EFFECT OF THE DYE MALACHITE GREEN ON SOIL MYCOBIOTA

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Abstract : Effect of the dye malachite green (MG) on soil mycobiota was evaluated with an aim to mark out fungal strains which might be able to remove MG from effluents by adsorption. Soil treated with different concentrations of solution of malachite green were screened for fungal isolates. On the whole, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* could survive malachite green treatment in soil to a resonable extent and their sizeable populations were isolated from MG- amended soils throughout the period of study, even from the soil treated with as high as 2000 ppm concentration malachite green solution.

Keywords : Dye pollution, Malachite green, Dye-tolerant fungi, soil mycobiota.

INTRODUCTION

The dye-containing effluents from textile industries as also from dye-manufacturing industries have become alarming sources of dye pollution (GonClaves *et al.,* 2000; Singh *et al.,* 2010; Kalaiarasi *et al.,* 2012). Effluents from these industries are highly coloured and are difficult to treat since the normally used dyes are synthetic complex molecules which are not only resistant to the aerobic digestion, but are also stable to light, heat and oxidizing agents (Fu and Viraraghavan, 2001; Radha *et al.,* 2005; Crini, 2006). Their breakdown products such as benzidine, naphthalene and other aromatic compounds are toxic, carcinogenic or mutagenic to life forms (Ahmed *et al.,* 2012). Malachite green, an N-methylated diaminotriphenylmethane dye, is quite commonly used for the dyeing of cotton, silk, paper, leather and also for manufacturing paints and printing inks (Gupta *et al.,* 2004). Malachite green and its reduced form, leucomalachite green, may persist in edible fish tissues for extended periods of time. This might lead to bioaccumulation of malachite green and leucomalachite green in terrestrial and aquatic ecosystems (Mitrowska and Posyniak, 2004). Hence, the need for proper treatment of effluents (to remove these dyes from the effluents) before their release in the environment is quite obvious. Methodologies for the treatment of dye-containing wastewater fall into three categories: physical, chemical and biological. Many physico-chemical methods such as precipitation, ion-exchange, electrolysis, nanofiltration, electro-coagulation and ozonation (Reddy and Kotaiah, 2005; Kashefialasl *et al.,* 2006; Gharbani *et al.,* 2008; Sundrarajan *et al.,* 2007; Husseiny., 2008) have been in use for the purpose. However, owing to their lower cost and ecofriendliness, biological methods are definitely better than the physico-chemical methods (Banat *et al.,* 1996). Attempts have been made to exploit the ability of microbes to degrade and metabolize dyes (Bhaskar *et al.,* 2003; Toh *et al.,* 2003; Tetsch *et al.,* 2005). However, the live microbes have to be maintained under very specific conditions for eliciting optimum or reasonably fair activity. On the other hand, the ability of dead biomass to adsorb dyes offers a much better proposition. The use of fungal biomass as adsorbent or ion-exchanger for the removal of heavy metals from effluents is well documented (Kumar and Charaya, 2012). Similar strategies may be attempted with dyes also. However, it would require the isolation and maintenance of those fungal strains whose biomass is highly efficient in adsorbing a given dye. Antonovics *et al.* (1971) isolated mercury-resistant strains of bacteria and fungi from soil treated with mercury–containing fungicides. The present study was conducted with an aim to isolate those fungal species from soil which are capable of surviving malachite green pollution so that the biomass of such fungal strains might be utilized to develop effluent-treatment equipments for managing dye pollution.

MATERIAL AND METHOD

Thirty six pots of 150 ml capacity, each filled with 100 gm soil, were divided into four sets of nine pots each. Nine pots of set I were treated with 25 ml of distilled water at regular intervals of seven days for a total period of twelve weeks. This set (set I) served as control. The nine pots of set II, nine pots of set III and nine pots of set IV were treated similarly but with 1000 ppm, 1500 ppm and 2000 ppm malachite green solution respectively in lieu of distilled water.

