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RESEARCH ARTICLE

Isolation and Identification of endophytic fungi and evaluation of their interactions with plants from *Syzygium cumini* (Jamun) and *Ocimum tenuiflorum* (Tulsi)

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Abstract

Endophytic microorganisms are able to promote plant growth through various mechanisms such as production of plant hormones and anti-microbial substances, as well as to provide soil with nutrients, for instance, inorganic phosphate. Endophytes within a plant can be located at the entry point or they can be found spread throughout the plant. These microorganisms may reside: within cells, in intercellular spaces or in the vascular system. The aim of this study is to evaluate endophytic fungi isolated from leaves and stems of Jamun and Tulsi plants to produce substances involved in the promotion of plant growth and identified by some biochemical tests. Total 5 endophytic fungi were isolated, out of which three were positive for catalase, amylase, and cellulase production test. There are negative results of protease for all fungal isolates. This study play a significant role to stablish endophyte-host plant interactions.

Keywords: Endophytic, fungus, plant growth promoters, catalase test, endophyte-host plant interactions

Introduction

"Endophyte"term is derived from Greek word, endon means within and phyte means plant. It was first introduced in 1866 by de Bary (Arnold 2008). The endophyte refers the colonising the fungi and bacteria in intercellular and intracellular regions of healthy plant tissues at a particular time, whose presence is unremarkable and asymptomatic (Schulz and Boyle 2006; Baron and Rigobelo 2022). The unexplored group of microorganism are the endophytic fungi that are believed to be wealth of biologically and structurally active compounds (Tan and Zou 2001). These are beneficial to promoting the growth of host plant and nutrient uptake or inhibit the growth of pathogenic in host bodies and reduce stresses. (Redman *et al.* 2011). Endophytes colonize the tissues of healthier plants for all or at least a part of their life cycle (Clay and Schardl 2002). Endophytes are known to be present everywhere and have been found mostly in all plant species studied so far. However, in most of the cases the ratio between the endophyte and plant are not well understood (Azevedo 2000). Antibacterial, anti-insectic, anti-cancer and many other potential candidates act as reservoirs for new secondary bioactive metabolites such as alkaloids, phenolic acids, quinones, steroids, saponins, tannins and terpenoids (Gouda 2016).

These endophytes produces bioactive compound which help the host plant to improve the nutritional status, pest and disease resistance and physical stress tolerance (Ladoh*et al.* 2015, Jeffrey et al. 2008). Impact of microbiome on different aspects of host plants in recent studies, such as improving tolerance to heat, drought and reducing susceptibility to disease, saline stress and increasing vigor. (Weiß *et al.* 2016; Llorens *et al.* 2019). Moreover, it also described that certain species from wild herbs, when transferred to wheat and tomato, are capable of improving the growth of these plants under conditions of heat and salinity stress (Redman *et al.* 1986, Rodriguez *et al.* 2008).There are two mechanisms by which the endophytic microorganisms improve the performance and the resistance of the plants that is direct and indirect mechanisms. These mechanisms

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include the stimulation of Induced Systemic Resistance (ISR) and Systemic Acquired Resistance (SAR) or the stimulation of plant secondary metabolites (Fadiji and Babalola 2020).

Endophytes can also produce chemicals that inhibit the growth of many known competitors, including pathogens. It has been shown that some fungal endophytes help plants to cope with drought and heat (Germaine *et al.* 2004). The benefits of host-fungi endophytes are much known phenomena and have been the focus of research, especially on endophytes found in the grass (Barac *et al.* 2004). Noteworthy changes have been reported in endophytes both indigenous and introduced (Germaine *et al.* 2004). The members of Ascomycetes and some are nearly related to fungi that are known to cause some diseases in plants or animals (Rodriguez *et al.*2009). Unlike the mycorrhizae that colonize the roots of plants and grow in the rhizosphere, the endophytes reside completely in the tissues of plants and can grow inside the roots, stems, leaves that emerges only to sporulate during senescence of host plant or tissues (Rodriguez et al. 2009). Endophytic fungi under a specific cultural condition, fungus-host relationships and their metabolic mechanisms under cultural conditions are well understood (Kumaran *et al.* 2008, 2009).

