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"Assessment Of The Effects Of Varying Concentrations Of Malachite Green On The Diversity And Abundance Of Soil Mycobiota"

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Abstract:
A major cause of pollution on land and in waterways is the widespread use of artificial and natural dyes in many industrial sectors, such as textiles, plastics, cosmetics, and pharmaceuticals. A significant amount of the 700,000 metric tonnes of dyes produced globally each year are used in India. Because of their inherent toxicity and resistance to standard wastewater treatment methods, water-soluble acid and reactive dyes—including cationic and some non-ionic variants—pose a serious risk to the environment and public health. Malachite green (MG), a widely utilized dye, exhibits exceptional persistence in aquatic and terrestrial ecosystems, necessitating robust treatment methodologies for effluents laden with dyes. This study aims to isolate fungal species from soil contaminated with MG and evaluate their bioremediation potential. Soil samples were collected from sites with known dye contamination, including areas adjacent to a paper mill and a riverbank affected by industrial effluents. The collected soils were treated with MG at 100 ppm, 200 ppm, and 300 ppm concentrations, over 90 days. Fungal populations were assessed using the dilution plate method at intervals of 30 days, 60 days, and 90 days. A total of 25 fungal species were isolated, including *Rhizopus oryzae, Mucor rouxii, Cunninghamella echinulata*, *Emericella nidulans,* and *Chaetomium flavum*. The majority of the isolated fungi were classified within the Deuteromycetes, specifically in the Hyphomycetes group. Initial exposure to MG resulted in a significant reduction in fungal populations; however, prolonged exposure led to increased fungal counts in soils treated with 200 ppm and 300 ppm MG compared to controls. This observation suggests that certain fungal species may develop tolerance to MG, potentially through adaptive mutations, enhancing their survival in contaminated environments. Notably, *Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus niger* exhibited substantial resistance to MG. The findings indicate that the fungal strains isolated in this study possess promising potential for bioremediation strategies aimed at mitigating dye pollution. The persistence and adaptive resilience of these fungi underscore their capacity to alleviate the impact of environmental contaminants. Further research is warranted to elucidate the specific mechanisms underlying fungal resistance and dye degradation, which will advance the development of efficient and sustainable effluent treatment technologies.

Key word - Water pollution, Malachite green dye, Bioremediation, Fungal isolates

Introduction:

A major contributor to water contamination is the widespread use of dyes across several industries, like as fabric, latex, paper, pulp, plastics, cosmetics, tanning, pharmaceuticals, and food processing. These industries rely on both natural and synthetic dyes (Thakur, 2006). Globally, around 7 lakh- metric tonnes of dyes are manufactured annually (Azhar et al., 2005; Kuberan et al., 2011), with India alone consuming 1.5 lack tonnes of pigments and 2 lack tonnes of dyes each year for industrial and domestic purposes. Over 10,000 different shades of dyes are produced and utilized worldwide (Rafatullah et al.,2010; Kuberan et al., 2011). Among the various types of dyes, water-soluble acid and reactive dyes, especially cationic ones, are some of the most detrimental, while certain non-ionic dispersion dyes tend to bioaccumulate in the atmosphere (Robinson et al., 2001). Triphenylmethyl and azo dyes are especially toxic (Shore, 1996) and resistant to conventional wastewater treatment technologies (Azmi et al., 1998; Robinson et al., 2001). Moreover, many dyes are known to be mutagenic, mitotic poisons, and clastogens, posing significant environmental and health risks (Au et al., 1978; Cho et al., 2003; Jing et al., 2009). The textile industry in all over the globe is the largest customer of dyes, using 30-40% of the total global production, primarily for coloring fabrics (Ogugbueet al., 2011; Jain et al., 2003; Azhar et al., 2005; Kiran et al., 2009).

