

Qualitative Assessment of Fungal Species Diversity in the Rhizosphere of Chickpea (*Cicer arietinum*)

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Abstract

The Rhizosphere, a dynamic zone of soil surrounding plant roots, hosts a diverse community of microorganisms that significantly influence plant health and soil fertility. This study aimed to assess the qualitative percentage distribution of fungal species inhabiting the rhizosphere of *Cicer arietinum* (chickpea), a major legume crop grown globally. Soil samples were collected from chickpea-growing fields at various growth stages, and fungi were isolated using standard serial dilution and plating techniques on Potato Dextrose Agar (PDA). Morphological and microscopic identification revealed the presence of several dominant fungal genera, including *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, and *Trichoderma*. *Aspergillus* spp. constituted the highest percentage of the fungal community, followed by *Penicillium* and *Fusarium*, indicating their adaptability and competitive dominance in the rhizosphere environment. Qualitatively a total of 67 fungal species were identified from 30 different genera from the rhizosphere and soil of chickpea. The *Aspergillus flavus* and *A. niger* were also commonly present in the rhizosphere. The species those were dominant in the rhizosphere were *F. oxysporum*, *F. moniliforme*, *A. ustus*, *F. semitectum*, *Rhizopus stolonifer* and *Trichoderma* spp. The presence of beneficial fungi such as *Trichoderma* suggests potential plant growth-promoting and biocontrol activity. The results highlight the ecological diversity and functional potential of rhizospheric fungi associated with chickpea, which could be further explored for sustainable agricultural practices and biofertilizer development.

Keywords: Chickpea, Rhizosphere, biofertilizer PDA etc

1. Introduction

Chickpea (*Cicer arietinum* L.) is a rich source of high-quality protein. India is the largest producer of chickpea, accounting for 75 percent and 73 percent, respectively of the world's share in terms of the area under cultivation and production. The constraints to increasing the production of chickpea are twofold; one of them is fungal diseases. Chickpea wilt is a major fungal disease caused by *F. oxysporum* f. sp. *ciceris* is a major constrain to chickpea production globally. The disease is important where the chickpea-growing season is dry and warm. Although actual yield loss is estimated to be 10-12% globally (Nene and

Thapliyal, 1993). The use of antagonistic microorganisms is the best control of Fusarial wilt. Rhizosphere bacteria have proved to be effective biocontrol agents against root diseases of many crop plants (Weller, 1988; Manmeet *et al*, 2002), their antibiotic production now recognized as an important factor in disease suppression (Fernandez, D. and Tantaoui, A. (1994). Marathwada a part of Maharashtra State is well known for semi-arid crops, which is favorable for chickpea cultivation. Many times it is cultivated as a rain fed crop. The yield losses caused by the Fusarial wilt disease amounted to 10 – 15 % in Marathwada. Now-a-day's integrated disease management of various crop diseases has been advocated in order to avoid chemical application. Biological control is an important practice in Integrated Disease Management, which has relied heavily on pesticides, is no longer applicable in many cases due to the lack of reliable control alternatives. Therefore, an alternative approach of biological control of plant pathogens has been recommended in recent years by trends in agriculture towards greater sustainability. So to avoid heavy infection of chickpea wilt, rhizosphere microorganisms are found to be very helpful as biocontrol agents and hence selected in this study. In the present study the survey of chickpea wilt was done in 59 villages wilt was observed 2.00- 20.00 % level.

In this study the rhizosphere of chickpea variety susceptible to *Fusarium oxysporum* f.sp. *ciceri* was selected. There was a variation in the quantity and quality of fungal sp. in the rhizosphere of chickpea varieties. A total of 19 fungal species were isolated from the rhizosphere of chickpea cultivation. Qualitative analysis of fungal spp. in the rhizosphere of resistant and susceptible varieties was also noted. Further the virulence of *F. oxysporum* f.sp. *ciceri* were studied on susceptible variety and it was variable. In addition when the isolates were grown on different media and noted again variation in the growth of isolates.

