Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.7620

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **7 (1):** 496-506 (2019)

Research Article

Probing Potential of Native Antagonistic and Plant Growth Promoting Rhizobacteria against Collar Rot Disease of Brinjal

Bhanothu Shiva[1*], P. Srinivas[2] and Deepa Khulbe[3]

[1]Ph.D. Scholar Department of Plant Pathology, Faculty of Agriculture, BCKV, Mohanpur-741252

[2]Central Horticultural Experiment Station (ICAR-IIHR), Aiginia, Bhubaneswar-751019

[3]Regional Research and Technology Transfer Station, OUAT, Bhubaneswar-751003

*Corresponding Author E-mail: shiva.agri12@gmail.com

Received: 12.01.2019 | Revised: 16.02.2019 | Accepted: 23.02.2019

ABSTRACT

Collars rot disease of brinjal 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 brinjal plants was used for isolation and screening of native bacterial antagonists for probing their biocontrol efficacy and plant growth promotion potential. Among 54 bacterial strains isolated from rhizoplane and rhizosphere of brinjal 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 (77.8%) followed by isolate-24 (74.8%). In greenhouse experiments percent disease incidence (PDI) was lower in artificially inoculated brinjal plants treated with isolate-32 (6.3%) and isolate-24 (8.6%), with percent disease reduction over control of 88.5% and 84.3%, respectively. These isolates also exhibited plant growth promoting characteristics as evident by significant increase in plant height, fresh and dry weight of treated brinjal plants as evident by higher vigour index of 798 and 781.3 of the plants treated respectively with isolate-32 and isolate-24 as compared to vigour index of 297.2 in non-treated plants. The study concluded that the two native rhizobacteria isolated from root zone of healthy brinjal plants could successfully protect the brinjal plants from the lethal infection by Sclerotium sp. while enhancing the overall growth of the treated plants.

Key words: *Brinjal, Biocontrol, PGPR, Sclerotium rolfsii.*

INTRODUCTION

Brinjal (*Solanum melongena* L.), is an important solanaceous vegetable crop of tropical and subtropical regions and is considered to be the native of Indian subcontinent[20,6]. Soil borne plant pathogens are devastating in nature and are very difficult to manage due to their persistence nature in soil. The situation becomes more challenging due to simultaneous infection by multiple soil-borne plant pathogens that drastically reduce the yield and quality of the brinjal crop[11,9].

Cite this article: Shiva, B., Srinivas, P. and Khulbe, D., Probing Potential of Native Antagonistic and Plant Growth Promoting Rhizobacteria against Collar Rot Disease of Brinjal, *Int. J. Pure App. Biosci.* **7(1):** 496-506 (2019). doi: http://dx.doi.org/10.18782/2320-7051.7620

Among the soil borne diseases, fungal collar rot disease caused by *Sclerotium rolfsii* Sacc, is one of the major bottlenecks in the profitable production of brinjal. During recent years, the collar rot disease has attained its serious proportion, causing yield losses of about 20-40 per cent in brinjal[16]. During recent surveys in the various brinjal growing areas of Odisha it was observed that besides the ever troubling bacterial wilt disease, collar rot of brinjal is causing noticeable crop mortality in several areas (data not presented).

Currently, management of collar rot disease relies mainly on application of chemical fungicides which is not sustainable in the longer run as chemical fungicides are known to have residual toxicity in crop produce, toxicity to non-target organisms and cause other environmental hazards. Therefore, much emphasis has been given to manage the *Sclerotium* diseases by employing the native plant growth promoting rhizobacteria (PGPR)[4]. The native antagonistic microbes have a better chance to establish in the familiar rhizoclimate and to restrict the establishment and spread of soil borne pathogens[17]. These native microbial biocontrol agents are able to restrict the growth of phytopathogens due to various mechanisms including mycoparasitism, secretion of antibiotics, volatile and non-volatile metabolites[18].