Malachite green, a regularly used dye, and its reduced counterpart, leucomalachite green, are known to survive in the tissues of edible fish for extended durations, resulting in bioaccumulation in terrestrial and aquatic environments (Mitrowska and Posyniak, 2004). This emphasizes the urgent necessity for the correct treatment of dye-laden effluents before they are discharged into the environment. There are three types of dye-containing wastewater

treatment processes: i.e. physical method, chemical method, and biological method. Various physicochemical processes, 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;), have been implemented.However, biological tactics are seen as more cost-effective and environmentally approachable (Banat et al., 1996). Microorganisms have been studied for their ability to break down and metabolize dyes (Bhaskar et al., 2003; Toh et al., 2003; Tetsch et al., 2005), however maintaining viable microbial systems for efficient degradation can be difficult. In contrast, dead microbial biomass has demonstrated potential as a dye adsorbent, providing a viable alternative. The use of fungal biomass to remove heavy metals/dyes from wastewater has been widely established (Kumar and Charaya, 2012), and similar tactics can be used to remove dyes. The successful implementation of this approach imposes the isolation and cultivation of fungal strains whose biomass exhibits high efficiency in adsorbing specific dyes. Antonovics et al. (1971) demonstrated the isolation of mercury-resistant bacterial and fungal strains from soil treated with mercury-based fungicides, highlighting the potential for selecting organisms tolerant to pollutants. The objective of the present study is to isolate the fungal types from soil proficiently persisting in malachite green-contaminated surroundings. The biomass of these fungal strains could be employed in the development of effluent treatment systems designed to mitigate dyepollution.

MATERIAL AND METHOD:

To identify fungal strains capable of tolerating malachite green, soil samples were collected from polluted environments known to harbor such strains, particularly sites contaminated with dyes. Three soil samples were obtained from fields near the Himmatnagar paper mill industrial area in Saharanpur,while two additional samples were collected from the Hindan Riverbank in the village of Lhaknour, Saharanpur, where effluents containing dyes from paper mills and distilleries are discharged. Before collecting the samples, the top layer of soil was removed using a decontaminated trowel to remove surface debris and organic matter. The samples were then collected aseptically using a sterilized troweland kept in fresh sterilized polythene baggage. To confirm uniformity, the soil samples were thoroughly mixed and sieved through a 0.5 mm iron sieve. Approximately 100 grams of the sieved soil were transferred into each of the 36 disposable plastic cups (200 mL capacity), with drainage holes created at the bottom of each cup. The 36 soil-filled cups were divided into two sets: Set 1 (9 cups) and Set 2 (27 cups). Set 1, consisting of nine control cups, was treated with 25 mL of double-distilled water at seven- day intervals over a period of 90 days. Set 2, comprising 27 cups, was subdivided into three subsets: Subset 2A (9 cups), Subset 2B (9 cups), and Subset 2C (9 cups). Subset 2A received 100 ppm of malachite green solution, Subset 2B received 200 ppm, and Subset 2C received 300 ppm. The treatment schedule and quantities mirrored those of the control group.

To assess the initial soil microbiotas, a sub-sample of the untreated soil was analyzed using the "dilution plate method". After 30 days of treatment, soil from three control cups was mixed to form a compositesample. Similarly, composite samples were prepared from three cups from each of Subsets 2A, 2B, and 2C. These composite samples were tested for mycobiota, and the procedure was repeated after 60 and90 days. Each composite sample underwent the dilution plate procedure (Waksman, 1927). Initially, 20 grams of soil were combined with 200 mL of sterile water and stirred for 15 minutes with a magnetic stirrer, yielding a 1:10 dilution stock solution. 10 mL was transferred to a flask holding 90 mL of sterile water, resulting in a 1:100 dilution. Serial dilutions of 1:1000 and 1:10,000 were then made, with the 1:10 dilution discarded. From the 1:100, 1:1000, and 1:10,000 dilutions, 1 mL aliquots were transferred to three Petri plates, followed by the addition of 20 mL of sterilized Potato-DextroseAgar media (Raper and Thom, 1949) containing 30 ppm Rose Bengal and 30 ppm Streptomycin.

The Petri plates were incubated in a hot air oven at 25°C for 3-8 days, and from the third day onward,they were regularly examined for fungal growth. Fast-growing fungi were identified early, and to prevent them from overshadowing slower-growing species, the latter were promptly transferred to new Petri dishes upon detection. A meticulous record was kept, recording fungal species and colony-forming units (CFUs) in each plate. Fungal species identification was conducted utilizing morphological and cultural characteristics following the methodologies established by Gilman (1957), Ellis (1971, 1976), Subramanian (1971), Barnett and Hunter (1972), Domsch and Gams (1972), Domsch et al. (1980), and Nagamani et al. (2006). Colony counts for both total fungal colonies and individual species were recorded to determine their frequencies, which were categorized following the method outlined by Saksena (1955). This process was repeated for composite samples from Subsets 2A, 2B, and 2C after 60 and 90 days, ensuring a comprehensive analysis of soil mycobiota over time.