After thirty days, soils from the three pots of set I were mixed thoroughly to obtain a composite sample. 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 20 ml of sterilized distilled water and stirred for 30 minutes to wash fungal propagules from the soil. 10 ml of this suspension were immediately transferred to a conical flask containing 90 ml of sterilized distilled water. From this suspension (1:100 dilution), further dilutions (1:1000 and 1:10000) were obtained. From the suspension of each (1:100 , 1:1000 and 1:10000) 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 (Raper and Thom, 1949) with 30 ppm Rose Bengal and 30 ppm of Streptomycin (per litre of medium). The Petri dishes containing the medium and the inocula were incubated at 25ºC for 6-8 days. The total number of colonies of individual fungal species growing in each Petri dish were recorded. The fungal strains obtained were identified using standard key (Gilman, 1957; Nagmani *et al.*, 2006). Composite samples were obtained from the pots of sets II, III and IV also, and were processed similarly. The procedure was repeated after 60 and 90 days. A separate sub-sample from the initial soil sample was also analysed for soil mycobiota.

RESULT AND DISCUSSION

In all, 21 species of fungi were isolated in the present study (Tables 1 to 3). Out of these, only one *i.e. Rhizopus* sp. belongs to Zygomycota. The remaining 20 species were anamorphic fungi (deuteromycetes). The dominance of Deuteromycota observed in the present study is in full agreement with the earlier reports (Hudson, 1968; Dickinson and Pugh, 1974; Singh and Charaya, 1975; Dube *et al.*, 1980; Charaya, 2006; Tiwari and Charaya, 2006; Kumar and Charaya, 2012). The results also lend support to the contention of Galloway (1935), Singh and Charaya (1975), Dube *et al.* (2006), Kumar and Charaya (2012) that there is a paucity of mucoraceous fungi in the tropical regions of India. Amongst the Deuteromycota, the Hyphomycetes constituted the major fraction. The Aspergilli were represented by four species while the Penicillia were represented by only three species. Not only this, the number of isolates of the Aspergilli largely dominated the culture plates. On the other hand, the Penicillia constituted only a minor fraction. It is widely believed that Aspergilli are more abundant in the warmer regions of the world while the Penicillia are more common in the cooler regions of the world (Waksman, 1927; Jensen, 1975; Singh and Charaya, 1975; Sen *et al*., 2009; Kumar and Charaya, 2012). The results of the present investigation are in agreement with the above observations. Out of the initial isolates, *Penicillium fellutanum* , *Penicillium fuscum*, *Cladosporium herbarum*, *Curvularia lunata*, *Fusarium nivale*, *Fusarium* sp. and *Candida* sp. could be isolated from the initial sample only, and were not isolated subsequently even from the control samples (placed in pots). Obviously, the soil might have got substantially disturbed during transfer from field to pots-causing alterations in resource availability and system structure (Pickett and White, 1985) leading, in turn, to a decrease in the species-richness and system heterogeneity (Zak, 1992). Not only this, out of these seven species, *Penicillium fuscum*, *Cladosporium herbarum*, *Fusarium* sp. and *Candida* sp. were meagrely represented. It is quite possible that these six species might have been allochthonous to soil collected from the field and hence, disappeared rapidly (Atlas and Bartha, 1998).

All the concentrations of malachite green (MG) caused marked decrease in the total number of fungal isolates obtained from the soil after 30 days, the inhibitory effect increasing 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 2000 ppm of MG. After 90 days also, greater number of isolates were obtained from soil treated with 1500 ppm solution as compared to those treatment with 1000ppm and 2000ppm solutions. Babich and Stotzky (1982) suggested that the level of a 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 the pollutant. The subsequent survival and multiplication of these strains might have led to an increase in the population of such strains resulting in a total positive effect on the fungal population. As far as mycodiversity is concerned, the treatment with MG solution did not appear to have any appreciable inhibitory effect on the number of species isolated from the soil till 60 days. Rather, in some situations greater number of species could be isolated as compared to control. However, after 90 days lesser number of fungal species were isolated as compared to control.

