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Efficacy of Bioagents and Fungicides against *Sclerotium rolfsii* of Tuberose

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Tuberose (*Polianthes tuberose* L.) is one of the important ornamental crops being cultivated mainly for its cut and loose flower and oil trade in the tropical and subtropical regions of India. Tuberose is reported to be affected by many diseases of which stem rot caused by *Sclerotium rolfsii* is the most important one in the tuberose growing regions of Tamil Nadu. The commercial cultivation of tuberose has been hampered due to the occurrence of root rot (Das, 1961). Little work has been done on the management of diseases of tuberose. Hence, it is necessary to evaluate bioagents and fungicides *in vitro* condition to formulate management strategy against root rot of tuberose.

An intensive systematic survey was conducted in tuberose growing region of Tamil Nadu (Trichy, Perambalur and Dindigul) to assess the root rot disease incidence. In each field, five plots each with 5x5 m area were selected, among the five plots one plot was fixed at the centre of the field and the remaining four plots were fixed at random in different places in the field avoiding border rows. The root rot incidence was assessed by counting the number of affected plants out of total number of plants in each plot ($25m^2$). In each area,

three fields were assessed and the mean disease incidence was calculated. Per cent disease incidence was calculated by using the formula:

$$\text{Per cent disease incidence} = \frac{\text{Number of plants affected}}{\text{Total number of plants observed}} \times 100$$

Occurrence of root rot disease was ranged from 0 to 15 % in all of the surveyed areas in Trichy, Perambalur and Dindigul districts.

The pathogen, *S. rofsii* was isolated from the affected portion of the root rot/ stem rot affected tuberose plants collected from different places separately on sterile Potato Dextrose Agar (PDA) medium. The diseased tuberose plants were pulled out with intact root showing the presence of white mycelial mat with small round brown sclerotia near the collar region are collected and gently tapped to remove the soil and dirt particle. The infected portions of diseased plants collected from different area were cut into small pieces of 1 to 1.5 cm separately using sterilized scalpel and these were surface sterilized with 0.1 per cent mercuric chloride for thirty seconds and washed in sterile distilled water thrice and then placed in a Petri dish at equidistance onto previously poured and solidified Petri dish containing Potato dextrose agar (PDA) medium. These plates were incubated at room temperature (28 ± 2°C) for five days and observed for the growth of the fungus. The hyphal tips of fungus grown from the pieces were transferred aseptically to PDA slants for maintenance of the culture. The pathogen was identified based on the morphological characters as described by Punja (1985). *Sclerotium rolfsii* was isolated from disease infected plants and identified as the pathogen.

The pathogen, *S. rolfsii* were multiplied on sand-maize medium (Riker and Riker, 1936). The medium containing 1900 g of sand and 100 g of maize powder (19:1) was mixed, moistened with 400 ml of water kg^{-1} and then packed in sterilized polypropylene covers. The covers were sterilized at 1.4 kg cm^{-2} pressure for two hours for two alternate days and inoculated each with two nine mm PDA culture disc of actively growing *S. rolfsii* and incubated for 15 days at room temperature (28±2°C), till the sclerotia turned brown indicating maturity.

The pathogenicity test of *S. rolfsii* was proved by following Koch's postulates. Sieved garden soil was taken in earthen pots with uniform size of 15 cm diameter each with two kg of garden soil and sterilized in an autoclave at 1.4 kg cm^{-2} pressure for 2 h on two successive days. Tubers of tuberose were planted in each pot and replicated three times. Thirty days old seedlings were inoculated by mixing 5 per cent of the pathogen inoculum (multiplied on sand maize medium). The pots were maintained in green house by regular, uniform and judicious watering and then plants were constantly observed for development of the disease symptoms. The per cent wilt incidence of each isolate was recorded after 25 days of inoculation. They were maintained for isolation of the pathogen. The pathogenicity test was conducted in the pot culture experiment

Antagonistic fungi and bacteria were isolated from the rhizosphere soil collected from different tuberose growing areas of Tamil Nadu. The plants were pulled out gently with intact tubers and the excess soil adhering on roots was removed gently. Ten gram of rhizosphere soil was transferred to 250 ml Erlenmeyer flask containing 100 ml of sterile distilled water. After thorough shaking, the antagonist in the suspension was isolated by serial dilution plate

method (Pramer and Schmidt, 1956). From the final dilutions of 10^{-3}, $10^{-5}$and 10^{-6}, one ml of each aliquot was pipetted out, poured in sterilized Petri dish containing *Trichoderma* selective medium (TSM), King's B medium and nutrient agar medium separately and they were gently rotated clockwise and anti-clockwise for uniform distribution and incubated at room temperature (28±2°C) for 24 hours. The pure cultures were maintained on respective agar slants at 4° C. Native isolates of *Trichoderma* spp, *Bacillus* spp. and *Pseudomonas* spp. were isolated from rhizosphere soil.