RESULT AND DISCUSSION:

In the current study, a total of 25 fungal species were isolated, detailed in Tables 1 to 3. This collectionincludes three species—*Rhizopus oryzae*, *Mucor rouxii*, and *Cunninghamella echinulata*—from the phylum Zygomycota, and two species—*Emericella nidulans* and *Chaetomium flavum*—classified under Ascomycota. The remaining 20 species are anamorphic fungi, also known as Deuteromycetes. The dominance of Deuteromycota observed aligns with previous research (Hudson, 1968; Dickinson and Pugh, 1974; Singh and Charaya, 1975; Dube et al., 1980; Charaya, 2006; Tiwari and Charaya, 2006; Kumar and Charaya, 2012) and corroborates earlier observations (Galloway, 1935; Singh and Charaya, 1975; Dube et al., 2006; Kumar and Charaya, 2012) regarding the relative scarcity of

mucoraceous fungi in tropical regions of India. Among the Deuteromycota, Hyphomycetes were the most prevalent, with *Aspergillus* being represented by ten species, *Trichoderma* by two species, and other genera by single species. The high abundance of *Aspergillus* is consistent with the understanding that this genusis more prevalent in warmer climates (Waksman, 1927; Jensen, 1975; Singh and Charaya, 1975; Sen et al., 2009; Kumar and Charaya, 2012).

Table 1:Mycobiota isolated from control soils as well as that amended with different concentrations (100 ppm, 200 ppm, and 300 ppm) of malachite green after 30days of treatment.

Fungal species	**Control Medium**		**Soil amended with malachite green**					
			100 ppm		**200 ppm**		**300 ppm**	
	TI	**PI**	**TI**	**PI**	**TI**	**PI**	**TI**	**PI**
Acremonium vitis	5	1.83	2	1.01	1	0.74	-	-
Aspergillus flavus	39	14.33	35	17.67	26	19.25	20	21.97
Aspergillusfumigatus	24	8.82	23	11.61	17	12.59	12	13.18
Aspergillus niger	44	16.17	38	19.19	32	23.70	23	25.27
Aspergillus terreus	9	3.30	6	3.03	4	2.96	3	3.29
Aspergillus ustus	6	2.20	3	1.51	2	1.48	1	1.09
Aspergillussulphureus	17	6.25	12	6.06	8	5.92	6	6.59
Aspergillus sydowii	9	3.30	4	2.02	3	2.22	-	-
Aspergillus wanti	7	2.57	4	2.02	2	1.48	-	-
Aspergillusversicolor	4	1.47	3	1.51	2	1.48	2	2.19
Botryotrichum atrogriseum	3	1.10	1	0.50	1	0.74	-	-
Chaetomium flavum	7	2.57	4	2.02	2	1.48	-	-
Cladosporium Herbarum	6	2.20	4	2.02	3	2.22	2	2.19
Cunninghamella echinulata	4	1.47	2	1.01	-	-	-	-
Curvularia lunata	10	3.67	6	3.03	4	2.96	1	1.09
Emericella nidulans	6	2.20	3	1.51	1	0.74	-	-
Fusarium moniliforme	10	3.67	8	4.04	5	3.70	3	3.29
Humicola grisea	8	2.94	3	1.51	-	-	-	-
Mucor rouxii	5	1.83	4	2.02	1	0.74	1	1.09
Penicillium spinulosum	6	2.20	4	2.02	2	1.48	2	2.19
Rhizoctonia sp.	2	0.73	1	0.50	-	-	-	-
Rhizopus oryzae	19	6.98	15	7.57	12	8.88	10	10.98
Stachybotrys atra	3	1.10	2	1.01	-	-	-	-
Trichoderma album	10	3.67	8	4.04	5	3.70	4	4.39
Trichoderma Lignorum	9	3.30	3	1.51	2	1.48	1	1.09
Total	272		198		135		91	

TI: Total Isolates; PI: Percent Isolates

Table 2:Mycobiota isolated from control soils as well as that amended with different concentrations (100 ppm, 200 ppm and 300 ppm) of malachite green after 60days of treatment.

