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Evaluation of *Trichoderma* spp. and Fungicides in the Management of Collar Rot of *Gerbera* Incited by *Sclerotium rolfsii*

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An extensive study was undertaken to manage the most vigorous, polyphagous pathogen *Sclerotium rolfsii* causing collar rot disease in *Gerbera* by utilizing nine commercial fungicides and eight novel *Trichoderma* spp. Under *in vitro* conditions, there was 61.11 per cent reduction of pathogen (*S. rolfsii*) by *T. harzianum* NVTH2 and *T. viride* TV1 over control and was followed by *T. citrinoviride* NVTC1, *T. citrinoviride* NVTC2 and *T. asperellum* NVTA1 with per cent inhibition of 55.55, 54.44 and 53.33 respectively. On the other hand, commercial fungicides, tebuconazole 50%+ trifloxystrobin 25%, tebuconazole 250 EC, propioconazole 25% EC, fenamidone 10%+ mancozeb 50%WG and propineb 70WP reduced the growth of *S. rolfsii* to an extent of 100 per cent inhibition in all the tested concentrations. Combination of most effective *Trichoderma* spp. and fungicides had resulted in the best treatment T_2 against the collar rot pathogen of *Gerbera* under protected cultivation.

Keywords: Fungicides, *Gerbera, Trichoderma* spp., collar rot.

Floriculture is recognized as a highly competitive and a profitable sector. Indian flower export markets are estimated as 11 billion US dollars at present and expected to grow up to 20 billion US dollars by 2020 (Gian Aggarwal, 2011). India has a vast potential to grow good quality *Gerbera*. Area under *Gerbera* cultivation in Tamil Nadu is around 25 ha with production of 53 lakh cut flowers at an estimated value of Rs. 15 lakhs (INDIASTAT, 2013).

There is a continuous exploitation of the soils in polyhouses which makes *Gerbera* highly susceptible to soil borne diseases. Jamwal and Jamwal (2012) observed foot rot, wilt, root rot complex, blight and grey mold in *Gerbera*. In India, collar rot disease in *Gerbera jamesonii* Bolus ex Hook was recorded for the first time caused by *Sclerotium rolfsii* (Suneeta *et al.,* 2016).

Biological control of soil borne pathogens by using antagonistic fungi, *Trichodema* spp. has been under investigations from several years. At the same time, chemical agents also carry the huge capacity to control the soil borne diseases with their quick mode of action. Integration of different treatments including seedling dip with carbendazim+mancozeb, addition of vermicompost, drenching with fungicide and application of *Trichoderma harzianum* (7%) were found to be effective in management of dry root rot (*Sclerotium rolfsii*) of chilli in comparison with individual treatments (Madhavi and Bhattiprolu, 2011). Seetharamulu et al. (2012) evaluated the efficacy of *Trichoderma viride* against *Fusarium solani* in *in-vitro* system while in *in-vivo* system it was effective against the disease in combination

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with fungicide Mancozeb. Comparison of bio-efficacy and combination of *Trichoderma* spp. and fungicidal molecules against collar rot pathogen (*Sclerotium rolfsii*) of *Gerbera* results in the better control of the pathogen.

MATERIALS AND METHODS

Isolation of the collar rot pathogen

The isolation technique of *Sclerotium rolfsii* was adapted from Rangaswami and Mahadevan (1999). The infected crown bits were surface sterilised by using 0.1% mercuric chloride ($HgCl_2$) for 30 seconds and placed on to the PDA medium amended with 100 ìg/ml of streptomycin sulphate which were incubated at temperature (20 ± 2°C) for 5 days.

Identification of the pathogen

The pathogen was identified according to the morphological characteristics based on size, shape and colour of sclerotia and mycelium growth.

Pathogenicity test

The mycelium and sclerotia of the pathogen *Sclerotium rolfsii* were inoculated in the collar portion of 30 days old *Gerbera* (var. Donavan yellow and Bellwater white) plants and maintained in the polyhouse at 22 ± 2°C. After 7 days of inoculation typical symptoms of browning and rotting were observed and the pathogen was re-isolated. Similar methodology was followed to prove pathogenicity in tomato plants by Xie *et al.* (2014).

Collection of fungal antagonists (*Trichoderma* spp.)

Eight isolates of *Trichoderma* spp. were collected from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India (Table 1).

