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BIOCONTROL OF CHICKPEA WILT DISEASE BY *FUSARIUM OXYSPORUM F. SP. CICERI* WITH RHIZOSPHERE MYCOFLORA

MEENAKSHEE ANURAGI* AND TIRTHESH K. SHARMA

Department of Botany,
Bipin Bihari P.G. College,
JHANSI -284001(U.P.) INDIA
*Corresponding Author
Email: meenakshee.anuragi@yahoo.in

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ABSTRACT

Biological control of soil-borne plant pathogens is a potential alternative to the use of chemical pesticides, which are harmful to environment. The rhizospheric fungal isolates were undertaken for the biocontrol. The fungal isolates of chickpea rhizosphere were evaluated for their biocontrol potential against *Fusarium oxysporum f. sp. ciceri* under *in-vitro* study (dual culture method) and sick soil (pot) condition. Under *in-vitro* study, isolate *Trichoderma reesei* showed the strongest antagoinistic activity towards *Fusarium oxysporum f. sp. ciceri* in dual cultural followed by *Trichoderma viride*, *Trichoderma harzianum*, *Aspergillus nidulans*, *Rhizopus oryzae*, *Curvularia clavata*, *Alternaria alternata* and *Mucor* spp.

Figure : 00 References : 22 Tables : 04

KEY WORDS : Biocontrol, Chickpea, *Fusarium oxysporum f. sp. ciceri*, Isolation.

Introduction

The wilt disease by *Fusarium oxysporum f. sp. ciceri* is a serious disease of Chickpea[1,13] (*Cicer arietinum* L.). This disease is seed borne as well as soil born and causes considerable crop losses in India[14]. Inspite of their effectiveness, chemicals are not advocated for controlling the soil borne plant pathogen in view of their prohibitive cost and threat to beneficial soil microflora, hence the interest is shifting towards biological control. The rhizosphere microflora is ideal as biocontrol agents because the rhizosphere provides the first line of defence to root surface against invading pathogen[20]. In the present investigation, the biocontrol potential of chickpea rhizosphere mycoflora has been studied in relation to their effect on the growth and sporulation development of wilt disease by *Fusarium oxysporum f. sp. ciceri*.

Materials and Methods

Isolation and Purification of Rhizospheric mycoflora:

The soil dilution was carried out[8]. The pH was adjusted at 7.3 either with by 0.1N Sodium hydroxide or hydrochloric acid. The dilution was prepared upto (10^5) was obtained. Standard protocols was followed for the cleaning and sterilization of glasswares. *Trichoderma* species were isolated on *Trichoderma* Specific Media (TSM)[6].

25ml of above medium was poured in sterile Petri plates of 90mm diameter and 1ml of soil suspension was added to it and allowed to solidify. The plates were then incubated at room temperature for one week. The *Trichoderma* species colonies developed on the medium were carefully taken out with the help of inoculation needle and transferred to PDA medium. The culture were further purified by single spore culture technique and transferred to PDA slant and kept in the refrigerator for further studies. The identifications were made[12].

Cultural characteristics :

Culture characteristics of each isolate of fungi were studied on PDA plate. All the Petri plates were incubated for five days at 25±2ºC.

Observations

Data were recorded from 48hrs of

TABLE - 1: Cultural characteristic of isolated bioagents.

