

*Full Length Research Paper*

# Efficacy of few plant extracts against *Fusarium oxysporum* f. sp. *gladioli*, the cause of corm rot of gladiolus

Sobia Chohan<sup>1</sup>, Rashida Atiq<sup>1</sup>, Mirza Abid Mehmood<sup>1\*</sup>, Safina Naz<sup>2</sup> and Bushra Siddique<sup>3</sup> and Ghazala Yasmin<sup>4</sup>

<sup>1</sup>Department of Plant Pathology, University College of Agriculture, Bahauddin Zakariya University, Multan-60800, Pakistan.

<sup>2</sup>Department of Horticulture, University College of Agriculture, Bahauddin Zakariya University, Multan-60800, Pakistan.

<sup>3</sup>Department of Agriculture and Environmental Sciences, The Islamia University, Bahawalpur-63100, Pakistan.

<sup>4</sup>Department of Chemistry, Bahauddin Zakariya University, Multan-60800, Pakistan.

Accepted 21 June, 2011

Fungi toxic effects of five medicinal plants namely, *Azadirachta indica*, *Ocimum basilicum*, *Datura stramonium*, *Tagetes erecta* and *Allium sativum* were tested at 2, 4, 6 and 8% concentration against *Fusarium oxysporum* f. sp. *gladioli* *in vitro*. Out of five medicinal plants, extract of *A. indica* showed maximum mycelial growth inhibition both at 8% concentration (83.5) and 2% concentration (34.5%) followed by *T. erecta*, *A. sativum* and *D. stramonium* that suppressed the mycelial growth at 8% concentration viz. 58.5, 35 and 28.5% respectively. *O. basilicum* was the least effective in suppressing the mycelial growth of *F. oxysporum*.

**Key words:** Fungitoxic, corm rot, medicinal plants.

## INTRODUCTION

Gladiolus hybrids belong to the family Iridiaceae and are characterized by 250 species (Anonymous, 1976). It is native to South Africa. It has been cultivated in different parts of the world. Out of many cut flowers, gladiolus hybrids are preferable due to its different shades, sizes and marvelous vase life (Bose et al., 2003). According to Bose and Yadav (1989), gladiolus occupies fourth place in international trade of cut flowers. In Punjab Province the total area under cut flowers such as Gladiolus, Jasmine, Roses and Tuberoses is estimated to be around 9000 acres. In Punjab, Gladiolus alone is cultivated over 450 acres (Anonymous, 2003). Gladiolus is attacked by various diseases caused by fungi, bacteria, nematodes and viruses. Among many diseases of gladiolus, corm rot is one of the fungal diseases caused by *Fusarium oxysporum* f. sp. *gladioli*.

Other members of Iridiaceae family are also infected by *F. oxysporum* f. sp. *gladioli* (Infantino and Rumine, 1993). Mirza and Shakir (1991) reported first time the fungal

pathogens of gladiolus from Pakistan. Chandel and Bhardwaj (2000) reported that the quality and market value of corm is deteriorated by *Fusarium oxysporum* Schlecht Fr f. sp. *gladioli* (Massey) Snyd. and Hans. Corm rot disease is also known as yellows and wilt. According to Sunita (1999), the symptoms of corm rot/wilt of gladiolus are leaf tip yellowing that gradually covered the whole leaf and later turned brown. At advanced stage of disease, the plant suddenly wilted or yellow and resulted in premature death.

The bulb turned rotted in the centre and remained mummified. In infected corms and soils fungus survives as mycelium, chlamydospores, macroconidia and microconidia. Due to the more risk of pest resistance and environmental hazards of pesticides, scientists diverted to search new antimicrobial agents from other sources like medicinal plants that should be safe to human and environment. According to Tomova et al. (2005), high levels of active ingredients are present in leaves, flowers, seeds and twigs of higher plants that are used for pathogen control. Many studies revealed that medicinal plants have antimicrobial and antioxidant properties. Wojdylo et al. (2007) suggested that there is a need to identify different types of medicinal plants with anti

\*Corresponding author. E-mail: [mirzaabidpp@gmail.com](mailto:mirzaabidpp@gmail.com). Tel: 92-322-6188042.

microbial activity. Obongoya et al. (2010), used *Azadirachta indica*, *Nicotiana tabacum* and *Vinca rosea* for the control of *Fusarium oxysporum* Schl. f. sp. phaseoli. Plant diffusates are low priced, non-polluting and easy to prepare as compared to commercial fungicides. The study was carried out with the following objectives: (i) To isolate and identify causal organism of corm rot, (ii) To determine the efficacy of different plant extracts against corm rot pathogen.

