Evaluation of Antagonistic Potential of Bacillus spp. and Trichoderma spp. against Sclerotium rolfsii Sacc. causing Collar Rot Disease in Solanum melongena L.

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ABSTRACT

Collar rot caused by Sclerotium rolfsii Sacc. is one of the catastrophic diseases of brinjal causing in negligible yield loss globally. The present study investigated the effect of Bacillus spp. and Trichoderma spp. on the growth of collar rot pathogen S. rolfsii under in vitro. The highest disease incidence was noticed in Vilangudi village which recorded 42.4% followed by Thirumangalam village (39.5%) and least incidence was noticed in Kottampatty village (8.8%) of Madurai district in Tamil Nadu. Results showed that the mycelial growth of the S. rolfsii was significantly inhibited by the Trichoderma isolate T-AG with 84.44 per cent growth reduction followed by the Bacillus isolate B-VI with 55.55 per cent growth reduction.

Keywords

Solanum melongena, collar rot, Sclerotium rolfsii, Bacillus spp., Trichoderma spp., Dual culture technique

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Introduction

Brinjal (Solanum melongena L.) shares many other names such as eggplant/aubergine and belongs to the Solanaceae family. It is originated in the Indian subcontinent and China as reported by (Martin and Rhodes, 1979). It is one of the principal renowned crops acclimatizing tropics and subtropics. It fits into distinct agro climatic zones all-round the year proving its versatility (Singh et al., 2014). India is recorded with the production of 12,779.54 thousand tonnes of Brinjal. Correspondingly, Tamil Nadu occupies
eleventh place with regard to production of brinjal in India (Apeda, 2017-18). Brinjal is cultivated under an area of 728.00 thousand hectares resulting for an yearly yield of 12,660.00 thousand metric tonnes and productivity of 17.7 metric tonnes per hectare (Indiastat, 2018-19). Brinjal extracts have been reported to victoriously quash the growth and development of tumours, lung cancer (Matsubara et al., 2005), inhibit inflammation (Keli et al., 1996) and cardiovascular diseases (Knekt et al., 1996; Knekt et al., 1997). Being a nutrient dense food, it can provide at least 5% of a person’s routine requirement of fiber, flavonoids, copper, vitamin B-6 and thiamine. Extracts of the purple skinned brinjal has been shown to exhibit a lofty ability in scavenging superoxide radicals and inhibition of hydroxyl radical production by chelating ferrous iron (Kaneyuki et al., 1999; Noda et al., 2000). All in all, brinjal has received a skyrocketing zest among consumers and researchers throughout and is ranked among top 10 vegetables with regard to antioxidant capacity (Cao et al., 1996). It is decimated by multiple pathogens categorized into fungi, bacteria and viruses. Among fungal diseases, collar rot caused by Sclerotium rolfsii Sacc. is turning out be a profuse menace under nursery and field cultivated brinjal crop. It can infect seeds, seedlings and mature plants in the field, cause diseases to fresh vegetables and rhizomes, while in storage and transit. Collar rot may cause up to 30-50 % loss in fruit yield in eggplant (Siddique et al., 2016). The pathogen invades the collar zone of the host adjacent to the soil level causing death by disrupting translocation of food from top to root zone (Begum et al., 1985). It is a facultative saprophyte and can maintain its generation even under drastic set up by formation of sclerotia. So, it is ineffective and uneconomical to control the pathogen with chemical, which being soil-borne and omnivorous. Hence, biological control which comprises the employment of various microorganisms to control plant pathogens is seemed to be very beneficial as it may be economically as well as environmentally useful and safer option for modern agriculture practice today. Suryawanshi et al., (2015) reported that Bacillus megaterium exhibited the highest mycelial growth inhibition (87.85%) against S. rolfsii. Doley and Jite, (2012) reported that the Trichoderma isolate inhibited the radial growth of S. rolfsii by 75%. Henis et al., (1982) reported mycoparasitism (penetration and infection) of Trichoderma spp. against S. rolfsii. Jadon and Tiwari, (2011) showed that T. viride was found most effective in inhibiting both mycelial growth (81.2%) and sclerotia production(14.15) of S. rolfsii. So keeping this in view, the present study was carried out to study the effect of fungal and bacterial biocontrol agents against the deadly pathogen S. rolfsii causing collar rot disease in brinjal.

Materials and Methods

Collection and isolation of Sclerotium rolfsii Sacc.

