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Fungal antagonist against black gram root rot caused by *Macrophomina phaseolina* (Tassi) goid

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Abstract
Black gram output accounts for about 10 per cent of India's total pulse production India and it 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. Root rot caused by *Macrophomina phaseolina* (Tassi) Goid is one of the most important fungal diseases of Black gram. It inflicts series 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 abilities 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* (Tv_3) recorded the maximum inhibition zone (73.74%), followed by *T. viride* (Tv_1) which recorded 71.74 percent inhibition on the growth of pathogen over control. The isolate *T. viride* (Tv_5) recorded the minimum inhibition (69.37%).

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

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 series 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) [15] 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) [16]. 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 candidate of biocontrol agents (Scala *et al.,* 2007) [14]. *Trichoderma* species are effective biocontrol agents for several soil borne fungal plant pathogens including *M. phaseolina* and some species are also known for their abilities to enhance systemic resistance to plant disease as well as overall plant growth. Sundravadana (2002) [13] 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 of blackgram in Thiruvannamalai district
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 work out using

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. The other information's 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) [12] and these were maintained on PDA slants for subsequent experiments.

Isolation of native antagonistic fungi from rhizosphere soil

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 used for the studies. Micrometric measurements of conidia and phialides were done by mounting four days old culture stained with lactophenol cotton blue and observed under high power of research microscope.

Effect of antagonists on mycelial growth by dual culture technique

The antagonistic activity of bio control agents (Tv_1-Tv_5 and Pf_1-Pf_2) 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 9mm 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).

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

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 in Thiruvannamalai district of Tamil Nadu

The data presented in table 1 the survey in different locations of Thiruvannamalai district revealed endemic nature of the root rot disease incidence. Among the different locations of Thiruvannamalai district surveyed for blackgram root rot incidence, Kailasapuram registered the maximum incidence of the disease (28.28%) followed by Arriyapadi with (25.95%), Ananthapuram with (23.82%) and the minimum root rot incidence of (12.89%) 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) [17] 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 *Macrophomina 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 MP_{10} recorded the maximum (90 mm) mycelial growth on 5 days after inoculation while it was the minimum (76.33 mm) in the case of MP_{10}. This was followed by MP_7, MP_3, MP_5, and MP_1 in the decreasing order of merit (Table 2).

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 MP_9 significantly recorded the maximum mycelial growth, while it was the minimum in the case of MP_{10} (Table 3). Similar such variation in the cultural characteristics of *M. phaseolina* on PDA was reported by Tandel *et al.* (2012) [11]. 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) [10, 8].

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) [7] reported that the isolates of *M. phaseolina* with faster mycelial growth were found more pathogenic to cluster beans. These earlier reports corroborate with the present findings.

Efficacy of native fungal biocontrol agents against *M. phaseolina* by Dual culture technique

In general all the native *Trichoderma viride* isolates (Tv_1 – Tv_5) tested significantly inhibited the mycelial growth of *M. phaseolina*. However, among the isolates, the isolate Tv_3 showed the maximum inhibition and significantly inhibited the growth of *M. phaseolina* (23.63 mm), which was 73.74 per cent reduction on the growth of the pathogen when compared to control. This was followed by the isolates Tv_1 and Tv_5 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 (69.37%) was exhibited by the isolate Tv_5 (Table 3).

All the native isolates of *Trichoderma viride* significantly inhibited the mycelial growth of *M. phaseolina* in dual culture. However, the isolate Tv_3 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 Tv_4. The results of the present study correspond with Sreedevi *et al.* (2011) [6] 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* was made by several earlier workers (Jite, 2012; Vasebi *et al.*, 2013) [4, 5]. 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) [3]. 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 Tv_3 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* (Raoof *et al.*, 2006) [2] and *Aspergillus niger* (Gajera *et al.*, 2012) [1] have also been reported under *in vitro*. The results of the *in vitro* studies by Elham *et al*. (2016) [18] 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*.