## MATERIALS AND METHODS

The study was conducted in Laboratory of Department of Plant Pathology, University College of Agriculture, Bahauddin Zakariya University (B.Z.U), Multan during 2009-2010. The main focus was to isolate the causal organism of corm rot and its management by using plant extracts in poison food technique.

### Collection of corms and plant materials

Corms with symptoms of rot were collected from the experimental field of Bahauddin Zakariya University, Multan. The corms with softness of tissues were identified as being rotted. *Azadirachta indica* (leaves), *Ocimum basilicum*, *Datura stramonium*, *Tagetes erecta* (flowers) and *Allium sativum* (bulb) were collected from different parts of the Multan district. Voucher specimens were brought to laboratory and stored in refrigerator at 4°C till use.

### Isolation of fungi from rotted corm

For isolation of fungi artificial growth medium Potato Dextrose Agar (PDA) was used which was prepared by taking ingredients like: Peeled potato (250 g), dextrose (20 g), Agar-Agar (20 g) and distilled water to make the volume 1000 ml which was sterilized in an electric operated autoclave at 15 pounds pressure per square inch (PSI) for 20 min. In each Petri plate of 9 cm diameter, 20 ml of melted-sterilized medium was poured and solidified at room temperature under safety cabinet. Pieces of corm tuber 3×3×2 mm in dimension cut from advancing margin of a rot were disinfected with (2%) sodium hypochlorite solution for about 1 to 2 min at room temperature and then dipped the disinfected portions in sterilized distilled water thrice. A minimum of four replicate pieces were plated out. The Petri plates were incubated at 25±2°C for eight days with alternate light and dark 12 h cycle. Colonies of fungi were examined regularly under stereomicroscope and identified using a compound microscope at 40X magnification and identification keys described by Nelson et al. (1983).

### Preparation of crude extracts of the plant part

Fresh bulbs of *A. sativum*, fresh leaves of *A. indica*, *O. basilicum*, *D. stramonium* and flowers of *T. erecta* were washed thoroughly under running tap water and soaked in 2% solution of sodium hypochlorite for 1 to 2 min, rinsed thoroughly with sterilized distilled water and air dried at room temperature for 2 h. Four different water extract concentrations were prepared by weighing 20, 40, 60 and 80 g of each plant part and 100 ml of sterilized distilled water added. Extract concentrations of 20, 40, 60 and 80% were thus obtained. The extracts were sieved through four layers of sterile muslin-cloth. One ml of each extract concentration was added with 9 ml of molten PDA. Thus we obtained 2.0, 4.0, 6.0 and 8.0% extract concentrations to prepare a PDA-extract mixture (Sangoyomi,

2004). The Petri plates were gently swirled to ensure even distribution of the extracts. The agar-extract mixture was allowed to solidify.

### Effect of the extract on fungal growth

The modified method of Sangoyomi (2004) was used to determine the effect of the extracts on fungal growth. This was done by inoculating at the centre of the Petri plates with a mycelial disc obtained from the colony edge of 8 days old culture of fungi. The control was set up using blank agar plates (no extracts). Three replicate plates of PDA-extract per isolate were incubated at 25±2°C and radial growth was measured daily for 8 days. Colony diameter was taken as the means along two directions on two perpendicular lines drawn on the reverse of the plates. The percentage of inhibition was calculated by using the formula:

$$\text{Percentage inhibition } I = 100 \times (C - T)/C$$

Where I is percentage inhibition (mm), C is growth of fungus in the control and T is growth of fungus in the treatment.

