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Screening of antagonistic potential of *Trichoderma* species against seed-borne pathogenic fungi in seeds of *Sorghum vulgare*

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ABSTRACT

Antagonistic activity of three species of *Trichoderma* and their isolates viz., *T. viride*,*T. harzianum* and *T. hamatum* against *Aspergillus flavus, A. fumigatus, A. niger, A. terreus and Fusarium oxysporum* of *Sorghum vulgare* was studied. *In vitro* studied were carried out in both dual culture technique and blotter test method. In dual culture technique the *T. harzianum* was most antagonistic ones to the seed-borne fungi followed by *T. viride* and *T. hamatumin vitro* condition. The result shows that maximum inhibition zone was created by *T. harzianum*against *A. terreus, F. oxysporum, A. niger, A. Fumigatus* and *A. flavus.* The less effective antagonistic activity in dual culture technique was recorded *T. viride* against all seed-borne fungi. *T. harzianum* antagonistic isolate as well as the commercial biocide was applied as seed treatment for controlling seed-borne mycoflora under Blotter test *in vitro* and Pot experiment *in vivo* conditions. It was observed that maximum seed germination and maximum shoot and root length recorded with *A. flavus* and *T. harzianum* combination in Pot experiment. Experiment shows, that *T. harzianum* antagonistic isolate was able to significant reduction in seed-borne mycoflora than *T. viride*and *T. hamatum* in *Sorghum vulgare*.

1) INTRODUCTION

Sorghum vulgare is the fifth most important cereal crop in the world after wheat, rice, maize and barley [1]. It is found in the arid and semi-arid parts of the world, due to its feature of being extremely drought tolerant. Sorghum is grown from seed and sorghum seed has been found to be readily infected by various pathogens. Sorghum seeds caused by fungi are of poor quality [2,3], reducing their acceptability and thus, the market value of the produce. Grain mold causes crop loss by reducing seed size and weight, the food value and keeping quality of grains [4,5}. Many of the diseases that cause reduced yields in sorghum have seed-borne phases. Seed borne inoculum therefore, has severe implications for yield, seed production and distribution systems, trade, human nutrition and germplasm. The management of these pathogens during the seed-borne phase is considered to be the cheapest disease control strategy [6].Through, seed-borne mycoflora can be reduced by seed treatment with fungicides but they do not persist for the whole cropping season.

The use of chemical fungicides is being discouraged in recent year due to environmental pollution and rising costs. Methyl bromide is a good example for a very efficient soil fumigant that has a great impact on the environment and has been recently phased out to the public concern and international agreements [7]. Therefore, the use of bio fungicides and an integrated approach to pathogenic fungi control have become necessary. Bio fungicides are biodegradable (environment-friend), non-toxic, cost-effective and helps in increasing the nutritional value of soil.

The use of antagonistic fungi against seed-borne mycoflora like *A. flavus, A. fumigatus, A. niger, A. terreus and F. oxysporum* has been investigated as one of the alternative control methods. Three antagonistic fungi are wild spread throughout the world and have been recognized as the most successful biocides agents for pathogenic mycoflora several mod of action of efficient bioagents on reducing diseases have been described, including competition for nutrients, antibiosis, resistance, mycoparasitism, plant growth promotion and rhizospheric colonization capability [7-12]. Studies on the antagonistic effect of fungi (*T. viride, T. harzianum* and *T. hamatum*) were employed against seed-borne mycoflora on seeds of *S. vulgare*. It was investigated that *T. harzianum* showed most effective antagonistic effect against seed-borne mycoflora while *T. hamatum* and *T. viride*, showed no inhibition against the above seed-borne mycoflora. Management of toxigenic mycoflora associated with seeds of *S. vulgare* through biocontrol agent *T. harzianum* may be safe, long lasting and ecofriendly. Therefore, in the present investigation, relative efficacy of biocontrol of seed-borne mycoflora was assessed under laboratory conditions.

2) MATERIALS AND METHODS

Seed-borne fungal culture: Seed samples of *pennisetusm americanum* (CSH 6) were collected Bikaner, Jaipur, Jodhpur,

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Nagaur and Sikar districts of Rajasthan. Detection of internal and external seed-borne mycoflora was carried out by standard Blotter techniques and Agar plate (PDA) methods [13]. Preliminary microscopic examination of the mycoflora isolated showed that they could be classified under two genera, *i.e. Aspergillus* and *Fusarium*. *Aspergillus* and *Fusarium* isolates were purified by plating single conidial spores [14].

