Corm rot and yellows of gladiolus and its biomanagement

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Summary. A corm dressing containing *Trichoderma harzianum* (T014) and *Pseudomonas fluorescens* (PS07) cultured on a bagasse-soil-molasses mixture was tested for its efficacy against corm rot and yellows caused by *Fusarium oxysporum* f. sp. *gladioli* on the gladiolus (*Gladiolus psittacinus* L.) cv. White Prosperity (WP), King Lear (KL), Friendship (FR), Her Majesty (HM) and American Beauty (AB) in a pot culture experiment. The effectiveness of the biocontrol agents was compared with that of the fungicide carbendazim (200 ppm). All cultivars were susceptible to the pathogenic fungus and developed the characteristic symptoms of corm rot and yellows. Cultivars HM and AB were highly susceptible, scoring 2.9–3.2 on a corm rot and yellows scale (0–5 scale; compared with 1.5–2.9 for the other cultivars). Fungal infection reduced plant growth and flowering significantly, with a 15–28% decrease in the number of florets/spike. Application of carbendazim, *T. harzianum* (P=0.001) and *P. fluorescens* (P=0.05) decreased the corm rot and yellows scores and the soil population of the pathogen, and increased plant growth and flowering. The greatest improvement in the flower variables of infected plants was recorded with *P. fluorescens* (+18–31% over control). The soil population of the bioagents increased significantly over time, both in the presence and in the absence.

Key words: Fusarium oxysporum f. sp. gladioli, Trichoderma harzianum, Pseudomonas fluorescens, carbendazim, corm dressing.

Introduction

Flowers may seem merely an expression of spontaneous beauty that brightens the natural terrain, but a closer look reveals that the world of flowers is very competitive against a multitude of pathogens that threaten their economic value. Gladiolus (*Gladiolus psittacinus* L.) is a seasonal but longduration flowering plant that furnishes a stable microclimate for an array of diseases. *Fusarium oxysporum* f. sp. *gladioli* (Masey) Synder and Hans is the single most dreaded pathogen affecting gladiolus, on which it causes corm rot and yellows. The pathogen may cause as much as 60–100% damage to gladiolus depending on varietal response (Pathania and Misra, 2000).

Fusarium oxysporum f. sp. *gladioli* causes three types of rot: vascular corm rot, brown rot and basal rot (Partridge, 2003). Vascular rot is also called yellows and is characterized by a brown discoloration in the centre of the corm and extending into the flesh (Fig. 1). The foliage of plants with vascular rot gradually turns yellow from the tip downwards. When vascular rot is severe, all the foliage becomes yellow and the plant dies (Heimann and Warf, 1997). Brown rot, also called dry rot, is a nonvascular infection of the corm, characterized by thick, tan to black lesions that commonly develop near the base of corm, but may appear anywhere,

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and that extend all the way through the fleshy tissue (Fig. 2) (Partridge, 2003). Infected corm tissue remains firm but may shrivel, become scaly and have a light coloured mould cover on the surface (Heimann and Warf, 1997). Basal rot is a form of brown rot that is restricted to the base of corm (Fig. 3). This rot appears on the surface as sunken dark spots that are hard and rough and constitute the chief symptom on the corm (Fig. 2). Roots attached to the infected corms may also be brown and decayed. There may be bending or arching of



Fig. 1. Longitudinal section of healthy corm of gladiolus cv. Her Majesty, and corm infected with *Fusarium oxysporum* f. sp. *gladioli* and showing vascular rot.

leaf stalks. The flower stems may be greener than normal, crooked and curved away from the infected side of the corm. Flower petals may be darker than normal, narrow and have less ruffling along the edges. Florets are small, may be tulip shaped,



Fig. 2. Corm of gladiolus cv. American Beauty infected with *Fusarium oxysporum* f. sp. *gladioli* and showing concentric lesions of brown rot.



Fig. 3. Corm of gladiolus cv. White Prosperity infected with *Fusarium oxysporum* f. sp. *gladioli* and showing basal rot and also concentric lesions of brown rot.

and do not open as wide as normal ones (Partridge, 2003).