Due to their anti-pathogenic properties the effective native microbes are considered as potential alternative to chemical fertilizers and pesticides[1,8]. The mechanism of action of rhizobacteria include, exerting direct antibiosis, inducing resistance against plant pathogens, regulating hormonal and nutritional balance in plants, and solubilizing nutrients for easy uptake by plants[21]. The PGPR inoculants have added advantage to be used as biofertilizers, as these produce different plant growth promoting substances, enzymes and antifungal and nutrient chelating substances[23]. The objective of present study was to isolate native rhizobacteria from the rhizosphere and rhizoplanes of brinjal crop and screen them for their bio-control potential against the devastating collar rot pathogen of brinjal crop and for their plant growth promoting capabilities.

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 brinjal 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 brinjal 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[10] 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[5,3]. 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[22].

$$\text{Percentage inhibition} = \frac{(\text{Control-Test})}{\text{Control}} \times 100$$

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

To study the efficacy of rhizobacterial isolates selected through *in-vitro* screening, the surface sterilized brinjal seeds (cv. Utkal Anushree) were planted in the potrays containing standard soil media inoculated with *S. rolfsii*. After one week and one day before transplanting the brinjal seedlings, selected rhizobacterial isolates were incorporated in soil media at the rate of 5 ml per well at 10^9cfu/ml. Three weeks old seedlings were root dipped in bacterial suspension of selected antagonistic bacteria (10^9cfu/ml) for 45 min and transplanted into pathogen-rhizobacteria mixture coir pith[12]. 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 brinjal 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 10^9cfu/ml. Three weeks old brinjal seedlings were root dipped in bacterial suspension of antagonistic bacteria (10^9cfu/ml) for 45 min and transplanted into pathogen-antagonist mixture coir pith[12]. Treatments were replicated four times. Appropriate positive and negative controls were maintained. The disease incidence and biocontrol efficiency were calculated as follows:

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

Screening of rhizobacteria for plant growth response

Selected rhizobacterial isolated were tested for plant growth response by seed treatment through rolled towel paper method, *in vitro*, and root dip method in pot culture under greenhouse condition.

Effect of seed treatment with antagonistic rhizobacteria on plant growth response assessed by Rolled paper towel method

One hundred seeds treated with respective selected isolates were randomly taken from each treatment with four replications and were placed uniformly between a pair of moist germination roll paper towels. The towels were rolled and the two ends were closed with rubber band. Then the rolled papers containing seeds were placed in an upright position for 7-10 days at room temperature under normal 12/12 light and darkness cycle. After incubation the shoot and root portions were

blotted dry with fine tissue paper and fresh weight was taken. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root was measured from the starting point of the root to the largest available lateral root tip. Germination percentage was calculated by the formula given below:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

The seedling vigour index was calculated by using the formula as described by Baki and Anderson[2]:

Seedling Vigor Index = (Root length + Shoot length) x Seed germination (%).

***In vivo* effect of root dip treatment with antagonistic rhizobacteria on plant growth response**

Greenhouse study was conducted for *in vivo* evaluation of antagonistic potential of selected rhizobacteria by root dip treatment in brinjal. Seedling root dip treatment was done as per the method described by Srinivasan *et al*.[19]. Seedlings of brinjal were grown in protrays. Two weeks old seedlings were taken out and roots were gently washed and dipped in the inoculum of respective rhizobacterial culture broth for 10 min. The treated seedlings were then grown in pots under greenhouse condition. Plant growth promotion was assessed after two weeks in terms of growth parameters like root length, shoot length, fresh root weight, fresh shoot weigh, dry root weight and dry shoot weight.

Statistical analysis

The data obtained in the experiments was analysed using appropriate analysis programme -Statistical Methods for Agricultural Workers, ICAR, New Delhi[15].

Experimental Results:

Isolation and identification of soil borne pathogen

The soilborne pathogen *S. rolfsii* was isolated from diseased samples of brinjal 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*[14,7]. *S. rolfsii* produced typical symptoms of collar rot on brinjal (cv. Utkal Anushree). 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.

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.

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 77.8 to 58.9 per cent. Maximum per cent inhibition over control was shown by isolate-32 (77.8 per cent) followed by isolate-24 (74.8).