Isolate	Isolate No.	Mycelial Growth (mm) after 48 hrs	Spore population after		Colony Colour Surface/ reverse
			48 hrs	120 hrs	
Trichoderma reesei	T_1	85.0	Moderate	Abundant	White pale/yellow
	T_2	85.0	Moderate	Abundant	White/yellow
	T_3	85.0	Scanty	Abundant	White/ yellow
Trichoderma viride	T_4	80.0	Nil	Abundant	Dark green
	T_5	80.0	Nil	Abundant	Dark green
	T_6	55.0	Scanty	Abundant	Dark green
Trichoderma harzianum	T_7	75.0	Nil	Moderate	Dark green
	T_8	75.0	Nil	Abundant	Dark green
	T_9	55.0	Scanty	Abundant	Dark green
Aspergillus nidulans	T_{10}	65.0	Scanty	Moderate	Yellow
	T_{11}	65.0	Scanty	Moderate	Purplish red
	T_{12}	65.0	Scanty	Abundant	Yellow
Rhizopus oryzae	T_{13}	60.0	Nil	Moderate	Grey
	T_{14}	60.0	Nil	Moderate	Dark brown grey
	T_{15}	60.0	Scanty	Abundant	Grey
Curvularia clavata	T_{16}	55.0	Scanty	Abundant	Dark grey
	T_{17}	55.0	Scanty	Abundant	Black
	T_{18}	55.0	Nil	Abundant	Black
Alternaria alternata	T_{19}	50.0	Nil	Moderate	Dark Grey
	T_{20}	50.0	Scanty	Abundant	Blackish Brown
	T_{21}	50.0	Scanty	Moderate	Blackish Brown
***Mucor* spp.**	T_{22}	50.0	Nil	Moderate	Grey
	T_{23}	50.0	Nil	Abundant	Dark grey
	T_{24}	50.0	Nil	Moderate	Dark grey

incubation untill 120hrs. Growth rate and sporulation was also recorded. Spore intensity was rated as: nil = 0 spores/plate, scanty = 0.35×10^4 spores/plate, moderate = 0.7×10^4 spores/plate and abundant = 1.05×10^4 spores/plate.

***In-vitro* study of Antagonistic Potential of Rhizospheric fungal isolates by Dual culture technique:**

The efficacy of rhizospheric fungal isolates on radial growth inhibition of test pathogen *Fusarium oxysporum f. sp. ciceri* was studied *in-vitro* by dual culture technique.The bioagents undertaken were *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp.

The studies on the antagonism was carried out by applying 'Direct Bit Placement Method[4]. The experiment was conducted with four replication and one control plate containing only test fungus. These plates were then incubated at 25±1ºC. Observation were recorded after seven days of inoculation on area covered by the *Trichoderma* species and pathogen and percent growth inhibition was calculated[3] given below:

Growth inhibition (%) =

$$\frac{\text{(Colony growth in Control plates)} - \text{(Colony growth in intersecting plate)}}{\text{Colony growth in control plate)}} \times 100$$

Pot Culture:

Efficacy of Rhizospheric Bioagents Against *Fusarium oxysporum f. sp. ciceri.*

24 isolates of bioagents namely *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp. were studied for the control of wilt disease of chickpea. The chickpea plants were raised in 9inches earthen pots containing sterilized heavy black and red soil. Three to five chickpea seeds were sown in these pots and were later thinned to three plants per pot. These were maintained at 22±2ºC at Bipin Bihari College, Jhansi. Each treatment involved 45 plants. Three set as control were taken for each *species* (Control I = sterilized soil + bioagent; Control II = sterilized soil + *F. oxysporum* f. sp. *ciceri* and Control III = sterilized soil + chickpea).

The mass culture of the test pathogen was prepared. Mycelia mats from 7 days old colony were scrapped and transferred to 500ml flask containing 100ml of PDA broth. The flasks were incubated at 25±2ºC for five days. These flasks were then placed on shaker operating at 96 r.p.m. for two hours. The content were then filtered through two layers of cheese cloth. The filtrate was diluted with distilled water to obtain enough quantity of inoculums. A haemocytometer was used to quantify inoculums. Similarly the inoculums of bioagents were also prepared. It was ensured that 12-15 fragments of the fungi were present in a microscopic field.

These pots were inoculated with *Trichoderma reesei* strains T_1, T_2 and T_3, *Trichoderma viride* strains T_4, T_5, and T_6, *Trichoderma harzianum* strains T_7, T_8 and T_9, *Aspergillus nidulans* strains T_{10}, T_{11} and T_{12}, *Rhizopus oryzae* strains T_{13}, T_{14} and T_{15}, *Curvularia clavata* strains T_{16}, T_{17} and T_{18}, *Alternaria alternata* strains T_{19}, T_{20} and T_{21} and *Mucor* spp. strains T_{22}, T_{23} and T_{24} collected from field and maintained in the laboratory.

