

Original Research Article

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In-Vitro Evaluation of Bio-control Agents against Alternaria Leaf blight of Sunflower and Vascular Wilt of Beans

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ABSTRACT

Four fungal bio-control agents, viz., *Trichoderma harzianum*, *T. viride*, *Aspergillus* spp. and *Tricothecium* spp. were evaluated *in vitro* through dual culture technique against *Alternaria helianthi*, the causative agent of leaf blight of sunflower and *Fusarium oxyporum* f. sp. *phaseoli*, the causative agent of vascular wilt of beans. The observations were taken after fourth and seventh day for both the pathogens. After fourth day, observations recorded in case of *A. helianthi*, the minimum colony diameter (0 cm) and maximum inhibition percentage over control (100%) were observed in *Trichoderma harzianum* and *T. viride* which were statistically at par with *Aspergillus* spp. and *Tricothecium* spp. while after seventh day, the result was more or less similar. The minimum colony diameter (0 cm) of pathogen were observed in *Trichoderma harzianum* and *T. viride* with maximum inhibition per cent (100%) over control, which differed statistically from *Aspergillus* spp. *Tricothecium* spp. showed least effectiveness against the target pathogen showing colony diameter (2.3 cm) and inhibition (23.33%) after fourth day and colony diameter (4.5 cm) and inhibition (35.71%) after seventh day over control. After fourth day, the observations taken in case of *F. oxyporum* f. sp. *phaseoli*, the minimum colony diameter (1.6 cm) and maximum inhibition per cent over control (77.14%) were observed in *T. harzianum* which were statistically at par with *T. viride*, *Aspergillus* spp. and *Tricothecium* spp. After seventh day the result was more or less similar. The minimum colony diameter (1.2 cm) of pathogen was observed in *T. harzianum* with maximum inhibition percentage (86.66%) over control, which differed statistically from *T. viride* and *Aspergillus* spp. *Tricothecium* spp. showed least effectiveness against the target pathogen showing colony diameter (3.5 cm) and inhibition per cent (50%) after fourth day and colony diameter (3.0 cm) and inhibition per cent (66.66%) after seventh day over control.

Keywords

Bio-control agents,
Beans, Fungus,
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Introduction

India has rich diversity of annual oilseed crops on account of diverse agro-ecological conditions. Nine annual oilseeds, which include seven edible oilseeds, viz., groundnut, rapeseed-mustard, soybean, sunflower, sesame, safflower and niger and two non-edible crops viz., castor and linseed are grown in the country. Cultivated sunflower (*Helianthus annuus* L.) is the main source of

edible oil. It ranks second to soybean in worldwide vegetable oil production. Commercially available sunflower contains 39 to 49 per cent oil in the seed.

It is extensively grown in Argentina, France, Spain, USA, China, Ukraine and India.

It is primarily grown for edible oil characterized by a high concentration of linoleic acid and moderate level of oleic acid.

The total area under sunflower in the world is about 25.34 million hectare with the production of 40.04 million tonnes (Anonymous 2012). In India, sunflower occupies the fourth place among oilseed crops in terms of acreage and production. The area under sunflower cultivation in India was 0.72 m ha, with a total production of 0.50 million tonnes and productivity of 692 kg ha⁻¹ (Anonymous, 2013). Over 70 per cent of the sunflower crop is being grown across Karnataka, Maharashtra and Andhra Pradesh. In Karnataka it occupies an area of about 3.84 lakh ha with a production of 1.93 lakh tonnes and productivity of 503 kg ha⁻¹. Karnataka is the leading state in the country accounting for more than 50 per cent of the total acreage and contributing to 38 per cent of the national sunflower production.

