

Evaluation of fungal antagonist *Trichoderma* species against *Macrophomina phaseolina* in Black gram

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ABSTRACT

India is the world's largest producer and consumer of black gram. India produces about 1.5 to 1.9 million tones of black gram annually from about 3.5 million hectares of area, with an average productivity of 500 kg per hectare. Black gram output accounts for about 10 per cent of India's total pulse production. Root rot caused by *Macrophomina phaseolina* (Tassi) Goid is one of the most important fungal diseases of Black gram. It inflicts serious economic loss to the crop. It was reported to result in a loss of 28.6 per cent in black gram yield. *Trichoderma* species are effective biocontrol agents for several soil borne fungal plant pathogens including *M. phaseolina* and some species are also known for their to enhance systemic resistance to plant disease. Soil application of *T. viride* significantly controlled the blackgram root rot caused by *M. phaseolina* by means of several antagonistic mechanisms such as nutrient composition, antibiotic production and mycoparasitism. Results showed in vitro efficiency of antagonist inhibited the mycelial growth. *T. viride* (Tv₃) recorded the maximum inhibition zone (73.74%), followed by *T. viride* (Tv₁) which recorded 71.74 per cent inhibition on the growth of pathogen over control. The isolate *T. viride* (Tv₅) recorded the minimum inhibition (69.37%).

Keywords: Root rot, *Macrophomina phaseolina*, Trichoderma, Root rot.

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INTRODUCTION

Vigna mungo commonly referred to as urd bean, black gram, black lentil or white lentil is a bean grown in southern Asia. Black gram is very nutritious as it contains high levels of proteins, potassium, calcium, iron, niacin (B3), thiamine (B1) and Riboflavin (B2). India is the world's largest producer and consumer of black gram. India produces about 1.5 to 1.9 million tonnes of black gram annually from about 3.5 million hectares of area, with an average productivity of 500 kg per hectare. Blackgram output accounts for about 10 per cent of India's total pulse production. Root rot caused by *Macrophomina phaseolina* (Tassi) Goid is one of the most important fungal diseases of Black gram. It inflicts serious economic loss to the crop. It was reported to result in a loss of 28.6 per cent in black gram yield. It is an important disease of broad range

of crops (Srivastava *et al.*, 2001) particularly in regions with warm and dry weather conditions. *M. phaseolina* is reported to produce charcoal rot disease over 500 species of plants (Sinclair, 1982). *M. Phaseolina* is primarily soil borne in nature, with heterogeneous host specificity *i.e.*, the ability to infect monocots as well as dicots and non-uniform distribution in the soil (Mayek-Perez *et al.*, 2001). Biological control of soil borne plant pathogen by addition of antagonistic microorganism to the soil is a potential non-chemical means and is known to be a cheapest and effective method for the management of soil diseases. Biological control is eco-friendly, does not leave any residual toxicity, besides being cost effective and can be successfully exploited in the framework of integrated disease management. *Trichoderma*, a saprophytic fungus is known to be one of the best candidates of biocontrol agents (Scala *et*

al., 2007). *Trichoderma* species are effective biocontrol agents for several soil borne fungal plant pathogens including *M. phaseolina* and some species are also known for their ability to enhance systemic resistance to plant disease as well as overall plant growth. Sundravadana (2002) reported that the seed and soil application of *T. viride* significantly controlled the blackgram root rot caused by *M. phaseolina*. The biocontrol exercised by *Trichoderma* can occur by means of several antagonistic mechanisms such as nutrient composition, antibiotic production and mycoparasitism.

MATERIALS AND METHODS

Survey on the root rot incidence

A field survey was conducted to assess the extent of root rot occurrence of blackgram in Thiruvannamalai district of Tamil Nadu state. Ten locations representing irrigated and rainfed situations were selected for the study. The per cent disease index was worked out according to "Phytopathometry" by Mayee and Datar (1986) as mentioned below:

$$\text{Per cent Disease Incidence (PDI)} = \frac{\text{No. of diseased plants}}{\text{No. of plants observed}} \times 100$$

Also, the infected plants showing the typical symptoms of root rot due to infection with *M. phaseolina* were collected along with rhizosphere soil for isolation of the pathogen. Additional information regarding the soil type in which the crop is grown and the variety of blackgram cultivated were also recorded in the respective survey fields.

Isolation of the pathogen

The pathogen *Macrophomina phaseolina* (Tassi) Goid. was isolated from the diseased roots of blackgram plants showing the typical root rot symptoms by tissue segment method on potato dextrose agar (PDA) medium. The axenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) and these were maintained on PDA slants for subsequent experiments.

Isolation of native antagonistic fungi

Blackgram rhizosphere soil samples collected from five different locations were used for the isolation of *Trichoderma* isolates by serial

dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). These *Trichoderma* cultures were purified by single hyphal tip method and were used for the studies. Micrometric measurements of conidia and phialides were done by mounting the four day old culture stained with lactophenol cotton blue and were observed under high power of research microscope.