The inoculations with bioagents were immediately followed with the inoculations with *Fusarium oxysporum* f. sp. *ciceri* and repeated at 15, 30 and 45 days separately in each treatment. The disease symptoms appeared on the plants were recorded after the inoculation with pathogen and concluded at 90 days.

There were three replications in each case. The percentage of disease incidence and average yield was recorded after 90 days.

To measure the percentage of infection or increase over healthy chickpea seed the percent reduction was calculated by the following formula

$$\text{Percent reduction} = \frac{\text{Healthy} - \text{Infected}}{\text{Healthy}} \times 100$$

Result

Isolation, Identification and Purification of Rhizospheric Fungal Bioagents:

The rhizospheric soil from different chickpea cultivated fields, frequently yielded fungal antagonists. During the study 24 fungal antagonists were isolated and identified. These fungal antagonist yielded 3 strains each of *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae,*

Colony growth in Colony growth in

TABLE- 2: *In vitro* antagonist efficacy of mycoflora against *Fusarium oxysporum* f. sp. *ciceri* by dual culture technique after 120hrs.

Antagonist	Isolate No.	*Fusarium oxysporum* f. sp. *Ciceri*		
		Radial growth of colony (mm)	Average Colony growth (mm)	Inhibition %
Trichoderma reesei	T_1	12.8	13.76	79.76
	T_2	13.6		
	T_3	14.9		
Trichoderma viride	T_4	15.7	17.53	74.22
	T_5	18.3		
	T_6	18.6		
Trichoderma harzianum	T_7	20.5	22.56	66.82
	T_8	23.7		
	T_9	23.5		
Aspergillus nidulans	T_{10}	26.7	27.73	59.22
	T_{11}	28.5		
	T_{12}	28.0		
Rhizopus oryzae	T_{13}	28.8	29.4	56.76
	T_{14}	29.5		
	T_{15}	29.9		
Curvularia clavata	T_{16}	33.5	33.86	50.20
	T_{17}	33.9		
	T_{18}	34.2		
Alternaria alternata	T_{19}	35.8	35.96	47.12
	T_{20}	35.4		
	T_{21}	36.7		
***Mucor* spp.**	T_{22}	37.2	38.06	44.02
	T_{23}	38.4		
	T_{24}	38.6		
Control		68		

TABLE - 3: Disease incidence (%) after 90 days on plant growth in pots per inoculated with strains and *Fusarium oxysporum f. sp. ciceri.*

Disease incidence (%) with strains	Strains type	Inoculated with Fusarium oxysporum f. sp. ciceri on days					
		0	15	30	45	Only pathogen	Check
Trichoderma reesei	T_1	24.62	23.53	20.83	18.09	97.45	40.79
	T_2	24.70	22.35	18.25	15.43	97.45	40.79
	T_3	20.23	22.32	16.06	18.14	97.45	40.79
Trichoderma viride	T_4	35.73	29.73	26.51	22.50	97.45	40.79
	T_5	30.47	28.42	25.17	23.27	97.45	40.79
	T_6	29.56	26.17	23.32	18.10	97.45	40.79
Trichoderma harzianum	T_7	38.06	35.91	32.50	29.47	97.45	40.79
	T_8	36.78	34.19	33.10	29.43	97.45	40.79
	T_9	36.35	35.01	28.37	25.28	97.45	40.79
Aspergillus nidulans	T_{10}	40.23	39.90	37.68	35.12	97.89	40.79
	T_{11}	41.83	38.59	37.51	34.02	97.89	40.79
	T_{12}	41.51	38.20	36.39	33.34	97.89	40.79
Rhizopus oryzae	T_{13}	45.37	44.80	43.56	41.68	97.89	40.79
	T_{14}	44.81	44.62	42.19	41.24	97.89	40.79
	T_{15}	44.29	43.70	42.11	40.12	97.89	40.79
Curvularia clavata	T_{16}	49.35	47.60	45.59	43.81	97.89	40.79
	T_{17}	48.76	47.35	45.20	42.12	97.89	40.79
	T_{18}	48.46	43.70	41.62	40.20	97.89	40.79
Alternaria alternata	T_{19}	51.62	49.40	48.57	46.71	97.89	40.79
	T_{20}	51.21	49.09	49.02	45.49	97.89	40.79
	T_{21}	50.34	47.28	46.10	42.19	97.89	40.79
***Mucor* spp.**	T_{22}	54.90	53.18	51.80	49.57	97.89	40.79
	T_{23}	51.66	52.49	50.63	49.44	97.89	40.79
	T_{24}	50.45	51.72	49.06	47.10	97.89	40.79

