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Exploring Endophytic Fungal Diversity of *Aegle Marmelos*

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ABSTRACT

Aegle marmelos, a well-known Indian plant with medicinal and religious importance, has been extensively used in Indian traditional medicine. These medicinal properties are supposed to be the effect of bioactive compounds synthesised by endophytes present in it. In the present research study, endophytic fungi were isolated from healthy, symptomless tissue of stem, root, fruit, and leaves of a well-known plant of *Aegle marmelos* (Bael) growing in different locations of Washim city area. The samples were processed under sterilized conditions, inoculated on Potato Dextrose Agar and Sobouraoud Dextrose Agar plates. The plates were incubated at room temperature for7 to 12 days. Total 20 isolates were purified and subjected for conventional identification viz. structural morphology, growth rate and conidial morphology. The predominant isolates were identified as belonging to genus *Fusarium, Altrenaria, Aspergillus, Rhizopus, Cladosporium, Trichoderma* etc. Most of the fungal endophytes were found as fast growing and some are moderate to slow growing endophytes. As a medicinal plant, *Aegle marmelos* exhibited diverse variety of fungal endophytes. Hence, *Aegle marmelos* plant could be explore for bioactive compounds synthesized from fungal endophytes which can be further use for therapeutic purposes.

Key words: *Aegle marmelos*, fungal endophytes, bioactive compounds

INTRODUCTION:

Endophytes are the class of microorganisms that reside in living plant tissues forming a symbiotic association with the host. Endophytes can be found in every plant species forming a non-pathogenic effect in them. However, some may exhibits infectious property in different genera of plants (Carroll and Carroll 1978; Petrini, O. & Fisher, P. J. 1986). Endophytes are microbes that inhabit plant tissues at some stages in their life cycle without harming to the host (PetriniO. 1991). Medicinal plants have also been recognized as a repository of fungal endophytes with novel metabolites of medicinal and pharmaceutical importance (Strobel *et al.,* 2004). Endophytes present in colonized plants often grow faster than non-colonized ones (Cheplick *et al.* 1989). These endophytes help the plant by providing them secondary metabolites that benefit the plants in

enhancing their defence against the pathogens, growth stimulation etc. many studies revealed that these metabolites are responsible for the medicinal properties of the plants.

Plants have been playing an important role in earth's ecosystem and nutrition of all living organisms on it. Humans are the one that have gained most of the benefits from plants. They are utilizing plants for their diet as well as for medicinal purpose. Anciently most medications have been developed by natural ingredients or ingredients prepared from natural compounds. (Katiyar *et.al.,* 2012). In daily life of some underprivileged human population's medicinal plants has been proved of utmost importance in sense of primary medical care (Shinwari, Z. K., & Qaiser, M. 2011). Approximately 80% of total countries trust on these conventional plant extract or herbal treatments (Li *et.al.,* 2017). Indian subcontinent has one of the oldest histories of treatment using various medicinal plants in the world. Till date they are utilizing these traditional methods to treat many disorders and diseases. Around 25000 plant based remedies are still used in rural communities (Süntar, I. 2020*). Aegle marmelos*, also known as Bael has been often utilized in the indigenous Indian system of medicine because of its abundant medicinal properties. It belonged to the family of *Rutacea*. It is also called as Bengal quince, Bilva, Indian quince, Golden apple, Holy fruit, Bel, Belwa, Sriphal, Stone apple, and Maredo in India (Neeraj, V. B., & Johar, V. 2017). Ayurveda has many references over utilization of *Aegle marmelos* for medicinal treatment (Rahman, S., & Parvin, R.2014). Traditionally *Aegle marmelos* is used to treat different disorders and diseases. It has been studied for presence of bioactive compounds helpful in field of medicine by many researchers that exhibits antimicrobial, antioxidant, anticancerous, anti-inflammatory, radio protective and antidiabetic activities (Badam *et.al.,* 2002;Gupta, A. & Tandon, N. 2004; .Jagetia, G. C., & Venkatesh, P. 2007; Subramaniam, D. *et.al.,* 2008; Jagetia, G. *et.al.,* 2005).Green leaves of *Aegle marmelos* possess the property lowering the blood glucose level in diabetic patients and animals (Chakrabarti et al. 1960; Vyas et al.1979). Methanolic extracts of unripe fruit from *Aegle marmelos* is effective in treating diarrhoea in mice caused by castor oil. (Shoba & Thomas 2001). However, studies revealed that the endophytic community present in the *Aegle marmelos* also helps in the medicinal activity of the host plant. Even though the endophytic population of the plant is yet to be explored especially the fungal endophytes. Hence this study is focused to enumerated diversified fungal endophytes present in the *Aegle marmelos*.

