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In-Vitro study on tolerance of heavy metals by some endophytic fungi

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Abstract: **The present study reports on tolerance study of heavy metals by endophytic fungi. Endophytic fungi isolated from agriculture field used for the study were *Phomopsis sp*, *Fusarium sp.1*, *Colletotrichum sp*, *Fusarium sp.2* and *Phoma sp*. To test the effect of different metal on varying concentration of heavy metal, the heavy metal of different concentration were added to the PDA media range varying from 50-600ppm. Fungal endophytes on PDA medium without heavy metal served as control. PDA plates with metal ions were inoculated with fresh culture of fungus and were allowed to incubate at 27 °C for 7 days. The different test isolates at the different concentrations of the test metals exhibited varying degree of effect on mycelia formation. The results indicated at 600 ppm metal concentration, the most heavy metals tolerant endophytic fungus was *Colletotrichum sp* (resistant to all metals under study) followed by *Fusarium sp.1* and *Phoma sp* (resistant to Cu (II), Zn (II), Pb (II) and Cd (II)). *Phomopsis sp* and *Fusarium sp.2* were tolerant to Cu (II), Zn (II) and Pb (II) and Zn (II), Pb (II) and Cr (VI) respectively. The ability of fungal endophytes to grow in the presence of heavy metals is always be an added advantage in the treatment of waste water where role of microorganisms is to degrade organic matter through biological process.**

Keywords: **Endophytic fungi, Heavy metal Tolerance, Bioremediation.**

1. INTRODUCTION

Due to increased industrialization and technological development, contamination of soil and water environments by heavy metals also increases significantly leading to heavy metal pollution. Such pollution has serious impact on the environment and public health as heavy metals are toxic in nature, non-biodegradable and are bio-accumulator. Failures of conventional treatment systems for heavy metal removal mainly pointed for high expenses, low efficient, labor-intensive operational or lack of selectivity in the treating process [1], [2], [3], [4].

In order to detoxify and degrade environmental pollutants, alternatives strategies has been necessitated for environmental clean-up. Recently, remediation with use of microorganisms has gained considerable attention for the restoration of contaminated environments. This remediation includes the phytoremediation, bacterial bioremediation and mycoremediation and effectiveness of these options has been well reported [5], [6], [7], [8]. Among these options, mycoremediation has been explored more recently because of its low cost inputs and significant outputs in the bioremediation of environments polluted with heavy metals [9], [10].

Plant-associated fungi isolated from the internal tissues, where reside and cause asymptomatic infection in the host, are called endophytes. Such fungal endophytes are often found to have beneficial effects on plant growth by providing essential elements, resisting colonization by pathogenic microorganisms, or by assisting the plant to adapt to environment [11], [12], [13]. Metal tolerance/resistance referred to as the capacity of a microorganism to survive to lethal effect of metal by means of involvement of their adaptation mechanisms when exposed to the metal(s) concerned [7]. Endophytic fungi have been known for their distinct attributes such as ability to adapt into host to the competitive, high-stress environment of the soil, and therefore with this advantage, these fungi serve as effective bioremediator [14], [15], [16], [17]. The aim of this study is to analyse the effect of heavy metals on growth response of fungal endophytes under laboratory conditions.

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2. MATERIALS AND METHODS

Fungi used

Endophytic fungi isolated from agriculture field used for the study were *Phomopsis sp*, *Fusarium sp.1*, *Colletotrichum sp*, *Fusarium sp.2 and Phoma sp.*

Preparation of reagents and Growth medium

Cd (II), Pb (II), Cu (II), Cr (VI) and Zn (II) stock solution (1000 mg/L) were prepared by adding the exact quantities of the $3CdSO_4.8H_2O$, $PbSO_4$, $CuSO_4.5H_2O$, $K_2Cr_2O_7$ and $ZnSO_4.7H_2O$ in distilled water. This stock solution will be used for preparing the working concentration of Cd (II), Pb (II), Cu (II), Cr (VI) and Zn (II) solution. Fungi were cultivated on Potato Dextrose Agar (PDA).

Screening of Fungal endophytes for Tolerance to Heavy Metals

To assess the effect of varied concentrations of five heavy metals on endophytic fungi, the heavy metal of different concentration were added to the PDA (pH 5.1-5.3) media range varying from 50-600ppm. Fungal isolates on normal PDA medium served as control. The PDA plates with heavy metals were inoculated with small pieces of agar from fresh fungus culture and were given 7 days of incubation at 27 °C. The experiments were carried out in three replicates [18].

