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In vitro and *In vivo* efficacy of culture filtrate of bacterial antagonists against *Sclerotium rolfsii* causing collar rot of peppermint

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Abstract
Eight native *Pseudomonas* species (I_1 to I_8) and one introduced *P. fluorescens* (I_9) were evaluated to test the antagonism against *Sclerotium rolfsii* under *in vitro* conditions. Of the eight antagonists tested, isolate-I_7 (PFP) recorded the maximum inhibition zone of 13.33 mm and a minimum of 23.00 mm mycelial growth of *S. rolfsii* accounting for 74.25 per cent reduction in the mycelial growth over control (89.33 mm) and it was on par with I_9 (PFC). This was followed by isolate-I_4 (PFK). The least mycelial growth inhibition was observed with the isolate-I_3 (BSE). Biochemical tests were conducted for all the isolates. All the isolates produced similar result with regard to gram staining and KOH test (I_1, I_4, I_6, I_7, and I_8) showed negative, whereas fluorescent pigment test showed positive results. The isolates-I_2, I_3, I_5, showed positive in gram staining and KOH test, whereas fluorescent pigment showed negative results. Generally all the isolates showed positive results in motility test. The eight bacterial isolates tested in this experiment showed variation with colony type, colour, growth type and reaction to UV light. The effect of different concentration of culture filtrate of bacterial isolates on the mycelial growth of *S. rolfsii* under *in vitro* conditions revealed that the culture filtrate of the isolate-I_7 totally inhibited the mycelial growth of *S. rolfsii* at 15% concentration under *in vitro* conditions followed by the isolate-I_4.. Studies on the effect of antagonist under pot culture condition revealed that the combined application of *P. fluorescens* (PFP) through seedling dip (SD) and soil application (SA) recorded the minimum incidence of collar rot (13.66 per cent). The maximum disease incidence was recorded in inoculated control treatment (93.33 per cent).

Keywords: Bacterial antagonists, *Sclerotium rolfsii*

Introduction
Peppermint (*Mentha piperita L.*) is an important aromatic perennial herb grown throughout the world, belonging to the family Labiatae. It consists of four commonly cultivated species namely Japanese mint (*Mentha arvensis* L.); Peppermint (*Mentha piperita* L); Spear mint (*Mentha spicata* L.) and Bergamot mint (*Mentha citrata* L). The mint crop is extensively cultivated in India and about 70% of the International annual requirement is met from crops raised in the central region of the Indo-Gangetic plains (Singh *et al*., 1999) [34]. *Mentha* is cultivated in Himalaya-hills, Haryana, Uttar Pradesh, Punjab and Bihar. Of these, Uttar Pradesh is the largest producing state in the country contributing 80-90% of the total production followed by Punjab, Haryana, Bihar and Himachal Pradesh (Anonymous, 2011) [3]. In Tamil Nadu, it is grown in Coimbatore, Dharmapuri, Dindigul, Erode, Krishnagiri, Namakkal, Salem and Theni. The annual world production of *Mentha arvensis L.* and *Mentha piperita L.* oils are 40,000 tons, while India is producing 17000 tons per year (Anonymous, 2014) [4]. Peppermint, serves as a source of menthol, menthone, isomenthone, menthofuran, linanool, linalyl acetate, methyl acetate, terpenes carvone piperitenone oxide, and other aromatic compounds. The oil and by-product (menthol and dementholized oil) of this plant have the highest share in the global mint trades. These constituents are used in medicinal preparation, toothpaste, mouthwash, perfumery, cosmetics and as flavoring agents, Mint oils are mainly produced in Argentina, Angola, Australia, Brazil, Bulgaria, China, Czechoslovakia, France, Hungary and India.

In India, peppermint is grown throughout the year and it is affected by fungal diseases caused by *Rhizoctonia bataticola*, *Verticillium dahliae*, *Colletotrichum cocodes*, *Rhizoctonia solani* and *Sclerotium rolfsii* (Singh and h Bahadur Singh, 2004) [2]. Of these, collar rot caused by *Sclerotium rolfsii* Sacc. is a major constraint in the pepper mint cultivation in Tamil Nadu. The collar rot disease caused by *S. rolfsii* is a soil borne disease which causes considerable damage to the crop and the disease intensity in the field ranged from 5 to 20% (Singh and Bahadur Singh, 2004) [2].

