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STUDY OF RHIZOSPHERIC MYCOFLORA OF TURMERIC (CURCUMA LONGA L.) GROWING AREAS FROM JHANSI VICINITY

Gazala Rizvi, Priyanka Sinha
Department of Botany
Bundelkhand University Jhansi
U.P. India

Abstract- **Turmeric (Curcuma longa L.) is an ancient and sacred spice of India. It is used in diversified forms as a condiment, flavouring, colouring agents, as curry powder and as a medicine. It is grown by its finger rhizome. Turmeric grows on diversified soil types, but it thrives best in sandy loam well drained soil. Soil is the habitat of a complex microbial community where interaction and the struggle for existence in this microbial world are equally complex. Rhizosphere is a region around roots; it is complex environment inhabiting microorganisms where roots interact with physical, chemical and biological properties of soil. To explore the soil mycoflora from turmeric growing areas of Jhansi vicinity, experiment were conducted during 2018-2019 in Department of Botany, Bundelkhand University Jhansi. The most promising genera/specie abundantently present in the soil mycoflora was also estimated. For this experiment. Soil samples were collected from 15 different turmeric growing areas of Jhansi vicinity. The soil mycoflora was obtained by soil dilution technique. About 25 different fungal species belonging to 18 genera were isolated. These species were member of Phycomycetes, Ascomycetes and Deuteromycetes.**

Keywords: **Turmeric, Phycomycetes, Ascomycetes, Deuteromycetes.**

I. INTRODUCTION

Turmeric (Curcuma longa L.) is an ancient and sacred spice of India. It is used in diversified forms as a condiment, flavouring, colouring agents, as curry powder and as a medicine. It is also used as natural preservatives for fresh fish to extend shelf life (1). India is the largest producer, consumer and exporter of turmeric in the world. The global production of turmeric is around 11 lakh tonnes per annum. India dominates the world production scenario contributing 80% followed by China (8%), Myanmar (4%), Nigeria (3%) and Bangladesh (3%). Turmeric can be grown in diverse tropical with an annual rainfall of 1500 mm or more, under rainfed or irrigated conditions. Though it can be grown on different types of soils, it thrives best in well-drained sandy or clay loam soils with a pH range of 4.5-7.5 with good organic status. Soil health is representing by its continuous capacity to function as a vital living system. Soil microbes directly or indirectly promotes plant growth though the production of phytohormones, enzyme, and by suppressing phytopathogen and insects, so microbes are soil health indicators (2,3) and among these microorganism's fungi are an important component of soil microbiota depending on soil depth and nutrient conditions (4). Large number of rhizospheric microbial species associated with turmeric rhizomes. These microbes involve in modulation of morphological growth, secondary metabolites production, curcumin content and antioxidant properties and disease management (5) so these microorganism play important roles in growth and ecological fitness of their host (6).

II. MATERIAL METHOD

Collection of soil samples-

Soil samples were collected from selected villages belonging to Jhansi vicinity. Soil samples were collected from rhizosphere of turmeric plants, removing the top soil and from 5-6inches in depth. These samples were and kept in sterilised plastic bag and brought to the Department of Botany laboratory for further analysis until experiments the samples were stored at 4ºC. At different growth stages of the crop, sample collection was done from 15 different villages. Five samples were collected from different fields of each village. Overall 125 samples were taken. Sampling was done thrice during crop season in the year 2018-2019 at regular intervals. These samples were used for microbial analysis within twelve hour of the sampling.

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Table 1: Soil samples collected from different pulse fields

S. No.	Site	Villages
1	A	Baruasagar
2	B	Majra
3	C	Jugbai
4	D	Rasoi
5	E	Maharajpura
6	F	Mutra
7	G	Sijwaha
8	H	Raksa
9	I	Ambabay
10	J	Lahar
11	K	Sijwaha
12	L	Marora
13	M	Panhari
14	N	Chakarpur
15	O	Papauni

Isolation and identification of soil fungi-
Soil mycoflora was isolated by soil dilution method as per Johnson et al. (7) and dilution of 10-5 was used to isolate fungi on potato dextrose agar (PDA) medium. The isolated fungi were identified by morphology, colour, and diameter of colony and microscopically by shape of conidia. The identification and classification of fungi was done from identification key of Alexopolus et al (8), Ainsworth and Bisby's (9) and Gillman (1975). It is simple and widely accepted.

