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Corm Treatment and Soil Solarization for the Management of Wilt (*Fusarium oxysporum*) in Gladiolus (*Gladiolus grandiflorus*)

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ABSTRACT

Aqueous extracts of leaves, seed and cloves of seven different plants [eucalyptus (*Eucalyptus* hybrid), aonla (*Phyllanthus emblica*), ginger (*Zingiber officinale*), aloe (*Aloe barbadensis*), neem (*Azadirachta indica*), darek (*Melia azedarach*) and garlic (*Allium sativum*)], three commercial neem (*Azadirachta indica*) formulations, five *Trichoderma* species and two bacterial antagonists (*Bacillus subtilis*, *Pseudomonas fluorescens*) were evaluated against the wilt pathogen (*Fusarium oxysporum*) of gladiolus (*Gladiolus grandiflorus*), var. 'Peter Pears'. Among these, neem formulation Neemazal, oil of *E.* hybrid, *Trichoderma viride*, *B. subtilis* and *P. fluorescens* were found to be effective with 100, 68.9, 62.0, 59.2 and 57.0% inhibition, respectively of the mycelial growth of the wilt pathogen. These effective treatments were used as corm treatment and integrated with soil solarization for the management of gladiolus wilt. Soil solarization with transparent polyethylene mulch (25 µm thick) for 40 days resulted in an increase of 8.3°C at 5 cm soil depth and the average maximum soil temperature during the period was 40.3°C. Corm treatment with *T. viride* formulation followed by their plantation in solarized plots resulted in a 72.2% reduction in wilt incidence in comparison to untreated corms sown in unsolarized soil. This treatment also resulted in improved growth as well as quality parameters with an increase of 32.3, 40.6, 84.4, 96.3, 38.4 and 42.2% in plant height, spike length, number of florets per spike, number of cormels per corm, corm size and corm weight, respectively and flowering was also recorded in 17.4% fewer days.

Keywords: biological control, cultural practices, mulching, soil-borne pathogen, *Trichoderma viride*
Abbreviations: EC, emulsifiable concentrate

INTRODUCTION

The area under flower production is around 126,235 ha in India, out of which 27,618 ha are under cut flowers; gladiolus (*Gladiolus grandiflorus*) is one of the important cut flowers grown over an area of more than 17,000 ha (Singh 2009). The crop is infected by many pathogens, primarily wilt or yellows caused by *Fusarium oxysporum* Schl. f.sp. *gladioli* (Massey) Sny. and Hans, which is a severe disease of the crop in India (Kaur *et al.* 1989; Tomar *et al.* 1997). Due to repeated growth of the crop on the same land, soil-borne pathogens continue to grow and perpetuate in the soil, aggravating the disease. Gladiolus wilt can not be effectively controlled with drenching of chemical fungicides as it is neither cost-effective nor eco-friendly. The present study was therefore conducted to test the efficacy of some botanicals, biological control agents and physical methods against the wilt pathogen. Soil solarization is an effective method to control different soil-borne pathogens in different soil eco-systems and crops (Raj 2008). Soil solarization has been found to be more effective against soil-borne pathogens when integrated with seed/root treatment with fungicides and biological control agents (Gamliel and Stapleton 1993; Stevens *et al.* 2003). Hence, the present investigation was carried out to integrate effective corm treatments with botanicals and biological control agents with soil solarization for the management of the wilt.

MATERIALS AND METHODS

In vitro evaluation of botanical formulations and fungal and bacterial antagonists

1. Evaluation of botanical formulations

Water extracts of four-months old leaves of 10-year old plants of eucalyptus (*Eucalyptus hybrid*), aonla (*Phyllanthus emblica*) var. 'Banarsi', neem (*Azadirachta indica*) local land race, and darek (*Melia azedarach*) local land race; three-month old leaves of ginger (*Zingiber officinale*) var. 'Himgiri' and aloe (*Aloe barbadensis*) local land race; freshly harvested cloves of garlic (*Allium sativum*) var. 'Agrifound Parvati'; essential oil of eucalyptus and three commercial registered formulations of neem of different companies namely Bioneem (0.03% azadirachtin) (Ajay Bio-Tech India Ltd. Pune, India), Neemazal (1% azadirachtin) (EID Parry (India) Ltd., Chennai, India) and Nimbicidine (0.03 azadirachtin) (T. Stanes & Co. Ltd. Coimbtore, India) were evaluated against the wilt pathogen of gladiolus under *in vitro* conditions. Fresh leaves and cloves of different plants as mentioned above were first washed with tap water and then with sterilized distilled water (SDW). The samples were then ground in an electric mixer and blender of Gopi (Super model) for 5 min by adding 100 ml SDW. Water extracts of different samples were prepared by adding different plant parts to water in a 2: 1 ratio (w/v). These samples were then homogenized in an orbital shaker at 2000 rpm for 30 min, filtered through double-layered muslin cloth, then placed in 500 ml conical flasks, plugged with cotton and steam sterilized for 5 min. *In vitro* evaluation of these botanical extracts and commercial neem formulations was done by the poisoned food technique. In this method, potato dextrose agar medium was prepared with double strength of agar and sterilized at 1.05 kg/cm^2 for 20 min in an autoclave. Fifty ml of this melted medium was added aseptically