A survey was conducted in prominent brinjal growing districts of Tamil Nadu. The plants exhibiting collar rot including sclerotial germination which may measure 1-3mm with mustard like appearance upon surfaces of the infected plant parts were collected (Koike, 2004). Then the pathogen was isolated through tissue segment method where the infected tissues along with adjacent small unaffected tissues were cut into small pieces, surface sterilized and plated on potato dextrose agar medium. The infected portion along with healthy portion of plant was cut into small pieces and surface sterilized with 1% Sodium hypochlorite for 1 minute, washed shortly in sterile distilled water and dried on sterile filter paper. The dried pieces were plated onto sterile Petri plate containing
PDA (potato dextrose agar) medium and incubated at 25±1°C for seven days. Pure culture of the pathogen was acquired following hyphal tip method and subsequently multiplied on PDA medium in test tubes and Petri dishes and stored at 4°C for further studies (Mian, 1995).

**Assessing the virulence of the pathogen**

The ten purified isolates of *S. rolfsii* were tested for pathogenicity under in vivo. The isolates were mass multiplied in sand maize medium. Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content and filled in polythene bags. These bags were autoclaved at 1.4 kg/cm² pressure for 20 minutes. Then seven days old actively growing mycelial discs (6 mm) of the pathogen isolate was inoculated into each bag under aseptic condition and incubated at room temperature (28 ± 2°C) for 10 days. Then thirty days old brinjal seedlings were transplanted to the pot containing sand maize medium mass multiplied with *S. rolfsii*. Symptoms expression was observed five days after inoculation and percent disease incidence was derived.

\[
\text{Percent Disease Incidence} = \left( \frac{\text{Number of plants rotted}}{\text{Total number of plants observed}} \times 100 \right)
\]

**Isolation of *Trichoderma* spp.**

The soil samples were collected from the root zones of brinjal crop at different locations in Tamil Nadu. Before isolation, the roots were gently shaken to remove excess soil and vortexed for 10 min in sterile distilled water (1g per 10 ml). Samples were serially diluted with sterile distilled water from 10⁻¹ to 10⁻⁴ dilutions and 1ml of the aliquot from 10⁻³ and 10⁻⁴ dilutions were plated on Trichoderma selective medium. The Petri dishes were rotated clockwise and anticlockwise for uniform distribution and incubated for five days at room temperature (25 ± 3°C). Colonies of *Trichoderma* isolates were identified following a standard key. Then isolates of *Trichoderma* were purified on PDA plates following single hyphal tip technique. After purification, all of the isolates were preserved on PDA slants at 4°C as stock culture for successive use.

**In vitro screening of different isolates of *Bacillus* spp. against the mycelial growth of *S. rolfsii***

Eleven isolates of *Bacillus* spp. were screened against the virulent isolate of *S. rolfsii* IS(VIL)-9 under in vitro by adapting dual culture technique (Dennis and Webster, 1971). Each bacterial isolate was streaked at one side of the Petri dish containing PDA at one cm away from the periphery of the Petri dish. A nine mm mycelial disc of *S. rolfsii* was placed at a contrary side of the Petri dish at one cm away from the periphery of the Petri dish and perpendicular to the bacterial streak. Control plates were maintained without...
bacterial streak. Three replications were maintained at room temperature for four days. After attaining complete growth of the pathogen in the control plate, percent inhibition over control was calculated using the formula proposed by (Pandey and Upadhyay, 2000).

$$\frac{D_c - D_t}{D_c} \times 100$$

$D_c =$average diameter of fungal growth (cm) in control

$D_t =$average diameter of fungal growth (cm) in treatment

**Statistical analysis**

Experimental data were statistically analyzed using analysis of variance (ANOVA) and the Statistical Package for the Social Sciences version 16.0. The treatment means were separated at 5% significant level using Duncan’s Multiple Range Test (DMRT).

**Results and Discussion**

**Collection and isolation of the pathogen**

The data obtained during the survey conducted in major brinjal growing areas of Tamil Nadu was presented in the table 1. Isolates of *S. rolfsii* were isolated from diseased plants showing the typical symptoms of collar rot and presence of sclerotia on the root surface using PDA medium. The fungi were observed under microscope, identified as *S. rolfsii* based on the morphological characters of the fungus and sclerotal structures. The isolated fungus produced dark, white coloured fluffy mycelium and brown sclerotia on the PDA medium. Similarly, Morton, (1969) observed that growth and branching of *S. rolfsii* filamentous fungi occurred at the apex of mycelium and pointed out that growth was regulated by a delicate balance between cell wall synthesis and degradation. Zarani and Christias, (1997); Sarma et al., (2002) reported the production of small, spherical, tan to dark brown and black colored sclerotia in the Petri plates. Thus, the purified fungal culture was sent to the Indian Type Culture Collection, PUSA, IARI, and was confirmed as *Sclerotium rolfsii* with the I.D.No 11,305.20.