MATERIAL AND METHOD:

Plant sample collection: -

Healthy plants parts of *Aegle marmelos* were collected from the different locations of Washim city area. The parts viz. (leaves, stem, roots and fruit) were cut and sealed into plastic bag and labelled and proceed immediately. The collected plant part was washed under a tap water to remove soil and further washed by the distilled water. Later they were subjected to then surface sterilization process as described by Petrini *et al.,* 1992. Surface sterilization was carried out by immersing the samples into 70% ethanol for 1-3min followed by aqueous solution of sodium hypochlorite for 2-5 min followed by washing with 70% ethanol for 5min. The tissue was then rinsed with sterile distilled water thrice & allowed to surface dry in sterile conditions.

Isolation and Identification

All the sterilized plant samples were inoculated separately on potato dextrose agar and Sobouraoud Dextrose Agar plates supplemented with tetracycline under aseptic condition to isolate fungal endophytes from plant. The plates were incubated at room temperature for 7 to 12 days. The isolation of fungal endophytes was confirmed by observing the fungal growth on the plates. The pure culture of all the fungal isolates was subculture and kept for incubation at room temperature for 2 days. After the growth of fungal isolates, the identification of endophytic fungi was carried on the basis of their morphological characters and its microscopic view using Lactophenol blue staining. (Barnett and Hunter (1998), Ellis (1976), Vonarx (1978), Raper and Thom (1949), Ainsworth *et al.*1973).

RESULT AND DISCUSSION:

In the present research study, total 20 endophytic fungal isolates were isolated from different plant samples of *Aegle marmelos*. The isolates were differentiated by their colony morphology, growth rate and conidial septation (table 1 and Fig 1). Most of the identified fungal endophytic culture belongs to Ascomycota while few belong to Mucoromycota. The Ascomycota fungi however, belong to 6 different orders. Highest number of fungal endophytes was found from root samples followed by stem samples. Most of which were identified as *Fusarium* and *Aspergillus* and some are identified as *Penicillium, Trichoderma, Monolinia, Cladosporium, Altrenaria, and Rhizopus.* Cloudy cottony white coloured colony was shown by the *Fusarium* sp. From top view, whereas creamy white to orange colour was observed in the back view of the isolates. Oval shaped conidia were observed with pointed ends with conidial septations when projected microscopic view. Different isolates of *Aspergillus* sp. showed diverse colony morphology. However, the texture of the colony was constant between velvety to woolly texture. Also the back view showed creamy white colour colonies. Microscopic examination of the isolates showed globose structured with no septations. Conidia were fast growing in some cases it turned into darker in colour with increase in time of incubation. Few isolates of *Trichoderma* were also isolated some of which showed greenish woolly textured colony with white periphery. The back view was observed as creamy white colony. It showed branched structure on performing microscopy. Some of *Altrenaria* was seen from it stop of having greenish grey with velvety texture with leaf shape conidia. *Penicillium* was found with white centre and green colour with velvety texture at its starting phase of growth after completing its incubation period it turned into brown coloured with woolly texture having microscopic conidial structure. Gond*et.al.,* 2007 also reported the predominant presence of *Fusarium* fungal species among the 79 endophytic isolates from *Aegle marmelos*. They also mentioned that all the isolated fungal species belong to Ascomycota. Mani *et.al.*in 2015 also mentioned that endophytic fungal population of *Aegle marmelos* had *Altrenaria* isolates along with the isolates of *Curvularia* species. They also mentioned that these endophytes showed high antioxidant activities.

