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Trichoderma and Intercropping Impact Production, Quality and Corm Rot Disease of *Gladiolus grandiflorus*

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ABSTRACT

The effects of *Trichoderma harzianum* Rifai application and intercropping with *Gazania rigens* L. (treasure flower) or *Calendula officinalis* L. (pot marigold), members of the Asteraceae family, were studied on growth, yield, quality and controlling corm rot disease of gladiolus. Two experiments were conducted; first using healthy corms of two gladiolus cultivars, 'Fado' and 'Cantate' and second on both healthy and artificially infected (with *Fusarium oxysporum*) corms of cultivar 'Fado' in order to compare the individual treatment effects. The addition of *Trichoderma* to the medium increased growth, production and quality as well as reduced disease incidence when applied to healthy corms, although intercropping had a similar effect. When individual treatment effects were evaluated, use of both *Trichoderma* and intercrops, applied to infected corms, significantly improved plant height (65%), foliage growth (122%), reduced crop harvest time (23%), and disease (corm rot) incidence (78%), increased leaf area (70%), total leaf chlorophyll contents (162%), number of florets (71%), stem length (69%), stem diameter (68%), and cormel diameter (158%) and were similar to those of grown from healthy corms. However, when both *Trichoderma* and intercropping were applied individually to healthy corms, the results were statistically similar. In both experiments, plants treated with *Trichoderma* and intercropped had up to 67% less corm rot disease on average than control (plants grown without *Trichoderma* and intercropping). In summary, *Trichoderma* application to soil and intercropping with members of the Asteraceae family can be effective not only for improving growth, yield and quality of cut gladiolus, but also controlling corm rot disease of gladiolus. Moreover, intercropped species can also be a source of additional income to the growers.

Keywords: allelopathy, cut flowers, *Fusarium oxysporum*, gladiolus, *Trichoderma harzianum*

INTRODUCTION

Gladiolus grandiflorus L., one of the most widely cultivated flowers worldwide, occupies fourth position in global cut flowers trade after roses (*Rosa hybrida* L.), carnations (*Dianthus caryophyllus* L.), chrysanthemums (*Dendranthema grandiflorum* Ramat.), and tulips (*Tulipa hybrida* L.) (Singh 2006; Anonymous 2011). It has great economic value and is considered as king amongst bulbous cut flowers. In Pakistan, it is the second most-widely grown cut flower after rose and has high demand in local markets (Ahmad *et al.* 2008). It is gaining popularity among local farmers on account of its beautifully colored spikes, easy production, and higher yields than roses in a shorter time period. During the last decade, an increased awareness and recognition of higher returns on investments, rising living standards and increase in floriculture business in the country has made it a lucrative enterprise. However, the gladiolus crop is attacked by a wide array of diseases including corm rot, leaf and neck rot, leaf chlorosis and necrosis, flower mottle, etc. (Beute 1970).

A soil-born fungus, *Fusarium oxysporum* Schlecht. f. sp. *gladioli* (Nelson *et al.* 1981), which causes yellowing and corm rot, is an important pathogen that reduces corm and flower production of gladiolus (Dallavalle *et al.* 2002; Nasir and Riazuddin 2008). During recent times, it has become a serious threat to this economically profitable crop and has caused great losses to the growers in Pakistan. Agrochemicals are indispensable to fight against plant diseases and maintain higher crop yields. However, indiscriminate use of these chemicals has often led to adverse environmental effects, disturbing the ecological balance of soils and making plants even more susceptible to pests and diseases (Nasir and Riazuddin 2008; Riaz *et al*. 2008). Increased public concern on environmental issues and need of alternate technologies for organic cut flower industry require integrated disease management systems, which are less pesticide-dependant and/or based on naturally occurring compounds (Cuthbertson and Murchie 2005). Corm rot disease of gladiolus can be managed by mixed cropping of *Helianthus annuus* and *Tagates erectus* or cultivation of *Tagates erectus* and *Brassica campestris* in rotation (Riaz *et al.* 2009).

Biological disease control is a nature-friendly, ecological approach to overcome the side-effects of chemical plant protection. *Trichoderma harzianum*, a beneficial fungi, was first recognized as bio-control agent of plant diseases in the 1930s (Weindling 1932) and since then, it has been effectively used for controlling various diseases of many important crops (Harman 2000). Corm dressing with *T. harzianum* and *Pseudomonas fluorescens* cultured on a bagasse-soil-molasses mixture decreased corm rot and yellow scores of gladiolus as well as soil population of the pathogen and increased plant growth and flowering (Khan and Mustafa 2005). *Trichoderma* can be integrated into a disease management program for Fusarium root rot in coneflower (*Echinacea purpurea*) (Wang *et al.* 2005).

