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Effect of heavy metals and xenobiotic compound on growth of some endophytic fungi isolated from Achanakmar –Amarkantak biosphere reserve, India

Manoj Kumar Mahish*, R.V. Shukla, Anil Choubey and Amit Sharma
Microbial Physiological Laboratory, Department of Botany, C.M. Dubey Post Graduate College Bilaspur (Chhattisgarh), India
manoj.mahish2016@gmail.com

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Abstract

The present study reports effect of heavy metals and Xenobiotic compound against the growth of some endophytic fungi. The endophytes were isolated from Sal (Sorea robusta) and Bahera (Terminallia bellerica) tree of Achanakmar-Amarkantak biosphere reserve, India. In view of the diversity of fungi Fusarium sp.1 and Colletotrichum sp.1 was screened for present work. The fungi were treated with soluble and insoluble heavy metals and Benxyl hexa chloride. The Fusarium sp. 1 was found resistant towards $FeCl_2$ and $ZnSO_4$ while 75% growth inhibition was observed with $CuSO_4$. Inhibition of growth of 66.67% was found with the treatment of $ZnSO_4$ and $CuSO_4$ to Colletotrichum sp. 1. Both the fungi demonstrated most extreme growth inhibition with insoluble Cu and Al while tolerance towards Cr and Pb. Fusarium Sp. 1 was found more delicate with the Benxyl hexa chloride with half hindrance of development in 500 mg/l concentration while just 25% development of Colletotrichum sp. 1 was restrained with the same. The P value for use of different concentration of heavy metals has been found P 0.44 for Fusarium sp. 1 while for Colletotricum it was recorded P 0.79 indicates no significant difference. Now it is concluded that endophytic fungi endure the heavy metals and xenobiotic compounds yet impact on the development was seen when concentration increases.

Keywords: Endophytic fungi, Achanakmar-Amarkantak biosphere reserve, Heavy metals, Xenobiotic compounds, Sal (*Sorea robusta*) and Bahera (*Terminallia bellerica*).

Introduction

Every one of the living beings possessing plant organs that sooner or later in their life can colonize inner plant tissues (e.g root, stem, leaves) without bringing about evident damage to the host[1-5]. The term endophyte was gotten from the Greek word "endo" implies inside; "phyte" implies plant, since the revelation of endophytic fungus in darnel (*Lolium temulentum*) Germany[6]. About 4000 secondary metabolites from different fungal species were depicted as organically dynamic[7]. Endophytes give a wide assortment of bioactive secondary metabolites with one of a kind structure including alkaloids, benzopyranones, chinones, quinones, steroids, flavonoids, phenolic acids, terpenoids, tetralones, xanthones and so forth[6]. Such bioactive metabolites uses as agrochemicals, antibiotics, immunosuppressants, antiparasitics, antioxidants, anticancer, antiviral, antioxidants, insecticidal and immunomodulatory[6,8,9,10].

The Achanakmar-Amarkantak biosphere reserve has a place with Chhattisgarh and Madhya Pradesh state, India. The Chhattisgarh is newly established state and it is rich in the mineral and vegetation. The vegetation of Chhattisgarh is essentially overall known for the Sal timberland which covers more than 40% zone of the state. Rapid industrialization and mining in Chhattisgarh is under progress after becoming as a new state because of substantial accessibility of coal, iron, bauxite, clay, aluminum etc. The industrialization is additionally thriving in the woodland cover ruled with Sal tree. The industrialization, urbanization, farming to a great extent contributes heavy metals, xenobiotic compounds and numerous other organic and inorganic pollutants to atmosphere, water assets and soil of the region. Along these lines, it is extremely important to ensure plants which are the wellspring of nourishment and shelter of tribal population.

Endophyte infected plants have been accounted for to have expanded resistance to drought, heat, metal toxicity, low pH, and high Salinity[11,12]. Xenobiotic compound incorporates engineered polymer, polychlorinated biophenyls, alkylbenzyl sulphonates, oil blend, manure, hydrocorbun and so on. Subsequently they are non biodegradable and endure for long time in environment[13]. This compound affects the fish, insects and other animal's when come to natural way of life furthermore influences the ripeness of soil[14,15]. The fungi were known to degrade DDT (Dichlorodiphenyldichlorotyluvine), hexa chlorocyclohexen, endosulfan, endrin etc.[16,17]. Similarly as xenobiotic compounds heavy metals are additionally harmful to flora and fauna originated from anthropogenic sources in forest area including mining and industrial applications. Fungi absorb the metal ions in to cell and also chelate on cell, so that they tolerant heavy metal compounds[18-20].

Along these lines, endophytic organisms are presently one alternate to protect the Sal and other medicinal plants against environment contaminated from heavy metals and Xenobiotic compounds. It is necessary to obtain fungi from plant and to investigate its tolerance towards such pollutants. The present concentrate in this manner means to test some Xenobiotic compound and substantial metals against the development of endophytic growths isolated from Sal and bahera tree of Achanakmar – Amarkantak Biosphere reserve, India.