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to an equal volume of steam-sterilized (100°C for 5 min in an autoclave) botanical formulations, which were made from different plant parts or oil, and mixed thoroughly. This mixture was poured aseptically in Petri dishes and the medium was allowed to solidify. The Petri dishes were then inoculated with three-day old culture of the wilt pathogen and incubated at 25°C. Radial growth of the wilt pathogen was recorded after six days to record the inhibitory effect of different botanical formulations. The botanical extracts made of different plant samples were tested at 50 and 100% concentrations against the wilt pathogen. In the case of eucalyptus oil, emulsifier (Tween-80 at 0.1%, v/v) (Himedia Laboratories, Mumbai) was added and evaluated at 100% concentration. Commercial neem formulations were tested at 1, 5, 10, 15, 20 and 25% concentration.

2. Evaluation of fungal and bacterial antagonists

Five native species of fungal antagonist *Trichoderma* i.e. *T. harzianum*, *T. hamatum*, *T. viride*, *T. virens* and *T. polysporum* were obtained from the biological control section of the Department of Mycology and Plant Pathology, Dr. Y. S. Parmar University of Horticulture and Forestry and two native bacterial antagonists, *Bacillus subtilis* and *Pseudomonas fluorescens* were obtained from the microbiology section of the Department of Basic Sciences, Dr. Y. S. Parmar University of Horticulture and Forestry. These were evaluated under *in vitro* conditions for their antagonistic activity against the wilt pathogen by dual culture technique in the case of fungal antagonist and streak plate method in the case of bacteria (Huang and Hoes 1976; Utkhede and Rahe 1983). The *Trichoderma* species that proved to be most effective in the *in vitro* studies was multiplied on talc-based formulation. The species was multiplied in potato dextrose broth for 10 days (Mathivanan *et al*. 1998). One part of the culture broth was mixed into the two parts of the talc powder (white stone powder of 750 mesh) (Marvellore Mining and Allied Industries Pvt. Ltd., Surat, India). Similarly, best bacterial antagonist was raised in nutrient broth culture for 48 hours at 30°C and then used as corm dresser.

Integration of effective botanicals, fungal and bacterial antagonists with soil solarization

A field experiment was laid out in randomized block design at the experimental farm in the university during 2004 and 2005. The texture of the soil of the farm was clay loam with pH 6.8. The experiment was conducted in two set of conditions with similar treatments i.e. solarized and unsolarized plots. The plots in both the treatments were irrigated to saturation level. In the solarized conditions, the plots were covered with thin (25 µm thick) transparent polyethylene mulch for 40 days during summer months (March-April) (**Fig. 1**). The polyethylene sheet was removed after 40 days of solarization. In unsolarized conditions, the plots were not covered.

Among the extracts of botanicals, neem formulations, fungal and bacterial antagonists, the treatments which proved most effective in inhibiting the growth of the wilt pathogen under *in vitro* conditions were used as corm treatment and integrated with soil solarization to observe their effect on the incidence of the wilt in the field. Effective fungal (talc powder based formulation) and bacterial (broth) bio-agents were used as corm dresser at the rate of 0.5% on a w/w and v/w basis, respectively. Water-based extracts of botanicals and neem formulation (Neemazal) were applied as corm dip for 30 min. The treated corms were shade-dried for 1 day prior to showing. In this study, gladiolus corms of var. 'Peter Pears' were used. Corms were sown at a distance of 60 × 15 cm. Data pertaining to wilt incidence was recorded after 60 days of sowing. Data related to different growth parameters i.e. plant height, spike length, size of corm, weight of corm, number of cormels per corm, number of florets per spike and number of days to flowering were also recorded by selecting 5 plants per replication in each treatment.

Fig. 1 Mulching with transparent polyethylene mulch (25 µm) in field.