Antagonistic fungal culture: The antagonistic fungal culture namely*Trichoderma harzianum*, *T. viride* and *T. hamatum* were obtained from culture collection centre, Department of Plant Pathology, IARI, New Delhi. The cultures were maintain on PDA medium by monthly subculturing and prevent in the refrigerator at 4^{o}C.

In vitro screening antagonistic effect: The study was carried out employing a "Dual culture test" method [15]. The isolates of the seed-borne fungi and antagonistic fungi were grown in petri-dishes. In this study, the agar discs of 3 mm diameter size were cut from the margins of three days old vigorously growing cultures of antagonistic and seed-borne fungi and were inoculated 3 cm apart in triplicate in petri-dishes containing 15 ml each of PDA medium and incubated for 5 days at 28°C. Controls were also maintained along with them.

The interactions between seed-borne fungi and antagonistic fungi were assayed using a key based on the observations of Skidmore and Dickinson and categorized into five separate modes of interaction of colony growth [16].

Grade

A = Homogenous

B = Overgrowth

C = Intermingling growth

D = Checking of growth at line of contact

E = Aversion

Interaction between the two fungal colonies was examined with the help of microscope. The comparisons were made with control and percent inhibition of fungi was calculated by the following formula.

C = Growth in control (mm)

T = Growth in treatment (mm)

I = Inhibition of fungal growth

Effect of biocontrol agents on seed-borne mycoflora: In this experiment, technique for suspension preparation in the same as used in dual culture test. Seed pelleting method – fungal spore were count using hemocytometer and spore concentration adjusting to 15X10^{3} conidia/ml 10 seeds were pelleted with 3 ml. Spore suspension for each seed-borne fungi for 30 minutes following by carboxyl methyl cellulose (0.2%w/v) for 50 second and them dried in shade, After drying, the seeds were pelleted with 3 ml of spore suspension of antagonistic fungi for 30 minutes followed by carboxymethyl cellulose (0.2% W/V) for 50 seconds and dried in shade. In case of control unionculated seeds were dipped only in carboxyl methyl cellulose solution,

One hundred seeds of *S. vulgare* (for each treatment and uninoculated control) were placed on moisture blotter paper in sterilized Petri-plate@ 10 seeds *et al* per plate and incubated at 28^{o}C for 10 days [13]. After incubation percent germination of seeds, root and shoot length of seedling were measured.

Experiment: *S. vulgare* seeds were pelleted by the seed-borne fungi individually and in combination with the antagonistic fungi as described earlier. Treated seeds were sown in earthen pots containing garden soil. The soil was sterilized by autoclaving. The antagonistic treated seeds (four per pot) were shown in each pot at a depth of 3 cm. pot were out treatment served as control. Four replicated pots were for each treatment. Pots were water daily to maintain the field capacity. Effect of seed coating was recorded on seed germination. The plants were harvest after 90 days and growth parameter like root and shoot length, root and shoot dry weight were recorded.

Simultaneously, population colony forming unit (cfu) of seed-borne fungi and antagonistic fungi individually, per gm of soil was determined at a dilution of 10^{-3} by dilution plate technique on PDA medium. The number of individual colonies appearing on each culture plate on the 4th day determined the number of colony forming unit (cfu) per gm of soil.

3) RESULT AND DISCUSSION

In vitro studies indicated that only antagonistic bacterium *Trichoderma* inhibited the growth of the seed-borne fungi with different degrees of inhibition. The maximum inhibition of mycelial growth of *A. flavus*77.77% (grade D) has been with *T. harzianum* followed by 71.42% (grade D) with *T. viride* and 65.71% (grade D) with *T. hamatum. T. harzianum* showed maximum inhibition of mycelial growth of *A. fumigatus*75.71% (grade B). Similarly, inhibition of mycelial growth of same fungus was recorded 71.42% (grade D) with *T.viride*and 67.77% (grade D) with *T. hamatum*. The maximum inhibition of mycelial growth of *A. niger* 71.42% (grade D) was obtained with *T. harzianum* followed by *T. viride* and *T. hamatum* which inhibited the mycelial growth by 64.44% and 54.28% respectively (grade D). *T. harzianum* showed maximum inhibition of mycelial growth of *A. terreus*55.55% (show grade B). Similarly, 51.11% of mycelial growth inhibition was recorded with *T. viride* (grade B) and 46.66% (grade B) with *T. hamatum.* Maximum inhibition of mycelial growth of *F. oxysporum*68.88% (grade B) was recorded with *T. viride*, 64.66% (grade B) with *T. harzianum* and 57.14% (grade B) with *T. hamatum*. Results were also supported by other workers too [17, 18].

