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Isolation and Identification of Soil Mycoflora in Agricultural Fields of Aurangabad District, Maharashtra, India

Shaikh Firdous Shaikh Habeeb, Khan Farheen Kauser, Irfana Begum
Department of Botany,
Dr. Rafiq Zakaria Centre for Higher Learning & Advanced Research, Aurangabad, Maharashtra, India
firdoushabeeb9718@gmail.com

Abstract: *Soil Mycoflora play an important role in soil environment.The soil samples were collected from agricultural fields at Aurangabad district during Kharif season (July 2022 to October2022) to Rabi season (Nov 2022 to Feb2023) in twointervals. The soil sample of sugarcane (Saccharum officinarum), corn(Zea mays), gram(Cicer arietinum),cotton(Gossypium herbaceum),andground nut(Arachis hypogaea) were isolated.The Mycoflora were isolated by using soil dilution method and soil plate technique on Potato Dextrose Agar medium supplemented by suitable antibiotics such as streptomycin.A total of 11 species belonging to 5 genera of fungi were isolated from agricultural field. The isolated species belongs to Deuteromycotina, Zygomycotina,and Ascomycotina. No species of Basidiomycotina was found in soil. Mycoflora were identified and characterized with the help of relevant literature and manuals of fungi.The dominant genera in all the agricultural fields were Aspergillus, penicillium and Fusarium.The most frequent identified genera are Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Penicillium frequentans,andFusarium oxysporum.The percentage frequency of the Mycoflora were statistically analyzed.*

Keywords: Aurangabad, Kharif, Mycoflora, Soil sample, Rabi season

I. INTRODUCTION

Soil is a main component of the earth's ecosystem which comprises of organic matter, minerals, gases, and large numbers of macro and microorganisms *(Chandrashekar et al., 2014)*. Fungi are an important component of the soil Mycoflora typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions *(Ainsworth and Bisby, 1995)*. Fungi are fundamental for soil ecosystem functioning. Fungi are ubiquitous and important because of their presence in all climates and on all substrates. The soil serves as a reservoir for many microbial communities of plants and herbs which can be producing Carbon dioxide and Nitrogen. The fungi play important role in soil ecosystem *(Jadhav et al., 2017).*

A recent study on the diversity of soil fungi revealed around 80,500 operational taxonomic units (OTUs) occurring in soils worldwide *(Tedersoo et al., 2014)*. The aim of the present study is isolation and identification of fungal species from different agricultural field of Aurangabad district of Maharashtra.

II. MATERIAL AND METHOD

2.1 Study Location and Area

Aurangabad is a city in the Indian state of Maharashtra .The study area lies on 19° 53′ 48″ N latitude and 75° 23′ 54″ longitude which has an average elevation of 568 m (1,864 ft). The city is surrounded by hills on all directions. Aurangabad features a semiarid climate under the Koppen climate classification. Annual mean temperatures range from 17 to 33 °C and the annual rainfall is 710 mm. Most of the rainfall occurs in the monsoon season from June to September.

The soil is mostly formed from igneous rocks and is black, medium black, shallow, and calcareous types having different depths and profiles. Maize, Cotton, Groundnut, Sorghum,Sugarcane,Pulses, and Vegetables are the crops cultivated (Fig.1).

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Fig .1 map of Aurangabad

2.2 Method for Collection of Soil Samples

The soil samples were collected from five different crop fields in various locations of Aurangabad district. Soil samples of five crop fields were collected during July to Feb 2022-23 in two intervals. The soil samples were collected from different crop fields (up to 15cm depth) into a small, sterilized polythene bags and brought to laboratory for further studies (TABLE).

TABLE 1. Agricultural soil samples collected from different places at Aurangabad.

Sample No.	Agricultural field	Place
1	Sugarcane	Jatwada
2	Corn	Phulambri
3	Groundnut	Sultanpur
4	Gram	Harsul
5	Cotton	Daulatabad

2.3 Preparation of Potato Dextrose Agar (PDA) medium

Peeled potato pieces were boiled in 500 ml of distilled water in a 1000 ml beaker till the pieces got softened and the extract were collected in a beaker by sieving through a double layered muslin cloth. 15g Agar - agar was melted in another 500 ml of distilled water in 1000 ml beaker into which 20g dextrose was added. The final volume of the medium was made up to 1000 ml by adding sterile distilled water. The medium was sterilized in an autoclave at 121^0 C for 15-20 minutes.

2.4 Isolation of fungi from the soil sample

The soil fungi were isolated by soil dilution method (Waksman, 1927), on Potato Dextrose Agar media. About 1 gm of soil sample was suspended in 9 ml of distilled water to make microbial suspensions (10^{-1} to 10^{-4}).Dilution of10^{-4}were used to isolate fungi. Streptomycin was added to the medium before pouring into Petri plates for preventing bacterial growth. About 20 ml of the medium was distributed to sterile Petri plate under aseptic condition. 1 ml of microbial suspension of 10^{-4} concentration was added to sterile Petri plates.

