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In-vitro and *In-vivo* Management of *Sclerotium rolfsii* Sacc. causing Stem Rot in Chrysanthemum

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ABSTRACT: Chrysanthemum (*Dendranthema grandiflora* Ramat), is one of the most beautiful and perhaps the oldest flowering plants commercially grown in different parts of the world. *Sclerotium rolfsii*, the soil-borne saprophytic fungus which causes different types of diseases like collar-rot, sclerotium wilt, stem-rot, charcoal rot, *etc*., in more than 500 plants species. Recently, *S. rolfsii* associated with stem rot disease on chrysanthemum has been observed in few farmer fields of Bengaluru Rural and Hassan districts in Karnataka. The disease incidence was up to 20-30 per cent in 2018-19. The effect of 12 different fungicides against the growth of *Sclerotium rolfsii* was studied under *in-vitro* conditions using poisoned food technique. Among the contact fungicides Captan 50%WP at 1000 ppm showed the highest inhibition per cent (80%) of mycelial growth. Among the systemic fungicides, Tebuconazole 25.9% EC showed 100% inhibition of mycelial growth at 100ppm itself. Among combi products Pyraclostrobin 133g/l +Epoxiconazole 50g/l SE, Carboxin 37.5%+Thiram 37.5% WP showed 100% inhibition of mycelial growth at 300ppm itself. Integrated disease management studies were conducted under polyhouse conditions, and among the fungicides, soil application of Tebuconazole (0.1%) suppress the growth of *S. rolfsii* with 0% mortality. Among the organic amendments, Neem cake (30g/plant) was effective on the suppression of *S. rolfsii* with PDI of 20.83%. Among the bioagents *P. fluorescence* (10g/plant) and *T. harzianum* (10g/plant) suppress the growth of *S. rolfsii* with PDI of 22.60% and 16.24 % respectively.

Keywords: *Sclerotium rolfsii*, Percent disease incidence, *Trichoderma harzianum*, *In- vitro*, *In-vivo*, Management.

INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora* Ramat.), the golden flower, is one of the beautiful and oldest commercial flowering plants grown in different countries of the world and belongs to the family Asteraceae. In chrysanthemums, there are several species *viz*., *Chrysanthemum grandiflorum, C. boreale, C. carinatum, C. coronarium, C. cinerariifolium, C. rubellum, C. satsumense, C. sibiricumetc*, are ornamental and grown in gardens for their beautiful flowers. Among the diverse species, the autumn flowering garden chrysanthemum *i.e*., *Chrysanthemum morifolium* is commercially cultivated throughout the world, and recently it is renamed botanically as a *Dendranthema grandiflora* Tzevelev (Bhattacharjee, 2003).

The crop is being cultivated throughout the world as a cut flower, especially in countries like China, Japan, Europe, USA and India (Broerties *et al*., 1980). In India, the Chrysanthemum growing states are Karnataka, West Bengal, Maharashtra, Tamil Nadu, Punjab, Madhya Pradesh, Andhra Pradesh and Bihar. It is also cultivated around big cities like Delhi, Bengaluru, Kolkata, Allahabad, Lucknow and Kanpur, because mainly for the reason of beautification and exhibitions (Devaraja, 2011). In India, during 2017-18 the total cultivated area occupied by chrysanthemum was 20.09 thousand hectares and production of cut flowers and loose flowers was 185.24 MT and 14.94 MT, respectively (Anon., 2018). In Karnataka, chrysanthemum cultivated extensively in Bengaluru urban and Bengaluru rural, Ramanagara, Hassan, Mysuru, Tumakuru, Kolar, Chikballapura, Dharwad, Bellary and Belgaum districts with an area of 30,612 ha producing 2,33,377 MT and productivity of 7.62 tonnes per hectare during the year 2017-18 (Anon., 2018).

