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Control of Wilt and Blight Diseases of Cumin through Antagonistic Fungi under in Vitro and Field Conditions

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Abstract

The experiment was carried out to assess their possible use as bio-agents for several antagonistic fungi on growth of two cumin fungal pathogens under in vitro and field conditions. Under in vitro conditions maximum inhibition (82.86%) of radial growth of Fusarium oxysporum f. sp. cumini was observed with the treatment of *Trichoderma harzianum* strain I, whereas maximum inhibition (85.45%) of the mycelial growth of *Alternaria burnsii* was observed in the presence of *Trichoderma harzianum* strain II. The antagonists who showed maximum inhibition of the pathogen in laboratory conditions were applied in field conditions as soil treatment/seed treatment or as foliar spray. The incidence of wilt disease was found to be lowest (PDI 27.40%) when soil was treated with *Trichoderma harzianium* strain I at the rate of 24g / 6m2 (weight of fungus with sorghum seeds). Minimum blight disease incidence was observed when *T. harzianum* strain II was applied to the soil at the rate of 24g / 6m2 (36.15%) or when 10% spore suspension of *T. harzianum* strain II was applied as seed treatment at the time of sowing and as spray at the time of flowering (PDI 35.10%). Thus treatments of *Trichoderma harzianum* strain I for wilt and *Trichoderma harzianum* strain II for blight diseases of cumin under both the conditions @ 24g / 6m2 or 40kg / ha seems promising for sustainable management of crop diseases.

Keywords: antagonistic, wilt disease, blight disease, *Cuminum cyminum* L., *Fusarium oxysporum* , *Trichoderma harzianum*

Introduction

Cumin (Cuminum cyminum L.) belongs to the family Umbellifereae and is believed to be a native of the Mediterranean and Near Eastern regions. It is mainly cultivated in India, Egypt, Libya, Iran, Pakistan and Mexico. Cumin is cultivated on 264 thousand hectares with a production of 108.7 thousand tones in the country (Peter and Nybe, 2002). In India, cumin is mainly cultivated in the states of Rajasthan, Gujarat, Madhya Pradesh, Haryana, Punjab, Uttar Pradesh and Bihar. Among these Rajasthan contribute maximum area as well as production. Major cumin cultivation areas in the state of Rajasthan are Jalore, Barmer, Nagaur, Jodhopur, Pali, Ajmer and Tonk districts (Arora et al., 2004).

The seeds are used as a condiment or spice in curries, pickles and in cooking. In view of the great economic importance of cumin, it is imperative that a coherent and comprehensive programmer be meticulously undertaken on the study of wilt and blight diseases of cumin with a view to control these diseases. This can be achieved only through a pave way for devising management strategies through bio-control. The main objective of the present study was disease control with eco-friendly environment by investigating host-pathogen interaction under both in vitro and in vivo conditions.

Materials and methods

The experiment was conducted during 2004-05 and 2005-06 at Department of Botany, University of Rajasthan, Jaipur. Five isolates of *Trichoderma* and two isolates of *Gliocladium* and three species of *Aspergillus* were screened for their antagonistic activity against the test pathogens by dual culture technique (Denis and Webster, 1971). Required quantity of PDA (20.0 ml) was poured in each of the sterilized Petri plates.

Composition of the in vitro culture medium (PDA)

Peeled potatoes	200 g
Dextrose	20.0 g
Agar	20.0 g
Distilled water to make	1000 ml

On solidification, 2 mm disc of seven days old culture of test pathogen and the antagonists were inoculated separately at the opposite end of each other in a Petri plate containing media. The Petri plates thus prepared were incubated at 25.0±2.0°C temperature. Zone of inhibition between the antagonists and the test pathogens were recorded after seven days of incubation and the percent inhibition was calculated from the following formula (Fokkema, 1973).

$$Percentage\ inhibition = \frac{r_1 - 2}{r_1} \times 100$$

r_1 = diameter growth of pathogen fungus
r_2 = diameter growth of antagonistic fungus

Field experiments were conducted by using the 2 × 3 m2 plot size. The bio-agent which showed maximum growth inhibition of test pathogens under in vitro conditions were applied at various concentrations with four replicates of each as soil treatment and as spray, on both blight and wilt disease. For soil treatment four levels of bio-agents with Sorghum seeds (10, 20, 30 and 40 kg/ha or 6, 12, 18, 24g / 6m2) were applied. The inoculums of bio-agents were grounded and finally mixed with soil at the time of seed sowing.

