Biodegradation of Chlorpyrifos by Microbial Strains Isolated from Agricultural Wastewater

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Abstract: Biodegradation of chlorpyrifos in mineral salt liquid (MSL) medium under different environmental factors such as pH of media, temperature and different concentrations of pesticide were investigated to optimize the conditions for biodegradation of chlorpyrifos by microbial strains. Isolating the microbial strains for identifying those having high chlorpyrifos degradation capability in liquid culture was undertaken as well. Out of thirteen microbial isolates from agricultural wastewater samples collected from different pesticides-polluted locations in Egypt, three isolates were only capable of degrading chlorpyrifos and utilizing it as a sole source of carbon and phosphorus. Isolates' biomass and chlorpyrifos degradation were found to be optimum at 30 °C and pH 7 (for bacteria and actinomycete), and pH 6 for fungi. These strains were identified as *Bacillus* sp. SMF5, *Penicillium* sp. F09-T10-1 and *Streptomyces thermocarboxydus* strain A-B based on morphological and biochemical tests as well as 16S rDNA analysis. It was also confirmed that biodegradation potential of the microbial isolates influenced by a range of abiotic factors such as pH of media, temperature and different concentrations of the tested pesticide. The rate of degradation for chlorpyrifos was faster in all inoculated samples relative to the corresponding control. Results showed also that chlorpyrifos was degraded faster by bacteria than fungi followed by actinomycete compared to its respective uninoculated water after 28 days. The dissipation of chlorpyrifos was coinciding with increasing biomass growth in chlorpyrifos contaminated water. Thus, the results from the present study confirmed that the newly isolated chlorpyrifos-degrading isolates can be successfully used for bioremediation of chlorpyrifos-contaminated water.

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1. Introduction

Environmental pollution caused by pesticides and their degradation products is a major ecological problem (Guliy et al., 2003). It has been documented that organophosphorus pesticides (OP) constitute the largest group of pesticides used globally account for about 38% of the total pesticides used worldwide (Singh and Walker, 2006). Chlorpyrifos is a broad-spectrum insecticide which considers as one of the most frequently used chlorinated organophosphorus pesticides (Maya et al., 2011). It represents the highest consumed pesticide in Egypt (about 1280 ton annually according to 2011' Agricultural Pesticides Committee statistics, Ministry of Agriculture & Land Reclamation). Its massive application has led to the contamination of water and soil, and disruption of biogeochemical cycles (Chishti et al., 2013). In addition, its residues have been detected in various ecological systems (Xue et al., 2005). Therefore, early detection and subsequent decontamination and detoxification of the polluted environment are essential. A number of methods, including chemical treatment, volatilization, photodecomposition and incineration, can be applied for the detoxification of chlorpyrifos (Muhammad, 2010). However, most of them are not applicable for diffused contamination at low concentration because they are expensive, inefficient and not always environmental friendly. Bioremediation offers several advantages over the conventional chemical and physical treatment technologies, especially for diluted and widely spread contaminants (Iwamoto and Nasu, 2001). Bioremediation, which involves the use of microbes to detoxify and degrade pollutants, has received increased attention as an effective biotechnological approach to clean up polluted environments (Belal et al., 2008). Studies of microbial degradation are useful for the development of bioremediation processes to detoxify pesticides to concentrations lower than the standards established by regulatory authorities (Vidali, 2001).

Microbial degradation of OP pesticides has been recognized as the most important process controlling their environmental fate (Eissa et al., 2006; Karpouzas and Singh, 2006). Therefore, biodegradation using native microorganisms for

pesticides removal from the environment is quite attractive (Maya et al. 2011). Success or failure of bioremediation depends on several factors such as the competitive ability of the bioremedial agents, bioavailability of pollutants and abiotic factors such as pH (Singh et al., 2003), temperature and moisture (Awasthi and Prakash, 1997), oxygen, redox potential and nutrients (Holden and Firestone, 1997), inoculated density and pesticide formulation (Duquenne et al., 1996). Biodegradation of chlorpyrifos in water is of high significance because of its toxicity to aquatic life and the complex nature of persistent metabolites. However, very little is known about the biodegradation of chlorpyrifos in water (Lu et al., 2006).

Therefore, the objectives of this study were to isolate the microbial strains from contaminated agricultural wastewater samples, characterize their degradation potential of chlorpyrifos in the liquid culture medium, and to investigate effect of the tested compound concentrations, and other factors affecting the microbial adaptation.

2. Materials and methods:

2.1. Chemicals:

Analytical grade standard of chlorpyrifos [O,O-diethyl O-(3,5,6-trichloro-2-pyridinyl) phosphorothioate] with a purity of 99% was provided by the U.S. EPA (Fig. 1). All other organic solvents and chemicals were of analytical grade and purchased from standard commercial suppliers.

