INDIAN JOURNAL OF
ARID HORTICULTURE
2016, Vol. 11 (1-2):64-69

Efficacy of *Trichoderma harzianum* and *Pseudomonas fluorescens* against wilt of cumin (*Cuminum cyminum* L.)

S.L. Godara, Suresh Kumar* and N.K. Pareek
Agricultural Research Station, SK Rajasthan Agricultural University, Bikaner-334006
*Corresponding author email: lorask77@gmail.com
(Received: 14.10.2015; Accepted: 4.01.2017)

Abstract

Efficacy of bioagents like *T. harzianum* and *P. fluorescens* alone or with combined inoculation as seed treatment and soil application along with or without vermicompost were evaluated against wilt of cumin caused by *Fusarium oxysporum* f. sp. *cumini* under field conditions *Rabi* 2013-14 and 2014-15. Among all the treatments, combined inoculation of *T. harzianum* + *P. fluorescens* as seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost resulted minimum disease incidence (15.82%) and highest per cent disease control (64.42%) followed by *T. harzianum* + *P. fluorescens* combined application as seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost. These treatments were also found effective on the dry weight of cumin plants. The shoot, root lengths and cumin seed yield were significantly increased in response to bioagent treatments. Highest shoot, root lengths and seed yield were also recorded in combined inoculation of bioagent treatments i.e. *T. harzianum* + *P. fluorescens* as seed treatment (4+4 gm/kg seed) and soil application along with vermicompost (2.5+2.5 kg/ha.) closely followed by *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost. Whereas, rest of the treatments were found least effective on shoot, root lengths and seed yield of the cumin.

Keywords: Cumin, wilt, *Fusarium oxysporum* f. sp. *cumini, T. harzianum* and *P. fluorescens*.

Introduction

Cumin (*Cuminum cyminum* L.) locally known as Zeera, belonging to the family Apiaceae and believed to have originated from Egypt. It is grown extensively in South-Eastern Europe and North Africa bordering the Mediterranean sea and in India and China (Chattopadhyay and Maiti, 1990). In India, cumin is mainly grown in the states of Rajasthan, Gujarat, Uttar Pradesh and Tamil Nadu. Rajasthan and Gujarat states contribute 56% of total cumin production in the country (Sree Kumar, 1994). India is the largest producer and consumer of cumin crop. India contributes about 70% of world production followed by Syria 11%, Iran and Turkey 6% each respectively (Anon, 2008). The cumin seeds have an aromatic fragrance and used in food beverages, pharmaceuticals and perfumery. The cumin oil contains cuminaldehyde, hydrocarbons and oxygenated compounds (Chattopadhyay and Maiti, 1990). Wilt caused by *Fusarium oxysporum* (Schlecht) Snyd. and Hans. f. sp. *cumini* (Prasad and Patel, 1963) is an endemic problem in most of cumin growing areas of the country. Vyas and Mathur (2002) recorded loss in seed yield to the extent of 35% due to this disease in Rajasthan. The pathogen is seed as well as soil-borne in nature. In absence of host crops, it survives in the soil mostly as chlamydospore (Mathur and Mathur, 1970).

With the increasing awareness of possible deleterious effects of ecosystem due to pesticide usage and growing interest in pesticide free agricultural products, the biological control of plant pathogens have received considerable attention. Biological control of plant pathogens by microorganisms has been considered as a natural and environmentally acceptable alternative to the existing chemical treatment methods (Baker and Paulitz, 1996). Hence, use of suitable bioagents alone and in combinations with organic substrates stands good alternatives for effective management of this pathogen (Mukhopadhyay, 1994; Chawla and Gangopadhyay, 2009; Gangopadhyay and Ram Gopal, 2010). Therefore, the present investigation was to evaluate combined effect of seed treatment and soil application of bioagents along with organic substrate against wilt of cumin. In the present experiment we carried out with alone and combined inoculation of *T. harzianum* with *P. fluorescens* to evaluate its efficacy against this pathogen.

Materials and Methods

Isolation and identification of the pathogen

Wilt infested cumin plants were collected from the field and gently washed in tap water to remove the soil and other extraneous materials adhering on root surface. The washed plant roots were cut into small pieces and surface

sterilized in 0.1 % $HgCl_2$ solution in Petri dishes for 1 to 2 minutes followed by washing in sterilized distilled water. The surface sterilized plant root pieces were transferred to potato dextrose agar (PDA) medium in Petri dishes. The Petri dishes were incubated in BOD incubator for seven days at 25 ±1°C for growth of the pathogen. The culture was observed under microscope and identified according to its spores, morphology and colony characteristics. The stock culture was kept in refrigerator at 4°C for further studies.

