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Isolation of mycoflora and Antibacterial Activity isolated fungi from *Madhuca indica*

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Abstract
The total 18 fungal *sp*. were isolated from rhizoplane and rhizosphere soil of *Madhuca indica*. Fungi present in the rhizoplane and rhizosphere soil of *Madhuca indica* were isolated by serial dilution method and identified on the basis of morphological characters. The fungi were *Aspergillus niger, A. fumigatus, A. clavatus, Mucor hiemalis, Alternaria triticina, rstolonifer* and *Mucor racemosus* were present in both the rhizoplane and rhizosphere soil where *P. oxalicum, A. herbarum* and *Penicillium frequentans A. clavatus, Penicillium chrysogenum* species only present in rhizosphere soil but they were absent in rhizoplane soil. The rhizosphere soil contains more fungal species than the rhizoplane. The dominent fungal species were used for the antibacterial activity. *Aspergillus niger* and *Mucor hiemalis* fungal culturature were extracts and used for the antibacterial activity against the *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli, Xanthomonas citri, Ralstonia solanacearum.* The maximum zone of inhibition *Mucor hiemalis* shows 21 mm against *Ralastonia solancearum* and the *Mucor hiemalis* minimum zone of inhibition against *Bacillus subtilis* as compare *Mucor hiemalis.*

Keywords: *Madhuca indica,* Rhizoplane, Rhizosphere, antibacterial activity.

1. Introduction
Rhizosphere is the narrow region of soil adjacent the root where microorganism populations are stimulated by root activities. Rhizosphere is unique biological niche with a diverse micro flora comprising bacteria, fungi, protozoa and algae. Fungi are standard as secondary metabolite producers. Microorganism growing on the plant roots can influence plant growth + vely or – ve ly. Microorganisms are helpful in growing the soil fertility and plant growth as they are involved in several biochemical change and mineralization activity in soil. There are plenty reports on rhizosphere fungal diversity like *Aesculus indica* (Sagar A. and Kaur R. 2010). Antagonistic activities of several microbial populations in the rhizosphere influences plant development and health. (Weller, *et al*., 2002) [12]. Many fungi create bioactive compound and their secondary metabolites having pharmaceutical significance. The rhizosphere soil is said to have a lower water potential, lower oxygen pressure and highs of carbon dioxide than the bulk soil (Suresh and Bagyaray 2002) [8] soil fungi play an main role nutrient cycling, plant health and development (Thorn 1997) [9].
These secondary metabolites of *Aspergillus flavus* and *Mucor hiemalis* species have been identified as well as proved their biological activities. The purpose of this study was to invistagate the identification and anti-bacterial activity of *Aspergillus flavus* and *Mucor hiemalis.*

Material and Method
Collection of soil sample: The soil sample was collected at botanical garden of Yeshwant Mahavidalata Nanded during the month of February 2017. The soil sample was collected in sterile plastic bag on root surface (Rhizoplane) and a depth of 10-15 cm (Rhizosphere region), immediately brought to the laboratory and for further experiment.

Isolation and Identification of fungal species: The soil sample was subjected to serial dilution method describe by (Aneja, 2003) [1] 10 gm of soil sample was suspended in 100 ml sterilized distilled water and mixed well and suitable in serial dilutions 0.100 ul aliquots was poured in sterilized PDA poured in Petri dishes containing the streptomycin (0.2 g^{-1}) Each dilution was incubated at 30 ^{0}C. After

6-7 days incubations the total fungal colonies forming unit (CFU/g) soil were recorded.

Staining technique for fungi: A small portion of the growth on the culture plate was transferred into the drop of lacto phenol in cotton blue on the side. The specimen is observed under the microscope for microscopic identification.

Identification of fungi: Identification of the fungal species is based on morphological characteristic of colony and microscopic examination. The fungal species were identified on the basis of cultural characteristics and morphology of fruiting bodies and spores by using standard texts and keys. The following morphological character was evaluated: colony growth, colony color, presence or absence of mycelium, pigment production, presence of wrinkles and furrows' he fungi were observed under the microscope and placed in appropriate genera and species of fungi using standard taxonomic keys. (Ellis 1971, Domsch *et al.* 1980) [3]

Test of bacterial organisms: A entire five bacterial strains *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Xanthomonas citri, Ralstonia solanacearum* used for the study were obtained from School of life Sciences S. R. T. M. University, Nanded. The bacterial strain subculture on nutrient agar medium at 37 ^{0} C.

