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Antifungal activity of plant extracts against *Fusarium oxysporum* – the cause of corm-rot disease of *Gladiolus*

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Abstract

Fusarium oxysporum f.sp. *gladioli* (Massey) Snyd. & Hans. was isolated from diseased corms of gladiolus (*Gladiolus grandiflorus* sect. Blandus) cv. Aarti. Antifungal activity of different concentrations (2, 4, 6 and 8% w/v) of leaf extracts of wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), sunflower (*Helianthus annus* L.), chilies (*Capsicum annum* L.), onion (*Allium cepa* L.) and marigold (*Tagetes erectus* L.) was evaluated against *in vitro* growth of *F. oxysporum.* Extract of marigold, sunflower and chilies were found highly effective where all the employed extract concentrations significantly reduced fungal biomass by 54-79%, 33-85% and 45-57%, respectively. In case of onion, only the highest concentration of the extract i.e. 8% significantly suppressed fungal biomass by 73%.

Keywords: Antifungal, corm rot, *Fusarium oxysporum, Gladiolus grandiflorus,* plant extract.

Introduction

Owing to their different sizes, shades, and excellent vase life, *Gladiolus* hybrids are among the preferred cut flowers (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. The causal organism is *Fusarium oxysporum* Schlecht. f.sp. *gladioli* (Masey) Snyder & Hans., which deteriorates its quality and market value (Remotti *et al*., 1997; Chandel and Bhardwaj, 2000).The fungus survives in infected corms and in the soil as mycelium, chlamydospores, macroconidia 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).

Agrochemicals are indispensable to fight against plant diseases and to maintain high crop yields. However, indiscriminate use of these chemicals has often resulted in adverse environmental effects, disturbing the ecological balance of soils and making plants even more susceptible to pests and diseases. (Mancini *et al.,* 2008). Increasing public concern on environmental issues requires alternative disease management systems, which are less pesticide dependant or based on naturally occurring compounds (Cuthbertson and Murchie, 2005). The plant world comprises a rich storehouse of biochemicals to be used as pesticides which are more environmentally safe than synthetic chemicals (Hashim and Devi, 2003). There are reports that phytochemicals of *Melia azedarach*, *Euclyptus citriodora* and *Alstonia scholaris* showed fungicidal activity against pathogenic fungi (Charmaine *et al.,* 2005). In a recent study Bajwa et al. (2008) reported that extracts of some rice cultivars exhibit antifungal activity against *Macrophomina phaseolina* and *Ascochyta rabiei.* The present study was carried out to investigate the antifungal activity of aqueous extracts of some plant species against *F. oxysporum* isolated from diseased corm of *G. grandiflorus.*

Materials and Methods

Isolation of *F. oxysporum*

Infected corms of *Gladiolus* were collected from fields in district Kasur, Punjab, Pakistan and kept in dry cellulose envelopes during transportation to the laboratory. The diseased portions of the specimens were cut into small pieces and surface disinfected by immersing in 1% sodium hypochlorite solution for one minute and then rinsed thrice in sterilized water. The surface sterilized pieces were placed on the Malt Extract Agar (MEA) medium in 9 cm diameter Petri plates and incubated at 25 °C for 7 days. Pure cultures of *F. oxysporum* f.sp. *gladioli* were isolated and maintained on 2% MEA medium.

Preparation of aqueous extracts

Leaves of wheat, maize, sunflower, chilies, onion (fleshy leaves of bulb) and marigold were

thoroughly washed with sterilized water several times. Leaves were surface sterilized with 1% sodium hypochlorite solution for one minute followed by several washings with sterilized water. Thirty gram of plant material of each of the six test plant species was taken in 100 ml of sterilized distilled water and blended for five minutes and left for one hour. The blended materials were then passed through muslin cloth and finally filtered through Whatman filter paper No.1 to obtain a 30% w/v stock solution.

Aqueous extract bioassays

Malt extract broth was sterilized by autoclaving at 121 °C for 30 min. and was cooled to 40 °C. In 250 ml flasks, 80 ml of malt extract medium was poured. Appropriate quantities of stock solutions and distilled water were mixed to make 2, 4, 6 and 8% (w/v) media with final volume of 100 ml in each flask. Control received 20 ml of distilled water to make the final volume of the growth medium to 100 ml. Actively growing mycelial discs of *F. oxysporum* were prepared using a cork borer of 5 mm diameter and transferred to the flasks aseptically. Each treatment was replicated three times. Flasks were incubated at 25 °C for 10 days on an electric shaker. After 10 days, fungal biomass in each flask was filtered, dried to constant weight and weighed.