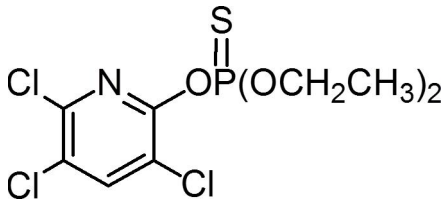

Fig. 1: Chemical structure of the chlorpyrifos pesticide

2.2. Wastewater samples:

Four agricultural wastewater samples were collected from four drainage canals located at two Egyptian governorates [i.e., El Sharkiya (Bilbes centre) and El Beheira (Eldelengat centre)]. Samples were transferred to the laboratory immediately, then extracted, cleaned up and analyzed as described afterwards. As shown in Table (1) chlorpyrifos was the most frequently detected pesticide since it was detected in 75% of wastewater samples collected from four agricultural drainage canals located in El Sharkiya and El Beheira governorates, Egypt. Accordingly, this pesticide was selected for conducting this research work.

Table 1: The concentration of detected pesticides in wastewater samples collected from four agricultural drainage canals located in two Egyptian governorates.

Sample No. (Location)	Detected pesticides	Concentration (mg l^{-1})
1 (El Sharkiya)	Oxamyl	0.002
	Chlorpyrifos	0.01
2 (El Sharkiya)	Chlorpyrifos	0.005
3 (El Beheira)	Aldicarb	0.004
4 (El Beheira)	Chlorpyrifos methyl	0.007
	Chlorpyrifos	0.005
	Pirimiphos methyl	0.001
	Diazinon	0.01

2.3. Isolation and purification of the most common microbes:

The populations of the total viable bacteria, fungi and actinomycetes were isolated by inoculating 1ml of the three analyzed samples that contain chlorpyrifos pesticide on nutrient agar medium (Difco, 1985), Dox medium (Allen, 1959) and starch nitrate agar medium (Waksman, 1961) for isolation and purification of bacteria, fungi and actinomycetes, respectively. Six replicates for each sample were incubated at 30°C for 3, 5 and 7 days for bacteria, fungi and actinomycetes, respectively (Reinhold et al., 1985). The most common microbes were selected according to their populations and characteristics.

2.4. Biodegradation of chlorpyrifos by the selected isolates:

Mineral salt liquid (MSL) medium supplemented with chlorpyrifos (10 mg l^{-1}) was used for biodegradation test**.** Cells were pre-cultured in broth medium harvested by centrifugation and washed 3 times with sterilized distilled water. For all preliminary experimental tests, the cells were used at a concentration of 10^6 cells ml^{-1} and samples were incubated on rotary shaker at 150 rpm and 30 ºC for 3, 5 and 7 days for bacteria, fungi and actinomycetes,

respectively. Medium without inoculation was maintained under the same conditions and served as controls. Further tests were carried out to select the microbes that have chlorpyrifos-degrading capability; dry weights of each microbial isolate and turbidity were measured according to Nephelometry A-12 method (APHA, 1995).

Afterwards, three selected microbial strains were cultured onto MSL medium supplemented with chlorpyrifos at three concentrations (20, 30 and 40 µg ml^{-1}) for 28 days. The cultures were incubated at optimum pH and temperature for each isolate on rotary shaker at 150 rpm. The percentage of degradation and the half-life of chlorpyrifos were determined as described afterward. Control flasks of equal volume of MSL medium and chlorpyrifos without any microbial population were incubated in parallel at all intervals to assess abiotic loss. During the experiment, samples were collected periodically at 0, 7, 14, 21 and 28 days intervals of time for estimation of chlorpyrifos degradation.

2.5. Optimization of the cultivation conditions (pH and temperature):

2.5.1. pH :

The isolates were grown on MSL medium containing chlorpyrifos (10 mg l^{-1}) and tested for their ability to grow at different pH values 6, 7 and 8 for both bacteria and actinomycetes isolates and 4, 5 and 6 for the fungal isolates according to the method described by Zajic and Supplisson, (1972) to reach the optimum pH values for microbial growth.

2.5.2. Temperature:

To obtain the best growth, the isolates were maintained on the MSL medium containing chlorpyrifos (10 mg l^{-1}) and incubated at different temperature degrees i.e., 20, 30 and 40 °C for each microbial isolate.

The most active degrading isolates from each microbial group were further purified to be identified.