The stem rot caused by *S. rolfsii*, is a soil borne pathogen with more than 500 plant species as hosts including vegetable, flower, ornamental, pulse, oil seed and medicinal crops (Farr *et al.*, 1989). The pathogen is a well-known polyphagous soil borne plant pathogenic

fungus (Aycock, 1966), generally distributed in tropical and subtropical regions, where soil temperature prevails around 30°C (Harlapur, 1988). Several management options are available for control of soil borne stem rot disease. It is also important to evaluate novel fungicides and bio agents and identifying suitable molecules for managing the stem rot of chrysanthemum on regional basis. However, disease outbreaks of stem rot disease are still not uncommon. Presently, the stem rot disease is managed through application of chemical fungicides under *in-vitro*, and application of chemicals, biocontrol agents, organic amendments, under polyhouse conditions that have several concerns in the areas of environmental safety, pathogen resistance to fungicides, groundwater pollution and escalated costs. Notable success on disease control through the combined use of antagonistic microorganisms and fungicides have been experimented during recent years. In the present study, an effort is made to identify potential antagonists of *Sclerotium rolfsii*, efficiency of chemicals and their compatibility, when applied under *in-vitro* and *in- vivo* management for the newly reported pathogen from Karnataka, India.

MATERIAL AND METHODS

A. Isolation and preservation of S. rolfsii infecting chrysanthemum

Freshly infected collar region and root region of chrysanthemum samples (Plate 1) collected from diseased fields were washed thoroughly, with sterile water. These were cut into small bits/pieces of 20-40 mm size with the help of sterilized blade and the same were surface sterilized in 0.1 per cent sodium hypochlorite solution for 1-2 minutes and washed three times consecutively in sterile distilled water to remove the traces of sodium hypochlorite. After surface sterilization, diseased specimens were kept in sterilized bags along with wet cotton under room temperature for about 8-10 days of incubation period, slight mycelial growth upon observation and that was transferred on Potato Dextrose Agar (PDA) medium. The inoculated plates were incubated at room temperature (25° C±2°C) for 3-5 days to facilitate growth of the fungus (Plate 2). The pure culture of *Sclerotium rolfsii* was obtained by hyphal tip culture technique and further maintained on PDA Petri plate incubated at 25ºC for further work.

B. Identification of Sclerotium rolfsii causing stem rot disease in chrysanthemum

The fungus was cultured on PDA plates for the detection of the pathogen. A white radiating mycelial mat was formed within seven days after incubation and covered the entire petri plate. Sclerotial bodies were developed at the periphery of the plates. The pathogenicity also was proved using giant culture and the culture was used for the subsequent studies.

C. In- vitro efficacy of fungicides by poisoned food technique

The efficacy of different chemicals was tested on PDA medium against *S. rolfsii* by poisoned food technique. Required concentrations of fungicides were prepared by dissolving a known quantity of fungicides in sterile distilled water separately under aseptic conditions. The poisoned medium was equally distributed into three Petri plates each, comprising 13 treatments. The mycelial growth of the pathogen *S. rolfsii* was cut into 5 mm discs from the periphery of actively growing colony with sterilized cork borer and transferred to the centre of each plate containing poisoned medium. Control was maintained by placing fungal discs in plates containing untreated medium. All the inoculated Petriplates were incubated at 28± 2ºC in BOD incubator. The diameters of fungal colonies in the treatments were measured when the growth in control plate was full. Per cent inhibition in the growth of the organism in different chemical treatments over the control was calculated. The percentage inhibition of radial growth was calculated by using equation:

Percentage of inhibition I = (C-T)/C × 100

Where, C= growth of pathogen in control, T = growth of pathogen in treatment

D. In-vivo management of stem rot disease of chrysanthemum caused by S. rolfsii under polyhouse conditions

The organic amendments, bioagents and few fungicides which were found effective under *in vitro* studies were evaluated under polyhouse conditions. Polyhouse studies were conducted at Department of Plant Pathology, College of Horticulture, Bengaluru, during 2019-2020. For the pot culture study sterilized soil was uniformly mixed with 10g inoculum of *S. rolfsii* which was grown in sand sorghum medium and filled in 40 × 35cm size pot. The soil was previously mixed with FYM, vermicompost, fungicides, bio–agents and organic amendments. For each treatment, three replications were maintained; Observations were recorded after 15 days of planting. Per cent disease incidence was calculated by using the formula given by Maiti and Sen (1979).

