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Evaluation of Bio-control Agents against *Macrophomina phaseolina* Causing Root and Stem Rot Disease of Sesamum

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A B S T R A C T

Sesame (*Sesamum indicum* L.) is aprimitive oilseed crops cultivated in semi-arid Tropics and sub tropics regions in India. *Macrophomina phaseolina* a soil borne fungus cause root and stem rot in sesame crop and pathogen attacks plant at all growth stages. Due to soil borne nature practically no effective field control and no source of resistant is available. A field experiment was conducted with twenty cultivars of sesame during *kharif* sesame 2017. *Trichoderma viride and Pseudomonas fluorescens* was evaluated *in vivo and in vitro,* to assess their antagonistic potential against *M. phaseolina* causing root and stem rot of sesame. Treatment (T_5-Seed treatment *T. viride* + *P. fluorescens* @ 10 g /kg + Soil application of *P. fluorescens* @ 2.5 kg/ha + *T. viride* @ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing) and Treatment (T_3-Seed treatment *T. viride* @ 10g/kg + soil application of *T. viride* @2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing) were found highly effective reducing the disease intensity (12.3%,14.0%) as compared to control (37.5%). The sesame cultivars TKG-306 (9.23%), TKG-21(10.0%) were found resistant to root and stem rot disease.

Introduction

Sesame (*Sesamum indicum* L.) is the oldest among the oilseeds crops cultivated in semi-arid tropics and sub tropics to temperate regions in India. Sesame which was originated in Africa is probably the most ancient oil seed plant cultivated in many parts of the world. Myanmar, India, China are the world's largest producers of sesame in the major sesame growing states are Uttar Pradesh, Rajasthan, Madhya Pradesh Chhattisgarh, Andhra Pradesh, Maharashtra, Gujarat, Tamil Nadu, West Wangal and Orissa Uttar Pradesh, Rajasthan, Madhya Pradesh or Chhattisgarh and contribute about half of the total sesame production of the country. However, a distressing feature is that the productivity of sesame in these states is very low. The main reason for low

productivity of this crop is due to the attack of various weather factors and diseases, such as root and stem rot (*Macrophomina phaseolina*), Alternaria leaf spot (*Alternaria sesam*e), Bacterial blight (*Xanthomonas campestris* pv. *sesam*e), Powdery mildew (*Erysiphe cichoracearum),* Cercospora leaf spot *(Cercospora sesame*) and phyllody *(Mycoplasma)* Gupta *et al.,* (2018). Root and stem rot caused by (*M. phaseolina*) affects severely at all the stages of the crop growth in the country it is grown in 17.14 lakh hactares area with production of 7.84 lakh tonnes and productivity of 457kg/ha during 2016 (Anon, 2017).Stem and root rot is caused by *(M. phaseolina)* is one of the important diseases of this crop all over the world (Rajput *et al.*, 1998; Dinakaran and Mohammed, 2001). It is very serious and destructive in all sesame growing areas and cause 5-100% yield loss and 57% yield loss at about 40% of disease incidence (Vyas, 1981; Maiti *et al.*, 1988). The pathogen attacks root stem, leaf, pod and seeds (Mukharji and bhasin, 1986). The most common symptoms of the disease is sudden wilting of growing plant mainly after the flowering stage, stem portion near the ground level show dark brown and dark black lesion at the collar region show shreding and to destroy the vascular bundles by causing the plant death. Stem portion can be easily pulled out leaving the rotten rot portion in the soil (Avila *et al.,* 1999). *Macrophomina phaseolina* is a seed and soil borne pathogen causing root and stem rot on sesame crops. The disease is very important as infection occurs from seed germination and emergence to adult stage. *M. phaseolina* is an important phytho pathogen distributed worldwide and causes charcoal rot on more than 500 plant species (Das *et al.,* 2008). It is a mithosporic 2 fungal species (Menezes, 1993). It is a member of Phylum- Deteromycetes, Class-Coelomycetes, Order- Sphaeropsidales, and Family Sphaeropsidaceae. Pycnidia are 100-200 µm in diameter, dark brown to greyish, becoming black with age, globose or flattened globose, membranous to sub carbonaceous with an inconspicuous or definite truncate ostiole. The pycnida bear simple, rod-shaped conidiophores, 10-15 µm long. Conidia are 14-33 × 6-12 µm in diameter, single celled, hyaline, and elliptic or oval. The pycniospores are elliptical, thin walled, hyaline, and measure 10-42 x 6-10 µm. Micro sclerotia of fungus are jet black in colour and appear smooth and round to oblong or irregular. Colonies in culture range in colour from white to brown or gray and darken with age. Hyphal branches generally form at right angles to parent hyphae, but branching is also common at acute angles. The fungus is greatly influenced by environmental factors and produces the pycnidia when the atmospheric temperature ranges of 25°C to 35°C. The fungus survives in the form of sclerotia in soil and crop residues and also been reported to be seed born (Maiti *et al.,* 1988). The available literature revealed that not much research work is carried out on sesame disease in Madhya Pradesh particularly on root and stem rot.

