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Promissory Action of *Trichoderma* spp. and Fungicides in the Management of *Fusarium* Wilt of *Gerbera*

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Novel *Trichoderma harzianum* isolate NVTH2, effective against *Fusarium oxysporum* f. sp. *gerberae* (FOG) causing wilt in *Gerbera* showed growth tolerance against azoxystrobin 23 % SC, kresoxim methyl 44.3% SC and carbendazim 50% WP at all the tested concentrations (50ppm, 100 ppm, 250 ppm, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm) followed by fosetyl Al 50% WP, difenoconazole 25% EC and tebuconazole 250 EC (effective against FOG) moderately tolerant at lower concentrations. The fungicides like propioconazole 25% EC, propineb 70 WP and tebuconazole 50%+ trifloxystrobin 25% WG (effective against FOG) completely resisted the growth of NVTH2. Finally, a field experiment was designed by utilizing 3 most efficient *Trichoderma* spp. and 4 fungicides in combination treatments. As a result, root dipping+ soil drenching with NVTH2 @5ml/lit followed by soil drenching of tebuconazole @1ml/lit at fortnight interval alternately resulted in highest yield, growth promotion and percent inhibition of wilt incidence.

Keywords: Fungicides, *Gerbera*, growth promotion, *Trichoderma*.

Gerbera jamesonii Bolus ex Hook has very huge demand globally. The production of cut flowers has gone from 2,071 million stems in 2007 to 6,667 million stems in 2011, which ultimately increased the growth of domestic and export markets in India[1]. Deterioration of soil health makes *Gerbera* highly susceptible to soil borne diseases under protected cultivation. The major soil borne diseases of *Gerbera* are foot rot, wilt, root rot complex and blight[2].

Trichoderma harzianum is among the various species of the Trichoderma that is considered to be the utmost effective biocontrol agent[3]. The additive effects of *Trichoderma* and compatible fungicides were studied from many years. Arunasri *et al.*[4] reported that *Trichoderma* sp. which was effective against collar rot of crossandra were highly compatible with thiram (32% growth inhibition) and followed by captan (47.5%). Integration of Captan + Metalaxyl with *Trichoderma harzianum* and *T. virens* were found to be the superior to control the wilt complex of bell pepper caused by four wilt pathogens viz. *Fusarium oxysporum*, *Phytopthora capsici*, *Rhizoctonia solani* and *Sclerotium rolfsii* and promoted seedling growth[5].

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MATERIALS AND METHODS

Isolation, pathogenicity and identification of FOG

Pathogen was isolated from the infected roots of *Gerbera* (var. Donavan yellow) on potato dextrose agar (PDA) medium amended with 1000 ppm of streptomycin sulphate. Surface sterilisation of infected root bits was done by 0.1% mercuric chloride ($HgCl_2$) solution for 30 seconds and

subsequently washed in sterile distilled water and were incubated at room temperature (27±2°C) for 5 days. The phenotypic characterization was done according to Burgess *et al.*[6] using a light microscope (Labomed – IVU 5100) and photographed using a Labomed camera model LX400 with an image analyser - pixelpro programme.

The pathogen *F. oxysporum* multiplied in potato dextrose broth, consisting of 10^7conidia/ml was inoculated @ 1% to sterilized potting mixture (laterite soil: sand: compost) in 3:1:1 ratio filled @ 5kg/pot.

Collection of fungal antagonists

Three most effective isolates of *Trichoderma* spp. against FOG were selected from the Department of Plant Pathology, Tamil Nadu Agricultural University, Coimbatore, India and were taken for further experimentation (Table 1).

Compatibility of *Trichoderma harzianum* NVTH2 with fungicides

Compatibility of *Trichoderma harzianum* NVTH2 with fungicides viz. difenoconazole 25% EC (Score), tebuconazole 50% + trifloxystrobin 25% WG (Nativo), azoxystrobin 23 % SC (Amistar), 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) was tested by Poisoned food technique[7]. The minimum inhibitory concentrations (50ppm, 100 ppm, 250 ppm, 500 ppm, 1000 ppm, 1500 ppm and 2000 ppm) of systemic and contact fungicides were amended in 100 ml of Potato Dextrose Agar medium @ 15 ml/plate. The medium without fungicide served as control. The plates were incubated at room temperature (28±2°C). Three

