

Acceleration of Fenamiphos Pesticide Degradation in Liquid Culture and Soil by some Microorganisms

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ACCCELERATED microbial degradation of fenamiphos pesticide (nema-cur) in liquid culture and uncultivated and cultivated soil by the efficient strains of *Paenibacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens* were investigated. Fenamiphos amounts in liquid cultures of the three microorganisms decreased with elapsed time when it was used as a sole source of carbon and nitrogen. *P. polymyxa* proved to be able to decompose the pesticide at a higher rate than did *Ps. fluorescens* and *S. aureofaciens*. In pot experiment, soil amended with fenamiphos and inoculated with the aforementioned biodegrading strains showed higher values of N₂-ase, dehydrogenase and phosphatase activity than uninoculated soil. The mixture of strains gave higher enzyme activities than when each strain was inoculated individually. Furthermore, soil cultivated with tomato showed higher activities of these enzymes than uncultivated soil. Uncultivated soil inoculated with only *S. aureofaciens* showed the most rapid disappearance of fenamiphos compared to soil inoculated with *P. polymyxa*, *Ps. fluorescens* or the mixture of the three organisms. However the rate of fenamiphos decomposition by the bacterial strains was higher in cultivated soil than in uncultivated soil, and cultivated soil exhibited the most rapid disappearance of fenamiphos with the mixture of the three strains. When soil samples were analyzed for the fenamiphos biodegradation after 22 days of the pesticide addition and 15 days after inoculation with the biodegradants, three metabolites of fenamiphos were detected. Results show that fenamiphos degradation can be effectively accelerated in soil by these microorganisms.

Keywords: Biodegradation, Pesticide, Fenamiphos, *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Streptomyces aureofaciens*, Persistence.

Application of pesticides has greatly contributed to crop production. Despite this success, harmful side effects of pesticides include toxicity to humans and environment pollution (Andrea *et al.*, 2000). Organophosphorus compounds are the most widely used insecticides, accounting for an estimated 34% of world-wide insecticide sales. These compounds possess high mammalian toxicity and therefore it is essential to remove them from the environment (Singh and Walker, 2006). Fenamiphos (manufactured and marketed under the trade name, Nema-cur®)

is an organophosphate insecticide/ nematicide used to control pest nematodes and insects in a wide variety of crops.

Biological removal of chemo-pollutants is a method of choice since microorganisms can use a variety of xenobiotic compounds, including pesticides, for their growth and can mineralize and detoxify them. Kanekar *et al.* (2004) mentioned that when organophosphates are released in the environment, their fate is decided by various environmental conditions and microbial degradation. Microbial degradation is the major reason for disappearance of these pesticides.

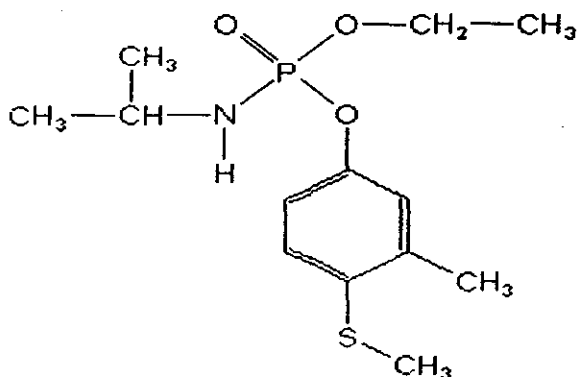
The biological efficacy of fenamiphos has been reported to be significantly reduced by enhanced biodegradation (Stirling *et al.*, 1992). It is oxidized rapidly in soil to fenamiphos sulfoxide (FSO) and fenamiphos sulfone (FSO₂). Chung and Ou (1996) reported that in soils showing enhanced biodegradation of fenamiphos, the parent compound is oxidized to FSO, which is then rapidly hydrolyzed to FSO-phenol (FSO-OH) which is subsequently mineralized to CO₂. A number of isolates capable of carrying out some form of degradation of fenamiphos have been isolated from soils and several genera of bacteria are represented; *Pseudomonas*, *Flavobacterium*, *Caulobacter*, *Bacillus* and *Streptomyces* (Singh *et al.*, 2003 and Abd El-Rahman, 2004).

The aim of the present study is to test strains of three bacterial species (*Pseudomonas fluorescens*, *Paenibacillus polymyxa* and *Streptomyces aureofaciens*) for acceleration of fenamiphos pesticide biodegradation in liquid culture and cultivated and uncultivated soil.

Material and Methods

Pesticide

Fenamiphos [Nemacur: ethyl 3-methyl-4-(methylthio) phenyl (1-methylethyl) phosphoamidate] is an organophosphorus nematicide used to control soil borne nematodes and some insects.



Chemical formula. C₁₃H₂₂O₃NSP

Bacterial strains

Three bacterial strains previously isolated and identified as *Paenibacillus polymyxa*, *Pseudomonas fluorescens* and *Streptomyces aureofaciens* which are highly efficient in the degradation of organophosphorus pesticides (Abd El-Rahman, 2004), were used in this work.