Sunflower is prone to severe damage by diseases especially when there is well spread rainfall and high humidity, efforts to stabilize crop production has not been successful owing to number of diseases encountered on this crop, viz., *Alternaria* leaf spot/blight, Rust, Downy mildew, Powdery mildew, *Phoma* leaf spot/blight, *Botrytis* grey mold, *Rhizopus* head spot, *Macrophomina* charcoal rot, *Sclerotium* root rot, *Fusarium* and *Verticillium* wilts are sporadic in nature and location specific (Gulya and Masirevic 1991). *Alternaria* leaf spot/blight caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara were first described in 1943 from Uganda. Since then this disease has been reported from almost all sunflower growing countries throughout the world. Two species of *Alternaria* cause leaf and stem spots on sunflower *Alternaria helianthi* and *A. zinniae*, of which *A. helianthi* is the more prevalent and more serious. These diseases can be serious in warm humid environments. Yield losses may occur through reduced head diameters, number of seeds per head, and oil content or quality. The disease is known to

cause more than 80 per cent of yield loss under severe epiphytotic conditions in northern Karnataka (Shankergoud *et al.*, 2006) in India. The disease severity is known to increase or decrease depending upon the changing environmental conditions during the crop growth period. *A. helianthi* is recognized as a major disease in more humid areas of India as well as in Central Europe, Australia, South America and parts of Africa. It occurred in an epidemic form during 1988 in Southern India, especially in Karnataka destroying major area of the crop due to heavy unexpected rain during flowering and grain filling stage of the crop. According to Allen *et al.*, (1981), the nature of yield reduction is determined to some extent by stage of plant growth when disease epidemic develops.

The disease generally first appears on the lower leaves in the form of dark-brown to black spots measuring 0.2 to 0.5 mm in diameter. The circular to oval spots are surrounded by chlorotic zone with grey-white necrotic Centre marked with concentric rings. The disease spread to middle and upper leaves with the growth of the plants. In advanced stages, elongated spots are formed on petioles, stem and ray florets. Particularly under humid conditions, the spots enlarge in size and coalesce resulting in blighting of leaves and sometimes the flower heads are also infected (Amaresh and Nargund, 2001).

The *Alternaria* leaf blight is known to infect all aerial parts of plant viz., leaf, petiole, stem, floral parts and seeds. Initially, the disease appears in the form of small, scattered brown spots on the leaf lamina. Later, these spots increase in size and coalesce covering larger leaf area (1 to 2.5 cm in diameter), with dark brown margin with yellow halo. Linear necrotic lesions also appear on stem, petioles and sepals. In severe cases, the head and seeds also get infected (Kolte and Mukhopadhyay 1973, AnilKumar *et al.*, 1974).

Pathogen survives on seed, host debris and weed host. Successive crop of sunflower may also help to multiply the pathogen. Hot weather and frequent rain during the milk and wax stages of development favour infection. Disease development is favoured by 25-27°C temperatures with at least 12 hours of wet foliage and is reported to spread rapidly during the rainy season (Basappa and Santha Lakshmi Prasad, 2005).

Common bean (*Phaseolus vulgaris* L.) belongs to family Leguminaceae and is a native to South Mexico and Central America. Dry bean is truly a “new world crop” originating 7000 years ago in different parts of North and South American continents (Landon 2008). It is a major grain legume consumed worldwide for its edible seeds and pods and is an important source of human dietary protein and calories (Pachico, 1993). The crop is consumed principally for its dry beans and green pods. It provides 15 per cent of the protein and 30 per cent of the caloric requirement to the world’s population and represents 50 per cent of the grain legume consumed worldwide (McConnell *et al.*, 2010). Dry beans are an important functional food containing high levels of chemically diverse components *viz.*, phenols, resistance starch, vitamins and fructo-oligosaccharides giving protection against such conditions as oxidative stress, cardiovascular diseases, diabetes, metabolic syndrome and many types of cancers (Camara *et al.*, 2013). Beans are eaten as boiled, baked, fried, or ground into flour. Crop residues such as dried pods, stems and processing by-products can be used as fodder. Although *Phaseolus vulgaris* is less efficient in fixing N than other legumes, yet it is able to fix up to 125 kg N ha⁻¹ and nodulates with several rhizobia (Wortmann, 2006).

According to FAO, production of dry beans was 26.83 million tonnes during 2016 in an area of 29.39 million ha and productivity was

0.91t ha⁻¹ with Brazil, China, India, Mexico, Myanmar, Tanzania and USA are main producer of beans in the world Brazil is the leading producer of bean in the world. In India it is grown in an area of 9.47 million ha with a production of 3.90 million tonnes and productivity 0.41 t ha⁻¹ in the states of Himachal Pradesh, Jammu and Kashmir, Kerala, Karnataka, Maharashtra, Tamil Nadu, Uttar Pradesh, and West Bengal and production of green beans was 23.60 million tonnes during 2016 in an area of 1.56 million ha with productivity of 15.5t ha⁻¹ (Anonymous, 2016).

