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In vitro evaluation of different bioagents against *Corynespora cassiicola* causing target leaf spot disease of cotton under South Gujarat of India

Nirva Patel, Prashant B Sandipan, Nishi Saini, PS Patel and RK Patel

Abstract
Cotton (*Gossypium hirsutum* L.) is one of the most important fiber crops playing a key role in the economic and social scenario of the globe. India is one of the major cotton growing countries in the world. India ranks first in area and second in the total production of cotton in the world. Cotton is grown worldwide for its natural fiber and oil. Cotton is primarily a raw material for a thriving textile industry and is also one of the most ancient and essential commercial crops, second only to food grains. In the present experiment, five different bioagents were screened *in vitro* for the growth inhibition of *C. cassiicola* by dual culture method, which inhibited the growth of pathogen by 65.55 to 80.97 percent. Among the *Trichoderma* isolates, *T. viride* followed by *T. harzianum* showed the most promising results compared to other isolates. The highest growth inhibition of *C. cassiicola* was recorded by *T. viride* (80.97%) followed by *T. harzianum* (76.25%). The least growth inhibition was recorded in *T. fasciculatum* (65.55%). *B. subtilis* (67.92%) and *P. fluorescens* (68.88%) were proved less effective as compared to fungal bioagents.

Keywords: *Gossypium hirsutum, Corynespora cassiicola*, target spot, fungicide, cotton

Introduction
Cotton, "The White Gold" or the "King of Fibres" enjoys a pre-eminent status among all cash crops in the country and is the principal raw material for flourishing textile industry (Patel *et al*., 2021) [5]. Cotton is affected by a number of important diseases that limit production in all locations where, the cotton crop is grown. As a subtropical to tropical crop that is grown over a wide range of latitudes as well as a perennial plant grown as an annual crop, cotton is often under stress that may exacerbate specific disease problems (Rothrock *et al*., 2015) [10]. 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), Leaf curl (Cotton leaf curl virus), Corynespora leaf blight (*Corynespora cassiicola* (Berk. & M. A. Curtis) C. T. Wei, Boll rot and physiological disorders as Para wilt, Leaf reddening and sometimes leaf elongation due to improper use of weedicides etc. Corynespora leaf blight of cotton (*Gossypium hirsutum* L.) also referred as "Target spot", is caused by *Corynespora cassiicola* (Berk & Curt.) Wei. Other than cotton the pathogen attacks on several crop plants. The disease was also reported for the first time on soybean in Bolivia in 1994 and on cotton in the State of Mato Grosso, Brazil in 1995 (Mehta and Barea, 1994) [6] and Mehta *et al*., 2005 [7]. During the year 2021 from the survey result, it is evident that the disease intensity of Surat district was ranged from 1.50 to 26.50 percent. Maximum disease intensity of 0.00-26.50 percent was observed in LRA 5166 followed by G. Cot. Hy. 12 BG II with 0.00-8.50 PDI, G. Cot. Hy. 8 BG II with 0.00-6.50 PDI and G. Cot. Hy. 10 BG II with 0.00-5.50 PDI of Choryasi taluka of Surat Patel *et al*., (2023) [8]. In South Gujarat condition, the bacterial blight is the most wide spread and destructive disease reported to cause yield losses of about 10 to 30 percent (Kalpana *et al*., 2004 [3] and Sandipan *et al*., (2017a) [13] and Sandipan *et al*., (2017b) [14]). However, over the years the extensive and continuous use of pesticides and fertilizers has not only posed an imperative risk to human health and ecosystems but also been disastrous for soil microorganism and humans too. However, the production of agricultural crops is continuously getting vulnerable and weak due

to attack of pests and diseases such as insects, bacteria, fungi, nematodes, virus etc. Plant diseases are among the main constraints affecting the production and productivity of crops both in terms of quality and quantity. Crop losses are creating a major threat to the food production with about 27 to 42% loss in global food production attributed to plant disease caused by plant pathogens which otherwise would have been doubled if no disease management strategies are applied (Singh, 2014, Alizadeh *et al*., 2020) [9, 1]. In recent times, various approaches are being used to manage and alleviate a variety of pathogens to control of plant diseases. The use of microbial pesticides is one of the best strategies available to battle the diseases in an eco-friendly and safe manner. A variety of bacterial and fungal based biopesticides have been identified and developed but required an effective adoption and approach and further development of such bioagents. Moreover, consumers are becoming more and more concerned about pesticide-free safer foods which, results in emergence of eco-friendly strategies for the plant disease management. Nowadays, several beneficial bacterial based biopesticides are widely used in agriculture at commercial level. Hence, this experiment was framed to control the *C. cassiicola* disease of cotton under *in vitro* condition.

