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Research Article

Biodegradation Of Herbicide Using Fungi Isolated From Paddy Fields Of Thanjavur District

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Abstract

Herbicide use raises a number of environmental concerns. Day by day there is a increase in the use of herbicide due to labour demand. Ninety five percentage 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 herbicide 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 two genera among the 22 species 19 species belongs to *Aspergillus* species and 3 species belongs to *Penicillium* species. Out of twenty two isolates, only two isolates *Aspergillus fumigatus* (90.5, 82.5%) in both concentration T1 and T2 and *A. niger* (87, 79%) were highly degrading the herbicide (butachlor) and form high level of biomass respectively.

Keywords: Herbicides, Pollution, Fungi and Biodegradation.

Introduction

Herbicides, also commonly known as weed killers, are pesticides used to kill unwanted plants[1]. Selective herbicides kill specific targets, while leaving the desired crop relatively unharmed. Some of these act by interfering with the growth of the weed and are often synthetic "imitations" of plant hormones. Herbicides have widely variable toxicity. In addition to acute toxicity from high exposures there is concern of possible carcinogenicity[2].

As farmers continue to realize the usefulness of herbicides, larger quantities are applied to the soil. But the fate of these compounds in the soils is becoming increasingly important since they could be leached; in which case groundwater is contaminated or immobile, and persists on the top soil[3]. These herbicides could then accumulate to toxic levels in the soil and become harmful to microorganisms, plant, wild life and man[4].

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[5]. 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[6].

Herbicides remain the most effective, efficient and economical way to control weeds; and its market continues to grow even with the plethora of generic products. With the development of herbicide-tolerant crops, use of herbicides is increasing around the world that has resulted in severe contamination of the environment. The strategies are now being developed to clean these substances in an economical and eco-friendly manner[7].

For this reason, several biological techniques involving biodegradation of organic compounds by microorganisms have been developed[8]. The use of microorganisms, either naturally occurring or introduced, to degrade pollutants is called bioremediation[9]. In addition, they are robust organisms and are generally more tolerant to high concentrations of polluting chemicals than bacteria[10]. Therefore, certain fungi represent a powerful prospective tool in soil bioremediation and some species have already been patented[11].

Therefore the present study is directed towards isolating the fungal species which are capable of carrying out the herbicide 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,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 cm^3. 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 4ºC, 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.

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

Isolation of soil fungi

The soil sample was serially diluted using distilled water. One ml of the diluted sample (10^3 and 10^4 dilution) was poured in to sterile Petri plates containing potato dextrose agar medium (PDA). The plates were incubated at room temperature (27±2º 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[12].

Effect of herbicide

The mycelia plugs of isolated fungi were inoculated in to potato dextrose agar medium along with different concentrations of herbicide, butachlor 0.70 ml/100 ml (T1), 0.77 ml/100 ml (T2) separately and control also maintained. The plates were incubated at 27º 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

$$\frac{w1}{w2}x100$$

Whereas,

W2= Wet weight of mycelium in control; W1= Wet weight of mycelium in pesticide utilization trails.

Results and Discussion

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 [13].

In the present study, totally 22 isolates were identi-

fied 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 fungal culture were treated with 0.70 ml/100ml and 0.77 ml/100 ml concentration of herbicide (Butachlor). Among the twenty two isolates, *Aspergillus fumigatus* highly degrade the herbicide and form high level of biomass (90.5, 82.5%) in both concentration T1 and T2 and next is *A. niger* 87, 79% respectively. Among the twenty two isolates, five isolates were highly degrade the herbicide and form high level of mycelial biomass in T1 concentration such as *Aspergillus fumigatus* 90.5%, *A. niger* 87%, *A. repens* 86.5% *A. sparsus* 84.6 and *A. arenarius* 82.2% were recorded. Other isolates were degrade the herbicide and form mycelial biomass at moderate level some of them at minimum level (Table-3)

Among the twenty two isolates, Five isolates were highly degrading the herbicide and form high level of mycelial biomass in T2 concentration such as *Aspergillus fumigatus* 82.5%, *A. niger* 79%, *A. repens* 77.6%, *A. arenarius* 74.1% and *A. terricola* 68.4% recorded. Other isolates were degrading the herbicide and form mycelial mat in moderate level some of them at minimum level. Aspergilli group are commonly found in soil and on decaying organic material. By converting resistant organic chemicals such as pesticides into simplified metabolites and eventually into soluble benefit molecules, fungi such as *Aspergillus* spp. play an important role in carbon cycling [14].