The plant leaves dried in oven at 20°C temperature over night and finally powdered with the help of grinder. Various quantities (6, 12, 18, 24g / 6m^2) of leaf powder, NPK and bio-agents in 1:1:1 ratios were applied for soil treatment.

For spray experiments the percentage spore suspension of suspending various quantities of mycelium in sterilized distilled water made up bio-agent. The aqueous suspension of mycelium was spread on the plant at different concentrations when disease symptoms appeared. Four replicates of each treatment were taken. The PDI and yield were recorded. The inoculums of *F. oxysporum* were applied as soil treatment and *A. burnsii* were applied as seed treatment.

Experimental Design:

The in vitro data were calculated by using CRD and in vivo data were calculated by using RBD with three representative samples.

Results and discussion

Bio-agents used were found to be effective for the inhibition of mycelial growth of both Alternria burnsii and Fusarium oxysporum f. sp. cumini under in vitro conditions (Table1 and Figure 1). The maximum inhibition (82.86%) of radial growth of *F. oxysproum* f. sp. *cumini* was observed with the treatment of *Trichoderma harzianum* strain I followed by *T. harzianum* strain II (75.00%), *T. viride* strain II (74.05%), *T. viride* strain II (72.83%) and *Aspergillus nigr* (65.98%). Least inhibition was shown by *Gliocladium virens* strain I (38.23%) and *Trichoderma hamatum* (51.00%), whereas, *Gliocladium virens* strain II, *Aspergillus flavus* and *Aspergillus fumigatus* showed moderate inhibition. Vyas and Mathur (1999) and Aghnoom et al., (2002) who observed *Trichoderma harzianum* as a potential biocontrol agent against *Fusarium wilt* of cumin through antibiosis and hyper-parasitism.

The mycelial growth of *Alternaria burnsii* was inhibited maximum (85.45%) by *Trichoderma harzianum* strain II followed by *T. harzianum* strain I (75.65%), *T. viride* strain II (71.47%), *T. viride* strain I (70.24%), *A. niger* (68.39%), *T. viride* strain III (66.90%) and *A. flavus* (64.79%). Minimum inhibition was shown by *T. hamatum* (37.36%) and *Aspergillus fumigatus* (35.42%), whereas, the strains of *Gliocladium virens* showed moderate inhibition (Table 2 and Figure 2). Monaco et al., (2004) reported that *Trichoderma* species inhibited the mycelial growth of *Alternaria alternata in vitro* conditions.

The antagonists which showed maximum inhibition of the pathogen in laboratory conditions were applied in field conditions as soil, seed and spray treatment. The results of field experiments indicate that per cent disease incidence of blight and wilt in all the treatments, where antagonist fungi were used was significantly lower than control. The incidence of wilt disease was found to be lower (PDI 27.40%) when soil was treated with *Trichoderma harzianum* strain I at the rate of 40 kg/ha followed by 32.26 and 38.76% respectively under the treatment of *Trichoderma viride* strain I and *Aspergillus niger* both at the rate of 40 kg/ha. A significant increase in yield was recorded under all the treatments in comparison to control. Maximum yield of 298.12 kg/ha was recorded in soil amended with *T. harzianum* strain I at the rate of 40 kg/ha (Table 3). Jayalakshmi *et al.,* (2003) also observed that

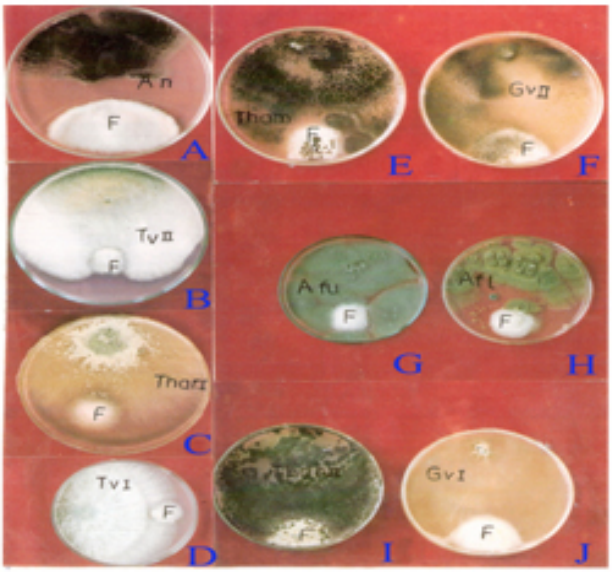

Figure 1 Antagonistic effect of various bioagents on F*usarium oxysporum* f. sp. *cumini (In vitro)*