Materials and Method

To determine the antagonistic action of various known species of fungal and bacterial bioagents (Table: 1) the dual culture test was carried out. 20ml of PDA medium poured aseptically in each of the Petri plates and allowed to solidify. Mycelial disc of 5mm diameter of both *i.e*., each antagonist and test fungus was placed on the solid PDA medium in the same Petri plate approximately 4cm away from each other. All the inoculated plates were incubated at 27±2 ºC and observed after 10 days for the growth of antagonist and test pathogen.

Index of antagonism was determined in each treatment by following the standard formula as given by Asalmol *et al*., (1990) [2] and similar, formula was used by Sandipan, (2014) [11] and Sandipan *et al*., (2015) [12].

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

Where,

P G I - Percent Growth Inhibition

C - Growth in control (mm)

T - Growth in treatment (mm)

Table 1: List of different bioagents used against the *Corynespora cassiicola* under *in vitro* conditions

Treatment No.	Bioagents
T_1	*Trichoderma harzianum*, Navsari isolate
T_2	*Trichoderma viride*, Navsari isolate
T_3	*Trichoderma fasciculatum*, Navsari isolate
T_4	*Pseudomonas fluorescens*, Navsari isolate
T_5	*Bacillus subtilis*, Navsari isolate
T_6	Control

Design: Completely Randomized Design

Treatments: 6

Repetitions: 4

Method: Dual culture technique

Location: Department of Plant Pathology, Post Graduate Laboratory, N. A. U., Navsari, Gujarat

Result and Discussion

The dual culture method was approached under *in vitro* conditions to investigate an effective bioagents for the control of Corynespora leaf spot disease of cotton.

For this purpose, different bioagents *viz., T. viride* (Navsari isolate), *T. harzianum* (Navsari isolate), *T. fasciculatum (*Navsari isolate), *P. fluorescens* (Navsari isolate), *B. subtilis* (Navsari isolate) has been selected. The observations on the Percent Growth Inhibition (PGI) are presented in Table: 2 and Photo: 1 with Fig. 1.

The data revealed that all the antagonists showed different results in inhibition of the pathogen. The maximum growth inhibition of pathogen (80.97%) was observed in the presence of *T. viride* followed by *T. harzianum* (76.25%) over the control while, *T. fasciculatum* gave minimum growth inhibition (65.55%).

The results of the present investigation are corroborated with the research findings obtained by Manju *et al.* (2014) [5] and Kochuthresiamma (2006) [4] as they tested different bioagents *viz., T. viride*, *T. harzianum*, *P. fluorescens* and *B. subtilis* and reported that the maximum growth inhibition of test pathogen was observed by *T. viride.*

Table 2: Inhibitory effect of different bioagents against the *Corynespora cassiicola* under *in vitro* condition

Sr. No.	Bioagents	Average colony diameter of pathogen (mm)	Growth Inhibition (%)
T_1	*Trichoderma harzianum*, Navsari isolate	21.37	76.25
T_2	*Trichoderma viride*, Navsari isolate	17.12	80.97
T_3	*Trichoderma fasciculatum*, Navsari isolate	31.00	65.55
T_4	*Pseudomonas fluorescens*, Navsari isolate	28.00	68.88
T_5	*Bacillus subtilis*, Navsari isolates	28.87	67.92
T_6	Control	90.00	-
	SEm±	0.56	
	CD at 5%	1.69	
	CV%	3.13	