2.6. Identification of microbes:

The efficient chlorpyrifos degrading microbial strains were identified depending upon microscopic, Biolog and PCR (16S rDNA) assays. The complete sequences of the 16S rRNA genes (rDNAs) of three isolates were determined and compared by using several tree-making algorithms (1, 4, 17, 26) to establish their position within the evolutionary radiation encompassed by similar microorganisms. PCR amplification of 16s rDNA was performed on the three isolates as described by Kim et al., (1991). The amplified fragments were directly sequenced by using a *Taq* Dye Deoxy. Sequencing gel electrophoresis was carried out and nucleotide sequences were automatically obtained using an Applied Biosystems DNA sequencer (model 37314) and software provided by the manufacturer.

2.7. Analytical procedure:

2.7.1. Extraction:

A known volume of a mineral salt liquid (MSL) medium (80 ml), for each time interval, was transferred into 500 ml separatory funnel and partitioned successively three times with 50 ml dichloromethane each and 40 ml of sodium chloride solution (20%). The combined extracts were filtered through a pad of cotton and anhydrous sodium sulfate then evaporated to dryness using a rotary evaporator at 30 ^{o}C, then the residue was quantitatively transferred to standard glass stopper test tube with ethyl acetate, and the solvent was evaporated just to dryness and the residue became ready for chromatographic determination. The residue half life value (RL_{50}) was calculated using the equation of Moye et al., (1987).

2.7.2. Chromatographic determination:

A Hewlett-Packard, USA serial 6890 gas chromatograph (GC) equipped with Flame Photometric Detector (FPD) operated in the phosphorus mode (526 nm filter) was used for determination of chlorpyrifos residues under the following conditions: Column: PAS-1701, 30 m length x 0.32 mm i.d. x 0.52 µm film thickness. Temperature (^{o}C): Detector: 260, Injector: 240, Column: 185. Gases flow (ml/min.): Nitrogen carrier gas: 3, Hydrogen: 75, Air: 100. Retention time for chlorpyrifos under these conditions was 5.027 minute as shown in Fig. (2).

2.8. Calculation of chlorpyrifos biodegradation:

The chlorpyrifos degradation was calculated by the following equation:

$$X\% = \frac{C_{ck} - C_x}{C_{ck}} \times 100$$

where, X is chlorpyrifos degradation; C_x is the concentration of chlorpyrifos (mg l^{-1}) in the medium that has chlorpyrifos degrading microbial strain; C_{CK} is the concentration of chlorpyrifos (mg l^{-1}) in the medium that does not contain chlorpyrifos degrading strain.

2.9. Statistical analysis:

Data were analyzed by one-way analysis of variance (ANOVA) and Duncan's multiple range test using SPSS (version 16.0; SPSS Inc. Chicago, IL, USA) to determine the significance of difference between the treatments on chlorpyrifos degradation in the MSL medium, and probability of 0.05 or less was considered significant. All the experiments were done in triplicates, and data were calculated as mean ± standard deviation (SD) using Excel (Microsoft, USA).

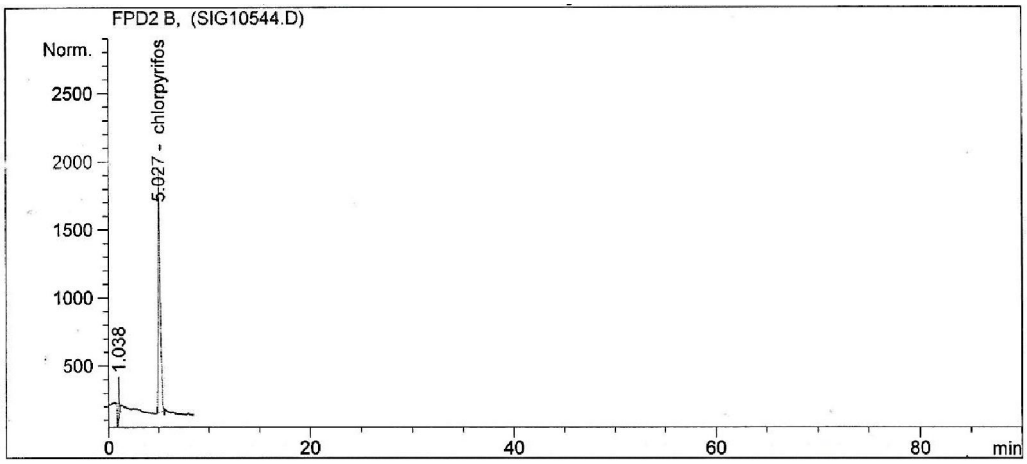

Fig. 2: GC- FPD chromatogram of the chlorpyrifos pesticide

3. Results and discussion:

3.1. Total count of the most common microorganisms:

Results in Table (2) indicated that the total microbial count of bacteria, fungi and actinomycetes in wastewater sample S_1 were higher than those in S_4. In addition, the populations of bacteria in S_1 were higher than fungi and actinomycetes recording $30x10^6$, $9 x10^6$ and $4 x10^6$ cfu/ml, respectively.