Two isolates of *T. viride* were screened against *S. rolfsii* by dual culture method (Dennis and Webster, 1971). A nine mm mycelial disc of *S. rolfsii* and test antagonists were placed opposite to each other near the periphery of the Petri plate and incubated at room temperature (28 ± 2°C). The Petri dishes were maintained for each antagonist separately. The medium inoculated with the pathogen alone was served as control. When the control plate reached full growth, the radial growth of the pathogen was measured in the other treatments. The results were expressed as per cent inhibition over control by using the formula of Pandey and Upadhyay (2000). The overgrowth of antagonists over the pathogen was measured seven days after incubation.

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Dc = average diameter of fungal growth (cm) in control

Dt = average diameter of fungal growth (cm) in treatment.

PI = Per cent inhibition over control

Seven isolates of *Pseudomonas fluorescens* and two isolates of *Bacillus* spp. were streaked in a four cm line (one cm away from the edge of the plate) on each PDA medium. A nine mm mycelial disc of *S. rolfsii* was placed to the most distal end of the Petri dish perpendicular to the bacterial streak (Vidhyasekaran *et al*., 1997). The plates were incubated at room temperature for four days and mycelial growth of the pathogen was measured (Table 1).

$$PI = \frac{Dc - Dt}{Dc} \times 100$$

Dc = average diameter of fungal growth (cm) in control

Dt = average diameter of fungal growth (cm) in treatment.

PI = Per cent inhibition over control

Arunasri (2003) made similar observation with various bacterial isolates where *Pseudomonas* spp. was found effective in inhibiting the mycelial growth and sclerotial production of *S. rolfsii* with 43.10 and 71.0 per cent respectively. However, in the present study we observed that *T. asperellum* (Tv1) was found to be superior in inhibiting the mycelial growth *in vitro* conditions as compared to *P. fluorescens* (Pf1). Similarly, Karthikeyan *et al*. (2006) reported that *P. Fluorescens* inhibited mycelial growth of *S. rolfsii* of groundnut.

Sclerotia of *S.rolfsii* were collected from groundnut shell medium, washed well and culture filtrates of the above biocontrol agents were collected and filtered through Whatman No 41 filter paper under aseptic conditions. In 200 ml of the culture filtrates, 100 sclerotia of *S. rofsii* were soaked and allowed undisturbed for 48 hours. Soaking of sclerotia in distilled water served as control. After 2 days of incubation, these sclerotia were collected and put at

the centre of Petri plates filled with sterile Czapeks dox agar medium aseptically and incubated for 5 days. Observation on germination was made by counting the no. of sclerotia germinated in each Petri plate. Results indicated that, sclerotial germination was less collected from the culture filtrates of *P. fluorescens* 1 (22.0) and *T. viride* (20.33) as compared to the other treatments and control (97.33) (Table 2).

The efficacy of different fungicides were tested against *Sclerotium rolfsii* using poisoned food technique (Schmitz, 1930). Required concentration of fungicides (1000 PPM) were added with 100 ml sterilized PDA medium. The medium was poured on sterilized Petri dish and then allowed to solidify. A nine mm mycelial disc of *Sclerotium rolfsii* was taken from actively growing culture and placed at the centre of each Petri dish and incubated at room temperature. The medium without fungicide served as control. The radial growth of *Sclerotium rolfsii* was recorded after seven days of incubation. It was expressed in per cent growth inhibition (in cm) over control (Table 3).

The efficacy of different fungicides were tested against *Sclerotium rolfsii* using poisoned food technique (Schmitz, 1930). Required concentration of fungicides (1000 PPM) were added in conical flasks with 100 ml sterilized Potato Dextrose liquid medium.

A nine mm mycelial disc of *Sclerotium rolfsii* was taken from actively growing culture and put in each conical flask incubated at room temperature. The medium without fungicide served as control. The mycelia dry weight (in mg) of *Sclerotium rolfsii* was recorded after seven days of incubation (Table 4). Among the six fungicides, Propiconazole

was found to be the best in inhibiting the growth of pathogen (100 %), followed by carbendazim, tebuconazole and penconazole which recorded 97.64, 97.11 and 96.85 per cent reduction respectively (Table 4).

