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Efficacy of biocontrol agents against *Fusarium oxysporum* F. SP. *Corianderi* causing wilt of coriander under *in vitro* condition

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Abstract
A laboratory experiment was conducted to find out the efficacy of different fungal and bacterial biocontrol agents against coriander wilt pathogen *Fusarium oxysporum* f. sp. *corianderi.* Among the seven antagonistic fungi tested using dual culture technique, *Trichoderma harzianum* JAU isolate 1 showed maximum reduced mycelial growth of *Fusarium oxysporum* f. sp. *corianderi* (46.23) than rest of antagonistic fungi. Whereas, the least effective biocontrol agent found was *Trichoderma hamatum* (NBA11 Tha 1) with 28.69 per cent mycelial growth inhibition of test fungus *in vitro.* The antagonistic actions of selected seven bacterial biocontrol agents were evaluated against the test fungus by dual culture technique (ring method). Among different bacterial antagonists, *Pseudomonas fluorescens* found significantly superior over rest of the biocontrol agents and showed maximum inhibition of mycelial growth of *F. oxysporum* f. sp. *corianderi* (99.76%). While, the least effective biocontrol agent found was *Bacillus subtilis* (KT894726) with 72.08 per cent mycelial growth inhibition of test fungus *in vitro.*

Keywords: *Fusarium oxysporum* f. sp. *corianderi,* Coriander, biocontrol agents, coriander wilt

Introduction
Coriander is the second most important seed spice with respect to exports and getting foreign exchange earnings after cumin (Peter *et al*., 2006) [10]. Coriander is affected by many diseases which may be fungal, bacterial or viral. Among these, the wilt of coriander is a serious problem and affected plants grew poorly and were stunted. Root infection results in dropping of terminal shoots, followed by withering and drying of leaves. Partial infection shows yellow to pink foliage as disease progressed, plants eventually died (Prakasam *et al*., 1987) [12]. The seed yield losses caused by Fusarium wilt ranges from 5 to 60 per cent in Rajasthan and 15 to 25 per cent in Gujarat (Prasad and Patel, 1963) [13]. At present, the most of cultivated cultivars are susceptible to wilt disease causes up to 60 per cent yield loss in coriander (Manoranjitham *et al*., 2003) [6]. Use of biocontrol agents against wilt pathogen regarded as an effective tools for successful management of this disease. In order to evaluate efficacy of different fungal and bacterial biocontrol agents against test pathogen, present study was carried out *in vitro*.

Materials and Methods
Isolation and purification of pathogen
The plant showing typical characteristic symptoms of wilt disease were collected from the research farm and brought to the laboratory. The isolation of the fungus was made by tissue isolation technique on potato dextrose agar (PDA) and incubated at 28±2 ºC. The resulting fungal culture was purified in aseptic condition by hyphal tip method. The pure culture obtained was used for testing the efficacy of different fungal and bacterial biocontrol agents *in vitro*.

In vitro evaluation of fungal biocontrol agents against test pathogen
The antagonistic effect of seven different Trichoderma spp. *viz*., T. viride (NBAIITv23), Trichoderma harzianum JAU isolate 1, T. virens (NBAII Tvs12), T. hamatum (NBAII Tha 1), T. koningii (MTCC 2051), T. harzianum improved JAU isolate 1 and Trichoderma isolate DGR were tested against F. oxysporum f. sp. corianderi using dual culture technique (Morton

and Stroube, 1955) [9] in Completely Randomized Design (CRD) with three repetitions. Twenty milliliters of sterilized melted potato dextrose agar media (PDA) was poured aseptically in each 90 mm Petri plates and allowed to solidify. Mycelial disc of four millimeter diameter of each biocontrol agents and test fungus was cut with the help of sterilized cork borer from the edges of actively growing culture and was placed on the PDA medium in the same Petri plates, on opposite corners by keeping one cm distance from distal ends of Petri plates. The inoculated plates were incubated at 28±2 °C. The plates were observed for growth of biocontrol agents and test fungus periodically. The growth of biocontrol agents and test fungus was measured by linear measurement. Control plate was also maintained by placing two pathogen in the same plate, on opposite corner.