***In vitro* screening by *Trichoderma* spp**

The antagonistic activity of *Trichoderma* spp. against the test pathogen was evaluated by dual culture technique (Dennis and Webster, 1971). The radial growth of mycelium of antagonist in mm and pathogen were measured and Percent Inhibition (PI) was calculated: PI= C-T/C X 100

Where, C is the growth of test pathogen (mm) in the absence of the antagonist; T is the growth of test pathogen (mm) in the presence of the antagonist.

***In vitro* evaluation of fungicides**

The efficacy of 9 commercial fungicides namely difenoconazole 25% EC (Score), tebuconazole 50% + trifloxystrobin 25% WG (Nativo), fenamidone 10%+mancozeb 50%WG (Sectin), propineb 70 WP (Antracol), fosetyl aluminium 50% WP (Alliete), propioconazole 25% EC (Tilt), tebuconazole 250 EC (Folicur), kresoxim-methyl 44.3% SC (Ergon) and carbendazim 50% WP (Benfil) at 25ppm, 50ppm, 100ppm, 250ppm, 500ppm, 1000ppm and 1500ppm concentrations were tested against the root pathogens by Poisoned food technique (Grover and Moore, 1962) using Potato dextrose agar medium. The treatments were replicated thrice and were incubated at room temperature (28±2°C) and the diameter of colonies were recorded on 7th day and expressed in centimeter (cm). The per cent inhibition (PI) of growth was calculated by using the formula:

PI = C-T/C x 100, Where I = Inhibition percentage, C = Rate of growth of pathogen in control and T = Rate of growth of pathogen in treatment.

Development of liquid formulation of *Trichoderma* spp.

The fungal antagonists (TV1, NVTH1 and NVTH2) were cultured on 1000ml of Potato Dextrose broth and incubated in an orbital shaker at 150 rpm at room temperature (28±2°C) for 48hr. Later the liquid biomass along with fungal mycelia were mixed with 1% glycerol (10ml), tween 20 (10ml) and poly vinyl pyrrolidone – 40000 ml. wt (10g) each separately (Somasegaran and Hoben, 1985). The resultant mixture was kept in orbital shaker at 200 rpm for 5 minutes to ensure uniform blending and was standardized to obtain one ml of formulation consists of 10^6cfu/ml. The liquid formulation was stored at 5°C for further study.

Integration of *Trichoderma* spp. and fungicides for the management of collar rot under protected cultivation

Field experiment was conducted during 2013-2014 in *Gerbera* (var. Donavan yellow) fields located at Spic Agro Biotech centre, Ooty, to assess the efficacy of liquid formulation of *Trichoderma* spp. (10^6 cfu/ ml) @ 5ml/litre individually and in combination with commercial fungicides @ 0.5 to 1ml/lit (moderate dosages) against collar rot under protected condition (polyhouse). Thirty days old

plants of *Gerbera* were used and the experiment was laid out with 7 treatments and 3 replications in RBD. The bed size of each replication was $5m^2$ with 30 × 30 cm spacing (Table-2).

Statistical analysis

All the experiments were statistically analyzed independently. The treatment means were compared by Duncan's Multiple Range-Test (DMRT) (Gomez and Gomez, 1984). The package used for analysis was IRRISTAT version 92-1 developed by the International Rice Research Institute, Biometrics unit, The Philippines.

RESULTS AND DISCUSSION

Symptomatology of collar rot

Initially, the infected plants exhibited brown necrotic lesions on the petioles near collar region. Subsequently, the affected leaves droop and resulted in death of the infected plants. Examination showed the presence of white cottony mycelium and plenty of round, brown sclerotial bodies on the affected collar portion. Similar symptomatology was described by Arunasri *et al.* (2011) in collar rot of crossandra.

Morphological characterization of the pathogen

Pathogen was isolated from *Gerbera* variety Donavan (yellow). The mycelium of the fungal culture on PDA medium was white and fluffy. Small white tufts were formed on mycelium which later turned to dark brown, round, sclerotia and measured 1-2 mm in diameter. Based on phenotypic characters, the pathogen was confirmed as *Sclerotium rolfsii*. Similar morphology was described by Sennoi *et al.* (2010) in the stem rot of Jerusalem artichoke (*Sclerotium rolfsii*).

Pathogenicity of *S. rolfsii*

Inoculation of *S. rolfsii* into the collar region of 30 days old healthy *Gerbera* plants (var. Bellwater white) expressed the typical symptoms within 5 days after inoculation. Typical rot in the collar portion with numerous brown, mustard seed like sclerotia, followed by blightening and girdling of the affected plants was seen.

