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Studies on Rhizosphere soil mycoflora in different agriculture crop field's in Karnataka's Northern dry zone

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Abstract

The Rhizosphere soil samples were collected from various agricultural crop fields of Bajra and Pigeon pea in seven districts of the Northern Dry Zone Karnataka,during the rabbi season.By using a serial dilution method, those collected samples were inoculated in Martins Rose Bengal Agar (MRBA) medium and supplemented by suitable antibiotics such as chloramphenicol which inhibits the growth of bacteria.The main objective of this study was to investigate the fungal diversity in various agricultural crop fields of Bajra and Pigeon pea in Karnataka's Northern Dry Zone (India). There were a total of 1021 colonies isolated. About 34 species of fungi comprising 7 families and 11 genera were isolated and identified, while 6 strains remained unknown. Morphological identification and characterization of the soil isolates were carried out by using authentic soil fungi manuals. Deuteromycotina will have the most fungal colonies, while zygomycotina had the lowest. *Aspergillus* flavus, *A. fumigatus, A. nidulans, A. niger, A. terreus, Trichoderma* sps, *Paecilomyce* sps, and *Verticillium* sps were most common isolates.The mycoflora's percentile contribution was visibly and statistically investigated.

Key words : Culture media, Isolate, Fungal Diversity, Northern Dry Zone, Rhizosphere.

The earth is home to a vast microbial flora, which can be found in all varieties of soils. These microorganisms may interact with plants, resulting in beneficial effects in some cases and disastrous outcomes in others. Fungi are present as mycelia fragments, rhizomorphs, or spores and constitute an essential component of the soil microbiota. They have an important role in the nourishment of soils and plants. As they secrete a wide range of degraded enzymes that attack virtually any organic substance, fungi are saprophytic, meaning they live on dead and decaying organic matter, breaking it down and transforming it into forms that become available to higher plants. Fungi are primary stakeholders in the

recycling of natural waste in our environment because of their degradative activities. Unfortunately, their degradative ability also promotes the growth of undesirable fungi that degrade beneficial materials.

Fungi are worldwide in distribution and thrive in a variety of settings in nature, requiring various essential ingredients for development and reproduction. These are isolated in the laboratory and placed on a special culture medium for cultivation, preservation, microscopy, and biochemical and physiological characterization. The species richness of a fungal community and the relative abundance of specific species have been used as indicators of the group's functional activities in a given habitat. Fungi, bacteria, and actinomycetes occupy a variety of environments and substrates, and in addition to causing disease, they play an important role in plant health and productivity. Fungi play a crucial function in the soil ecology, and their job is exceedingly complicated. They are widely spread geographically and have been found in a variety of environments, primarily in soils and decaying plants. A nutrient-rich soil is necessary for growing a robust and nutritious crop. Chemical, physical, and biological approaches can all be used to assess soil parameters. These qualities are frequently related to the parent material, the atmosphere, the relief, the living creature, and the passage of time. Nutrient compositions in the ecosystem are gradually gathered through the bio resorbable organisms, atmosphere, and litter fall, chemical, physical and biological. The crop structure and agricultural management systems being used by local people have a big impact on the physico-chemical properties of agro ecosystem soils.

The physical features of the soil and the productivity of agricultural areas are influenced by soil organic matter (SOM). Continuous cultivation, on the other hand, results in significant losses of SOM and other nutrients. Soil permeability is reduced as a result of cultivation practises that damage soil structure, and these changes have an impact on soil quality and agricultural output. However, considering agricultural productivity is mostly dependent on soil quality, agricultural soil characteristics are the most essential link between farming practises and sustainable agriculture. Agricultural goods (food, fibre) and crop residues extract soil nutrients (nitrogen, phosphorus, and potassium) from the soil.The preservation of mycoflora variety in farming areas is becoming increasingly important for the development of sustainable agriculture. Farmers, agronomists, researchers, and microbiologists can use the studies on fungal diversity and percentile contribution, as well as the periodic occurrence of soil mycoflora, for future activities related to soil ecosystem conservation, soil microbial variety conservation, and sustainable agriculture[23]. Banana (*Musa paradisiaca*) is a widely produced and valuable commercial fruit crop in tropical and subtropical regions of the world. It is grown in 120 countries on a total area of 5014.06 hectares. Bananas are India's second most significant cash crop, with an area of 830.5 thousand hectares under cultivation and a total yield of roughly 29, 779.91 thousand tonnes. It is a beloved fruit of all classes of society because to its availability, affordability, varietal range, flavour, nutritional and medicinal value[12].