Statistical analysis

The experiment was conducted using a completely randomized design. Standard errors of means of three replicates were computed using computer software Microsoft Excel. All the data were subjected to analysis of variance followed by mean separation through Duncan's Multiple Range Test (Steel and Torrie, 1980) using computer software COSTAT.

Results and Discussion

Aqueous extracts of marigold were found highly effective in suppressing the growth of *F. oxysporum*. All the concentrations of the extracts significantly reduced the *in vitro* growth of the target fungal pathogen. There was 54-79% reduction in fungal biomass due to different concentrations of the extracts (Fig. 1). Marigold has fungicidal properties due to the presence of thiophenes in its tissues (Gomez-Rodríguez *et al.*, 2003; Montes and García, 1997). Earlier studies reported that when intercropped with marigold, tomato achieved 80–93% reduction in foliar damage caused by *Alternaria solani* (Ellis and Martin) Jones. and 70–75% reduction in fruit damage, compared to non-intercropped tomato (Zavaleta-Mejía and Gómez, 1995). Rojas-Martínez *et al.* (1999) suggested that microclimate in tomato associated with *T. erectus* is less favorable for *A. solani* infection than in single cropped tomato.

Aqueous extract of chilies was also found highly effective in controlling the growth of the tested fungal pathogen. All the employed extract concentrations of this plant species significantly reduced fungal biomass by 45-57% (Fig. 1).

Aqueous extracts of sunflower exhibited remarkable antifungal activity against *F. oxysporum.* Maximum inhibition in fungal biomass production (85%) was recorded in 2% extract treatment. Effect of other extract concentration was also significant but less pronounced as compared to 2% concentration. Extract of 4, 6 and 8% concentrations resulted in 51, 54 and 33% reduction in fungal biomass as compared to control (Fig. 1). Giudici *et al.* (2000) reported the presence of 16-kDa protein as an antifungal agent in sunflower. This protein displays an associated activity of trypsin inhibitor, caused the complete inhibition of *Sclerotinia sclerotiorum* ascospores germination at a concentration of 5 $\mu g \cdot mL^{-1}$ (0.31 μM) and a clear reduction of mycelial growth at lower concentrations.

Lower concentrations of 2-4% of aqueous extract of onion did not exhibit any significant antifungal activity. In contrast to that, highest extract concentration of 8% showed a significant suppression of 73% in fungal biomass as compared to control (Fig. 1). Antifungal activity of onion may be attributed to the powerful sulfur and other numerous phenolic compounds (Griffiths, *et al.,* 2002).

Between the two cereals, 2 and 4% extracts of wheat significantly reduced the fungal biomass by 39 and 48%, respectively. Conversely, effect of higher concentrations of 6 and 8% was insignificant. None of the extract concentrations of maize expressed antifungal activity against the targeted pathogen (Fig. 1).

The present study concludes that aqueous extracts of sunflower, marigold and chilies contain antifungal constituents for the control of *F. oxysporum* f.sp. *gladioli.* Further green house and field experiments are suggested to investigate the *in vivo* effects of these extracts as compared to some commercial chemical fungicides for the management of corm-rot disease of *Gladiolus.*

