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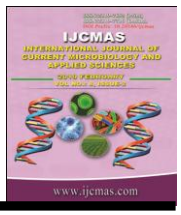

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Isolation and Morphological Characterization of Endophytic Fungi Isolated from Ten Different Varieties of Mango

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A B S T R A C T

Endophytes are the microbes that colonize the healthy tissues of plant. Endophytes associated with *Mangifera indica* (mango) are less understood. In this study, endophytic fungi were isolated fromten different mango varieties *viz.*, Alphonso, Totapuri, Neelam, Anfas, Willard, Badam model, Khaderi, Pancharasi, White Sari and KisanBhog from Bengaluru, Karnataka, India. Endophytic fungi were isolated fromleaf and stem tissues. Nine endophytic fungi were isolated from Alphonso out of which four were from leaf and five were from stem tissues. Two endophytic fungi were isolated from the leaf tissue of Totapuri, and five from the stem tissue. From Neelam, only four endophytic fungi were isolated from stem tissue and no endophytic fungi grew from the leaf tissue. One endophytic fungi was isolated from leaf and stem tissue each from Anfas, Willard, Badam Model, Kadari, Pancharasi and White sari, whereas two fungi were isolated from leaf tissue of KisanBogh and one from stem tissue. The isolates were studied for to their morphological characters and naming was done based on the variety and the part isolated.

Introduction

Mango (*Mangifera indica* L.) is considered as one of the choicest fruitcrops grown all around the world (Shad *et al.*, 2002). It is grown in more than 100 nations, however nowhere it is enormously esteemed as in India where 40 per cent of the total fruits grown is mango (Swamy, 2012). In India, mango is grown in 21.63 lakh hectares with a production of 185.27 lakh MT. In Karnataka, it is grown in 1.78 lakh hectares with a production of 1.78 metric tonnes and productivity is 10 t/ha (Anon., 2014). Mango is affected by a number of diseases at all the stages of its development right from seedling in nursery to the fruit in storage or transit

Endophytes are bacterial or fungal microorganisms that colonize plant tissue

intercellularly as well as intracellularly without causing any clear manifestations (Wilson, 1995). Endophyte, by definition, is one which lives in the tissues underneath the epidermal cell layers and makes no evident damage to the host (Stone *et al.,* 2000). They are present in all the plants parts, colonize all plants, and have been found from all plants analyzed till date (Nair and Padmavathy, 2014). To keep up consistent beneficial interaction, endophytes deliver different compounds that advance development of plants and help plant to better environmental adoption. Plants growing in areas of great biodiversity also have the prospect of housing endophytes with great biodiversity (Strobel *et al.,* 2003).Out of 1.5 million fungal species evaluated to be available, only 97,861 fungal species have been studied (Hawksworth, 1991). They deliver an immense range of compounds which are valuable for plants for their development, insurance to natural circumstances and supportability (Nair and Padmavathy, 2014). They additionally assume an essential part in nutrient cycling, biodegradation and bioremediation (Das and Varma, 2009; Lee *et al.,* 2004). Hence in this study we aimed at studying the diversity of endophytic fungi associated with ten different varieties of mango in terms of presence and their characteristics.

Materials and Methods

The foregoing investigations were conducted at the Department of Plant Pathology, College of Horticulture, Bangalore, University of Horticultural Sciences, Bagalkot.

Isolation of endophytic fungi

Collection of sample

For isolation of endophytic fungi, mature, healthy, green, asymptomatic leaves and stem tissues were collected from ten mango varieties. Healthy leaf and stem tissues of 10 mango varieties *viz.,* Alphonso, Totapuri and Neelam were collected from the orchards of University of Horticultural Sciences, Bagalkot, Regional Horticulture Research Station, Bengaluru. Similarly, healthy leaf and stem tissues of Anfas, Willard, Khaderi, Pancharasi, Kisanbhog, White Sari and Badam model were collected from Indian Institute of Horticultural Research Station, Bengaluru. Samples were collected from three randomly selected plants from each variety and collected leafs are clubbed and collected in a plastic bags, labeled, transported to lab within 12 hours and used for isolation. Although rapid changes in endophyte colonization probably do not occur immediately following collection, all samples were handled carefully and processed as quickly as possible. Samples were air-dried to remove any surface moisture before transport or storage. During transport, samples were kept cool and dry. Samples of Alphonso, Totapuri, Neelam were collected from six year old trees and the samples from the other remaining varieties *viz.*, Anfas, Willard, Khaderi, Pancharasi, KisanBhog, White Sari and Badam model were collected from 40 years old trees.

