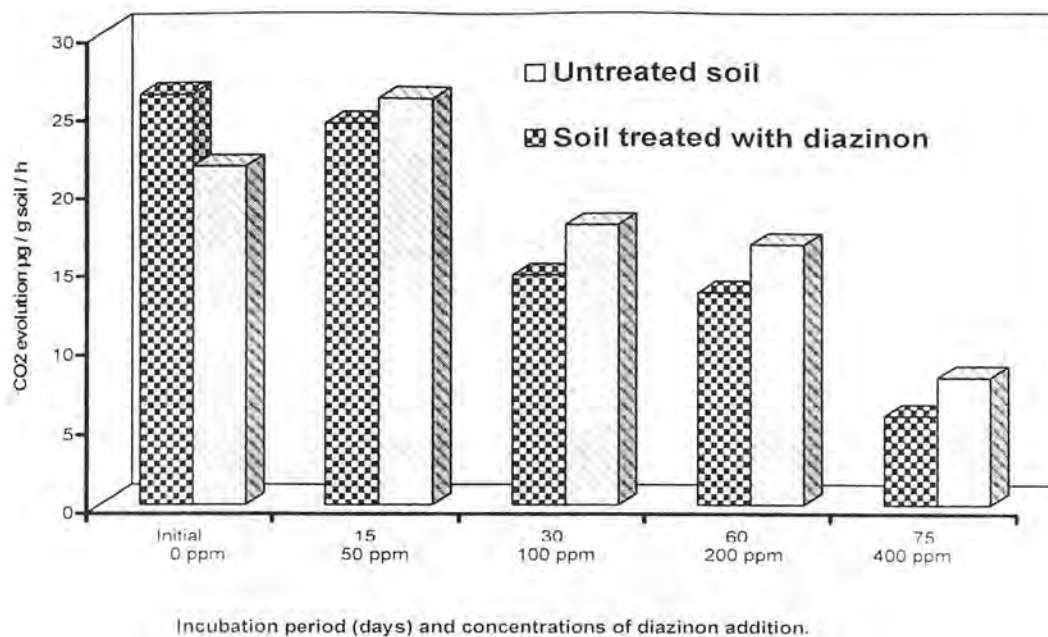


Fig.2. Changes in CO₂ evolution in diazinon treated soil during incubation period

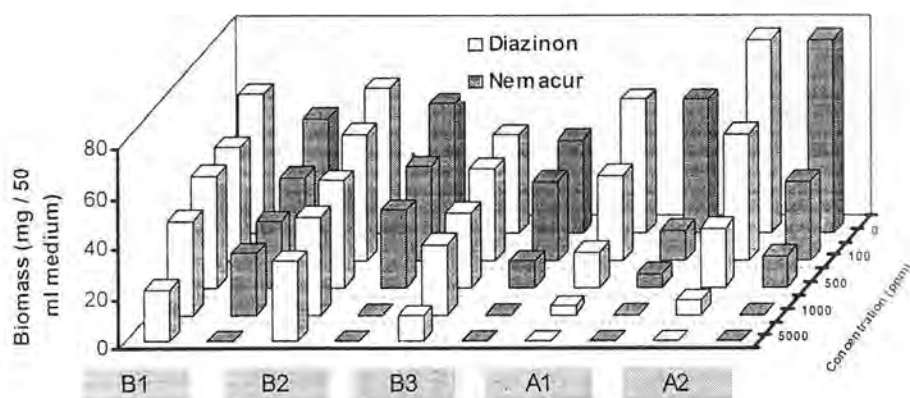
Screening of resistant isolates for tolerance of pesticides

All bacterial and actinomycetes purified isolates were tested for their ability to grow in presence of diazinon and nemacur on nutrient broth (for bacterial isolates) and starch nitrate broth media (for actinomycetes isolates), respectively. Four concentrations namely 100, 500, 1000 and 5000 ppm were applied with control.

Biomass of bacterial and actinomycetes growth were recorded at the end of the experiment. Obtained data illustrated by Fig (3). Results showed that all tested isolates normally grew in the control liquid media. But, the growth of different isolates was decreased with the increasing of pesticides concentration.

Increasing the concentration more than 500 ppm was accompanied by inhibitive effect and the biomass of growth became scanty. When the concentration of pesticides reached 5000 ppm, more inhibitive effect on the growth of some isolates was observed whereas, the other isolates did not grow.

Generally, data illustrated by **Fig (3)** showed that nemacur pesticide has more inhibitive effect than diazinon at different concentrations. These results are in harmony with **Ingham & Coleman (1984)** and **Kaul *et al.* (1986)** who found that diazinon reduced microbial populations whereas; the microbial activity was adversely affected by nemacur application.



Bacterial isolates

Fig3. Biomass (mg / 50 ml medium) of isolates under different concentrations of diazinon and nemacur pesticides

Data in **(Fig 3)** also revealed that the bacterial isolates (B1 and B2) and the actinomycetes isolate (A2) were more potent in growth on liquid media at different concentrations of diazinon and nemacur pesticides.

Therefore, these three isolates were chosen and identified. These isolates were used in further studies on biodegradation of diazinon and nemacur pesticides either in liquid culture or in soil.

Identification of the isolates

Three selected isolates capable of degrading diazinon and nematic pesticides were purified and subjected to detail morphological and physiological studies according to **Bergey's Manual of Determinative Bacteriology (2001)**.

Selected isolates were identified as *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*.

Biodegradation of diazinon and nematic pesticides by efficient microorganisms in liquid culture

Identified microorganisms which showed higher tolerance of diazinon and nematic pesticides were tested for their abilities to degrade these pesticides *in vitro*. Samples were investigated at zero, 3, 7, 15, 21 and 30 days from inoculation to determine the following:

- 1- pH values, phosphatase activity and microbial biomass.
- 2- Persistent amount of pesticides and their metabolites.

