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Biodegradation of the Organophosphorus Insecticide Diazinon by *Pseudomonas aeruginosa* Isolated from Agricultural Drainage Ditches

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DIAZINON is an organophosphorous insecticide that is commonly used to control various agricultural and household pests and is frequently found as contaminant in water bodies. In the present study, a diazinon degrading bacterium was isolated from agricultural drainage ditches (Fayoum, Egypt) by enrichment technique. Based on morphological, biochemical and 16S rDNA gene sequencing, it was identified as *Pseudomonas aeruginosa*. A pure culture of *P. aeruginosa* was grown in minimal medium supplemented with diazinon as sole carbon source. The influence of diazinon concentration, temperature and pH on the bacterial growth and rate of diazinon degradation was investigated. The maximum capability of diazinon degradation (83.6 %) was achieved at concentration 400 ppm of diazinon at pH value 7.0 and temperature 30°C within 14 days. Therefore, *P. aeruginosa* can be used efficiently for the environmental cleanup of agricultural wastewater contaminated with high levels of diazinon.

Keywords: *Pseudomonas aeruginosa*, Diazinon, Biodegradation, 16S rDNA.

Pesticides that are used for crop protection are considered to be most widely distributed contaminants in the environment. Huge amounts of pesticides are produced and spread annually worldwide (Courdouan *et al.,* 2004). The excessive use of pesticides has resulted in problems caused by their interaction with biological systems in the ecosystem (Kanekar *et al*., 2004). The quality of soils, ground water, surface water and air is negatively affected by pesticide contamination (Surekha *et al*., 2008). As a result of the accumulation of pesticides in water supplies, there is an increasing necessity to develop environmentally safe, convenient and cost-effective techniques for the removal of pesticides (Zhang and Quiao, 2002 and Hussaini *et al*., 2013).

Currently, the pesticides cleaning up methods such as chemical treatment, volatilization and incineration have several handicaps such as the production of large quantities of chemicals and emission of toxic compounds (Liu *et al*., 2003).

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As a result of that the biological techniques such as bioremediation via utilizing microorganisms have been developed (Levin and Forchiassin, 2003 and Schoefs *et al.*, 2004). Microorganisms have a chief impact on the persistence of most pesticides in soil (Surekha *et al*., 2008). Isolation of indigenous bacteria capable of metabolizing organophosphate pesticide compounds has received considerable attention in situ detoxification (Richins *et al*., 1997 and Mulchandani *et al*., 1999). Microorganisms can utilize organophosphorus pesticides as carbon and/or phosphorus sources after their degradation via specific pathways (Aislabie and Lloyd-Jones, 1995; Ibrahim and Essa, 2010).

Diazinon (O,O-diethyl O-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate) is organophosphorous insecticide (Fig. 1). It is commonly used to control agricultural and household pests (Tomlin, 2006). The toxicity of diazinon is attributed to the inhibition of acetylcholinesterase, a vital enzyme involved in neurotransmission, in the form of acetylcholine substitutes (Bakry *et al*., 2006).

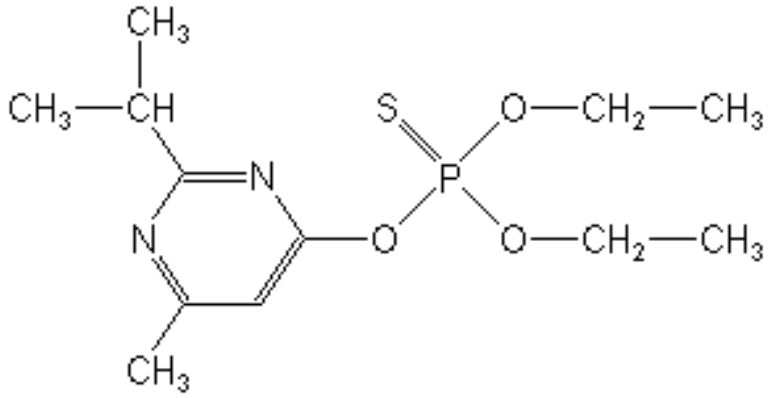

Fig. 1. Structure of the organophosphorus insecticide diazinon.

Diazinon can be hazardous as a result of runoff from areas of application into nearby drains or ditches, which typically transport water to streams and lakes. Subsequently it is subjected into degradation via biotic and abiotic processes (Getzin, 1967). Diazinon has a relatively short half-life in water, ranging from 70 hr to 12 weeks depending on pH, temperature, and sunlight as well as the presence of microorganisms while in soil it is influenced by the pH conditions in the soil and the soil type (Sethunathan and Yoshida, 1973).

Previous studies have showed that several bacterial species can utilize diazinon as a source of carbon and/or phosphorus such as *Serratia marcescens* (Abo-Amer and Aly 2011); *Pseudomonas* sp. (Ramanathan and Lalithakumari, 1999), *Agrobacterium* sp. (Yasouri, 2006); *Arthrobacter* sp. (Ohshiro *et al*., 1996) and *Flavobacterium* sp. (Ghassempour *et al*., 2002). Moreover, the some of these bacterial species might be contributed in the biotransformation of other organophosphorus insecticides (Lakshmi *et al*., 2008; Ortiz-Hernandez and Sanchez-Salinas, 2010).

Diazinon is one of the common water pollutants in Egypt (Masoud *et al*., 2007). Thus the objectives of the present study were (i) to isolate diazinon tolerant bacteria from agricultural wastewater, (ii) to investigate the optimum conditions of diazinon biodegradation.

Material and methods

Chemicals

Diazinon was purchased from Riedel-de Haën, Sigma-Aldrich, Seelze, Germany. All other chemicals purchased are of analytical grade from Fluka AG, Buchs, Switzerland.

