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Bioremediation of Certain Organophosphorus Pesticides by Two Biofertilizers, Paenibacillus*(Bacillus) polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck)

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ABSTRACT

Continuous and excessive use of organophosphorus compounds has led to the contamination of water and soil ecosystems. The degradation of organophosphorus insecticides, chlorpyrifos, chlorpyrifos- methyl, cyanophos and malathion in mineral salts media were studied. The effect of additional biofertilizers, singly or combined with organic amendments, on chlorpyrifos and cyanophos degrading activity in soil were investigated. *Paenibacillus (Bacillus) polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck) were found to degrade the organophosphorus insecticides, chlorpyrifos, chlorpyrifos- methyl, cyanophos and malathion in mineral salts media as a carbon and phosphorus source. *Paenibacillus (Bacillus) polymyxa* (Prazmowski) appeared to be more effective than *Azospirillum lipoferum* in degrading all the tested organophosphate pesticides in mineral salts media. The half-life values ($t_{1/2}$) of chlorpyrifos, chlorpyrifos – methyl , cyanophos and malathion were found to be undetectable , undetectable , 2.4, and undetectable days in mineral salts media inoculated by *Paenibacillus (Bacillus) polymyxa* (Prazmowski),while they reached 1.6, 0.1, 5.2, and 0.8 days by *Azospirillum lipoferum* (Beijerinck) compared to 4.4, 1.8, 8.8, and 1.4 days in non-inoculated mineral salts media. Chlorpyrifos and cyanophos degraded in soil samples inoculated by *Azospirillum lipoferum* (Beijerinck) plus peat- moss more rapidly than in the other treatments. Dual inoculation of *Azospirillum lipoferum* (Beijerinck) and *Paenibacillus (Bacillus) polymyxa* (Prazmowski) improved the rate of degradation of chlorpyrifos and cyanophos in soil. *Azospirillum lipoferum* (Beijerinck) appeared to be more effective than *Paenibacillus (Bacillus) polymyxa* (Prazmowski) in degrading soil-applied chlorpyrifos and cyanophos. These results highlight the potential of these bacteria to be used in the clean- up of contaminated pesticides – waste in the environment.

Keywords: Biofertilizer, Insecticides, Microbial degradation, Mineral soil.

INTRODUCTION

Organophosphorus pesticides are widely used worldwide to control agricultural and household pests. Overall, organophosphorus compounds account for about 38% of the total pesticides used globally (Singh and Walker, 2006). Continuous and excessive use of organophosphorus compounds has led to the contamination of several ecosystems in different parts of the world (Cisar and Snyder, 2000; Tse *et al*., 2004).

The metabolic fate of pesticides is dependent on abiotic factors (temperature, moisture, soil pH, etc.), microbial community and/or plant species, pesticide characteristics (hydrophilicity, pKa/b, etc.), and biological and chemical reactions (Kazemi *et al*., 2012). The environmental fate of chlorpyrifos has been studied extensively. Degradation in soil involves

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both chemical hydrolysis and microbial activity. The half-life of chlorpyrifos in soil varies from 10 to 120 days with 3, 5, 6-trichloro-2-pyridinol (TCP) as the major degradation product. This large variation in half-life has been attributed to different environmental factors, the most important of which are soil pH, temperature, moisture content, organic carbon content, and pesticide formulation (Racke *et al*., 1988). Initially, the high rate of chlorpyrifos degradation in soils with alkaline pH was attributed to chemical hydrolysis. Later, Racke *et al*. (1996) concluded that the relationship between high soil pH and chemical hydrolysis was weak and that other factors like soil silt content might be important in determining environmental fate. Biotic degradation is one of the most viable options for the remediation of chlorpyrifos in soil and water. Several researchers have focused on the microbial degradation, which has been reported as a primary mechanism of pesticide dissipation in the soil and water environment (Awad *et al*., 2011; Massiha *et al*., 2011). In some earlier studies, chlorpyrifos was reported to be resistant to biodegradation due to accumulation of the antimicrobial degradation products in soil (Racke *et al*., 1990). Later, several studies revealed that many microorganisms are capable of degrading chlorpyrifos efficiently (Singh *et al*., 2004, 2006; Zhu *et al*., 2010; Kulshrestha and Kumari, 2011; Liu *et al*., 2012). According to Floesser-Mueller and Swack (2001), cyanophos is not easily hydrolyzed and, thus, it is highly persistent and accumulates in various aquatic compartments such as rivers and lakes. Desmethyl-cyanophos, 4-Cyanophenol and desmethyl-cyanophos oxon are degradation products in soil (Chiba *et al*., 1976). Malathion is of low persistence in soil with reported field half-lives of 1 to 25 days (Wauchope *et al.,* 1992). Degradation in soil is rapid and related to the degree of soil binding. Breakdown occurs by a combination of biological degradation and non-biological reaction with water. If released to the atmosphere, Malathion will break down rapidly in sunlight, with a reported half-life in air of about 1.5 days (Howard, 1991). Hence, the removal of Malathion from water is one of the major environmental concerns. Chlorpyrifos-methyl is relatively stable in neutral media, but it is hydrolyzed under both acidic (pH 4–6) and more readily under alkaline (pH 8–10) conditions (Worthing and Hance, 1991).

