

Management of root rot of black gram caused by *Macrophomina phaseolina* (Tassi) Goid using *Trichoderma viride*

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Abstract

Black gram is one of the important legume crops in India. It is a favorable pulse crop since it thrives better in all the seasons as a sole and intercrops or fallow crop. It is being infected by many phytopathogens. Among the phytopathogens, the root rot/ charcoal rot disease caused by *Macrophomina phaseolina* (Tassi.) Goid is a major disease, distributed worldwide and considered as the most serious one its management with chemical fungicides exhibit residual toxicity in the soil. It is therefore felt that, it is very essential to develop an effective, cheap and environmentally safe non-chemical method for the management of dry root rot of the pathogen. The biological control using antagonistic microorganisms offer a practical and economical alternative for management of plant pathogens. Hence the present investigation was conducted to test the efficacy of native antagonists of *Trichoderma viride* for the management of *M. phaseolina*. The results showed the *in vitro* efficiency of antagonist inhibited mycelial growth of *M. phaseolina*. *T. viride* (Tv₆) which recorded the maximum inhibition zone of 81.55%. The culture filtrate of *T. viride* (Tv₆) at a concentration of 30% was found to be maximally reduced in agar well method and poison food technique. Their culture filtrates were also found to be effective in promoting the *in vitro* growth. Tv₆ also promote growth (root length, shoot length, plant biomass), numbers of pods and minimum disease incidence compared to the other isolates under *in vivo* condition. The findings reported in the present study supported the applicability of Tv₆ isolate as a possible alternative to fungicides for the control of root rot in black gram.

Key words: *Vigna mungo*, Root rot, Bio control agents

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INTRODUCTION

Black gram (*Vigna mungo* L.) is one of the most important pulse crops all over the world. It is commonly referred as the Urd bean or black lentil. It is widely cultivated in India, Pakistan, Iran, East Africa, South East Asia and Greece (Ahmed *et al.*, 2015). It is known as the "poor man's meat" and is a major dietary protein of the vegetarian population of the world in India and highly priced among other pulses. Black gram grows normally in 90-120 days and is very nutritious as it contains high levels of Protein, Potassium, Calcium and Iron

helping the reduction of cholesterol and supporting the blood circulation (Khairnar *et al.*, 2019).

In India, it is around 4.47 million hectares with a production of 2.83 million tones and a productivity of 632 kg ha⁻¹. In Tamil Nadu, black gram is cultivated in 4.30 lakh hectares with a production of 2.74 lakh tones and an average productivity of 637 kg ha⁻¹ (Indiastat, 2019). It is affected by number of diseases caused by fungi, bacteria and viruses.

Among the fungus, the root rot/stem rot/charcoal rot disease is caused by *M. phaseolina* (Tassi.) Goid which is a major disease, distributed worldwide occurring from seedling stage to the maturity stage (Meena, 2018). Various cultural and chemical applications are currently employed to control the *M. phaseolina* disease. However, the continuous, inappropriate and non-discriminative use of synthetic chemicals are reported to cause undesirable effects such as residual toxicity, development of resistance, environmental pollution, health hazards to humans and animals and increased the cost of expenditure for plant protection. Hence, organic control seems to be a potential alternative to chemical fungicide for the suppression of root rot diseases.

Among different organic methods of plant disease management, bio control assumes special significance, being an eco-friendly and cost effective strategy which can be used in integration with other strategies for a greater level of protection with sustained rice yields. Presently, root rot disease management is done using the bacterial bio-control representatives which also acts as plant growth enhancing rhizobacteria (PGPR) presents a promising way of safe guarding from plant diseases (Mew and Rosales, 1986; Singh *et al.*, 2018). In this study, experiments have been undertaken to assess the efficacy of biocontrol agent *T. viride* isolates against black gram root rot diseases.

MATERIALS AND METHODS

Isolation of pathogen

The Pathogen *M. phaseolina* was isolated from the root bark tissues of mung bean bearing fungal sclerotia. Typical root rot symptoms were collected for isolation of the pathogen. The tissues were cut into small pieces of 5-10 mm length and 2-3 mm thickness, surface sterilized with 1% sodium hypochlorite (NaOCl) for 2 minutes and then rinsed thrice in sterile distilled water and plated on to PDA medium in sterilized Petri plates and then incubated at room temperature ($28\pm 2^{\circ}\text{C}$) for five days and were

observed for fungal growth. The Purification of fungus was done by single spore isolation technique (Rangaswami, 1958).

