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EFFICACY OF MYCOENDOPHYTES FROM BACOPA MONNIERI L. FOR HEAVY METAL TOLERANCE

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ABSTRACT The present study is related with the study of the isolation, identification of fungal endophytes from the metal tolerant plant Bacopa monnieri L. study is also related with the studying the efficacy of the mycoendophytes like Aspergillus oryzae, Fusarium oxysporum , Cladosporium herbarum, Curvularia lunata isolated from this plant. All the isolates were tolerant to copper and zinc heavy metals when tested invitro. Fusarium oxysporum and Curvularia lunata showed maximum tolerance index of Cu and Zn heavy metals. The highest tolerance level was observed in case of Fusarium oxysporumm.

KEYWORDS : Mycoendophytes, Bacopa Monneri L. Tolerance Index

INTRODUCTION:

The soil is an important life-supporting system and is central to essential planetary functions such as primary production, the regulation of biogenic gases and the earth's climate, biogeochemical and water cycling, and the maintenance of biodiversity (Abhilash et al. 2012).However, continued worldwide industrialization, modern agriculture, and urbanization have introduced heavy metals (HMs) into the soil and caused serious environmental issues (Rajkumar et al. 2010; Wei et al. 2014). Among all remediation methods, phytoremediation is considered as the most promising technology for its sustainability, cost effectiveness, and environment-friendliness (Weyens, van der Lelie, Taghavi, Vangronsveld 2009; Li, Wei, et al. 2012). To overcome these problems, microbial-assisted phytoremediation is being explored. Microorganisms could promote plant growth by transformation of nutrient elements, production of phytohormones, or provide iron to reduce the deleterious effects of metal contamination to plants (Rajkumar et al. 2010).

Microorganisms play a significant role in bioremediation of heavy metal contaminated soil and wastewater. Microorganisms including fungi have been accounted to remove heavy metals from contaminated sites through bioaccumulation at low cost and in eco-friendly way. (Jyoti Verma, Arun Bhatt and Pavan Kumar Agrawal 2016). An Endophyte is endosymbiont, often a bacterium or fungus that lives within the plants for a least part of its life cycle without causing apparent disease. Endophytes are ubiquitous and have been found in all species of plant studied to date; however, some endophytes may enhance host growth, nutrients acquisition and improve the plants ability to tolerate abiotic and biotic stresses by enhancing plant resistance to insects, pathogens and herbivores. (Petrini et.al., 1993).Removal of heavy metal ions using bioaccumulation is a promising technology. As a result, the interest in this area has grown substantially during recent years. Removal of heavy metals contamination by using bacteria, fungi, algae and yeasts has been reported by several workers (Roohan et al., 2006). Fungal cell walls and their components have major role in the sequestering of metals because they contains the different functional groups i.e. carboxyl, hydroxyl, sulphydryl, amino and phosphate groups which help them in binding of the heavy metals (Frurest and Volesky, 1989).Fungi constitutes a versatile group which can adapt and grow under various extreme conditions (Anand et al., 2006).

Zn is a transition metal that occupies central position in the Periodic Table. This metal is used in galvanizing and alloying and in the manufacture of electric goods, dying, insecticides and cosmetics. The heavy metals concentrations found in the sediment is contributed by natural geological processes and man-induced activities. Therefore, it is interesting to know if the metal concentrations in the sediments are contributed to nature or anthropogenic sources. This metal exists as a soluble, hydrated species in acidic environments. However, it may also exist as a component of insoluble complexes in neutral and alkaline soils and adsorbed to colloids in seawater (Nriagu, 1980).In addition, presence of Zn in the natural environmental of the coastal area, the anthropogenic inputs could cause elevated Zn concentration in coastal environment due to high human activities. It can cause health problem in living organisms: symptoms of dehydration, electrolyte imbalance, abdominal pain, nausea, vomiting, lethargy, dizziness, acute renal failure, and muscular incardination in human. Thus, it is listed as one of the hundred and twenty-nine priority pollutants. Zn is considered dangerous for organisms at 5 mg/L. The most important information reported is its interference with Cu metabolism. Concentration of Zn in tissue of marine plants and animals throughout the world usually are much higher and more variable than concentration of other metals. In comparison with Cu, Zn is readily taken up by plants from Zn–contaminated soils, whereas Cu resemble Pb in being reluctantly taken up by plants from contaminated soils, and nutritionally undesirable level of Cu in plants at the levels of soil contamination normally found in urban area (Purves, 1985).