Bioagents collection and preparation of formulations

Two bioagents *viz*., *Trichoderma harzianum* and *Pseudomonas fluorescens* obtained from the culture collection of the Department of Plant Pathology, College of Agriculture, SKRAU, Bikaner were used in the present study. These bioagents were isolated from the rhizosphere soils of this region. Antagonistic potentiality of these bioagents against *Fusarium oxysporum* f. sp. *cumini* was tested under laboratory conditions. Talc based formulations of these bioagents were prepared in the laboratory for testing their efficacy against cumin wilt under field conditions.

Trichoderma harzianum

T. harzianum was mass cultured on potato dextrose broth medium. For this purpose, mycelial discs (5 mm diameter) taken from periphery of actively growing colony of fungus raised on potato dextrose agar media in Petri dishes were transferred in 250 ml Erlenmeyer flasks containing 70 ml potato dextrose broth media and incubated at 26±1°C for 15 days. The biomass was homogenized and mixed with talc powder (1:2 ratio) and allowed to dry in shade. Carboxymethyl cellulose was added at 6 g kg^{-1} talc preparation as a sticker and mixed thoroughly. The colony forming units (CFU) of the preparation was determined using Trichoderma selective medium (TSM). The CFU of the bioagents prepared was nearly 10^9 g^{-1} formulation.

Pseudomonas fluorescens

The culture of *P. fluorescens* was maintained on Pseudomonas agar fluorescens (PAF) medium in slants. A loop full of culture was transferred to Erlenmeyer flask containing 70 ml sterilized King's B broth (King, *et al.*, 1954) and incubated at 27±1°C for 72 hrs. The media containing the bacterial growth was mixed with the talc powder in 1:2 ratio and dried in shade. The mixture was sieved to obtain fine powder. Carboxymethyl cellulose was added at 6 g kg^{-1} talc preparation and mixed thoroughly. The CFU of the preparation was determined in Pseudomonas agar fluorescens media which was approximately 10^{12} g^{-1} formulation.

Management of cumin wilt under field conditions

The field trials were conducted on management of wilt of cumin using bioagents alone or with combination during the cropping season *Rabi*-2013-14 and 2014-15 using cumin variety RZ-19. Talc based formulations of *viz*., *T. harzianum* and *P. fluorescens* were used for seed treatment and soil application along with vermicompost at 5 tonnes/ha. was applied in field soil. Nine treatments *viz*., *T. harzianum* seed treatment (8 gm/kg seed), *T. harzianum* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost, *T. harzianum* + *P. fluorescens* combined application as a seed treatment (4+4 gm/kg seed), *T. harzianum* + *P. fluorescens* combined application as a seed treatment (4+4 gm/kg seed) and soil application (2.5 +2.5 kg/ha.) along with vermicompost, *P. fluorescens* alone seed treatment (8 gm/kg seed), *P. fluorescens* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost, *T. harzianum* + *P. fluorescens* combined application as seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost, *T. harzianum* alone seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) without vermicompost and control (without any bioagents treatment) were tested randomized block design having plot size 4.5x3 m^2. Each treatment was replicated thrice. The trial was conducted under artificial soil inoculation conditions. For this purpose, the *F. oxysporum* f. sp. *cumini* isolate were multiplied on sand maize meal (2:1) medium in Erlenmeyer flasks. These flasks containing the sterilized media were inoculated with respective *F. oxysporum* f. sp. *cumini* cultures and incubated at 25±1°C for 15 days. Sand maize meal inocula of *F. oxysporum* f. sp. *cumini* was applied at 50 g per plot (4.5x3 m^2) and mixed thoroughly on top surface of soil using a hand rack. Standard agronomic practices recommended for its cultivation in this region was followed. In case of control, the untreated seeds were sown. Observations on wilt incidence were recorded periodically. The shoot length, root length and dry weight of cumin plants were recorded at maturity and seed yield was recorded at harvest. The shoot length, root length and dry weight of five plants for each replication were recorded.