Sr. No	Isolates	Growth	Front view	Back view	Conidial Morphology	Possible Species
1	SI1	Fast	Cottony white	Orange/ yellowish	Oval shape, septated conidia	*Fusarium*
2	SI2	Moderate	Greenish, grey, Cottony colony	Blackish centre, white periphery	Circular unseptated conidia	,
3	SI3	Moderate	Greyish blue colony white corner velvety	Yellowish white	Flower shaped conidia, microscopic spores	*Penicillium*
4	SI4	Fast	white colour, cottony texture	Yellowish white	Oval shaped, septated conidia microscopic spores	*Fusarium*
5	SI5	Fast	Greenish white, woolly texture, waves present	Creamy white	Leaf shape conidia, microscopic structure, without septations	*Trichoderma*
6	RI1	Slow	Blackish grey, white periphery, velvety	Blackish grey	Shield shaped conidia, highly dispersed.	*Cladosporium*
7	RI2	Fast	Brownish centre, white periphery, cottony	Orange	Oval shaped septated conidia	*Fusarium*
8	RI3	Fast	Green colour, white periphery, cottony white	Yellowish white	Dense conidia, branched conidiophores	*Trichoderma*
9	RI4	Moderate	Cloudy white, velvety texture	Creamy white	Leaf shape conidia, microscopic	*Altrenaria*
10	RI5	Fast	Black colour, woolly texture	Creamy white	Unbranched conidia, globose in shape, non-septate.	*Aspergillus*
11	LI1	Fast	Greyish, velvety texture	Black	Shield shape conidia	*Cladosporium*
12	LI2	Moderate	White, cottony texture	Creamy white	Oval shaped septated conidia	*Fusarium*
13	LI3	Fast	Greenish centre, white periphery, velvety texture	Yellowish white	Oval shape septated conidia	*Fusarium*
14	LI4	Moderate	Greyish white, velvety texture, cloud shape	Creamy white	Oval shape septated conidia microscopic spores	*Fusarium*
15	LI5	Fast	Reddish white in colour with cottony texture	Dark red	Microscopic conidia, oval shaped, septated	*Fusarium*
16	FI1	Fast	Green/grey colony, woolly texture	Creamy white	Circular conidia, macroscopic unbranched structure	*Aspergillus*

17	FI2	Moderate	Greenish grey colour, velvety texture	Blackish grey	Leaf shape conidia, macroscopic structure	*Altrenaria*
18	FI3	Fast	Cottony white, black spores	Creamy white	Umbrella shaped conidia, macroscopic structure	*Rhizopus*
19	FI4	Fast	White colony, cottony texture	Orange	Sickle shaped, septated conidia microscopic structure	*Fusarium*
20	FI5	Moderate	Brown centre, white periphery, cottony texture	Creamy white	Tube like conidia, with septations, macroscopic structure	*Altrenaria*

Table 1: Morphological characteristics of endophytic fungal isolates

Figure 1 represents the diversity of fungal endophytes. It is evident that *Fusarium* is predominantly present followed by *Altrenaria* and *Aspergillus*. Some isolates of *Cladosporium* and *Trichoderma* were also present. *Rhizopus, Monolinia, and Penicillium* were found in last number.

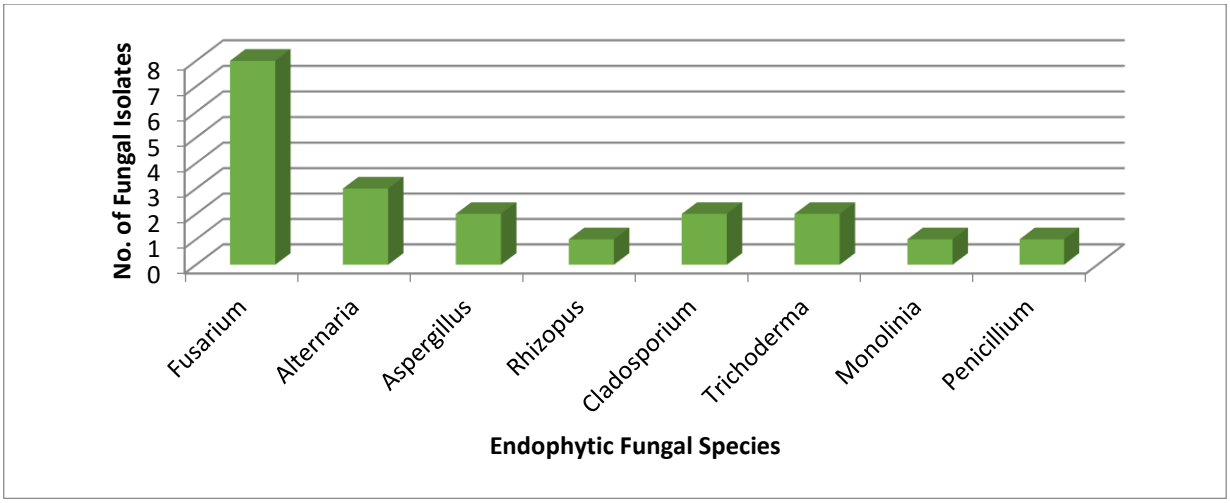

Figure 1.Diversity of endophytic fungal isolates.

CONCLUSION

Aegle marmelos has a diverse form of fungal endophytic population. Total 20 isolates of fungi have been identified. These isolates can be further subjected to production of bioactive secondary metabolites. These metabolites could be further studied for therapeutic activities of economical interest. The secondary metabolites isolated from these fungal endophytes can be utilized to produce industrially important products.

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