ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

Isolation of potential biological strains from soil and endophytes from *Azadirachta indica* against foot rot disease of *Piper nigrum* L.

Shobha M.S.[1*] and Mahadeva Murthy S.[2]

[1]Department of Microbiology, Government College for Womens, Chintamani Chikkballapur Karnataka 563125
[2]Department of Microbiology, Yuvaraja's College, Mysuru 570 006

Abstract

Endophytic organisms are the one which are capable of producing plant associated metabolites and their analogs with therapeutic value. To identify the potential endophytic isolates producing bioactive compounds, screening of all isolated endophytes is necessary which may run up to hundreds. Isolation of endophytic organisms is relatively a cumbersome process; but screening of the isolated fungi for required metabolite production is a cumbersome process. Endophytic organisms producing plant associated metabolites may contain genes that are involved in the entire biosynthetic pathways. The purpose of the present study was to investigate the endophytic diversity from medicinal plant *Azadirachta indica* occurring in Mysore region. The traditional identification was based on morphology of spores and reproductive structures. Some of those showed no reproductive structures or distinctive features were grouped into mycelia sterilia and some endophytes could not be identified to genus or species level. Among the plant parts, the colonization as well as the isolation rates of endophytic fungi was higher in bark followed by leaf samples.

Keywords: Endophytes, *Azadirachta indica, Piper nigrum,* PGPR, Biochemical test

Introduction

The control of plant diseases using antagonistic bacteria is now considered as a promising alternative method that could reduce the use of hazardous chemical fungicides or bactericides. Rhizobacteria, saprophytic bacteria that live in the plant rhizosphere and colonize the root system have been studied as plant growth promoters for increasing agricultural production and as biocontrol agents against plant diseases (Kloepper, 1992; Chen *et al*., 2013). Colonization of the plant root system can lead to reduced pathogen attack directly through the production of antimicrobial substances or competition for space, nutrients and ecological niches and indirectly through induction of systemic resistance. They are eco-friendly in nature, have a high cost-

benefit ratio and do not pose the risk of the pathogen developing resistance. Many root-rot colonizing bacteria are known to promote plant growth by producing gibberllins, cytokinin and indole acetic acid. Several plant growth promoting rhizobacteria (PGPR) strains have been reported to control various fungal, bacterial and viral diseases (Raupach *et al*., 1996). The mechanisms of biological control by PGPR strains generally involve the production of siderophores.

Endophytic fungi have been reported from various plant species, which contribute to the diversity of microorganisms in innate environment and produce various bioactive compounds and novel metabolites (Strobel *et al.,* 2004; Sun *et al.,* 2008). The composition of the fungal community usually differs between host species (Frommel, 1991), among the geographically separated individuals of the same host species (Jackson and Taylor, 1996), and also within the various tissue or organs of a host plant (Kumar *et al*., 2004). The novel microbial flora from unique plants could offer bioactive and chemically novel metabolites with huge medicinal potential (Strobel *et al*., 2004). Endophyte *Taxomyces andreanae* produced bioactive compound like Taxol, a diterpenoid compound with anticancer properties, which was first isolated from *Taxus brevifolia* (Mayer *et al.,* 1965). Some endophytic fungi belonging to different genera such as *Pestalotiopsis microspora*, *Alternaria alternata*, *Periconia* sp., *Pithomyces* sp., *Monochaetia* sp. and *Seimatoantlerium nepalense* are reported to produce Taxol. Likewise, few other endophytic fungi are found to produce economically important bioactive compounds like Phomol from *Phomopsis* sp. Endophytes have gained importance as components of screening program for therapeutics. These organisms have the ability to produce and mimic the secondary metabolites produced by the plant. Thus, if a microbial source of the compound would be available, it would eliminate the need to harvest and extract the slow growing and relatively rare trees for the compound. The endophytic fungi are of biotechnological importance as sources of new biological control and other useful characteristics.

The purpose of the present study was to investigate the endophytic diversity from medicinal plant *Azadirachta indica* occurring in Mysore region. The endophyte of *Azadirachta indica* has been reported by Banerjee (2006). The traditional identification was based on morphology of spores and reproductive structures. Some of those showed no reproductive structures or distinctive features were grouped into mycelia sterilia and some endophytes could

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

not be identified to genus or species level. This is a common problem concerning the identification of endophytes.

MATERIALS AND METHODS

Isolation of PGPR

PGPR strains like *P. fluorescens* and *B. subtilis* were isolated from pepper growing regions and named as (strain A and B). The soil samples (Figure 1) were serially diluted on nutrient medium and PGPR strains were confirmed by biochemical characterizations.

Fig. 1. Soil samples collected from pepper growing regions to isolate PGPR strains

Biochemical characterization

The gram reaction was determined following the staining procedure. First, thin bacterial smear was prepared on a clean glass slide, dried in air and fixed by heating. The dried smear was flooded with crystal violet solution for 30 sec and washed in tap water for few seconds. It was again flooded with Grams iodine solution for 1 min and washed and blot dried. It was then decolourized with 95% ethyl alcohol by applying drop by drop until no more colour flows from smear, washed and blot dried. Finally, slides were counter stained for about 1 min with safranin, washed and examined under microscope using oil immersion objective.