Growth Media and Culture conditions

The Mineral salt medium (MSM) used in isolation of bacteria from soil and diazinon degradation studies was consisting of $(NH_4)_2SO_4$ (2.0 g/L); KH_2PO_4 (1.5 g/L); Na_2HPO_4 (1.5 g/L); $MgSO_4$.$7H_2O$, (0.2 g/L); $CaCl_2$.$2H_2O$ (0.01 g/L); $FeSO_4.7H_2O$ (0.001 g/L). The pH of the medium was adjusted to 7.0 ± 0.1 with 2 M NaOH. Diazinon was added to the liquid MSM medium after sterilization. For solid medium, 2% (w/v) agar was added to the same diazinon containing liquid mineral salt medium. Stock solution of diazinon was prepared with a concentration 500 ppm in acetone and was diluted to the required concentrations for the degradation studies.

Isolation of diazinon degrading bacteria

This experiment was performed to exclude sensitive bacteria and selecting the most tolerant bacteria to be acclimatized to diazinon. Five hundred milliliter of the agriculture wastewater was collected from El-Batts drain, Fayoum, Egypt. The samples were centrifuged under 10,000 rpm for 10 min and reconstructed with 10 mL Milli-Q water. Forty five milliliter of liquid MSM medium supplemented with diazinon (100 ppm) was inoculated with 5 mL of bacterial suspension and incubated for 48 hr inside shaking incubator (120 rpm) at 30^{o}C. Aliquots were sub-cultured every 3 days for a total of five passes. The final culture was diluted and plated on diazinon agar plates. Developed colonies were repeatedly streaked on diazinon agar plates for isolation of pure cultures. The bacterial isolate designated AAD was chosen for this work because it was the most tolerant strain and can grow under elevated levels of diazinon up to 500 ppm. A pure culture of AAD isolate was then stored in solid MSM containing 100 ppm diazinon for further studies.

Morphological and biochemical tests

The AAD isolate was tested for morphology, motility and Gram stain by phase contrast microscopy. Biochemical identification of the AAD isolate was performed according to Selim *et al*. (2014) using commercially available miniaturized multitest identification systems API (BioMérieux, France). Prior to inoculation of each identification system, a 24hr NA culture of AAD isolate was re-inoculated onto Nutrient agar plates to obtain isolated colonies for testing

purposes. The API 20NE identification system is designed for identifying non-fastidious, non-enteric Gram-negative rods. Test strips were inoculated and incubated according to the instructions provided. Sterile 0.85% saline solution was used as a negative control. APIWEB software was used for identification and was considered acceptable when given a probability of 85% or greater.

16S rDNA identification

The AAD diazinon degrading bacterial isolate was identified using 16S rDNA gene sequencing technique. The genomic DNA was extracted according to Essa (2012). The primers used in the amplification of the 16S rDNA gene are forward primer (F1; AGA GTT TGA TCC TGG CTC AG) and reverse primer (R1; GGT TAC CTT GTT ACG ACT T). The PCR mixture was prepared as described by Reyad *et al.* (2014). PCR were carried out for 35 cycles under the following conditions: denaturation step at 94°C for 40 sec, annealing step at 55°C for 1 min., extension step at 72°C for 2 min. and final extension at 72°C for 10 min. An aliquot of the PCR products (10 µL) was mixed with 2 µL of DNA loading buffer and analyzed by electrophoresis (15 V/cm, 60 min.) on 0.7% horizontal agarose gel in TBE buffer containing 0.5 µg/mL ethidium bromide, then visualized on an UV transilluminator. Sequencing of the amplified fragments was performed at GATC Biotech, Constance, Germany. DNA Sequences were aligned at NCBI DataBase (www.ncbi.nlm.nlh.gov). Phylogenetic tree was then constructed by neighbour-joining method using TREEVIEW software (1.6.6) based on 16S rRNA gene sequences of some strains phylogenetically close to the isolated strain.

Optimization of the growth conditions and diazinon biodegradation

Experiments aiming to study the effect of diazinon concentration on the growth of the bacterial strain were conducted in 250 mL flask containing 50 mL MSM supplemented with different concentrations of diazinon (100 – 500 ppm). The medium was inoculated by five milliliter of bacterial cell suspension (OD_{600} = 0.2) and incubated on a rotary shaker (120 rpm) at 30°C. Cell growth in liquid media was determined spectrophotometrically by measuring the cultural optical density at 600 nm at 24 hr intervals over 21 days. The protein content of the bacterial cultures was assayed using Bradford assay (Bradford, 1976) in order to confirm the bacterial growth. To investigate the effect of pH value on the bacterial growth, experiments were carried out at different pH values. Cultures supplemented with 400 ppm diazinon as a sole carbon source were incubated as mentioned above. At the same time, the effect of temperature on the bacterial growth was studied using MSM medium supplemented with 400 ppm diazinon at the optimum pH. Bacterial cultures were incubated at different temperatures. All the experiments were done in triplicates. Cell growth in liquid media was determined spectrophotometrically as mentioned above. The MSM supplemented with the same concentration of diazinon without bacterial inoculums were prepared and incubated under the same conditions in order to measure abiotic degradation of diazinon.

