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BIOLOGICAL CONTROL OF *MACROPHOMINA PHASEOLINA* CAUSING SESAME (*SESAMUM INDICUM* L.) ROOT ROT

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ABSTRACT

In vitro evaluation of different fungal and bacterial bioagents revealed that all the bioagents showed more than 64 % growth inhibition and also reduce the sclerotial formation. Among them *Bacillus subtilis* showed maximum mycelial growth inhibition (87.03%) and scanty sclerotial formation followed by *Trichoderma viride* and *T. hamatum* with 71.48 % and 70 % growth inhibition respectively. While *Pseudomonas fluorescens* gave poor antagonistic activity and least growth inhibition (64.07 %) and moderate sclerotial formation against test pathogen. *T. koningii* and *T. harzianum* was also found less effective with 68.14 % and 67.40% mean mycelial growth inhibition. *B. subtilis* also proved their worthiness in field conditions by reducing disease. The least disease incidence (18.93%) and higher yield (680 kg/ha) was recorded in treatment of *B. subtilis* which is followed by the treatment of *T. koningii* (25.43%) and *T. hamatum* (26.90 %). *T. viride* and *T. harzianum* found less effective in field condition with 28.42 % and 30.02 % disease incidence and gave 579 kg/ha and 510 kg/ha yield respectively.*P. fluorescens* were also found least effective under *in vivo* condition which showed 30.93% disease incidence.

INTRODUCTION

Sesame (*Sesamum indicum*L)is important oilseed crop of India. Sesame cultivation has existed for several hundred years as a sustainable form of agriculture in India and many other countries. This crop is significant source of income, making India the world's single largest producers and exporters country in the world. Sesame crop is affected by many diseases like as; *Alternaria* leaf blight, *Cercospora* leaf spot, wilt, stem blight, powdery mildew, bacterial leaf spotand Phyllody *etc*. Among all the diseases, root rot caused by *Macrophomina phaseolina*is a major problem in India and one or more significant economic constraints to sesame production worldwide. This disease also causes severe losses right from seedling to maturity of the crop (Khan, 2007).

Sesame root rot pathogen *M. phaseolina* has wide host range and its capacity to produce sclerotia, pycnidia and pycnidiospores that may persist in soil for several years (Dhingra and Sinclair, 1973; Rettinasababady *et al.,* 2000; Pande *et al*., 2004). Hence management of sesame root rot is difficult to achieve only with chemically. Frequent application of fungicides also causes environmental pollution. In this context bioagents can be used as analternative source for controlling the diseases sincethey comprise a rich source of bioactive substance and also eco-friendly to environment.

Several workers have attempted to control *M. phaseolina* in various crops by using different biocontrol agents (Ahmad and Shrivastava, 2000; Indra and Tribhuvanmala, 2002). However, information is lacking for effectively management of root rot disease of sesame by various bioagents. Hence the present investigation was under taken. The objective of this study was to evaluate various fungal and bacterial bio agents against *M. phaseolina in vitro* condition for testing their efficacy to inhibit mycelial growth and sclerotial formation and also *in vivo* condition to control the disease.

MATERIALS AND METHODS

In vitro evaluation of bioagents against M. phaseolina

Under *in vitro* conditionfour fungal bioagents *viz.*, *Trichoderma harzianum*, *T. viride*, *T. koningii, T. hamatum* and two bacterial bioagents *Pseudomonas fluorescens* and *Bacilus subtilis* were tested against sesame *M. phaseolina* causing sesame root rot by using dual culture technique (Sagar and Sugha, 1997; Bhaliya and Jadeja, 2013).

Twenty milliliter of sterilized and cooled potato dextrose agar was poured into sterile petriplates and allowed to solidify. Themycelial disc of test fungus was inoculated at one end and antagonistic fungus opposite to it. In case of evaluation of bacterial antagonist, the bacterium was streaked one day earlier at one end of the petriplate and the test fungus was placed at the other end. Each treatment was replicated thrice. The plates were incubated at 30 ± 1ºC for five days and zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism (Sinclair and Dhingra, 1985). The colony diameter of pathogen in control platewas also recorded. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947).

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

Where,

I = Per cent growth inhibition

C = Colony diameter in control (mm)

T = Colony diameter in respective treatment (mm)

Sclerotial formations were counted in fungal culture suspensions under the microscope at low power (10X). The fungal culture suspension was prepared by vigorously shaking the 4 mm mycelial disc of the fungus in 10 ml sterilized distilled water. The relative degree of formation of sclerotia was recorded as described by Sinclair and Dhingra, 1985. (Table. 1)

In vivo* (Field) evaluation of bioagents against *M. phaseolina

A field trial was conducted in the sick plot at Plant Pathology Department farm, JAU, Junagadh.

