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Soil fungi associated with cotton, cucumber plant fields of Koppal region in Karnataka, India

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Abstract
A study was conducted for the isolation and identification of soil fungi associated with cotton and cucumber plants cultivated in Koppal region of Karnataka. Soil samples were collected from cotton and cucumber plants growing fields during the months of January 2018 to November 2020, at Koppal region of Karnataka, India. Soil fungi were isolated by using soil dilution technique on PDA medium containing strepto - penicillin. Identification of the soil fungal isolates was made with the help of relevant literature and standard manuals of soil fungi. The number of fungal isolates present in cotton plants field is 51,550 per gram of soil. The number of fungal isolates present in cucumber plants field is 8, 21,800 per gram of soil. In cotton plants field, the percentage contribution of Basidiomycotina is more with 18.60%, In cucumber plants field, the highest percentage contribution is given by *Aspergillus niger* with 27.5%, In cotton plants field, 14 different species belonging to 9 genera. Among which *Aspergillus* species is dominating over other species. In cucumber plants field, 9 different species belonging to 5 genera were observed. Among which *Fusarium* sp. is dominating over other species. The most frequently isolated fungi from the cotton plants and cucumber plants fields of Koppal region is *Aspergillus* sp and *Fusarium* sp. In cotton plants field, amongst the isolates, 6 *Aspergillus* species (*A. niger, A. flavus, A. fumigatus, A. awamori, A. terreus, Aspergillus* sp.) 2 *Fusarium* species (*Fusarium oxysporum F. pallidoroseum*) and in cucumber plants fields among the isolates 3 *Aspergillus* species (*A. niger, A. flavus, A. awamori,*) 3 *Fusarium* species (*F. oxysporum F. pallidoroseum, Fusarium* sp.) were characterized and percentage occurrence of the soil fungi was statistically evaluated.

Keywords: fungal diversity, soil fungi, Koppal, Karnataka, cotton plants, cucumber plants

Introduction
Soil harbors diverse groups of organism such as bacteria, fungi, actinomycetes, protozoa etc, and dead and decaying materials in various stages (Hoorman and Islam, 2010) [6]. The soil biomass is predominantly constitutes microbial world including the large proportion of fungi which are widely distributed in soils and associated land-use (Ritz and Young, 2004) [7]. Fungi is found in almost every environment and lives in a wide range of pH and temperature (Frąc et al., 2015) [8]. Fungal diversity in soil is strongly influenced by diversity and composition of plant community (Hannula et al., 2017) [11]. The soils with high organic matter, little physical disturbance or low nutrient inputs observed to be fungal dominated, whereas the biomass is frequently tilled in soils and receives high inorganic fertilizer supply shows more number of bacteria (Ritz and Young 2004) [7]. In general the fungal pathogens are carried through the infected seeds to the crop fields and these pathogens are accumulated in the soil of crop fields (Ramesh et al, 2013)[12]. The distribution of fungi in different types of crop fields depends on soil structure, vegetation, temperature, humidity etc (Gaddeyya et al, 2012) [13]. Fungi play an important role in the conversion of dead matter into biomass, carbon dioxide and organic acids (Baldrian, 2003) [14]. Fungi are used for bioremediation, as they have the capacity to degrade pollutant molecules (Kirk and Farrell, 1987) [15]. Many of the fungal species are potential absorbers of toxic metals like zinc and cadmium (Baldrian, 2003) [13]. Fungi helps in growth of the plant through mutualism, pathogenicity, making the availability of nutrient and recycling the different organic materials (Hannula et al., 2017)[11]. Pesticide residues in the soil can affect species differently, some species can tolerate and degrade the molecules and therefore thrive in the soil (Rohilla and Salar 2012) [16]. The study of fungal community in the soils of crop fields from Koppal district is lacking; so the present study aim to observe the fungal diversity in soil samples of cotton and cucumber fields,

Materials and Methods
Study area
The area of Koppal district is located between 15º-21'N to 15º -45'N Latitude, 76º-10' E to 76º-32' E Longitude, the types of soils are ranges from well drained red sandy loam to medium deep black soils (Fig, A). Different soils such as black cotton soil, red soils, deep black soils, red sandy soil are predominantly present in the Koppal region, Karnataka, India (Ramamurthy et al., 2009) [21]. The study area comes under the hot arid region with

deep loamy saline and alkali soils, and having low to medium available water holding capacity to it. The pH of the different soil types observed to be neutral. The district is witnessed regular showers with an average rainfall of around 612.6 mm; maximum rainfall is observed from July to October. The tree species like Bellary jali (*Prosopis juliflora*), banni (*Acacia ferugenia*) and neem (*Azadiracta indica*) were predominantly found in this area (Prathibha et al. 2019) [22].