Calculation and statistical analysis

Disease incidence (%) and disease control (%) in field experiments were calculated as follows:

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total No. of plants germinated}} \times 100$$

$$\text{Disease control (\%)} = \frac{\text{Disease incidence in inoculated control (\%)} - \text{Disease incidence in treatment (\%)}}{\text{Disease incidence in inoculated control (\%)}} \times 100$$

The data of per cent disease incidence in the experiment was transformed to their Arcsin values (Fisher

and Yates, 1963). The statistical analyses of the data of all field experiments were analyzed following Randomized Block Design (Cochran and Cox, 1957).

Results and Discussion

The results revealed that all the treatments significantly suppress the wilt incidence of cumin with the application of respective bioagents as compare to control (Table 1). Treatment *T. harzianum* + *P. fluorescens* combined application as a seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost resulted minimum disease incidence (15.82%) and highest per cent disease control (64.42%) followed by seed treatment with *T. harzianum* + *P. fluorescens* (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) as a combined application without vermicompost. The seed treatment of *T. harzianum* + *P. fluorescens* (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost was statistically at par with treatment *T. harzianum* + *P. fluorescens* combined application as seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost. The disease control efficacy in this combined bioagent treatment i.e. seed treatment by *T. harzianum* + *P. fluorescens* (4+4 gm/kg seed) and soil application along with vermicompost (2.5+2.5 kg/ha.) was significantly higher than seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) of *T. harzianum* along with vermicompost alone treatment. Further, the treatments *P. fluorescens* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost, combined seed treatment with *T. harzianum* + *P. fluorescens* (4+4 gm/kg seed), *T. harzianum* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) without vermicompost and alone *T. harzianum* seed treatment (8 gm/kg seed) were also provide more than 40 per cent disease control. While, the seed treatments with *P. fluorescens* alone (8 gm/kg seed) were found least effective in controlling of the wilt incidence of cumin on the basis of pooled data.

Effect of bioagent treatments on the dry weight of cumin plants was also evaluated. The highest dry weight of cumin plants were recorded in the combined bioagent treatment with *T. harzianum* + *P. fluorescens* as a seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost. Maximum dry weight of cumin plants were recorded in this treatment was statistically significant as compare to the combined treatment of *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost and rest of the treatments. The treatment *T. harzianum* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost, seed treatment and soil application of *T. harzianum* along with vermicompost, *P. fluorescens* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost, *T. harzianum* + *P. fluorescens* combined bioagent seed treatment (4+4 gm/kg seed) and *T. harzianum* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) without vermicompost were also found effective on dry weight of cumin plants. Whereas, rest of the treatments were recorded least effective on cumin plants dry weight (Table 1).

Table 1. Effect of bioagents against disease incidence and dry weight of cumin plants (Pooled 2013 and 14)

Treatments	Disease incidence (%)		Pooled	(%) Disease Control	Dry weight (gm/plant)		Pooled	(%) Increase over control
	2013	2014			2013	2014		
T. harzianum (ST @ 8 gm/kg seed)	25.42 (30.27)	27.33 (31.50)	26.38 (30.89)	40.67	1.31	1.26	1.28	33.12
T. harzianum (ST @ 8 gm/kg seed and SA- 5 kg/ha.) along with vermicompost	18.64 (25.42)	21.58 (27.67)	20.11 (26.54)	54.76	1.57	1.53	1.55	44.50
T. harzianum + *P. fluorescens* (ST @ 4+4 gm/kg seed)	23.28 (28.84)	24.75 (29.82)	24.01 (29.33)	45.99	1.38	1.33	1.36	36.65
T. harzianum + *P. fluorescens* (ST @ 4+4 gm/kg seed and SA- 2.5+2.5 kg/ha.) along with vermicompost	14.75 (22.58)	16.89 (24.27)	15.82 (23.42)	64.42	1.75	1.71	1.73	50.48
P. fluorescens (ST @ 8 gm/kg seed)	28.44 (32.22)	30.44 (33.48)	29.44 (32.85)	33.77	1.26	1.19	1.23	30.03
P. fluorescens (ST @ 8 gm/kg seed and SA- 5 kg/ha.) along with vermicompost	20.78 (27.09)	22.39 (28.22)	21.58 (27.65)	51.45	1.49	1.46	1.48	41.87