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

KOH solubility test

A loopful of bacterial strains (strain A and B) from a well grown colony was mixed in a drop of 3% aqueous KOH solution for not more than 10 sec with the help of a toothpick. Toothpick was raised few centimeters from the glass slide and was observed for the formation of a mucoid thread.

Starch hydrolysis

The strains (A and B) were streaked on starch agar medium to evaluate their ability to hydrolyze starch (amylase production). The plates were incubated at 28°C and for 3-4 days. Starch hydrolysis was observed by flooding the plates with Gram's iodine solution for 30 sec. The appearance of clear zone around the growth of each bacteria indicated starch hydrolysis.

Kovac's test

A loopful of bacteria (strain A and B) was rubbed on a filer paper with drops of aqueous N,N,N,N- tera methyl-p-phenylenediamine dihydrochloride solution. Based on the standard procedure, isolates which developed purple colour within 10 sec were taken as positive, purple colour in 10-60 sec were taken as slow positive and those with no colour for more than 60 sec taken as negative to oxidase test.

Catalase test

A 24 h old culture of the bacteria (strain A and B) was flooded with 1 ml of 3% hydrogen peroxide and observed for the production of gas bubbles.

Lipase activity

Tween 80 agar medium was autoclaved and poured into sterile petri plates. The plates were streak inoculated with bacterial strains (strain A and B). The plates were incubated for three days and observed for the development of a milky precipitate around the colony. Cultures are positive for the lipase test, if granular precipitate is seen around the colonies.

Arginine dihydrolase test

A fresh culture tube containing 5 ml of sterilized Thornely's medium was stab inoculated with the bacterial strains (strain A and B). The surface of the medium was sealed with sterile

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

molten Vaseline. Uninoculated tube served as negative control. The tubes were incubated at 26 ± 2°C for three days and observed for the change in colour. Change in colour from orange to red indicates positive result.

Casein hydrolysis

Double strength nutrient agar medium with skimmed milk powder solution (10%) was sterilized in two different flasks. Both were mixed well before pouring into sterile petri plates. The bacterial strains (strain A and B) were streak inoculated with the test isolate and incubated at 26 ± 2°C for 48 h. Bacterial strains were recorded positive if the zone of hydrolysis was seen around the colonies.

H_2S production from peptone

Test tubes containing reagent were autoclaved at 121°C for 20 min. Filter paper strips (0.5 × 7.5 cm) soaked in saturated solution of lead acetate were sterilized and dried in an oven at 60°C and placed at the mouth of the test tube in such a way that one half of the strip was hung below the cotton plug and the other half remained outside. Tubes were inoculated with bacterial strains (strain A and B). Uninoculated tube was maintained as negative control. Tubes were incubated at 26 ± 2°C for three days for the production of H_2S.

Levan formation

Nutrient agar medium supplemented with 5% sucrose and 0.2% (w/v) yeast extract was prepared, sterilized and poured into sterile petri plates. The medium was streak inoculated with the test bacterial strains (strain A and B) and incubated for 3-4 days. The plates were observed for the development of white, domed, shining, mucoid colonies, which is due to the formation of levan by the enzyme levan sucrose produced by the bacteria.

Protease activity

Nutrient agar medium supplemented with Difco gelatin dissolved in distilled water was prepared and dispensed into sterile petri plates. Plates were inoculated with test bacterial strains (strain A and B) and incubated for two days at 26 ± 2°C after the incubation period; the plates were flooded with saturated ammonium sulphates solution to observe the zone of hydrolysis.

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

Cellulase activity

Cellulase medium was prepared and autoclaved. The plates were point inoculated with the bacterial strains (strain A and B) and incubated for two days at 26 ± 2°C. After the incubation, the plates were flooded with 0.5% Congo red for 15 min and then bleached with 1 M NaCl.

Gelatin liquefaction

The media were stab inoculated with bacterial strains (strain A and B) were grown for 48 h on yeast peptone sucrose agar medium and incubated at 26 ± 2°C After 3, 7 and 21 days of incubation, each isolate was evaluated for gelatin liquification. The bacterial strains in the test tubes were kept at 4°C for 30 min.

Litmus milk test

Litmus milk broth supplemented with skimmed milk was inoculated by PGPR strains Using sterile technique and appropriately labeled tube by means of a loop inoculation. The last tube will serve as a control. After inoculation of test cultures it was incubated for 24 to 48 hours at 37˚C.

Identification of medicinal plants

Medicinal tree *Azadirachta indica* belonging to Meliaceae, growing in natural habitats was identified based on taxonomic parameters. The natural habitat of medicinal plant is presented in figure 2.

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

Fig. 2. *Azadirachta indica tree*

Kingdom : Plantae
Division: Angiospermae
Class: Dicotyledonaceae
Order: Sapindales
Family: Meliaceace
Genus: *Azadirachta*
Species: *A. indica*

Habitat and Distribution: This is a deciduous, medium-sized tree, abundantly growing in all over the places of India.

