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 abilities 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 in inoculated medium. 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-l-methyl-6- methylpyrimidine and l,3-dimethyl-2-nitrobenzene. While, the compounds produced from the biodegradation of nemacur (fenamiphos) pesticide are fenamiphos phenol, fenamiphos sulfone and fenamiphos sulfoxide.

INTRODUCTION

Organophosphorus pesticides are widely used in several countries in the world. They are relatively long-lived, its activity in soil lasting for several years **(Franzmann *et al*, 2000).**

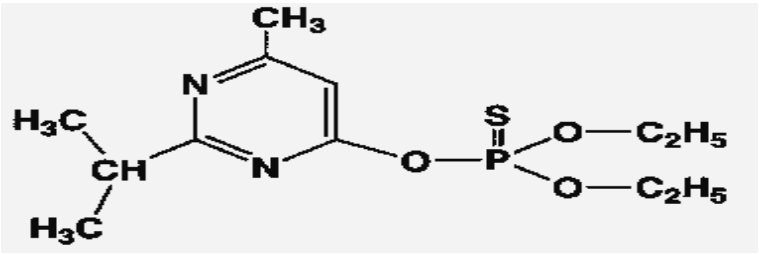
Organophosphorus pesticides are more toxic to mammals than organochlorine one. In addition organophosphorus pesticides such as diazinon and nemacur have been reported to be associated with chronic neurobehavioral effects. It is worthy to mention that after repeated application of pesticides, soil microorganisms become capable of detoxify the pesticides. Some soil microorganisms become adapted to use the pesticides as carbon and nitrogen source.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 N_2-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 dirverse group of bacteria including species of Bacillus, Pseudomonas, Alcaligenes, Flavobacetrium and Streptomyces genera are able to metabolize of pesticides. This research aimed to select an Egyptian microbial strains from soil 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.

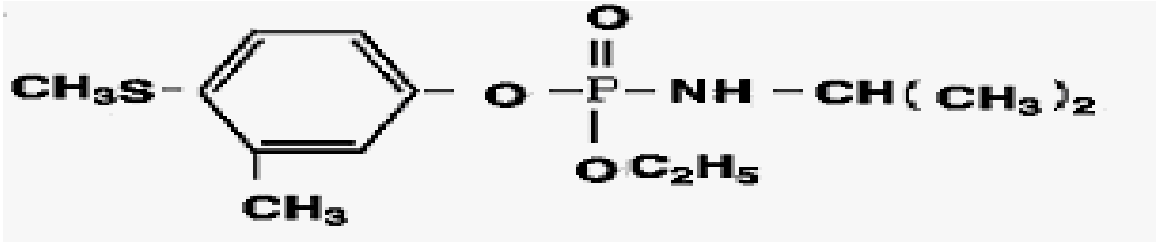
Two organophosphorus pesticides which are widely used in controlling many pests in Egyptian agricultural farms were chosen. These pesticides are:

Diazinon (Dimpylate). 0,0-Diethyl-0-(2-isopropyl-4-methyl-6-pyrimidinyl) phosphoro-thioate.



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 CO_2 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 procedures 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 viz medium alone, medium supplemented with pesticides (500 ppm) and medium supplemented with pesticides (500 ppm) and inoculated. 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 chromatographic analysis to determinate diazinon and nemacur residues.

3-GC/Mass to identify diazinon and nemacur metabolites.

Microbiological determinations.

Total bacterial counts, counts of Actinomycetes and counts of fungi were determined according to **Labeda (1990), Waksman & Lechevolier (1961)** and **Martin (1950)** respectively.

Dehydrogenase, Phosphatase and Nitrogenase activity in soil were estimated according to **Thalmann (1967)**, **Drobnikova (1961)** and **Diloworth (1970)** respectively.

CO2 evolution were estimated using the method of **Maswadeh (1976)**.

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, nemacur and their metabolites.

Diazinon, nemacur 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 and 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, CO_2 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.

