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Evaluation of different biocontrol agents against *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) under *in vitro* condition of South Gujarat

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Abstract
Cotton (*Gossypium* spp.) is one of the most important fiber crops playing a key role in economic globe. The symptoms of *Fusarium oxysporum* f. sp. *vasinfectum* is both seed borne and soil borne and colonizes the roots and vascular system of susceptible cotton cultivars, causing root and vascular discoloration, wilting and sometimes death of the plant. As the wilt disease is becoming an important and destructive disease in the cotton growing areas. Here in this experiment, *in vitro* evaluation of native isolated antagonists were tested under dual culture method revealed the growth inhibition of test fungus (*Fusarium oxysporum* f. sp. *vasinfectum*) by the test antagonists. Five different biocontrol agents were screened *in vitro* for the growth inhibition of *Fusarium oxysporum* f. sp. *vasinfectum* by dual culture method and found that all the Trichoderma isolates were more effective than bacterial isolates. Among the Trichoderma isolates *Trichoderma harzianum* showed most promising results compared to other isolates. The highest growth inhibition of *Fusarium oxysporum* f. sp. *vasinfectum* was caused by *Trichoderma harzianum* (71.70%). While, the least growth inhibition was showed by *Bacillus substillis* (47.29%).

Keywords: Cotton, bioagent, inhibition, FOV

Introduction
In India, the productivity of cotton is very low due to many constraints including diseases. The most common cotton diseases reported in India are wilt (*Fusarium oxysporum* f. sp. *vasinfectum* (G.F. Atk.) W.C. Snyder & H.N. Hansen), root rots (*Rhizoctonia bataticola* (Taubenh.), verticillium wilt (*Verticillium dahliae* Kleb.), anthracnose (*Colletotrichum gossypii* Southworth. or *C. capsici* (Syd.) Butler & Bisby), grey mildew (*Ramularia areola* G.F. Atk.), blackarm (*Xanthomonas campestris* pv. *malvacearum* (Pammel) Dowson), leaf blight (*Alternaria macrospora* Zimm) and leaf curl (*Cotton leaf curl virus*). Among them wilt, root rot and verticillium wilt are soil borne diseases of cotton and anthracnose, grey mildew, black arm and leaf blight are foliar diseases of cotton (Ramod, 2016) [10].

Among these diseases, *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *vasinfectum* (FOV) is one of the most important and serious diseases. It was the first vascular wilt described by Atkison (1892) [2] and this disease is still causing enormous yield losses in several parts of the world and remains a threat to cotton production in the future (Feng *et al*., 2000) [6]. The disease is responsible for serious losses to the crop in the central and western India on a large scale and on almost all the cultivated varieties of both *G. arboreum* and *G. herbaceum*, the two indigenous species, especially in black cotton soils of Maharashtra, Madhya Pradesh, Karnataka and Gujarat. At present the most of cultivated cultivars are susceptible to wilt disease (*Fusarium oxysporum* f. sp. *vasinfectum*) and caused 54-60% yield loss (*Anon*., 2003) [1] because of reduced stand, stunted growth, small bolls and poor lint quality. The symptoms of *Fusarium oxysporum* f. sp. *vasinfectum* is both seed borne and soil borne, and colonizes the roots and vascular system of susceptible cotton cultivars, causing root and vascular discoloration, wilting and sometimes death of the plant (Chen *et al*., 1985, Hillocks, 1992 and Davis *et al*., 1996) [4, 9, 5].

Material and Methods

To determine the antagonistic action of various known species of fungal and bacterial bioagents, the dual culture test was carried out. Twenty millilitre of media poured aseptically in each of the Petri plates and allowed to solidify. Mycelia disc of 5mm diameter of both *i.e.* each antagonist and test fungus was placed on solid media in the same Petri plates approximately 4cm away from each other. All the inoculated plates were incubated at 27±2 ºC and observed after 10days for the growth of antagonist and test pathogen. Index of antagonism was determined in each treatment by following standard formula as given by Vincent (1947) [14].

$$PGI = \frac{100\ (DC–DT)}{DC}$$

Where,
PGI = Per cent growth inhibition
DC = Average diameter of mycelial colony in control set (mm)
DT = Average diameter of mycelial colony of treated set (mm)

Table 1: List of fungal and bacterial biocontrol agents

Treat. No.	Biocontrol agent
T_1	*Trichoderma harzianum*, Navsari isolate
T_2	*Trichoderma viride*, Navsari isolate
T_3	*Trichoderma fasciculatum*, Navsari isolate
T_4	*Pseudomonas flueroscence*, Navsari isolate
T_5	*Pseudomonas aeurogenosa*, Navsari isolate
T_6	*Bacillus substillis*, Navsari isolates
T_7	Control

Design: CRD
Repetitions: 03

Result and Discussion

In vitro evaluation of native isolated antagonists was tested under dual culture method revealed the growth inhibition of test fungus (*Fusarium oxysporum* f. sp. *vasinfectum*) by the test antagonists *viz.*, *Trichoderma harzianum, T. viride, T. fasciculatum, Pseudomonas flueroscence, P. aeurogenosa and Bacillus subtilis.* The observations on per cent growth inhibition are presented in Table 2 and depicted in Plate 1 with Fig. 1.

The results revealed that all the antagonists showed different results. Maximum reduction of pathogen (71.70%) was observed in the presences of *T. harzianum* followed by *T. viride* (67.44%). The *T. fasciculatum* was also found better with 62.40 per cent reduction in growth of the pathogen. Among the bacterial antagonists *P. flueroscence* and *P. aeurogenosa* found 60.46 and 54.65 per cent growth inhibition over control while, *B. subtilis* gave minimum growth inhibition (47.29%).