Statistical analysis-
Data regarding number of colonies of different fungal flora per gram soil were recorded. The number of colonies per plate in one gram of soil was calculated. The contribution percent of each isolate was calculated by using the following formula**:**
Contribution percentage =

$$\frac{\text{Total no.of CFU of an individual specie}}{\text{Total no.of CFU of all species}} \times 100$$

The data was analysed by the standard statistical methods. The principal component analysis (PCA) of soil fungal community was calculated by using IBM SPSS Statistics 20.

III. RESULT

Rhizospheric mycoflora was isolated from fifteen different villages of turmeric growing areas by soil dilution method. The different fungal species present in the rhizospheric soil were isolated and recorded at all three different stages (early stage, mid stage and harvesting stage) of turmeric. Seventeen fungal species belonging to nine genera were obtained from in all the selected villages. In our study we observed the dominance of ascus fungi belonging to Ascomycotina over the others. Estimation of fungal population was done for all the villages. Amount of the fungi colony forming unit per soil sample at different growth stage of the crop was carried out. Total number of colony forming unit was 1902, 2681and 1757 at early stage of the crop, mid stage of the crop and harvesting stage of the crop respectively (Table 3). Highest contribution percent of fungal population was found in Baruasagar in mid and harvesting stage of the crop (Table 3, Graph1). Maximum fungal species were from genera Aspergillus and Trichoderma. Morphological and cultural characteristic of isolated fungi was recorded in table 2.

The principal component analysis (PCA) of soil fungal community-
The PCA analysis was done at each growth stage of the crop separately. In our study correlation above 0.5 was deemed significant. Interpretation of the principal components was based on finding which fungal species was most strongly correlated with each component. These larger correlations are in boldface in the mentioned table 4, 5 and 6 at early stage, mid stage and harvesting stage of the crop. This suggests that these significantly correlated fungal species possessed a significant impact on the growth of each other. Each principal component indicates that the different growth stage of the crop affects fungal community. The scree plot displays the component number and eigenvalues in the order from largest to smallest. The scree plot shows that the eigenvalues start to from a straight line after five, seven and five principal component in graph 2, 3 and 4 respectively. Therefore, the remaining principal component accounts for a very small proportion of the variability and are probably not significant.

Table 2: Morphological and cultural characterisation of isolated mycoflora

S. No.	Rhizospheric mycoflora	Fungal Species code	Spore Population after		Category of the fungi	Colony Colour	Reverse plate colour
			48 hr	120 hr			
1	Alternaria alternate	FS1	Scanty	Abundance	Pathogenic	Pale brown to olive brown	Grey
2	Aspergillus flavus	FS2	Nil	Moderate	Pathogenic	Yellow-citron green	Golden to red-brown

3	Aspergillus fumigates	FS3	Nil	Moderate	Pathogenic	White at first then Smoky grey green	Olive grey
4	Aspergillus niger	FS4	Scanty	Abundance	Pathogenic	Black	Light yellow
5	Aspergillus oryzae	FS5	Scanty	Abundance	Pathogenic	Greenish yellow or olive green.	Yellow to pale brown
6	Carvularia lunata	FS6	Nil	Moderate	Pathogenic	Grey to deep black	Dark grey
7	Cladosporium sp.	FS7	Nil	Slow	Pathogenic	Blackish brown	Dirty white
8	Emiricella nudilance.	FS8	Scanty	Abundance	Saprophytic	Dark grass green	Pink to violet brown with age
9	Mucor sp.	FS9	Scanty	Abundance	Saprophytic	White to yellow becoming dark-grey	Pale brown to apricot in colour
10	Penicillium citrium	FS10	Nil	Moderate	Saprophytic	White becoming green as conida develop	Bright yellow
11	Penicillium crysogenum	FS11	Nil	Moderate	Saprophytic	Green color center of colony was white yellow	Pale yellow
12	Pythium sp.	FS12	Nil	Moderate	Pathogenic	White	Off white
13	Trichoderma harzianum	FS13	Scanty	Abundance	Saprophytic	Dark green	Light green
14	Trichoderma koningii	FS14	Nil	Slow	Saprophytic	Greenish white to dark/yellow green	Colourless to yellow
15	Trichoderma pseudokoningii	FS15	Nil	Slow	Saprophytic	White pale, yellow to green/ uncoloured to pale yellow	Light yellow.
16	Trichoderma virens	FS16	Scanty	Abundance	Saprophytic	Whitish to light green to deep grass green	Off White to light yellow
17	Trichoderma viride	FS17	Scanty	Abundance	Saprophytic	Dark bluish green to blackish green colour	Dark green

