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Diversity of the Endophytic Fungi Isolated from Acalypha Indica Linn - A Promising Medicinal Plant

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Abstract- Altogether 700 segments from 15 plants of *Acalypha indica Linn.* were collected from Karnatak University Botanical Garden; Dharwad. And they were screened for the presence of endophytic fungi. A total of 12 fungal species viz, *Aspergillus candidus* Link ex. Fries., *Aspergillus flavipes* Bainer and Sart*ory. Aspergillus niger* Tiegh., *Aureobasidium pullulans* (de Bary) Arnaud. Les., *Bipolaris nodulosa* (Bert and Curt. ex. Sacc.) Shoemaker., *Cladosporium epiphyllum* Person., *Cunninghamella blacksleeana* Lender., *Fusarium heterosporum., Fusarium oxysporum* Schlechtendahl., *Penicillum purpurogenum* stoll., *Rhizopus nigricans* Ehrenberg., *Unidentified* I isolated and identified based on the morphology of the fungal culture and spores with the help of manuals. The present investigation was the diversity of endophytes and colonization frequency was more dominated in host leaves had been determined. Importance of endophytes in medicinal plant has been discussed.

Index Terms- Endophytes, Host Specificity, Colonization Frequency, *Acalypha indica* Linn., *Rhizopus nodusus* Namyslowski.

I. INTRODUCTION

Endophytic fungi, defined as those species that occur within the living tissues of plants, without causing symptoms and have been isolated from every organ of almost every plant species sampled (Stone *et al*., 2000). Fungal endophytes colonize within plant organs and recently endophytes are viewed as outstanding source of secondary metabolites, bioactive compounds, antimicrobial natural products and nutrients uptake process. One of the most compelling features of fungal endophytes is their exceptional diversity. Several fungi showed differences in the same plant (Jayashree *et al*., 2011), but these differences were not consistent between sites. The aim of this paper is to provide recent data about diversity of fungal endophytes, colonization frequency of particular site of plant and their dominancy in their host.

Acalypha indica Linn. is an erect annual herb, about 2ft high, branches numerous, long, angular softly hairy leaves upto 3 in long, ovate, thin, globrous, margins toothed, leaf-stalk longer than the blade, slender. Flower sessile, monoecious, in erect long spikes, male flowers very small, clustered near the summit, female flowers, scattered, each or a group of 3-5 with a large, leafy, truncute bract, usually one- seeded, seeds minute, ovoid, pale brown.Chemical composition of plant contains kaempferol, sitosterol,triacetonamine. Leaves and twigs, acalyphine, acalyphamide and other amides, quinone, sterols and cyanogenic glycoside. Active principle HCNand an unknown substance, extremely poisonous to rabbits; causes discoloration of blood and gastro-intestinal irritation.

Medicinal uses *A. indica* are haritamanjari plant is bitter, acrid and possesses diuretic, cathartic, expectorant, emetic, anthelmentic, anodyne and hypnotic properties. But it causes gastro-intestinal irritation. It is used as a substitute for ipecac and senega. A decoction of the herb is used as a cure for tooth and ear ache and is safe and speedy laxative. It is useful in bronchitis, pneumonia and asthama. Root is cathrtic. Leaves are laxative, used in scabies and in snake bite. Fresh leaf juice useful in rheumatoid arthritis and skin affections. Juice with salt applied on eczema. Paste of leaves applied on burns; with juice of lime, useful in early cases of ringworm. Power of leaves for bed-sores and maggot-infested wounds. Extract found useful in cardinal symptoms of wheezing cough; the raised eosionophil count also came down to normal. Herb is used as a remedy for severe cough associated with bleeding from lungs, haemoptysis and incipient phthisis. It has produced a number of symptoms in the alimentary tracts characterised by burning sense of weight in stomach, flatulence and spduttering diarrhoea.

II. MATERIALS AND METHODS

*Acalypha indica Linn.*is a most promising medicinal plants was selected for the present investigation of endophytic fungal diversity, where plants are growing in Karnatak University Botanical Garden Dharwad. Location of the studied site is lying in between 14°15¹ to 15°5¹ North longitude and 74°49¹ and 76° 21¹ east latitude. There is a marked diurnal temperature difference. That can be below as 20.2°C in June and high as 34.42°C in March. The annual rain fall is 600-850 mm. The climate is semi humid to humid. Soil is covered with a hard, compact crust having dark brown colour.

Collection of plant samples.

