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IN VITRO EFFICACY VARIOUS ISOLATES OF *TRICHODERMA VIRIDE* AND *PSEUDOMONAS FLUORESCENS* AGAINST *MACROPHOMINA PHASEOLINA* CAUSING SESAME ROOT ROT

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Abstract

Sesame (*Sesamum indicum* L.) is one of the oldest oil seed crop and is affected by a number of diseases, of which the root rot caused by *Macrophomina phaseolina* is the most serious disease. In this study, five native *Trichoderma viride* and *Pseudomonas fluorescens* antagonists isolated from healthy sesame rhizosphere soil in different regions, were assessed for their ability to reduce the growth of *M. phaseolina* as well as sclerotial germination to a greater extent by dual culture assay and Poision food technique. Among these five isolates tested, isolate *Tv3* exhibited maximum growth inhibition (79.23%) against *M. phaseolina* followed by *Tv1* (77.18%) and minimum mycelial growth inhibition was recorded with *Tv4* (60.64%) and also most effective in reducing sclerotial formation (74.21), Sclerotial germination (74.21%), No. of. Germ tubes (4.92) while Tv4 was least effective. Among bacterial antagonists the isolate *Pf55* exhibited maximum growth inhibition (75.2 2%) against *M. phaseolina* followed by *Pf3* (72.91%) and minimum mycelial growth inhibition was recorded with *Pf2* (68.67%) also most effective in reducing sclerotial formation (64.78), Sclerotial germination (39.63 %), No. of. Germ tubes (5.09) while Pf2 was least effective. Similarly, the different concentration of the culture filtrates of the native isolates of *T. viride* and *P. fluorescens* were tested and the isolates *T. viride* (*Tv3*) and *P. fluorescens* (*Pf5*) appeared to be most effective against *M. phaseolina* showing nill growth at 40, 50 % concentration on test fungi when tested by Poison food technique. Thus the present study revealed that the *T. viride* (*Tv3*) and *P. fluorescens* (*Pf4*) could be further exploited for field testing for the management of sesame root rot disease.

Keywords: *Macrophomina phaseolina, Trichoderma viride, Pseudomonas fluorescens*

Introduction

Sesame (*Sesamum indicum* Linn.), belongs to family Pedaliaceae and it is an important oilseed crop known to mankind for edible and medicinal applications (Akbar *et al.,* 2012). It is regarded as the queen of oilseeds because of its superior quality oil and grown in many parts of the world. 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).

The main reason for the low productivity of sesame is due to the attack of various diseases. Among the fungal diseases, stem and root rot also called charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is widely distributed and most destructive necrotrophic pathogens that infect more than 500 plant species across 75 families. Chattopadhyay *et al*., 2002 reported that disease incidence was up to 50 per cent or more in field resulting in heavy yield losses. The fungus causes many diseases like seedling blight, collar rot, stem rot, charcoal rot and dry root rot, under moisture stress condition. The sudden wilting of plants throughout the crop growth mainly after the flowering phase was the most common symptoms of these disease. The pathogen attacks mostly at the basal region of the plant (Kumar *et al*., 2011). Its microsclerotia, formed in senescing shoot tissues, survive well in soil (Mayek-Perez *et al*., 2002). The fungus is soil-borne and poses great problem in managing the disease. Soil borne nature coupled with complex survival capacity of sclerotia makes its chemical control reliable, difficult and uneconomical (Gul *et al*., 1989; Ahmed and Burney, 1990) and also having several disadvantages *viz.,* pesticide residues in soil and crop produce, ground water pollution, emergence of resistant races and killing of non-target

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beneficial microbes, etc. Hence, biological agents provide an alternative to chemicals in the management of soil borne diseases as they are eco-friendly in nature (Ramesh and Korikanthimath, 2006). The most commonly used biocontrol agents are *Trichoderma* spp. against many soilborne pathogens, nowadays. Recently, *P. fluorescens* is the most importance of Plant growth promoting rhizobacteria (PGPR) in disease management has been realized.

Isolation of native antagonists from rhizosphere soil

Trichoderma spp.

Sesame rhizosphere soil samples collected from five different locations (Eggoor, Maganoorpatti, Pudhuchathiram, Singaraipettai and Karapattu) were used for the isolation of Fungal and Bacterial isolates by serial dilution plating technique using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983) and King's B medium (King *et al.,* 1954).