Gladiolus cultivation in India is largely done for commercial purposes to satisfy the great demand for flowers in the country and abroad. The government encourages growers because gladioli production can reach a 100% export rate (Kapoor, 2001). Local consumption is also economically attractive. The Indian climate favours gladiolus cultivation of high yield and quality, but frequent infection by soil or bulb-borne pathogens means that plants usually fail to produce a quality spike. Moreover, growers involved in ornamental cultivation in India are generally small and have little knowledge of disease management. Consequently, when a disease becomes severe they feel concerned and simply apply fungicides. But quite apart from the high price of chemical control agents, growers generally avoid them because of their toxic effects. The present study, therefore, investigated the feasibility of the biological control of corm rot and yellows on five commonly grown cultivars of gladiolus in India by means of bulb dressings with Pseudomonas fluorescens (Threvesan) Migula strain PSO7 and Trichoderma harzianum Rifai strain TO14 in a pot culture experiment. The effectiveness of the bioagents was compared with that of the fungicide carbendazim.

Materials and methods

Pure culture of *F. oxysporum* f. sp. *gladioli* was obtained from I.A.R.I., New Delhi, India, and cultured on sorghum seeds. Seeds of gladiolus were soaked overnight in water containing 50 g sucrose and 30 mg chloramphenicol per litre. The soaked seeds were transferred to 500-ml conical flasks and autoclaved twice at 121°C for 15 min. The flasks were inoculated with pure culture of *F. oxysporum* f. sp. *gladioli* and incubated at $25\pm2°C$ for 8 days. During incubation, the flasks were shaken daily for a few minutes and the duration of incubation, if required, was extended until there was full and uniform colonization of the seeds in the flask.

Strain PS07 of *P. fluorescens* was isolated from chickpea fields, and strain T014 of *T. harzianum* from tomato fields. Strain PS07 was tolerant to 2.275 mg l⁻¹ tetracycline per litre (maximum tolerance concentration), and strain T014 to 1.25 g metalaxyl l⁻¹. Both bioagents were cultured on a

sawdust, soil and 5% molasses mixture (3:1:1). The mixture was autoclaved in 500 ml conical flasks at 121°C for 15 min. Thereafter, the mixture was inoculated with a 6-mm PDA disc colonized by T. harzianum, or with 5 ml of an overnight pure culture broth (King's B) of P. fluorescens. The flasks were incubated at 27±2°C for 8– 10 days. During incubation, the flasks were shaken manually for a few min each day to facilitate uniform colonization by the bioagents. After incubation the CFU load of the bioagents was determined on PDA supplemented with 1.25 g metalaxyl l⁻¹ and on King's B agar supplemented with 2.275 mg l⁻¹ tetracycline using the dilution plate method. The CFU counts were $6-9 \times 10^8$ of *T. harzianum* and 5–8×10¹² of *P. fluorescens* in a 1 g sawdust-soil-molasses mixture.

Clay pots (15 cm diameter) filled with a mixture of loam soil and farmyard manure (3:1) were autoclaved at 121°C for 15 minutes. Sorghum seeds colonized by *F. oxysporum* f. sp. *gladioli* were macerated with distilled water in an electric grinder to make an inoculum suspension. A 25-ml suspension containing 4 g colonized sorghum seeds was mixed with 2 kg autoclaved soil placed in a pot.

Certified corms of G. psittacinus cv. American Beauty (AB), King Lear (KL), White Prosperity (WP), Her Majesty (HM) and Friendship (FR) were obtained from Sandeep Nusery, Lucknow, India. The papery scales covering the corms were removed. Thereafter 2% gum arabic was sprayed on the corms followed by dusting with the bioagentcolonized sawdust-soil-molasses mixture at the rate of 1 g material per corm. Another set of corms was dipped in a 200 ppm carbendazim solution for 2–3 min. Immediately after application, the corms were planted in pots at a depth three times the corm diameter. The treatments given are indicated in Table 1. Each treatment was replicated five times and the pots were arranged in a completely randomized block design in an open field (16–28°C, 57– 83% RH). The plants were grown from mid-November to mid-March. Plant height, spike length, number of florets per spike, number of spikes or corms, number of cormels, and number of corm rot and yellows infections were recorded on 4-monthold plants. The corm rot and yellows indices were determined on a 0–5 scale (0, 0% rot and yellows; 1, 1-19%; 2, 20-39%; 3, 40-59%; 4, 60-79%; 5, 80-100%). The soil population of the pathogen and the bioagents was determined at planting and at a plant age of 2 and 4 months by the dilution plate method using PDA supplemented with 1.5 g l⁻¹ pentachloronitrobenzene (PCNB) (*F. oxysporum* f. sp. *gladioli*), or with 1.25 g l⁻¹ metalaxyl (*T. harzianum*), or King's B agar supplemented with 2.37 mg l⁻¹ tetracycline (*P. fluorescens*).