In order to manage the control 4 isolates of *F. oxysporum* f.sp. *ciceri* were studied using fungicides captan, carbendazim, difenoconazole, thiram, thiophanate methyl and vitavax in the agar medium. It was observed that some isolates of *F. oxysporum* f.sp. *ciceri* were resistant while some of them were sensitive. In physiological studies on different amino acids, carbon, nitrogen, oxides, phosphates and salts, etc. found again variation in the growth. Molecular characterizations of *F. oxysporum* f.sp. *ciceri* were studied by RAPD method. Here it was observed that three groups were present in the population of *F. oxysporum* f.sp. *ciceri* in Marathwada. In order to avoid the use of chemical, biological management of *F. oxysporum* f.sp. *ciceri* isolates was done by using *Gliocladium virens*, *Trichoderma* sp., *Pseudomonas* sp., *Bacillus* sp. and Actinomycetes. In addition, altogether 22 plant extracts were used at both *in vitro* and *in vivo*.

Studies on Rhizosphere

From the very beginning in the 19th century, rhizosphere-research was characterized by multidisciplinary approaches and paralleled diversification and development of novel disciplines in natural sciences. The term 'Rhizosphere' was introduced by Lorenz Hiltner (1904) as soil compartment, influenced by root excretions with impact on activity of beneficial and pathogenic microorganisms. It is closely linked with the development of soil microbiology and phytopathology. Many microbial interactions, which are regulated by specific molecules/signals (Pozo, M.J et al 2004). Many studies have demonstrated that soil-borne microbes interact with plant roots and soil constituents at the root–soil interface (Bowen and Rovira, 1999, Barea *et al.*, 2002b). The great array of root–microbe interactions results in the development of a dynamic environment known as the rhizosphere where microbial communities also interact. The differing physical, chemical, and biological properties of the root-associated soil, compared with those of the root-free bulk soil, are responsible for changes in microbial diversity and for increased numbers and activity of

microorganisms in the rhizosphere micro-environment (Kennedy, 1998). Microbial activity in the rhizosphere affects rooting patterns and the supply of available nutrients to plants, thereby modifying the quality and quantity of root exudates (Bowen and Rovira, 1999; Gryndler, 2000; Barea *et al* 2002). There are three separate, but interacting, components recognized in the rhizosphere. These are the rhizosphere (soil), the rhizoplane, and the root itself. The root itself is a part of the system, because certain microorganisms, the endophytes, are able to colonize root tissues (Kennedy, 1998; Bowen and Rovira, 1999). Microbial colonization of the rhizoplane and/or root tissues is known as root colonization, whereas the colonization of the adjacent volume of soil under the influence of the root is known as rhizosphere colonization (Kumar, B.H. *et al.*, 2006; Kumar, D 2001, Kumar, 2002). Now a day the side effects of agrochemicals, there is an increasing interest in the understanding of co-operative activities among rhizosphere microbial populations and how these might be applied to agriculture (Barea *et al.*, 2004; Lucy *et al.*, 2004). Certain co-operative microbial activities can be exploited as a low-input biotechnology, and form a basis for a strategy to help sustainable, eco-friendly practices fundamental to the stability and productivity of both agricultural systems and natural ecosystems (Kennedy and Smith, 1995). An analysis of the co-operative microbial activities known to effect on chickpea wilt pathogen *F. oxysporum* f.sp. *ciceri*

Studies on Chickpea Rhizosphere

Mycofloral populations were determined in the rhizospheres and control soils. Altogether five varieties were procured from ICRISAT and used in this study. Amongst them, three were resistant and two were susceptible to FOC. The data were collected after 15 days of intervals up to 90 days. Number of fungi in the rhizosphere (R), control soil (S) and the corresponding ratios are given in respective tables. Occurrence of microorganisms in rhizosphere of chickpea germplasm are presented in Table 5.1 to 5.15, was studied at various growth periods. Antagonistic fungi and bacteria were isolated and used for further study. From the data it is clear that quantitatively there were significant variation in the rhizosphere and soil mycoflora and their corresponding R/S ratios among five varieties of chickpea. However, significant variation between growth periods of these varieties was found in case of rhizosphere mycoflora.

Material and Methods

Studies on Rhizosphere

There are many techniques for evaluation of rhizosphere microflora, the soil dilution and plate count method is widely followed the rhizosphere studies. The effect can also be noted by microscopic examinations. The chickpea is carefully uprooted from the field and the superfluous soil dislodged by gentle agitation. The root and adhering soil are placed in sterile petriplates and dilution series is prepared and plate counts are made.

