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Lokesh Yadav
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Naresh Kumar Yadav
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Vinod Kumar Malik
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Pankaj Yadav
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Nisha Yadav
College of Agriculture, CCS Haryana Agricultural University, Hisar, Haryana, India

Preeti Vashisth
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Sheetal Dhariwal
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Corresponding Author:
Naresh Kumar Yadav
Department of Plant Pathology, CCS Haryana Agricultural University, Hisar, Haryana, India

Evaluation of biological control agents against *Colletotrichum gossypii* under *in vitro* condition

Lokesh Yadav, Naresh Kumar Yadav, Vinod Kumar Malik, Pankaj Yadav, Nisha Yadav, Preeti Vashisth and Sheetal Dhariwal

Abstract
Cotton (*Gossypium* spp.) is the most versatile and important fibre crop next to food grains and grown throughout the tropical and sub-tropical regions of the world. Globally, cotton is known as 'White Gold' and 'King of Fibers' and it has immense importance in providing basic raw material to textile industries. The anthracnose disease caused by *Colletotrichum gossypii* is becoming serious threat for cotton production. In current study, *in vitro* efficacies of five biological control agents were examined during year 2021 against *C. gossypii*. The radial growth was recorded minimum (31.16 mm) in the Petri plate inoculated with *Trichoderma harzianum* followed by *Trichoderma viride* (36.17 mm), hence providing mycelium growth inhibition of 65.37 and 59.81per cent, respectively as compared to untreated control. *Pseudomonas fluorescens* was found least effective and 50.63 per cent mycelial growth inhibition was recorded, however the radial growth of 44.43 mm was observed as maximum among all tested antagonists.

Keywords: Cotton, *Colletotrichum gossypii*, biocontrol, *in vitro*

Introduction
Cotton (*Gossypium* spp.) is the most ancient and important fibre belonging to the Malvaceae family, under the genus *Gossypium* which includes 50 species. Cotton is one of the extensively grown cash crop and it is an industrial commodity of worldwide significance. Globally, cotton is known as 'White Gold' and 'King of Fibers' and it has gigantic importance in providing basic raw material to textile industries. Among 50 species, only four are cultivated (Percival and Kohel, 1990) [1]. Among these four cultivated species, *Gossypium hirsutum* and *Gossypium barbadense* are tetraploids (2n=4x=52) and frequently known as new world cotton. Whereas, *G. arboreum* and *G. herbaceum* are diploids (2n=4x=26) and frequently known as old world cotton. From ancient times, the solitary country known for cotton fabrics is India, predominantly for *G. arboreum* and *G. herbaceum* while, the rest of the world is known for cotton silk, flax and wool. There is no clear evidence available to validate the exact centre of origin of cotton. Various countries including Nubia, South America, Pakistan, Egypt, Mexico and India are linked on the basis of theoretical considerations and archaeological evidences (Lee and Fang, 2015) [2]. India is considered as home of domestication and development of the Asiatic cultivated cottons including *G. arboreum* and *G. herbaceum* (Narayana *et al.*, 2002) [3]. Cotton has immense importance and utilized in processing and fibre, feed, oil, food, fuel and ethanol production industries. It is also utilized as medicinal and industrial purposes. Cotton seed cake is used as feed for farm animals. The dried cotton sticks are utilized as fuel (Siwach and Sangwan, 2012) [4]. Major cotton growing countries are India, China, United States, Pakistan, Brazil and Australia. India ranks first in cotton production (6188000 tons) followed by China and USA in world. In India, cotton is grown over an area of 12.35 million hectares compared to 32.91 million hectares globally. The major cotton growing states of India are Gujrat, Maharashtra, Telangana, Andhra Pradesh, Madhya Pradesh, Karnataka, Haryana, Rajasthan, Punjab and Odisha (Anonymous, 2022) [5]. Various biotic and abiotic factors are responsible for reducing the quality and quantity of produce. Among several diseases caused by various pathogens, the anthracnose disease of cotton incited by *Colletotrichum gossypii* is becoming serious impediment for cotton production. Anthracnose disease was first time reported In India, it was first time reported from Bihar state (Butler, 1918) [6] and afterwards severe outbreaks were reported from Maharashtra, West Bengal, Tamil Nadu and Madhya Pradesh (Dastur, 1934; Kulkarni *et al.*, 1958) [7, 8]. With the passage of time, it become serious obstruction for cotton production and yield losses up to 48.4 per cent are also reported

(Sundararaman, 1930) [9]. The chemical fungicides have undesirable toxic effects on living beings and to overcome these negative impacts, the use of biological control agents is a safer approach for eco-friendly plant disease management. It is more economical as compared to application of fungicides. Therefore, an attempt was made in the year 2021, to evaluate the antagonistic activities of five biocontrol agents under *in vitro* conditions against *Colletotrichum gossypii* causing anthracnose disease of cotton.