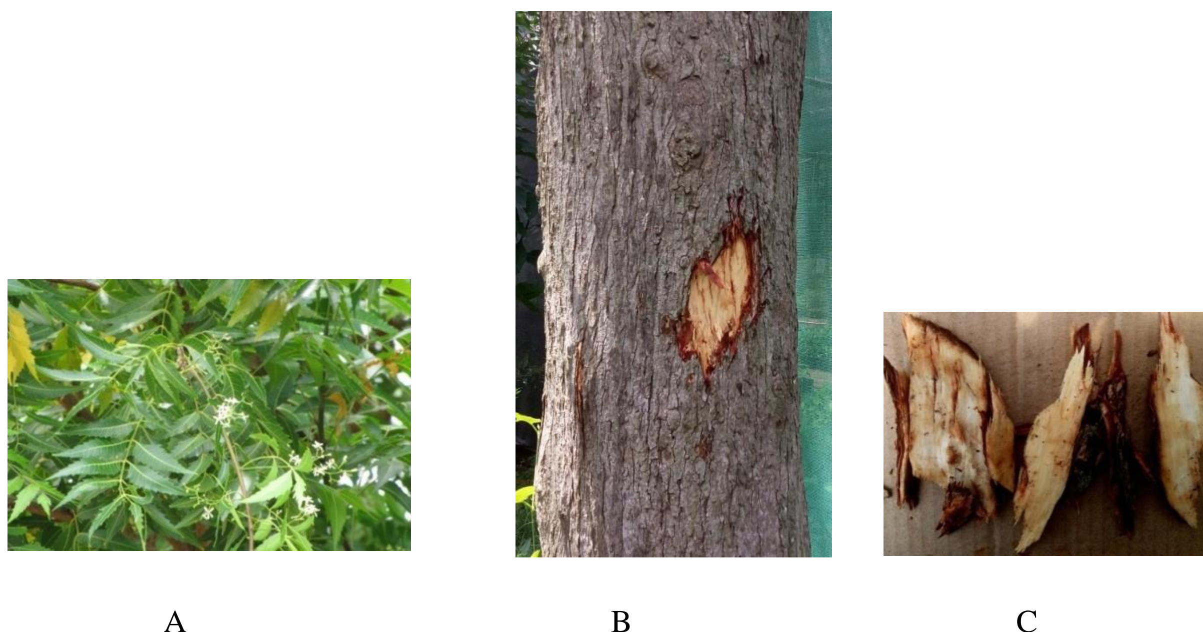
Taxonomic description: (Juss, 1830) Brandis, *Azadirachta indica* is a moderate-to-large branching tree, growing to a height of 14-20 m (49-66 ft), with a circumference of 35-40 m (Fig. 2) found in India, Pakistan, Northern Africa and in Middle East of Australia. The branches are wide and spreading. The fairly dense crown is roundish and grows straight. The leaves are pinnate and are 20-40 cm in long, with 20-31 medium to dark green leaflets about 3-8 cm in long and the petioles are short. Flowers are arranged in more-or less drooping axillary panicles which are up to 25 cm long. Fruit is a smooth, olive-like drupe which varies in shape from elongate oval to nearly roundish, and white, hard inner shell of the fruit encloses one, rarely two or three elongated seeds having brown seed coat.

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

Collection of plant material

Bark and leaf samples of *A. indica* were collected from five different regions in Mysore, Karnataka *viz*., Nanjangudu, Chamundi hill, Mandakalli, Hunsur and Heggadadevana Kote has the deciduous vegetation.

Bark pieces (5.0 × 5.0 cm) from the trunk were cut 1.5-2.0 m above the ground level with the help of sterile machete. The outer part was considered as bark and leaves were placed in polythene bags, labelled, transferred in ice box to the laboratory and placed in a refrigerator at 4°C. The samples were processed within 24 h of collection as shown in figure 3.



A B C

Fig. 3. Samples of leaves (A), Tree trunk (B) and bark (C) of *Azadirachta indica*

Isolation and preservation of endophytes

The samples were washed thoroughly in the running tap water before processing. Bark, inner bark, twig and leaf samples were surface sterilized by dipping in 70 % ethanol (v/v) for one min and 3.5% NaOCl (v/v) for 3 min, rinsed thrice with sterile water and dried. Bits of 1.0 × 1.0 cm size were excised with the help of a sterile blade. Three hundred segments representing bark, inner bark and leaf of *A. indica* were placed on water agar (15 g/ l) (WA) medium supplemented with streptomycin (100 mg/l; Sigma, St. Louis, MO, USA) contained in 9 cm diameter Petri dishes. Ten to fifteen segments were placed on solidified 20 ml WA medium in each Petri dish.

The Petri dishes were incubated at 22°C with 12 h light and dark cycles up to four weeks (Schulz *et al.,* 1993). After sporulation, individual fungal colonies were picked from the edge with a sterile fine tipped needle and transferred onto potato dextrose agar (PDA) medium for further identification. The identification was done based on the morphological and conidial characteristics. All isolates were maintained in cryovials on PDA layered with 15% glycerol (v/v) at -80°C in an Ultrafreezer.