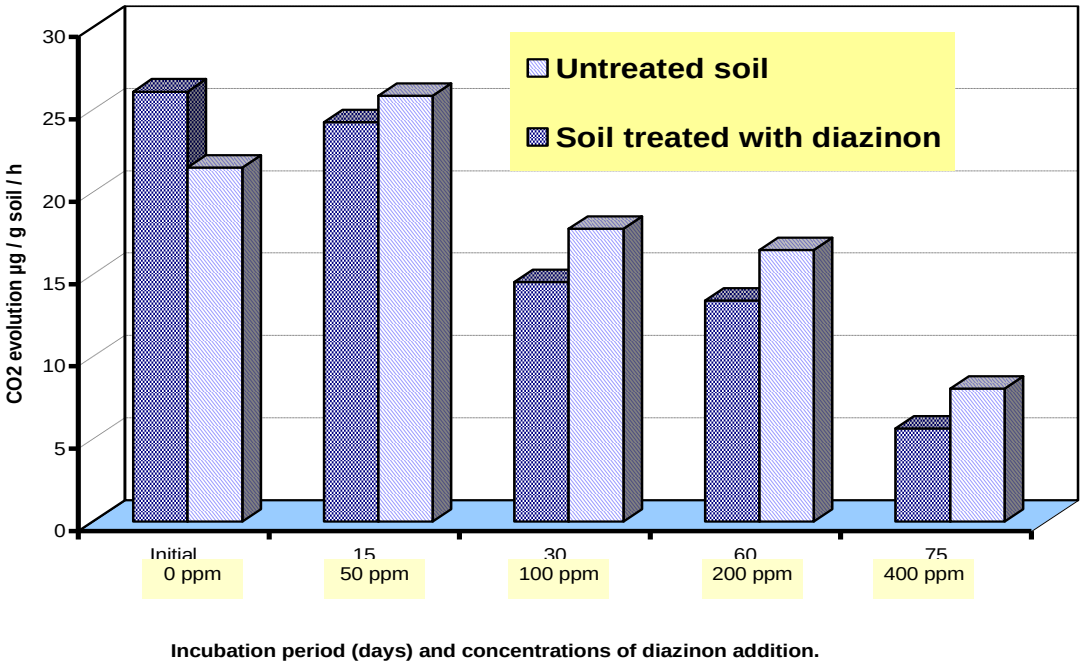


Fig.2. Changes in CO2 evolution in diazinon treated soil during incubation period.

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.

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.

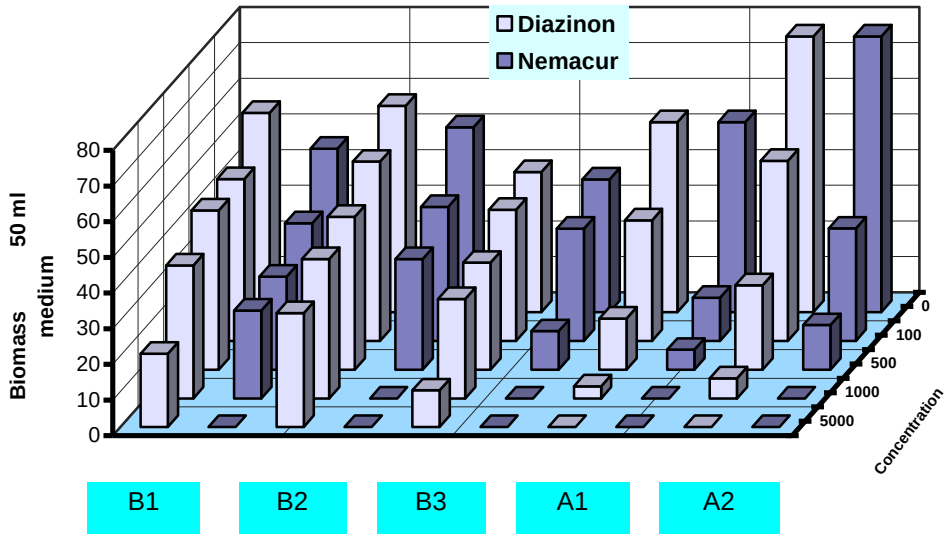


Fig3. Biomass (mg / 50 ml medium) of isolates under different concentrations of diazinon and nemacur pesticides.