T. harzianum + *P. fluorescens* (ST @ 4+4 gm/kg seed and SA- 2.5+2.5 kg/ha.) without vermicompost	17.67 (24.52)	18.92 (25.78)	18.29 (25.15)	58.86	1.63	1.61	1.62	47.02
T. harzianum (ST @ 8 gm/kg seed and SA- 5 kg/ha.) without vermicompost	22.25 (28.12)	26.43 (30.94)	24.34 (29.53)	45.25	1.36	1.32	1.34	36.10
Control (without bioagents treatment)	42.75 (40.83)	46.17 (42.80)	44.46 (41.82)		0.90	0.82	0.86	
S.Em. ±	(1.33)	(0.76)	(0.76)		0.01	0.02	0.01	
C.D (p=0.05)	(3.98)	(2.28)	(2.20)		0.04	0.06	0.04	

* Figures in parentheses are angular transformed values. Note: ST- Seed treatment, SA- Soil application

The shoot and root lengths of cumin plants was significantly increased in response to bioagent treatments. Highest shoot and root lengths were recorded in combined bioagent treatment with *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost followed by the combined use of bioagent treatment *T. harzianum* + *P. fluorescens* as a seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost (table 2). The treatment *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed) and soil application along with vermicompost (2.5+2.5 kg/ha.) was statistically significant as compared to treatment *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost and rest of the treatments. Both the shoot and root lengths of cumin plants were also positively influenced by the *T. harzianum* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost, *P. fluorescens* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) used along with vermicompost and *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed).

Results also revealed that cumin seed yield was significantly enhanced in response to bioagent treatments as compare to control. Highest seed yield was recorded in the combined bioagent treatment *T. harzianum* + *P. fluorescens* as seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost which was closely followed by seed treatment *T. harzianum* + *P. fluorescens* (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost and *T. harzianum* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost (table 2). The seed yield recorded in combined bioagent treatment *T. harzianum* + *P. fluorescens* used as a seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost was statistically at par with the treatment *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) without vermicompost. However, the combined bioagent *T. harzianum* + *P. fluorescens* seed treatment (4+4 gm/kg seed) and soil application (2.5+2.5 kg/ha.) along with vermicompost was statistically significant as compare with *T. harzianum* seed treatments (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost treatment and all other treatments. Seed yield of cumin was also considerably higher in the bioagent treatments *P. fluorescens* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) along with vermicompost, alone seed treatment by *T. harzianum* + *P. fluorescens* (4+4 gm/kg seed) and *T. harzianum* seed treatment (8 gm/kg seed) and soil application (5 kg/ha.) without vermicompost as compare to all other treatments (Table 2).

Table 2. Effect of bioagents on shoot, root length and grain yield of cumin (Pooled 2013 and 14)

Treatments	Shoot length (cm)		Pooled	Root length (cm)		Pooled	Grain yield (q/ha)		Pooled
	2013-14	2014-15		2013-14	2014-15		2013-14	2014-15	
T. harzianum (ST @ 8 gm/kg seed)	26.33	22.37	24.35	13.23	11.77	12.50	3.63	3.89	3.76
T. harzianum (ST @ 8 gm/kg seed and SA- 5 kg/ha.) along with vermicompost	32.50	30.30	31.40	15.30	14.00	14.65	4.88	5.25	5.07
T. harzianum + *P. fluorescens* (ST @ 4+4 gm/kg seed)	29.97	26.00	27.98	14.00	12.70	13.35	4.21	4.83	4.52

T. harzianum + *P. fluorescens* (ST @ 4+4 gm/kg seed and SA- 2.5+2.5 kg/ha.) along with vermicompost	35.43	34.27	34.85	16.77	15.43	16.10	5.41	5.89	5.65
P. fluorescens (ST @ 8 gm/kg seed)	23.80	20.50	22.15	12.43	10.03	11.23	3.16	3.44	3.30
P. fluorescens (ST @ 8 gm/kg seed and SA- 5 kg/ha.) along with vermicompost	31.70	27.43	29.57	15.40	13.30	14.35	4.76	5.06	4.91
T. harzianum + *P. fluorescens* (ST @ 4+4 gm/kg seed and SA- 2.5+2.5 kg/ha.) without vermicompost	33.67	31.20	32.43	15.70	14.43	15.07	5.21	5.58	5.40
T. harzianum (ST @ 8 gm/kg seed and SA- 5 kg/ha.) without vermicompost	26.60	23.47	25.03	13.67	12.20	12.93	4.38	4.43	4.41
Control (without bioagents treatment)	19.83	17.60	18.72	8.50	7.30	7.90	2.83	3.50	3.17
S.Em. ±	0.57	0.73	0.46	0.39	0.36	0.27	0.10	0.26	0.14
C.D (p=0.05)	1.71	2.20	1.34	1.18	1.08	0.77	0.30	0.77	0.40