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring beneficial soil bacteria that colonize the rhizosphere and plant roots resulting in enhancement of plant growth and protection against certain plant pathogens (EL- Kabbany, 2002; EL-Mancy and Kotb, 2006; Van Loon, 2007; Myresiotis and Vryzas, 2012). Currently, there is an increasing interest in testing PGPR-based products in agricultural crop production systems. These products are mainly applied as seed treatment, soil amendment, or soil drench at the time of seeding or immediately after transplanting, to promote plant growth and effectively suppress several diseases in a number of crops (Kloepper *et al*., 2004). Furthermore, much attention has recently been paid to bioremediation of contaminated soils with PGPR (Huang *et al*., 2004; Jiang *et al*., 2008). *Pseudomonas, Azospirillum, Agrobacterium, Bacillus, Enterobacter, and Flavobacterium* are some of the genera that include PGPR strains able to degrade organic and inorganic contaminants in soil (Zhuang *et al*., 2007; Hong *et al*., 2011). Phosphorus solubilizing microorganisms (PSM) such as *Paenibacillus (Bacillus) polymyxa* (Prazmowski) enhance P-availability in soil. Organic P is catalyzed by PSM through hydrolysis of C-O-P ester bonds by phosphatase or phytase to release soluble phosphorous, which are very important in the nutrition of plants (Illmer and Schinner, 1995). Co-inoculation of *Azospirillum* with PSM has been reported to improve growth and yield of many plants (Krishna, 2002). The addition of organic amendments to agricultural soil improves the efficiency of biofertilizers, when estimated in terms of plant growth and yield

(Requena *et al.*, 1997). Soil amended with organic nutrients, straw composts, rice straw and peat enhanced the population of *Azospirillum spp.* (Joseph and Dube, 1988).

To the best of our knowledge, limited data are available on *in vitro* biodegradation of soil-applied pesticides by PGPR strains and their effects on bacterial growth (Osman *et al.*, 2008). On the other hand, several works have focused on the effect of pesticides on the indigenous soil microbial community (Bending *et al.*, 2007; Wang *et al.*, 2008) but little is known regarding the effect of soil-applied pesticides on the introduced PGPR populations. Therefore, the present investigation was carried out to compare the capability of *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck) to degrade chlorpyrifos, chlorpyrifos- methyl, cyanophos and malathion in mineral salts media as a carbon and phosphorus source. Also, the interactions of biofertilizers, singly or combined with organic amendments, on chlorpyrifos and cyanophos degrading activity in soil were investigated.

MATERIALS AND METHODS

Pesticides and Biofertilizers

Chlorpyrifos (O, O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothionate), Cyanophos (O, O-dimethyl O-4-cyanophenyl phosphorothioate), Chlorpyrifos- methyl (O,O-dimethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate) and Malathion S-(1,2-dicarbethoxyethyl)-O,O-dimethyl-dithiophosphate) were obtained from the Central Agriculture Pesticide Laboratory, Agriculture Research Center, Dokki, Gaiza, Egypt.

Two strains of *Paenibacillus (Bacillus) polymyxa* (Prazmowski), phosphate dissolving bacteria, and *Azospirillum lipoferum* (Beijerinck), a symbiotic nitrogen fixing bacteria that exerts phyto-hormonal effects, were obtained from Biofertilizers Production Unit, Genetic Engineering Department, Faculty of Agriculture, Menia University, Egypt.

