STUDY OF FUNGAL ENDOPHYTES FROM PLANTS WITH ANTIOXIDANT PROPERTIES USING FOLDSCOPE

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Abstract: in the present work in all five medicinal plants like*Ocimum basilicum* (L.) *Aloe vera* (L.) *Momordica charantia* (L.) *Momordica charantia* (L.) *Camellia sinensis* (L.) having antioxidant properties were selected for presence of mycoendophytes which may be having a role in production of antioxidants inside the cells of plants. It was found that total 24 endophytic fungi were inhabitansof these plants. Specifically from 5 mycoendophytes were isolated from *Ocimum basilicum* (L.), 4 from *Aloe vera* (L.), 5 is from *Momordica charantia* (L.), 6 from *Centella asiatica* (L.), and 4 from *Camellia sinensis* (L.) were found.The Frequency of mycoendophytes from root of *Ocimum basilicumos* (L) of *Alternaria alternata* was found to be more and *Penicillium notatum, Aspergillus terrus* found to be less. Similarly, Frequency from stem of *Ocimum basilicum* (L) was more of *Alternaria alternata* and less of *Aspergillus terrus* .when leaves of *Ocimum basilicumos* (L) were accessed for incidience of mycoendophytes it was found that *Penicillium notatum* was found to be more and, *Alternaria alternata, Cladosporium sp., Aspergillus fumigatus* found to be less.The Frequency from root of *Aloe vera* (L) of *Fusarium* Was found to be more and *Aspergillus terrus* found to be less. Frequency from stem of *Aloe vera* (L) of *Aspergillus fumigatus* was found to be more and *Cladosporium sp., Fusarium sp.* found to be less. Frequency from leaves of *Aloe vera* (L) of *Trichoderma viridae* was found to be more and *Aspergillus fumigatus* found to be less. Frequency from root of *Momordica charantia* (L) of *Fusarium oxysporium* was found to be more and *Phoma sp., Fusarium verticilliodes* found to be less. Frequency from stem of *Momordica charantia* (L) of *Aspergillus terrus*, chaetomium sp., *Fusarium oxysporium, Fusarium verticilliodes* was found to be equal. Frequency from leaves of *Momordica charantia*(L) of *Aspergillus terrus*, chaetomium sp., *Phoma sp.* was found to be equal. Colonization Frequency from root of *Centella asiatica* (L) of *Curvularia lunata* was found to be more and *Nigrospora sp., Penicillium sp., Cladosporium sp.* found to be less. Frequency from stem of *Centella asiatica* (L) of *Rhizoctonia* was found to be more and *Penicillium sp., Cladosporium sp.*, found to be less. % Colonization Frequency from leaves of *Centella asiatica* (L) of *Rhizoctonia sp, Nigrospora sp., Phytophthora sp.*,was found to be equal. Frequency from leaves of *Camellia sinensis* (L) of *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Cladosporium sp.*was found to be equal.

This study of incidience of mycoendophytes in antioxidants plants can be further explored for accessing and upgrading the knowledge of antioxidants production further.

Key words: Mycoendophytes, antioxidants, Foldscope.

Introduction:

" Endophytes" are most commonly defined as those organisms whose "infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonisation can be demonstrated to be internal" (Stone *et al.*, 2000).

Variety of relationships exists between fungal endophytes and their host plants, ranging from mutualistic or symbiotic to antagonistic or slightly pathogenic. Endophytes may produce overabundance of substances of potential use to agriculture, industry and modern medicine such as novel antibiotics, antimycotics, immunosuppressant and anticancer compounds. In addition, the studies of endophytic fungi and their relationships with host plants will shed light on the ecology and evolution of both the endophytes and their hosts: the evolution of endophyte plant symbioses; the ecological factors that influence the direction and strength of the endophyte host plant interaction. Since natural products are likely adapted to a specific function in nature, so search for novel secondary metabolites should concentrate on organisms that inhabit novel biotopes(Laxmipriya Padhi *et al.*,2013).