Biodegradation of fenamiphos in liquid culture

The three bacterial strains were tested for their ability to degrade fenamiphos when it was used as a sole source of carbon and nitrogen in liquid culture. One ml of standard inoculum containing 8×10^6 viable cells (2 days old) for *P. polymyxa*, *Ps. fluorescens* and 19×10^5 spores (7 days old) for *S. aureofaciens* was inoculated into basal mineral salt medium supplemented with fenamiphos at a rate of 500 ppm and incubated on a rotary shaker (200 rpm) for 30 days at 30°C. The basal medium (Smith and Adkins, 1996) contained, per liter, 3.5g K_2HPO_4 ; 1.5g KH_2PO_4 ; 0.27g $MgSO_4 \cdot 7H_2O$; 0.03g $Fe_2(SO_4)_3 \cdot 9H_2O$ and 0.03g $CaCl_2 \cdot 2H_2O$. Levels of fenamiphos were assayed 0,3,7,15,21 and 30 days.

Biodegradation of fenamiphos in uncultivated and cultivated soil

A pot experiment was carried out to study the efficiency of the bacterial strains on biodegradation of fenamiphos in uncultivated and cultivated soil. The study was conducted using clayey loam soil obtained from different fields at Faculty of Agriculture, Moshtohor, Qualubia Governorate, Egypt. Pots (30 cm diam.) were filled with soil (3 kg/pot). The pots were divided into two groups; the first was left without cultivation, while the second group of pots was amended with NPK fertilizers and planted with seedlings of tomato c.v. Super Strain B. After 15 days of planting each group was divided into two categories. One was left as a control (without fenamiphos application) while, the other group was mixed with fenamiphos to give a final concentration (50 ppm). After 7 days of pesticide application, soil was inoculated with 20 ml/ pot of *P. polymyxa*, *Ps. fluorescens* and *S. aureofaciens* individually or mixture of them, each ml containing about 12×10^6 c.f.u., 32×10^6 c.f.u. and 25×10^5 spores, respectively.

After initial, 3, 7, 15 and 35 days post inoculation, samples were taken to determine activity of dehydrogenase (Casida *et al.*, 1964), phosphatase (Drobnikova, 1961) and nitrogenase (Dilworth, 1970). Persistence of fenamiphos and its metabolites were determined by gas chromatography (GC) and GC-mass spectrometry (GCMS) (Finnigan mat SSQ 7000, digital DEC 3000). Fenamiphos was extracted from liquid culture and soil according to the method described by Atmakuru and Muthukrishnan (1999).

Results and Discussion

Rate of fenamiphos loss in liquid culture

Persistence data of fenamiphos in liquid culture of *P. polymyxa*, *Ps. fluorescens* and *S. aureofaciens* were estimated as detectable percentage amounts of the initial concentration (500 ppm) and illustrated by Fig. 1. Results showed that fenamiphos amounts decreased with elapsed time in both inoculated and

uninoculated media. The rate of decrease in inoculated medium was faster than that in uninoculated medium, especially 7 days after inoculation. These results are in agreement with those obtained by Megharaj *et al.* (2003) and Singh *et al.* (2003) who reported that fenamiphos fate in the environment is affected by microbial activity. At the end of the experiment (30 days), only 35, 40 and 47% of the added fenamiphos was detected in inoculated media with *P. polymyxa*, *Ps. fluorescens* and *S. aureofaciens*, respectively compared to 84% in the uninoculated medium. It can be concluded that the three bacterial strains can grow and use fenamiphos as a sole source of carbon and nitrogen. Furthermore, *P. polymyxa* decomposed fenamiphos in liquid culture faster than did the other two bacteria. This result is in harmony with those obtained by Abd El-Rahman (2004).