RESULTS

All the botanical extracts inhibited the mycelial growth of the pathogen in comparison to the control (**Fig. 2**). Leaf extract on aonla was most effective with 61.6% inhibition of the wilt pathogen followed by eucalyptus oil and darek leaves with 61.1 and 57.4% mycelial inhibition, respectively. Among the three neem formulations evaluated against the wilt pathogen, Neemazal was most effective with 100% reduction of the wilt pathogen followed by Bioneem with 61.4% inhibition (**Fig. 3**). All the fungal and bacterial antagonists inhibited the growth of the wilt pathogen ranging from 48.0 to 62.1%. Out of the five native species of *Trichoderma* evaluated, *T. viride* was found to be most effective with 62.0% inhibition (**Fig. 4**). Among bacterial antagonists, *B. subtilis* was most effective with 59.2% inhibition followed by *P. fluorescens* with 57.0% inhibition. Soil solarization with transparent polyethylene mulch resulted in an 8.3°C increase in the average maximum soil temperature which was 40.3°C in comparison to 31.9°C in unsolarized soil at 5 cm soil depth. However, an increase in the average maximum soil temperature was only 2.9°C at 20 cm soil depth. The range of maximum soil temperature at 5 cm soil depth during the period of soil solarizarion was 33.0 to 47.5°C.

Integration of different effective treatments of botanical extracts/oil, neem formulation, fungal and bacterial antagonists used as corm treatment with soil solarization proved effective with 12.3 to 16.8% wilt in comparison to 21.5 to 30.0% in unsolarized soil (**Table 1**). However, the incidence of wilt without any treatment of corms in control plots of solarized soil was 18.6% and in unsolarized soil plots was 45.3%. Corms treated with *T. viride* formulation then sown in solarized plots was most effective with 12.3% incidence of the disease in comparison to 45.3% in the control. Corms treated with *P. fluorescens* and Neemazal in solarized soil were statistically similar with a wilt incidence of 14.7 and 14.9%, respectively. Treatment of corms with a *T. viride* dressing then sown in solarized plots was found most effective and resulted in maximum plant height (112.0 cm), spike length (94.6 cm), number of florets per spike (16.6), corm weight (54.5 g), corm size (51.5 mm), number of cormels per corm (17.6) and flowering was recorded in least number of days (94.3). However, in control (unsolarized and without any treatment) minimum plant height (84.6 cm), spike length (67.3), number of florets per spike (9.0), corm weight (38.3 g), corm size (37.3 mm), number of cormels per corm (9.0) and flowering was also recorded in maximum number of days (115).

DISCUSSION

Extract of aonla leaves (100%) was most effective with 72.7% inhibition of the wilt pathogen followed by eucalyptus oil, extract of darek leaves and extract of garlic cloves. Aqueous extract of *Azadirachta indica* and *Allium sativum* have been reported to completely inhibit the germination of *Fusarium* spores (Tripathi *et al.* 1999). Aqueous extracts of leaves (aonla and eucalyptus), seed (darek) and

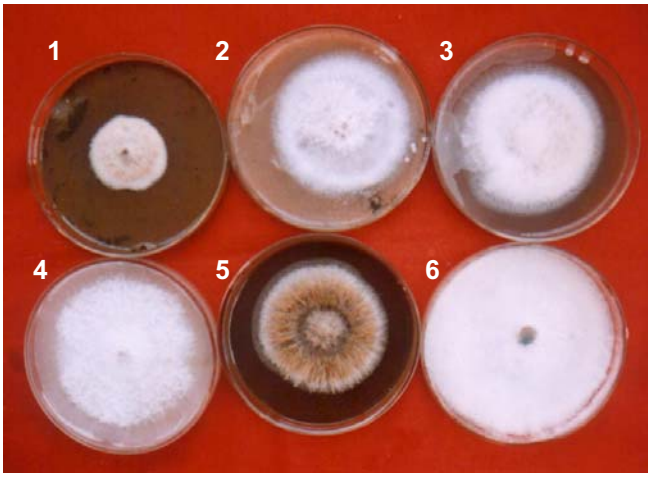

Fig. 2 Effect of different botanical extracts (100%) on the growth of *Fusarium oxysporum* f sp. *gladioli* under *in vitro* conditions. 1. darek leaves; 2. darek seed; 3. ginger leaves; 4. aloe leaves; 5. neem leaves; 6. control.