T. harzianum was most effective inhibitor of the above seed-borne *aspergillis* showing antagonism by checking the growth of fungi at the line of contact. The effect of *T. harzianum* was followed by *T. viride*and *T. hamatum* in inhibiting the seed-borne fungi.

The results obtained exhibited two types of antagonistic properties *viz;* Grade-D, checking of growth at the line of contact and grade-B, overgrowth exhibited by the antagonistic fungi against the seed-borne fungi. All the tested antagonistic fungi minimized the growth of the seed-borne fungi. Among all, *T. harzianum* was most effective in controlling the growth of *Aspergillus* spp. and *F. oxysporum* by suppressing the sporulation at the site of influence. The study reveals that a biological control mechanism exists between the tested antagonists and seed-borne fungi and that the selected antagonists (*T. harzianum*) could be used for inoculating soil

Table-1: Antagonistic Behavior of *Trichoderma* Spp. against the Fungal Pathogens Associated With Seeds of *Sorghum Vulgar*

Antagonistic fungi	**Seed-borne fungi**	**Growth of seed-borne fungi in Control (cm)**	**Growth of seed-borne fungi in Treatment (cm)**	**Inhibition of growth (%)**	**Type of antagonism**	**Grade of antagonism**
Trichoderma harzianum	*Aspergillus flavus*	9.0	2.0 ±0.89	77.77	Checking of growth at line of contact	D
	A. fumigatus	9.0	2.1±0.38	75.71	Over growth	B
	A. niger	9.0	2.5±1.02	71.42	Checking of growth at line of contact	D
	A. terreus	9.0	4.0±0.01	55.55	Over growth	B
	Fusarium oxysporum	9.0	3.2±0.50	64.66	Checking of growth at line of contact	D
Trichoderma viride	*Aspergillus flavus*	9.0	2.4±0.10	71.42	Checking of growth at line of contact	D
	A.fumigatus	9.0	2.3±0.05	74.28	Checking of growth at line of contact	D
	A.niger	9.0	3.2±0.80	64.44	Checking of growth at line of contact	D
	A. terreus	9.0	4.4±1.2	51.11	Over growth	B
	Fusarium oxysporum	9.0	2.8±0.25	68.88	Checking of growth at line of contact	D
T. hamatum	*Aspergillus flavus*	9.0	3.0±0.05	65.71	Checking of growth at line of contact	D
	A.fumigatus	9.0	2.9±1.00	67.77	Checking of growth at line of contact	D
	A.niger	9.0	4.1±0.02	54.28	Checking of growth at line of contact	D
	A. terreus	9.0	4.8±0.35	46.66	Over growth	B
	Fusarium oxysporum	9.0	3.8±0.05	57.14	Over growth	B

or seeds for effective control of the incidence of seed-borne *Aspergillus* spp. and *Fusarium oxysporum* [19-27].

In the present study, the bioagent evaluated under DCT were further tested in blotter test as biological seed dressing agents against seed-borne mycoflora of *S. vulgare* [22]. Several combinations of *T. harzianum, T. viride, T. hamatum* with *A. flavus, A. niger, A. fumigatus*and *A. terreus* were experimented. Results revealed that the combination of *Trichoderma harzianum*+ *A. niger* recorded highest seed germination (68.0%) and growth in terms of shoot length (7.4 cm) and root length (5.8 cm) in comparison to single inoculation treatment of *A. niger* and uninoculated control. The next best performance of seed germination was recorded in the combination of *A. niger* + *T. viride* (61.0%) followed by *A. flavus*+ *T. harzianum* (60.0%) and *A. fumigatus*+ *T. harzianum* (56.0%). The combination of *T. hamatum* with *Aspergillus* species did not contribute much to seed germination and growth.

