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Jaywant Kumar Singh
Research Scholar, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Manoj Kumar
Research Scholar, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Sanjeev Kumar
Scientist, Indian Institute of Soybean Research, Indore, Madhya Pradesh, India

Anil Kumar
Professor, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Naresh Mehta
Professor, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Correspondence
Manoj Kumar
Research Scholar, Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Effect of chemicals and antagonists on growth and sporulation of *Fusarium oxysporum* causing wilt of chilli (*Capsicum annuum* L.)

Jaywant Kumar Singh, Manoj Kumar, Sanjeev Kumar, Anil Kumar and Naresh Mehta

Abstract
Efficacy of fungicides and antagonists was tested under *in vitro* conditions for the mycelial growth inhibition and reduction in sporulation of *Fusarium oxysporum* (*i.e.*, Isolate Fo8). The fungicides carbendazim 50% and carbendazim 12%+mancozeb 63% were quite effective which completely inhibited the mycelial growth and the sporulation at all the concentrations (50 ppm to 1000 ppm); other fungicides *viz*., propiconazole 25%, captan 70%+hexaconazole 5%, carboxin 37.5%+thiram 37.5% showed effectiveness relatively at higher concentrations (*i.e.*, 1000 ppm), whereas, captan 50% was least effective. The rate of sporulation decreased linearly with an increase in fungicides concentration and with the type of fungicidal treatment. The statistical analysis ($P \geq 0.05$) showed that rate of sporulation measured at 100 ppm was significantly affected with the fungicides. Complete inhibition in sporulation was revealed by carbendazim 50% and carbendazim 12% + mancozeb 63% that was statistically at par with rest of the fungicides. However, the minimum inhibition in sporulation was recorded in captan 50% (*i.e.*, 20.01%) that was significantly lower than rest of the treatments. Among the four antagonists, *Trichoderma harzianum* was better which inhibited 45.9 per cent mycelial growth, followed by *T. viride* (31.9%), whereas, the bacterial antagonist *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were ineffective. The maximum reduction in sporulation (92.66%) was exhibited by *T. harzianum* and *T. viride* (86.85%), whereas, *P. fluorescens* and *B. subtilis* were least effective with 49.85 and 34.86% reduction in sporulation, respectively. The effective chemicals and antagonists may form a part of integrated management of the disease.

Keywords: *capsicum annuum*, *fusarium oxysporum*, chemicals, antagonists, growth inhibition, sporulation

Introduction
Chilli under genus *Capsicum* and family solanaceae, is an often cross-pollinated annual herb with diploid chromosome number (2n= 2X= 24). It is most widely used universal spice, known with several synonyms as wonder spice, pepper, hot pepper, *etc.* The worldwide area of cultivation is 1.99 million hectare, with production of 3.35 million tonnes on dry weight basis (FAO, 2012) [2]. India is the leading producer and consumer of chilli with production capacity of 1.49 million tones, area 0.77 milllion hectare and productivity 1.92 MT/ha (Anonymous, 2014) [4]. Fungi, bacteria and viruses have drastically restricted the yield potential and quality (Ochoa and Ramirez, 2001; Egea *et al*., 2002) [38, 18]. Fusarium wilt has emerged as a serious problem in past decade with the disease incidence of 2-85 per cent in different regions of India (Anonymous, 2005) [3]. The yield loss due to the disease is 10-80 per cent worldwide (Loganathan *et al*., 2013) [26] on different cultivars depending on the prevailing climatic conditions. *Fusarium oxysporum, F. solani, F. moniliforme* and *F. pallidoroseum* are important species causing wilt of chilli in India (Naik, 2006) [34]. The pathogen is typically soil-borne (Booth, 1971) [11] and produce different types of spores, *i.e.*, macro-conidia, micro-conidia and chlamydospores (Nelson *et al.*, 1981) [35] which help in survival of the pathogen. Generally, dry weather condition and excessive soil moisture are conducive factors for the disease development. Daami-Remadi *et al.* (2006) [14] observed that the temperature range from 25 to 30°C was optimum for maximum mycelial growth and sporulation of *F. oxysporum* f.sp. *tuberose.* Different fungicides are used worldwide at large scale against *Fusarium* wilt diseases in economically important crops (Haware *et al*., 1990; Foster and Hausbeck, 2010; Ragab *et al*., 2012; Abdel-Monaim *et al*., 2014; Arunodhayam *et al*., 2014) [23, 21, 41, 1, 5].

In practice, many fungicides are used to control the wilt disease of chilli, like di-thiocarbamates, benzimidazoles, sterol biosynthesis inhibitors but the new generation fungicides *viz*., strobilurins, azoxystrobins and trifloxistrobin are most effective against the disease (Kelaniyangoda *et al*., 2011; Rather *et al*., 2012; Wani and Najar, 2012) [24, 43, 55]. Amany *et al*. (2003) [2] revealed a progressive reduction in growth and sporulation of *F. oxysporum* grown on media amended with increased dose of benlate and zineb. Management through antagonists *viz*., *Trichoderma* spp., *Bacillus* spp., *Pseudomonas* spp. have shown limited success in few crops but are otherwise promising in nature (Cook and Baker, 1983; Hallman *et al*., 1997; Madhukar and Naik, 2004; Rini and Sulochana, 2006; El-Hassan *et al*., 2013; Wani *et al*., 2014; Srideepthi and Krishna, 2015) [28, 44, 49, 55]. The effect of *Trichoderma* spp. and fluorescent *Pseudomonad* on the incidence and intensity of Fusarium wilt and growth improvement in chilli has been reported by Rini and Sulochana (2006) [44]. As, limited work has been done in management of *Fusarium* wilt of chilli by the use of fungicides, botanicals and antagonists. Keeping in view these research gaps, the present investigation was undertaken to sort out the most effective chemicals and antagonists against the growth and sporulation of *F. oxysporum* to make them an integral part of integrated management of Fusarium wilt disease of chilli.

