Studies on Biodiversity of Fungal Endophytes of Indigenous Monocotaceous and Dicotaceous Plants and Evaluation of their Enzymatic Potentialities

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***Abstract*-** Fungal species that establish an endophytic role inside the tissues of plants are known to produce a wide range of biologically active metabolites and enzymes. In the present study, Endophytic fungi were isolated from the stems and leaves of monocot and dicot plants species collected from Jabalpur region, (M.P.) and were screened for their ability to produce amylase, cellulase, protease, lipase, and laccase activity. Each of endophytic fungal isolates showed a wide range of enzyme activity. Total ninety five fungal strains were isolates from plants and total thirty strains tested for their ability to produce amylase, cellulase, protease, lipase, and laccase activity. The ability to produce these enzymes was distributed amongst the strains tested. The implication of enzyme production in relation to lifestyle abilities of the endophytes is discussed.

***Index Terms*-** Endophytic microorganism, Enzyme, Fungi, Monocot, Dicot.

I. INTRODUCTION

The term endophyte (Greek: endo = within + phyte=plant) include all organism that during a variable period of their life colonies the living internal tissues of their hosts. Bacteria, actinomycetes and fungus can be found in inside the plants [10] defined endophytes as "the organisms inhabiting plant organs some time in their life internal plant tissues without causing apparent harm to the host". Endophytes are found in a wide verity of plant tissue types such as seeds, ovules, fruits, stems, roots, leaves, tubers, buds, xylem and bark [2][14]. Endophytic microorganisms are those which inhabitant the internal part of plants, causing apparently no visible changes to their hosts. Although known since long time, their importance become evident only more recently when it was shown that they play specific roles as for instance, protecting the host-plants against insects and diseases. It has been found also that some endophytic microorganisms can produce valuable pharmaceutical substances of biotechnological interest [12] [13].Fungal endophytes live internally, either intracellularly or intracellularly without causing overt sign of damage [2] within the plant tissue. Fungal endophyte usually occurs in monocot and dicot plant part such as stem, leaves and root accourding to previous reports [1]. Several studies have shown the presence of fungal endophytes in host speices belonging virtually to all plant divisions from mosses and ferns to monocotyledons and dicotyledons. Limited evidence also indicates that Endophytic fungi may influence population dynamics, plant community diversity & Ecosystem function. Many scientist proved that Monocot and Dicot Plant is rich in nutrient & they are beneficial host for the Endophytic fungi, for example *Callicarpa tomentosa,Withania somnifera, Clotropis procera and Cynodon dactylon* [1] [8] [9]. Resent report indicates that the endophytic fungi are responsible for the production of secondary metabolites [6] [11] [13]. Presence of Endophytic fungi in monocot and dicot plant is in the most cases beneficial for the plant. The aim of the present study was to identify the endophytic fungi form dicot and monocot plants part and screened their enzymetic activity.

II. METHODS

1. Source of endophytes-

Fungal endophytes were isolated from the monocot and dicot plant parts (Table 1). The plants samples were collected from the diverse region of Jabalpur. The samples was collected in sterile plastic bags, sealed and carefully brought to the laboratory.

2. Isolation of Fungal Endophytes-

The fungal endophytes isolated from various plant parts i.e. root, stem and leaf from monocot and dicot plants [1] [14].

Isolation from leaves-In the laboratory, the sample leave surfaces were sterilized to remove all microbial epiphytes by soaking them in 1:5 dilutions of NaOCl (Sodium Hypochlorite solution for 15 minutes. Then, rinsed in sterile distilled water and dipped in 70% ethanol for 10 minutes. After that the samples were washed with distilled water. Then from the surface sterilized leaves, segments approximately 2mm X 2mm were aseptically cut with sterile scalpel in a laminar-flow hood, the outer tissues of the sample were cut so as to expose the interior surface to PDA plates and incubated at 28±1ºC for the appearance of the fungal growth. After about 15 days of incubation mycelial growth appeared on the plates. Then small bits of these growths from periphery were transferred onto new PDA plates and repetitive re-plating of the fungal colonies was continued until the pure cultures were obtained. Differences in morphology shape and color helped to distinguish between different microbial entities.