In India it is grown in an area of 0.23 million ha with a production of 0.66 million tonnes and productivity 2.81 t ha⁻¹ (Anonymous 2016). It is the premier green legume crop of Jammu and Kashmir where its cultivation is mainly confined to rainfed and Karewa areas covering an area of about 26.75 thousand ha with an annual production of 14.2 thousand metric tonnes. As vegetable crop, common bean is grown in Kashmir over an area of 2000 ha with an annual production of 400 metric tonnes (Bhat *et al.*, 2017). The common bean is a warm season legume that does better under tropical, subtropical and temperate conditions and under wet conditions suffers from fungal attacks (Wortmann 2006).

A number of biotic and abiotic stresses like diseases, pests, soil and environmental conditions result in reduction of crop yield. Among diseases, bean rust, angular leaf spot, powdery mildew, halo blight, anthracnose, white mold and Fusarium wilt of bean are prevalent (Junior *et al.*, 2001). Fusarium wilt is one of the most important economic diseases of common beans world over (Okungbowa and Shittu, 2014). In China, Fusarium wilt has caused significant economic losses in common bean production due to increased land pressure to grow crops in monoculture (Xue *et al.*, 2015). The Fusarium

wilt was first identified on common bean in the USA in 1929 (Harter 1929). It is ubiquitous fungal pathogen causing wilt diseases in a broad range of important crop species such as cotton, banana, tomato, flowers and legumes (Harter 1929). The pathogen can perpetuate in soil for pretty long periods of time and continued cropping of beans result in build-up of fungal pathogen population in soil. Being a seed-borne pathogen, there occurs distant dissemination of the disease (Kendrick and Snyder, 1942).

The mode of infection of pathogen takes place through penetration of plant root tissue, subsequent colonization of the vascular tissues, stem causing xylem blockage, internal stem discoloration and total plant wilt. The main symptoms of beans infected with pathogenic isolates of *Fusarium oxysporum* f. sp. *phaseoli* are severe developmental alterations like stunting and complete wilting along with extensive chlorosis and necrosis leading to plant death (Harter, 1929).

The pathogen has been detected in most of the bean-growing regions of the world. Fusarium wilt is also a problem in many other intensively cropped areas which witness high rainfall or rains become more frequent early in the season with climate change and weather variability. High moisture, excessive irrigation or poorly drained fields and a lack of crop rotation are prone predisposing factors for the disease (Harter 1929). The pathogen is soil-borne, introduced through infected seeds or contaminated farm implements. The primary symptom is characterized by discoloration of the vascular tissues, loss of turgidity and leaf chlorosis. This is occasionally followed by leaf abscission and/or plant death and manifested on one side of host plant (Schwartz *et al.*, 2005). Infection occurs at seedling stage, impair development and result in stunted growth of plants particularly if infected at a younger age. Fusarium wilt

usually appear on medium-aged or older plants and appear as yellowing and wilting of lower leaves that progress until plant turns yellow and are readily observed in the field (Toledo-souza *et al.*, 2012). Optimum temperature for disease development is 20°C. The roots and stem show discoloration of vascular system that is a diagnostic symptom for Fusarium wilt and after cutting the lower stem a red-brown streak in vascular tissues is observed. The discoloration is usually noticed in plants mostly in lower stem and Optimum temperature for disease development is 20°C. The roots and stem show discoloration of vascular system that is a diagnostic symptom for Fusarium wilt and after cutting the lower stem a red-brown streak in vascular tissues is observed. The discoloration is usually noticed in plants mostly in lower stem and petiole nodes. Moreover, the roots are partially or totally reddish-brown in color (Kendrick and Snyder, 1942).