Growth response of tested fungal strains in heavy metal-rich media

Fungal endophytes were checked for their Copper, Lead, Chromium, Cadmium and Zinc tolerance. PDA plates added with 50-600ppm of heavy metal were inoculated with fungal endophytes. The inoculated plates were allowed to incubate. Growth pattern of the fungi obtained after the treatment with each heavy metal was calculated individually by measuring the diameter of fungal growth extension against the control (without metal) [16], [19].

Statistical analysis:

All experiments were performed in three replicated and the data was presented as Mean (SD). The data were analyzed for statistical significance using analysis variance (One way ANOVA) by Tukey test using SPSS 20.0 and Microsoft excel 2007 to compare the treatment group with their respective controls. The results are significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

Analysis of Fungal endophytes for tolerance to heavy metals

The effect of heavy metals on fungal growth was assessed on the basis of mycelia diameter. The different test isolates at the different concentrations of the test metals exhibited varying degree of effect on mycelia formation. The *Colletotrichum sp* was found to be most heavy metals tolerant endophytic fungus, tolerated all metals at high concentration of 600 ppm. *Phomopsis sp* was tolerant to Cu (II), Zn (II) and Pb (II), *Fusarium sp.1* and *Phoma sp* exhibited tolerance to Cu (II), Zn (II), Pb (II) and Cd (II) whereas *Fusarium sp.2* showed tolerance to Zn (II), Pb (II) and Cr (VI) at 600 ppm.(TABLE 1.) Observation showed maximum growth of fungal endophytes at low concentration of heavy metals but as the concentration of heavy metals increased, there were decrease in growth of fungi. All the tested isolates showed strong colony growth on Zn (II) and Pb (II) media at 600µg/mL in comparison to the control. Similar studies of L. Ezzouhri *et. al* [20] reported *Aspergillus* and *Penicillium*, the most resistant isolates, were found to exhibit tolerance and strong growth in presence of heavy metals (Pb, Cr, Cu and Zn, Cd). A. Sani *et al*. [21] documented *Cheatomium sp* showed highest resistance to Co, Cu, Zn and Cd metals.

TABLE 1: Growth of the fungi observed at 600 ppm concentration of heavy metals (Cu, Zn, Pb, Cr and Cd)

Endophytic fungi	Cu	Zn	Pb	Cr	Cd
Phomopsis sp	+	+	+	-	-
Fusarium sp.1	+	+	+	-	+
Colletotrichum sp	+	+	+	+	+
Fusarium sp.2	-	+	+	+	-
Phoma sp	+	+	+	-	+

+: Indicate the presence of growth, -: Indicates absence of growth, Cu: Copper, Zn: Zinc Pb: Lead, Cr: Chromium, Cd: Cadmium.

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Growth response of tested fungal strains in heavy metal-rich media

Effect of Copper, Zinc, Lead, Chromium and Cadmium on the tested fungal endophytes is presented in TABLE 2. On exposure to zinc and lead concentrations, all endophytic fungi exhibited mycelia growths compared to the control. With respect to *Phomopsis sp, Fusarium sp.1 Colletotrichum sp* and *Phoma sp*, no statistical ($p > 0.05$) differences were obtained in the radial growth of the fungi compared to their controls in Zn (50-600 ppm) and Pb (50-600 ppm) enriched media. When exposed to Zn and Pb-enriched media, *Fusarium sp.2* revealed statistical ($p < 0.05$) difference in radial growth compared to the control at 600 ppm. M. K. Mahish *et. al*. [22] reported the *Fusarium sp.1* was found significantly tolerant to Cr, Pb, Fe and Zn while its growth was inhibited with Cu. *Colletotrichum sp.1* exhibited inhibited growth in presence of Zn, Cu and Al while tolerance towards Cr and Pb with no significant difference. In the studies of A. K. Vala *et. al*. [23] indicated *Aspergillus* isolates were resistant a wide range of Cr (VI).