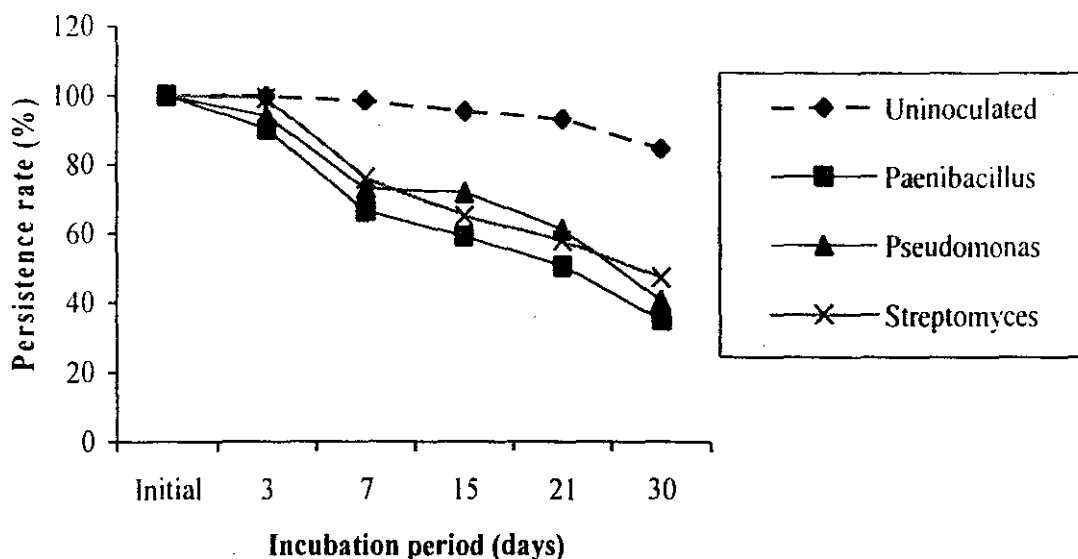


Fig. 1. Persistence rate of fenamiphos in liquid culture of three bacterial species.

Biodegradation of fenamiphos in uncultivated and cultivated soil

Dehydrogenase activity

Dehydrogenase activity was periodically determined as an indication of respiration rate and total microbial activity in the soil under different treatments. Fig. 2 shows that untreated soil gave higher values of dehydrogenase activity than soil treated with fenamiphos. A similar trend was obtained with both uncultivated and cultivated soil. This may be because pesticide application to the soil had adverse effects on microbial populations and consequently the enzyme activities decreased (Lan *et al.*, 2006). Also, the lowest values of dehydrogenase activities in uncultivated and cultivated soil were obtained when the soil was treated with the pesticide and uninoculated with the degrading microorganisms. In both uncultivated and cultivated soil, inoculation with the mixture of strains of *P. polymyxa*, *Ps. fluorescens* and *S. aureofaciens* showed higher dehydrogenase activity than the soil inoculated with any of the strains individually. Furthermore, cultivated soil exhibited greater activity of dehydrogenase as compared to uncultivated soil. This may be due to the beneficial effect of root exudates and root debris which represents nutritional substances for different soil

microorganisms. The results were in agreement with those obtained by Zaghloul *et al.* (2007).

Phosphatase activity

Phosphatase activity was periodically determined for its importance in hydrolysis of organic phosphorus compounds included in the pesticide under current study. Fig. 3 shows that soil treated with fenamiphos exhibited a slightly decrease in phosphatase activity compared to untreated soil after 15 days, especially in cultivated soil. This result can be attributed to the hydrolysis and biodegradation of the pesticide either by the tested strains or by the native microorganisms of the soil (Li *et al.*, 2002). Also, Megharaj *et al.* (1999) reported that fenamiphos showed no negative effect on phosphatase levels in the soil.