Materials and Methods

Biocontrol agents *viz.*, *T. harzianum*, *T. viride*, *Aspergillus* spp. and *Trichothecium* spp. were screened for their efficacy against *A. helianthi*, the causative agent of leaf blight of sunflower and *F. oxysporum* f. sp. *phaseoli*, the causative agent of vascular wilt of beans in the Division of Plant Pathology, SKUAST, Kashmir. The bioassay was done by the dual culture technique given by Morton and Stroube (1955). Seven day old culture discs of 5 mm diameter each of fungal antagonist and the pathogen were placed at equidistance from the margin of Petri plates (90 mm) containing Potato Dextrose Agar medium on opposite sides. The plates were incubated for 7 days at 25±2°C in the BOD incubator after inoculation of the pathogen (Wang *et al.*, 2006). A control having only test pathogen was also kept side by side for comparison. Each treatment was replicated thrice. Observations on linear growth (cm) of the test organism up to the

inhibition zone were recorded after fourth and seventh day and the per cent growth inhibition of test pathogen over control was calculated. Radial growth of the mycelium in Petri plates was measured in two directions at right angles to each other and average of two was expressed as diameter of colony (Lilly *et al.*, 1951). In case of wavy, irregular growth of colony average of the largest and shortest diameter was taken as the growth diameter (Brown, 1923). The radial growth of mycelium was recorded in each treatment after the pathogen covered the media in control and per cent inhibition over control was calculated using the formula (Vincent, 1947).

$$\text{Per cent growth inhibition} = \frac{C-T}{C} \times 100$$

Where,

C= Colony diameter of pathogen in control.

T= Colony diameter of pathogen in treatment.

Results and Discussion

Four bio agents *viz.*, *Trichoderma harzianum*, *T. viride*, *Aspergillus* spp. and *Tricothecium* spp. were evaluated by dual culture technique against *Alternaria helianthi*. The data revealed that all bioagents inhibited the growth of *Alternaria helianthi* varied from 35.71 per cent to 100 per cent (Table 1). Minimum colony diameter (0 cm) and maximum inhibition per cent over control (100 %) were observed after fourth day in case of *T. harzianum* and *T. viride* which were found statistically at par with each other while *Aspergillus* spp. showed 33 per cent inhibition (2 cm diameter) and *Tricothecium* spp. 23.33 per cent inhibition (2.3 cm diameter). After seven days the results were more or less similar to the results of fourth day. The minimum colony diameter (0 cm) of pathogens was observed in case of *T. harzianum* and *T. viride* with the inhibition

percentage of 100 per cent over control which were observed statistically different from *Aspergillus* spp. and *Tricothecium* spp. The data also revealed that *T. harzianum* and *T. viride* were actively inhibiting the growth of pathogen in dual culture up to seven days and also the inhibition was more or less same to the inhibition after four days. *Tricothecium* spp. was least effective biological agent against the target pathogen with 2.3 cm diameter and 23.33 per cent inhibition after fourth day and with 4.5 cm diameter and 35.71 per cent inhibition after seventh day over control (Plate 1). This may due to the slow growth of biocontrol agent. The present findings are in accordance with other workers like Vijayalakshmi *et al.*, (2018).

The data given from Table 2 reveals that all bioagents significantly inhibited the growth of *F. oxysporum*, varied from 66.66 to 86.66 per cent. Minimum colony diameter (1.6 cm) and maximum inhibition per cent (77.14 %) over control was observed after fourth day in case of *T. harzianum*, 76.28 per cent inhibition (1.7 cm diameter) in case of *T. viride*, 62.8 per cent inhibition (2.6 cm) with *Aspergillus* spp. and 50% inhibition (3.5cm) with *Tricothecium* spp. After seven days, the results were more or less similar to the results of fourth day. The minimum colony diameter (1.2 cm) of pathogen was observed in case of *T. harzianum* with the inhibition per cent of 86.66 over control which were statistically significant than *T. viride* *Aspergillus* spp. and *Tricothecium* spp. The data also revealed that *T. harzianum* and *T. viride* were actively inhibiting the growth of pathogen in dual culture up to seven days and also the inhibition was more or less same to the inhibition after four days. *Tricothecium* spp. was least effective biological agent against the target pathogen with 3.5 cm diameter and 50 % inhibition after fourth day and with 3.5cm diameter and 66.66% inhibition after seventh day over control (Plate 2).