Materials and Methods

Evaluation of Fungicides: The fungicides were tested against *Fusarium oxysporum* (virulent isolate Fo8) by using poisoned food technique (Nene and Thapliyal, 1973) [36] at 50, 100, 200, 500 and 1000 ppm, respectively. A 0.2 per cent (= 2000 ppm) stock solution was prepared for each fungicide and the required concentration was achieved through dilution technique and plated in Czapek's Dox agar (CZA) plates. The mycelial discs of five mm size from actively growing (4 days old) culture of the *F. oxysporum* (isolate Fo8) were cut by using sterile cork-borer and one such disc was placed at the centre of each CZA plate and incubated at 27±1°C in BOD incubator with alternate light and dark for 12 hrs. The mean radial growth was measured in terms of diameter of mycelial growth in Petri plate, till fungus in the control plate attained maximum growth (90 mm). The per cent inhibition of the radial mycelial growth of the pathogen over control was calculated as per formula given by Vincent (1947) [54], as depicted below.

$$I = \frac{C - T}{C} X100$$

Where,
I = Per cent inhibition; C = Growth in control; T = Growth in treatment

Evaluation of Antagonists: The antagonists *viz*., *Trichoderma harzianum*, *Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their antagonistic effect against *F. oxysporum* (virulent isolate Fo8) by dual culture technique (Morton and Stroube, 1955) [31]. The cultures of antagonists used in the present study were obtained from the Department of Plant Pathology, CCS HAU, Hisar. The fungal antagonists were revived on potato dextrose agar (PDA) plates, whereas, the bacterial antagonists were revived on the nutrient agar (NA) plates by incubating at 27±1°C in BOD incubator for 3-4 days after inoculation. For evaluation of fungal antagonists, mycelial disc (5mm) of test fungus (*i.e.*, isolate Fo8) was inoculated at one end of the Petri plate and antagonistic fungus *viz*., *Trichoderma* was placed opposite to it on the other end. In case of evaluation of bacterial antagonist, the bacterium *viz*., *B. subtilis/ P. fluorescens* was streaked with inoculating loop at one end of the Petri plates and mycelial disc (5mm) of the test fungus (*i.e.*, isolate Fo8) was placed at the other end. The plates were incubated at 27±1°C in BOD incubator with alternate light and dark for 12 hrs. The zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The radial growth (towards the antagonists) of the test pathogen in dual culture was recorded till complete radial growth occurred in the control plate (maximum radial growth= 45 mm from the mycelial disc). The per cent inhibition of radial mycelial growth of the pathogen over control was calculated as per formula given by Vincent (1947) [54], as mentioned earlier.

Measurement of Sporulation: The conidial density and sporulation pattern of the isolate Fo8 with different concentrations of chemicals and the plated antagonist, was studied in the incubated CZA plates. Ten ml of sterile distilled water was added to culture plate and using a sterile glass slide, the culture surface was gently scrapped to make a conidial suspension. The number of conidia were counted using Neubauer haemocytometer. Conidia produced per unit surface area and spore density (= Number of conidia/ml suspension) were estimated using the formula given below.

$$\text{Conidia produced per unit surface area (mm}^2\text{)} = \frac{\text{Number of conidia ml}^{-1}\text{ suspension x Total surface area from which conidial suspension was derived}}{\text{Volume of water to make suspension}}$$

Spore density (spores/ml) = (n) x 25 x 10^4
Where, n = the average cell count per small square in a central large square
Three replications were maintained for each concentration of the treatments and the plated antagonists in a completely randomized design. The data was analyzed by OPSTAT package of programs (Sheoran, 2006) [48] after arcsine transformation.

Results

Fungicides: There was a significant decrease in mycelial growth with an increase in concentration of fungicides. The extent of growth inhibition, however, varied amongst the six fungicides. All the fungicides strongly inhibited the growth of the test fungi at 1000 ppm concentration. Perusal of data indicated complete growth inhibition with carbendazim 50% and carbendazim 12 %+mancozeb 63% at 100 ppm (*i.e.*, 0.01%) that was statistically at par with propiconazole 25%, captan 70%+hexaconazole 5%, carboxin 37.5%+thiram 37.5% (Table 1; Figure 1), while growth inhibition recorded in captan 50% was significantly lower than rest of the treatments. Intermediate level of growth suppression was observed with propiconazole 25%, captan 70%+hexaconazole 5%, carboxin 37.5%+thiram 37.5%, whereas, captan 50% was ineffective. At lower concentration (100 ppm), carbendazim 50% and carbendazim 12%+mancozeb 63% caused complete