Isolation from stem and roots-The roots of each plant were first of all washed in sterile water to remove the debris and epiphytic microorganisms attached to outer surfaces surface then subjected to sequential 1-min washes with solutions containing 1% sodium hypochlorite, 70% ethanol and sterile distilled water. These root sections were then chopped into 1-cm lengths and inoculated into PDA plates as such to expose their internal

surfaces onto the plates and incubated at 28±1ºC till the growth appeared in the plates. Further the organisms were purified in the same way as mentioned above in the case of isolation from leaves by repeated sub culturing method.

3. Identification of Isolated fungal organisms-

The identification of fungi was done using the culture characteristics and microscopic characteristics of fungal culture such as shape, color, pattern and arrangement of the mycelium, conidial arrangement, types of spore etc [4]. All the isolated fungi were identified up to genus level on the bases of detailed culture and microscopic study and by consulting relevant literature. The pure culture of isolated fungal strains was maintained in PDA slants at 28^0C during the study.

III. STUDIES ON ENZYME PRODUCING ABILITY

The isolated fungal cultures were subjected to their enzymatic activity i.e amylase, cellulase, laccase, lipase and protease.

Assessment of enzyme producing efficiencies of fungal endophytes:-

- ✓ A total of five types of enzymes viz, Amylases, Cellulases, Laccases, Lipases and Proteases producing activity were assessed on solid medium as per methods of [5] [7] [8] [9].
- ✓ Amylase Activity: The amylase producing ability of all the isolated fungal endophytes were assessed by growing the fungal organisms on Glucose Yeast Peptone Agar (Glucose: 1g; Yeast extract: 1g; Peptone: 0.5g; Agar: 16g; D.W.:1000ml; pH 6) medium supplemented with 2% starch as substrate for enzyme activity and incubating the plates for 7 days at 28±1ºC for appearance of the fungal growth. After incubation the pates were flooded with1% iodine. The clear zone formed surrounding the respective fungal colonies were considered positive for amylase activity.
- ✓ Cellulase Activity: The cellulose producing ability of all the isolated fungal endophytes were assessed by growing the fungal organisms on Yeast Peptone Agar medium (Yeast extract: 1g; Peptone: 0.5; Agar: 16g; D.W. 1000ml; Supplemented with 0.5% Na-carboxymethyl cellulose (CMC) and supplemented with 0.5% Na-carboxymethyl cellulose (CMC) as enzyme substrate and incubating the plates for 7 days at 28±1ºC for appearance of the fungal growth. After incubation the plates were flooded with 0.2 aqueous Congo red reagents and destained with 1M NaCl for 15 min. The clear zone surrounded the colony indicated the cellulase activity.
- ✓ Laccase Activity: The laccase producing ability of all the isolated fungal endophytes were assessed by growing the fungal organisms on Glucose yeast peptone agar medium (Glucose: 1g; Yeast extract: 1g; Peptone: 0.5g; Agar: 16g; D.W.: 1000ml; pH: 6; amended with 1-naphthon-0.005%) amended with 0.005% 1-naphthol as enzyme substrate and incubating the plates for 7 days at 28±1ºC for appearance of the fungal growth. On oxidation of 1-naphthol by laccase the medium changed from clear to blue. The colour change indicates laccase positive test.
- ✓ Lipase Activity: The lipase producing ability of all the isolated fungal endophytes were assessed by growing the fungal organisms on peptone agar medium (Peptone: 10 g; .NaCl: 5g.; $CaCl_2$ $2H_2O$: 0.1g.; Agar: 16 g; .D.W.: 1000ml; pH: 6 supplemented with Tween20) supplemented with Tween 20 as enzyme substrate and incubating the plates for 7 days at 28±1ºC for appearance of the fungal growth. After incubation a clear zone around the colony indicated lipase positive activity by the fungi.
- ✓ Protease Activity: The protease producing ability of all the isolated fungal endophytes were assessed by growing the fungal organisms on glucose yeast peptone medium (glucose: 1g; Yeast extract; 1g; Peptone; 0.5g; Agar; 16gD.W.; 1000ml; pH: 6; amended with gelentin-0.4%) supplemented with 0.4% gelatin as enzyme substrate and incubating the plates for 7 days at 28±1ºC for appearance of the fungal growth. After incubation plates were flooded with saturated aqueous ammonium sulphate. The undigested gelatin precipitated with ammonium sulphate and the digested area around the colonies appeared clear and thus indicating the positive protease activity by the fungi.

