Screening of fungicides and bio-control agents against *Sclerotium rolfsii* (Sacc.) causing collar rot in Lentil

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Abstract

Lentil is an important pulse crop, contributes about 8-9% of the total pulse production in India and is also a major source of protein, minerals and vitamins. Collar rot of lentil is an important seedling disease caused by *Sclerotium rolfsii* Sacc. In this study, twenty isolates (Th1-Th20) of *Trichoderma harzianum*, one isolate of *Pseudomonas fluorescens* and nine commercially available fungicides were tested in vitro for their efficacy in inhibiting the growth of the pathogen. *Trichoderma harzianum* isolate Th16 and Th14 were found to inhibit maximum mycelial growth of the pathogen. The complete inhibition of mycelial growth of *Sclerotium rolfsii* was found with Propiconazole, Tabuconazole, Hexaconazole, Ridomil and Carbendazim+ Mancozeb at all (0.03%, 0.05% and 0.1%) concentrations. Other fungicides (Captan, Mancozeb and Carbendazim) also showed significant inhibition of the mycelial growth of the pathogen. No inhibition was recorded in the treatment with copper oxychloride even at 0.2% concentration.

Keywords: Lentil, *Sclerotium rolfsii*, *Trichoderma harzianum*, *Pseudomonas fluorescens* and fungicide

Introduction

Lentil, (*Lens culinarius*) is a bushy, annual shrub plant that is popular for its lens shaped seeds and nutritious value of the seeds that is rich in protein, fibers having second highest levels of proteins and fiber after soybeans. The tap root system of the plant usually grows to a depth of around 15 cm that makes it a moderately drought resistant crop. India is second top producer of lentil in the world after Canada. In India, total area covered by lentil producers is about ~1.3 million hectares (APEDA, 2015) [1]. World lentil production has been increasing in recent years with most of the production coming from North American and Asian countries. Productivity of lentil in India varies from region to region due to variation in environment and biotic factors.

Lentil is attacked by fungal, viral and bacterial pathogens. *Sclerotium rolfsii* is one of the important soil borne fungal pathogens having a wide host range and world-wide distribution (Punja, 1988) [9], causing collar rot, root rot, stem rot and wilt on more than 500 plant species including almost all the agricultural and horticultural crops. The fungus is a soil borne pathogen of very aggressive nature and causes considerable damage to young seedlings causing collar rot resulting in substantial yield losses. *S. rolfsii* produces sclerotia which is the principal structure to survive under adverse conditions. Control of soil borne pathogens has become one of the major concerns in agriculture. However effective and efficient management of crop diseases is generally achieved by the use of synthetic pesticides. These pesticides are known to pollute the environment, soil and water, besides causing deleterious effects on human health and biosphere. The present investigation is related to the study of the management of collar rot disease of Lentil by different fungicides and bioagents and comparing the efficacy of both.

Material and Methods

Experimental site, Location and Collection of diseased specimens-
The experiments were conducted at Department of Plant Pathology, Sardar Vallabhbhai Patel University of agriculture and Technology, Meerut (Uttar Pradesh).
Isolation and identification of pathogen - Infected lentil plants showing the typical symptoms of collar rot collected from Crop Research Centre (CRC) of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut and from farmers’ field in the vicinity of Meerut. All specimens were collected and placed in a moist chamber at 25°C. Besides this the collar portion of the infected plants were cut in 3-5 mm thick tissue sections and sterilized with 0.1% sodium hypochlorite solution for 30 seconds, rinsed thrice in sterilized distilled water (SDW) and dried on sterilized filter paper at room temperature. The tissue sections were then placed on potato dextrose agar (PDA) amended with 100 µg/ml streptomycin sulphate and incubated at 25°C for fifteen days. The pure culture of the pathogen was isolated and subsequently maintained on PDA.

Isolation of biocontrol agent - Soil samples were collected from the rhizospheric region of healthy plants from different locations of Meerut district for isolation of biocontrol agents. By using dilution plate techniques, Bio control agents were isolated, purified and identified on the basis of their morphological characters (Nashwa et al., 2008; Rifai, 1969) [6]. The obtained Bioagents (Fungi- Trichoderma Spp.) were maintained in PDA and (Bacterial- P. fluorescens) maintained in King’s B agar slants.