Materials and Methods

The present investigation, all the experiments were carried out during *kharif* 2017 in the Department of Plant Pathology and experimental area of PC Unit, AICRP Sesame and Niger, JNKVV, Jabalpur (Madhya Pradesh). For all laboratory experimental studies Corning and Borosil glasswares were used. The glass wares were kept for 24 hrs in cleaning solution containing 60 g of potassium dichromate ($K_2Cr_2O_7$), 60 ml of concentrated sulphuric acid (H_2SO_4) in 1000 ml of water and were washed with detergent powder followed by washing in running tap water and then finally rinsed with distilled water. All the glasswares were sterilized in hot air oven at 160^0C for two hrs. Sterilization of both solid and liquid media was done by

autoclaving at 1.1 kg/cm^2 (121.6^{0}C) pressure for 20 minutes for all the laboratory studies. The earthen pots were sterilized by dipping them in 10 per cent formaldehyde solution for 5 minutes.

Two bio agents viz. *Trichoderma viride* and bacterial bio-gents *Pseudomonas fluorescens* and its combination *tested* against (*M. phaseolina*) by using dual culture technique (Sagar and Sugha, 1997). This test was conducted to evaluate the effect of biocontrol agent's *viz. Trichoderma viride and Pseudomonas fluorescens* against the growth and sclerotia formation of the pathogen *(M. phaseolina)* in *in vitro.* Dual culture technique was used to see the effect of bio control agents in which culture discs of 5 mm diameter of both antagonists and pathogen was cut from 7 days old culture using sterilized cork borer. A disc of pathogen culture was placed in plate poured with PDA opposite to antagonist at a distance 6 cm. In control plate PDA disc were used in place of antagonistic culture. The plates were incubated at 27 ± 20°C. The radial growth of the pathogen was measured after five days. Sclerotia from 10 microscopic fields (100 x) were observed at the interaction zone between the bio-agent and *(M. phaseolina).* Ten sclerotia from each of the field were measured by micrometry. The per cent inhibition was calculated as suggested by Vincent (1947).

$$I = \frac{C - T}{C} \times 100$$

I = Per cent growth inhibition
C = Colony diameter in control (mm)
T = Colony diameter in respective treatment (mm)

This test was conducted to evaluate the most effective bio control agent under field condition, in which various bio control agents was used as seed treatment with recommended dose (Table 1).

Results and Discussion

In vitro efficacy of bio-agents

Among Two isolates of *Trichoderma viride* using dual culture technique Tv-1, (JNKVV Jabalpur isolate) showed 60.22% inhibition on radial growth of *M. phaseolina* (Table 2) whereas other isolate of *Trichoderma viride* Tv-2, (TNAU, Tamilnadu) showed 43.18% inhibition over control. Among two isolates of *Pseudomonas fluorescence* testing using the dual culture technique Pf-1, (JNKVV Jabalpur isolate) showed 48.86% inhibition on radial growth on *M. phaseoli* (Table 2) whereas other isolate of *Pseudomonas fluorescence* Pf-2 (TNAU, Tamilnadu) showed 44.31% inhibition over control hence all tested bio agents were significant effective.