Table 2: Isolation of microorganisms from the Herbicide applied soil samples

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

+ present; -absent

Table 3: Effect of Herbicide (Butachlor 0.70 ml/100 ml and 0.77 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	*Aspergillus aureolatus*	3.2	2.1	1.9	65.6	59.4
2	*A.avensceus*	4.9	3.6	3.4	73.4	69.4
3	*A.arenarius*	6.2	5.1	4.6	82.2	74.1
4	*A.candidus*	3.0	2.2	1.4	73.3	46.6
5	*A.duricaulis*	2.9	2.1	1.6	72.4	55.1
6	*A.fumigatus*	6.3	5.7	5.2	90.5	82.5
7	*A.janus*	2.6	1.4	1.2	53.8	46.1
8	*A.niger*	6.2	5.4	4.9	87	79
9	*A.puniceus*	3.6	2.9	2.2	80.5	61.1
10	*A.panamensis*	2.5	1.6	1.3	64	52
11	*A.repens*	6.7	5.8	5.2	86.5	77.6
12	*A.sparsus*	1.3	1.1	0.7	84.6	53.8
13	*A.speluneus*	2.9	1.8	1.6	62	55.1
14	*A.subolivaceus*	3.9	3.1	2.5	79.4	64.1
15	*A.tamarii*	2.9	1.7	1.1	58.6	37.9
16	*A.terreus*	5.9	3.8	3.4	64.4	57.6
17	*A.terricola*	3.8	3.1	2.6	81.5	68.4
18	*A.ustus*	1.8	0.6	0.3	33.3	16.7
19	*A.wentii*	3.5	2.6	2.0	74.2	57.1
20	*Penicillium* sp.	3.8	2.5	2.3	65.7	60.5
21	*Penicillium frequentans*	4.7	3.5	3.1	74.4	65.9
22	*P. granulatum*	3.9	2.4	2.0	61.5	51.2

Note: T1 - *Butachlor (0.70 ml/100 ml);* ***T2*** *- Butachlor (0.77 ml/100 ml)*

Marecik *et al* [15] observed the highest level of degradation of atrazine contaminated site by culturing microorganisms of the sweet flag rhizosphere for a longer time can play an important role in the bioremediation of atrazine-contaminated sites.

The present study, concluded that the herbicides when applied in field, do not destroy the microbial biomass, but reduce the population level. Excess applications of herbicides leads to the eradication of beneficial microbes. In this study some fungal isolates were found to gow well on herbicide applied media and form biomass up to 90.5%. So studies on these fungal isolates could be further carried out to degrade the herbicides.

References

1. Kellogg RL, Nehring R, Grube A, Goss DW, Plotkin S. 2000. Environmental indicators of pesticide leaching and runoff from farm fields. United States Department of Agriculture Natural Resources Conservation Service.
2. Howard I. Morrison, Kathryn Wilkins, Robert Semenciw, Yang Mao, Don Wigle Herbicides and Cancer. *Journal of the National Cancer Institute* .1992; 84 (24): 1866–1874.
3. Ayansina ADV, Ogunshe OAA, Fagade OE. 2003. Environment impact assessment and microbiologist: an overview. Proc. of 11th annual national conference of environment and behavior association of Nig. Nig. (EBAN), pp. 26-27.
4. Amakiri MA. Microbial degradation of soil applied herbicides. *Nig. J. Microb.* 1982;2:17-21.
5. Gupta PK. Pesticide Exposure-Indian Scene.*Toxicol*. 2004;198:83-90.
6. Shelton DR, Somich CJ. Isolation and identification of coumaphos-metabolising bacteria from cattle dip. *AppI. Environ. Microbiol*. 1988; 54:2566-2571.
7. Baljinder Singh, Kashmir Singh. Microbial degradation of herbicides, *Critical Reviews in Microbiology* . 2016;42(2):112-119.

8. Schoefs O, Perrier M, Samson R. Estimation of contaminant depletion in unsaturated soils using a reduced-order biodegradation model and carbon dioxide measurement. *App. Microbiol. Biotechnol.* 2004; 64: 256-61.
9. Pointing SB. Feasibility of bioremediation by white-rot fungi. *Appl. Microbiol. Biotechnol.* 2001; 57:20 -33.
10. Evans C, Hedger J. 2001. Degradation of cell wall polymers. In: Fungi in bioremediation. Gadd G. Ed Cambridge University Press. Cambridge, U.K.
11. Sasek V. 2003. Why mycoremediations have not yet come to practice. In Sasek V. *et al.*, (Eds.) In: The utilization of bioremediation to reduce soil contamination: Problems and solutions,. Kluwer Academis Publishers. pp:247-276
12. Waksman S. A method of counting the number of fungi in the soil *J.Bact*. 1922;7: 339-117.
13. Hernandez M, Morgante V, Avila M, Villalobos P, Miralles P, Gonzalez M, Seegers M. Novel s-triazine-degrading bacteria isolated from agricultural soils of central chile for herbicide bioremediation. *Electron. J. Biotechn*. 2008; 11(5): 1-6.
14. Tournas VH. Spoilage of vegetable crops by bacteria and fungi and related health hazards. *Crit. Rev. Microbiol*. 2005; 31:33-44.
15. Marecik R, Kroliczak P, Czaczyk K, Wojciech Białas, Anna Olejnik, Paweł Cyplik. Atrazine degradation by aerobic microorganisms isolated from the rhizosphere of sweet flag (*Acorus calamus* L.) *Biodegradation.* 2008; 19: 293. https://doi.org/10.1007/s10532-007-9135-5