Data in Fig (3) also revealed that the bacterial isolates (B1and 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 nemacur 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 nemacur pesticides by efficient microorganisms in liquid culture.

Identified microorganisms which showed higher tolerance of diazinon and nemacur 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 nemacur 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 nemacur

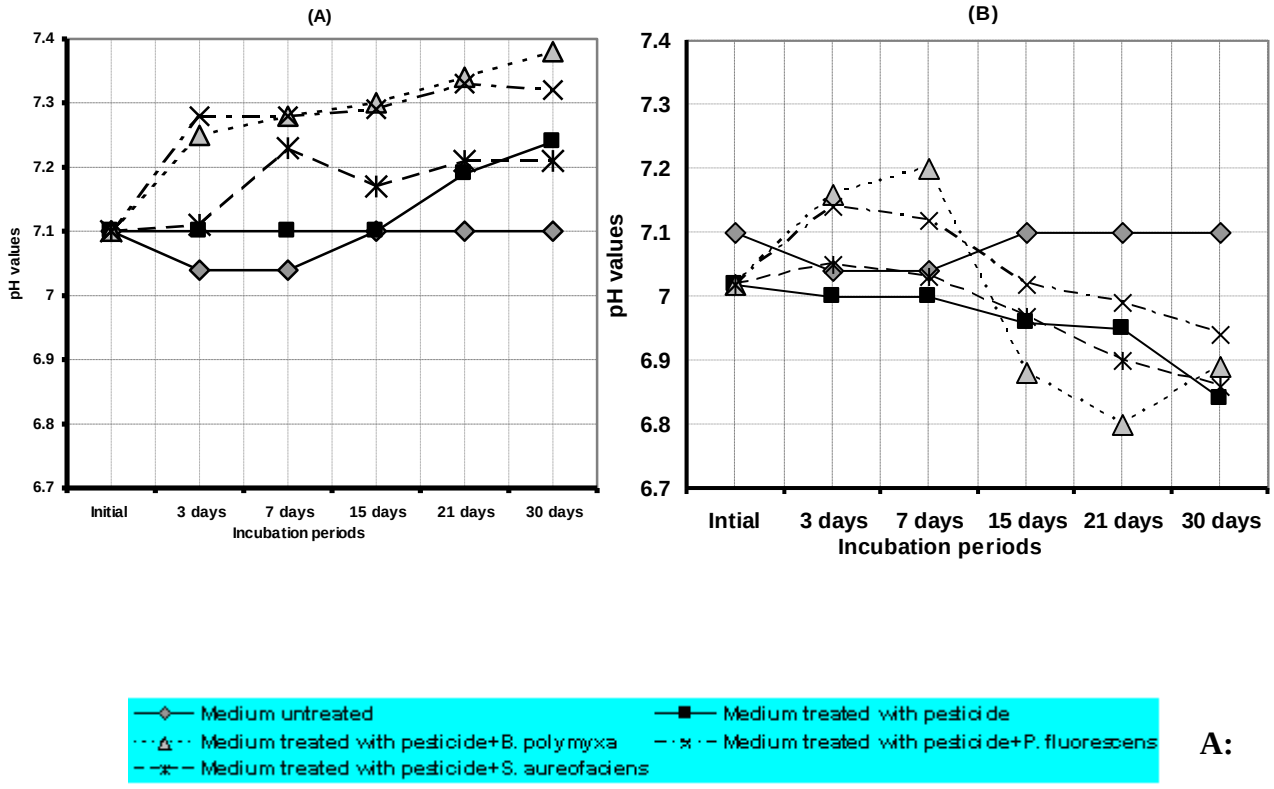
persistence determination. Data in Fig (4) showed that pH values in uninoculated media ranged from 7.1 to 7.24 and from 6.80 to 7.02 for diazinon and nemacur, respectively.