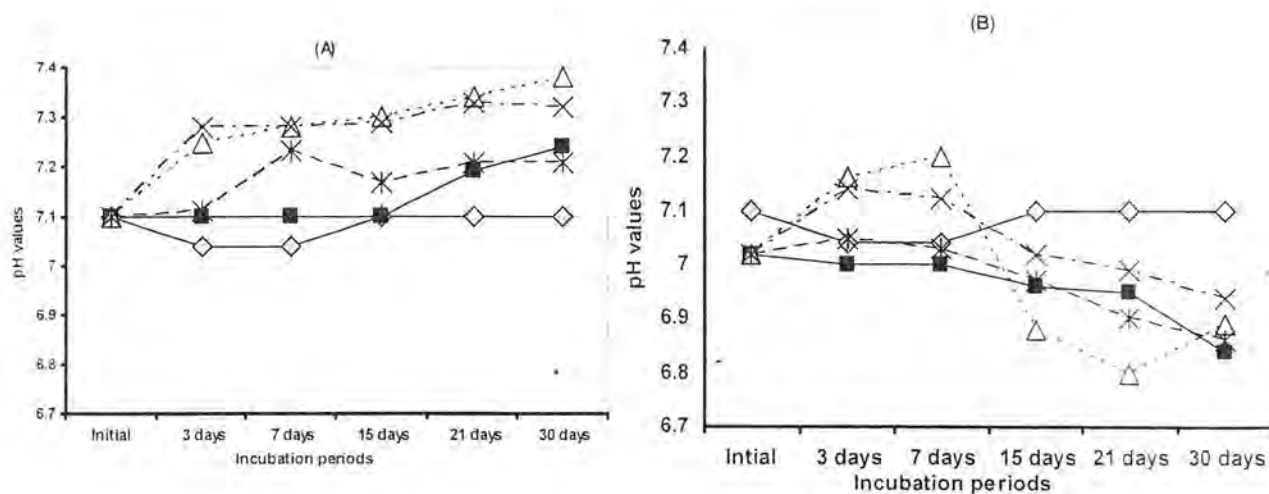
Periodical changes in pH values

Throughout the experiment of diazinon and nematic persistence in liquid cultures of *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, pH values were determined whether in uninoculated or inoculated media at each time of sampling for diazinon and nematic persistence determination. Data in **(Fig 4)** showed that pH values in uninoculated media ranged from 7.1 to 7.24 and from 6.80 to 7.02 for diazinon and nematic, respectively.

Data in **(Fig 4 a)** also showed that the pH values whether in uninoculated or inoculated media were still in the range of neutrality throughout the experiment and not affect the stability of pesticides.

As well, obtained results indicated that the changes in pH values of uninoculated media were very little so far it has no effect on the persistence of pesticides. While, in the media amended with diazinon and inoculated with various tested microorganisms, pH values slightly tended to alkaline state. This may be due to diazinon hydrolysis by microorganisms.

On the contrary, media amended with nemacur and inoculated with tested microorganisms results revealed that pH values slightly tended to acidic state. This result indicates that nemacur metabolites are low acidic compounds



A: diazinon

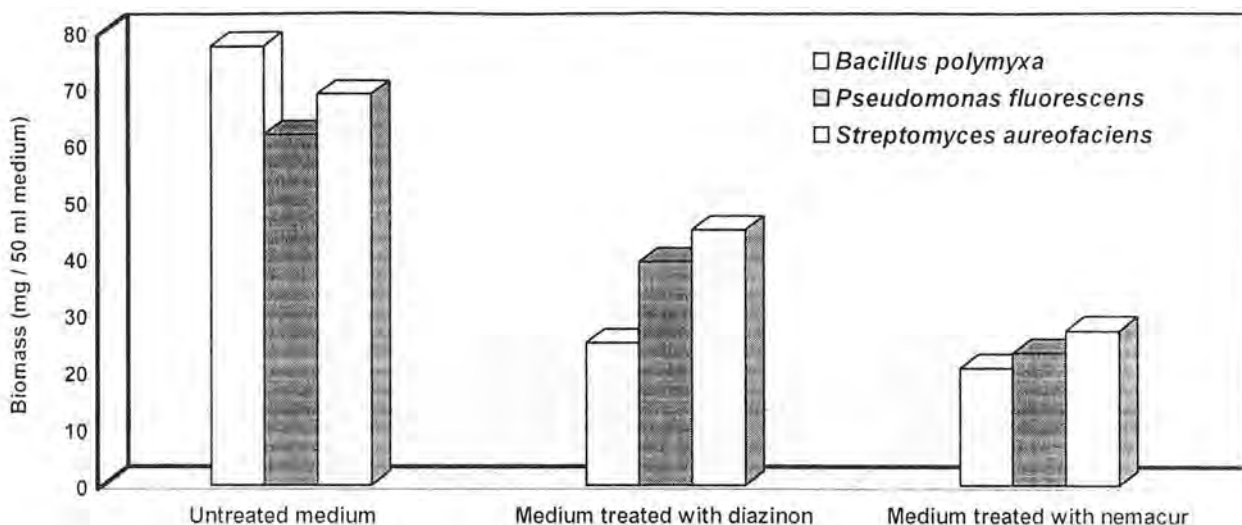
B: nemacur

Fig 4 a. Periodical changes in pH value during incubation periods

Biomass of investigated microorganisms in mineral salt medium

Obtained results (Fig 4b) showed that the biomass of different microorganisms was higher when the microorganisms were grown in untreated medium with either diazinon or nemacur. *Streptomyces aureofaciens* recorded the highest biomass in untreated medium followed by *Bacillus polymyxa* then *Pseudomonas fluorescens*. *Bacillus polymyxa* gave the highest biomass values in treated medium with pesticides. This result was observed with the two pesticides. While, the lowest values of biomass were recorded in case of *Streptomyces aureofaciens*. It is clear that the pesticides affected the proliferation of *Streptomyces aureofaciens* more than *Bacillus polymyxa* since the growth in control was higher in *Streptomyces aureofaciens* than *Bacillus polymyxa*.

Generally, the biomass values of all microorganisms were lower with nemacur application compared to diazinon one.



Biomass produced (mg / 50 ml medium) in inoculated mineral salt medium amended with either diazinon or nemacur pesticides.

Periodical changes in phosphatase activity

Data in Fig (5) showed that the phosphatase activity exhibited some fluctuations in various treatments. Either untreated or treated media with pesticides showed lower values of phosphatase activity compared to the media that treated with pesticides and inoculated with microorganisms under study. Results also showed that phosphatase activity in diazinon treated medium were increased when compared to nemacur applied treatments. This trend of results was observed at all determination periods. The highest records of phosphatase activity during incubation period were observed in case of diazinon treated medium inoculated with *Bacillus polymyxa*. Except control treatments, the lowest records of phosphatase activity during incubation periods were observed in case of nemacur treated medium inoculated with *Streptomyces aureofaciens*.