Table 1. List of *Trichoderma* spp. used in the study

S. No.	Name of isolate	Name of antagonist	Accession number of the isolate in NCBI
1.	NVTA1	*T. asperellum*	KJ803854
2.	NVTA2	*T. asperellum*	KJ803855
3.	NVTH1	*T. harzianum*	KJ803856
4.	NVTH2	*T. harzianum*	KJ803857
5.	NVTE1	*T. erinaceum*	KJ813823
6.	NVTC1	*T. citrinoviride*	KJ813824
7.	NVTC2	*T. citrinoviride*	KJ813825
8.	TV1	*T. viride*	Commercial strain (not submitted)

Table 2. Treatment schedule of *Trichoderma* spp. and fungicides

S. No.	Treatment	Treatment details
1.	T_1	RD- NVTH2+ TV1 @ 5ml/lit **SD- NVTH2+ TV1 @ 5ml/lit+ SD- tebuconazole 250 EC @ 500ppm
2.	T_2	RD- NVTH2+ TV1 @ 5ml/lit **SD- NVTH2+ TV1 @ 5ml/lit+ SD- tebuconazole 50%+ trifloxistrobin 25% WG @ 500ppm
3.	T_3	RD- NVTH2+ TV1 @ 5ml/lit **SD- NVTH2+ TV1 @ 5ml/lit+ SD-propioconazole 25% @ 500ppm
4.	T_4	RD- NVTH2+ TV1 @ 5ml/lit **SD- NVTH2+ TV1 @ 5ml/lit+ SD- propineb 70 WP @ 500ppm
5.	T_5	RD- NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+ SD- fenamidone 10%+ mancozeb 50% WG @ 500ppm
6.	T_6	RD- NVTH2+ TV1 @ 5ml/lit **SD- NVTH2+ TV1 @ 5ml/lit+ SD- difenoconazole 25%EC @ 500ppm
7.	T_7	RD- NVTH2+ TV1 @ 5ml/lit **SD- NVTH2+ TV1 @ 5ml/lit
8.	T_8	Control

RD- Root dipping at the time of planting; ** SD- Soil drenching at 15 days interval.

In vitro* screening of *Trichoderma* spp. against *S. rolfsii

The efficacy of *in vitro* antagonism of different isolates of *Trichoderma* spp. against *S. rolfsii* by dual culture technique revealed that the growth of *S. rolfsii* was suppressed to an extent of 61.11 per cent by *T. harzianum* NVTH2 and *T. viride* TV1 over control and was followed by *T.*

Table 3. Antifungal activity of *Trichoderma* spp. against *S. rolfsii* under *in vitro*

S. No.	Isolates	Mycelial growth (mm)*	Per cent Inhibition over control
1	*T. erinaceum*-NVTE1	43.00^{de}	52.22^{de} (46.27)
2	*T. citriniviridae*-NVTC1	40.00^{b}	55.55^{b} (48.18)
3	*T. citrinoviridae*-NVTC2	41.00^{bc}	54.44^{bc} (47.54)
4	*T. asperellum*-NVTA1	42.00^{cd}	53.33^{cd} (46.90)
5	*T. asperellum*-NVTA2	45.00^{f}	49.99^{f} (44.99)
6	*T. harzianum*-NVTH1	44.00^{ef}	51.11^{ef} (45.63)
7	*T. harzianum*-NVTH2	35.00^{a}	61.11^{a} (51.42)
8	*T. viride* (TV1)	35.00^{a}	61.11^{a} (51.42)
9	Control	90.00^{g}	-

*Values are mean of three replications.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
Values in parentheses are arc sine transformed values

Table 4. *In vitro* antagonism of fungicides against *S. rolfsii*

S. No.	Fungicides	Per cent inhibition over control						
		25ppm	50ppm	100 ppm	250 ppm	500 ppm	1000 ppm	1500 ppm
1	Tebuconazole 250 EC	86.67 (68.32)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)
2	Propioconazole 25% EC	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)
3	Difenoconazole 25% EC	70 (57.18)	74.44 (59.51)	78.89 (62.43)	86.67 (68.6)	100 (89.53)	100 (89.53)	100 (89.53)
4	Fenamidone 10%+Mancozeb 50% WG	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)
5	Propineb70 WP	80 (63.91)	83.33 (65.91)	88.89 (69.74)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)
6	Fosetyl aluminium 50% WP	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)
7	Kresoxim-Methyl 44.3% SC	4.44 (12.01)	7.78 (15.7)	16.67 (24.27)	20 (27.03)	23.33 (28.87)	40 (39.42)	42.22 (40.45)
8	Tebuconazole 50% + Trifloxystrobin 25%WG	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)	100 (89.53)
9	Carbendazim 50% WP	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	8.89 (16.75)	13.33 (21.55)
10	Control	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)	0 (0.46)