Materials and Methods

Five bio-control agents' *viz.*, *Aspergillus flavus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Trichoderma viride* were tested against *C. gossypii* for their efficacy by performing dual culture technique. The complete randomized design was followed with three replications of each treatment with untreated control plate. The PDA was poured in sterilized Petri dishes. Mycelial discs (5 mm) of antagonist and *C. gossypii* fungus were placed in straight line at periphery of Petri plates. The plates were sealed with parafilm and incubated at 25±2 °C temperature. Mycelial growth was recorded when there is 90 mm growth in control plates at 25±2 °C and per cent growth inhibition was calculated by using the formula given by Vincent (1927) [10].

$$\text{Growth Inhibition (\%)} = \frac{\text{Growth in control} - \text{growth in treatment}}{\text{Growth in control}} \times 100$$

Statistical analysis of laboratory and field experiments of the data were carried out using OPSTAT software from CCSHAU, Hisar, web site using appropriate programme as per requirement of the experiment. The critical difference (CD) was calculated at 5% level of significance (p=0.05) for comparison of difference between the means of treatment (Anonymous, 2022) [11].

$$CD = \sqrt{\frac{2 \text{ Error Variance}}{n}} \times t \text{ at } 5\% \text{ probability level}$$

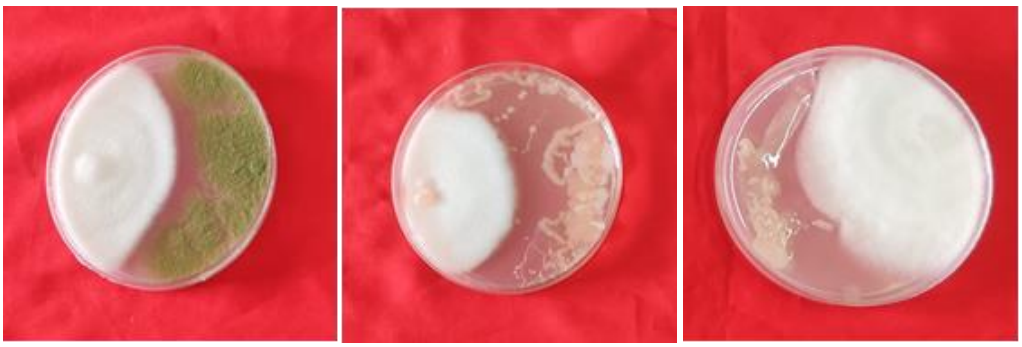
Where,
CD = is the critical difference.
n = is the number of observations of that factor which CD is to be calculated.
$^{t}0.05\%$ = is the value of percentage point of 't' distribution for error degree of freedom at 5% level of significance.

Results and Discussion

The antagonistic activities of five biocontrol agents *viz.*, *Aspergillus flavus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Trichoderma harzianum* and *Trichoderma viride* were recorded against *C. gossypii* by dual culture method under *in vitro* conditions. All the tested antagonists effectively inhibited mycelial growth of test fungus. The result of tested bio-agents has been presented (Table 1, Fig. 1 and Plate 1) and it was observed that *Trichoderma harzianum* was most effective which showed 65.37 per cent mycelium growth inhibition followed by *Trichoderma viride* (59.81%) as compared to untreated control. *Bacillus subtilis* was also effective in inhibiting mycelial growth by 58.37 per cent. The least effective biological control agent was *Pseudomonas fluorescens* (50.63%). However, the radial growth was observed maximum in case of *Pseudomonas fluorescens* (44.43 mm) followed by *Aspergillus flavus* (40.10 mm). The radial growth was recorded minimum (31.16 mm) in the Petri plate inoculated with *Trichoderma harzianum* followed by *Trichoderma viride* (36.17 mm) under *in vitro* conditions. Similarly, Singh *et al.* (2012) [12] also revealed that *T. viride* and *P. Fluorescens* inhibited the mycelial growth by 88.00 and 86.00 per cent, respectively. The results obtained by Sushmitha and Zacharia, (2021) [13] during evaluation of bio-agents against anthracnose causing pathogen were also in concordance with present study and it was concluded that *T. harzianum* and *P. fluorescens* inhibited by mycelial growth by 93.59 per cent and 61.92 per cent respectively, as compared to untreated control. It is concluded that the tested antagonists efficiently inhibited the mycelial growth of test fungus and these can be utilized for further use for management of disease in integrated manner and to avoid the hazardous effects of chemical fungicides.

Table 1: Efficacy of biological control agent against *C. gossypii* under *in vitro* conditions

Bioagents	Radial Growth* (mm)	Growth inhibition (%)
T_1: *Aspergillus flavus*	40.10	55.44
T_2: *Bacillus subtilis*	37.47	58.37
T_3: *Pseudomonas fluorescens*	44.43	50.63
T_4: *Trichoderma harzianum*	31.16	65.37
T_5: *Trichoderma viride*	36.17	59.81
T_6: Control	90	0.00
C.D. (p=0.05)	1.18	
SE (m)±	0.38	



Aspergillus flavus *Bacillus Subtilis* *Pseudomonas fluorescens*

Trichoderma harzianum *Trichoderma viride* Control

Plate 1: Growth inhibition of *C. gossypii* by biological control agents

Conclusion

Among five biological control agents, *Trichoderma harzianum* was most effective which recorded 65.37 per cent mycelium growth inhibition followed by *Trichoderma viride* (59.81%) and *Bacillus subtilis* (58.37%). It is concluded that all the tested antagonists efficiently inhibited the mycelial growth of test fungus and these can be utilized for further use for management of disease in integrated manner and to avoid the hazardous effects of chemical fungicides.

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