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Full Length Research Paper

Management of *Fusarium* corm rot of gladiolus (*Gladiolus grandiflorus* sect. Blandus cv. Aarti) by using leaves of allelopathic plants

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Two pot experiments were conducted to investigate the potential of different plant materials to manage the corm rot disease of gladiolus (*Gladiolus grandiflorus* sect. Blandus cv. Aarti) caused by *Fusarium oxysporum* f.sp*. gladioli* (Massey) Snyd. & Hans*.* In the first experiment, leaves of five allelopathic plant species viz. *Eucalyptus citriodora* Hook*., Syzygium cumini* (L.) Skeels*., Coronopus didymus* (L.) Smith*., Chenopodium album* L. and *Cyperus rotundus* L. were incorporated in the soil at 2, 4 and 6 g 100 g^{-1} of soil. In the second experiment, leaves of five plant species namely *Azadirachta indica* A. Juss*., Alstonia scholaris* (L.) R. Br*., Parthenium hysterophorus* L., *Ageratum conyzoides* L. and *Allium cepa* L. were spread on the surface of the pot soil at 4 g 100 g^{-1} of soil. All the leaf incorporation and spreading treatments significantly reduced the disease incidence and number of infection lesions on corms. Incorporation of all the dosages of 2 – 4% of *C. rotundus* significantly enhanced shoot biomass. Similarly 2% *E. citriodora* and 4 – 6% *C. album* incorporation also enhance shoot biomass significantly over *Fusarium* control. All the leaf spreading treatments significantly enhanced shoot length and biomass. The present study concludes that corm rot disease of gladiolus can be effectively managed by using allelopathic plants.

Key words: Allelopathic plants, corm-rot, disease management, *Fusarium oxysporum, Gladiolus grandiflorus.*

INTRODUCTION

Gladiolus hybrids are among the preferred cut flowers due to their different sizes, shades, and excellent vase life (Bose et al., 2003). It is native of South Africa and has been cultivated globally. Fusarium rot is one of the most serious diseases of *Gladiolus*, affecting plants in the field and corms in storage. Corm rot is also called "yellows" on infected plants in the field. The causal organism is *Fusarium oxysporum* Schlecht. f. sp. *gladioli* (L. Masey) Snyder & Hans., which deteriorates its quality and market value (Armitage, 1993; Remotti et al., 1997; Chandel and Bhardwaj, 2000). The fungus survives in infected corms and in the soil as mycelium, chlamydospores, macro-conidia and microconidia. The infected corms show brownish to black dry rot symptoms. Foliage of affected plants first turns yellow and then brown. Infected roots remain small and are gradually killed. Despite many attempts to control this disease, the problem is still widespread (Roebroeck and Mes, 1992).

Soil fumigation is the most common approach to control soil-borne diseases for many years. Unfortunately, certain fumigants possess negative attributes, such as health hazards, environmental pollution, and even potential atmospheric ozone depletion (Gamliel et al., 2000). Increased environmental concern has been a major factor in triggering regulatory restrictions on the use of soil fumigants. In many countries, the use of fumigants such as 1,2-dibromochloropropane and ethylene dibromide has been discontinued or suspended, and a phase-out of methyl bromide, which is the most widely used soil fumigant, is underway (Gamliel et al., 2000; Pavlou and Vakalounakis, 2005). Furthermore, the high cost associated with the use of fungicides is a limiting factor in the profitability of the production (Partridge et al., 2006).

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For more sustainable, eco-friendly integrated disease management strategies, there is a growing trend toward the search for alternatives to chemical pesticides, which are less pesticide dependant or based on naturally occurring compounds (Singh et al., 2003; Cuthbertson and Murchie, 2005). One such alternative for protecting crops from diseases is biocontrol with extracts and residue of allelopathic plants (Javaid and Amin, 2009; Riaz et al., 2009a, b). Soil organic amendments are known to affect soil aeration, structure, drainage, moisture holding capacity, nutrient availability and microbial ecology (Davey, 1996). These practices influence pathogen viability and distribution, nutrient availability and the release of biologically active substances from both crop residues and soil microorganisms. Developing disease suppressive soils by introducing allelopathic crop residue management takes time, but the benefits accumulate across successive years by increasing beneficial soil microorganism population and structure (Huang et al., 2006). Many plant diseases viz. *Fusarium* crown and root rot in tomato (Benhamou and Theriault, 1994), *Verticillium* wilt of cotton (Huang et al., 2006), *Phytophthora* root rot of alfalfa (Wiggins and Kinkel, 2005), and *Fsarium* root and stem rot of cucumber (Pavlou and Vakalounakis, 2005) have been controlled with the incorporation of plant materials to the soil. The purpose of the present pot study is to evaluate the effects of different allelopathic plant's leaf materials, to be used either as soil incorporation or spreading on the soil surface, for the management of corm rot of gladiolus [*Gladiolus grandiflorus* var. Aarti] caused by *F. oxysporum* f.sp. *gladioli*.