Tamil Nadu, Maharashtra, Gujarat, Andhra Pradesh, and Karnataka are the main banana-growing states. Jalgaon district ranks

first in Maharashtra for banana output, with a total of 48000 hector under this crop. Jalgaon generates more than 16 percent of India's bananas and consequently 3% of global banana production. According to Mahabanana, a Maharashtra-based organization of banana producers, Jalgaon is home to 66 percent of Maharashtra's banana-growing territory. As a result, the current study focused on fungal diversity in the rhizosphere soil of a banana crop field.

Figure 1. Agro climatic zones of Karnataka state

Study site and location: Northern Dry Zone:

Geography: At an altitude of 300m-460m above the mean sea level, 76065' E longitude, and 17025N latitude situated at the Northern Dry Zone. On the north, it is bordered by the North Eastern dry zone; on the south, it is bordered by the Central zone; on the west, it is bordered by the Northern transition zone; and on the east, it is bordered by Andhra Pradesh. The climate is dryness and the Annual rainfall ranges from 500mm to 625mm and about 420mm or about 52%of yearly rainfall is received during Kharif season (Jun-Sep) and an average normal rainfall of 91mm in the pre-monsoon (Jan-May) and 149mm in the northeast monsoon (Oct-Dec). It is essential to understand the basic rhizosphere and rhizoplane microbial ecology in cultivated Agricultural crop fields. The agroclimatic zones of Karnataka are shown in Fig. 1.

Rhizosphere soil samples were collected from 14 different agricultural crop fields in 7 different districts in Northern Dry Zone Karnataka (INDIA) during the end of the kharif season. Rhizosphere soil was dug up to a depth of 15 cm to 20 cm and a mass has been placed in small sterilised Polythene bags and brought to the laboratory for further investigation. The location of the sample collection is depicted in (Table-1).

Table-1. Site of Soil sample collection and season

Sl/No	Collection site and District	Season	Rhizosphere soil samples of agricultural Crops fields	
			Bajra crop	Pigeon pea crop
1.	Bijapur	Rabbi season	B-1	P-1
2.	Baglkot	Rabbi season	B-2	P-2
3.	Gadag	Rabbi season	B-3	P-3
4.	Belagavi	Rabbi season	B-4	P-4
5.	Koppal	Rabbi season	B-5	P-5
6.	Bellary	Rabbi season	B-6	P-6
7.	Davangere	Rabbi season	B-7	P-7
Total 14 Soil Samples			7	7

Isolation of fungi from rhizosphere soil samples :

The Rhizosphere soil fungi were isolated using methods, namely the serial dilution technique on two different media, chiefly Martin's Rose Bengal agar (MRBA) and Potato dextrose Agar (PDA) medium supplemented with 1 percent antibiotic, chloramphenicol, which restricts bacterial growth.Method of soil dilution:[27,28] to create a microbial suspension 10-1 to 10-5, 1 gram of soil sample was diluted in 10 ml of sterilized distilled water. To isolate fungus, dilutions of 10-2 to 10-5 were utilized. In a petriplate, 1 ml of dilution was obtained from each serial dilution sample in triplicate and 15 ml medium was added. The Petriplates were incubated for 72 hours at 28°C.