Two isolate of *Trichoderma viride*Tv1 and Tv2 and two isolates of *Pseudomonas fluorescens*Pf1 and Pf2 were tested for the antagonistic effect against *M. phaseolina* by dual culture method. The native isolate Tv1 and pf1 (JNKVV Jabalpur) *Trichoderma viride* and *Pseudomonas fluorescens* was found most effective in inhibiting the radial growth (60.22%) and (48.86%). Significant antagonistic property of *Trichoderma viride* against *M. phaseolina* has been reported by many workers (Deshmukh and Raut (1992), Sindhan *et al.*, (2002) and Karthikeyan *et al.*, (2006), Anju and Verma (2007)). Jeyarajan (1996) reported that *Trichoderma* isolate also reduced the number and size of sclerotia as observed in the present investigation. Native isolate was most effective and has not been reported so far hence, seems to be the first report in this aspect. The antagonistic effect of *Trichoderma spp.* against *M. phaseolina* also has been reported by Parakhia and Vaishnav (1986). The present findings were in consonance with the results of Ahmad and Shrivastava (2000); Vyas and Patel (2015) as

they reported that *T. virid e*and *B. subtilis* were effective against *M. phaseolina* causing dry root rot of chickpea. Similar result was also found by Khirood and Paramjit (2012) while working with *M. phaseolina.* Zape *et al.,* (2014) recorded that *Trichoderma* spp. was very effective in inhibiting the mycelial growth and sclerotial production of *M. phaseolina* and Singh and Verma (2015) also achieved effective growth inhibition of *M. phaseolina* by using bacterial bioagents *B. subtilis.*

Table.1 Detailed of the treatments were included

T_1	Seed treatment *T. viride* 10 g/kg +soil application of *T.viride* 2.5 Kg/ha enriched in 100 Kg of FYM at sowing.
T_2	Seed treatment *P. fluorescens 10* g/kg + Soil application of *P. fluorescens*2.5 Kg/ha enriched in 100 Kg of FYM at sowing.
T_3	Seed treatment *T. viride* 10 g /kg + soil application of *T.viride* 2.5 Kg/ha enriched in 100 Kg of FYM + Oil cake @ 250 kg/ha at sowing.
T_4	Seed treatment *P. fluorescens*10 g /kg + soil application of *P. fluorescens* 2.5 Kg/ha enriched in 100 Kg of FYM + Oil cake 250 kg/ha at sowing.
T_5	Seed treatment *T. viride* + *P. fluorescens*10 g /kg + Soil application of *P. fluorescens*@ 2.5 Kg/ha + *T. viride* 2.5 Kg/ha enriched in 100 Kg of FYM + Oil cake @ 250 kg/ha at sowing.
T_6	Seed treatment with Thiram 2 g /kg + Carbindazim 1 g / kg (2:1) 0.3%.
T_7	Untreated check.

Table.2 Effect of the bio agents against *Macrophomina phaseolina* by Dual Culture technique

Treatments	**Bio-agents**	**Source**	**Mean colony diameter (mm)**	**Percent Inhibition (%)**
T1	*Trichoderma viride (Tv-1)*	JNKVV., Jabalpur	35	60.22
T2	*Pseudomonas fluorescence (Pf1)*	JNKVV., Jabalpur	45	48.86
T3	*Trichoderma viride (Tv-2)*	TNAU (Tamilnadu)	50	43.18
T4	*Pseudomonas fluorescence (Pf2)*	TNAU (Tamilnadu)	49	44.31
T5	Control		88	
S. Em ±			0.57	
CD at (P=0.05%)			1.83	

Table.3 Field performances of various bio-control agents on root rot, yield and B.C. ratio

