KAVAKA 41: 15-17 (2013)

Fungi in Chlorpyrifos contaminated soil

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(Received 13 June 2013; Accepted on 17 September 2013)

ABSTRACT

Chloropyrifos (O,O-diethylO-3,5,6-trichloro-2-pyridyl phosphorothioate) is a broad spectrum, moderately toxic organophosphorous insecticide which is widely used in India on grain, cotton, vegetable crops etc.. as well as a termicide. There is growing concern about the toxicological and environmental risks associated with chlorpyrifos residues. The persistent nature of the insecticide is a health hazard, and thus, there is a need to detoxify this moiety. The present study deals with soil contaminated with chlorpyrifos and its mycoflora. A soil sample collected from the field was analyzed for chlorpyrifos concentration by HPLC and fungal diversity by soil dilution and pour plate technique. Fungal diversity was dominated by species of *Aspergillus* and other filamentous fungi. Actinomycetes were also common. All 7 fungi tested grew in the presence of different concentrations of CPF in culture media, ranging in concentrations from 0.001 to 0.1 mg / ml, indicating the bioremediation potential of these fungi.

Keywords: Chloropyrifos; pesticide; toxicology; fungi; *Aspergilli*; bioremediation

INTRODUCTION

Pesticides constitute the key control strategy for crop disease and pest management and have been making significant contribution in India towards improving the crop yield per hectare. However, the widespread use of these pesticides has resulted in problems caused by their interaction with biological systems in the environment (Singh and Walker, 2006), as the residues of applied pesticides stay in the environment for variable periods of time.

Chlorpyrifos (*O*, *O*-diethyl-*O*-3,5,6 trichloro-2-pyridyl phosphorothioate), a non-systemic, broad-spectrum, moderately toxic, soil-applied organophosphorus insecticide is used to control aphids, white fly, *Leptinotarsa* sp., *Dociostaurus maroccanus* and other insects in crop such as corn, fruits and vegetables (Linan 1994). It is also a termiticide at higher application dose of 1,000 mg kg"1 (Racke et al. 1994; Xu et al. 2008). It has high soil-absorption coefficient, but low water solubility (2mgL-1) (Racke 1993). The environmental fate of chlorpyrifos has been studied extensively and its degradation may involve a combination of photolysis, chemical hydrolysis and microbial degradation (Xu et al. 2008). Chlorpyrifos was resistant to biodegradation and remained effective for up to 5–17 years (Baskaran et al. 1999). It was suggested that the accumulation of 3,5,6-trichloro-2-pyridinol (TCP) which is the hydrolytic product of chlorpyrifos has anti-microbial properties and this prevents the proliferation of chlorpyrifos degrading microorganisms (Racke, 1993). Subsequently Jones and Hastings (1981) reported the metabolism of 50 parts per million (ppm) Chlorpyrifos to 3, 5, 6-trichloro-2-pyridinol (TCP) in cultures of several forest fungi (*Trichoderma harzianum*, *Penicillium vermiculatum*, and *Mucor sp*.).

Isolation and characterization of pesticide degrading microorganisms is crucial for enhancing our understanding of the variety of mechanisms and biodegradative pathways relating to their enhanced degradation in the environment. Chlorpyrifos, which was previously thought to be immune to enhanced biodegradation, has now been shown to undergo enhanced biodegradation by bacterial and fungal species. Bioremediation technologies are in the process of development for this toxic compound and related nerve agents using organophosphorus hydrolase enzyme. Future, studies on the genes responsible for enhanced biodegradation will enable us to elucidate the exact degradative pathway involved in its microbial biodegradation.

MATERIALS AND METHODS

Chlorpyrifos contaminated soil was collected from an agriculture field where chlorpyrifos had been used for

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several years on vegetables to protect against insects. Soil was analysed for the presence of insecticide chlorpyrifos by means of GC-MS. Serial dilution (1:10) was made in sterile water using 1gm of contaminated soil sample. The pour plate method was used, whereby 1 ml of each dilution was mixed with Potato dextrose agar and Saboraud's agar each and poured in Petri plates. Plates were incubated for a week at a room temperature of approximately 37ºC.