From the observation, five endophytic fungi showed different radial fungal growth against individual heavy metal (Fig.1 to 5) The difference in radial growth may be due to the presence of various strategies of resistance mechanism exhibited by the fungi [3], [24]. Studies on fungi like *Aspergillus*, *Fusarium*, *Humicola*, and *Nannizzia* have been documented to exhibit tolerance against heavy metals [20], [25]. The results noted that some native fungi (*Phoma* spp., *Peyronellaea* sp., and *Alternaria* sp.) have been adapted to constant metal stress enviornment for a long time. Therefore, beneficial effects of these stress-adapted fungi might be employed for phytoremediation in heavy metal contaminated soils [26]. It is observed that as the concentration of heavy metal increased, the growth of the fungi decreased due to toxicity of heavy metals. On the whole, a growth response order of Pb = Zn > Cu > Cd > Cr was shown among the endophytic fungi to elevated heavy metal levels. Similarly their response in media composed with heavy metals, an order showing *Colletotrichum sp* > *Fusarium sp.1*=*Phoma sp* > *Phomopsis sp* = *Fusarium sp.2* was observed.

TABLE 2: Effect of varied concentrations of heavy metal on endophytic fungi growth

Endophytic fungi	Conc. of heavy metals (μg/mL)	Cu	Zn	Pb	Cr	Cd
Phomopsis sp	0	40.00	40.00	40.00	7.00***	40.67(0.577)
	50	40.00	40.00	40.00	17.33(0.577)	39.33(0.577)
	100	40.00	40.00	40.00	17.67(0.577)	21(1.000)***
	200	19.33(0.577)***	40.00	40.00	40.00***	14.67(0.577)***
	400	9.33(0.577)***	40.00	40.00	0.00	0.00
	600	13.33(0.577)***	40.00	40.00	0.00	0.00
Fusarium sp.1	0	40.00	40.00	40.00	40.00	41.67(0.577)***
	50	40.00	40.00	40.00	40.00	29.33(0.577)
	100	38.00***	40.00	40.00	40.00	27.33(1.528)
	200	19.00***	40.00	40.00	40.00	20(1.000)***
	400	12.00***	40.00	40.00	0.00	10.00
	600	15.67(0.577)***	40.00	40.00	0.00	9.00
Colletotrichum sp	0	40.00	40.00	40.00	40.00	40.67(0.577)*
	50	40.00	40.00	40.00	40.00	37*
	100	40.00	40.00	40.00	40.00	28(1.000)***
	200	15.33(0.577)***	40.00	40.00	40.00	15.67(0.577)*
	400	9.33(0.577)***	40.00	40.00	27***	12.67(0.577)*
	600	12***	40.00	40.00	31.67(0.577)***	9.67(1.528)*
Fusarium sp.2	0	16***	32.33(0.577)	32.00	40.00	16.67(0.577)***
	50	12.67(1.155)*	32.33(0.577)	32.00	40.00	13.33(0.577)

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	100	10.67(1.155)*	30.00	36.33(0.577)***	40.00	12.33(0.577)
	200	0.00	28.67(0.577)	32.00	40.00	12(1.000)
	400	0.00	27.33(0.577)	29.33(0.577)***	40.00	0.00
	600	0.00	21.67(0.577)***	33.33(0.577)*	23.67(0.577)***	0.00
Phoma sp	0	40.00	40.00	40.00	18.67(0.577)*	40.67(0.577)***
	50	40.00	40.00	40.00	18*	30.33(0.577)***
	100	23.33(0.577)***	40.00	40.00	22***	26(1.000)***
	200	18.00	40.00	40.00	40***	15.33(0.577)***
	400	14.67(1.155)***	40.00	40.00	0.00	11.67(0.577)
	600	17.00	40.00	40.00	0.00	10.00

The data represent Mean of diameter of fungus growth and Standard deviation (parenthesis) of three independent experiments.

*Significance at P ≤ 0.05, ** Significance at P ≤ 0.01, *** Significance at P≤ 0.005.