Antagonists on mycelial growth

The antagonistic activity of bio control agents (Tv₁-Tv₅ and Pf₁-Pf₂) against *M. phaseolina* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile Petri dish containing 15 ml of sterilized and solidified PDA medium a 9 mm mycelial disc obtained from five-day old culture of *Trichoderma viride* was placed under aseptic conditions. Similarly, at the opposite end approximately 75 mm away from the *Trichoderma viride* culture disc, a 9 mm culture disc of *M. phaseolina* was placed and incubated. A control was maintained by inoculating *M. phaseolina* alone at one end of the Petri dish. The plates were incubated at room temperature (28 ± 2°C) for three days. The radial growth (mm) of the pathogen and the test antagonists and the extent of the inhibition zones (mm) developed between the two colonies were measured. The effective antagonists were identified based on the inhibition of the growth of the pathogen. The radial mycelial growth of the pathogen and per cent reduction over control was calculated by using the formula (Vincent, 1927):

$$\text{Per cent inhibition (I)} = \frac{C-T}{C} \times 100$$

Where,

C- mycelial growth of pathogen in control (mm)

T- Mycelial growth of pathogen in dual plate (mm)

RESULTS AND DISCUSSION

Survey on the dry root rot incidence of blackgram

The data presented in Table 1 of the survey in different locations of Thiruvannamalai district Revealed endemic nature of the root rot disease incidence. Among the different

Table 1. Survey on the incidence of blackgram root rot disease in Thiruvannamalai district of Tamil Nadu

Isolate No.	Village	Soil type	Variety	Situation	Root rot incidence (%)
MP ₁	Padavedu	Clay loam	VBN5	Irrigated	19.95 ^e
MP ₂	Palakollai	Clay loam	CO6	Rain fed	18.19 ^f
MP ₃	Murugapadi	Sandy loam	VBN 7	Rain fed	22.29 ^d
MP ₄	Pudhur	Clay loam	VBN 4	Irrigated	17.19 ^g
MP ₅	Ananthapuram	Sandy loam	VBN 5	Irrigated	23.82 ^e
MP ₆	Kalpattu	Clay loam	ADT 5	Irrigated	15.35 ^h
MP ₇	Arriyapadi	Sandy loam	VBN 4	Irrigated	25.95 ^b
MP ₈	Samanthipuram	Red sandy	VBN 5	Rain fed	13.49 ⁱ
MP ₉	Kailasapuram	Sandy loam	TVM1	Rain fed	28.28 ^a
MP ₁₀	Vazhiur	Red sandy	VBN 5	Rain fed	12.89 ^j

locations of surveyed for blackgram root rot incidence, Kailasapuram registered the imum incidence of the disease followed by Arriyapadi with, Ananthapuram with and the minimum root rot incidence was recorded in Vazhiur.

The survey conducted to assess the root rot incidence of blackgram in the major blackgram growing areas in Thiruvannamalai district of Tamil Nadu revealed the endemic nature of the disease with root rot incidence ranging from 12.89 to 28.28 per cent (Table 1). The variation in the extent of the disease incidence observed in the present study might be due to the prevalence of the isolates of the pathogen differing in their virulence. Soil texture also had a significant impact on root rot infections. In the present survey, severe root rot disease incidence was observed in sandy loam as compared to red sandy or clay loam. Similar to the present results, Cruz Jimenez (2011) observed highest *M. phaseolina* root populations in sandy soils, followed by seedlings planted in loamy sand and loam soil

textures. Likewise, increased populations of *M. phaseolina* and root rot severity of soybean, sorghum and mungbean in sandy soils was also reported (Dhingra and Sinclair, 1973; Collins *et al.*, 1991; Hooda and Grover, 1990). Higher incidence of the disease in sandy soils might be attributed to the less competitive saprophytic ability (CSA) of the pathogen at high moisture holding capacity (MHC) associated with heavy soils like clay (Umamaheswari, 1991) and reduction in the germination of sclerotia of *M. phaseolina* at high MHC (Ali and Ghaffar, 1991).

Mycelial growth of *M. phaseolina* isolates

All the ten isolates of the root rot pathogen *M. phaseolina* produced white, whitish grey, grey, and black scanty to profuse aerial mycelial growth on Potato Dextrose Agar (PDA) medium. The isolates MP₁₀ recorded the maximum (90 mm) mycelial growth on 5 days after inoculation while it was the minimum in the case of MP₁₀. This was followed by MP₇, MP₃, MP₅, and MP₁ in the decreasing order of merit (Table 2).