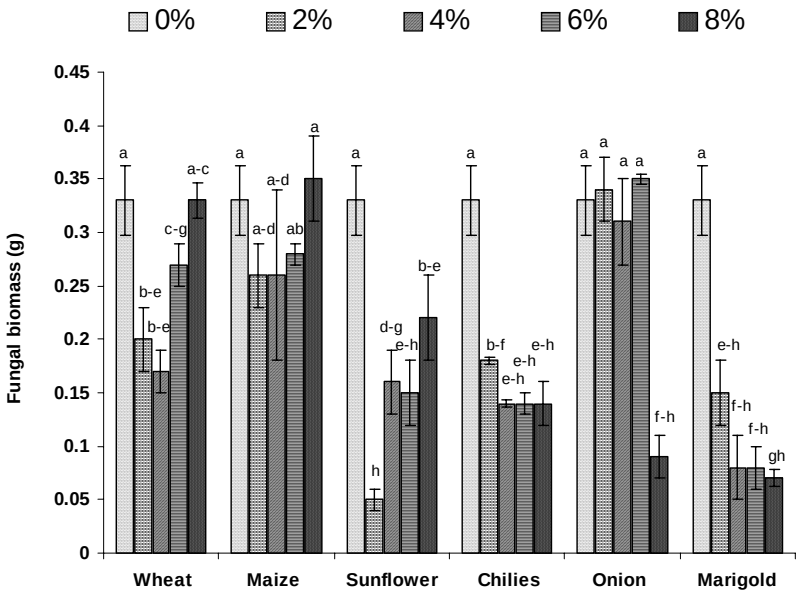

Fig. 1: Effect of different concentrations of plant extracts on biomass of *Fusarium oxysporum.* Vertical bars show standard error of means of three replicates. Values with different letters show significant difference at (P≤0.05) as determined by Duncan's Multiple Range Test.

References

Bajwa R, Javaid A, Shafique S, Javaid A, Jabeen K, Shafique S, 2008. Fungistatic activity of aqueous and organic solvent extracts of rice varieties on phytophathogenic fungi. *Allelopathy J.,* **22:** 363-370.

Bose TK, Yadav LP, Pat P, Parthasarathy VA, Das P, 2003. Commercial Flowers, Vol. II. Naya Udyog, Kolkata, India.

Chandel SS, Bhardwaj LN, 2000. Effect of sowing dates and fungicidal treatment on the management of *Fusarium* wilt of gladiolus. *Plant Dis. Res.,* **15**1: 24-27.

Charmaine LAC, Menon T, Umamaheshwari K, 2005. Anticandidal activity of *Azadirachta indica*. *Indian J. Pharamcol.,* **37**: 386-389.

Cuthbertson AGS, Murchie AK, 2005. Economic spray thresholds in need of revision in Northern Irish Bramley orchards. *Bio. News.,* **32**: 19.

Gómez-Rodríguez O, Zavaleta-Mejía E, González-Hernández VA, Livera-Muñoz M, Cárdenas-Soriano E, 2003. Allelopathy and microclimatic modification of intercropping with marigold on tomato early blight disease development. *Field Crops Res.,* **83**: 27-34.

Griffiths G, Trueman L, Crowther T, Thomas B, Smith B, 2002. Onions—a global benefits to health. *Phytoth. Res.,* **16**: 603-615.

Hashim MS, Devi KS, 2003. Insecticidal action of the polyphenolic rich fraction from the stem bark of *Sterlus asper* on *Dysdercus cingulatus*. *Fitoterapia*, **74**: 670-676.

Mancini F, Termorshuizen AJ, Jiggins JLS, van Bruggen AHC, 2008. Increasing the environmental and social sustainability of cotton farming through farmer education in Andhra Pradesh, India. *Agric. Systems,* **96**: 16-25.

Montes BR, García LR, 1997. Efecto de extractos vegetales en la germinación de esporas y en los niveles de daño de *Alternaria solani* en tomate. *Fitopatología* **32**: 52-57.

Remotti PC, Loffler HJM, Volten DLU, 1997. Selection of cell line and regeneration of plants resistance to fusaric acid from *Gladiolus grandiflorus* cv. peter pears. *Euphytica*, **96:** 237-245.

Roebroeck EJA, Mes JJ, 1992. Physiological races and vegetative compatibility groups within *Fusarium oxysporum* f.sp. *gladioli. Netherlands J. Plant Pathol.,* **98**: 57-64.

Rojas-Martínez RI, Zavaleta-Mejía E, Gómez RO, 1999. Efecto de la asociación cempazúchil-jitomate en el daño producido por *Alternaria solani* en jitomate. *Fitopatología*, **34**: 83-89.

Steel RGD, Torrie JH, 1980. *Principles and Procedures of Statistics.* McGraw Hill Book Co., Inc, New York, USA.

Zavaleta-Mejía E, Gómez RO, 1995. Effect of *Tagetes erecta* L.–Tomato (*Lycopersicon esculentum* Mill.) intercropping on some tomato pests. *Fitopatología,* **30**: 35-45.