**Assessing the virulence of the pathogen**

The isolated pathogen was proved to be pathogenic on brinjal plants. Inoculated plants produced rotting and disruption in translocating the food from top to root zone.
with sclerotia on the root surface. No such symptoms were observed on the uninoculated control plants. Among the different isolates tested, the isolate from Vilangudi was found to be most virulent in inducing the collar rot symptoms. Similarly, Bhuiyan et al., (2012) tested 10 isolates of S. rolfsii for their ability to cause foot and root rot disease of soybean by soil infestation method in pot culture experiment under shade condition. Then the causal agent of pre-emergent seedling mortality was confirmed after re-isolation of the pathogen from un-germinated seeds, infected root and stems.

**Table.1** Survey for assessing the collar rot disease incidence in brinjal in different location of Tamil Nadu

| S. No | Place of collection | Districts | Isolate code | Geo co ordinates | Percent Disease Incidence(%) |
|-------|---------------------|-----------|--------------|------------------|-----------------------------|
|       |                     |           |              | Latitude Longitude |                             |
| 1     | Thirumangalam       | Madurai   | IS (THI)-1   | 9.8216°N 77.9891°E | 39.5                        |
| 2     | Kanakiliyanallur    | Trichy    | IS (KAN)-2   | 10.9956°N 78.8800°E | 18.8                        |
| 3     | AC & RI             | Madurai   | IS (AGR)-3   | 9.9699°N 78.2040°E | 10.2                        |
| 4     | Palamedu            | Madurai   | IS (PAL)-4   | 10.1043°N 78.1130°E | 35.2                        |
| 5     | Vandalaikudalur     | Trichy    | IS (VAN)-5   | 10.9701 N 78.8878 E | 12.5                        |
| 6     | K.K.Patty           | Theni     | IS (KKP)-6   | 9.7386° N 77.3181°E | 15.8                        |
| 7     | Pudhupatty          | Theni     | IS (PUD)-7   | 9.4356° N 77.9996°E | 21.5                        |
| 8     | Ayanpannapatty      | Trichy    | IS (AYA)-8   | 9.6313° N 77.7666°E | 24.6                        |
| 9     | Vilangudi           | Madurai   | IS (VIL)-9   | 9.9498° N 78.0879°E | 42.4                        |
| 10    | Kottampatty         | Trichy    | IS (KOT)-10  | 10.6228°N 78.4471°E | 8.8                         |

**Table.2** Bacillus spp. isolated from rhizosphere region of brinjal plant in different locations in Tamil Nadu

| S. No | Place of collection | Districts | Isolate code | Geo co ordinates | Colony characters |
|-------|---------------------|-----------|--------------|------------------|-------------------|
|       |                     |           |              |Latitude Longitude|                   |
| 1     | Thirumangalam       | Madurai   | B-TH         | 9.8216°N 77.9891°E | Dull white colonies |
| 2     | Kanakiliyanallur    | Trichy    | B-KA         | 10.9956°N 78.8800°E | Regular white colonies |
| 3     | AC & RI             | Madurai   | B-AG         | 9.9699°N 78.2040°E | Bright white colonies |
| 4     | Palamedu            | Madurai   | B-PA         | 10.1043°N 78.1130°E | Irregular creamy colonies |
| 5     | Vandalaikudalur     | Trichy    | B-VA         | 10.9701°N 78.8878 E | Thin white colonies |
| 6     | K.K.Patty           | Theni     | B-KK         | 9.7386°N 77.3181°E | Regular bright white colonies |
| 7     | Pudhupatty          | Theni     | B-PU         | 9.4356°N 77.9996°E | Irregular dull white colonies |
| 8     | Ayanpannapatty      | Trichy    | B-AY         | 9.6313°N 77.7666°E | Light yellow colonies |
| 9     | Vilangudi           | Madurai   | B-VI         | 9.9498°N 78.0879°E | Flat bright white colonies |
| 10    | Kottapatty          | Trichy    | B-KO         | 10.6228°N 78.4471°E | Creamy white colonies |
| 11    | CR Palayam          | Trichy    | B-CR         | 11.2000°N 78.4900°E | Bright white colonies |
### Table 3
*Trichoderma* spp. isolated from rhizosphere region of brinjal plant indifferent locations of Tamil Nadu