***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. All the isolates gave significant control of collar rot and wilt diseases when compared with inoculated control. Incidence of the diseases reduced to the level of 6.3% with isolate-32 which gave 88.5 % disease control over inoculated control. Effect of seed treatment with native rhizobacterial isolates on seed germination in artificially inoculated protrays under greenhouse conditions was also evaluated (Table 2).

The results (fig.1) showed that the percent germination of brinjal seeds treated with five rhizobacterial isolates ranged between 85% and 95% as compared to 20% germination in control treatment plants inoculated with collar rot pathogen alone. Among individual isolates isolate-32 effected highest germination (95%) followed by isolate-24 (93%).

Plant growth response

Based on the screening for antagonistic potential revealed through dual culture technique and other characteristics, five isolates were selected and studied in detail for plant growth response through *in-vitro* seed treatment by using rolled towel method and by root dip method under greenhouse condition.

***In-vitro* seed treatment with selected native bacterial isolates on seedling growth assessed by roll towel method**

Effect of seed treatment with selected rhizobacterial isolates on germination, root length, shoots length, and vigour index were recorded and showed in below Table 3. Significant variation in germination was recorded. It varied from 95% to 83.75%, with highest germination of 95% in seeds treated with isolate-32 as compared to 83.75% germination in untreated seed control. Seed treatment with different rhizobacterial isolates, showed significant variation with respect to root length, shoot length and seedling vigour index. The maximum shoot length (4.17cm) was recorded in isolate-24 as against minimum shoot length (1.71 cm) in control. The maximum root length (4.37 cm) was recorded in isolate-32 as compared to root length of 1.83 cm in control seedlings. The highest vigour index (798) could be achieved by the seedlings treated with Isolate-32 closely followed by vigour index 781.3 in seedlings treated with Isolate-24.

Efficacy of rhizobacterial treatment by root dip method on plant growth parameters under greenhouse condition

Results of pot experiment conducted in green house for *in vivo* evaluation of effect of root dip treatment of rhizobacterial isolates on the plant growth parameters such as shoot length, root length, and fresh and dry weight are given below Table 4.

The highest root length of 18.3cm was observed in treatment with Isolate-32 followed by 14.8 cm in Isolate-17. The highest shoot length was recorded in the Isolate-32 (12.1 cm) and Isolate-17 (12.1 cm). The maximum fresh root weight was observed in the Isolate-23 (1.53 g), which was statistically at par with Isolate-32 (1.40 g). Similarly, maximum root dry weight was observed in the Isolate-23 (0.71 g) followed by Isolate-32 (0.64 g), significantly higher than control (0.36 g). Highest shoot fresh weight was recorded in the isolate-24 treatment (1.79 g) followed by Isolate-32 (1.59 g), which were significantly higher as compared to the control (0.87 g). The maximum shoot dry weight was recorded in the Isolate-24 (0.81 g) followed by Isolate-32 (0.80 g). All the treatments were statistically at par with each other.

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	**37.0**	**58.9**	**16.0**
Iso-17	**30.7**	**65.9**	**18.0**
Iso-23	**34.0**	**62.2**	**14.7**
Iso-24	**22.7**	**74.8**	**20.0**
Iso-32	**20.0**	**77.8**	**23.0**
Control	**90.0**	**0.0**	**0.0**
SE(m)±	**0.6**	**0.6**	**0.5**
C.D.≤ (0.05)	**1.7**	**1.9**	**1.5**

Table 2: Effect of seed 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	**11.4**	**79.3**
Iso-17	**11.4**	**79.2**
Iso-23	**12.0**	**78.1**
Iso-24	**8.6**	**84.3**
Iso-32	**6.3**	**88.5**
Control	**55.0**	**00.0**
SE(m)±	**1.5**	
C.D. (≤0.05)	**4.5**	