A soil suspension was prepared from rhizosphere sample by shaking 1 g of soil sample in 10 ml of sterile distilled water and serial dilutions were made. From the 10^{-3} and 10^{-5} soil dilution 1 ml was transferred into sterile Petri dish under aseptic condition to which 15 ml of sterile TS medium and King's B medium was poured separately, gently rotated for uniform mixing of the soil dilution with the medium and incubated at room temperature (28 ± 2^0C) for 5-7 5ays for fungi and 48 hours for bacteria. These colonies were marked and sub- cultured. The pure cultures of these isolates were obtained by single colony method. and used for further studies

Effect of antagonists on mycelial growth (Dual culture technique)

The antagonistic activity of bio control agents (Tv_1-Tv_5; Pf_1-Pf_5) against *M. phaseolina* was tested by culture technique (Dennis and Webster, 1971). At one end of the sterile plate dish containing 15 ml sterilized and solidified PDA medium mycelial disc obtained from five days old culture of *T. viride* was placed under aseptic conditions. Similarly at the opposite end approximately 75 mm away from the *Trichoderma* culture disc, a 9 mm culture disc of the pathogen *M. phaseolina* alone at one end of the Petri dish. The plates were incubated at room temperature (28+2^0C) for three days. In case of *P. fluorescens* one cm long streak was gently made onto the medium using two days old culture. The radial growth (mm) of the pathogen and the antagonists and the extent of the inhibition zones (mm) developed between the colonies were measured. The effective antagonists and the extent of 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 mycelia growth of the pathogen of the pathogen and percent reduction over control was calculated by using the formula (Vincent, 1927).

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

Where,

C – Mycelial growth of pathogen in control (mm)

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

Effect of antagonists on sclerotial production, size and germination

The culture disc (four discs of 9mm size) obtained at the point pathogen and antagonist interacted from the above experiment were used in the estimation of the sclerotial number and size. The culture discs cut from the plates inoculated with *M. phaseolina* alone served as control.

For assessing the effect of antagonists on the sclerotial germination, twenty five sized sclerotia from dual culture plates were picked and placed on a cavity slide containing sterile water. The observations on the germination and the number of germ tubes produced were recorded 24 h. after incubation (Dhingra and Sinclair, 1978).

Bioassay of culture filtrate of the antagonist on the mycelial growth of *M. phaseolina*

Preparation of the culture filtrate of *T. viride*

The effective *T. viride* and *P. fluorescens* isolates (Tv_1-Tv_5; Pf_1-Pf_5) were inoculated in to the Erlenmeyer flasks containing 50 ml of sterilize potato dextrose broth and King's B broth and kept on a rotary shaker at 100 rpm. The culture filtrated under vacuum through bacteriological filter and thus filtrate thus obtained was used for the studies.

Effect of culture filtrates of antagonists on the mycelia growth of *M. phaseolina*

The culture filtrate of the antagonists (Tv_1-Tv_5; Pf_1-Pf_5) were separately incorporated into sterilized PDA medium at 10, 20, 30, 40 and 50 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 the sterile Petri dishes separately @ 15ml and allowed to solidify. Each plate was inoculated at the centre with five days old (9 mm) PDA culture disc of *M. phaseolina.* Five replication were maintained for each treatment. The diameter of the mycelia growth (mm)

of *M. phaseolina* was measured when the mycelia growth fully covered the control plates.

Results and Discussion

In general all the native *T. viride* and *P. fluorescens* isolates tested (Tv_1- Tv_5; Pf_1-Pf_5) significantly inhibited the mycelial growth of *M. phaseolina* by Dual culture technique (Table 1). However, among the isolates tested, the isolate Tv_3 of *T. viride* collected from Singarapettai and Pf_5 of *P. fluorescens* (Pudhuchathiram) showed maximum inhibition and significantly inhibited the growth of *M. phaseolina* to the tune of 79.23 and 75.22 percent, respectively. The least growth inhibition of the pathogen (60.64 % and 68.67 %) was exhibited by the isolate Tv_4 from Karapattu village and Pf_2 from Eggoor village. The results of the present study correspond with Abdel-lateif and Bakr (2018) stated that all three *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 (Vasebi *et al.,* 2013; Manjunatha *et al.,* 2013). *P. fluorescens* strain effectively inhibited the mycelial growth of *M. phaseolina,* causing dry root rot in blackgram and sesame (Jayashree *et al.,* 2000: Zilli *et al.,* 2018).