Treatment	Root and stem rot incidence (%)	Yield (kg/ha)	B:C ratio
T1-Seed treatment *T. viride*@ 10 g/kg + soil application of *T. viride*@ 2.5 kg/ha enriched in 100 kg of FYM at sowing.	20.5 (26.89)	312.77 (312.73)	1.23
T2-Seed treatment *P. fluorescens*@ 10 g/kg + Soil application of *P. fluorescens* @ 2.5 kg/ha enriched in 100 kg of FYM at sowing.	26.6 (31.03)	268.9 (268.96)	0.75
T3-Seed treatment *T. viride*@ 10g/kg + soil application of *T. viride*@ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing.	14 (21.96)	385.4 (385.4)	1.77
T4-Seed treatment *P. fluorescens*@ 10 g/kg + soil application of *P. fluorescens*@2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing.	28 (31.93)	287.2 (287.26)	0.82
T5-Seed treatment *T. viride* + *P. fluorescens*@ 10 g /kg + Soil application of *P. fluorescens*@ 2.5 kg/ha + *T. viride*@ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing.	12.3 (20.45)	402.23 (402.23)	1.81
T6-Seed treatment *T. viride* + *P. fluorescens* @ 10 g /kg + Soil application of P. fluorescens @ 2.5 kg/ha + *T. viride* @ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing.	16.5 (23.95)	380.63 (380.63)	1.71
T-7 Untreated check	37.1 (37.5)		133.33
S.Em ±	4.03		1.71
CD at (P = 0.05%)	11.34		3.06
Cv at (5%)	1.98		9.44

In vivo efficacy of bio-agents

Data Presented in (Table 3) reveal that all tested treatments were significantly effective and highly seed yield over, T5-Seed treatment with *T. viride* + *P. fluorescence* @ 10 g /kg + Soil application of *P. fluorescence* @ 2.5 kg/ha + *T. viride* @ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing recorded minimum disease incidence (12.3%), maximum yield (402.23kg/ha) and Benefit cost ratio (1.81) followed by Treatment T3-Seed treatment *T. viride* @ 10g/kg + soil application of *T. viride* @ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing recorded minimum disease incidence (14%) maximum yield (385.4 kg/ha) cost benefit ratio 1.77 over control.

Field performance of Effective biocontrol agent under field condition was studied during 2017-2018.The result indicated that all the treatment had higher germination performance as compared to control treatment T5 (Seed treatment *T. viride*+ *P. fluorescence* @ 10 g /kg + Soil application of *P. fluorescence* @ 2.5 kg/ha + *T. viride*@ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake @ 250 kg/ha at sowing respectively) recorded minimum incidence (12.3%), maximum yield (402.23kg/ha) and higher Benefit cost

ratio1.81 followed by T3 (Seed treatment *T. viride* @ 10g/kg + soil application of *T. viride* @ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing) recorded minimum incidence (14%) maximum yield (385.4 kg/ha) and cost benefit ratio 1.77 over control. Kheri and Chandra (1991) recorded that *T. konigii* was inhibitory effect against dry root rot of mung caused by *M. phaseolina.* Chung and Choi, (1990) also observed that *T. viride, T. harzianum* and *B. subtilis* have strong biocontrol effect against *Macrophomina phaseolina* causing stem and root rot in sesame. Afouda *et al.*, (2012) in cowpea against root rot (*M. phaseolina)* pathogen in field, who reported that *B. subtilis* showed strongest antagonistic activity against *M. phaseolina*. In the field experiment seed treatment and soil application of *T. viride* reduced the incidence of root and stem rot. The present result is in accordance with reports of Dinakaran *et al.,* (1995), Sanker and Jeyarajan (1996), Nair *et al.,* (2006).Gupta *et al.,* (2016).

In conclusion the efficacy of bioagentsTv-1 and pf_1 (JNKVV Jabalpur isolate) showed maximum inhibition on radial growth of *M. phaseolina.* Field performance of bioagents ware found significantly effective and higher seed yield. Treatment T_5-Seed treatment *T. viride* + *P. fluorescence* @ 10 g /kg + Soil application of *P. fluorescence* @ 2.5 kg/ha + *T. viride* @ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing was recorded minimum disease incidence (12.3%),maximum yield (402.23kg/ha) and Benefit cost ratio (1.81) followed by Treatment T_3-Seed treatment *T. viride* @ 10g/kg + soil application of *T. viride* @ 2.5 kg/ha enriched in 100 kg of FYM + Oil cake) @ 250 kg/ha at sowing recorded minimum disease incidence (14%) maximum yield (385.4 kg/ha) and cost benefit ratio 1.77 over control.

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