The composition of Martins rose Bengal streptomycin agar medium as follows:

Dextrose - 10 gms
Peptone - 5 gms
KH₂PO₄ - 1 gms
MgSO₄ - 0.5 gms
Rose bengal - Trace
Streptomycin – 0.03 gms
Agar-Agar – 20 gms

Distill water -1000 ml

Dissolve 1 gms of streptomycin sulphate in 100 ml sterile distilled water, after opening the viol aseptically, add 0.3 ml of streptomycin in solution to each 100 ml of the basal medium after it is cooled. All these constituents were mixed well in conical flask (1000 ml) and the medium is transferred to four conical flasks (500 ml). The medium is sterilized in autoclave at 15pp for 20 minutes. All the glasswares, petri-plates, pipettes were sterilized. When medium gets cooled down it is transferred work were conducted there.

The ‘**Soil serial dilution plate techniques**’ was employed by following protocol.

1. Collect the root sample with soil of the plant under study in sterile polythene bag and brought to the laboratory.
2. Separate rhizosphere soil from 5 to 6 roots with the help of brush in a sterile petri-plate.
3. 1 gm. of soil is transferred to 9 ml. of sterile water blank. Shake it well for about 15 minutes. It make a the 1/10 dilution.
4. Take 1ml. of 1/10 diluted soil sample and transfer it to another 9 ml. sterile water blank so that the dilution will be 1/100.
5. Take another 1ml. of 1/100 dilution soil sample and transfer it to 9 ml. water blank so that the dilution will be 1/1000, from this again 1 ml. was taken and transfers it to 9 ml. sterile water blank to have 1/10,000 sample called as serial dilution sample technique.
6. Same procedure is used for isolation of fungi from non- rhizosphere soil also.

Qualitative composition:

It was seen that quantitative fungal organisms were more in number in the rhizosphere, when compared with soil. Hence the rhizosphere effect was always more than one. The rhizosphere mycoflora reached the highest values when chickpea plants were at the flowering stage noted in ICC 2072 & ICC 14669. It was high before flowering stage in ICC 4495, ICC 4951 & ICC 12475. There was also variation in the number of fungi in the rhizosphere and / or soil. Number was found to be higher at the flowering stage and the maturation stage of chickpea plants. Qualitatively a total of 67 fungal species were identified from 30 different genera from the rhizosphere and soil of chickpea. Interestingly it was also seen that the pathogens such as *Aspergillus flavus* and *A. niger* were also commonly present in the rhizosphere. The species those were dominant in the rhizosphere were *F. oxysporum*, *F. moniliforme*, *A..ustus*, *F. semitectum*, *Rhizopus stolonifer* and *Trichoderma* spp. They occurred more than 20% of the total fungal colonies. There was also a variation in the occurrence of their different fungal species at different growth period of chickpea. The R / S ratio in all the varieties is more than one. It was 5.97 in ICC 2072 in resistant category and 5.00 in ICC 12475 in susceptible category. In order to study the rhizosphere mycoflora amongst three varieties; it was observed that twenty-four different fungal genera were isolated from resistant varieties however it was twenty in two susceptible varieties.

Qualitative percentage of fungal species in the rhizosphere of chickpea at various growth periods in variety ICC 2072 resistant to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus aculeatus</i>	--	--	01.59	--	--	--
2	<i>A. flavus</i>	--	--	--	12.20	--	--
3	<i>A. fumigatus</i>	06.25	--	--	--	--	--
4	<i>A. nidulans</i>	--	--	--	02.44	--	--
5	<i>A. niger</i>	--	01.69	09.53	--	04.34	46.15
6	<i>A. terreus</i>	37.50	--	--	14.63	--	--
7	<i>A. ustus</i>	--	28.81	07.93	17.07	--	--
8	<i>Cladosporium oxysporum</i>	--	--	03.17	04.88	--	--
9	<i>Fusarium moniliforme</i>	25.00	--	--	--	--	--
10	<i>F. oxysporum</i>	09.37	66.10	49.21	04.88	86.96	15.38
11	<i>F. semitectum</i>	--	--	--	07.32	--	23.07
12	<i>Mucor circinelloides</i>	--	--	03.17	--	--	--
13	<i>M. varians</i>	--	03.39	--	--	--	--
14	<i>Penicillium miczynskii</i>	--	--	--	02.44	--	--
15	<i>P. rubrum</i>	15.62	--	01.59	--	--	--
16	<i>Phoma herbarum</i>	--	--	--	12.20	--	--
17	<i>Rhizoctonia albus</i>	--	--	03.17	--	--	--
18	<i>R. bataticola</i>	--	--	--	07.32	--	--
19	<i>Rhizopus stolonifer</i>	06.25	--	19.04	04.88	--	15.38
20	<i>Torula herbarum</i>	--	--	--	02.44	--	--
21	<i>Trichoderma harzianum</i>	--	--	--	--	08.70	--
22	Sterile mycelium	--	--	01.59	07.32	--	--
Total species		32	59	63	41	23	13

