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POT CULTURE EXPERIMENTS ON EVALUATION OF BIOCONTROL AGENTS AND FUNGICIDES AGAINST *FUSARIUM OXYSPORUM* F. SP. *GERBERAE*

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ABSTRACT

The effect of biocontrol agents and chemical fungicides were studied under glasshouse conditions against *Fusarium oxysporum* f. sp. *gerberae* (FOG bearing Accession no.**KJ570974**) causing wilt in *Gerbera*. The pathogen was initially isolated from infected root portion of *Gerbera* and confirmed the identification through morphological study. The pathogen FOG was mass multiplied in sterilized sand maize media (sand and maize powder at the ratio of 19:1) which was inoculated into the potting mixture (laterite soil, sand and compost in the ratio 3:1:1) at the rate of 10 g per kg of soil. Then the pots were kept in completely randomized design (CRD) arranged in 8 treatments & 3 replications for biocontrol agents trial and 7 treatments & 3 replications for fungicides trial under glasshouse conditions. As a result, T_4 was found to be the best biocontrol treatment and T_3 as best fungicidal treatment against FOG with increased growth parameters of *Gerbera*.

KEYWORDS: Fungicides, *Gerbera, Fusarium, Bacillus*, Glasshouse Conditions

INTRODUCTION

Gerbera jamesonii is an attractive cut flower commonly known as African Daisy. It is grown widely in protected cultivation. Due to continuous utilization of the soils under polyhouses, *Gerbera* is widely infected by soil borne diseases like *Fusarium* wilt.

Gerbera wilt was reported in Italy and identified as *Fusarium oxysporum* f. sp. *chrysanthemi* (Garibaldi *et al.*, 2004). In gladiolus, corm rot caused by the fungal pathogen *Fusarium oxysporum* f. sp. *gladioli* is a serious problem in gladiolus production, causing huge financial losses to growers (Ramani *et al*., 2006).

Raiz *et al*. (2007) reported 100% disease incidence and 20% plant mortality with reduction in shoot and root biomass of 63 and 100% respectively, when *Gladiolus grandiflorus* corms were grown in a pot culture system inoculated with *F. oxysporum* f. sp. *gladioli*. High inoculum density of *F. o.* f. sp. *chrysanthemi* caused greater disease incidence, hasty disease advancement and low flower yield in chrysanthemum (Singh and Kumar, 2014).

MATERIALS AND METHODS

Isolation 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.* (1994) using a light microscope (Labomed – IVU 5100) and photographed using a Labomed camera model LX400 with an image analyser - pixelpro programme.

Pathogenicity

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. Then, *Gerbera* (var, Bellwater white) plants were planted and an uninoculated control was also maintained. After symptom development, re-isolation was done and compared with the original culture for confirmation of the pathogen identity.

Formulation Development of *Bacillus* Spp

The method adopted from Somasegaran and Hoben (1985). The bacterial antagonists (*Bacillus licheniformis* isolate BSD1, *Bacillus subtilis* isolate PSB5 and *Ochrobactrum* spp. isolate BSD5) were cultured on Luria Bertani medium from the stock culture maintained at -80ºC and incubated at room temperature (28±2°C) for 48hr. From these plates loopful of bacteria were inoculated into 1000 ml of NB which were incubated in an orbital shaker at 150rpm at room temperature (28±2°C) for 48hr. These liquid biomass along were mixed with 1% glycerol (10ml), tween 20 (10ml) and poly vinyl pyrrolidone – 40000 ml.wt (10g) each separately. 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 10^7cfu/ml and was stored at 5°C for further study.

Development of Liquid Formulation of *Trichoderma* Spp

The fungal antagonists (*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 (Somasegaran and Hoben, 1985). 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.