Cu is a transition metal, one of several elements found in rows 4 through 7 between Groups 2 and 13 in the Periodic Table. Copper is a reddish metal that occurs naturally in rock, soil, water, sediment, and, at low levels, air. Its average concentration in the earth's crust is about 50 parts copper per million parts soil (ppm). In other words, copper also occurs naturally in all plants and animals. It is an essential element for all known living organisms including humans and other animals at low levels of intake. At much higher levels, toxic effects can occur. The term copper in this profile not only refers to copper metal, but also to compounds of copper that may be in the environment. Metallic copper can be easily molded or shaped.The reddish color of this element is most commonly seen in the electrical wiring, and water pipes. It is also found in alloys, such as brass and bronze. Compounds of copper include naturally occurring minerals as well as manufactured chemicals.The most commonly used compound of copper is copper sulfate. Many copper compounds can be recognized by their blue-green color. Copper is extensively mined and primarily used as the metal or alloy in the manufacture of wire, sheet metal, pipe, and other metal products. Copper compounds are most commonly used in agriculture to treat plant diseases, like mildew, or for water treatment and as preservatives for wood, leather, and fabrics.

Copper can enter in our body when we drink water or eat food or other substances that contain copper. Copper can also enter in our body when we breathe air or dust containing copper. It may enter the lungs of workers exposed to copper dust or fumes. Copper rapidly enters the bloodstream and is distributed throughout the body. It is essential for good health. However, exposure to higher doses can be harmful. Long-term exposure to copper dust can irritate our nose, mouth, and eyes, and cause headaches, dizziness, nausea, and diarrhea. If we drink water that contains higher than normal levels of copper, it may cause nausea, vomiting, stomach cramps, or diarrhea, Higher intakes of copper can cause liver and kidney damage and even death (Chen et al., 2007).

Low concentration of soil heavy metals does not affect the growth of plants within certain range. However, if the concentration is too high, the content of heavy metals enriched by the plant exceeds its tolerance threshold, and thus, the plant will be poisoned and may even die. In a study in Florida (US), it was observed that if the copper content in soil was more than 50 mg/ kg, it would affect citrus seedlings; if soil copper content reached 200mg/ kg, wheat would wither (Zhang et al., 1989).

Microbial remediation refers to using some microorganisms to perform the absorption, precipitation, oxidation and reduction of heavy metals in the soil. Siegel et al., (1986) found that fungi could secrete amino acids, organic acids and other metabolites to dissolve heavy metals and the mineral containing heavy metals. Microorganisms are omnipresent that dominate in heavy metal contaminated soil and can easily convert heavy metals into non-toxic forms. Microorganisms remove the heavy metals from soil by using chemicals for their growth and development. Keeping in view the significant role of fungi in bioremediation, the present studies has been designed with the aim of isolating and identifying heavy metal tolerant fungi from the industrial effluents and waste water dumping sites. The proposed study has the following objectives.Isolation and identification of heavy metal tolerant fungi from phytoremediation plants metal dumping sites and waste water region.Determination of the heavy metal tolerance limits of different fungal isolates.

MATERIALS AND METHODS:

Collection of plant material: -

Naturally growing *Bacopa monnieri* L. plant were collected from the banks of Ghodnadi river of Shirur Tehsil. *Bacopa monnieri* L. plant was freely found at Waste water sites. The plants which was healthy, fresh and disease free were selected for the studies.

Isolation of fungal endophytes:

First of all, plant parts (Root, Stem, leaves) were washed and cut in to small pieces so that they can be inoculated in petri dishes. Explants were washed thoroughly 2-3 times with distilled water followed by a wash for 2-3 seconds with 7% sodium hypochlorid. The explants were again washed with Distilled water for 1-2 times. 2-3 explants of each plant were placed in one petri dish. Inoculated petri dishes were sealed with the help of Para film. Petri dishes were allowed for incubation for 7 days at 27˚c temperature. After the desired incubation period the growth of fungal colonies were observed. 1 to 2 colonies were observed in each inoculated petri dish. Within aseptic conditions desired colonies or mycelia or hyphae of fungal body were picked from petri dishes and it was transferred into test tubes slants of PDA for further studies. Fungal colonies were observed and identified on the basis of morphology of fungal spores and colonies. Fungal isolates were identified by the slide culture technique.

Preparation of slide cultures:

The slides culture technique was used to observe microscopic structure of fungal isolates and to identify them by their morphological structures. Generally, in slide culture technique the PDA media was put on slide and spread it over with the help of sterile coverslip under aseptic condition. Fungal mycelia or spores are kept on PDA media contained slides. Slides were incubated in the moist chamber for 7 days. After 7 days slides were observed under microscope for their identification. Morphological (vegetative and reproductive) structures were examined and identification was done.

