In vitro effect of bio-agents for control of Alternaria blight of Indian mustard incited by *Alternaria brassicicola*

Abstract

Indian mustard [*Brassica juncea* (L.) Czern and Coss.] is one of the most popular *rabi* oil seed crop in India. It's belongs to family Cruciferae (Brassicaceae). It is mostly cultivated *rabi*, crop of Northern as well as North eastern region of India. Indian mustard contributes about 85 per cent of total rapeseed-mustard production in India (Kumar and Chauhan, 200). Crucial Indian mustard growing states are Rajasthan (50%), Uttar Pradesh (12.3%), Haryana (11.2%), Madhya Pradesh (9.8%), Gujrat (6.5%) and West Bengal (5.1%). Indian mustard is affected by many major diseases like Fungal, Bacterial, Viral, and also parasitic nematode. In mustard maximum losses in yield occurs due to microbial diseases. Among the major disease *viz.*, Alternaria blight (*Alternaria brassicae*, *Alternaria brassicicola*, *Alternaria alternate*), White rust (*Albugo candida*), Downey mildew (*Peronospora parasitica*), Powdery mildew (*Erysiphe cruciferarum*), Bacterial blight (*Pseudomonas cannabina*), and Sclerotinia stem rot (*Sclerotinia sclerotiorum*). Alternaria blight of Indian mustard is one of the most important disease which reduce quantity and quality of seed yield. Alternaria blight disease is one of the damaging disease among the major diseases of mustard causing up to 70 per cent losses in yield with no proven source of registance against the disease reported till date in any of the hosts. To control the losses caused by pathogens many farmers are using fungicides to obtain very good yield. The present study was carried out to recognize the effect of different bioagents on control against *Alternaria brassicicola* under *in-vitro* condition. Four antagonists *viz.*, *Trichoderma viride*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis*, checked the growth of *Alternaria brassicicola* at point of their contact on culture medium. Among these, maximum inhibition (78.05%) of *Alternaria brassicicola* was observed in *Trichoderma harzianum*.

Keywords: Indian mustard, bioagents, per cent inhibition, *Trichoderma harzianum*.

Introduction

Indian mustard [*Brassica juncia* (L.) Czern and Coss.] is one of the most popular *rabi* oil seed crop in India. It's belongs to family Cruciferae (Brassicaceae). It is mostly cultivated *rabi*, crop of Northern as well as North eastern region of India. It was emanated in china and from there

it was introduced to India. According to ancient scripture, it has been grow as early as 5000 BC (Kumar *et al*., 2014).

Indian mustard contributes about 85 per cent of total rapeseed-mustard production in India (Kumar and Chauhan, 2005).

Mustard seed oil is popularly used for edible purpose, but also it used as industrial lubricant. Mustard cake is used as organic manure and as a protein cource in animal feeds (Bhowmik, 2003).

Indian mustard is affected by many major diseases like Fungal, Bacterial, Viral, and also parasitic nematode. In mustard maximum losses in yield occurs due to microbial diseases. Among the major disease *viz.,* Alternaria blight (*Alternaria brassicae*, *Alternaria brassicicola*, *Alternaria alternate*), White rust (*Albugo candida*), Downey mildew (*Peronospora parasitica*), Powdery mildew (*Erysiphe cruciferarum*), Bacterial blight (*Pseudomonas cannabina*), and Sclerotinia stem rot (*Sclerotinia sclerotiorum*).

Alternaria blight of Indian mustard is one of the most important disease which reduce quantity and quality of seed yield. In Varuna cultivar under unprotected conditions 20.8 per cent seed yield loss observed by Prasad *et al*. (2003a).

According to Chadar *et al*. (2016) 23.9 to 62 per cent disease intensity of Alternaria leaf blight of mustard which occurred extensively in Jhansi region of India.

Symptoms of disease appear on leaves and stems and adult plants. Due to Alternaria disease dark brown spots on the leaves and siliquae reduce the photosynthetic capacity and induce immature ripening, which causes decreased the quality seed production in both vegetable and *oleiferous Brassicas* (Kumar *et al*., 2014).

Chemicals are not only costly but also they are creating problems on the environment, human health in all areas of the world (Rahmatzai *et al*. 2017). Now a day bioagents also used for control of many fungal diseases because those are not hazardous to crop as well as humans.

The Present studied was carried out to find the best botanical bioagent on control against *Alternaria brassicicola* causing Alternaria blight of Indian mustard.

Materials and methods

Isolation of fungus cultures

The diseased plants were collected from the mustard grown in the field. The Alternaria blight affected leaf and stem portion was cut into small pieces and surface sterilized with 0.1% sodium hypochlorite solution then afterward three times thoroughly washed with water. Potato dextrose agar (PDA) media were prepared and sterilized in an autoclave at 120°C, 15 lbs pressure for 20 minutes. The media were poured into Petri plates and allowed to cool for sometimes. Then, the leaf pieces were inoculated into the media and kept for incubation. After seven to eight days the fungal tips were transferred to PDA slants in order to obtain pure cultures. The isolates were confirmed as *Alternaria* (Van Bruggen 1984) [20] by microscopic observation of fungal culture.