The definition of antioxidants, given in 1995 by Halliwell and Gutteridge, stated that an antioxidant is "any substance that when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate". In 2007, Halliwell gave a more specific definition, stating that an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule"(Yevgenia Shebi *et al.,* 2013).

Antioxidant is a substance that reduces damage due to oxygen, such as that caused by free radicals. Well-known antioxidants include enzymes and other substances, such as vitamin C, vitamin E, and beta carotene, which are capable of counteracting the damaging effects of oxidation. Other naturally occurring antioxidants include flavonoids,tannins, phenols and lignin. Antioxidants may possibly reduce the risks of cancer. Antioxidants clearly slow the progression of age-related maculardegeneration.Your body uses antioxidants to balance free radicals. This keeps them from causing damage to other cells. Antioxidants can protect and reverse some of the damage. They also boost your immunity.

In addition to dietary antioxidants, the body relies on several endogenous defense mechanisms to help protect against free radical-induced cell damage. The antioxidant enzymes glutathione peroxidase, catalase, and superoxide dismutase (SOD) – metabolize oxidative toxic intermediates and require micronutrient cofactors such as selenium, iron, copper, zinc, and manganese for optimum catalytic activity.Exogenous antioxidants can derive from natural sources (vitamins, flavonoids, anthocyanins, some mineral compounds), but can also be synthetic compounds, like butylhydroxyanisole, butylhydroxytoluene, gallates, etc. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs(Anuj Yada *et al*., 2016).

Free radicals (ROS/RNS) are produced by normal metabolism and are involved in various physiological and pathological conditions. When there is an imbalance between the antioxidants and oxidants, the fee radicals accumulate leading to vigorous damage to macromolecules such as nucleic acids, proteins and lipids. This leads to tissue damage in various disease conditions such as diabetes mellitus, neurodegenerative diseases, cancer, cardiovascular diseases, cataracts, rheumatoid arthritis, asthma etc. and thus severely hastening the disease progression.

Materials and Methods:

Collection of plant material

Aloe vera(L.), *Ocimum basilicum*(L.), *Momordica charantia*(L.)were collected from shirur place and *Centella asiatica*(L.) were collectedfrom Manipur region. *Camellia sinensis*(L.) product was bought from medical store. The disease free parts of the plant were collected in a sterile polythene bag.

Isolation of endophytic fungi

Plant part sample were rinsed with water and then 75% ethanol for 1 min (3 time wash), 1 min in 5% sodium hypochlorite solution (2 time wash), and 1 min in sterile distelled water for three times. The samples were then surface-dried with sterile filter paper. Plant part sample were cut into 0.5 cm x 0.5 cm pieces and placed in petri dishes with potato dextrose agar (PDA) medium and culture put in dark condition at 25 ^{o}C. for 7 days. (JF Leslie*et al*., 2006) (TJ White*et al*.,1990)

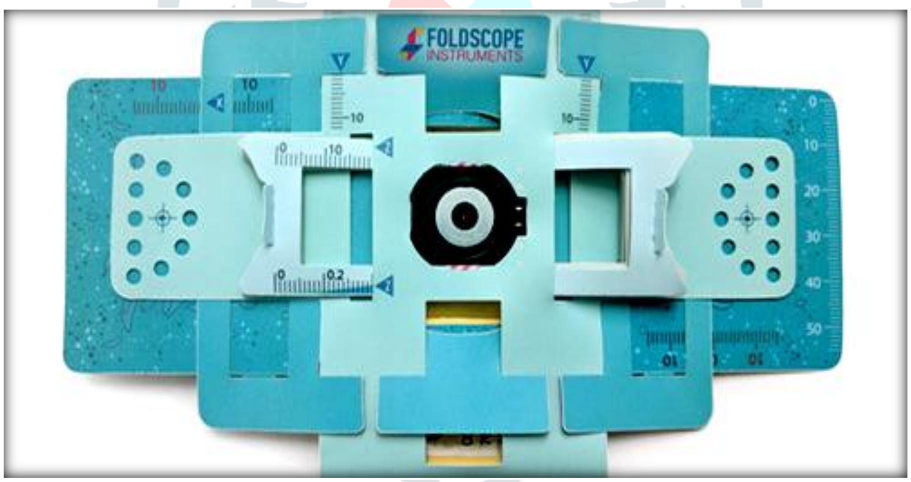
The purified endophytic fungi isolates were transferred separately to PDA slants. Finally, all the purified entophytes were maintained in refrigerator.