Intercropping is practiced to obtain higher yields by making the best use of available resources that would otherwise not be utilized by a single crop (Trenbath 1976). Common practice to control Fusarium rot during storage is disinfection with chemical fungicides. However, due to the new development of physiological races of pathogens, these synthetic chemicals are becoming ineffective in many cases (Spots and Cervante 1986). Allelopathy can reduce yield loss caused by fungi or other pests. Interplanting lettuce

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(*Lactuca sativa* L.) with a spring tomato crop is used successfully in many countries for allelopathic control of foot and root rot caused by *F. oxysporum* f. sp. *radicis-lycopersici* (Jarvis and Thorpe 1981; Jarvis 1989).

Pakistan has a better scope in the future for ornamental crop production as there is a shift in trend towards tropical and sub-tropical flowers that can be gainfully exploited by the country with reasonable diversity in indigenous flora and climatic conditions in different regions. The suitable agro-climatic conditions of the country clearly indicate that a wide range of ornamental crops can be grown, which can improve the economic livelihood of the growers. As gladiolus is cultivated in rural areas, its potential for generating employment is an added advantage to improve the economic conditions of the weaker sections of the society. On the basis of the popularity of gladiolus in the country, it may be considered as the most cultivated crop in the future as it can be grown outdoors throughout the year in different parts of the country. There is also a great scope for export of gladiolus spikes to Gulf countries and other international markets. However, to export gladiolus spikes, crop quality standards needs to be maintained higher as required by international markets. Despite the agro-climatic conditions of Pakistan favoring good quality gladiolus production, frequent infection by soil or bulb-borne pathogens results in the failure of plants to produce quality spikes. Moreover, flower growers are generally poor with limited resources and have little knowledge of disease management. Consequently, when a disease becomes severe, they apply chemicals which do not prove effective and rather increase production cost.

The present study, therefore, attempted to find a biological and cultural means to control corm rot disease of gladiolus, which is a major problem in quality gladiolus production. The specific objectives of the study were to evaluate the effectiveness of *Trichoderma* application and intercropping with members of the Asteraceae family (which contain a wealth of secondary metabolites; Teixeira da Silva 2003, 2004; Teixeira da Silva *et al.* 2005), to inhibit the corm rot disease caused by *F. oxysporum* in gladiolus and to compare the individual effects of intercropping and *Trichoderma* application on growth, yield, quality and controlling corm rot disease of gladiolus.

MATERIALS AND METHODS

The experiments were conducted at the University of Agriculture, Faisalabad, Pakistan (latitude 31° 30′ N, longitude 73° 10′ E and altitude 213 masl). Cut 'Fado' and 'Cantate' gladioli corms were obtained from a local distributor of Stoop flower bulbs, the Netherlands. *T. harzianum* isolate FCBP-325 was obtained from the stock cultures of the First Fungal Culture Bank of Pakistan (FCBP), Institute of Mycology and Plant Pathology, University of the Punjab, Lahore, Pakistan, maintained on Potato Dextrose Agar (PDA), stored at 4ºC and used as the biological control agent for Fusarium rot disease after sub culturing. *Trichoderma* was applied in the experimental plots before planting the crop at 200 kg ha^{-1} after thorough mixing in the soil. Two annual flowering species of the Asteraceae family, *Gazania rigens* L. (treasure flower) and *Calendula officinalis* L. (pot marigold), were used as inter-crops. In the first experiment, the effect of *Trichoderma* and intercropping was studied on production, quality and disease inhibition on healthy corms of 'Fado' and 'Cantate', while in the 2nd experiment, the same treatments were compared on healthy and infected 'Fado' corms to compare their individual effects. Experiments were laid out in a randomized complete block design with a factorial arrangement in which eight treatments were replicated three times. Each replicate had five plants. In the first experiment, treatments included intercropping with *Gazania* or *Calendula* either with or without *Trichoderma* application on both gladiolus cultivars; while in the second experiment, treatments were 1) no *Trichoderma* and no intercrop, 2) only *Trichoderma* application without any intercrop, 3) no *Trichoderma* and intercropping with gazania, and 4) no *Trichoderma* and intercropped with calendula, applied on healthy as well as infected corms of 'Fado'. For this study, the isolate of *Fusarium oxysporum* f. sp. *gladioli* was isolated from infected gladiolus corms, procured from a local commercial grower. Diseased corms were sectioned into 4-5 mm pieces, followed by surface-sterilization with 1% sodium hypochlorite solution for 5 min, and rinsed thrice with double distilled water. *F. oxysporum* inocula were prepared using 2-3 small pieces of diseased samples placed in 90 mm Petri dishes, on autoclaved potato dextrose agar (PDA) medium. Colonies were grown at 25 ± 2°C for one week, sub-cultured again on PDA medium, isolated, and were cultured on boiled chick peas packed in plastic bags, 250 g chick peas and one *Fusarium* colony per bag, for two weeks at 25 ± 2°C with a 12-h photoperiod under a PAR flux of 15-20 mol m^{-2}s^{-1} at bench level, which were applied at 20 g m^{-2} and mixed uniformly in planting beds before planting corms. Corms were spaced at 15 cm between plants in 60 cm spaced rows.

