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Short Note

Evaluation of bioagents against *Sclerotinia sclerotiorum* causing Sclerotinia rot of Indian mustard

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Abstract

Among the fungal diseases, Sclerotinia rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is the most destructive disease of Indian mustard [*Brassica juncea* (L.) Czern & Coss.], and its management with chemicals is not economically viable, and environmentally safe. Therefore, the present investigations were aimed to carry out the test of bioagents against *S. Sclerotiorum* under *in-vitro* and *in-vivo* conditions. *In-vitro,* screening of bioagents (*Bacillus subtilis, Trichoderma harzianum* and *T. viride*) was done by dual culture technique. All the bio agents viz., *Bacillus subtilis, T. harzianum,* and *T. viride* were antagonistic to the growth of *S. sclerotiorum.* Maximum 69.8 % mycelial growth inhibition of pathogen was recorded with *T. viride,* and minimum mycelial growth inhibition was recorded by *Bacillus subtilis* (42.2%). Results under field conditions revealed that minimum disease intensity (26.0 %) was recorded with seed + soil application of *T. viride* followed by soil application of *T. viride* (35.7 %) as compared to control (68.3 %).

Key words: *Bio-agents, evaluation, Indian mustard, Sclerotinia rot*

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important oilseed crop of family *Crucifereae* (*Brassicaceae*). This family contains many important species yielding high quality edible, and industrial oils, common vegetables, and weeds. The largest cultivation of *Brassica* crops is done for edible vegetable oil production. Mustard is the second most important oilseed crop of India after groundnut in terms of area and production. The black and brown seeds possess about 40 per cent oil content. Indian contribution in global rapeseed and mustard production is 7.8 million tonnes with an area of 6.5 million hectares, and average productivity of 1208 kg/ha (Anonymous, 2013-14a). Rapeseed-mustard crops are extensively grown in northern and western parts of India viz., Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, Gujarat, West Bengal, Assam, Bihar, Punjab, and Jammu & Kashmir. Among these, Rajasthan state ranks first both in area, and production i.e., 2.78 million ha, and 3.62 million tonnes, respectively with an average productivity of 1301 kg / ha (Anonymous, 2013-14b).

The attack of diseases and pests is the most important factor causing yield instability in rapeseed and mustard. The severe attack of many diseases and insects not only deteriorates the quality of the seed, but also reduces the oil content considerably. More then thirty diseases are known to occur on *Brassica* crops in India (Saharan *et al*., 2005). High incidence and severity of the disease has led to discouragement of the farmers. Among these diseases, Sclerotinia rot (SR) is the most destructive. Sclerotinia rot incited by *Sclerotinia sclerotiorum* (Lib.) de Bary, is the most ubiquitous, omnivorous, soil-borne, and destructive plant pathogen, more than 500 plant species (Saharan and Mehta, 2008 and Sharma, 2014). Yadav *et al*. (2013) reported 17.4 per cent diseases incidence from six districts of Rajasthan. Management of this disease mainly

depends on fungicides. However, fungicidal application is hazards to human health, and increases environmental pollution. Therefore, alternative eco-friendly approaches for control of Sclerotinia rot of Indian mustard were required. Keeping in view all these facts, the investigations were carried out with the use bioagents for the management of Sclerotinia rot of Indian mustard.

Materials and Methods

Isolation

Stem rot infected plants of Indian mustard were collected from farmer's field, and isolations were made on potato dextrose agar (PDA) medium from black sclerotia present inside the diseased stem as well as from individual stem rot lesion as per procedure, and purified by hyphal tip method. The pure culture of pathogen was maintained on PDA slants in a refrigerator at 20 ± 2ºC, and renewed after every fifteen days for further studies.

In vitro efficacy of bioagents

In vitro, screening of bio agents (*Bacillus subtilis, Trichoderma harzianum,* and *T. viride)* was done by dual culture technique (Dennis and Webster, 1971). All the bioagents were obtained from Department of Plant Pathology, RARI, Durgapura, Jaipur, India. Single colonies of the isolate were sub-cultured on PDA, and stored in refrigerator to maintain their genetic purity. Fifteen ml of PDA medium was poured into sterile Petri plate, and allowed for solidification. 5 mm dia. discs from actively growing colony of pathogen was cut with a sterile cork borer, and placed near the periphery of PDA plate. Similarly, bio agents were placed on the other side *i.e.,* at an angle of 180º. Plates with no antagonists served as control for the pathogen. The plates were incubated at 25 ± 1ºC for seven days. In each treatment five replications were maintained. The extent of antagonistic activity by bioagent was recorded after incubation period of 7 days by measuring the growth of the test pathogen in dual culture, and in control plates. The bioagent found effective were used in *in-vivo* studies. The per cent inhibition of pathogen was calculated using formula as suggested by Bliss, 1934.