TABLE- 4: Impact of strains of mycoflora on chickpea yield after 90 days, pre inoculated with *Fusarium oxysporum f. sp. ciceri*.

Strains	Strains type	*Fusarium oxysporum f. sp. ciceri* on days									
		0		15		30		45		Natural	
		Yield gms/pot	% reduction	Yield gms/pot	% reduction	Yield gms/pot	% reduction	Yield gms/pot	% reduction	Yield gms/pot	% reduction
Trichoderma reesei	T_1	210	64.34	289	50.93	456	22.75	565	4.07	589	-
	T_2	210	64.52	295	50.16	480	18.92	568	4.05	592	-
	T_3	215	63.24	289	50.59	495	15.38	572	2.22	585	-
Trichoderma viride	T_4	190	67.68	230	60.88	448	23.89	505	14.11	580	-
	T_5	195	66.60	245	58.04	468	19.86	520	10.95	584	-
	T_6	202	65.76	260	55.93	490	16.95	565	4.23	590	-
Trichoderma harzianum	T_7	189	67.74	215	63.31	429	26.79	500	14.67	586	-
	T_8	185	68.21	225	61.34	438	24.74	520	10.65	582	-
	T_9	190	67.74	240	59.25	460	21.90	535	9.16	589	-
Aspergillus nidulans	T_{10}	180	67.85	195	65.17	240	57.14	410	26.78	560	-
	T_{11}	185	67.42	210	63.02	260	54.22	458	19.36	568	-
	T_{12}	190	66.66	225	60.52	295	48.24	490	14.03	570	-
Rhizopus oryzae	T_{13}	160	71.58	190	66.25	230	59.14	343	39.07	563	-
	T_{14}	168	70.31	198	65.01	245	56.71	365	35.51	566	-
	T_{15}	172	69.93	206	63.98	269	52.17	390	31.81	572	-
Curvularia clavata	T_{16}	155	72.32	186	66.78	219	60.89	290	48.21	560	-
	T_{17}	160	71.68	190	66.37	240	57.52	310	45.13	565	-
	T_{18}	168	70.42	205	63.90	260	54.22	315	44.54	568	-
Alternaria alternata	T_{19}	155	77.22	160	71.32	217	61.11	250	55.19	558	-
	T_{20}	158	71.93	168	70.16	220	60.93	265	52.93	563	-
	T_{21}	163	71.20	170	69.96	235	58.48	295	47.87	566	-
Mucor spp.	T_{22}	154	72.50	185	66.96	230	58.92	275	50.89	560	-
	T_{23}	158	71.93	198	64.83	245	56.48	285	49.37	563	-
	T_{24}	150	73.02	165	70.32	215	61.33	235	57.73	556	-

Curvularia clavata, Alternaria alternata and *Mucor* spp.

Cultural characteristics of isolates bioagents:

Rhizospheric mycoflora study isolates from Chickpea field revealed 24 fungal isolates belonging to six genera and eight species. These bioagents were grown on PDA and TSM. It was observed that *species* of *Trichoderma* fast grow in media.