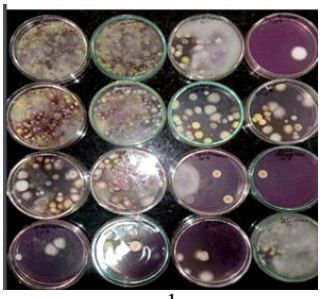
Identification of soil fungus :

Literature was used to identify the fungi.(Soil fungi handbook, Manual of soil fungi-Gillman[10], (The illustration of imperfect fungi-Barnet[2]), Hypomycetes- An account of Indian species C.V. Subramanian Statistical study of isolated soil fungi: with the assistance of KUVEMPU University resource persons With dilution factors, population density is represented in colony forming units (CFU) per gram soil. Each isolate's percent contribution was computed using

$$\% \text{ Contribution} = \frac{\text{Total no. of an individual}}{\text{Total No. of CFU of all sps}} \text{ X } 100$$

CFU stands for colony forming units:

Species diversity indices were calculated using the following formula: The following formula was used to construct species diversity indices: Simpson diversity index: $D = € (n / N)$ and Shannon diversity index: $Hs = - € (Pi) (logPi)$.



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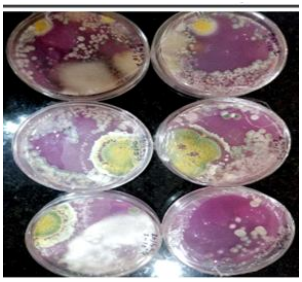

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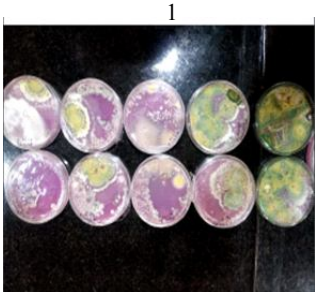

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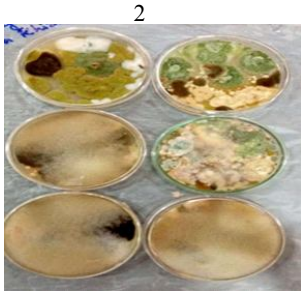

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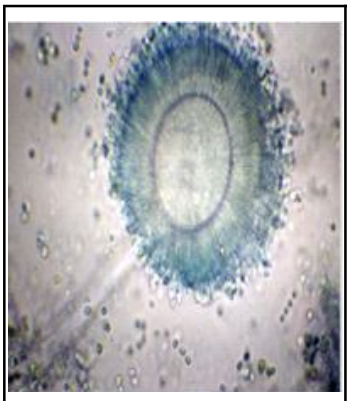
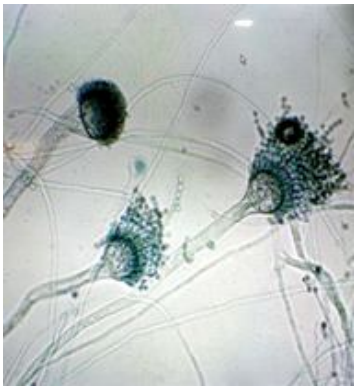
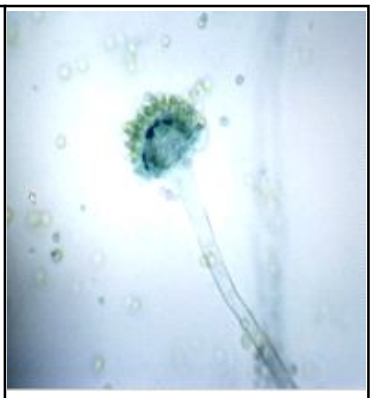
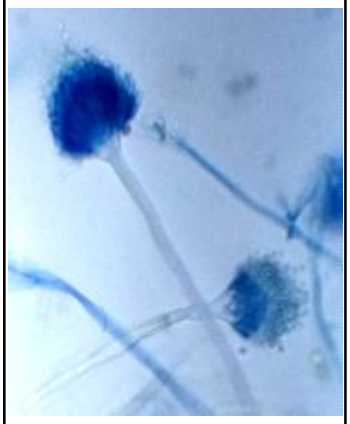
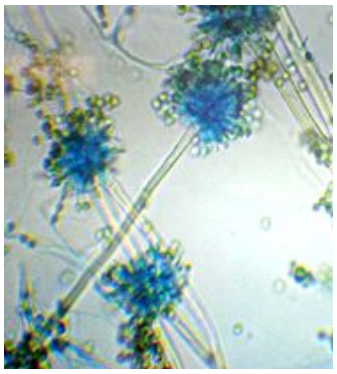
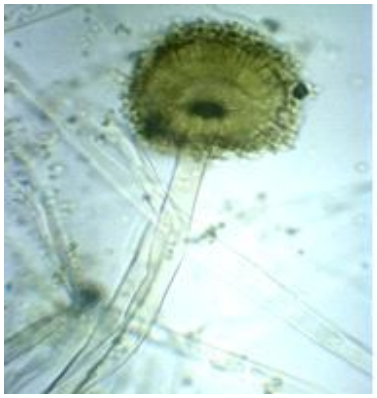
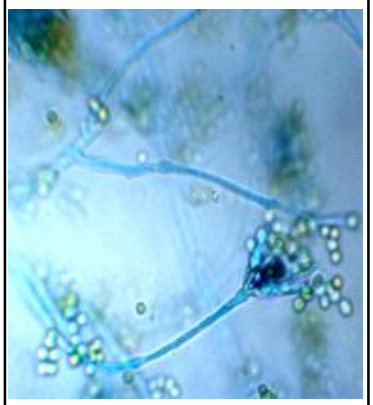
Fig. 1-4. Serial dilution of rhizosphere soil of *Bajra* & Pigeon pea crop flelds (10^3-10^5) in Martin's Rose Bengal Agar medium.

