The Asian Journal of Experimental Chemistry, **(June, 2010) Volume 5 Issue 1 : 36-39**

Research Paper :

Efficacy of four microbial cultures in dissipation of chlorpyriphos in mollisols

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Accepted : ***April, 2010***

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ABSTRACT

The efficacy of four microbial cultures strains *i.e.* P (*Fluorescent pseudomonas* sp.), S8 (*Sphingomonas* sp.), Cf (*Gordonia* sp.), S9P (*Consortia* of *Bacillus* and *Pseudomonas* sp.) was evaluated in dissipation of chlorpyriphos (O,O-diethyl-O-3,5,6–trichloro-2-pyridinyl phosphorothioate), an organophosphate insecticide in mollisols under laboratory conditions. The data obtained in dissipation of chlorpyriphos treated with four cultures in (0–15cm) depth soil was evaluated after eight consecutive samplings (0, 1, 3, 5, 7, 10, 15 and 30 days). The results indicate that the degradation of chlorpyriphos was at slower rate during the first 5 days but thereafter became faster from 7 to 30 days. Dissipation studies could be better accounted by biphasic pattern involving an initial slower and a later faster phase. The kinetics of dissipation of chlorpyriphos from soil was accounted by first order kinetics. As per the data obtained indicates that S9P consortia have shortest half life of 5.37 d with chlorpyriphos in intial phase while P, S8, Cf and control showed half life of 6.53, 9, 8.45 and 9.62 d. While in later phase culture P and S8 showed more effectiveness followed by S9P and Cf in comparison to control in dissipation of chlorpyriphos. Thus S9P consortia was found to be most potent in degradating chlorpyriphos in comparison with rest of cultures.

Key words : Microbial culture, Chlorpyriphos, Mollisols, Organophosphate insecticide

Chlorpyriphos (O,O-diethyl o-3,5,6 – trichloro-2-pyridinyl phosphorothioate) is used worldwide as an agricultural organophosphate insecticide .It is prominent acetylcholine inhibitor Chlorpyriphos alters cholinesterase, preventing it from inactivating Acetylcholine. As the acetylcholine builds up, the muscles of the body become over stimulated, leading to paralysis and death. Its environmental fate has been extensively studied and the reported half life in soil varies from 10 to 120 days (Singh, 2003). It is widely used for insect pest control on grain, cotton, fruit, nut and vegetable crops as well as lawn and ornamental plants, which has caused a wide range of soil contamination (EPA, 1997). The dissipation of Chlorpyriphos after a single application in soil and its effect on soil microbial biomass C and N, microbial population, microbial respiration and enzyme activity has been well investigated (Pandey and Singh, 2004). Soil microorganisms collectively decompose various xenobiotic compounds and return elements to the mineral state utilized by plants. They also play important roles in the dissipation of pesticides in the soil. Organophosphates have been extensively applied as alternatives to organochlorine compounds which possess long term persistence and high toxicity. Organophosphorus compounds such as chlorpyriphos rapidly undergo degradation by soil microorganisms, so they do not persist in the environment. To understand and control the enhanced biodegradation of organophosphates, it is important to know the individual degradation ability of microorganisms, the process of their acquisition of the ability, and their behavior in soil (Kazufumi *et al.,* 1996). Racke and Coats reported that the enhanced degradative phenomenon of microbes may exhibit much better degree of specificity in degradation of pesticides. Since, chlorpyriphos had shown adverse impacts on the environment therefore, it is critically important to develop different methods to enhance its degradation. The aim of this study is to determine the efficacy of four microbial cultures strains *i.e.* P (*Fluorescent pseudomonas* sp.), S8 (*Sphingomonas* sp.), Cf (*Gordonia* sp.), S9P (*Consortia* of *Bacillus* and *Pseudomonas* sp.) in dissipation chlorpyriphos in mollisols.

MATERIALS AND METHODS

Culture preparation:

Microorganisms were isolated from soil and were identified using various biochemical tests and prepared microbial cultures were provided by Department of Microbiology, G.B.Pant.University of Agriculture and Technology, Pantanagar, for studying potential of four bacterial cultures in dissipation of chlorpyriphos. The standard chlorpyriphos of 99.5% purity grade was obtained

from Dow Agro Sciences, Bangalore and the other solvents used were of analytical grade and purchased locally.