The samples of 15 *Acalypha indica* Linn. plants were collected from medium sized healthy plants from Karnatak University Botanical Garden Dharwad. The plant materials were collected in closed sterile polythene bag with labeled and brought to the laboratory and they were processed within 24 hours of collection.

Isolation of endophytic fungus:

The samples were rinsed gently under running tap water to remove dusts and debris. The leaves, petiole, stem and roots cut into segments (0.5-1cm). The samples were surface sterilized accordingly proposed by Dobranic *et al*., (1995). The samples were immersed in 70% ethanol for 5 seconds followed by 4%

sodium hypochlorite for 90 seconds and then rinsed in sterile distilled water for 10 seconds. The excess moisture was blotted in a sterile filter paper. The surface sterilized segments were placed in petridishes containing PDA and MEA medium. The petridishes were sealed using parafilm type and incubated at 26 ± 1^0c at 12 hours light and dark cycle. The petridishes were monitored every day to check the growth of endophytic fungal colonies from the segments.

$$\% \text{ of CF} = \frac{\text{Number of segments colonized by an endophyte}}{\text{Total number of segments analyzed}} \times 100$$

The hyphal tips which grew out from the segments were isolated and sub cultured on PDA and MEA medium. The pure cultures were maintained on PDA slants. The endophytic fungi were identified according to their macroscopic and microscopic characteristics such as the morphology of fruiting structures and spore morphology with the help of identification manuals (Ellis, 1971; Subramanin; 1971; Sutton, 1980; Nag Raj, 1993).

III. RESULTS

Altogether 700 segments (225 segments are leaves, 225 segments are petiole, 225 segments are stem, 25 segments are roots) of *Acalypha indica* Linn, and they were processed for the isolation of endophytic fungus. A total of 12 fungus was obtained (Table 1) in root samples there is no significance occurrence of fungi. All the isolated and identified fungus was stored in Microbiology Laborotary Karnatak University Dharwad (MLKUD).

Description of Endophytic Fungi:

Species 1: *Aspergillus candidus* Link ex Fries.

Colonies on media heads, white, globose, radiate, conidiophores 500-1000µm long 10-20µmn in diameter, with vesicles thick, smooth, colourless, globose, fertile once the entire surface, 35µm in diameter; phialides born on metulae, usually colourless. Meyulae 4-5µm in length. Phialides 4.5×2-2.5µm. Conidia hyaline, globose to subglobose, thin walled, smooth 3-3.5µm. in diameter.

Species 2: *Aspergillus flavipes* Bainier and Sartory.

Colonies on MEA or PDA media white at first, becoming yellowish, in some strains forming more or less abundant, closely woven, yellow masses containing many helicoids to horseshoe-shaped, thick-walled cells. Heads mostly columnar or calyptri form masses, commonly persistently white, but with some strains in pale avellaneous shades to deep avellaneous. Conidiophores300-500× 4-5µ, or up to 2-3mm. in length and 8-10 µ in diameter, smooth; vesicles subglobose or elliptical up to 20×30 µ; phialides in two series; primary 4-7 µ or 8×2µ or 3µ, secondary 5-8 ×1.5-2µ.Conidia 2-3 µ. Smooth, subglobose, colorless or nearly so.

Species 3: *Aspergillus niger* Tiegh.

Colinies growing moderately on PDA or MEA, 3.5-4.5cm in 10 days, with aboundant submerged mycelium, conidial heads carbon black, exudates lacks, conidial heads large and black, at first globose and then radiate or splitting in well defined columns in age, up to700-800µmin diam; conidiophores arising directly from the substratum, smooth, nonseptate, thick walled, 1-2mm ×15-20µm; vesicles globose, walls thick, commonly 45-75µm in diam, occasionally longer, bearing two series of fully packed phialides, brownish;metulae mostly 20-30×5-6µm, often reaching 60-80×8-10µm,rarely septate; phialides7-10×3-3.5µm;conidia globose, spinulose with colouring substance, black, 4-5µm; globose to subglobose.

Species 4*: Aureobasidium pullulans* (de Bary) Arnaud, Les.

Colonies growing fast, reaching 4cm in 7 days at 24C on MEA, mid to dark brown; Vegitative hyphae hyaline, up to 12µm wide; pigmented hyphae distinctly constricted at the septa; Conidiophores mostly 6-8µm wide, dark brown, with small lateral protuberances which become short, open ended necks of phialides; conidia hyaline ellipsoidal, often with in distinct basal apiculation, variable in size and shape, straight, mostly (7.5-)9-11X(3.5-)4-5.5(-7)µm , but may be bigger in old colonies; secondory conidia and endoconidia similor, but smaller.