Identification of endophytic fungi

Identification of fungal endophytes was carried out based on the morphology of surface texture and spores at the hyphal tips with standard manual. The fungal isolates on sterile slides were stained with Lactophenol, Cotton Blue and observed in research microscope.(F Sanger*et al*.,1977)(GL Maria *et al*., 2005).

Isolation and identification of the mycoendophytes was done by using Foldscope a new invention in microscopy. The foldscope is an optical microscope with small spherical glass lens and resolution upto 140X. The foldscope microscope is invented by Manu Prakash et.al in Stanford university,USA in 2014 .

All slides were observed under foldscope instrument and microphotographs were taken using Smartphone.



FOLDSCOPE

Statistical Analysis

The percentage of colonization frequency (CF) was calculated as follows:

$$CF\ (\%) = \frac{\text{Number of species isolated}}{\text{Number of segment screened}} \times 100$$

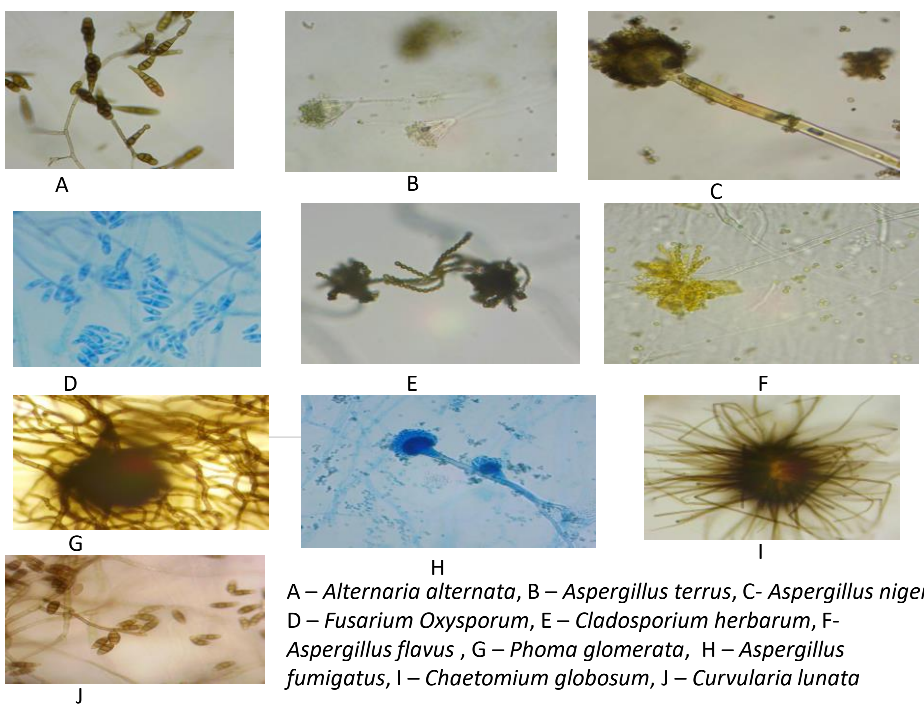
Fungal Cultivation

The endophytic fungi were cultured in 250 ml flasks, each containing 100 ml potato dextrose liquid medium (g/l; dextrose-20, potato infusion-200). Each funguswasinoculated and cultured with shaking (50 rpm) for 1 week.

After that, the cultures were filtered. The mycelia were filtered and transferred to a glass petri plate and dried overnight in a hot air oven at 40°C.