The Petri plates were incubated at 28^0Cfor a period of 4-7 days. During incubation plates were observed regularly and fungal growth were noted. After 7 days of incubation, photographs of plates were taken. The colony forming units (CFU) of the fungal isolates were calculated. Isolated fungal colonies were used for preparation of slides. Slides were prepared by using cotton blue stain and lactophenol as mounting medium. Slides were observed under the light microscope and photography of fungi was also taken.

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2.5 Identification of Fungal colonies

Fungal morphologies were studied macroscopically by observing colony features like hyphal structure, spore size, shapes and spore bearing structure and microscopically by staining with lacto phenol cotton blue and observe under compound microscope. Fungi were identified by using standard protocols, literature,and manuals of fungi *(Nagamani, Kunwar and Manoharachary2006, joseph C Gilman 2008*).All the results were calculated and statistically analyzed.

2.6 Pure Culture and identification

Purification of the fungi was made by single spore culture method. A portion of the growing edge of each colony was picked up with the help of needles and transfer to PDA medium. The Petri plates were incubated at 28^0Cfor a period of 3-7 days. During incubation plates were observed regularly and fungal growth were noted. After 5 days of incubation, photographs of plates were taken.

2.7 Physio-chemical analysis of soil

The collected soil was characterized for its physio-chemical properties. The physio-chemical parameters were measured by standard methods. Physical and chemical parameters of soil such as pH, salinity, organic carbon, nitrogen, phosphorous and potassium were analyzed. The physio-chemical parameters of the soil samples were analyzed at Shivaji Arts, Commerce and Science College, Kannaddistrict, Aurangabad.

TABLE 2. Physio-chemical properties of soil samples collected from different agricultural fields at Aurangabad.

SL. No	Crop field	Place	Soil color	Soil type	pH	Salinity	Organic carbon %	NKg /h	PKg/ h	K Kg/h
1	Sugarcane	Jatwada	Dark grey	SCL	6.9	0.48	Low	77	24	64
2	corn	Phulambri	Brown	SL	6.2	0.51	Medium	71	39	86
3	groundnut	Sultanpur	Black	SCL	6.3	0.15	Low	65	28	41
4	gram	Harsul	Black	SL	5.4	0.52	Medium	93	36	105
5	cotton	Daulatabad	Black	SICL	6.9	0.22	Low	82	45	193

SCL-Sandy Clay Loam, SICL- Silt Clay Loam, SL -Sandy Loam
Organic content: 0.3-0.5= low, 0.5-0.75 = medium, 0.75-1 = high

2.8 Statistical Analysis

The number of colonies per plate in 1gm of soil was calculated. The percentage of contribution of each isolate was calculated by using the following formula:
= Total no. of CFU of an individual species X100
Total no. of CFU of all species
* CFU-Colony Forming Unit

III. RESULTS AND DISCUSSION

The present study reveals a total of 11 fungal species were identified by different soil samples from agricultural fields of Aurangabad. During the investigation period 73 fungal colonies of 11 fungal species were observed (TABLE). The fungal species belongs to Deuteromycotina (64 colonies), Ascomycotina (6 colonies), and Zygomycotina (3 colonies) were observed. Among the isolate genera Aspergillus, Penicillium and Fusarium was dominant (Table.III). Trichoderma and Rhizopus were found occasionally (Table.III). The present study shows that, the Deuteriomycotina Fungi frequently found in soil because of their rapid growth rate of asexual reproduction, as they cause various diseases to crop plants and their rich flora in soil. Among the isolated colonies *Aspergillus*, *Penicillium*, and *Fusarium* were dominant in all the agricultural soil of all the areas. They may prevent the growth of other fungal species due to high sporulation and production of different types of toxins from Aspergillus and Fusarium species.

The Soil pH, organic content and water are the main factors affecting the fungal population and diversity. The organic carbon, nitrogen, phosphorus, potassium is important for fungi. In the absence of any of these the growth and