This result is in accordance with Mukherjee *et al.* (2001) reported that Propiconazole was found to be best against *S. rolfsii*. Similarly, Manu *et al.* (2012) reported that Tebuconazole + Trifloxystrobin and Mancozeb showed better antagonistic activity against *S. rolfsii* causing foot rot of ragi.

The present observations show that the *T. asperellum* (TNAU) and *P. fluorescens*1 (TNAU) are the most effective antagonist compared to other antagonists which were tested against the *Sclerotium rolfsii*. The inhibition shown by the antagonists may be due to the hyphal parasitism and antibiosis mechanisms of the antagonists. Most of the soil borne pathogens isolated from the soil are known to survive saprophytically in nature. Release of secondary metabolites by *Trichoderma* spp. into the host organism is known to result in direct inhibition of growth of the pathogen by cell lysis (Campbell, 1989; Ekefan *et al*., 1990). Various plant diseases have been successfully controlled through bacterial and fungal antagonists (Campbell, 1989). The exploitation of biocontrol agents for the management of plant diseases have achieved greater significance in the recent time due to its readily available nature, antimicrobial activity, easy biodegradability, non phytotoxicity, besides inducing resistance to the host.

From this study, it can be suggested that fungicide, Propiconazole and bioagents such as, *T. asperellum* and *P. fluorescens* can be used in the integrated disease management

strategy against the *S.rolfsii* of tuberose. However, further work should be taken up to explore the possibility of the use of these antagonists and fungicide under field condition for the management of the root rot/stem rot caused by *S. rolfsii* in tuberose.

BIBLIOGRAPHY

[1]. Arunasri, P. (2003). Managemant of collar rot disease of crosandra incited by *Sclerotium rolfsii* Sacc. *M.Sc. Thesis*. Acharya N.G Ranga Agricultural University, Hyderabad, India.

[2]. Campbell, R. (1989). Biological control of microbial plant pathogen. *Cambridge University Press*, Cambridge 8-24.

[3]. Das, A, C. (1961). Diseases of rajanigandha (*Polianthes tuberosa* L.) and larkspur (*Delphinium ajacis* L.) caused by *Sclerotium rolfsii* Sacc. *Science and Culture*, 27,543-550.

[4]. Dennis, C., & Webster, J. (1971). Antagonistic properties of species groups of *Trichoderma* 1. Production of non-volatile antibiotics. *Transactions of the British Mycological Society*, 57, 25-39.

[5]. Ekefan, E.J, Jama, A. & Gowen, S.R. (2009). Potential of *Trichoderma harzianium* isolates in biocontrol of *Colletotrichum capsici* causing anthracnose of pepper (*Capsicum* spp.) in Nigeria. *Journal of applied bioscience*, 20,1138-1145.

[6]. Karthikeyan, V., Sankaralingam, A., & Nakkeeran, S. (2006). Biological control of groundnut stem rot caused by *Sclerotium rolfsii* Sacc. *Archives of Phytopathology and Plant Protection*, 39(3), 239-246.

[7]. Manu, T.G., Nagaraja, A., Chetan, S., Janawad, & Vinayaka, H. (2012). Efficacy of fungicides and biocontrol agents against *Sclerotium rolfsii* causing foot rot disease of finger millet, under *in vitro* conditions. *Global Institute for Research and Education***,** 1(2), 46-50.

[8]. Mukerjee, S., Tripathi, H.S., & Rathi, Y.P.S. (2001). Integrated management of wilt complex in French bean (*Phaseolus vulgaris* L.). *Journal of Mycology and Plant Pathology*, 31(2),213-215.

[9]. Pandey, K. K. & Upadhyay, J. P. (2000). Microbial population from Rhizosphere and Non rhizosphere soil of pigeonpea: Screening for resident antagonist and mode of mycoparasitism. *Journal of Mycology and Plant Pathology*, 30, 7-10.

[10]. Pramer, D. E., & Schmidt, E.L. (1964). *Experimental Soil Microbiology Burges publishing company Minneapolis*, 15. Minnesota, USA. Sons Ltd. Beecles and London. pp.31-32.

[11]. Punja, Z.K. (1985). The biology, ecology and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology*, 23, 97-127.

[12]. Riker, A. J. & Riker A. S. (1936). *Introduction to Research on Plant Diseases*. John Swilt, C.M.C., New York, 117p.

[13]. Schmitz, H. (1930) Poisoned food technique. *Industrial and Engineering Chemistry Analytical Edition*, 2, 361–363.

[14]. Upadhay, J.P. & Mukhyopadhayay, A.N. (1986). Biological control of *Sclerotium rolfsii* by *Trichoderma harzianum*in sugarbeet. *Indian phytopathology*, 39, 394-396.