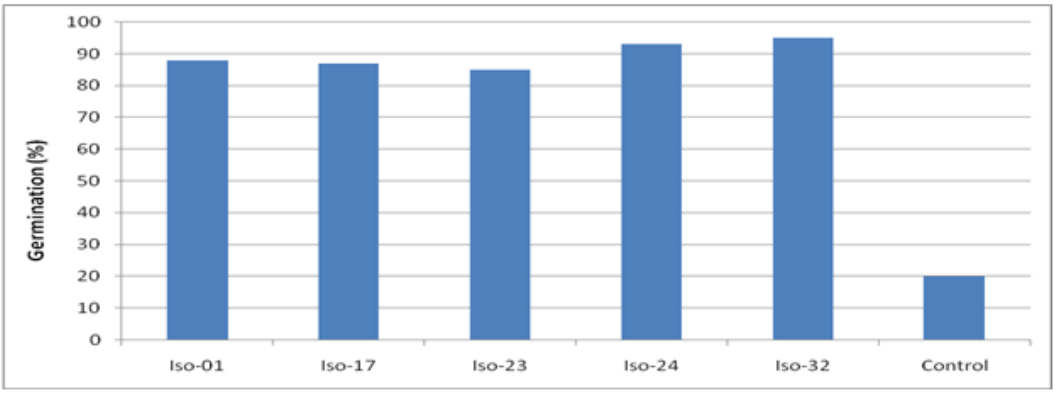


Fig. 1: Effect of seed treatment with native rhizobacterial isolates on germination (%) under *in vivo* conditions

Table 3: Effect of seed treatment with native rhizobacteria on seedling growth parameters

Treatment	Germination %	Root length (cm)	Shoot length (cm)	**Vigour Index**
Iso-01	**90.00**	**2.51**	**2.36**	**437.6**
Iso-17	**91.25**	**3.25**	**3.53**	**618.2**
Iso-23	**92.50**	**3.66**	**3.60**	**670.9**
Iso-24	**93.75**	**4.16**	**4.17**	**781.3**
Iso-32	**95.00**	**4.38**	**4.04**	**798.6**
Control	**83.75**	**1.84**	**1.71**	**297.2**
SE(m)±	**1.98**	**0.05**	**0.08**	**13.3**
C.D. (≤0.05)	**5.92**	**0.16**	**0.24**	**39.9**

Table 4: Efficacy of potential antagonistic bacteria on enhancing seedling growth parameters of brinjal crop under greenhouse condition

Treatment	Root length (cm)	Root fresh weight (gm)	Root dry weight (gm)	Shoot length (cm)	Shoot fresh weight (gm)	Shoot dry weight (gm)
Iso-01	**14.13**	**1.14**	**0.57**	**11.49**	**1.30**	**0.65**
Iso-17	**14.80**	**1.23**	**0.56**	**12.10**	**1.33**	**0.66**
Iso-23	**11.92**	**1.53**	**0.71**	**11.40**	**1.25**	**0.63**
Iso-24	**10.72**	**1.24**	**0.54**	**10.80**	**1.79**	**0.81**
Iso-32	**18.30**	**1.40**	**0.64**	**12.13**	**1.59**	**0.80**
Control	**9.35**	**0.81**	**0.36**	**8.92**	**0.87**	**0.40**
SE(m)±	**0.39**	**0.06**	**0.03**	**0.40**	**0.05**	**0.03**
C.D. (≤0.05)	**1.18**	**0.18**	**0.08**	**1.20**	**0.16**	**0.10**