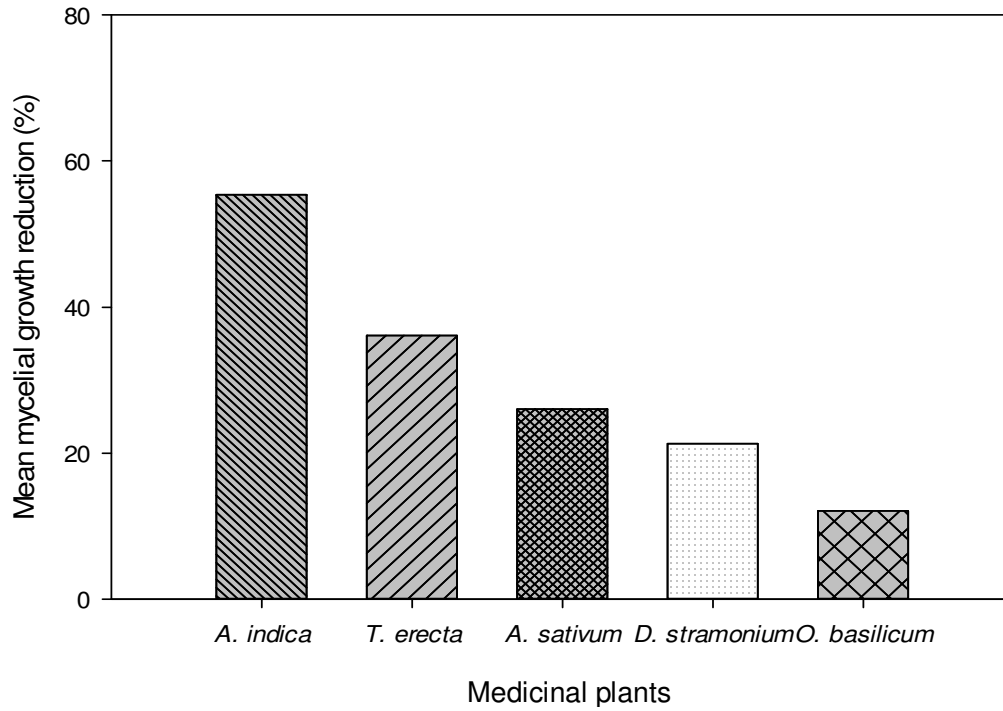
### Statistical analysis

All the analysis was done using sigmaplot 12. systat software Inc.

