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Soil Mycobiota Influenced by Different Concentration of Basic Fuschin Dye

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ABSTRACT

Aim: The present study was done to evaluate the effect of the dye basic fuchsin (BF) on soil mycobiota with an aim to mark out the fungal strains which might be able to remove triphenylmethane dyes from effluent by adsorption.

Methodology: Pot experiments were conducted during the study and different concentration (500, 750 and 1000 ppm) of Basic fuchsin dye were used on soil mycobiota. Soils treated with different concentration of the solution of basic fuchsin were screened for fungal isolates.

Results: *A. flavus* and *A. niger* could survive basic fuchsin treatment in the soil to a reasonable extent and their sizable populations were isolated from BF treated soil throughout the period of 90 days, even from the soil treated with as high as 1000 ppm concentration of the dye.

Discussion: The genus *Aspergillus* and *Aspergillus niger* could survive in the higher concentration of dye. It can tolerate the 1000 ppm of Basic fuchsin dye and it may be helpful to overcome water pollution by removing color contaminants from water bodies through biosorption.

Key Words: Basic fuchsin, Dye-tolerant fungi, Soil mycobiota

INTRODUCTION

Pollution is the worldwide problem and it's potential to affect the health of human population. The major effort that has been made over years to clean up the environment, pollution still remains a drastic problem and poses continuing risk to health. The pollution problem is undoubtedly great in the growing population (Fereidown et al. 2007). Soil pollution is one the major form of environmental disaster our world is facing today (Khan, 2004). The basic sources of pollution are emission from industry, inadequate waste management, contaminated water supply, extreme uses of chemical fertilizers etc. Besides these factors, drastically increasing population and growing industries, and volcanic ash from Iceland (World Health Organization, 2010) are the other source of soil pollution (Briggs, 2003). Rapid industrialization and the lack of public awareness towards the environment invites natural disaster (Carter, 1985; Helpppart and Sparks, 2006).

Soil pollution causes cancer including leukemia and it can cause developmental damage to the brain of young children.

The trace amount of mercury present in soil increases the risk of neuromuscular blockage which can cause headache, kidney failure depression of the central nervous system and can also cause eye irritation, skin, nausea and fatigue. Soil pollution is closely associated with air and water pollution that's why it's numerous effects come out as similar as caused by water and air contamination. Soil pollution can also alter the metabolism of plants and reduce crop yield and same process with microorganisms in given soil environment; this may eliminate some layers of the key food chain and thus have a negative effect on animals. Small life forms can consume harmful chemicals which can then be passed up the food chain to larger animals; this might be lead to increased mortality rates and even animal extinction. (Khan and Ghouri, 2011)

Industrial effluent alters the number and activity of microorganisms and also affects physico-chemical process and fertility of the soil. Microorganisms are of assistance in increasing the soil fertility and plant growth as they are related with certain biochemical activities in soil. The microorgan-

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isms sometimes affect soil environment more quickly than abiotic stress (Titljanova and Tesarova, 1991). Extreme uses of chemicals or effluent can also damage the beneficial microorganisms. (Hemanth et al, 2016) Hence, the microbial community may be useful as a highly sensitive bioindicator of soil disturbance and process of remediation. (Gremion et al, 2004). Nematodes, bacteria and fungi are the main microorganisms present in rhizosphere. Fungi are major components for soil microbiota, it constitutes more of the soil biomass than bacteria which depends upon soil depth and nutrient conditions.

Chemical contamination can cause a shift in microbial population (Doelman et al 1994, Roane and Kellogg, 1996, Elis et al, 2001; Kelly et al, 2003; Lugauskas et al, 2005). The physicochemical processes that occur naturally in certain biomass allow it to passively concentrate and bind contaminants into its cellular structure. (Sameera, 2011). Different kinds of biomasses as fungal and yeast, bacterial biomass, algal biomass have special surface properties to accumulate chemicals (Shankar et al 2014).

Fungi play a crucial role in nutrient cycling by regulating soil biological activity; these fungi grow in different pH, moisture, temperature, and nutrient availability. Fungi also benefits most plant by suppressing plant root disease and promoting healthier plant by attacking plant pathogens with fungal enzymes. Fungi get influence over other microorganisms by secreting enzyme and they also have the ability to survive and propagate in extreme condition environment.

