

Original Research Article

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Biodegradation of Pesticide using Fungi Isolated from Paddy Fields of Thanjavur District, India

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ABSTRACT

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Pesticide use raises a number of environmental concerns. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, including non-target species, air, water and soil. Although they improve the quality and yield of the agricultural product but do have certain serious effect on the environment. This study focuses on development of a method to reduce the environmental burden of the pesticide by way of biodegradation. In the present study, totally twenty two fungal isolates were screened. The isolates from agricultural soils were identified as filamentous fungi belonging to the phyla Ascomycota (7 genera), Deuteromycota (2) and Zygomycota (1). Out of twenty two isolates, only two isolates were highly degrade the pesticide (chlorpyrifos) and form high level of biomass by *Aspergillus fumigatus* (93.6, 93.3%) and *A. niger* (82.2, 77.4%) respectively.

Introduction

The pesticides belong to a category of chemicals used worldwide as herbicides, insecticides, fungicides, rodenticides, molluscicides, nematocides, and plant growth regulators in order to control weeds, pests and diseases in crops as well as for health care of humans and animals. The positive aspect of application of pesticides renders enhanced crop/food productivity and drastic reduction of vector-borne diseases (Damalas, 2009 and Agrawal *et al.*, 2010).

As a result of human activities, currently a large number of pollutants and waste are

eliminated to the environment. Worldwide, more than one billion pounds of toxins are released into the air and water. Approximately 6×10^6 chemical compounds have been produced; annually 1,000 new products are synthesized and between 60,000 and 95,000 chemicals are commercially used (Shukla *et al.*, 2010).

Worldwide approximately 9,000 species of insects and mites; 50,000 species of plant pathogens, and 8,000 species of weeds damage crops. Different pests such as insects and plants causing losses estimated

in 14% and 13% respectively. Pesticides are indispensable in agricultural production. About one-third of the agricultural products are produced by using pesticides. Without pesticide application the loss of fruits, vegetables and cereals from pest injury would reach 78%, 54% and 32% respectively. Crop loss from pest's declines to 35% to 42% when pesticides are used (Pimentel, 2007).

Pesticides can be classified by target organisms (e.g. herbicides, insecticides, fungicides, rodenticides, and pediculicides), chemical structure (e.g. organic, inorganic, synthetic, or biological, although the distinction can sometimes blur), and physical state (e.g. gaseous). Biopesticides include microbial pesticides and biochemical pesticides. Plant-derived pesticides or "botanical", have been developing quickly. These include the pyrethroids, rotenoids, nicotinoids and a fourth group that includes strychnine and scillirosides (Johnson and Ware, 1991).

Pesticides exposure inflicts chronic and acute threats to human health. For example, long term low dose exposure to pesticides cause immune suppression, hormonal disruption, diminished intelligence, reproductive abnormalities and carcinoma (Gupta, 2004). Among most of the important problems associated with pesticides application are their possible persistence in the environment and therefore, their possible incorporation in to the food chain affect ecosystem and human beings (Shelton and Somich, 1988).

Native microorganisms from soil and sediment are capable of degrading pesticides (Eizuka *et al.*, 2003) evaluated ipaconazole (triazole fungicide) degradation by soil microorganisms. They reported bacteria, actinomycetes and fungi were responsible

for the degradation of this compound. Singh *et al.*, (2008) isolated a native fungal strain from corn field soil able to use atrazine as source of nitrogen. The fungus degraded 44% of atrazine in 20 days.

Biodegradation that involves the capabilities of microorganisms in the removal of pollutants is the most promising, relatively efficient and cost-effective technology. Biodegradation is a process that involves the complete rupture of an organic compound in its inorganic constituents. The microbial transformation may be driven by energy needs, or a need to detoxify the pollutants, or may be fortuitous in nature (co-metabolism). Because of the ubiquitous nature of microorganisms, their numbers and large biomass relative to other living organisms in the earth, wider diversity and capabilities in their catalytic mechanisms (Paul *et al.*, 2005), and their ability to function even in the absence of oxygen and other extreme conditions the search for pollutant-degrading microorganisms, understanding their genetics and biochemistry, and developing methods for their application in the field have become an important human endeavour (Megharaj *et al.*, 2011).