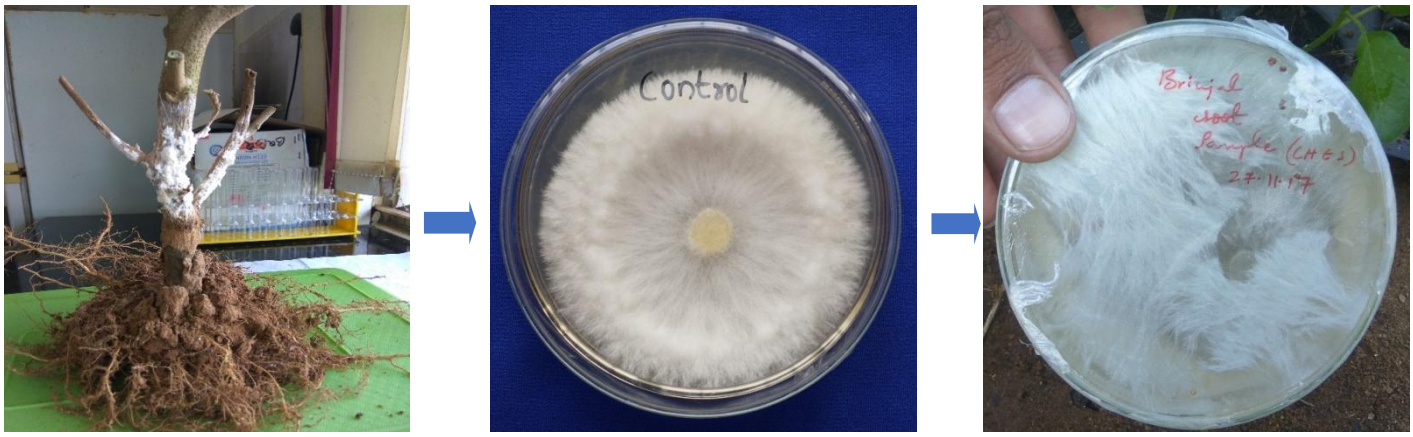


Plate 1: (a) Collar rot infected brinjal plant (b) Mycelial growth *Sclerotium rolfsii* (c) Sclerotial bodies

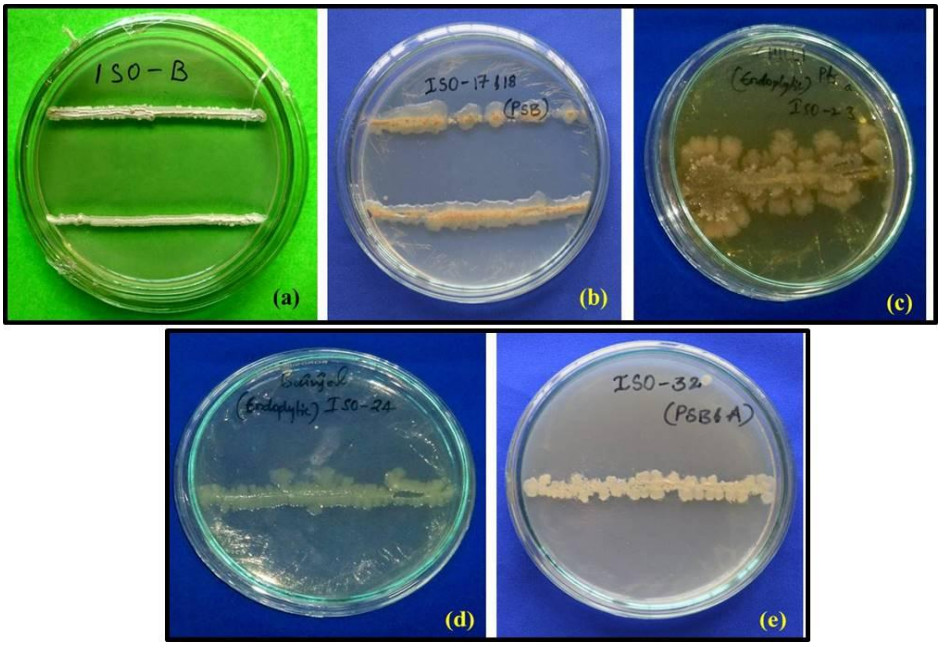


Plate 2: Pure cultures of selected rhizobacterial isolates

Plate 3: Dual culture of selected rhizobacterial isolate with *Sclerotium sp.*

Plate 4: (a) Inoculation of pathogen at collar region (b) initial symptom with white sclerotial bodies (c) complete collapse of the plant