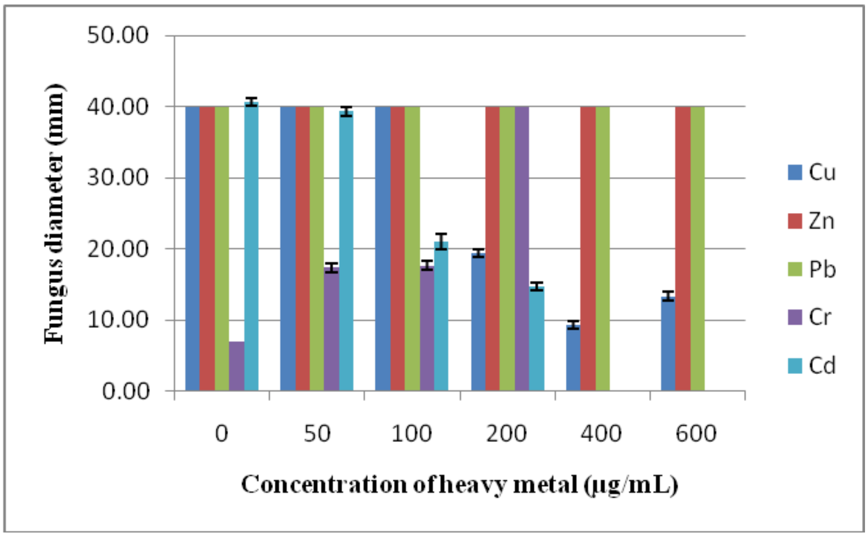

Fig. 1: Effect of varied concentrations of heavy metals on fungus *Phomopsis sp* radial growth (mm) over 7 days

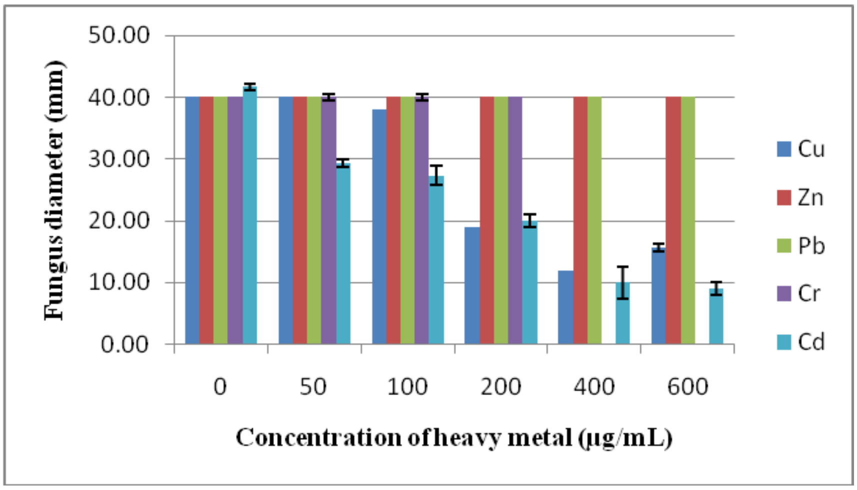

Fig. 2: Effect of varied concentrations of heavy metals on fungus *Fusarium sp.1*radial growth (mm) over 7 days.

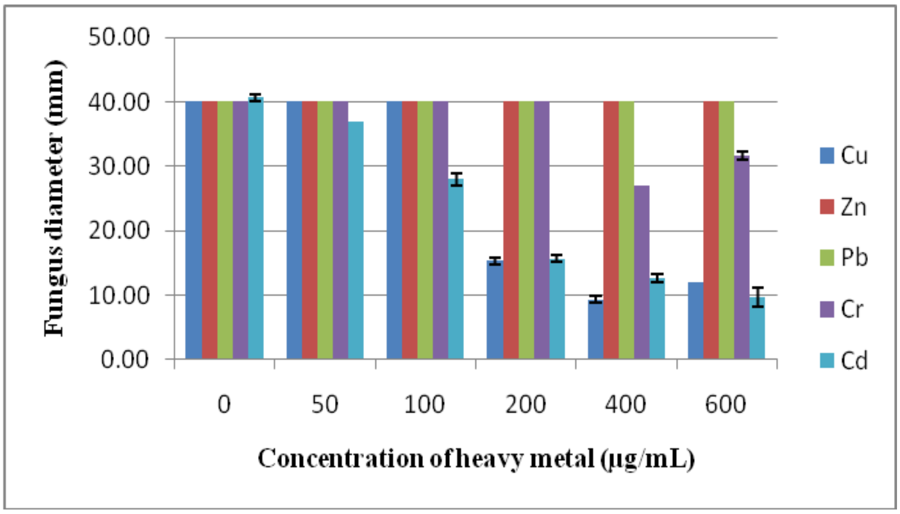

Fig. 3: Effect of varied concentrations of heavy metals on fungus *Colletotrichum sp* radial growth (mm) over 7 days.