Qualitative percentages of fungal species in the non rhizosphere of chickpea at various growth periods in variety ICC 2072 resistant to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus flavus</i>	--	--	--	--	--	16.66
2	<i>A. fumigatus</i>	--	--	06.06	--	--	--
3	<i>A. niger</i>	28.57	50.00	03.03	20.00	60.00	--
4	<i>A. nidulans</i>	--	--	06.06	--	--	--
5	<i>A. terreus</i>	--	--	--	10.00	--	--
6	<i>A. ustus</i>	--	10.00	06.06	--	10.00	--
7	<i>Fusarium moniliforme</i>	28.57	--	--	--	--	--
8	<i>F. oxysporum</i>	42.85	--	39.39	50.00	--	50.00
9	<i>F. semitectum</i>	--	--	--	--	--	33.33
10	<i>Cunninghamella echinulata</i>	--	--	06.06	--	--	--
11	<i>Cladosporium oxysporum</i>	--	10.00	06.06	--	--	--
12	<i>Penicillium chrysogenum</i>	--	--	03.03	--	--	--
13	<i>P. funiculosum</i>	--	10.00	09.09	10.00	--	--
14	<i>Phoma glomerata</i>	--	20.00	03.03	--	--	--
15	<i>Torula herbarum</i>	--	--	03.03	--	--	--
16	Sterile mycelium	--	--	09.09	10.00	20.00	--
Total species		7	10	33	10	10	6

Qualitative percentage of fungal species in the rhizosphere of chickpea at various growth periods in variety ICC 14669 resistant to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus flavus</i>	06.06	--	01.32	--	--	--
2	<i>A.niger</i>	--	11.32	--	25.93	26.32	42.10
3	<i>A. terreus</i>	--	--	--	07.41	07.89	--
4	<i>A. ustus</i>	12.12	--	--	03.70	--	--
5	<i>Absidia spinosa</i>	--	--	10.52	--	--	--
6	<i>Blastomyces dermatitides</i>	--	--	--	03.70	--	--
7	<i>Cladosporium oxysporum</i>	--	05.66	--	14.81	15.79	--
8	<i>Fusarium moniliforme</i>	36.36	22.64	17.10	--	31.58	--
9	<i>F.oxysporum</i>	30.30	60.37	43.42	--	02.63	31.58
10	<i>F.semitectum</i>	--	--	--	--	--	15.78
11	<i>Gliocladium virens</i>	--	--	--	03.70	--	--
12	<i>Humicola fuscoatra</i>	--	--	--	03.70	--	--
13	<i>Helminthosporium hawaiiense</i>	--	--	--	03.70	--	--
14	<i>Penicillium funiculosum</i>	06.06	--	--	--	--	--
15	<i>Papulaspora pallidula</i>	--	--	01.32	--	--	--
16	<i>Rhizoctonia bataticola</i>	--	--	--	--	13.16	--
17	<i>Rhizopus stolonifer</i>	09.09	--	18.42	25.92	02.63	10.52
18	<i>Staphylotrichum coccosporum</i>	--	--	--	03.70	--	--
19	Sterile mycelium	--	--	07.89	03.70	--	--
Total species		33	53	76	27	38	38

Qualitative percentages of fungal species in the nonrhizosphere of chickpea a various growth periods in variety ICC resistant susceptible to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus flavus</i>	12.50	11.76	10.00	--	--	03.84
2	<i>A.niger</i>	08.30	08.82	--	--	13.33	11.53
3	<i>A. terreus</i>	--	--	--	07.69	20.00	--
4	<i>A. ustus</i>	08.30	--	--	07.69	--	--
5	<i>Cladosporium herbarum</i>	--	--	--	03.85	06.66	--
6	<i>C.oxysporum</i>	--	08.82	05.00	--	--	--
7	<i>Fusarium moniliforme</i>	25.00	38.23	--	15.38	--	--
8	<i>F.oxysporum</i>	37.50	23.52	50.00	42.30	46.66	38.46
9	<i>F. semitectum</i>	--	--	--	--	06.66	26.92
10	<i>Humicola fuscoatra</i>	--	--	--	03.85	--	--
11	<i>Helminthosporium hawaiiense</i>	--	--	05.00	--	--	--
12	<i>Phoma glomerata</i>	--	--	10.00	--	--	--
13	<i>Penicillium rugulosum</i>	--	--	20.00	--	--	--
14	<i>Rhizopus stolonifer</i>	08.30	--	--	03.85	--	07.69
15	<i>Trichoderma atroviride</i>	--	08.82	--	03.85	--	11.53
16	<i>Verticillium puniceum</i>	--	--	--	03.85	--	--
17	Sterile mycelium	--	--	--	07.69	06.66	--
Total species		24	34	20	26	15	26