The growth of isolated strains T_1, T_2, T_3 of *Trichoderma reesei*, T_4, T_5 of *Trichoderma viride* and T_7, T_8 of *Trichoderma harzianum* is very fast growth in Petri plates in 48 hrs. Isolates of *Trichoderma viride* T_6 and *Trichoderma harzianum* T_9 had comparatively slow growth. Among the isolates *Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp. showed comparatively slow growth. The spore formation was very fast in 48 hrs strains of T_1, T_2, T_3 *Trichoderma reesei*, T_6 *Trichoderma viride*, T_9 *Trichoderma harzianum*, T_{10}, T_{11}, T_{12} *Aspergillus nidulans*, T_{15} *Rhizopus oryzae*, T_{16}, T_{17} *Curvularia clavata* and T_{20}, T_{21} *Alternaria alternata* (Table -1).

With time, spore intensity increased in all the isolates of the bioagents *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp. and reached at peak after 120hrs of incubation. *Trichoderma reesei* showed fast growth than *Trichoderma viride, Trichoderma harzianum , Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp.

***In-vitro* antagonistic efficacy of rhizospheric fungal isolates against wilt diseases of chickpea:**

Dual culture technique:

The antagonistic potential of 24 rhizospheric fungal isolates have been worked out. Among the various isolates of rhizospheric fungi 3 strains each of *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp.

Isolated of *Trichoderma* spp.:

The radial growth of the colony, average growth of the colony and inhibition percentage was estimated at regular intervals and finally after 120hrs of inoculation, reults are presented (Table- 2). The maximum inhibition percentage was in *Trichoderma reesei* followed by *Trichoderma viride* > *Trichoderma harzianum* > *Aspergillus nidulans* > *Rhizopus oryzae* > *Curvularia clavata* > *Alternaria alternata* > *Mucor* spp.

Evaluation of antagonistic potential of rhizospheric fungal isolates wilt pathogen (*Fusarium oxysporum f. sp. ciceri*). The present study revealed that all fungal isolates significantly inhibited the radial growth of test pathogen in comparison to control. Among the rhizosplarheric bioagents, isolate of *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp. bioagents to control wilt in *Cicer arietinum*.

Disease incidence on plants pre inoculated with bioagent strains and *Fusarium oxysporum f. sp. ciceri*:

The result of the interactions of rhizospheric fungi *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp. and pathogen on plants grown in pots were recorded after 90 days of plants growth. It is evident from data recorded in (Table- 3) that the impact of the interactions of bioagents on disease incidence percentage by *Fusarium oxysporum* f. sp. *ciceri* varied among the strains of *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp. The strain T_3 of *Trichoderma reesei* was superior in reducing the disease intensity of gram. The maximum disease incidence after 90 days due to this strain has been 8.14% after inoculation with pathogen as compared to control 97.45%.

The strain T_6 of *Trichoderma viride*, T_9 of *Trichoderma harzianum*, T_{12} of *Aspergillus nidulans*, T_{15} of *Rhizopus oryzae*, T_{18} of *Curvularia clavata*, T_{21} of *Alternaria alternata* and T_{24} of *Mucor* spp. was superior in reducing the disease intensity of gram. The maximum disease incidence after 90 days due to these strain has been 18.10%, 25.28%, 33.34%, 40.12%, 40.20%, 42.19%, 47.10% after inoculation with pathogen as against 97.45% and 97.89% in control.

The fungicidal potential of all the strains of the bioagents were in the descending order of *Trichoderma reesei* >*Trichoderma viride*> *Trichoderma harzianum*> *Aspergillus nidulans*>

Rhizopus oryzae> Curvularia clavata> Alternaria alternata > *Mucor* spp.