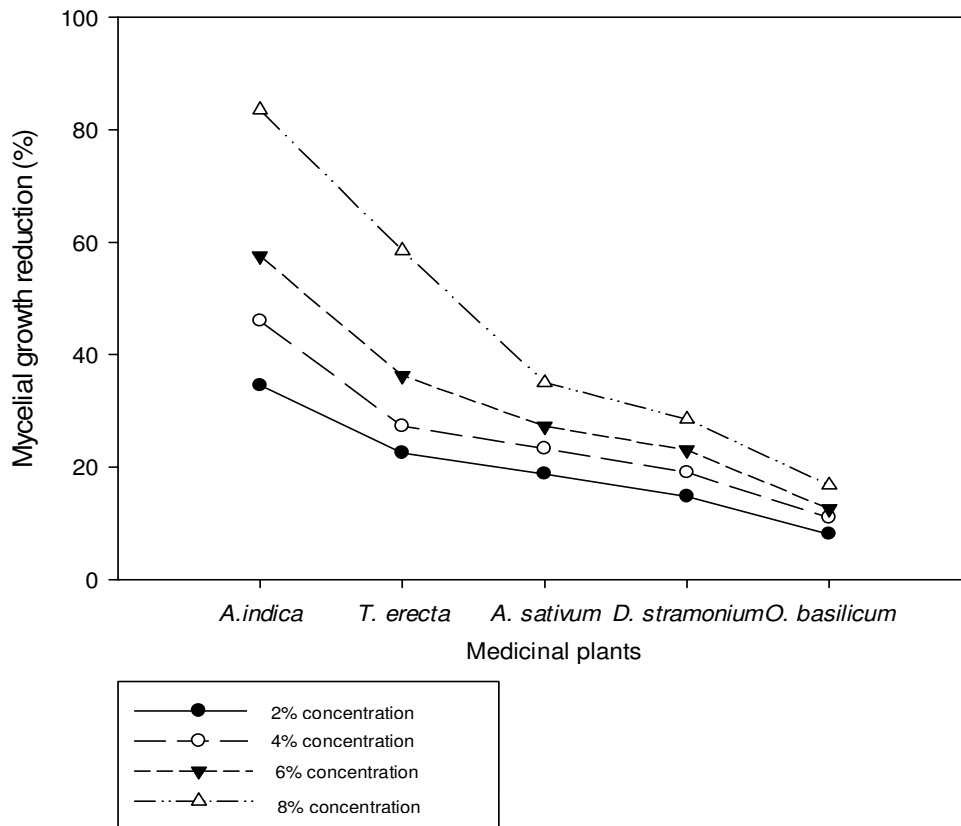
## RESULTS AND DISCUSSION

A total of five medicinal plants namely, *A. sativum*, *A. indica*, *O. basilicum*, *D. stramonium* and *T. erecta* were used against the corm rot disease caused by *F. oxysporum* Schlecht Fr f. sp. *gladioli* (Massey) Snyd. and Hans. Out of five medicinal plants, *A. indica* showed good result by inhibiting the mean radial mycelial growth of the fungi (55.4%) followed by *T. erecta* (36.13%), *A. sativum* (26.06%), *D. stramonium* (21.31%) and *O. basilicum* (12.09%) as shown in (Figure 1). *A. indica* and *T. erecta* inhibited the mean radial mycelial growth of the *F. oxysporum* at all concentrations viz. (83.5 and 58.5%), (57.5 and 36.25%), (46 and 27.25%) and (34.5 and 22.5%) at 8, 6, 4 and 2% concentrations respectively (Figure 2). When applied as a spray *A. indica* is systemically translocated in the vascular system of the plant therefore results in the inhibition of mycelial growth (Vyas et al., 1999). Present study indicated that *A. indica* inhibited the growth of *F. oxysporum* at higher concentrations as well as at lower concentrations. According to Okigbo and Nmeko (2005), diseases of plants and tuber crops can be successfully controlled by using plant extracts.

Okigbo and Emoghene (2004) reported that plant extracts have fungicidal activity against different pathogens of crop plants. Aqueous leaf extracts of *A. indica* and *Melia azedarach* inhibited the mycelial growth of *A. solani* and *F. oxysporum*, whereas extracts of *A. indica* showed high values of inhibition compared to *M. azedarach* (Hassanein et al., 2008). According to Vyas (1984), the mycelial growth of the fungus showed sensitivity to various plant extracts (biopesticides) may be



**Figure 1.** Mean mycelial growth reduction percentage of *F. oxysporum* f. sp. *gladioli* by using five medicinal plants.



**Figure 2.** Mycelial growth reduction percentage of *F. oxysporum* f. sp. *gladioli* at different concentrations of five medicinal plants.

due to their active ingredients and chemical configuration. Different *Tagetes* species showed fungicidal, insecticidal and nematicidal properties. Obongoya et al. (2010) studied that *A. indica* followed by *Tagetes minuta* inhibited the growth of *F. oxysporum* at different concentrations and is according to the findings of the present study. Lokhande et al. (1998), indicated the effectiveness of antifungal activity of neem extracts and oils. Neem leaves inhibited the soil borne fungi *F. oxysporum* when used as a soil treatment (Kannaiyan and Prasad, 1981).

Similarly *A. sativum* and *D. stramonium* gave best results at 8% concentration by inhibiting the 35 and 28.5% mean radial mycelial growth of the *F. oxysporum* and least at 2% concentration that is 18.75 and 14.75%, respectively. *O. basilicum* were the least effective among the five medicinal plants used and inhibited only 16.75% mean radial mycelial growth at 8% concentration (Figure 2). *A. sativum* was found to be most effective in inhibiting the growth of *F. oxysporum* compared to *O. gratissimum*. Extracts of *A. sativum* and *O. gratissimum* showed inhibitory effect on mycelial growth of *F. oxysporum* and the inhibition percentage is directly proportional to the concentration of the extract that is, inhibition percentage is increased with increasing the concentration of the extracts (Okigbo et al., 2009), and the results were cognizant with the findings of the present study. Due to the cheapness, easy availability and environment friendly effect, it is recommended that *A. indica* and *T. erecta* would be used to control corm rot of gladiolus.

The control of pathogens by using the fungicides is the most common and instant mean. Besides the development of resistance in pathogens against fungicides, environmental pollution and residual hazardous effects of fungicides on non target hosts are the main drawbacks in their use. Different plants having the fungicidal, insecticidal and nematicidal properties are present in the universe. There is a need to identify their principle ingredients and active ingredients and use them for the benefits of mankind.