inhibition, whereas propiconazole 25%, captan 70%+hexaconazole 5%, carboxin 37.5%+thiram 37.5% and captan 50% led to 84.44, 78.15, 36.30 and 6.30% inhibition of mycelia, respectively. The systemic fungicides either alone or in combination with contact fungicide *viz*., carbendazim 50% and carbendazim 12%+mancozeb 63% were generally effective at lower concentrations (50-100 ppm), whereas, the non-systemic fungicides *viz*., captan 50% and carboxin 37.5%+thiram 37.5% were effective at higher concentration of 500 and 1000 ppm. The rate of sporulation of *F. oxysporum* (*i.e*., isolate Fo8) decreased linearly with an increase in fungicides concentration and with the type of fungicidal treatment. The statistical analysis ($P \geq 0.05$) showed that rate of sporulation measured at 100 ppm was significantly affected with the fungicides. Complete inhibition in sporulation was revealed by carbendazim 50% and carbendazim 12% + mancozeb 63% that was statistically at par with rest of the fungicides. However, the minimum inhibition in sporulation was recorded in captan 50% (*i.e*., 20.01%) that was significantly lower than rest of the treatments (Table 1; Figure 2).

Table 1: *In vitro* evaluation of fungicides against mycelial growth and sporulation of *F. oxysporum*

Fungicides	Mycelial growth inhibition (%)						Mean sporulation† [spores/ml x 10^4]	Reduction in sporulation (%)
	Concentration (ppm)					Mean		
	50	100	200	500	1000			
Carbendazim 50% WP	100.00(89.39)	100.00(89.39)	100.00(89.39)	100.00(89.39)	100.00(89.39)	100.00(89.39)	0.0	100.00
Captan 50% WP	0.74(4.22)	6.30(14.44)	13.33(21.39)	50.37(45.19)	80.37(63.69)	30.22(29.79)	223.3	20.01
Propiconazole 25% EC	78.15(62.12)	84.44(66.75)	88.52(70.17)	91.85(73.39)	93.70(75.45)	87.33(69.58)	13.3	95.22
Carbendazim 12 % + Mancozeb 63% WP	100.00(89.39)	100.00(89.39)	100.00(89.39)	100.00(89.39)	100.00(89.39)	100.00(89.39)	0.0	100.00
Captan 70% + Hexaconazole 5% WP	64.07(53.16)	78.15(62.12)	85.93(67.96)	91.11(72.65)	92.96(74.65)	82.44(66.11)	50.0	82.09
Carboxin 37.5% + Thiram 37.5% WP	23.70(29.11)	36.30(37.03)	74.07(59.38)	90.37(71.92)	91.85(73.39)	63.26(54.17)	115.8	58.51
Control	---	---	---	---	---	---	279.2	---
Mean	61.11(54.57)	67.53(59.85)	76.98(66.28)	87.28(73.66)	93.15(77.66)			
	Fungicides (F)	Concentration (C)				Interaction (F x C)		
SEm ±	0.28	0.26				0.63	1.58	---
CD (p = 0.05)	(0.80)	(0.73)				(1.79)	4.82	---
CV (%)	---	---				1.65	2.80	---

*Figures in parentheses indicate arcsine transformed values; †Sporulation at 100 ppm concentration (1000 ppm= 1000 µg/ml); All values represent means of three replicates.

Note: 0.01 value is added to zero per cent of values, while 0.01 value is reduced from hundred per cent of values for each observation for the statistical analyses.