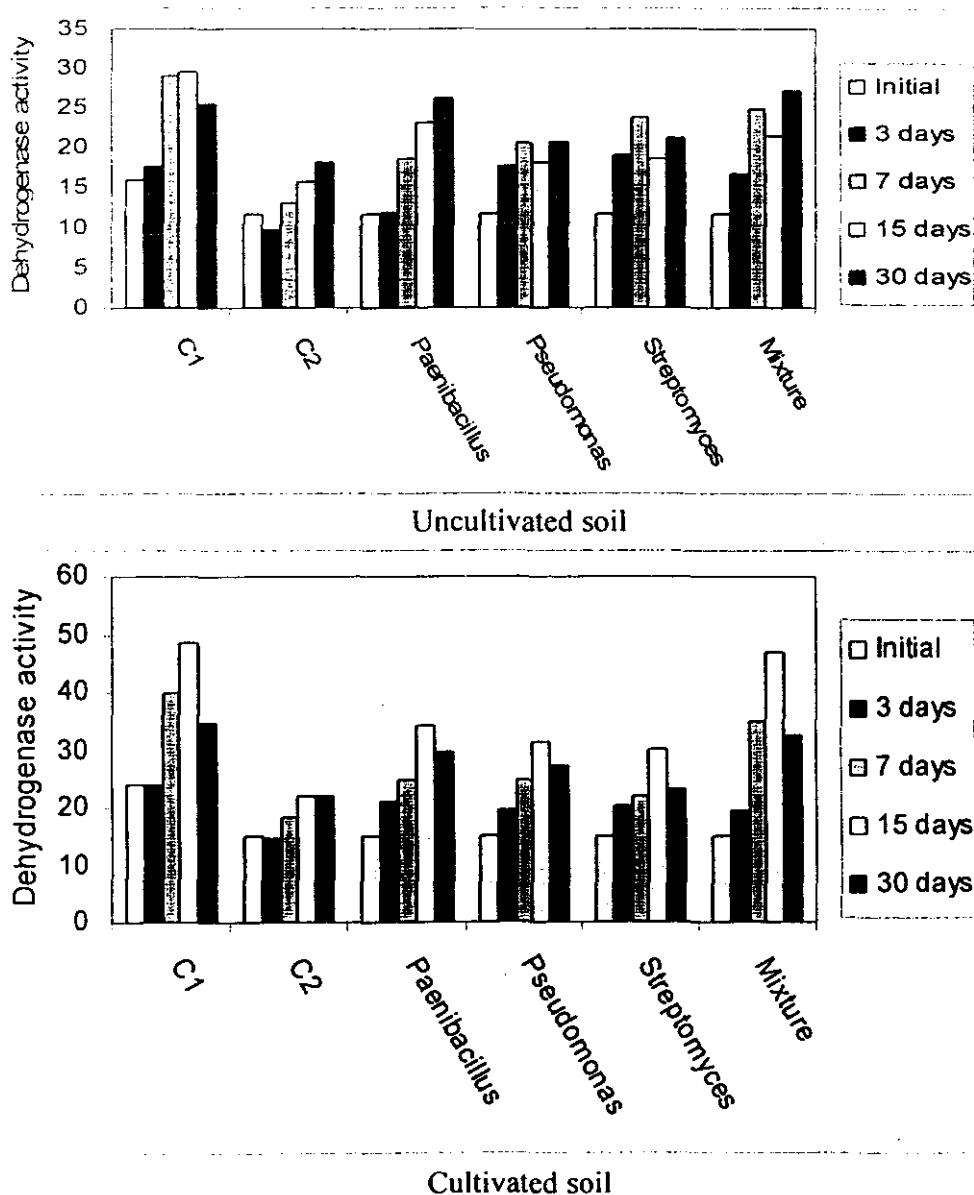
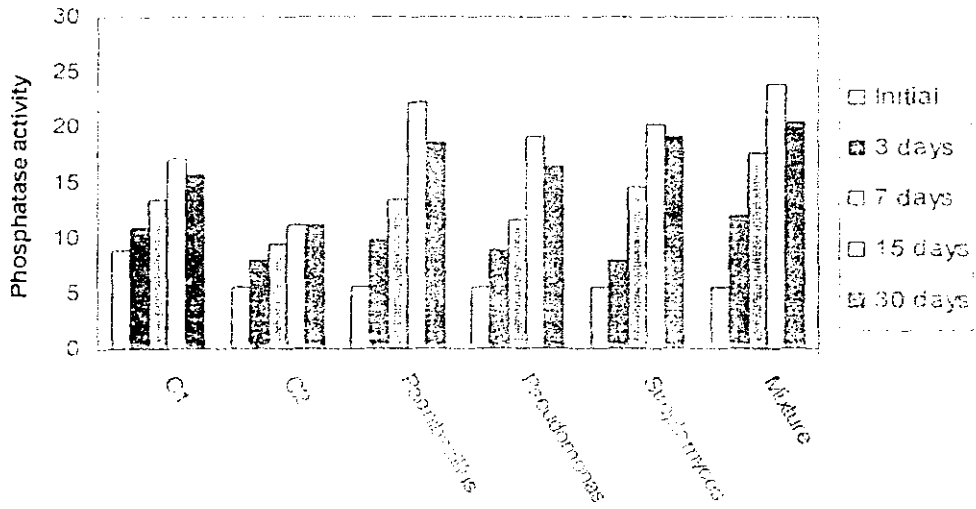


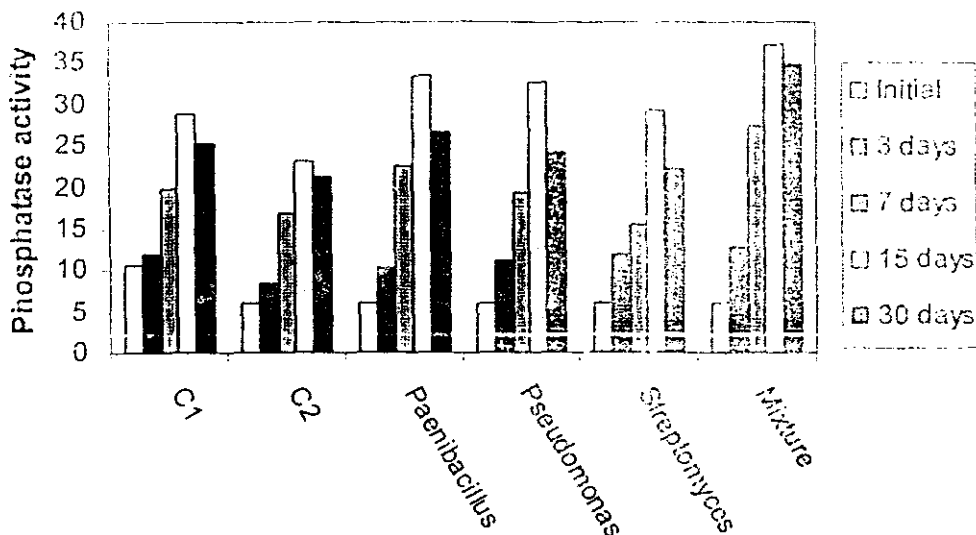
Fig. 2. Dehydrogenase activity ($\mu\text{l TPF/g dry soil/24h}$) in uncultivated and cultivated soil treated with fenamiphos and inoculated with the degrading bacteria.