Seed treatment with different seed-borne fungi and biological agents *Trichoderma* greatly influenced the germination of *S. vulgare* seeds as compared to control (Table 3). Maximum average seed germination of 72.0%, growth in terms of shoo; t root length (78.3 cm, 15.2 cm) and dry weight of shoot; root (1.7678 gm, 1.075 gm) was recorded with combination of *A. flavus*+ *T. harzianum*as compared to *A. fumigatus* + *T. viride, A. niger*+ *T. harzianum* and *A. terreus*+ *T. harzianum* combinations respectively. Further, the population count of *Trichoderma* species after the experimental duration was found to be more in all the treatments as compared to the population count of *Aspergillus* spp. However in rest of the dual culture experiments both in pot trials and blotter test, the effect of *T. harzianum* on seed growth was much superior to *T. viride* and *T. hamatum.*

In pot culture experiments, after 90 days of harvest, the higher population count of *Trichoderma* and correspondingly lower count of *Aspergillus* suggested that the former was inhibitory to the growth of the latter. Further, the population count of *Aspergillus* in combination with *Trichoderma* decreased 5-6 folds in comparison to single inoculations with species of *Aspergillus* alone. This proves that both *T. harziamum* and *T. viride* are true antagonists (biological control agent) significantly suppressing the growth of *Aspergillus*.

The possible reasons for microbial antagonism have been described by Dennis and Webster, Upadhyay and Rai and Fravel [15, 28, 29]. According to them, the occurrence of antagonism between the antagonists *Trichoderma* and *Aspergillus* could be as a result of mechanical obstruction to growth and hyphallysis, the production of antibiotics, pH changes and competition for nutrients. In the present study also microbial antagonism between *Trichoderma* and *Aspergillus* spp. could be attributed to some such mechanism operating within the system.

The reasons for microbial antagonism has been previously work out by the following workers [17, 22]. According to them *Trichoderma* spp. treatment reduced seed colonization and root rot caused by pathogenic fungi and it was suggested in the form of antibiotics that inhibit the seeds-borne mycoflora. In the present study, the lower counts of *Aspergillus* spp. and *Fusarium* sp. in the rhizosphere of test seedling indicate the prevalence of some such mechanism operating inhibiting the growth of seed-borne mycoflora.

Table-2: Effect of Seed Pelleting of Seed-borne fungi and Antagonistic Fungi on Seed Germination and Growth of *Sorghum vulgare* (Blotter Test)

Treatment	Seed germination (%)	Shoot length (cm)	Root length (cm)
Control (uninoculated)	57	4.8±0.64	3.4±0.07
Aspergillus flavus alone	38	0.8±0.94	1.9±0.35
A.flavus+T.harzianum	60	7.2±0.67	10.1±0.16
A.flavus+T.viride	58	7.0±0.05	9.5±0.09
A.flavus+T. hamatum	52	6.6±0.03	8.3±0.85
Aspergillus fumigatus alone	40	0.9±0.84	1.1±0.01
A.fumigatus+T.harzianum	56	6.3±0.68	6.3±0.54
A.fumigatus+T.viride	50	5.8±0.45	3.3±0.94
A.fumigatus+T. hamatum	44	4.6±0.32	6.0±0.15
Aspergillus niger alone	33	0.5±0.25	2.9±0.24
A.niger+T.harzianum	68	7.4±1.20	5.8±1.50
A.niger+T.viride	61	7.2±1.39	5.3±0.22
A.niger+T. hamatum	40	4.2±1.29	3.4±0.35
Fusarium oxysporum alone	37	0.7±0.25	6.0±0.14
F. oxysporum +T.harzianum	48	5.3±0.00	8.2±0.81
F. oxysporum +T.viride	53	6.0±0.37	9.5±0.79
F. oxysporum +T. hamatum	45	4.9±1.80	6.5±0.06

Table-3: Effect of Seed Pelleting of Seed-borne fungi and Antagonistic Fungi on Seed Germination and Growth of *Sorghum vulgare* (Pot Experiment)