Similarliy, all the isolates of *Trichoderma* and *P. fluorescens* si8gnificantly reduced the sclerotial production, their size and germination. The maximum per cent inhibition in the sclerotial production (74.21), size (70.24), germination (42.62) and the number of germ tubes produced by the sclerotium (4.92) was caused by the isolate Tv_3. Regarding to *P. fluorescens,* the maximum inhibition on the sclerotial production (59.65), size (41.35 per cent reduction), germination (56.37) and the number of germ tubes produced by the sclerotium (5.09) was caused by the isolate Pf_5 (Table 7). The isolate Tv_4 and Pf_2 was proved to be the least effective.

Several workers have reported about the inhibitory effect of *T. viride* and *P. fluorescens* on the number, size and sclerotial germination of *M. phaseolina* (Krishnaveni, 1991; Laha *et al.,* 1996; Rettinasababady and Ramadoss, 2000). Reduction in the production, size and sclerotial germination of *M. phaseolina* by *T. viride* was observed earlier by Aly *et al.,* (2007).

P. fluorescens formed inhibition zones and reduced sclerotial formation, sclerotial size, germination and germ tube formation by *M. phaseolina* (Selvarajan and Jeyarajan, 1996). *P. fluorescens* effectively colonized and reduced the germination of sclerotia of *M. phaseolina* and it was believed that they may act as a potential bio-control agent against root rot (Srivastava *et al*., 2001; Manjunatha *et al.,* 2013).

Table 1: Screening of native isolates of *T. viride* (Tv_3) and *P. fluorescens* (Pf_5) against *M. phaseolina* by Dual culture Technique.

S. No.	Isolates	*T. viride*(Tv_3)		*P. fluorescens* (Pf_5)			
		Mycelial growth of *M. phaseolina* (mm)	Percent inhibition over control	Isolates	Mycelial growth of *M. Phaseolina* (mm)	Per cent inhibition over control	Inhibition zone (mm)
1	Tv_1	20.53	77.18	Pf_1	27.33	69.63	7.53
2	Tv_2	30.56	65.93	Pf_2	28.19	68.67	6.75
3	Tv_3	18.69	79.23	Pf_3	24.38	72.91	9.26
4	Tv_4	35.42	60.64	Pf_4	26.40	70.66	8.42
5	Tv_5	23.56	73.82	Pf_5	22.30	75.22	10.04
6	Control	90.00	—	Control	90.00	-	-
	S. Ed	0.51		S. Ed	0.13		
	CD (p=0.05)	1.21	—	CD (p=0.05)	0.28	—	—

Table 2: Effective of native *T. viride* isolates on number, size and sclerotial germination.

S. No.	Isolates	No. of. Sclerotia	Per cent inhibition	Sclerotial size (m)	Per cent reduction	Sclerotial germination(%)	Per cent inhibition	No. of. Germ tube per Sclerotium^{-1}	Percent reduction
1	Tv_1	94.90	44.13	75.11	37.50	49.46(44.69)	45.73	7.05	49.17
2	Tv_2	105.73	37.75	88.32	26.51	64.85(53.63)	30.22	9.39	32.29
3	Tv_3	74.21	56.31	70.24	41.55	42.62(40.75)	54.14	4.92	64.52
4	Tv_4	134.92	20.56	102.13	15.01	68.78(56.03)	25.99	10.03	27.68
5	Tv_5	102.02	39.93	85.79	28.92	56.09(48.49)	39.64	6.85	50.61
6	Control	169.86	-	120.18	-	92.94(74.59)	-	13.87	-
	S. Ed	0.42		0.62		0.45		0.12	
	CD (p=0.05)	1.091	—	1.65	—	0.99	—	0.28	—

Table 3: Effect of culture filtrate of *T. viride* and *P. fluorescens* the mycelial growth and mycelial dry weight of *M. phaseolina* by Poison food technique.

