



ISOLATION, CULTURAL CHARACTERIZATION AND ANTAGONISTIC ACTIVITY OF *TRICHODERMA VIRIDE* AGAINST *MACROPHOMINA PHASEOLINA*

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Abstract

The aim of present investigation was to assess cultural characters and *in vitro* antagonistic potential of native isolates of *Trichoderma viride* against *Macrophomina phaseolina*. Among the native isolates tested, isolate Tv₁ significantly reduced the mycelial growth, size, number, germination and germ tube production of sclerotia of *Macrophomina Phaseolina*. Further, the same isolates tested at different concentrations. Where, isolate Tv₁ @ 30% was significantly inhibits the growth (22.51mm) of test pathogen. Also, all the isolates were showed variation in their cultural characters like colony character (initially moderate white and later become as colony fluffy and green) and length (3.05-3.70 µm) and breadth (2.25-3.30 µm) of conidia.

Key words: Root rot; *Macrophomina phaseolina*; Characterization; Survey.

Introduction

Sesame (*Sesamum indicum* Linn.) an important oilseed crop grown in many parts of the world is regarded as the queen of oilseeds because of its superior quality oil. The seeds and oil from sesame find an important place in human diet in the Asian countries since ancient times and the oil is used in the preparation of ayurvedic and siddha medicines (Ramasamy, 2001). In world, Sesame is grown in area of 11.25 million hectares with production of 6235.53 thousand tones and productivity of 576.3 kg/ha (FAO, 2015). Globally, India contributes the highest sesame acreage of above 17.73 lakh ha with a production 8 lakh tones and productivity of 445kg/ha (Gupta *et al.*, 2018). The major states producing sesame are West Bengal, Madhya Pradesh, Rajasthan and Gujarat representing more than 70 percent of total country's production. In Tamil Nadu, Erode, Villupuram, Thanjavur, Karur, Cuddalore, Thoothukudi and Salem are the major sesame producing districts (www. agrigalance.com). India's share to world's sesame production is 13.1%, but the productivity is very low with 0.38 ton/ha as against to the maximum productivity of 1.23 ton/ha in China (www.nmoop.gov.in). To date, various disease management methods, viz., cultural, regulatory, physical,

chemical (fungicides) and biological have been implemented to combat and eradicate the phytopathogenic fungi. However, these methods are effective only when employed well in advance as precautionary measures (Ganeshamoorthi *et al.*, 2010). Moreover, as the soil-borne nature chemical fungicides for the control of *M. phaseolina* may be less effective (Anis *et al.*, 2010).

Biological agent for the control of plant disease is an alternative method of chemical control (Cook and Baker, 1983) and had attained importance in modern agriculture for disease control (Gupta *et al.*, 2018). Among various biocontrol agents that have been tested against root rot causing fungi, *Trichoderma* spp. have proven to be more effective in controlling the root-rot pathogens for having a greater ability to survive under a wide range of temperatures (Pan and Bhagat, 2008), soil types (Mohiddin *et al.*, 2010) and microbial communities (Huagn *et al.*, 2003). *Trichoderma* spp. can suppress the target pathogens through antibiosis by producing fungitoxic metabolites, trichodermin, gliotoxin, viridin etc. (Harman, 2006). *Trichoderma* spp. produce glucanase, cellulase, protease and chitinase enzymes to cause lysis (Vinale *et al.*, 2009). Several studies were clearly indicated that *Trichoderma* spp. was effectively inhibits the growth of *M. phaseolina* under *in vitro* condition

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Table 1: Cultural characteristics of *T. viride* native isolates.

Sl. No.	Isolates	Colony character		Conidia	
		Third day after inoculation	Seventh day after inoculation	Length (μm)	Breadth (μm)
1.	Tv ₁	Moderate white mycelium	Colony fluffy and green	3.05-3.70 ^a	2.25-3.30 ^a
2.	Tv ₂	Profuse mycelium	Dark green sporulation	2.60-3.40 ^c	2.10-2.97 ^c
3.	Tv ₃	White cotton mycelium	Complete dark green sporulation	2.80-3.50 ^b	2.25-3.30 ^b
4.	Tv ₄	Thin white cotton mycelium	Colony fluffy and green sporulation	2.26-3.30 ^d	2.30-3.10 ^d

Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

(Rettinababady and Ramadoss, 2000; Manjunatha *et al.*, 2013; Khaledi and Taheri, 2016; Arya *et al.*, 2017; Thombre and Kohire, (2018). Hence, the aim of present investigation was to assess the cultural characters and antifungal activity of *T. viride* against *M. phaseolina* under *in vitro* condition.