MATERIALS AND METHODS

Soil analysis

Field soil used in the experiment was collected from the experimental station of the Mycology and Plant Pathology Department (MPPL), University of the Punjab, Lahore, Pakistan. The city of Lahore is located on latitude 31.57 N and longitude 74.31 E. The EC of the soil was 0.14 .S m^{-1} and its pH was 7.8 (Soil Testing Laboratories. Lahore, Pakistan). The amount of organic matter was 0.69% and quantities of N was 0.05%, and that of P and K were 6.3 and 100 mg kg^{-1}, respectively. The concentrations of micronutrients viz. boron, manganese, iron; copper and zinc were 1.06, 22.8, 10.8, 1.9 and 1.3 mg kg^{-1}, respectively, in the soil sample. No additional chemical fertilizers were added to the soil in the present experiment.

Preparation of inoculum

Mass inoculum of different *F. oxysporum* f.sp. *gladioli* was raised on chickpea (*Cicer arieinum* L.). Healthy chickpea seeds were boiled for 45 min and packed in transparent polythene bags (40 x 28 cm) whose open ends were passed through a plastic pipe of 6 with 3 cm diameter plugged with cotton. Each bag containing 250 g of boiled chickpeas was autoclaved at 121°C under 1.035 $\times 10^5$ Pa pressure for 30 min and cooled at room temperature. A 5 mm disc from periphery of actively growing seven days old *F. oxysporum* f.sp. *gladioli* culture was introduced into each bag under aseptic conditions. These bags were kept at room temperature in the dark for 8 days. After 8 days, all chickpea bags were completely filled with the fungal growth. These bags were maintained at 4°C until used in the further studies.

Experiment 1: Incorporation of leaves of allelopathic plants

Experiment was conducted in plastic pots of 7.5 cm diameter and 12 cm deep each containing one 1 kg soil. Five putative allelopathic plants (*Eucalyptus citriodora*, *Syzygium cumini, Coronopus didymus, Chenopodium album* and *Cyperus rotundus*) were selected for the experiment. Fresh leaves of these plants were cut into small pieces of about 1 cm and mixed in the pot soil at 2, 4 and 6 g 100 g^{-1} of soil. Pots were watered and left for 15 days to allow decomposition of the plant materials. Four grams of *F. oxysporum* f.sp. *gladioli* inoculum was thoroughly mixed in each pot three days prior to sowing. Control treatment was without *F. oxysporum* inoculation and leaf materials incorporation.

Gladiolus corms of uniform size were sown in each pot 15 days after leaf mixing and three days after *Fusarium* inoculation. One corm was sown in each pot. Each treatment was replicated three times. There were 10 pots in each replicate. Pots were arranged in a completely randomized manner on a bench in a green house. Plants were harvested 65 days after sowing and data regarding length and dry weight of both root and shoot was recorded. Data regarding disease incidence and mortality were recorded as follows:

$$\text{Disease incidence } (\%) = \frac{\text{No. of diseased plants}}{\text{Total No. of plants}} \text{ x } 100$$

$$\text{Mortality}(\%) = \frac{\text{No. of plants died due to disease}}{\text{Total No. of plants}} \text{ x } 100$$

Disease severity was assessed with a 0 – 3 visual scale, where 0 = no symptoms, 1 = yellowing of leaves, 2 = drying of leaves, and 3 = dead or almost dead plants.