All other cultural practices like production medium (garden soil: silt: farm yard manure; 1:1:1 v/v/v), fertilization (17-17-17 NPK at 100 kg ha^{-1} biweekly application from planting to spike emergence), irrigation (at 5-7 days interval depending on weather conditions), earthing up (twice; after 30 and 60 days of planting), staking (at spike emergence, as needed), etc. were similar for all treatments. Data were collected on time to 50% sprouting, plant height (cm), number of leaves plant^{-1}, leaf area (cm^2), total leaf chlorophyll contents (mg g^{-1}), days to harvest (from planting), number of florets spike^{-1}, floret diameter (mm), stem length (cm), stem diameter (mm), fresh and dry weight of a stem (g), vase life (days), disease (corm rot) incidence (%), number of cormels and cormel diameter (mm).

Total leaf chlorophyll (chl) content was measured using the method of Arnon and Hoagland (1949). Fresh leaves were chopped into 0.5 cm long segments and 0.5 g sample was extracted with 5 mL of 80% acetone at 10°C overnight. The material was centrifuged at 14000 rpm for 5 min and the absorbance of the supernatant was measured at 645, 652 and 663 nm for chl *a*, *b* and total chl, respectively using a spectrophotometer (Hitachi Model-U 2001, Japan). Afterwards, chlorophyll *a*, *b* and total chl were calculated according to the Litehtenhaler and Wellburn (1983) equations:

$$\text{Chl } a \text{ (mg g}^{-1}\text{ FW)} = [12.7\,(OD_{663}) - 2.69\,(OD_{645})] \times V/1000 \times W$$

$$\text{Chl } b \text{ (mg g}^{-1}\text{ FW)} = [22.9\,(OD_{645}) - 4.68\,(OD_{663})] \times V/1000 \times W$$

$$\text{Total chl (mg g}^{-1}\text{ FW)} = [20.2\,(OD_{645}) - 8.02\,(OD_{663})] \times V/1000 \times W$$

where 'OD' is optical density, 'V' is volume of the sample and 'W' is weight of the sample (Ghani 2010).

For vase life determination, cut spikes were harvested at commercial harvest maturity when 1-2 lowermost florets started showing color, with sharp secateurs, leaving two lower most leaves intact for onward corm and cormel development. Stems were immediately put in buckets containing distilled water and shifted to laboratory within 1 h of harvest. Stems were graded on the basis of stem caliper and number of florets, labeled, re-cut to 45 cm and put individually in vases containing 400 mL distilled water. Stems were arranged on benches at 25 ± 2°C temperature, and 60-80% RH with 12 h photoperiod. Cut stems were daily observed for their visual appeal and were considered dead when more than 50% of florets were wilted, faded, or dried (Ahmad *et al.* 2011). For measuring disease incidence, plants were monitored throughout the study and number of wilted plants was recorded weekly from sprouting till harvest. Disease severity was accessed on a 0-3 scale where 0 = no symptoms, 1 = leaf chlorosis, 2 = leaf drying, and 3 = dead or likely to be dead plant (Riaz *et al.* 2010). All wilted plants were documented and percentage of fusarium wilt was calculated by dividing number of infected plants by total number of plants × 100 (Riaz *et al.* 2010; Barakat and Al-Masri 2012). Three way analysis of variance (ANOVA) on data was performed using the GLM program of STATISTICA 5.6 and means were compared using Tukey's multiple-comparison test at $P \leq 0.05$ (Steel *et al.* 1997).

Table 1 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy corms, on time to 50% sprouting, plant height, and number of leaves plant^{-1} of cut 'Fado' and 'Cantate' gladioli. Means are an average of data from 15 plants.

Treatments			Time to 50% sprouting (days)	Plant height (cm)	Number of leaves plant^{-1}
Cultivar	***Trichoderma***	**Intercrop**			
Fado	Yes	Gazania	10.3 b[a]	43.8 c	11.9 a
		Calendula	9.3 b	52.6 a	10.4 ab
	No	Gazania	10.0 b	45.2 bc	9.6 b
		Calendula	13.3 a	33.5 d	6.9 c
Cantate	Yes	Gazania	8.9 b	49.4 abc	11.2 ab
		Calendula	10.5 b	44.0 bc	11.0 ab
	No	Gazania	13.0 a	35.2 d	6.6 c
		Calendula	10.7 b	50.0 ab	11.3 ab
Significance[b]					
Cultivar (C)			NS	NS	NS
Trichoderma (T)			0.0009	NS	NS
Intercrop (I)			NS	NS	NS
C*T			NS	NS	NS
C*I			NS	0.0004	0.0001
T*I			NS	NS	NS
C*T*I			0.0008	0.0000	0.0007

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of corm type, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Table 2 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy corms, on total leaf chlorophyll contents, time to harvest and number of florets of cut 'Fado' and 'Cantate' gladioli. Means are an average of data from 15 plants.