Though collar rot and wilt disease of mint were reported way back in 1933 from Japan (Goto, 1993) [12], no further studies were undertaken on this disease. The pathogen produces sclerotia which over winter in soil and on plant debris and can survive for a long period causing disease in the following season (Punja, 1985) Thus, the control of the disease is very difficult by conventional means (Punja, 1998, Sarma *et al.*, 2000) [27]. The first attempt to control the disease is by using chemical means (Pandotra and Ganguly, 1964) [22]. Although fungicides have shown promising results in controlling the fungal diseases, phytotoxicity and fungicide residues are major problems leading to environmental pollution and human health hazards. Thus, existing control measures are not effective for the control of collar rot disease. Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and eco-friendly method for plant disease management. Soil has enormous untapped potential antagonistic microbes *viz.*, *Trichoderma* spp. *Bacillus* spp. and fluorescent pseudomonads which show antagonistic effects against soil borne plant pathogenic organisms. The use of bio-control agents is gaining importance for their plant growth promotion and disease reduction abilities (Muthukumar *et al*., 2010; Muthukumar *et al*. 2011; Thahir Basha *et al*., 2012) [20, 19, 35].

The successful application of antagonistic microorganisms for the control of *S. rolfsii* has been reported by several workers in various crops (Rahel Ratnakumari *et al*., 2011; Muthukumar and Venkatesh, 2014) [29, 18]. With this background, the present study has been undertaken with the following objectives.

2. Materials and Methods

2.1. Isolation and maintenance of pathogen

The collar rot symptoms were collected from major mint growing tracts of Tamil Nadu pertaining to districts such as Coimbatore, Dindigul, Erode, Hosur, Krishnagiri, Namakkal, Salem and Theni. The infected plant materials brought back from the field were washed, cut into 5 mm segments including the advancing margins of infection. The segments were surface sterilized in 0.5% sodium hypochlorite solution for 5 min. and rinsed in three changes of sterile distilled water. The segments were separately dried in between sheets of sterile filter paper and placed (3 pieces per plate) on fresh potato dextrose agar (PDA) medium (Ainsworth, 1961) impregnated with streptomycin, and incubated for seven days at 28±2 °C.

A total of eight isolates (I_1 to I_8) causing collar rot was isolated from infected plant samples collected from different tracts of Tamil Nadu. The fungal growth on 5th day, which arose through the sclerotial bodies was cut by inoculation loop and transferred aseptically to the PDA slants and allowed to grow at room (28±2 °C) temperature to obtain the pure culture of the fungus. The culture thus obtained was stored in refrigerator at 5 °C for further studies and was sub cultured periodically. The purified isolates were identified based on morphological and colony characteristics (Punja and Damini, 1996; Sarma *et al*., 2002; Watanabe, 2002) [26, 32, 39]. They were identified as *Sclerotium rolfsii*. Based on the pathogenicity test the highly virulent isolate of (I_1) was used for my further studies.

2.2. Isolation of bacteria from rhizosphere of mint plants

Bacterial isolates were collected from rhizosphere region of mint plants from eight different mint growing tracts of Tamil Nadu. After removing the loosely adhering soil from freshly excised roots, root segments (1g) were taken and suspended in 10 ml of sterile distilled water to get 10^{-1} dilution. Serial dilutions were made to get dilutions up to 10^{-6}. One ml of 10^{-5} and 10^{-6} dilution were pipetted out into sterile Petri plate and 15 ml of King's B medium (King *et al*., 1954**)** was added and rotated clockwise and anti-clockwise. The plates were incubated at room temperature (28±2 °C) for 48 hours for the development of bacterial colonies. These isolates were subjected to various biochemical tests for the identification. Totally eight bacterial isolates were obtained from mint plants.

The identified isolates were designated as *P. fluorescens* (Alangudi) PFA; *B. subtilis* (Attupalam) BSA; *B. subtilis* (Edappadi) BSE; *P. fluorescens* (Therkupalayam) PFT; *B. subtilis* (Kattuvalasu) BSK; *P. fluorescens* (Morepalayam) PFM; *P. fluorescens* (Pochampalli) PFP and *P. fluorescens* (Vattur) PFV. For *in vitro* studies comparison, *P. fluorescens* was obtained from Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore was designated as PFC. Based on the dual culture technique the effective bacterial isolates were used for further studies.

2.3. Biochemical tests for identification of native bacterial isolates

For the identification of native bacterial isolates certain biochemical tests were conducted.