Qualitative percentage of fungal species in the rhizosphere of chickpea at various growth periods in variety ICC 4495 resistant to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus flavus</i>	--	--	02.94	--	--	05.13
2	<i>A.niger</i>	55.55	14.63	07.35	15.87	08.00	--
3	<i>A. nidulans</i>	--	--	--	--	--	20.51
4	<i>A. terreus</i>	--	--	--	14.28	24.00	--
5	<i>A. ustus</i>	--	12.19	08.82	--	--	--
6	<i>Curvularia brachyspora</i>	--	--	01.47	--	--	--
7	<i>Cladosporium oxysporum</i>	--	01.21	04.41	--	--	--
8	<i>Fusarium moniliforme</i>	14.81	02.43	--	01.59	--	--
9	<i>F.oxysporum</i>	14.81	69.51	51.47	47.61	60.00	33.33
10	<i>F.semitectum</i>	--	--	--	--	--	35.89
11	<i>Humicola fuscoatra</i>	--	--	--	03.17	--	--
12	<i>Helminthosporium hawaiiense</i>	07.41	--	--	--	--	--
13	<i>Mucor circinelloides</i>	--	--	04.41	--	--	--
14	<i>Penicillium funiculosum</i>	--	--	02.94	--	--	--
15	<i>Rhizoctonia solani</i>	--	--	--	01.59	--	--
16	<i>Rhizopus stolonifer</i>	07.41	--	10.29	--	04.00	--
17	<i>Tetracocco sporium sacchari</i>	--	--	02.94	--	--	--
18	<i>Torula caligans</i>	--	--	02.94	--	--	--
19	<i>Torula herbarum</i>	--	--	--	03.17	--	--
20	<i>Trichoderma pseudokoningii</i>	--	--	--	06.34	--	05.13
21	Sterile mycelium	--	--	--	06.34	04.00	--
Total species		27	82	68	63	25	39

Qualitative percentages of fungal species in the non rhizosphere of chickpea at various growth periods in variety ICC 4495 resistant to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Alternaria alternata</i>	--	--	--	15.15	--	--
2	<i>Aspergillus flavus</i>	--	--	--	--	--	11.11
3	<i>A. fumigatus</i>	--	--	15.38	06.06	--	--
4	<i>A. niger</i>	55.55	35.29	10.25	09.09	33.33	--
5	<i>A. nidulans</i>	--	--	10.25	06.06	--	--
6	<i>A. petratii</i>	11.11	--	--	--	--	--
7	<i>A. terreus</i>	--	--	--	03.03	--	--
8	<i>A. ustus</i>	--	05.88	05.12	--	--	--
9	<i>Cladosporium oxysporum</i>	--	02.94	--	--	11.11	--
10	<i>Fusarium moniliforme</i>	11.11	--	--	--	--	--
11	<i>F. oxysporum</i>	14.81	08.82	30.76	--	--	55.55
12	<i>F. semitectum</i>	--	41.17	--	18.18	--	--
13	<i>Mucor circinelloides</i>	--	--	07.69	--	--	--
14	<i>Penicillium corylophilum</i>	--	--	05.12	--	--	--
15	<i>P. funiculosum</i>	07.40	--	--	--	--	--
16	<i>P. oxalicum</i>	--	05.88	--	09.09	11.11	--
17	<i>Phoma glomerata</i>	--	--	--	09.09	--	--
18	<i>Rhizopus arrhizus</i>	--	--	--	12.12	--	--
19	<i>R. stolonifer</i>	--	--	12.82	--	--	--
20	<i>Syncephalastrum racemosum</i>	--	--	--	--	11.11	--
21	<i>Trichoderma koningii</i>	--	--	--	03.03	--	33.33
22	Sterile mycelium	--	--	07.69	09.09	33.33	--
Total species		27	34	39	33	09	18