Treatments			Total leaf chlorophyll contents (mg g^{-1})	Time to harvest (days)	Number of florets
Cultivar	***Trichoderma***	**Intercrop**			
Fado	Yes	Gazania	58.7 a[a]	104.3 b	11.9 a
		Calendula	56.2 a	107.0 b	12.6 a
	No	Gazania	57.9 a	106.9 b	11.6 a
		Calendula	37.6 b	126.7 a	5.7 b
Cantate	Yes	Gazania	59.2 a	106.8 b	11.1 a
		Calendula	55.1 a	107.0 b	10.8 a
	No	Gazania	35.3 b	125.7 a	6.0 b
		Calendula	55.5 a	103.0 b	11.0 a
Significance[b]					
Cultivar (C)			NS	NS	NS
Trichoderma (T)			0.0001	0.0001	0.0001
Intercrop (I)			NS	NS	NS
C*T			NS	NS	NS
C*I			0.0001	<0.0001	0.0006
T*I			NS	NS	NS
C*T*I			<0.0001	<0.0001	0.0001

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

RESULTS

Trichoderma and intercropping effects on healthy corms

Trichoderma application enhanced sprouting by reducing the time to reach 50% sprouting in both cultivars (9.8 and 9.7 d, respectively) than plants without *Trichoderma* application (11.6 and 11.8 d, in 'Fado' and 'Cantate', respectively) (**Table 1**). Cultivars and intercrops had similar sprouting time. *Trichoderma* application and intercropping had no effect on plant height, and number of leaves plant^{-1} and averaged 44.2 cm, and 9.9, respectively (**Table 1**). Application of *Trichoderma* to the medium increased leaf chl contents as compared with no *Trichoderma*, while intercrops and cultivars were statistically similar (**Table 2**). Plants grown in *Trichoderma* added medium had higher total leaf chl contents (57.3 mg g^{-1}) than without *Trichoderma* (52.6 mg g^{-1}). Among individual treatments, 'Cantate' plants supplied with *Trichoderma* and intercropped with gazania had higher total chl contents (59.2 mg g^{-1}). Intercrops and cultivars had no effect on total leaf chl contents and averaged 52.7 mg g^{-1} for gazania, 51.1 mg g^{-1} for calendula, 52.6 mg g^{-1} for 'Fado' and 51.3 mg g^{-1} for 'Cantate'.

Trichoderma application produced an early crop while intercropping and cultivars had no effect (**Table 2**). Plants grown in medium containing *Trichoderma* were harvested after 106.2 days, while those without *Trichoderma* after 115.6 days of planting. Intercropping and cultivars had similar harvest time. Plants supplied with *Trichoderma* had higher number of florets spike^{-1} (11.6; **Table 2**), longer stem length (94.6 cm), greater stem diameter (8.3 mm), floret diameter (10.2 mm), and cormel diameter (50.8 mm) than without *Trichoderma* (8.6 florets, 74.5 cm long stems, 6.6 mm stem diameter, 7.7 mm floret diameter, and 42.6 mm cormel diameter) (**Table 3**). Among intercrops and cultivars, no statistical differences were recorded as they had similar stem length, stem diameter, floret diameter and cormel diameter. *Trichoderma* application and intercropping had no effect on leaf area and number of cormels clump^{-1} and averaged 59.1 cm^2, and 4.7, respectively (**Table 4**).

Trichoderma application had positive effect on vase life extension, reduction of disease incidence, caused by *Fusarium oxysporum*, and an increase in fresh and dry weight of individual gladiolus stems (**Table 5**). Plants grown in medium having *Trichoderma* had longer vase life (5.5 d), lower corm rot disease incidence (9%), higher fresh (69.1 g) and dry weight (6.4 g) of a stem than without *Trichoderma* (3.8 d vase life, 45% disease incidence, 50.2 g fresh weight, and 4.3 g dry weight of a stem. No differences were recorded among intercrops and cultivars regarding vase life, disease control, and fresh and dry weight of a stem.

Table 3 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy corms, on stem length, stem diameter, floret diameter and cormel diameter of cut 'Fado' and 'Cantate' gladioli. Means are an average of data from 15 plants.

Treatments			Stem length	Stem diameter	Diameter of a floret	Diameter of a
Cultivar	***Trichoderma***	**Intercrop**	**(cm)**	**(mm)**	**(mm)**	**cormel (mm)**
Fado	Yes	Gazania	93.1 a[a]	8.3 ab	10.3 a	50.3 ab
		Calendula	98.9 a	7.8 b	9.3 a	49.6 ab
	No	Gazania	95.6 a	8.2 ab	9.4 a	52.1 ab
		Calendula	55.8 b	4.8 c	5.8 b	28.4 c
Cantate	Yes	Gazania	95.5 a	8.8 a	10.7 a	47.0 b
		Calendula	90.8 a	8.3 ab	10.6 a	56.1 a
	No	Gazania	51.3 b	4.9 c	6.1 b	21.9 c
		Calendula	95.2 a	8.6 ab	9.4 a	46.8 b
Significance[b]						
Cultivar (C)			NS	NS	NS	NS
Trichoderma (T)			<0.0001	<0.0001	<0.0001	<0.0001
Intercrop (I)			NS	NS	NS	NS
C*T			NS	NS	NS	NS
C*I			0.0001	<0.0001	0.0001	<0.0001
T*I			NS	NS	NS	NS
C*T*I			<0.0001	<0.0001	0.0011	0.0001

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Table 4 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy corms, on leaf area, and number of cormels clump^{-1} of cut 'Fado' and 'Cantate' gladioli. Means are an average of data from 15 plants.