Table 2: Total microbial count in wastewater samples contaminated with chlorpyrifos.

Sample	Bacteria $x10^6$ cfu/ml	Fungi $x10^6$ cfu/ml	Actinomycetes $x10^6$ cfu/ml
S_1	30	9	4
S_4	28	8	2

Table 3: Effect of chlorpyrifos (10 mg l^{-1}) on growth of microbial isolates.

Microbial isolates	Dry weight (g)	Turbidity
B1	0.150	++
B2	0.140	++
B3	0.050	+
B4	0.201	+++
B5	0.179	++++
B6	0.322	+++++++
F1	0.222	+++++
F2	0.143	++++
F3	0.033	+
F4	0.154	++
F5	0.031	+
A1	0.179	+++
A2	0.060	++

B, F and A stand for bacteria, fungi and actinomycetes, respectively; whereas + represents intensity of turbidity.

3.2. Selection of the most common isolates:

The results in Table (3) revealed that the bacterial isolates B6 (0.322 g dry weight), B4 (0.201 g d.w.), B5 (0.179 g d.w.), fungal isolates F1 (0.222 g d.w.), F4 (0.154 g d.w.), F2 (0.143 g d.w.) and actinomycetes isolates A1 (0.179 g d.w.), A2 (0.060 g d.w.) were selected according to their highest dry weight and turbidity to carry out subsequent experiment to reach the optimum growth condition in terms of pH values and temperature degrees for each isolate in MSL medium containing chlorpyrifos (10 mg l^{-1}).

3.3. Effect of some environmental factors (pH and temperature) on chlorpyrifos degradation by the isolated microorganisms:

3.3.1. Optimum pH:

The optimum pH value for growth of bacterial isolates was pH 6 since the biggest dry weight as an indicator of the growth recording 0.153 g dry cells weight with B5 isolate (Table 4). At pH 7, B6 achieved the highest dry weight 0.149 g whereas at pH 8, B5 recorded the highest dry weight 0.117 g. As for fungi, the isolate F1 achieved the best dry mycelial weight (0.467 g) in comparison to the isolates F3 and F5 at pH 5. Regarding actinomycetes, the same trend like bacteria was recorded with isolate A1 at pH 6 achieving 0.845 g dry weight. Generally, the isolates B6 and A1 at pH 7, and F1 at pH 5 were considered the best degrading isolates. Vidali, (2001) reported that pH 5-8.8 was required for activities of most microbial strains in polluted soil and water with an optimum value for degradation properties often between pH 5 and 8.

Table 4: Effect of different pH values on the dry weight (g) of selected microbial isolates incubated with chlorpyrifos at 10 mg l^{-1}.

Microbial isolates	pH values				
	4	5	6	7	8
B4	-	-	0.081	0.104	0.072
B5	-	-	0.153	0.070	0.117
B6	-	-	0.080	0.149	0.077
F1	0.412	0.467	0.141	-	-
F3	0.091	0.046	0.102	-	-
F5	0.093	0.137	0.105	-	-
A1	-	-	0.845	0.172	0.186
A2	-	-	0.160	0.029	0.301

B, F and A stand for bacteria, fungi and actinomycetes, respectively.

3.3.2. Optimum temperature:

The highest dry weight of bacterial cells that grown on MSL medium was 0.833 g dry weight at 30 ºC with B6 isolate as presented in Table (5). At both 20 and 40 ºC the bacterial dry weight cells was low compared to 30 ºC. The optimum growth of the fungal isolates was recorded at temperature 30 ºC where the isolate F1 gave the highest dry weight of fungal mycelium compared with the two other isolates F3 and F5. At 20 and 40 ºC the mycelial dry weight of all isolates obtained less values. The same trend was recorded with isolate A1 where it also achieved the highest dry weight at 30 ºC (0.650 g). Many authors reported that the most rapid degradation of chlorpyrifos was observed at 30 ºC (Singh et al., 2004 and Racke et al., 1990), which is exactly similar to our results. Recently, it has been reported by many researchers that optimization of different environmental factors can play a vital role in accelerating the process of chlorpyrifos biodegradation (Vijayalakshmi and Usha, 2012; Liu et al., 2012).

Table 5: Effect of different temperature degrees on the dry weight (g) of selected microbial isolates incubated with chlorpyrifos at 10 mg l^{-1}.