Therefore the present study is directed towards isolating the fungal species which are capable of carrying out the pesticide degradation.

Materials and Methods

Collection of soil samples

The study area is situated in Thanjavur District of Tamilnadu state (Lat. 20° 0' - 10° 23' N-S and Long. 77° 0' - 78° 49' E-W). The present investigation was carried out by the collection and examination of soil samples from paddy fields of three different

stations viz., station I (Kayavur), station II (Kollakadu) and station III (Pathirankottai) in Thanjavur District.

Soil samples were collected from paddy field of the three villages, viz., Kayavur, Kollakadu and Pathirankottai of Thanjavur District, Tamil Nadu (Table-1)

The soil samples were collected from grids defined by 8 columns and 5 rows. The width of the column and rows were 8 m and 10 m respectively. The soil samples were taken as cubes to a depth of 20 cm, and the other two dimensions were 20 cm; thus the sampled spaces were 8000 cm³. In each grid, two soil cubes were taken. The cubes were one meter apart from each other, and the midpoint was the centre of the grid.

The soil cubes were immediately placed in a plastic bag, mixed and passed through a 2 mm sieve, brought to the laboratory and stored at 4°C, and then used for microbial analysis within 12 h of the sampling. For physico-chemical analysis, 1 kg of soil samples were air-dried and then sieved through a 2 mm sieve.

Isolation of soil fungi

The soil sample was serially diluted using distilled water. One ml of the diluted sample (10³ and 10⁴ dilution) was poured in to sterile Petri plates containing potato dextrose agar medium (PDA). The plates were incubated at room temperature (27±2° C) for 7 days. Three replicates were maintained for each sample. After incubation period, the mycelial growth was observed on PD agar plates then the organism was identified by using standard methods (Walksman, 1922).

Effect of pesticide

The mycelia plugs of isolated fungi were inoculated in to potato dextrose agar

medium along with different concentrations of pesticide, Chlorpyriphos 0.33 ml/100 ml (T1), 0.38 ml/100 ml (T2) separately and control also maintained. The plates were incubated at 27° C for 14 days.

After incubation, the mycelia mat was harvested, weighed using an electronic balance and to find out the wet weight of the fungal mycelium. Wet biomass of the fungal mycelium is accounted as the growth of parameter of the fungi. From the duplicate values, the mean value were derived and recorded.

Growth of the fungal isolates was calculated with the following formula

$$\frac{W_1}{W_2} \times 100$$

Whereas,

W2= Wet weight of mycelium in control

W1= Wet weight of mycelium in pesticide utilization trails.

Results and Discussion

Pesticides are usually applied simultaneously or one after another for crop production and this type of pesticide application often leads to a combined contamination of pesticide residues in the soil environment. A laboratory study was conducted to investigate the influence of chlorothalonil on chlorpyrifos degradation and its effects on bacterial, fungal, and Actinomycete soil populations (Chu *et al.*, 2008).

Repeated application of pesticides in the same field for a certain number of years developed an active microbial population in soil with the ability to degrade determined compounds (Hernandez *et al.*, 2008). Rohila and Salar (2012) reported that the total of 59