In vitro evaluation of bio-agents against S. rolfsii
The tested isolates of Trichoderma spp. were grown on PDA medium at 20°C, for 6-days and used as inocula. Culture discs from each isolate of Trichoderma spp. and the pathogen (S. rolfsii) were inoculated opposite to each other at 4cm apart in the petriplate containing 20 ml PDA and the antagonistic properties of the test bioagents were exhibited (dual culture technique). Five replicates were used for each isolate of Trichoderma spp. The inoculated plates with culture discs of pathogen without bioagents served as control. After 24, 48, 72 and 96 hrs of incubation at 20°C, radial growth of pathogen and percent inhibition was recorded. Inhibition percent of pathogen growth was calculated using the following formula:

Vincent (1927).

\[ I = \frac{C - T}{C} \times 100 \]

Where,
I = Per cent inhibition
C = Growth in control
T= Treatment

Table 1: List of Isolates of Bio-agents

| S. N. | Bioagent | Isolated from crop | Location |
|-------|----------|--------------------|----------|
| 1. | T1 | Wheat | Budaun |
| 2. | T2 | Wheat | Budaun |
| 3. | T3 | Wheat | Budaun |
| 4. | T4 | Wheat | Meerut |
| 5. | T5 | Berseem | Budaun |
| 6. | T6 | Berseem | Budaun |
| 7. | T7 | Berseem | Meerut |
| 8. | T8 | Berseem | Meerut |
| 9. | T9 | Chickpea | Budaun |
| 10. | T10 | Chickpea | Budaun |
| 11. | T11 | Chickpea | Meerut |
| 12. | T12 | Chickpea | Meerut |
| 13. | T13 | Lentil | Budaun |
| 14. | T14 | Lentil | Budaun |
| 15. | T15 | Lentil | Budaun |
| 16. | T16 | Lentil | Meerut |
| 17. | T17 | Lentil | Meerut |
| 18. | T18 | Sugarcane | Budaun |
| 19. | T19 | Sugarcane | Budaun |
| 20. | T20 | Sugarcane | Meerut |

In vitro evaluation of fungicides against S. rolfsii
Effect of eight fungicides belonging to different groups (listed in Table-2) with different concentrations (viz., 0.03, 0.05 and 0.1 per cent for systematic fungicide and 0.05, 0.1 and 0.2 per cent for non-systemic fungicides) were tested in vitro for evaluating their efficacy to inhibit the growth of the pathogen. Effect on the growth of S. rolfsii was studied using poisoned food technique (Nene and Thapliyal, 1982) [7]. 20 ml of poisoned medium was poured into each of the 90 mm sterilized petriplates. Each plate was inoculated with five mm disc of mycelium at the center and incubated at 27± 1°C. Three replications were maintained for each treatment. Potato dextrose agar medium without any fungicide served as control. All plates were incubated at 27± 1°C till the growth completed in control plate. The per cent inhibition of the growth over control was calculated by following the equation given by Vincent (1927).

\[ I = \frac{C - T}{C} \times 100 \]

Where,
I = Per cent inhibition
C = Growth in control
T = Growth in treatment
Table 2: List of fungicides used

| S. No. | Common Name               | Chemical name    | Concentrations       |
|--------|---------------------------|------------------|----------------------|
| T₁     | Carbendazim (Bavistin)    | C₉H₉N₃O₂        | 0.03, 0.05 and 0.1 %  |
| T₂     | Propiconazole (Tilt)      | C₉H₉Cl₂N₃O₂     | 0.03, 0.05 and 0.1 %  |
| T₃     | Tebuconazole (Torque)     | C₆H₆ClN₂O       | 0.03, 0.05 and 0.1 %  |
| T₄     | Hexaconazole (Contaf)     | C₆H₆ClN₂O       | 0.03, 0.05 and 0.1 %  |
| T₅     | Ridomil (Metalaxyl)       | C₆H₆NO₄         | 0.03, 0.05 and 0.1 %  |
| T₆     | Mancozeb (Dithane M-45)   | C₆H₆MnN₃S₂Zn    | 0.05, 0.1 and 0.2 %   |
| T₇     | Captan                    | C₆H₇ClNO₂S      | 0.05, 0.1 and 0.2 %   |
| T₈     | Copper Oxy Chloride       | CuCl₂3CuH₂O₂    | 0.05, 0.1 and 0.2 %   |
| T₉     | Carbendazim + Mancozeb    | ------------------|----------------------|