Endophytic fungi are different polyphyletic groups of microorganisms, and can thrive asymptomatically in different healthy tissues of living plants above and/or under the ground, including stems, leaves, and/or roots. It is estimated that over one million endophytic fungal species occurring in the nature (Faeth and Fagan 2002). Schultz classified the fungal endophytic fungi into three main biological groups: (a) mycorrizal; (b) balansicaeous or pasture endophytic fungi; and (c) non pasture endophytic fungi (Faeth and Fagan 2002). The various bioactive compounds produced by endophytic fungi, exclusive for their host plants these compounds can induce the production of biologically active secondary metabolites (Zhang *et al.* 2006, Firakova *et al.* 2007, Rodriguez *et al.* 2009) that can be exploited and applied by human as important medicinal purposes. Inside the tissues of a host-plant, the endophytic fungi assumed a dormant state, either for the lifetime of the host plant (neutralism) or for a prolonged period of time (mutualism or antagonism) until environmental conditions are favorable for endophytic fungi or the ontogenetic state of the host changes to the advantage of the fungi (Sieber 2007). The nutrient level and genetic background ecological habitats of the host plants are considered as the important factors on the population of the endophytic fungi that, in turn, confer some kinds of benefits, such as the induced growth, increased resistance to disease or herbivore (Rodriguez *et al.*2009), as well as accumulated bioactive components (Firakova *et al.* 2007), some of which can be used by human as medicines. Therefore, the mutual interrelation between endophytic fungi and their host plants can execute certain effects on the preparation of some types of bioactive compounds that can be used by human.

This study is design to evaluate endophytic fungi isolated from leaves and stems of Jamun and Tulsi plants. Endophytic microorganisms are able to promote plant growth through various mechanisms such as production of plant hormones and anti-microbial substances, as well as to provide soils with nutrients, for instance, inorganic phosphate and identified by some biochemical tests.

Material and method

Isolation of endophytic microorganisms

The endophytic microorganisms were obtained from surface sterilized plants of Jamun and Tulsi plants collected from campus of Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala Haryana in the month of March 2022. The part of stem along with leaves were sterilized. In this protocol samples were washed several time with sterile water and disinfected by using 70% ethanol for two minutes. Then, samples were cut into small pieces using a sterile razor and placed on potato dextrose agar medium plates. The sealed petri dish kept in incubator for 4 days for growth of fungi (Abdelwahab *et al.* 2021, Abdelkader *et al.* 2022). Then sub-culturing of isolated pure culture in incubator at suitable temperature for proper growth.

After that some test were performed with isolated sub-culturing fungus to check the most efficient endophytic fungus from all isolated fungi.

Catalase test

The test in which catalase is a common enzyme found in bacteria, plants, and animals. It catalyses the degradation of hydrogen peroxide into water and oxygen. It is a very important enzyme to protect the cell against oxidative damage of reactive oxygen species.

Anti-microbial test

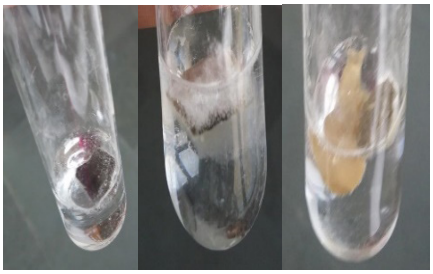
The test was done to check whether the isolated endophytes are able to inhibit the test pathogenic microorganisms, it was done by agar well diffusion technique.

Amylase test

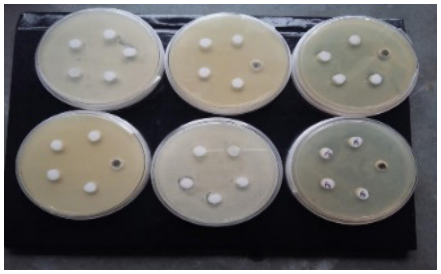
The amylase is an enzyme that catalyses the hydrolysis of starch in sugar. The role of amylase in the plant is for the demolition of starches.

Protease test

The protease is an enzyme that performs proteolysis, i.e protein catabolism begins with the hydrolysis of the peptide bond that binds amino acids into a polypeptide chain. Protease plays a key role in plants, maintains strict quality control of proteins and degrades a specific set of proteins in response to various environmental and developmental stimuli**.**

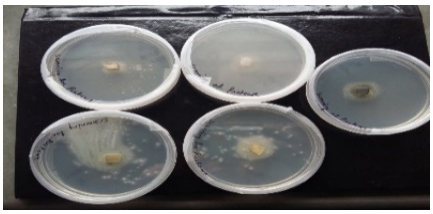


Catalyse test: bubble formation took place in when mycelia was put into the test tube containing hydrogen peroxide, F3, F4 and F5 were catalase positive

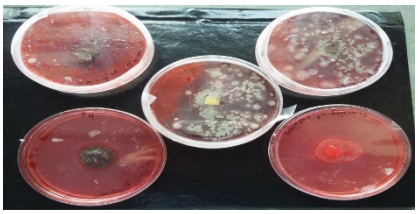


Anti-Microbial Test: Antimicrobial activity of fungal endophytic isolates: No inhibition observed.

