Int.J.Curr.Microbiol.App.Sci (2021) *10*(05): 672-679

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 05 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1005.075

Efficacy of Bio - Agents and Botanical Extracts against Anthracnose (*Colletotrichum lindemuthianum*) of Black Gram (*Vigna mungo* L.)

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Keywords

Bio-agents, carbendazim 50WP, Colletotrichum lindemuthianum, plant extracts

Article Info

Accepted:
22 April 2021
Available Online:
10 May 2021

A B S T R A C T

An experiment was conducted during *Kharif* season of 2017-2018 at the Central Research Farm of Sam Higginbottom University of Agriculture Technology and Sciences, to evaluate the efficacy of bio-agents and botanical extracts against anthracnose (*Colletotrichum lindemuthianum*) of black gram (*Vigna mungo* L.) both *in vitro* and *in vivo*. *In vitro* studies showed significant difference in the inhibition per cent of mycelial growth of the pathogen among all the treatments. Among the bio-agents, *Trichoderma harzianum* was found most effective and recorded 5.33 mm mean colony diameter and recorded significantly highest growth inhibition (93.59 %) of the test pathogen as compared to carbendazim (treated check) and untreated control which was followed by *Psuedomonas fluorescens* with 61.92 per cent growth inhibition. Among the plant extracts, datura leaf extract @ 5% showed the minimum percent of inhibition (10.22 %) of the pathogen followed by neem leaf extract @ 5% (20.84). In field evaluation, a significant difference in the percent disease intensity was observed among all the treatments, the minimum disease intensity (%) was recorded in *Trichoderma harzianum* @ 8g (23.53%), as compared to carbendazim (treated check) and untreated control respectively, followed by *Trichoderma harzianum* @ 6g (24.68 %), *Trichoderma harzianum* @ 4g (28.76 %), *Psuedomonas fluorescens* (29.21 %) and neem leaf extract @ 5% (33.9 %) and maximum disease intensity (%) was recorded in datura leaf extract @ 5% (35.97).

Introduction

Anthracnose of black gram (*Vigna mungo* L.) caused by *Colletotrichum lindemuthianum*, is a serious disease in almost all green gram growing areas. In India, the black gram anthracnose was first reported from Uttar Pradesh state in 1984 (Saxena, 1984). Anthracnose diseases cause an estimated yield losses of 24 to 67 per cent. The per cent viability of conidia of *Colletotrichum lindemuthianum* in crop debris was significantly affected by duration of storage as well as different storage conditions. The conidia survival for a maximum of 360 days under freeze (4-50C) conditions and least

survivability of 90 days under field condition (28-300C) (Kulakarni and Benagi, 2012). The disease is soil as well as seed-borne. The fungus surviving as conidia and dormant mycelium. The conidia are hyaline, unicellular, falcate or lunette (sickle shaped) or cylindrical, more or less guttulate, muticate or with the apex prolonged into a simple cellular appendage, produced from phialides (conidiogenous cells) enteroblastically (Agrios, 2006).

Biological control is an alternative to the management of diseases caused by soil-borne microorganisms (Zavaleta, 2000). Botanicals are biodegradable and their use in crop protection is a practical sustainable alternative (Delvin and Zettel, 1999). It reduces environmental contamination and health hazards (Grange and Ahmed, 1988).

Materials and Methods

Isolation of pathogen

Black gram leaves with symptoms of anthracnose lesions were collected from field. The section of 4-5 mm was cut from the margin of the infected lesions and sterilized for one minute in 0.1% mercuric chloride solution and rinsed with several changes of Sterile Distilled Water. The sterile pieces were blotted on sterilized Petri plates containing solidified Potato Dextrose Agar (PDA) in aseptic conditions. The plates were incubated at ambient temperature (30±2°C) for 7 days after incubation according to the Nduagu *et al.,* 2008.The tip of hyphal growth radiating from the infected tissue was transferred onto PDA. Culture was confirmed by microscopic examination and comparison with reference cultures (Boxter *et al.*, 1983).

Dilution plate technique was employed for the isolation of biocontrol agents i.e. *Trichoderma harzianum* on selective medium (TSM) (Elad *et al.,* 2011), whereas *Pseudomonas fluorescens* was isolated on King's B medium. (Rini and Sulochana, 2007). Isolated anatagonists were screened against *Colletotrichum lindemuthianum* using dual culture technique. For dual culture, fungal antagonist and *Colletotrichum lindemuthianum* on potato dextrose agar media were used. Whereas for bacterial antagonist King's B medium was employed for all above mentioned pathogens. Fifteen ml of medium was poured in sterilized Petriplates and allowed to settle for 15-20 minutes. Five mm disc of pathogen was placed at one end of Petridish and fungal antagonist was placed opposite to this. For bacterial antagonist, they were streaked at the other end of Petridish over the medium. A control seeded only with pathogen was also maintained. Antagonistic activity was tested 4 days after incubation by measuring the radius of the, *Colletotrichum lindemuthianum* colony in the direction of the antagonist colony and the radius of the, *Colletotrichum lindemuthianum* colony in the control plate. The two readings were transformed into percentage inhibition of radial growth (PIRG) using the formula developed by Vincent (1947).