Qualitative percentage of fungal species in the rhizosphere of chickpea at various growth periods in variety ICC 4951 susceptible to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus flavus</i>	--	--	--	03.84	--	--
2	<i>A.niger</i>	57.69	06.66	14.13	07.69	16.66	06.66
3	<i>A. nidulans</i>	--	--	--	30.76	--	--
4	<i>A. terreus</i>	--	--	--	--	16.66	--
5	<i>A. ustus</i>	--	28.33	01.08	19.23	05.55	--
6	<i>Colletotrichum dematium</i>	--	--	01.09	--	--	--
7	<i>Cunninghamella echinulata</i>	--	--	02.17	--	--	--
8	<i>Cladosporium herbarum</i>	--	--	--	07.69	--	--
9	<i>Drechslera australiensis</i>	--	--	--	--	05.55	--
10	<i>Fusarium moniliformae</i>	--	20.00	13.04	--	--	--
11	<i>F.oxysporum</i>	23.07	--	44.56	23.07	05.55	56.66
12	<i>F.semitectum</i>	--	48.33	--	--	--	36.66
13	<i>Penicillium funiculosum</i>	02.00	--	08.69	--	--	--
14	<i>Phytophthora palmivora</i>	--	--	--	--	05.55	--
15	<i>Rhizopus stolonifer</i>	11.53	--	05.43	07.69	33.33	--
16	<i>Sclerotium rolfsii</i>	--	--	01.09	--	--	--
17	<i>Torula herbarum</i>	--	--	07.61	--	--	--
18	<i>Thermomyces lanuginosus</i>	--	--	01.09	--	--	--
19	<i>Trichoderma longibrachiatum</i>	--	--	--	--	16.66	--
20	Sterile mycelium	--	--	--	--	05.55	--
Total species		26	120	92	26	18	30

Qualitative percentage of fungal species in the non rhizosphere of chickpea at various growth periods in variety ICC 4951 susceptible to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus aculeatus</i>	11.11	--	--	--	--	15.00
2	<i>A. flavus</i>	05.55	--	04.17	19.04	--	10.00
3	<i>A. fumigatus</i>	--	--	08.33	--	--	05.00
4	<i>A.niger</i>	11.11	14.28	25.00	19.04	30.76	25.00
5	<i>A.nidulans</i>	--	--	--	04.76	23.07	--
6	<i>A.terreus</i>	--	--	--	--	15.38	--
7	<i>A. ustus</i>	33.33	09.52	20.83	--	--	--
8	<i>Cladosporium herbarum</i>	--	--	--	04.76	--	--
9	<i>Cladosporium oxysporum</i>	--	--	08.33	--	--	--
10	<i>Fusarium moniliforme</i>	11.11	--	--	09.52	15.38	--
11	<i>F.oxysporum</i>	16.66	09.52	08.33	04.76	07.69	45.00
12	<i>F.roseum</i>	--	--	--	09.52	--	--
13	<i>F. semitectum</i>	--	23.80	--	04.76	--	--
14	<i>Humicola fuscoatra</i>	--	--	--	04.76	--	--
15	<i>Helminthosporium hawaiiense</i>	--	04.76	04.16	--	--	--
16	<i>Phoma herbarum</i>	--	--	12.50	--	--	--
17	<i>Penicillium oxalicum</i>	--	--	--	04.76	--	--
18	<i>Rhizoctonia bataticola</i>	--	--	--	09.52	--	--
19	<i>Rhizoctonia solani</i>	--	--	--	--	07.69	--
20	<i>Rhizopus stolonifer</i>	11.11	--	--	--	--	--
21	<i>Trichoderma harzianum</i>	--	38.09	--	--	--	--
22	<i>Torula herbarum</i>	--	--	08.33	--	--	--
23	Sterile mycelium	--	--	--	04.76	--	--
Total species		18	21	24	21	13	20

Qualitative percentage of fungal species in the rhizosphere of chickpea at various growth periods in variety ICC 12475 susceptible to FOC