These fungal isolates were identified morphologically by slide culture technique, and the identification was confirmed at Agharkar Research Institute, Pune. The fungi were screened for survival in minimal media supplemented with chlorpyrifos at concentration 0.01mg/ml. The composition of the minimal medium was magnesium sulphate (0.20gm), Calcium chloride (0.02g/L), monopotassium phosphate (1.0gm/L), dipotassium phosphate (1.0gm/L), ammonium nitrate (1.0gm/L) and ferric chloride (0.05 gm/L). Those which survived were further tested for their tolerance level and growth at CPF concentrations of 0.001, 0.005, 0.01, 0.05 and 0.01 mg ml^{-1}.

RESULTS AND DISCUSSION

Analysis from freshly sprayed vegetable farm soil showed presence of chlorpyrifos at the concentration of 150mg/kg. The test soil was black, clayey and near neutral (7.6pH).

No	Name of organism	No. of colonies	% frequency
1	*Aspergillus niger*	16	13.22
2	*Aspergillus flavus*	22	18.18
3	*A. terreus*	02	1.65
4	*A. glaucus grp*	08	6.611
5	*A. glaucus grp*	08	6.611
6	*A. nidulans*	18	14.87
7	*A. oryzae*	03	2.47
8	Non sporulating hyaline form	04	3.30
9	*Penicillium funiculosum*	11	9.09
10	*Curvularia pallescens*	18	14.87
11	Actinomycete*s*	11	9.09

Table 1: Percent frequency of fungi in chlorpyrifos contaminated soil.

Mycoflora was dominated by species of *Aspergillus* (63.61%), followed by *Curvularia* (14.87). *Penicillum* and actinomycetes occurred in equal frquency (9.09%) (Table 1). Among the aspergilli, *A. flavus* topped the list with 22%, *A. nidulans* and *A.niger* were close behind at 18% and 16% respectively. Other species of this genus included *A. glaucus grp* 13.22%, *A.terreus* 1.65% and *A.oryzae* 2.47%. Abundance of aspergilli in soil contaminated with various pollutants is a common

FUNGI	Growth in different concentration(mg/ml) of CPF				
	0.001	0.005	0.01	0.015	0.1
Aspergillus niger	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+
A. terreus	+	+	+	+	-
A. glaucus grp	+	+	+	+	-

Table 2: Growth response of fungi in different concentrations of CPF

observation (Abd et al,2003; Mario et al,2002; Suman Kumari et al, 2010) and its wider reach in the soil has made the species of this genus a favourable biological system for various applications. *Curularia pallescens* was reported the next most abundant (14.87%) species in the present investigation. Frequency of *Penicillium funiculosum* (9.09%) was also appreciable. Nonsporulating hyaline forms constituted a small proportion of the fungal polulation. Beside fungi, Actinomycetes also showed healthy existence (9.09%) in chlorpyrifos contaminated soil. Out of the eleven species isolated, seven showed survival in simulated condition i.e. growth in presence of chlorpyrifos in medium. These seven fungi were grown in different concentrations of chlorpyrifos (Table 2). The isolates of *Aspergillus niger* and *Aspergillus flavus* both tolerated the maximum concentration 0.1mg/ml tested.

The results indicate that soil contaminated with insecticide chlorpyrifos forms a unique ecological niche for the fungal growth. Several fungi are known to grow on contaminated soil. Fungi could be potential candidates for bioremediation of chlorpyrifos contaminated soils due to their ability to degrade this pesticide .

However, the use of pesticide-degrading fungi for removal of organophosphorus compounds from contaminated environments requires a better understanding of ecological requirements of chlorpyrifos-degrading fungi. Further research is needed on biochemical and genetic aspects of chlorpyrifos-degradating fungi for bioremediation of chlorpyrifos-contaminated environments.

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