Microbial isolates	Temperature degree		
	20 °C	30 °C	40 °C
B4	0.381	0.690	0.116
B5	0.030	0.706	0.108
B6	0.592	0.833	0.154
F1	0.211	0.830	0.148
F3	0.151	0.776	0.015
F5	0.100	0.349	0.092
A1	0.101	0.650	0.208
A2	0.310	0.461	0.163

B, F and A stand for bacteria, fungi and actinomycetes, respectively.

3.4. Identification of most potent microbes:

According to Biolog GN program and the 16S rDNA analyses, the phylogenetic tree of the chlorpyrifos degrader bacteria (B6), fungi (F1) and actinomycete (A1) based on the 16S rDNA sequence was provided in Fig. 3, 4 and 5, respectively. It was evident from the phylogenetic analyses that the bacteria isolate was most closely related to *Bacillus* sp. SMF5 and the fungi isolate was most closely related to *Penicillium sp* F09-T10-1, whereas the actinomycete isolate was most closely related to *Streptomyces thermocarboxydus* strain A-B since they showed the highest sequence similarities.

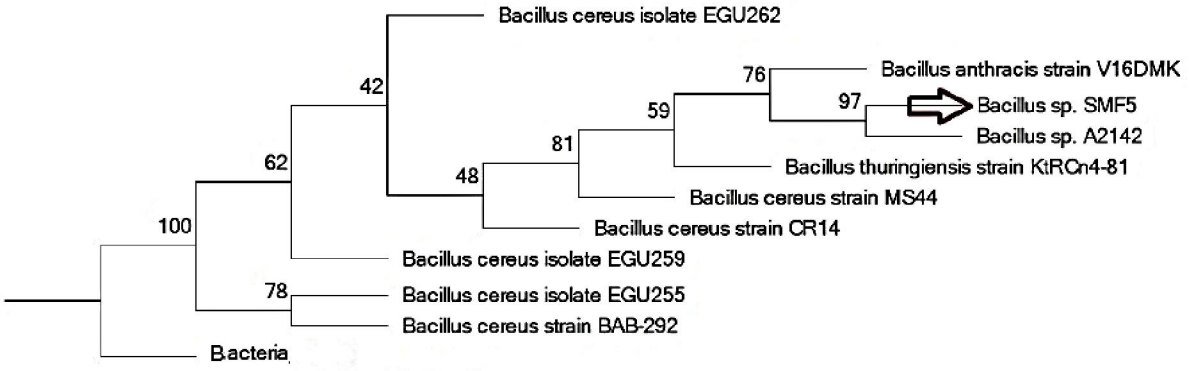

Fig. 3: Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain *Bacillus* sp. SMF5 among phylogenetic neighbors.

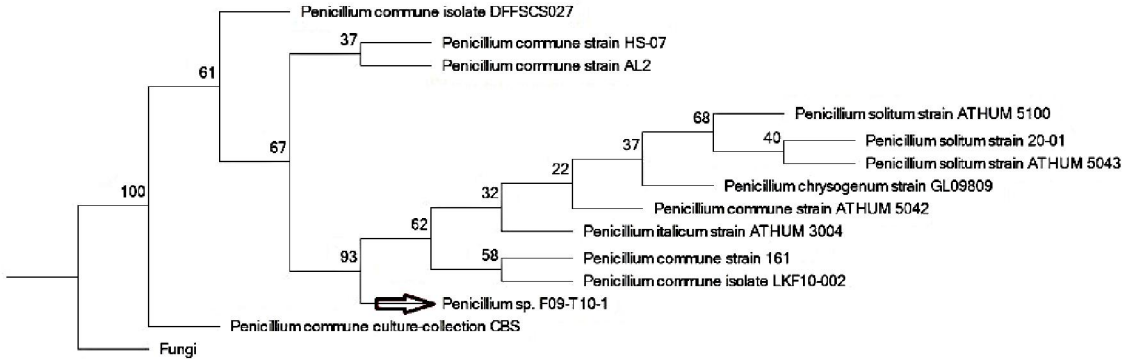

Fig. 4: Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain *Penicillium sp* F09-T10-1 among phylogenetic neighbors.