Data in Fig (4a) 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**

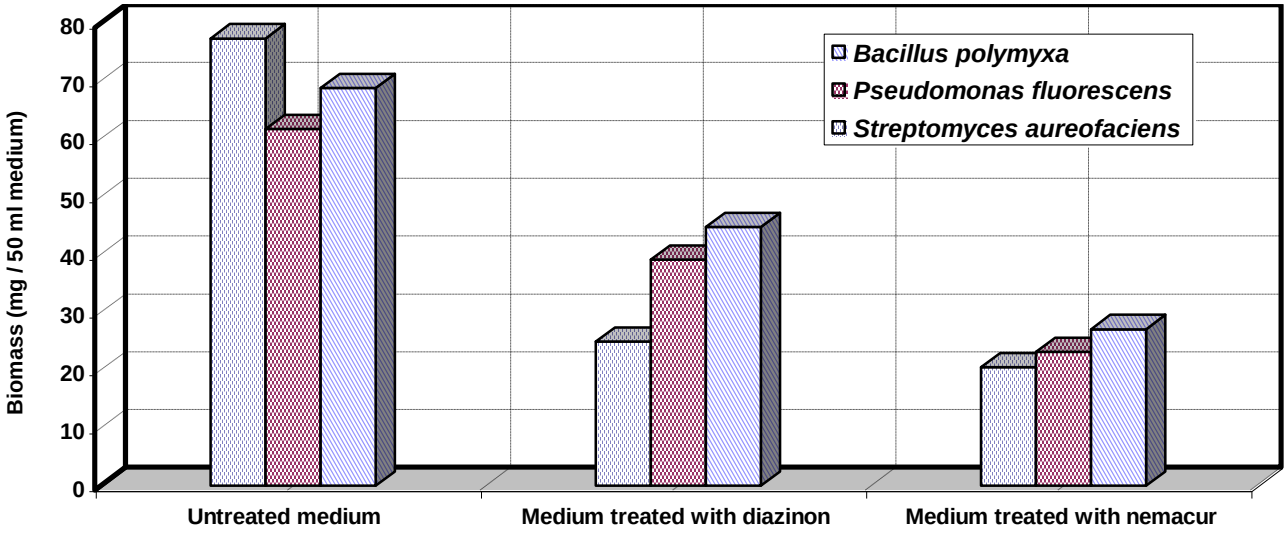
Fig 4 a. Periodical changes in pH value during incubation periods.

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.

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

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*.

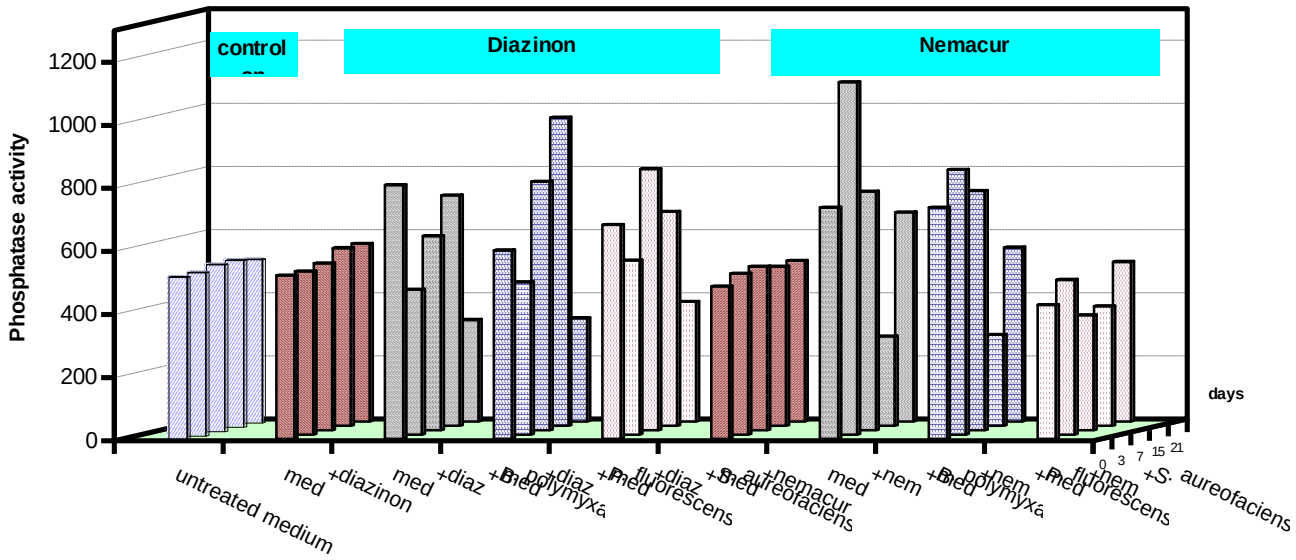


Fig.5. The Phosphatase activity (by µg inorganic phosphate / ml / 24 hrs enzyme activity on nucleic acid) changes during isolates incubation with diazinon and nemacur in liquid culture .