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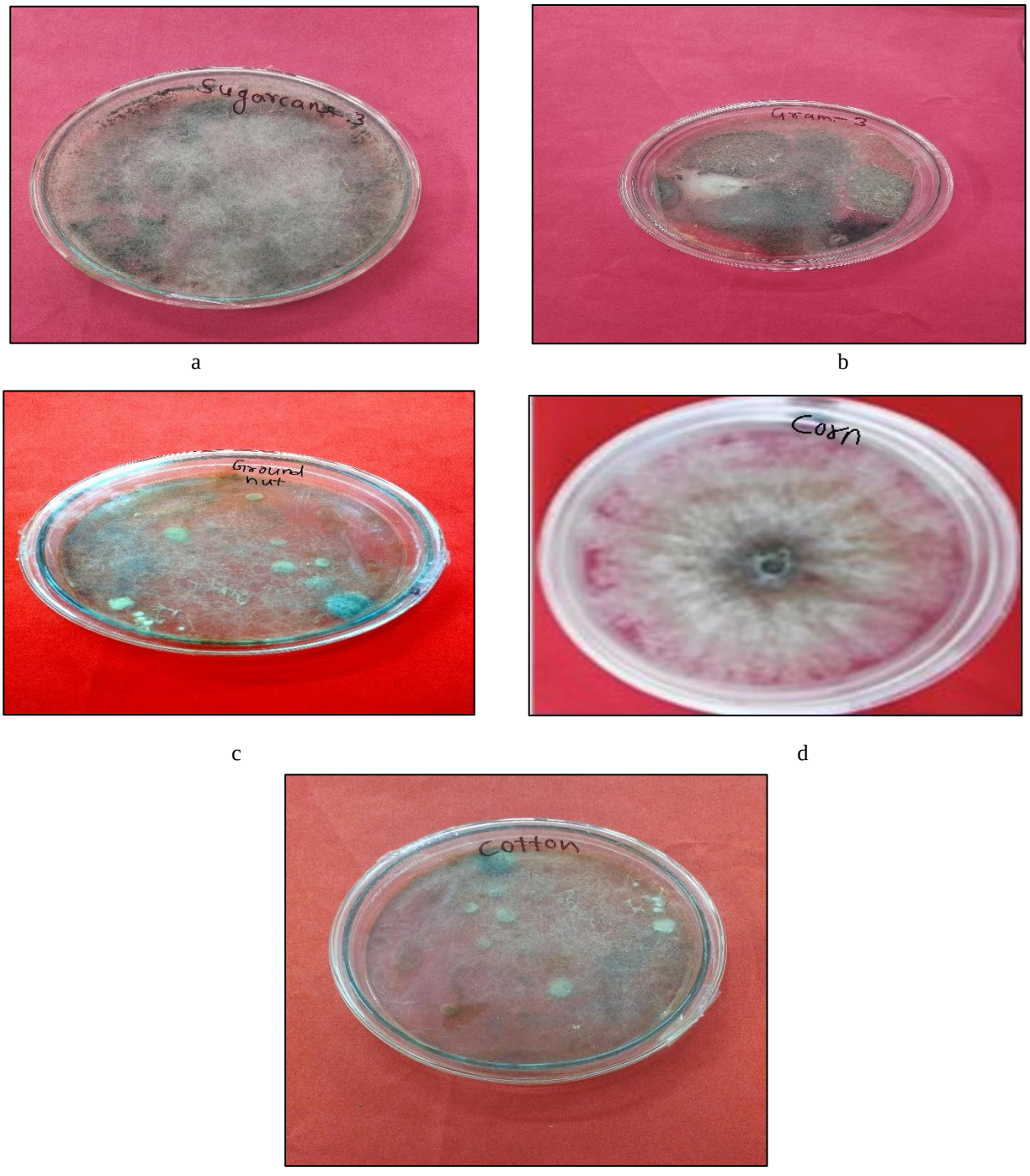
sporulation of fungi as well as other microorganisms are hampered a lot. It has been reported that the density of fungal population occurred during the monsoon (rainy) season when the soil moisture was significantly high *(Gaddeya et al., 2012).* The diversity of fungi in soil is mainly affected positively as well as negatively on growth of crop plants *(Ratna Kumar et al., 2015).*



a b c d e

Fig.2 Cultural view of Mycoflora on PDA [a-sugarcane, b-gram, c-ground nut, d-corn, e-cotton]

The most common Mycoflora in sugarcane, corn, ground nut, gram, and cotton were *Aspergillus niger* (13.6%) *Aspergillus nidulans* (6.8%), *Aspergillus flavus* (10.5%) *Aspergillus fumigatus* (10.9%), *Aspergillus terreus* (6.8%), *Penicillium frequentans* (5.4%), *Penicillium chrysogenum*(12.3%), *Trichoderma viride* (12.3%), *Fusarium oxysporum*

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(8.2%), *Fusarium solani* (8.2%), and *Rhizopus stolanifer* (4.1%). The seasonal variation and percentage frequency of the Mycoflora were statistically analyzed (TABLE)

TABLE. 3 Frequency of Mycoflora in different crop fields at Aurangabad.