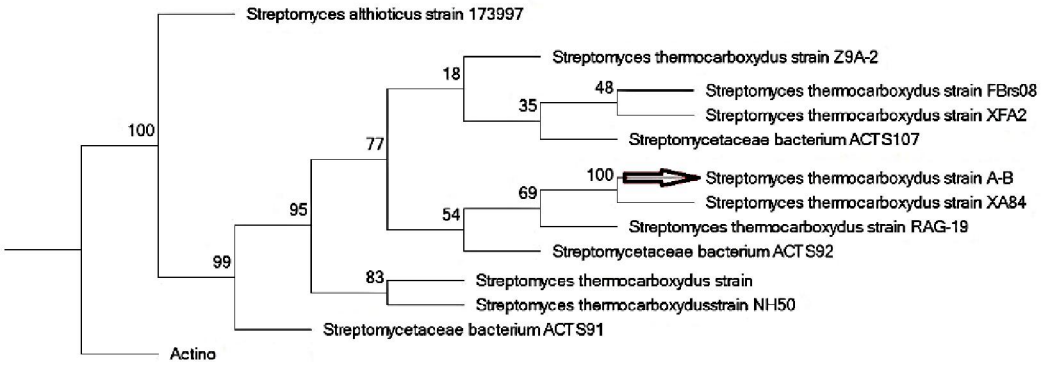

Fig. 5: Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain *Streptomyces thermocarboxydus* strain A-B among phylogenetic neighbors.

3.5. Degradation of chlorpyrifos in liquid medium by microorganisms:

The degradation of chlorpyrifos at different concentrations (20, 30 and 40 mg l^{-1}) was examined in the MSL medium on rotary shaker at 150 rpm, 30 °C and optimum pH for each isolate. As shown in Table 6, 7 and 8, there was an initial phase of slower degradation, which was longer at high concentrations. The chlorpyrifos-degrading isolates degraded concentrations of chlorpyrifos with a longer lag phase for the higher concentrations especially with *Bacillus sp.* SMF5 and *Streptomyces thermocarboxydus* strain A-B. There was also little degradation of chlorpyrifos at all concentrations in the uninoculated controls during 28 day incubation period since the hydrolysis percentages of chlorpyrifos were less than 15% in all controls. This difference on degradation performance of different strains to chlorpyrifos may be attributed to

microbial activity, incubation conditions and degradation pathway (Fang et al., 2008).

The inoculation of *Bacillus* sp. SMF5 resulted in a higher chlorpyrifos degradation rate than that of the control. In the first 7 days, the chlorpyrifos concentration dropped from 20 to 13.15 mg L^{-1}. After 14 and 21 day, chlorpyrifos was declined to 5.59 and 3.28 mg l^{-1}, respectively and by day 28 only 1.93 mg l^{-1} remained in the liquid medium.

Inoculation of chlorpyrifos-contaminated medium with *Penicillium* sp. F09-T10-1 resulted in a degradation of 29.51, 42.43, 61.17 and 91.25% within 7, 14, 21 and 28 day, respectively as compared to uninoculated medium.

Addition of *Streptomyces thermocarboxydus* strain A-B into the contaminated medium (20 mg l^{-1}) resulted in a more rapid rate of chlorpyrifos degradation compared to an uninoculated control. Table 6. demonstrates that approximately 15.44, 33.52 and 58% of chlorpyrifos were degraded within 7, 14 and 21 days, respectively. However, after incubation for 28 days, less than 3.85 mg l^{-1} chlorpyrifos was left in the MSL medium compared with 17.15 mg l^{-1} in uninoculated medium (control) and was statistically significant.

Table 6: Biodegradation of chlorpyrifos at initial concentration of 20 mg l^{-1} in mineral salt liquid (MSL) medium inoculated with microorganisms at 30 °C for different time intervals.

Incubation time (day)	*Bacillus* sp. SMF5		*Penicillium* sp. F09-T10-1		*Streptomyces thermocarboxydus* strain A-B		Control	
	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %
Initial	20.00	00.00	20.00	00.00	20.00	00.00	20.00	00.00
7	$13.15^{c}\pm$ 0.49	33.46	$13.93^{c}\pm$ 0.39	29.51	$16.71^{b}\pm$ 1.03	15.44	$19.76^{a}\pm$ 0.23	1.23
14	$5.59^{d}\pm$ 0.56	70.26	$10.82^{c}\pm$ 0.49	42.43	$12.50^{b}\pm$ 1.04	33.52	$18.79^{a}\pm$ 0.21	6.03
21	$3.28^{c}\pm$ 0.45	81.80	$6.99^{b}\pm$ 0.58	61.17	$7.55^{b}\pm$ 1.26	58.00	$17.99^{a}\pm$ 0.13	10.05
28	$1.93^{c}\pm$ 0.11	88.77	$1.5^{c}\pm$ 0.17	91.25	$3.85^{b}\pm$ 0.65	77.57	$17.15^{a}\pm$ 0.45	14.28
Degradation rate	0.059		0.044		0.046		0.005	
Half life (day)	11.57		15.75		15.07		128.36	

Means accompanied by different letters are significantly different for each treatment within the same row at the level ($P < 0.05$)

Table 7: Biodegradation of chlorpyrifos at initial concentration of 30 mg l^{-1} in mineral salt liquid (MSL) medium inoculated with microorganisms at 30 °C for different time intervals.