Treatments			Leaf area (cm^2)	Number of cormels clump^{-1}
Cultivars	***Trichoderma***	**Intercrop**		
Fado	Yes	Gazania	66.2	6.8 a[a]
		Calendula	64.3	4.7 b
	No	Gazania	60.1	5.6 ab
		Calendula	59.7	2.4 c
Cantate	Yes	Gazania	57.2	4.7 b
		Calendula	56.0	5.0 b
	No	Gazania	56.0	2.7 c
		Calendula	53.5	5.5 ab
Significance[b]				
Cultivar (C)			NS	NS
Trichoderma (T)			NS	NS
Intercrop (I)			NS	NS
C*T			NS	NS
C*I			NS	0.0001
T*I			NS	NS
C*T*I			NS	0.0001

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Table 5 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy corms, on vase life, disease incidence, and fresh and dry weight of a stem of cut 'Fado' and 'Cantate' gladioli. Means are an average of data from 15 plants.

Treatments			Vase life	Disease incidence	Fresh weight of a stem	Dry weight of a stem
Cultivars	***Trichoderma***	**Intercrop**	**(days)**	**(%)**	**(g)**	**(g)**
Fado	Yes	Gazania	5.5 a[a]	7 c	71.7 ab	5.8 ab
		Calendula	5.1 a	3 c	70.9 ab	6.6 ab
	No	Gazania	5.5 a	13 bc	61.3 abc	5.3 bc
		Calendula	2.3 b	76 a	45.0 cd	3.2 d
Cantate	Yes	Gazania	5.8 a	17 bc	77.2 a	7.4 a
		Calendula	5.7 a	10 bc	56.6 bc	5.7 ba
	No	Gazania	1.6 b	63 a	37.7 d	3.7 cd
		Calendula	5.7 a	27 b	57.0 bc	5.2 bc
Significance[b]						
Cultivar (C)			NS	NS	NS	NS
Trichoderma (T)			<0.0001	<0.0001	0.0006	0.0003
Intercrop (I)			NS	NS	NS	NS
C*T			NS	NS	NS	NS
C*I			<0.0001	<0.0001	NS	NS
T*I			NS	NS	NS	NS
C*T*I			<0.0001	<0.0001	0.0056	0.0020

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Table 6 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy and/or infected corms, on time to 50% sprouting, plant height and time to harvest of cut 'Fado' gladiolus. Means are an average of data from 15 plants.

Treatments			Time to 50% sprouting	Plant height	Time to harvest
Corm type	***Trichoderma***	**Intercrop**	**(days)**	**(cm)**	**(days)**
Healthy	No	No	10.7 bc[a]	45.3 a	91.7 b
	Yes	No	8.9 d	49.6 a	91.2 b
	No	Gazania	10.0 bcd	49.7 a	95.3 b
	No	Calendula	9.7 bcd	49.9 a	97.1 b
Infected	No	No	15.3 a	30.1 b	125.0 a
	Yes	No	10.0 bcd	50.6 a	99.1 b
	No	Gazania	11.3 b	47.1 a	95.6 b
	No	Calendula	9.3 cd	51.3 a	94.8 b
Significance[b]					
Corm type (C)			NS	0.0021	0.0014
Trichoderma (T)			0.0006	0.0184	0.0010
Intercrop (I)			0.0081	NS	0.0485
C*T			0.0070	NS	0.0030
C*I			0.0032	0.0076	0.0027
T*I			0.0060	0.0070	0.0001
C*T*I			<0.0001	0.0008	0.0007

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Table 7 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy and/or infected corms, on number of leaves plant^{-1}, leaf area and total leaf chlorophyll contents of cut 'Fado' gladiolus. Means are an average of data from 15 plants.

