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Isolation of Root Surface (Rhizoplane)Mycoflora From Medicinal Plants of Akola

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ABSTRACT:

Medicinal plants from a numerically large group are economically important plants which provide basic raw materials for medicines, perfumes, flavors and cosmetics. There plants and their products not only serve as valuable source of income for small holders and entrepreneurs but also help the country to earn valuable foreign exchange by way of export. These plants are those plants which are rich in secondary metabolites and are potential source of drugs. India is one of the few countries where almost all the known medicinal plants can be cultivated in some part of the country or the other. A plant species usually has its specific flora around its roots and on the root surface. Micro-organisms when they growing proximity exert various kinds of influences on one another, some of which are favorable or associative others are antagonistic. Some root diseases cause considerable economic losses of plants. Rood diseases are in several ways different from the diseases of the aerial parts. Acknowledge of the complex microbial interactions occurring in the soil and of the ecology of soil microorganisms, saprophytic and parasitic are essential for a proper understanding of root diseases and for a rational approach to their control.

KEYWORDS: Medicinal plants, Root mycoflora, Rhizoplane.

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INTRODUCTION:

Roots are an important part of the plant which absorbs the water and inorganic nutrients. These roots may decline and die from a variety of causes. Unfavorable environments that result in either waterlogged or drought conditions are common causes of poor health of plants. There are also fungi in the soil that have the ability to infect roots and cause root rot.[1,2]If major roots or the crown are affected by root rots or other problems the entire plant can wilt and die rapidly. If only the small feeder rootlets are affected, the plant may decline slowly and appear generally sickly and unproductive. Sick or damaged roots may be present only on part of plant's root system, resulting in a one-sided appearance of symptoms on leaves and stems. Infection may first appear as brown streak in the wood under the bark. Later, cankers may form and become well defined.[3] Infected wood is often brick red or brown and there is a sharp line of demarcation between healthy and infected tissue.[4]

Medicinal plants have the ability to synthesize a wide variety of chemical compounds that are used to perform important biological functions. Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs.[5,6]

Akola is a city in Vidarbha region in the state of Maharashtra in Central India. It is located at latitude 20.7° North and longitude 77.07° East and has a tropical savanna climate. Annual temperatures range from a high of 48°C to a low of 10°C. The annual rainfall averages goes upto 800 mm. In this region the increasing demand of medicinal plants has resulted in the rapid dwindling of these natural resources. There is urgent need of systematic approach for conservation and sustainable production of medicinal plants.Present investigation focus on some fungi present on root surface from medicinal plants of Akola.

MATERIALS AND METHODS

1) Collection of Materials & isolation of fungi:

A regular survey of infected roots of medicinal plants was made from gardens, fields and Panjabrao Krishi Vidyalpeeth, Akola from Nov. 2014 to April, 2015.More than 25 different hosts were collected in different polythene bags and carried out in laboratory.Symptoms of collected diseased plant roots were recorded in different seasons on same host to know the stages of disease development. The serial washing technique of Harley and Waid was employed to isolate fungi from the root surface[7]. After the final wash, the roots cut into lengths of 0.5 cm each, were placed on Asthana and Hawker's medium[7] 'A' (5.0 gm glucose, 3.5 gm KNO_3, 1.75 gm KH_2PO_4, 0.75 gm $MgSO_4$, $7H_2O$ and 15 gm agar agar), four rootlengths placed at equal distances apart from one another in each petri dish. The dishes were incubated at the temperature 27°C ($\pm$ 2°C).

All isolations of fungi from root samples were carried out immediately on reaching the laboratory. After 2 or 3 days of inoculation, the mycelium coming out from the diseased tissue was picked up and reinoculated to another fresh slant.

Identification of Isolates:

All the fungal isolates were morphologically identified according to colony colour and texture pigmentation Microscopic observation of spores and spore bearing structures were performed by the use of lacto-phenol cotton blue mounts.

Identification was made with the help of H.L. Barnett and Barry B. Hunters[8] "Illustrated Genera of Imperfect Fungi" and some already identified cultures which were identified cultures which were identified from PDKV, Akola.

Culture and taxonomical studies of different genera were carried out.