SL. No	Crop field	Average no of total colonies	Average no of individual colonies										
			Aspergillus					*Penicillium*		*Fusarium*		*Trichoderma*	*Rhizopus*
			Aspergillus n	Ani	Afl	Afu	At	Pch	Pf	Fo	Fs	Tv	Rs
1	Sugarcane	14	2	1	2	1	1	-	2	1	2	1	1
2	Corn	16	2	1	1	3	1	1	2	2	1	2	-
3	Groundnut	15	2	1	1	2	1	1	2	2	2	1	-
4	Gram	13	1	1	2	2	1	1	2	2	-	1	-
5	Cotton	15	3	1	2	-	1	1	1	2	1	1	2
Total		73	10	5	8	8	5	4	9	9	6	6	3
% Contribution			13.6	6.8	10.5	10.9	6.8	5.4	12.3	12.3	8.2	8.2	4.1

The soil Mycoflora in different crop fields were observed. The percent contribution of *Aspergillus niger* (20%) was higher in cotton as compared to other agricultural soil (TABLE). The percent contribution of *Penicillium frequentans* (15.38) was higher in gram as compared to other agricultural soil (TABLE). The percent contribution of *Fusarium oxysporum* (15.28) was higher in gram as compared to other agricultural soil (TABLE)

TABLE 4 Percent contribution of Fungal species in different crop fields at Aurangabad.

SL. No.	Fungal species	% contribution				
		Sugarcane	Corn	Groundnut	Gram	Cotton
1	*Aspergillus niger*	14.28	12.25	13.33	7.69	20
2	*Aspergillus nidulans*	7.14	6.25	6.66	7.69	6.66
3	*Aspergillus flavus*	14.28	6.25	6.66	15.38	13.33
4	*Aspergillus fumigatus*	7.14	18.75	13.33	15.38	
5	*Aspergillus terreus*	7.14	6.25	6.66	7.69	6.66
6	*Penicillium chrysogenum*		6.25	6.66	7.69	6.66
7	*Penicillium frequentans*	14.28	12.25	13.33	15.38	6.66
8	*Fusarium oxysporum*	7.14	12.25	13.33	15.38	13.33
9	*Fusarium solani*	14.28	6.25	13.33		6.66
10	*Trichoderma viride*	7.14	12.25	6.66	7.69	6.66
11	*Rhizopus stolanifer*	7.14				13.33

Our findings are in accordance with the results of Ratna Kumar et al., (2019) studied Soil Mycoflora in different soil samples in crop fields of Chintalapati Mandal in West Godavari District, Andhra Pradesh, India. They were isolated genera like Aspergillus, Mucor, Curvularia, Fusarium, Penicillium and Rhizopus. Similar genera were isolated during our investigation.

Chandrashekar et al., (2014), isolated and identified Fungal diversity in rhizospheric soils from paddy, pulses, ragi, sugarcane, vegetables, and banana fields of Nanjangud taluk of Mysore district, Karnataka, and recorded 10 fungal species representing 7 genera. They have reported Aspergillus, Alternaria, Mucor, Curvularia, Fusarium, Penicillium and Rhizopus. We have isolated 11 species belongs to 5 genera in that Aspergillus and Fusarium and Penicillium were dominant.

Jadhav et al., (2017), isolated 18 species belongs to 4 genera from Kadegaon Tehsil, Sangli District, Maharashtra, India. The Aspergillus genera were dominant. Similar results were reported during our investigation. In our

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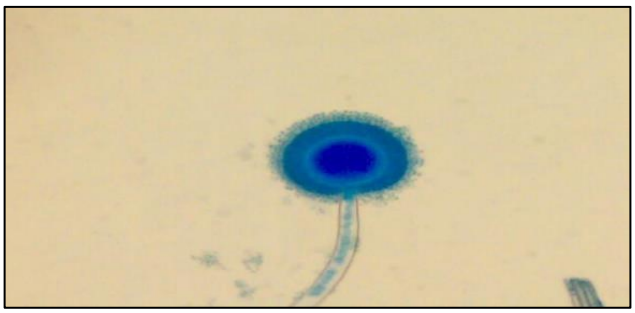
investigation total of 11 fungal species belongs to 5 genera were isolated and the dominant genera in all the crops were Aspergillus,Penicillium and Fusarium.

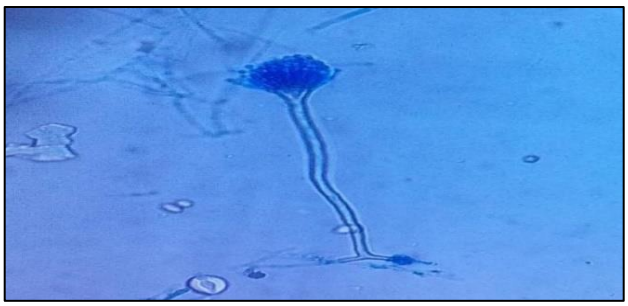
Ch Ramesh et al., (2021), investigated Mycoflora of total 27 species belong to 19 genera from Soil in Different Crop fields at Hubli Taluk, Karnataka, India. Aspergillus and Fusarium were dominant in their study. In our investigation total of 11 fungal species belongs to 5 genera were isolated and the dominant genera in all the crops were Aspergillus, Penicillium, and Fusarium.