Preparation of fungal cultrate filtrates extracts: Mycelia discs (8 mm) from 7 days old cultures of *Aspergillus niger* and *Mucor hiemalis* spp. were inoculated in 100 mL of 2% Nutrient Agar (NA) broth. The fungal cultures were incubated without agitation for 15 days at room temparature ºC. At the end of incubation the cultures mycelia was filtered through Whatman paper No: 1 to remove the mycelium. Culture extracts of each *Aspergillus niger* and *Mucor hiemalis* sp. were tested against bacterial strains for antimicrobial activity using the Agar well diffusion assay.

Culture medium and inoculums

Antibacterial activity of crude extract of the rhizospheric fungi by Agar well diffusion method: The extracted secondary metabolite was dissolved in ethyl acetate of concentrations of 50µg/ml poured into the 5mm diameter well made in Petri dishes containing NA for bacteria, inoculated with a fixed amount of test-microorganisms (10 ^{6} cells /ml). The cultures were kept for 12 hours at 2- 8 °C for the antimicrobial metabolite diffusion and there after they were incubated at an appropriate temperature for the growth of test-microorganisms. The zone of inhibition was measured in mm.

Result and discussion: The total 18 fungal sp. were isolated from rhizoplane and rhizosphere soil of *Madhuca indica*. The rhizoplane and rhizosphere soil sample was serially diluted and colonies were observed on potato dextrose agar plate after 6-7 days. Uninoculated plate with medium was maintained as control**.** The isolated fungi were identified on the basis of morphological characters. (Table1)

Table 1: Rhizosphere and rhizoplane mycoflora isolated from of *Madhuca indica.*

Sr. no	Name of fungi	Rhizosplane	Rhizosphere
1	*Aspergillus niger*	++	+++
2	*A. fumigatus*	+	++
3	*A. claratus*	++	+
4	*A. herbarum*	-	+
5	*Penicillium frequentans*	-	+
6	*Mucor racemosus*	++	++
7	*Penicillium oxalicum*	-	+
8	*Rhizopus stonifer*	+	+
9	*Mucor hiemalis*	++	+++
10	*Alternaria triticina*	+	+
11	*Penicillium chrysogenum*	-	+

+ Fungi observed, **-** Fungi not observed

The dominent fungal species were used for the antibacterial activity. *Aspergillus niger* and *Mucor hiemalis* fungal culturature were extracts and used for the antibacterial activity against the *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli, Xanthomonas citri, Ralstonia solanacearum.* The maximum zone of inhibition *Mucor hiemalis* shows 21 mm against *Ralastonia solancearum* and the *Mucor hiemalis.* minimum zone of inhibition against *Bacillus subtilis* as compare *Mucor hiemalis*. (Table 2).

Table 2: Antibacterial activity of ethyl acetate extract of isolated fungi.

Rhizosphere fungi	Zone of inhibation against pathogen (mm)				
	Gram + ve bacteria		Gram - ve bactera	Plant pathogenic bacteria	
	Bs	Sa	Ec	Xc	Rs
Aspergillus niger	17	19	15	17	17
Mucor hiemalis	0	7.1	8	16	21

Bs=*Bacillus subtilis*, Sa= *Staphyloccus aureus*, Ec=*Escherichia coli*, Xc=*Xanthmonas citri*, Rs=*Ralastonia solancearum.*