Isolation of Fungi from Soil Samples

A survey was done during pre-monsoon period of 2019- 2021 for collection of soil samples in the cotton plants and cucumber plants growing fields in Koppal region of Karnataka, India. Approximately one kg of soil sample is collected from minimum five different areas of the crop fields in Polythene bags and brought to the laboratory for further study. The soil samples were dried, powdered and sieved; to get fine soil sample. Isolation of fungi from soil samples is done by soil dilution method. One gram of fine soil sample was weighed and dissolved in the 10 ml of distilled water containing test tube and labeled as 10^{-1}. Dilution of 10^{-2}, 10^{-3}, 10^{-4} and10^{-5}were prepared by serial dilution method. Approximately 15 ml of cooled PDA (Potato Dextrose Agar) medium supplemented with strepto-penicillin is poured in to plates and labeled as 10^{-2} to 10^{-5}. Few drops of inoculums are added to the plate and then the inoculum is spread by gentle rotation of the petridish with the help of an 'L' shaped glass rod. The triplicates of petridishes with each dilution were incubated in an inverted position at 27℃ in an incubator for 2 to 7 days. Organism per gram of soil is calculated by formula: No. of colonies × dilution factor /dry wt of soil.

% frequency = (No of observations in which a species appeared/ total no of observations) X 100

% Contribution = Total No of colonies of species in all observations taken together/Total no of colonies in all the species X 100

Identification of Fungi

A micro preparation slide of each fungal species was stained cotton blue stain and observed under the microscope. The size, colour, shape, structure of the conidia or spores, branching of mycelium were considered for identification of fungal species. They were compared with the standard works of manual of soil fungi (Gillman, 1957) [1], Hyphomycetes (Subramanian, 1971) [2], A Manual of *Penicillium* (Raper and Thom, 1949) [3], Manual of *Aspergillus* (Raper and Fennell, 1965) [4] and Soil fungi (Domsch et al., 1980) [5].

Results and Discussion

The number of fungal isolates present in cotton field is 51,550 per gram of soil, i.e., the 10^{-2} dilution have 2950 fungal isolates per gram of soil, the 10^{-3} dilution have 31600 fungal isolates per gram of soil, 10^{-4} dilution have 23000 fungal isolates per gram of soil. The number of fungal isolates present in cucumber field is 8,21,800 per gram of soil, i.e., the 10^{-2} dilution have 800 fungal isolates per gram of soil, the 10^{-3} dilution have 11,000 fungal isolates per gram of soil, the 10^{-4} dilution have 1,10,000 fungal isolates per gram of soil, the 10^{-5} dilution have 7,00,000 fungal isolates per gram of soil (Table 1). In cotton plants growing field, the percentage contribution of *Basidiomycotina* is more with 18.60%, whereas the lowest contribution is given by *Trichoderma* sp and *Penicillium chrysogenum* (Fig. G) with 1.744%. In cucumber plants growing field, the highest percentage contribution is given by *A. niger* with 27.5%, whereas the lowest contribution factor was given by *Fusarium* sp with 2.5%. Overall, when both the plants growing fields are considered, the highest percentage of contribution is observed by *F. palidorosium* with 18.49% and least percentage contribution is observed by *Fusarium* sp with 1.02%. In the cotton plants growing fields the highest percentage frequency was shown by *Basidiomycotina*; whereas lowest percentage frequency was shown by *Trichoderma* sp and *P. chrysogenum.* In cucumber plants field the highest percentage frequency was shown by *A. niger*; whereas lowest percentage frequency was shown by *Fusarium* sp. In cotton plants field, 14 different species belonging to 9 genera was observed. Among which, *Aspergillus* sp is dominating over other species; amongst the isolates, 6 *Aspergillus* species (*A. niger, A. flavus, A. fumigatus, A. awamori, A. terreus, Aspergillus* sp. (Fig. C, F)), 2 *Fusarium* species (*F. oxysporum F. pallidoroseum* (Fig. H)*)* were characterized and percentage occurrence of the soil fungi was statistically evaluated. In Cucumber plants field, 9 different species belonging to 5 genera were observed. Among which *Fusarium* sp. is dominating over other species; among the isolates 3 *Aspergillus* species (*A. niger, A. flavus, A. awamori*), 3 *Fusarium* species (*F. oxysporum F. pallidoroseum Fusarium* sp.) were characterized and percentage occurrence of the soil fungi was statistically evaluated (Table 2).

