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ISOLATION, CHARACTERIZATION AND MANAGEMENT OF *SCLEROTINIA SCLEROTIORUM* (LIB.) DEBARY: CAUSING WHITE MOLD DISEASE ON EGGPLANT

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ABSTRACT

Sclerotinia blight caused by *Sclerotinia sclerotiorum* is a major threat to Brinjal production. To manage this disease, a study was conducted to evaluate the efficacy of different treatments, including biocontrol agents, fungicides, intercropping and mulching. All the treatments showed significant efficacy in reducing disease severity and increasing yield. The most effective treatment was Carbendazim 50WP at 0.1% concentration with a minimum disease severity of 9.50% after the first foliar spray. The other treatments, including *Trichoderma harzianum*, *Pseudomonas fluorescens*, Fenugreek intercropping, Neem leaf mulching and Foliar Spray with Panchgabya, also showed promising results. After the second spray, all treatments were found to be significantly superior to the control group. Maximum disease control (64.10%) was observed in Carbendazim 50WP at 0.1% concentration, followed by biocontrol agents and intercropping. These findings suggest that Carbendazim is a promising treatment for managing Sclerotinia blight of Brinjal and other treatments can also be effective. Biocontrol agents and intercropping can be used as an alternative to chemical fungicides to manage Sclerotinia blight, which can reduce the harmful effects of chemical treatments on the environment and human health.

Key words : Sclerotinia blight, Fungicides, *Trichoderma harzianum*, *Pseudomonas fluorescens*, Panchgabya.

Introduction

Brinjal, also known as eggplant, is an important vegetable crop grown throughout the year in many Asian countries, including India, where it is the second largest producer after China. It provides vital vitamins, minerals, and dietary fiber to the human diet and is also used in Ayurvedic medicine for curing diabetes. During 2017-2018, it was cultivated in 0.73 million hectare area in India with a production of 12.80 million tones and productivity of 19.15 tones/ha. In Uttar Pradesh, the productivity was higher, where it was cultivated in 0.03 million hectares with a production of 1.06 million tons (Anonymous, 2017). Sclerotinia blight is a significant disease that affects the quality and quantity of brinjal fruits. The disease incidence can be as high as 47.3% in greenhouse conditions. Effective measures need to be taken to control and prevent the spread of this disease for a healthy and productive crop yield (Iqbal *et al*., 2003 and Bairwa *et al*., 2020). It is reported that treatment with isolate of *Trichoderma harzianum* and *Allium sativum* clove extract caused significant increase in seed germination and radical length of Indian mustard by reducing Sclerotinia rot (Chattopadhyay *et al*., 2007). It is found that *Allium sativum* and *Eucalyptus globosus* are effective in preventing the spread of stem rot in Indian mustard caused by *S. sclerotiorum*; suggests that botanicals may be a viable alternative to chemical fungicides for controlling plant diseases (Yadav, 2009). Sclerotinia rot in plants has been difficult to manage due to resistance against chemical methods. However,

alternative options like garlic bulb extracts and bio-agents/ biological control can be effective. As research continues, sustainable methods that promote plant and environmental health should be explored (Meena *et al.*, 2013).

Materials and Methods

Collection of Diseased Plant material

Infected Brinjal plants showing typical symptoms of white mold including white cottony fungal mycelial growth, sclerotia on the leaves, petiole and stems were collected from the Brinjal fields of the Main Experiment Station (MES), Ayodhya (U.P) for isolation, characterization, inoculation and severity assessment.

Fig. 1 : Infected brinjal plant showing typical symptoms of sclerotinia white mould.