Observations from plants for each treatment were averaged and are presented in Tables 1–3. The data (five replicates) were analyzed by two– factor analysis of variance (ANOVA) considering pathogen inoculation (2 treatments) as the first factor, and the control agents (5 treatments) as the second factor. The least significance difference (LSD) was calculated at three probability levels, P=0.001, 0.01 and 0.05 (Dospekhov, 1984) to identify any significant treatments.

Results

Symptoms

Soil infestation with F. oxysporum f. sp. gladioli caused the characteristic symptoms of brown rot and yellows in all plants. Plants of all cultivars except WP exhibited leaf yellowing and rot in the centre core of the corms (Fig. 1). The non-vascular corm rot (brown rot) appeared as concentric brown to black lesions on the corm surface. This is a primary symptom of the disease and it occurred on the corms of all cultivars, being most severe on the cultivar AB (Fig. 3). Corms of all cultivars from the inoculated pots showed basal rot, and the emerging roots were partially or fully rotted (Fig. 2, 4). The foliage emerging from infected corms was usually yellow, and dried at later stage, especially in cultivars AB, HM, and KL (Fig. 2). The severity of symptoms varied with the cultivar. Cultivar HM

exhibited the most severe yellows (3.2). In other cultivars the yellows index was 2.2–2.9 except in WP, where it was 1.7. The response of the cultivars to corm rot was different from their response to yellows. The highest corm rot index occurred in the cv. AB (2.9), followed in order by HM (2.6), KL (2.0), FR (1.9) and WP (1.5). Application of the various treatments decreased the severity of both yellows and corm rot (Table 1). The greatest decrease in the yellows index was in the cv. FR: -21% compared with the control. In the other cultivars P. *fluorescens* was more effective in controlling yellows, with decreases of 18% (KL), 17% (WP) and 13% (HM). Carbendazim was relatively more effective than the bioagents but the difference was not significant. Corm rot was not reduced as much by the various treatments as was yellows (Table 1). A significant decline in the rot index was achieved by carbendazim for the cv. HM (-27%) and KL (-15%). Treatment with T. harzianum and P. fluorescens reduced rot in HM by 23 and 12% respectively.

Plant growth

Pathogen-infected soil significantly reduced plant height in all cultivars except WP (Table 2). The reduction was 20% in HM (compared with the control), followed by KL (15%), AB (11%) and FR (10%). *T. harzianum* significantly decreased the growth-suppressive effect of the wilt fungus, increasing plant height of gladiolus cv. AB by 11%, of HM by 10%, and of KL by 8% over the control. *P. fluorescens* significantly increased the growth variables of the cv. AB, KL and HM (8–12%) compared with the control. Carbendazim also increased plant height in these three cultivars. The disease reduced cormel production by 10–19% (Table 2). *P. fluorescens* significantly increased

Table 1. Effect of application of *Fusarium oxysporum* f. sp. *gladioli* (Fog), *Pseudomonas fluorescens* (Pf), *Trichoderma harzianum* (Th) and carbendazim (Carb) on the yellows index and the corm rot index in 5 gladiolus cultivars.

| Treatment | Yellows / Corm rot index (0–5 scale) | | | | |
|--------------------------------------|--|---|--|---|---|
| | White Prosperity | King Lear | Friendship | Her Majesty | American Beauty |
| Fog Fog+Pf Fog+Th Fog +Carb | $1.7 / 1.5 \\ 1.6 / 1.4 \\ 1.5 / 1.5 \\ 1.5 / 1.5$ | $\begin{array}{c} 2.7 \ / \ 2.0 \\ 2.2^{\rm b} \ / \ 1.9 \\ 2.1^{\rm a} \ / \ 1.6^{\rm b} \\ 2.0^{\rm a} \ / \ 1.6^{\rm b} \end{array}$ | $\begin{array}{c} 2.9 & / 1.9 \\ 2.6^{\rm c} / 1.9 \\ 2.3^{\rm b} / 1.8 \\ 2.2^{\rm a} / 1.8 \end{array}$ | $\begin{array}{c} 3.2 \ / \ 2.6 \\ 2.8^{\rm c} / \ 2.3 \\ 2.7^{\rm b} / \ 2.0^{\rm b} \\ 2.4^{\rm a} / \ 1.9^{\rm a} \end{array}$ | 2.2 / 2.9 2.2 / 2.8 2.1 / 2.7 2.0° / 2.6 |

a,b,c Significantly different from the control at *P*=0.001 (^a), *P*=0.01 (^b), and *P*=0.05 (^c); otherwise not significant at *P*=0.05.