S. No.	Biochemical tests	References
1	Gram staining	Buchanan and Gibbson, 1974 [8]
2	Motility	Karuna Vishunavat and Kolte, 2005[15]
3	Fluorescent pigment	Karuna Vishunavat and Kolte, 2005[15]
4	KOH solubility test	Karuna Vishunavat and Kolte, 2005 [15]

2.4. *In vitro* testing of bacterial antagonists

The antagonistic activity of nine bacterial antagonists against *S. rolfsii* was tested by dual culture technique (Dennis and Webster, 1971) [10] using PDA medium. At one end of the sterile Petri plate containing fifteen ml of sterilized and solidified PDA medium, a six mm culture disc of pathogen obtained from seven days old culture of *S. rolfsii* was placed at 1.5 cm away from the margin of the Petri plate. Similarly, one cm long streak was gently made onto the medium using 48h old culture of bacterial isolates just opposite to the pathogenic culture at equidistance under aseptic condition. A control was maintained by inoculating *S. rolfsii* alone at one end of the Petri plate. The plates were incubated at room temperature (28±2°C) for 48 h. The radial growth (in mm) of the pathogen was measured after incubation. The effective antagonists were selected based on the inhibition of the growth of pathogen. The per cent inhibition of mycelial growth was calculated according to Vincent (1929) [37].

Per cent inhibition (I) = C-T / C × 100

Where, C - Radial growth in control

T - Radial growth in treatment

I - Inhibition per cent

2.5. Morphological studies of native bacterial isolates

Pure cultures of the bacterial isolates were streaked on King's B agar Petri plates separately for colony development. The individual colonies were examined for shape, size, structure of colonies and pigmentation. The gram reactions of all the selected isolates were recorded according to Buchanan and Gibbson (1974) [8]. Two loopful of the bacterial suspension were smeared on grease free clean micro slide, stained

accordingly and observed under compound microscope. Gram positive or negative reactions, shape of cells were observed and recorded.

2.6. Bioassay of culture filtrates of bacterial isolates on the mycelial growth of *S. rolfsii* Preparation of the culture filtrates of bacterial isolates

The bacterial isolates were inoculated into Erlenmeyer flasks containing 50 ml of sterile King's B broth and kept on a rotary shaker at 100 rpm for 48 h. Then the cultures were filtered through bacteriological filter under vacuum and the filtrates thus obtained were used for the studies.

2.6.1. Effect of culture filtrates on the mycelial growth of *S. rolfsii*

The culture filtrates of the bacterial antagonists were separately incorporated into sterilized PDA medium at 5, 10 and 15 per cent by adding the calculated quantity of the culture filtrate to the medium by means of a sterile pipette. The amended media were transferred to sterile Petri plates separately @15 ml and allowed to solidify. Each plate was inoculated at the centre with seven days old (six mm) culture disc of *S. rolfsii* grown on PDA. Three replications were maintained for each treatment. Sterile water served as control. The diameter of the mycelial growth (in mm) of *S. rolfsii* was measured when the mycelial growth fully covered the control plates.

2.7. Pot culture studies

2.7.1. Efficacy of seedling dip and soil application with bacterial antagonists on the incidence of collar rot of mint

Sterilized soil (1.0 kg) was mixed with the pathogen inoculum @ 5g (multiplied on sand maize medium) and filled in 15 x 30 cm diameter earthen pots. Surface sterilized mint cuttings were separately treated with the talc based formulation of the antagonists and planted in pots. The treatment schedule followed is mentioned below.

2.7.2. Treatment schedule

T_1 - *P. fluorescens* SD @8×10^8 cfu/ml
T_2 - *P. fluorescens* SA @2.5kg/ha
T_3 -T1+T2
T_4 - Propiconazole @0.1% as soil drenching
T_5 - Inoculated control
T_6 - Healthy control

Seedling dip with Propiconazole @ 0.1 % was used for comparison and pathogen alone inoculated pots served as control. The experiment was conducted with three replications in a randomized block design. The treated seedlings were sown in pathogen inoculated soil @ 8 seedlings per pot and irrigated daily. The observations on the incidence of collar rot were recorded at 20 days after inoculation and calculate the per cent disease incidence as per the formula.

2.8. Statistical analysis

The data on the effect of the treatments on the growth of pathogen and disease incidence were analyzed by analysis of variance (ANOVA) and treatment means were compared by Duncan's multiple range test (DMRT). The data on disease incidence was arcsine transformed before undergoing statistical analysis (Gomez and Gomez, 1984) [11]. The package used for analysis was IRRISTAT version 92-1eveloped by the Biometrics Unit of the International Rice Research Institute, The Philippines.