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 nemacur) 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 nemacur 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 nemacur pesticide. Since the persistent amount at the end of the experiment was higher in case of nemacur 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, only23.79, 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 nemacur 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					
			Bacillus polymyxa		*Pseudomonas fluorescens*		*Streptomyces aureofaciens*	
	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 from the biodegradation of diazinon by investigated microorganisms. The limited data are presented in Fig (7).

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.

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
	Bacillus polymyxa					
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
Metabolite 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	-	-	-	-
	Pseudomonas fluorescens					
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
Metabolite 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	-	-
	Streptomyces aureofaciens					
Diazinon	4.8	4.8	4.8	4.8	4.8	4.8
metabolite 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	-	-	-	-

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 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 nemacur by microbial strains in liquid culture.

Chromatographic analysis of nemacur 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 nemacur. 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 nemacur by investigated microorganisms. The limited data are presented in Fig (9). Data in Table (3) emphasize that the tested microorganism began to degrade nemacur 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. Nemacur 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
	Bacillus polymyxa					
Nemacur	2.65	2.65	2.65	2.65	2.65	2.65
metabolite 1	-	1.19	1.19	1.19	1.19	1.19
2	-	1.89	-	-	-	1.89
3	-	5.12	5.12	5.12	-	-
	Pseudomonas fluorescens					
Nemacur	2.65	2.65	2.65	2.65	2.65	2.65
metabolite 1	-	1.19	1.19	1.19	1.19	1.19
2	-	-	1.89	-	1.89	1.89
3	-	-	-	-	-	-
	Streptomyces aureofaciens					
Nemacur	2.65	2.65	2.65	2.65	2.65	2.65
metabolite 1	-	-	-	-	-	-
2	-	1.89	1.89	1.89	1.89	1.89
3	-	-	-	-	-	-

From data presented in Table (3) it is worthy 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

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 SO_2). 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 nemacur and its oxides persisted in the mineral salt medium, but were transitory in soil and groundwater due to their further metabolism by indigenous microorganisms.

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التحلل الحيوي لبعض مبيدات الفوسفور العضوية

بواسطة ميكروبات التربة

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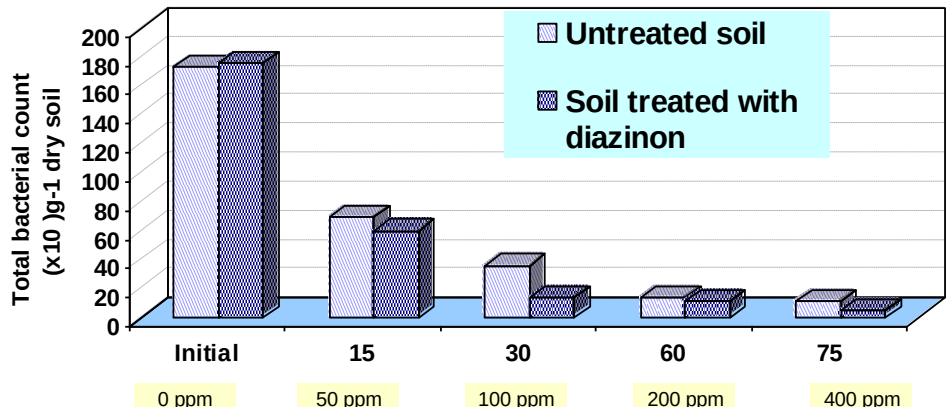
تم إجراء هذا البحث بهدف عزل بعض ميكروبات التربة التي لها القدرة علي تحليل مبيدات الفوسفور العضوية مثل الديازينون والنيماكور. في هذا البحث تم التعرف علي ثلاث عزلات لها القدرة والكفاءة العالية في تحليل المبيدات سابقة الذكر واستخدامها كمصادر للكربون والنيتروجين . أوضحت تجارب التعريف لهذه العزلات أنها *Bacillus polymyxa, Pseudomonas fluorescens and Streptomyces aureofaciens.*