Experiment 2: Spreading of leaves on soil surface

Fresh leaves of *Melia azadirechta*, *Alstonia shoraris* (trees), *Parthenium hysterophorus, Ageratum conyzoides* (weeds) and *Allim cepa* (crop) were collected from University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan. Leaves were thoroughly washed with tap water and were cut into 1 cm pieces. Fresh plant materials were left on the surface of the pot soil at 4 g 100 g^{-1} of soil. Ground water was added and materials were left to allow the release of allelochemicals. After two weeks, chickpea based *Fusarium* inoculum was added into the soil at 4 g 100 g^{-1} of soil. Sowing of corms and data regarding various vegetative and disease parameters were done as in experiment 1.

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test ($P \leq 0.05$) to separate the treatment means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Effect of leaf incorporation on corm-rot disease

Highest disease incidence of 80% with maximum disease

Table 1. Effect of incorporation of green manures of allelopathic plants on growth and fusarium rot disease of *Gladiolus grandiflorus.*

Treatments	Dosage g/100 g of soil	Days to sprout	Shoot length (cm)	Shoot dry wt. (g)	Root length (cm)	Root dry wt. (g)	No. of roots	Disease incidence (%)	Mortality (%)	Disease rating	No. of lesions/ corm
Control	0	21ab	96.3a	23ab	27a	0.08a	160a	0.0c	0.0c	0	0.0f
Fusarium oxysporum	0	18a-d	68c	1.17e	10.2d	0.012e	74ef	80a	16.6a	2-3	33a
Eucalyptus citriodora	2	14d	84.6b	2.36a	17.8c	0.046bc	94c-e	30b	0.0c	1-2	27ab
	4	16b-d	78b	1.4d-e	14.3cd	0.04cd	98c-e	23.3bc	0.0c	0-1	22bc
	6	17a-d	80.5b	1.8a-d	23.1ab	0.063ab	136ab	13.3bc	3.3bc	0-2	9e
Syzygium cumini	2	17a-d	75bc	1.56c-e	14.1cd	0.033c-e	90d-f	27b	0.0c	0-2	13d-e
	4	15cd	80b	1.66c-e	14.1cd	0.02de	118bc	13.3bc	0.0c	1-2	16c-e
	6	15cd	82.8b	1.77a-e	18.9bc	0.04cd	83.3ef	13.3bc	0.0c	1-2	14c-e
Coronopus didymus	2	16b-d	79.8b	1.7b-e	15.2cd	0.046bc	87d-f	23.3bc	3.3bc	1-2	14c-e
	4	14d	82.6b	1.9a-d	13.7cd	0.033c-e	98.3c-e	10.0bc	3.3bc	1-3	14c-e
	6	20abc	84.3b	1.53c-e	15.3cd	0.04cd	74ef	13.3bc	0.0c	0-2	14c-e
Chenopodium album	2	20abc	74.1bc	1.6c-e	14.5cd	0.036cd	64f	26.6b	0.0c	0-1	19cd
	4	17a-d	82.8b	2.13a-c	16.2c	0.046bc	73ef	7bc	16.6a	0-2	10e
	6	16b-d	80.4b	1.83a-d	13.8cd	0.033c-e	96c-e	13.3bc	0.0c	0-2	14c-e
Cyperus rotundus	2	15cd	83.4b	2.0a-d	15.5cd	0.04cd	112b-d	26.6b	10ab	1-3	13de
	4	19a-d	80.9b	2.0a-d	14.8cd	0.03c-e	93c-e	20bc	0.0c	0-1	13de
	6	22a	79.1b	2.13a-c	15.5cd	0.03c-e	111b-d	10bc	0.0c	1-2	12de

In each column, values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

severity of 2 - 3 rating was recorded in *Fusarium* control as compared to 0% disease incidence in un-inoculated control. Highest mortality of 16.6% and maximum number of infection lesions on corms (33 corm^{-1}) were also recorded in this treatment. Incorporation of leaves of different plant species managed the disease to variable extents. In *E. citriodora* leaf incorporation treatments, disease incidence was 13 – 30% that was significantly lower than *Fusarium* control. Mortality was reduced to 0 – 3.3%, disease severity to 0 – 2 and infection lesions to 9 – 27 corm^{-1}. Earlier**,** Fiori et al. (2000) reported that crude extracts of *E. citriodora* were very effective in suppressing the growth of fungus *Didymella bryoniae.* Essential oils are considered responsible for fungistatic activity of *E. citriodora* (Fiori et al., 2000; Ramezani et al., 2002).