The radial growth of the test pathogen was measured when control plate pathogen contacted to each other. The per cent growth inhibition of the fungus by biocontrol agents in each treatment was calculated by using the following formula (Vincent, 1947).

$$I = \frac{C - T}{C} \times 100$$

Where,
I = Per cent reduction in growth of test pathogen
C = Radial growth (mm) in control
T = Radial growth (mm) in treatment

In vitro evaluation of bacterial biocontrol agents against test pathogen

The antagonistic effect of seven different bacterial isolates *viz*., Bacillus subtilis (KT894724), B. subtilis (KT894725), B. subtilis (KT894726), B. subtilis (KT894727), Pseudomonas fluorescens, B. subtilis and B. cereus and were tested against F. oxysporum f. sp. coriander in CRD with three repetitions. The mycelia of F. oxysporum f. sp. corianderi was dual-culture plated with different seven bacterial isolates as described by Montealegre *et al*. (2003) [8]. Twenty milliliters of sterilized melted nutrient agar (NA) media was poured aseptically in each 90 mm Petri plates and allowed to solidify. Mycelial disc of four millimeter diameter of test fungus was cut with the help of sterilized cork borer from the edges of actively growing culture and was placed in the centre of NA media containing Petri plates. A circular line made with a 60 mm diameter Petri plate dipped in a suspension of different bacterial species (6×108 cfu ml−1) surrounding the fungal inoculum. Plates were cultured for 72 h at 28+2 °C and the fungal growth was measured and compared with control growth where the bacterial suspension was replaced by sterile distilled water. The radial growth of the test pathogen was measured when control plate attain full growth of fungal pathogen. The per cent growth inhibition of the fungus by biocontrol agents in each treatment was calculated using the

% Inhibition = [1 - (Diameter of the lawn /Control growth)] × 100

Results and Discussion

In vitro evaluation of fungal biocontrol agents against test pathogen

The data presented in Table 1 indicated that among different fungal biocontrol agents tested *in vitro*, maximum reduction of pathogen (46.23 per cent) was observed in the presences of Trichoderma harzianum JAU isolate 1 followed by Trichoderma harzianum improved JAU isolate 1(42.92 per cent), Trichoderma viride (NBA11Tv23) (40.20 per cent), Trichoderma virens (NBA11 Tvs12) (37.41 per cent), Trichoderma koningii (MTCC 2051) (36.70 per cent) and Trichoderma isolate DGR (34.20 per cent). Trichoderma hamatum (NBA11 Tha 1) gave minimum growth inhibition with 28.69 per cent.

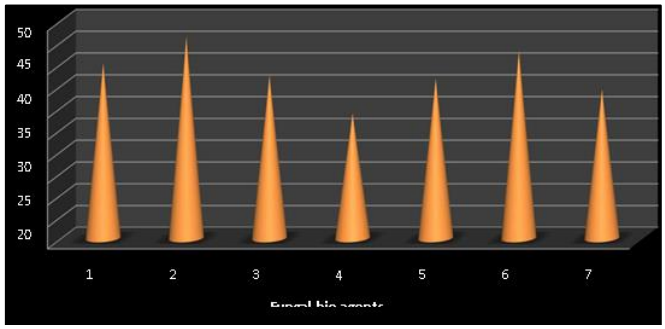

Fig 1: Mean inhibition per cent of fungal bio agents against *F. Oxysporum F*. SP. *corianderi in vitro*

1.	*Trichoderma viride* (NBA11Tv23)	5.	*Trichoderma koningii* (MTCC 2051)
2.	*Trichoderma harzianum* JAU isolate 1	6.	*Trichoderma harzianum* improved JAU isolate 1
3.	*Trichoderma virens* (NBA11 Tvs12)	7.	*Trichoderma* isolate DGR
4.	*Trichoderma hamatum* (NBA11 Tha 1)		

Table 1: *In vitro* evaluation of fungal biocontrol agents against test pathogen

Sr. No.	Fungal bio agents	#Growth inhibition (%)
1	*Trichoderma viride* (NBA11Tv23)	*39.35 (40.20)
2	*Trichoderma harzianum* JAU isolate 1	42.84 (46.23)
3	*Trichoderma virens* (NBA11 Tvs12)	37.71 (37.41)
4	*Trichoderma hamatum* (NBA11 Tha 1)	32.39 (28.69)
5	*Trichoderma koningii (MTCC 2051) 37*	.29 (36.70)
6	*Trichoderma harzianum improved JAU isolate 1 40*	.93 (42.92)
7	*Trichoderma isolate DGR 35*	.79 (34.20)
S.Em. ±	0.	
CD at 5%	0.	
CV%	1.39	