Results
Isolation and identification of pathogen: On PDA plates, the fungus produced silky, white mycelium, which gradually loses its luster and became somewhat dull in appearance. Aerial hyphae grow fast, were not uniformly distributed. The hyphae were septet, hyaline, clamp connections occurred in the broader threads and branching was at right angles behind the cross wall. The individual hyphal cells were 60-350 µm in length and 2-8 µm in diameter. After the mycelial mat develops, the sclerotial initials formed from hyphal strands lying parallel. A spherical shape was assumed by the loose mass of hyphae. The fully matured sclerotia were spherical, light to dark brown in colour, embedded in fuzzy mycelial mat and measured 1.5 to 4.2 mm in diameter. Initiation of sclerotial bodies was observed from fifth day onwards after inoculation. Sclerotia may be seen in the mycelium, on diseased tissues above or below ground, on soil surfaces, or in soil crevices also (West, 1961) [11].

In vitro evaluation of bio-agents against S. rolfsii:
Antagonistic activities of twenty isolates of Trichoderma sp. and one strain of Pseudomonas sp. were evaluated against Sclerotium rolfsii in vitro as indicated in Table: 4.2, Fig-1, Plate-4. The results from the table revealed that, significant difference in per cent inhibition of mycelial growth of Sclerotium rolfsii by all the tested bioagents. Among them maximum inhibition per cent (70.7%) of Sclerotium rolfsii were recorded in Trichoderma isolate T16,which is significantly superior from all the tested isolates and it is followed by T14 (70.0%), T2 (69.3%), T11 (68.5%), T8 (68.1%), and other isolates T1, T4, T7, T3, T5, were found 67.7% mycelial inhibition. whereas T10, T18, T15, T20, T17, T12, T16, T19 and T13 inhibited the mycelial growth of the pathogen between the range of 61.1% to 66.6% and the growth was significant as compared to control. However, the other bioagent Pseudomonas fluorescence inhibits 52.5% mycelial growth of the pathogen. The minimum inhibition per cent of mycelial growth was recorded in Trichoderma isolates T19 (47.0%). All the tested bioagents were statistically significant but among the isolates T1, T4, T7, T3, T5 and T6, T12, T17, T20, T15 were non-significant among each other, but significant over control.
**Fig 2**: *in-vitro* evaluation of bio agents (left to right 1<sup>st</sup> three rows as T1 – T20, 4<sup>th</sup> row pure bioagent and control)