Species 5: *Bipolaris nodulosa* (Bert. And Curt.ex Sacc.) Shoemaker.

B.nodulosa (Bert. And Curt.ex Sacc.) Shoemaker. Conidiophores simple, branched, hyaline at tip dark brown at lower parts usually broader toward the apex, swollen and geniculate at the conspicuous circular conidial scars occurring in close succession in the upper poprtion; conidia straight, ellipsoid or ovate or typically obclavate,. Thin-walled pale to moderately dark brown or olivaceous brown, with a circular basal hilum included within the coutous of theroundedbasal wall and often surrounded by a hyaline area, 3-7 septate, 28-70× 10-18µ, mostly 48.4 ×56.6 ×13-15.7µ(21.64× 12-17 µ mean 48.6× 14µ) in the type 1 produced singly and acrogenously at the tips of the conidiophores. And successive growing points, with alength/ breadth ratio of about 5.5-3.7.

Species 6: *Cladosporium epiphyllum* Person.

Colonies greenish-black, large, thick; conidiophores at first erect, then falling, pale green; conidia very numerous, soon falling from the chain, at first one-celled, then two-to more-celled, olive-green, 10-22µ long×4-6µ thick.

Species7: *Cunninghamella blakesleeana* Lender.

Colonies growing rapidly on PDA and MEA, white, later becoming yellow, loose, erect,2-4cm in height; sporangiophores long, simple or regularly verticillately branched, lateral branches of the sporangiophores variable in length and number, usually less than 50µm long; terminal vesicals, globose to subglobose, 40-60µm; lateral vesicles usually smaller than terminal vesicles, 19-28µm;sporangioles hyaline, echinulate or smooth, ovoid ones 7.5-10µ; in diam(excluding spines), ellipsoidal ones 10-12.5×7-8µm(excluding spines);.

Species 8: *Fusarium heterosporum***.**

Both sterile and fertile hyphae creeping, at first hyaline, then dark; sterile hyphae septate 18µ in diameter; fertile hyphae septate, 4µ in diameter, much branched bearing swollen jar-like cells terminally and laterally on which are borne single the sub spherical, smooth, black conidia. Conidia one-celled, 11-14µ in diameter. (Plate II-6)

Species 9: ***Fusarium oxysporium*** **Schlechtendahl.**

Stroma brownish-white, plectenchymatic smooth, extended or colored green to blue black by erumpent sclerotial hard bodies, and 0.5-3mm. or 3-6 mm. in thickness more or less wrinkled under moist conditions usually covered by fascicled medium high aerial mycelium, later forming sporodochia, more seldom pionnotes with three septste, spindle –sickle –shaped conidia, curved or almost straight, rarely or weakly pedicillate. Smaller conidia one or two celled, ovel to reniform, or numerous in the aerial mycelium but are lacking in typical fruiting layers of the macroconidia.

Species 10: ***Penicillum purpurogenum*** **Stoll.**

Colonies on MEA or PDA agar slowly spreading very closely flocosse to almost velvety, white at first, becoming yellow to pinkish shades , and finally light gray-green; reverse and medium colored deep red to purple. Conidiophores arise from aerial mycelium, up to 100µm or 300µm long. Conidial fructifications consist of long, divergent chains, up to 100µ long, in two stages; metulae 10-16 x 2- 2.5µ; phialides 11-12 x 2.5µ . Conidia elliptical, 3.4-3.8 x 2-2.5µ, smooth, pale green.