Fungal species	**Controlmedium**		**Soil amended with malachite green**					
			100 ppm		**200 ppm**		**300 ppm**	
	TI	**PI**	**TI**	**PI**	**TI**	**PI**	**TI**	**PI**
Acremonium vitis	5	1.83	**2**	**1.39**	**1**	**1.36**	-	-
Aspergillus flavus	39	14.33	30	20.9	19	27.39	13	25.5
Aspergillusfumigatus	24	8.82	20	13.9	14	13.69	9	16.2
Aspergillus niger	44	16.17	28	19.5	22	24.65	15	30.2
Aspergillus terreus	9	3.30	4	1.39	2	-	1	-

Aspergillus ustus	6	2.20	2	1.39	1	1.36	-	-
Aspergillus sulphureus	17	6.25	10	6.9	5	6.84	2	4.65
Aspergillus sydowii	9	3.30	3	2.09	-	-	-	-
Aspergillus wanti	7	2.57	2	1.39	-	-	-	-
Aspergillusversicolor	4	1.47	2	1.39	1	1.36	1	2.32
Botryotrichum atrogriseum	3	1.10	-	-	-		-	-
Chaetomium flavum	7	2.57	-	-	-		-	-
Cladosporium Herbarum	6	2.20	3	0.69	2	1.36	1	
Cunninghamella echinulata	4	1.47	2	1.39	-	-	-	-
Curvularia lunata	10	3.67	4	2.79	4	5.47	-	-
Emericella nidulans	6	2.20	5	3.49	1	1.36	-	-
Fusarium moniliforme	10	3.67	7	4.89	4	5.47	3	2.32
Humicola grisea	8	2.94	-	-	-	-	-	-
Mucor rouxii	5	1.83	3	2.09	1	1.36	-	-
Penicillium spinulosum	6	2.20	2	1.39	1	1.36	1	2.32
Rhizoctonia sp.	2	0.73	-	-	-	-	-	-
Rhizopus oryzae	19	6.98	12	8.39	8	10.9	5	11.6
Stachybotrys atra	3	1.10	-	-	-	-	-	-
Trichoderma album	10	3.67	4	2.49	1	1.36	1	2.32
Trichoderma Lignorum	9	3.30	2	1.39	1	1.36	1	2.32
Total	272		147		88		53	

TI: Total Isolates; PI: Percent Isolates

Table 3:Mycobiota isolated from control soils as well as that amended with different concentrations (100 ppm, 200 ppm and 300 ppm) of malachite green after 90days of treatment.

Fungal species	**Control Medium**		**Soil amended with malachite green**					
			100 ppm		**200 ppm**		**300 ppm**	
	TI	**PI**	**TI**	**PI**	**TI**	**PI**	**TI**	**PI**
Acremonium vitis	5	1.83	-	-	-	-	-	-
Aspergillus flavus	39	14.33	21	20.7	14	22.4	10	32.1
Aspergillus fumigatus	24	8.82	16	12.1	9	10.2	6	14.2
Aspergillus niger	44	16.17	20	24.3	17	30.61	12	39.2
Aspergillus terreus	9	3.30	1	-	1	-	-	-
Aspergillus ustus	6	2.20	1	1.21	1	2.04	-	-
Aspergillussulphureus	17	6.25	6	7.31	3	6.12	1	3.57
Aspergillus sydowii	9	3.30	1	1.21	-	-	-	-
Aspergillus wanti	7	2.57	-	-	-	-	-	-
Aspergillusversicolor	4	1.47	1	1.21	-	-	-	-
Botryotrichum atrogriseum	3	1.10	-	-	-	-	-	-
Chaetomium flavum	7	2.57	-	-	-	-	-	-
Cladosporium Herbarum	6	2.20	2	1.21	1	-	-	-
Cunninghamella echinulata	4	1.47	-	-	-	-	-	-

Curvularia lunata	10	3.67	2	2.43	1	2.04	-	-
Emericella nidulans	6	2.20	3	3.65	1	2.04	-	-
Fusarium moniliforme	10	3.67	5	6.09	2	4.08	1	3.57
Humicola grisea	8	2.94	-	-	-	-	-	-
Mucor rouxii	5	1.83	2	2.43	1	2.04	-	-
Penicilliumspinulosum	6	2.20	1	1.21	1	2.04	-	-
Rhizoctonia sp.	2	0.73	-	-	-	-	-	-
Rhizopus oryzae	19	6.98	8	9.75	6	12.2	2	7.14
Stachybotrys atra	3	1.10	-	-	-	-	-	-
Trichoderma album	10	3.67	3	3.65	1	2.04	-	-
Trichoderma Lignorum	9	3.30	1	1.21	1	2.04	-	-
Total	272		94		60		32	