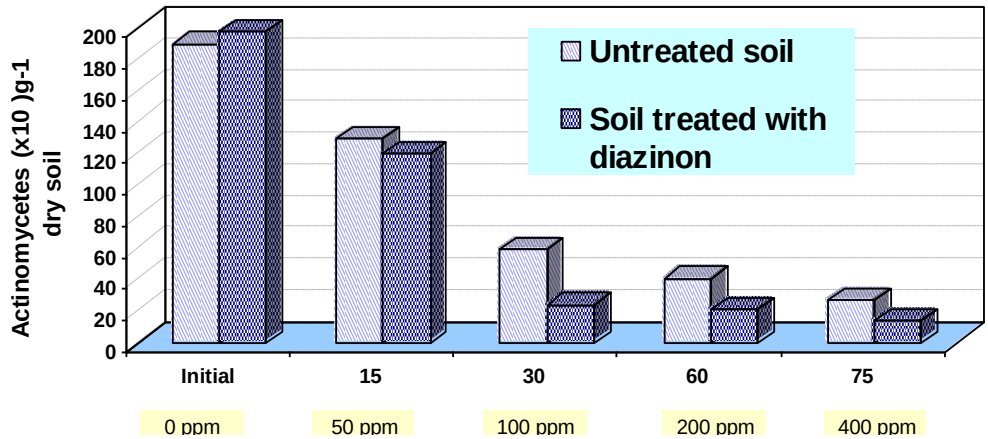
أوضحت نتائج هذا البحث أن كمية المبيدات المضافة إلي بيئة النمو لهذه الميكروبات أخذت في التناقص تدريجيا. معدل النقص في المبيدات كان أسرع في البيئات الملقحة بالمقارنة بغير الملقحة. كما أظهرت النتائج أن كمية مبيد الديازينون التي تحللت خلال الخمس عشر يوما من التجربة هي ٢٢.٧١، ٦٣.٨٢، ٦٢.٦٠ و ٤٧.٥١% من الكمية 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 من التجربة هي ٤.٨، ٢٨.٠٤، ٢٨.٣٠و 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- methylpyrimidine and 1,3-dimethyl-2-nitrobenzene. بينما المركبات الناتجة من تحلل النيماكور (الفيناميفوس) هي fenamiphos phenol, fenamiphos sulfone and fenamiphos sulfoxide.



(A)



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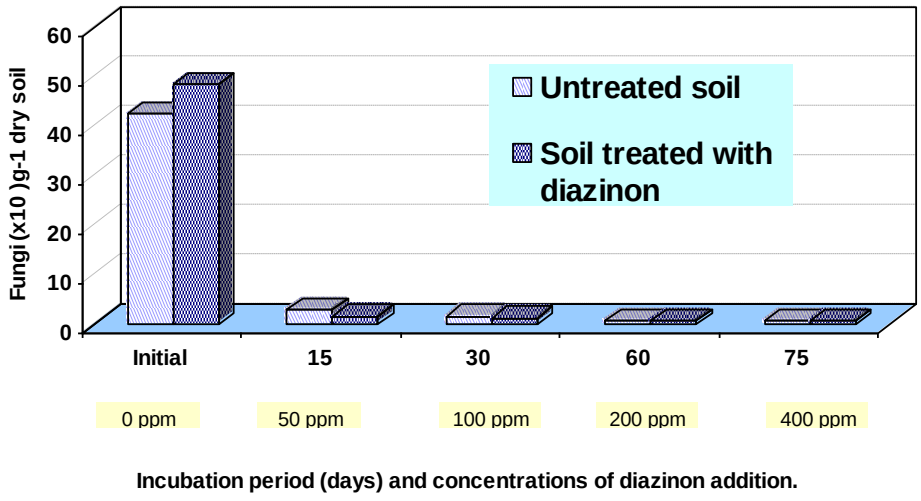