Species 11: ***Rhizopus nigricans.***

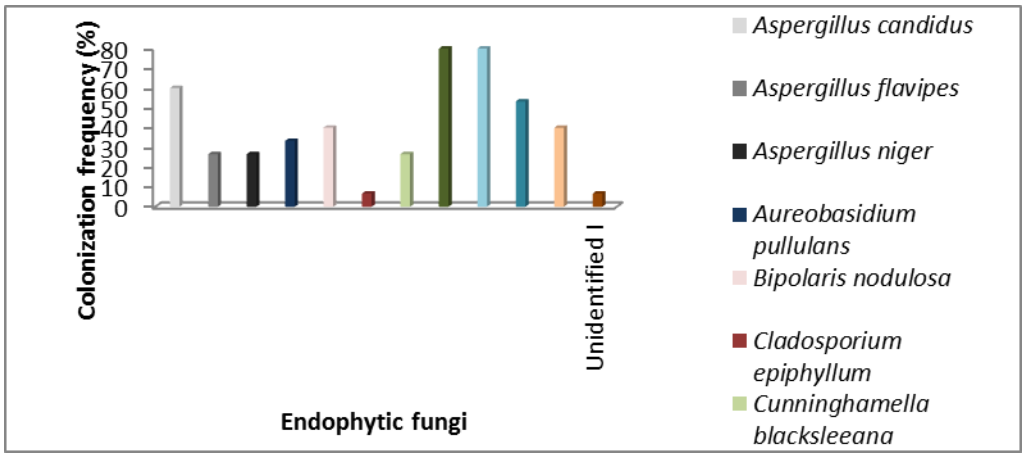
Stolon creeping, recurving to the substrate in the form of arachnoid hyphae, which are strongly raosed and distant from the substrate and implanted at each node by means of rhizoids.

Table 1: Colonization frequency (%) of endophytes in *Acalypha indica* on different media

S.N	Endophytic fungi	Colonization frequency (%)						Total isolates
		MEA			PDA			
		L	P	S	L	P	S	
1	*Aspergillus candidus* Link ex. Fries.	-	-	20	13.3	-	26.6	59.9
2	*Aspergillus flavipes* Bainer and Sart*ory.*	-	-	-	26.6	-	-	26.6
3	*Aspergillus niger* Tiegh.	-	-	-	-	-	26.6	26.6
4	*Aureobasidium pullulans* (de Bary) Arnaud. Les.	-	20	-	-	13.3	-	33.3
5	*Bipolaris nodulosa* (Bert and Curt. ex. Sacc.) Shoemaker.	13.3	-	-	20	-	-	39.96
6	*Cladosporium epiphyllum* Person.	6.66	-	-	-	-	-	6.66
7	*Cunninghamella blacksleeana* Lender	-	-	6.66	6.66	-	13.3	26.6
8	*Fusarium heterosporum*	-	6.66	26.6	-	26.6	20	79.86
9	*Fusarium oxysporum* Schlechtendahl.	6.66	13.3	13.3	13.3	20	13.3	79.86
10	*Penicillum purpurogenum* stoll	-	26.6	-	-	26.6	-	53.2
11	*Rhizopus nigricans* Ehrenberg.	-	-	26.6	-	-	13.3	39.9
12	*Unidentified* I	-	-	-	-	-	6.66	6.66
	Total							479.12

* L-Leaf, P-petiole, S-stem

Colonization frequency (%) of endophytes in *Acalypha indica* Linn.



IV. DISCUSSION

Medicinal plants are one of the oldest forms of health care known, every plant on earth is known to harbor at least one endophytic microbe. These are one of the most unexplored and diverse group of organisms having symbiotic association with higher life forms and may produce benificial substances for host (Weber,1981). Present studies revealed that the leaves of the host plant exhibited the highest endophytic diversity than the petiole and stem samples. Thus the present finding have suported that the endophytes isolated from leaf samples exhibited greater diversity and high colonization frequincy compared to the endophytes of the other plant parts examined.

Leaves, petiole, stem and roots of a single plant often differ greatly in the dominant members of their endophytic communities (Chaverri *et al*.,2010, Gazis *et al*.,2010, Hoffman *et al*.,2008, Pocasangre.,2000). and may even show functional differences. As in case of Alfalfa plants of leaves, stems and roots are colonized by distinct fungi that produce different ranges of secondary metabolites (Weber *et al*., 2006). Even with a single plant different leaves may differ significantly in community composition (Gamboa *et al*.,2001, Fisher *et al*., 1996). Single leaves of a tropical forest tree, *manilkara bidentata*, showed fine scale variation of endophyte isolation rates and identity. In this respect, plants are genetic mosaics because each organ may have a unique combination of genes in its micro biome (Herre *et al*., 2007.). However, some endophytes are restricted to single cell and tissues in the leaf endophytes in different tissues may not interact (Stone JK. 1987). This potential goldmine of undescribed biodiversity has the issue of host specificity, particularly in tropical plants. A recent meta-analysis found that leaf endophytes are indeed more species-rich in the tropics than in temperate regions (Arnold *et al*., 2007). This report consistent to earlier workers as, leaf samples finding more number of endophytic diversity in the plants. One of the possible reasons for the differences in the colonization rates between plants is the structure and substrate which influence the colonization and distribution of endophytic fungi (Okane *et al*., 1997). Kumar and Hyde (2004) also stated that the overall colonization rate in the leaves was found to be significantly higher than those in root, stem and petiole. Present studies clearly exhibited that the number of endophytic fungi was higher in leaves followed by petiole, stem and roots. However, the overall colonization frequencies differed with different organs. Similar results have been observed in the endophytic diversity of Thalavaipandian *et al*., 2011.