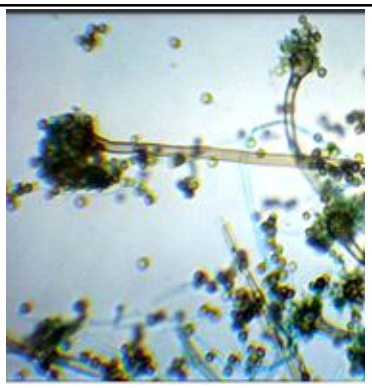
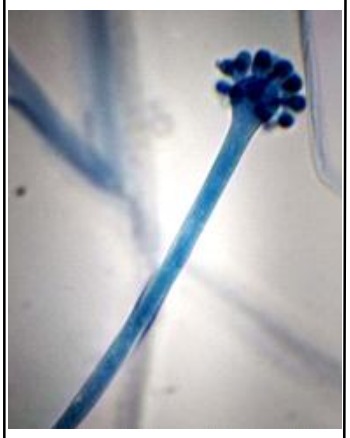
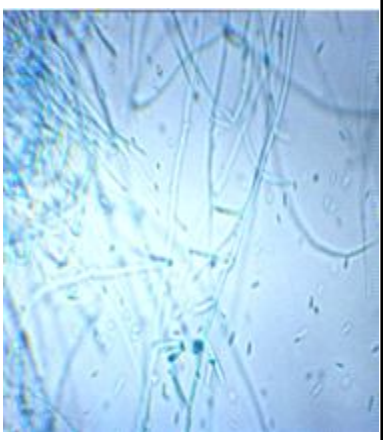
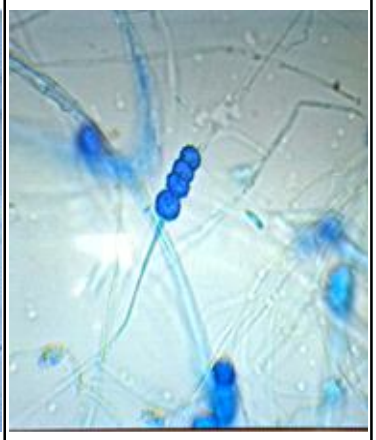
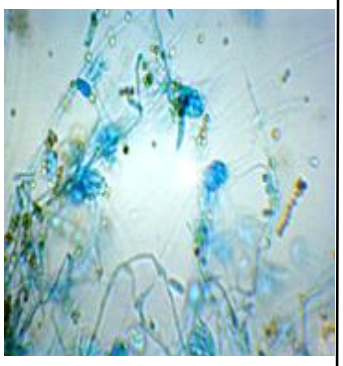
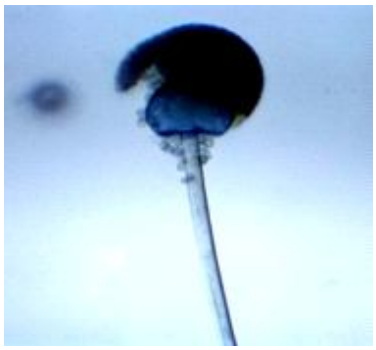
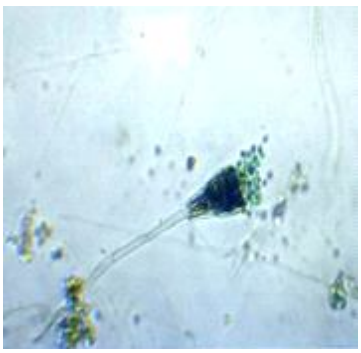
Table-2. Frequency of rhizosphere soil in different Bajra crop fields on Martin's Rose Bengal Agar medium