Treatment	Seed germination (%)	Shoot		Root		Population of antagonistic fungi (cfux10^3/g)	Population of seed-borne fungi (cfux10^3/g)
		Length (cm)	Dry weight (g)	Length (cm)	Dry weight (g)		
Control (uninoculated)	60	48.0±0.60	1.2215±0.62	10.9±0.70	0.702±0.85	-	-
Aspergillus flavus *alone*	31	32.2±0.93	0.8905±0.00	11.6±0.64	0.701±0.26	-	8.2±0.53
A.flavus+T.harzianum	72	78.3±0.85	1.7678±0.09	15.2±0.10	1.075±0.14	35±0.042	1.5±0.62
A.flavus+T.viride	57	64.2±0.60	1.5783±0.32	13.6±0.04	0.821±0.39	26±0.27	3.2±0.60
A.flavus+T. hamatum	52	62.2±0.03	1.5450±0.22	12.0±0.25	0.792±0.40	20±0.00	4.2±0.45
Aspergillus fumigatus alone	28	29.0±0.25	0.9032±0.54	9.90±0.96	0.673±0.25	-	11.5±0.32
A.fumigatus+ T.harzianum	68	73.5±0.40	1.6801±0.15	16.1±1.00	1.004±0.60	38±0.25	1.2±0.30
A.fumigatus+ T.viride	63	69.6±0.22	1.6514±0.59	15.7±0.20	0.983±0.78	32±0.13	1.9±0.21
A.fumigatus+T. hamatum	48	58.1±0.50	1.4257±0.14	11.5±0.36	0.715±0.20	16±0.62	5.1±0.20
Aspergillus niger alone	34	35.6±0.84	0.7651±0.62	10.0±0.22	0.503±0.01	-	7.0±0.92
A.niger+T.harzianum	65	72.4±0.64	1.6720±0.35	15.0±0.94	0.924±0.94	36±0.74	1.6±0.76
A.niger+T.viride	52	60.0±1.54	1.5303±0.00	12.8±0.10	0.724±0.14	22±0.35	4.0±0.28
A.niger+T. hamatum	45	56.8±0.00	1.4026±0.84	11.2±0.00	0.690±0.00	15±0.02	5.8±0.30
Fusarium oxysporum alone	32	33.8±1.00	0.6951±0.75	10.3±0.58	0.497±0.51	-	8.9±0.03
F. oxysporum +T.harzianum	53	61.3±0.56	1.5530±0.69	12.0±0.14	0.724±0.40	22±0.36	4.2±0.00
F. oxysporum +T.viride	59	64.5±0.28	1.5872±0.56	14.8±0.25	0.910±0.25	28±0.15	2.7±0.07
F. oxysporum +T. hamatum	50	58.5±0.36	1.4837±0.84	11.2±0.28	0.695±002	18±0.08	4.8±0.1.

There are many mechanisms suggested to clarify the role of antagonistic organisms in suppression of growth pathogens and thus to control diseases. Their action could be through antibiosis by Trichoderma metabolites, mycoparasitism, and competition for nutrients and/or space. Also, the other mechanisms involved are induction of resistance in plants

through increased of oxidative enzymes, *i.e.* polyphenoloxidase, peroxidase, enhanced lignifications, induction of pathogeneses related protein (PR-1), chitinase, chitobiosidase and β, 1-3, gluconase in addition to increase salicylic acid (SA) level in plants [9, 17, 29-42].

4) CONCLUSION

On the bases of the above observations it can be concluded that management of seed-borne mycoflora of *S. vulgare* could be based on antagonistic effect of *Trichoderma* increase of plant growth under field conditions and significant reduction of seed-borne mycoflora. Also, the obtained bioagent *T. harzianum* proved to be a commercial biocide product, but this needs further studies on this fungal isolates before using in the biological control programs.