Isolation of *Trichoderma viride*

Ten isolates of *T. viride* were isolated from the Namakkal and Cuddalore districts and tested for their antagonistic effect on *M. phaseolina* by three different methods at Annamalainagar, Mutloor, Sivapuri, Bhuvanagiri, Puthoor, Sellapampatty, Thalambadi, Pudhansanthai, Thengalpalayam and Agaram. Isolation of native antagonistic fungi was from the Black gram rhizosphere soil. *T. viride* isolated by *Trichoderma* selective medium (TSM) and purified by single hyphal tip method (Rangaswami, 1972).

Dual culture technique

The antagonistic activity of *Trichoderma viride* against *Macrophomina phaseolina* was tested by dual culture technique. A 9 mm mycelial disc from five days old PDA culture of *M. phaseolina* was placed at one side of petri dishes and *Trichoderma viride* from 5 days old culture was placed on the other side and incubated at $28\pm 2^{\circ}\text{C}$ for 5-7 days. Petri dishes inoculated with fungal discs alone served as control (Dennis and Webster, 1971).

Three replications were maintained for each isolate. Observation on width of inhibition zone and mycelia growth of the test pathogen was recorded and per cent inhibition of pathogen growth was calculated by the formula proposed.

Preparation of the culture filtrate of *T. viride*

In Erlenmeyer flasks, the effective *T. viride* isolates were grown for 10 days at room temperature ($28\pm 2^{\circ}\text{C}$) containing 100 ml of sterilize potato dextrose broth. The culture was filtered under vacuum through Whatman filter to remove the mycelium and spores. The filtrate thus obtained was used for the Poison food technique (Maheshwari *et al.* 2001).

Poison food technique

The culture filtrate of the fungal antagonist, *T. viride* was separately incorporated into

sterilized PDA medium at 10, 20, 30 per cent by adding a calculated quantity of the culture filtrates to the medium of 90, 80, 70 ml by means of a sterile pipette. The PDA medium without the culture filtrate served as the control. The amended media were transferred to sterile Petri dishes separately @ 15ml and allowed to solidify. Each plate was inoculated at the centre with five days old (9 mm) PDA culture discs of *M. phaseolina*. Three replications were maintained for each treatment. The diameter of the mycelia growth (mm) of *M. phaseolina* was measured when the mycelial growth fully covered the control plates (Schmitz, 1930).

The per cent inhibition of the fungus over control was calculated using the following formula:

$$PI = (A - B) / A \times 100$$

Where, A is the colony diameter of the fungus in control plates (mm) and B is colony diameter of the fungus in treated plates (mm).

Agar well method

Antifungal activity of *Trichoderma* poison was tested using agar well method. Twenty ml of PDA medium was seeded with 3 ml of spore suspension (5×10^{-5}). Wells were made on the agar surface with a 5mm cork borer. 1 ml of poison was poured separately into the well using a sterile syringe at different concentrations viz., 10, 20, 30 per cent. The plates were incubated at $28 \pm 2^\circ\text{C}$ for seven days and observed for fungal growth. Three replications were maintained for each treatment. The plates were observed for zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter (Bauer *et al.*, 1996).

Plant growth-Roll towel method

The plant growth-promoting activity of the bio control agents was assessed based on the seedling vigour index by the standard roll towel method (ISTA 1993). The germination paper was soaked in water for 2 to 4 h to moist it evenly and to remove water soluble toxic substances present in it. The black

gram seeds were treated with different fungicides mentioned above (@ 2g/ kg each) for the treatment of seeds. The treated seeds were equidistantly placed between the two sheets of paper towel (27×20 cm), rolled carefully ensuring to pressure on seeds, wrapped with a polythene sheet to reduce surface evaporation and kept in germination chambers in an upright position and incubated in a growth chamber for 5 days. Three replications were carried out for the treatment. The root length and shoot length of individual seedlings were measured and the per cent germination of the seeds was calculated. The seedling vigour index was calculated using standard formula (Abdul Baki and Anderson, 1973),

Pot culture technique

Totally 1kg of soil and 1kg of sand was taken into polythene bags and sterilized at 121°C for 30min at 15lbs pressure for two successive days. Earthenware pots were taken, sterilized sandy soil was added into the pots. Surface sterilized blackgram seeds were sown in pots filled with sandy soil containing *M. phaseolina* and 10 isolates of *T. viride* were added separately in earthen pots. Replicates were maintained for each treatment. Control pots were also maintained without any fungal culture. On germination, disease incidence, root/shoot length and yield parameters were recorded.