IV. RESULTS

In order to collect different monocot and dicot plant species for isolation of endophytic fungi , an extensive survey from different biodiversity regions of Jabalpur. This resulted in collection of a total of 22 plant species of monocot and dicot and their different plant parts i.e. Root, Stem, leaf and bark used for isolation of endophytic fungi that is summarized in Table No 1.

Table: 1 Endophytic fungal isolates from monocot and dicot plant parts.

Fungi	Plant name with plant parts	
	Monocot	**Dicot**
Aspergillus sp.	*Triticum turgidum(L), Aloe barbadensis (L), Cyperace sp.(S), Agrostis sp.(S)*	*Rouwolfia serpentine (S), Syzygium aeromaticum (S), Gymmnema sylvestre (S),Datura alba (L)*
Nigrospora sp.	*Triticum turgidum (L,R.), Cynodon sp. (L,B), Pennisetum sp.(L,S)*	*Datura alba (R), Magnifera indica,(L,S), Acacia arabica (L)*
Mucor sp.	*Zea mays (L,R), Aloe barbadensis (L), Pennisetum sp. (S)*	*Datura alba (S), Calotropis Procera (S), Brassica compestris,(S)*
Curvularia sp.	*Zea mays (L), Allium cepa (L), Acorus calamus (L), Cyperace sp.(L), Tradescontin*	*Rouwolfia serpentine (L), Magnifera indica (L), Brassica compestris (L)*

	sp.(B)	
Fusarium sp.	*Acorus calamus (L), Zea mays (R), Agrostis sp.(L)*	*Rouwolfia serpentine (L,S), Gymmnema sylvestre (S)*
Alternaria sp.	*Cyperace sp.(L), Zea mays (L), Allium cepa (L),*	*Gymmnema sylvestre (L), Aegle marmelos (S), Acacia arabica (S),*
Stemphylum sp.	*Tradescontin sp.(L,B, Zea mays (L),*	*Lycopersicum esculentum (L,S)*
Chetomium sp.	*Tradescontin sp.(L), Agrostis sp.(L)*	*Aegle marmelos (L), Osimum sanctum (L,S)*

Note: L-leaf, B-bark, R-root, S-stem.

A total of 86 isolates belonging to eight different fungi namely *Nigrospora sp., Aspergillus sp., Mucor sp., Curvularia sp.,Alterneria sp.,Stemphylum sp.,Fusarium sp.,Chetomium sp.* have been isolated from different plant parts of monocot and dicot plants. Fig 1 showed total no of endophytic fungi isolated from monocot and dicot plants.