Among all the microorganisms, fungal cell wall is a complex macromolecular structure consisting of chitin, glucans, mannans, proteins also containing other polysaccharides, lipids and pigments like melanin (Gadd, 1993). Different functional groups are able to bind dyes and other chemicals to different degrees (Bailey et. al. 1999). Chitin is a very important structural component of fungal cell wall which is an effective biosorbent for chemicals and radionuclides (Gadd, 2008). Micro-organisms (fungi) can develop high resistance to dye and metals through adsorption to cell surface, complexation by exo-polysaccharides, intracellular accumulation, and precipitation, (Saxena, 2006).

The present communication was conducted with an aim to isolate those fungal species from the soil which are capable of surviving basic fuchsin pollution and to obtain basic fuchsin resistant fungal strains which might facilitate the management of dye level in soil and effluents.

MATERIALS AND METHODS

Thirty six pots of 150 ml capacity, each filled with 100 gm soil were taken for the present study. Nine pots from these thirty six pots were treated with 25 ml of distilled water at

regular intervals of seven days for a total period of twelve weeks. These nine pots served as control. The remaining 27 pots were treated with different concentrations of basic fuchsin dye solution. Nine pots were treated with 500 ppm, nine pots were treated with 750 ppm and the remaining nine pots with 1000 ppm concentration of basic fuchsin dye solution.

After 30 days, soil from the three pots of control were mixed thoroughly to obtain a composite sample. Similarly, three composite samples were prepared from the soil treated with basic fuchsin dye (one composite sample each for 500 ppm, 750 ppm and 1000 ppm concentration). Each composite soil sample so obtained was analyzed for mycobiota, using dilution plate method (Waksman, 1927). 20 gm of soil from the composite sample were transferred to 200 ml of sterilized distilled water and stirred well. 10 ml of this suspension were immediately transferred to a conical flask containing 90 ml of sterilized distilled water. From this suspension, 1:100, 1:1,000, 1:10000 and 1:100000 were prepared. From the suspension of each dilution, 1 ml aliquots were transferred to each of a set of three Petri dishes followed by the addition of 20 ml of cooled and sterilized Potato Dextrose Agar medium amended with 30 ppm Rose Bengal and 30 ppm streptomycin (per liter of medium). After inoculation, the Petri dishes were incubated at $25^{\circ}\text{C} \pm 2$ for 4 to 5 days. The total number of colonies of individual fungal species growing in each Petri dish were recorded at a regular interval of time. The fungal strains obtained were identified using standard keys (Gilman, 1957; Nagamani *et al.*,2006). For the preparation of axenic culture, the fungal strains were transferred to the Petri plates containing fresh medium.

Composite samples were obtained from the basic fuchsin treated soil with different concentration were processed similarly. The procedure was repeated after 60 and 90 days.

RESULTS

A total of 35 species of fungi were isolated from the control as well as those treated with basic fuchsin dye using dilution plate method. Out of these, only one belongs to Zygomycota and one belongs to Ascomycota while remaining 33 species were anamorphic fungi. Eight fungal species belonged to the genus *Aspergillus*. The number of isolates of the aspergilli largely dominated the culture plates. The result of the present study indicates that *A. niger* is the most dominant species that could tolerate basic fuchsin dye even at 1000 ppm concentration. Madhuri and Vijyalakshmi (2014) could obtain 19 fungal species from the dye amended soil and observed the dominance of aspergillus species. Also, *A. niger*, *A. fumigatus* and *A. flavus* were the most dominant fungal species isolated from trypan blue treated soil. On the other hand, the genus *Chaetomium* was represented by 5 species and other fungal species constituted only a minor fraction. It

is believed that aspergilli are more abundant in the warmer regions as compared to the other fungal species (Waksman, 1917; Jensen, 1975; Singh and Charaya, 1975; Sen *et al.*, 2009; Kumar and Charaya, 2012; and Choudhary *et al.*, 2015).