Soil samples:

Surface (0 – 15 cm) soil sample was collected from Practical Crop production (PCP) block located in G.B. Pant University of Agriculture and Technology, Pantnagar, India. The soil sample was air-dried in shade and passed through a sieve having openings of 2 mm. soil The samples were analyzed for organic C, pH and electrical conductivity in 1:2 soil water suspension following standard methods (Jackson, 1973). The experimental soil had silty clay loam texture, 14.5 g organic C and 25.2 g $CaCO_3$ kg^{-1} soil and a pH of 8.16.

Dissipation experiment:

50 g soil sample (0-15 cm depth) was taken in five plastic bags and 100 µg of chlorpyriphos was added in each bag. Then 2ml. of each microbial culture (P, S8, CF, S9P) was incubated individually in four bags. One bag was maintained as control (without culture). Thereafter 5 g of soil was taken after 0(1hr), 1, 3, 5, 7 10, 15 and 30 d of insecticide and microbial culture application and subjected to extraction and residue estimation by GC.

Extraction and cleanup:

Soil: 5 g of soil samples (0-15 cm depth) was taken in conical flask and extracted with 15 ml. of acetone for one hour by using mechanical shaker. It was then filtered by using Buchner funnel and the residue was washed thoroughly with 10 ml of acetone again. The combined filterate was concentrated in a rotary flash evaporator at 50°C and the residue was dissolved in n-hexane for clean up by column chromatography. For cleanup process the column was packed with 5g silica gel having a small quantity of anhydrous sodium sulphate at the bottom of centered disc in the column. The dissolved residue from above was loaded on to the column and eluted with n-hexane. The eluates were collected in a conical flask, concentrated in a rotary flash evaporator. The residue obtained was re-dissolved in 1 ml of n-hexane for the estimation of chlorpyriphos by gas chromatography.

Quantification analysis:

Chlorpyriphos residues was estimated by the Gas chromatograph (Chemito Tech. Ceres 800 plus) equipped a with packed column 10% SE 30 (8' length and 1.8' i.d) and an Electron Capture Detector for quantitative analysis of chlorpyriphos in the samples. The flow rate of N_2 (UHP grade) as carrier gas was maintained at a flow rate of 30 ml min^{-1}. The temperatures of column, injector and detector were maintained at 180^0C, 230^0C and 300^0C, respectively. One µl aliquot was injected for each analysis. The retention time of chlorpyriphos under the above conditions was 8.7 min. A calibration curve was plotted between peak area and known concentrations of chlorpyriphos solution (0-5 µg ml^{-1}). The concentration of chlorpyriphos in the eluates of dissipation experiments was calculated with the help of the calibration curve.

RESULTS AND DISCUSSION

The results obtained from the present investigation are below :

Effect of microbial cultures on the kinetics of chlorpyriphos dissipation:

The dissipation data were fitted to a first order kinetic equation and dissipation rate coefficients were computed.

$\ln Ct = -k t + \ln C_0$

$t_{1/2} = 0.693/k$

C = conc. of chlorpyriphos in mg kg^{-1} at time t.

C_0 = initial conc. of chlorpyriphos in mg kg^{-1}

k = degradation rate constant in d^{-1}

$t_{1/2}$ = half life of chlorpyriphos

The pattern of distribution of values clearly indicated that the dissipation of chlorpyriphos occurred in two phases, an initial phase which was noted during first 0-5 d (slower phase) followed by another phase which lasted up to 7- 30 d (faster phase). Therefore data were fitted to double phase first order kinetics. The computed half life as per the double phase first order kinetics is mentioned in Table 2. Comparatively values of coefficient of determination (R^2) obtained in case of double phase dissipation kinetics revealed that the dissipation of chlorpyriphos could be better accounted by an initial slower dissipation and later faster dissipation phases each one obeying first order kinetics. Initial dissipation is attributed to surface losses (runoff, volatilisation, and photodegradation) and leaching, whereas subsequent slower disappearance is related to abiotic and microbial