Identification of endophytic fungi

The morphological identification of endophytic fungal strains was studied using Zeiss Advanced Stereo Discovery V20 Binocular Microscope, based on the colony characteristics, spores and the reproductive structure (Eziashi *et al*., 2006; Bowers *et al.*, 2007). The non-sporulating fungal endophytes were inoculated onto sterilized banana leaf bit (1 cm^2) impregnated on agar to facilitate sporulation (Gherbawy *et al*., 2014).

The cultures were stained with lactophenol blue and sealed with nail enamel. Those cultures which were failed to sporulate were grouped as mycelia sterilia

RESULTS

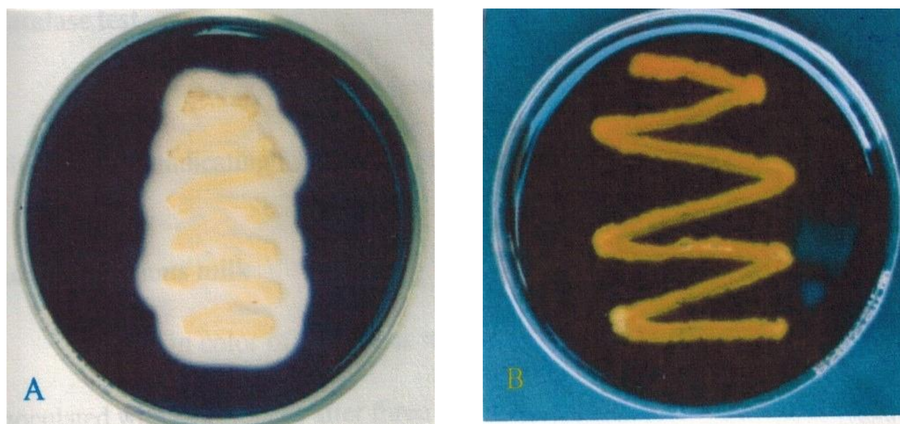
Table 1: Biochemical characterization of PGPR strains

Sl. No.	**Biochemical test**	***P. fluorescens*** **(Strain A)**	***B. subtilis*** **(Strain B)**
1	Grams test	**Negative**	Positive
2	KOH solubility test	+	-
3	Kovac’s hydrolysis	+	+
4	Catalase test	+	+
5	Casein hydrolysis	**-**	+
6	Action on litmus milk	**-**	-
7	Lipase activity	**-**	+
8	Arginine dihydrolase	**-**	+

9	H_2S production	+	+
10	Levan formation	+	+
11	Protease activity	-	+
12	Cellulase activity	-	+
13	Starch hydrolysis	-	+
14	Gelatin hydrolysis	+	-

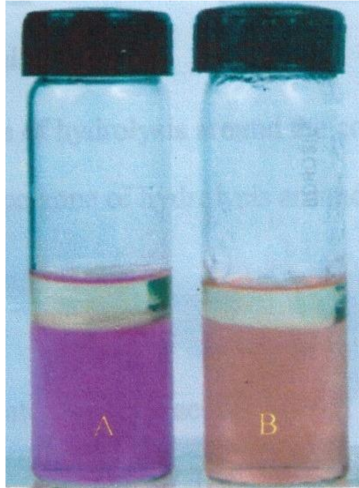
+: positive and -: negative

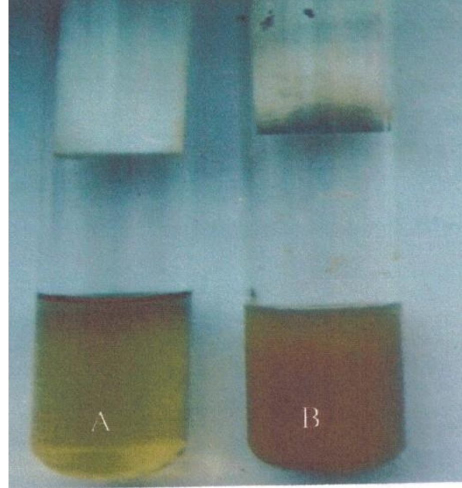
A. Kovac's test



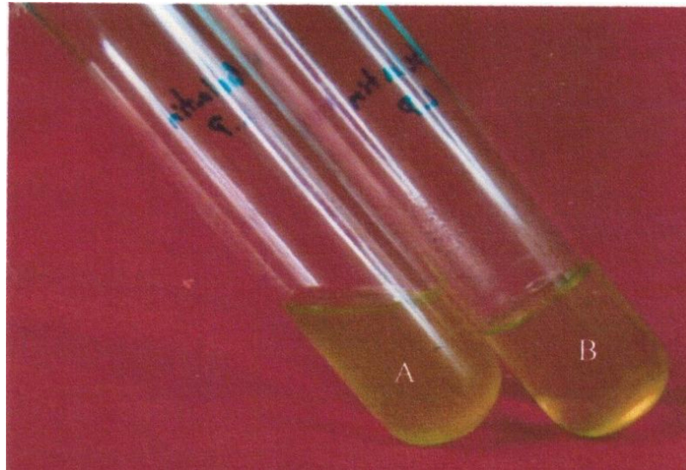
B. Starch hydrolysis

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019





C. Arginine dihydrolase **D. H_2S production**



E. Gelatin hydrolysis

Fig. 4. Biochemical characterization of PGPR strains A. Kovacs test, B. Starch hydrolysis, C. Arginine dihydrolase, D. H_2S production

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

Isolation of endophytes (colonization frequency)

A total of 28 endophytic fungi were isolated from the *A. indica* among them 10 species were identical in all the regions of plant tissues with different colonization rate (CR) (Tables 2).