Bhattacharya (1995) reported that the infection in fish by *Aspergillus flavus* could be reduced by MG. Bragulat *et al.* (1991) reported that even 1 ppm concentration of MG in culture medium could reduce the colony diameter of *Aspergillus flavus* by 4.5%. In the present study, the treatment with 1000ppm solution of MG resulted in increase rather than decrease in the number of isolates of *Aspergillus flavus*. However, by 90 days, it caused discernible reduction in their number. 1500 ppm and 2000ppm concentration were clearly inhibitory. Of course, in 2000ppm MG treated soil, the isolates of *Aspergillus flavus* were more than that in control after treatment after 60 days.

Sadasivan (1947) observed that 2 ppm concentration of MG could inhibit *Aspergillus niger* completely. However, in the present study complete inhibition of *Aspergillus niger* was not observed though, in general, there was a marked reduction in the number of isolates in MG-treated soil as compared to control. Bergheim and Asgared (1996) reported that MG is not very soluble in water and it binds to sediment. It is quite possible that in the present study, a substantial amount of MG added to the soil might have got bound to the soil particles and only a small fraction of it could exert its effect on soil microbiota .

On the whole, *Aspergillus flavus*, *Aspergillus fumigatus* and *Aspergillus niger* could resist MG to a reasonable extent and their populations were able to survive MG in the soil throughout the period of study even in the soil treated with 2000 ppm MG. It is expected that these fungal strains are able to either degrade the dye or adsorb it so as to prevent its entry into the hyphae. Further studies are in progress to evaluate the potential of these strains to adsorb MG to resolve this issue.

Table 1: Mycobiota isolated from control soils as well as that amended with different concentrations (1000 ppm, 1500 ppm and 2000 ppm) of malachite green after 30 days of treatment.

Fungal Species	Initial		Control		1000 ppm MG		1500 ppm MG		2000 ppm MG	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Rhizopus sp.	3	0.26	-	-	-	-	-	-	2	1.15
Candida sp.	2	0.17	-	-	-	-	-	-	1	0.57
Acremonium vitis	4	0.35	-	-	-	-	-	-	-	-
Aspergillus flavus	389	34.21	290	30.85	378	50	219	53.15	87	50.28
Aspegillus fumigatus	357	31.39	320	34.04	198	26.19	110	26.69	53	30.63
Aspergillus niger	304	26.73	312	33.19	180	23.80	73	17.71	23	13.29
Aspergillus terreus	4	0.35	-	-	-	-	7	4.69	-	-
Aspergillus ustus	13	1.14	18	1.91	-	-	-	-	4	2.31
Aspergillus versicolor	7	0.61	-	-	-	-	3	0.72	-	-
Sporotrichum pruinosum	18	1.58	-	-	-	-	-	-	1	0.57
Penicillium fellutanum	3	0.26	-	-	-	-	-	-	-	-
Penicillium fuscum	1	0.08	-	-	-	-	-	-	-	-
Penicillium sp.	2	0.17	-	-	-	-	-	-	-	-
Trichoderma album	2	0.17	-	-	-	-	-	-	2	1.15
Trichoderma lignorum	11	0.96	-	-	-	-	-	-	-	-
Botryotrichum atro-griseum	4	0.35	-	-	-	-	-	-	-	-
Cladosporum herbarum	2	0.17	-	-	-	-	-	-	-	-
Curvularia lunata	3	0.26	-	-	-	-	-	-	-	-
Fusarium equiseti	4	0.35	-	-	-	-	-	-	-	-
Fusarium nivale	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	2	0.17	-	-	-	-	-	-	-	-
No of species	21	-	4	-	3	-	5	-	7	-
Total isolates	1137	-	940	-	756	-	412	-	173	-

TI= Total isolates; PI= Percentage of isolates

Table 2: Mycobiota isolated from control soils as well as that amended with different concentrations (1000 ppm, 1500 ppm and 2000 ppm) of malachite green after 60 days of treatment.

