International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 674-676

Received: 13-07-2019
Accepted: 15-08-2019

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Efficacy of biocontrol agents against *Fusarium oxysporum* f. sp. *cumini* causing wilt of cumin 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 cumin wilt pathogen *Fusarium oxysporum* f. sp. *cumini.* Among different fungal biocontrol agents tested using dual culture technique, *Trichoderma harzianum* improved JAU isolate 1 exhibited significantly the maximum inhibition of mycelial growth of *F. oxysporum* f. sp. *cumini* (25.22 per cent). Whereas, the least effective biocontrol agent found was *Trichoderma koningii* (MTCC 2051) with 13.18 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. *cumini* (95.11%). While, the least effective biocontrol agent found was *B. subtilis* (KT894726) with 47.22 per cent mycelial growth inhibition of test fungus *in vitro*.

Keywords: Cumin, *Fusarium oxysporum* f. sp. *cumini,* biocontrol agents, cumin wilt

Introduction
Cumin is one of the most important *rabi* spice crop of Saurashtra and North Gujarat regions. The crop is reported to affect by several fungal diseases. Among them, cumin wilt caused by *Fusarium oxysporum* f. sp. *cumini* is the most destructive and widespread disease of cumin. Wilt results in yield losses up to 35 per cent in cumin in some districts of Rajasthan (Vyas and Mathur, 2002) [12]. Dange. (1992) [4] also reported yield losses of 7.0 to 30.6 per cent in cumin due to wilt disease under Gujarat condition. The disease manifests from the seedling stage itself and continues till the maturity of the crop.
The severity of wilt increases with the plant age. Under severe condition, the tips of the leaves and branches droop down leading to drying and mortality of the plants. 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., Trichoderma harzianum* JAU isolate 1, *T. viride* (NBAIITv23), *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. *cumini* using dual culture technique (Morton and Stroube, 1955) 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) [11].

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

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), *B. tequilensis* (KT894729), *B. cereus* and *Pseudomonas fluorescens* were tested against *F. oxysporum* f. sp. *cumini* in CRD with three repetitions. The mycelia of *F. oxysporum* f. sp. *cumini* was dual-culture plated with different seven bacterial isolates as described by Montealegre *et al.* (2003) [7]. 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 (6×10^8 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 following formula (Montealegre *et al.*, 2003) [7].

% 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*, *Trichoderma harzianum* improved JAU isolate 1 showed significantly the maximum inhibition of mycelial growth of *F. oxysporum* f. sp. *cumini* (25.22 per cent). *Trichoderma hamatum* (NBAII Tha 1) found moderately effective bicontrol agent with 22.50 per cent mycelial growth inhibition of test pathogen. Whereas, *Trichoderma viride* (NBAIITv23) exhibited 21.13 per cent mycelial growth inhibition of *F. oxysporum* f. sp. *cumini*. While, *Trichoderma* isolate DGR and *Trichoderma harzianum* JAU isolate 1 found equally effective in inhibiting the mycelial growth of test fungus with 18.63 and 18.40 per cent, respectively. The least effective biocontrol agent found was *Trichoderma koningii* (MTCC 2051) with 13.18 per cent mycelial growth inhibition of test fungus *in vitro.*

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

Sr. No.	Fungal biocontrol agents	Mycelial growth inhibition (%)
1.	*Trichoderma harzianum* JAU isolate 1	25.40 (18.40)*
2.	*Trichoderma viride* (NBAII Tv23)	27.37 (21.13)
3.	*Trichoderma virens* (NBAII Tvs12)	24.03 (16.59)
4.	*Trichoderma hamatum* (NBAII Tha 1)	28.32 (22.50)
5.	*Trichoderma koningii* (MTCC 2051)	21.29 (13.18)
6.	*Trichoderma harzianum* improved JAU isolate 1	30.14 (25.22)
7.	*Trichoderma* isolate DGR	25.57 (18.63)
	S. Em. ±	0.26
	C. D. at 5%	0.81
	C. V. %	1.79

*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 Bardia and Rai (2007) [1], Deepak *et al*. (2008) [5] reported that *Trichoderma harzianum* exhibited maximum inhibition of mycelial growth of the pathogen. Similarly, Gangopadhyay *et al*. (2009) [6], Chawla *et al*. (2012) [3] and Sharma *et al.* (2015) [9] also found that both antagonists *Trichoderma harzianum* and *Trichoderma viride* inhibited the mycelial growth of test pathogen *in vitro*.

***In vitro* evaluation of bacterial biocontrol agents against test pathogen**

The perusal of data in Table 2 revealed that among different bacterial biocontrol agents tested *in vitro*, *Pseudomonas fluorescens* found significantly superior over rest of the biocontrol agents and showed maximum inhibition of mycelial growth of *F. oxysporum* f. sp. *cumini* (95.11%). The biocontrol agent *Bacillus subtilis* (KT894724) exhibited 77.33 per cent mycelial growth inhibition of test fungus. Whereas, *B. subtilis* (KT894725) and *B. tequilensis* (KT894729) found equally effective in inhibiting the mycelial growth of *F. oxysporum* f. sp. *cumini* with 60.88 and 59.67 per cent. The least effective biocontrol agent found was *B. subtilis* (KT894726) with 47.22 per cent mycelial growth inhibition of test fungus *in vitro*.

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

Sr. No.	Bacterial biocontrol agents	Mycelial growth inhibition (%)
1.	*Bacillus subtilis* (KT894724)	61.57 (77.33)*
2.	*Bacillus subtilis* (KT894725)	51.29 (60.88)
3.	*Bacillus subtilis* (KT894726)	43.41 (47.22)
4.	*Bacillus subtilis* (KT894727)	47.80 (54.89)
5.	*Bacillus tequilensis* (KT894729)	50.57 (59.67)
6.	*Bacillus cereus*	44.49 (49.12)
7.	*Pseudomonas fluorescens*	77.25 (95.11)
	S. Em. ±	0.32
	C. D. at 5%	0.99
	C. V. %	1.06

*Data outside the parenthesis are arcsine transformed whereas inside are re-transformed values.

The present findings are in corroborate with the results obtained by different workers. Sobhanipour *et al.* (2008) [10] reported *Pseudomonas aeruginosa strain B-28* as the most effective antagonist in inhibiting the mycelial growth of the test pathogen *in vitro*. Similarly, Chawla and Gangopadhyay (2009) [6], Chawla *et al*. (2012) [3] and Sharma *et al.* (2015) [9] also revealed maximum inhibition of mycelial growth of *F. oxysporum* f. sp. *cumini* in presence of *P. fluorescens*.

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