Biodegradation Experiments

Biodegradation of Pesticides in Liquid Medium

To study the degradation of chlorpyrifos, chlorpyrifos-methyl, cyanophos and malathion in liquid media, a stock culture of each *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck) was grown in nutrient medium for 48 hours to mid-log phase of growth. Each pesticide was added to pre-sterilized 100-ml Erlenmeyer flask at the concentration of 50 µg/ml in acetone. After evaporation of acetone, 50 ml of mineral salts medium (Mg $SO_4 7H_2O$, 0.2 g; $CaSO_4$, 0.4 g; $FeSO_4 7H_2O$, 0.001 g, and distilled water, 1 L, pH 6.5) were placed in 100 ml Erlenmeyer flasks and the flasks were shaken for two hours. The medium was inoculated with a suspension of the cells of *Paenibacillus (Bacillus) polymyxa* (Prazmowski) or *Azospirillum lipoferum* (Beijerinck) grown on nutrient media for 48 hours. The bacterial cultures were centrifuged at 8,000 rpm for 10 minutes and the precipitate was resuspended in sterile distilled water to obtain a final density of about 1×10^8 CFU (colony forming units) ml^{-1}. Bacterial concentration was determined by the plate counting method, in terms of CFU. Medium not inoculated with a bacterial suspension served as control. Both inoculated and uninoculated samples were incubated under intermittent shaking to provide aerobic condition. After 0.083, 1, 3, and 6 days , duplicate flasks from inoculated and uninoculated samples were withdrawn aseptically and analyzed for pesticide residues by HPLC after its extraction in hexane (Barcelo, 1991).

Interactions of Biofertilizers and Soil Applied Pesticides

Soil experiment was conducted with un-sterilized soil in order to study the interaction of biofertilizers *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck), singly or combined with organic amendments, with the endogenous microbial community on the degradation of soil applied chlorpyrifos and cyanophos as it happens in the field. Air-dried sieved clay loam soil (organic matter, 1.71%, pH 7.71, electrical conductivity 2.34 dS/m) was obtained from Aboutwala, Menia EL-Kamh province, Sharkia governorate, Egypt, and placed in plastic pots. Subsamples (500 g) of dry soil were weighed, placed in pots, and then treated separately with chlorpyrifos and cyanophos at the concentration of 10 µg g^{-1}. Soil experiment was divided into three sets. The first set was inoculated separately with the suspensions of *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) , *Azospirillum lipoferum* (Beijerinck) and mixture of the two bacterial cultures. The second set was amended with peat moss at the rate of 0.05 g kg^{-1} and mixed thoroughly, then inoculated separately with suspensions of *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck). The third set was prepared as a control without inoculum of bacterial culture and without peat- moss but treated with the pesticide. The bacterial suspension of each strain was inoculated into soil to give a final concentration of about 2×10^8 CFU g^{-1}. The inoculum was thoroughly mixed into the soil and the moisture content was adjusted by the addition of sterile distilled water to 60% of its maximum water holding capacity. All soil samples were incubated at 28±2ºC. Pesticide residues in soil samples from duplicate plastic pots were extracted 3, 7, and 14 days after incubation periods. Soil samples were extracted and cleaned up according to the method of Krause *et al.* (1986). Soil samples (25 g) were shaken mechanically with 50 ml of acetone-water (3:1) for one hour in 500 ml glass stopper bottle. The extract was filtered through a clean pad of cotton, then, 50 ml of filtrate was concentrated by using a rotary evaporator on water bath at 40ºC to remove acetone and then extracted twice with 50ml chloroform. The combined chloroform extract was dried using anhydrous sodium sulfate and then evaporated to dryness at 40°C using a rotary evaporator for HPLC determination.