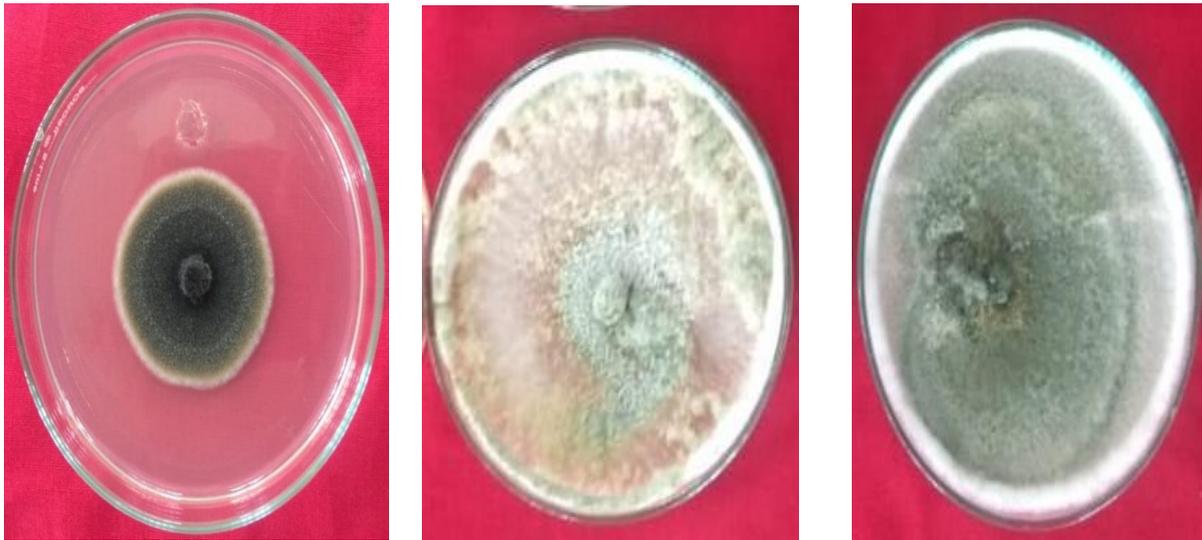
Table.1 *In-Vitro* evaluation of Bio-control Agents against *A. helianthi*

Treatments	After 4 Days		After 7 Days	
	Colony Diameter (cm)	% Inhibition over control	Colony Diameter (cm)	% Inhibition over control
<i>T. harzianum</i>	0	100	0	100
<i>T. viride</i>	0	100	0	100
<i>Aspergillus spp.</i>	2.0	33	4.0	42.85
<i>Tricothecium spp.</i>	2.3	23.33	4.5	35.71
Control	3	----	7.0	----
CD	0.23	-----	0.48	---
SE(d)	0.11	----	0.22	---
SE(m)	0.08	----	0.15	----
CV	8.19	----	8.32	----

Table.2 *In-vitro* evaluation of bio-control agents against *F. oxysporum* f. sp. *phaseoli*

Treatments	After 4 Days		After 7 Days	
	Colony Diameter (cm)	% Inhibition over control	Colony Diameter (cm)	% Inhibition over control
<i>Trichoderma harzianum</i>	1.6	77.14	1.2	86.66
<i>Trichoderma viride</i>	1.7	76.28	1.33	85.22
<i>Aspergillus</i>	2.6	62.85	2.0	77.77
<i>Tricothecium</i>	3.5	50.00	3.0	66.66
Control	7.0	----	9.0	-----
CD	0.45	----	0.51	----
SE(d)	0.19	----	0.22	----
SE(m)	0.14	----	0.15	----
CV	7.29	----	8.32	----

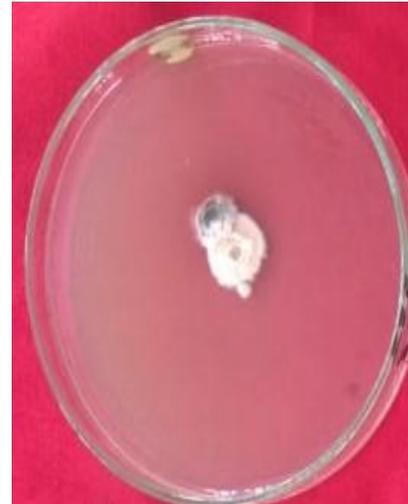
Plate.1 *In-vitro* evaluation of Bio-control agents against *A. helianthi*