The results of present investigation corroborate with the research results obtained by Hossain *et al*. (2013) [8], Singh *et al.* (2017) [12].

They reported that *T. harzianum* showed strong antagonistic activity towards *Fusarium oxysporum* under *in vitro* conditions. Various workers *viz.*, Basak and Basak (2011) [3], Gandhi *et al.* (2015) [7], Patil *et al*. (2015), Shankar *et al.* (2016) [11] and Vahunia *et al.* (2017) [13] found *Trichoderma* spp. more effective against *Fusarium* spp.

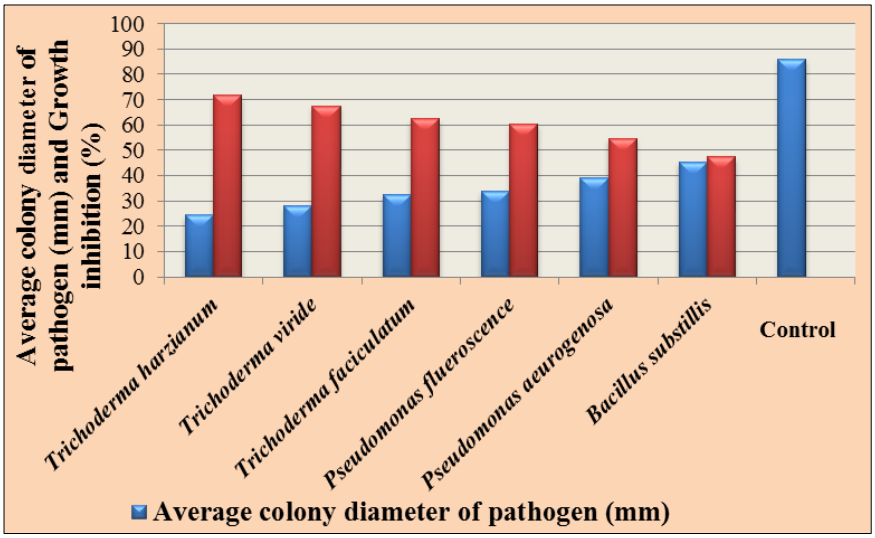

Fig 1: Average colony diameter of pathogen (mm) and per cent growth inhibition

Table 2: Inhibitory effect of different biocontrol agents against pathogen *in vitro*

Sr. No.	Name of Bio-agents	Average colony diameter of pathogen (mm)#	Growth Inhibition (%)
T_1	*Trichoderma harzianum*, Navsari isolate	4.982* (24.33)**	71.70
T_2	*Trichoderma viride*, Navsari isolate	5.338 (28.00)	67.44
T_3	*Trichoderma faciculatum*, Navsari isolate	5.729 (32.33)	62.40
T_4	*Pseudomonas flueroscence*, Navsari isolate	5.872 (34.00)	60.46
T_5	*Pseudomonas aeurogenosa*, Navsari isolate	6.284 (39.00)	54.65
T_6	*Bacillus substillis*, Navsari isolates	6.769 (45.33)	47.29
T_7	Control	9.300 (86.00)	-
	S. Em. ±	0.08	
	CD at 5%	0.24	
	CV %	2.14	

(Average of three repetitions)
*Figures outside the parentheses indicate √x + 0.5 transformation value
**Figures in parentheses indicate original values

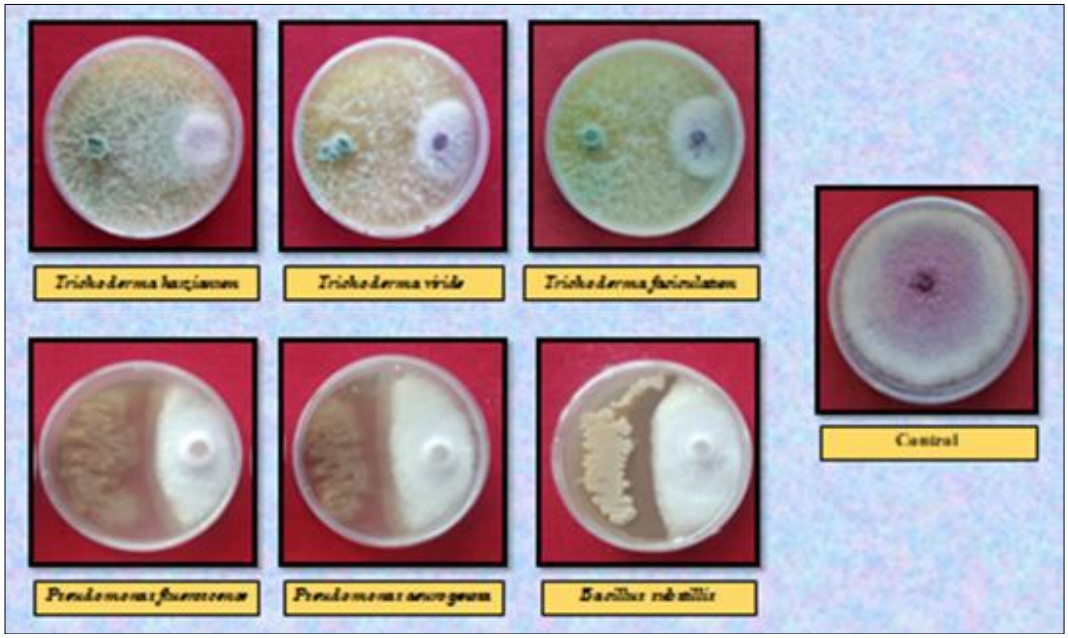

Plate 1: Evaluation of different biocontrol agents against *Fusarium oxysporum* f. sp. *Vasinfectum*

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