| S. No | Place of collection | Districts | Isolate code | Geo co ordinates | Culture characters |
|-------|---------------------|-----------|--------------|------------------|-------------------|
| 1     | Thirumangalam       | Madurai   | T-TH         | 9.8216ºN 77.9891ºE | Scattered in dull green |
| 2     | Kanakiliyanallur    | Trichy    | T-KA         | 10.9956ºN 78.8800ºE | Dull white to dark green |
| 3     | AC & RI             | Madurai   | T-AG         | 9.9699ºN 78.2040ºE | Tufts of white mycelium tuning green |
| 4     | Palamedu            | Madurai   | T-PA         | 10.1043ºN 78.1130ºE | Yellowish green tufts |
| 5     | Vandalaikudalur     | Trichy    | T-VA         | 10.9701 N 78.8878 E | White tufts of mycelium turning into dark green |
| 6     | K.K.Patty           | Theni     | T-KK         | 9.7386ºN 77.3181ºE | White tufts of mycelium becoming green from the centre |
| 7     | Pudhupatty          | Theni     | T-PU         | 9.4356º N 77.3996ºE | Dark green in concentric rings |
| 8     | Ayanpannapatty      | Trichy    | T-AV         | 9.6313º N 77.7666ºE | Scattered in minute tufts dark green |
| 9     | Vilangudi           | Madurai   | T-VI         | 9.9498º N 78.0879ºE | Complete dark green |
| 10    | Kottapatty          | Trichy    | T-KO         | 10.6228ºN 78.4471ºE | White tufts of mycelium gradually turning into yellowish green |
| 11    | CR Palayam          | Trichy    | T-CR         | 11.2000ºN 78.4900ºE | Yellowish green |
| Ck    | TNAU T.V.1 (Check)  |           |              | 11.0152 ºN 76.9326ºE | White mycelium turning into Yellowish green |

### Table 4
Efficacy of *Bacillus* spp. against the mycelial growth of *S. rolfsii* IS (VIL)-9 in *in vitro*

| S.No. | Treatments | Mycelial growth(cm)* | Percent inhibition over control |
|-------|------------|----------------------|---------------------------------|
| 1     | B-TH       | 6.20                 | 31.11                           |
| 2     | B-KA       | 6.90                 | 23.33                           |
| 3     | B-AG       | 6.40                 | 28.88                           |
| 4     | B-PA       | 5.90                 | 34.44                           |
| 5     | B-VA       | 5.90                 | 34.44                           |
| 6     | B-KK       | 6.60                 | 26.66                           |
| 7     | B-PU       | 6.40                 | 28.88                           |
| 8     | B-AV       | 5.50                 | 38.88                           |
| 9     | B-VI       | 4.00                 | 55.55                           |
| 10    | B-KO       | 4.70                 | 47.77                           |
| 11    | B-CR       | 5.80                 | 35.55                           |
| Control |           | 9.00                 |                                 |

CD(.05) 0.24

*Mean of three replications*
Smith *et al.*, (1986) proved that *S. rolfsii* isolate from sorghum was found to be pathogenic on greenhouse grown host plants like bean, sugar beet and carrot. Sultana *et al.*, (2012) evaluated *S. rolfsii* for its pathogenicity in a pot culture experiment under the shady atmosphere by soil and seed infestation. Expression of pre-emergence and post-emergence seedling mortality were observed and recorded frequently after sowing. Re-isolation of the pathogen was done to confirm the causal agent of seedling infection as *S. rolfsii*

### Isolation and confirmation of biocontrol agents

Eleven isolates of *Bacillus* spp. and twelve isolates of *Trichoderma* spp. were isolated from the rhizosphere region of brinjal plant collected at various places in Tamil Nadu. They were assigned different names as per the biochemical analysis results which confirmed the fungal isolates as *Trichoderma* spp. and bacterial isolates as *Bacillus* spp. Similarly, *Bacillus* spp. were identified based upon their colony characters, microscopic characteristics and biochemical characters viz., starch hydrolysis (Iverson and Millis, 1974), catalase test (Schaad, 1992) and oxidase test (Gordon and McLeod, 1928). *Trichoderma* spp. were identified based on the morphological features like colony colour, concentric rings with green conidial production, irregular yellow zone without conidia and white pustules on the green mat of conidia depending upon species. *Trichoderma* spp. isolates were identified based on mycological characters.