| Table 2. Effect of application of <i>Pseudomonas fluorescens</i> (Pf), <i>Trichoderma harzianum</i> (Th) and carbendazim (Carb) |
|---|
| on plant height and number of cormels of gladiolus cultivars grown in the soil with or without Fusarium oxysporum |
| f. sp. gladioli (Fog). |

| Treatment | Plant height (cm) / No. of cormels per plant | | | | |
|-----------|--|-----------------------|-----------------------------|---------------------|-----------------------------|
| | White Prosperity | King Lear | Friendship | Her Majesty | American Beauty |
| Control | 127 / 223 | 148 / 176 | 114 / 201 | 118 / 67 | 136 / 121 |
| Pf | 135 / 236 | 156 / 188 | 120 / 201° | 123 / 76 | $184 / 149^{a}$ |
| Th | 136 / 221 | 151 / 184 | 120 / 208 | 123 / 72 | 138 /137° |
| Carb | 130 / 220 | 150 / 177 | 117 / 216 | 117 / 70 | 134 / 120 |
| Fog | 120° / 219 | $125^{\rm a} / 148$ | $102^{ m c}$ / $180^{ m c}$ | $95^{ m b}/54$ | $114^{ m b}$ / 105 |
| Fog+Pf | $129^{ m c}/209$ | $131^{ m b}$ / 155 | 107 / 188 | $107^{ m c}/63$ | $127^{ m c}$ / $118^{ m c}$ |
| Fog+Th | 128 / 212 | $137^{ m c}/151$ | 110° / 185 | $105^{ m c} / 61$ | 131° / 112 |
| Fog +Carb | 122 / 214 | 136° / 150 | 110° / 186 | $105^{\circ}/62$ | 125° / 110 |

a,b,c, Significantly different from the control at *P*=0.001 (^a), *P*=0.01 (^b) and *P*=0.05 (^C); otherwise not significant at *P*=0.05.

(P=0.05) the number of cormels per plant in the cultivars AB and HM over the control (P=0.05), and both *T. harzianum* and carbendazim significantly reduced the number of cormels per plant in the cv. HM.

Infection with *F. oxysporum* f. sp. gladioli significantly reduced spike length and the number of florets and spikes per corm compared with the control (Table 3). The greatest decrease was in the number of florets per spike (18–31%) in the cultivars HM and AB. The treatments improved the flowering variables of fungus-infected plants. The spike length of the cultivars AB, KL and HM increased significantly (P=0.05) with application of all control agents. Application of *P. fluorescens* significantly improved the number of florets per spike in cultivars AB (P=0.001) and FR (P=0.01) over the control. The increase in florets due to *T. harzianum* was significant at P=0.001 with KL and HM, and at P=0.05 with WP and AB. An increase of 33% in the number of spikes per plant (P=0.001) was recorded in the cv. HM with all three treatments. In the cultivars KL and HM the increase in the number of spikes was significant (P=0.05) with carbendazim only, although P. *fluorescens* also increased the number of spikes in cv. HM. Some of the flower variables of the gladiolus cultivars (but not WP) that were not inoculated with the rot fungus were significantly improved by treatment with P. *fluorescens* (Table 3).