A total of 12 species belonging to 6 genera of fungi were isolated from crop fields at Hegga dadevana Kote (H.D. Kote) of Mysore district. (Sharma and Raju, 2013)[17]. A total of 15 species belonging to 6 genera of fungi were isolated from agricultural fields at Salur Mandal. (Gaddeyya et al, 2012)[13]. In the present study the crop fields like cotton, cucumber soil samples were used to study soil mycoflora, which shows the presence of 14 different species of 9 genera in cotton plants field and 9 different species of 5 genera in cucumber plants field. The most common fungi isolated from agricultural fields at Salur Mandal was *A. flavus, A. fumigatus, A. niger, A. nidulans, A. terreus, P. chrysogenum, P. frequentans, P. funiculosum, Trichoderma viride, T. harzianum, F. oxysporum, F. solani, Curvularia clavata, C. lunata*, and *Rhizopus stolanifer* were isolated and characterized (Gaddeyya et al, 2012)[13]. In the present study the most frequently isolated fungi from the cotton and cucumber

plants fields of Koppal region is *Aspergillus* sp and *Fusarium* sp. 32 different fungal species associated with soil collected in cotton plant growing area. This indicates that all fungi found in the soil and/or in the rhizosphere of cotton were not associated with cotton plants. These fungal species have specific preference of host existence. (khaskheli et al, 2014) [18]. in the present study the soil samples of the cotton plants field showed 14 species of different fungi.

Table 1: Fungal isolates from Soil Samples collected in Cotton plants and Cucumber plants Field

S. No.	Area	Dilution factor	No of isolates	Average	Organisms per gram of soil
1	cotton	10^{-2}	*Aspergillus fumigatus* (3)	1	100
			Penicillium chrysogenum (3)	1	100
			Aspergillus flavus (6)	2	200
			Trichoderma sp (3)	1	100
			Aspergillus terreus (9)	4	400
			Cladosporium sp (24)	8	800
			Aspergillus sp. (9)	3	300
			Absidia sp (12)	4	400
			Fusarium oxysporum (17)	6	550
2	cotton	10^{-3}	*Actinomycetes* (15)	5	5000
			Fusarium palidorosium(6)	2	2000
			Aspergillus fumigatus (14)	4	4000
			Basidiomycotina (32)	11	10600
			Aspergillus niger (12)	4	4000
3	cotton	10^{-4}	*Fusarium palidorosium* (7)	2	23000
			Colletotrichum gleosporoides (12)	4	40000
4	cucumber	10^{-2}	*Cladosporium* sp (8)	3	300
			Aspergillus awamori (6)	2	200
			Aspergillus niger (6)	2	100
			Fusarium oxysporum (3)	1	100
			Fusarium palidorosium (3)	1	100
5	cucumber	10^{-3}	*Aspergillus niger* (15)	5	5000
			Fusarium palidorosium (9)	3	3000
			Fusarium sp (3)	1	1000
			Trichoderma sp (6)	2	2000
6	cucumber	10^{-4}	*Aspergillus flavus* (25)	6	60000
			Fusarium palidoroseum (3)	1	10000
			Penicillium chrysogenum (6)	2	20000
			Fusarium oxysporum (6)	2	20000
7	cucumber	10^{-5}	*Aspergillus niger* (12)	4	400000
			Fusarium pallidoroseum (9)	3	300000

Table 2: Percentage frequency and contribution of different fungal species in cotton and cucumber fields