The most prevalent endophytes were recorded among *Fusarium heterosporum* and *Fusarium oxysporum* Schlechtendahl. was dominant followed by *Aspergillus candidus* Link ex. Fries. The total colonization frequency higher from *Fusarium heterosporum* and *Fusarium oxysporum* Schlechtendahl. and low from *Nigrospora sphaerica* (Saccardo) Mason followed by *Bipolaris Cunninghamella blacksleeana* Lender, *Aspergillus flavipes* Bainer and Sarto*ry*., *Aspergillus niger* Tiegh., *Cladosporium epiphyllum* Person. These endophytes have also been reported as endophytes in earlier studies.

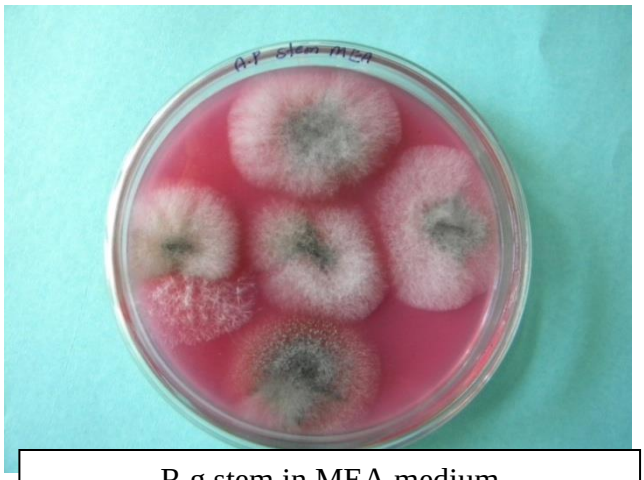
In conclusion; the understanding of the number of endophytic diversity associated with a *Acalypha indica Linn.* plant of different sites of leaves, petiole and stem parts finding is a significant variation was detected in the colonization frequency of endophytic species with 15 medicinal plants. However, high colonization frequency was in leaves. Endophytic flora in roots examined but do not get results, in some samples shows results as only one or two endophytic fungi. Similarly made an attempt to culture in different culture media results shows as endophytes grows more in MEA compare to PDA media. In Other finding have supported that the number of endophyte species present in *Acalypha indica* Linn we now has an accumulation of studies suggesting that their diversity among there fungal endophytes were immense.