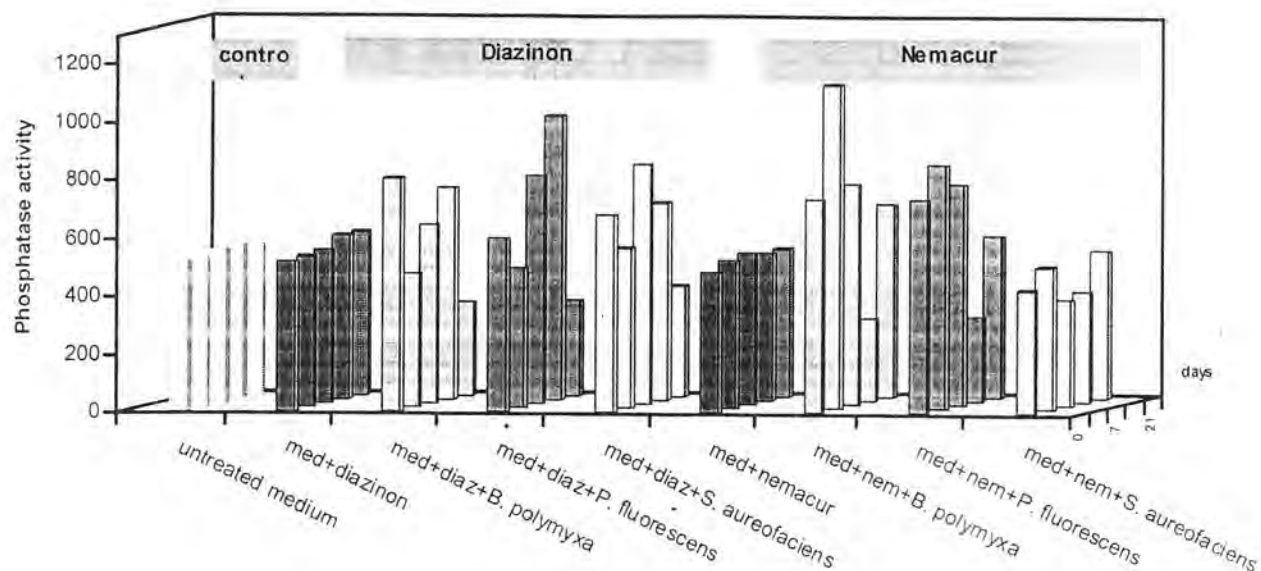


Fig.5. The Phosphatase activity (by µg inorganic phosphate / ml / 24 hrs enzyme activity on nucleic acid) changes during isolates incubation with diazinon and nemacur in liquid culture.

These results emphasized the ability of tested microorganisms to produce considerable quantities of phosphatase enzyme. The adaptive enzyme clearly had the ability to degrade the pesticide as will be shown in further studies.

Persistence rate of diazinon and nemacur in liquid culture

Data presented in **Table (1)** showed the diazinon and nemacur persistence detected as percent amount of initial concentration (500 ppm). Obtained data showed that diazinon and nemacur amounts decreased with elapsed time in both inoculated and uninoculated media. The rate of decrease in inoculated medium was faster than that in uninoculated one since, 22.71, 63.82, 62.60 and 47.51 % of the added diazinon disappeared from the uninoculated, inoculated media with *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively throughout the first 15 days of diazinon addition. Whereas, the disappearance rates of nemacur at 15 days of incubation period were 4.8, 28.04, 28.30 and 12.11 % for uninoculated, inoculated media with *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively.

This result is in agreement with those obtained by **Aislabie & Lloyd-jones (1997)** who reported that pesticide fate in the environment is affected by microbial activity. Some pesticides are readily degraded by microorganisms, other have proven to be recalcitrant. **Sharom et al. (1980)**; **Chapman & Cole (1982)**; **Schoen & Winterlin (1987)**; **Frank et al. (1991)**; **Ferrando et al. (1992)** and **Scheunert et al. (1993)** reported that diazinon has a relatively short half-life in soil, ranging from 70 hours to 12 weeks depending on pH, temperature and the presence of microorganisms. **El-Sebae (1985)** mentioned that organophosphorus pesticides (such as nematicur) the residual life is longer.

At the end of the experiment (30 days) obtained results showed that only 71.25, 23.79, 26.04, and 50.35 % of the added diazinon were detected in uninoculated, inoculated media with *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively. While, the detectable amounts of nematicur at the end of the experiment were 84.28, 43.95, 50.32 and 56.90 % for uninoculated, inoculated media with *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively.

From the obtained results, it could be concluded that the diazinon pesticide is easily decomposed rather than nematicur pesticide. Since the persistent amount at the end of the experiment was higher in case of nematicur pesticide rather than diazinon one. Obtained data also revealed that *Bacillus polymyxa* is able to decompose the two pesticides with higher degree rather than those occurred with *Pseudomonas fluorescens* and *Streptomyces aureofaciens*. Since, only 23.79, 26.04 and 50.35 % of the added amounts were detected as diazinon in the cultures of *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively at the end of the experiment. Whereas, the detectable amounts of nematicur at the end of the experiment were 43.95, 50.32 and 56.90 % for *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*, respectively.

This result is in harmony with those obtained by **Keller (1981)** who reported that diazinon (97% pure) applied at 10 ppm rapidly degraded to 2-isopropyl-4-methyl-6-hydroxypyrimidine (IMHP) with a half-life of less than one month. Within 14 days, only 12.3% of the activity was found as the parent; 72.9% was identified as IMHP. **Megharaj**

et al. (2003) found that a bacterium identified as *Brevibacterium sp* MM1, is able to readily hydrolyse nemacur, widely used organophosphorus insecticide and its toxic oxides (nemacur sulfoxide, nemacur sulfon), which all contain a common P-O-C bond, in mineral salts medium.

Table (1). Persistence rate of diazinon and nemacur in uninoculated and inoculated liquid culture.

Incubation periods (days)	Recovery of pesticides (%)							
	Uninoculated medium		Medium inoculated with					
			<i>Bacillus polymyxa</i>		<i>Pseudomonas fluorescens</i>		<i>Streptomyces aureofaciens</i>	
	Diazinon	Nemacur	Diazinon	Nemacur	Diazinon	Nemacur	Diazinon	Nemacur
Initial	100	100	100	100	100	100	100	100
3	84.11	99.81	59.51	90.18	58.03	93.87	64.6	99.17
7	78.88	98.27	39.45	76.32	57.01	72.81	54.19	91.79
15	77.29	95.20	36.18	71.91	37.40	71.70	52.49	87.89
21	76.36	93.09	34.15	55.41	27.64	60.96	51.48	67.71
30	71.25	84.28	23.79	43.95	26.04	50.32	50.35	56.90

Initial concentration = 500 ppm

Metabolism of diazinon by microbial strains in liquid culture

Chromatographic analysis of diazinon and its metabolites in the liquid culture extracts of different investigated microorganisms in current study was carried out periodically throughout 30 days of incubation initially and at 3, 7, 15, 21 and 30 days after culture providing with 500 ppm of diazinon. The obtained results are presented in **Table (2)** and illustrated by (**Fig 6 a ,b**).

Gas mass spectrometer was used to determine the values of M/e for each compound to be as reference to use these values for the identification of the compounds produced

Table (2). Identification of diazinon and its metabolites in the liquid cultures of isolated strains by gas liquid chromatography (GLC).