Isolation of fungal endophytes

Endophytic fungi were isolated from leaf and stem tissues collected from the different mango varieties like Anfas, Willard, Khaderi, Pancharasi, KisanBhog, Alphanso, Totapuri, Neelum, White Seri and Badam Model.

All the leaf and stem samples were washed under running water prior to surface sterilization. Leaf discs were cut from leaf using a sterile blade to isolate endophytes. Stem segments were cut from internal tissues of stems. Size of the sampling unit and surface sterilization procedures vary according to the preferences of the

investigator, the species of host plant, and host tissue type sampled. In our study we followed the modified method of Michereff *et al.,* (2014) as discussed below:

Leaf segments were surface sterilized in 75 per cent ethanol for 1 minute and 2 per cent sodium hypochlorite for 1 minute. The stem fragments were surface sterilized in 75 per cent ethanol for 1minute and 2 per cent sodium hypochlorite for 2 minute. Sterilized segments were rinsed in sterilized distilled water and then dried on sterilized paper. The effectiveness of the sterilization procedure was tested using the imprint method (Schulz *et al.,* 1993). Three fragments were placed evenly in petri dishes (9 cm dia.) containing potato dextrose agar (PDA) medium amended with streptomycin to suppress bacterial growth and incubated at 28ºC. The fungi growing out of the segments during the incubation period were recorded as endophytes and then those endophytes were pure cultured.

Characterization of fungal endophytes

Morphological study

Cultures on PDA media were assessed according to their morphology. Colony character of the fungal growth, topography was noted and characters of mycelial colour, type and spore production were observed.

Results and Discussion

Isolation of fungal endophytes from different mango varieties

Endophytic fungi were isolated from leaf and stem of different mango varieties like Alphanso, Totapuri, Neelum, Anfas, Willard, Khaderi, Pancharasi, KisanBhog, White Sari and Badam Model. Totally 35 fungal endophytes were isolated from mango varieties, out of which 14 isolates were from leaf and 21 isolates were from stem tissues (Table 1). The result showed that more number of endophytic fungi was isolated from stem tissue as compared to the leaf tissues. The isolates were designated with first two alphabets denoting endophytic fungi (EF), next letter the first letter of the variety from which the fungi was isolated (Eg.:A-Alphonso), the fourth letter the tissue from which it was isolated, wherein L stands for leaf and S stands for stem. The endophytes isolated are designated as A, B, C, D and so forth.

Similar isolations from plants were done by other workers like Nayak (2015) who isolated 17 fungal endophytic species from the ornamental mango (*Mangiferaindica*) and Michereff*et al.,* (2014) isolated 169 fungal endopytes from noncommercial mango plant; Amin *et al.,* 2014 isolated endophytes from cultivars which were resistant and susceptible to vascular streak disease.

Morphological characterization of fungal endophytes

Morphological characteristics of all the 35 isolates like mycelial characters and spore production were examined using cultures grown on PDA. There was a lot of variation in the morphological characters of all the endophytic fungi isolated from different varieties (Table 2).

Out of nine fungal endophytes isolated from Alphonso variety, two endophytes EFAL-A and EFAS-E exhibited slow growth and two isolated EFAL-B and EFAS-C exhibited medium growth. Five endophyes isolated from leaf as well as stem, EFAL-C, EFAL-D, EFAS-A, EFAS-B and EFAS-D exhibited fast growth. Five endophytes EFAL-A, EFAL-C, EFAL-D, EFAS-C and EFAS-D showed greyish mycelium. All the isolates were non-

sporulating except EFAS-E. Four endophytes isolated from leaf EFAL-A, EFAL-B, EFAL-C and EFAL-D showed septate mycelium and five endophytes isolated from stem EFAS-A, EFAS-B, EFAS-C, EFAS-D and EFAS-E showed aseptate mycelium.