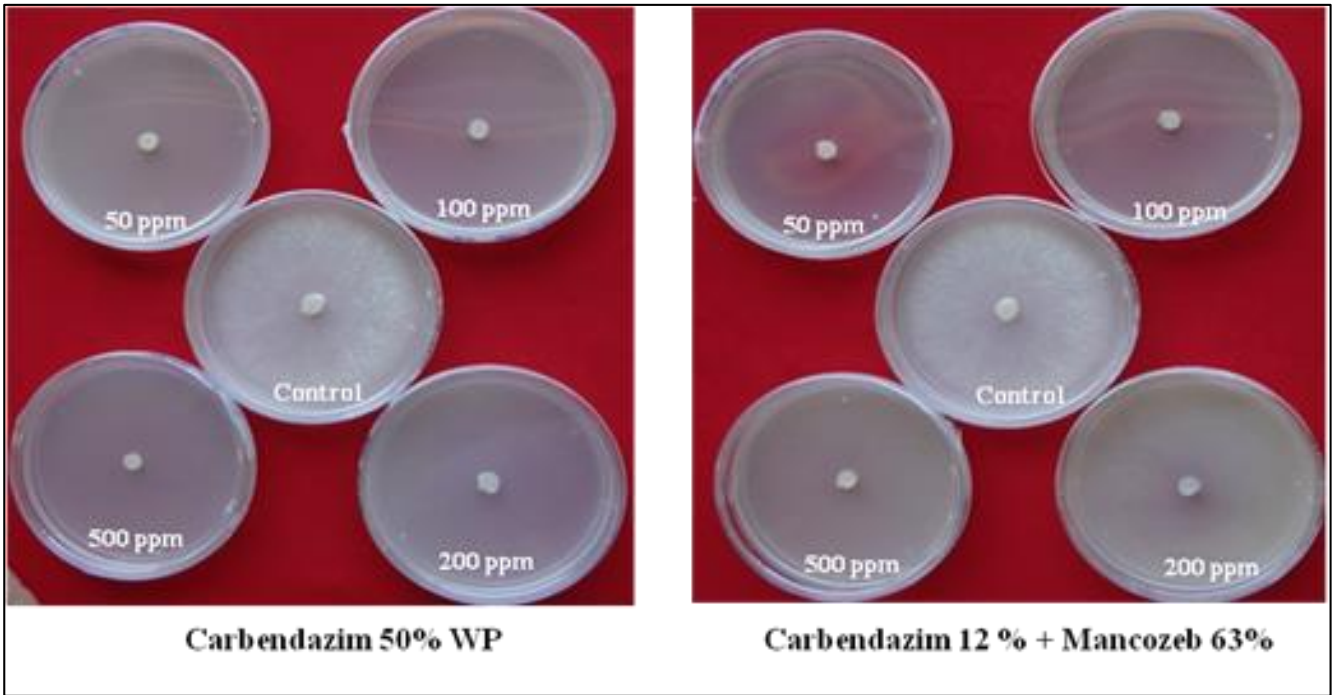

Fig 1: Mycelial growth inhibition of *F. oxysporum* (Isolate Fo8) with different fungicides at different concentrations (1000 ppm= 1000 µg/ml).

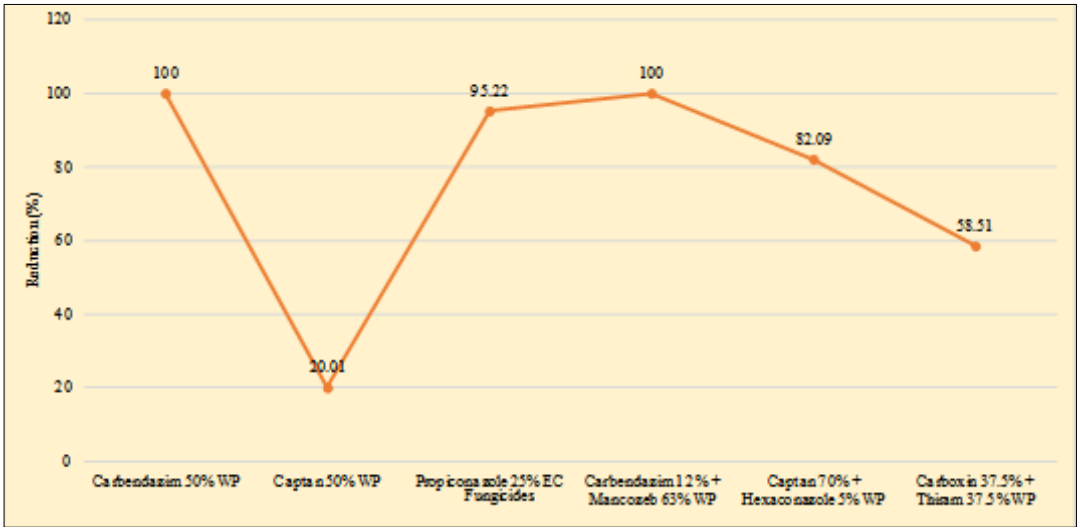

Fig 2: Reduction (%) in sporulation of *F. oxysporum* with different fungicides at 100 ppm concentration; (1000 ppm= 1000 µg/ml).

Antagonists: The antagonists significantly checked the growth of *F. oxysporum* (*i.e.*, isolate Fo8) either by over-growing or by exhibiting inhibition zones. Both the species of *Trichoderma* showed considerable antimycotic activity compard to the bacterial antagonist *B. subtilis and P. fluorescens.* Among the four antagonists, *Trichoderma harzianum* was better which inhibited 45.9 per cent mycelial growth, followed by *T. viride* (31.9%), whereas, the bacterial antagonist *viz*., *Pseudomonas fluorescens* and *Bacillus subtilis* were ineffective (Table 2; Figure 3). In comparison to *T. viride*, the *T. harzianum* isolates showed faster growth with maximum inhibition of the fungus. Among the antagonists, *T. harzianum* supported minimum sporulation (20.0 x 10^4 conidia/ml), which accounted for 92.66% reduction in sporulation compared to control that was at par with *T. viride.* The sporulation in *T. viride* treated plates was higher (35.8 x 10^4 conidia/ml), which exhibited 86.85% reduction in sporulation. However, *B. subtilis* and *P. fluorescens* proved least effective that accounted for only 49.85 and 34.86 per cent reduction in sporulation, respectively (Table 2; Figure 4).

Table 2: *In vitro* evaluation of antagonists against mycelial growth and sporulation of *F. oxysporum*

Antagonist	Growth and inhibition of mycelia		Mean sporulation [spores/ml (x 10^4)]	Reduction in Sporulation over control (%)
	Mean Radial growth (mm)	Percent Inhibition over control		
Trichoderma harzianum	24.3	45.9	20.0	92.66
Trichoderma viride	30.7	31.9	35.8	86.85
Pseudomonas fluorescens	45.0	0.0	136.7	49.85
Bacillus subtilis	45.0	0.0	177.5	34.86
Control (Isolate Fo8)	45.0	---	272.5	---
SEm ±	0.56	---	1.54	---
CD (p = 0.05)	1.78	---	4.90	---
CV (%)	2.54	---	2.07	---