fungus isolates were obtained from the analyses of 23 soil samples taken from pesticide contaminated soils through soil dilution agar plating. Thirty three fungus isolates were identified as, *Aspergillus allhabadii*, *A. aultaceus*, *A. candidus*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. ochraceus*, *A.oryzae*, *A. sclerotiorum*, *Aspergillus* sp., *A. sulphureus*, *A. sydowii*, *A. terreus*, *Alternaria brassicola*, *A. exserohilum turcicum*, *Drechslera australiensis*, *D. halodes*, *D. hawaiiensis*, *Drechslera* sp., *Humicola insolens*, *H. brevis*, *Fusarium acuminatum*, *Fusarium* sp., *Rhizopus* sp., *Torula herbarum* and *Ulocladium* sp. The isolates were identified as filamentous fungus belonging to the class Ascomycotes (7 genera), Deuteromycotes (2) and Zygomycotes (1). Most of the fungus isolates were identified to the species level. *Alternaria*, *Aspergillus*, *Drechslera*, and *Fusarium* were predominant genera. *Curvularia*, *Exserohilum*, *Humicola*, *Rhizopus* and *Torula* were the most frequently isolated genera.

In the present study, totally 22 isolates were identified from all the three sampling station. Out of 22 isolates, 18 isolates were identified from Kayavur and Pathirankottai soil samples, 20 isolates were screened from Kollakadu soil samples (Table-2)

In the present study, to evaluate above mentioned fungus culture were treated with 0.33 ml/100ml and 0.38 ml/100 ml concentration of Pesticide (Chlorpyrifos). Among the twenty two isolates, *Aspergillus fumigatus* highly degrade the pesticide and form high level of biomass (93.6, 93.3%) in both concentration T1 and T2 and next to *A. niger* 82.2, 77.4% respectively. Among the twenty two isolates, five isolates were highly degrade the pesticide and form high level of mycelial biomass in T1

concentration such as *Aspergillus fumigatus* 93.6%, *A. repens* 89.4%, *A. terricola* 82.8%, *A. wentii* 89.5% and *A. niger* 82.2% were recorded. Other isolates were degrade the pesticide and form mycelial biomass at moderate level some of them at minimum level (Table-3)

Among the twenty two isolates, Five isolates were highly degrade the pesticide and form high level of mycelial biomass in T2 concentration such as *Aspergillus fumigatus* 93.3%, *A. avensceus* 81.6%, *A. niger* 77.4%, *A. terreus* 76.5% and *Penicillium granulatum* 71.8% recorded. Other isolates were degrade the pesticide and form mycelial mat in moderate level some of them at minimum level. Hussaini *et al.* (2013) also reported that four (4) fungus isolates were showed significant ability to carry out the degradation of selected pesticides, these cultures were identified as *Aspergillus niger*, *Ganoderma austral*, *Trichosporon*, *Verticillium dahaliae*. *Aspergillus niger*, has showed maximum (59%) degradation of Endosulfan, and it has degradaed (29%) of Lindane, it was not effective against other pesticides.

Ganoderma austral, has showed highest (61%) degradation for Lindane whereas for Chlorpyrifos degradation was only 16%. *Trichosporon* sp., has given highest degradation of Chlorpyrifos at 55%, and for Endosulfan and, Lindane was 10% and 7% respectively. For *Verticillium dahaliae*, maximum degradation (64%) of Chlorpyrifos, whereas for Endosulfan was 8%, for Malathion was 10% and for Lindane was 10%.

Similar work was reported by Thabit, *et al.*, (2014), the chlorpyrifos degradation was not significantly altered by its combination with chlorothalonil.

Table.1 Collection of soil sample from different irrigation paddy field of Thanjavur Dt.