RESULTS AND DISCUSSION

Taxonomical Study of the Isolates :

1)*Mucor sp.* Fresen (Mucoraceae) :

Collected on *Ficus religiosa* L. (Moraceae) from Chawre plot, Akola 24 December, 2014; *Azadirachta indica*. A. Juss (Meliaceae) from Ramdaspeth, Akola, 24 January, 2015, *Ocimum basilicum* L. (Lamiaceae) from Chaure Plot, Akola on 24 March 2015, *Asparagus racemosus* Willd (Asparagaceae) from Shivaji College, Akola. 24 February 2015, *Datura metel* L. (Solanaceae) from Chaure Plot, Akola 24 March, 2015, *Calotropis procera* (Aiton) W.T. Aiton (Asclepiadaceae) 30 March, 2015, *Ricinus communis* L. (Euphorbiaceae) from Durga Chauk, Akola, 30 March, 2015. *Hibiscus rosa-sinensis* L. (Malvaceae) from Mata Nagar, Akola, 7 April, 2015, *Aloe barbadensis* Mill (Liliaceae) from Mata Nagar, Akola, 7 April, 2015; *Polyalthia longifolia* Sonn. (Annonaceae) from Shivaji College, Akola, 7 April, 2015; *Tamarindus indica* L. (Fabaceae) from Dr. P.D.K.V., Akola 8 November, 2014, *Vitex negundo* L. (Lamiaceae) from Dr.P.D.K.V., Akola 18 April, 2015. *Bryophyllum pinnatium* (Lam.) Oen Crassulaceae from Dr.P.D.K.V., Akola 18 April, 2015; *Ocimum tenuiflorum* L. (Lamiaceae) from Shivaji College, Akola 18 April, 2015.

Morphology of Isolate : Colonies cottony to fluffy, white to yellow, becoming dark grey with the development of sporangia; Sporangiophores erect, simple or branched, terminal, globose to spherical, multi spored sporangia, without apophyses and with well-developed subtending columelae, Collarette present at the base of the columella after sporangiospore dispersed, sporangiospores hyaline, grey or brownish, globose to ellipsoidal, smooth or finely ornamented.

2) *Rhizopus sp.* Ehrenb (Mucoraceae) :

Collected on *Azardirachta indica* A. Juss (Meliaceae) from Ramdaspeth, Akola 24 January 2015; *Ocimum basilicum* L. (Lamiaceae) from Chaure Plot, 24 March, 2015; *Asparagus racemosus* Willd (Asparagaceae) from Shivaji College, Akola, 24 February, 2015; *Datura metel* L. (Solanaceae) from Chaure Plot, Akola, 24 March 2015; *Polyalthia longifolia* Sonn. (Annonerceae) from Shivaji College, Akola 24 April, 2015; *Catharanthus roseus* (L) G. Don (Apocynaceae) from Shivaji College, Akola 18 April, 2015; *Ficus racemosa* L. (Moraceae) from Durga Chauk, Akola 18 April, 2015.

Morphology of Isolate :

Colonies cottony, white become grey or yellowish brown, Sporangiophores singly or in groups from nodes directly above the rhizoids and apophyses columellate, multispore, sporangia globose apophyses and columella form an umbrella like structure. Sporangiaspores globose to avoid one celled hyaline to brown and striate.

3) *Bipolaris sp.*Shoemaker:

Collected on root surface of *Aloe barbadensis* Mill (Xanthorrhoeaceae) from Mata Nagar, Akola, 7 April, 2015.

Morphology of Isolate:

Colonies effuse, grayish to olivaceous black, mycelium well branched, septate, brown, conidiophores flexous, densely geniculate. Conidia oblong, ellipsoidal to cylindrical, 3 to 5 celled (mostly 3), central 1 to 3 cells coloured and peripheral cell hyaline, central cell large, darker, globular.

4) *Alterneria sp.* Nees ex Fries:

Collected on root surface of *Azadirachta indica* A. Juss (Meliaceae) from Ramdaspeth, Akola on March2 015; *Asparagus racemosus* Willd (Liliaceae) collected from Shivaji College, Akola on February, 2015; *Ricinus communis* (Euphorbaceae) collected from Durga Chowk, Akola on 3 March, 2015.