C1: without inoculation or fenamiphos C2: fenamiphos without inoculation

In addition soil treated with fenamiphos and inoculated with the mixture of *P. polymyxa*, *Ps. fluorescens* and *S. aureofaciens* showed higher phosphatase activity than did the soil inoculated with each strain individually. This may be due to a synergistic effect between the degrading strains. Maximum values of phosphatase activity were evidently obtained in cultivated soil; this may be attributed to the beneficial effect of root exudates on the soil microorganisms. Similar results were observed by Lui *et al.* (2005) and Zaghoul *et al.* (2007).



Uncultivated soil



Cultivated soil

Fig. 3. Phosphatase activity (μl inorganic phosphate/g dry soil/24h) in uncultivated and cultivated soil treated with fenamiphos and inoculated with the degrading bacteria. C1: without inoculation or fenamiphos C2: fenamiphos without inoculation

Nitrogenase activity

Nitrogenase activity in uncultivated and cultivated soil treated with fenamiphos is presented in Fig. 4. Uncultivated or cultivated soil amended with *Egypt. J. Appl. Agric.Res. (NRC), Vol. 2, No. 1 (2009)*

fenamiphos without the degrading strains showed adverse effect on nitrogenase activity compared to untreated soil with the pesticide. This result was confirmed by Niewiadomska and Klama (2005) who found that the pesticides caused decrease in growth rate of nitrogen fixers and nitrogenase activity. Also, addition of degrading bacteria to the uncultivated or cultivated soil gave an increase in nitrogenase activity compared to the control. Inoculation with *P. polymyxa* individually or in mixture inoculum gave the maximum values of nitrogenase activity after 15 days from pesticide addition. This may be attributed to the hydrolysis and biodegradation of fenamiphos by either indigenous or introduced microorganisms which led to improvement of microbial growth rate, Abd El-Rahman (2004).