Materials and Method

Isolation of the pathogen

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

Isolation and identification of *T. viride*

Sesame rhizosphere soil samples collected from four different locations *viz.*, Kothatti, Virudhachalam, Bhuvanagiri and Palur of Cuddalore district, Tamil Nadu, India were used for the isolation of *T. viride* native isolates by soil dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983). The isolates were further purified by single hyphal tip method and used for the subsequent studies. Micrometric measurements of conidia and phialides were done by mounting four days old culture stained with lactophenol cotton blue and observed under Phase-contrast microscope. All the isolated was subjected for identification based on the key to species suggested by (Domsch *et al.*, 1980). The identified isolates were designated as Tv₁ (Virudhachalam), Tv₂ (Kothattai), Tv₃ (Bhuvanagiri) and Tv₄ (Palur).

Dual culture technique

The antagonist activity of native *T. viride* isolates against *M. phaseolina* was tested by dual culture technique (Dennis and Webster, 1971). At one end of the sterile plate dish containing 15ml sterilized and solidified PDA medium a 9mm mycelia disc obtained from five days old culture of *T. viride* was placed under aseptic condition. Similarly at the opposite end approximately 75 mm away from the antagonist culture disc, a 9mm culture

disc of the test pathogen was placed and incubated. A control was maintained by inoculating *M. phaseolina* alone at one end of the Petri dish. The radial growth of the pathogen and the antagonists and the extent of the inhibition zone was developed between the two colonies were measured. The radial growth of the pathogen and percent reduction over control was calculated by using the formula (Vincett, 1927)

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

Where,

C- Mycelial growth of pathogen in control

T- Mycelial growth of pathogen in dual plate.

Poisoned food technique

The culture discs (four discs of 9mm size) obtained at the point where the pathogen and antagonist interacted from the above experiment were used in the estimation of the sclerotial number and size. The culture disc cut from the plates inoculated with test pathogen alone was served as control. For assessing the effect of antagonists on the sclerotial germination, twenty five uniform sized sclerotia from dual culture plates were picked and placed on a cavity slide containing sterile water. The observations on germination and the number of germ tubes production were recorded after 24h. (Dhingra and Sinclai, 1978).

With regard to effect of culture filtrates of *T. viride* against *M. phaseolina*, the isolates of *T. viride* were grown for 10 days at room temperature (28±2°C) in Erlenmeyer flasks containing 50ml of sterilized potato dextrose broth. The cultures were filtered under vacuum through bacteriological filter to remove the mycelium and spores. The filtrate thus obtained was used for further studies.

The culture filtrate of the antagonist were separately incorporated into sterilized PDA media at 10, 20 and 30 percent by adding the calculated quantity of the culture filtrates to the medium by means of a sterile pipette. The PDA medium without the culture filtrate served as 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 (9mm) PDA culture disc of *M. phaseolina*. Three replications

Table 2: Efficacy of native *T. viride* isolates against *M. phaseolina* (Dual culture).

Isolates	Growth of <i>M. phaseolina</i>	
	Linear growth (mm)	Percent inhibition
Tv ₁	27.40 ^a	69.55
Tv ₂	41.24 ^c	54.17
Tv ₃	33.26 ^b	63.04
Tv ₄	49.72 ^d	44.75
Control	90.00 ^e	-
Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).		

were maintained for each treatment. The diameter of the mycelial growth was measured and recorded when the fungus covered entire plate in the control treatment.

Results and Discussion

Cultural characteristics of native *T. viride* isolates

The colony characters of the four native isolates of *T. viride* on PDA were observed visually on third and seventh day after inoculation and the results were presented in table 1. The colony morphology of all the four isolates was almost similar showing sparse to thin cottony mycelial mass with whitish colour. The colonies on PDA reached 80-90mm dia., in three days at the temperature of 28±2°C.

Sporulation started after 48h., of incubation for all the four isolates. The conidiophores showed typical pyramidal branching *viz.*, short branches near the tip and longer branches with frequent branching at the bottom. Phialides were irregularly bent and found in group of 2-3. The conidia of Tv₁ were almost globes and measured 3.05-3.70µm length and 2.25-3.30 µm breath. The cultural and morphological characteristics of antagonist was agreed with those described by Domsch *et al.*, (1980) and therefore identified as *T. viride*.

Efficacy of native *T. viride* isolates against *M. phaseolina* (Dual culture)

The native *Trichoderma* isolates tested significantly inhibited the mycelial growth of *M. phaseolina* (Table 2). Among the isolates tested, the isolate Tv₁ showed the

maximum inhibition and significantly inhibited the growth (27.40 mm) of *M. phaseolina*, which was 69.55 percent reduction on the growth of the pathogen as against the growth of 90 mm in control. This was followed by the isolates Tv₃ and Tv₂ in the decreasing order of merit, which inhibited the growth of *M. phaseolina* by 63.04 and 54.17 percent reduction on the mycelial growth over control. The least growth inhibition of the pathogen (44.75%) was exhibited by the isolate Tv₄. The results of the present study correspond with several workers (Jagannathan, 1993; Singh Band Mujumdar, 1995). Efficacy of *T. viride* against various pathogens *viz.*, *Foxysporum* f. sp. *ricini* (Raof *et al.*, 2006) and *Aspergillus niger* (Gajera *et al.*, 2012) have also been reported under *in vitro*. 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* was made by several earlier workers (Jite, 2012; Vasebi *et al.*, 2013). *Trichoderma* spp. was reported to be a potential antagonist against *M. phaseolina* through colony interaction and producing non-volatile metabolites (Biswas and Sen, 2000). All these earlier reports are in line and lend support with the present findings.