Table 2. Isolation of *M. phaseolina* from different places of Thiruvannamalai district

Isolate number	Location	Variety
MP ₁	Padavedu	VBN5
MP ₂	Paiiakollai	CO6
MP ₃	Murugapadi	VBN 7
MP ₄	Pudhur	VBN 4
MP ₅	Ananthapuram	VBN 5
MP ₆	Kalpattu	ADT 5
MP ₇	Arriyapadi	VBN 4
MP ₈	Samanthipuram	VBN 5
MP ₉	Kailasapuram	TVM1
MP ₁₀	Vazhiur	VBN 5

All the ten isolates of the root rot pathogen *M. phaseolina* produced white, whitish grey, grey, black scanty to profusely arial mycelial growth on PDA. The isolate MP₉ significantly recorded the maximum mycelial growth, while it was the minimum in the case of MP₁₀ (Table 3). Similar such variation in the cultural characteristics of *M. phaseolina* on PDA was reported by Tandel *et al.* (2012). Also, several earlier workers have reported about the variations in the mycelial growth among the isolates of *M. phaseolina* (Edraki and Banihashemi, 2010; Ijaz *et al.*, 2012; Mohanapriya *et al.*, 2017). Further, it was observed that the isolates of *M. phaseolina* with faster mycelial growth were more pathogenic and produced higher root rot incidence. The virulence of the isolates of *M. phaseolina* was positively correlated with their growth rate. Sharmishha *et al.* (2004) reported that the isolates of *M. phaseolina* with faster mycelial growth were found to be more pathogenic to cluster beans. These earlier reports corroborate with the present findings.

Efficacy of native fungal biocontrol agents

In general all the native *Trichoderma viride* isolates (Tv₁-Tv₅) tested significantly inhibited the mycelial growth of *M. phaseolina*. However, among the isolates, the isolate Tv₃ showed the maximum inhibition and significantly inhibited the growth of *M. phaseolina*, which was 73.74 per cent reduction on the growth of the pathogen when compared to control. This was followed by the isolates Tv₁ and Tv₅ in the decreasing order of merit, which inhibited the growth of

M. phaseolina by 71.74 and 69.37 per cent over control. The standard isolate used for comparison recorded 66.41 per cent reduction on the growth of the pathogen over control. The least growth inhibition of the pathogen was exhibited by the isolate Tv₅ (Table 3).

Table 3. Screening of native *T. viride* isolates against *Macrophomina phaseolina* by Dual Culture Technique

Isolate number	Mycelial growth of <i>M. phaseolina</i> (mm)	Percent (%)inhibiti on over control
<i>T. viride</i> (Tv ₁)	27.56 ^c	71.74
<i>T. viride</i> (Tv ₂)	29.39 ^d	67.34
<i>T. viride</i> (Tv ₃)	23.63 ^a	73.74
<i>T. viride</i> (Tv ₄)	32.72 ^f	63.66
<i>T. viride</i> (Tv ₅)	25.43 ^b	69.37
<i>T. viride</i> (Comparison Isolate)	30.23 ^e	66.41
Control	90.00 ^g	0.00

All the native isolates of *Trichoderma viride* significantly inhibited the mycelial growth of *M. phaseolina* in dual culture. However, the isolate Tv₃ of *T. viride* significantly inhibited the growth of *M. phaseolina* to the tune of 73.74 per cent. The least growth inhibition of the pathogen (63.66%) was exhibited by the isolate Tv₄. The results of the present study correspond with those of Sreedevi *et al.* (2011) who stated that all five *Trichoderma* spp. were very effective against *M. phaseolina* in dual culture technique. Similar observations on the *in vitro* inhibitory effect of *Trichoderma* spp. against *M. phaseolina* have been made by several earlier workers (Jite, 2012; Vasebi *et al.*,



2013). All these earlier reports are in line with the present findings. *Trichoderma* spp. was reported to be a potential antagonist against *M. phaseolina* through colony interaction (Biswas and Sen, 2000). Bell *et al.* (1982) classified *Trichoderma* isolates based on their ability to overgrow the hyphae of *R. solani*. They considered an isolate of *Trichoderma* to be antagonistic to the pathogen only if it overgrew on the pathogen in the dual culture. In the present study also, *Trichoderma* isolate Tv₃ put forth copious overgrowth and sporulated on *M. phaseolina* in dual culture. Efficacy of *T. viride* against various pathogens viz., *F. oxysporum* f. sp. *ricini* (Raouf *et al.*, 2006) and *Aspergillus niger* (Gajera *et al.*, 2012) have also been reported under *in vitro*. The results of the *in vitro* studies by Elham *et al.* (2016) revealed that all three isolates of *T. harzianum* significantly inhibited the growth of *M. phaseolina*. These earlier reports lend support to the present findings. A multiplicity of mechanisms involving mycoparasitism, antibiosis, lysis and hyphal interference could be attributed to the reduction in the mycelial growth of *M. phaseolina*.

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