Note: ST- Seed treatment, SA- Soil application

The antagonistic potentiality of *T. harzianum*, *T. viride*, *T. hamatum* and bacterial antagonists like *P. fluorescens*, *B. subtilis*, etc. against *F. oxysporum* pathogenic to cumin and many other host crops have been reported by several workers (Mukhopadhyay, 1987; Dubey *et al.*, 2007; Deepak *et al.*, 2008 and Khan and Gangopadhyay, 2012). The antagonistic effect of many bacterial antagonists like *P. fluorescens* and *B. subtilis* against different *Fusarium* spp. *viz.*, *F. oxysporum* f. sp. *ciceri*, *F. oxysporum* f. sp. *udum*, *F. oxysporum* f. sp. *cubense*, *F. oxysporum* f. sp. *vasinfectum*, etc. was reported by many workers (Ushamalini *et al*., 1997; Haq *et al*., 2001 and Prameela *et al*., 2005).

Godwin-Egein and Arinzae (2001) concluded that growth inhibition of *F. oxysporum* by *T. harzianum* was due to competition, lysis and hyperparasitism. Besides, induction of systemic resistance by *Trichoderma* and *Pseudomonas* species against soil-borne pathogens have been reported (Van Loon *et al*., 1998; Karthikeyan *et al*., 2006 and Jayalakshmi *et al*., 2009).

In another study, Yigit and Dikilitas (2007) tested the ability of biocontrol agents, fluorescent *Pseudomonas*, non-pathogen *Fusarium* strain and *T. harzianum* T-22 applied in combination and alone, to control of *F. oxysporum* f. sp. *lycopersici* was studied in the greenhouse. Tomato roots were treated with combination of *T. harzianum* T-22 + fluorescent *Pseudomonas* isolate gave the best control. Similarly, Garkoti, *et al*., (2014) observed that seed treatment with *T. harizanum* + *P. fluorescens* significantly reduced the *Fusarium* wilt disease incidence of lentil and increase in grain yield. In field conditions, seed and soil treatment with *T. harzianum* and *P. fluorescens* were found to enhance the root/shoot length and seed yield.

In conclusion, the combined bioagent seed treatment and soil application by *T. harzianum* + *P. fluorescens* isolates along with vermicompost reduced wilt severity in cumin plants.

References

Anonymous, 2008. Cumin_profile- www.icexindia.com.

Baker, R. and Paulitz, T.C. 1996. Theoretical basis for microbial interactions leading to biological control of soil borne plant pathogens In: Principles and Practice of Managing Soil borne Plant Pathogens. Hall, R. (ed.). *The* American Phytopathol. Soc. St. Paul, MN. pp. 50- 79.

Chattopadhyay, S.B. and Maiti, S. 1990. Diseases of Betelvine and Spices. ICAR, Krishi Anusandhan Bhavan, New Delhi. pp. 122-129.

Chawla, N. and Gangopadhyay, S. 2009. Integration of organic amendment and bioagents in suppressing cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini. Indian Phytopath.,* 62 (2): 209-216.

Cochran, W.G. and Cox, G.M. 1957. *Experimental Designs*, Second Edition, New York: John Wiley & Sons, Inc.

Deepak P., Saran, L. and Lal, G. 2008. Control of wilt and blight diseases of cumin through antagonistic fungi under *in vitro* and field conditions. *Not. Bot. Hort. Agrobot. Cluj.*, 36 (2): 91-96.

Dubey, S.C., Suresh, M. and Singh, B. 2007. Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. *Biol. Control*, 40 (1): 118-127.

Duffy, B.K. and Defago, G. 1999. Environmental factors modulating antibiotic and siderophore biosynthesis

by *Pseudomonas fluorescens* biocontrol strains. *Appl. Environ. Microbiol,* 65 (6): 2429-2438.

Fisher, R.A. and Yates, F. 1963. Statistical Tables for Biological, Agricultural and Medical Research. Oliver and Boyd, Edinburgh, London. pp. 146.