Sl. no	Name of the fungal species	Average no. of total colonies	Bijapur	Baglkot	Gadag	Belagavi	Koppal	Bellary	Davangere
			Bajra	Bajra	Bajra	Bajra	Bajra	Bajra	Bajra
1	*Aspergillus niger*	34	7	5	4	7	6	2	3
2	*A. ustus*	15	-	6	-	7	-	-	2
3	*A. candidus*	13	-	6	4	-	-	-	3
4	*A. repens*	20	5	4	-	7	4	-	-
5	*A. flavipes*	8	-	-	-	-	3	-	5
6	*A. versicolor*	17	7	3	-	5	-	2	-
7	*A. sydowii*	19	-	-	4	7	-	3	5
8	*A. terrus*	9	-	-	6	-	-	-	3
9	*A. nidulans*	7	-	-	-	7	-	-	-
10	*A. flavus*	25	6	4	7	-	5	3	-
11	*A. ochraceus*	20	6	-	-	8	-	6	-
12	*A. tamarii*	14	8	6	-	-	-	-	-
13	*A. fumigatus*	14	-	7	-	4	-	3	-
14	*Fusarium soloni*	10	-	-	6	-	-	4	-
15	*F. oxysporum*	21	7	5	-	9	-	-	-
16	*F. redolens*	8	-	-	-	-	-	3	5
17	*Cinninghamella.echinulata*	13	7	-	-	4	-	2	-
18	*C. bertholletiae*	15	-	6	-	4	-	3	2
19	*Absidia reflexa*	10	-	-	4	6	-	-	-
20	*Paecilomyces variotii*	17	-	5	3	6	-	3	-
21	*Penicillium citrinum*	19	6	4	6	-	3	-	
22	*P. aurantigorisum*	15	-	7	-	4	4	-	-
23	*P. chrysogenum*	11	7	-	-	-	-	4	-
24	*Mucor hiemalis*	20	-	-	6	9	-	-	5
25	*Mucor racemosus*	29	7	5	6	-	7	-	4
26	*Rhizopus microsporus*	29	5	7	4	9	4	-	-
27	*Rhizopus oryzae*	9	-	-	-	-	4	-	5
28	*Rhizopus oligosporus*	18	-	7	-	-	6	-	5
29	*Trichoderma atroviride*	10	4	-	-	-	-	-	6
30	*T. pseudokononGii*	27	6		5	4	4	5	3
31	*T. viride*	11		2		5	4		
32	*T.harzianum*	34	4	6	4	4	6	3	7
33	*Syncephalastrum* sp.	18	5		4		6	-	3
34	*Verticillium dahliae*	10	-	6	-	4	-	-	—
	Total	569	97	101	73	120	66	46	66
	% of Contribution		17.047	17.75	12.83	21.09	11.6	8.08	11.6

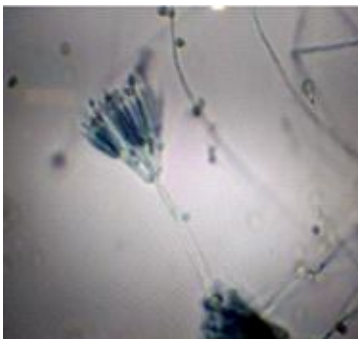
Table-3. Frequency of rhizosphere soil mycoflora in different Pigcon pea crop fields grown on Martin's Rose Bengal Agar medium