Analysis of the residual diazinon

The capability of *P. aeruginosa* for diazinon biodegradation was determined under different temperatures and pH values after 14 days incubation in order to identify the optimum condition for diazinon degradation. The residual diazinon was extracted according to the method of Mahiudddin *et al*. (2014). About 50 mL of 90% methanol was added to 20 mL liquid culture and was set overnight. Then the mixture was filtrated and extracted twice with 50 mL CH_3Cl. The received solution was concentrated under nitrogen flow to 1 mL to be determined by gas chromatography. A Hewlett-packard, USA serial 6890 gas chromatograph equipped with electron detector (ECD, Radioisotope Nuclide 63Ni) and HP PAS-1701 column 25 m length x 0.32 mm x 0.52 thickness. Pure nitrogen was used as carrier gas (2 mL/min). Detector, injector and column temperature was 250, 240 and 225°C, respectively. diazinon detection limit was set at concentration where the analyte signal was three times higher than background noise and it was 0.1 µg/L. The diazinon degradation rate was calculated according to Lin *et al*. (2008) by the following formula:

$$\mathbf{A = [C_a - C_b / C_a] \times 100}$$

where, (A) is the percentage of diazinon degradation, (C_a) is the concentration of diazinon (mg/L) in the medium in absence of diazinon degrading strain, (C_b) is the concentration of diazinon (mg/L) in presence of diazinon degrading strain.

Statistics

The data presented here are the mean values of three replicates. Standard errors were calculated for all the values using MS Excel 2007.

Results

Isolation and identification of diazinon degrading bacteria

Some bacterial species capable of degrading diazinon were isolated from agricultural drainage ditches in May, 2013 (Fayoum, Egypt) using enrichment technique. The bacterial isolate AAD was the most tolerant strain against high levels of diazinon (500 ppm). A variety of morphological and biochemical assays were carried out to have a comprehensive view of the phenotypic characteristics of the bacterial isolate AAD as shown in Table 1. AAD isolate was Gram negative motile non-spore forming rods. This isolate demonstrated positive results with β-galactosidase, arginine dihydrolase, lysine decarboxylase, orenthine decarboxylase, urease, lipase, amylase, gelatinase, catalase, cytochrome oxidase, nitrate reduction and acetoin production tests. Meanwhile, negative results were recorded for tryptophane deaminase, H_2S production, indole production tests. Simultaneously, the AAD isolate showed the capability to utilize glucose, sucrose, mannitol, amygdalin, inositol, starch and citrate as carbon source.

TABLE 1. Morphological and biochemical characters of the diazinon degrading bacterial isolate (AAD).

Morphological characters	**Result**	**Sugar fermentation**	**Result**
Gram staining	Negative	Glucose	Positive
Motility	Motile	Sucrose	Positive
Cell shape	Rod	Mannitol	Positive
Endospore formation	Negative	Inositole	Negative
Enzyme profile		Rhamnose	Negative
β-galactosidase	Positive	Melibiose	Negative
Arginine dihydrolase	Positive	Amygdalin	Positive
Lysine decarboxylase	Positive	Arabinose	Negative
Orenthine decarboxylase	Positive	Starch	Positive
Urease	Positive	Citrate utilization	Positive
Tryptophane deaminase	Negative	Sorbitol	Negative
Gelatinase	Positive	**Other tests**	
Catalase	Positive	H_2S production	Negative
Amylase	Positive	Acetoin production	Positive
Lipase	Positive	Indole production	Negative
Cytochrome oxidase	Positive		
Nitrate reduction	Positive		
-To nitrite	Positive		
-To N_2	Negative		

The AAD isolate was identified as *Pseudomonas aeruginosa* using 16S rDNA gene sequencing technique with maximum homology of 87% *Pseudomonas aeruginosa* PAO1 strain PAO1 (accession number: NR0748281; Fig. 2). The phylogenetic tree of the diazinon degrading bacterial strain AAD and related bacterial species based on the 16S rDNA sequence was provided in Fig. 3. It can be clearly seen that the AAD diazinon degrading strain was included in the genus *Pseudomonas* and closely related to the species *aeruginosa*.

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AAD     93    GCCTGGGAACGAATTCACCAAAACATTCTGATTCTTAATTGGGTCCGATTTTTACTTCA-  151
              ||| ||||||| |||||||   ||||||||||||   |||     |||||  |||||||
Sbjct   1310  GCCCGGGAACGTATTCACCGTGACATTCTGATTCACGATTACTAGCGATTCCTACTTCAC  1251

AAD     152   AAATTCGAGTTGCAGACTGAAATCGGGAAGA-GATGGGTTTTGTGGAATTACTTCCACCT  210
                ||||||||||||||||  ||| |||  | ||| |||||| ||| ||||  |||||||
Sbjct   1250  GCATTCGAGTTGCAGACTGCGATCCGGACTACGATCGGTTTTATGGGATTAGCTCCACCT  1191

AAD     211   CGGTGCTTGGCAACCCTTTGT-CCGA-ATTTGTACAACGTGTGAAGCCCTGGCCGTAAGG  268
              ||  |||||||||||||||| ||||   |||||  ||||||| ||||||||||||||||
Sbjct   1190  CGCGGCTTGGCAACCCTTTGTACCGACCATTGTAGCACGTGTGTAGCCCTGGCCGTAAGG  1131

AAD     269   GCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAG  328
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   1130  GCCATGATGACTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTCTCCTTAG  1071

AAD     329   AGTGCCCACCCGAGGTGCTGGTAAATAAGGACAAGGGTTGCGCTCGTTACGGGACTTAAC  388
              |||||||||||||||||||||||| ||||||||||||||||||||||||||||||||||
Sbjct   1070  AGTGCCCACCCGAGGTGCTGGTAACTAAGGACAAGGGTTGCGCTCGTTACGGGACTTAAC  1011

AAD     389   CCAACATCTCACGACACGAGCTGACGACCGCCATGCAGCACCTGTGTCTGAGTTCCCAAA  448
              |||||||||||||||||||||||||||| |||||||||||||||||||||||||||| ||
Sbjct   1010  CCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTCTGAGTTCCCGAA  951

AAD     449   GGCACCAATCCATCTCTGGAAAGTTCTCAGCATGTCAAGGCCAGGTAAGGTTCTTCGCGT  508
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   950   GGCACCAATCCATCTCTGGAAAGTTCTCAGCATGTCAAGGCCAGGTAAGGTTCTTCGCGT  891

AAD     509   TGCTTCAAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGT  568
              |||||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   890   TGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGT  831

AAD     569   TTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTATCGCGTTAGCTGCGCCACTAAG  628
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct   830   TTTAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTATCGCGTTAGCTGCGCCACTAAG  771

AAD     629   ATCTCAAGGATCCCCACGGCTAGTCAACATCGTTTACGGCGTGGACTACCAGGGTATCTA  688
              ||||||||||||||| |||||||||| ||||||||||||||||||||||||||||||||||
Sbjct   770   ATCTCAAGGATCCCAACGGCTAGTCGACATCGTTTACGGCGTGGACTACCAGGGTATCTA  711

AAD     689   ATCCTGTGTTGGTCCCCCCACTTTCGCACCCCCAGTGTGTGTATCAGTACAGGTGGTCCC  748
              ||||||| ||| |||||| | |||||||||| | |||||  |||||||| ||||||||| |
Sbjct   710   ATCCTGT-TTGCTCCCCACGCTTTCGCACCTC-AGTGTCAGTATCAGTCCAGGTGGTCGC  653

AAD     749   CCTTCTCCCACTGGTGTTTCCCTCCTATATCTACACATTTCACCCCCTACACAGGAGAAA  808
              | ||| || ||||||| |||| |||||||||||| ||||||||| | ||||||||| ||
Sbjct   652   C-TTCGCC-ACTGGTG-TTCCTTCCTATATCTACGCATTTCACCGC-TACACAGGA-AAT  598

AAD     809   TCCCCCACCCCTCCTACCGTACTCTAGCTACTGTAGTTTTGGATGCCGATTCCCCAGGGT  868
              ||| |||||| | |||||||||||||||| | |||||||||||||| | ||||| ||| |
Sbjct   597   TCCACCACCC-T-CTACCGTACTCTAGCT-CAGTAGTTTTGGATGCAG-TTCCC-AGG-T  544

AAD     869   GTGAGACCCCGGGAGTATATCATCCAAACATTGCTGCAGTCACCCACGCCGGCGCTTTTA  928
               |||| ||| |||| | |  ||||||| | |||||| |  |||| |||| | |||||| |
Sbjct   543   -TGAG-CCCGGGGATT-TCACATCCAA-C-TTGCTGAAC-CACCTACGC-G-CGCTTT-A  493

AAD     929   TGCCCAGATATTACCGA-TATCGCTTGCACCCCTTCTTATTAACCCCGGCCTGCTGGGAG  987
               |||||| || | |||| || |||||||||| ||| ||||| || |||| |||||| |
Sbjct   492   CGCCCAG-TAATTCCGATTAACGCTTGCACCC-TTCGTATTA-CCGCGGC-TGCTGGCA-  438

AAD     988   CGAAAATTAGACCGGGGCGTACTCCTGTCTGGTTGACCGTCCAAACAAGCACGGCGAGTG 1047
              ||||  |||| |||| || || || ||| ||||  || ||| ||||| ||| ||  | |
Sbjct   437   CGAAG-TTAG-CCGGTGCTTATTC-TGT-TGGTA-AC-GTCAAAACA-GCAAGGT-ATT-  387

AAD     1048  AAGATGAGTGTCCCTTCCCTCTCCAGACTTAATGTGCATTACAATCCGA-GAC  1099
              ||  | | || |||||||| || ||| ||||||| |||| ||||||||||| |||
Sbjct   386   AACTT-ACTG-CCCTTCC-TC-CCA-ACTTAAAGTGCTTTACAATCCGAAGAC  339
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Fig. 2. Partial DNA sequences of the 16S rDNA gene of the bacterial strain AAD isolated from agricultural wastewater and the corresponding gene of *Pseudomonas aeruginosa* strain PAO1 (accession number: NR0748281).

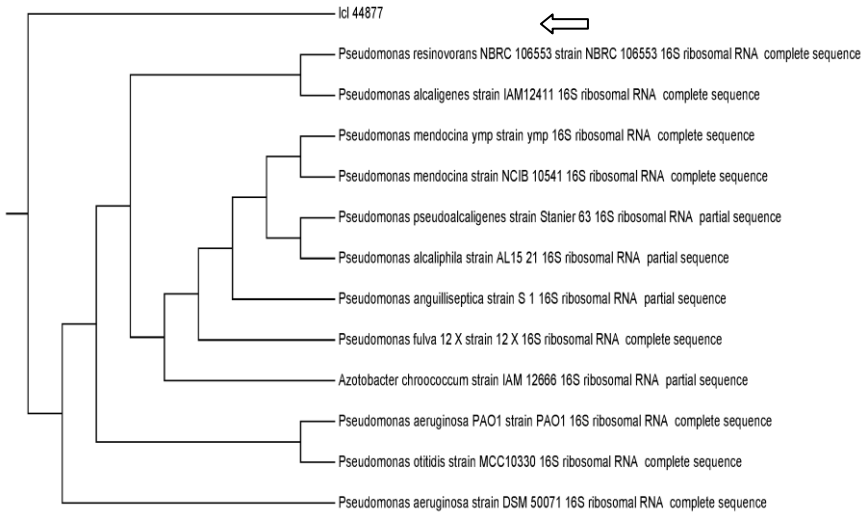

Fig. 3. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences showing the position of the bacterial isolate AAD among phylogenetic neighbors. The black arrow indicates the position of AAD strain.