Effect of native *T. viride* isolates on the number, size and sclerotial germination of *M. phaseolina*

The results clearly revealed that all the native isolates of *T. viride* considerably reduced the number, size, germination and germ tube production of sclerotia was presented in table 3. Among the isolates tested, Tv₁ was found to be the most effective isolate in inhibition of test pathogen over control with minimum number (78.20), size (72.55 µm), germination (44.25 %) and germ tube (7.31%) production of sclerotia which accounting 51.18, 36.63, 51.90 and 56.35 percent reduction over control respectively. This was followed by the isolate Tv₃ and Tv₂. The least effect was found with isolate Tv₄ recorded with number (26.20%), size (83.47µm), germination (32.16%) of sclerotia and germ tube (10.48) per sclerotia (Table 3).

Table 3: Effect of the native *T. viride* isolates on number, size and sclerotial germination of *M. phaseolina*.

Isolate	Number of sclerotia	Percent reduction	Sclerotial size (µm)	Percent reduction	Sclerotial germination (%)	Percent inhibition	No. of germ tubes per sclerotium ⁻¹	Percent reduction
Tv ₁	78.20 ^a	51.18	72.55 ^a	36.63	44.25 ^a	51.90	7.31 ^a	56.35
Tv ₂	98.70 ^c	38.38	78.43 ^c	31.50	55.00 ^c	39.27	9.47 ^c	43.46
Tv ₃	86.55 ^b	45.97	74.14 ^b	35.24	49.45 ^b	46.25	8.23 ^b	50.86
Tv ₄	118.50 ^d	26.20	83.47 ^d	27.10	58.20 ^d	32.16	10.48 ^d	37.43
Control	160.20 ^e	-	114.50 ^e	-	92.00 ^e	-	16.75 ^e	-
Values not sharing a common superscript differ significantly at P < 0.05 (DMRT)								

Table 4: Effect of culture filtrate of native *T. viride* isolates on the growth of *M. phaseolina*.

Isolates	Growth of <i>M. phaseolina</i> (mm)			Mean
	Concentration of culture filtrate (%)			
	10	20	30	
Tv ₁	52.13 ^a	31.73 ^a	22.51 ^a	35.45
Tv ₂	61.43 ^c	39.63 ^c	26.26 ^c	42.44
Tv ₃	54.72 ^b	34.47 ^b	24.51 ^b	37.90
Tv ₄	64.35 ^d	42.62 ^d	29.17 ^d	45.38
Control	90.00 ^e	90.00 ^e	90.00 ^e	90.00

Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

Reduction in the production, size and sclerotial germination of *T. viride* caused the maximum inhibition on the spore germination of *C. capsici* causing fruit rot of chillies (Mandeep Kaur et al., 2006). The metabolites produced by *T. viride* might have reduced the number, size and sclerotial germination of *M. phaseolina* as observed by Kapil and Kapoor, (2005). Reduction of *M. phaseolina* by *T. viride* was observed by (Aly et al., 2007). *T. viride* inhibited the growth, reduced the number and sclerotial size of *M. phaseolina* infecting black gram (Retinasababady and Ramadoss, 2000). *T. viride* and *T. harzianum* were observed as potential antagonists and reduced the number size and germination of sclerotia of *M. phaseolina* causing charcoal rot in sunflower (Suriachandraselvan et al., 2004).

Effect of culture filtrate of native *T. viride* isolates on the mycelia growth of *M. phaseolina* (in vitro)

The results presented in table 4, showed that all the *T. viride* isolates significantly inhibited the growth of *M. phaseolina* when compared to control and generally an increase in the concentration of the culture filtrate reduced the mycelial growth of the pathogen. Among the isolate tested, the isolate Tv₁ was found to be most inhibitory to the growth of *M. phaseolina* by recording the least mycelia growth with 52.13, 31.73 and 22.51 mm at 10, 20 and 30 percent concentration of the culture filtrate, respectively. This was followed by Tv₃ and Tv₂ in the decreasing order of merit. The isolate Tv₄ exhibited the least inhibitory effect. *T. viride* and *T. harzianum* were observed as potential antagonists and inhibited the mycelial growth of *M. phaseolina* causing charcoal rot in sunflower (Suriachandraselvan et al., 2004). Culture filtrates of *T. viride* inhibited the growth of the *M. phaseolina* as well as sclerotial germination to a greater extent (Karthikeyan et al., 2006). Similarly, 30 percent conc. of culture filtrate of *T. harzianum* showed maximum inhibition of *M. phaseolina* (Rashmi Singh et al., 2012). These earlier reports are in line with the present observations.

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