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Prospects of native antagonistic rhizobacteria for the management of collar rot of chilli caused by *Sclerotium rolfsii* Sacc.

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Abstract

Chilli (*Capsicum annuum* L.) belongs to the family Solanaceae is mainly cultivated for green fruits as table purpose and dry chilli as spice. Diseases of chilli act as the chief limiting factor to its economic production. Recently, the collars rot disease of chilli caused by *Sclerotium rolfsii* is a threatening disease in eastern coastal regions of Odisha. Due to its soil borne nature, this disease is very difficult to be managed by chemical fungicides. Rhizosphere soil of healthy chilli plants was used for isolation and screening of native bacterial antagonists for their biocontrol efficacy and seed germination potential. Among 34 bacterial strains isolated from rhizoplane and rhizosphere of chilli roots, five isolates viz. isolate-01, isolate-17, isolate-23, isolate-24 and isolate-32 were found highly inhibitory against mycelial growth of *S. rolfsii* in dual cultures. Highest inhibition of radial mycelial growth of pathogen in dual culture was induced by isolate-32 (73%) followed by isolate-24 (69.6%). In greenhouse experiments percent disease incidence (PDI) was lower in artificially inoculated chilli plants treated with isolate-32 (7.4%) and isolate-24 (9.9%), with percent disease reduction over control of 85.9% and 81%, respectively. These isolates also exhibited efficient seed germination characteristics as evident by significant increase in germination (%) of treated chilli seeds of 94.5 and 89 of the plants treated respectively with isolate-32 and isolate-24 as compared to inoculated control. The study concluded that the two native rhizobacteria isolated from root zone of healthy chilli plants could successfully protect the chilli plants from the lethal infection by *Sclerotium sp.* while enhancing the germination of the treated plants.

Keywords: Chilli, biocontrol, germination (%), *Sclerotium rolfsii*

Introduction

Chilli (*Capsicum annuum* L.) is mainly cultivated for its vegetable green fruits and for dry chilli as the spice of commerce. It is a rich source of Vitamin C, A and B. In India, it is an important cash crop. Chilli crop suffers with many fungal, bacterial and viral diseases resulting in huge yield losses. Among the fungal diseases, in recent, the collar rot caused by *Sclerotium rolfsii* is becoming sever disease of chilli in India. Crop losses up to 16-80 per cent due to collar rot disease have been reported by many researchers in this crop (Singh and Dhancholia, 1991) [13]. During 1985 the collar rot of chilli caused by *Sclerotium rolfsii* was observed in Maharashtra in Vidarbha region (Wangihar *et al.* 1985). In the year (Kalmesh and Gurjar (2001) [7] root rot of chilli caused by *S. rolfsii* was first time reported from Rajasthan near Jaipur chilli growing areas, (Mathur and Gurjar, 2001) [9] where the severe mortality of chilli plants during March-April was observed.

Sclerotium rolfsii Sacc. is widely distributed in tropical and sub-tropical areas of the world, coupled with the large number of hosts attacked by it indicate that, economic losses are substantial every year due to infection of *S. rolfsii* (Aycock, 1966) [2]. The pathogen preferentially infects stem, but it can also infect any parts of the plant including root, leaf, flower and fruit. On erect plant, yellowing and wilting symptoms are usually preceded by light to dark brown lesions at collar region of the plant adjacent to the ground. Drying or shriveling of the foliage and ultimately death of the plants occurs after wilting. Sclerotia are white and later they become brown to black, which are produced on mats of mycelium on the plant or soil (Ansari, 2005) [11].

Collar rot of chilli is a destructive soil borne disease causing 100 per cent mortality of the infected plants and it can occur at any stage of the crop. Management of this disease is difficult by chemical fungicides, as it produces sclerotia which over winter in the soil and serve as primary source of inoculum in the following season. Biological control of soil borne pathogens offers environmentally safe, durable and cost-effective alternative to chemicals (Papavizas and Lumsden, 1980) [12]. The objectives of this study are to isolates the rhizosphere bacteria

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from root zone of healthy chilli crop, for bio-control potential against major collar rot pathogen of chilli crop.

Materials and Methods

The study was carried out during 2017-18 in the Department of Plant Pathology, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha, India, which Agro-climatically falls under East & South East Coastal Plain zone.