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

RESULTS AND DISCUSSION

A. Isolation and preservation of S.rolfsii

The fungus produced white, dense, radiating mycelial growth on potato dextrose agar medium. In the early stages, the fungus produced white mycelium and gradually lost its luster and became somewhat dull in appearance. Aerial hyphae were not uniformly distributed. Formation of sclerotial bodies was observed from seventh day onwards after incubation. In the beginning, the sclerotial bodies were white which gradually turned to golden yellow, buff brown colour and then to chocolate brown colour at maturity. The fully matured sclerotial bodies were spherical to ellipsoidal and measured 0.5 mm to 0.70 mm in diameter. The culture was maintained at 4°C in the refrigerator for the subsequent study.

The experimental results presented in Table 1 revealed that, the per cent mean inhibition of stem rot pathogen differed significantly among the treatments. Among the contact fungicides-Captan 50%WP at 1000 ppm showed the highest inhibition per cent (79.41%) of mycelia growth (Plate 3a and Fig. 1). Among the systemic fungicides-Tebuconazole 25.9% EC showed

100% inhibition of mycelial growth at 100ppm itself followed by Propiconazole 25% EC showed 100% inhibition of mycelial growth at 300ppm (Plate 3b and Fig. 1) and among the combi-products Pyraclostrobin 133g/l + Epoxiconazole 50g/l SE, Carboxin 37.5% + Thiram 37.5% WP showed 100% inhibition of mycelial growth at 100ppm itself (Plate 3c and Fig. 1).

Table 1: *In vitro* efficacy of different fungicides against *Sclerotium rolfsii* causing stem rot of chrysanthemum.

Treatment No.	Chemical name	% Inhibition over control (*)		
		10% recommended field concentration	20% recommended field concentration	30% recommended field concentration
Contact fungicides				
T_1	COC 50% WP @ 0.3%	00.00 (0.28)*	00.00 (0.28)	00.00 (0.28)
T_2	Copper hydroxide 75% WP@0.2%	17.31 (24.58)	20.24 (26.72)	25.33 (30.21)
T_3	Mancozeb 75% WP@0.2%	34.16 (35.77)	53.08 (46.77)	60.16 (50.87)
T_4	Captan 50% WP@0.2%	64.28 (53.30)	66.52 (54.50)	79.41 (63.02)
T_5	Chlorothalonil 75% WP@0.1%	43.24 (41.12)	50.27 (45.05)	72.28 (58.23)
Systemic fungicides				
T_6	Propiconazole 25% EC@0.1%	90.00 (71.66)	95.00 (77.50)	100 (89.71)
T_7	Tebuconazole 25.9% EC@0.1%	100 (89.71)	100 (89.71)	100 (89.71)
T_8	Carbendazim 50% WP@0.2%	00.00 (0.28)	00.00 (0.28)	00.00 (0.28)
Combi products				
T_9	Mancozeb 63% + Carbendazim 12% WP@0.2%	42.16 (40.48)	54.60 (47.64)	71.58 (57.80)
T_{10}	Carbendazim 25% + Mancozeb 50% WS@0.2%	31.33 (34.03)	52.28 (46.20)	55.05 (47.90)
T_{11}	Carboxin 37.5 + Thiram 37.5% WP@0.1%	100 (89.71)	100 (89.71)	100 (89.71)
T_{12}	Pyraclostrobin 133g/l + Epoxiconazole 50g/l SC@0.1%	100 (89.71)	100 (89.71)	100 (89.71)
T13	Control	00.00 (0.28)	00.00 (0.28)	00.00 (0.28)
S. Em±		**0.943**	**1.257**	**0.419**
CD @ 1 %		**2.861**	**3.731**	**1.257**