$$PIRG=R_1-R_2/R_1 \text{ X } 100$$

Where,

R_1, Radius of *Colletotrichum lindemuthianum* colony in control plate;

R_2, Radius of *Colletotrichum lindemuthianum* colony in dual culture plate

The plant extracts @ 5 % *viz.* Neem leaf extract and Dhatura leaf extract were evaluated *in-vitro* against *Colletotrichum lindemuthianum* using Poisoned Food Technique and Potato Dextrose Agar according to (Nene and Tapliyal, 1993). Twenty ml of medium with desired

concentration of plant extracts was poured in each sterilized petri plate. Suitable checks were kept for comparison. Five mm mycelial disc of *C. lindemuthianum* was taken from periphery of ten days old culture and sclerotia taken from one month culture was placed at centre of the separate plates and All the treatment (inoculated) and control petri plates where then incubated at 24 ± 2°C in BOD incubator till the control plates were fully covered with mycelial growth of the test pathogen. Observations on radial mycelial growth of *C. lindemuthianum* where recorded in each treatment and per cent growth inhibition of the test pathogen over control was worked out (Vincent, 1927) as follows.

$$I = \frac{(C - T)}{C} \times 100$$

Where,

C = Radial growth in control.

T= Radial growth in treatment.

***In – vivo* experiment**

Field preparation

An experiment was conducted during *Kharif* season of 2017 at the central research farm of Sam Higginbottom Institute of Agriculture Technology and Sciences. The selected field area was prepared by ploughed and harrowing during summer season, Experimental plots were laid out as per statistical Randomized Block design. Total area was divided into 21 plots and plot size 2 x 1 m^2. To evaluate the efficacy of plant extracts against *Colletotrichum lindemuthianum* with Randomized Block Design in three replications. The crop was raised as per recommended package of practices and protective irrigation was given as and when required. The treatments *viz. Trichoderma harzianum* @ 4 g, *Trichoderma harzianum* @ 6 g, *Trichoderma harzianum* @ 8 g, *Psuedomonas fluorescens* @ 0.6 %, Neem leaf extract 5 %, Dhatura leaf extract 5 %, and treated (carbendazim 50 WP (@ 0.1%), were evaluated under field condition.

Three sprays of all the treatments were undertaken at intervals of 15 days, starting first spraying at 30 days after sowing of the crops. One plot/ replication was maintained as unsprayed control without receiving any treatments. Observations on foliage anthracnose disease were recorded after each sprayings and last observation on anthracnose were recorded at 15 days after last spraying. Disease severity of leaves was determined by the diagrammatic keys according to the scale described by (Singh, 2006) per cent of the surface of the leaf affected by anthracnose. The infection on leaves were graded in 0-9 scale on the basis of severity of infection on leaves.

The used scale was: 0 = no affected; 1 = 1% leaf area affected; 3 = 2-10% leaf area affected; 5 = 11-25% leaf area affected; 7 = 26-50 % leaf area affected; and 9 = 50 % > leaf areas affected. Five plants per treatment per replication were selected randomly and tagged for recording the observations. Three trifoliate leaves (bottom, middle and top) from main branch on each observation plant were selected for recording observations and per cent anthracnose disease intensity was worked out as detailed under table no 2.

Per cent disease intensity/index was calculated by applying following formula,

$$\text{Disease intensity (\%)} = \frac{\text{sum of all disease ratings}}{\text{Total no. of leaves per plant} \times \text{maximum disease grade}} \times 100$$

Results and Discussion

The data presented on % inhibition of mycelial growth as influenced by treatments are given in table 1. The maximum inhibition was observed in *Trichoderma harzianum* (T_1 - 93.59 %) as compared to the treated carbendazim (T_5 - 100 %) and untreated check, followed by *Psuedomonas fluorescens* (T_4-61.92 %), Neem leaf extract (T_7 - 20.84 %) and Datura leaf extract (T_6 - 10.22 %) showed the least per cent of inhibition. All the treatments are statiscally significant and over the control also. Similar findings have been reported by Shovan *et al.*, (2008) and Dey *et al.*, (2013) they reported that among the bio-agents *Trichoderma harzianum* showed the highest per cent of inhibition followed by *Psuedomonas fluorescens*. Marinus *et al.*, (2010) found that among the botanicals, Neem leaf extract showed the highest per cent of inhibition followed by Datura leaf extract. The same results are corroborated by Dey *et al.*, (2013).

The data pertaining to influence of bio-agents and plant extracts on anthracnose disease development are given in table 2. The data indicated that all the treatments were significantly superior over control.