Isolation of pathogen and native rhizobacteria

Several diseased chilli plants were collected during the field surveys. The pathogen was isolated from diseased plant part in water agar and sub-cultured on Potato Dextrose Agar (PDA). The fungus, *Sclerotium rolfsii*, produced white, dense radiating mycelial growth in early stages of its growth on PDA and later produced matured spherical to ellipsoidal sclerotia. The pathogenicity of the isolate of *S. rolfsii* was proved under artificial condition on chilli seedlings. The inoculum of the pathogen was grown on milled maize grain seeds, added to the moistened coir pith @ 10g kg⁻¹ and mixed thoroughly. Suitable check was maintained without addition of inoculum to the coir pith. The seedling crates were watered at regular interval to maintain soil moisture. The seedlings were observed after 15 days for symptom development. Re-isolation of the fungus (*Sclerotium* spp.) was done from infected seedlings and the cultures obtained were compared with initial cultures to confirm the identity and pathogenicity of pathogens.

Isolation of native rhizobacteria from collected soil samples was carried out by dilution plate technique as described by Islam (2009) [6] on nutrient agar (NA). The Plates were incubated at 25°C+2 for 2-4 days in inverted position so that vapours condensed from the lid may not hamper the growth of the isolated bacteria. After incubation bacterial colonies were counted and representative colonies were selected, isolated, purified and maintained in NA slants for further use.

Screening and evaluation of selected antagonistic native rhizobacteria against *S. rolfsii*

In vitro screening of rhizobacterial isolates for their antagonist properties against *S. rolfsii*

The antagonistic potential of the rhizobacterial native isolates against soil borne fungal pathogens was investigated by dual culture method (Dennis and Webster, 1971a, Buysens and Scheffer, 1992) [4, 3]. The extent of antagonistic activity by rhizobacterial isolates against *S. rolfsii* pathogen was recorded on fifth day by measuring the radial growth of the pathogen in dual culture plates and in control plate. The per cent inhibition of radial growth of *S. rolfsii* over control was calculated (Vincent (1927) [14].

$$\text{Percent incidence} = \frac{(\text{Control-Test})}{\text{control}} \times 100$$

In-vivo screening of antagonistic rhizobacteria against *S. rolfsii*

To study the efficacy of rhizobacterial isolates selected through *in-vitro* screening, the surface sterilized chilli seeds

(cv. Agni Jwala) were planted in the potrays containing standard soil media inoculated with *S. rolfsii*. After one week and one day before transplanting the chilli seedlings, selected rhizobacterial isolates were incorporated in soil media at the rate of 5 ml per well at 10⁹cfu/ml. Three weeks old seedlings were root dipped in bacterial suspension of selected antagonistic bacteria (10⁹cfu/ml) for 45 min and transplanted into pathogen-rhizobacteria mixture coir pith (Lemessa and Zeller, 2007) [8]. The seedlings were maintained in green house at 24-28°C temperature and 75-90% relative humidity. The seedlings were watered with sterile water when necessary.

In-vivo evaluation of selected antagonistic rhizobacteria for biocontrol of *S. rolfsii*

Five selected rhizobacterial isolates with higher inhibition under *in vitro* tests were further tested in green house on chilli plants to evaluate their ability to control soil borne diseases. Portrays containing standard soil mix and milled maize grains inoculated with *S. rolfsii*. After one week and one day before transplanting the seedlings, antagonists were incorporated in the coir pith at a rate of 5 ml per well at 10⁹cfu/ml. Three weeks old chilli seedlings were root dipped in bacterial suspension of antagonistic bacteria (10⁹cfu/ml) for 45 min and transplanted into pathogen-antagonist mixture coir pith (Lemessa and Zeller, 2007) [8]. Treatments were replicated four times. Appropriate positive and negative controls were maintained. The disease incidence and biocontrol efficiency were calculated as follows:

$$\text{Percent incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Statistical analysis

The data obtained in the experiments was analyzed using appropriate analysis programme -Statistical Methods for Agricultural Workers, ICAR, New Delhi (Panse and Sukhatme, 1989) [11].

Experimental Results

Isolation and identification of soil borne pathogen

The soilborne pathogen *S. rolfsii* was isolated from diseased samples of chilli plants collected from the OUAT fields, RRTTS farm, CHES farm and local farmer's fields during survey. The fungus produced white, dense radiating mycelial growth on PDA. In early stages, the mycelium was silky white which later became dull in appearance. Sclerotial initials were observed from 6th day onwards. At the initial stage, the sclerotial bodies were white in colour later they turned buff brown colour to chocolate brown at maturity. On the basis of these characters the fungus was identified as *S. rolfsii* (Mordue, 1974; Farr *et al.* 1995) [10, 5]. *S. rolfsii* produced typical symptoms of collar rot on chilli (cv. Agni Jwala). Profuse white mycelial growth was found on the soil surface after 24 hours of inoculation. White cottony growth at collar region and root zone was observed in wilted plants. Numerous round brown and mustard seed like sclerotia were seen on soil surface and root region of the infected plants at 9 days after inoculation.