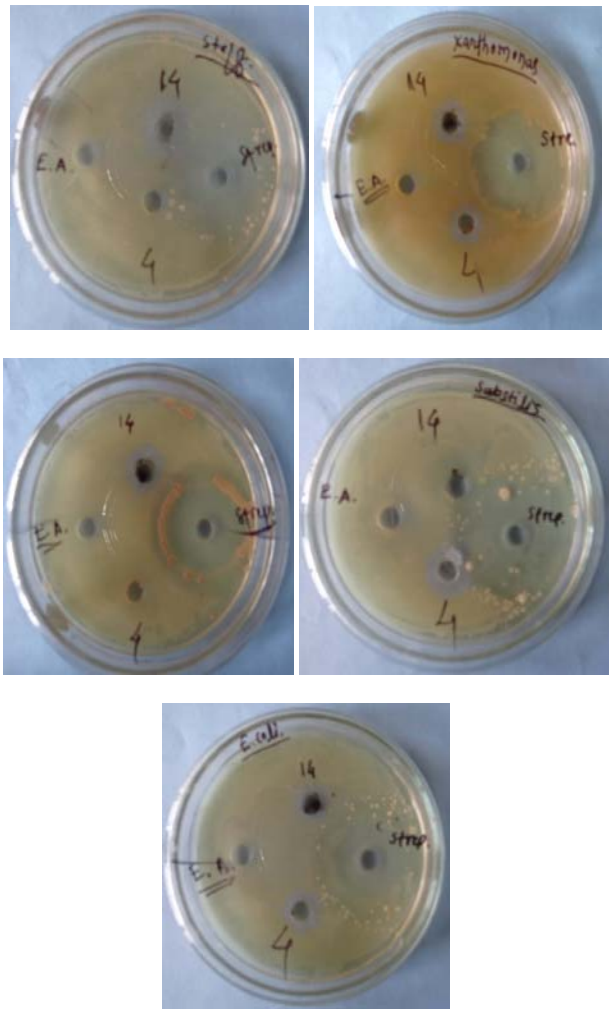

Fig 1: Antibacterial activity of fungal cultrate extract of isolated fungi.

Studied rhizosphere mycoflora of two verities of sugarcane (*Saccharum officinarum* L.) Viz.CO 86032 and CO 0265 were investigated. Isolated fungi from both rhizosphere and non-rhizosphere soil were domineneated by Aspergillus, *Alternaria*, and *Rhizopus*. Shivanna & Vasanthakumari (2011) [11] reported that *C. barbata* could provide shelter for a wide range of fungal species in the rhizosphere and presence vary depending on the season, root region and soil nutrient situation. *A. spergillus* spp. particalary *A. flavious, A. niger. A. ustus* and *A. wentii* were isolated from rhizospheric soil of all there wild plants out of which 100 %.sample showed presence of *A. niger* in *C. barbata*. Pathogenic fungi like *F. oxisporium, F. solani, M. phaseolina, P. eupyrena* species also isolated of *C. lunta*. Tariq *et al*. **(**2008) [10] in which serial dilution method showed maximum number of fungi (20) species & serial dilution method and due to its simple method the results obtained are more manageable. Frisvad and Samson, (2004). Present results showed that culture filtrates of *P. verrucosum* (FCBP 025), *P. citrinum* (FCBP 580) and *P. expansum* (FCBP 1101) were weakly effective to control the growth of *A. xylinum* (FCBP 239) as compared to *P. viridicatum* (FCBP 025) and *P. digitatum* (FCBP 726) which exhibited maximum inhibitory activity against this bacterial species. However, culture filtrates of all tested *Penicillium* species were found the most effective to control *S. gallinarum* (FCBP 038). Several *Penicillium* species have also been reported to suppress the bacterial growth (Samane *et al.*, 1991) [7].

Conclusion

In present investigation the fungal isolation were done from the rhizosphere soil of *Madhuca indica.* In *Aspergillus niger* and *Mucor hiemalis* were isolated from rhizosphere soil of *Madhuca indica.* The fungi were identified on the basis of morphological characters. For the study of my research work it was concluded that the extract of *Aspergillus flavous* and *Mucor hiemalis* have compound which ability to inhibit the growth of bacterial strains. It is recommended that further study should make available to discover new antifungal drug and antibiotic also in other ascents. Isolated fungal species *Aspergillus flavus* against *E. coli, Bacillus substillus, Straphyloccus aureus.* The maximum zone of inhibition *Mucor hiemalis* shows 21 mm against *Ralastonia solancearum*. The culture filtrate of *Mucor hiemalis* showed antibacterial activity against all tested pathogenic bacteria the zone of inhibition was less as compare to standard streptomycin.

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