Sl. no	Name of the fungal species	Average no. of total colonies	Bijapur	Baglkot	Gadag	Belagavi	Koppal	Bellary	Davangere
			Pigeon pea	Pigeon pea	Pigeon pea	Pigeon pea	Pigeon pea	Pigeon pea	Pigeon pea
1	*Aspergillus niger*	26	7	5		7	3	4	
2	*A. ustus*	17		5	3		4		5
3	*A. candidus*	12			5	4			3
4	*A. repens*	15	4	3		5		5	
5	*A. flavipes*	12		3		6	3		
6	*A. versicolor*	17			5		6		6
7	*A. sydowii*	10					6		4
8	*A. terrus*	19	7	4	4	4			
9	*A. nidulans*	10		4				6	
10	*A. flavus*	18	3		3	3	5	4	
11	*A. ochraceus*	8				3			5
12	*A. tamarii*	10			5		3		2
13	*A. fumigatus*	19	4	3		5	3	4	
14	*Fusarium soloni*	18		3	4	5	6		
15	*F. oxysporum*	22	5	3	4	6	4		
16	*F. redolens*	7			3			4	
17	*Cinninghamella echinulata*	9	3		2				4
18	*C. bertholletiae*	6		3				3	
19	*Absidia reflexa*	7			4		3		
20	*Paecilomyces variotii*	8	3			5			
21	*Penicillium citrinum*	9			5	4			
22	*P. aurantigorisum*	7		4			3		
23	*P. chrysogenum*	14		3	4			7	
24	*Mucor hiemalis*	16					6	5	5
25	*M. racemosus*	11	5		6				
26	*Rhizopus microsporus*	21		5	7	4	5		
27	*Rhizopus oryzae*	17		4		3		6	4
28	*Rhizopu soligosporus*	8	4		4				
29	*Trichoderma atroviride*	6				3	3		
30	*T. pseudokononGii*	17	3	4	5	5			
31	*T. viride*	7				4	3		
32	*T.harzianum*	26	7	5	3		6		5
33	*Syncephalastrum*	7		2					5
34	*Verticillium dahliae*	16			4	6	4		2
	Total	452	55	63	80	82	76	48	50
	% of Contribution		12.16	13.93	17.7	18.14	16.81	10.61	11.06