Gaddeya et al., (2012), isolated a total of 15 species belonging to 6 genera of fungi from agricultural fields at Salur Mandal. Genera Aspergillus and Penicillium were dominant in their study. In our investigation total of 11 fungal species belongs to 5 genera were isolated and the dominant genera in all the crops were Aspergillus, Penicillium and Fusarium.

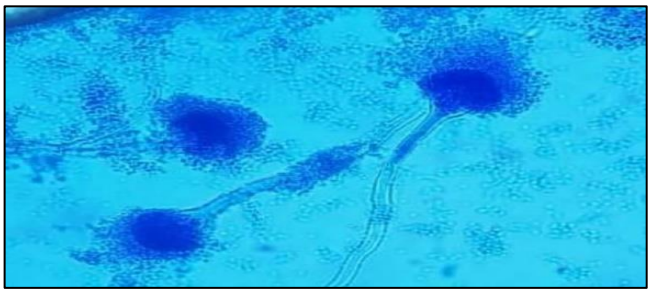
IV. MICROSCOPIC VIEW OF DIFFERENT MYCOFLORA IN CROP FIELDS AT AURANGABAD.



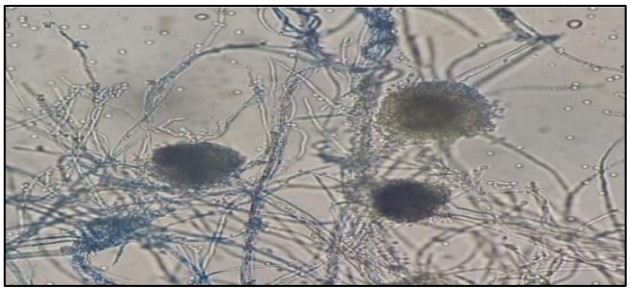
a- Aspergillus niger