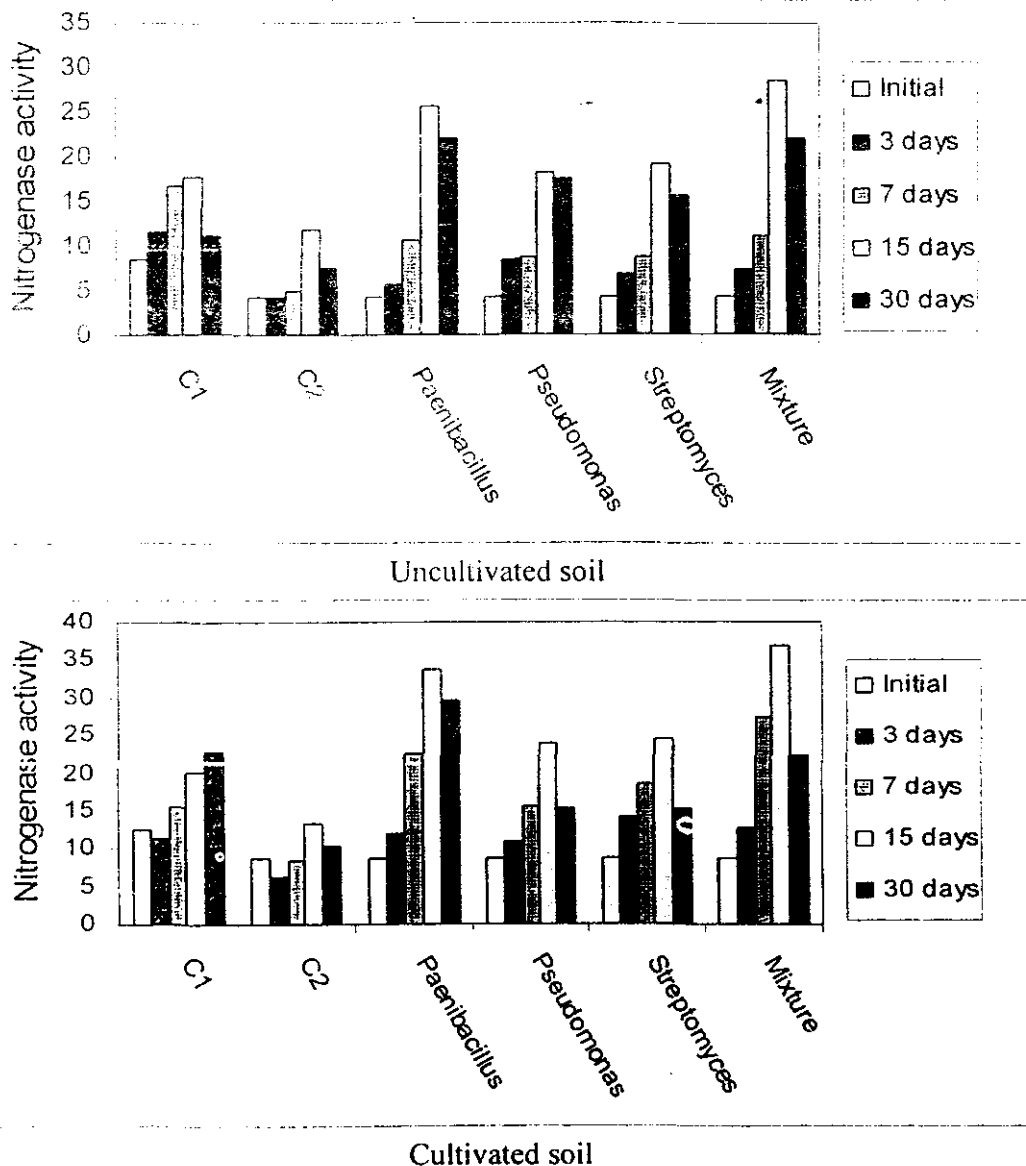


Fig. 4. Nitrogenase activity ($\mu\text{L C}_2\text{H}_4/\text{g dry soil/h}$) in uncultivated and cultivated soil treated with fenamiphos and inoculated with the degrading bacteria. C1: without inoculation or fenamiphos C2: fenamiphos without inoculation

Rate of fenamiphos loss in soil

Efficiency of *P. polymyxa*, *Ps. fluorescens* and *S. aureofaciens* to accelerate fenamiphos degradation was studied in uncultivated soil and soil cultivated with tomato. Residual fenamiphos estimated by GC (Fig. 5), indicates that fenamiphos degradation was accelerated in soil inoculated with the tested strains individually or in mixture, especially in cultivated soil. These results revealed that all tested strains are capable of hydrolyzing fenamiphos and they can use the pesticide as a carbon and nitrogen source. Megharaj *et al.* (1999), Pattison *et al.* (2000) and Abd El-Rahman (2004) similarly found a significant increase in the rate of fenamiphos degradation in inoculated soil compared to uninoculated soil. The percentages of residues recovered 30 days in uncultivated soil were 18.2, 15.4, 8.7 and 9.2% for *P. polymyxa*, *Ps. fluorescens*, *S. aureofaciens* and mixture of them, respectively. Whereas in cultivation soil, percentage of fenamiphos detected after 30 days of inoculation (37 days from pesticide addition) were 4.5, 2.8, 3.4 and 1.4% for the aforementioned treatments, respectively. This difference in disappearance rate of fenamiphos might be due to the tomato exudates, which may promote the proliferation of soil bacteria, both native and introduced. Soil inoculation with the mixture of strains exhibited slight increase in disappearance rate of fenamiphos as compared to the soil inoculated with any of the tested microorganisms alone.