Morphology of Isolate:

Colonies usually black, Hyphae dark brown, branched; conidiophores arising singly or in small groups, branched, flexous, geniculate, pale to olivaceous or golden brown, smooth, thick with one or several conidial scare; Conidia formed in branched chain obclavate, obpyri form, ovoid or ellipsoidal often with a short conical or cylindrical beak, pale to mid golden brown, smooth or verruculose with upto 8 transverse and usually several longitudinal or oblique septa.

5) *Fusarium sp.* Link ex Fries:

Collected on the root surface of *Asparagus racemosus* Willd (Asparagaceae) from Shivaji College, Akola, 24 February, 2015.

Morphology of Isolate:

Mycelium pale to dark brown, branched septate; conidiophores simple, short, branched, bearing a whorl phialides; conidig hyaline, vociable and of two kinds in moist heads; macro conidia 5 to 6 celled, micro conidia one celled, ovoid or oblong borne, singly on conidiophores, intermediate conidia 2 to 3 celled oblong or slightly curved.

6) *Phoma sp.* Sacc. :

Collected on *Ficus religiosa* L. (Moraceae) from Chaure Plot, Akola, 24 December, 2014. *Tamarindus indica* L. (Fabaceae) from Dr. P.D.K.V., Akola, 18 November, 2014.

Morphology of Isolate:

Colonies black, spreading, hyphae hyaline, branched, septate, pycnidium, globose to sub-globose, glabrous, globose pycnidium, conidia 1 celled, hyaline globose.

7) *Aspergillus sp.* Micheli ex Link :

Recovered from *Azadirachta indica* A. Juss. (Meliaceae) from Ramdaspeth, Akola, 24 January 2015; *Ocimum basillium* L. (Lamiaceae) 24 March, 2015, *Datura metal* L. (Solanaceae) 24 March, 2015; *Hibiscus rosa-sinensis* L. (Malvaceae) Mata Nagar, Akola 7 April, 2015; *Aloe barbadinesis* Mill (Liliaceae) Mata Nagar, Akola, 7 April, 2015; *Tamarindus indica* L. (Fabaceae), 18 November, 2014 from Dr. P.D.K.V., Akola; *Vitex negundo* L. (Verbenaceae) 18 April, 2015 from Dr. P.D.K.V., Akola, *Catharanthus roseus* (L.) G. Don (Apocynaceae) 18 April, 2015 from Shivaji College, Akola; *Bryophyllum pinnatum* (Lam.) open (Crassulaceae) 18 April, 2015 from Dr. P.D..V., Akola; *Ocimum tenuiflorum* L. (Lamiaceae) 18 April, 2015; from Shivaji College, Akola.

Morphology of Isolates:

Colonies white initially but soon turning black due to production of conidia. Hyphae hyaline, branched septate. Conidiophores unbranched, septate hyaline, terminating in globose vesicles; sterigmata flask shaped, producing conidia in acropetal succession in chains, conidia globose, one celled, verrucose, dark brown to grayish black.

8)*Penicillium sp.* Link :

Collected on *Ocimum basilium* L. (Lamiaceae) 24 March, 2015 from chaure plot, Akola, *Asparagus racemosus* willd (Asparagaceae); *Tamarindus indica* L. (Fabaceae) 18 November, 2014 from Dr. P.D.K.V., Akola.

Morphology of Isolate :

Colonies broadly spreading, velvety, heavily sporulating forming conidia in chains. Mycelium submerged, septate, penicillia biverticillate and asymmetrical borne on smooth walled Metulae forming chains phialides borne in terminal clusters, conidia globular, elliptical smooth.

9)*Trichoderma sp*. Pers :

Collected on *Ficus racemosa* L. (Moraceae) from Durga Chowk, Akola, 18 April, 2015; *Ricinus communis* L. (Euphorbaceae) from Durga Chowk, Akola, 30 March, 2015.

Morphology of Isolate :

Conidiaphores hyaline, much branched not verticulate phialides single or in groups. Conidia (phialospores) hyaline, 1 celled, ovoid, borne in small terminal clusters, usually easily recognized by its rapid growth and green patches or cushions of conidia.

