

## **Antifungal potential of *Brassica campestris* against *Macrophomina phaseolina*, *Fusarium oxysporum* and *Drechslera australiensis***

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### **Abstract**

Antifungal potential of aqueous extracts of *Brassica campestris* L. was evaluated against three plant pathogenic fungi namely *Fusarium oxysporum* Schlechtend, *Drechslera australiensis* (Bugnic) Subram & B.L. Jain. and *Macrophomina phaseolina* Tassi Goid. Aqueous extract of leaves of *B. campestris* was prepared and added in the potato dextrose broth medium to get 2, 4, 6, 8 and 10% concentrations (w/v). Growth of all the test fungi was significantly suppressed by higher concentration of aqueous extract of root and shoot of the test plant. Maximum biomass reduction (44%) was due to 10% root extract against *M. phaseolina* followed by 30% reduction in biomass of *F. oxysporum*. Minimum reduction in biomass (14%) was recorded in *D. australiensis* at 10% concentration. Shoot extract also reduced fungal biomass which was 38% in *M. phaseolina*, 26% in *F. oxysporum* and 9% in *D. australiensis*.

**Keywords:** Antifungal potential, *Brassica campestris*, *Drechslera australiensis*, *Fusarium oxysporum*, *Macrophomina phaseolina*.

### **Introduction**

Phytopathogenic fungi are one of the major biotic stresses that contribute substantially to the overall yield loss in crop plants (Ribera and Zuñiga, 2012). Fungal species such as *M. phaseolina*, *F. oxysporum* and *D. australiensis* have been considered to be major plant pathogens worldwide. They have wide host range and cause rots, wilt and spot diseases in their host crops (Bowers and Locke, 2000; Khan 2007; Rabbani *et al.*, 2011).

Traditionally synthetic fungicides are used to control fungal diseases of plants. The overzealous and indiscriminate use of most of the synthetic fungicides has created different types of toxicological problems. These fungicides are not only expensive, but also hazardous to the environment. The toxic effect of synthetic chemicals can be overcome only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective (Mohana *et al.*, 2011). Recently, plant materials are being used against many plant pathogenic fungi and their antifungal action has gained much attention (Javaid and Saddique, 2011; Rauf and Javaid, 2013). Members of the family Brassicaceae including *Brassica* species were found to possess antimicrobial

compounds, while antifungal activity of *Brassica* phytoalexins was also reported. Green tissues of *Brassica* species when incorporated into soil can reduce population density of some fungal and nematode pathogens (Subbarao *et al.*, 1994). Several research works have revealed that use of *Brassica* spp. as soil amendment can reduce the disease incidence or inoculum level of *M. phaseolina*, *Pythium ultimum* Trow and *F. oxysporum* (Lohda and Sharma, 2002). *Brassica* amendments are reported to reduce the population of *Verticillium dahliae* Beyma and *Melodiogyne chitwoodi*, and decreased root rot of pea caused by *Aphanomyces euteiches* Drechsler in field. Fresh leaf tissue of *Brassica* species suppressed root rot of bean and sesame caused by *Thielaviopsis basicola* (Hilary *et al.*, 1995). The mechanism involved in the disease control or suppression of a pathogen is considered to be the production of allyl isothiocyanate (AITC) by the tissues of *Brassica* spp. AITC can show its antimicrobial activity on fungal propagules by vapor action, thus acting as a fumigant. This volatile compound is produced in an aqueous medium through enzymatic hydrolysis of sinigrin, which is the predominant glucosinolate in the tissue of *Brassica* spp. (Dastan *et al.*, 2011). Purpose of the present study was to investigate the antifungal

potential of the aqueous extracts of *B. campestris* against some phytopathogenic fungi.

## Materials and Methods

### Procurement of fungal species

*M. phaseolina* was isolated from sesame plants infected with charcoal rot while *F. oxysporum* and *D. australiensis* were obtained from First Fungal Culture Bank, University of the Punjab, Lahore, Pakistan.

### Collection of plant material

Fresh and healthy plants of *B. campestris* were collected from experimental field of Institute of Agricultural Sciences, University of the Punjab, Quaid-e-Azam Campus Lahore.

### Processing of plant material

Plants materials were brought in the laboratory and washed with sterilize water to remove dust and debris. Leaves were surface sterilize with 1% sodium hypochlorite for 2 min and washed 2-3 times with sterilize distilled water then shade dried. Roots and shoots (leave+stem) of the test plant were cut into small pieces.

### Preparation of plant extract

Fifty grams of dried root and shoot of *B. campestris* were blended in 50 mL of distilled water to get 100% w/v stock solution. The blended material was left for 1 hour and filtered through double layered autoclaved muslin cloth and finally through Whatman filter paper No. 1.