All values represent means of three replicates

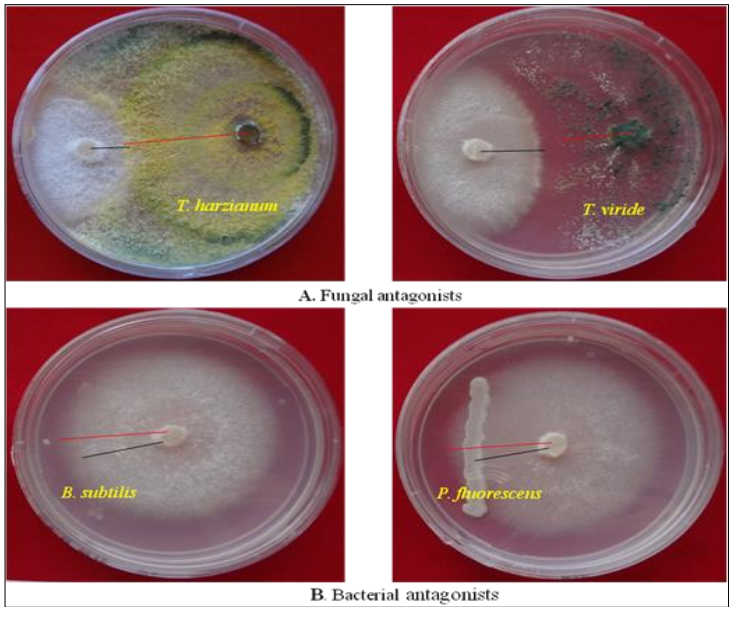

Fig 3: Mycelial growth inhibition of F. oxysporum with different antagonists (A) Fungal antagonists and (B) Bacterial antagonists under dual culture.

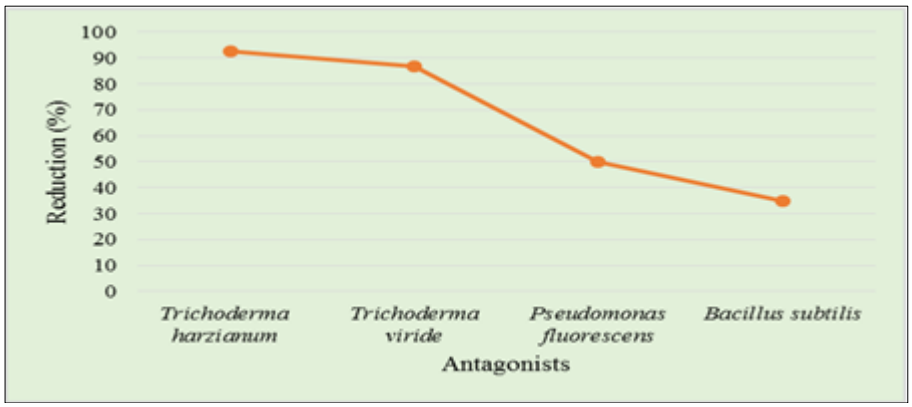

Fig 4: Reduction (%) in sporulation of *F. oxysporum* with different antagonists in dual culture.

Discussions

Fungicides: In the present investigation, the data revealed that carbendazim 50% and carbendazim 12%+mancozeb 63% completely inhibited the mycelial growth at 500 and 1000 ppm concentrations and these fungicides were highly effective even at low concentration (*i.e*., 50 ppm). In a similar study, Chavan (2007) [12] reported complete inhibition of the *F. solani* mycelial growth *in vitro* at 250, 500 and 1000 ppm with carbendazim and carbendazim 12%+mancozeb 63%. Nikam *et al.* (2007) [37] reported carbendazim (1000 ppm) alone and in combination with thiram was most effective against growth inhibition of *Fusarium oxysporum* f.sp. *ciceris*. The results are also in conformity with the findings of several other workers in case of *F. solani and F. oxysporum* (Madhavi and Bhattiprolu, 2011; Rather *et al.*, 2012; Wani and Nazar, 2012; Bashar and Chakma, 2014; Mamun *et al*., 2016) [43, 7, 29, 27 55]. The fungicides carbendazim 50% and carbendazim 12%+mancozeb 63% in the present study also caused complete inhibition in sporulation of the test fungi. These findings are in agreement with Amany *et al*. (2003) [2] who found that these benzamidazole group fungicides significantly reduced the sporulation in *F. oxysporum*. Similar finding were reported by Bhat (2002) [10], Taskeen-Un-Nisa *et al*. (2011) [51] and Mamza *et al*. (2012) [30] who reported a reduction in sporulation of the *F. oxysporum* isolates by carbendazim 50% and carbendazim 12%+mancozeb 63%. The fungitoxic effect of carbendazim 50% and carbendazim 12%+mancozeb 63% (benzimidazoles) is provided by interfering with a number of cellular processes such as mitosis, meiosis, intracellular transport of molecules and the maintenance of cell shape (Orbach *et al.*, 1986; Peterbauer *et al.*, 1992; Tikhomirova and Inge-Vechtomov, 1996) [39, 52, 40] leading to the eventual cell death in benzimidazole-treated fungi.