**Table**: 3 Effect of different Bioagents on radial growth of *sclerotium rolfsii* in *vitro.*

| Treatments | Inhibition after 24 hr. | Inhibition after48 hr. | Inhibition after 72hr. | Inhibition after 96 hr. |
|------------|-------------------------|------------------------|------------------------|------------------------|
|            | colony diameter (mm)    | colony diameter (mm)   | colony diameter (mm)   | colony diameter (mm)   |
|            | Per cent inhibition     | Per cent inhibition    | Per cent inhibition    | Per cent inhibition    |
| T1         | 8.0                     | 38.4                   | 15.0                   | 55.8                   | 22.0                   | 64.5                   | 29.0                   | 67.7                   |
| T2         | 9.0                     | 30.7                   | 15.3                   | 55.0                   | 25.0                   | 59.6                   | 27.6                   | 69.3                   |
| T3         | 9.0                     | 30.7                   | 18.7                   | 45.0                   | 28.0                   | 54.8                   | 29.3                   | 67.4                   |
| T4         | 8.0                     | 38.4                   | 19.0                   | 44.1                   | 25.7                   | 58.5                   | 29.0                   | 67.7                   |
| T5         | 8.0                     | 38.4                   | 17.0                   | 30.0                   | 25.3                   | 59.1                   | 29.3                   | 67.4                   |
| T6         | 10.0                    | 23.1                   | 18.7                   | 45.0                   | 31.3                   | 49.5                   | 33.0                   | 63.3                   |
| T7         | 7.7                     | 40.7                   | 15.0                   | 55.8                   | 21.0                   | 66.1                   | 29.0                   | 67.7                   |
| T8         | 10.0                    | 23.1                   | 13.7                   | 59.7                   | 22.0                   | 64.5                   | 28.7                   | 68.1                   |
| T9         | 9.0                     | 30.7                   | 16.0                   | 52.9                   | 27.0                   | 56.4                   | 31.0                   | 65.5                   |
| T10        | 9.7                     | 25.3                   | 18.7                   | 45.0                   | 30.3                   | 51.1                   | 35.0                   | 61.1                   |
| T11        | 8.3                     | 36.1                   | 11.7                   | 65.5                   | 20.0                   | 67.7                   | 28.3                   | 68.5                   |
| T12        | 7.7                     | 40.7                   | 16.0                   | 52.9                   | 25.0                   | 59.6                   | 33.0                   | 63.3                   |
| T13        | 9.0                     | 30.7                   | 17.3                   | 49.1                   | 25.3                   | 19.1                   | 30.0                   | 66.6                   |
| T14        | 8.0                     | 38.4                   | 12.0                   | 64.7                   | 19.0                   | 69.3                   | 27.0                   | 70.0                   |
| T15        | 11.0                    | 15.3                   | 21.0                   | 38.2                   | 32.3                   | 47.9                   | 34.3                   | 61.8                   |
| T16        | 9.0                     | 30.7                   | 13.0                   | 61.7                   | 24.0                   | 61.2                   | 26.3                   | 70.7                   |
| T17        | 8.0                     | 38.4                   | 20.0                   | 41.1                   | 29.3                   | 52.7                   | 33.0                   | 63.3                   |
| T18        | 9.0                     | 30.7                   | 20.0                   | 41.1                   | 30.0                   | 51.6                   | 35.0                   | 61.1                   |
| T19        | 9.0                     | 30.7                   | 18.0                   | 47.0                   | 30.7                   | 50.4                   | 47.7                   | 47.0                   |
| T20        | 10.0                    | 23.1                   | 19.3                   | 43.2                   | 30.3                   | 51.1                   | 33.3                   | 63.0                   |
| Tp         | 8.7                     | 33.1                   | 19.0                   | 44.1                   | 29.3                   | 52.7                   | 42.7                   | 52.5                   |
| Control    | 13.0                    | 34.0                   | 62.0                   | 90.0                   |                        |                        |                        |                        |

**CD at 5% level**

| Interval (A) | Treatments (B) | Factor (A X B) |
|--------------|----------------|----------------|
| 0.3397       | 0.7966         | 1.593          |

**SE(m)**

| Interval (A) | Treatments (B) | Factor (A X B) |
|--------------|----------------|----------------|
| 0.1217       | 0.2854         | 0.5708         |
In vitro evaluation of systemic and non-systemic fungicides

Efficacy of five systemic and four non systemic fungicides were tested at different concentrations by poisoned food technique. The results thus obtained have been presented in Table-4, Fig-5 and Plate-6. The results from table – revealed that there is significant difference in per cent inhibition of mycelial growth of *Sclerotium rolfsii* with the fungicides which were tested. The per cent inhibition of mycelial growth of *Sclerotium rolfsii* was found highest (100%) in the treatment with Propiconazole, Tabuconazole, Hexaconazole and Ridomil at all three concentrations (0.03%, 0.05% and0.1%) and Carbendazim+ Mancozeb at all three concentrations (0.05%, 0.1%, and 0.2%). Other fungicides also inhibited the growth of the pathogen significantly. Captan (0.2%) and Mancozeb (0.2%) showed 88.5 per cent and 48.9 per cent inhibitions respectively and were found significantly superior over remaining treatment tested. No inhibition was recorded in the treatment with copper oxy chloride at all concentrations.

Table: 4 Effect of different Fungicides on the growth of *Sclerotium rolfsii* in vitro.