Materials and methods

Study area: The Achanakmar Amarkantak Biosphere Reserve (ABR) has an area of 552 sq. km. It is situated between 22°15' to 22°58' N latitude and 81°25' to 82°5' E longitudes. The ABR is spread in Madhya Pradesh (Anuppur and Dindori district) and partly in Chhattisgarh state (Mungeli district). The reserve falls in almost northern part of bio-geographic zone and bio-geographic province (Deccan peninsula, central highlands). About 68.10% out of the sum area of this reserve lies in the Mungeli district in Chhattisgarh. ABR is a 14th Biosphere Reserve which is representing for numbers of endangered medicinal tree species. The forest covers of the ABR correspond to tropical deciduous vegetation and can be classified into northern tropical moist deciduous and southern dry mixed deciduous forests[21]. The area is "Genetic Express Highway" connecting two biological hot spots that is, the Western Ghats and the Eastern Himalayas that serves as meeting point for the northern and the southern kind of vegetation.

Isolation and Identification of Endophytic Fungi: The tree *Terminalia bellerica* (Bahera), and *Shorea robusta* (Sal) trees were selected for the isolation of endophytic fungi. The mature leaves of above tree species were gathered from various woodland destinations of ABR in Mungeli area. Sampling of plant tissues (leaf) were done early in the morning. The leaves of Bahera and Sal trees were gathered from the tallness of 10-15 ft. The segments were then surface cleaned taking after the strategy from previous work[22]. Segments of each sample were placed on growth medium of potato dextrose agar (PDA). The Parafilm - sealed Petri dishes were then incubated at 27 ± 1°C for 25 days supplied with 12-h. light/dark cycle. Standard taxonomic guidebooks were used to recognize the fungal genera[23-26].

Effect of heavy metals and Xenobiotic compound on growth of Endophytic fungi: Benzyl hexa chloride (BHC) was used as xenobiotic compound and soluble and insoluble Iron, Copper, Chromium, Lead and Zinc were used as heavy metal compound for its impact on development of some endophytic fungi. $FeCl_2$, $CuSo_4$ and $ZnSo_4$ were dissolved in potato dextrose agar media to make 50, 100 and 500 mg/l in PDB (potato dextrose broth) medium. The liquid medium containing xenobiotic compound and heavy metals were taken in 150 ml of conical flasks. One flask was additionally taken as control without metal ion and BHC. After autoclaving of flasks fungi were incubated on it and incubated for 27±1°C for 7 days. After incubation period mycelium was measured using pre weighted filter paper. The effect of heavy metal and xenobiotic compounds in endophytic fungi was calculated by comparing the dry weight of mycelium with control one. The above methodology was taken from previous work with some modification[27,28]. The insoluble heavy metal powder was also used to study the effect of metal ions in growth of endophytic fungi. Chromium, Copper, Aluminum and Lead metal powder were placed in PDA medium plate by making whole in borer. The fungi were inoculated in medium and allow growing around well containing metal powder. The impact of metal particles on development of fungi was ascertained by measuring the width of zone around well.

Results and discussion

The effect of soluble and insoluble heavy metals on growth of *Fusarium* sp. 1 and *Colletotricum* sp. 1 is introduced here with tables and figures. The *Fusarium* sp. 1 was able to tolerate 100 mg/l concentration of $FeCl_2$ and $ZnSO_4$ with no hindrance of development, while at 500 mg/l concentration 25 percent of growth was inhibited. Also, contemplated with the $CuSO_4$ 25 and 75 percent growth was repressed at 100 and 500 mg/l concentration (Table-1). The mean weights of triplicate mycelium of control alongside tests were subjected to one way anova at significance level P 0.05. The P value was found 0.44 which is higher than the table value. So, there is no significant different among treatment of different heavy metals on growth of *Fusarium* sp. 1. The mean value of dry weight of mycelium was found higher in $FeCl_2$ and $ZnSO_4$ treated mycelium (0.375 mg/l) than $CuSO_4$ treated (0.300 mg/l). So also, soluble heavy metals compounds $FeCl_2$, $CuSO_4$ and $ZnSO_4$ were additionally subjected to study against growth of *Colletotricum* sp. 1. The 500 mg/l concentration of $CuSO_4$ and $ZnSO_4$ could repress the 66.67% development of fungus (Table-2). One way Anova was also performed to find out the significance of effect of different heavy metal on growth of *Colletotricum* sp. 1 at P 0.05 levels. The P value was found 0.79 which is higher than the significance level. So, there is no significant different among treatment of different heavy metals on growth of *Colletotricum* sp. 1. The mean value of dry weight of mycelium was found higher in $CuSO_4$ treated mycelium (0.225 mg/l) followed by $Fecl_2$ (0.200 mg/l) and $ZnSO_4$ (0.175 mg/l).