Table 3: Colony forming units (Cfus) and contribution percent of isolated rhizospheric mycoflora at different stages of the crop

S. No	Site	Early stage of the crop		Mid stage of the crop		Harvesting stage of the crop	
		Total Cfus	Contribution Percent	Total Cfus	Contribution Percent	Total Cfus	Contribution Percent
1	A	138	7.26	201	7.50	131	7.46
2	B	120	6.31	178	6.64	118	6.72
3	C	122	6.41	197	7.35	131	7.46
4	D	113	5.94	170	6.34	111	6.32
5	E	126	6.62	174	6.49	114	6.49
6	F	132	6.94	173	6.45	118	6.72
7	G	122	6.41	166	6.19	110	6.26
8	H	127	6.68	176	6.56	114	6.49

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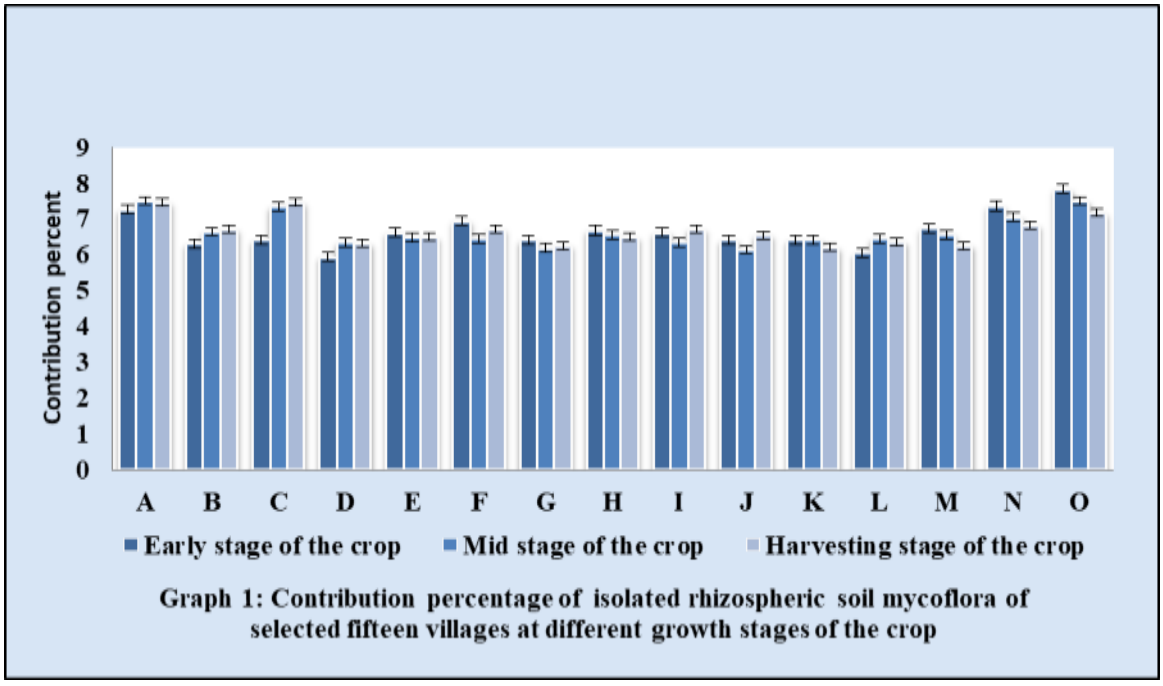
9	I	126	6.62	170	6.34	118	6.72
10	J	122	6.41	165	6.15	115	6.55
11	K	122	6.41	172	6.42	109	6.20
12	L	115	6.05	173	6.45	112	6.37
13	M	128	6.73	176	6.56	110	6.26
14	N	140	7.36	189	7.05	120	6.83
15	O	149	7.83	201	7.50	126	7.17
16	Total	1902		2681		1757	