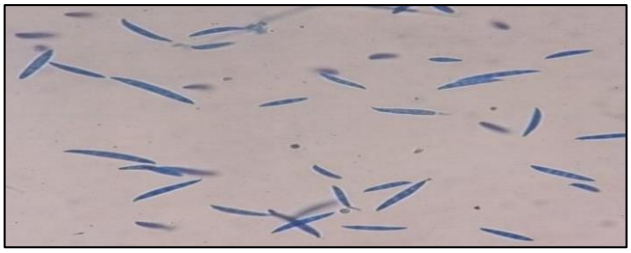
b-Aspergillus nidulans



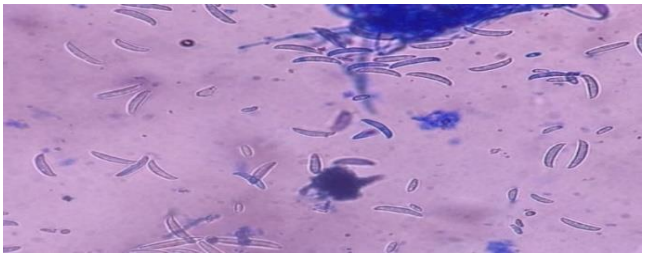
c-*Aspergillusfumigatus*



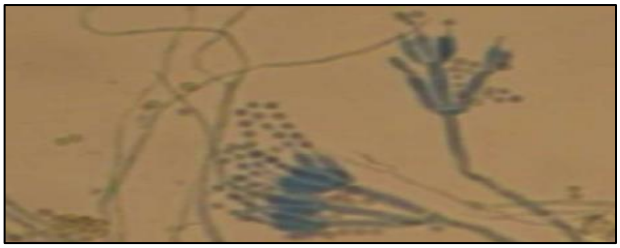
d-Aspergillus flavus



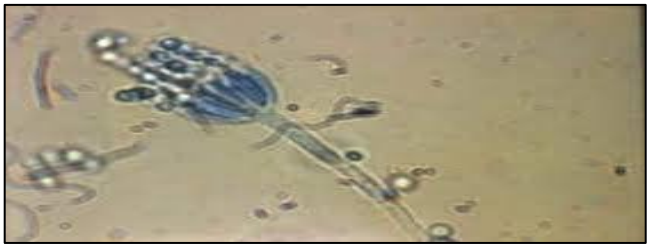
e-*Fusarium oxysporum*



f-*Fusarium solani*



g-*penicillium chrysogenum*



h-*penicillium frequentans*

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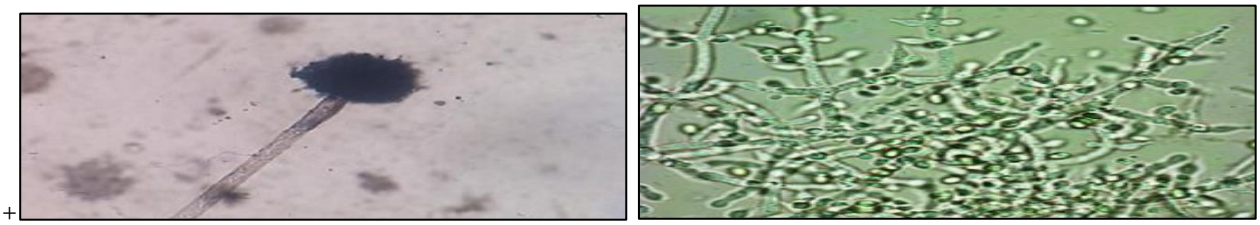
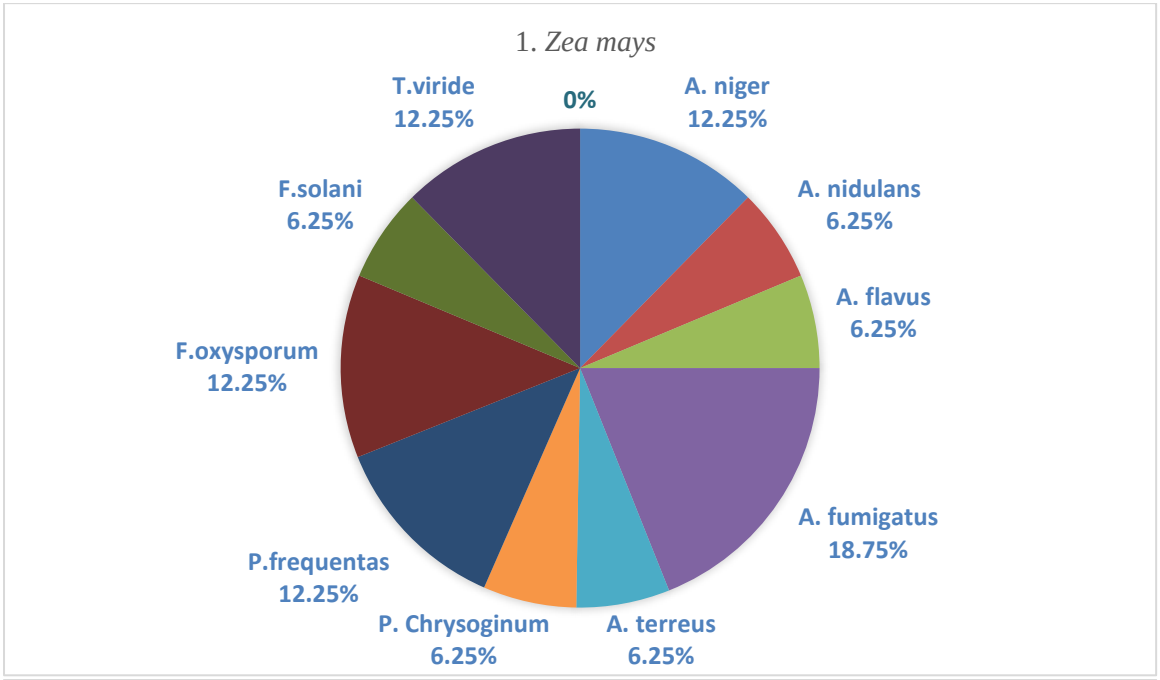
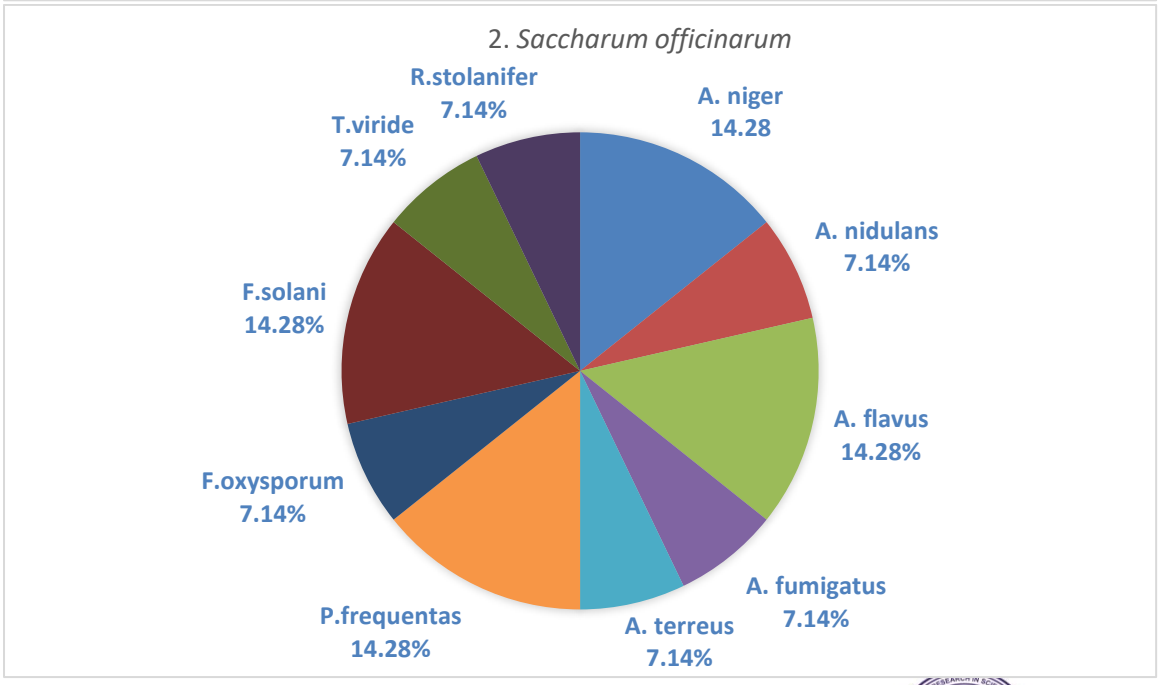