Fungal Species	Initial		Control		1000 ppm MG		1500 ppm MG		2000 ppm MG	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Rhizopus sp.	3	0.26	-	-	-	-	-	-	-	-
Candida sp.	2	0.17	-	-	-	-	-	-	-	-
Acremonium vitis	4	0.35	-	-	-	-	-	-	-	-
Aspergillus flavus	389	34.21	182	45.12	218	61.93	175	63.17	283	57.75
Aspegillus fumigatus	357	31.39	169	41.21	103	29.26	85	30.68	177	43.17
Aspergillus niger	304	26.73	56	13.65	22	6.25	11	3.97	19	3.87
Aspergillus terreus	4	0.35	-	-	1	0.28	-	-	-	-
Aspergillus ustus	13	1.14	-	-	-	-	-	-	-	-
Aspergillus versicolor	7	0.61	-	-	-	-	-	-	-	-
Sporotrichum pruinosum	18	1.58	-	-	-	-	-	-	-	-
Penicillium fellutanum	3	0.26	-	-	-	-	-	-	-	-
Penicillium fuscum	1	0.08	-	-	-	-	-	-	-	-
Penicillium sp.	2	0.17	1	0.08	-	-	-	-	-	-
Trichoderma album	2	0.17	-	-	-	-	-	-	-	-
Trichoderma lignorum	11	0.96	-	-	-	-	-	-	-	-
Botryotrichum atro-griseum	4	0.35	-	-	8	2.27	6	2.16	11	2.24

Cladosporum herbarum	2	0.17	-	-	-	-	-	-	-	-
Curvularia lunata	3	0.26	-	-	-	-	-	-	-	-
Fusarium equiseti	4	0.35	-	-	-	-	-	-	-	-
Fusarium nivale	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	2	0.17	-	-	-	-	-	-	-	-
No of species	21	-	4	-	5	-	4	-	4	-
Total isolates	1137	-	410	-	352	-	277	-	490	-

TI= Total isolates; PI= Percentage of isolates

Table 3 : Mycobiota isolated from control soils as well as that amended with different concentrations (1000 ppm, 1500 ppm and 2000 ppm) of malachite green after 90 days of treatment.

Fungal Species	Initial		Control		1000 ppm MG		1500 ppm MG		2000 ppm MG	
	TI	PI	TI	PI	TI	PI	TI	PI	TI	PI
Rhizopus sp.	3	0.26	-	-	-	-	-	-	-	-
Candida sp.	2	0.17	-	-	-	-	-	-	-	-
Acremonium vitis	4	0.35	-	-	-	-	-	-	-	-
Aspergillus flavus	389	34.21	284	50.35	218	62.10	196	35.89	198	53.08
Aspegillus fumigatus	357	31.39	62	10.99	90	25.64	255	46.70	145	38.89
Aspergillus niger	304	26.73	198	35.10	35	9.97	86	15.75	19	5.09
Aspergillus terreus	4	0.35	-	-	-	-	-	-	-	-
Aspergillus ustus	13	1.14	-	-	-	-	-	-	-	-
Aspergillus versicolor	7	0.61	-	-	-	-	-	-	-	-
Sporotrichum pruinosum	18	1.58	-	-	-	-	-	-	-	-
Penicillium fellutanum	3	0.26	-	-	-	-	-	-	-	-
Penicillium fuscum	1	0.08	-	-	-	-	-	-	-	-
Penicillium sp.	2	0.17	-	-	-	-	-	-	-	-
Trichoderma album	2	0.17	-	-	-	-	-	-	-	-
Trichoderma lignorum	11	0.96	7	1.24	8	2.27	5	0.91	11	2.94
Botryotrichum atro-griseum	4	0.35	12	2.12	-	-	-	-	-	-
Cladosporum herbarum	2	0.17	-	-	-	-	-	-	-	-
Curvularia lunata	3	0.26	-	-	-	-	-	-	-	-
Fusarium equiseti	4	0.35	1	0.17		-	4	0.73	-	-
Fusarium nivale	-	-	-	-	-	-	-	-	-	-
Fusarium sp.	2	0.17	-	-	-	-	-	-	-	-
No of species	21	-	6	-	4	-	5	-	4	-
Total isolates	1137	-	564	-	351	-	546	-	373	-

TI= Total isolates; PI= Percentage of isolates

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