*Values are mean of three replications. **SD-soil drenching at 15 days interval.
In a column, means followed by a common letter are not significantly different at the 5% level by DMRT
Values in parentheses are arc-sine transformed values

Table 5. Effect of *Trichoderma* spp. and fungicides on collar rot incidence, growth characters and flower yield under protected cultivation

S. No.	Treatment module	Collar rot incidence*	Root length (cm)*	Plant height (cm)*	No. of flowers/m^2*
T_1	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-tebuconazole 250 EC @500ppm	4.30^{a}(56.52)	20.23^{a}	41.46^{b}	50.10^{b}
T_2	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-tebuconazole 50%+trifloxistrobin 25% WG @500ppm	4.00^{a}(59.55)	20.40^{a}	42.11^{a}	51.80^{a}
T_3	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-propioconazole 25% @500ppm	5.53^{b}(45.99)	19.22^{b}	39.20^{c}	48.60^{c}
T_4	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-propineb 70 WP @500ppm	6.11^{c}(38.22)	18.12^{c}	37.23^{e}	46.10^{e}
T_5	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-fenamidone 10%+mancozeb 50% WG @500ppm	5.69^{b}(42.46)	18.70^{c}	38.27^{d}	47.30^{d}
T_6	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-difenoconazole 25%EC @500ppm	7.91^{d}(20.02)	17.82^{d}	36.71^{f}	45.20^{f}
T_7	RD-NVTH2+ TV1 @5ml/lit**SD- NVTH2+ TV1 @5ml/lit	8.23^{e}(16.78)	17.22^{d}	36.36^{f}	44.10^{g}
T_8	Control	9.89^{f}	16.83^{e}	34.69^{g}	39.30^{h}

*Values are mean of three replications. **SD-soil drenching at 15 days interval
Means followed by a common letter are not significantly different at 5% level by DMRT
Data in the parenthesis are per cent reduction over control

Table 6. Effect of *Trichoderma* spp. and fungicides on flower characters of *Gerbera* under protected cultivation

S. No.	Treatments	Days taken for flower bud initiation*	Days taken for flower bud opening*	Length of flower stalk (cm)*	Flower diameter (cm)*
T_1	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-tebuconazole 250 EC @500ppm	83.40^{b}	106.00^{bc}	36.20^{bc}	8.80^{b}
T_2	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-tebuconazole 50%+trifloxistrobin 25% WG @500ppm	81.10^{a}	104.33^{a}	36.40^{ab}	9.00^{a}
T_3	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-propioconazole 25% EC @500ppm	84.00^{bc}	107.66^{cd}	35.60^{ef}	8.60^{c}
T_4	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-propineb 70 WP@500ppm	85.66^{cd}	110.00^{f}	34.80^{gh}	8.00^{e}
T_5	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-fenamidone 10%+mancozeb 50% WG@500ppm	86.20^{de}	108.00^{de}	35.90^{de}	8.40^{d}
T_6	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit+SD-difenoconazole 25% EC @500ppm	88.30^{f}	112.00^{g}	34.60^{hi}	7.90^{fg}
T_7	RD-NVTH2+ TV1 @5ml/lit **SD- NVTH2+ TV1 @5ml/lit	90.10^{g}	115.33^{h}	34.40^{ij}	7.80^{gh}
T_8	Control	92.33^{h}	119.00^{i}	32.20^{k}	7.10^{i}

*Values are mean of three replications
Means followed by a common letter are not significantly different at 5% level by DMRT

citrinoviride NVTC1, *T. citrinoviride* NVTC2 and *T. asperellum* NVTA1 with per cent inhibition of 55.55, 54.44 and 53.33 respectively (Table-3).