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} (26.52)
MP_2	Palakollai	Clay loam	CO6	Rain fed	18.19^{f} (25.24)
MP_3	Murugapadi	Sandy loam	VBN 7	Rain fed	22.29^{d} (28.17)
MP_4	Pudhur	Clay loam	VBN 4	Irrigated	17.19^{g} (24.49)
MP_5	Ananthapuram	Sandy loam	VBN 5	Irrigated	23.82^{c} (29.21)
MP_6	Kalpattu	Clay loam	ADT 5	Irrigated	15.35^{h} (23.06)
MP_7	Arriyapadi	Sandy loam	VBN 4	Irrigated	25.95^{b} (30.62)
MP_8	Samanthipuram	Red sandy	VBN 5	Rain fed	13.49^{i} (21.54)
MP_9	Kailasapuram	Sandy loam	TVM1	Rain fed	28.28^{a} (32.12)
MP_{10}	Vazhiur	Red sandy	VBN 5	Rain fed	12.89^{j} (21.04)

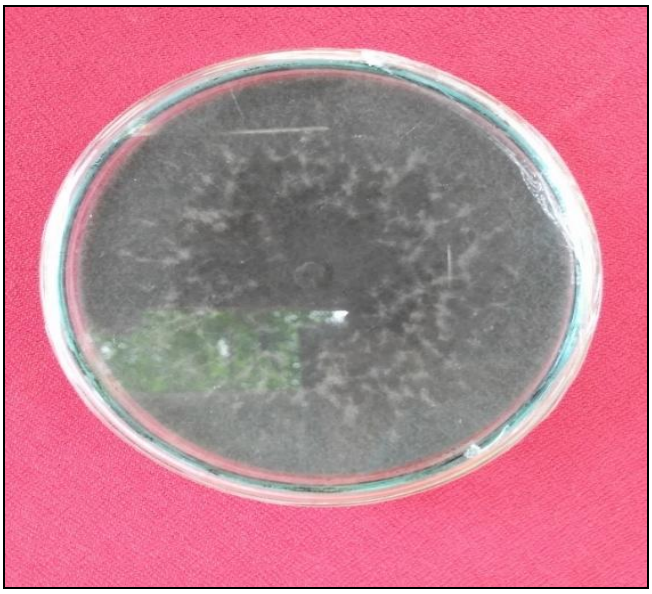
Table 2: Isolation of *M. phaseolina* from different places of Thiruvannamalai district

S. No	Isolate number	Location	Variety
1	MP_1	Padavedu	VBN5
2	MP_2	Paiiakollai	CO6
3	MP_3	Murugapadi	VBN 7
4	MP_4	Pudhur	VBN 4
5	MP_5	Ananthapuram	VBN 5
6	MP_6	Kalpattu	ADT 5
7	MP_7	Arriyapadi	VBN 4
8	MP_8	Samanthipurm	VBN 5
9	MP_9	Kailasapuram	TVM1
10	MP_{10}	Vazhiur	VBN 5

Table 3: Screening of native *T. viride* isolates against *Macrophomina phaseolina* by Dual culture technique

S. No	Isolate number	Mycelial growth of *M. phaseolina* (mm)	Per cent (%) inhibition over control
1	*T. viride* (Tv_1)	27.56^{c}	71.74
2	*T. viride* (Tv_2)	29.39^{d}	67.34
3	*T. viride* (Tv_3)	23.63^{a}	73.74
4	*T. viride* (Tv_4)	32.72^{f}	63.66
5	*T. viride* (Tv_5)	25.43^{b}	69.37
6	*T. viride* (Comparison Isolate)	30.23^{e}	66.41
7	Control	90.00^{g}	0.00