Antagonists: In present investigation, *T. harzianum* revealed highest inhibition of mycelial growth (45.9%), followed by *T. viride* (31.9%), however, *B. subtilis and P. fluorescens* proved ineffective. Rini and Sulochana (2007) [45] reported *T. harzianum* as more effective than *P. fluorescens* against *F. solani* and *F. oxysporum.* The findings are in conformity with the work of Devi and Singh (2012) [15] where inhibition of the *Fusarium* spp. was maximum with *T. harzianum* followed by *T. viride*, *P. fluorescens* and *B. subtilis*. Srideepthi and Krishna (2015) [49] reported 68.4 and 34.2 per cent of mycelial inhibition by *T. viride* and *T. harzianum* against *F. oxysporum* f.sp. *capsici*, respectively. Similar findings of higher antagonistic effect of *Trichoderma* spp. against *Fusarium* spp. have been recorded previously by various workers (Sahi and Khalid, 2007; Mustafa *et al.*, 2009; Rajeswari and Kannabiran, 2011; Wani *et al*., 2014; Sudarma *et al.*, 2015) [56, 46, 33, 65, 50, 42]. Maximum reduction in sporulation in present study was shown by *T. harzianum*, which reduced 92.66 per cent sporulation, followed by *T. viride* (86.85%), however, *B. subtilis* and *P. fluorescens* were least effective. Similarly, Dey *et al.* (2013) [16] reported reduction in sporulation and germination of uredospores of *Puccinia sorghi*, where *T. harzianum*, *B. subtilis* and *P. fluorescens* reduced the germination of uredospores by 32.29, 41.30 and 46.66 per cent, respectively. This variation in effectiveness might be due to difference in nature, quality and quantity of the inhibitory substances produced by these antagonistic soil fungi (Kexiang *et al.*, 2002; Shafiquzzaman *et al.*, 2009; Barakat *et al.*, 2013) [26, 6, 47]. The higher antimycotic activity of *Trichoderma* spp. can be attributed to different mechanisms *viz.*, competition, lysis, antibiosis, siderophore production and hyperparasitism adopted by these antagonists (Vidyasekaran, 1999) [53]. High antagonistic activity of the *Trichoderma* spp. observed against the test fungus might be due to their fast growing nature, rapid sporulation and toxic metabolite producing capacity. The bacterial antagonists, *i.e*., *B. subtilis* produces a wide range of antifungal compounds, such as subtilin, TasA, subtilosin, bacilysin, mycobacillin and some enzymes, which can degrade fungal cell wall (Berg *et al*., 2001), similarly, *P. fluorescens* produces siderophores and antibiotics like phenazine-1 carboxylic acid and 2,4-diacetyl-phloroglucinol (2,4-DAPG) preventing further advancement of the fungus (Beckman *et al*., 1982; Mueller and Beckmann, 1988) [8, 32] by inducing severe cell disturbances in pathogenic fungi (Dowling and O'Gara, 1994) [17].

Conclusions

Among the different available options for the management, chemicals are neither economically viable, nor safe for the environment, still the chemicals due to the immediate effect and higher antifungal activity are used at large scale for controlling this menace pathogen. Carbendazim 50%, carbendazim 12% + mancozeb 63% and *T. harzianum* found effective against *F. oxysporum* are promising to control the disease. These chemicals and antagonists may provide an effective measure for management of Fusarium wilt of chilli that may form an integral part of integrated management.

References

1. Abdel-Monaim MF, Abdel-Gaid MA, Zayan SA. Effectiveness of organic compounds in controlling root rots/wilts diseases, growth and yield parameters of