Growth optimization and diazinon degradation of P. aeruginosa

The obtained data highlighted the prominent capability of *P. aeruginosa* isolated from agricultural wastewater, to tolerate elevated levels of diazinon up to 500 ppm. Data in Fig. 4 showed that the growth of *P. aeruginosa* in the minimal media supplemented with diazinon as a sole carbon source was increased by increasing diazinon concentration up to 500 ppm then the growth decreased at higher concentrations. Within 14 days incubation, the maximum cell density (1.15) was recorded with 500 ppm while the maximum protein content (217.6 mg/L) was achieved with 400 ppm of diazinon.

Moreover, the obtained results demonstrated the effect of temperature on the growth of *P. aeruginosa* (Fig. 5) and biodegradation rate of diazinon (Fig. 7 and Table 2). The maximum optical density (1.09) and protein content (349.4 mg/L) and diazinon degradation (83.6%) was demonstrated at 30^{o}C after 14 days of incubation. At higher temperature, a clear inhibition in the optical density, protein contents and percentage of diazinon degradation was recorded. At the same time, moderate bacterial growth and diazinon biodegradation rate was demonstrated at 20^{o}C.

Concurrently, the change in the pH value demonstrated a remarkable outcome on the growth of *P. aeruginosa* (Fig. 6). The maximum optical density (1.15) and protein content (328.5 mg/L) and diazinon degradation (83.6%) was recorded at pH 7.0 within 14 days. In the meantime, the recorded growth parameters and diazinon degradation were significantly reduced at the pH levels 5.0 and 9.0 (Fig. 7 and Table 2).

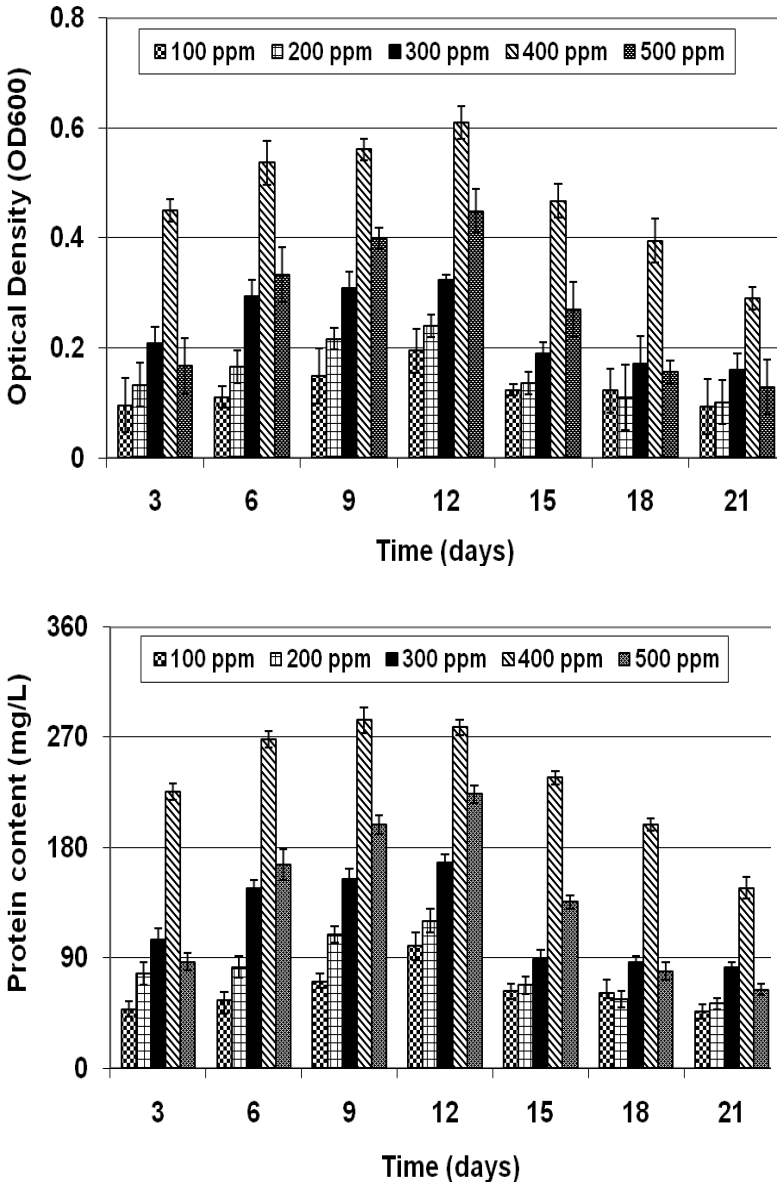

Fig. 4. Effect of diazinon concentration on the growth of *Pseudomonas aeruginosa*, (A) represents the optical density (OD_{600}) while (B) represents the protein content (mg/L) of the bacterial growth. Data are the means of three replicates and error bars represent the standard errors of the means.