A susceptible variety GT-3 (Gujarat Til-3) was used for the study. Six treatments of all bioagents with one check were laid out in randomized block design. The treatments were randomized block wise having plot area of 5.0 x 2.7 sq.m. and replicated thrice. The inoculum of pathogen was applied in furrow at time of sowing @ 50gram/plot (13.59 sq.m) (10^5cfu/g) mixed in 500gram of farm yard manure. Sesame seeds were sown to each plot at recommended seed rate (2.5 kg/ha) keeping distance of 45 x 15 cm. The crop was thinned after plants attained age of 21 days to maintain appropriate plant stand. The talc base formulation of fungal bioagents and bacterial antagonists were applied in furrow just before the time of sowing @ 2.5 kg/ha mixed in 300 kg FYM. The *Trichoderma* spp. was grown on sterilized half cooked sorghum seed and incubated for six days. This culture was mixed in talc and soluble starch @ 1:1 to get approximately 10^6cfu/g in the formulation. While bacterial antagonists were grown in nutrient broth for 72 hrs and mixed to get approximately 10^9cfu/g in the formulation. The disease incidence was calculated as per following formula (Vidhyasekaran and Muthamilan, 1995). Seed yield was recorded after harvesting the crop.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \times 100$$

RESULTS AND DISCUSSION

In vitro* evaluation of bioagents against *M. phaseolina

Six bioagents were evaluated for their efficacy against *M. phaseolina* through dual culture techniques as explained in material and methods. The results of the study are presented in Table 2. The perusal of results showed that (Table 2) all the bioagents were effective and gave more than 64 per cent mycelial growth inhibition of test fungus. *B. subtilis* gave maximum growth inhibition (87.03%) tested in present investigation. *T. viride*(71.48%) was found next in order of mycelial growth inhibition followed by *T. hamatum* (70%). Similarly scanty sclerotial formation found in the treatment of *B. subtilis* while moderate sclerotial formation recorded in treatment of *T. viride*. *T. koningii* and *T. harzianum* was showed week antagonistic activity toward the pathogen with 68.14 % and 67.40 % mycelial growth inhibition respectively. Among all the bacterial bioagents *P. fluorescens* gave least growth inhibition (64.07 %) with moderate sclerotial formation. Sclerotial formation was reduced in each bioagents treatment. This results is in agreement with finding of Jharia and Dunoon (2000), they found that *B. subtilis* gave maximum mycelial growth inhibition of *M. phaseolina* causing root rot of sesame. Similarly, Singh *et al.* (2008) recorded that *B. subtilis* exhibit strong antagonistic activity against *M. phaseolina*. The antagonistic effect of *Trichoderma* spp. against *M. phaseolina* also reported by Parakhia and Vaishnav (1986). This finding is also in consonance with the results of Ahmad and Shrivastava (2000); Vyas and Patel (2015) as they reported that *T. viride* 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 *Rhizoctonia solani*. Singh and Verma (2015) also achieved effective growth inhibition of *M. phaseolina*by using bacterial bioagents *B. subtilis*.

In vivo* (field) evaluation of bioagents against *M. phaseolina

Total six biocontrol agents were evaluated under field condition. The observed data are presented in Table 3. The data present in table showed thatsoil application of *B. subtilis* at the time of sowing reduce disease incidence. Minimum disease incidence (18.93%) and maximum yield 680.47 kg/ha was recorded in the treatment of *B. subtilis* as compare to control. Among the treatments of various *Trichoderma* spp. the maximum reduction of the root rot incidence (25.43%) was recorded in *T. konigii* which is followed by *T. hamatum* with 26.90 % disease incidence and 609 kg/ha yield. The maximum disease incidence (30.93%) was found in the treatment of *P. fluorescens* with minimum yield (536.89 kg/ha) as compared with other biocontrol agents. The *P. fluorescens* and *T. harzianum* were found ineffective in field condition, as they were at par with control. Our finding are in consonance with those reported by Afouda *et al.* (2012) in cow pea against root rot (*M. phaseolina*) pathogen in field, who reported that *B. subtilis* showed strongest antagonistic activity against *M. phaseolina* and treatment of *B. subtilis* strain A11 reduced disease incidence 89.29% over the untreated control plants. Similarly, Kheri and Chandra (1991) recorded that *T. konigii* effective 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

Table 1: Different Grade and Sign given as per to Sclerotia formed by *M. Phaseolina*

No. of sclerotia per microscopic field (10X)	Grade	Sign
0	Absent	-
1-4	Scanty	+
5-8	Moderate	+ +
9-15	Good	+ + +
>15	Abundant	+ + + +

Table 2: Effect of different bioagents on the growth and sclerotial formation of *M. phaseolina*

Sr. No.	Biocontrol agents	Sclerotial formation	Per cent inhibition over control
1	*Bacillus subtilis*	+	87.03
2	*T. viride*	+ +	71.48
3	*T. hamatum*	+ + +	70.00
4	*T. koningii*	+ + +	68.14
5	*Trichoderma harzianum*	+ +	67.40
6	*Pseudomonas fluorescens*	+ +	64.07
7	Control		-
	S. Em. ±	1.602	
	CD at 5%	4.862	
	CV %	4.54	

Table 3: Field performance of various biocontrol agents against *M. phaseolina*

Sr. No.	Biocontrol agents	Per cent disease incidence	Yield (kg / ha)
1	*Bacillus subtilis*	18.93(10.53)	680
2	*Trichoderma konigii*	25.43(18.44)	585
3	*T. hamatum*	26.90(20.48)	609
4	*T. viride*	28.42(22.65)	579
5	*T. harzianum*	30.02(25.02)	510
6	*Pseudomonas fluorescens*	30.93(26.42)	537
7	Control	32.96(29.61)	507
	S. Em. ±	1.30	28.36
	C.D. at 5%	3.89	87.38
	C.V.%	10.46	8.59

potential against *M. phaseolina* causing root rot of sesame and also reduced the disease incidence significantly. Soil application of *Bacillus* spp. reduce root rot (*M. Phaseolina*) disease severity (62.90 %) in the green house as well as filed test (Bhattacharyya *et al.,* 2014; Singh and Verma, 2015). Similarly Vyas and Patel (2015) reported that soil application of talc base formulation of *B. subtilis* reduce root rot disease in chick pea caused by*M. phaseolina*.

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