h- *Rhizopus stolonifera* *e-Trichoderma viride*

V. PERCENTAGE CONTRIBUTION OF MYCOFLORA IN DIFFERENT CROP FIELDS AT AURANGABAD.





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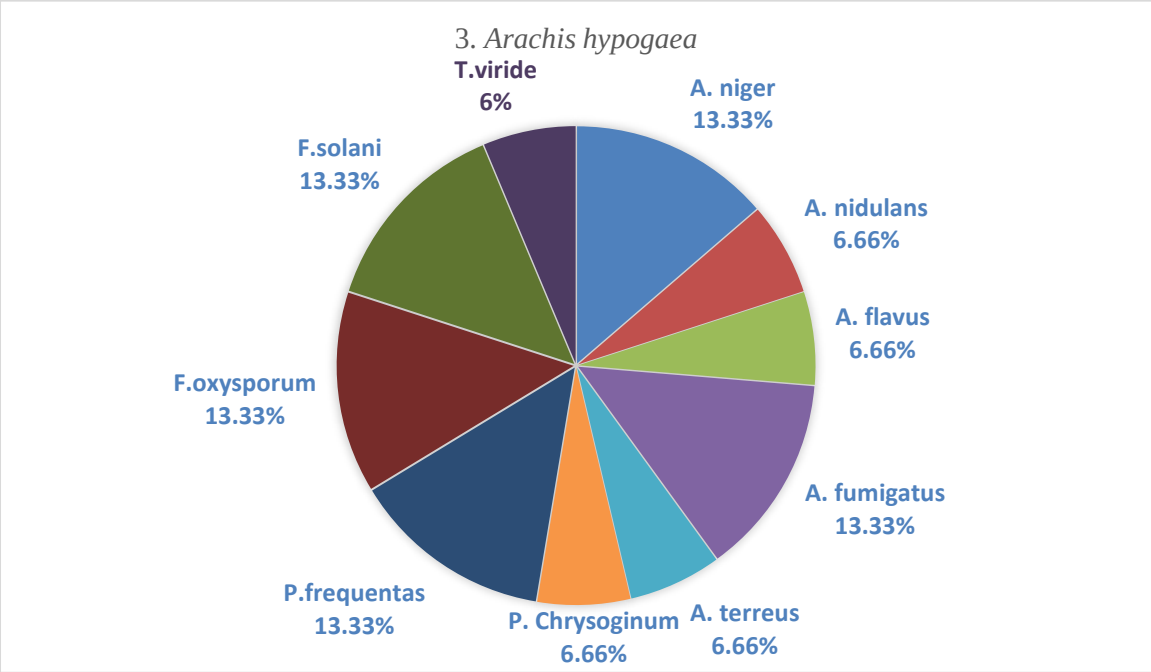