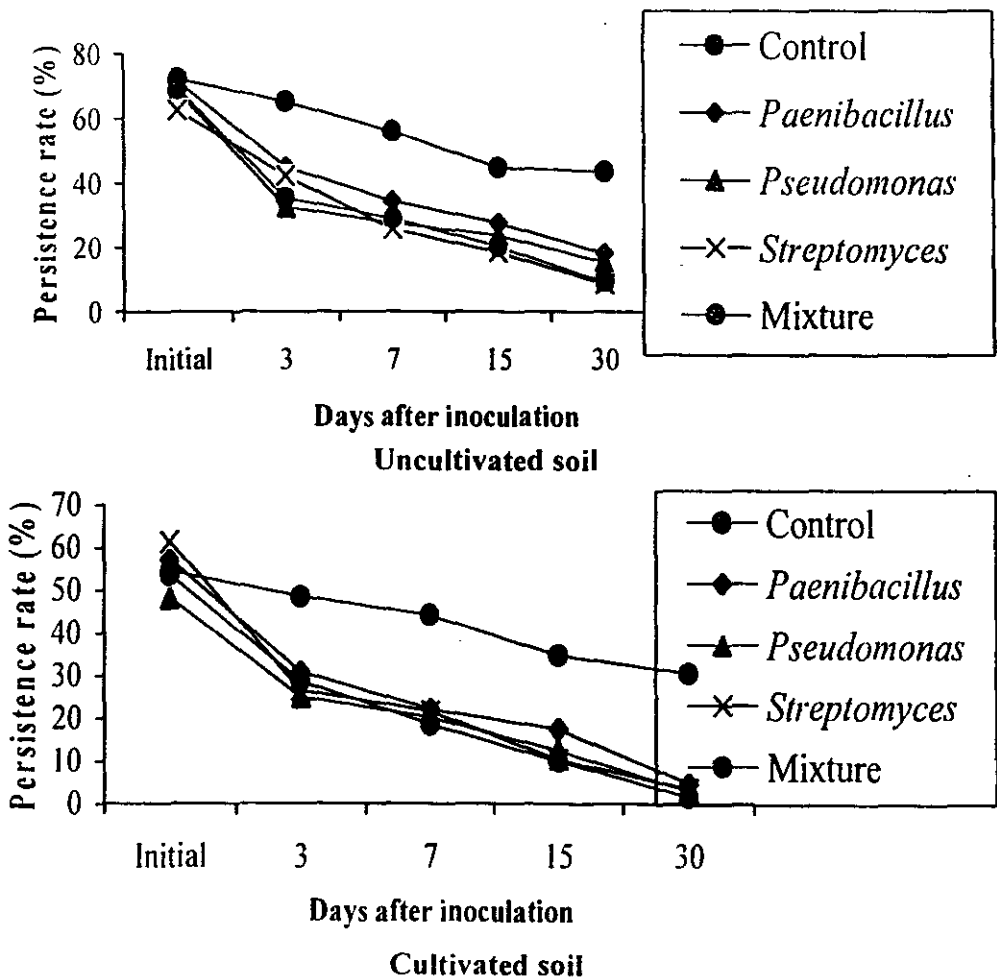


Fig. 5. Kinetics of fenamiphos loss in uncultivated and cultivated soil.

Analysis of samples was achieved by GC after 22 days of fenamiphos treatment (15 days after inoculation) to determine products produced from fenamiphos degradation in uncultivated and cultivated soil. The data obtained are presented in Table 1. Results show that uninoculated soil revealed two compounds which were detected in both uncultivated and cultivated soil. Concerning the inoculated soil, it is clear that two or three compounds were detected from the extracts of soil inoculated with *P. polymyxa* or *S. aureofaciens* in uncultivated and cultivation soil, respectively. Moreover, inoculation of uncultivated and cultivated soil with *Ps. fluorescens* or a mixture of the bacteria showed in chromatographic analysis three compounds. It is worth notice that inoculation with mixture of the tested bacteria or *Ps. fluorescens* were more potent in fenamiphos degradation than inoculation with either *P. polymyxa* or *S. aureofaciens*.

From the Gas/Mass analysis, it can be noticed that the metabolites produced from the biodegradation of fenamiphos by investigated microorganisms have molecular weights and m/z values equivalent with the molecular weights of fenamiphos and its dominant metabolites which observed and identified by Megharaj *et al.* (2003) and Abd El-Rahman (2004). Therefore, the metabolites produced from the biodegradation of fenamiphos were most likely fenamiphos phenol (FP), fenamiphos sulfone (FSO₂) and fenamiphos sulfoxide phenol (FSOP) (Table 2). This results are in harmony with those obtained by Sing *et al.* (2002) who noticed that fenamiphos was rapidly converted into fenamiphos sulfoxide which was further oxidized to fenamiphos sulfone.

TABLE 1. Identification of fenamiphos and its metabolites in uncultivated and cultivated soil by GC.

Co mpounds	Retention time (min.)				
	Control	<i>P. polymyxa</i>	<i>Ps. fluorescens</i>	<i>S. aureofaciens</i>	Mixture
Uncultivated soil					
Fenamiphos	5.9	5.9	5.9	5.9	5.9
Metabolites 1	-	-	-	1.63	1.63
2	-	1.72	1.72	-	1.72
3	1.86	1.86	1.86	1.86	1.86
4	1.95	-	1.95	-	-
Cultivated soil					
Metabolites 1	-	-	-	1.63	-
2	1.72	1.72	1.72	1.72	1.72
3	-	1.86	1.86	1.86	1.86
4	1.95	1.95	1.95	-	1.95

TABLE 2. GCMS analysis of fenamiphos and related compounds in soil.

Compounds	Molecular weight	m/z	Chemical formula
Fenamiphos	303	303	C ₁₃ H ₂₂ O ₃ NPS
Fenamiphos sulfone	335	335	C ₁₃ H ₂₂ O ₅ NPS
Fenamiphos sulfoxide phenol	154	154	C ₈ H ₁₀ OS
Fenamiphos phenol	138	138	C ₈ H ₁₀ S