Pot Culture Experiment

A pot culture experiment was laid out in Completely Randomized Design (CRD) to test the efficacy of biocontrol agents and 6 commercial fungicides viz. difenoconazole 25% EC (Score), tebuconazole 50% + trifloxystrobin 25% WG (Nativo), propineb 70 WP (Antracol), propioconazole 25% EC (Tilt), tebuconazole 250 EC (Folicur) & carbendazim 50% WP (Benfil) in controlling the wilt disease and changes in growth promotion and yield parameters of *Gerbera*. Potting medium (laterite soil, sand and compost in the ratio 3:1:1 w/w/w) was autoclaved for 1 h for two consecutive days. The virulent strain of *Fusarium oxysporum* f. sp. *gerberae* was mass multiplied in sand maize medium (sand and maize powder at the ratio of 19:1) and incorporated in the soil at the rate of 10 g per kg of soil. The *Gerbera* seedlings were planted in the inoculated pots.

Table 1: Different Treatments of Biocontrol Agents against FOG under Glasshouse Conditions

Treatment	Treatment Details
T_1	RD + *SD with *B. licheniformis* BSD 1 (10^7cfu/ml) @ 5ml/litre
T_2	RD + *SD with *B. subtilis* PSB 5 (10^7cfu/ml) @ 5ml/litre
T_3	RD + *SD with *Ochrobactrum* spp. BSD 5 (10^7cfu/ml) @ 5ml/litre
T_4	RD + *SD with *T. harzianum* NVTH1 (10^6cfu/ml) @ 5ml/litre
T_5	RD + *SD with *T. harzianum* NVTH2 (10^6cfu/ml) @ 5ml/litre
T_6	RD + *SD with *T. viride* TV1 (10^6cfu/ml) @ 5ml/litre
T_7	RD + *SD with Carbendazim @ 1ml/litre
T_8	Control

RD-Root Dip at planting; SD-Soil Drenching; * Soil Drenching given at 15 days interval

Table 2: Different Treatments of Fungicides against FOG under Glasshouse Conditions

Treatment	Treatment Details
T_1	RD + *SD with Propineb 70WP@ 1ml/litre
T_2	RD + *SD with Tebuconazole 250EC@ 1ml/litre
T_3	RD + *SD with Tebuconazole 50%+Trifloxystrobin 25%WG @ 1ml/litre
T_4	RD + *SD with Propioconazole 25%EC @ 1ml/litre
T_5	RD + *SD with Difenoconazole 25%EC @ 1ml/litre
T_6	RD + *SD with Carbendazim 50%WP @ 1ml/litre
T_7	Control

RD-Root Dip at planting; SD-Soil Drenching; * Soil Drenching given at 15 days interval

The wilt incidence of *Fusarium oxysporum* f. sp. *gerberae* was recorded and expressed as percentage of disease incidence. Plant height and root length were recorded at monthly interval until harvesting. Flowering started after 30 days of transplanting and yield was recorded at the end.

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 DISCUSSIONS

Symptomatology of Fusarium Wilt

Wilt symptoms were observed in seedlings and in older plants. The symptoms were yellowing of the leaves and subsequently spread to entire plant. Affected leaves droop down and finally wilted. Wilting of the entire plant occurred within 2 weeks after infection. Examination of the infected plants showed the presence of black discolouration in collar areas and brownish discolouration in petioles. Similar observations of yellowing of leaves, stunting, wilting and death of the infected *Gerbera* plants in patches were seen by Garibaldi *et al.* (2004) and Garibaldi & Minuto (2007).

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 (2007).

Phenotypic Characterization 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 oxysporum f. sp. gerberae (KJ570974). The morphological characters were similar with the descriptions made by Booth (1971).

Pot Culture Study of Biocontrol Agents on Wilt Incidence and Growth Parameters of *Gerbera*

Among all the treated biocontrol agents, *Trichoderma harzianum* NVTH1 (T_4) was found to be best treatment in reducing the wilt incidence (21.50%) and growth promotion of *Gerbera* (43.50cm plant height and 21.30cm root length) followed by treatments T_5 and T_6 over the control. Moreover, *Ochrobactrum* spp. (T_3) was found to be significantly different among antagonistic bacterial strains which increased the yield and decreased the wilt incidence over the control (Table-3).