| Treatment                        | Inhibition after 24 hrs. | Inhibition after 48 hrs. | Inhibition after 72 hrs. |
|----------------------------------|-------------------------|--------------------------|--------------------------|
|                                  | Colony diameter (mm) | % inhibition | Colony diameter (mm) | % inhibition | Colony diameter (mm) | % inhibition |
| Carbendazym 0.03%                | 8.0                     | 61.9 | 24.0                     | 54.9 | 65.3                     | 27.4         |
| Carbendazym 0.05%                | 7.0                     | 75.0 | 19.7                     | 63.0 | 57.0                     | 36.7         |
| Carbendazym 0.1%                 | 6.0                     | 71.4 | 16.0                     | 70.0 | 49.0                     | 45.5         |
| Propiconazole 0.03%              | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Propiconazole 0.05%              | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Propiconazole 0.1%               | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Tabuconazole 0.03%               | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Tabuconazole 0.05%               | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Tabuconazole 0.1%                | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Hexaconazole 0.05%               | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Hexaconazole 0.1%                | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Ridomil 0.03%                    | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Ridomil 0.05%                    | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Ridomil 0.1%                     | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Mancozeb 0.05%                   | 8.0                     | 61.9 | 20.7                     | 61.1 | 69.3                     | 23.0         |
| Mancozeb 0.1%                    | 5.7                     | 72.8 | 17.0                     | 68.1 | 59.0                     | 34.5         |
| Mancozeb 0.2%                    | 0.0                     | 100  | 13.7                     | 74.3 | 46.0                     | 48.9         |
| Captan 0.05%                     | 0.0                     | 100  | 8.0                      | 75.7 | 30.3                     | 66.3         |
| Captan 0.1%                      | 0.0                     | 100  | 5.7                      | 82.7 | 26.3                     | 70.8         |
| Captan 0.2%                      | 0.0                     | 100  | 0.0                      | 100  | 10.3                     | 88.5         |
| Blitox 0.05%                     | 15.0                    | 28.6 | 57.0                     | -6   | 90.0                     | 0            |
| Blitox 0.1%                      | 13.3                    | 36.7 | 45.0                     | 15.6 | 90.0                     | 0            |
| Blitox 0.2%                      | 10.0                    | 52.4 | 34.0                     | 36.2 | 90.0                     | 0            |
| Carbendazym+ Mancozeb 0.05%      | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Carbendazym+ Mancozeb 0.1%       | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Carbendazym+ Mancozeb 0.2%       | 0.0                     | 100  | 0.0                      | 100  | 0.0                      | 100          |
| Control                          | 21.0                    | 53.3 | 90.0                     | 90.0 | 90.0                     | 90.0         |
**Discussion**

Bhuiyan *et al.*, 2012 [2] studied the effect of six fungicides namely Provax-200, Bavistin, Ridomil, Dithane M-45, Rovral 50 WP and Tilt (at 100, 200 and 400 ppm concentration) for their efficacy against the radial colony growth of *S. rolfsii* and found that, complete inhibition was obtained with Provax-200 and Tilt at all concentrations.

These findings for Provax-200 in this study were supported by the findings of Rubayet (2011) who concluded that complete inhibition of sclerotia formation of the tested pathogen was achieved highest at 250 ppm and 500 ppm concentration of Provax-200 but sclerotia formation of *S. rolfsii* was completely inhibited at 100 ppm, 250 ppm and 500 ppm concentration.

For management of plant disease, Biological control is an efficient, environment friendly and alternative approach. Many microbial species have been reported that act efficiently against different pathogens. *Trichoderma* spp., *Bacillus* spp. and other microbial spp. have showed efficient inhibition of pathogens in-vitro and in-vivo. Kumar *et al.*, 2012 studied the antagonistic properties of *Trichoderma* isolates and found that *Trichoderma* have higher potential antagonism against *S. rolfsii* with percent inhibition of 44.67 and 47.88.

Similarly, Kulkarni (2007) [4], found maximum inhibition of mycelial growth in *T. harzianum* (59.81%), followed by *T. harzianum* (57.97%) and least inhibition of mycelial growth was observed in Bacillus subtilis (10.74%). Bisht *et al.*, 2013 tested different strains of *Trichoderma* spp. against *Curvularia* leaf spot of maize and found that *Trichoderma harzianum*, Th-13 showed maximum mycelial growth inhibition (83.82 %) followed by Th-9 (80.29 %) and Th-3 (79.12 %).

**Fig 2:** Effect of different Fungicides on *Sclerotium rolfsii*

**Table 1:**

| CD at 5% level | Interval (A) 0.2173 | Treatments (B) 0.5143 | Factor (A X B) 1.150 |
| --------------- | ------------------- | --------------------- | ------------------- |
| SE(m)           | Interval (A) 0.0781 | Treatments (B) 0.1847 | Factor (A X B) 0.4131 |

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