Sr. No.	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus flavus</i>	13.15	00.98	09.20	14.04	22.92	16.28
2	<i>A. niger</i>	15.78	12.75	01.15	01.75	--	--
3	<i>A. terreus</i>	--	--	--	08.77	04.16	--
4	<i>A. ustus</i>	--	--	--	08.77	08.33	--
5	<i>Cladosporium herbarum</i>	--	--	--	01.75	02.08	--
6	<i>C. spongiosum</i>	--	--	--	--	06.25	--
7	<i>Fusarium moniliforme</i>	40.78	30.39	40.23	35.09	52.08	51.16
8	<i>F. oxysporum</i>	27.63	06.86	--	--	--	--
9	<i>F. semitectum</i>	--	--	--	--	--	32.56
10	<i>Humicola fuscoatra</i>	--	--	--	01.75	--	--
11	<i>Helmenthosporium hawaiiense</i>	--	--	05.75	--	--	--
12	<i>Phoma eupyrena</i>	--	--	06.89	--	--	--
13	<i>Penicillium funiculosum</i>	--	--	10.34	03.51	02.08	--
14	<i>Papulaspora pallidula</i>	--	--	01.15	--	--	--
15	<i>Rhizopus stolonifer</i>	02.63	--	13.79	10.53	--	--
16	<i>Staphylotrichum coccosporum</i>	--	--	--	01.75	----	--
17	<i>Torula caligans</i>	--	--	09.20	--	--	--
18	<i>Trichoderma viride</i>	--	49.02	--	07.02	02.08	--
19	Sterile mycelium	--	--	02.29	05.26	--	--
Total species		76	102	87	57	48	43

Qualitative percentage of fungal species in the nonrhizosphere of chickpea at various growth periods in variety ICC 12475 susceptible to FOC

Sr no	Fungal species	Days					
		15	30	45	60	75	90
1	<i>Aspergillus flavus</i>	--	--	--	08.77	04.16	--
2	<i>A. fumigatus</i>	--	--	--	--	07.14	--
3	<i>A. niger</i>	15.38	09.09	06.66	21.43	42.86	--
4	<i>A. terreus</i>	--	--	--	08.77	08.33	--
5	<i>A. ustus</i>	15.78	12.75	01.15	01.75	--	--
6	<i>Cladosporium herbarum</i>	--	--	--	01.75	02.08	--
7	<i>C. oxysporum</i>	--	--	06.66	--	--	--
8	<i>C. spongiosum</i>	--	--	--	--	06.25	--
9	<i>Fusarium moniliforme</i>	11.54	13.64	20.00	07.14	--	--
10	<i>F. oxysporum</i>	40.78	30.39	40.23	35.09	52.08	51.16
11	<i>F. semitectum</i>	--	--	--	--	--	42.86
12	<i>Humicola grisea</i>	--	--	--	--	07.14	--
13	<i>Mucor racemosus</i>	--	--	--	--	07.14	--
14	<i>Myrothecium gramineum</i>	07.69	--	--	--	--	--
15	<i>Penicillium digitatum</i>	--	--	06.66	--	--	--
16	<i>P. funiculosum</i>	11.54	04.54	--	--	--	--
17	<i>Phoma eupyrena</i>	--	18.18	10.00	--	--	--
18	<i>Rhizoctonia bataticola</i>	--	09.09	--	--	--	--
19	<i>Rhizopus stolonifer</i>	--	--	13.33	42.86	14.29	--
20	Sterile mycelium	38.46	--	06.66	--	--	--
Total species		26	22	30	14	14	14

Result and discussion

Altogether 58 villages were selected randomly for the observation of chickpea wilt in Marathwada. Quantitative data showed significant variation in the rhizosphere and soil mycoflora and their corresponding R/S ratios. However, significant variation between growth periods of these varieties was found in case of rhizosphere mycoflora. Qualitatively a total of 67 fungal species were identified from 30 different genera from the rhizosphere and soil of chickpea. The *Aspergillus flavus* and *A. niger* were also commonly present in the rhizosphere. The species those were dominant in the rhizosphere were *F. oxysporum*, *F. moniliforme*, *A. ustus*, *F. semitectum*, *Rhizopus stolonifer* and *Trichoderma spp.* They occurred more than 20% of the total fungal colonies. There was also a variation in the occurrence of their different fungal species at different growth period of chickpea. The R / S ratio in all the varieties is more than one. It was 5.97 in ICC 2072 in resistant category and 5.00 in ICC 12475 in susceptible category. In order to study the rhizosphere mycoflora amongst three varieties; it was observed that twenty-four different fungal genera were isolated from resistant varieties however it was twenty in two susceptible varieties.

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