A - *Aspergillus nige*r on *Fusarium oxysporum f. sp. cumini*; B - *Trichoderma viride* (Strain-II) on *Fusarium oxysporum f. sp. cumini;* C - *Trichoderma harzianum* (Strain-I) on *Fusarium oxysporum f. sp. cumini;* D - *Trichoderma viride* (Strain-I) on *Fusarium oxysporum f. sp. cumini;* E - *Trichoderma hamatum* on *Fusarium oxysporum f. sp. cumini*; F - *Gliocladium virens* (Strain-II) on *Fusarium oxysporum f. sp. cumini;* G - *Aspergillus fumigatus* on *Fusarium oxysporum f. sp. cumini*; H - *Aspergillus flavus* on *Fusarium oxysporum* f. sp. *cumini*;mI - *Trichoderma harzianum* (Strain-II) on *Fusarium oxysporum* f. *sp. cumini;* J - *Gliocladium virens* (Strain –I) on *Fusarium oxysporum f. sp. cumini.*

Table 1 Antagonistic effect of certain bio-agents on *Fusarium oxysporum* f. sp. *cumini* and *Alternaria burnsii* (*in vitro*)

Antagonists	*Fusarium oxysporum* f. sp. *Cumini*			*Alternaria burnsii*		
	Ave. mycelial radial growth of antagonist (mm)	Ave. mycelial radial growth of pathogen (mm)	Growth inhibition%	Ave. mycelial radial growth of antagonist (mm)	Ave. mycelial growth of pathogen (mm)	Average inhibition %
Trichoderma hamatum	50.00±0.28	24.50±0.28	51.00±0.29	45.50±0.28	28.50±0.28	37.36±0.23
Gliocladium virens (St. I)	21.33±0.33	13.16±0.16	38.23±1.50	46.16±0.16	22.16±0.44	51.97±1.09
Trichoderma viride (St. III)	49.83±0.44	15.16±0.16	69.56±0.22	45.83±0.16	15.16±0.16	66.90±0.48
Trichoderma viride (St. II)	52.16±0.16	14.16±0.16	72.83±0.37	46.16±0.16	13.16±0.16	71.47±0.42
Trichoderma viride (St. I)	31.50±0.76	8.16±0.16	74.05±0.49	42.16±1.42	12.50±0.28	70.24±1.61
Trichoderma harzianum (St. II)	40.66±0.66	10.16±0.16	75.00±0.00	35.50±0.28	5.16±0.16	85.45±0.36
Gliocladium virens (St. II)	54.66±0.16	24.00±0.28	56.09±0.41	46.83±0.16	24.16±0.16	47.67±1.25
Trichoderma harzianum (St. I)	30.16±0.16	5.16±0.16	82.86±0.60	44.50±0.28	10.83±0.16	75.65±0.25
Aspergillus niger	41.66±0.33	14.16±0.16	65.98±0.67	32.16±0.16	10.16±0.16	68.39±0.35
Aspergillus flavus	30.50±0.28	14.33±0.92	53.04±2.65	29.83±0.44	10.50±0.28	64.79±0.93
Aspergillus fumigatus	31.50±0.28	12.50±1.32	60.33±4.11	29.16±0.16	18.83±0.16	35.42±0.50
CD	4.68	1.53	1.18	1.44	0.711	2.40
SEM	1.58	0.52	0.40	0.49	0.24	0.81
CV	4.32	6.36	1.76	2.10	2.68	2.29

the seed treatment with *Trichoderma viride* followed by *T. harzianum* was found to be effective in reducing the wilt disease incidence in coriander.

When the bio-agents were applied in combination with NPK and plant leaves powder at different quantities, it resulted in better control of wilt incidence over individual application. The soil treatment with a mixture of similar ratio (1:1:1:1) of NPK + *T. viride* strain I + *T. harzianum* strain I and *Aspergillus niger* at the rate of 40kg/ha showed significant reduction of wilt disease severity and enhanced the yield (366.25 kg/ha) when compared to control. When *Trichoderma harzianum* strain I and leaf powder of *Datura stramonium* (1:1) were mixed in the soil at the rate of 40 kg/ha, it showed significant reduction in wilt disease incidence. Disease incidence was 30.33%, while in control it was recorded 53.39%. A mixture of *T. harzianum* strain I and *Azadirchta indica* (neem) leaves powder at the rate of 40 kg/ha retarded the disease incidence up to 31.06% followed by 33.72% disease incidence with the treatment of *T. harzianum* strain I + *Lantana camera* leaves powder which was at per with *T. viride* strain I + neem leaves powder both at (40 kg/ha). The yield was also increased significantly when compared to control. The lower concentrations of bio-agents and plant leaves powder were observed almost at par with each other to control disease incidence and for the enhancement of yield as given in table 4.