Graph 1: Contribution percentage of isolated rhizospheric soil mycoflora of selected fifteen villages at different growth stages of the crop

Table 4: Results of principal component analysis showing principal components (PC) with their Eigenvalues proportion of variance (in percent) explained and communalities of FS in early stage of crop

Fungal species	PC-1	PC-2	PC-3	PC-4	PC-5	Communalities
FS1	0.468	-0.239	0.490	-0.425	-0.090	0.704
FS2	**0.682**	0.119	-0.237	0.413	0.182	0.739
FS4	**0.744**	-0.212	0.148	-0.306	0.243	0.774
FS5	-0.140	-0.430	**0.516**	**0.512**	0.198	0.772
FS6	-0.456	0.188	0.070	0.250	-0.143	0.331
FS7	-0.439	0.095	**0.617**	0.119	0.410	0.765
FS9	0.210	**0.561**	-0.294	0.174	-0.331	0.585
FS11	**0.815**	-0.125	0.209	0.387	-0.091	0.881
FS12	0.229	**0.663**	0.388	0.015	0.100	0.653
FS13	-0.087	-0.058	-0.467	-0.283	**0.715**	0.821
FS14	0.001	**0.700**	0.288	0.112	0.222	0.634
FS15	0.060	0.498	0.258	**-0.539**	-0.074	0.614
FS17	0.176	0.298	-0.323	0.202	0.378	0.407

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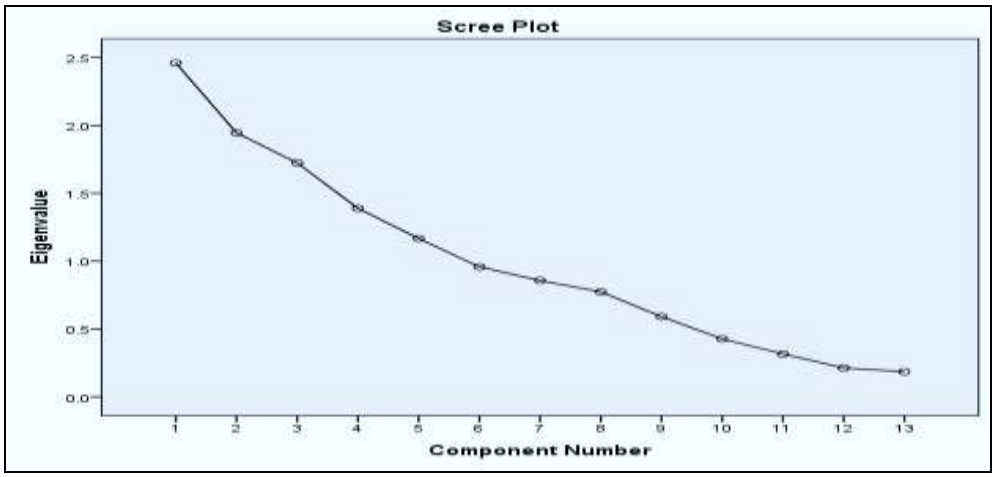
Eigenvalues	2.460	1.944	1.723	1.388	1.166	
Variance explained (%)	18.921	14.957	13.252	10.680	8.969	
Cumulative variance explained (%)	18.921	33.878	47.131	57.811	66.779	



Graph 2- Scree Plot between Principal components and eigenvalue at early stage of the crop

Table 5: Results of principal component analysis showing principal components (PC) with their Eigenvalues proportion of variance (in percent) and communalities of FS in mid stage of crop