Different concentrations of *S. cumini* leaf incurporation significantly suppressed disease incidence to 13**-**27% as compared to 80% in *Fusarium* control. Mortality was significantly reduced to 0%. Disease severity was also low as compared to *Fusarium* control. Disease lesions on corms significantly declined to 13 – 15 corm^{-1} in different leaf incorporation treatments (Table 1). Earlier**,** Chandrasekaran and Venkatesalu (2004) reported that aqueous seed extract of *S. cumini* was very effective against certain other fungal species viz. *Candida albicans*, *Aspergillus flavus* and *Aspergillus fumigatus*, *Aspergillus niger*.

The effect of *C. didymus* leaf incorporation on disease suppression was also significant. Different doses of this cruciferous weed species reduced disease incidence to 13 – 23%, mortality to 0 – 3.3%, disease severity to 0 – 3 rating and infection

lesions on corms to 13 – 14 corm^{-1} (Table 1). *C. didymus* is a cruciferous (Brassicaceae) weed. Control of plant pathogens by crucifers is attributed to their production of glucosinolates (Lewis and Papavizas, 1971). Glucosinolates are sulfur compounds. Allyl glucosinolate is one of the predominant glucosinolates in many brassicaceous species. In soil, this compound is hydrolyzed into allyl isothiocyanate, a volatile compound that is as toxic to fungi as methyl isothiocyanate, an active ingredient in commercial soil fumigants (Lewis and Papavizas, 1971; Vaughn et al., 1993; Mayton et al., 1996).

Disease suppressive effect of *C. album* was also significant as compared to *Fusarium* control. Disease incidence of only 6.6 – 26%, mortality 0 – 16.6%, disease severity 0 – 2 rating and number of infection lesions 10 – 19 corm^{-1} were recorded in different leaf incorporation treatments of this weed (Table 1). Shafique et al. (2006) have reported 60% reduction in incidence of *Alternaria alternata* on wheat due to aqueous leaf extract of *C. album.* Tahara et al. (1994) isolated a highly fungitoxic metabolite mucondialdehyde (trans-2, trans-4-hexa-dienedial) from leaves of this species. Some other compounds have also been isolated from different parts of this species viz. betalain alkaloids, phenolic acids in fruits, betain and oxalic acid in leaves (Hegnauer and Hegnauer, 1964), and oleanolic acid and sitosterol in flowers (Nicholas et al., 1955), which could be responsible for antifungal activity against *F. oxysporum*.

Different doses of *C. rotundus* leaf incorporation significantly reduced disease incidence to 10 – 26%, mortality to 0 – 10%, disease severity to 0 – 3 rating and number of infection lesions to 12 – 13 corm^{-1} (Table 1). The antifungal effects of *C. rotundus* leaf incorporation may be attributed to the presence of phenolic substances in leaves of this weed species. Quayyum et al. (2000) have isolated 19 phenolic compounds in leaves and tubers of *C. rotundus*.

Effect of leaf incorporation on plant growth

Fusarium inoculation significantly suppressed length as well as dry weight of root and shoot. Incorporation of leaves of different plant species variably alleviate the biotic stress of *Fusarium* and enhanced root and shoot growth of gladiolus. All the dosages of *E. citriodora, C. didymus* and *C. rotundus* significantly enhanced shoot length over *Fusarium* control. The effect of lower dosages of 2% of *C. album* and *S. cumini* was not significant while higher dosages of 4 and 6% significantly enhanced shoot length (Table 1). Response of shoot dry weight to different leaf incorporation treatments under *Fusarium* stress was different than that of shoot length. The effect of 2% *E. citriodora* leaf incorporation was more pronounced than higher concentrations of 4 and 6%. In case of *C. didymus* and *C. album,* 4% leaf incorporation was found to be the most effective in improving shoot dry weight. In contrast, in the case of *S. cumini* and *C. rotundus,* there was a gradual increase in shoot biomass as the leaf incorporation dosage was increased from 2 – 6% (Table 1). The variable response of shoot dry weight production to different leaf incorporation treatments could be attributed to different nature of allelochemicals present in different test allelopathic plant species.