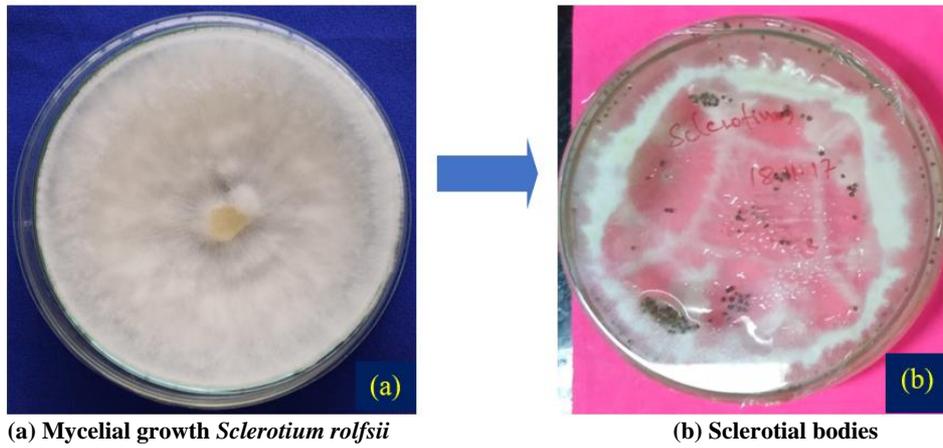


Plate 1: Pure culture of *Sclerotium rolfsii*



Plate 2: Pathogenicity test

**After artificial inoculation of pathogen, seedlings show less vigour with chlorotic leaves, stem girdling
Screening of isolated rhizobacteria for antagonistic potential**

Preliminary *in-vitro* bioassay of isolated rhizobacterial isolates was carried out against *Sclerotium* sp. by the dual culture method. The intensity of the antagonism by various

isolates against the pathogens was recorded as percent inhibition of mycelial growth by scoring in a scale from 0 (no inhibition) to >75% as (+++++) (Data not presented). The efficiency of isolates 01, 17, 23, 24 and 32 was highest (55% inhibition or more), while other strains were either inferior or inefficient in checking the mycelial growth of the pathogens.

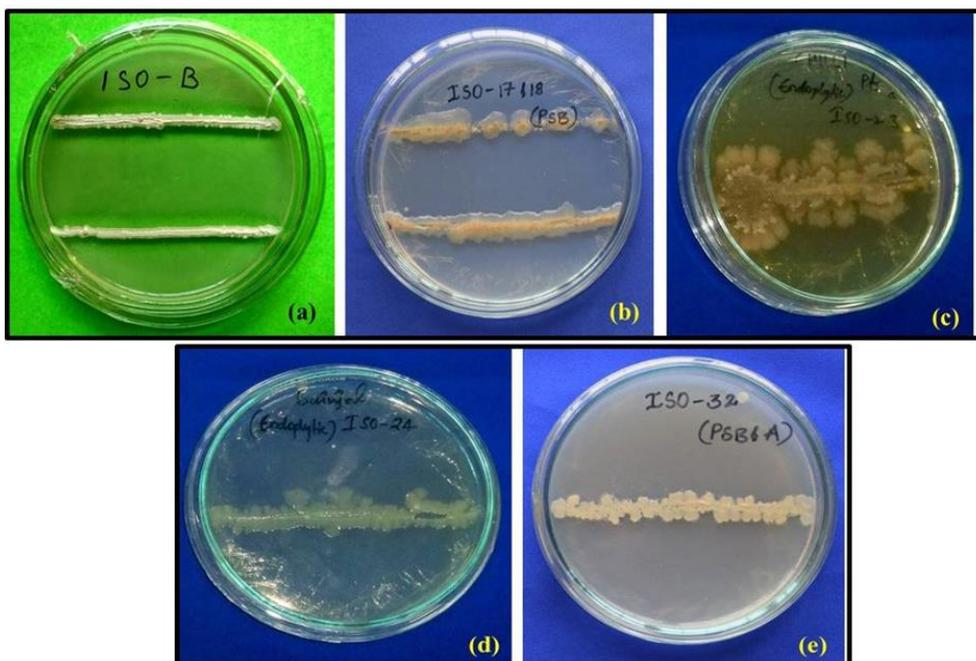


Plate 3: Pure cultures of selected rhizobacterial isolates

In vitro* evaluation of selected rhizobacterial isolates against *S. rolfsii

In vitro evaluation of selected rhizobacterial isolates (isolate-01, isolate-17, isolate-23, isolate-24 and isolate-32), against *Sclerotium* spp. was carried out using dual culture method to test their efficiency to inhibit the mycelial growth of isolated fungal plant pathogen.