The content of the dry mycelia was powdered using sterilized mortar and pestle. 0.1 g of dry powder was extracted in 10 ml of water and methanol separately and designated as aqueous and methanolic extracts respectively. (Poorani Kandasamy *et al*., 2015)

The metablolites from the fungal cultures were extracted as per the procedure of Wicklow., (1998). The endophytic fungi were cultured in 250 ml flasks, each containing 100 ml potato dextrose liquid medium. Each fungus was inoculated and cultured with shaking (50 rpm) for 2 weeks. After attaining full growth, each fungal culture was immersed in 250 ml of ethyl acetate for 24 h. The contents were mixed thoroughly with hand blender and then filtered. The filtrate was extracted thrice with ethyl acetate and filtered. The ethyl acetate extract was dried on rotary evaporator. The dried ethyl acetate extract was further subjected to bioassays.



A – *Alternaria alternata*, B – *Aspergillus terrus*, C- *Aspergillus niger* D – *Fusarium Oxysporum*, E – *Cladosporium herbarum*, F- *Aspergillus flavus* , G – *Phoma glomerata*, H – *Aspergillus fumigatus*, I – *Chaetomium globosum*, J – *Curvularia lunata*

Table 1: Incidence of Fungal endophytes on antioxidant plants

Sr no.	Plants	f endophytes isolated
1	*Ocimum basilicum* (L.)	5
2	*Aloe vera* (L.)	4
3	*Momordica charantia* (L.)	5
4	*Centella asiatica* (L.)	6
5	*Camellia sinensis* (L.)	4

A total 24 endophytic fungi isolated from the root, stem, leaves, of *Ocimum basilicum* (L.),*Aloe vera* (L.), *Momordica charantia* (L.), *Centella asiatica* (L.), *Camellia sinensis* (L.) 5 isolated from *Ocimum basilicum* (L.), 4 from *Aloe vera* (L.), 5 from *Momordica charantia* (L.), 6 from *Centella asiatica* (L.), and 4 from *Camellia sinensis* (L.)

Table 2 : Colonization Frequency of fungal endophytes from *Ocimum basilicum* (L.) roots.

Part of plant	Sr.No.	Name of fungal endophyte	No. of segments occupied by fungus	Total no. of segments studied	% C.F
Root	1	*Alternaria alternata*	1	9	33.33
	2	*Penicillium notatum*	1	9	11.11
	3	*Aspergillus terrus*	1	9	11.11

Table 3.

Colonization Frequency of fungal endophytes from *Ocimum basilicum* (L.) stem.

Part of plant	Sr.No.	ame of fungal endophyte	No. of segments occupied by fungus	Total no. of segments studied	% C.F
Stem	1	*Alternaria alternata*	3	9	33.33
	2	*Cladosporium sp*	2	9	22.22
	3	*Aspergillus terrus*	1	9	11.11

Table 4. Colonization Frequency of fungal endophytes from *Ocimum basilicum* (L.) leaves.

Plant part	Sr.No.	Name of endophyte	No. of segments occupied by fungus	Total No. of segments studied	% C.F
Leaves	1	*Alternaria alternata*	1	9	11.11
	2	*Cladosporium herbarum*	1	9	11.11
	3	*Penicillium notatum*	2	9	22.22
	4	*Aspergillius fumigatus*	1	9	11.11

% C.F

Table 5. Colonization Frequency of fungal endophytes from *Aloe vera* (L.) roots

Part of plant	Sr.No.	Name of fungal endophyte	No. of segments occupied by fungus	Total no. of segments studied	% C.F
Root	1	*Aspergillus terrus*	1	9	11.11
	2	*Fusarium oxysporum*	3	9	33.33

Table 6. Colonization Frequency of fungal endophytes from *Aloe vera* (L.) stem.

Part of plant	Sr.No.	Name of fungal endophyte	No. of segments occupied by fungus	Total no. of segments studied	% C.F
Stem	1	*Cladosporium herbarum*	1	9	11.11
	2	*Fusarium oxysporum*	1	9	11.11
	3	*Aspergillus fumigatus*	3	9	33.33

Table 7: Colonization Frequency of fungal endophytes from *Aloe vera* (L.) leaves.