The blight disease incidence was observed minimum (PDI 36.15%) when *T. harzianum* strain II was applied at the rate of 40 kg/ha followed by 39.19 and 43.47% disease incidence under soil treatment with *Trichoderma viride*

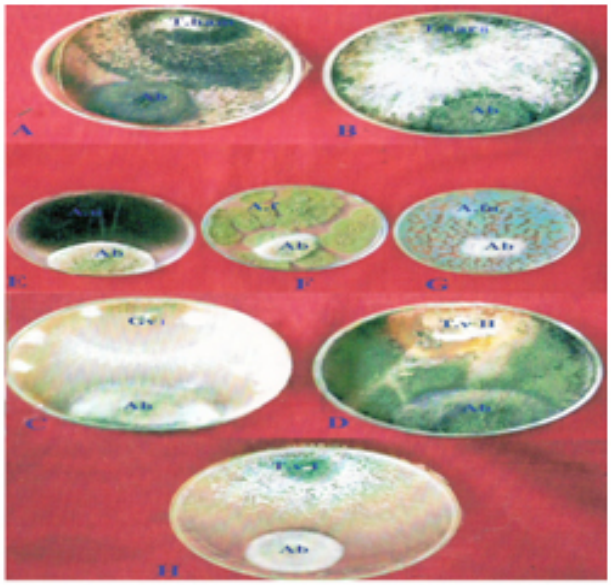

Figure 2 Antagonistic effect of various bioagents on *Alternaria burnsii (In vitro)*

A - *Trichoderma hamatum* on *Alternaria burnsii*; B - *Trichoderma harzianum* (Strain-II)on *Alternaria burnsii;* C - *Aspergillus niger* on *Alternaria burnsii;* D - *Aspergillus flavus* on *Alternaria burnsii;* E - *Aspergillus fumigatus* on *Alternaria burnsii;* F - *Gliocladium virens* (Strain-I) on *Alternaria burnsii;* G - *Trichoderma viride* (Strain-II) on *Alternaria burnsii;* H - *Trichoderma viride* (Strain-I) on *Alternaria burnsii*

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Table 2 Effect of soil treatment with bio-agents on wilt disease of cumin (*in vivo*)

S. No.	Bio-agent	Quantity (g / m^2)	Per cent Disease Incidence (Average, Mean Value)			Yield (Kg/ha)		
			2004-2005	2005-2006	Pooled	2004-2005	2005-2006	Pooled
1.	*Trichoderma viride* (St-I)	6	43.45 ±0.71	46.45±0.81	45.10	191.75	200.75	196.25
		12	39.37±0.15	41.19±0.86	40.28	210.75	221.75	216.25
		18	35.38±1.34	33.06±0.45	34.22	242.50	223.75	233.12
		24	33.21±0.54	31.32±0.69	32.26	271.50	292.50	282.00
2.	*T.harzianum* (St-I)	6	41.44±1.39	43.61±0.79	42.52	201.25	204.00	202.62
		12	37.02±1.43	40.29±0.74	38.65	211.25	215.00	213.12
		18	33.64±0.95	32.97±0.22	33.30	221.75	241.25	231.50
		24	29.99±1.16	24.82±125	27.40	291.25	305.0	298.12
3.	*Aspergillus niger*	6	51.85±0.71	46.49±0.87	49.17	181.00	192.50	186.75
		12	45.13±2.11	42.96±0.67	44.04	211.25	215.25	213.25
		18	42.20±2.66	38.68±0.75	40.44	230.00	225.00	227.50
		24	42.23±1.18	35.29±1.58	38.76	256.25	262.50	259.37
4.	Control		50.47±0.84	50.11±1.36	50.29	87.50	93.75	90.62
	CD		3.94	2.72	5.12	4.51	5.37	16.09
	SEM		1.37	0.94	1.64	1.56	1.86	5.16
	CV		6.77	4.85	5.86	5.45	6.67	3.33

Table 3 Effect of soil treatment with bio-agents, NPK and plant leaf powder on wilt disease of cumin (*in vivo*)