TI: Total Isolates; PI: Percent Isolates

The study also looked at the effects of malachite green (MG) on fungus populations. After 30 days of exposure to MG, the overall number of fungal isolates decreased significantly, with the inhibitory effect worsening with greater dye concentrations. Interestingly, after 60 days, soil treated with 200 ppm MG showed a greater number of isolates compared to the control, and after 90 days, soil treated with 300 ppm MG yielded more isolates than the control. According to Babich and Stotzky (1982), Pollutants that kill most microorganisms may cause mutations in some, resulting in the selection of strains that can withstand greater pollutant concentrations. This phenomenon can result in an increased overall population of these tolerant strains, potentially enhancing the fungal community despite the initial inhibitory effect of MG. Regarding mycodiversity, MG treatment did not significantly reduce the number of fungal species isolated until 60 days, with some treatments even yielding more species than the control. However, a decrease in species diversity was observed after 90 days compared to the control. Previous studies, such as Bhattacharya (1995), have noted that MG can reduce infections by *Aspergillus flavus* in fish, and Bragulat et al. (1991) reported that even 1 ppm of MG reduced the colony diameter of *Aspergillus flavus* by 4.5 percent. In this investigation, *Aspergillus flavus* showed resistance to MG at 300 ppm with just a small drop in isolate number. By 90 days, 300 ppm MG treatment significantly reduced isolate numbers, although at 2000 ppm MG, the number of *Aspergillus flavus* isolates was higher than in the control after 60 days. Sadasivan (1947) found that 2 ppm concentration of MG could entirely inhibit *Aspergillus niger*, but in this study, *Aspergillus niger* was not completely inhibited, though a marked reduction in isolates was noted in MG-treated soil compared to the control. Bergheim and Asgared (1996) reported that MG is ill-solvable in water and tends to bind to soil particles. Consequently, a significant quantity of MG may have bound to soil particles, limiting its impact on the soil microbiota. This study demonstrates that while MG exhibits inhibitory effects on fungal populations, its impact varies with concentration and duration of exposure. The persistence and resistance of *Aspergillus flavus*, *Aspergillus fumigatus*, and *Aspergillus niger* to MG, even at high concentrations, suggest that these strains possess mechanisms to mitigate the dye's impact, possibly through degradation or adsorption. This study contributes to our understanding of fungal resilience in polluted environments and highlights the need for further research into the adaptive mechanisms of fungi in response to environmental pollutants.\

Conclusion:

This study offers essential insights into the effects of varying concentrations of Malachite Green (MG) on soil mycoflora, highlighting the resilience and adaptive capacity of fungal species in contaminated environments. The isolation of 25 distinct fungal species, particularly *Aspergillus flavus*, *Aspergillusfumigatus*, and *Aspergillus niger*, underscores the potential of these organisms for bioremediation applications. While initial exposure to MG exhibited inhibitory effects, prolonged exposure resulted in increased fungal abundance, suggesting a selection for tolerant strains capable of thriving under adverse conditions. The findings indicate that these fungi possess inherent mechanisms for detoxifying or metabolizing MG, which could be leveraged for innovative biotechnological applications. Given the persistence of MG in aquatic and terrestrial ecosystems, developing fungal-based bioremediation strategies presents a promising approach for mitigating dye pollution in industrial wastewater. This strategy not only aligns with sustainable environmental practices but also offers cost-effective solutions for industries that heavily rely on synthetic dyes, such as textiles and plastics.

Future research should focus on characterizing the specific biochemical pathways and genetic adaptations that confer resistance to MG in these fungal strains. Such insights will be invaluable for engineering enhanced bioremediation systems capable of efficiently degrading a broader spectrum of recalcitrant dyes and other industrial pollutants. Additionally, integrating these fungal species into existing wastewater treatment frameworks

could significantly improve effluent quality while reducing the ecological footprint of dye-intensive industries. Lastly, this study emphasizes the dual role of fungi as both indicators of environmental health and agents of bioremediation, paving the way for innovative industrial applications aimed at restoring contaminated ecosystems and promoting sustainability.

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