ORIGINAL RESEARCH PAPER

Botany

KEY WORDS:
Mycoendophytes, *Catheranthus Roseus*, Foldscope, Anticancer Plant.

INCIDENCE OF THE MYCOENDOPHYTES FROM *CATHERANTHUS ROSEUS* – AN ANTICANCER PLANT FROM MAHARASHTRA.

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ABSTRACT

The endophytic fungi are one of the most unexplored and diverse group of symbiotic organisms shows great association with higher plants. The production of some essential bioactive compounds from these mycoendophytes plays very important role in development of drugs and results in cure of some types of cancers in human beings as well as in animals. In present investigation *Catheranthus roseus* has been studied for the occurance of mycoendophytes from Maharashtra and it was found that the plant harbors seven endophytic fungi. 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 . It is clear from the study that all the parts of the *Catheranthus roseus* show the presence seven mycoendophytes in more or less frequency. In particular mycoendophytes like *Aspergillus flavus* showed the maximum colonization where as *Penicillium notatum* and *Aspergillus niger* was found less with moderate frequency of *Penicillium chrysoginum*, *Cladosporium cladosporioidis* and *Alternaria alternata* in the roots of *Catheranthus roseus.* Similaraly, in leaves and stem the colonization of Penicillium notatum was found to be maximum with very less colonization of *Cladosporium cladosporioidis, Penicillium chrysoginum* and *Aspergillus flavus*. These organs showed the moderate growth of *Alternaria alternate.*

INTRODUCTION:

Fungal endophytes are the microorganisms that reside inside the plant tissue that can be root, leaves, or stem. They are basically belongs to ascomycota and basidiomycota group.(Arnold and Lutzoni 2007; Selosse et al .2009).

There are one million species of endophytic fungi associated with plants worldwide that can provide a variety of secondary bioactive compounds such as alkaloids, benzopyrenones, flavanoides, phenols, phytochemicals and anticancer agents. (Aly et al. 2010).

Basically these endophytic fungi have been recognized as a repository of novel compounds of immense value in agriculture industry and medicine. The nature of relationship established by the endophytes with the host plant however, varies from symbiotic to pathogenic. They may be transmitted either vertically (offspring) or horizontally. Vertically transmitted fungal endophytes are normally considered as sterile and transmit via fungal hyphae penetrating the host's seed. (Tan and Zou 2001; Strobel and Daisy 2003).

Cancer is a group of disease characterized by unregulated growth and spread of abnormal cell, which can result in death if not controlled (Pimentel et al. 2010).

It has been considered as one of the major causes of death worldwide (about 13% of all deaths). Evidences are present about bioactive compounds produced by endophytes and could be an alternative approach for discovery of novel drugs, since many natural products from plants, microor ganisms, and marine sources were identified as anticancer agents (Firakova et al. 2007). The anticancer properties of several secondary metabolites from endophytes and could be an alternative approach for discovery of novel drug, since many natural products from plants, microorganisms and marine sources were identified as anticancer agents (Firakova et al. 2007).

The anticancer properties of several secondary metabolites from endophytes have been investigated recently. The first anticancer agent produced by endophytes was Taxol and its derivatives. Taxol is highly functionalized diterpenoid, isolated from yew (Taxus) species (Bacon and White 1994).

Camptothecin another potent antineoplastic agent was firstly isolated from the wood of *Camptotheca acuminata* decasine (nyssaceae) in china (Wall et al. 1996). Camptothecin and 10-hydroxycamptothecin are two important precursors for the synthesis for clinically useful anticancer drugs, topothecan , and irinotecan (Uma et al. 2008). The products were obtained from the endophytic fungi *Fusarium solani* isolated from *Camptotheca acuminate* (Kusari et al. 2009).

It is obvious that mycoendophytes serve as a source of poten tially useful medicinal compounds. For example, 3-Nitropropionic acid was isolated from *Phomopsis* species which inhibited *Mycobacterium tuberculosis* and harbors anti-tuberculosis activity (Copp and Pearce 2007).

The present study was conducted to determine the biodiversity of fungal endophytes from anticancer plants of various places of Maharashtra.