S. No.	Treatment	Quantity (g / m^2)	Per cent Disease Incidence (Average Mean Value)			Yield (Kg/ha)		
			2004-2005	2005-2006	Pooled	2004-2005	2005-2006	Pooled
1.	NPK + *Trichoderma viride (St.-I)* + *T. harzianum* (St.-I)	6	46.49±1.62	39.09±0.24	42.79	310.0	193.75	251.87
		12	39.94±1.02	33.35±1.99	36.64	341.25	293.75	317.50
		18	32.53±0.65	25.84±1.78	29.18	355.00	351.25	353.12
		24	27.53±1.62	22.27±3.18	24.90	368.75	363.75	366.25
2.	*T. viride* (St.-I) + *Azadirachta indica*	6	48.66±3.36	38.16±1.10	43.41	215.00	194.25	204.62
		12	45.04±1.36	32.89±2.09	38.96	223.75	221.25	222.50
		18	43.47±1.16	30.67±1.01	36.77	231.25	245.00	238.12
		24	39.31±1.20	27.96±3.93	33.63	251.25	260.00	255.62
3.	*T. harzianum* (St.-I) + *Azadirachta indica*	6	43.68±1.33	43.68±2.19	43.68	200.75	204.25	202.50
		12	41.44±1.04	37.71±1.25	39.57	212.50	223.75	218.12
		18	39.25±0.05	.32.41±1.69	35.83	251.25	262.50	256.87
		24	34.24±1.55	27.88±1.84	31.06	283.75	292.50	291.12
4.	*T. viride* (St.-I) + *Datura stramonium*	6	49.37±2.21	41.75±0.85	45.56	192.50	200.75	196.62
		12	44.25±3.12	37.14±1.25	40.69	210.00	205.25	207.62
		18	40.75±1.11	34.45±2.15	37.60	240.75	238.50	239.62
		24	36.99±1.74	32.52±3.26	34.75	252.50	252.75	252.62
5.	*T. harzianum* (St.-I) +*Datura stramonium*	6	47.14±2.11	40.51±2.64	43.82	192.50	194.00	193.25
		12	44.48±3.12	33.65±2.84	39.06	210.52	235.00	222.75
		18	36.28±0.15	32.16±1.35	34.22	242.50	242.50	242.50
		24	32.10±1.15	28.57±1.85	30.33	273.75	271.25	272.37
6.	*T. viride* (St.-I) + *Lantana camara*	6	53.44±3.12	46.13±1.15	49.78	190.50	200.75	195.62
		12	49.30±2.79	41.20±0.25	45.25	201.25	216.50	208.87
		18	43.68±1.72	38.84±1.15	41.26	220.75	222.75	221.75
		24	38.50±0.28	36.00±1.09	37.25	241.05	250.00	245.62
7.	*T. harzianum* (St.-I) + *Lantana camara*	6	49.40±0.31	32.97±3.10	41.18	215.25	226.00	220.62
		12	44.33±3.21	42.67±2.12	43.50	194.00	197.25	195.62
		18	36.89±1.22	38.67±1.85	37.78	266.00	263.75	264.87
		24	31.90±2.85	35.55±1.10	33.72	290.25	294.00	292.12
8	Control		53.80±0.15	52.98±0.21	53.39	108.00	121.25	114.62
	CD		3.86	3.16	6.47	4.69	4.83	37.14
	SEM		1.37	1.12	2.22	1.66	1.71	1275
	CV		6.55	6.41	8.10	5.38	7.43	7.51

Table 4 Effect of soil treatments with bio-agents and NPK on blight disease *(in vivo)*