Under greenhouse conditions, the application of *Trichoderma harzianum* (ANR-1) exhibited the least disease incidence (by 15.33%) of *Fusarium* wilt in tomato and also the plants treated with *Trichoderma harzianum* (ANR-1) isolate showed a significant stimulatory effect on plant height (by 73.62cm) and increased the dry weight (by 288.38 g) of tomato plants in comparison to other 14 isolates and untreated control (Sundaramoorthy and Balabaskar, 2013).

Table 3: Effect of Biocontrol Agents on Wilt Incidence and Growth Parameters of *Gerbera* under Glasshouse Conditions

S. No.	Treatment Schedule	Wilt Incidence*(%)	Plant Height* (cm)	Root Length* (cm)	No. of Flowers/Plant*
T_1	RD+SD* with BSD1	42.46^{e} (36.02)	40.30^{d}	19.17^{c}	7.10^{f}
T_2	RD+SD* with PSB5	40.10^{d} (39.57)	40.10^{d}	19.66^{c}	8.20^{e}
T_3	RD+SD* with BSD5	35.26^{c} (46.36)	41.60^{c}	20.44^{b}	9.33^{d}
T_4	RD+SD* with NVTH1	21.50^{a} (67.60)	43.50^{a}	21.30^{a}	12.12^{a}
T_5	RD+SD* with NVTH2	22.10^{a} (66.69)	42.30^{b}	20.13^{b}	11.33^{b}
T_6	RD+SD* with TV1	24.30^{b} (63.38)	42.00^{b}	20.26^{b}	10.01^{c}
T_7	RD+SD*-Carbendazim	48.30^{f} (27.21)	39.30^{e}	18.13^{d}	6.23^{g}
T_8	Control	66.36^{g}	38.20^{f}	17.26^{e}	4.46^{h}

***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 (%).

Likewise, among 6 different treatments of commercial fungicides, Tebuconazole 250EC (Score) and Tebuconazole 50%+ Trifloxystrobin 25%WG (Nativo) were found to be highly significant in reducing the wilt incidence upto 20% and promoted the growth parameters like plant height, root length & yield of *Gerbera* (Table-4).

Carbendazim and Thiophanate-methyl suppressed the *Fusarium* wilt of chickpea caused by *F. o.* f. sp. *ciceris* (*Foc*) with 8-10% disease incidence and enhanced the plant growth, followed by Aliette and Nativo which reduced the impact of pathogen as well as enhancing the plant growth in greenhouse experiment (Maitlo *et al.,* 2014).

Table 4: Effect of Fungicides on Wilt Incidence and Growth Parameters of *Gerbera* under Glasshouse Conditions

S. No.	Treatment Schedule	Wilt Incidence*(%)	Plant Height* (cm)	Root Length* (cm)	No. of Flowers/Plant*
T_1	RD+SD*-Propineb 70WP	24.89^{d} (63.52)	38.46^{c}	18.86^{b}	10.06^{c}
T_2	RD+SD*-Tebuconazole 250EC	21.33^{b} (68.74)	39.12^{b}	18.26^{b}	13.48^{a}
T_3	RD+SD*- Tebuconazole 50%+ Trifloxystrobin 25%WG	20.60^{a} (69.81)	40.32^{a}	19.21^{a}	13.33^{a}
T_4	RD+SD*-Propioconazole 25%EC	22.66^{c} (66.79)	38.13^{c}	18.13^{b}	11.18^{b}
T_5	RD+SD*-Difenoconazole 25%EC	29.36^{e} (56.97)	36.21^{d}	18.72^{b}	8.81^{d}
T_6	RD+SD*-Carbendazim	47.41^{f} (30.52)	34.69^{e}	17.92^{c}	6.21^{e}
T_7	Control	68.24^{g}	33.23^{f}	17.20^{c}	5,11^{f}

***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 (%).

CONCLUSIONS

The study reveals the detailed picture on effects of biocontrol agents and fungicides on *Fusarium* wilt of *Gerbera* in pot culture under glasshouse conditions. The pathogen FOG is a highly devastating pathogen with great diversity; also it has limited ways to be controlled. So, an attempt was done in order to get the most efficient strains of *Trichoderma* spp. and *Bacillus* spp. Moreover, all the 6 commercial fungicides were found to be highly efficient in controlling the pathogen FOG under controlled conditions.

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