Fig. 3 Effect of different commercial neem formulations (10%) and neem leaf extract (100%) on the growth of *Fusarium oxysporum* f.sp. *gladioli* under *in vitro* conditions. 1. Bioneem; 2. Neemazal; 3. Nimbicidine; 4. neem leaves; 5. control.

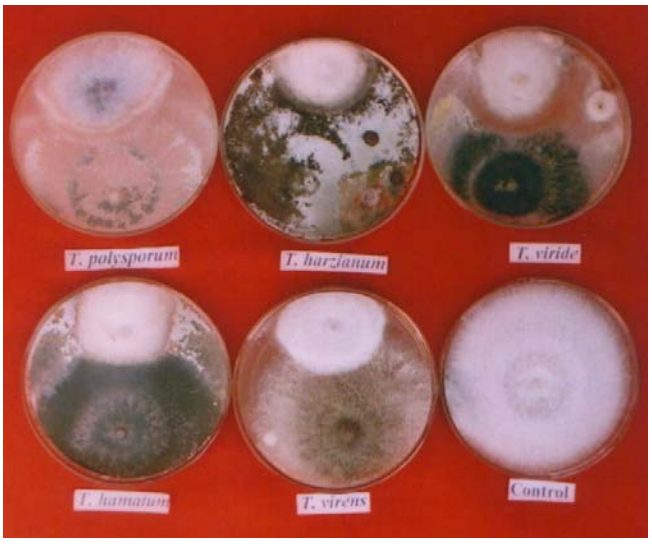

Fig. 4 Inhibition in mycelial growth of *Fusarium oxysporum* f.sp. *gladioli* by different *Trichoderma* species in dual culture.

cloves (garlic) are reported to be effective under *in vitro* conditions against *Alternaria alternata* and *Rhizoctonia solani*. Neemazal (25%) was found to be most effective with complete inhibition of the wilt pathogen of gladiolus. Neem oil has been reported to be effective against *Fusarium* wilt of tomato and onion (Eswaramurthy *et al.* 1989; Raj and Kapoor 1993).

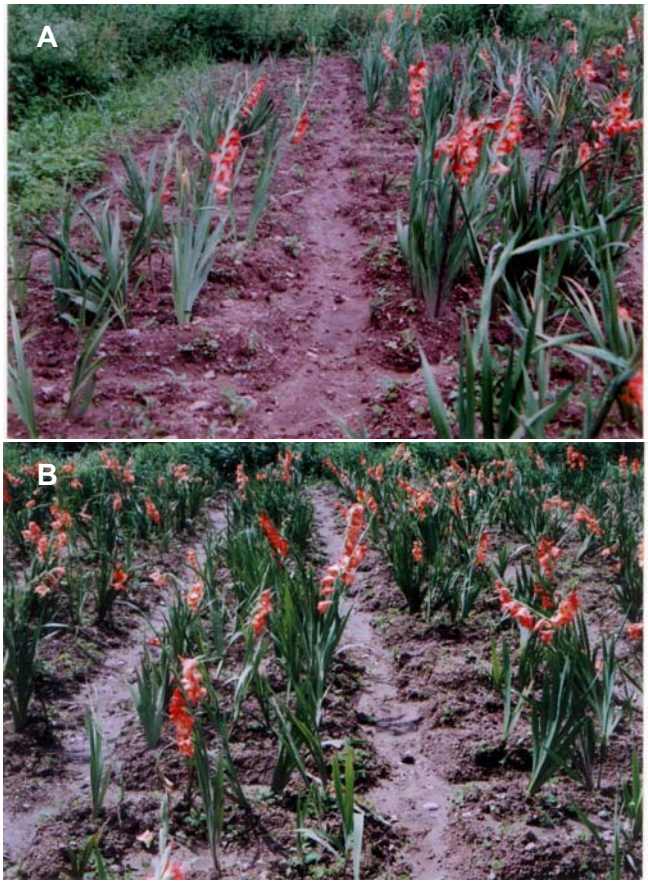

Fig. 5 Gladiolus crop in unsolarized (A) and solarized (B) plots.

Biological control agents play an important role in the management of soil-borne pathogens and can become an important component in the integrated management strategy of these pathogens. In the present study, *T. viride* was most effective with 62.1% inhibition of the wilt pathogen followed by *B. subtilis* with 59.2% inhibition. Native isolates of *P. florescens* and *Trichoderma* spp. have been reported to be effective under *in vitro* conditions against the *Fusarium* wilt of gladiolus (var. 'White Friendship') and carnation (Vaidya *et al*. 2004). Karimi *et al*. (2007) also reported the treatment of carnation cuttings with *P. florescens* and *Bacillus subtilis* effective against the *Fusarium* wilt of carnation under field conditions. Application of *B. subtilis* to the substrate has also been found effective for the management of wilt of carnation (Obiegilo 1992).