Sl. No	Species Name	Cotton		Cucumber		Total	
		A	B	A	B	A	B
1	*Absidia*	6.97	12	-	-	4.10	12
2	*Actinomycetes*	8.72	15	-	-	8.72	15
3	*Aspergillus sp.*	5.23	9	-	-	3.08	9
4	*Aspergillus awamori*	-	-	5	6	2.05	6
5	*Aspergillus flavus*	3.48	6	20.83	25	10.61	15.5
6	*Aspergillus fumigatus*	9.88	17	-	-	5.82	17
7	*Aspergillus terreus*	5.23	9	-	-	3.08	9
8	*Aspergillus niger*	6.97	12	27.5	33	11.30	22.5
9	*Basidiomycotina*	18.60	32	-	-	10.95	32
10	*Cladosporium sp*	13.95	8	6.66	8	10.95	8
11	*Colletotricum gleosporoides* (Fig. B)	6.97	12	-	-	4.10	12
12	*Fusarium sp*	-	-	2.5	3	1.02	3
13	*Fusarium oxysporum*	15.22	17	7.5	9	3.08	13
14	*Fusarium palidorosium*	17.44	13	20	24	18.49	18.5
15	*Penicillium chrysogenum*	1.74	3	5	6	3.08	4.5
16	*Trichoderma sp*	1.74	3	5	6	3.08	4.5

A= % Contribution, B= % Frequency

Many factors influence the development of *Fusarium* wilt in a field, including virulence of the population of *F. oxysporum f. vasinfectum*, susceptibility of the cotton cultivar, climatic conditions, soil type, soil fertility, and interactions with nematodes and other soilborne microorganisms. All these factors may impact the severity of the disease and subsequent yield losses (Davis et al., 2014) [19]. In the present study the soils of cotton field from koppal district showed the presence of *F. pallidorosium*, which is responsible for causing wilt disease in cotton. F*usarium* species present in the sample collected are second dominant species, which may affect the plant adversely and affect the production. Though *Aspergillus sp* and *Fusarium sp.* were dominant there were other species observed, which are *Penicillium sp, Trichoderma sp, Cladosporium sp*(Fig. E) and *Absidia sp* (Fig. D). Which are not dominantly observed, they may affect growth of the plant to varying degrees also the metabolites or toxins produced by fungi may also affect people working in the field by causing allergies or affecting other health conditions. This soil mycoflora study can further help in learning relationships between plants, soil, and fungi. This can also help in studying antifungal properties in plants if there are any, and also, we can infer the fungi species diversity. The predatory myxobacterium *Corallococcus* sp. strain EGB controlled cucumber *Fusarium* wilt by migrating to the plant root and regulating the soil microbial community (Ye et al 2020) [20]. In the present study, the biocontrol agent like *Trichoderma* was isolated from the soil samples of cotton and cucumber crops of koppal district, which may be controlling the wilt disease in cotton and cucumber.