Isolation and purification of Pathogen

The pathogen was isolated on Potato dextrose agar (PDA) medium followed surface sterilized with 0.1% $HgCl_2$ for 30 second and inoculated at 20 to 25^{0}C for 5-6 days. When the fungal colony developed a small cut of single mycelium was transferred on another Petriplate containing PDA medium to obtain pure culture. The fungus was purified by hyphal tip method (Riker and Riker, 1936). The culture was maintained in refrigerator at 4^{o}C and renewed after every fifteen days. Identification of isolated associated fungi was identified as *Sclerotinia sclerotiorum* on the basis of morphological and colony characters. Sorghum grains were used to multiply the test isolates of *Sclerotinia sclerotiorum*. Sorghum grains were used to multiply the test isolates of *Sclerotinia sclerotiorum*. Sorghum grains were initially soaked in water (1-2 hrs.), then drained off the excess water, filled 100 g sterilized sorghum grain in 500 ml Erlenmeyer flasks, plugged with non-absorbent cotton and autoclaved at 15 psi at 121°C for 30 minutes. After cooling at room temperature, these Erlenmeyer flasks were inoculated separately with mycelial discs of 7 days old culture of *Sclerotinia sclerotiorum* under aseptic condition (Laminar-airflow Cabinet) and inoculated at 25 ± 1^{0}C temperature for one week or until the sorghum grains completely covered with *S. sclerotiorum* growth.

Morphological characterization of the isolated Fungal pathogen

Morphological characteristics of the isolated pathogens such as mycelial growth pattern, sclerotial characters, conidia and conidiophores production, nature of apothecia and ascospores were studied for the identification of the pathogen. Single hyphal tips of fungal isolate were transferred onto new PDA dishes and cultured for a week. Colony characteristics and sclerotial morphology of the isolate were studied with naked eye. Permanent slides were prepared from colony and examined under light microscope to observe the fungal structures and growth pattern at different magnification.

Pathogenicity assessment

For testing the pathogenicity, isolated *S. sclerotiorum* was purified in fresh culture medium of PDA. The mycelial disc of this fresh culture was inoculated near rhizospheric of the Brinjal host plant. Pots containing sterilized soil were kept as a control. Observations regarding the symptoms of sclerotinia blight were weekly observed from leaves, stems and fruits.

Isolation of *Trichoderma* and *Pseudomonas* isolates

Soil samples were collected from various ecological habitats of Brinjal crops from Ayodhya district to isolate *Trichoderma* and *Pseudomonas* species (Fig. 2). For isolation of the *Trichoderma* spp., sieved soil samples in sterilized distilled water were serially diluted up to five-fold (10^{-5}), 0.5 ml of the diluted sample was poured onto the surface of the Trichoderma Specific Medium (TSM) and incubated at 28 ± 2°C for 96 hours (Kumar *et al.*, 2012). Morphologically distinct colonies that appeared on the plates were further purified in the Potato Dextrose Agar (PDA) medium.

For *Pseudomonas* spp., 1 ml of the suspension-sieved soil sample and serially diluted up to 10^{-7} dilution using a sterile saline solution. They inoculated the diluted samples into tubes containing 10 ml of nutrient broth and incubated at 35°C for 24 hours (De Britto *et al.*, 2020). After the incubation, an aliquot of 100 µl was spread plated on Nutrient Agar (NA) medium and incubated at 35°C for 24 hours. Finally, they purified the fully grown colonies using the quadrant streak method. Purified culture was stored at 4°C until needed for further use.

Management of Sclerotinia Blight of brinjal

The present investigations were carried out during *Rabi* (2019-2020) at Students Instructional Farm (SIF), Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya (U.P.), India. The experiment was designed using a randomized block design (RBD) with plot size of 2 × 3 m^2 with 3 replications included 7 treatments with the spacing of 60 × 45 cm. The effectiveness of different treatment combination against Sclerotinia blight of Brinjal (Table 1) were applied as foliar sprays at 15 days intervals with first spray at 75 after transplanting and second sprays were given at 90