Compounds	Retention time (Rt min.)					
	Days					
	Initial	3	7	15	21	30
<i>Bacillus polymyxa</i>						
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
Metabolites 1	-	-	1.3	1.3	1.3	1.3
2	-	1.7	1.7	1.7	1.7	1.7
3	-	1.8	1.8	1.8	1.8	1.8
4	-	-	1.9	1.9	1.9	1.9
5	-	4.3	-	-	-	-
<i>Pseudomonas fluorescens</i>						
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
Metabolites 1	-	-	-	1.3	1.3	1.3
2	-	-	1.7	1.7	-	-
3	-	-	-	1.8	-	-
4	-	1.9	1.9	1.9	1.9	1.9
5	-	-	4.3	4.3	-	-
<i>Streptomyces aureofaciens</i>						
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
Metabolites 1	-	1.3	1.3	1.3	1.3	1.3
2	-	1.7	1.7	1.7	1.7	1.7
3	-	1.8	-	1.8	-	-
4	-	1.9	1.9	1.9	1.9	1.9
5	-	4.3	-	-	-	-

from the biodegradation of diazinon by investigated microorganisms. The limited data are presented in (Fig 7).

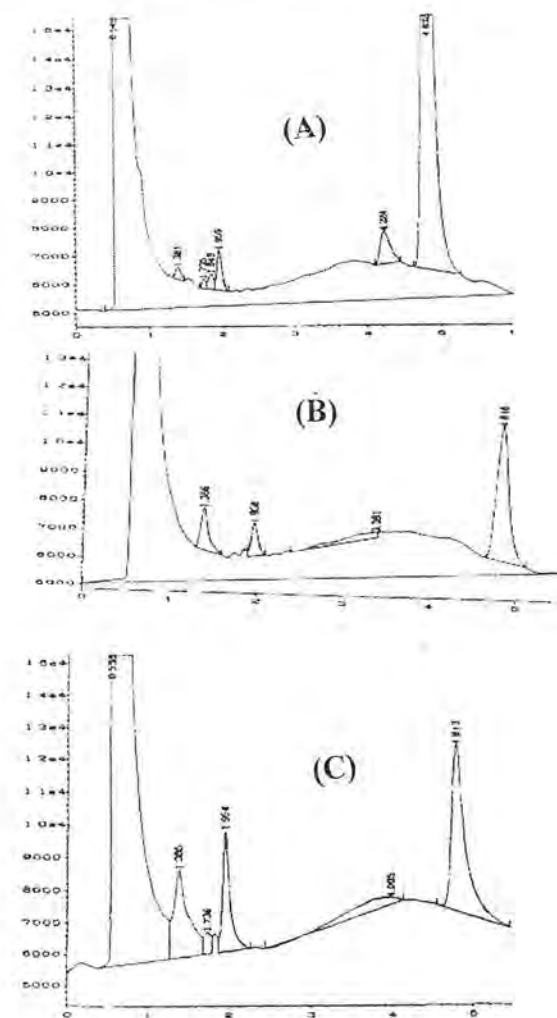
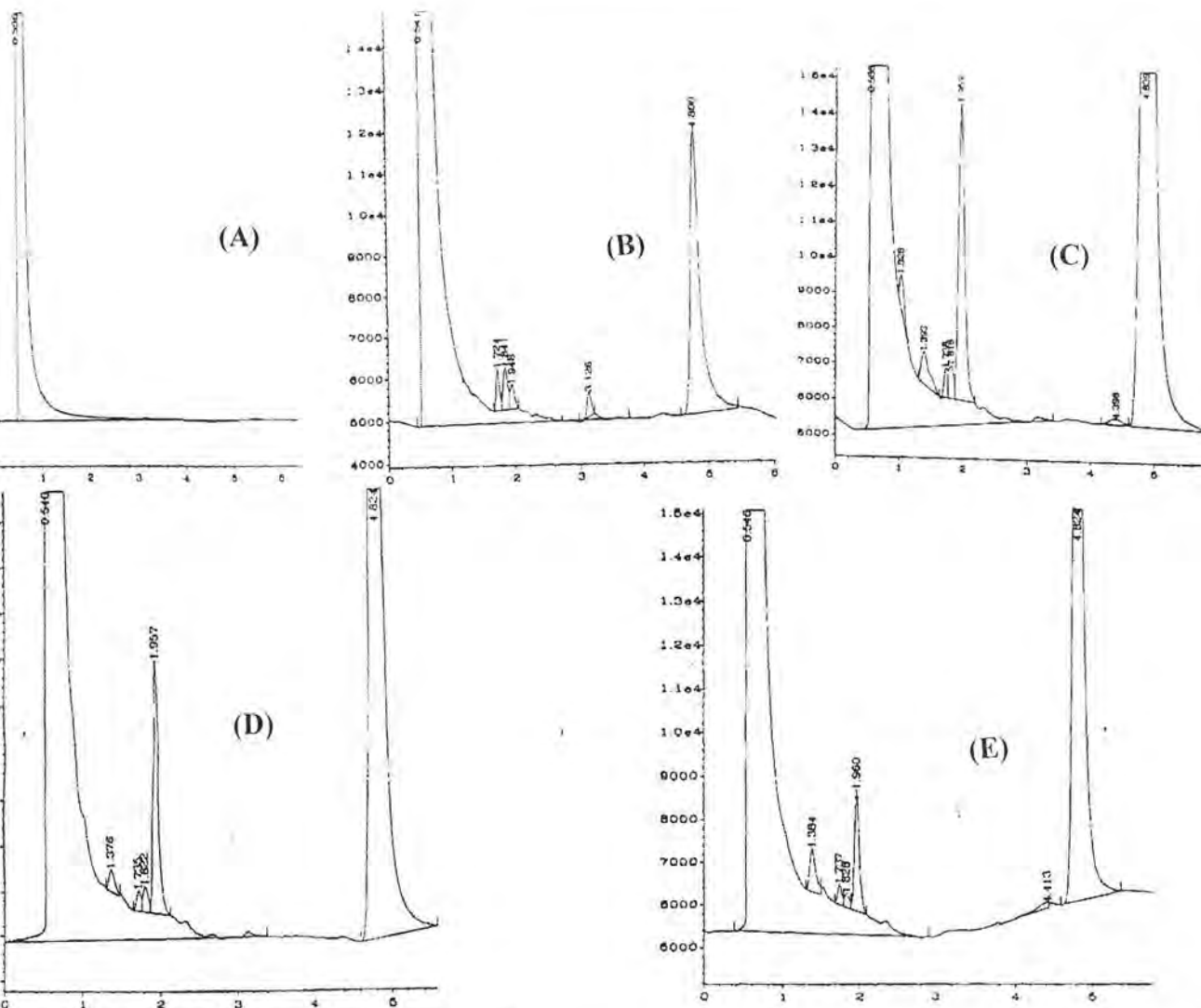


Fig 6a . Gas liquid chromatography spectra of:
 A) Diazinon free liquid culture extract.
 B) Liquid medium + diazinon extract.
 C) Liquid culture extract of *P. fluorescens* after 7 days of treatment with diazinon.
 D) Liquid culture extract of *B. polymyxa* after 7 days of treatment with diazinon.
 E) Liquid culture extract of *S. aureofaciens* after 7 days of treatment with diazinon.