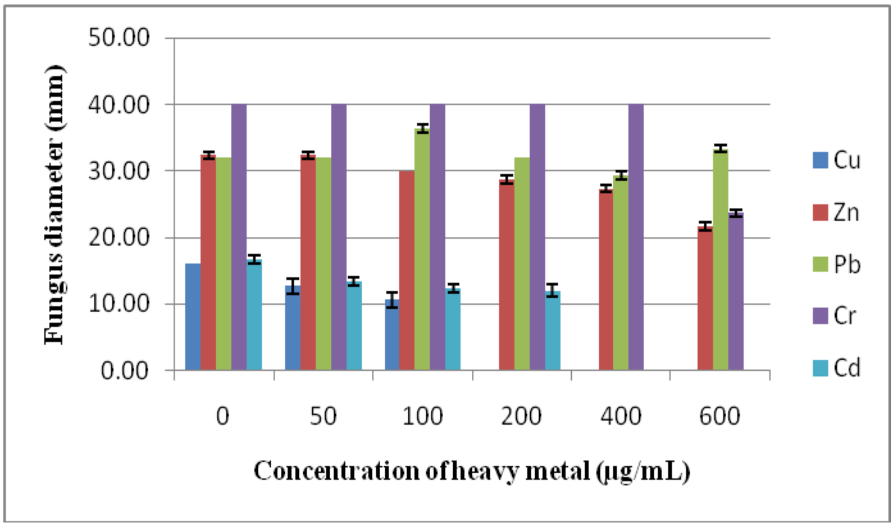

Fig. 4: Effect of varied concentrations of heavy metals on fungus *Fusarium sp.2* radial growth (mm) over 7 days.

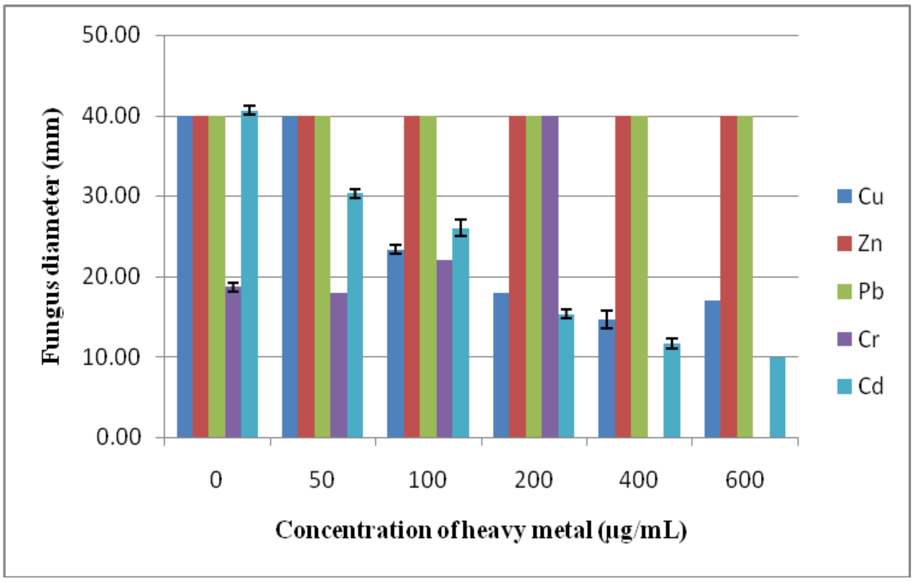

Fig.5: Effect of varied concentrations of heavy metals on fungus *Phoma sp* radial growth (mm) over 7 days.

4. CONCLUSION

The results showed that the fungal population has the ability to resist higher concentrations of metals. Among different fungal endophytes, the *Colletotrichum sp* was the most resistant to all the metals tested, this exceptional character indicate the bioremediative potentials. Such investigation could provide a new direction for applying the endophytic fungi as prospective bioremediation agents for decontamination of pollutants favouring more environmental and economical concern. Also the plant having endophytic fungi may survive against the metal pollutant atmosphere. Also in the treatment of waste water, by taking advantage to grow in the presence of heavy metal, fungal endophytes would be helpful in the decomposition of organic matter present in waste water contaminated with heavy metals.

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CONFLICT OF INTEREST

There is no any conflict of interest exist.

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