Table 1. Various isolates of *Trichoderma* collected from Department of Plant Pathology

S.No	Name of isolate	Name of antagonist	Accession number of the isolate in NCBI
3.	NVTH1	*T. harzianum*	KJ803856
4.	NVTH2	*T. harzianum*	KJ803857
3.	TV1	*T. viride*	Commercial strain (not submitted)

Table 2. Treatment schedule for *Fusarium* wilt management

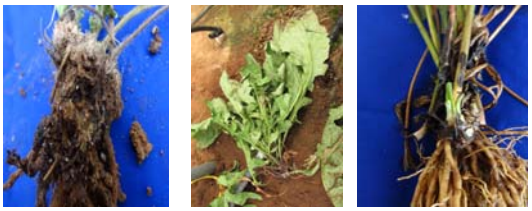
Treatment	Treatment details
T_1	RD of seedlings with *T. harzianum* NVTH2 (10^6cfu/ml) @ 5ml/litre during planting + *SD with NVTH2 @ 5 ml/litre at 15 DAP+ **SD with Tebuconazole 250 EC @ 1ml/lit at 30 DAP
T_2	RD of seedlings with *T. harzianum* NVTH2 (10^6cfu/ml) @ 5ml/litre during planting + *SD with NVTH2 @ 5 ml/litre at 15 DAP+ **SD with Difenoconazole 25% EC @ 1ml/lit at 30 DAP
T_3	RD of seedlings with *T. harzianum* NVTH2 (10^6cfu/ml) @ 5ml/litre during planting + *SD with NVTH2 @ 5 ml/litre at 15 DAP+ **SD with Fosetyl Al 50% WP @ 1ml/lit at 30 DAP
T_4	RD of seedlings with *T. harzianum* NVTH2 (10^6cfu/ml) @ 5ml/litre during planting + *SD with NVTH2 @ 5 ml/litre at 15 DAP+ **SD with Carbendazim 50% WP @ 1ml/lit at 30 DAP
T_5	RD of seedlings with *T. harzianum* NVTH2 (10^6cfu/ml) @ 5ml/litre during planting + *SD with NVTH2 @ 5 ml/litre at 15 days interval.
T_6	RD of seedlings with *T. harzianum* NVTH2 (10^6cfu/ml) @ 5ml/litre during planting + *SD with *T. harzianum* NVTH1 @ 5 ml/litre at 15 DAP+ **SD with *T. viride* TV1 @ 5 ml/litre at 30 DAP
T_7	Untreated control

SD-Soil Drenching; RD-Root Dip; DAP-days after planting
*Soil Drenching given at 15 days interval alternately with **Soil Drenching.

replications were maintained for each treatment @ 10 plates per replication.

Development of liquid formulation of *Trichoderma* spp

The fungal antagonists *viz*., *T. harzianum* strain NVTH1, *T. harzianum* NVTH2 and *T. viride* TV1 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 was mixed with 1% glycerol (10ml), tween 20 (10ml) and poly vinyl pyrrolidone – 40000 ml. wt (10g) each separately[8]. The resultant mixture was kept in orbital shaker at 200 rpm for 5 minutes to ensure uniform blending and homogenization of the bacterial cells. Then the formulation was standardized to obtain one ml of formulation consists of 10^6 cfu/ml. The liquid formulation was stored at 5°C for further study.



a. Discoloured crown portion; b. Wilting of the plant; c . Browning of petioles

Fig. 1. Symptoms of *Fusarium* wilt of *Gerbera*

Fig. 2. Pathogenicity of FOG on *Gerbera* (var. Bellwater white)