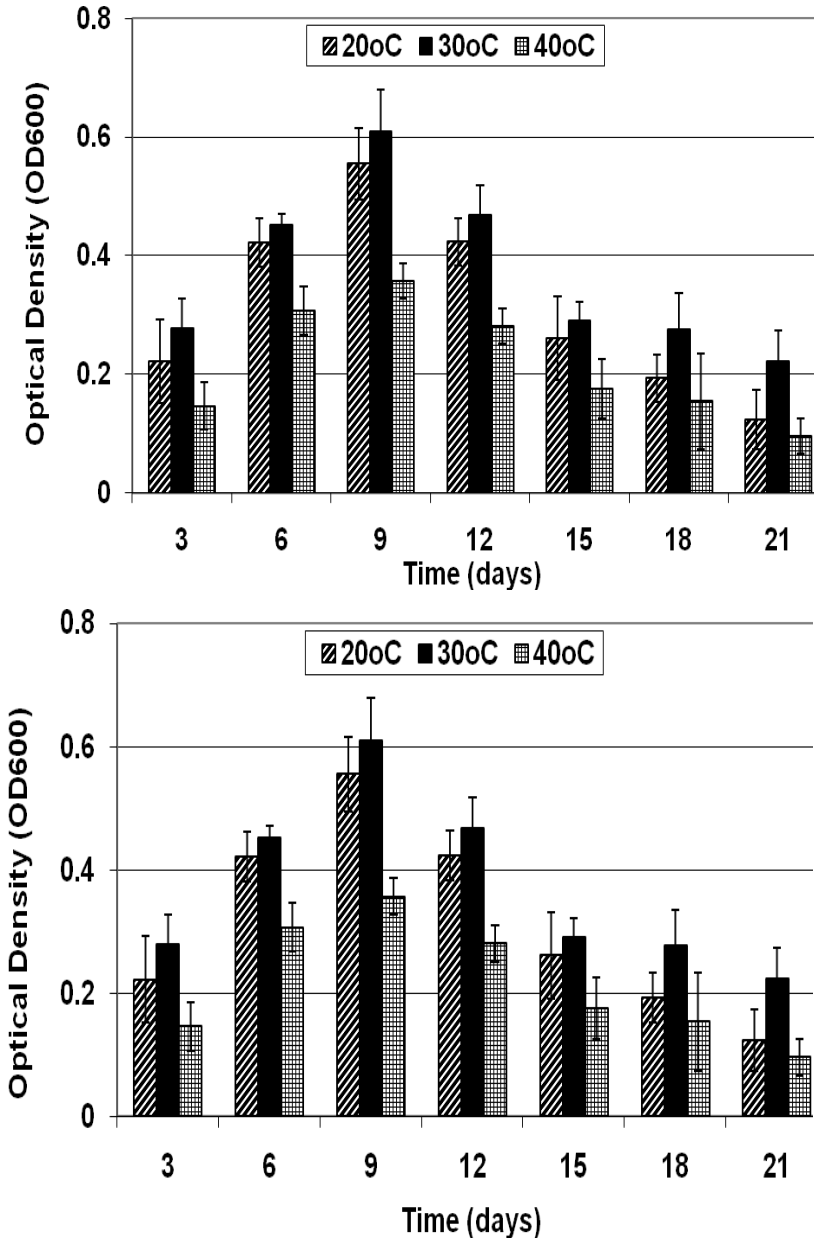

Fig. 5. Effect of temperature on the growth of *Pseudomonas aeruginosa*, (A) represents the optical density (OD_{600}) while (B) represents the protein content (mg/L) of the bacterial growth. Data are the means of three replicates and error bars represent the standard errors of the means.