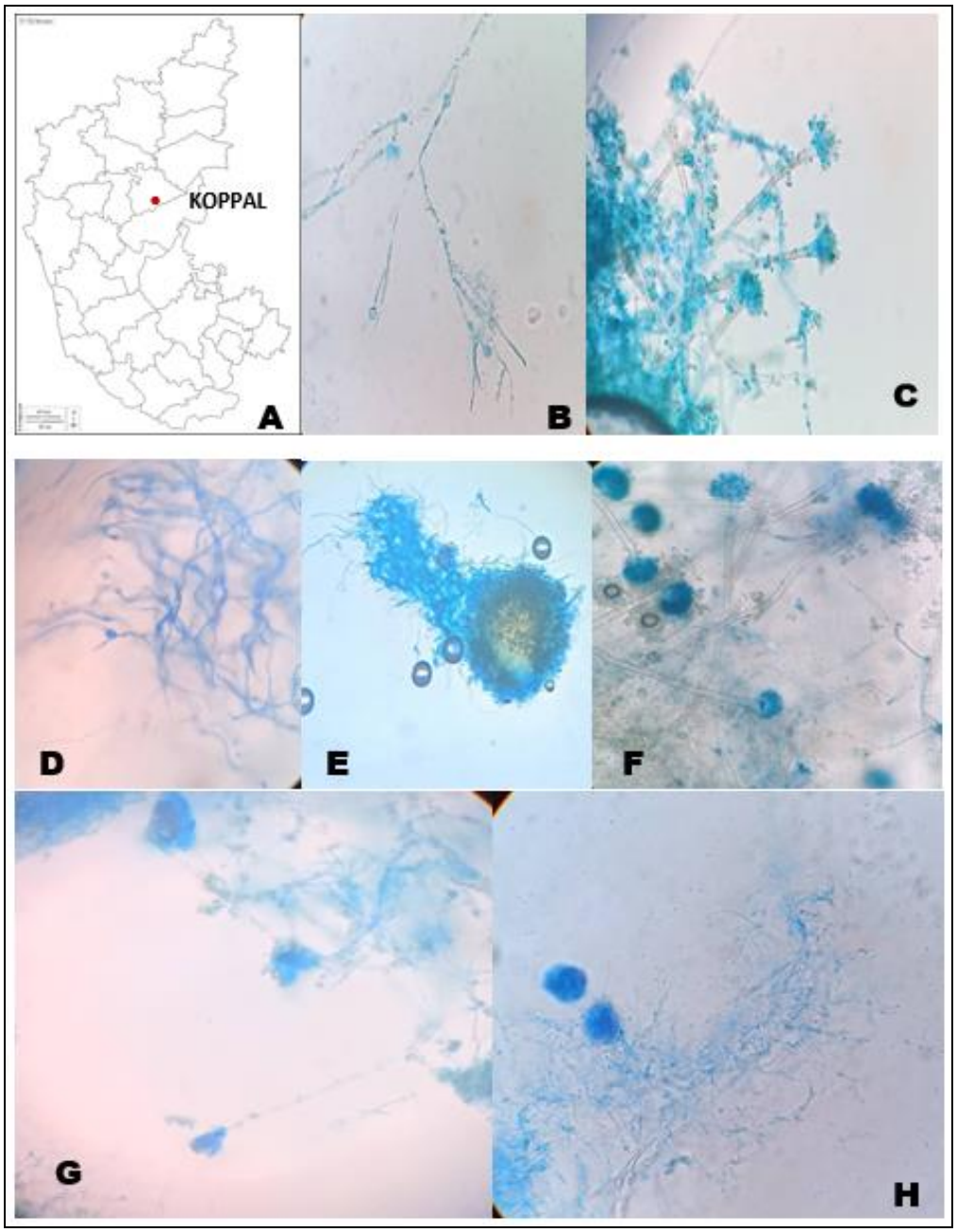

Fig 1: A. Map of karnataka showing the study area of Koppal district, B: The microscopic picture of *colletotrichum gleosporoides*, Fig. C: The microscopic picture of *Aspergillus fumigates*, D: Microsccopic picture of *Absidia* sp. E: microscopic picture of *Cladosporium* sp. F: Microscopic picture of *Aspergillus terreus* G: microscopic picture of *Penicillium chrysogenum* H: microscopic picture of *Fusarium pallidoroseum*

Conclusion

Fungi are an important constituent of the soil micro biota along with soil biomass and bacteria varies depending on soil depth and nutrient conditions. This study deals with the primary screening, and characterization of mycoflora, isolated from two soil samples. The soil samples were collected from agricultural fields like cotton and cucumber of five different locations in the months of January 2018 to November 2020, at Koppal District, Karnataka, India. In the cotton field, the total number of fungal isolates is 51,550 per gram of soil; where as in cucumber field, the total number of fungal isolates is 8, 21, 800 per gram of soil. In cotton field, the highest percentage contribution was shown by Basidiomycetes (18.60%); whereas in cucumber field, the highest percentage contribution is shown by *Aspergillus niger* (27.5%). In cotton field, 14 different species belonging to 9 genera was observed. Among which *Aspergillus* species is dominating over other species. In Cucumber field, 9 different species belonging to 5 genera were observed. Among which *Fusarium* sp. is dominating over other species. *Aspergillus* sp. is dominating over other species in both cotton and cucumber field. The second dominant species in both the fields is *Fusarium* sp. In cotton fields, amongst the isolates, 6 *Aspergillus* species (*A. niger, A. flavus, A. fumigatus, A. awamori, A. terreus, Aspergillus* sp.) and In cucumber fields among the isolates 3 *Aspergillus* species (*A. niger, A. flavus, A. awamori*,) were characterized and percentage occurrence of the soil mycoflora was statistically evaluated. the *Trichoderma* sp was isolated from the soil samples of cotton and cucumber plants growing areas of koppal district, may be controlling the wilt disease in cotton and cucumber plants fields and act like bio-control agent.

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