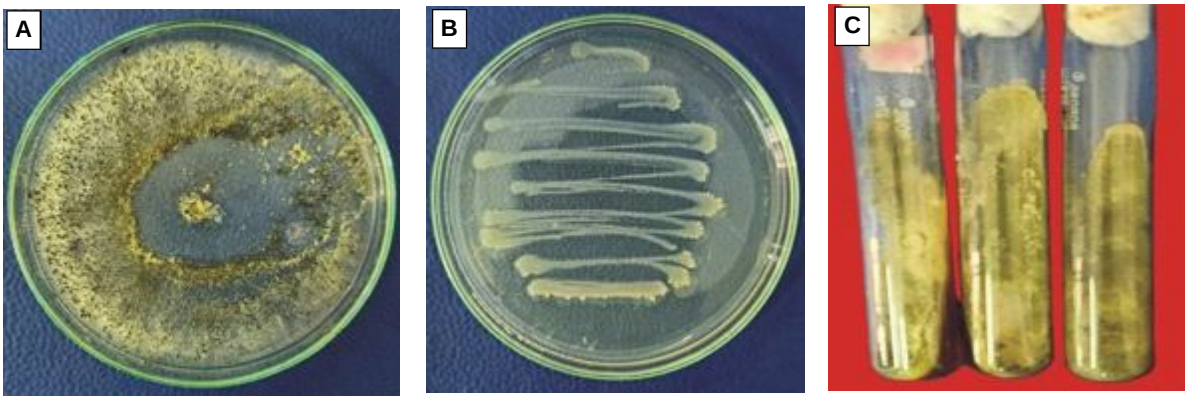

Fig. 2 : Pure culture of *Trichoderma* (A) and *Pseudomonas* spp. (B and C).

days after transplanting except control plot. Percent disease severity and Percent disease control was recorded in all the treatments with fallowed recommended agronomical field practices. The percent disease control was calculated by using following formula:

Disease Control (%) = C-T/C × 100

Where,

C = Per cent disease incidence in control (without control).

T = Per cent disease incidence in treatment.

Table 1 : Details of different treatment combination.

S. no.	Treatment	Concentration
T_1	Soil application with *Trichoderma harzianum*	0.4%
T_2	Soil application with *Pseudomonas fluorescens*	0.5%
T_3	Foliar spray with Carbendazim 50 WP	0.1%
T_4	Inter cropping with Fenugreek	N/A*
T_5	Neem leaf mulching	N/A*
T_6	Foliar spray of Panchgabya (Cow urine + cow dung + curd + milk + ghee)	0.3%
T_7	Control	-

*No applicable concentration.

Results and Discussion

Pathogenicity and characterization isolated pathogen

For testing the pathogenicity, the isolated *S. sclerotiorum* was purified in fresh culture. The mycelial disc of this fresh culture was inoculated on the Brinjal host plant near the rhizospheric area. Pots containing only the sterilized soil were kept as a control. After some days the suspected pathogen produced the same symptom (Fig. 3), which was present in the original host plant. The pathogen was re-isolated from these infected seedlings on PDA under aseptic condition and culture of the re-isolated fungus was found identical to original one. Similar findings were observed by Iqbal *et al*. (2003) and Bairwa *et al*. (2020).

Fig. 3 : Infected Eggplant stems showing white fluffy mycelial growth.

Sclerotinia sclerotiorum, the pathogen responsible for sclerotinia blight of Brinjal was isolated on PDA show uniform growth of whitish to gray-colored mycelium without zonation with rapid coverage of the entire Petri plate within 72 hours. Glossy water droplets were appeared frequently around the mycelial tuft in culture plates and the formation of hard black oval to irregular shape sclerotia (Fig. 4) were formed and measured 2.3-8.5mm (length) × 2-5.5 mm (width) in size. Microscopic characteristics included hyaline and branched hyphae, barrel-shaped asci arranged on the periphery of ascocarp, and elliptical to oval ascospores. Pones *et al*. (1979), pathogen was isolated on brinjal and observed that white mycelium with hyaline, branched with septate hyphae, Husain and Choudhary (2018).