Amylase Test: Formation of zone of clearance depicts the production of amylase in plate F2, F3 and F4.

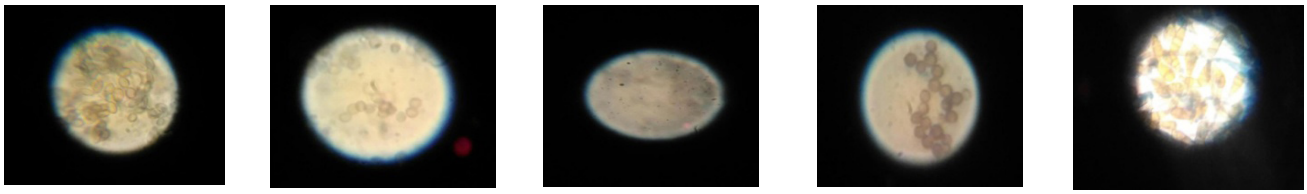


Protease Test: No zone formation took place in plates, hence all the fungus were negative for the protease test.



Cellulase Test: Zone formation took place in plate F1,F2 and F3 depecting production of cellulase.

Figure 1: Interactions of fungal endophytes



F1 **F2** **F3** **F4** **F5**

Figure 2: Identification of isolated fungus was also done in which isolate F1, F2, F3 and F4 was found as *Asperigillus* sp. and F5 was found as *Curvularia* sp.

Cellulase test

Cellulase is an enzyme that catalyses cellulolysis, the breakdown of cellulose and some related polysaccharides. Cellulase can be produced by endophytes and can promote the growth of plants when grown in association withendophytes that produce cellulase. Identifying the isolates with the help of two standard manuals of soil fungi (Gilman 1994, Nagmani *et al.* 2006). The complete record of the fungal isolates and their species was maintained (Singh *et al.* 2021).

Result and discussion

In the present study, a total of 5 fungal endophytes F1, F2, F3, F4 & F5 were isolated from tulsi and jamun by using surface sterilization method (Table 1). These endophytic isolates were taken for screening for amylase production in which 3 isolates were found positive, 3 isolates were positive for catalase test, none of isolate was found positive for protease test while for cellulase 3 isolates were found positive (Table 2)

Table 1: Isolated endophytic fungus from Jamun and Tulsi

Sr. No	*Culture Code of Fungal Isolates*	*Part of Plant used*	*Fungi*
1	F1	Jamun Leaf	*Asperigillus* sp
2	F2	Tulsi Leaf	*Asperigillus* sp
3	F3	Jamun Stem	*Asperigillus* sp
4	F4	Jamun Leaf	*Asperigillus* sp
5	F5	Tulsi Stem	*Curvularia* sp.

and (Figure 1). These findings are full of conformity of Pavithra *et al.*(2012) isolated 40 endophytic fungi out of which 13 isolates were catalase positive, 20 isolates were found positive for both amylase and protease test and for cellulose test 18 were found positive. Anti-microbial test was also done by extracting the metabolites from fungal endophytes, no zone of inhibition was seen in these case. Whereas Devi *et al.* (2020) reported antimicrobial activities of different parts of *Syzium cumini*.The identified isolate F1,

Table 2: Interactions results of fungal endophytes

Sr. No.	*Catalyse Test*	*Anti-Microbial Test*	*Amylase Test*	*Protease Test*	*Cellulase Test*
F1	-	-	-	-	+
F2	-	-	+	-	+
F3	+	-	+	-	+
F4	+	-	+	-	-
F5	+	-	-	-	-

F2, F3 and F4 was found as *Asperigillus* sp. and F5 was found as *Curvularia* sp. (Table 2) and (Figure 2).

Catalase test was found +ve in fungal isolates F3, F4 and F5.No anti-microbial activity was observed from isolated fungus against any test micro-organisms. Amylase test was found +ve in fungal isolates F2, F3 and F4. Protease test was found -ve for all the fungal isolates. Cellulose test was found +ve in fungal isolates F1, F2 and F3. After screening fungal isolate No. F3 was considered as most efficient fungal endophyte

Conclusion

Endophytes are the endosymbionts, which live inside the plant body for their life time, without any apparent disease. They are beneficial for plant growth and development through a variety of mechanism, several mechanism such as production of plant growth promoters, activation of phosphate solubilisation and nitrogen fixation, IAA production etc. There are total 5 fungal endophytic funguses were isolated out of which three were positive for catalase, amylase, and cellulose production test. There was negative result of protease for all fungal isolates. This study will play a significant role in the research of antibiotics, lytic enzymes and phyto-hormones production and endophytes-host plant interactions.

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