pepper. International Journal of Agricultural Sciences. 2014; 4(4):143-150.
2. Amany H, Ellil A, Sharaf EF. Growth, morphological alterations and adaptation of some plant pathogenic fungi to benlate and zineb - A New Look. Journal of Biological Sciences, 2003; 3(3):271-281.
3. Anonymous. Annual report of Network Project on Wilt of chilli with special reference to cultural, morphological, molecular characterization and pathogenic variability of isolates of India, submitted to ICAR, New Delhi. 2005, 7.
4. Anonymous. http://nhb.gov.in/area-pro/Indian Horticulture Database. National Horticulture Board, Ministry of Agriculture, Government of India, 2014.
5. Arunodhayam K, Eswara-Reddy NP, Madhuri V.). Pathogenicity and management of Fusarium wilt of chickpea, *Cicer arietinum* L. – A review. Current Biotica. 2014; 7(4):343-358.
6. Barakat FM, Abou-Zeid NMA, El-Gammal YHE. Effect of volatile and nonvolatile compounds of *Trichoderma* spp. on *Botrytis fabae* the causative agent of faba bean chocolate spot. WebPub Journal of Agricultural Researc. 2013; 1(3):42-50.
7. Bashar MA, Chakma M. *In vitro* control of *Fusarium solani* and *F. oxysporum* the causative agent of brinjal wilt. Dhaka University Journal of Biological Sciences. 2014); 23(1):53-60.
8. Beckman CH, Mueller WC, Tessier BJ, Harrisson NA. Recognition and callose deposition in response to vascular infection in Fusarium wilt-resistant or susceptible tomato plants. Physiological Plant Pathology. 1982; 20:1-10.
9. Berg G, Fritze A, Roskot N, Smalla K. Evaluation of potential biocontrol rhizobacteria from different host plants of *Verticillium dahliae* Kleb. Journal of Applied Microbiology. 2001; 91:963-971.
10. Bhat ZA. Comparative efficacy of bio-control agents, botanical extracts and fungicide in the management of chickpea wilt caused by *Fusarium oxysporum*. M.Sc. (Agri.) Thesis, Allahabad Agriculture Institute (Deemed University), Allahabad-211007, (U.P) India. 2002, 65.
11. Booth C. The Genus Fusarium. Commonwealth Mycological Institute, Kew Surrey, England. 1971, 237.
12. Chavan SS. Studies on fungal diseases of patchouli with special reference to wilt caused by *Fusarium solani* (Mart.) sacc. M.Sc. (Agri). Thesis, Univ. Agri. Sci., Dharwad. 2007, 98.
13. Cook RJ, Baker KF. The nature and practice of biological control of plant pathogens. American Phytopathological Society, St.Paul, MN, USA. 1983, 539.
14. Daami-Remadi M, Jabnoun-Khiareddine H, Ayed F, El-Mahjoub M.). Effect of temperature on aggressivity of Tunisian *Fusarium* spp. causing potato *Solanum tuberosum* L. tuber dry rot. Journal of Agronomy. 2006; 5(2):350-355.
15. Devi TN, Singh MS.). Evaluation of suitable fungicide for integration with *Trichoderma* isolates for the control of tomato wilt. Journal of Mycopathological Research. 2012; 50:223-228.
16. Dey U, Harlapur SI, Dhutraj DN, Suryawanshi AP, Jagtap GP, Apet KT, *et al*. Effect of fungicides, botanicals, bioagents and Indigenous Technology Knowledge (ITK's) on germination of urediniospores of *Puccinia sorghi in vitro.* African Journal of Agricultural Research. 2013; 8(39):4960-4971.
17. Dowling DN, O'Gara F.). Metabolites of *Pseudomonas* involved in the biocontrol of plant disease. Trends in Biotechnology. 1994; 3:121-141.
18. Egea C, Dickinson MJ, Candela M, Candela ME. β-1,3-glucanase isoenzyme and genes in resistant and susceptible pepper (*C. annuum*) cultivars infected with Phytophthora capsici. Physiologia Plantarum, 2002; 107:312-318.
19. El-Hassan SA, Gowen SR, Pembroke B. Use of *Trichoderma hamatum* for biocontrol of lentil vascular wilt disease: efficacy, mechanisms of interaction and future prospects. Journal of Plant Protection Research. 2013; 53(1):12-26.
20. FAO. http:/faostat.fao.org/site/567/default. aspx# anccor. FAOSTAT, 2012.
21. Foster JM, Hausbeck M Managing Phytophthora crown and root rot in bell pepper using fungicides and host resistance. Plant disease. 2010; 94:697-702.
22. Hallman J, Quadt-Hallman A, Mahafee WF, Kloepper JW. Bacterial endophytes in agricultural crops. Canadian Journal of Microbiology. 1997; 43:895-914.
23. Haware MP, Jimenez-Diaz RM, Amin KS, Phillips JC. Halila H. Integrated management of wilt and root rots of chickpea. In: Chickpea in the nineties: Proceedings of the second international workshop on chickpea improvement. Patencheru, India: ICRISAT Centre. 1989, 1990, 129-133.
24. Kelaniyangoda DB, Salgadoe ASA, Jayasekera SJBA, Gunarathna-Banda RM. Wilting of bell pepper *C. annuum* L. causal organism isolation and a successful control approach. Asian Journal of Plant Pathology. 2011; 5(4):155-162.
25. Kexiang G, Xiaoguang L, Youghong L, Tianbo Z, Shuliang W. Potential of *Trichoderma harzianum* and *T. atroviride* to control *Botryosphaeria berengeriana* f.sp. *piricola*, the cause of apple ring rot. Phytopathology. 2002; 150:271-276.
26. Loganathan M, Venkataravanappa V, Saha S, Sharma BK, Tirupathi S, Verma MK. Morphological, cultural and molecular characterizations of Fusarium wilt infecting tomato and chilli. In: National Symposium on Abiotic and Biotic Stress Management in Vegetable Crops, Indian Society of Vegetable Science, IIVR, Varanasi, 2013.
27. Madhavi GB, Bhattiprolu SL.). Evaluation of fungicides, soil amendment practices and bioagents against *Fusarium solani* - causal agent of wilt disease in chilli. Journal of Horticultural Sciences. 2011; 6(2):141-144.
28. Madhukar HM, Naik MK.). Evaluation of bioagents against Fusarium wilt of chilli (*Capsicum annuum* var. *annuum*). In: Proc. 15th International Plant Protection Congress on Plant Protection towards 21st Century held in Beijing, China. 2004, 540.
29. Mamun MA, Shamsi S, Bashar MA. *In vitro* evaluation of fungicides and plant extracts against pathogenic fungi of jute seeds. Bioresources and Communications. 2016; 2(1):189-192.
30. Mamzaa WS, Zarafib AB, Alabib O. Effect of six fungicides on sporulation of *Fusarium pallidoroseum* isolated from castor (*Ricinus communis*) in Samaru, Nigeria. IOSR Journal of Agriculture and Veterinary Science, 2012; 1(5):40-42.
31. Morton DT, Stroube NH. Antagonistic and stimulatory effects of micro-organism upon Sclerotium rolfsii. Phytopathology. 1955; 45:419-420.