Although all the leaf incorporation treatments enhanced root length, however, effect varied with the test plant species. All the dosages of *C. rotundus* and *C. didymus* exhibited insignificant effect in improving the studied parameter. By contrast, the effect of 6% leaf incorporation of *E. citriodora* and *S. cumini,* and 4% that of *C. album* was significant (Table 1). Similar to that of shoot dry weight, response of root dry weight to different leaf incorporation treatments was variable. The effect of *E. citriodora* was most pronounced where all the dosages of leaf incorporation significantly increased root dry weight over *Fusarium* control (Table 1).

Effect of leaf spreading on corm-rot disease

No disease symptoms were recorded in negative control treatment. Highest disease incidence of 47% was recorded in *Fusarium* inoculated (positive of *Fusarium* control) treatment. Similarly, maximum number of infection lesions on corm (51corm^{-1}) was recorded in this treatment. Spreading of leaves of all the five allelopathic test plant species significantly reduced disease incidence as well as number of infection lesions on corms. Lowest disease incidence of 20% was recorded in treatment where *A. scholaris* leaves were used followed by 23% in *A. cepa* and *P. hysterophorus* leaf spreading treatments (Table 2). Various indole alkaloids have been identified in leaves of *A. scholaris* (Macabeo et al., 2005), which might be responsible for antifungal activity against *F. oxysporum*. In the present study, mortality percentage due to *Fusarium* inoculation was low, that is, only 6.6%. Spreading of different plant materials exhibited insignificant effect on this parameter. The effect of spreading leaves of different plant species on disease severity was variable (Table 2).

Effect of leaf spreading on plant growth

The effect of *Fusarium* inoculation as well as spreading of leaves of different plant species on days to sprouting of corms was insignificant (Table 2). All the five test plant species significantly enhanced shoot length and dry weight as compared to *Fusarium* control. Highest stimulatory effect on shoot length and dry weight was recorded due to *A. scholaris* and *A. cepa*, respectively. Spreading of leaves of *A. indica, A. scholaris* and *A. cepa* significantly enhanced root length over *Fusarium* control. However, root dry weight was significantly enhanced only

Table 2. Effect of mulching of various crops on growth and *Fusarium* rot disease of *Gladiolus grandiflorus* in pot cultures.

Treatments	Days to sprout	Shoot length (cm)	Shoot dry wt. (g)	Root length (cm)	Root dry wt. (g)	No. of roots	Disease incidence (%)	Mortality (%)	Disease rating	No. of lesions/ corm
Control	20a	96.3a	2.6ab	31.9a	0.1a	177a	0c	0.00a	0	0d
Fusarium oxysporum	18a	74.7e	0.8d	14c	0.007de	86.6c	47a	6.6a	0-1	51a
Azadirachta indica	19a	87.5bc	1.9bc	33.6a	0.05bc	165a	30b	6.6a	0-2	17.3bc
Alstonia scholaris	17a	89.4b	2.5ab	26.2b	0.06b	156a	20b	3.3a	1-2	22b
Parthenium hysterophorus	18a	83.2cd	2.2bc	12.3c	0.03b-d	128b	23b	16.6a	0-1	12.6c
Ageratum conyzoides	20a	81.1d	1.4cd	13.4c	0.0002e	129.6b	30b	10a	1-2	13c
Allium cepa	21a	84.4cd	3.0a	24.1b	0.02c-e	128b	23b	10a	1-2	14c

In each column, values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

by *A. indica* and *A. scholaris* leaves spreading on the soil surface (Table 2).

The present study concludes that incorporation of leaves of *C. rotundus, E. citriodora* and *C .album* can effectively be used in the management of *Fusarium* corm rot as these plant materials not only provide significant protection against the pathogen but also enhanced the shoot dry weight significantly over *Fusarium* control. Similarly, corm rot disease of gladiolus can also be managed by spreading leaves of allelopathic plant species on the surface of the soil prior to cultivation of gladiolus. However, further studies should be carried out to confirm the efficacy of these plant materials on the management of *Fusarium* corm rot of gladiolus under field conditions.

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