Fig 6b . Gas liquid chromatography spectra of:
 A) Liquid culture extract of *B. polymyxa* after 15 days of treatment with diazinon
 B) Liquid culture extract of *P. fluorescens* after 15 days of treatment with diazinon
 C) Liquid culture extract of *S. aureofaciens* after 15 days of treatment with diazinon

Data revealed that the tested microorganisms began to degrade diazinon during the first three days of incubation. At third day, the extract of *Streptomyces aureofaciens* culture showed five metabolites having Rt 1.3, 1.7, 1.8, 1.9 and 4.3 minutes. Whereas, the extract of *Pseudomonas fluorescens* showed only one metabolite having Rt 1.9 minutes.

Generally, from data presented in **Table (2)** and illustrated by **(Fig 6 a ,b)** it could be concluded that the investigated strains in the current study are able to metabolize or degrade the diazinon to five compounds in their liquid cultures. These metabolites having Rt 1.3, 1.7, 1.8, 1.9 and 4.3 minutes.

All the metabolites could not be identified by GLC analysis because their authentic materials (active ingredients) are not available. But by the Gas/Mass analysis spectrometer, it could be noticed that the metabolites produced from the biodegradation of diazinon by investigated microorganisms showed (M/e) values equivalent with the molecular weights of diazinon and its dominant metabolites which were noted and identified by **Robert et al. (2000)** in their study. They reported that diazinon pesticide metabolized by microorganisms to diazoxone, diethylphosphate, HMMP, IMHP and 1,3-dimethyl-2-nitrobenzene. These compounds were obtained from Gas/Mass analysis on the cultures extract of *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens* and illustrated in **(Fig 7)**.

Therefore, the compounds produced from the biodegradation by various investigated microorganisms in this study are likely be diazoxon, diethylphosphate, HMMP, IMHP and 1,3-dimethyl-2-nitrobenzene.

The identification deduction for these metabolites was based on the relation between retention time obtained from GLC analysis and the values of (M/e) obtained from Gas/Mass analysis which is equivalent with molecular weights of diazinon and its metabolites

The chromatographic analysis of the extract of *Bacillus polymyxa* culture showed two metabolites having Rt 1.7 and 1.8 minutes which appeared at the third day of incubation and still detected to the end of the experiment. These metabolites are likely be

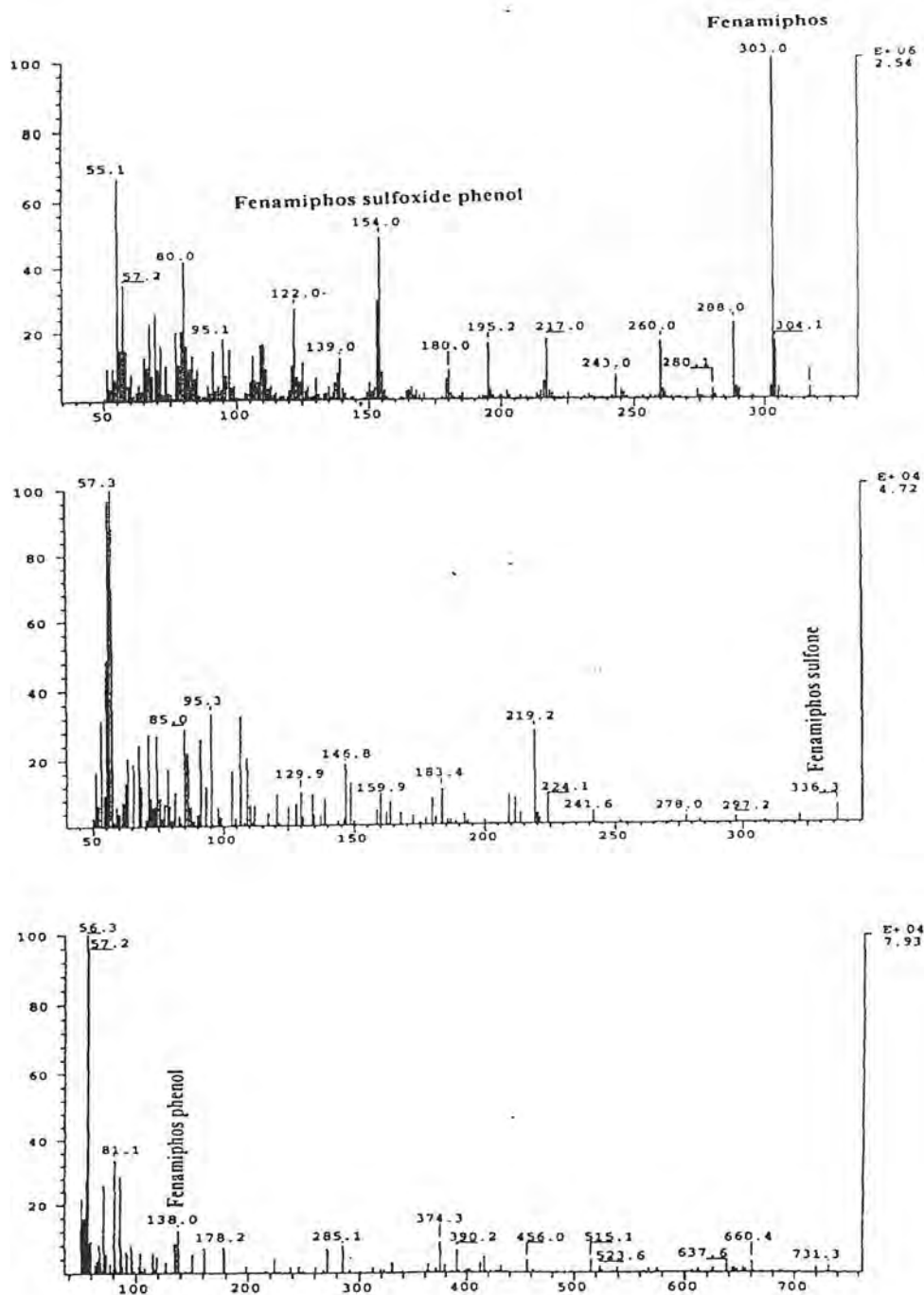


Fig 9. Mass spectrum of nemacur and its metabolites in liquid culture.