The method used for isolation of endophytic fungi which is described by Petrini O. in 1986.

Identification of isolated endophytic fungi by their microphotographs was done by using the novel microscope known as foldscope. The invention was carried out in 2014 at Stanford university in California, USA by Manu Prakash et.al .The foldscope is an optical microscope that can be assembled from punched sheet of cardsheets , spherical glass lense and light emitting diode and diffuser panel along with a watch battery that powers the LED. The foldscope weighs 8 grams and comes in multiple lenses that provide magnification of 140X the kit also includes magnets that can be stuck on the foldscope to attach it to smartphone for microphotography.

In nature a foldable microscope- foldscope is highly durable because is made up of paper and is origami based with a cost of goods less than one U.S. dollar.

MATERIAL AND METHODS

SAMPLE COLLECTION

Healthy leaves Stem and roots of anticancer plants were collected from respective selected fields in pre-sterilized zip lock bags. All samples were carried to the laboratory for further processing. (Bhattacharyya et al. 2016)

STERILIZATION OF PLANT MATERIAL

Asymptomatic healthy plant materials were thoroughly

washed in tap water to remove debris as well as soil particles, and surface sterilized.(Petrini O.1986) Selected leaf, stem and root segments were immersed in 95% ethanol for 30 sec, 7 % sodium hypochlorite solution for 15 sec. and again in 95% ethanol for 30 sec (as per the plant material) after above treatment segments were rinsed by sterile distilled water three times for 10 sec. and allowed to dry on sterile blotting paper under sterile conditions. After drying each segment was cut into approximately 0.5 cm in diameter and up to 1 cm in length with the help sterilized knife.
(Petrini O.1986)

INOCULATION

Surface sterilized segments were placed on petriplate containing potato dextrose agar medium supplemented with streptomycin (100mg/L) to suppress the bacterial growth. (Petrini O.1986)

Petri plates were properly sealed and incubated at 27°C in dark conditions for 15 days .they were monitored every day for growth of Endophytic fungal colonies .

The selected isolates were further grown on potato dextrose agar plates in order to obtain pure cultures of Endophytic fungi. (Chow and Ting, 2015).

Colonization frequency was calculated as described by Sury anarayanan et al., (2003).briefly, proper time of incubation was given for CF counting.

$$Colonization\ frequency\ (\%) = \frac{Number\ of\ segments\ colonized\ by\ fungi}{Total\ number\ of\ segments\ observed} \times 100$$

FUNGUS IDENTIFICATION

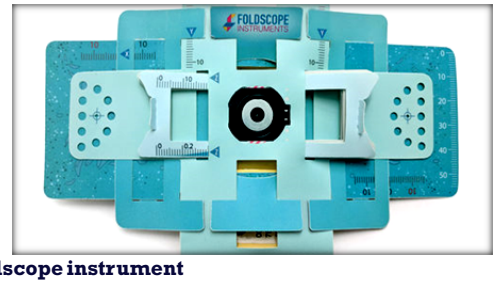
The morphological characteristics of fungal isolates were examined.Taxonomic identification keys such as colony color and morphology of hyphae and conidia were recorded. (Photita W et al. 2005 and Ainsworth GC et al. 1973).

For Morphological identification of pure fungal colonies the services of identification were hired from NFCCI – Agharkar Research Institute, Pune.

MORPHOLOGICAL CHARACTERIZATION OF ENDO PHYTIC FUNGI USING SLIDE CULTURE TECHNIQUE-

The slide culture technique is used to observe morphological characteristics of fungi without disturbing the arrangement of spore's conidiogenous cells.The fungal strains were grown on PDA media above a sterile glass slide and stained using lacto phenol cotton blue and then observed (Aneja, 1996)

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



Foldscope instrument

RESULTS:

Table No.1: Incidence of mycoendophytes in *Catheran thus roseus* roots

Sr.No.	Name of the place	mycoendophytes	No. of root segments occupied by fungus	Total no. of root segments studied	% C.F.
1.	Mahabaleshwar	*A.flavus*	4	24	16.66
2.	Nashik	*Penicillium notatum*	0	16	00.00
3.	Shirur	*Penicillium chrysogenum*	2	16	12.05
		A.niger	0	16	00.00
		A.flavus	1	16	6.25
4.	Bhimashankar	*Cladosporium cladospriodes*	2	17	11.76
		Alternaria alternata	2	18	11.11

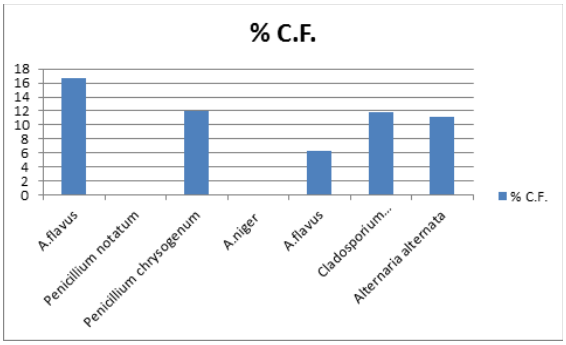


Table 2 :Incidence of mycoendophytes in *Catheranthus roseus* Stem

Sr.No.	Name of the place	mycoendophytes	No. of stem segments occupied by fungus	Total no. of stem segments studied	% C.F.
1.	Mahabaleshwar	*A.flavus*	2	24	8.33
2.	Nashik	*Penicillium notatum*	2	16	12.05
3.	Shirur	*Penicillium chrysogenum*	0	16	00.00
		A.niger	0	16	00.00
		A.flavus	0	16	00.00
4.	Bhimashankar	*Cladosporium cladospriodes*	1	17	5.88
		Alternaria alternata	0	18	00.00

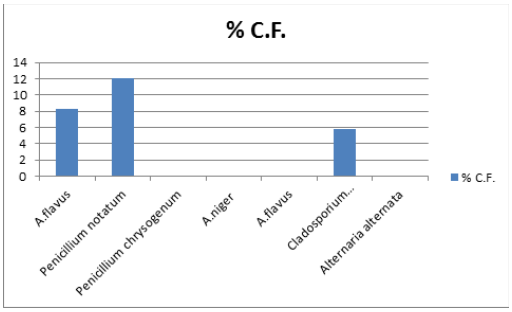
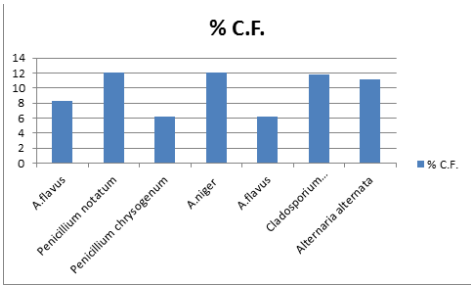


Table 3: Incidence of mycoendophytes in *Catheranthus roseus* leaves

Sr.No.	Name of the place	mycoendophytes	No. of leaf segments occupied by fungus	Total no. of leaf segments studied	% C.F.
1.	Mahabaleshwar	*A.flavus*	2	24	8.33
2.	Nashik	*Penicillium notatum*	2	16	12.05
3.	Shirur	*Penicillium chrysogenum*	1	16	6.25
		A.niger	2	16	12,05
		A.flavus	1	16	6.25
4.	Bhimashankar	*Cladosporium cladospriodes*	2	17	11.76
		Alternaria alternata	2	18	11.11



From the observations it is clear that all the parts of the *Catheranthus roseus* show the presence of almost all seven mycoendophytes in more or less frequency. In particular mycoendophytes like *Aspergillus flavus* showed the maximum colonization where as *Penicillium notatum* and *Aspergillus niger* was found less with moderate frequency of *Penicillium chrysoginum*,*Cladosporium cladosporioidis* and *Alternaria alternata* in the roots of *Catheranthus roseus.* Similaraly, in leaves and stem the colonization of Penicillium notatum was found to be maximum with very less colonization of *Cladosporium cladosporioidis, Penicillium chrysoginum* and *Aspergillus flavus*. These organs showed the moderate growth of *Alternaria alternate.*

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