***In vitro* management of disease through bioagents**

Two fungal and two bacterial bioagents were evaluated in vitro against *Alternaria brassicicola* applying Dual Culture Technique (Dennis and Webster, 1971). Seven days old cultures of the test bioagents and test pathogen (*Alternaria brassicicola*) grown on PDA were used for the study. Two 5 mm culture discs, one each of the test pathogen and test bioagent were cut out with sterilized cork borer and placed at equidistance, exactly opposite to each other on autoclaved and solidified PDA medium in Petri plates and three plates were incubated at 28 ± 2 °C PDA plates inoculated alone with pure culture disc (5 mm) of the test pathogen were maintained as untreated control.

Experimental details

Design: C.R.D. (Completely Randomized Design)

Replication: Four

Treatments: Four

S. No.	Bioagents
1	*Trichoderma viride*
2	*Trichoderma harzianum*
3	*Pseudomonas fluorescens*

4	*Bacillus subtilis*
5	Control

The extent antagonistic activity by bioagent was recorded after full growth covers in the control plate.

The per cent mycelial inhibition zoon of pathogen was calculated using formula of Bliss (1934).

I =

Where, I = Per cent mycelial inhibition

C = Growth of fungal pathogen in Control (mm)

T = Growth of fungal pathogen in dual culture plate (mm)

Results and discussion

The results of fungal mycelial growth and *Alternaria brassicicola* suppression with two fungal and two bacterial bioagents are shown in (Table 1 and Plate 1).

Results showed that all four bioagents evaluated exhibited antifungal activity against *Alternaria brassicicola* and significantly inhibited pathogen growth over control. *Trichoderma harzianum* was superior to *Trichoderma viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* which produced 78.05 per cent inhibition of mycelial growth of pathogen in dual culture plates (Table 1 and Fig. 1) These findings were in accordance with Zafar *et al*. (2013) and Selim (2015). Sivapalan (1993) observed that *Gliocladium roseum* and *Trichoderma harzianum* reduced leaf infection of *Alternaria brassicicola* on broccoli. Prasad and Kulshrestha (2002) observed that *Pseudomonas fluorescens* showed lowest incidence of blight infected seedling of sunflower followed by *Bacillus subtilis*.

Table – 1: *In vitro* evaluation of bioagaints against *Alternaria brassicicola*

S. No.	Bioagaints	Mycelial Growth (mm)	Per cent inhibition of mycelial growth
1	*Trichoderma viride*	28.50	68.33 (55.73)

2	*Trichoderma harzianum*	19.75	78.05 (62.04)
3	*Pseudomonas fluorescens*	54.75	39.16 (38.72)
4	*Bacillus subtilis*	52.50	41.67 (40.18)
5	Control	90	0.00
	SEm±	0.671	
	CD (p=0.05)	2.091	
	CV	2.363	

*Mean of four replications

**Figures in the parenthesis are square root transformed values

Fig – 1: *In vitro* evaluation of bioagaints against *Alternaria brassicicola*

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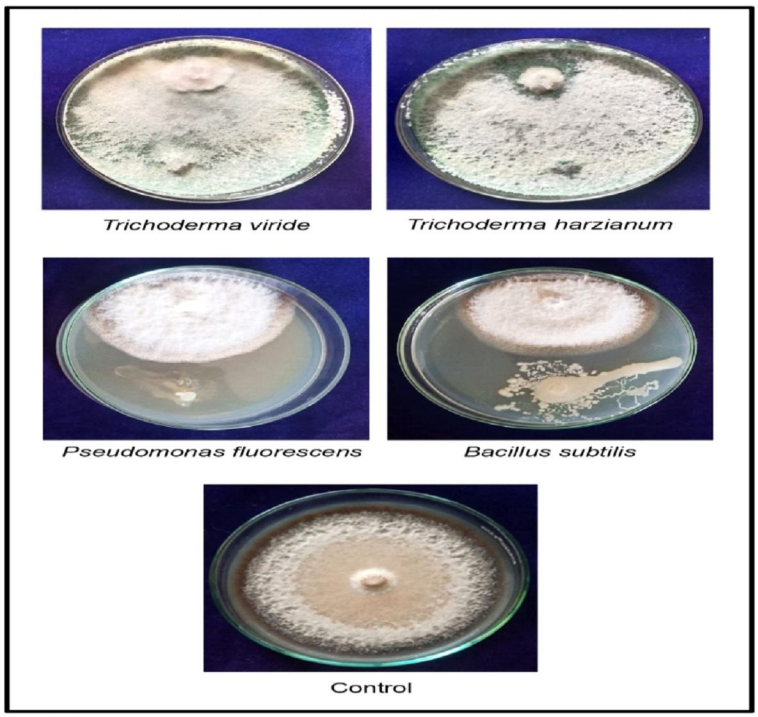

Plate :-

Plate – 1: *In vitro* evaluation of bioagaints against *Alternaria brassicicola*