Sample no	Irrigation	Place
1	Bore well	Kayavur
2	River	Kollakadu
3	Pond	Pathirankottai

Table.2 Isolation of microorganisms from the pesticide applied soil samples

S.no	Organisms	Kayavur	Kollakadu	Pathirankottai
1	<i>Aspergillus aureolatus</i>	+	+	+
2	<i>A.avensceus</i>	+	+	+
3	<i>A.arenarius</i>	+	+	+
4	<i>A.candidus</i>	+	+	+
5	<i>A. duricaulis</i>	-	+	+
6	<i>A.fumigatus</i>	+	+	+
7	<i>A.janus</i>	+	-	-
8	<i>A.niger</i>	+	+	+
9	<i>A.puniceus</i>	+	+	+
10	<i>A.panamensis</i>	+	+	+
11	<i>A.repens</i>	+	+	+
12	<i>A.sparsus</i>	+	+	-
13	<i>A.speluneus</i>	+	+	+
14	<i>A.subolivaceus</i>	-	+	+
15	<i>A.tamarii</i>	+	-	+
16	<i>A.terreus</i>	+	+	+
17	<i>A.terricola</i>	+	+	+
18	<i>A.ustus</i>	+	+	+
19.	<i>A.wentii</i>	+	+	-
20.	<i>Penicillium</i> sp.	-	+	+
21.	<i>Penicillium frequentans</i>	-	+	+
22.	<i>P. granulatum</i>	+	+	-

+ present

-absent

Table.3 Effect of pesticide (Chloropyriphos 0.33 ml/100 ml and 0.38 ml/100 ml) on various fungal growth

S.no	Name of the Species	Mycelial biomass (g)			Percentage of biomass	
		Control	T1	T2	T1	T2
1	<i>Aspergillus aureolatus</i>	3.2	1.7	0.5	53.1	15.1
2	<i>A.avenseus</i>	4.9	4.2	4.0	76.5	81.6
3	<i>A.arenarius</i>	6.2	5.1	4.9	85.7	25.7
4	<i>A.candidus</i>	3.0	1.9	0.4	63.3	11.1
5	<i>A.duricaulis</i>	2.9	2.1	1.4	72.4	48.2
6	<i>A.fumigatus</i>	6.3	5.9	5.6	93.6	93.3
7	<i>A.janus</i>	2.6	1.3	1.0	50.0	38.4
8	<i>A.niger</i>	6.2	5.1	4.8	82.2	77.4
9	<i>A.puniceus</i>	3.6	2.9	2.2	80.5	50.0
10	<i>A.panamensis</i>	2.5	1.7	1.5	68.0	60.0
11	<i>A.repens</i>	6.7	6.0	5.1	89.5	65.3
12	<i>A.sparsus</i>	1.3	1.0	0.4	76.9	22.2
13	<i>A.speluneus</i>	2.9	0.9	0.3	31.0	10.3
14	<i>A.subolivaceus</i>	3.9	3.1	2.6	79.5	44.0
15	<i>A.tamaritii</i>	2.9	1.5	1.0	51.7	30.3
16	<i>A.terreus</i>	5.9	3.9	3.6	66.1	76.5
17	<i>A.terricola</i>	3.8	3.4	2.9	89.4	54.7
18	<i>A.ustus</i>	1.8	0.5	0.2	27.7	4.2
19	<i>A.wentii</i>	3.5	2.9	2.2	82.8	26.5
20	<i>Penicillium</i> sp.	3.8	2.7	2.2	71.0	44.8
21	<i>Penicillium frequentans</i>	4.7	3.8	3.0	80.9	50.8
22	<i>P. granulatum</i>	3.9	2.6	2.2	66.6	71.8

A review of Briceno *et al.*, (2007) showed that various bacteria and fungi present in soils has the capacity to degrade or mineralize several pesticide groups. The studies showed that addition of organic amendment and nutrients can affect mainly the adsorption, movement, and biodegradation of pesticides. Repeated application of pesticides in the same field for a certain number of years developed an active microbial population in soil with the ability to degrade determined compounds.

The present study, concluded that the pesticides when applied in field rate, do not destroy the microbial biomass, but reduce the population level. Inadequate applications

of pesticides most of the sensitive beneficial microbes are eradicate by the pesticide. In this study some fungal isolates were well grew on pesticide applied media and form biomass up to 93.6%. So this research was preliminarily to degrade the pesticides using native fungal isolates.

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