Manu *et al*. (2012) observed the maximum inhibition of mycelial growth of 61.88% in *T. harzianum,* which was followed by *T. viride* (Tv-27 isolate) (57.77%) and *T. harzianum* (Th-55 isolate) (56.33%) against *S. rolfsii* causing foot rot of fingermillet. Patro and Madhuri (2013) evaluated the antagonistic effect of biocontrol agents *viz., T. harzianum* - 1, *T. harzianum* – 2, *T. viride*, *Pseudomonas fluorescens* and *Bacillus subtilis* against *S. rolfsii* causing foot rot of finger millet and observed maximum inhibition of mycelial growth (61.88%) in *T. harzianum* - 2, which was followed by *T. viride* (57.77%).

In vitro* screening of fungicides against *S. rolfsii

Among the nine fungicides, tebuconazole 50%+ trifloxystrobin 25%, tebuconazole 250 EC, propioconazole 25% EC, fenamidone 10%+mancozeb 50%WG and propineb70WP recorded 100 per cent inhibition of pathogen growth in all the concentrations tested. Difenoconazole 25% EC recorded 100 per cent inhibition of pathogen at 500, 1000 and 1500ppm concentrations. Other fungicides like carbendazim 50%WP, fosetyl aluminium 80%WP and kresoxim-methyl 44.3% SC were not so effective in inhibiting the mycelial growth of *S. rolfsii* (Table-4).

Arunasri *et al*. (2011) reported that the combi products containing triazoles *viz.,* avatar, merger and nativo were highly inhibitive to the growth of *Sclerotium rolfsii* in crossandra. Sangeetha and Jahagirdar (2013) reported that mancozeb, carbendazim, thiophanate methyl, hexaconazole, propioconazole completely inhibited the growth of *S.rolfsii, R. bataticola* and *Fusarium* sp. causing root rot and wilt complex in soybean.

Effect of *Trichoderma* spp. and fungicides on management of collar rot and plant growth promotion of *Gerbera*

Field experiment resulted in T_2 as best treatment module in reducing collar rot incidence, growth and yield parameters of *Gerbera* which was a combination of most effective *Trichoderma* spp. strains @ 5ml/lit and commercialized combi fungicide, Nativo @ 0.5ml/lit applied at 10ml/plant in the polyhouse. This was followed by treatment T_1 which was significantly effective against collar rot disease and improved the plant growth parameters of *Gerbera* either over the control (Table 5 & 6).

Triazole compounds are generally used as fungicides for the management of both soil borne and foliar diseases of crop plants, which also have plant growth regulating properties (Fletcher *et al*., 1986). In the triazole fungicide (difenoconazole), thirteen novel triazole analogs containing 1, 3-dioxolane rings have been synthesized and they express plant-growth regulatory activity similar to those of a difenoconazole (Xu *et al*., 2004).

There are several reports demonstrating control of a wide range of plant pathogens including *Sclerotium rolfsii* by *Trichoderma* spp. by elicitation of induced systemic or localized resistance which occur due to the interaction of bioactive molecules such as proteins avr-like proteins and cell wall fragments released by the action of extracellular enzymes during mycoparasitic reaction (Thangavelu & Mustaffa, 2010). The possible mechanisms involved in the reduction of collar rot severity of *Gerbera* due to *Trichoderma* spp. treatment might be the mycoparasitism, spatial and nutrient competition, antibiosis by enzymes and secondary metabolites, and induction of plant defense system.

Integration of different treatments like carbendazim+metalaxyl, captan+ metalaxyl+ *G. virens* 3 and captan+ metalaxyl+ *T. viride* 2 was confirmed as higher disease reduction of wilt complex caused by four wilt pathogens viz, *Fusarium oxysporum, Phytopthora capsici, Rhizoctonia solani* and *Sclerotium rolfsii* to 59.8, 58.6 and 58.0 % over control and maximum yield of 138.6, 137.0 and 135.6 q/ha was observed respectively in Bell pepper (Rather *et al.,* 2012).

Hence, the combination of the efficient *Trichoderma* spp. and fungicidal molecules resulted in the collar rot disease reduction significantly and subsequently increased the plant growth parameters in *Gerbera jamesonii*.

CONCLUSION

S. rolfsii is a polyphagous, highly vigourous and destructive soil borne pathogen and is a difficult task to control this pathogen once established. This study was undertaken to obtain the efficient strains of *Trichoderma* spp. and the effective fungicides in order to get a novel integrated management module against the

most destructive collar rot pathogen of *Gerbera* under polyhouse. This occurrence of collar rot in *Gerbera jamesonii* is a first report in Tamil Nadu, India. This might be due to the unknowing transmission of propagules through implements and tools used in the soils of polyhouse.

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