After 30 days, the number of fungal species were reduced with increase in dye concentration. After 60 days of treatment, greater number of isolates as compared to control were obtained from the soil treated with 1000 ppm of basic fuchsin dye. After 90 days, lesser number of isolates were obtained with 500 ppm and 1000 ppm as compared to 750 ppm solution. In the present study, an overall inhibitory effect of basic fuchsin was observed on soil mycobiota *i.e.* with the increasing concentration of pollutants (dye) the diversity of microflora decreased (with few exceptions). This is further approved by calculation of Diversity indices (D and 1-D).

DISCUSSION

A. niger is the most dominant species that could tolerate basic fuchsin dye up-to 1000 ppm concentration. This is probably due to the capacity of *A. niger* to produce toxins that may prevent the growth of other fungal species (Chandrasekar *et al.*, 2014). In the present study, 35 different species of fungi were obtained. However, Choudhary *et al.*, 2015 obtained as many as 52 different fungal species, possibly because of a different approach been followed. Choudhary *et al.*, 2015 followed an approach in which *in-situ* treatment of pollutants (in the field itself) was given to the soil. In the present study, the soils were filled in the pots and probably the transfer of the soil to the pots might have disturbed the mycobiota during drying, sieving and transfer. (Pickett and White, 1985; Kumar and Charaya, 2012).

Babich and Stotzky (1982) observed that the level of pollutant which is lethal to a majority of microbes may only cause mutation in some and thereby increase the selection of such strains which can tolerate the higher concentration of pollutant. The subsequent multiplication and survival of these strains may have led to an increase in the population of such strains resulting in a total positive effect in the fungal population. As far as mycodiversity is concerned, the treatment with dye solution did not appear to have any appreciable inhibitory effect on the number of species isolated from the soil till 90 days. After 90 days of treatment lesser number of fungal species was isolated as compared to control (Kumar and Charaya, 2012).

Bragulat *et al.*, (1991) observed that even 1 ppm concentration of basic dye in culture medium could reduce the colony diameter of *Aspergillus flavus* by 4.5%. In the present finding, the treatment with 1000 ppm solution of basic fuchsin resulted in increase rather than decrease in the number of some fungal isolates. On the whole, *Aspergillus niger*, *Asper-*

gillus flavus and *Fusarium* sp. could resist basic fuchsin to a reasonable extent and their populations were able to survive basic fuchsin in the soil throughout the period of study, even the soil treated with 1000 ppm. It is expected that these fungal strains are able to degrade the dye or adsorb it and could be used to remove environmental pollution.

CONCLUSION

Present study was conducted to evaluate the effect of basic dye on soil mycobiota to obtain fungal strains which might be able to remove basic dyes by the process of adsorption. In the present observation soil were treated with different concentration *i.e.* 500 ppm, 750 ppm and 1000 ppm for basic dye over the total period of 90 days to screened the fungal isolates. Out of total 35 species eight fungal species belonged to the genus *Aspergillus* and *Aspergillus niger* is the most dominant species that could tolerate triphenylmethane dye even at 1000 ppm concentration and it may be helpful to remove color contaminants from water bodies in future with the help of biosorption. Besides the genus *Aspergillus*, *Chaetomium* were represented by 5 species and other one constituted the minor fraction throughout the period of three month.

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Table 1: Qualitative and quantitative distribution of mycobiota in soils–control as well as treated with 500 ppm, 750 ppm and 1000 ppm concentrations of basic fuchsin over a period of 90 days (as obtained by dilution plate method).