DISCUSSION:

Regular surveys were undertaken for study of the medicinal plants root surface mycoflora in Akola from November, 2014 to April, 2015. During the survey medicinal plants grown in Akola were concentrated and it was observed the most of the fungi associated with root surface belonging to class Deuteromycetes.During these investigations 16 medicinal plants root specimens were collected, out of which 8 fungal species belonging to genera Deuteromycetes and 2 from Zygomycetes on different hosts.On the members of Lamiaceae like *Ocimum basilicum*, *Vitex negundo, Ocimum tenuiflorum* species of *Rhizopus, Mucor, Penicillium, Aspergillus* were found.In members of Moraceae like *Ficus religiosa* and *Ficus racemosa* species of *Mucor, Phoma, Trichoderma, Rhizopus* were found.On member of Meliaceae like *Azadirachta indica* species of *Rhizopus, Mucor, Aspergillus, Alternaria* were found.On Member of Liliaceae like *Asparagus racemosus* species of *Rhizopus, Alterneria, Penicillium* were found. On member of Solanaceae like *Datura metel* species of *Mucor, Rhizopus, Aspergillus* were found and on member of Asclepediaceae like *Calatropis procera* species of *Mucor* were found. On member of Euphorbiaceae like *Ricinus communis* species of *Mucor, Alterneria, Trichoderma* were found and member of Malvaceae like *Hibiscus rosa-sinensis* species of *Mucor* and *Aspergillus* were found.On member of Liliaceae like *Aloe barbaidensis* species like *Bipolaris, Mucor, Aspergillus* were found while on members of Annonaceae like *Polyalthia longifolia* species like *Mucor* and *Rhizopus* were found. On member of Fabaciae like *Tamarindus indica* species of *Mucor, Aspergillus, Penicillium, Phoma* were found while on member of Apocyenaceae like *Catharanthus roseus* species of *Rhizopus* and *Aspergillus* were found. On member of Crassulaceae like *Bryophyllum pinnatum* species like *Aspergillus* and *Mucor* were found.

There are obligate saprophytes which include many species of *Mucorales, Aspergillus, Penicillium* and other fungi such as *Fusarium* which infect roots under favorable condition. These are mainly soil-inhabiting fungi which have the capacity to parasitize.[9] Root infecting fungi also include more highly specialized parasites with little or no ability to survive as free living saprophytes in soil, although they often survive in the form of resistant propagules of various roots. [10,11,12]Root diseases cause considerable economic losses to the medicinal plants. Dissemination of soil borne pathogens can take place by wind, water, living vectors and man.[13]

wavelength was selected for estimation of Ketoprofen and Thiocolchicoside as shown in fig.3

CONCLUSION:

Medicinal plants contain rich resources of ingredients which can be used in drug development and synthesis.[14] Besides that these plants play a critical role in the development of human cultures around the whole world.[15,16] Some plants consider as important source of

nutrition and as a result of that these plants recommended for their therapeutic values.[17] Therefore it is very important point to encourage researchers and clinicians to clarify their role in the treatment of present diseases and how they can be used to produce or synthesis more effective control measure to cure fungal diseases.[18]

Control of root diseases is closely bond with agricultural practices which keep the soil in good condition and thus encourage vigorous growth of the medicinal plant.[19,20] Root diseases can be controlled by sanitation, palliative measures to hinder the spread of infection, soil treatment, biological control and control by breeding disease resistant varieties.[21]The most important is the biological control method in which the disease can be control through some biological agency such as by green manuring, by fumigating the soil with carbon disulphide, organic amendments and also by using some micro-organisms or microbial products, which can reduced the environmental pollution.[22]