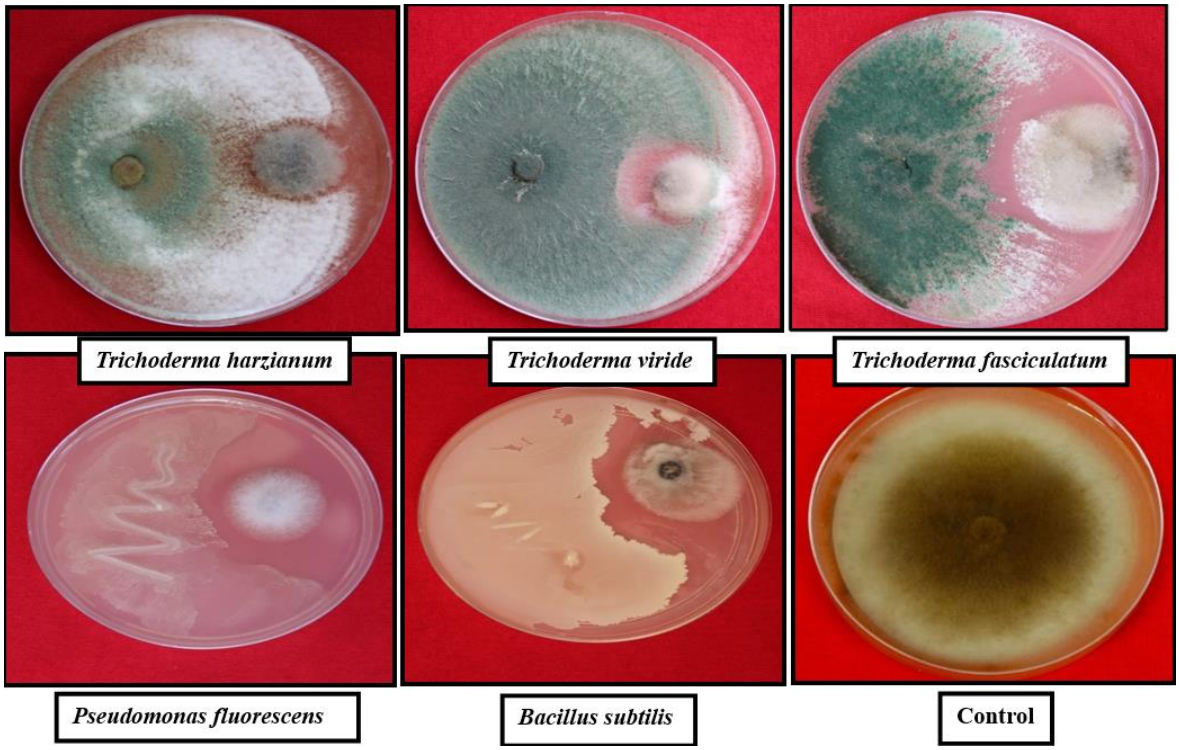

Photo 1: Evaluation of different bioagents against the *Corynespora cassiicola*

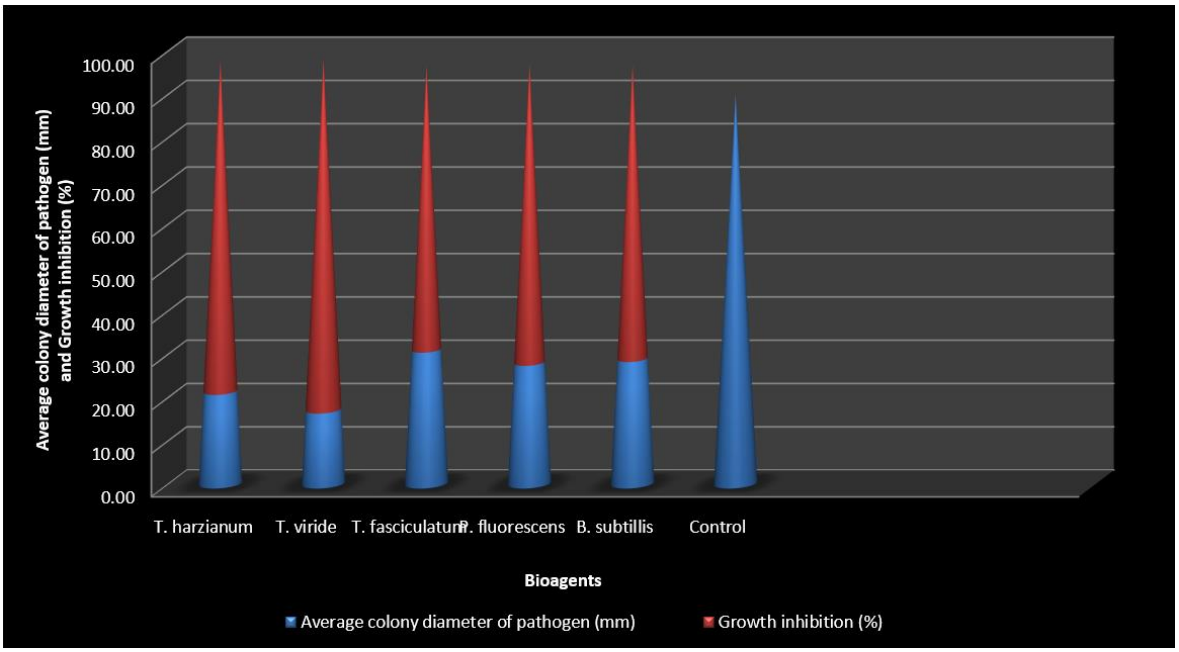

Fig 1: Inhibitory effect of different bioagents against the *Corynespora cassiicola* under *in vitro* condition

Conclusion

It was concluded from all the conducted experiments that the target leaf spot is one of the serious and devastating disease of cotton with characteristic symptoms such as small, round to irregular, dark red, small and many lesions observed on the leaves of cotton plant. From the above experiment it is clearly stated that the *Trichoderma viride* was the most effective bioagent in controlling *C. cassiicola* pathogen of cotton.

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