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

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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* ≥ 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 *Bacillus 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 million 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) [39] 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) [14]. 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. *tuberosum*. 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, azoxystrobin and trifloxystrobin are most effective against the disease (Kelaniyangoda et al., 2011; Rather et al., 2012; Wani and Najar, 2012) [24, 43, 59]. 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; Sridheepthi 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.

\[ I = \frac{C - T}{C} \times 100 \]

Where, 

\[ I = \text{Per cent inhibition}; \ C = \text{Growth in control}; \ T = \text{Growth in treatment} \]

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.

\[ \text{Spore density (spores/ml)} = \frac{(n) \times 25 \times 10^4}{\text{conidia produced per unit surface area (mm²)}} = \frac{\text{Number of conidia ml}^{-1} \text{ suspension} \times \text{Total surface area from which conidial suspension was derived}}{\text{Volume of water to make suspension}} \]

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* ≥ 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*

<table>
<thead>
<tr>
<th>Fungicides</th>
<th>Mycelial growth inhibition (%)</th>
<th>Mean sporulation† [spores/ml x 10⁴]</th>
<th>Reduction in sporulation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (ppm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>Carbendazim 50% WP</td>
<td>100.00(89.39)</td>
<td>100.00(89.39)</td>
<td>100.00(89.39)</td>
</tr>
<tr>
<td>Captan 50% WP</td>
<td>0.74(4.22)</td>
<td>6.30(14.44)</td>
<td>13.33(21.39)</td>
</tr>
<tr>
<td>Propiconazole 25% EC</td>
<td>78.15(62.12)</td>
<td>84.44(66.75)</td>
<td>88.32(70.17)</td>
</tr>
<tr>
<td>Carbendazim 12 % + Mancozeb 63% WP</td>
<td>100.00(89.39)</td>
<td>100.00(89.39)</td>
<td>100.00(89.39)</td>
</tr>
<tr>
<td>Captan 70% + Hexaconazole 5% WP</td>
<td>64.07(53.16)</td>
<td>78.15(62.12)</td>
<td>85.93(67.96)</td>
</tr>
<tr>
<td>Carboxin 37.5% + Thiram 37.5% WP</td>
<td>23.70(29.11)</td>
<td>36.30(37.03)</td>
<td>74.07(59.38)</td>
</tr>
<tr>
<td>Control</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Mean</td>
<td>61.11(54.57)</td>
<td>67.53(59.85)</td>
<td>76.98(66.28)</td>
</tr>
</tbody>
</table>

*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).](image-url)
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 antifungal activity compared to the bacterial antagonist *B. subtilis* and *P. fluorescens*. Among the four antagonists, *Trichoderma harzianum* was better which inhibited 45.9% mycelial growth, followed by *T. viride* (31.9%), whereas, the bacterial antagonist *B. subtilis* and *P. fluorescens* 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*

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

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.
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, 28, 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) [1] who found that these benzimidazole group fungicides significantly reduced the sporulation in F. oxysporum. Similar finding were reported by Bhat (2002) [10], Taskeen-Un-Nisa et al. (2011) [31] 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-Veichtomov, 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) [53] 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; Shafiuzzaman et al., 2009; Barakat et al., 2013) [56, 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 (Beckmann et al., 1982; Mueller and Beckmann, 1988) [8, 32] by inducing severe cell disturbances in pathogenic fungi (Dowling and O’Gara, 1994) [13].

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.

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