Table 2: Colonization rate of fungal endophytes in *A. indica*

Region	Number of samples yielding fungi	Number of isolates
Region 1	04	05
Region 2	10	10
Region 3	03	03
Region 4	05	05
Region 5	06	05

Identification of endophytic fungi

A total of 10 species with 28 isolates of endophytic fungi were recovered from 300 tissue segments of bark and leaf from *A. indica* recovered from 5 regions and 20 were identical in all the regions of plant tissues. Among the endophytes, *Acremonium strictum* was 1.33%. The other endophytes recovered from *A. indica* are *Acremonium strictum*, *Fusarium oxysporum*, *Pestalotipsis* sp., *Aspergillus niger, Trichoderma viride*, *Trichoderma asperellum*, *Colletotrichum gloeosporioides*, *Fusarium solani, Aspergillus flavus* and *Phomopsis* sp. as shown in table 2.

Acremonium strictum

The mycelia are white to light orange bearing round, watery heads on conidiophores arising perpendicular to ropy hyphae; small, hyaline, ellipsoidal to cylindrical, straight, single-celled conidia with rounded ends (Figure 4) (Mathur and Olga, 2003).

Fig. 4. *Acremonium strictum* growing in a culture plate and spore

Fusarium oxysporum

The growth is sparse to abundant. Mycelium white to cream coloured. Microconidia are generally produced in abundance, they vary a lot in size and are oval-shaped, elliptical or reniform, usually non-septate but 1 septate conidia may be found. Macroconidia are hyaline, thin walled, 3-5 septate, falcate to almost straight (Figure 5) (Mathur and Olga, 2003).

Fig. 5. *Fusarium oxysporum* growing in a culture plate and spore

***Pestalotiopsis* sp.**

Produces sooty, black, long, curly, thick and thread like structures with white mycelium at the base. These thread like structures are full of conidia. Conidia ellipsoidal, broadest in the middle, narrow at both ends, 4 septate, the central three cells are brown to dark brown, end cells hyaline. The basal hyaline cell sometimes bears a short, hyaline stalk. The apical hyaline cell is

conical, provided with 2-3 long, hyaline, filiform, frequently branched setulae (Mathur and Olga, 2003) as shown in figure 6.

Fig. 6. *Pestalotiopsis* sp. growing in a petriplate and conidia

Aspergillus niger

Aspergillus niger has been classified as a member of Ascomycota. The major difference between other species of *Aspergillus* is the production of carbon black or very dark brown spores from biseriate phialides. Colonies are usually fast growing, black or shades of green, mostly consisting of an erect conidiophores as shown in figure (**2.6**). Conidiophores terminate in a vesicle covered with either a single palisade-like layer of phialides (uniseriate) or a layer of subtending cells (metulae) which bear small whorls of phialides. The vesicle, phialides and conidia form the conidial head. Conidia are one-celled, smooth or rough-walled, hyaline or pigmented, are produced in long dry chains which may be divergent (radiate) or aggregated in compact columns (columnar; figure 7) (Mathur and Olga, 2003).

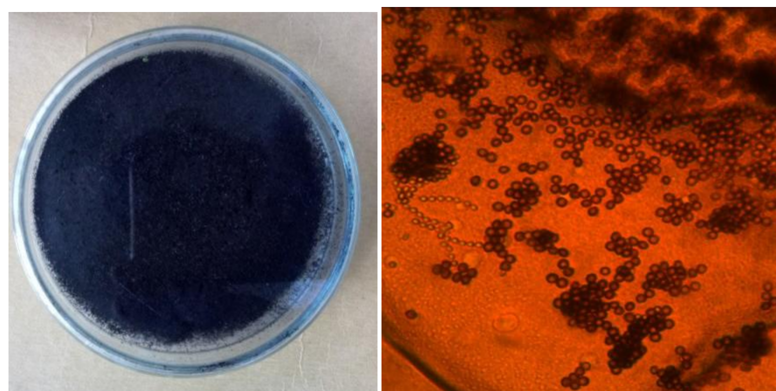


Fig. 7. *Aspergillus niger* growing in a petriplate and conidia

Trichoderma viride

Trichoderma viride was identified based on the colour of the colony, formation of chlamydospores, conidiophores and phialides characters, shape of conidia as the main characters to identify the species (Bisset, 1992). Colony showed dark green to dark bluish green sporulation, conidiophore usually long, infrequently branched, verticillate conidiophores. Phialides frequently paried, lageniform divergent conidia shape was globose to ellipsoidal Chlamydospore formation was infrequent, terminal and intercalary in structure as shown in figure 8.