S. No.		*T. viride* (Tv_3)				*P. fluorescens* (Pf_5)			
Concentration of cultural filtrates		Mycelial growth (mm)	Percent inhibition over control	Mycelial dry weight (mg)	Percent inhibition over control	Mycelial growth (mm)	Percent inhibition over control	Mycelial dry weight (mg)	Percent inhibition over control
1	10	24.33	72.96	200.86	36.10	29.57	65.84	176.96	43.50
2	20	19.46	78.37	120.95	61.52	17.39	75.23	154.92	50.54
3	30	10.13	88.74	49.93	84.12	12.64	80.37	57.65	81.60
4	40	NG	100.00	1.68	99.46	NG	100.00	1.32	99.57
5	50	NG	100.00	1.25	99.60	NG	100.00	1.05	99.67
6	Control	90.00	-	314.34	-	90.00	—	313.25	—
S. Ed		0.54		0.98		0.49		0.52	
CD (p=0.05)		1.96	—	2.13	—	1.11	—	1.45	—

NG-Nil Growth

Table 4: Effective of native *P. fluorescens* isolates on number, size and sclerotial germination.

S. No.	Isolates	No. of. Sclerotia	Percent inhibition	Sclerotial size (m)	Percent reduction	Sclerotial germination(%)	Percent inhibition	No. of. Germ tube per sclerotium^{-1}	Percent reduction
1	Pf_1	80.33	49.55	77.46	28.91	51.03 (45.59)	43.79	10.14	37.62
2	Pf_2	84.25	47.09	79.74	26.85	54.86 (47.78)	39.61	12.94	20.32
3	Pf_3	72.91	52.33	70.06	35.70	45.02 (42.14)	50.44	7.01	56.83
4	Pf_4	81.25	48.97	78.95	27.54	51.96 (46.12)	42.80	9.94	38.79
5	Pf_5	64.78	59.65	63.91	41.35	39.63 (39.01)	56.37	5.09	68.65
6	Control	159.25	-	108.97	-	90.85 (72.39)	-	16.24	-
S. Ed				0.12		0.11		0.13	
CD (p=0.05)		0.150.45	—	0.26	—	0.23	—	0.28	—

While regarding to the culture filtrate, all the culture filtrate of *T. viride* and *P. fluorescens* isolates significantly inhibited the growth of *M. phaseolina.* Generally an increase in the concentration of the culture filtrate reduced the growth of the pathogen. Among the isolates tested the isolate Tv_3 and Pf_5 was found to be most inhibitory to the growth of *M. phaseolina* by recording the least mycelial growth with 24.33, 19.46, 10.13, 0.00, 0.00 mm and 29.57, 17.39, 12.64, 0.00 and 0.00 mm at 10, 20, 30, 40 and 50 percent concentration of the culture filtrate, respectively as against the maximum growth of 90 mm in the control in Poison food technique.

Similarly to the present observations, Suriachandraselvan *et al*., 2004 observed that *T. viride* and *T. harzianum* have the potential antagonists and inhibited the mycelial growth of *M. phaseolina* causing charcoal rot in sunflower. 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, 40 per cent conc. of culture filtrate of *T. harzianum* showed maximum inhibition of *M. phaseolina* (Rashmi Singh *et al.,* 2012). .The fungistatic activity of the fluorescent *Pseudomonas* based on the inhibition of mycelial growth of the pathogen was well established by workers (Josic *et al.*, 2012: Tewari and Arora, 2016 and Meena *et al.,* 2018). Ashwini *et al.,* (2014) reported that among the bio agents test, *Pseudomonas fluorescens* proved best antagonist against *M. phaseolina* (62.41%) by inhibition of mycelial growth. Das *et al.,* (2008) have found that the cell free culture filtrates of fluorescent *Pseudomonas* strains at 20 per cent conc. significantly reduced the formation and germination of microsclerotia of *M. phaseolina* as observed in the present study.

Trichoderma spp 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 (Athira, 2017). The mode of action of these *Trichoderma* includes mycoparasitism, production of antibiotics and secondary metabolites, competition for space and nutrients, and induction of defense responses including systemic resistance responses in the plant (Pastrana *et al*., 2016). Such fungistatics metabolites and some of the enzymes produced *T. viride* could be attributed as the reason for reduction in the number, size and sclerotial germination of *M. phaseolina*.

Production of antibiotics and lytic enzymes by *P.*

fluorescens against fungal pathogens was also reported (Mishra and Arora, 2017) which might also be attributed as the reason for the reduction in the number, size and sclerotial germination of *M. phaseolina*. Hence the production of fungistatic metabolites and antibiotics by the native isolates of *P. fluorescens* could be attributed as the reason for reduction in the number, size and sclerotial germination of *M. phaseolina*.

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