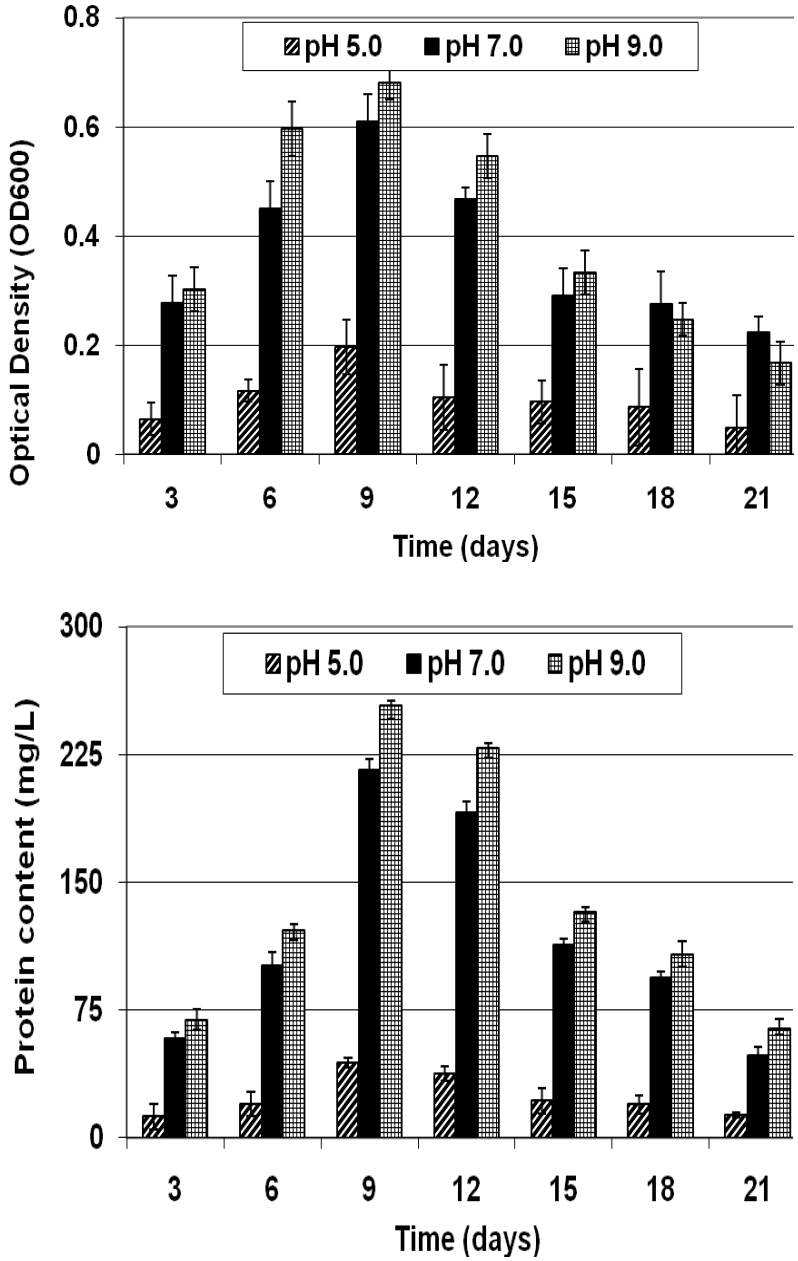

Fig. 6. Effect of pH value on the growth of *Pseudomonas aeruginosa*, (A) represents the optical density (OD_{600}) while (B) represents the protein content (mg/L) of the bacterial growth. Data are the means of three replicates and error bars represent the standard errors of the means.

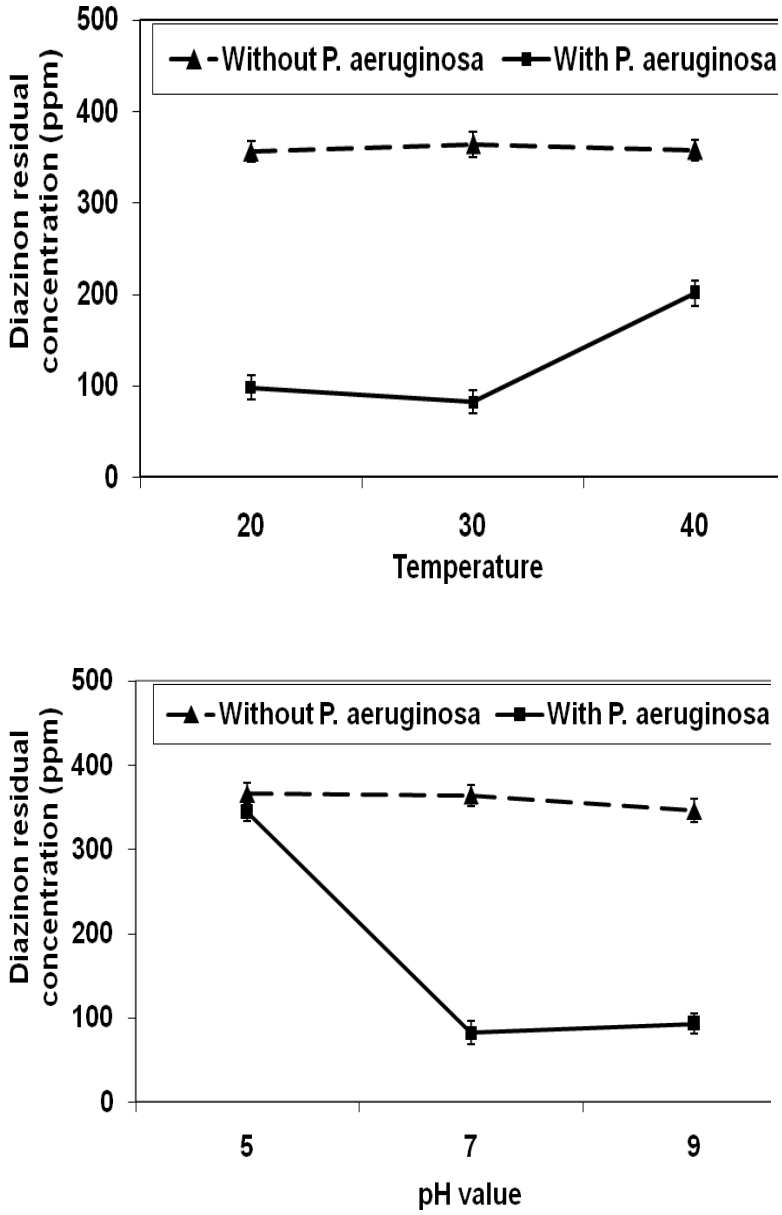

Fig. 7. Optimization of diazinon degradation by *Pseudomonas aeruginosa* where (A) represents the effect of pH value and (B) represents the effect of temperature on the biodegradation process after 14 days. Initial concentration of diazinon was 368 ppm. Data are the means of three replicates and error bars represent the standard errors of the means.

TABLE 2. The percentage of diazinon removal by *Pseudomonas aeruginosa* under different temperature and pH values after 14 days of incubation. The initial concentration was 368 ppm.

Treatment		Retention time (min)	Residual concentration of diazinon (ppm)	
			with *P. aeruginosa*	without *P. aeruginosa*
pH value	5.0	2.051	344 ± 8	366 ± 12
	7.0	2.048	82 ± 11	364 ± 10
	9.0	2.040	93 ± 9	346 ± 8
Temperature	20°C	4.616	88 ± 13	356 ± 12
	30°C	4.609	82 ± 10	364 ± 9
	37°C	4.638	201 ± 11	358 ± 8

Discussion

Isolation and identification of diazinon degrading bacteria

Microbial degradation is the primary route of the dissipation of the insecticide diazinon from the environment (Karpouzas and Singh, 2006). The bacterial isolate AAD that was isolated from agricultural drainage was highly tolerant to elevated concentrations of diazinon. Most agricultural fields are well drained, whereas hydrologic conditions in ditches can shift widely. Passage of agricultural runoff through vegetated drainage ditches has been shown to accumulate high levels of pesticides (Moore *et al*., 2008 and Moore *et al*., 2011). The microbial communities of the agricultural wastewater usually contain certain bacterial strains that can tolerate elevated concentrations of the toxic pesticides and might have the capability to mineralize these compounds (Tyler *et al*., 2013). Based on the morphological, biochemical assays in addition to 16S rDNA gene sequencing, AAD isolate was included in the genus *Pseudomonas* and closely related to the species *aeruginosa*.