Two endophytes EFAL-D and EFAS-A showed cottony type mycelium and three endophytic fungi *viz*., EFAL-B, EFAL-C and EFAS-D showed fluffy mycelium on PDA (Figure 1).

The results revealed that, out of total 35 fungal isolates, 17 endophytes EFAL-A, EFAL-B, EFAL-C, EFAL-D, EFTS-A, EFTS-B, EFTS-D, EFTS-E, EFNS-A, EFNS-B, EFNS-C, EFWL-A, EFWS-A, EFBML-A, EFKAL-A, EFPL-A, EFWSL-A, EFKBL-B and EFKBS-A showed septate mycelium which was dark coloured and the remaining 18 endophytes showed aseptate mycelium and were hyaline in colour.

Majority of them did not produce spores and only two endophytes EFTS-E and EFKBL-A isolated from the stem tissue of totapuri and leaf tissue of Kisanbhog respectively produced hyaline spores (Figure 2).

The fungal isolates EFAL-A, EFAS-E, EFATL-A, EFATL-B, EFTS-A, EFTS-B, EFTS-C, EFWDL-A, EFBML-A, EFKAL-A and EFKAS-B were slow growing and EFAL-B, EFAS-C, EFTS-D, EFTS-E, EFNS-B, EFNS-C, EFNS-D, EFANS-A, EFBMS-A, EFPL-A, EFPS-A, EFWSL-A, EFKBL-A and EFKBL-B showed medium growth rate and EFAL-C, EFAL-D, EFAS-A, EFAS-B, EFAS-D, EFNS-A, EFWS-A, EFANFL-A, EFWS-A and EFKBS-A were reported to be fast growing.

Six isolates out of 35 showed fluffy cottony growth of mycelia (EFAL-B, EFAL-C, EFAS-D, EFNS-B, EFNS-C and EFKBS-A) on PDA and 11 isolates out of 35 showed cottony growth (EFAL-D, EFAS-A, EFATL-A, EFATL-B, EFTS-A, EFTS-C, EFTS-D, EFTS-E, EFNS-D, EFWS-A and EFKBL-B). Nine endophytic fungi (EFAL-A, EFAL-C, EFAL-D, EFAS-C, EFAS-D, EFNS-B, EFWS-A, EFBML-A and EFKL-A) out of 35 isolates showed greyish colony on PDA media, three (EFTL-A, EFTS-B and EFTS-E) showed blackish growth on PDA. Six (EFAS-B, EFTL-B, EFTS-C, EFNS-C, EFWDL-A and EFKBS-A) endophytes showed mixed colony colour on PDA media.

Table.1 The endophytic fungi isolated from ten different varieties of mango

Sl. No	Varieties	Fungal strains isolated	
		Leaf	Stem
1	Alphonso	4	5
2	Totapuri	2	5
3	Neelam	0	4
4	Anfas	1	1
5	Willard	1	1
6	Badam model	1	1
7	Khaderi	1	1
8	Pancharasi	1	1
9	White Sari	1	1
10	KisanBogh	2	1
	Total	**14**	**21**

Table.2 Morphological characteristics of endophytic fungi isolated from ten different varieties of mango