32. Mueller WC, Beckman CH. Correlated light and EM studies of callose deposits in vascular parenchyma cells of tomato plants inoculated with *Fusarium oxysporum* f.sp. *lycopersici*. Physiological and Molecular Plant Pathology, 1988; 33:201-208.
33. Mustafa A, Khan MA, Inam-ul-Haq M, Pervez MA, Umar U. Usefulness of different culture media for *in vitro* evaluation of *Trichoderma* spp. against seed borne fungi of economic importance. Pakistan Journal of Phytopathology. 2009; 21:83-88.
34. Naik MK. Wilt of chilli with special reference to cultural, morphological, molecular characterization and pathogenic variability of *Fusarium* isolates of India. In: Proc. Midterm Review Meeting of the Project held at Indian Institute of Vegetable Research, Varanasi, 2006.
35. Nelson PE, Toussoun TA, Cook RJ.). Fusarium*:* Diseases Biology and Taxonomy. Pennsylvania State University Press, University Park, Pennsylvania, USA. 1981, 457.
36. Nene YL, Thapliyal BN.). Fungicides in plant disease control. 3rd edition, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 1973, 325.
37. Nikam PS, Jagtap GP, Sontakke PL. Management of chickpea wilt caused by *Fusarium oxysporium* f.sp. *ciceris*. African Journal of Agricultural Research. 2007; 2(12):692-697.
38. Ochoa NA, Ramirez MR. *In vitro* chilli pepper biotechnology. In vitro Cellular and Developmental Biology-Plant. 2001; 37:701-729.
39. Orbach MJ, Porro EB, Yanovsky C. Cloning and characterization of the gene β-tubulin and its use as a dominant selectable marker. Molecular and Cellular Biology, 1986; 6:2452-2461.
40. Peterbauer AH, Heidenreich R, Baker RT. Kubuick CP. Effect of benomyl and benomyl resistance on cellulase formation by Trichoderma reesei and Trichoderma harzianum. Canadian Journal of Microbiology. 1992; 38:1297.
41. Ragab MMM, Ashour AMA, Abdel-Kader MM, El-Mohamady R, Abdel-Aziz A. *In vitro* evaluation of some fungicides alternatives against *Fusarium oxysporum* the causal agent of wilt disease of pepper *Capsicum annuum* L. International Journal of Agriculture and Forestry, 2012; 2(2):70-77.
42. Rajeswari P, Kannabiran B. *In vitro* effects of antagonistic micro-organism on *Fusarium oxysporum* (Schlecht. Emend. Synd and Hans) infecting *Arachis hypogea* L. Journal of Phytology. 2011; 3:83-85.
43. Rather TR, Razdan VK, Tewari AK, Shanaz E, Bhat ZA, Hassan MG, *et al*. Integrated management of wilt complex disease in bell pepper (*Capsicum annuum* L.). Journal of Agricultural Science. doi: 10.5539/jas. 2012; 4(7):141.
44. Rini CR, Sulochana KK. Biocontrol of *Fusarium solani* in chilli by *Trichoderma* spp. and fluorescent *Pseudomonads.* Department of Plant Biotechnology, Mar Athanasios College for Advanced Studies, Tiruvalla. Journal of Tropical Agriculture. 2006; 44:79-82.
45. Rini CR, Sulochana KK. Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. Journal of Tropical Agriculture. 2007; 45:21-28.
46. Sahi IY, Khalid AN. *In vitro* biological control of *Fusarium oxysporum* causing wilt in Capsicum annuum. Mycopathology. 2007; 5(2):85-88.
47. Shafiquzzaman S, Yusuf UK, Hossain K, Jahan S. *In vitro* studies on the potential *Trichoderma harzianum* for antagonistic properties against *Ganoderma boninense.* Journal of Food, Agriculture and Environment. 2009; 7(3-4):970-976.
48. Sheoran OP. Online statistical analysis tool (OPSTAT), 2006. www.hau.ernet.in/about/opstat.php. CCS HAU, Hisar.
49. Srideepthi R, Krishna MSR. Antimycotic effect of *Trichoderma* species on *Fusarium oxysporum* f.sp. *capsici* inciting vascular wilt in chilli. In: New Horizons in Biotechnology. (Eds. Viswanath, B. and Indravathi, G.). Paramount Publishing House, India. 2015, 29-31.
50. Sudarma IM, Puspawati NM, Suniti NW, Wijaya IN, Bagus IGN. Utilization of rhizosphere fungi to control *Fusarium oxysporum* f.sp. *capsici in vitro.* International Journal of Bioscience and Biotechology. 2015; 2(2):83-92.
51. Taskeen-Un-Nisa, Wani AH, Bhat MY, Pala SA, Mir RA. *In vitro* inhibitory effect of fungicides and botanicals on mycelia growth and spore germination of *Fusarium oxysporum*. Journal of Biopesticides. 2011; 4:53-56.
52. Tikhomirova VL, Inge-Vechtomov SG. Sensitivity of sup 35 and sup 45 suppressor mutants in *Saccharomyces cerevisiae* to the anti-microtubule drug benomyl. Current Genetics. 1996; 30:44-49.
53. Vidyasekaran P. Biotechnological approaches to suppress seed to plant transmission of seed-borne pathogens. In: National Symposium on Seed Science and Technology 5-7th August, University of Mysore, Manasagangotri, Mysore, India. 1999, 41.
54. Vincent JM. Distortion of fungal hyphae in presence of certain inhibitors. Nature. 1947; 159:850.
55. Wani FA, Najar AG. Integrated management of Fusarium wilt [*Fusarium pallidoroseum* (Cooke) Sacc.] in chilli *Capsicum annuum* L. SKUAST Journal of Research, 2012; 14(1-2):40-45.
56. Wani SA, Mohiddin FA, Hamid B, Rizvi G, Bhat KA, Hamid A, *et al*. Incidence of Fusarium wilt of chilli *Capsicum annuum* L. in Kashmir valley and its management by Trichoderma spp. Mycopathology. 2014; 12(1):1-8.