Fungal species	**PC-1**	**PC-2**	**PC-3**	**PC-4**	**PC-5**	**PC-6**	**PC-7**	**Communalities**
FS1	**0.704**	-0.385	0.037	0.036	0.051	-0.080	0.115	0.669
FS2	0.120	0.319	**0.799**	0.057	0.049	0.016	-0.121	0.776
FS3	-0.091	0.255	-0.254	**0.691**	-0.009	-0.070	**-0.537**	0.908
FS4	**0.717**	0.001	0.375	0.011	0.213	0.136	-0.199	0.758
FS5	-0.332	-0.419	0.152	0.281	0.316	0.216	**0.545**	0.830
FS6	-0.460	-0.160	-0.079	0.493	0.307	0.476	0.038	0.809
FS7	-0.003	-0.281	-0.184	0.310	**-0.828**	0.021	0.117	0.909
FS8	**0.776**	-0.267	-0.250	0.058	0.002	-0.048	-0.048	0.744
FS9	-0.205	0.051	0.212	0.490	0.026	**-0.598**	0.350	0.811
FS10	**0.725**	0.267	-0.204	0.362	0.035	0.130	0.142	0.809
FS11	0.395	0.084	**0.730**	0.292	-0.126	0.002	-0.085	0.805
FS12	**0.666**	0.006	0.028	0.008	0.131	-0.397	0.148	0.640
FS13	**0.795**	-0.099	-0.167	-0.052	-0.083	0.139	0.207	0.741
FS14	-0.161	**0.573**	-0.469	-0.002	0.334	-0.343	0.050	0.806
FS15	**0.726**	0.278	-0.368	0.078	0.150	0.220	0.016	0.816
FS16	-0.008	**0.817**	-0.173	0.021	-0.208	0.183	0.257	0.840
FS17	0.005	**0.762**	0.307	-0.093	-0.154	0.182	0.265	0.811
Eigenvalues	4.307	2.411	2.099	1.379	1.174	1.090	1.024	
Variance explained (%)	25.335	14.182	12.345	8.110	6.905	6.411	6.024	
Cumulative variance explained	25.335	39.517	51.862	59.972	66.877	73.288	79.312	

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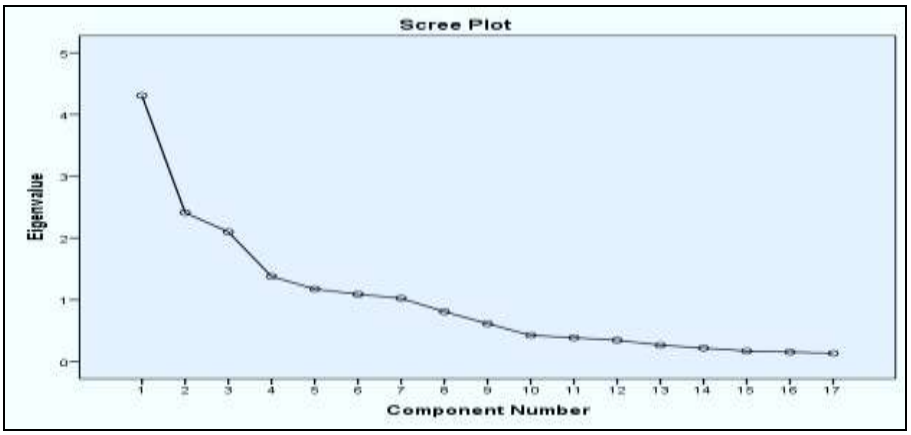

Graph 3- Scree Plot between Principal components and eigenvalue at mid stage of the crop

Table 6: Results of principal component analysis showing principal components (PC) with their Eigenvalues proportion of variance (in percent) and communalities of FS in harvesting stage of crop