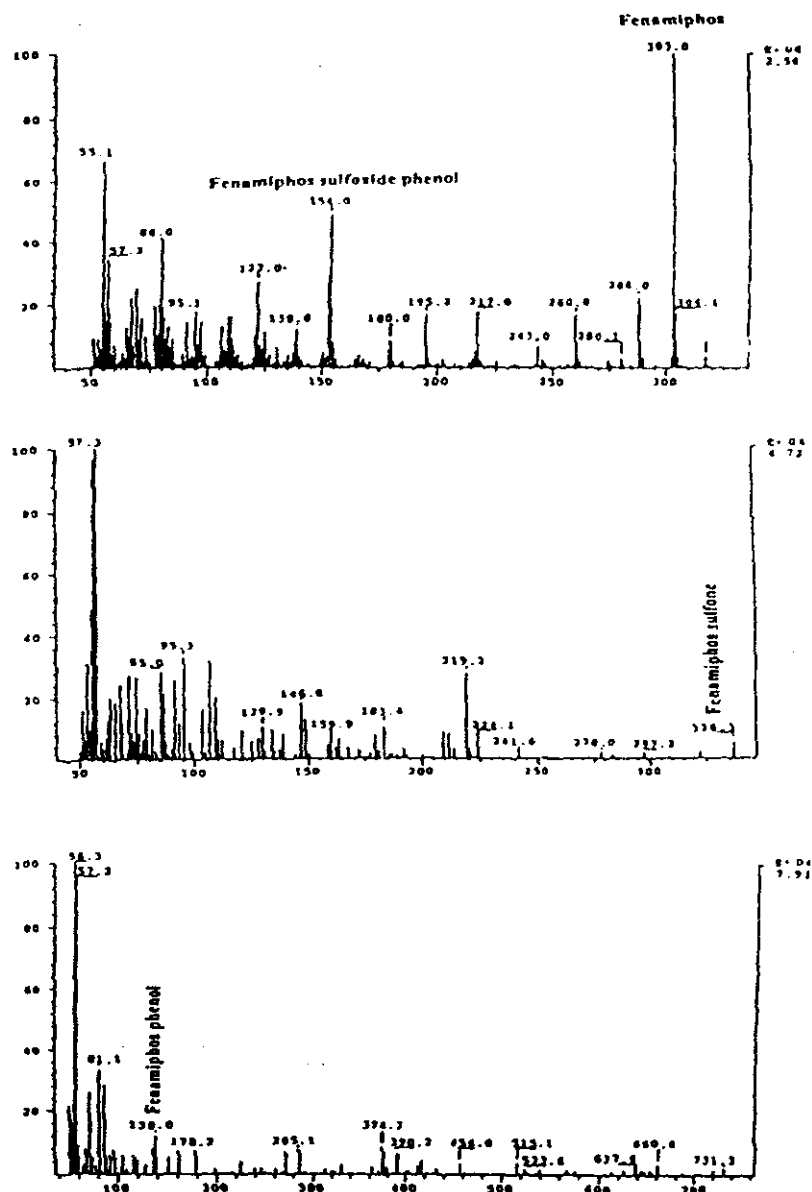


Fig. 6. Mass spectrum of fenamiphos and its metabolites.

Generally, from the results obtained in the current study, it can be concluded that fenamiphos degradation can be accelerated and produce some metabolites in both liquid culture and uncultivated and cultivated soil by *P. polymyxa*, *Ps. fluorescens*, *S. aureofaciens* or a mixture of these bacterial strains. Also, fenamiphos was degradable in cultivated soil faster than in uncultivated soil.

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إسراع تحلل مبيد الفيناميفوس فى المزرعة السائلة والتربة باستخدام بعض الميكروبات

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تم استخدام سلالات فعالة من *Paenibacillus polymyxa* و *Pseudomonas fluorescens* و *Streptomyces aureofaciens* فى إسراع تحلل المبيد فيناميفوس (نيماكور) فى كل من المزرعة السائلة والتربة غير المنزرعة والمنزرعة.

وقد أوضحت النتائج أن كمية المبيد المتبقى فى المزرعة السائلة الملقحة بكل سلالة قد انخفض تدريجيا عند استخدامه كمصدر وحيد للكربون والنيتروجين. كذلك تبين أن التلقيح بميكروب *P. polymyxa* أعطى معدل أسرع لتحلل المبيد بالمقارنة بالميكروبات الأخرى.

وفى تجربة الأصص أوضحت النتائج أن التربة المضاف إليها الفيناميفوس والملقحة بالميكروبات السابقة أعطت زيادة فى نشاط إنزيمات الديهيدروجينيز والنيتروجينيز والفوسفاتيز مقارنة بالتربة المضاف إليها المبيد وغير الملقحة. وكان أعلى نشاط إنزيمى عند تلقيح التربة بخليط من الميكروبات الثلاثة مقارنة بالتلقيح الفردى. علاوة على ذلك أعطت التربة المنزرعة بالطماطم والمضاف لها المبيد والملقحة بالميكروبات أعلى نشاط إنزيمى مقارنة بالتربة الغير منزرعة.

وكان معدل اختفاء المبيد أسرع عند التلقيح بالسلالة *S. aureofaciens* مقارنة بالتلقيح الفردى أو مخلوط السلالات الثلاثة فى التربة غير المنزرعة أما فى التربة المنزرعة فقد كان لمخلوط الميكروبات الثلاثة أكبر الأثر فى معدل اختفاء المبيد مقارنة بالتلقيح الفردى. وعند تحليل عينات التربة للمركبات الناتجة من التحلل الحيوى لمبيد الفيناميفوس بعد ١٥ يوم من التلقيح (٢٢ يوم من اضافة المبيد)، أظهرت النتائج وجود ثلاثة مركبات وسطية. وكانت التربة المنزرعة أفضل فى معدل تحلل المبيد مقارنة بالتربة غير المنزرعة. والنتائج المتحصل عليها توضح مدى كفاءة الميكروبات الثلاثة المستخدمة فى إسراع تحلل مبيد الفيناميفوس.