Table 3. Effect of application of *Pseudomonas fluorescens* (Pf), *Trichoderma harzianum* (Th) and carbendazim (Carb) on flowering variables of gladiolus cultivars grown in the soil with or without *Fusarium oxysporum* f. sp. *gladioli* (Fog).

| Treatment | Spike length (cm) / No. of florets / No. of spikes | | | | | |
|-----------|--|---|--|---|--|--|
| | White Prosperity | King Lear | Friendship | Her Majesty | American Beauty | |
| Control | 99 / 13 / 2.6 | 129 / 18 / 2.1 | 96 / 17 / 2.0 | 110 / 19 / 2.4 | 88 / 18 / 7.7 | |
| Pf | 106 / 14 / 2.8 | 116 / $21^{ m b}$ / 2.2 | 101° / 18 / 2.0 | $116/23^{ m b}/3.0$ | $89/23^{ m a}\!/2.0^{ m b}$ | |
| Th | 103 / 14 / 2.7 | 114/19/2.1 | 100 / 18 / 2.0 | 110 / $21^{ m c}$ / 2.5 | $90 / 21^{ m b} \! / 1.9^{ m c}$ | |
| Carb | 102 / 14 / 2.6 | 114 / 19 / 2.0 | $97 / 17 / 8.0^{\circ}$ | 112 / 20 / 2.3 | 87 / 19 / 1.7 | |
| Fog | 92 / $11^{ m c}$ / 2.3 | $94^{ m c}$ / 14 / $1.8^{ m c}$ | $86^{ m c}$ / $14^{ m b}$ / $2.2^{ m c}$ | $89^{ m b}$ / $13^{ m a}$ / $1.8^{ m b}$ | 78 / 13 / 1.5° | |
| Fog+Pf | $98 / 13^{ m a} / 2.3$ | $103^{ m c}/19^{ m a}/1.9$ | $92/17^{ m b}/2.3$ | $99^{ m c}$ / $16^{ m a}$ / $2.0^{ m c}$ | $85^{ m c}$ / $17^{ m a}$ / $2.0^{ m a}$ | |
| Fog+Th | 99 / $12^{ m c}$ / 2.4 | $103^{ m c}$ / $18^{ m a}$ / 1.9 | 93 / 16° / 2.3 | $98^{\circ}/13/1.9$ | $87^{ m c}$ / $15^{ m c}$ / $2.0^{ m a}$ | |
| Fog +Carb | 100° / 12 / 2.3 | $103^{ m c}$ / $18^{ m a}$ / $2.0^{ m c}$ | $92/17^{ m b}/2.3$ | $98^{\rm c}$ / $16^{\rm a}$ / $2.0^{\rm c}$ | $87^{\circ}/17^{a}/2.0^{a}$ | |

^{a,b,c} Significantly different from the control at *P*=0.001 (^a), *P*=0.01 (^b) and *P*=0.05 (^c), otherwise not significant at *P*=0.05.

| Treatment - | Colony forming units per g soil | | | | | |
|-------------|---------------------------------|------------------------------|--------------------------------|------------------------------|-------------------------------|--|
| | White Prosperity | King Lear | Friendship | Her Majesty | American Beauty | |
| Control | | | | | | |
| Planting | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | |
| 2 months | $5.9{	imes}10^{ m 4c}$ | $9.8	imes10^{4\mathrm{b}}$ | $8.0 	imes 10^{4 \mathrm{b}}$ | $1.6{	imes}10^{5\mathrm{a}}$ | $2.7{	imes}10^{5\mathrm{a}}$ | |
| 4 months | $7.3{	imes}10^{ m 4c}$ | $2.7{	imes}10^{5{ m a}}$ | $1.6{	imes}10^{5\mathrm{a}}$ | $5.9{	imes}10^{5\mathrm{a}}$ | $9.4{	imes}10^{5\mathrm{a}}$ | |
| Pf | | | | | | |
| Planting | $3.1{	imes}10^4$ | $3.1{	imes}10^4$ | $3.1{	imes}10^4$ | $3.1{	imes}10^4$ | $3.1{	imes}10^4$ | |
| 2 months | $5.1{	imes}10^4$ | $7.2{	imes}10^{4	ext{c}}$ | $7.5{	imes}10^4$ | $9.7{	imes}10^{4	ext{c}}$ | $1.5{	imes}10^{5{ m c}}$ | |
| 4 months | $5.6{	imes}10^{ m 4c}$ | $1.1{	imes}10^{5	ext{c}}$ | $9.9{	imes}10^{4	ext{c}}$ | $2.4{	imes}10^{5	ext{c}}$ | $3.2{	imes}10^{5{ m c}}$ | |
| Th | | | | | | |
| Planting | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | $3.2{	imes}10^4$ | |
| 2 months | $4.7{	imes}10^{ m 4c}$ | $8.9 	imes 10^{4 	ext{ b}}$ | $7.1{	imes}10^{4	ext{c}}$ | $8.8	imes10^{4\mathrm{b}}$ | $9.4{	imes}10^{4\mathrm{a}}$ | |
| 4 months | $5.2{	imes}10^{ m 4c}$ | $9.5{	imes}10^{4	ext{c}}$ | $8.6	imes10^{4\mathrm{b}}$ | $1.9{	imes}10^{5\mathrm{b}}$ | $2.6{	imes}10^{5{	ext{a}}}$ | |
| Carb | | | | | | |
| Planting | $3.0{	imes}10^4$ | $3.0{	imes}10^4$ | $3.0{	imes}10^4$ | $3.0{	imes}10^4$ | $3.0{	imes}10^4$ | |
| 2 months | | $5.2{	imes}10^{4{ m b}}$ | $6.2{	imes}10^{4\mathrm{a}}$ | $8.4{	imes}10^{4\mathrm{a}}$ | $7.8{	imes}10^{4\mathrm{a}}$ | |
| 4 months | | $7.3{	imes}10^{4\mathrm{b}}$ | $8.2{	imes}10^{4\mathrm{b}}$ | $9.3 \times 10^{4 a}$ | $1.1{	imes}10^{5{	extsf{a}}}$ | |