Table 1: General properties of soil

Soil type	Soil properties							
	Depths (cm)	Sand (%)	Silt (%)	Clay (%)	pH (1:2, soil water suspension)	EC (dS m^{-1} 1:2, soil water suspension)	Organic C (g kg^{-1})	Free iron oxide (g kg-1)
PCP	0-15	16.4	44.0	39.6	8.16	0.130	14.5	9.7

Table 2 : Regression equation and half life for first order dissipation of chlorpyriphos with four cultures

Treatments	Dissipation phases (Biphasis) (K)	Dissipation rate coefficient	Half life ($t_{½}$, d)	Regression coefficient (R^2) **
Control	Slower	0.072	9.62	0.83
	Faster	0.190	3.64	0.79
P culture	Slower	0.106	6.53	0.88
	Faster	0.247	2.80	0.84
S9 culture	Slower	0.077	9.00	0.91
	Faster	0.272	2.54	0.99
Cf culture	Slower	0.082	8.44	0.90
	Faster	0.218	3.17	0.93
S9P culture	Slower	0.129	5.37	0.92
	Faster	0.202	3.43	0.96

degradation in the soil. As per the data obtained indicates that S9P consortia have shortest half life of 5.37 d with chlorpyriphos in intial phase while P, S8, Cf and control showed half life of 6.53,9, 8.45 and 9.62 d (Table 2). While in later phase culture P and S8 showed more effectiveness in degrading chlorpyriphos. Cf and S9P represented slight effectiveness in dissipation of insecticide in later phase in comparision with control. Thus S9P contortia was found to be most potent in degrading chlorpyriphos in comparison to rest of cultures (Fig. 1). Bioremediation is a cost-effective method for degrading toxic compounds into innocuous products. Successful removal of pesticides by inoculation of bacteria had been previously reported for many compounds (Karpouzas and Walker,2000). Pesticide degradation in soil can be influenced by both biotic and abiotic factors, which act in tandem and complement one another in the microenvironment. Environmental conditions play an important role in the survival and proliferation of microorganisms as well as affecting chemical stability (Singh *et al.,* 2003) . Repated applications of pesticides may have adverse effect on soil microbial functional diversity and subsequently influence soil fertility and plant growth, which poses serious threat to sustainability of agricultural soils (Johnsen *et al.,* 2001). These results have implication in managing chlorpyriphos residues by application of microbial consortium although all bacterial strains utilize insecticides as their carbon source but it was observed from the earlier studies that the consortium is found to be better in degradation of any chemical rather than the single strain (Soni *et al*., 2008). Thus encouraging faster dissipation of insecticide from soil surface can further diminish the possibilities of chlorpyriphos leaching to groundwater.

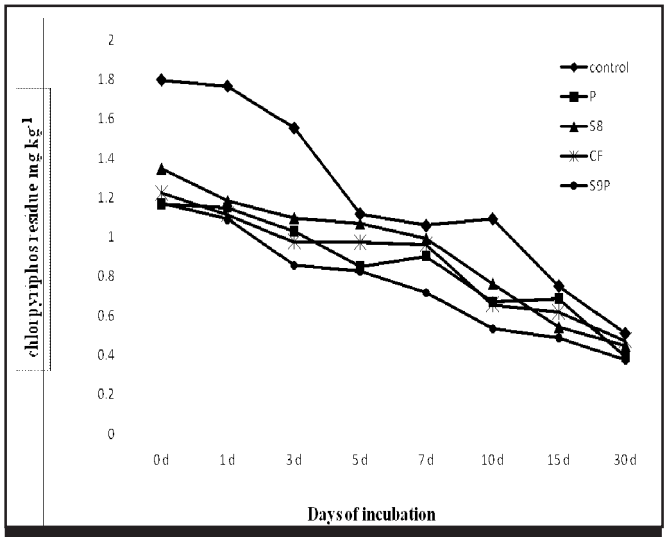

Fig. 1 : Dissipation of chlorpyriphos with four different culture treatments in soil

Acknowledgement:

Authors are pleased to acknowledge Dr. Anita Sharma, Assistant Professor, Department of Microbiology, G.B. PantUniversity of Agri and Tech. Pantanagar, for providing of four bacterial cultures which were used in dissipation study of chlorpyriphos.

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