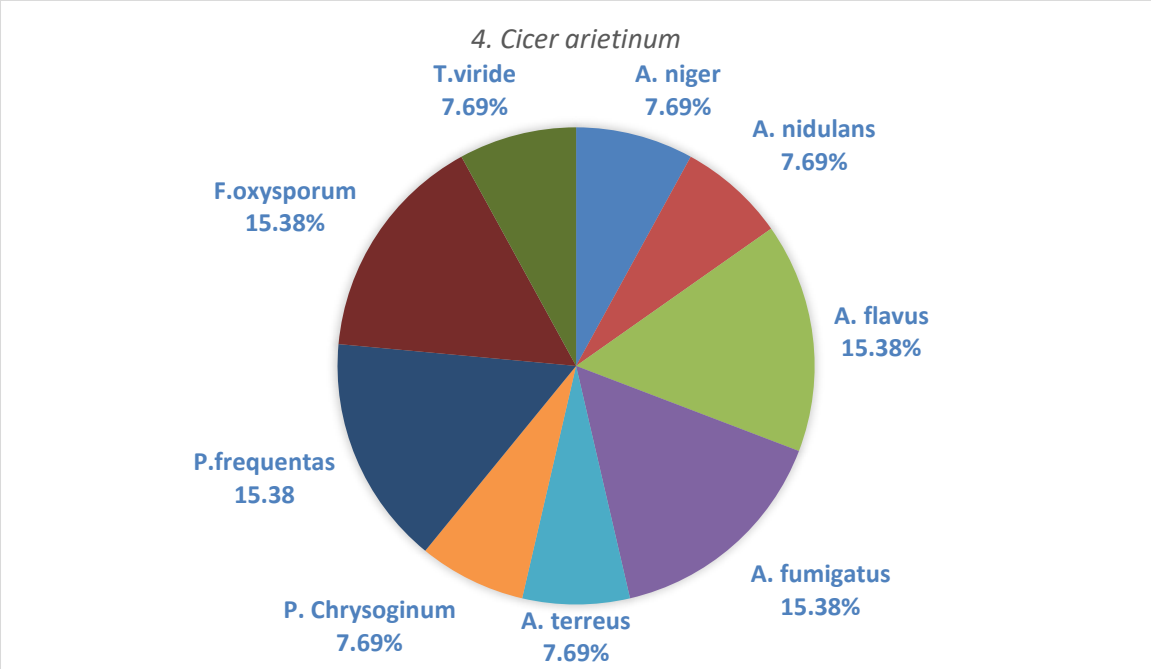

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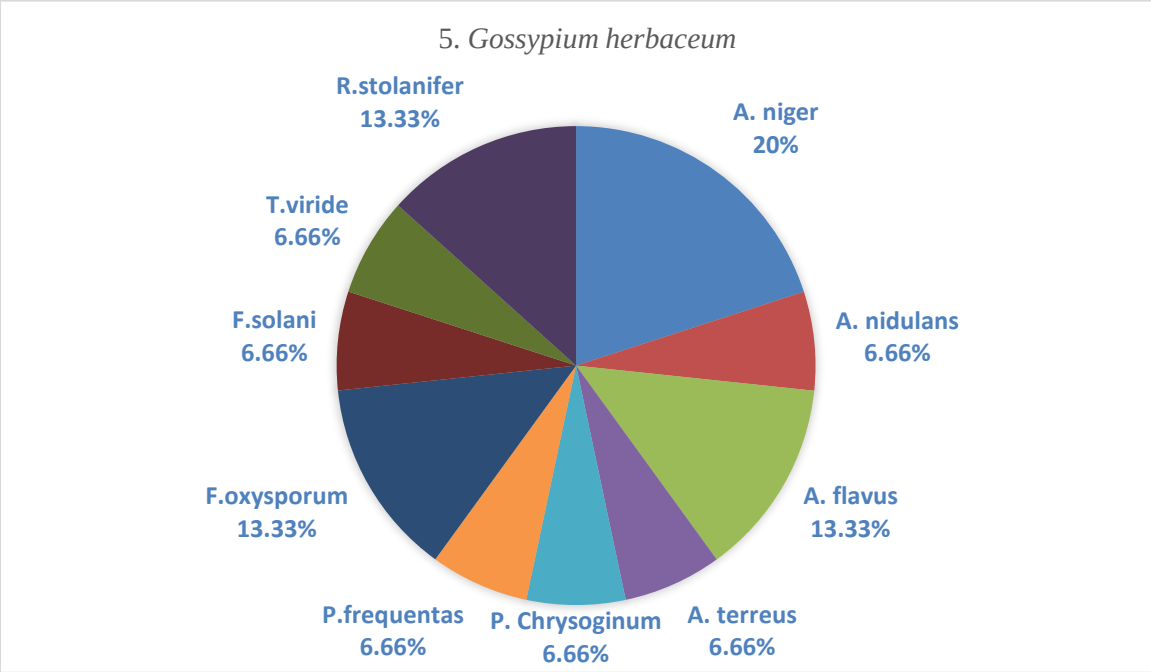

VI. CONCLUSION

In the present study soil samples of five crop fieldsviz. sugarcane (*Saccharum officinarum*), corn (*Zeamays)*, gram (*Cicer arietinum)*, ground nut (*Arachis hypogaea)*cotton (*Gossypium herbaceum*) was isolated and studied. The obtained results clearly shows that *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium frequentans, and fusarium oxysporum* were of high occurrence in all agricultural crop fields and some other fungi like Trichoderma, and Rhizopus were negligible. Among the isolates Aspergillus, Penicillium, and Fusarium were dominant in all agricultural fields due to high sporelation capacity. The Penicillium spp producing fungal and bacterial antibiotics and the Aspergillus spp producing different kinds of toxins such as aflotoxins. These toxins may prevent the growth of other fungal species. The frequency ofMycoflora in agricultural fields were found to be regulated by many factors like temperature, humidity, vegetation, organic and inorganic materials, soil type and texture. The fungi were mostly observed in month of June to September due to suitable temperature and humidity.

VII. ACKNOWLEDGEMENT

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