3.0. Results and Discussion

3.1. *In vitro* inhibition of mycelial growth of *S. rolfsii* (I_1) by native bacterial isolates

The results presented in the table 1 revealed varying degree of antagonism by the bacterial isolates against *S. rolfsii*. Among the isolates tested isolate-I_7 recorded the maximum inhibition zone of 13.33 mm and a minimum of 23.00 mm mycelial growth of *S. rolfsii* accounting for 74.25 per cent reduction in the mycelial growth over control and it was on par with I_9 (PFC). This was followed by isolate-I_4. The least mycelial growth inhibition was observed with the isolate-I_3.

Similar observations on variation in antagonistic efficacy between isolates were recorded by several workers. Kamalakannan *et al*. (2003) [13] [reported that *P. fluorescens* (PFMMP) recorded the highest inhibition zone against *Rhizoctonia solani* causing stem and stolon rot of peppermint. Pastor *et al*. (2010) [23] reported that *Pseudomonas* spp. isolated from rhizosphere soil of groundnut plants showed highest antagonistic activity against *S. rolfsii*. Rakh (2011) [30] isolated 11 *Pseudomonas spp*., from rhizospheric soil, were evaluated for their antagonistic activity against *Sclerotium rolfsii*. A soil bacterium identified as, *Pseudomonas cf. monteilii 9*, showed highest antagonistic activity against the pathogen *Sclerotium rolfsii*. He reported, the *Pseudomonas cf. monteilii 9* inhibited the *Sclerotium rolfsii* to up 94 % in terms of dry weight. Similarly, Prasada Babu and Paramageetham (2013) [25] reported that *P. fluorescens* isolate PATPT 6 was found to be potential antagonist against *S. rolfsii*. Recently, the bacterial endophytes RB-KK-6 (40.78%), SB-BS-6 (50.08%) and LB-BU-1 (47.02%) were found effective against *S. rolfsii* and the isolates SB-DG-11 (47.41%), LB-BiN-8 (41.22%) were effective against *R. bataticola* (Brunda *et al*., 2018) [7].

Table 1: *In vitro* inhibition of mycelial growth of *S. rolfsii* (I_1) by native bacterial isolates

I. No.	Isolates	Mycelial growth (mm)	Per cent inhibition over control	Inhibition zone (mm)
I_1	PFA	27.00 b	69.77	10.33 d
I_2	BSA	31.33 d	64.92	8.33 f
I_3	BSE	32.00 d	64.17	8.00 g
I_4	PFK	25.00 b	72.01	12.00 b
I_5	BSK	30.66 c	65.67	9.66 e
I_6	PFM	26.00 b	70.89	11.0 c
I_7	PFP	23.00 a	74.25	13.33 a
I_8	PFV	29.66 c	66.79	9.66 e
I_9	PFC	23.66 a	73.51	13.00 a
	Control	89.33 e	-	-

Mean of three replications
Values in each column followed by the same letter are not significantly different according to the DMRT method (p = 0.05)

Production of siderophores and chitinases are two factors that may be involved in biological control activity. Indeed, it is known that chitinolytic activity and siderophore production are correlated with antifungal activity (Kamensky *et al*., 2003; Quecine *et al*., 2008) [13]. In addition, *P. fluorescens* is capable of solubilizing phosphate and producing IAA, characteristics that may enhance its potential use as an effective biological control agent to contribute to the control of *S. rolfsii*. Mahesh (2007) [17] suggested that fungal growth is mainly inhibited by HCN production and siderophore production. All these earlier results lend support to the present findings. In addition to this, *Pseudomonas* spp. are well known for production of broad spectrum antibiotics such as phenazine-1-carboxylic acid (PCA), 2, 4-diacetylphloroguoinol (2,4-DAPG), Pyoluteorin, Pyrrolnitrin and antibiosis which proved to be a major mechanism involved in

their biocontrol activity (O' Sullivan and O'Gara, 1992) [21].

3.2. Biochemical tests for the identification of native bacterial isolates

The results of the gram reaction and biochemical tests performed for the identification of native bacterial isolates showed that all the isolates produced similar result with regard to gram staining and KOH test (I_1, I_4, I_6, I_7, and I_8) showed negative, whereas fluorescent pigment test showed positive results. Hence they are identified as *P. fluorescens.* The isolates-I_2, I_3, I_5, showed positive in gram staining and KOH test, whereas fluorescent pigment showed negative results. Hence, they are identified as *B. subtilis* (Table 2). Generally all the isolates showed positive results in motility test.