		
1 *Aspergillus niger*	2 *Aspergillus fumigatus*	3 *Aspergillus flavus*
		
4 *Aspergillus versicolor*	5 *Aspergillus terrus*	6 *Aspergillus tamarii*
		
7 *Aspergillus ustus*	8 *Aspergillus ochraceus*	9 *Aspergillus sydowii*

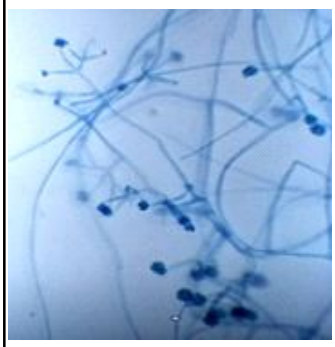
		
10 *Aspergillus flavipes*	11 *Aspergillu snidulans*	12 *Abisidia reflexa*
		
13*Cunninghamella echinulata*	14 *Fusarium soloni*	15 *Fusarium oxysporum*
		
16 *Fusarium redolens*	17 *Mucorr acemosus*	18 *Penicillium chrysogenum*

19 *Paecilomyces variotii*

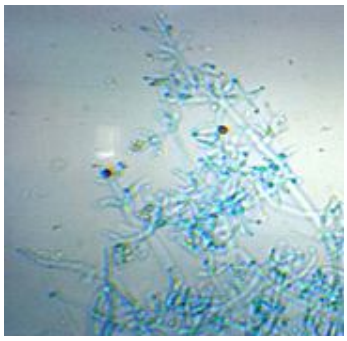


20 *Penicillium citrinum*

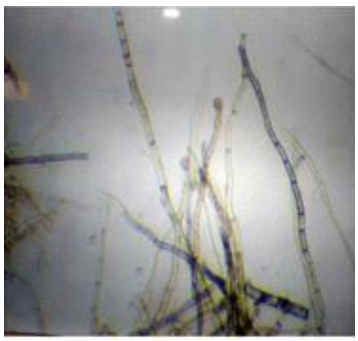
21 *Rhizopus oryzae*



22 *Trichoderma pseudokoningii*

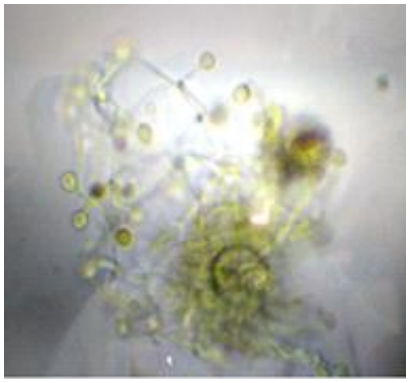


23 *Trichoderma harzianaum*



24 *Trichoderma atroviride*

25 *Syncephalastrum racemomus*



26 *Verticillium dahliae*

Fig. 1-26. Isolated fungi from rhizosphere soil of different *Bajra* & Pigeon pea crop fields.

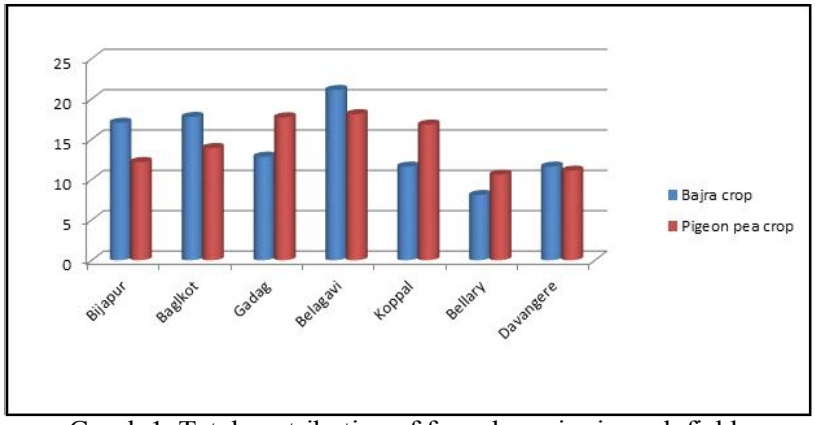
Rhizosphere soil samples were collected from different agricultural crop fields of seven district in northern dry zone of Karnataka Bijapur , Baglkot , Gadag, Belagavi, Koppal, Bellary and Davangere respectively (Table-1) the present investigation Total 1021 fungal colonies were isolated from both Bajra and pigeon pea crops (Figure 1). The isolated and identified fungal colonies and microscopic structure. (Photoplate 1 and 2). Contribution of each isolates were observed as follows (figure 2).

In present investigations four genera were dominant in bajra crop *i.e. Trichoderma* harzianum, *Fusarium oxysporum* and *Aspergillus niger* and *A. flavus*. And in pigeon pea crop three genera were dominant *ie. Trichoderma* harzianum, *Fusarium oxysporum* and *Aspergillus niger* Earlier Ratna Kumar, *et. al*.,[23] also reported *Aspergillus, Fusarium* and *Penicillium* as dominant genera in soil. In 2015 Gnanasekaran, *et.al*.[11] recorded 26 genera from banana field. Out of 26 fungal genera, *Aspergillus* genus was dominant followed by *Penicillium, Trichoderma, Absidia,* and *Fusarium.*