Screening of heavy Metal tolerance by using solid agar media:

To test the effect of different metals of varying concentration of heavy metals, the heavy metals of different concentration were added to the PDA media range varying from 50-1000ppm.

Fungal isolates on normal PDA medium served as control. The metal ions treated plates were inoculated with 6mm agar plates from young fungal colonies grown on normal PDA medium and were incubated at 27°C for at least 7 days. Tolerance of fungal endophytes was studied by the determination of tolerance index.

Metal Tolerance Index:

Fungal endophytes were checked for their copper (Cu++) and zinc (Zn++) tolerance.PDA plates supplemented with 50 to 1000ppm of heavy metals were inoculated with fungal isolate.

The inoculated plates were incubated at 27˚C for 7 days. The effect of each heavy metal on the growth of the isolates was estimated individually by measuring the radius of fungal growth extension against the control (without metal).

Metal Tolerance Index (Ti) was calculated as the ratio of the extended radius of the treated colony to that of the untreated colony (Akhtar et al., 2013).

$Ti = Dt/Du$

Where Dt is the radial extension (cm) of treated colony and Du is the radial extension (cm) of untreated colony.

RESULTS:

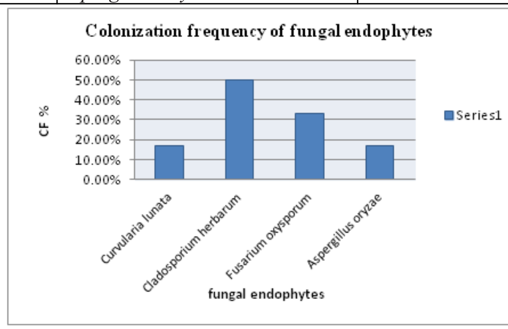
Isolation and identification of the fungi

From plant *Bacopa monnieri* following Endophytic fungi were isolated. The isolates were identified on the basis of their morphological characteristics on potato dextrose agar (PDA) and their colonization frequency was calculated. To find out colonization frequency of mycoendophytes isolated the following formula was used: -

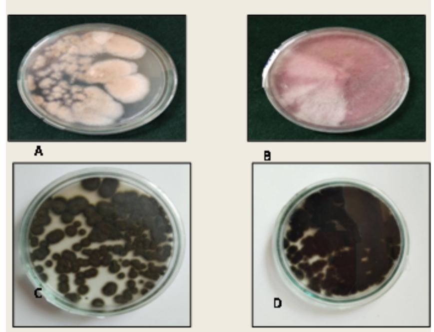
$$CF\% = \frac{\text{No. of segments colonized By endophytic fungi}}{\text{Total no. of segments}} \times 100$$

Table No. 1: Colonization frequency of fungal endophytes.

Sr. No.	Fungalisolates	CF
1	*Curvularialunata*	16.66%
2	*Cladosporium herbarum*	50%
3	*Fusarium oxysporum*	33.33%
4	*Aspergillus oryzae*	16.66%



Fungal endophytes from *Bacopa monneri* L.



A) *Aspergillus oryzae*, B) *Fusarium oxysporum* , C) *Cladosporium herbarum* ., D) *Curvularia lunata*.

Table 2: Efficacy of fungal endophytes against 'cu++' metal tolerance.

Sr. No.	Fungal isolates	control	CuSo4 conc. in ppm/radius in cm			
			50	100	500	1000
		radius in cm				
1	*Curvularia lunata*	1.5cm	1.5	1.4	1.1	0.9
2	*Cladosporium herbarum*	0.6cm	0.6	0.6	0.5	0.4
3	*Fusarium oxysporum*	2.2cm	2.2	1.8	1.3	0.5
4	*Aspergillus oryzae*	1cm	1	0.8	0.5	0.5

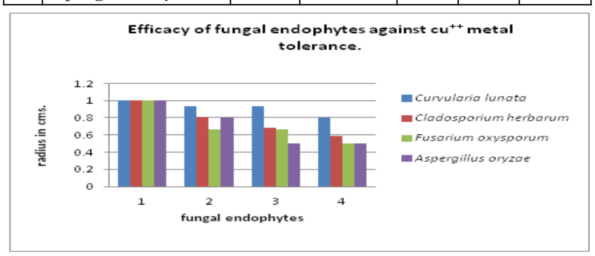
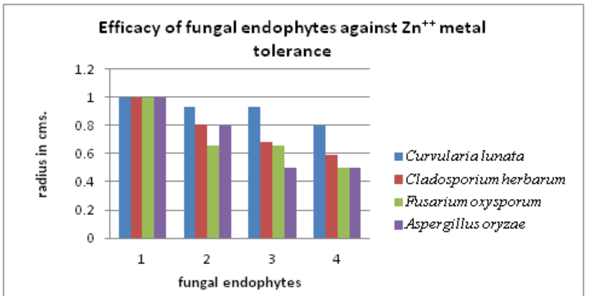