Fig. 8. *Trichoderma viride* growing in a petriplate and conidia

Trichoderma asperellum

Trichoderma asperellum phialides are usually single, verticillate and straight. They are almost cylindrical rather than swollen in the middle, regularly branched, with lateral branches being more or less uniformly spaced and paired, the longest branches occurring the farthest from the tip. Conidia are globose to subglobose or ovoidal, with 3.7-6.0 × 3.0-5.0 µm as shown in figure 9.

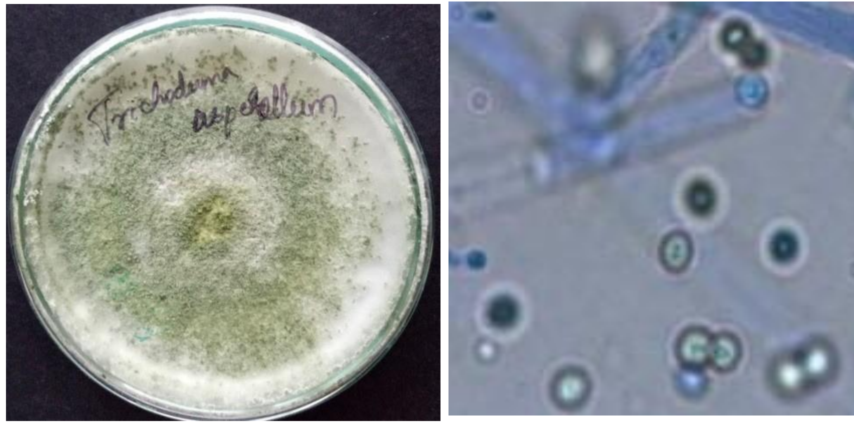


Fig. 9. *Trichoderma asperellum* growing in a petriplate and conidia

Colletotrichum gloeosporioides

Conidial mass appears dull white to dull orange or at times bright orange. Mycelium mostly absent, if present, white and shiny. Conidia are hyaline, 1-cellled, straight, cylindrical, ends rounded (Mathur and Olga, 2003) as shown in figure 10.

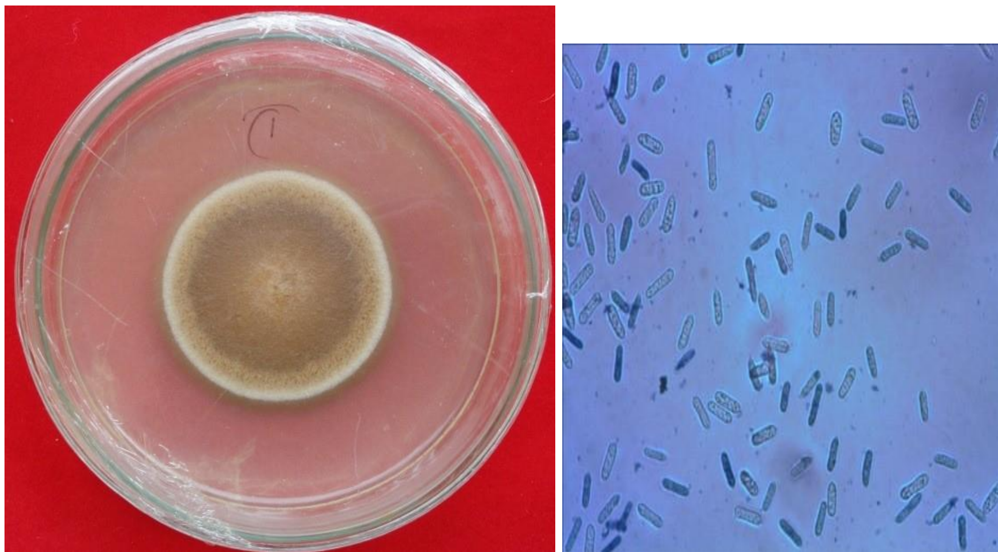


Fig. 10. *Colletotrichum gloeosporioides* growing in a petriplate and conidia

Fusarium solani

The mycelium produces white to cream to coloured, usually sparse, floccose mycelium. Microconidia are 1-2 celled, hyaline, oval, ellipsoid or reniform. Macroconidia are 3-4 septate,

hyaline, thick walled with short rounded and sometimes hooked apical cell and a notched base in the basal cell (Mathur and Olga, 2003) as shown in figure 11.