(# Average of three replications *Data outside the parenthesis are arcsine transformed whereas inside are re-transformed values)

The present findings are in close agreement with the results obtained by workers *viz*., Baradia and Rai (2007) [1], Lodha and Singh (2007) [5] and Deepak *et al*. (2008) [2] against Fusarium oxysporum F. SP. cumini, Suman and Biswas (2017) [16], Praful and Mane (2017) [11] and Mayur *et al*. (2001) [7] against Fusarium oxysporum f. sp. ciceri and Jat *et*

al. (2017) [4] against Fusarium oxysporum f. sp. corianderi.

In vitro evaluation of bacterial biocontrol agents against test pathogen

The perusal of data in Table 2 Among the seven antagonistic bacteria tested Pseudomonas fluorescens showed maximum reduced mycelial growth of Fusarium oxysporum f. sp. corianderi than rest of antagonistic bacteria. Maximum reduction of pathogen (99.76) was observed in the presences of Pseudomonas fluorescens followed by Bacillus subtilis (KT894727) (94.75 per cent), Bacillus subtilis (84.63 per cent), Bacillus cereus (83.68 per cent), Bacillus subtilis (KT894724) (79.43 per cent), Bacillus subtilis (KT894725) (73.84 per cent). Bacillus subtilis (KT894726) gave minimum growth inhibition with 72.08 per cent.

The present findings are in collaborate with the results obtained by different workers. The antagonistic effect of Pseudomonas fluorescens against Fusarium oxysporum f. sp. ciceri (Rajan *et al*., 2013) [14], Pseudomonas aeruginosa against Fusarium oxysporum f. sp. cumini (Sobhanipour *et al*., 2008) [15] and Bacillus subtilis against F. oxysporum f. sp. vasinfectum, Rhizoctonia solani and Macrophomina phaseolina (Hoda-Ahmed *et al*. 2000) [3] was reported by several workers.

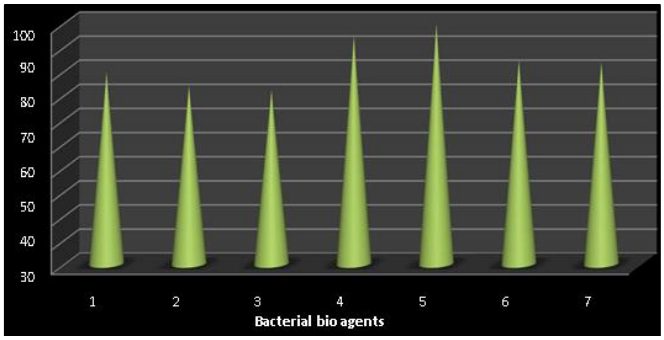

Fig 2: Mean inhibition per cent of bacterial bio agents against F. *oxysporum F*. SP. *corianderi in vitro*

1.	*Bacillus subtilis* (KT894724)	5.	*Pseudomonas fluorescens*
2.	*Bacillus subtilis* (KT894725)	6.	*Bacillus subtilis*
3.	*Bacillus subtilis* (KT894726)	7.	*Bacillus cereus*
4.	*Bacillus subtilis* (KT894727)		

Table 2: *In vitro* evaluation of bacterial biocontrol agents against test pathogen

Sr. No.	Bacterial bio agentsGr	Owth inhibition (%)
1.	*Bacillus subtilis* (KT894724) 63	.04* (79.43)
2.	*Bacillus subtilis* (KT894725) 59	.24 (73.84)
3.	*Bacillus subtilis* (KT894726) 58	.10 (72.08)
4.	*Bacillus subtilis* (KT894727) 76	.80 (94.75)
5.	*Pseudomonas fluorescens* 87	.27 (99.76)
6.	*Bacillus subtilis* 66	.93 (84.63)
7.	*Bacillus cereus* 66	.18 (83.68)
S.Em. +	0.50	
CD at 5% 1.		
CV%	1.29	

(*Data outside the parenthesis are arcsine transformed whereas inside are re transformed values)

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