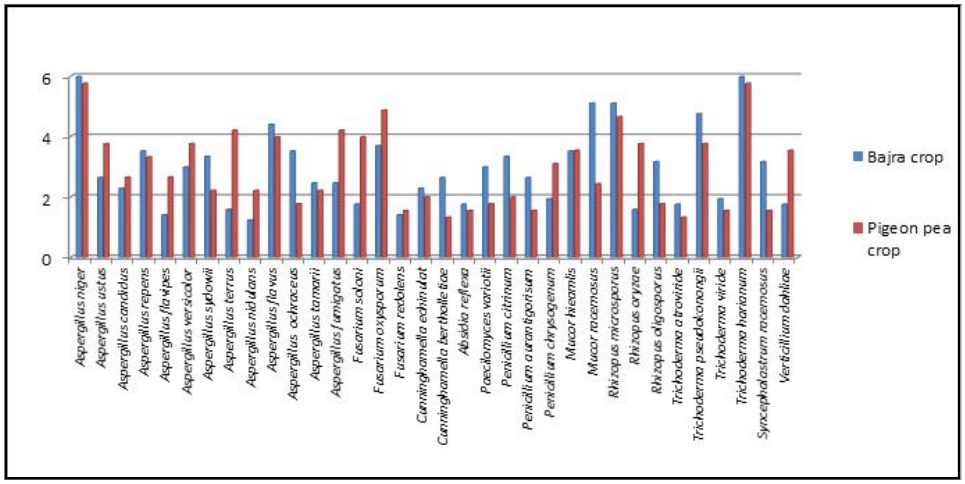
Shannon diversity index was recorded highest fungal diversity in bajra crop found in Belagavi followed by Baglkot, Bijapur (*i.e.* 2.950, 2.907 and 2.754 respectively.) were observed same diversity index. Gadag and Koppal and Davangere showed 20713 2.680 2.605 were found equal diversity index. Minimum diversity index were noted in Bellary 2.586 (Table-2). In Simpson diversity index, Maximum diversity index were observed in Belagavi and Baglkot, Bijapur as well as Gadag, Davangere showed equal diversity index, then it decreased in Bellary and Koppal respectively. (Table-2).

Species Richness: Maximum species richness was noted in Belagavi, Baglkot and Bijapur. Species richness was same in Gadag Koppal and Davangere. It was minimum in Bellary (Table-2). The maximum species richness specifies high species diversity present in fields.

Species Evenness: Highest evenness was occurred in Bijapur and then it decreased as following sequences, Bgalkot, Gadag It was equal range in Koppal, Belagavi. Lowest evenness recorded in Davangere. (Table-2). Highest evenness indicates low species diversity observed in fields.



Graph 1. Total contribution of fungal species in each field



Graph 2. Percentage contribution of each species in Seven District

Table-4. Statistical analysis of isolated soil fungi

Sr. No.	Sampling location	Richness		Shannon Diversity indices		Simpson Diversity indices		Evennes	
		Bajra crop	Pigeon pea crop	Bajra crop	Pigeon pea crop	Bajra crop	Pigeon pea crop	Bajra crop	Pigeon pea crop
1.	Bijapur	17.04	12.17	2.754	2.429	0.9450	0.9239	0.9933	0.9777
2.	Baglkote	17.75	13.9	2.907	2.803	0.9532	0.9528	0.9875	0.9896
3.	Gadag	12.83	17.7	2.680	2.907	0.9425	0.9553	0.9857	0.98732
4.	Belagavi	21.09	18.1	2.950	2.857	0.9533	0.9524	0.9847	0.9887
5.	Koppla	11.59	16.8	2.605	2.845	0.9379	0.9519	0.9874	0.9846
6.	Bellary	8.08	10.6	2.586	2.273	0.9410	0.9131	0.9799	0.9873
7.	Davangere	11.59	11.1	2.713	2.438	0.9445	0.9281	0.9785	0.9812

According to the present findings, *Aspergillus niger* (5.97 percent), *Trichoderma harzianum* (5.97 percent), *Rhizopus microsporus* (5.09 percent), and *Aspergillus flavus* (4.393 percent), *Aspergillus niger* (5.46 percent), and *Fusarium* (4.65 percent) contributed the most to the bajra crop in all districts. The statistical study of soil mycoflora (Table-4) clearly shows that species richness and fungal species diversity were both high in the field. However, when species evenness was greatest, species diversity was lowest.

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