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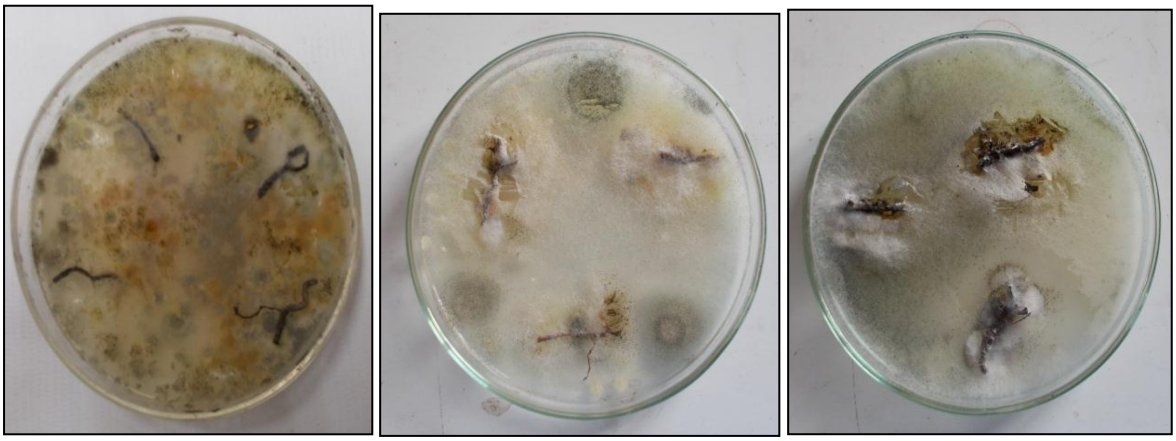
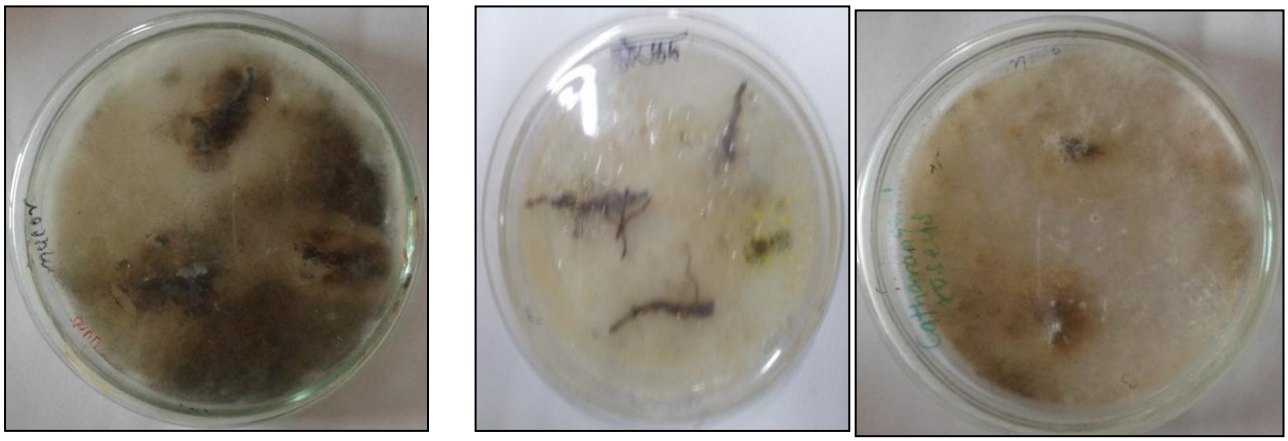
Photo Plate 1



Petriplate culture showing fungal colonies caused by infected root of *Tamarind indica* L.

Petriplate culture showing fungal colonies caused by infected root of *Ficus reliogiosa* L

Petriplate culture showing fungal colonies caused by infected root of *Azardirachta indica* A.Juss



Aloe barbadensis Mill. rootshowing fungal infection

Petriplate culture showing fungal colonies caused byinfected root of *Aloe barbadensis* Mill.

Petriplate culture showing fungal colonies caused by infected root of*Catharanthus roseus* L.

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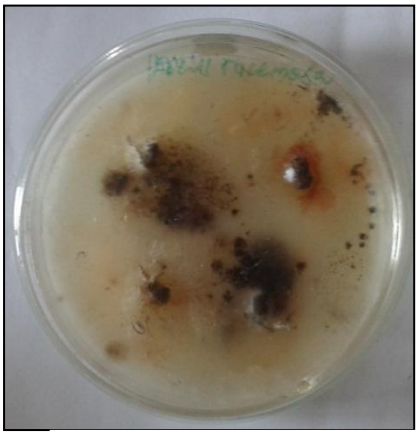
Petriplate culture showing fungal colonies caused by infected root of *Ficus racemosa* L.



Petriplate culture showing fungal colonies caused by infected root of *Bryophyllum pinnatum* Lamk.

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