2-isopropyl-6-methyl-4-hydroxypyrimidine (IMHP) and diethylphosphate, respectively according to the values of (M/e) obtained from Gas/Mass analysis (Fig 7).

GLC analysis of *Pseudomonas fluorescens* extract showed only one metabolite having Rt 1.9 minutes which appeared at the third day of incubation and still detected up to the end of the experiment, this metabolite may be 2-hydroxy-1-methyl-6-methyl-4-hydroxy pyrimidine (HMMP) according to the values of (M/e) obtained from Gas/Mass analysis.

Extract of *Streptomyces aureofaciens* showed three metabolites having Rt 1.3, 1.7 and 1.9 minutes which appeared at the third day of incubation and still detected up to the end of the experiment (30 days), these metabolites are likely be 1,3-dimethyl-2-nitrobenzene, HMMP and IMHP, respectively. From data presented in Table (2) it could be noticed that *Bacillus polymyxa* is more efficient in diazinon degradation. Since, it produced metabolites more than both *Pseudomonas fluorescens* and *Streptomyces aureofaciens*.

These results are in accordance with Keller (1981) and Allender & Britt (1994) who reported that diazinon application at 10 ppm rapidly degraded to 2-isopropyl-6-methyl-4-hydroxypyrimidine (IMHP) with a half life of less than one month. Within 14 days, only 12.3 % of the activity was found as the parent, 72.9 % was identified as IMHP.

Diazinon pesticide degraded by abiotic and biotic processes, so that the parent compound is not persistent. Microbial degradation appears to be the major pathway for the degradation (EPA, 1990). The main compounds produced from the biodegradation of diazinon are IMHP, diazoxone and diethylphosphate (Frank *et al.*, 1991; Ferrando *et al.*, 1992; Scheunert *et al.*, 1993 and Seyfried, 1994).

In other studies on the degradation of diazinon, identification of the metabolites showed the IMHP is a major degradation product when low concentration of diazinon was applied in compost (Sumner *et al.*, 1987), soil (Michel *et al.*, 1997) and water (Ku *et al.*, 1998). Although IMHP is found to be potentially leachable, it is less toxic than diazinon (Sumner *et al.*, 1987).

In another study concluded by Li *et al.* (2002) obtained results on the biodegradation of diazinon showed that the diazinon degraded to 1,3-dimethyl-2-nitrobenzene.

Metabolism of nematicur by microbial strains in liquid culture

Chromatographic analysis of nematicur and its metabolites in the liquid culture extracts of different investigated microorganisms was carried out periodically. Determinations intervals were initially and at 3, 7, 15, 21 and 30 days after culture providing with 500 ppm of nematicur. The obtained data are presented in **Table (3)** and illustrated by **Fig (8 a,b)**. Gas mass spectrometer was used to determine the values of M/e for each compound to be as references to use these values for identification the produced compounds from the biodegradation of nematicur by investigated microorganisms. The limited data are presented in **Fig (9)**. Data in **Table (3)** emphasize that the tested microorganism began to degrade nematicur during the first three days of incubation. At third day, the extract of *Bacillus polymyxa* culture showed three metabolites having Rt 1.19, 1.89 and 5.12 minutes. Whereas the extract of *Pseudomonas fluorescens* and *Streptomyces aureofaciens* cultures showed only one metabolite for each having Rt 1.19 and 1.89 minutes, respectively.

The chromatographic analysis of the extract of *Bacillus polymyxa* culture extract showed one metabolite having Rt 1.9 minute which appeared at the third day of incubation and still detected until the end of the experiment. Also, the same culture extract showed one metabolite having Rt 5.12 minute which appeared at the third day and still detected up to 15 day of incubation period. Meanwhile, GLC analysis extract of *Pseudomonas fluorescens* culture also showed one metabolite having Rt 1.19 minute which appeared at the third day of incubation and still detected till the end of the experiment. Extract of *Streptomyces aureofaciens* culture also showed only one metabolite having Rt 1.89 minute which appeared at the third day of incubation and still detected till the end of the experiment. Nematicur was also detected with small amounts having Rt 2.65 minutes in all analyzed sample.

Table (3). Identification of nemacur and its metabolites in the liquid cultures of isolated strains by gas liquid chromatography (GLC).

Compounds		Retention time (Rt min.)					
		Days					
		Initial	3	7	15	21	30
<i>Bacillus polymyxa</i>							
Nemacur		2.65	2.65	2.65	2.65	2.65	2.65
Metabolites	1	-	1.19	1.19	1.19	1.19	1.19
	2	-	1.89	-	-	-	1.89
	3	-	5.12	5.12	5.12	-	-
<i>Pseudomonas fluorescens</i>							
Nemacur		2.65	2.65	2.65	2.65	2.65	2.65
Metabolites	1	-	1.19	1.19	1.19	1.19	1.19
	2	-	-	1.89	-	1.89	1.89
	3	-	-	-	-	-	-
<i>Streptomyces aureofaciens</i>							
Nemacur		2.65	2.65	2.65	2.65	2.65	2.65
Metabolites	1	-	-	-	-	-	-
	2	-	1.89	1.89	1.89	1.89	1.89
	3	-	-	-	-	-	-

From data presented in **Table (3)** it is worthily to notice that *Bacillus polymyxa* is more potent in nemacur degradation. Since, it produced many metabolites more than either *Pseudomonas fluorescens* or *Streptomyces aureofaciens*

Generally, from the obtained data, it could be concluded that the investigated strains in the current study are able to metabolize or degrade nemacur to three metabolites. These metabolites having Rt 1.19, 1.89 and 5.12 minutes. Metabolites produced from nemacur decomposition can't be identified by GLC analysis because their authentic materials are not available.