Table 4. Populations of *Fusarium oxysporum* f. sp. *gladioli* (Fog) in relation to application of *Trichoderma harzianum* (Th), *Pseudomonas fluorescens* (Pf) or carbendazim (Carb) in the rhizosphere of gladiolus cultivars

^{a,b,c,} Significantly different from the planting population at *P*=0.001 (^a), *P*=0.01(^b) and *P*=0.05(^c), otherwise not significant at *P*=0.05.

Rhizosphere population of wilt fungus and bioagents

In the rhizosphere of all cultivars except WP the 2- and 4-month-old populations of *F. oxysporum* f. sp. *gladioli* increased (P=0.001 or P=0.01) over time in comparison to the planting popula-

tion. In WP this increase was significant at P=0.05 (Table 4). Application of carbendazim caused the greatest decline in the CFUs of the wilt fungus per g soil in the rhizosphere of the cultivars AB, HM and FR (P=0.001), and of KL and WP (P=0.01) com-

Table 5. Populations of *Pseudomonas fluorescens* (Pf) and *Trichoderma harzianum* (Th) in the rhizosphere of gladiolus cultivars grown in the soil with or without *Fusarium oxysporum* f. sp. *gladioli*.

| Treatment | Colony forming units per g soil | | | | | |
|-----------|---------------------------------|------------------------------|------------------------|---------------------------|------------------------|--|
| | White Prosperity | King Lear | Friendship | Her Majesty | American Beauty | |
| Pf | | | | | | |
| 2 months | $5.2{	imes}10^4$ | $5.1{	imes}10^4$ | $5.3{	imes}10^4$ | $5.0{	imes}10^4$ | $5.2{	imes}10^4$ | |
| 4 months | $8.6{	imes}10^{5\mathrm{b}}$ | $8.1{	imes}10^{5\mathrm{b}}$ | $8.4{	imes}10^{ m 5b}$ | $8.8{	imes}10^{5b}$ | $8.7{	imes}10^{ m 5b}$ | |
| Th | | | | | | |
| 2 months | $3.9{	imes}10^{3}$ | $3.8{	imes}10^3$ | $3.9{	imes}10^{3}$ | $4.0 	imes 10^{3}$ | $3.9{	imes}10^{3}$ | |
| 4 months | $4.1{	imes}10^{4	ext{b}}$ | $4.3{	imes}10^{4{ m b}}$ | $4.3{	imes}10^{ m 4b}$ | $4.4{	imes}10^{ m 4b}$ | $4.0{	imes}10^{ m 4b}$ | |
| Pf +Fog | | | | | | |
| 2 months | $8.1{	imes}10^4$ | $1.1{	imes}10^5$ | $9.8{	imes}10^4$ | $3.5{	imes}10^5$ | $7.4{	imes}10^5$ | |
| 4 months | $2.6{	imes}10^{ m 6b}$ | $8.7{	imes}10^{ m 6b}$ | $6.6{	imes}10^{ m 6b}$ | $1.4{	imes}10^{ m 6c}$ | $5.9{	imes}10^{ m 6b}$ | |
| Th +Fog | | | | | | |
| 2 months | $9.3{	imes}10^3$ | $2.7{	imes}10^4$ | $2.1{	imes}10^4$ | $7.5{	imes}10^4$ | $4.5{	imes}10^5$ | |
| 4 months | $1.0 	imes 10^{5a}$ | $9.3{	imes}10^{4a}$ | 9.2×10^{4a} | $2.9{	imes}10^{6	ext{a}}$ | $8.7{	imes}10^{6a}$ | |