Antagonistic activity of selected rhizobacteria against *S. rolfsii*. by dual culture method

The data presented in the given Table 1, have been revealed that antagonistic effect of all the selected isolates against *S. rolfsii* showed significant reduction in mycelial growth. The per cent inhibition over control in collar rot disease ranged from 73 to 60.7 per cent. Maximum per cent inhibition over control was shown by isolate-32 (73.0 per cent) followed by isolate-24 (69.6 per cent). Radial growth was recorded minimum by isolate-32 followed by isolate-24 (24.3 mm) and (27.3 mm) respectively, whereas in control plate (90.0 mm)

Table 1: Antagonistic activity of rhizobacterial isolates against *S. rolfsii* in dual plate

Treatment	Radial growth (mm)*	Per cent inhibition over control*	Inhibition zone (mm)*
Iso-01	33.7	62.6	14.0
Iso-17	34.7	61.5	13.0
Iso-23	35.3	60.7	12.7
Iso-24	27.3	69.6	16.7
Iso-32	24.3	73.0	19.6
Control	90.0	0.0	0.0
SE(m)±	0.5	0.5	0.4
C.D.≤ (0.05)	1.4	1.6	1.3



Plate 4: Dual culture of selected rhizobacterial isolate with *Sclerotium rolfsii*

***In vivo* evaluation of selected native antagonistic rhizobacteria for against collar rot disease**

The effect of selected antagonists was investigated for their biocontrol potential against collar rot disease was given in below Table 2. All the isolates gave significant control of collar rot diseases when compared with inoculated control. Incidence of the diseases reduced to the level of 7.4% with isolate-32 which gave 85.9% disease control over inoculated control followed by isolate-24 with 9.9% diseases incidence shown 81% disease reduction over control.

Table 2: Effect of seedling treatment with native rhizobacterial isolates on *in vivo* incidence of collar rot under artificial inoculation of pathogen

Treatment	Disease Incidence (%)	Disease reduction over control (%)
Iso-01	11.8	77.5
Iso-17	10.3	80.4
Iso-23	12.6	76.0
Iso-24	9.9	81.0
Iso-32	7.4	85.9
Control	52.5	00.0
SE(m)±	1.4	
C.D. (≤0.05)	4.3	



Effect of seed treatment with native rhizobacterial isolates on seed germination in artificially inoculated pro trays under greenhouse conditions

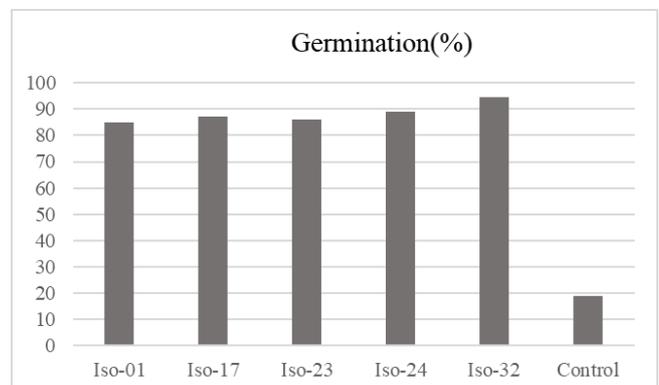


Fig 1: Effect of seed treatment with native rhizobacterial isolates on germination (%) under *in vivo* conditions

The results (fig.1) showed that the percent germination of chilli seeds treated with five rhizobacterial isolates ranged between 85% and 94.5% as compared to 19% germination in control treatment plants inoculated with collar rot pathogen alone. Among individual isolates isolate-32 effected highest germination (94.5%) followed by isolate-24 (89%).



Conclusion

Native microbes are best bets while bioprospecting agriculturally important microorganisms from any agro-ecological system. Vegetables being highly economically important crop receive more pesticides for management of several pest and diseases. However, as the fruits, the edible parts of the plants, come in direct contact with deadly pesticides, it is imperative to explore more native microbes which can counter pathogens more effectively. The present study concluded that native rhizobacterial strains isolated from the chilli crop can be successfully used for managing soil borne *Sclerotium* sp. affecting chilli crop besides enhancing the growth of the treated plants. Among five rhizobacterial isolates two isolates isolate-32 and isolate-24 identified as having highly potential antagonistic properties along with plant growth promotion ability, which would pave way for eco-friendly management of collar rot of chilli.

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