But from the Gas/Mass analysis which achieved in this study, it can be noticed that the metabolites produced from the biodegradation of nemacur by investigated

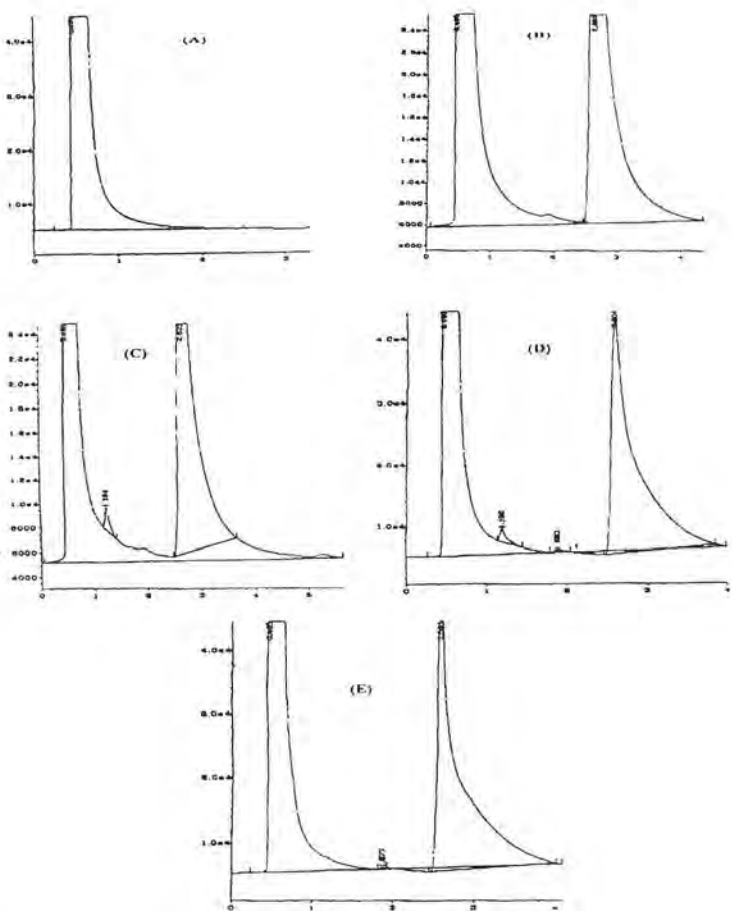


Fig 8a . Gas liquid chromatography spectra of:

- A) Nemacur free liquid culture extract.
- B) Liquid medium + nemacur extract.
- C) Liquid culture extract of *P. fluorescens* after 7 days of treatment with nemacur.
- D) Liquid culture extract of *B. polymyxa* after 7 days of treatment with nemacur.
- E) Liquid culture extract of *S. aureofaciens* after 7 days of treatment with nemacur.

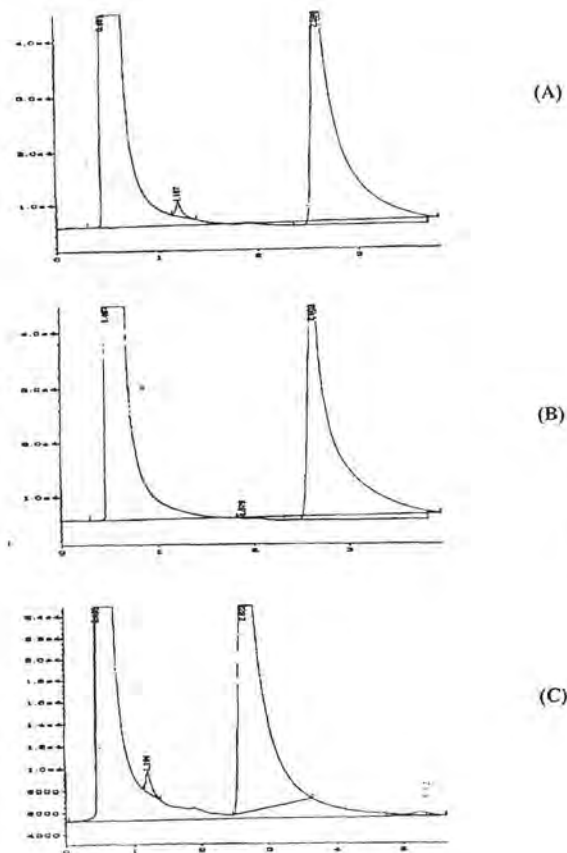


Fig 8b . Gas liquid chromatography spectra of:

- A) Liquid culture extract of *B. polymyxa* after 15 days of treatment with nemacur
- B) Liquid culture extract of *P. fluorescens* after 15 days of treatment with nemacur
- C) Liquid culture extract of *S. aureofaciens* after 15 days of treatment with nemacur

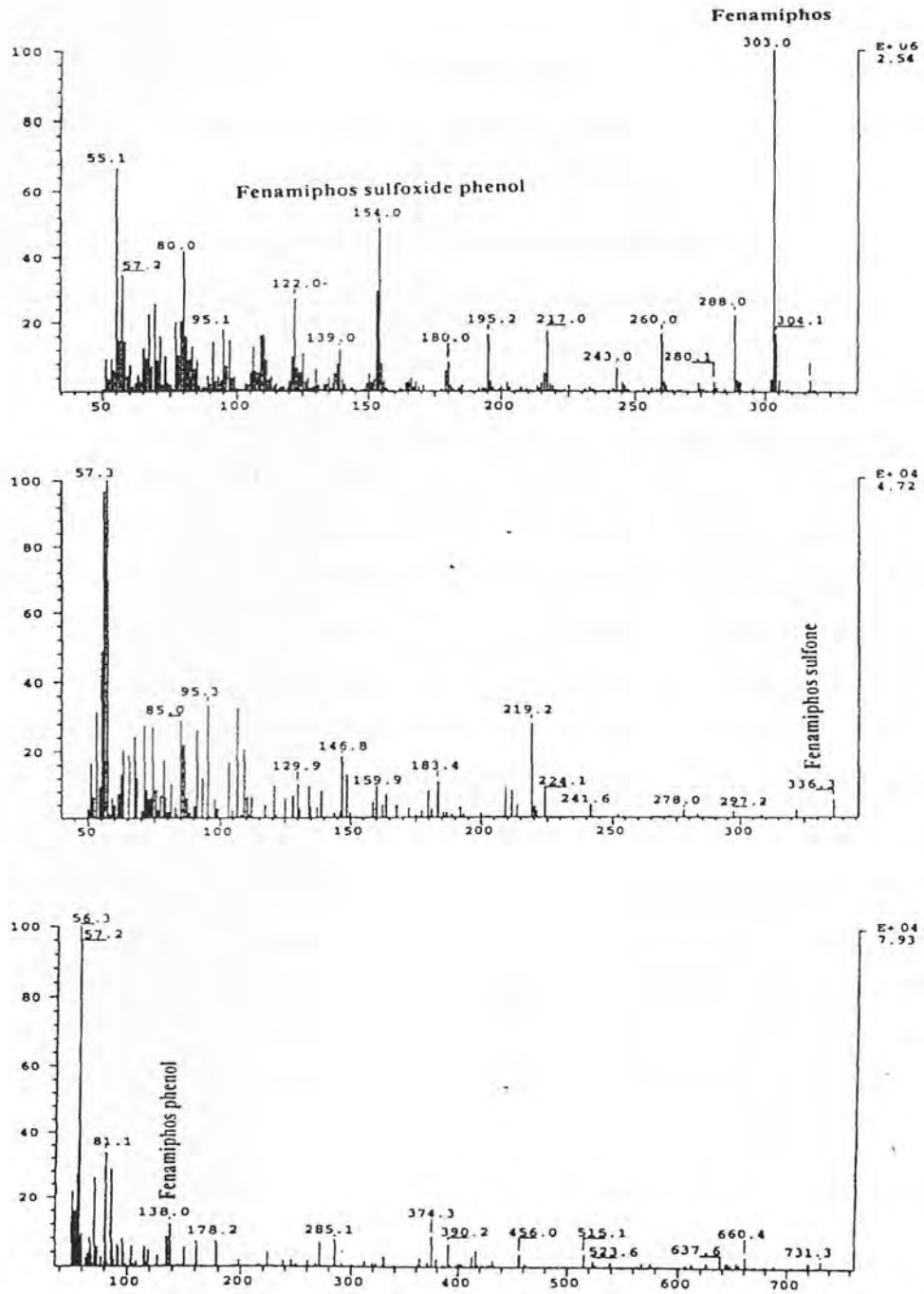


Fig 9. Mass spectrum of nematicur and its metabolites in liquid culture.