Fungal species	**PC-1**	**PC-2**	**PC-3**	**PC-4**	**PC-5**	**Communalities**
FS1	**0.743**	0.051	0.195	0.342	-0.035	0.711
FS2	-0.107	**0.700**	0.268	0.345	0.296	0.780
FS3	-0.192	0.424	**-0.551**	-0.335	0.316	0.732
FS5	-0.046	**-0.629**	0.284	-0.068	0.447	0.683
FS6	-0.401	-0.025	-0.488	0.159	**0.548**	0.725
FS8	**0.859**	0.055	-0.055	-0.087	0.107	0.762
FS9	-0.425	0.187	**0.513**	-0.183	-0.009	0.512
FS12	0.127	0.006	**0.503**	**-0.645**	0.378	0.828
FS13	**0.884**	0.135	0.112	0.060	0.177	0.847
FS14	-0.247	-0.157	0.265	**0.560**	0.382	0.615
FS15	**0.772**	0.076	-0.257	-0.084	0.188	0.710
FS16	-0.164	**0.647**	0.236	-0.042	-0.044	0.505
Eigenvalues	3.161	1.574	1.456	1.162	1.057	
Variance explained (%)	26.344	13.120	12.132	9.681	8.811	
Cumulative variance explained (%)	26.344	39.465	51.597	61.278	70.088	

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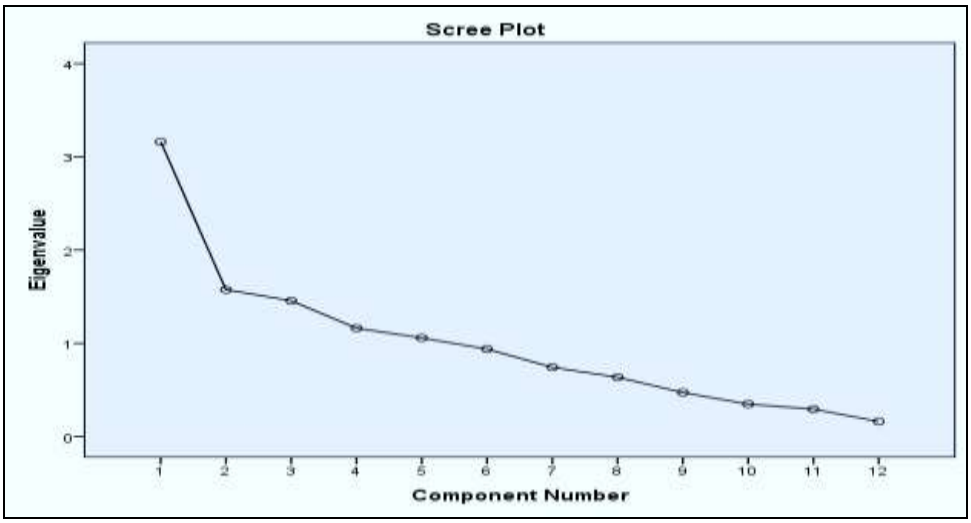

Graph 4- Scree Plot between Principal components and eigenvalue at harvesting stage of the crop

IV. DISCUSSION

Soil fungal diversity plays an important role in soil processes that determine plant productivity. Fungi are present in soil as mycelial bits, rhizomorphs or as spores. Many researchers found different fungal species at different crop field. Our result is consistent with Sarathi et al., (11) who recorded similar results from turmeric growing area of Tamil Nadu. Same fungal genera i.e., Trichoderma, Alternaria, Penicillium, Fusarium, Rhizopus, Mucor and Aspergillus, were reported from pulse field by Chandrashekar et al. (13) and Sinha et al (4). Shrivastava A., (13) accessed sixteen fungal species from rhizospheric soil of ginger growing areas of Baruasagar Jhansi in which maximum number of fungal species were from genera Aspergillus and Trichoderma. The results of the present study reveal important implications regarding the effects of different growth stages of the crop on fungial diversity in a turmeric field. This results indicate the crop growth stages also influence fungi diversity significantly, a result which is inconsistent with the claim that the plant only has a minor influence on the constitution of the rhizosphere fungal community (14, 15).

V. CONCLUSION

The soil fungi obtained from Rhizospheric soil of Turmeric (Curcuma longa L.) was classified into seventeen species belonging to nine genera Ascomycotina was dominant in all of the above. Species of Aspergillus and Trichoderma was dominantly present in all the fields and growth stages of the crop. The PCA of soil fungal community showed greater effect at mid stage of the crop than the early and harvesting stages of the crop. Recently there have been a few studies reports of turmeric disease fields, however to best of our knowledge, the research work on soil mycoflora in turmeric field are limited in Baruasagar, Jhansi.

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