Growth optimization and diazinon degradation of P. aeruginosa

The bacterial isolate *P. aeruginosa* demonstrated a marked potentiality to tolerate elevated levels of diazinon up to 500 ppm. It is well known that *Pseudomonas* is highly active bacteria and has the ability to utilize wide range of organic compounds. A number of studies recorded the isolation of *Pseudomonas* strains from different environments contaminated with diazinon and other organophosphorus pesticides (Sorensen *et al*., 2008; Ortiz-Hernandez *et al*., 2013).

This study showed that the growth of *P. aeruginosa* was increased by increasing diazinon concentration up to 500 ppm then the bacterial growth was decreased at higher concentrations.. These findings are in harmony with those obtained with Abo-Amer and Aly (2011) who studied the effect of diazinon concentration on the growth of a diazinon degrading bacteria *Serratia marcescens*. This strain showed a high ability for the utilization of diazinon as

carbon and phosphorus source. At low diazinon concentrations, complete degradation was achieved within 9 days while at higher concentrations only 40% was degraded within 16 days. Moreover, Mahiudddin *et al*. (2014) demonstrated similar results for the degradation of diazinon by the bacterial isolates *Pseudomonas peli*, *Burkholderia caryophylli* and *Brevundimonas diminuta* within 12 days of incubation. Also, Cycon *et al*. (2009) reported an elevated rate of diazinon degradation by *Serratia* sp. and *Pseudomonas* sp. within 14 days when it was added to MSM at low concentration.

The persistence of diazinon in the environment is strongly influenced with the existence and activity of microorganisms. As a result of the bacterial degradation of diazinon, some of the released products could be used as a carbon and phosphorus sources by the degrading bacteria (Thabit and El-Naggar, 2013). At the same time, the rate of the diazinon degradation is directly influenced with the physicochemical factors such as temperature, pH, organic carbon and moisture content (Gunther, 1974). The present investigation showed that the maximum bacterial growth and diazinon degradation were achieved at 30^{o}C after 14 days of incubation. Meanwhile a clear inhibition in the microbial growth and diazinon degradation rate were recorded at high temperature levels These results are in agreement with those reported by Abo-Amer (2012) who studied the effect of temperature on the degradation of the organophosporus pesticides by *Pseudomonas sp* isolated from agricultural soils. It was found that the maximum rate of pesticide degradation was recorded at 20°C and 37°C. At the same time, Abo-Amer and Aly (2011) showed that the optimal incubation temperature for diazinon degradation by *Serratia marcescens* was recorded between 25^{o}C and 30^{o}C while, a low degradation rate was demonstrated at extreme temperatures.

Similarly, the highest bacterial growth rate and diazinon degradation were recorded at pH 7.0 within 14 days. These outcomes are in harmony with the studies of Abo-Amer and Aly (2011) who recorded remarkable changes in degradation rates of diazinon by *Serratia marcescens* at different pH values. Diazinon was completely degraded on within 11 days at pH values between 7.0 and 8.0 while, the degradation rate was sharply inhibited at pH 5.0 and pH 10.0. In the same way, Drufovka *et al*. (2008) found that pH value had a clear influence on microbial degradation of chlorpyrifos, fenitrothion and parathion.

Conclusion

A diazinon tolerant bacterial strain was isolated from agricultural drainage ditches by enrichment technique. This strain that was identified by 16S rDNA techniques as *Pseudomonas aeruginosa*, showed a high capabilty to utilize diazinon as a sole carbon source. A remarkable rate of diazinon degradation was achieved at pH value 7.0 and temperature 30°C within 14 days. As a consequence, *P. aeruginosa* could be used effectively for the environmental cleanup of agricultural wastewater contaminated with high levels of diazinon and to minimize the levels of such these insecticides carried downstream ecosystems.

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التحلل البيولوجي للمبيد الحشري الفسفوري الديازينون باستخدام بكتريا *Pseudomonas aeruginosa* المعزولة من قنوات تصريف المياه الزراعية

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يعد الديازينون احد المبيدات الحشرية الفسفورية التي تستخدم عادة للسيطرة على مختلف الآفات الزراعية والمنزلية وكثيرا ما توجد كملوثات عضوية في المياه. في هذه الدراسة تم عزل بكتيريا ذات مقدرة لتحمل تركيزات مرتفعة من هذا المبيد من قنوات الصرف الزراعي (الفيوم، مصر). تم تعريف هذه البكتريا علي انها *Pseudomonas aeruginosa* استنادا لدراسة الخصائص المورفولوجية والبيوكيمائية والتسلسل الجيني 16S rDNA. لقد تم زراعة هذه البكتريا على بيئات معدنية مثراة بالديازينون كمصدروحيد للكربون. كما تناولت هذه الدراسة تأثير تركيز الديازينون وكذلك درجة الحرارة و والرقم الهيدروجيني على نمو البكتيريا ومعدل التحلل البيولوجي للديازينون. وقد تحققت القدرة القصوى لتحلل الديازينون (٨٣,٦٪) عند تركيز ٤٠٠ جزء في المليون والرقم الهيدروجيني ٧,٠ ودرجة الحرارة ٣٠° في غضون ١٤ يوما. لذلك توصى هذه الدراسة باستخدام هذه السلالة البكتيرية لتنظيف البيئة من مياه الصرف الزراعي الملوثة بمستويات عالية من المبيد الحشري الديازينون.