Soil solarization with thin polyethylene mulch is an effective method for the management of soil-borne pathogens (Katan 1981). Soil solarization has been reported to increase up to 14°C in average maximum soil temperature below the sheet and which is found lethal for the soil-borne pathogens (Pullman *et al.* 1979; Raj and Kapoor 1993; Raj and Gupta 1996). In the present study, soil solarization for 40 days resulted in an increase of 8.6°C in average maximum soil temperature at 5 cm soil depth.

Corm treatment with different botanical formulations and antagonists followed by their plantation in solarized soil significantly reduced the incidence of wilt in the field and also resulted in an increase in different growth and quality parameters of gladiolus. Corms treatment with *T. viride* followed by their plantation in solarized soil was found most effective with 72.2% reduction in the disease incidence in comparison to untreated corms sown in unsolarized plots. Corm treatment with *P. fluorescence* and Neemazal followed by their plantation in solarized soil were equally efficient (67.7 and 67.3%, respectively) in reducing the disease incidence. Corm treatment with biopesticides

Table 1 Effect of integration of corm treatment with bio pesticides/antagonists and soil solarization on disease incidence, growth as well as quality parameters of gladiolus crop.

Treatment	Concen-tration	Disease incidence (%)		Plant height (cm)		Spike length (cm)		№ of florets/plant		Days to flowering		№ of cormels/corm		Corm size (mm)		Corm weight (g)	
	(%)	S	US	S	US	S	US	S	US	S	US	S	US	S	US	S	US
T. viride	0.50	12.3 (22.69)	21.5 (27.23)	112.0	99.3	94.6	81.6	16.6	12.9	95.0	100.7	17.6	12.6	51.6	43.6	54.5	46.2
Neemazal	10	14.8 (22.69)	20.9 (27.23)	111.3	99.3	92.3	79.3	16.5	13.0	94.3	102.0	17.6	12.3	47.6	41.0	53.8	44.1
Eucalyptus oil	5	16.8 (22.93)	22.2 (28.15)	108.8	90.6	89.6	76.3	14.8	10.0	96.0	100.0	16.0	12.0	48.3	43.0	52.4	42.3
B. subtilis	0.50	16.5 (24.03)	25.0 (30.02)	111.0	99.0	91.6	79.0	15.5	11.6	95.0	102.3	17.3	13.0	49.0	41.0	52.3	41.5
P. fluorescens	0.50	14.7 (22.53)	21.3 (27.49)	109.0	97.6	91.6	79.3	15.5	11.3	97.0	105.0	15.6	13.0	49.3	40.6	52.3	41.8
Control	-	18.6 (25.58)	45.3 (42.34)	98.6	84.6	80.0	67.3	13.1	9.0	105.0	115.0	15.0	9.0	44.0	37.3	45.1	38.3
$LSD_{0.05}$		1.60		1.67		1.25		0.82		2.43		0.85		0.74		2.22	

S, solarized; US, unsolarized

like Neemazal and Nimbicidine has been reported to reduce the incidence of *Fusarium* wilt of gladiolus (var. 'Peach Blossom') (Chandel and Tomar 2007). Soil solarization with transparent polyethylene mulch has been reported to be effective for the management of different wilt pathogens in different crops (Katan 1981; Pinkerton *et al.* 2002). Freeman *et al*. (1986) reported that integration of soil solarization with *Trichoderma harzianum* gave better control of white root rot (*Rosselinia necatrix*) disease in apple orchards. Corm treatment with *T. viride* followed by their plantation in solarized soil also resulted in improved growth as well as quality parameters with an increase of 32.3, 40.6, 84.4, 96.3, 38.4 and 42.2% in plant height, spike length, number of florets per spike, number of cormels per corm, corm size and corm weight respectively and flowering was also recorded in 17.4% less days (**Fig. 5A, 5B**). Soil solarization and integration of soil solarization with other disease management methods have been reported to result in higher growth response in peach, walnut, tomato and many other crops (Katan 1981; Stapleton and DeVay 1982; Raj and Kapoor 1993). The mechanism for explaining increased growth responses and yield in plants has been attributed to chemical factors (like release of nutrients and other growth factors, nullification of toxins) and biological factors (elimination of minor and unknown pathogens and stimulation of beneficial microorganisms (Chen and Katan 1980; Stevens *et al*. 2003).

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