Table 2: Biochemical tests for identification of native bacterial isolates

I. No.	Isolates	Gram staining	KOH test	Motility	Fluorescent pigment
I_1	PFA	-ve	-ve	+ve	+ve
I_2	BSA	+ve	+ve	+ve	-ve
I_3	BSE	+ve	+ve	+ve	-ve
I_4	PFK	-ve	-ve	+ve	+ve
I_5	BSK	+ve	+ve	+ve	-ve
I_6	PFM	-ve	-ve	+ve	+ve
I_7	PFP	-ve	-ve	+ve	+ve
I_8	PFV	-ve	-ve	+ve	+ve

Similarly, Karabasappa (2016) [14] reported that the confirmation of the identity of bacterial isolates, colour of fluorescent pigment under UV light, gram reaction test, siderophore production (PLATE 2) and catalase tests (PLATE 3) were conducted. All the isolates were shown yellow-green colour under UV (Ultra-Violet) light and gram negative in gram reaction test. All isolates produced siderophore on *Pseudomonas* agar modified medium and shown positive in catalase test.

3.3. Cultural characteristics of native bacterial isolates

The eight bacterial isolates tested in this experiment showed variation with colony type, colour, growth type and reaction to UV light (Table 3). The isolates-I_1, I_4, I_6, I_7 and I_8 were short rod in shape and produced bright fluorescence when exposed to UV light. The isolates-I_2, I_3, and I_5 were rod shaped and it did not produce bright fluorescence when exposed to UV light. With regard to colony type varied from round to circular undulated margin. The colony colour varied from yellowish, cream colour and greenish yellow. Further, the growth type varied from fast to slow.

Table 3: Cultural characteristics of native bacterial isolates

I. No.	Isolates	Cell shape	Colony type	Colony colour	Growth type	Reaction to UV light fluorescence emission
I_1	PFA	Short rod	Round	Yellowish	Fast	Bright
I_2	BSA	Rod shape	Circular undulated margin	Cream colour	Slow	No
I_3	BSE	Rod shape	Circular undulated margin	Cream colour	Slow	No
I_4	PFK	Short rod	Round	Greenish yellow	Fast	Bright
I_5	BSK	Rod shape	Circular undulated margin	Cream colour	Fast	No
I_6	PFM	Short rod	Round	Yellowish green	Slow	Bright
I_7	PFP	Short rod	Round	Greenish yellow	Fast	Bright
I_8	PFV	Short rod	Round	Yellowish	Fast	Bright

3.4. Effect of culture filtrate of bacterial isolates on the mycelial growth of *S. rolfsii* (I_1)

The results on the effect of different concentration of culture filtrate of bacterial isolates on the mycelial growth of *S. rolfsii* under *in vitro* conditions revealed that the culture filtrate of the isolate-I_7 totally inhibited the mycelial growth of *S. rolfsii* at 15% concentration under *in vitro* conditions followed by the isolate-I_4 (Table 4). The antifungal metabolites produced by *P. fluorescens* might be attributed as the reason for the reduction in the growth of the pathogen. *P. fluorescens* was known to produce an array of low-molecular weight metabolites some of which were potential antifungal agents (O' Dowling and O 'Gara, 1994). Several studies indicated the production of lytic enzymes which was correlated with antagonistic potential of *P. fluorescens* against various soil-borne plant pathogens (Velazhahan *et al*., 1999; Meena *et al*., 2001) [36]. The cell free extracts of fluorescent pseudomonads effectively inhibited the growth of *R. solani* (Saxena *et al*., 1995) [33]. Culture filtrate of *P. fluorescens* isolate EBS 20 and EBR 4 totally inhibited mycelial growth of *Pythium aphanidermatum* at a concentration of 15% *in vitro* (Muthukumar *et al*., 2010) [20]. Chanutsa *et al.* (2014) [9] reported 100 per cent inhibition in the growth of *S. rolfsii* with culture filtrate of *P. Florescence.*

Table 4: Effect of culture filtrate of bacterial isolates on the mycelial growth of S. *rolfsii*