Statistical analyses

The experiments were conducted using completely randomized design (CRD) with three replications. The significant difference, if any, among the means were compared by Duncan's multiple range test (DMRT). Whenever necessary, the data were transformed before statistical analysis following appropriate methods.

RESULTS

The results for the screening of 10 isolates of *T. viride* against *M. phaseolina* on PDA plates are presented in Table 2. Among the isolates, *T. viride* (Tv₆) appeared to be the most effective against the test pathogen, showing 81.55 per cent (P=0.05) inhibition of colony growth and found to be the best. It

was followed by the isolate (TV₂) showing 79.45 per cent (P=0.05) inhibition. The isolates TV₅ and TV₁₀ showed minimum growth inhibition when compared to other isolates. All the native isolates of *Trichoderma spp.* significantly inhibited the mycelial growth of *M. phaseolina* (Table 2) in dual culture. However, the isolate TV₆ of *T. viride* significantly inhibited the growth of *M. phaseolina*. The least growth inhibition of the pathogen was exhibited by the isolate TV₅ (Table 2).

Ten *Trichoderma* isolates were tested against *M. phaseolina* in *in vitro* condition by agar well method. The culture filtrate of *Trichoderma viride* (TV₆) at a concentration of 30% was found to be maximally reduced in agar well method and recorded 14.4 per cent (P=0.05) inhibition zone, followed by

TV₂, which recorded 14.2 per cent (P=0.05) while the concentration of TV₅ showed a minimal per cent inhibition zone than all the others.

The ten fungal isolates were tested against, the root rot of black gram in *in vitro* condition by poison food technique. Among the isolates, *T. viride* (TV₆) appeared to be most effective in managing the mycelial growth of *M. phaseolina*. It recorded minimum mycelial growth at 30% conc. (2.9mm) and a maximum per cent inhibition over the control (96.7 percent (P=0.05)) which was followed by TV₂ which showed a minimum mycelial growth (4.0) and a maximum percent inhibition over the control (95.5percent (P=0.05)). The culture filtrate of all the *T. viride* isolates significantly inhibited the growth of *M. phaseolina*.

Table 1. Cultural characteristics of *T. viride*

Isolates	Colony character	
	Third day after inoculation	Seventh day after inoculation
TV ₁	White cottony mycelium	Deep green sporulation
TV ₂	Moderate white mycelium	White green to dull green sporulation
TV ₃	Profuse white mycelium	Dark green sporulation
TV ₄	Moderate white scanty mycelium	White green to dull green sporulation
TV ₅	Thin white cottony mycelium	Colony fluffy and green sporulation
TV ₆	Profuse white mycelium	Dark green sporulation
TV ₇	Thin white cottony mycelium	Colony fluffy and green sporulation
TV ₈	Moderate white scanty mycelium	White green to dull green spores
TV ₉	White cottony mycelium	Deep green sporulation
TV ₁₀	Moderate white mycelium	White green to dull green sporulation

Table 2. Evaluation of *T. viride* against *M. phaseolina* under *in vitro* condition