### Bioassays with plant extracts

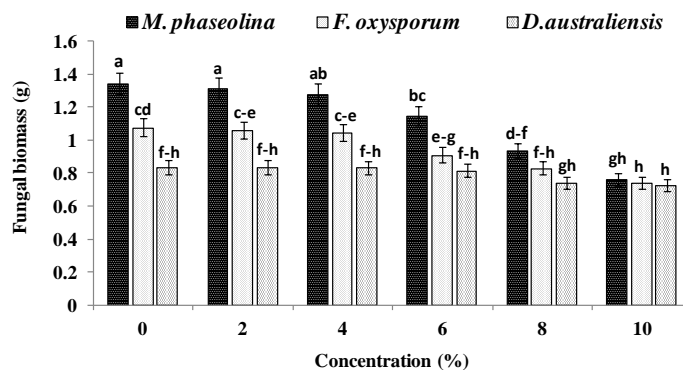
To determine the effect of plant extracts on fungal biomass, 80 mL of potato dextrose broth was prepared in 250 mL flask. Media was sterilized by autoclaving at 121 °C under  $1.035 \times 10^5$  Pa pressure for 30 min and was cooled to 40 °C. Leaf and root extract (20 mL) of the test plant were added to growth medium at concentration varied from 2, 4, 6, 8 and 10% (w/v). The medium with 20 mL of distilled water served as control. The mycelial discs of 5 mm from 7 days old culture of the test fungi were taken using a sterilized cork borer and transferred aseptically to the flasks. Each treatment was replicated thrice. Flasks were incubated at  $25 \pm 1$  °C for 10 days. After 10 days, fungal biomass in each flask was filtered, dried in oven at 60 °C for 24 hrs and weighed.

### Data analysis

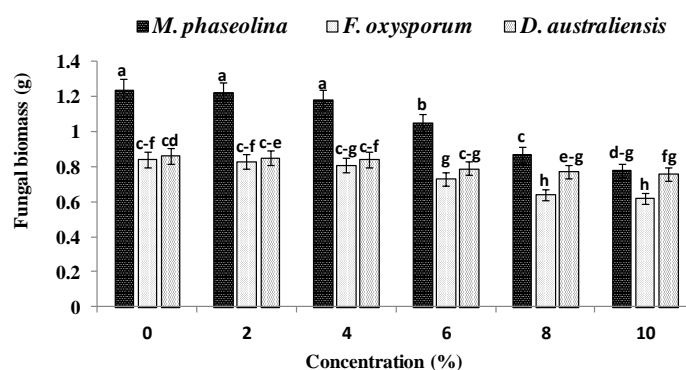
The effect of treatments was determined by using a completely randomized design. Standard errors of means of three replicates were computed using computer software Microsoft Excel. All the data were subjected to analysis of variance followed by mean separation through Duncan's Multiple Range Test at 5% level of significance.

## Result and Discussion

Aqueous extracts of root and shoot with various concentrations (2-10%) were used and data was recorded for dry fungal biomass after five days. Dry weight determination in different concentration showed both root and shoot extract caused reduction in biomass of all the fungi. However, variation was observed in fungitoxic effect of extract treatments against the test fungi. Root extract caused more biomass reduction of the test fungi than that of shoot extract. Significant reduction of dry fungal biomass of *M. phaseolina* (44%) *F. oxysporum* (30%) and *D. australiensis* (14%) was recorded at 10% concentration in root extract bioassay (Fig 1). In case of shoot extract, biomass reduction exhibited by *M. phaseolina* was 38% and by *F. oxysporum* was 26%. Loss in dry weight of *D. australiensis* was 9% which exhibited less susceptibility against shoot extract than the other two fungi (Fig. 2). In a similar kind of work, Bajawa *et al.* (2008) reported antifungal activity of root and shoot extracts of *Parthenium hysterophorus* and *Ageratum conyzoides* against *M. phaseolina* and dichloromethane fractions of *Cicer arietinum* against *D. tetraemera* and *D. hawaiiensis*. Aqueous extracts of sunflower exhibited remarkable antifungal activity against *F. oxysporum* (Riaz *et al.*, 2008). The extracts of *Polyalthia longifolia*, *Annona squamosa* and *Tridax procumbens* were found to be inhibitory for the growth of *Alternaria porri*, *Aspergillus niger*, *F. oxysporum* and *Cladosporium allii* (Ghangaonkar, 2007). Members of Brassicaceae have been used as soil amendments to control soil-borne pathogen (Riaz *et al.*, 2007). Present study also provides evidence that aqueous extracts of *B. campestris* contains antifungal constituents for the control of pathogenic fungi and suggests the possible alternative of hazardous chemicals. It is concluded from the results that among both extracts of *B. campestris* root extract were more effective. Further investigations on identification of active compounds in *B. campestris* may lead to chemical entities with antifungal potential which is best and economical way to control destructive plant pathogenic fungi.



**Fig. 1:** Effect of root extract of *B. campestris* on dry biomass of test fungi. Vertical bars with different letters show significant difference ( $P \leq 0.05$ ) as determined by DMR test.



**Fig. 2:** Effect of shoot extract of *B. campestris* on dry biomass of test fungi. Vertical bars with different letters show significant difference ( $P \leq 0.05$ ) as determined by DMR test.

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