Incubation time (day)	*Bacillus* sp. SMF5		*Penicillium* sp. F09-T10-1		*Streptomyces thermocarboxydus* strain A-B		Control	
	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %
Initial	30.00	00.00	30.00	00.00	30.00	00.00	30.00	00.00
7	$19.89^{c}\pm$ 0.63	31.55	$15.61^{d}\pm$ 0.59	46.28	$24.58^{b}\pm$ 0.43	15.40	$29.05^{a}\pm$ 0.05	3.17
14	$10.76^{c}\pm$ 0.74	62.42	$11.43^{c}\pm$ 0.46	60.08	$21.85^{b}\pm$ 0.65	23.69	$28.64^{a}\pm$ 0.25	4.55
21	$8.17^{c}\pm$ 0.39	70.60	$6.12^{c}\pm$ 0.11	77.97	$13.01^{b}\pm$ 4.11	53.18	$27.78^{a}\pm$ 0.01	7.42
28	$2.29^{c}\pm$ 0.54	91.25	$2.05^{c}\pm$ 0.28	92.17	$7.23^{b}\pm$ 3.23	72.39	$26.17^{a}\pm$ 0.16	12.78
Degradation rate	0.073		0.093		0.039		0.005	
Half life (day)	9.49		7.45		17.77		138.63	

Means accompanied by different letters are significantly different for each treatment within the same row at the level ($P < 0.05$)

The knowledge on degradation kinetic at naturally relevant low concentration is important in order to give qualified estimates for the time needed in order to decrease contaminant concentrations to below the accepted limit of 0.1 µg l^{-1} for pesticides. Degradation kinetics are complex and for a given chemical within any ecosystem, rate and extent of degradation will possible be subject to daily, seasonal, and spatial variations. The half-lives of chlorpyrifos were significantly shortened by increasing its concentrations from 20 to 30 and 40 mg l^{-1} in *Penicillium sp.* F09-T10-1 inoculated medium representing 15.75, 7.45 and 6.66 day, respectively. In contrast, the half-lives of chlorpyrifos were almost linearly proportional to its concentrations i.e., 20, 30 and 40 mg l^{-1} in *Streptomyces thermocarboxydus* strain A-B inoculated medium recording 15.07, 17.77 and 20.39 day, respectively. However, in case of *Bacillus sp.* SMF5 inoculated medium the half-lives of chlorpyrifos were significantly went down from 11.57 day at 20 mg l^{-1} to 9.49 day at 30 mg l^{-1} then increased again to 16.91 day at 40 mg l^{-1}. The degradation rates constant of chlorpyrifos in inoculated medium with *Bacillus sp.* SMF5, *Penicillium sp.* F09-T10-1 and *Streptomyces thermocarboxydus* strain A-B, and uninoculated medium were found to be (0.059, 0.044, 0.046 and 0.005), (0.073, 0.093, 0.039 and 0.005) and (0.041, 0.104, 0.034 and 0.005) at 20, 30 and 40 mg l^{-1}, respectively.

Table 8: Biodegradation of chlorpyrifos at initial concentration of 40 mg l^{-1} in mineral salt liquid (MSL) medium inoculated with microorganisms at 30 °C for different time intervals.

Incubation time (day)	*Bacillus* sp. SMF5		*Penicillium* sp. F09-T10-1		*Streptomyces thermocarboxydus* strain A-B		Control	
	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %	Conc. (ppm)	Degradation %
Initial	40.00	00.00	40.00	00.00	40.00	00.00	40.00	00.00
7	$32.85^{c}\pm$ 0.65	16.31	$19.32^{d}\pm$ 0.09	50.78	$37.93^{b}\pm$ 0.43	3.36	$39.25^{a}\pm$ 0.25	1.87
14	$22.48^{c}\pm$ 1.42	41.44	$13.62^{d}\pm$ 0.36	64.52	$30.37^{b}\pm$ 0.46	20.89	$38.39^{a}\pm$ 0.66	4.03
21	$9.10^{d}\pm$ 0.05	75.06	$12.04^{c}\pm$ 0.03	67.00	$19.71^{b}\pm$ 0.51	45.98	$36.49^{a}\pm$ 0.88	8.78
28	$4.72^{c}\pm$ 0.72	86.48	$6.87^{b}\pm$ 0.47	80.32	$6.67^{b}\pm$ 0.72	80.93	$34.92^{a}\pm$ 0.45	12.71
Degradation rate	0.041		0.104		0.034		0.005	
Half life (day)	16.91		6.66		20.39		138.62	

Means accompanied by different letters are significantly different for each treatment within the same row at the level ($P < 0.05$)