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

Where,
I = Per cent mycelial growth inhibition
C = Growth of fungal plant pathogen in control (mm)
T = Growth of fungal plant pathogen in dual culture plate (mm)

In vivo efficacy of *Trichoderma viride*

Field studies were conducted at Department of Plant Pathology (SKNAU, Jobner) to manage the Sclerotinia rot through *T. viride* during Rabi season of the year 2014-15 under artificial soil inoculation conditions. The field study consisted of four different treatments of *T. viride,* and control with five replications using mustard variety Varuna (T-59) in RBD design with a plot size of 1.0 m sq. *T. viride* was added @ 6 g/plot. The talc based formulation of *T. viride* formulation ($2x10^8$ cfu) procured from market was used as seed application, soil application, and seed + soil application. The pathogen multiplied on sorghum grains at 25 ± 1ºC for one week was used as soil inoculum. Sowing was done using *T. viride* as seed treatment @ 10 g/kg seed, and soil application @ 2.5 kg/ha pre-incubated in 50 kg well decomposed farm yard manure for fifteen days. The row spacing (30 cm), and plant spacing (10 cm) were maintained 10 days after sowing (DAS). To protect the crop from aphids, oxy-demeton methyl (0.1%) was sprayed. The observations for per cent disease intensity of Sclerotinia rot on stem lesions basis (0-4 scale) were recorded 20 days before the maturity of crop (Meena *et al*., 2013).

The plants showing even a minute lesion of stem rot symptoms were considered as a diseased plant. Disease rating (0-4) scale of Lesovoi *et al*. (1987), and Sansford (1995) with a slight modification was followed to assess disease intensity as: 0 = Healthy (no visible lesion); 1 = 0.1 – 2 cm lesion length on stem; 2 = 2.1 – 4.0 cm; 3 = 4.1 – 6.0 cm; 4 = > 6.1 cm lesion length on stem or complete dried plant. The length of lesion on infected stem was considered for recording the disease intensity. The infected area was calculated from 15 randomly selected plants in each plot, and the average for each treatment was

worked out. The per cent disease intensity was calculated using the formula of Wheeler (1969) :

$$\text{Per cent Disease Intensity} = \frac{\text{Sum of individual ratings}}{\text{No. of plants observed x Maximum disease rating}} \times 100$$

$$\text{Per cent disease control} = \frac{\text{Disease in control} - \text{Disease in treatment}}{\text{Disease in control}} \times 100$$

Results and Discussion

In vitro efficacy of bioagents

Efficacy of *Bacillus subtilis, Trichoderma harzianum,* and *T. viride* was tested against *S. sclerotiorum* (dual culture technique). After 7 days of incubation at 25 ± 1ºC mycelial growth inhibition was recorded. Results (Table 1) indicated that all the bio agents viz., *Bacillus subtilis, T. harzianum,* and *T. viride* were antagonistic to the growth of *S. sclerotiorum.* Maximum, 69.8% mycelial growth inhibition of pathogen was recorded by *T. viride* followed by 61.8 % in *T. harzianum,* and minimum by *Bacillus subtilis* (42.2%).

Our observations are in agreement with Srinivasan *et al*. (2001) and Shivpuri and Mali (2009). They reported that *T. viride* was significantly superior in reducing mycelial growth of *S. sclerotiorum.*

Table1: Efficacy of bioagents on mycelial growth inhibition of *S. sclerotiorum* by dual culture technique after 7 days at 25 + 1 ^{0}C (*in vitro*)

Bio agent	Per cent mycelial growth* inhibition
Bacillus subtilis	42.2 (40.5)
Trichoderma harzianum	61.8 (51.8)
Trichoderma viride	69.8 (56.7)
Control	0.0 (0.0)
SEm+	0.74
CD (p=0.05)	2.56

* Average of five replications
Figures given in parentheses are angular transformed values

In vivo efficacy of *T. viride*

Among treatments, seed-cum-soil treatment of *T. viride* was found most effective to control the disease with reduced disease intensity, followed by soil treatment, and seed treatment alone. Results (Table 2) revealed that minimum disease intensity (26.0%) was recorded with seed + soil application of *T. viride* @ 10kg/kg seed, and 2.5 kg/ha, respectively, followed by soil application of *T. viride* @ 2.5 kg/ha (35.7%) as compared to control (68.3%). Maximum reduction in disease intensity over control was observed with seed + soil application of *T. viride* (61.9%) followed by soil

Table 2 : Efficacy of *Trichoderma viride* against Sclerotinia rot of Indian mustard *(in vivo)*

Treatments	Disease* intensity (%)	Disease control (%)
Seed application @ 10 g/kg seed	44.3 (41.7)	35.1
Soil application @ 2.5 kg/ha	35.7 (36.7)	47.8
Seed @ 10 g/kg seed + soil application @ 2.5 kg/ha	26.0 (30.7)	61.9
Control	68.3 (55.8)	0.0
SEm+	1.04	-
CD (p=0.05)	3.20	-

* Average of five replications
Figures given in parentheses are angular transformed values

application of *T. viride* (47.8 %). Minimum per cent disease control (35.1%) was observed in seed treatment by *T. viride* , alone. These results are in agreement with the results of Rabeendran *et al*. (1998), Dutta *et al*. (2008), Gaur *et al*. (2010), and Yadav *et al*. (2015). They reported efficacy of various *Trichoderma sp*. for *S. sclerotiorum* disease control.

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