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REFERENCES

1) Panchal, V.H. and Dhale, D.A. 2011. Isolation of seed-borne fungi of sorghum (*Sorghum vulgare* pers.). Journal of Phytopathology, 3(12) 45-48.
2) Castor, L.L. and Frederiksen, R.A. 1980. Fusarium head blight, occurrence and effects on sorghum yields and grain characteristics in Texas. Plant Disease, 64, 1017-1019.
3) Gopinath, A. and Shetty, H.S. 1987. Comparison of field and Laboratory evaluation of head mould of sorghum with special reference to Fusarium. Indian Phytopathology, 40, 52-55.
4) Gopinah, A. 1984. Seed-borne Fusarium diseases of sorghum. Ph.D. Thesis, University of Mysore, India, 263.
5) Bandyopadhyay, R. 1986. Grain mold. In: Fredariksen, RA (ed) Compendium of sorghum diseases., Am. Phytopathol. Soc., St.Paul Minnesota. USA, pp. 36-38.
6) Shenge, K.C. 2007. Bacterial speck and bacterial spot disease of tomato in Tanzania: Pathogen characterization, epidemiology and management options. PhD Thesis, Sokoine University of Agriculture, Morogoro, Tanzania, p. 271.
7) ABDEL-MONAIM and Fawzy, M. 2011. Integrated Management of Damping-off, Root and/or Stem Rot Diseases of Chickpea and Efficacy of the Suggested Formula. Notulae Science Biologicase, 3(3) 80-88.
8) Bailey, B.A., Bae, H., Strem, M.D., Crozier, J., Thomas, S.E., Samuels, G.J., Vinyard, B.T. and Holmes, K.A. 2008. Antibiosis, mycoparasitism, and colonization success for endophytic *Trichoderma* isolates with biological control potential in *Theobroma cacao*. Bio Control, 46, 24-35.
9) Sharma, P. and Dureja, P. 2003. Evalution of *Trichderma harzianum* and *T. viride* Isolates at BCA Crop Interface. Journal of Mycology and Plant Pathology, 33(3) 467-468.
10) Weindling, R.S. 1932. *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology, 22,837-845.
11) Ramaiah, K. and Sampangi 1989. Elucidation of the antagonistic variations among isolates of *Trichoderma* sp. against *Fusariumoxysporum*. Journal of Phytology Research, 2(2) 139-144.
12) Gangwar, R.K., Bhushan, G., Singh, J., Upadhyay, S.K. and Singh, A.P. 2013. Combined effect of plant growth promoting Rhizobacteria and fungi on Mung bean (*Vigna radiate* L.). International Journal of Pharmaceutical Science and Research, 4(11), 4422-4426.
13) International seed testing association. International rules for seed testing, rules. (1976). Seed Science and Technology, 4(1) 3-49.
14) Singh, K., Frisvad, C.J., Thrane, U. and Mathure, S.B. 1991. An iiiustrated manual on identification of some seed-borne Aspergilli, Fusaria, Penicillia and their mycotoxins. (1Ed) Printing: AioTryk as Odense, Demnar, 1-132.
15) Dennis, C. and Webster, J. 1971. Antagonistic properties of species-groups of *Trichoderma* II. Production of volatile antibiotics. Transactions of the British Mycological Society, 57(1) 41-48.
16) Skidmore, F.M. and Dicknson, G.H. 1976. Colony interaction and hyphal interference between *Septorianodorum* and phylloplane fungi. Transactions of the British Mycological Society, 66, 57-64.
17) Deshmukh, P.P., Raut, J.G. and Khan, Y.D. 1992. Effect of *Trichoderma* species and fungicides on fungi of sorghum. Indian Journal of Agricultural Science, 64(3) 205-206.
18) Calistru, C., Mc Lean, M. and Berjak, P. 1995. Some aspects of the biological control of seed storage fungi. In basic and applied aspects of seed biology. Proceedings of the fifth international workshop on seeds.
19) Wiley, H.B. and Kommedahl, T. 1981. Biological seed treatment in sweet corn and wheat as a component of crop management. Phytopathology, 71, 265.
20) Locke, J.C., Marios, J.J. and Papaviza, G.C. 1982. Biocontrol of Fusariumwilt of greenhouse grown *Chrysanthemum*. Phytopathology, 72(6) 709.
21) Ramaiah, K. and Sampangi, 1989. Elucidation of the antagonistic variations among isolates of *Trichoderma* sp. against *Fusarium oxysporum*. Journal of Phytology Research, 2(2) 139-144.
22) Dhedhi, B.M., Gupta, O and Patel, V.A. 1990. Antagonistic effect of micro-organisms to *F. oxysporum* f. sp. *ciceri*. Indian Journal of Mycology and Plant Pathology, 20(1) 70-71.
23) Mane, S.B. and Raut, S.P. 1991. Biocontrol of soil-borne pathogenic fungi of black pepper with two *Trichoderma* species. Indian Phytopathology, Suppl. 45C XIIII.
24) Singh, S.K. and Rai, B. 1996. Screening of antagonistic potential of some micro-organisms against *Fusarium oxysporum* f. sp. *lini*. Acta Bot. Indica, 24, 165-168.
25) Chattopadhyay, C. and Sen, B. 1996. Integrated management of Fusarium wilt of muskmelon caused by *Fusarium oxysporum*. Indian Journal of Mycology and Plant Pathology, 26(2) 162-170.
26) Marois, J.J., Mitchel, D.J. and Sonoda, R.M. 1981. Biological control of Fusarium crown rot of tomato under field conditions. Phytopathology, 77, 1257-1260.
27) Purohit, R. 1998. Studies on the mycoflora and mycotoxin elaboration in grains of maize and minor millets cultivated in tribal belts of Rajasthan. Ph.D. Thesis, Jai Narayan-Vyas University, Jodhpur.
28) Upadhyay, R.S. and Rai, B. 1987. Studies on antagonism between *Fusarium udum* Butler and root region microflora of pigeon pea. Plant and Soil, 101, 79-93.
29) Fravel, D.R. 1988. Role of antibiotics in the biocontrol of plant diseases. Ann. Rev. Phytopathology, 26, 75-91.