Table 3. Compatibility of *Trichoderma harzianum* (NVTH2) with fungicides

S. No.	Fungicides	Mycelial growth (mm^2)*						
		Lower dosages		Moderate dosages		Recommended dosages		Higher dosages
		50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	1500 ppm	2000 ppm
1	Tebuconazole 250 EC	30.00 (66.67)	21.00 (76.67)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
2	Propioconazole25% EC	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
3	Difenoconazole 25% EC	14.00 (84.44)	8.00 (91.11)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
4	Azoxystrobin 23 % SC	67.00 (25.56)	62.00 (31.11)	53.00 (41.11)	47.00 (47.78)	33.00 (63.33)	26.00 (71.11)	19.00 (78.89)
5	Propineb70 % WP	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
6	Fosetyl aluminium 80% WP	63.00 (30.00)	55.00 (38.89)	41.00 (54.44)	36.00 (60.00)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
7	Kresoxim-Methyl 44.3%SC	90.00 (0.00)	78.00 (13.33)	61.00 (32.22)	58.00 (35.56)	51.00 (43.33)	49.00 (45.56)	42.00 (53.33)
8	Tebuconazole 50% + Trifloxystrobin 25% WG	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)	0.00 (100.0)
9	Carbendazim 50%WP	90.00 (0.00)	90.00 (0.00)	82.00 (8.99)	62.00 (31.11)	56.00 (37.78)	41.00 (54.44)	39.00 (56.67)
10	Control	90.00	90.00	90.00	90.00	90.00	90.00	90.00

*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 percent inhibition over control (%).

Root dip and soil drenching with liquid formulation of *Trichoderma* spp. against wilt of *Gerbera* 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 and fungicides @ 1ml/ lit against wilt 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) [9]. 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 *Fusarium* wilt

The symptoms associated with wilt were yellowing of lower most leaves, and subsequently spread to entire plant. Affected leaves droop down and finally wilted. In later stages of the crop the presence of black discolouration in collar areas and brownish discolouration in petioles was observed. Wilting of the entire plant occurred within 3 to 4 weeks after infection (Figure 1). Similar report was made by Garibaldi *et al*.[10]

Identification of the Pathogen

The mycelium of the fungal culture on PDA medium was initially white and later turned light pink to dark pink in different isolates. Macroconidia was sparse, and fusoid, 2-3 septate and measured 16.0-29.0 x 2.5-4.2 µm. Microconidia were abundant, hyaline, continuous, ovoid and measured 3.8-8.5 x 2.0-3.5 µm. Chlamydospores were hyaline and spherical, measured 4.0 – 7.5 µm in diameter. Based on these phenotypic characters, the pathogen was confirmed as *Fusarium*

Table 4. Effect of *Trichoderma* spp. + fungicides on wilt incidence, growth characters and flower yield of *Gerbera* under protected cultivation

S. No.	Treatment module	Wilt incidence*	Root length (cm)*	Plant height (cm)*	No. of flowers/m^2*
1.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-Tebuconazole@1ml/lit	3.28^{a}(73.33)	21.23^{a}	43.20^{a}	52.33^{a}
2.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-difenoconazole@1ml/lit	6.16^{d}(49.91)	19.30^{c}	39.93^{c}	47.00^{d}
3.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-Fosetyl Al @1ml/lit	9.25^{e}(24.79)	18.63^{d}	38.43^{d}	45.38^{e}
4.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-Carbendazim@1ml/lit	10.22^{f}(16.91)	18.66^{d}	37.56^{e}	44.20^{e}
5.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit	5.29^{c}(56.99)	19.13^{c}	39.20^{c}	49.00^{c}
6.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH1 @5ml/lit+ ***SD-TV1 @5ml/lit	4.23^{b}(65.60)	20.33^{b}	42.20^{b}	51.40^{b}
7.	Untreated Control	12.30^{g}	18.23^{d}	36.20^{f}	38.33^{f}

*Values are mean of three replications
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
RD-Root dipping; SD-soil drenching; **SD applied alternate to ***SD at fortnight interval

oxysporum f. sp. *gerberae* (**KJ570974**). The morphological characters were similar with the descriptions made by Booth[11].

Pathogenicity

Inoculation of *F. o.* f. sp. *gerberae* (FOG) in to the healthy *Gerbera* seedlings of var. Bellwater white (30 days old) expressed the typical symptoms of wilt of *Gerbera* after 15 days of inoculation. Infected plants showed typical stunting of the plants and yellowing of leaves with brown to black streaks noticed in the crown portion and petioles of the plant. No symptoms were observed in un-inoculated control plants. Similar pathogenicity results were recorded by Garibaldi and Minuto[12].

Compatibility of *Trichoderma harzianum* isolate NVTH2 with fungicides

In the present study, tebuconazole 250 EC which was the most effective fungicide against FOG[13] was compatible with biocontrol agent *T. harzianum* isolate NVTH2 only at lower concentrations and was highly compatible with azoxystrobin 23 % SC, kresoxim methyl 44.3% SC and carbendazim 50% WP at all the tested concentrations. It was followed by fosetyl Al 50% WP, difenoconazole 25% EC and tebuconazole 250 EC moderately tolerant at lower concentrations. The fungicides like propioconazole 25% EC, propineb 70 WP and tebuconazole 50%+ trifloxystrobin 25% WG (effective against FOG)[13] resisted the growth of NVTH2 to 100% (Table-3).