## REFERENCES

- Anonymous (2003). Directorate of Floriculture, Government of the Punjab, Lahore.
- Anonymous (1976). Hortus third a concise dictionary of plant cultivated in the United States and Canada. Macmillan publishing Co. inc. New York, pp. 511-523.
- Bose TK, Yadav LP, Pat P, Parthasarathy VA, Das P (2003). Commercial Flowers, Naya Udyog, Kolkata, India. Vol. 2.
- Bose TK, Yadav LP (1989). Commercial flowers. (Ed., Vayar Prakash), Calcutta Publication, pp. 267-350.
- Chandel SS, Bhardwaj LN (2000). Effect of sowing dates and fungicidal treatment on the management of *Fusarium* wilt of gladiolus. Plant Dis. Res., 151: 24-27.
- Dhingra OD, Sinclair JB (1985). 'Basic plant pathology methods.' (CRC Press, Inc.: Boca Raton, FL).
- Hassanein NM, AbouZeid MA, Youssef KA, Mahmoud DA (2008). Efficacy of Leaf Extracts of Neem (*Azadirachta indica*) and Chinaberry (*Melia azedarach*) against Early Blight and Wilt Diseases of Tomato. Aust. J. Basic Appl. Sci., 2(3): 763-772.
- Infantino A, Rumine P (1993). *F. oxysporum* f. *sp. gladioli* on montbretia cutflowers in Italy. Petria, 3: 65-68.
- Kannaiyan S, Prasad NN (1981). Effect of organic amendments on seedling infection of rice caused by *Rhizoctonia solani*. Plant Soil, 62: 131-133.
- Lokhande NM, Lanjewar RD, Newaskar VB (1998). Effect of different fungicides and neem products for control of leaf spot of groundnut. J. Soils Crops, 8: 44-46.
- Mirza JH, Shakir AS (1991). First report of fungal pathogens of Gladiolus from Pakistan. Pak. J. Phytopathol., 3: 1-2, 74-76.
- Nelson PE, Toussoun TA, Marasas WFO (1983). *Fusarium Species: An Illustrated Manual for Identification*. Pennsylvania State University. University Park, PA, USA, 193.
- Obongoya BO, Wagai SO, Odhiambo G (2010). Phytotoxic effect of selected crude plant extracts on soil-borne fungi of common bean. Afr. Crop Sci. J., 18(1): 15-22.
- Okigbo RN, Okorie RE, Putheti RR (2009). *In vitro* effects of garlic (*Allium sativum* L.) and African basil (*Ocimum gratissimum* L.) on pathogens isolated from rotted cassava roots. INCI, 34(10): 742-747.
- Okigbo RN, Nmeka IA (2005). Control of yam tuber with leaf extracts of *Xylopiya aethiopicica* and *Zingiber officinale*. Afr. J. Biotechnol., 4(8): 804-807.
- Okigbo RN, Emoghene AO (2004). Antifungal activity of leaf extracts of some plant species on *Mycosphaerella fijiensis* Morelet, the causal organism of black sigatoka disease of Banana (*Musa acuminata*) KMITL Sci. J., 4: 20-31.
- Sangoyomi TE (2004). Post- Harvest Fungal Deterioration of Yam (*Dioscorea rotundata* Poir) and its control. Thesis. International Institute of Tropical Agriculture. Ibadan, Nigeria, p. 179.
- Sunita CS (1999). Fungal and bacterial disease of bulbous ornamentals. In: Verma LR, Sharma RC (eds), diseases of horticultural crops – vegetables, ornamentals and mushrooms.. Indus Publishing Co., New Delhi, pp. 501-531.
- Tomova BS, Waterhouse JS, Doberski J (2005). The effect of fractionated *Tagetes* oil volatiles on aphid reproduction. Entomol. Exp. Appl., 115: 153-159.
- Viyas SC (1984). *Systematic fungicides*. Tata Mc Grah hill Co. limited. New Delhi, p. 36.
- Vyas BN, Ganesan S, Raman K, Godrej NB, Mistry KB (1999). Effects of three plant extracts and a chook: A commercial neem formulation on growth and development of three noctuid pests. In: Singh RP, Saxena RC (eds.). *Azadirachta Indica A. Juss.* Oxford and IBH Publishing, New Delhi, India, pp. 103-109.
- Wojdylo AJ, Oszmianski CR (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem., 105: 940-949.