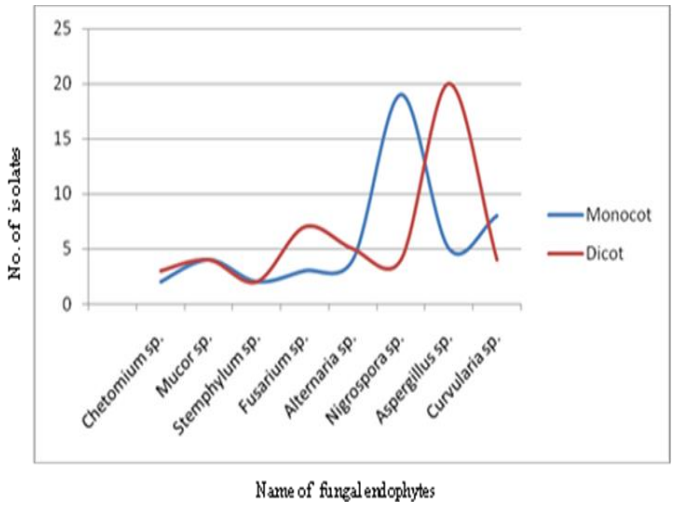
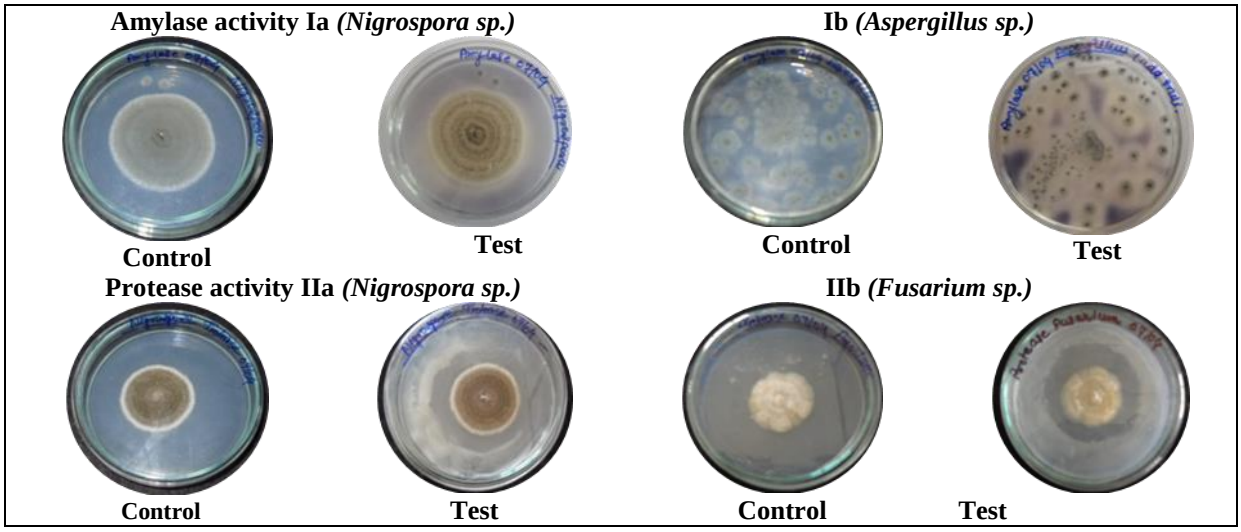


Fig1. Total no of Endophytic Fungi isolated from Monocot and Dicot plants.

It is known from the previous literature that endophytic microflora are the richest source of bioactive metabolites. On the virtues of these metabolites only these endophytic organisms impart useful effects on their respective hosts in which they survive. The results of qualitative estimation of endophytic fungal enzymes Keeping this fact on mind in view the enzyme producing capability of some of the important fungal endophytes were screened out on solid media. For this, the species of *Aspergillus sp., Fusarium sp, Nigrospora sp.* and *Curvularia sp.* were selected to screen out the amylase, cellulose, lipase, protease and laccase producing ability on solid medium with respective enzymatic substrates.

The result obtained revealed that all the four fungal endophytes possessed excellent enzymatic activity. However *Aspergillus sp., Nigrospora sp., Fusarium sp.* and *Curvularia sp.* Did not show the lipase activity as well as the fungal endophytes *Aspergillus sp.* did not show laccase activity.