Management of *Sclerotinia sclerotiorum* causing White Mold Disease on eggplant

A study was conducted to evaluate the efficacy of seven different treatments in controlling Sclerotinia blight in Brinjal. The treatments tested were *Trichoderma harzianum* (0.4%), *Pseudomonas fluorescens* (0.5%),

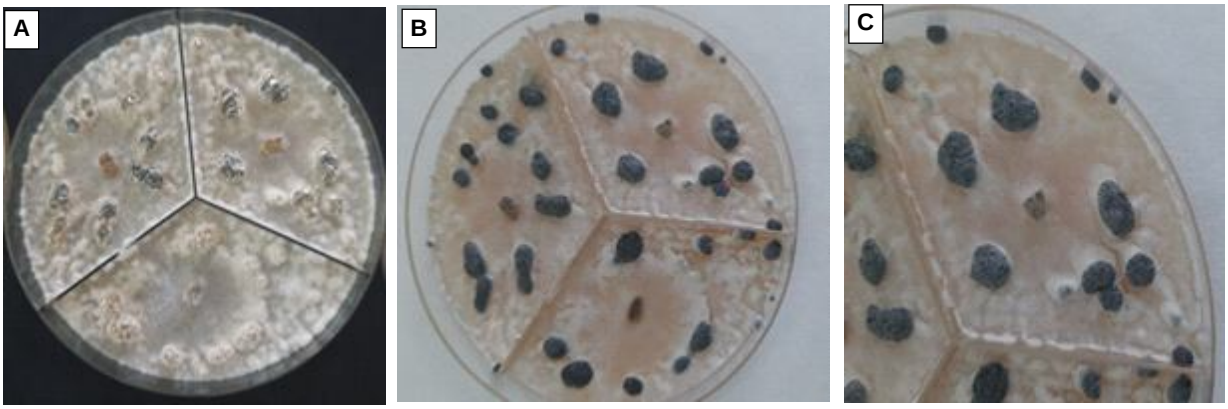

Fig. 4 : *Sclerotia sclerotiorum* (A) white fungal mycelium representing a colony of Sclerotinia and beginning of the formation of stone bodies gathering the fungal mycelium in the form of white clumps (B) black hard sclerotia (C) distribution of Sclerotia on culture medium.

Table 2 : Efficacy of different treatments against Sclerotinia blight of Brinjal (after first spray).

S. no.	Treatments	Percent Disease Severity	Percent Disease Control
1	**T_1**	11.99 (20.23)	54.32
2	**T_2**	14.88 (22.67)	43.31
3	**T_3**	9.50 (17.93)	63.80
4	**T_4**	16.87 (24.33)	35.73
5	**T_5**	15.66 (23.29)	40.34
6	**T_6**	16.33 (23.82)	36.79
7	**T_7**	26.25 (30.80)	0
	S Em	0.32	
	CD	**0.97**	

*Data in parenthesis are the transform value of mean data.

Table 3 : Efficacy of different treatment against Sclerotinia blight of Brinjal (after second spray).

Treatments	Percent disease severity	Percent disease control
T_1	14.11 (22.01)	55.29
T_2	16.5 (23.89)	47.71
T_3	11.33 (19.62)	64.10
T_4	20.25 (26.73)	35.83
T_5	17.25 (24.96)	45.34
T_6	19.88 (26.37)	37.00
T_7	31.56 (31.17)	0
SEm±	0.78	
CD at 5%	**2.37**	

*Data in parenthesis are the transform value of mean data.