Fungal Species	30 Days				60 Days								90 Days											
	Control		500ppm		750ppm		1000ppm		Control		500ppm		750ppm		1000ppm		Control		500ppm		750ppm		1000ppm	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
<i>Aspergillus niger</i>	6	5.21	15	17.64	8	17.36	8	8	3	2.29	7	7.21	29	19.33	5	4	126	70	204	80.63	35	61.4	287	80.16
<i>Aspergillus nidulans</i>	4	3.47	-	-	9	19.56	-	-	-	-	-	-	-	-	-	-	1	.55	5	1.97	2	3.50	-	-
<i>Aspergillus giganteus</i>	4	3.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus terreus</i>	-	-	7	8.23	-	-	-	-	-	-	-	-	-	-	1	.8	-	-	-	-	-	-	-	-
<i>Aspergillus fumigatus</i>	-	-	13	15.29	11	23.29	39	39	8	6.10	12	12.37	11	7.33	12	9.6	2	1.11	9	3.55	2	3.50	-	-
<i>Aspergillus flavus</i>	-	-	-	-	-	-	-	-	30	22.90	46	47.42	57	38	62	49.6	-	-	3	1.18	11	19.29	29	8.10
<i>Aspergillus luchuensis</i>	-	-	-	-	-	-	-	-	-	-	-	-	2	1.33	-	-	-	-	-	-	-	-	-	-
<i>Aspergillus humicola</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26	10.27	-	-	-	-
<i>Aulternaria alternata</i>	-	-	4	4.70	7	15.21	-	-	-	-	-	-	-	-	2	1.6	-	-	-	-	-	-	-	-
<i>Aulternaria sp.</i>	3	2.60	2	2.35	-	-	3	3	-	-	-	-	-	-	-	-	-	-	-	-	-	2	.55	
<i>Arthrimum euphorbiae</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1.66	-	-	-	-	-	-
<i>Auriobasidium pullulans</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	2.77	-	-	-	-	-	-
<i>Botryotrichum atrogriseum</i>	49	42.60	7	8.23	-	-	-	-	25	19.08	2	2.06	-	-	-	-	18	10	-	-	-	-	-	-
<i>Botryotrichum piluliferum</i>	15	13.04	-	-	-	-	-	-	15	11.45	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium mollicellum</i>	4	3.47	-	-	-	-	12	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium indicum</i>	4	3.47	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium sp.</i>	5	4.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Chaetomium globosum</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1.6	-	-	-	-	-	-	-	-

Table 1: (Continued)

Fungal Species	30 Days				60 Days								90 Days											
	Control		500ppm		750ppm		1000ppm		Control		500ppm		750ppm		1000ppm		Control		500ppm		750ppm		1000ppm	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
<i>Chaetomium bostrychodes</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	3.50	2	.55
<i>Curvularia clavata</i>	2	1.73	-	-	4	8.69	1	1	-	-	-	-	-	-	1	.8	-	-	-	-	-	-	2	.55
<i>Cladosporium sp.</i>	-	-	3	3.52	-	-	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Cladosporium cladosporioides</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	.55	-	-	-	-	-	-	-
<i>Fusarium sp.</i>	5	4.34	3	3.52	3	6.52	29	29	26	19.84	5	5.15	-	-	18	14.4	4	2.22	4	1.58	3	5.26	35	9.77
<i>Humicola grisea</i>	5	4.34	8	9.41	2	4.34	7	7	-	-	-	-	-	-	1	.8	-	-	-	-	-	-	-	-
<i>Monodictyts fluctuata</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	1.6	-	-	-	-	-	-	-	-
<i>Paecilomyces variotii</i>	1	.86	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Penicillium sp.</i>	-	-	5	5.88	-	-	-	-	-	-	-	-	-	-	-	-	20	11.11	-	-	-	-	-	-
<i>Penicillium vinaceum</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	.66	13	10.4	-	-	-	-	-	-	-	-
<i>Periconia hispidula</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1.75	1	.27
<i>Rhizoctonia sp.</i>	-	-	1	1.17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Rhizopus sp.</i>	-	-	4	4.70	2	4.34	-	-	24	18.32	25	25.77	48	32	5	4	-	-	-	-	-	-	-	-
<i>Stachybotrys sp.</i>	5	4.34	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Scytalidium lignicola</i>	-	-	2	2.35	-	-	-	-	-	-	-	-	2	1.33	-	-	-	-	-	-	-	-	-	-
<i>Trichoderma glaucum</i>	-	-	5	5.88	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Verticillium sp.</i>	3	2.60	6	7.05	-	-	-	-	-	-	-	-	-	-	-	-	-	2	.79	1	1.75	-	-	
Number of Species	15		15		8		8		7		6		7		12		9		7		8		7	
Total Isolates	115		85		46		100		131		97		150		124		180		253		57		358	
Simpson's index of Diversity (1-D)	0.791		0.914		0.855		0.745		0.827		0.693		0.714		0.711		0.489		0.339		0.589		0.343	