Where, * indicates 10%, 20%, 30% of recommended dose of field concentration

The findings are in support with the results of Chavala and Thammasak (1986), Hari *et al*. (1989); Bhise (2002); Kaur and Gupta (2003); Anju and Varma (2007); Prabhu and Patil (2004); Kolte and Raut (2007), mancozeb and copper oxy-chloride were found to be less effective against *S. rolfsii.* Similarly, Bhat and Srivastava (2003) evaluated *in vitro* efficacy of captan, thiophanate-methyl and propiconazole at 250, 500 and 1000 ppm concentrations against *S. rolfsii.* The findings of the present study were also in agreement with the reports of, many workers (Prabhu and Hiremath 2003; Kulkarni, 2007; Arunasri *et al*., 2011) who reported that the Triazoles (Hexaconazole, Propiconazole, Difenoconazole) were highly inhibitive to the growth of *S. rolfsii*. The present results are also in agreement with the reports of Mukhopadhyay and Thakur (1971), showed that vitavax and chloroneb significantly reduced the growth of *S. rolfsii*. Chouhan (1978) reported that fungicides showed better inhibition of the soil borne fungal pathogen, the best inhibition of *S. rolfsii* was observed with calixin and vitavax. Patil and Rane (1982) demonstrated vitavax to be effective in inhibiting the growth of *S. rolfsii.* Vineela *et al.* (2017) evaluated the fungicides and reported that, vitavax power significantly inhibited the growth of *S. rolfsii* against groundnut stem rot. Triazole fungicides interfere with the ergosterol biosynthesis of the fungus, the results are in agreement with Vanitha and Suresh (2002).

Plate 1: Chrysanthemum samples used for isolation of *S. rolfsii*

Plate 2: White, dense, radiating mycelial growth of *S.rolfsii* on potato dextrose agar medium and sclerotial bodies initiation in the potato dextrose broth

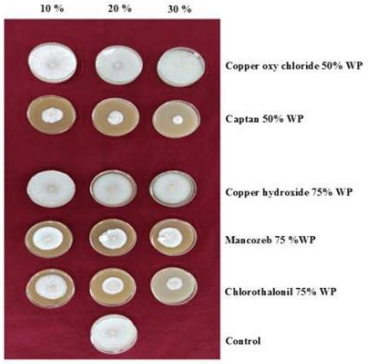

Plate 3a: *In-vitro* evaluation of contact fungicides against *S.rolfsii*

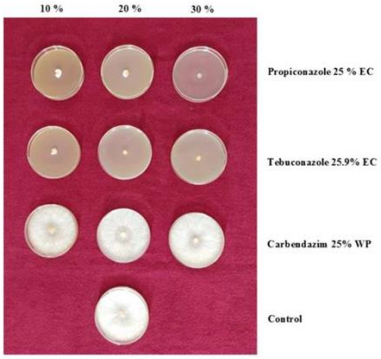

Plate 3b: *In-vitro* evaluation of systemic fungicides against *S.rolfsii*

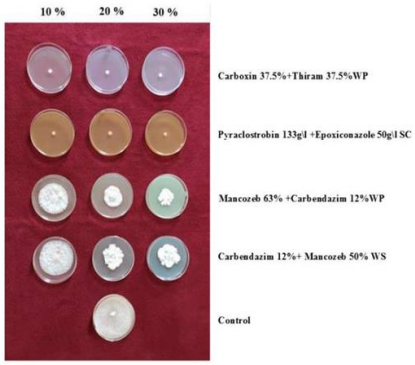

Plate 3c: *In-vitro* evaluation of combi products against *S.rolfsii*

Plate 4: *In-vivo* management of *S.rolfsii* causing stem rot in chrysanthemum under polyhouse conditions

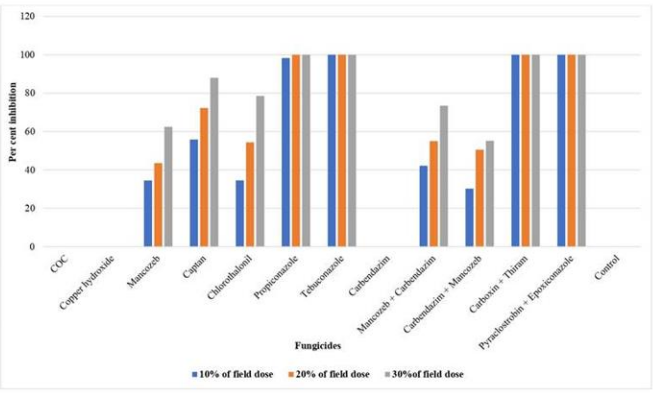

Fig. 1. *In-vitro* evaluation of fungicides against *S. rolfsii*.