Most OP compounds are degraded by microorganisms in the environment as a source of phosphorus or carbon or both (Karpouzas and Singh, 2006). Similar studies had been conducted by Yang et al., (2005) who reported that the degradation rate of chlorpyrifos (100 mg l^{-1}) by *Alcaligenes faecalis* DSP3 was 76.2% in the mineral salts medium of pH 7 at 30 °C after 18 days of incubation. *Bacillus pumilus* C2A1 degraded 89% of 1000 mg l^{-1} chlorpyrifos within 15 day (Anwar et al., 2009). *Sphingomonas* sp. utilized chlorpyrifos as its sole source of carbon for growth, by hydrolyzing chlorpyrifos to 3,5,6-trichloro-2-pyridinol (Li et al., 2007). Wang et al., (2006) reported that the degradation half-life of chlorpyrifos (1 and 10 mg l^{-1}) by *Bacillus latersprorus* DSP was measured to be 1.48 and 5.00 days in the mineral salts medium under conditions of pH 7 and 25 °C, and chlorpyrifos (100 mg l^{-1}) showed obvious inhibitive effect on degradation capability of the strain. Similar results were obtained by Shelton et al., (1996) who found that a strain of *Streptomyces* was able to grow on eight pesticides and also degraded them in soil. Biodegradation by fungi is also known as mycodegradation. Likewise, bioremediation in which fungi are employed is sometimes called mycoremediation (Singh, 2006). Only a few studies indicated that fungi degrade chlorpyrifos in liquid media. Bumpus et al., (1993) reported only 27.5% degradation of chlorpyrifos by *Phanerochaete chrysosporium* after 18 days incubation. Fang et al., (2008) found that the biodegradation rate of chlorpyrifos by the *Verticillium* sp. strain DSP was pH and temperature dependent and the maximum

degradation of 1 mg l^{-1} chlorpyrifos was achieved at pH 7.0 and 35 °C.

Chlorpyrifos degrading fungi *Acremonium* strain have utilized the compound as a source of both carbon and nitrogen (Kulshrestha and Kumari, 2011). Several fungi are known to produce enzymes that are able to degrade pesticides and have therefore been suggested as candidates for bioremediation (Entry et al., 1996). Fungi can account for up to 75% of soil microbial biomass (Harms et al., 2011) and their hyphae can grow to a length of 10^2-10^4 m g^{-1} soil (Ritz and Young, 2004). Fungi may play an important role in bioremediation of soil as the fungal hyphae can grow into micropores between soil aggregates and through air filled gaps (Wösten et al., 1999) and in this way gain a better access to the compound to be degraded.

Bacteria were the most enormously available decomposers and were able to degrade chlorpyrifos. Residual chlorpyrifos is considered to be critical as it can last for long periods of time in the environment depending on the initial concentration of pesticide and the biodegradation rate (Surekha et al., 2008; Nawaz et al., 2011). Bacteria and fungi can degrade chlorpyrifos either by using it directly as a source of C, N and P (Singh et al., 2004; Awad et al., 2011) or co-metabolically (Mallick et al., 1999). In co-metabolism, the target compound is transformed by a microorganism without using it or its constituent elements as a source of C and energy (Alexander, 1999). Various researchers have reported that chlorpyrifos in liquid media is degraded co-metabolically and attempts to isolate bacteria using chlorpyrifos as the sole source of C have not been successful (Guha et al., 1997; Mallick et al., 1999). Later on, several bacterial isolates capable of utilizing chlorpyrifos as the sole source of C, N or P were reported (Yang et al., 2006; Ghanem et al., 2007; Zhu et al., 2010).

The concentration, solubility and availability of pesticide to microbes are essential factors affecting the rate and extent of bioremediation. Fulekar and Geetha (2008) reported that *P. aeruginosa* (NCIM 2074) was well adapted to chlorpyrifos up to a concentration of 75 mg l^{-1} in a mineral salt medium, but higher concentrations were detrimental to the growth and survival of the bacterium. However, Li et al., (2007) demonstrated that the concentration of the chlorpyrifos had apparently no effect on the degradation rate, but when the concentration was higher than 200 mg l^{-1}, the bacterium stopped degrading its intermediate TCP and grew very slowly.

4. Conclusions

This study confirmed the diversity of chlorpyrifos-degrading microorganisms isolated from chlorpyrifos-contaminated agricultural wastewater in Egypt. As indicated by the kinetic constants, the three microbial isolates showed different capabilities for the biodegradation of chlorpyrifos. Moreover, this study would be helpful in the practical application of bioremediation of chlorpyrifos-contaminated water due to its low cost and less collateral destruction of indigenous organisms. Additional work is required for detecting the metabolites, mapping the degradation pathway and identifying the enzymes involved in the biodegradation process.

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