Sl. No	Variety	Isolate	Spore production	Mycelium characters	Topography of the fungal growth	Color of the colony	Growth rate
	Alphonso						
1	Leaf	AFAL-A	No	Septatecoloured	Flat uniform	Greyish	Slow
2		EFAL-B	No	Septatecoloured	Fluffy	Dull whitish	Medium
3		EFAL-C	No	Septatecoloured	Fluffy uniform	Greyish	Fast
4		EFAL-D	No	Septatecoloured	Cottony type mycelium	Greyish	Fast
5	Stem	EFAS-A	No	Aseptate hyaline	Cottony type mycelium	White and yellow	Fast
6		EFAS-B	No	Aseptate hyaline	Flat Silky	White and yellow	Fast
7		EFAS-C	No	Aseptate hyaline	Flat spongy	Greyish	Medium
8		EFAS-D	No	Aseptate hyaline	Fluffy uniform	Greyish black	Fast
9		EFAS-E	Yes	Aseptate hyaline	Flat milky growth of mycelium	Yellowish white	Slow
	Totapuri						
10	Leaf	EFTL-A	No	Aseptate hyaline	Cottony flat	Black	Slow
11		EFTL –B	No	Aseptate hyaline	Cottony flat	Centrally brown, whitish margin	Slow
12	Stem	EFTS –A	No	Septatecoloured	Cottony and slightly raised	Reddish brown	Slow
13		EFTS –B	No	Septatecoloured	Corky growth with wavy margin slightly undulated	Blackish grey	Slow
14		EFTS –C	No	Aseptate hyaline	Cottony flat	Reddish center with white margin	Slow
15		EFTS –D	No	Septatecoloured	Cottony flat type	Dark brownish centre with brown margin	Medium
16		EFTS –E	No	Septatecoloured	Cottony flat	Black	Medium
17	**Neelum**						
	Stem	EFNS-A	No	Septatecoloured	Flat uniform	Brownish yellow	Fast

18		EFNS –B	No	Septatecoloured	Fluffy uniform	Greyish	Medium
19		EFNS –C	No	Septatecoloured	Fluffy cottony growth raised at center	White and greyish	Medium
20		EFNS –D	No	Aseptate hyaline	Cottony uniform	Whitish purple	Medium
	Anfas						
21	Leaf	EFANL-A	No	Aseptate hyaline	Flat uniform	Dull brown	Fast
22	Stem	EFANS-A	No	Aseptate hyaline	Flat uniform growth	Whitish yellow	Medium
	Willard						
23	Leaf	EFWL-A	No	Septatecoloured	Undulated	Yellow and grey	Slow
24	Stem	EFWS-A	No	Septatecoloured	flat cottony growth	Grey	Fast
	Badam Modal						
25	Leaf	EFBML-A	No	Septatecoloured	Raised colony	Grey	Slow
26	Stem	EFBMS-A	No	Aseptate hyaline	Uniform undulated	Whitish yellow	Medium
	Kadari						
27	Leaf	EFKL-A	No	Septatecoloured	Raised undulated	Grey	Slow
28	Stem	EFKS-A	No	Aseptatehyaline	Flat wavy	Red releasing blackish brown	Slow
	Pancharasi						
29	Leaf	EFPL-A	No	Septatecoloured	Flat uniform	Brownish black	Medium
30	Stem	EFPS-A	No	Aseptatehyaline	Flat undulated	Yellowish white	Medium
	White Seri						
31	Leaf	EFWSL-A	No	Septatecoloured	Flat wavy	Dark brown	Medium
32	Stem	EFWSS-A	No	Aseptate hyaline	Flat undulated wavy growth	Yellow	Medium
	KisanBogh						
33	Leaf	EFKBL-A	Yes	Aseptate hyaline	Flat milky growth	Dull yellow	Medium
34		EFKBL-B	No	Septatecoloured	Flat cottony growth	Brownish black	Medium
35	Stem	EFKBS-A	No	Septatecoloured	Uniform raised fluffy growth	Grey and dull white	Fast