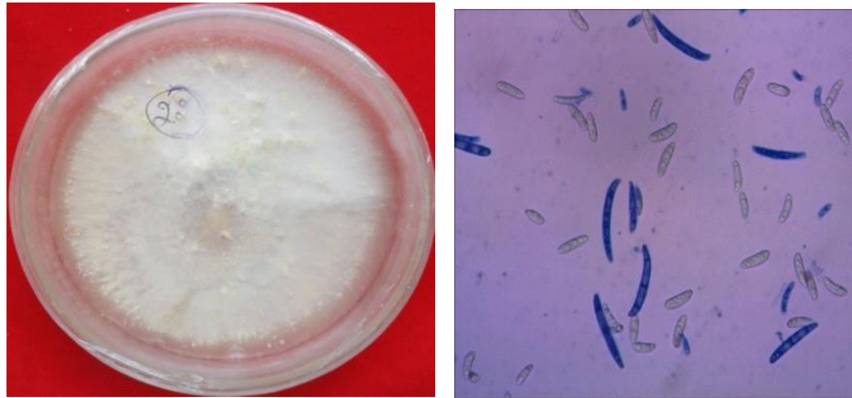


Fig. 11. Colony morphology, conidial characters of *Fusarium solani* and SEM photo of conidia

Aspergillus flavus

Brown to black growth consisting of conidia in long chains. Conidia are polymorphous, short to long, olive brown, highly variable in shape (Mathur and Olga, 2003) as shown in figure 12.

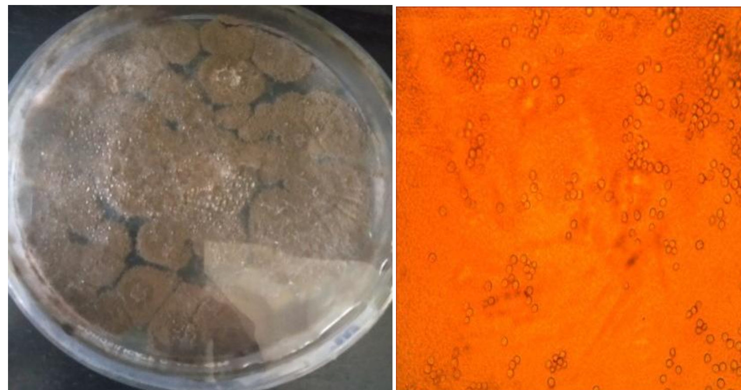


Fig. 12. *Aspergillus flavus* growing in a petriplate and conidia

Phomopsis vexans

Pycnidia solitary or in groups, have large ostioles and some may be seen with wet ooze of pycnidiospores. Pycnidiospores are of two types, alpha and beta. Alpha is fusoid to ellipsoidal, biguttulate, rarely 3, while beta is filiform, curved and rarely straight as shown in figure 13.

Fig. 13. *Phomopsis vexans* growing in a petriplate. Alpha conidia and beta conidia

DISCUSSION

Two strains of rhizobacterial isolates and 10 fungal endophytes were used for the efficacy study of foot rot disease.

Endophytes establish in the living internal tissues of their hosts without producing symptoms of disease (Rubeena *et al*., 2013). Endophytic fungi have been reported from various plant species, which contribute to the diversity of microorganisms in natural environments and produce various bioactive compounds that play a major role in inherent surroundings.

Endophytic microorganisms are a significant reservoir of novel bioactive secondary metabolites. The number of secondary metabolites produced by fungal endophytes is larger than that of any other endophytic microorganism class. These endophytic fungi are obviously a rich and reliable source of chemically novel bioactive compounds with huge medicinal and agricultural potential (Strobel *et al.,* 2004). The Western Ghats is one of the hot spots of plant biodiversity endowed with more than 4,700 different plant species. A new species of endophyte, *Muscodor kashayum* was recently isolated from the medicinal species *Aegle marmelos* (Bael

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

tree) from the Western Ghats of Muthanga region of Wayanad Wildlife Sanctuary, Kerala, India. *Azadirachta indica* is an important medicinal plant used in the traditional medicine and has a variety of pharmacological activities.

Previous reports showed that the endophytic colonization frequency exhibit host preference, tissue specificity and seasonal differences with reference to compositions of endophytic strains. These "seasonal" variations and locations are likely to the affect medicinal plant potency (Huang *et al.*, 2008). The present study showed region wise occurrence of endophytic fungi of *A. indica* medicinal plants.

Plants collected from different places were found to be colonized with different endophytic fungi and exhibited different relative frequencies. The dominance not only depends on the plant species but also on the location where biodiversity is more. This was also observed in many previous studies (Strobel *et al*., 2004, Huang *et al.,* 2008). From *A. indica* plant, region 2 (Chamundi Hills) yielded the greatest fungal diversity and 10 different endophytic fungal taxa were obtained from the bark.

Acremonium strictum and *Pestalotiopsis* sp*.,* were found in only one region. Whereas, *Trichoderma* was found in region 3 and *Phomopsis* in region 2**.** The findings proved that the number of endophytic fungi was higher in bark than in leaves. However, the overall colonization frequencies differed with different organs. The number of species occurring in the inner bark and the twig region was almost the same; yet, the twig was more densely colonized by the endophytes as evidenced by the total CF%. Similar results have been reported for *Azadirachta indica* (Rajakumar *et al*., 2012). However, the colonization frequency reported according to Meng and Chen (2001) was 62.5% where a total of 32 species belonging to 21 genera were isolated from inner bark of *Prosopis cineraria* tree.

Among the plant parts, the colonization as well as the isolation rates of endophytic fungi was higher in bark followed leaf samples. Study corroborates with the findings of Sun *et al*. (2008), who have reported that colonization frequency and isolation rates of endophytic fungi were conspicuously higher in bark than in leaves in the six plants examined.