PLATE I

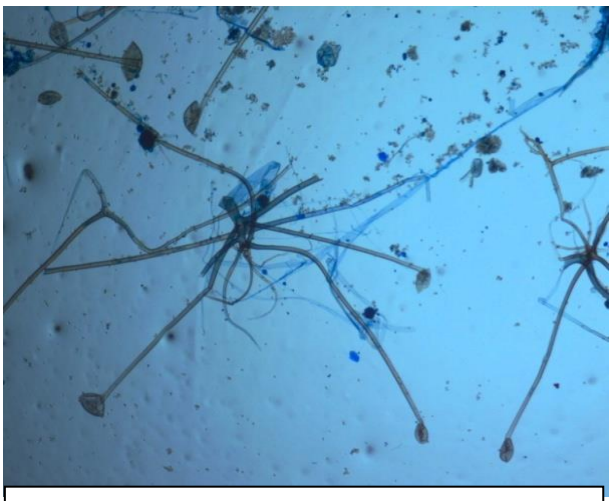
Ruta graveolens Linn

R.g leaf in MEA medium

R.g stem in PDA medium



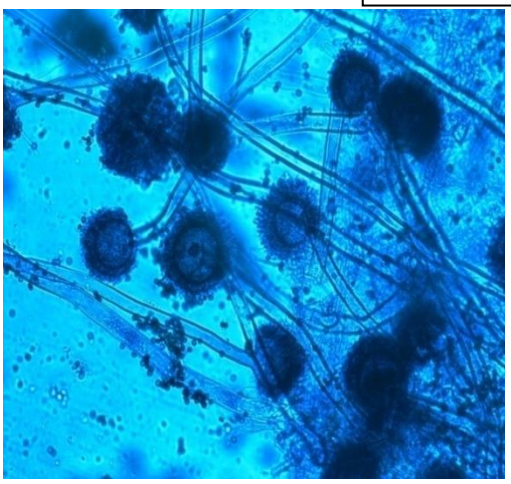
R.g stem in MEA medium



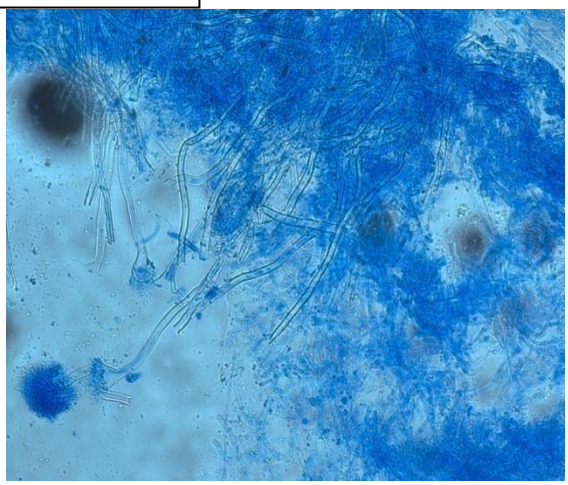
7 *Rhizopus nodusus* Namyslowski

Pure culture

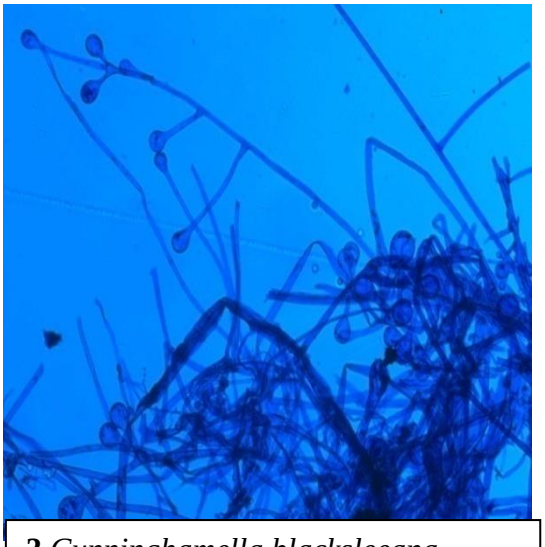
PLATE II



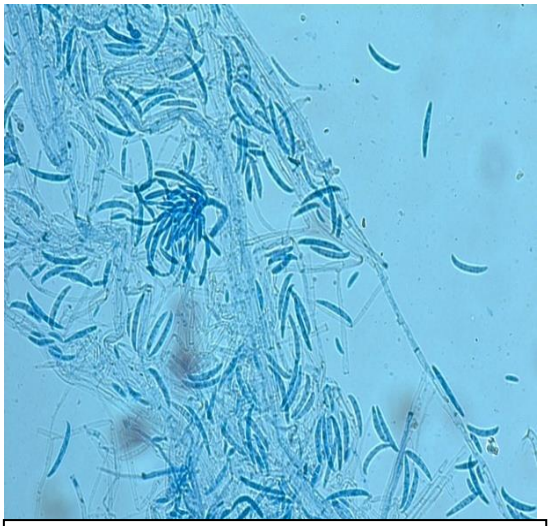
1 *Aspergillus niger* Tigeh



2 *Aspergillus flavipes* Bainer and Sartory

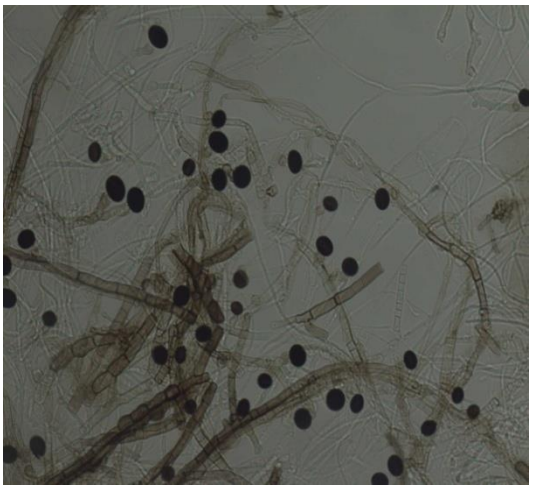


3 *Cunninghamella blacksleeana* lender



4 *Fusarium avenaceum* sacc

5 *Bipolaris nodulosa* (Bert&cart



6 *Nigrospora sphaerica* (saccurdo)

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