Plate 5: Mass multiplication of pathogens and bacterial isolates

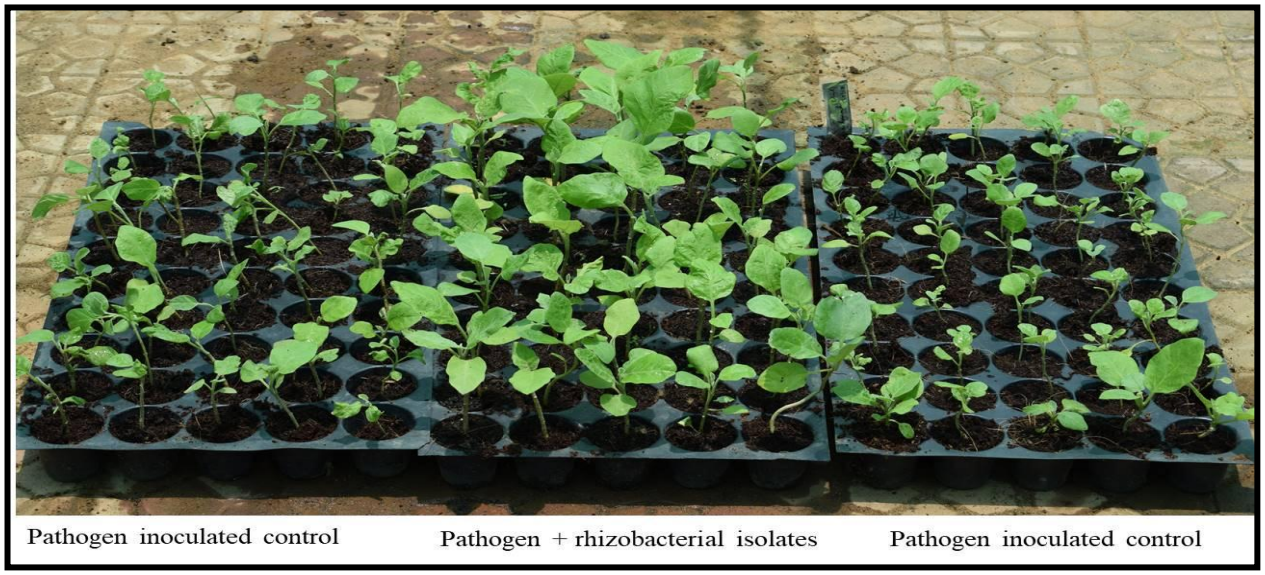


Plate 6: Effect of rhizobacteria on management of collar rot

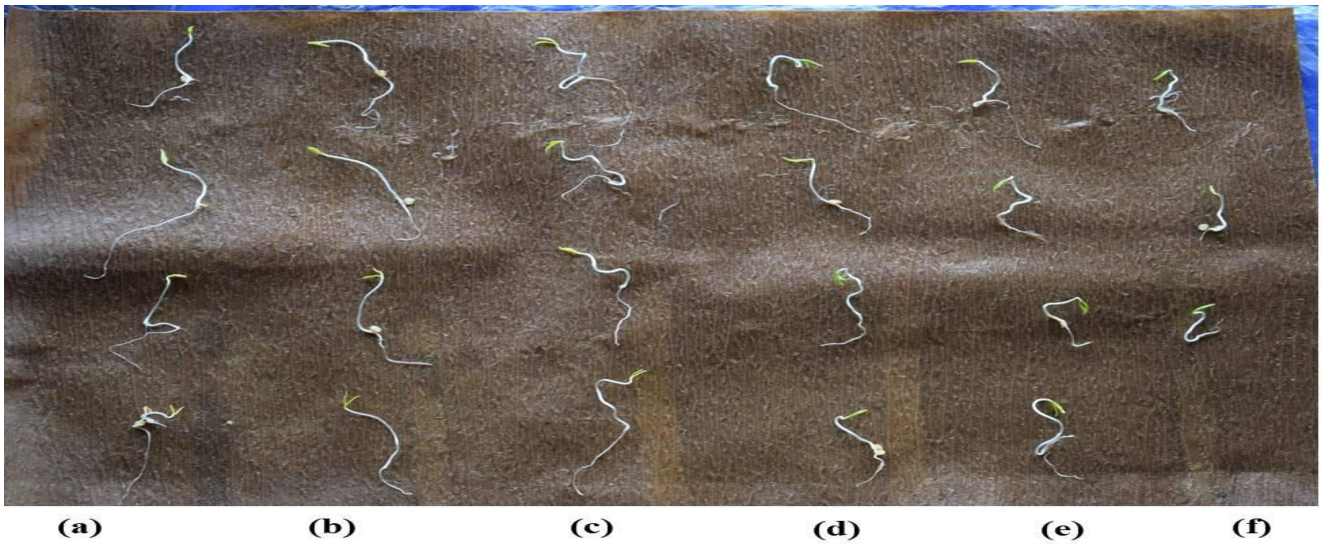
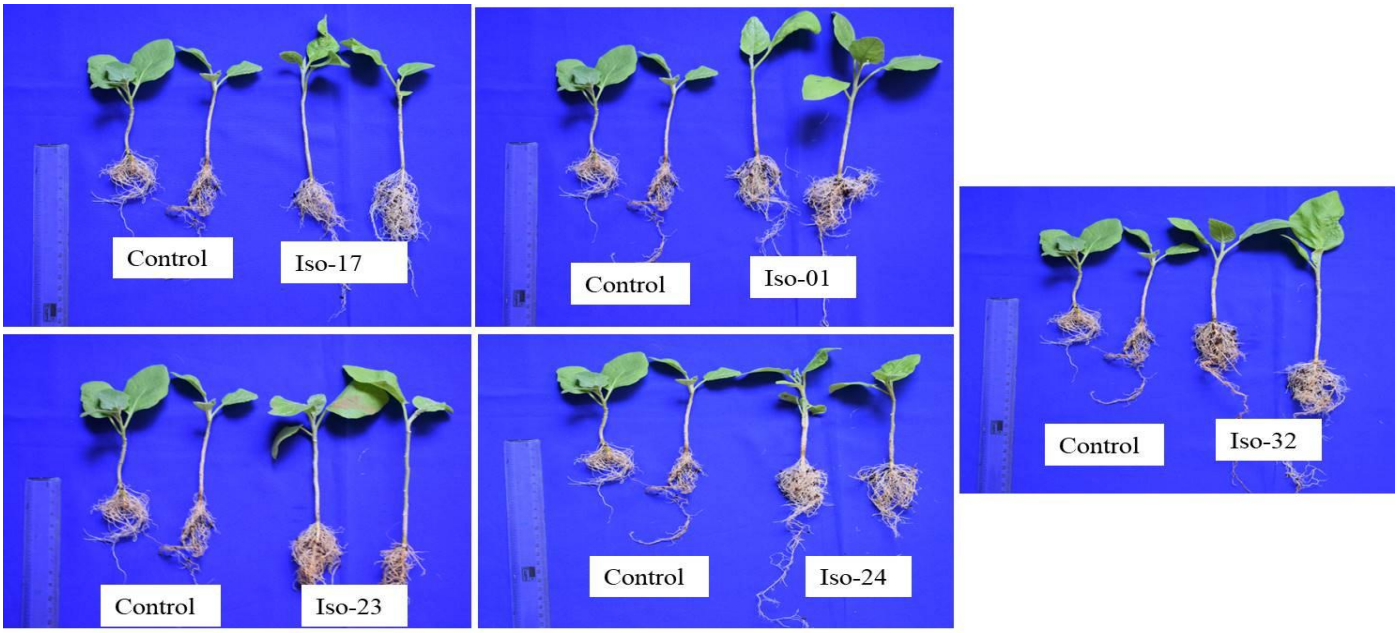


Plate 7: Seed treatment of brinjal seeds with rhizobacteria by roll paper towel method

Plate 8: Pot culture experiment for plant growth response in brinjal

Plate 9: Different growth parameters of brinjal



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 brinjal crop can be successfully used for managing soil borne *Sclerotium sp.* affecting brinjal 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 brinjal.

REFERENCES

1. Apastambh, A. R., Tanveer, K. and Baig, M. M. V., Isolation and Characterization of Plant Growth Promoting Rhizobacteria from Banana Rhizosphere. *Int. J. Curr. Microbiol. App. Sci.*, 5: 59-65 (2016).
2. Baki, A. A. and Anderson, J. D., Physiological and Biological deterioration of seeds. *In: Seed Biology*, Academic Press, New York. 11: 283-315 pp (1972).