Table 3: Efficacy of f ungal endophytes against Zn++ metal tolerance

Sr. No.	Fungal isolates	ZnSo4 conc. In ppm/radius in cm			
		50	100	500	1000
1	*Curvularia lunata*	1.5	1.5	1.4	1.2
2	*Cladosporium herbarum*	0.6	0.4	0.4	0.3
3	*Fusarium oxysporum*	2.2	1.8	1.5	1.3
4	*Aspergillus oryzae*	1	0.8	0.5	0.5



Screening of heavy metal-tolerant fungal isolates

The fungal isolates recovered from different petri dishes were screened for tolerance to 50-1000ppm concentration of each heavy metals (Cu and Zn). Their indices of tolerance were determined.All the isolates were tolerant to Cu++ and Zn++ heavy metals. Fusarium oxysporum and curvularia lunata shown maximum tolerance index for Cu++ and Zn++ heavy metals.

Table No. 4 : Tolerance index of fungal I solates to heavy metal Cu++.

Name of the mycoendophytes	CuSo4 conc. In ppm / radius in cm			
	50	100	500	1000
Curvularia lunata	1	0.93	0.7	0.6
Cladosporium herbarum	1	0.81	0.59	0.22
Fusarium oxysporum	1	1	0.83	0.66
Aspergillus oryzae	1	0.8	0.8	0.5

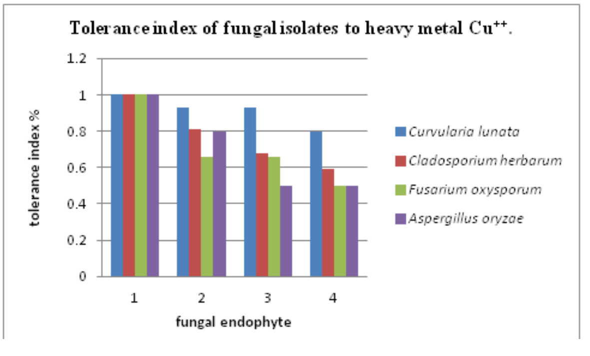
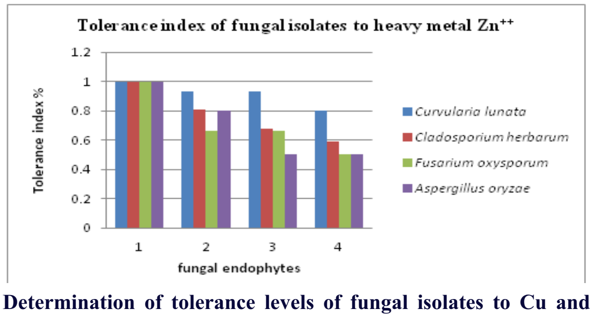


Table no. 5 Tolerance index of fungal isolates to heavy metal Zn++

Sr. No.	Fungal isolates	ZnSo4 conc. In ppm / radius in cm			
		50	100	500	1000
1	*Curvularia lunata*	1	0.93	0.93	0.8
2	*Cladosporium herbarum*	1	0.81	0.68	0.59
3	*Fusarium oxysporum*	1	0.66	0.66	0.5
4	*Aspergillus oryzae*	1	0.8	0.5	0.5



Determination of tolerance levels of fungal isolates to Cu and Zn

The ranges of heavy metal tolerance of all the fungal isolates are presented in Table no. 4 and 5.The highest tolerance limits was observed in case of Fusarium oxysporum. The heavy metal tolerance limit of fungal isolates is presented in Fig. 3 It is evident from the table that the increase in the conc. of heavy metal ions, a decrease in the diameter of zone of growth was observed.

2. CONCLUSIONS:

In the present study Bacopa monneri L. was used for phytoremediation which was collected from industrial effluents and wastewater sites in and around Shirur city of Pune district Maharashtra. A total of 4 fungal endophytes were isolated from the above plant and studied for the heavy metal resistance. The isolates were identified on the basis of morphological characteristics.Microscopic examination revealed that 4 endophytes were as, Curvularia lunata, Cladosporium sp., Fusarium oxysporum and Aspergillus oryzae. All the isolates were tolerant to all the heavy metals like copper and zinc. Fusarium oxysporum and Curvularia lunata showed maximum tolerance index to Cu and Zn heavy metals. The highest tolerance level was observed in case of Fusarium oxysporumm which is 2.2 cm for Zn and Cu respectively.

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