Treatments			Number of leaves plant^{-1}	Leaf area	Total leaf chlorophyll
Corm type	***Trichoderma***	**Intercrop**		**(cm^2)**	**contents (mg g^{-1})**
Healthy	No	No	11.3 a[a]	54.0 a	60.2 a
	Yes	No	11.9 a	46.9 ab	55.4 a
	No	Gazania	11.0 a	47.6 ab	56.4 a
	No	Calendula	10.8 a	54.2 a	54.0 a
Infected	No	No	5.0 b	26.7 c	22.4 b
	Yes	No	11.7 a	42.8 b	59.9 a
	No	Gazania	11.0 a	47.2 ab	58.7 a
	No	Calendula	10.5 a	46.6 ab	57.8 a
Significance[b]					
Corm type (C)			0.0268	0.0168	0.0005
Trichoderma (T)			0.0009	0.0068	0.0008
Intercrop (I)			0.0219	0.0168	0.0003
C*T			0.0012	0.0012	0.0011
C*I			0.0111	NS	0.0001
T*I			0.0118	NS	0.0001
C*T*I			0.0241	0.0208	0.0153

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Trichoderma and intercropping effects on healthy and infected corms

Trichoderma and intercropping with calendula reduced sprouting time of gladiolus, while corm type had no effect (**Table 6**). *Trichoderma* application decreased time for 50% sprouting by 5.3 d in infected and 1.8 d in healthy corms. Among intercrops, calendula had more pronounced effect on sprouting and reduced time for 50% sprouting of corms by 6.0 d in infected and 1.0 d in healthy corms. Infected corms took longer time (15.3 d) to reach 50% sprouting than healthy corms (10.7 d).

Plants supplied with *Trichoderma* had greater plant height than those without *Trichoderma*, while intercrops had similar plant height (**Table 6**). Healthy corms had greater and uniform height, while those from infected corms had less height with those without *Trichoderma* and intercropping having least (30.1 cm). Plants intercropped with calendula had comparatively greater plant height (50.6 cm) as compared with intercropped with gazania (48.4 cm), however, were statistically similar. Plants grown in a medium containing *Trichoderma* or intercropped with either gazania or calendula produced early spikes as compared with those grown from infected corms without *Trichoderma* and intercropping (**Table 6**). Among corm type, plants grown from healthy corms reduced crop time by producing early crop (93.8 d) than from infected corms (103.6 d).

Plants grown from infected corms and supplied with *Trichoderma* and/ or intercropped produced more leaves with larger area and higher total leaf chl contents than without *Trichoderma* and intercropping (**Table 7**). *Trichoderma* application in the medium around infected corms almost doubled foliage growth and total chl contents. Plants grown from infected corms without *Trichoderma* and intercropping had less number of leaves plant^{-1} (5.0), smaller area (26.7 cm^2) and less leaf chl contents (22.4 mg g^{-1}). However, plants grown from healthy corms had similar number of leaves (11.2), leaf area (50.7 cm^2) and leaf chl contents (56.5 mg g^{-1}).

Reproductive growth of gladiolus was also increased when plants grown from corms infected with *F. oxysporum* were supplied with *Trichoderma* or intercropped with gazania or calendula. Plants supplied with *Trichoderma* or intercropped with calendula or gazania had greater number of florets spike^{-1}, stem length and stem diameter than plants grown from infected corms and without *Trichoderma* or intercropping (**Table 8**). However, these treatments had no effect when plants were grown from healthy corms and averaged 10.8, 98.6 cm, and 8.6 mm for number of florets, stem length and stem diameter, respectively.

Plants raised from infected corms had longer vase life, similar with those healthy corms, when intercropped with

Table 8 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy and/or infected corms, on number of florets, stem length and stem diameter of cut 'Fado' gladiolus. Means are an average of data from 15 plants.

Treatments			**Number of florets**	**Stem length**	**Stem diameter**
Corm type	***Trichoderma***	**Intercrop**		**(cm)**	**(mm)**
Healthy	No	No	10.2 a[a]	95.5 ab	8.4 a
	Yes	No	10.6 a	98.2 ab	8.8 a
	No	Gazania	10.8 a	97.5 ab	8.5 a
	No	Calendula	11.6 a	103.4 a	8.8 a
Infected	No	No	6.8 b	57.9 c	4.9 b
	Yes	No	12.2 a	100.3 ab	8.2 a
	No	Gazania	11.0 a	91.3 b	8.0 a
	No	Calendula	11.6 a	102.0 ab	8.7 a
Significance[b]					
Corm type (C)			0.0433	0.0005	0.0061
Trichoderma (T)			0.0048	0.0004	0.0016
Intercrop (I)			0.0030	0.0154	0.0118
C*T			NS	0.0201	0.0031
C*I			0.0029	0.0009	0.0031
T*I			NS	0.0183	0.0223
C*T*I			NS	0.0001	0.0001

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Table 9 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy and/or infected corms, on vase life, disease incidence, floret diameter and cormel diameter of cut 'Fado' gladiolus. Means are an average of data from 15 plants.