(C)

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

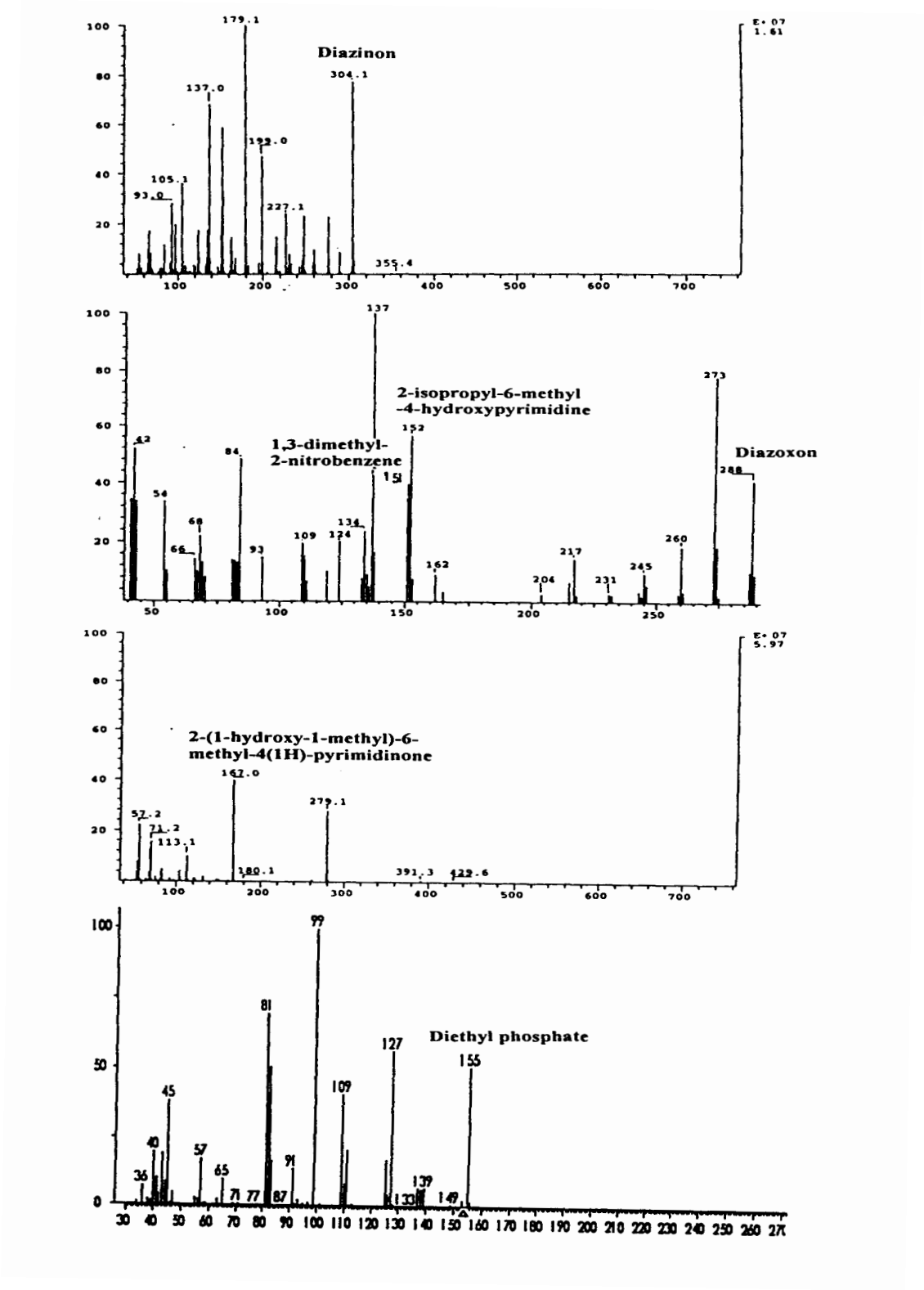

Fig 7. Mass spectrum of diazinon and its metabolites in liquid culture.