^{a,b,c} Significantly different from the control or planting population at P=0.001 (^a), P=0.01(^b) and P=0.05(^c); otherwise not significant at P<0.05.

pared to the 2- and 4-month-old controls. The decrease in the population of the wilt fungus due to treatment with T. harzianum was usually significant at P=0.01, except in AB (P=0.001) and WP (P=0.05). The effect of P. fluorescens treatment was significant at P=0.05 as compared with the planting populations (Table 4). The harvest population of the bioagents in the absence of the pathogenic fungus increased significantly (P=0.01) as compared with the 2-month-old population (Table 5). In the presence of *F. oxysporum* f. sp. gladioli, the 4-month-old populations of T. harzianum and P. *fluorescens* increased significantly (P=0.001, and P=0.01 or 0.05 respectively), as compared to the 2month-old population in the rhizosphere of all cultivars (Table 5).

Discussion

All the cultivars of gladiolus tested exhibited susceptibility to infection with *F. oxysporum* f. sp. *gladioli* in the order HM>KL>AB>WP, and developed the characteristic symptoms of corm rot and yellows. Corms of all cultivars showed brown to black concentric lesions and the subsequent yellowing of the leaves that are the primary symptoms of the disease (Moorman, 1998).

The control treatments considerably decreased the severity of corm rot and yellows, and improved subsequent plant growth and flowering. P. fluorescens was not as effective as T. harzianum or carbendazim in controlling infection, but it was more effective in enhancing plant flowering. P. fluorescens is basically a plant growth promoter and improves plant growth and yield through the solubilization of phosphates (Kloepper, 1992; Alabouvette et al., 1993) or the production of phytohormones (Glick, 1995). For this reason it increased some of the flowering variables of most of the cultivars not inoculated with the wilt fungus in our test. The growth stimulatory effects of *P. fluores*cens have also been reported on tomato (Khan and Khan, 2002). The strains of P. fluorescens used are also reported to have fungicidal effects (Khan et al., 2004) by producing antibiotics, such as phenazin (Toohey et al., 1965), pyrolnintrin (Burkhead et al., 1994) and phloroglucinol (Mazzola et al., 2002), and also siderophores (Perez et al., 2001). This may suggest that the promotion of plant growth and flowering that *P. fluorescens* brought about in gladiolus cultivars infected with the wilt fungus was due in part to plant growth promotion and in part to disease suppression. The data on the soil population of the wilt fungus support this explanation, since the decrease in *F. oxysporum* f. sp. *gladioli* CFUs produced with *P. fluorescens* was less than that produced with *T. harzianum* or carbendazim.

Trichoderma harzianum is an established mycoparasite of F. oxysporum (Papavizas, 1988), and its suppressive effects on other soilborne fungal pathogens are also well documented (Leben et al., 1987; De and Mukhopadhayay, 1994; Khan and Gupta, 1998). T. harzianum also suppressed F. oxysporum f. sp. gladioli, as shown by the significant decrease in the soil population of the wilt fungus and in the corm rot index achieved with T. harzianum treatment. Carbendazim is an effective fungicide against soilborne diseases (Satija and Hooda, 1987; Nene and Thapliyal, 1993) and it controlled corm rot and yellows in the gladiolus cultivars. The fact that the greatest decrease in the rhizosphere population of the wilt fungus was due to carbendazim, and also the rot and yellows scores recorded with this control agent, indicate that it was more effective than T. harzianum in controlling wilt infection in gladiolus.

The study demonstrated that *F. oxysporum* f. sp. *gladioli* suppresses flowering in gladiolus cultivars by 15–28%. Application of *P. fluorescens*, however, can compensate for this damage, leading to an 18–31% increase in the flowering. The application of this bacterium on corms is a handy and cost-effective means of treatment.

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