Treatments			**Vase life**	**Disease incidence**	**Diameter of a floret**	**Diameter of a cormel**
Corm type	*Trichoderma*	**Intercrop**	**(days)**	**(%)**	**(mm)**	**(mm)**
Healthy	No	No	5.6 a[a]	40 b	10.2 a	51.9 ab
	Yes	No	5.4 a	10 cd	9.9 a	50.9 b
	No	Gazania	5.7 a	3 d	9.4 a	50.4 b
	No	Calendula	5.7 a	3 d	10.7 a	52.6 ab
Infected	No	No	1.6 b	77 a	5.6 b	20.3 c
	Yes	No	5.5 a	10 cd	10.6 a	50.4 b
	No	Gazania	5.9 a	23 c	10.8 a	56.8 a
	No	Calendula	6.1 a	17 cd	9.9 a	49.9 b
Significance[b]						
Corm type (C)			0.0007	0.0056	0.0003	<0.0001
Trichoderma (T)			0.0063	0.0008	0.0012	0.0001
Intercrop (I)			0.0002	<0.0001	0.0048	<0.0001
C*T			0.0033	<0.0001	0.0076	<0.0001
C*I			0.0007	0.0056	0.0478	0.0012
T*I			0.0004	NS	0.0122	<0.0001
C*T*I			0.0007	<0.0001	NS	0.0005

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

Table 10 Effect of *Trichoderma* and intercropping, either with gazania or calendula, applied to healthy and/or infected corms, on fresh and dry weight of a stem, and number of cormels clump^{-1} of cut 'Fado' gladiolus. Means are an average of data from 15 plants.

Treatments			**Fresh weight of a stem (g)**	**Dry weight of a stem (g)**	**Number of cormels clump^{-1}**
Corm type	***Trichoderma***	**Intercrop**			
Healthy	No	No	81.5 a[a]	11.1 a	5.1 a
	Yes	No	92.3 a	11.2 a	5.5 a
	No	Gazania	66.1 a	10.5 a	5.0 a
	No	Calendula	78.8 a	12.2 a	4.9 a
Infected	No	No	33.3 b	3.7 b	2.6 b
	Yes	No	87.0 a	9.9 a	5.7 a
	No	Gazania	70.2 a	8.9 a	4.4 a
	No	Calendula	83.0 a	12.3 a	5.0 a
Significance[b]					
Corm type (C)			NS	NS	NS
Trichoderma (T)			0.0060	0.0255	0.0186
Intercrop (I)			0.0170	0.0255	NS
C*T			NS	NS	NS
C*I			NS	NS	NS
T*I			NS	NS	NS
C*T*I			NS	0.0038	NS

[a] Means followed by the same letter within a column are not statistically different according to Tukey's test at $\alpha \leq 0.05$.
[b] P values were obtained using General Linear Model (GLM) procedure of STATISTICA 5.6 for significant effects of cultivars, *Trichoderma* and intercropping.
[NS] Non-significant at $P > 0.05$.

calendula or gazania or supplied with *Trichoderma* (**Table 9**). While those grown from infected corms without *Trichoderma* or intercropping had shorter vase life (1.6 d) and were of unmarketable quality (pers. obs.). *Trichoderma* and intercropping proved very effective in controlling corm rot disease irrespective of the plants grown from infected or healthy corms (**Table 9**). *Trichoderma*-treated plants had less corm rot incidence (10%) in both infected and healthy corms, while infected corms without *Trichoderma* or intercropping had 77% corm rot and plants grown from healthy corms had 40% corm rot incidence. Among plants grown from healthy corms, plants intercropped with either gazania or calendula had greater disease control (3% each disease incidence) and from infected corms had 23 and 17% disease incidence. Plants raised from infected corms without *Trichoderma* and intercropping also had smaller floret and cormel diameter (5.6 and 20.3 mm, respectively), while those intercropped and having *Trichoderma* in their growing medium had higher floret and cormel diameter, which were statistically similar to the plants grown from *Fusarium* free corms. Intercropping had no effect on fresh and dry weight of a stem, and number of cormels clump^{-1} were also similar (**Table 10**), however, *Trichoderma* had a little effect on fresh and dry weight of the stems, while corm type also affected fresh and dry weight of a stem and number of cormels clump^{-1}.