DUAL METHOD			AGAR WELL METHOD						POISON FOOD TECHNIQUE					
			Mycelial inhibition zone (mm)*						Mycelial inhibition zone (mm)*					
Isolates	Mycelial growth of <i>M.phaseolina</i> (mm)	Percent inhibition over control	10%	Percent inhibition over control	20%	Percent inhibition over control	30%	Percent inhibition over control	10%	Percent inhibition over control	20%	Percent inhibition over control	30%	Percent inhibition over control
Tv ₁	30.36 ^f	66.26	8.7 ^{cde}	90.33	9.10 ^{efg}	89.88	10.9 ^{de}	87.88	22.40 ^e	75.11	17.12 ^{cde}	80.97	11.8 ^{ef}	86.88
Tv ₂	18.49 ^{ab}	79.45	11.4 ^{ab}	87.33	13.10 ^b	85.44	14.2 ^{ab}	84.22	19.10 ^{ab}	78.77	10.20 ^b	88.66	4.0 ^{ab}	95.55
Tv ₃	18.69 ^{ab}	79.23	10.3 ^{abc}	88.55	11.4 ^c	87.33	13.1 ^{bc}	85.44	20.12 ^{abc}	77.64	11.20 ^b	87.55	6.0 ^{bc}	93.33
Tv ₄	23.36 ^d	74.04	9.4 ^{abcde}	89.55	10.0 ^{ef}	88.88	11.5 ^d	87.22	21.80 ^{de}	75.77	15.76 ^c	82.26	10.7 ^{def}	88.11
Tv ₅	35.22 ^g	60.86	7.5 ^e	91.66	8.0 ^g	91.11	9.3 ^f	89.66	24.30 ^f	73.00	19.36 ^e	78.48	13.2 ^f	85.33
Tv ₆	16.69 ^a	81.55	11.5 ^a	87.22	14.3 ^a	84.11	14.4 ^a	84.0	18.50 ^a	79.44	7.20 ^a	92.00	2.9 ^a	96.7
Tv ₇	19.72 ^c	78.08	10.2 ^{abcd}	88.66	11.2 ^{cd}	87.55	12.9 ^c	85.66	20.23 ^{bcd}	77.52	11.10 ^b	86.44	8.1 ^{cd}	91.0
Tv ₈	26.96 ^e	70.04	8.8 ^{bcde}	90.22	9.3 ^{ef}	89.66	11.2 ^{de}	87.55	22.10 ^e	75.44	16.12 ^{cd}	82.08	10.9 ^{ef}	87.88
Tv ₉	20.06 ^c	77.71	9.5 ^{abcde}	89.44	10.2 ^{de}	88.66	11.7 ^d	87.0	21.70 ^{cde}	75.88	15.76 ^{cd}	82.26	9.2 ^{de}	89.7
Tv ₁₀	33.11 ^{gf}	63.21	7.6 ^{de}	91.55	9.0 ^{fg}	90.00	10.2 ^{ef}	88.66	24.10 ^f	73.22	18.36 ^{de}	79.06	12.2 ^f	86.44
Control	90.00 ^g	-	90.00 ^f	-	90.00 ^g	-	90.00 ^g	-	90.00 ^g	-	90.00 ^f	-	90.00 ^g	-

*Mean of three replications

Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

Table 3. Effect of *T. viride* on black gram seed germination and plant growth promotion under *in vitro* condition (Roll towel method)

Treatment	Seed germination %*	Shoot length (cm)*	Root length (cm*)	Vigour index
Tv ₁	95 ^{cd}	17.4 ^e	11.3 ^{bcde}	2726.5 ^h
Tv ₂	97 ^{ab}	18.2 ^{ab}	12.1 ^{ab}	2939.1 ^b
Tv ₃	97 ^{ab}	18.1 ^b	12.1 ^{ab}	2929.4 ^c
Tv ₄	96 ^{bc}	17.6 ^d	11.7 ^{abcd}	2812.8 ^f
Tv ₅	94 ^d	16.9 ^g	10.8 ^e	2538 ^j
Tv ₆	98 ^a	18.3 ^a	12.4 ^a	3008.6 ^a
Tv ₇	97 ^{ab}	17.9 ^c	11.9 ^{abc}	2890.6 ^d
Tv ₈	96 ^{bc}	17.5 ^{de}	11.6 ^{abcde}	2793.6 ^g
Tv ₉	97 ^{ab}	17.8 ^c	11.8 ^{abc}	2871.2 ^e
Tv ₁₀	95 ^{cd}	17.1 ^f	10.9 ^{de}	2660 ⁱ
Control	95 ^d	16.2 ^h	10.3 ^{ef}	2462.8 ^k

*Mean of three replications

Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

Ten isolates of *T. viride* were tested for the efficacy of black gram seed germination and plant growth promotion in single application using roll towel method. *T. viride* (Tv₆) recorded significantly (P=0.05) maximum seed germination, shoot length, root length and vigour index respectively. A minimum seed germination and plant growth promotion was exhibited by the isolate Tv₅. (Table 3).

In vivo condition by soil application

The experimental results tabulated in Table 4 revealed that, all treatments significantly enhanced growth and fruit yield when compared to control. Among the treatments, application of *T. viride* Tv₆ (soil application) significantly (P=0.05) increased the mean plant height, root length, biomass and number of pods/plant and minimum disease incidence 9.10 per cent when compared to all the other treatments.