S. No.	Bio-agent	Quantity (g / m²)	Per cent Disease Incidence (Average Mean Value)			Yield (Kg/ha)		
			2004-2005	2005-2006	Pooled	2004-2005	2005-2006	Pooled
1.	*T. viride* (St. II)	6	46.80±1.56	39.12±2.17	42.96	180.50	211.00	195.75
		12	45.86±1.51	38.21±1.12	42.03	189.75	215.25	202.50
		18	40.15±1.18	34.93±0.62	37.54	192.50	225.00	208.75
		24	42.54±2.65	35.84±2.93	39.19	185.75	230.00	207.87
2.	*T. harzianum* (St. II)	6	43.66±1.15	36.26±2.02	39.94	176.25	240.00	208.12
		12	40.64±0.83	35.06±1.04	37.85	184.25	252.75	218.50
		18	t38.57±1.70	34.98±0.45	36.77	192.50	261.25	226.87
		24	38.93±0.92	33.38±1.12	36.15	191.00	273.50	232.25
3.	*Aspergillus niger*	6	54.50±2.74	40.02±1.46	47.26	131.00	201.00	166.00
		12	53.02±1.16	39.38±1.83	46.20	134.25	216.25	175.25
		18	51.94±0.45	38.75±1.70	45.34	185.00	221.00	203.00
		24	48.34±2.64	38.61±0.26	43.47	190.00	224.75	207.37
4.	NPK + *T. harzianum* (St. II) + *T. viride* (St. II) + *A. niger*	6	33.81±2.21	29.45±1.88	31.63	172.50	310.50	241.25
		12	32.70±2.82	29.19±1.26	30.94	182.50	315.00	248.75
		18	31.21±2.88	28.18±1.40	29.69	200.75	315.00	257.87
		24	30.81±1.80	27.90±216	29.35	212.50	317.50	265.00
5	Control		59.55±1.88	54.23±1.25	56.89	90.00	110.00	100.00
	CD		5.63	4.19	5.61	4.35	5.53	56.65
	SEM		1.97	1.47	1.85	1.52	1.94	18.73
	CV		9.16	8.14	6.62	6.70	6.57	12.63

strain II and *Aspergillus niger* both at (40 kg/ha), respectively. A significant increase of yield was observed in comparison to control. The combined application of NPK, *T. harzianum* strain II, *T. viride* strain II and *A. niger* (similar ratio) at all concentrations viz. 10,20,30 and 40 kg/ha were found to be superior both for control of disease incidence and to enhance yield over control as given in table 5. EI Rafai et al. (2003) proposed that soil inoculum with *T. hamatum* spores completely controlled the blight disease of tomato, caused by *Alternaria alternata*.

The blight incidence was observed minimum (PDI 32.58%) in seed treatment with *Trichoderma harzianum* strain II at 10% spore suspension concentration at the time of sowing and as spray at the time of flowering. It was followed by 36.72 and 40.69% disease incidence under the treatment of *Trichoderma*

Table 5 Effect of seed treatment and spray with bio-agents on blight disease (*in vivo*)

S. No.	Bio-agent	Conc. (% Mycelium/ spore suspension)	Per cent Disease Incidence (Average Mean Value)			Yield (Kg/ha)		
			2004-2005	2005-2006	Pooled	2004-2005	2005-2006	Pooled
1.	Trichoderma viride (St. I)	1	51.48±1.53	35.07±2.63	43.27	180.75	210.75	195.75
		2	48.58±1.33	33.92±1.42	41.25	187.00	225.25	206.12
		5	45.14±1.66	31.88±1.50	38.51	201.00	226.50	213.75
		10	39.49±2.23	33.96±2.05	36.72	211.00	238.25	224.62
2.	T. harzianum (St. II)	1	42.17±2.98	38.37±1.72	40.27	218.50	217.25	217.87
		2	43.42±.2.40	38.10±1.00	40.76	223.75	240.00	231.87
		5	39.67±3.58	33.57±0.72	36.62	232.75	262.50	247.62
		10	31.05±6.99	34.11±1.39	32.58	244.75	257.50	251.12
3.	*Aspergillus niger*	1	52.39±0.62	40.89±2.46	46.64	127.75	215.50	171.62
		2	50.66±1.06	40.62±0.59	45.64	135.75	221.25	178.50
		5	48.27±2.54	39.70±0.78	43.98	179.75	225.50	202.62
		10	45.39±2.41	36.00±1.45	40.69	193.75	229.75	211.75
4	Control		59.55±1.88	54.23±1.25	56.89	94.00	102.50	98.25
	CD		7.92	3.96	8.10	5.63	6.44	41.48
	SEM		2.75	1.37	2.60	1.95	2.23	13.31
	CV		11.98	7.30	8.79	2.09	2.02	9.23

viride strain II and *Aspergillus niger* respectively. In control 56.89% disease incidence was observed. The maximum yield was observed in the seed and spray treatment with *Trichoderma harzianum* strain II at all concentrations as compared to other treatments and control (Table 6). Yield was observed minimum in seed and spray treatment with *Aspergillus niger* at all concentrations. When results were statistically analyzed was found significant. Similarly, Nakkeeran and Devi, (1997) observed that *Alternaria alternata* causing blight disease in pigeon pea was most effectively reduced by seed treatment with *T. harzianum*. Ghosh *et al*. (2002) also revealed that *Trichoderma viride*, *T. hamatum* and *Aspergillus awamori* inhibited the growth of *Alternaria alternata*.

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