[15]. Vidhyasekaran, P., Rabindran, R., Muthamilan, M., Nayar, K., Rajappan, K., Subramanian, N., & Vasumathi, K. (1997). Development of powder formulation of *Pseudomonas fluorescens* for control of rice blast. *Plant Pathology*, 46, 291-297.

TABLES

Table 1. Screening of biocontrol agents against *Sclerotiumrolfsiiin vitro* condition (Dual plate technique)

Sl. No.	Treatments	Mean mycelial growth (mm)*	Per cent reduction over control
1	*Pseudomonas* sp. Native isolate 1	59.3	34.1
2	*Pseudomonas* sp. Native isolate 2	52.7	41.4
3	*Pseudomonas* sp. Native isolate 3	67.3	25.2
4	*Pseudomonas* sp. Native isolate 4	56.0	37.8
5	*Pseudomonas* sp. Native isolate 5	48.3	46.3
6	*Pseudomonas* sp. Native isolate 6	49.7	44.8
7	*Pseudomonas* sp. Native isolate 7	56.0	37.8
8	*Bacillus sp.* Native isolate 1	57.3	36.3
9	*Bacillus sp.* Native isolate 2	59.3	33.8
10	*Trichoderma asperellum* (TNAU)	25.0	72.2
11	*Pseudomonas fluorescens* 1 (TNAU)	27.3	69.7
12	Control	90.0	
CD (P = 0.05)		**1.05**	

*Native and known biocontrol agents were screened against *Sclerotiumrolfsii* by dual culture method. Among the bioagents screened, *Trichoderma asperellum*(TNAU) (72.2) and *Pseudomonas fluorescens*1 (TNAU) (69.7) were found to be the best in the reduction of mycelial growth over the control (Table 4).

Table 2. Effect of culture filtrates of biocontrol agents on the sclerotial germination of *Sclerotiumrolfsii*

Sl. No.	Treatments	No. of Sclerotia germinated	No. of Sclerotia not germinated	Per cent reduction over control
1	*Pseudomonas* sp. Native isolate 1	84.00	13.33	13.7
2	*Pseudomonas* sp. Native isolate 2	80.33	17.00	17.5
3	*Pseudomonas* sp. Native isolate 3	74.67	22.66	23.3
4	*Pseudomonas* sp. Native isolate 4	79.67	17.66	18.1
5	*Pseudomonas* sp. Native isolate 5	66.00	31.33	32.2
6	*Pseudomonas* sp. Native isolate 6	65.33	32.00	32.9
7	*Pseudomonas* sp. Native isolate 7	81.00	16.33	16.8
8	*Bacillus sp.* Native isolate 1	79.00	18.33	18.8
9	*Bacillus sp.* Native isolate 2	80.33	17.00	17.5
10	*Trichoderma asperellum* (TNAU)	20.33	77.00	79.1
11	*Pseudomonas fluorescens* 1 (TNAU)	22.00	75.33	77.4
12	Control	97.33		
CD (P = 0.05)		**1.84**		

Table 3. Efficacy of fungicides against *Sclerotiumrolfsiiin vitro* condition (Poisoned food technique – agar medium)

Sl. No.	Treatments (1000 ppm)	Mean mycelial growth (mm)*	Per cent reduction over control
1	Copper oxy chloride	51.5	42.78
2	Carbendazim	2.5	97.22
3	Penconazole	2.3	97.44
4	Tebuconazole	5.5	93.89
5	Propiconazole	0.0	100.0
6	Chlorothalonil	48.3	46.33
7	Control	90.0	-
CD (P = 0.05)		**2.2**	

Table 4.Efficacy of fungicides against *Sclerotiumrolfsiiin vitro* condition (Poisoned food technique – liquid medium)

Sl. No.	Treatments (1000 ppm)	Mean mycelial dry weight (mg)*	Per cent reduction over control
1	Copper oxy chloride	468	38.58
2	Carbendazim	18	97.64
3	Penconazole	24	96.85
4	Tebuconazole	22	97.11
5	Propiconazole	0.0	100.0
6	Chlorothalonil	452	40.68
7	Control	762	-
CD (P = 0.05)		**2.8**	

Six fungicides were evaluated against *Sclerotiumrolfsiiin vitro* condition by poisoned food technique (liquid medium). Among them, Propiconazole was found to the best in inhibiting the growth of pathogen (100 %), followed by carbendazim, tebuconazole and penconazole which recorded 97.64, 97.11 and 96.85 per cent reduction respectively.