Gangopadhyay, S. and Ram Gopal 2010. Evaluation of *Trichoderma* spp. along with farm yard manure for the management of *Fusarium* wilt of cumin (*Cuminum cyminum* L.). *J. Spices Arom. Crops*, 19 (1&2): 57-60.

Garkoti. A., Kumar, V. and Tripathi, H.S. (2014). Control of wilt disease of lentil through bio control agents and organic amendments in Tarai region of Uttarakhand, India. *J. Environ. Biol.*, 35, 1067-1070.

Godwin-Egein, M.I. and Arinzae, A.E. 2001. Antagonism between *Trichoderma harzianum* Rifai and *Fusarium oxysporum* Schlecht Emend Sny and Hans. *J. Mycol. Pathol.* 31(1): 22-30.

Haq, M.I., Ahmed, R., Khan, S.M. and Javed, N. 2001. Use of different strains of *Pseudomonas fluorescens* alone and in combination to suppress chickpea wilt. *Pak. J. Phytopath*., 13 (2): 144-150.

Jayalakshmi, S.K., Raju, S., Usha Rani, S., Benagi, V.I. and Sreeramulu, K. 2009. *Trichoderma harzianum* L_1 as a potential source for lytic enzymes and elicitor of defense responses in chickpea (*Cicer arietinum* L.) against wilt disease caused by *Fusarium oxysporum* f. sp. *ciceri*. *Australian J. Crop Sci.,* 3 (1): 44-52.

Karthikeyan, M., Radhika, K., Mathiyazhagan, R., Bhaskaran, R., Samiyappan, R. and Velazhahan, R. 2006. Induction of phenolics and defense related enzymes in coconut (*Cocos nucifera* L.) roots treated with biocontrol agents. *Braz. J. Plant Physiol*., 18 (3): 367-377.

Khan, M.A. and Gangopadhyay, S. 2012. Effect of soil inhabiting antagonistic microflora against *Fusarium oxysporum* f. sp. *ciceri*, incitant of wilt in chickpea. *J. Mycol. Pl. Pathol*., 42 (3): 341-346.

King, E.O., Ward, M.K. and Raney, D.E. 1954. Two simple media for the demonstration of Pycocyanis and Fluorescin. *J. Lab. Clin. Med.,* 44: 301-307.

Mathur, B.L. and Mathur, R.L. 1970. Role of contaminated seed in dissemination of cumin wilt fungus *Fusarium oxysporum* f. sp. *cumini*. *Rajasthan J. Agril. Sci.*, 1 (2): 80-82.

Mukhopadhyay, A.N. 1987. Biological control of soil-borne plant pathogens by *Trichoderma* spp. *Indian J. Mycol. Pl. Pathol.*, 17 (1): I-X.

Mukhopadhyay, A.N. 1994. Biocontrol of soil-borne fungal plant pathogens – current status, future prospectus and potential limitation. *Indian Phytopath*., 47: 119-126.

Prameela, M., Rajeswari, B., Prasad, R.D. and Reddy, D.R.R. 2005. Bioefficacy of antagonists against *Fusarium oxysporum* f. sp. *carthami* isolates inciting safflower wilt. *J. Mycol. Pl. Pathol*., 35 (2): 272-274.

Prasad, N. and Patel, P.N. 1963. *Fusarium* wilt of cumin (*Cuminum cyminum* L.) in Gujarat State, India. *Plant Dis. Reptr*.,47 (6): 528-531.

Sree Kumar, B. 1994. Production and export of seed spices with special reference to Rajasthan. *Spices India,* 7: 6-8.

Ushamalini, C.K., Rajappan, and K. Gangadharan. 1997. Control of *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *tracheiphilum* by antagonists under field conditions. *Plant Dis. Res.,* 12 (2): 122-129.

Van Loon, L.C., Bakker, P.A.H.M. and Pieters, C.M.J. 1998. Systemic resistance induced by the rhizosphere bacteria. *Ann. Rev. Phytopahol*., 36: 453-483.

Vyas, R.K. and Mathur, K. 2002. Distribution of *Trichoderma* spp. In cumin rhizosphere and their potential in suppression of wilt. *Indian Phytopath.,* 55 (4): 451-457.

Yigit, F. and Dikilitas, M. 2007. Control of Fusarium wilt of tomato by combination of Fluorescent *Pseudomonas*, Non-pathogen *Fusarium* and *Trichoderma harzianum* T-22 in Greenhouse Conditions. *Pl. Patho. Journal*, 6: 159-163