I. No.	Concentration of culture filtrate (%)					
	5		10		15	
	Mycelial Growth (mm)	Per cent inhibition over control	Mycelial Growth (mm)	Per cent inhibition over control	Mycelial Growth (mm)	Per cent inhibition over control
I_1	38.00 d	57.61	28.00 d	68.53	19.66 d	77.99
I_2	43.00 e	52.04	36.33 e	59.17	25.00 e	72.01
I_3	41.33 e	53.90	35.00 e	60.67	26.00 e	70.89
I_4	29.66 b	66.9	20.00 b	77.52	8.33 b	90.67
I_5	36.66 d	59.11	25.00 c	71.91	24.66 e	72.39
I_6	33.33 c	62..82	24.66 c	72.29	11.33 c	87.31
I_7	21.66 a	75.84	10.00 a	88.76	0.00 a	100
I_8	39.33 e	56.13	30.33 d	65.92	22.00 d	75.37
Control	89.66 f	-	89.00 f	-	89.33 f	-

Mean of three replications

Values in each column followed by the same letter are not significantly different according to the DMRT method (p = 0.05) Culture filtrate of *P. fluorescens* was the most effective in inhibiting the mycelial growth of *S. rolfsii* (Revathy and Muthusamy, 2003) [31] and *C. gloeosporioides* (Vivekanandhan *et al*., 2004) [38]. The results of the present investigations are confirmed by the above reports.

3.5. Effect of seedling dip and soil application with antagonist on the incidence of collar rot of mint

Studies on the effect of antagonist under pot culture condition revealed that the combined application of *P. fluorescens* SD+*P. fluorescens* SA recorded the minimum incidence of collar rot (13.66 per cent). This was on par with the chemical treatment *viz.*, soil drenching with Propiconazole @ 0.1% which recorded an incidence of 12.66 per cent. These were followed by soil application with *P. fluorescens* which recording 20.00% collar rot incidence. The maximum disease incidence was recorded in inoculated control treatment (Table 5).

Soil application of *P. Fluorescence* was effective in controlling collar rot of groundnut incited by *S. rolfsii* (Patil *et al*., 1998) [24]. Bhatia *et al*. (2005) [6] reported that fluorescent *Pseudomonas* PS-I and PS–II coated seed sown in *S. rolfsii* infected soil significantly increased seed germination by 13.1 and 8.5 per cent respectively as compared to control. Belkar *et al*. (2013) [5] reported that the seed treatment with *Pseudomonas fluorescens* @ 10g/kg of seed+ *Bradyrhizobium japonicum* @ 20g/kg of seed + *Pseudomonas striata* @ 20g/kg of seed with minimum stem rot incidence, i.e. 8.86%, 13.33%, 20.00% at 20 DAS and 17.73%, 33.33% and 40.00% of flowering, respectively. Out of six isolates *P. fluorescens* under study, Isolates G45RII, G45RIII and G60RI were more superior in promoting seed germination, seedling growth of groundnut crop and antagonistic potential against soil borne pathogen *S. rolfsii* (Karabasappa, 2016) [14]. The above results lend support to the present findings.

Table 5: Effect of seedling dip and soil application with antagonists on the incidence of collar rot of mint

S. No.	Treatments	Disease incidence (%)
1.	T_1-*P. fluorescens* SD	24.00 d (29.32)
2.	T_2-*P. fluorescens* SA	22.0 c (27.95)
3.	T_3-*P. fluorescens* SD+ SA	13.66 b (25.59)
4.	T_4-Propiconazole @ 0.1% as soil drenching	12.66 a (20.81)
5.	T_5-Inoculated control	93.33 f (75.04)

Mean of four replications
SD- -Seedling dip with *P. fluorescens* @$8x10^8$ cfu/ml
SA-Soil application with *P. fluorescens* @2.5 kg/ha
Values in each column followed by the same letter are not significantly different according to the DMRT method (p = 0.05)

4. Conclusion

The findings from present study suggested that native isolate-I_7 (PFP-Pseudomonas fluorescens- Pochampalli) recorded maximum per cent inhibition on the growth of *Sclerotium rolfsii* under *in vitro*. Application of talc-based formulation of isolate-I_7 (PFP) through seedling dip (8×10^8 cfu/ml) and soil application (2.5 kg/ha) were found as best treatments to minimize collar rot disease of mint. Whereas effects on the environment as well as the person who handles it, while application in the field and consumers using the product, use of *Pseudomonas* in field condition could be considered as better, as it is beneficial and eco-friendly. Since, one year data is not sufficient to conclude concurrent results; further experimentations are required to confirm the findings.

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