High Performance Liquid Chromatography (HPLC) Analysis

Chlorpyrifos, chlorpyrifos- methyl, cyanophos and malathion residues were dissolved in 1.0 ml of methanol and an aliquot (10 µl) were analyzed by high-performance liquid chromatography (HPLC) with a UV-detector set at wavelength of 248 nm. A C18 column was used, and the mobile phase was a mixture of methanol and water (70:30, v/v). The flow rate was 0.7 ml min^{-1}. The retention time of chlorpyrifos, chlorpyrifos- methyl, cyanophos and malathion were 2.5, 3.20, 4.0, and 2.17 min, respectively. The extraction efficiency of the analytical procedure was evaluated via recovery experiments conducted in triplicate using the fortified blank liquid media and soil samples at one concentration level. Mean recovery values obtained for chlorpyrifos, chlorpyrifos- methyl, cyanophos and malathion were 95, 94, 96.31, and 94% in liquid media. Mean recovery values obtained for chlorpyrifos and cyanophos were 90.5 and 92.7% in the soil samples.

RESULTS AND DISCUSSION

As shown in Table 1, *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck) utilized chlorpyrifos , chlorpyrifos –methyl, cyanophos and malathion in mineral salts media as the sole carbon and phosphorus sources. *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski)appeared to be more effective than the *Azospirillum lipoferum* (Beijerinck)

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Table 1. Dissipation of insecticides in mineral salts media by *Bacillus polymyxa* and *Azospirillum lipoferum.*

Incubation times (days)	Uninoculated		*B. Polymyxa*		*A. Lipoferum*	
	(μg ml^{-1})	% loss	(μg ml^{-1})	% loss	(μg ml^{-1})	% loss
			Chlorpyrifos			
0.083	47.50	5.00	35.68	28.63	45.5	9.00
1	37.49	25.00	UND	100	29.67	40.65
3	29.49	40.42	UND	100	20.28	59.45
6	16.49	67.02	UND	100	4.45	91.10
Rate of degradation	9.69×10^{-7} sec^{-1}		Undetected		10.01×10^{-7} sec^{-1}	
$T_{1/2}$ (days)	4.4		Undetected		1.6	
			Chlorpyrifos- methyl			
0.083	43.22	3.36	33.29	33.42	38.12	23.76
1	24.56	50.87	UND	100	5.64	88.73
3	16.11	67.79	UND	100	UND	100
6	5.23	89.54	UND	100	UND	100
Rate of degradation	11.5×10^{-7} sec^{-1}		Undetected		54.18×10^{-7} sec^{-1}	
$T_{1/2}$ (days)	1.8		Undetected		0.1	
			Malathion			
0.083	44.15	3.7	34.95	30.09	40.44	19.11
1	29.75	40.5	6.62	86.76	11.76	76.46
3	11.69	76.63	UND	100	4.41	91.18
6	0.43	99.14	UND	100	UND	100
Rate of degradation	13.54×10^{-7} sec^{-1}		Undetected		32.89×10^{-7}sec^{-1}	
$T_{1/2}$ (days)	1.4		Undetected		0.8	
			Cyanophos			
0.083	46.81	6.38	35.83	28.34	39.56	20.88
1	42.76	14.47	28.90	42.21	32.94	34.11
3	36.98	26.03	18.49	63.01	28.89	42.21
6	28.89	42.21	12.14	75.73	21.38	57.24
Rate of degradation	7.01×10^{-7} sec^{-1}		7.74×10^{-6} sec^{-1}		8.2×10^{-7} sec^{-1}	
$T_{1/2}$ (days)	8.8		2.4		5.2	

in degrading all the tested organophosphorus insecticides. After 24 hours of incubation, chlorpyrifos and chlorpyrifos methyl were degraded 100 and 100% by *B. polymyxa* and, respectively, 40.65 and 88.73% by *Azospirillum lipoferum* (Beijerinck), compared to 25 and 50.87% in uninoculated control. *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck) caused 100% and 91.18% dislodge of malathion in mineral salts media in 3-days incubation period. During the same period, in the uninoculated control, the abiotic dissipation rates of malathion was 76.63%. Only 75.73 and 57.24% losses of cyanophos were recorded at the end of 6 days of exposure to *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) and *Azospirillum lipoferum* (Beijerinck) compared to 42.21% in uninoculated control (Table I). The half-life ($t_{1/2}$) values for chlorpyrifos, chlorpyrifos–methyl , malathion and cyanophos were found to be undetected, undetected, undetected, and 2.4 days in mineral salts media inoculated by *Paenibacillus (Bacillus) polymyxa* (Prazmowski), while the values reached 1.6, 0.1, 5.2, and 0.8 days by *Azospirillum lipoferum* (Beijerinck), compared to 4.4, 1.8, 8.8, and 1.4 days in uninoculated control (Table 1). Such rapid degradation indicated the enzymes involved in the degradation of