Bhai and Thomas[14] reported that mancozeb and copper oxychloride were highly compatible with *Trichoderma harzianum* which was effective against rhizome rot of cardamom. Tapwal *et al.*[15] reported that *T. viride* effective against *Armillaria mellea* is compatible with the fungicide captaf and blue copper only to some extent.

Effect of *Trichoderma* spp. and fungicides on the management of *Fusarium* wilt and Plant growth promotion in polyhouse

Delivery of *T. harzianum* isolate NVTH2 through root dipping & soil drenching @ 5ml/litre+ soil drenching of Tebuconazole @ 1ml/lit recorded

Table 5. Effect of *Trichoderma* spp. + fungicides on flowering in *Gerbera* under protected cultivation (wilt management trial)

S. No.	Treatment module	Days taken for flower bud initiation*	Days taken for flower bud opening*	Length of flower stalk(cm)*	Flower diameter (cm)*
1.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-Tebuconazole@1ml/lit	82.20^{a}	99.30^{a}	36.60^{a}	9.60^{a}
2.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-difenoconazole@1ml/lit	84.00^{b}	105.00^{cd}	30.40^{d}	8.30^{bc}
3.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-Fosetyl Al@1ml/lit	85.00^{c}	109.00^{e}	28.80^{e}	7.90^{cd}
4.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit+ ***SD-Carbendazim@1ml/lit	87.00^{d}	111.33^{f}	28.00^{e}	7.50^{de}
5.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH2@5ml/lit	83.66^{b}	104.00^{c}	32.10^{c}	8.60^{b}
6.	RD-NVTH2 @ 5ml/lit+ **SD-NVTH1 @5ml/lit+ ***SD-TV1 @5ml/lit	83.00^{b}	101.00^{b}	35.70^{b}	9.20^{a}
7.	Untreated Control	91.00^{e}	119.00^{g}	26.10^{f}	7.10^{f}

*Values are mean of three replications

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

RD-Root dipping; SD-soil drenching; **SD applied alternate to ***SD at fortnight interval

the lowest wilt incidence of 3.28% with highest mean flower yield of 52.33 numbers/m^2 and also increased the root length and plant height. It was found significant with the application of treatment T6 in growth promotion and yield parameters. Likewise, treatment T1 also favored the early flowering and increased flower parameters, followed by treatment T6 over the untreated control (Table-4 and 5).

The efficiency of managing soil borne pathogen FOG can be maximised with the combined use of fungicides and antagonistic fungi but this can be achieved only if both are compatible with each other. Gaur and Sharma[16] integrated *T. viride* -1 or *T. harzianum* (TG-1) with metalaxyl and cymoxanil 8% + mancozeb 64% to control root rot in cotton. Seed treatment with *T. harzianum* + soil treatment with neem cake powder + foliar spray with carbendazim had reduced the *Fusarium* wilt of tomato and increased the the yield and growth parameters like shoot & root length and fresh weight & dry weight of the plants over the control[17].

Many triazole compounds have good fungicidal and plant growth regulating activities. In the triazole fungicide (difenoconazole), thirteen novel triazole analogs of difenoconazole containing 1, 3-dioxolane rings have been synthesized and they express plant-growth regulatory activity[18]. Seedling inoculation with three isolates of *Trichoderma* spp. (1, 2 and 3) significantly increased the growth and controlled the wilt pathogen (*F. o.* f. sp. g*erberae*) of Gerbera plants as compared to control[19].

CONCLUSION

Present experiment was undertaken by keeping in view of the hasty incidence of *Fusarium oxysporum* f. sp. *gerberae* in *Gerbera* plants. Initially the antagonistic fungi (NVTH2) was tested for its compatibility with the chemical fungicides at all types of dosages. Mostly it was compatible at lower dosages with fungicides. Later, the management module was developed at field level by combining both the *Trichoderma* spp. and fungicides against the FOG which finally resulted in the efficient module of RD + SD with NVTH2 @ 5ml/lit+ SD with Tebuconazole @ 1ml/lit at fortnight interval alternatively.

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