Yield of chickpea inoculated with bioagent strains and *Fusarium oxysporum f. sp. ciceri*:

In the same experiment average yield of *Cicer* per pot was recorded to assess the loss due to interaction of pathogen *Fusarium oxysporum f. sp. ciceri* with the bioagent *viz.,* species of *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum, Aspergillus nidulans, Rhizopus oryzae, Curvularia clavata, Alternaria alternata* and *Mucor* spp. It is observed from the (Table-4), that the bioagents had supported chickpea yield in all the stages of growth. There has been a gradual reduction in the percentage of yield and gradual increase in yield per pot at different days of inoculations with pathogen. The maximum reduction in yield percentage has been 64.34, 64.52 and 63.24 due to bioagent strains T_1, T_2 and T_3 respectively. The strain T_3 of *Trichoderma reesei* was found superior over the strains in allowing highest yield per pot and also exhibiting comparatively low reduction in disease percentage at 0 and 45 days of inoculation with pathogen. It is also seen that the maximum yield at 0 day inoculation by the pathogen was found with T_3 of *Trichoderma reesei.* The minimum yield was found in case of strain T_{24} of *Mucor* spp. Importantly, the chickpea yield decreased many fold at the end of the experiment. This strongly suggests that the bioagents overtake the pathogen growth and thereby minimize its affect in affecting adversely the yield of chickpea.

Discussion

Trichoderma is known to be the potential antagonists of fungal plant pathogens[15]. Worker[16] established the *Trichoderma* specie from any substrate on PDA. First detailed descriptions of a *Trichoderma* species as a parasitic of the other soil fungi and concluded that under certain conditions[19], *Trichoderma* species might be used for the biological control of fungal diseases[22].

Bioagents like *Trichoderma reesei, Trichoderma viride, Trichoderma harzianum* and *Trichoderma hamahum* as effective in controlling pigeonpea wilt caused by *Fusarium oxysporum f. sp. ciceri,* of *Trichoderma viride* on growth and sporulation of *Fusarium oxysporum f. sp. ciceri* and reported maximum fungal growth inhibition by *Trichoderma viride[17].* 4 Strains of *Trichoderma aureoviride*, *Trichoderma harzianum*, *Trichoderma reesei* and *Trichoderma viride* antagonized the phytopathogens *Rhizoctonia solani* and *Fusarium oxysporum f. sp. dianthi*. *Trichoderma reesei* were more effective against *Fusarium oxysporum*[2].

Antagonistic fungi in controlling plant disease[18]; among the antagonistic fungi, *Trichoderma reesei* has been found to be highly efficient by virtue of producing chitinase to degrade cell well of tested plant pathogen[9,21] reported antagonistic activity of *Trichoderma reesei* and *Aspergillus nidulans* against *Fusarium oxysporum.* Under laboratory condition the co inoculation of *Trichoderma reesei* and *Aspergillus nidulans* showed significant increase in chickpea (*Cicer arietinum*) growth parameters including shoot length, root length and dry weight of shoot and root. Features were assigned to high phosphate solubility by *Aspergillus nidulans* and *Trichoderma reesei*.

The antagonistic activities of the *species* of *Aspergillus* and *Trichoderma* in antagonising the *Fusarium oxysporum f. sp. ciceri*, *in-vitro* and *in-vivo* as observed in the present studies have been in accordance with the findings[5,7,10,11].

References

1. ANDRABI, M., VAID, A. AND RAZDAN, V.K. (2011) Evaluation of different measures to control wilt causing Pathogens in Chickpea. *Journal* of *Plant Protection Research* **5** : 55-59.
2. ANTAL (2000) Colony growth *in-vitro* antagonism and Secretion of Extracellilar enzymes in cold tolerant strains of *Trichoderma* species. *Mycological Research,* **104**: 545-549.
3. ARORA, D.K. AND UPADHYAY, R.K. (1978) Effect of fungal staling growth substances on colony interaction *Pl. and Soil,* **49** : 685-690.
4. BRODBENT, P., BAKER, K.F. AND WATERWORTH, Y. (1971) Bacterial and actinomycetes antagonistic to fungal root pathogens in Australian soil. *Austr. Journal* of *Biol. Sci.* **24**: 925-944.
5. CHAUDHARY, R.G. AND PRAJAPATI, R.K. (2004) Comparative efficacy of fungal bioagents against *Fusarium udum. Ann. Pl Protc. Sci.,* **12**: 75-79.