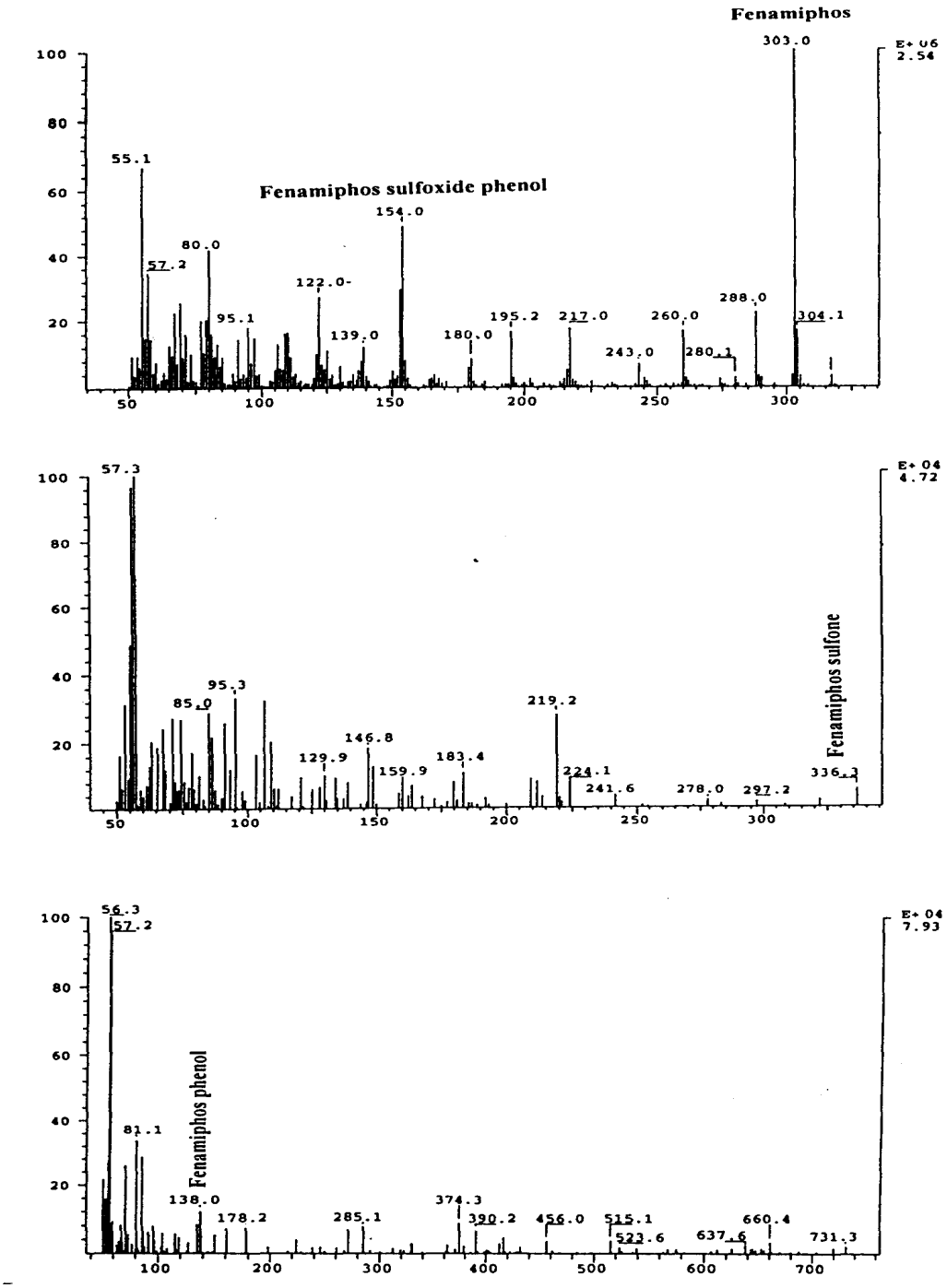

Fig 9. Mass spectrum of nemacur and its metabolites in liquid culture.