microorganisms have molecular weights and (M/e) values equivalent with the molecular weights of nemacur and its dominant metabolites which observed and identified by **Megharaj et al., (2003)** in their study. They mentioned that nemacur (fenamiphos) pesticide metabolized by microorganisms to fenamiphos phenol, fenamiphos sulfone and fenamiphos sulfoxide phenol. These compounds are recorded in Table (3) which obtained from Gas/Mass analysis on the culture extract of *Bacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens* which used in this study.

Therefore, the metabolites produced from the biodegradation of nemacur by various microorganisms are likely being fenamiphos phenol, fenamiphos sulfone and fenamiphos sulfoxide phenol. The identification deduction for abovementioned metabolites was based on the relation between retention time obtained from GLC analysis and molecular weight obtained from Gas/Mass analysis which achieved to confirm the obtained results.

These results are in harmony with **Kookana et al. (1997)** and **Rai et al. (1997)** who found that fenamiphos (Fen) was rapidly oxidized to fenamiphos sulfoxide (Fen SO), further oxidation of Fen SO to sulfone (Fen SO₂). The time taken for 50 % loss of the total residue of fenamiphos was found to be 50 days. Also, **Singh et al. (2002)** noticed that fenamiphos was rapidly converted into fenamiphos sulfoxide which was further oxidized to fenamiphos sulfone. Repeated application was associated with reduced rate of degradation and the half-life of the third application of fenamiphos was 10.6 days. The dissipation of total toxic residues (fenamiphos plus the sulfoxide and sulfone oxidation products) was affected by repeated application. The overall half-life was about 30 days for the first treatment, but there was little change in total toxic residues (TTR) concentration during the 30 days period following the third treatment. Sequential treatment of fenamiphos therefore suppressed the overall rate of change in TTR in this soil which contrasts with previous findings where repeated treatment has resulted in enhanced degradation of fenamiphos.

Megharaj et al. (2003) found that a bacterium identified as *Brevibacterium sp* MM1 readily hydrolyzed nemacur and its toxic oxides (nemacur sulfoxide, nemacur sulfone) which all contain a common P-O-C bond, in mineral salts medium. Interestingly, nemacur phenol, nemacur sulfoxide phenol, nemacur sulfone phenol formed during

bacterial hydrolysis of nematicur and its oxides persisted in the mineral salt medium, but were transitory in soil and groundwater due to their further metabolism by indigenous microorganisms.

Summarily, obtained data showed that diazinon and nematicur amounts decreased with elapsed time in inoculated media with tested microorganisms. Diazinon is easily decomposed rather than nematicur. *Bacillus polymyxa* is able to decompose the two pesticides with higher rate than those occurred with *Pseudomonas fluorescens* and *Streptomyces aureofaciens*. Metabolites produced from diazinon degradation were 2-isopropyl-6-methyl-4-hydroxy pyrimidine (IMHP), 1,3 dimethyl-2-nitrobenzene, diethyl phosphate, diazoxon and 2-(1-hydroxy-1-methyl)-6-methyl-4(1H) pyrimidine (HMMP). While, the metabolites produced from nematicur (fenamiphos) degradation were fenamiphos phenol, fenamiphos sulfone and fenamiphos sulfoxide phenol.

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التحلل الحيوي لبعض مبيدات الفوسفور العضوية بواسطة ميكروبات التربة

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تم إجراء هذا البحث بهدف عزل بعض ميكروبات التربة التي لها القدرة علي تحليل مبيدات الفوسفور العضوية مثل الديازينون والنيماكور. في هذا البحث تم التعرف علي ثلاث عزلات لها القدرة والكفاءة العالية في تحليل المبيدات سابقة الذكر واستخدامها كمصادر للكربون والنيتروجين. أوضحت نتائج التعرف لهذه العزلات أنها

Bacillus polymyxa, *Pseudomonas fluorescens* and *Streptomyces aureofaciens*.
أيضا"، أوضحت نتائج هذا البحث أن كمية المبيدات المضافة إلي بيئة النمو لهذه الميكروبات أخذت في التناقص تدريجيا وأن معدل النقص في هذه المبيدات كان أسرع في البيئات الملقحة بالمقارنة بغير الملقحة. كما أظهرت النتائج أن كمية مبيد الديازينون التي تحللت خلال الخمس عشر يوما الأولى من التجربة هي ٢٢,٧١، ٦٣,٨٢، ٦٢,٦٠ و ٤٧,٥١% من الكمية المضافة في بداية التجربة وذلك في حالة عدم التلقيح ، التلقيح بميكروب *Bacillus polymyxa* ، التلقيح بميكروب *Pseudomonas fluorescens* والتلقيح بميكروب *Streptomyces aureofaciens* علي التوالي. بينما أظهرت النتائج أن كمية مبيد النيماكور التي تحللت خلال الخمس عشر يوما الأولى من التجربة هي ٤,٨، ٢٨,٠٤، ٢٨,٣٠ و ١٢,١١% من الكمية المضافة في بداية التجربة وذلك في حالة عدم التلقيح ، التلقيح بميكروب *Bacillus polymyxa* ، التلقيح بميكروب *Pseudomonas fluorescens* والتلقيح بميكروب *Streptomyces aureofaciens* علي التوالي.

وأظهرت نتائج هذه الدراسة أن المركبات الناتجة من تحلل مبيد الديازينون هي diazoxon, diethylphosphate, 2-isopropyl-4-methyl-6-hydroxypyrimidine, 2-hydroxy-1-methyl-6-methyl-4(1H) pyrimidine and 1,3-dimethyl-2-nitrobenzene بينما المركبات الناتجة من تحلل مبيد النيماكور (الفيناميفوس) هي fenamiphos phenol, fenamiphos sulfone and fenamiphos sulfoxide phenol.