Dry root rot affected Blackgram plants



Axenic culture of *M. phaseolina*

Reference

1. Gajera HP, Bambharolia RP, Patel SV, Khatrani TJ, Goalkiya BA (Antagonism of *Trichoderma* spp. against *Macrophomina phaseolina*. Evaluation of coiling and cell wall degrading enzymatic activities. J Plant Pathol. Microb. 2012; 3:7.
2. Raoof MA, Ramabhadra R, Mehtab Y. Bio control potential and shelf life of *Trichoderma viride* for the management of castor wilt. Indian J Pl. Prot. 2006; 34(1):75-80.
3. Biswas KK, Sen C. Identification of effective isolate of *Trichoderma harzianum* (Rifai) for biocontrol of *Macrophomina phaseolina* (Tassi.) Goid. J Mycol. Pl. Pathol. 2000; 30:408-410.
4. Jite PK. *In vitro* efficacy of *Trichoderma viride* against *Sclerotium rolfsii* and *Macrophomina phaseolina*. Not. Sci. Biol. 2012; 4(4):39-44.
5. Vasebi Y, Naser Safaie, Azizollah Alizadeh. Biological control of soybean charcoal root rot disease using bacterial and fungal antagonists *in vitro* and greenhouse condition. J Crop Prot. 2013; 2(2):139-150.
6. Sreedevi B, Charitha Devi M, Saigopal DVR. Isolation and screening of effective *Trichoderma* spp. against the root rot pathogen *Macrophomina phaseolina*. J Agrl. Technol. 2011; 7(3):623-635.
7. Sharmishha P, Bhavneet Kaur, Neeraj Dilbaghi, Ashok Chaudhury. Cultural and pathogeinc variation in the charcoal root rot pathogen from cluster bean. Ann. Agri Bio Res. 2004; 9(2):217-221.
8. Mohanapriya R, Naveenkumar R, Balabaskar P. Survey, Virulence and Pathogenicity of Root Rot Incidence of Cowpea in Selected Districts of Tamilnadu caused by *Macrophomina phaseolina* (Tassi.) Goid. International Journal of Current Microbiology and Applied Sciences. 2017; 6(3):694-705.
9. Ijaz S, Sadaqat HA, Khan MN. A review of the impact of charcoal rots *Macrophomina phaseolina* on sunflower. J Agrl. Sci. 2013, 1-6.
10. Edraki V, Banihashemi Z. Phenotypic diversity among isolates of *Macrophomina phaseolina* and its relation to pathogenicity. Iran. J Plant Path. 2010; 46(4):93-100.
11. Tandel DH, Sabalpara AN, Patel RC. Evaluation of different solid and liquid media for the growth and sclerotial formation of *Macrophomina phaseolina* (Tassi) Goid *in vitro*. The Bioscan. 2012; 7(4):743-745.
12. Rangaswami G. Diseases of crop plants in India. Prentice Hall of India Pvt. Ltd., New Delhi, 1972, 520.
13. Sundravadana S. Management of blackgram [*Vigna mungo* (L.) Hepper] root rot *Macrophomina phaseolina* (Tassi) Goid with bio agents and nutrients. *M.Sc. (Ag.) Thesis*, Tamil Nadu Agricultural University, Coimbatore, India, 2002.
14. Scala F, Raio A, Zonia A, Lorito M. Biological control of fruit and vegetable diseases with fungal and bacterial antagonists: *Trichoderma* and *Agrobacterium*. In: Chincholkar SB, Mukerji KG (eds) Biological control of plant diseases. Howorth Press, Binghamton, 2007, 150-190.
15. Srivastava AK, Singh T, Jana TK, Arora DK. Microbial colonization of *Macrophomina phaseolina* and suppression of charcoal rot of chickpea. In Microbes and Plants, Sinha A (ed). Vedams eBooks (P) Ltd, New Delhi, 2001, 269-319.
16. Mayek-Perez N, Lopez-Castaneda C, Gonzalez-Chavira M, Garcia-Espinosa R, Acosta-Gallegos J, De-la-vega O *et al*. Variability of Mexican isolates of *Macrophomina phaseolina* based on pathogenesis and AFLP genotype. Plant Pathol. 2001; 59(5):257-264.
17. Cruz Jimenez. Influences of soils, nutrition, and water relations upon charcoal rot disease processes in Kansas. M.Sc. Thesis, Kansas State University, Kansas, 2011.
18. Elham khalili, Muhammad Arsad Javed, Fahrul Huyop, Siavosh Rayatpanah, Soleiman Jamshidi, Roswanira Abdul Wahab. Evaluation of *Trichoderma* isolates as potential biological control agent against soybean charcoal rot disease caused by Macrophomina phaseolina. Biotechnology & Biotechnological equipment. 2016; 30(3):479-488.