Conclusion

Endophytes are beneficial microbes that grow within the plants without showing any visible symptoms. Being ubiquitous in plant tissues, they can be isolated from leaves, stem, roots, seeds, fruits and flowers. The direct effect of endophytes in promoting plant growth are thought to include phytohormone production, asymbiotic nitrogen fixation, solubilization of inorganic phosphate and mineralization of organic phosphate/other nutrients. Endophytes have deleterious indirect effects on plant pathogens through siderophores production and through induced systemic resistance.

References:

Banerjee, S. 2006. Suppression of gross chromosomal rearrangements by yKu70-yKu80 heterodimer through DNA damage check points. *Proc. Natl. Acad. Sci.USA*. 103:1816-1821.

Bissett, J. 1992. *Trichoderma atroviride*. *Can. J. Botany* 70:639-641.

Bowers, J.H., Martin, F.N., Tooley, P.W. and Luz, E.D.M.N. 2007. Genetic and morphological diversity of temperate and tropical isolates of *Phytophthora capsici*. *Phytopathology* 9**7**:492-503.

Chen, J., Zhang, L.C., Xing, Y.M., Wang, Y.Q. and Xing, X.K. 2013.Diversity and taxonomy of endophyticXylariaceous fungi from medicinal plants of Dendrobium (Orchidaceae). 8(3):e58268.

Eziashi, E.I., Uma, N.U., Adekunle, A.A. and Airede, C.E. 2006. Effect of metabolites produced by *Trichoderma* species against *Ceratocystis paradoxa* in culture medium. *Afr. J. Biotechnol.* 5:703-706.

Frommel, M.I., Nowak, J. and Lazarovits, G. 1991. Growth enhancement and developmental modifications in *in vitro* grown potato (*Solanum tuberosum*) as affected by a non-fluorescent *Pseudomonas* sp. *Plant Physiol*. 96:928-936.

Gherbawy, Y.A., Hussein, N.A. and Al-Qurashi, A.A. 2014. Molecular characterization of *Trichoderma* populations isolated from Soil of Taif City, Saudi Arabia. *Intl. J. Curr. Microbiol. Appl. Sci.* 3:1059-1071.

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

Huang, H., Rong, H., Li, X., Tong, S., Zhu, Z., Niu, L.and Teng, M. 2008. The crystal structure and identification of NQM1/YGR043C, a transaldolase from *Saccharomyces cerevisiae*. *Proteins* 73:1076-1081.

Jackson, A.O. and Taylor, C.B. 1996. Plant–microbe interactions: life and death at the interface. *Plant Cell* 8:1651-1680.

Juss, A. 1830. Classification of *Azadiracta indica* 19, 220.

Kloepper, J.W. 1992. Plant growth-promoting rhizobacteria as biological control agents. In: Metting, F.B Jr. (Ed.), Soil Microbial Ecology: Applications in Agricultural and Environmental Management. Marcel Dekker Inc., N.Y. pp. 255-274.

Kumar, M., Yadav, V. and Tuteja, N. 2006. Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. *Microbiology* 155:780-790.

Mathur, S. B. and Olga, O. 2003. *Common laboratory seed health testing methods for detecting fungi*. Pp12-230.

Mayer, E., Harel, R.B. and Shaul, A.M. 1965. Assay of catechol oxidase a critical comparison of methods. *Phytochemistry* 5:783-789.

Meng, X. and Chen, W. 2001. Applications of AFLP and ISSR techniques in detecting genetic diversity in the soybean brown stem rot pathogen *Phialophora gregata. Mycol. Res.* 105**:**936.

Rajakumar, N., Ravindra Kumar, K. and Seshakiran, K. 2012. Management of phytophthora foot rot disease in black pepper. *Green Farm.* 3:583-585.

Raupach, G.S., Liu, L., Murphy, J.F., Tuzun, S. and Kloepper, J.W. 1996. Induced systemic resistance in cucumber and tomato against cucumber mosaic cucumo virus using plant growth-promoting rhizobacteria (PGPR). *Plant Dis*. 80:891-894.

Rubeena, M., Neethu, K. and Sajith, S. 2013. Lignocellulolytic activities of a novel strain of *Trichoderma harzianum*. *Adv. Biosci. Biotechnol*. 4:214-221.

Schulz, B., Wanke, U. and Draeger, S. 1993. Endophytes from herbaceous and shrubs: Effectiveness on surface sterilization methods. *Mycol Res.* 97:1447-1450.

Strobel, G.A., Daisy, B., Castillo, U. and Herper, J. 2004. Natural products from endophytic fungi. *J. Nat. Prod*. 67:257-268.

ISSN- 2394-5125 VOL 06, ISSUE 01, 2019

Sun, M.L.C., Mackey, L.V., Luo, J., Fuselier, J.A. and David H.C. 2008.Targeted chemotherapy using a cytotoxic somatostatin conjugate to inhibit tumor growth and metastasis in nude mice. *Clin. Med.: Oncol.* 2:491-499.