6. ELAD AND CHET (1983) Improved selective media for isolation of *Trichoderma* and *Fusarium spp. phytoparasitica.* **11**: 55-58.

7. GANGWAR, R.K., PRAJAPATI, R.K. AND KUMAR, K. (2004) Evaluation of fungal antagonists *Fusarium oxysporum* f. sp. *ciceri*. *Ann*. *PI*. *Protect*. *Sci.* **12** : 444-445.

8. JOHNSON, L.F., CURL, E.A., BOND, J.S. AND FRIBOURG, H.A. (1995) Methods for studing soil micro flora and plant disease relationships. *Burgess Publication Company*. *Minneapolis*. *Minn*.

9. KUCUK, C. AND KIVANC, M. (2004) *In-vitro* antifungal activity of strains of *Trichoderma harzianum. Turk. Journal Biol.* **28** : 111-115.

10. KURUNDKAR, B.P. AND MAHAJAN, S.B. (2005) Antagonistic effect of fungal isolates against *Fusarium udum. Indian Phytopathology.* **58** (3): 353.

11. LAL, M., KHAN, ANIS AND KAMALWANSHI, R.S. (2006) Efficacy of Bioagents against *Curvularia* leaf Spot of black gram. *Annals of Plant Protection Sciences.* **14** (1): 257-258.

12. NAGAMANI, A., MANOHARACHARY, C., AGARWAL, S.K. AND CHOWDHARY, P.N. (2002) Monographic contribution on Trichoderma associated. Publishing Co., Post Box No. 2679, Karol Bagh, New Delhi, pp. 47.

13. NIKAM, P.S., JAGTAP, G.P. AND SONTAKKE, P.L. (2011) Survey, Surveillance and Cultural characteristics of Chickpea Wilt caused by *Fusarium oxysporium* f. sp. *ciceri. African Journal* of *Agricultural Research*. **6** : 1913-1917.

14. PANDE, S., RAO, J.N. AND SHARMA, M. (2007) Establishment of the Chickpea wilt Pathogen *Fusarium oxysporum* f. sp. *ciceri* in the soil through seed transmission. *Plant Pathology J*. **23** : 3 – 6.

15. PAPAVIZAS, G.C. (1985) Characterization of alginate pellets formulated with *Trichoderma* and *Gliocladium* and their effect on the Proliferation of the fungi in soil. *Plant Pathology.* 34: 571-577.

16. PERSON, C.B. (1974) Dispostio methodical fungourm Romer's neues. *Mag Bot.* **1**. 81- 128.

17. SOMASKEKHARA, Y.M., KUMAR, T.B., SIDDARAD, A.H. (1996) Biocontrol of Pigeonpea wilt *Fusarium udum. Mysore journal* of *Agric.* **30** : 159-163

18. SOYTONG, K., SRINON, W., RATANACHERDCHAI, K., KANOKMEDHAKUL, S. AND KANOKMEDHAKUL, K. (2005) Application of antagonistic fungi to control anthracnose disease of grape. *Agricultural Technology.* **1**: 33-42.

19. WEINDLING, R (1932) *Trichoderma lignorum* as a parasite of other soil fungi. *Phytopathology*. **22** : 837-845.

20. WELLER, D.M. (1988) Biological control of soil borne plant pathogen in the rhizosphere with bacteria. *Annu. Rev. Phytopath.* **26** : 379-407.

21. YADAV, JANARDAN, VERMA, JAY PRAKASH AND TIWARI, KAVINDRA NATH (2011) Plant growth promoting activities of Fungi and their effect on Chickpea Plant Growth. *Asian Journal* of *Biological Sciences.* **4**: 291-299.

22. ZACHOW, CHRISTIAN, B., HENRY, M., REMO, M., KOMON -ZELAZOWSKA, M., DRUZHININA, I.S., KUBICEK, C.P. AND BERG, G. (2009) Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. *The ISME Journal.* **3**: 79-92.