DISCUSSION

Trichoderma sp produces a large variety of volatile secondary metabolites such as ethylene, hydrogen cyanide, aldehydes and ketones which play an important role in controlling the plant pathogens. The results of the present study correspond with Abdel and Bakr (2018) who stated that, all three *Trichoderma* spp were very effective against *M. phaseolina* in dual culture technique. Swamy *et al.* (2018)

reported that, among different fungal bio-agents tested, *T. harzianum* resulted in maximum mycelial growth inhibition (41.86%) of *M. phaseolina* causing stem canker of pigeon pea. Efficacy of *T. viride* against various pathogens viz., *Aspergillus niger* (Gajerja *et al.*, 2012); *M. phaseolina* (Suthin Raj *et al.*, 2008b; Tetali *et al.*, 2015), *R. bataticola* (Maruti *et al.*, 2017) and *F. oxysporum* f. sp. *sesami* (Mahmoud and Abdalla 2018) have also been reported under *in vitro*. *Trichoderma* sp was reported to be a potential antagonist against *M. phaseolina* through colony interaction (Biswas and Sen 2000). These earlier reports lend support to the present findings. A multiplicity of mechanisms involving antibiosis, mycoparasitism, lysis and hyphal interference could be attributed to a reduction in the mycelial growth of *M. phaseolina*. The antagonists of *T. viride* inhibited the growth of *M. phaseolina* than other *Trichoderma* sp.

Generally, an increase in the concentrations of culture filtrate reduced the growth of the pathogen. Among the isolates tested, Tv₆ was found to be most inhibitory to the growth of *M. phaseolina* and a least inhibition was found in Tv₅. *T. viride* and *T. harzianum* were observed as potential antagonists which inhibited the mycelial growth of *M. phaseolina* causing charcoal rot

in sunflower as reported by Suriachandraselvan *et al.* (2004). Similarly, 40 per cent conc. of culture filtrate of *T. harzianum* showed maximum inhibition of *M. phaseolina* (Suthin Raj *et al.*, 2008a; Rashmi Singh *et al.*, 2012). These earlier reports are in line with the present observation.

In the present study, the *Trichoderma viride* Tv₆ enhanced seed germination, promoted plant growth and increased the shoot length, root length and vigour index. This was confirmed by Kredics *et al.* (2001) and Yedidia *et al.* (1999) where the antagonist's culture filtrate of *T. viride* showed a maximum seed germination and growth parameters. Saxena *et al.* (2015) and Singh *et al.* (2016) reported a fungal isolate of *T. asperellum* BHUT8 and evaluated for plant growth promotion effect in pea and the results showed that there was a significant increase in shoot length, root length, number

of leaves, shoot fresh weight, root fresh weight, shoot dry weight and root dry weight as compared to the control and concluded that seed bio priming is a very effective method for seed treatment that ultimately resulted in enhancing the plant growth in pea. The results of the present experiment revealed the superiority of all the treatments in increasing the mean leaf area, mean plant height, mean no. of flowers/plant, mean no. of fruits/plant, mean fruit length and fruit yield g/plant over control. The same results were stated by Deshmukh *et al.* (2016), as the approach of using bio-agents and organic amendments for controlling pathogens has potential benefits in managing the disease with good plant health with significant enhancement in root and shoot lengths along with a dry weight of plants. Thilagavathi (2007) reported the application of biocontrol as sole and in combination increases the yield of the green gram plants.

Table 4. Evaluation of the efficacy of *Trichoderma viride* isolates as biological control agents against *M. phaseolina* under *In vivo* condition by soil application

Native isolates	Shoot length*	Root length*	Biomass g/plant*	No of pods per plant*	Root rot incidence*
Tv-1	25.10 ^h	14.80 ^e	4.2 ^f	10.99 ^g	14.40 ^h
Tv-2	31.10 ^b	18.20 ^{ab}	6.0 ^b	14.30 ^b	10.10 ^b
Tv-3	31.00 ^c	17.70 ^b	5.7 ^c	14.10 ^c	11.01 ^c
Tv-4	27.50 ^f	16.25 ^c	5.3 ^{de}	11.50 ^f	13.40 ^f
Tv-5	23.12 ^j	14.20 ^f	3.7 ^h	10.00 ^h	16.12 ^j
Tv-6	32.83 ^a	18.60 ^a	6.9 ^a	15.41 ^a	9.10 ^a
Tv-7	30.53 ^d	16.50 ^c	5.4 ^d	12.93 ^e	12.01 ^d
Tv-8	26.10 ^g	15.20 ^d	4.9 ^e	13.95 ^d	14.20 ^g
Tv-9	28.51 ^e	16.40 ^c	5.4 ^d	11.11 ^f	13.20 ^e
Tv-10	24.23 ⁱ	13.70 ^g	3.9 ^g	10.79 ^g	15.32 ⁱ
Control	22.06 ^k	12.70 ^h	3.0 ⁱ	9.12 ⁱ	28.60 ^k

*Mean of three replications

Values in the column followed by common letters do not differ significantly by DMRT (P=0.05).

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