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phosphorothioate in chlorpyrifos, chlorpyrifos–methyl and cyanophos or phosphorodithioate in malathion were constitutive. The primary mechanism of microbial attack in phosphorothioate and phosphorodithioate seems to be hydrolyses of the ester linkage which destroys the toxicity of the compound by enzymes known as hydrolases, phosphotriesterases, or aryl dialkyl phosphatases (Qiao *et al*., 2003). The bacterial phosphotriesterases were reported to be the most promiscuous of all enzymes (Scott *et al*., 2008). Generally, they have a broad substrate range, being able to hydrolyze a number of related compounds. In addition to the hydrolysis of P–O bonds in phosphotriesters, they also could catalyze the hydrolysis of P–S bonds (Lai *et al*., 1995), P–F bonds (Watkins *et al*., 1997), P–CN bonds (Raveh *et al*., 1992), and C–O bonds in esters and lactones (Roodveldt and Tawfik, 2005). Datta *et al*. (1992) found that phosphorus solubilizing microorganisms such as *B. polymyxa, Pseudomonas striata* and *B. firma* enhanced P-availability in Indian soil through hydrolysis of C-O-P-ester bonds from organic P by phosphatase or phytase , which are very important in the nutrition of plants. Qiao *et al.* (2003) mentioned that 70.5% of malathion was degraded after 60 minutes and 79% after 90 minutes, compared to 83% of parathion after 6 hours and 13.4% monocrotophos after 2 hours by genetically– engineered enzyme (carboxyl esterase). It has been proved that bacteria *Flavobacterium sp*. ATCC 27551 (Mallick *et al*., 1999), *Enterobacter strain* B-14 (Singh *et al*. 2004, 2005), *Alcaligenes faecalis* (Yang *et al*., 2005), *Klebsiella sp*. (Ghanem *et al*., 2007), fungal *Verticillium sp.* (Fang *et al*., 2008), ,*Pseudomonas aeruginosa* (Vidya *et al*., 2009) and *Synechocystis sp.*(Singh *et al*., 2011) can degrade and utilize chlorpyrifos as a nutritional source. The degradation of acibenzolar-S-methyl by all PGPR tested in low and high concentration was, respectively, 5.4 and 5.7 times faster than that in non-inoculated liquid culture medium (Myresiotis and Vryzas, 2012).

Data in Table 2 shows the effects of biofertilizers *Azospirillum lipoferum* (Beijerinck) and *Paenibacillus (Bacillus) polymyxa* (Prazmowski), singly or combined with organic amendments, on the degradation of the soil applied chlorpyrifos

Table 2. Degradation of soil applied chlorpyrifos and cyanophos by *A.lipoferum* and *B. Polymyxa*.

Treatments	Days after treatment					
	3		7		14	
	(μg g^{-1})±SD	% loss	(μg g^{-1})±SD	% loss	(μg g^{-1})±SD	% loss
			Cyanophos			
Without inoculum	7.34±0.41a	25.3	6.06±0.2a	39.4	3.41±0.11a	65.9
A. lipoferum	5.74±0.2c	48.3	2.91±0.04d	70.9	0.96±0.03d	90.4
A. lipoferum plus peat-moss	2.87±0.04e	51.3	0.64±0.01f	93.6	0.00±0.0e	100.0
B.polymyxa	6.38±0.31b	41.7	5.10±0.15b	49.0	2.51±0.04b	74.9
B. Polymyxa plus peat- moss	4.46±0.32d	43.8	3.47±0.1c	65.3	1.66±0.02c	83.4
A. lipoferum plus *B. Polymyxa*	4.42d±0.28d	46.8	1.28±0.02e	87.2	0.00±0.0e	100.0
	***		***		***	
			Chlorpyrifos			
Without inoculum	7.44±0.52a	25.3	6.34±0.42	36.6	4.41±0.26a	55.9
A. lipoferum	5.17±0.30e	48.3	3.78±0.22	62.2	1.51±0.02d	84.9
A. lipoferum plus peat-moss	4.87±0.20f	51.3	3.02±0.20	69.8	1.05±0.02f	89.5
B.polymyxa	5.83±0.22b	41.7	3.98±0.26	60.2	1.96±0.03b	80.4
B. Polymyxa plus peat- moss	5.62±0.26c	43.8	3.62±0.21	63.8	1.86±0.03c	81.4
A. lipoferum plus *B. Polymyxa*	5.32±0.18d	46.8	3.36±0.22	66.4	1.41±0.02e	85.9
	***		NS		***	