DISCUSSION

Trichoderma and intercropping effects on healthy corms

Public concerns on environmental issues and use of alternate technologies for organic cut flower production require integrated, less pesticide dependant disease management systems (Cuthbertson and Murchie 2005). Biological disease control is one of the nature-friendly, ecological approaches to overcome the side-effects of chemical plant protection (Nasir and Riazuddin 2008; Chandel and Deepika 2010). The study was conducted to compare a biological agent, *Trichoderma*, and intercropping with members of family Asteraceae. When *Trichoderma* and intercropping were used along with healthy, *Fusarium* free corms, *Trichoderma* improved growth, yield, quality as well as corm rot inhibition. However, intercropping was equally effective only when it was used along with *Trichoderma*, whereas, was not effective when used individually, without *T.* This might be due to competition of the intercrops along with gladiolus plants. Similar findings have also been reported by Riaz *et al.* (2009) who reported good corm rot disease control of gladiolus by mixed cropping of *Helianthus annuus* and *Tagates erectus* or cultivation of *Tagates erectus* and *Brassica campestris* in rotation. The two tested intercrop species, gazania and calendula, of family Asteraceae, were quite effective against the pathogen when used along with *Trichoderma* and significantly suppressed the disease incidence, mortality percentage, disease severity as well as number of diseased lesions on the corms along with improvement of the crop growth and yield. Two other Asteraceae family members, *H. annuus* and *T. erectus* suppressed corm rot disease by 74 and 43%, respectively, when planted as preceding crops to gladiolus (Riaz *et al.* 2009). *Trichoderma* also significantly suppressed corm rot disease of gladiolus. The bio-control mechanism of *T. harzianum* is a complex process mediated by the secretion of extra cellular enzymes, such as chitinases (de la Cruz *et al.* 1992), β-glucanases (Lorito *et al.* 1994) and proteinases (Geremia *et al.* 1993), as well as secondary metabolites (Sivasithamparam and Ghisalberti 1998). However, the role of *Trichoderma* antibiotics in bio-control is still not clear. Although some antibiotics may be the major factor for the bio-control activity of a certain strain, this may not be the case for others (Harman 2000). Plants which were not treated with *Trichoderma* and intercropping had greater disease incidence than treated ones. *Trichoderma* spp. has also been proved effective in controlling carnation wilt (Tramier *et al.* 1983).

Both gladiolus cultivars were positively affected by the *Trichoderma* and intercropping, however, 'Cantate' showed relatively better response towards growth as compared with 'Fado'. Among intercrops, gazania had relatively better response than calendula when used along with healthy corms for disease inhibition. *Trichoderma* treated plants inhibited corm rot disease, which was further improved by using intercrops. These results were in line with the findings of Riaz *et al*. (2009) who reported that the members of family Asteraceae, *H. annuus* and *T. erectus*, effectively suppressed the corm rot disease of gladiolus.

Trichoderma significantly reduced the disease incidence (up to 79% in 'Fado' and 63% in 'Cantate') when both *Trichoderma* and intercropping were used. These results confirmed the findings of Mishra *et al.* (2000), Nasir and Riazuddin (2008) and Chandel and Deepika (2010), who reported that biological agents are effective in controlling fusarium disease of gladiolus, and have significantly reduced disease incidence and severity on foliage and roots. These results suggested that use of *Trichoderma* in the medium is an effective biological tool for improving growth; yield, quality as well as controlling corm rot disease of gladiolus and may be used by gladiolus growers, particularly in areas susceptible to corm rot (Chandel and Deepika 2010).

Trichoderma and intercropping effects on healthy and infected corms

When *Trichoderma* or intercropping were compared by using either with healthy or infected corms, *Trichoderma* and intercropping with calendula were better as they reduced sprouting time and increased plant height. For many growth and yield indices, *Trichoderma* or intercropping with either crop, had positive effect even when applied on infected corms. Plants grown from artificially infected (inoculated with fusarium) corms had weak growth, delayed sprouting and harvest time, smaller spikes with less florets, smaller cormels and higher corm rot incidence than those supplied either with *Trichoderma* applied to the medium or intercropped with calendula or gazania. However, effect of *Trichoderma* was much more pronounced than intercropping. In this experiment, four treatments (corms) were artificially inoculated with *F. oxysporum* f. sp. gladioli (Massey), while other fours did not. *Trichoderma* application and intercropping effectively reduced corm rot disease and improved vegetative and reproductive growth of gladiolus even when used with infected corms. These results revealed that these treatments not only inhibited the corm rot incidence, but also controlled the already existing disease causing organisms (Akrami *et al*. 2011).

Intercropping was also effective in controlling corm rot disease of gladiolus with little superiority of calendula over gazania for disease control and improving reproductive growth of gladiolus, while gazania was relatively better for vegetative growth than calendula. Our results are consistent with the observation that the presence of *F. oxysporum* in the xylem increased resistance to water movement not only because of its physical presence, but also because of the apposition of elements in the vessel walls of susceptible cultivars (Nogués *et al.* 2002; Ouellette *et al.* 2004). Plants which were not treated with *Trichoderma* and intercropping had weak growth and higher disease incidence (Riaz *et al.* 2009, 2010).

The suppression of disease and improvement of vegetative and reproductive characters enhanced *Trichoderma* disease inhibitory and intercropping effects on gladiolus. These results suggested that application of *Trichoderma* and intercropping can be effectively used to control corm rot disease, to improve the growth and good quality yield of gladiolus spikes and to earn additional return from intercrops than only single main crop (Riaz *et al.* 2009; Chandel and Deepika 2010).

In summary, incorporation of *Trichoderma* in growth medium and intercropping had a positive effect on growth; yield and corm rot control of 'Fado' and 'Cantate' gladioli. Plants treated with either *Trichoderma* and/ or intercropped with either calendula or gazania had better growth and production as well as fewer corm rot than untreated. Therefore, *Trichoderma* and intercropping can be efficiently used to improve growth, productivity, quality and controlling corm rot disease of gladiolus (*gladiolus grandiflorus* L.). Moreover, profitability can also be increased by getting additional income from the intercrops.

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