The insoluble heavy metals Cr, Cu, Al and Pb were also studied against the growth of endophytes. The Table-3 demonstrating the detail of finding of the experiment. The Copper and the Aluminum were the heavy metal effected the development of fungi. *Colletotricum* sp. 1 was found more sensitive than *Fusarium* sp. 1. Endophytes endure the chromium and lead demonstrating whole development in agar medium. *Fusarium* sp. 1 was discovered more resistance limit against heavy metal ions compare with *Colletotrichum* sp. 1 species. The tolerance of Lead, Chromium, Copper and Zinc by *Fusarium* species was reported previously[29]. The fungi from genera of *Fusarium* likewise reported to resistant high concentration of heavy metal[27,30].

Table-1: Effect of soluble heavy metals on growth of *Fusarium* sp. 1

S. No.	Heavy metals	Concentration (mg/l)	Weight of mycelium (g) Mean of Triplicate	Percent of growth inhibition (C-T/C*100)
1	$FeCl_2$	Control	0.4	00
		50mg/l	0.4	00
		100mg/l	0.4	00
		500mg/l	0.3	25
2	$CuSO_4$	Control	0.4	00
		50mg/l	0.4	00
		100mg/l	0.3	25
		500mg/l	0.1	75
3	$ZnSO_4$	Control	0.4	00
		50mg/l	0.4	00
		100mg/l	0.4	00
		500mg/l	0.3	25

Table-2: Effect of soluble heavy metals on growth of Colletotrichum sp. 1 species

S. No.	Heavy metals	Concentration (mg/l)	Weight of mycelium (g) mean of triplicate	Percent of growth inhibition (C-T/C*100)
1	$FeCl_2$	Control	0.3	00
		50mg/l	0.3	00
		100mg/l	0.1	33.33
		500mg/l	0.1	33.33
2	$CuSO_4$	Control	0.3	00.00
		50mg/l	0.3	00.00
		100mg/l	0.2	33.33
		500mg/l	0.1	66.67
3	$ZnSO_4$	Control	0.3	00
		50mg/l	0.2	33.33
		100mg/l	0.1	66.67
		500mg/l	0.1	66.67

Effect of Xenobiotic compound BHC on growth of endophytic fungi was studies. Fifty percent growth was inhibited in 50 to 500 mg/l BHC supplement in medium for growth of *Fusarium* sp.1 while studies with *Colletotrichum* sp.1 just 25 percent growth was repressed in 500 mg/l BHC supplement in medium (Table-4). The significance of effect of different concentration of BHC on *Fusarium* sp. 1 and *Colletotrichum* sp. 1 was subjected to study using one way anova at level P 0.05. The P value was found 0.41 which is higher than the significance level. So, there is no significant different among treatment of different concentration of BHC on *Fusarium* sp. 1 and *Colletotrichum* sp. 1. The mean value of dry weight of mycelium was found higher in *Colletotrichum* sp. 1 (0.350 mg/l) than *Fusarium* sp. 1 (0.250 mg/l). The genera of *Fusarium* sp.1 were found to be degrading the Xenobiotic compound such as endosulfan and DDT[31,32]. The benzyle hexa chloride studied in the present work was also found to be degrade by some fungal species[33,34].

Table-3: Effect of Insoluble heavy metals on growth of some endophytic Fungi

S. No.	Heavy metals	Fungi	Diameter of Zone (Day 7)
1	Cr	*Fusarium* sp-1	00 mm
		Colletotrichum sp-1	00 mm
2	Cu	*Fusarium* sp-1	38 mm
		Colletotrichum sp-1	50 mm
3	Al	*Fusarium* sp-1	18 mm
		Colletotrichum sp-1	22 mm
4	Pb	*Fusarium* sp-1	00 mm
		Colletotrichum sp-1	00 mm

Table-4: Effect of Xenobiotic compound (BHC) on growth of some endophytic compounds

S. No.	concentration of Xenobiotic BHC on mg/l	Fungi	Wight of Mycelium (g) Mean of triplicate	Percentage of inhibition (C-T/C*100)
1	Control	*Fusarium* sp. 1	0.4	00
		Colletotrichum sp. 1	0.4	00
2	50 -mg/l	*Fusarium* sp. 1	0.2	50
		Colletotrichum sp. 1	0.4	00
3	100 -mg/l	*Fusarium* sp. 1	0.2	50
		Colletotrichum sp. 1	0.3	25
4	500 -mg/l	*Fusarium* sp. 1	0.2	50
		Colletotrichum sp. 1	0.3	25

Conclusion

Growth of a plant in pollutant atmosphere including heavy metals and Xenobiotic is so essential regarding its survival. Its expectation is concentrated in the present work. Relationship with endophytic fungi and plant benefited each other. Endophytes of Sal and Bahera were observed to tolerate soluble and insoluble heavy metals and Xenobiotic compounds while hindrance of development was discovered just in the high focus. The study now concluded tolerance of endophytes against heavy metal and Xenobiotic compound. So that, plant having endophytic fungi may survive against the metal and Xenobiotic pollutant atmosphere.

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