Table.1 Per cent inhibition of radial mycelial growth of *Colletotrichum lindemuthianum* as affected by treatments

Treatments	Radial mycelial growth of the pathogen (mm)	Per cent inhibition
Trichoderma harzianum	5.33	93.59
***Psuedomonas fluorescens* @ 10 g + ST**	31.67	61.92
Carbendazim @0.1 % ST + FS	0.00	100.00
Datura leaf extract @ 5 % + FS	74.67	10.22
Neem leaf extract @ 5 % + FS	65.83	20.84
Control	83.17	0.00
F-test	S	
S.Ed(±)	0.892	
CD (5%)	2.750	

*ST = Seed treatment, FS = Foliar spray

Table.2 Field efficacy of treatments on the intensity of anthracnose on black gram at 30, 45 and 60 days after sowing

S/No.	Treatments	Disease intensity		
		30DAS	45DAS	60DAS
T_1	*Trichoderma harzianum* @ 4g + ST	17.370^{bc}	24.343^{bc}	28.776^{b}
T_2	*Trichoderma harzianum* @ 6g + ST	16.563^{bcd}	23.290^{bcd}	24.680^{c}
T_3	*Trichoderma harzianum* @ 8g + ST	15.206^{cd}	21.226^{cd}	23.153^{c}
T_4	*Psuedomonas fluorescens* @ 10g + ST	18.723^{b}	25.300^{bc}	29.210^{b}
T_5	Carbendazim @ 0.1 % ST+ FS	14.376^{d}	18.926^{d}	22.343^{c}
T_6	Datura leaf extract @ 5 %+ FS	24.870^{a}	30.093^{a}	35.976^{a}
T_7	Neem leaf extract @ 5 % FS	22.326^{a}	27.003^{ab}	33.900^{a}
T_0	Control	28.133	36.563	43.523
F-test		**S**	**S**	**S**
S.Ed(±)		0.839	1.465	1.060
CD (5%)		2.516	4.392	3.177

*ST = Seed treatment, FS = Foliar spray

Table.3 Field efficacy of treatments on yield of black gram at the time of maturity

S/No.	Treatments	Yield (qt/ha)
T_1	*Trichoderma harzianum* @ 4g +ST	6.25[bc]
T_2	*Trichoderma harzianum* @ 6g +ST	6.38[b]
T_3	*Trichoderma harzianum* @ 8g +ST	6.76[a]
T_4	*Psuedomonas fluorescens* @ 10g +ST	6.20[c]
T_5	Carbendazim @ 0.1 % ST+FS	7.86
T_6	Datura leaf extract @ 5 %+FS	4.98[e]
T_7	Neem leaf extract @ 5 %FS	5.48[d]
T_0	Control	3.15[f]
Over all mean		5.86
F-test		**S**
S.Ed(±)		0.056
CD (5 %)		0.168

*ST = Seed treatment, FS = Foliar spray

Among all the treatments, the minimum disease intensity was recorded in *Trichoderma harzianum* @ 8g, followed by *Trichoderma harzianum* @ 6g, *Trichoderma harzianum* @ 4g, *Psuedomonas fluorescens,* Neem leaf extract and maximum disease intensity was observed in T_6 - Datura leaf extract as compared to treated Carbendazim and untreated control. The data on yield of black gram is presented in table 4. The perusal of the data indicated that all the treatments were significantly superior over control.

Among all the treatments, the maximum yield of black gram was recorded in T_3 - *Trichoderma harzianum* @ 8 g as compared to treated Carbendazim and untreated control.T_3 - *Trichoderma harzianum* @ 8 g, was followed by *Trichoderma harzianum* @ 6 g, *Trichoderma harzianum* @ 4 g, *Psuedomonas fluorescens*, Neem leaf extract and Datura leaf extract was least effective among all the treatments.

It was observed that *Trichoderma harzianum* @ 8 g gave the maximum yield (6.76 q/h) followed by *Trichoderma harzianum* @ 6 g (6.38 q/h). Similar findings were made by the Kendre *et al.,* (2017). It was also supported by Amin *et al.,* (2014).

Based on the result it was observed that *Trichoderma harzianum* proved to be most effective against anthracnose under field and *in vitro* compared to treated, Carbendazim 50WP and untreated control. Among the bio-agents *Trichoderma harzianum* found to be most effective and among the botanicals Neem leaf extract showed the best results in both *in vitro* and *in vivo*. There is a limitation in this experiment that, the trial was conducted only for one season.

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How to cite this article:

Badam Sushmitha and Sunil Zacharia. 2021. Efficacy of Bio - Agents and Botanical Extracts against Anthracnose (*Colletotrichum lindemuthianum*) of Black Gram (*Vigna mungo* L.). *Int.J.Curr.Microbiol.App.Sci.* 10(05): 672-679. doi: https://doi.org/10.20546/ijcmas.2021.1005.075