Carbendazim 50 WP (0.1%), intercropping with Fenugreek, Neem leaf mulching, Foliar Spray with Panchgabya and a control group. The results showed that all treatments were successful in reducing the severity of Sclerotinia blight and increasing the yield when compared to the control group. After the first foliar spray, the most effective treatment was found to be T_3 (Carbendazim 50WP @ 0.1%) with a minimum disease severity of 9.50% (Table 2; Fig. 5), followed by T_1 (*Trichoderma harzianum*; 11.99%), T_2 (*Pseudomonas fluorescens*; 14.88%), T_5 (Neem leaf mulching; 15.66%), T_6 (Foliar Spray with Panchgabya; 16.33%) and T_4 (Inter cropping with Fenugreek; 16.87%). The control group had the highest disease severity of 26.25%. However, other treatments such as *Trichoderma harzianum*, *Pseudomonas fluorescens*, Fenugreek intercropping, Neem leaf mulching and Foliar Spray with Panchgabya can also be effective in reducing disease severity and increasing yield as compare to control.

After the second spray of treatments, all treatments were found significantly superior as compared to check. Minimum per cent disease severity 11.33% (Table 3) was noted in treatment T_3 followed by T_1 (14.11%), T_2(16.50%), T_5(17.25%), T_6(19.88%) and T_4(20.25%).

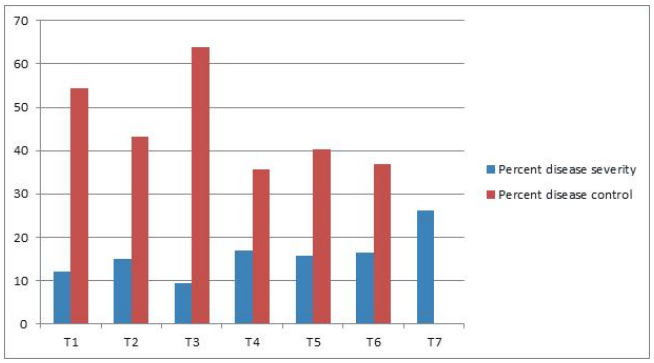

Fig. 5 : Effect of treatment on management of Sclerotinia blight of brinjal after first spray.

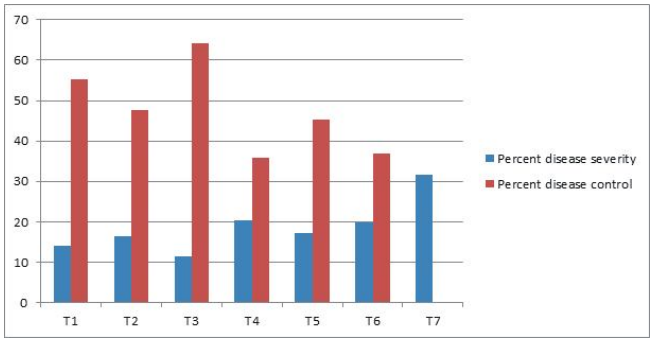

Fig. 6 : Effect of treatment on management of Sclerotinia blight of brinjal after second spray.

Maximum disease severity was found in T_7. Foliar spray of Carbendazim-50 WP (0.1%) was most effective with 11.33 per cent disease severity as compare to all treatment. Above findings suggest that Carbendazim is a promising treatment for managing Sclerotinia blight of brinjal. These findings are in agreement with the results of Krishnamoorthy *et al*. (2017), reported the effectiveness of fungicides in disease control against *S. Sclerotiorum.* Abdullah *et al*. (2008) and Upamanya and Dutta *et al.* (2019) conducted a study and find that, the effective bio-control agents against important pathogens of Brinjal.

Percent Disease control

Data presented in Table 2 indicated that all the treatments were more or less effective and exhibited reduction in disease and significantly increase the yield as compare to check. Maximum disease control (63.80%) was found in T_3, (Carbendazim 50WP @ 0.1%) followed by T_1 (54.32%), T_2(43.31%), T_5(40.34%), T_6(35.79%), $T_4$and (35.73%). After the second spray, maximum disease control (64.10%) was found in T_3 followed by T_1 (55.29%), T_2(47.71%), T_5 (45.34%), T_6 (37.00%), T_4(35.83%), respectively (Table 3). Where, T_3 (Carbendazim 50WP @ 0.1%) was most effective with (64.10%) disease control compared other treatments.

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