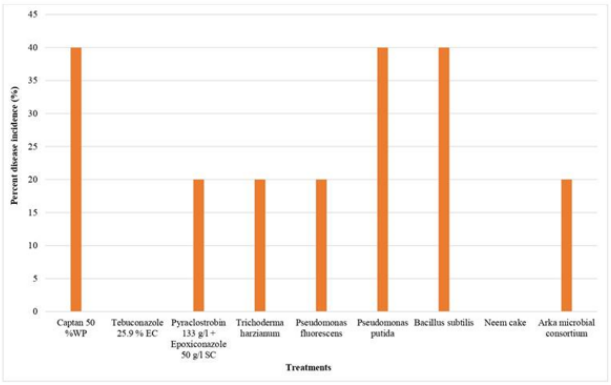

Fig. 2. *In-vivo* efficacy of different fungicides, organic amendments and bioagents stem rot of chrysanthemum.

B. In-vivo management of stem rot disease of chrysanthemum caused by S. rolfsii under polyhouse conditions

Table 2: Effect of different fungicides, organic amendments and bio-agents on the incidence of stem rot of chrysanthemum.

Treatments number	Treatments	Stem rot incidence (%)	Percent disease inhibited over control
T_1	Captan 50 %WP @0.3%	40.00 (39.23)	60.00
T_2	Tebuconazole 25.9 % EC@ 0.1%	00.00 (0.28)	100.00
T_3	Pyraclostrobin 133 g/l + Epoxiconazole 50 g/l SC @0.1%	20.00 (26.56)	80.00
T_4	*Trichoderma harzianum*@10g/pl	20.00 (26.56)	80.00
T_5	*Pseudomonas fluorescens*@10g/pl	20.00 (26.56)	80.00
T_6	*Pseudomonas putida*@10g/pl	40.00 (35.00)	60.00
T_7	*Bacillus subtilis*@10g/pl	40.00 (39.23)	60.00
T_8	Neem cake @30 g/kg of soil	00.00 (17.80)	100.00
T_9	Arka microbial consortium @5 ml/pl	20.00 (26.56)	80.00
T_{10}	Control	100.00(67.92)	
		S. Em±	**4.919**
		CD@1%	**14.615**

The results of the experiment revealed that Tebuconazole 25.9 % EC @ 1ml/lt. of water and Neem cake @30 g/kg of soil found to be very effective against chrysanthemum stem rot with zero PDI. Further, there was no significant difference among the four bioagents and AMC tested against stem rot pathogen of chrysanthemum, which ranged from 20 to 40 per cent PDI (Plate 4 and Fig. 2). Gandhi *et al.* (2017) conducted experiment on few fungicides and bio-agents on seed treatment and soil application to manage the collar rot of sunflower under screen house and field conditions. Results indicated that use of fungicides and bioagents as seed and soil treatments significantly reduced the incidence of collar rotunder screen house conditions, *Trichoderma harzianum* @ 20g/kg soil application showed maximum control of the disease (40.62%) as compared to untreated control. Bharathi and Narayanaswamy (2018) carried out an experiment on integrated disease management against stem rot of tuberose, Soil drenching with *Trichoderma viride* alone recorded lower disease incidence (28.00%). Among organic amendments, neem cake showed minimum disease incidence (32.66 %).

CONCLUSIONS

The *Sclerotium rolfsii* is a serious soil borne pathogen, affecting many crop plants including chrysanthemum. To manage this pathogen in chrysanthemum Tebuconazole 25.9 % EC @ 1ml/lt. of water is the best for soil drenching as a chemical means and utilization of Neem cake @30 g/kg of soil, followed by *Trichoderma harzianum*@10g/pl, *Pseudomonas fluorescens*@10g/pl and Arka microbial consortium @5 ml/pl as a organic means is the best as organic means.

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