30) Haran, S., Schickler, H. and Chet, I. 1996. Molecular mechanisms of lytic enzymes involved in the biocontrol activity of *Trichoderma harzianum*. Microbiology, 142, 2321-2331.
31) Ingle, R.W. and Raut, J.G. 1993. Fungi associated with glume and seed in pearl millet at different seed developmental stages. Seed Research, 21(2) 131-132.
32) Turner, D., Kovacs, W., Kuhis, K., Lieckfeldt, E. W., Peter, B., Arisan, A.I., Strauss, J., Samuels, G.J., Bomer, T. and Kubicek, C. 1998. Biogeography and phenotypic variation in *Trichoderma* sect. *Longibraclatum* and associated *Hypocrea* species. Mycological Research, 101, 449-549.
33) Singh, S.D., Swami, S.D and, Rawal, P. 2003. Evaluation of different plant protectants against seed mycoflora of pearl millet. Journal of Mycology and Plant Pathology, 33(1) 106-108.
34) Prasad, R.D., Rangeshwaran, R., Anuroop, C.P. and Rashni, H.J. 2002. Biological control of wilt and root rot of chickpea under field conditions. Annals of Plant. Protection Science, 10(1) 72-75.
35) Sallam, N.M.A., Abo-Elyousr, K.A.M., Hassan, M.A.E. 2008. Evaluation of *Trichoderma* species as biocontrol agent for damping-off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. Egyption Journal of Phytopathology, 36(1-2) 81-93.
36) Elad, Y. 1996. Mechanisms involved in the biological control of *Botrytis cinerea*incited diseases. European Journal of Plant Pathology, 102(8) 719-732.
37) Govindappa, M., Ravishankar, R.V. and Lokesh, S. 2011. In vitro and In vivo responses of different treating agents against wilt disease of safflower. Journal of Cereals and Oilseed, 2(1) 16-25.
38) De Meyer, G., Bigirimana, J., Elad, Y. and Hofte, M. 1998. Induced systemic resistance in *Trichodermaharzianum*T39 biocontrol of *Botrytis cinerea*. European Journal of Plant Pathology, 104 279-286.
39) Govindasamy, V. and Balasubramanian, R. 1989. Biological control of groundnut rust *Puccinia arachidis* by *Trichoderma harzianum*. Journal of Plant Diseases Protection, 96(4) 337-345
40). Lunge, A.G. and Patil, A.S. 2012.characterizationof efficient Chitinolytic enzyme producing Trichoderma species: A tool for baterantagonistic approach. Inter. Journal of Science Technology and Environment, 1(5) 377-385.
41) Waghundes, R.R., Shelake, M.R. and Sabalpara, N.A. 2016. Trichoderma: Asignificant fungus for agriculture and environment. African Journal of Agricultural Research, 11(22) 1952-1965.
42) Bhushan, G., Chhangani, S., Singh, J and Singh, A.P. 2013. Antagonistic Effects of *Bacillus subtilis* and *Pseudomonas fluorescens* Against Seed borne Mycoflora of *Pennisetum americanum,* G- Journal of Environmental Science and Technology, 1(3) 71-75.