Part of plant	Sr.No.	Name of fungal endophyte	No. of segments occupied by fungus	Total no. of segments studied	% C.F
Leaves	1	*Cladosporium*	2	9	22.22
	2	*Aspergillus terrus*	2	9	22.22
	3	*Aspergillus fumigatus*	1	9	11.11
	4	*Trichoderma viridae*	3	9	33.33

Table 8. Colonization Frequency of fungal endophytes from *Momordica charantia* (L.) roots.

art of plant	Sr.No.	me of fungal endophyte	No. of segments occupied by fungus	Total no. of segments studied	% C.F
Root	1	*Phoma glomerata*	1	9	11.11
	2	*Fusarium oxysporum*	2	9	22.22
	3	*Fusarium verticilliodes*	1	9	11.11

Table 9. Colonization Frequency of fungal endophytes from *Momordica charantia* (L.) stem.

art of plant	Sr.No.	me of fungal endophyte	No. of segments occupied by fungus	otal no. of egments studied	% C.F
Stem	1	*Fusarium oxysporum*	1	9	11.11
	2	*Fusarium verticilliodes*	1	9	11.11
	3	*Chaetomium globosum*	1	9	11.11
	4	*Aspergillus terreus*	1	9	11.11

Table 10. Colonization Frequency of fungal endophytes from *Momordica charantia* (L.) leaves.

Plant part	Sr,. No.	Name of the endophyte	No. of segment occupied by fungi	Total no of segment used	% C.F.
leaves	1.	*Phoma glomerata*	1	9	11.11
	2.	*Chaetomium globosum*	2	9	22.22
	3.	*Aspergillus terrus*	3	9	33.33

Results showed that the Frequency of mycoendophytes from root of *Ocimum basilicumos* (L) of *Alternaria alternata* was found to be more and *Penicillium notatum, Aspergillus terrus* found to be less. Similarly, Frequency from stem of *Ocimum basilicum* (L) was more of *Alternaria alternata* and less of *Aspergillus terrus* .when leaves of *Ocimum basilicumos* (L) were accessed for incidience of mycoendophytes it was found that *Penicillium notatum* was found to be more and*, Alternaria alternata, Cladosporium sp., Aspergillus fumigatus* found to be less.The Frequency from root of *Aloe vera* (L) of *Fusarium* Was found to be more and *Aspergillus terrus* found to be less. Frequency from stem of *Aloe vera* (L) of *Aspergillus fumigatus* was found to be more and *Cladosporium sp., Fusarium sp.* found to be less. Frequency from leaves of *Aloe vera* (L) of *Trichoderma viridae* was found to be more and *Aspergillus fumigatus* found to be less. Frequency from root of *Momordica charantia* (L) of *Fusarium oxysporium* was found to be more and *Phoma sp., Fusarium verticilliodes* found to be less. Frequency from stem of *Momordica charantia* (L) of *Aspergillus terrus,* chaetomium sp., *Fusarium oxysporium, Fusarium verticilliodes* was found to be equal. Frequency from leaves of *Momordica charantia*(L) of *Aspergillus terrus,* chaetomium sp., *Phoma sp.* was found to be equal. Colonization Frequency from root of *Centella asiatica* (L) of *Curvularia lunata* was found to be more and *Nigrospora sp., Penicillium sp., Cladosporium sp.* found to be less. Frequency from stem of *Centella asiatica* (L) of *Rhizoctonia* was found to be more and *Penicillium sp*., *Cladosporium sp.,* found to be less. % Colonization Frequency from leaves of *Centella asiatica* (L) of *Rhizoctonia sp, Nigrospora sp., Phytophthora sp.,*was found to be equal. Frequency from leaves of *Camellia sinensis* (L) of *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Cladosporium sp.*was found to be equal.

This study of incidience of mycoendophytes in antioxidants plants can be further explored for accessing and upgrading the knowledge of antioxidants production further.

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