3. Buysens, S. and scheffer, R. J., Screening systems for bio-control and growth promotion. *Bulletin Oilb/Srop*., 15: 145-146 (1992).
4. Compant, S., Duffy, B., Nowak, J., Clement, C. and Barka, E. A., Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. and Envir. Microbio.* 71: 4951-4959 (2005).
5. Dennis, C. and Webster, J., Antagonist properties of species group of *Trichoderma* II. Production of volatile antibiotics. *Trans British Mycology Society*, 57: 41 -48 (1971).
6. Doijode, S. D., Seed storage of horticultural crops. *Haworth Press.* p. 157 (2001).
7. Farr, D. F., Bills, G. F., Chamuris, G. P. and Rossman, A. Y., Fungi on plants and plant products in the united states *APS Press*. Pp. 526-528 (1995).
8. Glick, B. R., Plant Growth-Promoting Bacteria: Mechanisms and Applications. Hindawi Publishing Corporation, *Scientifica* (2012).
9. Gupta, V., Razdan, V. K., Kumar, D. and Gupta, V., Status of soil borne diseases of brinjal and the frequency of associated pathogens. *Vegetos*, 26: 27-35 (2013).
10. Islam T., Population dynamics of *Phomopsis vexans, Sclerotium rolfsii, Fusarium oxysporum* f. sp *lycopersici* and *Trichoderma* spp. in the soil of eggplant field. M.S. thesis submitted to the Dept. of Plant Pathology, *Bangladesh Agricultural University*, Mymensingh 48-57 pp. (2009).
11. Koike, S. T., Gordon, T. R. and Aegerter B. J., Root and Basal Rot of Leek caused by *Fusarium culmorum* in California. *Plant Dis*, 87: 601 (2003).
12. Lemessa, F. and Zeller, W., Screening rhizobacteria for biological control of *Ralstonia solanacearum* in Ethiopia, *Biological Control*, 42: 3- 336 (2007).
13. Liu, W., Lu, H. H., Wu, W., Wei, Q. K., Chen, Y. X. and Thies, J. E., Transgenic Bt rice does not affect enzyme activities and microbial composition in the rhizosphere during crop development. *Soil Biol. Biochem*. 40: 475-486 (2008).
14. Mordue, J. E. M., CMI descriptions of pathogenic fungi and bacteria. No. 410 (1974).
15. Panse, V. G. and Sukhatme, P. V., Statistical Methods for Agricultural Workers. 4th edn. ICAR. New Delhi (1989).
16. Singh, K., Tiwari, P. K. and Trimurthi, V. S., Physiological studies of *Sclerotium rolfsii*. causing root rot of brinjal. *J. Mycol. PI. Pathol*. 35(3): 521 (2005).
17. Srinivas, P. and Ramakrishnan, G., Management of rice seed borne pathogens by native bio-agents. *Annals of Plant Protection Sciences* 13(2): 328-337 (2005).
18. Srinivas, P. and Ramakrishnan, G., Effect of volatile and non-volatile metabolites produced by native microorganisms in biocontrol of rice seedborne pathogens. *Annals of Plant Protection Sciences* 11(1): 53-56 (2003).
19. Srinivasan, K., Gilardi. G. and Garibaldi, A., Bacterial antagonists from used rockwool soilless substrates suppress *Fusarium* wilt of tomato. *Journal Plant Pathology*, 91: 147–54 (2009).
20. Tsao, and Lo, Handbook of Food Science, Technology, and Engineering by Yiu H. Hui. In Vegetables: Types and Biology. *CRC Press*. pp. 928 (2006).
21. Vejan, P., Abdullah, R. R., Khadiran, T., Ismail, S. and Nasrulhaq, B. A., Role of Plant Growth Promoting Rhizobacteria in Agricultural Sustainability. *Molecules*, 21: 573 (2016).
22. Vincent, J. M., Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* 159: 850 (1927).
23. Weller, D. M., Biological control of soilborne plant pathogens in the rhizosphere with bacteria. *Annual Rev. Phytopath.* 26: 379-407 (1988).
24. Winstead, N. N. and Kelman, A., Inoculation techniques for evaluating resistance to *Pseudomonas solanacearum. Phytopathology*, 42: 628–634 (1952).