### Effect of antagonistic activity of *Bacillus* spp. against *S. rolfsii* under *in vitro*

Eleven isolates of *Bacillus* spp. were tested against *S. rolfsii* under *in vitro*. Among the isolates, the isolate B-VIdraconiany inhibited the mycelial growth of *S. rolfsii* (4.0 cm) with

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**Table.5 Efficacy of *Trichoderma* spp isolates against the mycelial growth of *S.rolfsii* IS(VIL)-9 in *in vitro***

| S.No | Treatments | Mycelial growth(cm)* | Percent inhibition over control |
|------|------------|----------------------|--------------------------------|
| 1    | T-TH       | 5.00                 | 44.44                          |
| 2    | T-KA       | 3.70                 | 58.88                          |
| 3    | T-AG       | 1.40                 | 84.44                          |
| 4    | T-PA       | 4.40                 | 51.11                          |
| 5    | T-VA       | 2.60                 | 71.11                          |
| 6    | T-KK       | 4.10                 | 54.44                          |
| 7    | T-PU       | 4.20                 | 53.33                          |
| 8    | T-AY       | 6.10                 | 32.22                          |
| 9    | T-VI       | 4.20                 | 53.33                          |
| 10   | T-KO       | 5.10                 | 43.33                          |
| 11   | T-CR       | 5.20                 | 42.22                          |
| Ck   | TNAUT.V.1 (Check) | 4.10 | 54.44 |
| Control |         | 9.00                 |                                |
| CD(.05) |            | 0.14                 |                                |

*Mean of three replications
55.55 per cent growth reduction followed by B-KO and B-AY which were successively effective and recorded 4.7 and 5.5 cm growth of the pathogen with 47.77 and 38.88 per cent growth reduction over control respectively. Similarly, Suryawanshi et al., (2015) reported that *B. Subtilis* and *B. megaterium* were fungistatic against *S. rolfsii*. Suneeta et al., (2017) reported that *B. subtilis* was expressed to be significantly different between antagonistic *Bacillus* strains which proliferated the yield and cut down the collar rot incidence (44.21%) over the control of gerbera collar rot. De Curtis et al., (2010) reported that *B. cepacia* significantly inhibited damping-off caused by *S. rolfsii*, reducing the disease index by 81% compared to the untreated control. Shifa et al., (2015) tested that *B. subtilis* strain G-1 was the most effective in inhibiting the mycelial growth of *S. rolfsii* and recorded an inhibition of 28%. *B. subtilis* secreted antifungal substance which is highly antagonistic against *S. rolfsii* (Nalisha et al., 2006). Gholami et al., (2014) reported that the *Bacillus* sp. and *S. cyaneofuscatus* isolates showed the same capacity for reducing the disease severity of *S. rolfsii* for over 50% (Table 2–4).

**Effect of antagonistic activity of Trichoderma spp. against S. rolfsii under in vitro**

Twelve isolates of *Trichoderma* spp. and check (TNAU T.v. 1) were tested against *S. rolfsii* under *in vitro* and tabulated in the table 5. Among the isolates, T-AG drastically inhibited the mycelial growth of *S. rolfsii* (1.40 cm) with 84.44 per cent growth reduction followed by T-VA and T-KA which were rigorously effective and recorded 2.60 and 3.70 cm growth of the pathogen with 71.11 and 58.88 per cent growth reduction over control respectively. Similarly, Doley and Jite, (2012) observed that the bioagent *T. Viride* inhibited 75% of *S. rolfsii* growth.

Bhuiyan et al., (2012) showed 83.06% significant reduction of mycelial growth of *S. rolfsii* in presence of *Trichoderma* spp. Madhavi and Bhattacharya (2011) reported the highest (57.5%) mycelial inhibition of *S. rolfsii* with *T. harzianum* resulting similar to Virupaksha Prabhu et al., (1997) who worked with *T. Harzianum* against collar rot of cotton. Singh and Singh, (1994) proved that *T. harzianum* showed highest antagonistic activity (73%) against *S. rolfsii* of brinjal.

In conclusion there are umpteen results available for the control of *S. rolfsii* under *in vitro* by bioagents like *Bacillus* spp. and *Trichoderma* spp. Present study revealed that the bioagents expressed antifungal activity through competition, antibiosis, mycoparasitism and induced systemic resistance. Moreover, the lofty cost connected with the use of chemical fungicides to control soil borne pathogenic fungi is an extensive limiting factor restraining the profitability of crop production. The use of bioagents in lieu of fungicides or bactericides with high potential is the need of the hour to protect the crop with no harm to environment.

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