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and cyanophos. These two compounds degraded in the inoculated soil samples by *Azospirillum lipoferum* (Beijerinck) plus peat- moss more rapidly than in the other treatments, probably due to enhanced population grwoth of *Azospirillum spp.* by peat moss. After 14 days of treatment, the dissipation of chlorpyrifos and cyanophos was 89.5 and 100% in the soil inoculated with *Azospirillum lipoferum* (Beijerinck) plus peat-moss, while it was 81.4 and 83.4% in the soil inoculated with *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski), compared to 55.9 and 65.9% in non-inoculated soil. Soil amended with organic nutrients, straw composts, rice straw, and peat enhanced the population of *Azospirillum spp.* (Joseph and Dube, 1988). Gu *et al.* (2003) reported that addition of compost increased the rate of mineralization of atrazine because compost with nitrogen content of 1.14% probably decreased C/N ratio, creating a shortage of C in the soil so that microbes resorted to the use of atrazine as an energy source. Frenich *et al.* (2005) reported that during the composting process, the organophosphorus pesticides chlorpyrifos-methyl and malathion were almost fully degraded (more than 99%) as well as the organochlorine pesticide lindan. On the other hand, Karpouzas and Walker (2000), and Singth *et al*. (2005) reported that the high organic matter resulted in reduced degradation. Weber and Huang (1996) suggested that high organic matter could lead to reduced bioavailability of substrate to the degrading microorganisms, especially when the compounds have a high sorption coefficient (K_{oc}) value. Hydrophobic compounds become unavailable because they get entrapped in the solid phase of organic matter and also in nano-pores at specific sites. However, many degrading microorganisms produce surfactants or other emulsifiers that desorb chemical compounds from soil and make them bioavailable (Aronstein *et al*., 1991). Data in Table 2 cleared that chlorpyrifos and cyanophos dissipated in non-inoculated soil rapidly may be due to the alkaline nature of the soil (pH, 7.71) attributed to chemical hydrolysis and presence of endogenous microbial community in the un-sterilized soil (Mulchaldani *et al*., 1999; Ortiz-hernández and Enrique, 2010; Vryzas *et al*., 2012). The chemical nature of the pesticides and some factors, such as pH, light, metal ions, and ozone, also impact the degradation of pesticide residues (Bo *et al*., 2011).

Dual inoculation of *Azospirillum lipoferum* (Beijerinck) and *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) improved the rate of degradation of chlorpyrifos and cyanophos in soil. After 14 days exposure, chlorpyrifos and cyanophos were degraded by 85.9 and 100% in amended clay loam soil with *Azospirillum lipoferum* (Beijerinck) plus *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski), compared to 55.9 and 65.9% in non-inoculated control soil, respectively. The activities of both fungi and bacterial components of soil microflora caused complete mineralization of chlorpyrifos in the Australian soil (Singh *et al*., 2003). Application of pesticides, monocrotophos and chlorpyrifos, singly and in combination with mancozeb and carbendazim, up to 5.0 kg ha^{-1}, significantly increased the population of *Azospirillum* sp. after 7 and 14 days of incubation in vertisol soil (Srinivasulu *et al*., 2012).

Azospirillum lipoferum (Beijerinck) appeared to be more effective than *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) in degrading soil-applied chlorpyrifos and cyanophos, while in mineral salts media supplemented with chlorpyrifos or cyanophos, *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) was more effective than *Azospirillum lipoferum* (Beijerinck) in degrading chlorpyrifos or cyanophos (Tables 1 and 2). The reason for this discrepancy in the chlorpyrifos and cyanophos degrading ability of the two bacteria in the soil and mineral salts media is not clear. Probably *Azospirillum lipoferum* (Beijerinck) was more efficient than *Paenibacillus* (Bacillus) *polymyxa* (Prazmowski) in competing with the indigenous microorganisms in the complex