Fig2 shows representative petri plates indicating the presence of enzymes from isolated endopytic fungi by qualitative test. In the figure 2, plate (I) showing a clear zone aroundthe fungal colony indicating the degradation of starch by the amylase enzyme produced by the fungi. Plate (II) represents the production of protease enzyme by endopytic fungi. The clear zone around the fungal colony indicates degradation of gelatin due to protease activity. Plate (III) represents the production of Cellulase enzyme from isolated endophytic fungi. Plate (IV) represents the production of Laccase activity from isolated endophytic fungi the medium colour get changed from yellow to reddish positive for laccase test.



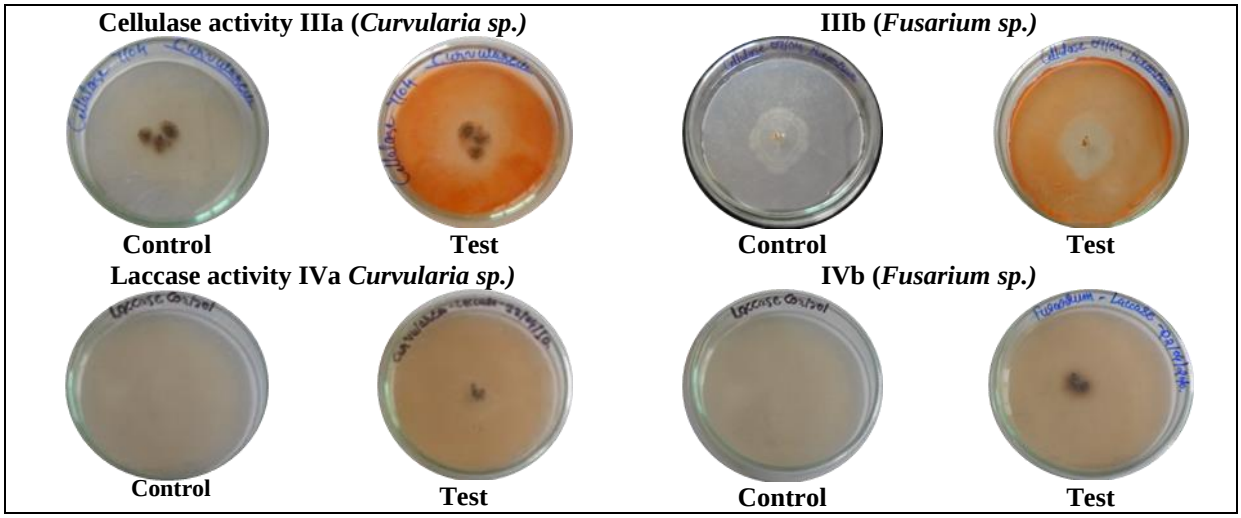


Fig: 2 Enzymatic activities of fungal endophytes isolated from monocot and dicot plant parts.

I Amylase activity – (Control)- (Without substrate) Test- (with starch (2%) as substrate) Zone of clearance around the colony indicate the positive amylase test.

II Protease activity- (Control)- (Without substrate) Test (with aqueous ammonium sulfate as substrte zone of clearance around the colony indicate the positive protease activity

III Cellulase activity – (Control)- (Without substrate) Test (With CMC (0.5%) as substrate) Zone of clearance around the Colony indicate the positive cellulase activity.

IV Laccase activity- (Control)- Control (with un-inoculated on organism on medium amended 0.005% 1-with 0.005% 1-naphthol as substrate of the medium remained unchanged.

Test (with inoculated organism medium amended with napthol as substrate) the colour the medium colour get changed from yellow to reddish positive laccase test

V. Conclusion

The present study leads to the need of further in depth studies on these isolated bioactive endophytic fungal isolates. On our study shows that monocot and dicot plants have good source of different kind of endophytic fungi. Our study indicates that these endophytic fungi are able to produce quite a good source of different types of industrially important enzymes. There are no reported studies on amylase, protease, laccase, lipase and cellulase enzymes to the present knowledge.

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