Fig.1 Colony morphology of fungal endophytes isolated from Alphonso variety

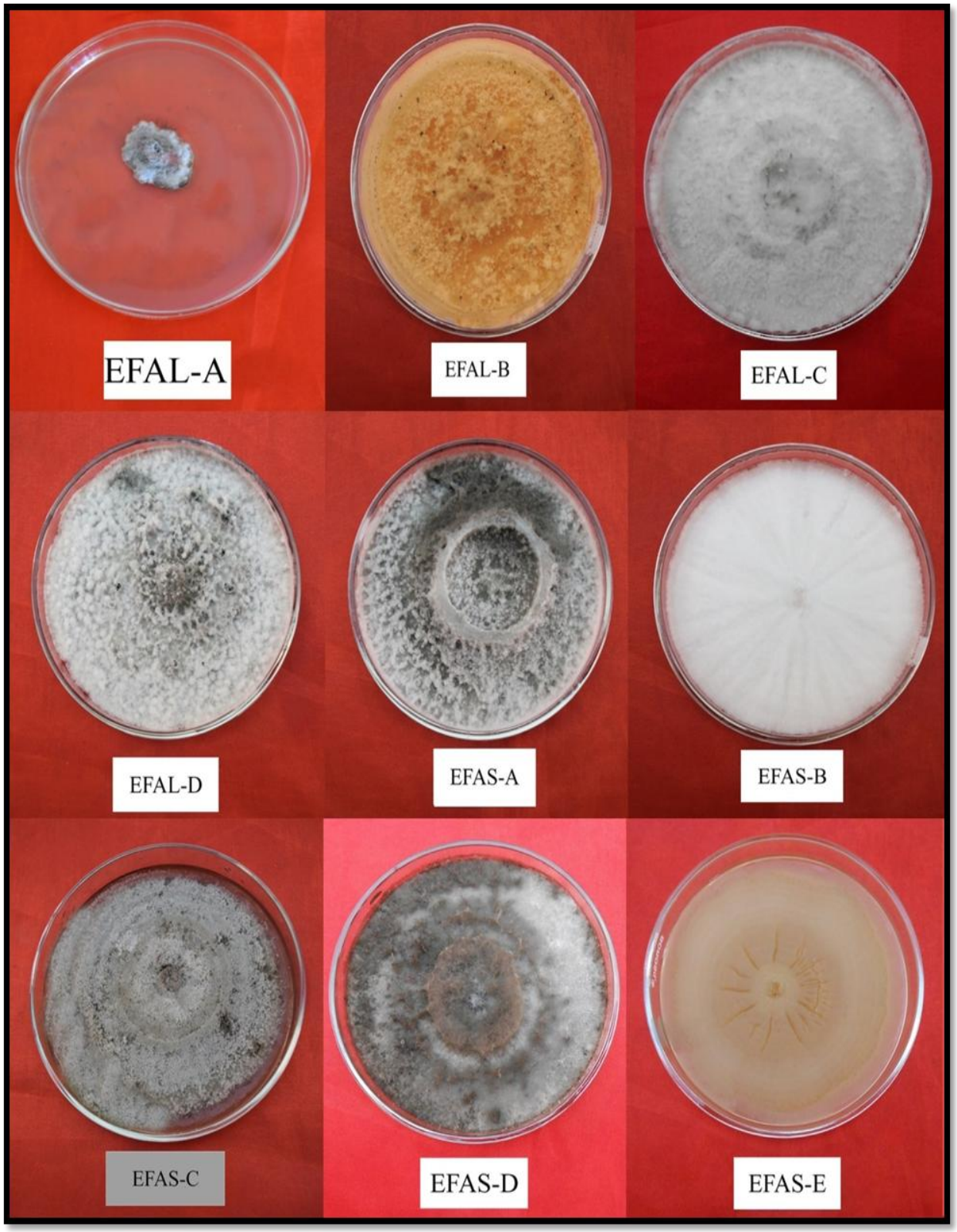
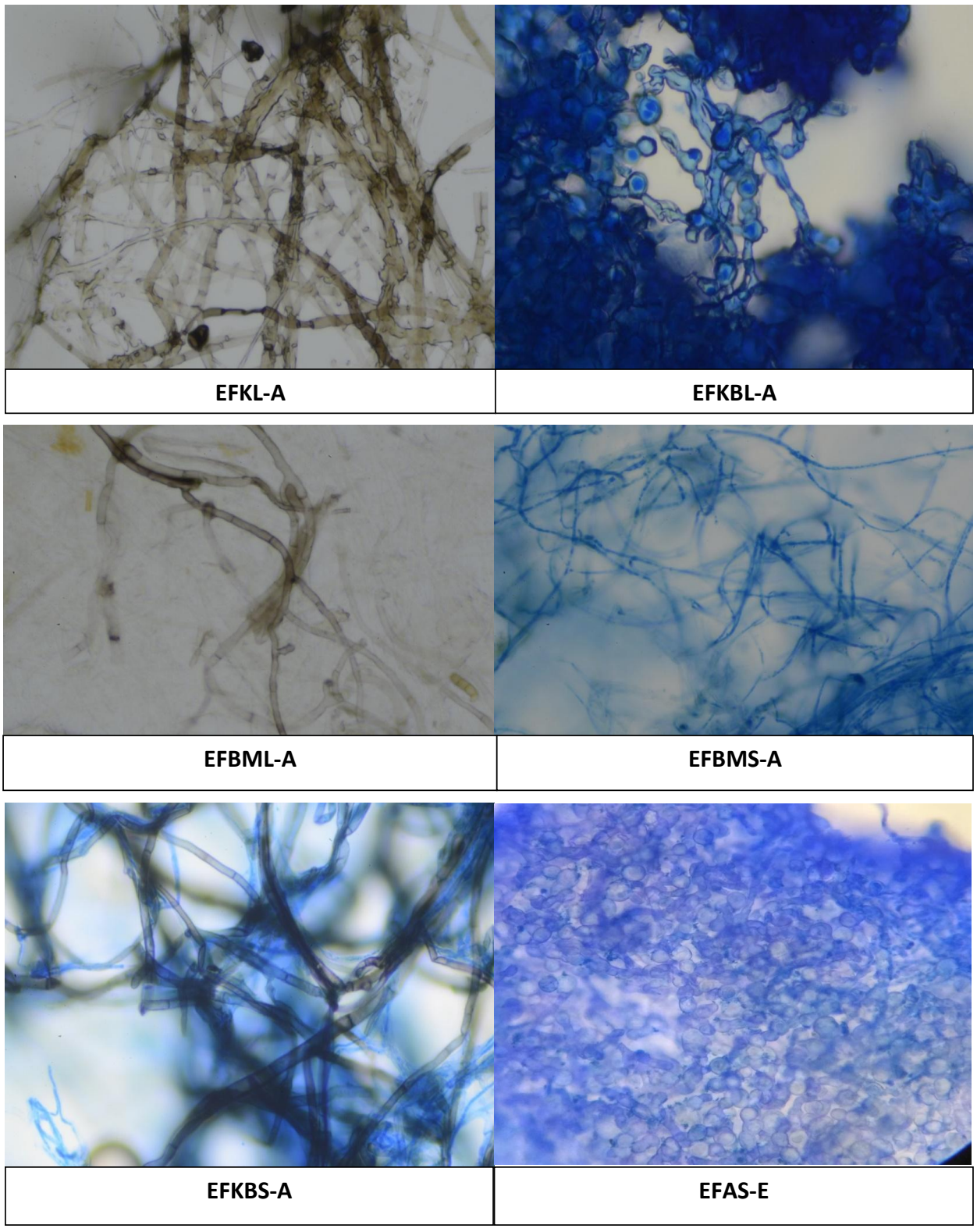


Fig.2 Microscopic characters of some fungal endophytes isolated from different mango tissues



Similar morphological studies in fungi done by Huang *et al.*, 2009 isolated 108 fungal isolates from three medicinal Artemisia species was first carried out according to colony or hyphal morphology of the fungal culture, characteristics of the spores; Carmichael *et al.*, (1980), Barnett and Hunter, (1998), Ainsworth *et al.*, (1973), Shekhawat *et al.*, (2010) studied morphological studies from endophytic fungi isolated from leaves of *Melia azedarach* L. (Meliaceae); Correa *et al.*, 2014 studied morphological studies on endophytic fungi from *Actinidia macrosperma*.

In conclusion, endophytic miccrobes are known to exist in unique ecological niches and influence the distribution, ecology, physiology and characteristic of the plants. The present investigation was undertaken to know the diversity and distribution of the endophytic fungi in ten different varieties of mango. The study revealed that there exists diversity in the number and morphological characteristics of the endophytic fungi isolated from the various tissue of the host. There was also variations in the mycelialcolour, sepatations and the colony characters similar to the variation in the host characters. Further studies on the identification of the fungi, their role in the growth and development of the plant and their ability to suppress the pathogens will play a significant role in horticulture.

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