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soil environment (Mallick *et al.*, 1999). Success or failure of bioremediation depends on several factors such as the competitive ability of the bioremedial agents (Gunalan and Fournier, 1993), bioavailability of pollutants (Alexander, 2000) and a biotic factors such as soil moisture, pH, and temperature (Van-Veen *et al.*, 1997). Inoculated biofertilizers (phosphoren, microbien, cerealin, and *Azospirillum*) may act as potential candidates for soil inoculation to bioremediate pesticides contaminated soil (EL-kabbany, 2002). Successful removal of pesticides by the addition of bacteria (bioagumentation) has been previously reported for many compounds including, coumaphos (Mulbry *et al.,* 1998), ethoprophos (Karpouzas and Walker, 2000), dicofol (Khaled *et al*., 2008) and malathion (Kanade *et al*., 2012). Under the soil microcosm experimental conditions, the half-lives of acibenzolar-S-methyl incubated in the presence of PGPR strains spiked at 1.0 and 10.0 mg kg^{-1} were 10.3–16.4 and 9.2–15.9 days, respectively, markedly lower compared with 34.2 days in the control (Myresiotis and Vryzas, 2012).

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پالایش زیستی محیط از بعضی آفت کش های آلی فسفره با کاربرد دوکود زیستی (**Paenibacillus(** ***Bacillus) polymyxa*** **(Prazmowski)** و ***Azospirillum lipoferum*** **(Beijerinck)**

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چکیده

کاربرد پیوسته وزیاده از حد سموم آلی فسفره منجربه آلودگی زیست بوم های آبی وخاکی شده است.در این پژوهش، تجزیه آفت کش های آلی فسفره مانند کلرپیریفوس، کلرپیریفوس متیل، سیانوفوس و مالاتیون در محیط نمک های معدنی مطالعه شد و اثر افزودن کودهای زیستی به تنهایی یا همراه با بهساز های آلی روی تجزیه کلرپیریفوس و سیانوفوس در خاک هم بررسی شد. نتایج نشان داد

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که دو کود زیستی *Paenibacillus (Bacillus) polymyxa (*Prazmowski*)*و *Azospirillum lipoferum (*Beijerinck*)* در محیط نمک های معدنی، آفت کش های آلی فسفره شامل کلرپیریفوس، کلرپیریفوس متیل، سیانوفوس و مالاتیون را به عنوان منبع تامین کربن و فسفر تجزیه کردند. در محیط نمک های معدنی، باکتری *Paenibacillus (Bacillus) polymyxa (*Prazmowski*)* در تجزیه همه آفت کش های آلی فسفره مورد آزمون، کارآیی بیشتری از *Azospirillum lipoferum(*Beijerinck) نشان داد. در محیط نمک های معدنی که با باکتری *Paenibacillus (Bacillus) polymyxa (*Prazmowski تلقیح شده بود، نیمه عمر($t_{1/2}$) کلرپیریفوس، کلرپیریفوس متیل، سیانوفوس و مالاتیون به ترتیب غیرقابل تشخیص، غیرقابل تشخیص، ۲/۴روز ، و غیرقابل تشخیص بود در حالی که این اعداد در مورد *Azospirillum lipoferum(*Beijerinck) برابر ۱/۶،۰/۱، ۵/۲، و۰/۸ روز بود و در محیط نمک های معدنی تلقیح نشده به ۴/۴، ۱/۸، ۸/۸، و ۱/۴ روز رسید. کلرپیریفوس و سایانوفوس در خاک هایی که با *Azospirillum lipoferum(*Beijerinck) تلقیح شده و به آنها پیتماس اضافه شده بود سریع تر از تیمارهای دیگر تجزیه شدند. تلقیح دوگانه *Azospirillum lipoferum(*Beijerinck) و *Paenibacillus (Bacillus) polymyxa (*Prazmowski) نرخ تجزیه کلرپیریفوس و سایانوفوس را درخاک بهبود داد. همچنین، به نظر می رسید که در تجزیه کلرپیریفوس و سایانوفوس افزوده شده به خاک، باکتری *Azospirillum lipoferum(*Beijerinck) کارآمد تر از *Paenibacillus (Bacillus) polymyxa (*Prazmowski) بود. این نتایج حاکی از پتانسیل و استعداد این باکتری ها برای کار برد در پالایش آلودگی ها وضایعات آفت کش ها در محیط است.

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