A

B

C

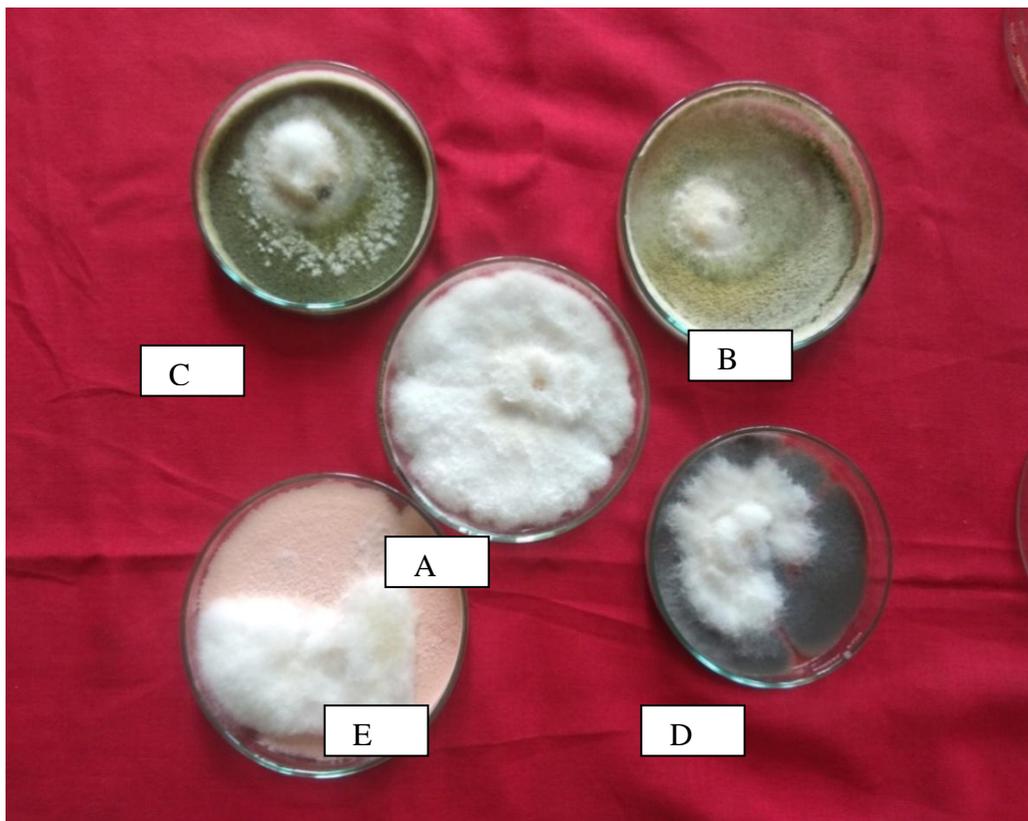


D

E

A) Control (Pathogen) B) *T. viride*. C) *T.harzianum*. D) *Aspergillus* spp. E) *Tricothecium* spp.

Plate.2 In-vitro evaluation of bio-control agents against *F. oxysporum* f. sp. *phaseoli*



A) Control (Pathogen) B) *T. viride* C) *T. harzianum* D) *Aspergillus* spp. E) *Tricothecium* spp.

This may be due to the slow growth of the biocontrol agent. The present findings are in accordance with other workers like Carvalho *et al.*, (2014, 2015).

The present investigation clearly reveals that the use of microbial inoculants offers a practical and alternative method for the management of *Alternaria* leaf blight in sunflower and vascular wilt of beans.

Use of *Trichoderma* spp. as a biocontrol agent is found most significant in controlling *Alternaria* leaf blight in sunflower as well as vascular wilt of beans as compared to the rest of the bioagents. Use of bioagents is therefore strongly recommended to control these diseases, keeping in view their eco-friendly nature and long-lasting effect on plants compared to chemical pesticides.

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