Antifungal activity of methanolic extracts of *Sorghum halepense* against *Macrophomina phaseolina*

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*Macrophomina phaseolina* (Tassi) Goid., the cause of charcoal rot disease, is the major biotic factor that limits cowpea [*Vigna unguiculata* (L.) Walp.] productivity worldwide. The present study was designed to investigate the antifungal potential of an allelopathic grass *Sorghum halepense* Pers. for the management of *M. phaseolina* isolated from charcoal rot infected cowpea plants. In laboratory bioassays, different concentrations (0, 0.5, 1.0,…, 3.0 g/ml) of methanolic extracts of shoot, root and inflorescence of the test allelopathic grass were evaluated for their in vitro antifungal activity against *M. phaseolina*. Extracts of all the three parts of the grass exhibited variable antifungal activity. Shoot extract was found to be the most effective where all the extract concentrations significantly reduced the fungal biomass by 14 to 61% over control. Similarly, all concentrations of the root extract except 0.5% significantly suppressed the fungal biomass. Inflorescence extract exhibited the least antifungal activity where only 2% and higher concentrations showed the significant effect. There was 9 to 48% and 4 to 39% reduction in fungal biomass due to various concentrations of methanolic root and inflorescence extracts, respectively.

**Key words:** Johnson grass, antifungal activity, *Macrophomina phaseolina*.

**INTRODUCTION**

Cowpea, also known as black eye beans (*Vigna unguiculata* L. Walp.) is an important leguminous crop in humid regions of tropic and sub-tropics. In Pakistan, cowpeas are ranked amongst the major legumes and planted as summer or spring crop. A decade ago, its cultivation area was about 17000 hectares with annual production of 8000 tons (Anonymous, 2001); however, currently, a significant increase has been recorded in its production (Zia et al., 2010). Nutritional value of cowpea lies in its protein (22 to 33%), especially of lysine and folic acid, carbohydrate (53.56 to 57.36%), phosphorus, iron and vitamins contents (Khan et al., 2010; Imran et al., 2010). Cowpea seeds, leaves and oil have several medicinal uses for treatment of common cold, amenorrhea, headaches, constipation, dysmenorrheal, cancer and cardiovascular diseases, etc., (Brink and Belay, 2006; Siddhuraju and Becker, 2007). Its well adaptability in low acidic soils, extreme condition of temperature and with capability to maintain nitrogen status of the soil has increased its wide utilization as vegetable, fodder, hay, grain or green manure (Singh, 2003).

Among the various constraints faced by cowpea crop, charcoal rot being the serious one is induced by *Macrophomina phaseolina* (Tassi) Goid. This pathogen is reported to cause significant loss in a more than 500 plant species, including legumes in many regions of the world (Wyllie, 1993; Das et al., 2008; Ma et al., 2010; Zveibil et al., 2012). *M. phaseolina* infects plants on almost all growth stages and are instigated by seed, soil and plant residues (Reuveni et al., 1983). Colonization of pathogen of charcoal rot resulted in death of seedling because of impediment of xylem vessels. Plant defoliation and wilting occurs due to formation of red to brown lesions on roots and stems with production of dark mycelia and black microsclerotia (Abawi and Pastor-Corrales, 1990). The disease may cause up to 100% yield losses (Bashir and Malik, 1988) varying according to severity and disease severity may be enhanced by hot...
and dry environment (Gaige et al., 2010).

Number of different disease control measurement practices like fungicides application, antagonists organism and crop rotation have been adopted (Pineda, 2001; Choudhary et al., 2004) with inherent problem in their employment. Amongst the alternative options, natural fungicides from allelopathic and medicinal plants have focused the attention of scientists from all over the world because of their environment and economic feasibility (Bajwa and Iftekhar, 2005; Hernandez et al., 2007; Javaid and Reham, 2011). *Sorghum halepense* is a rhizomatous perennial summer weed (Loddo et al., 2012). The allelopathic potential of *S. halepense* has been well-documented due to presence of number of phenolic and flavonoids in its different parts (Czarnota et al., 2003; Huang et al., 2010; Liu et al., 2011). The present investigation was conducted to evaluate the antifungal potential of *S. halepense* against *M. phaseolina*, isolated from charcoal rot infected cowpea plant.

**MATERIALS AND METHODS**

*Isolation and identification of fungal pathogen*

Cowpea plants showing the symptoms of charcoal rot were procured from National Agricultural Research Centre, Islamabad, Pakistan. Infected stem pieces of the cowpea plants were surface sterilized with 1% sodium hypochlorite solution for 30 s, then rinsed twice in autoclaved distilled water and were dried in a laminar flow cabinet. The surface sterilized infected stem pieces were plated on malt extract agar (MEA) medium under aseptic conditions. The plates were incubated at 28°C in the dark for 6 to 7 days. The isolated fungal pathogen was subcultured on MEA in 9 cm diameter Petri plates for culture purification. The pure cultures were maintained in refrigerator at 4°C. Plant samples were preserved in paper bags and stored at 4°C.

Morphological identification was done by studying cultural characters which were assessed through naked eye and by microscopic examination. Colony morphology was recorded from cultures grown on MEA. Pycnidia, conidia, microsclerotia and hyphal branching were assessed under microscope.

*Preparation of methanolic extracts*

Shoot, inflorescence and roots were collected from mature *S. halepense* plants from the University of Punjab, Quaid-e-Azam Campus Lahore, Pakistan. After rinsing with tap water, plant materials were dried in an electrical oven at 45°C, thoroughly crushed and ground to fine powder. Two hundred grams of each of the four powdered plant parts were soaked in 2 L methanol in air tight glass jars separately for 7 days at room temperature. Afterwards, extracts were obtained from soaked materials by filtering through muslin cloth followed by filter papers and preserved in plastic bottles. Filtrates were evaporated in rotary evaporator under vacuum to reduce the volume to 20 ml. Then, 20 ml of the extracts was poured in open wide mouth pots and put in the air dried oven at 40°C to completely evaporate the methanol.

*Bioassays with methanolic extracts*

In *vitro* bioassays were carried out with methanolic extracts of shoots, roots and inflorescence. Crude methanolic extracts (8.4 g) of each of the three different parts of the grass species were dissolved in sterilized distilled water to prepare 14 ml of stock solution. Seventy six milliliters ME was autoclaved at 121°C for 30 min in 250 ml conical flasks and cooled at room temperature. Chloromycetin at 50mg/100ml of the medium was added to avoid bacterial contamination.

Six concentrations, namely, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 g/100 ml were made by adding 0.67, 1.332, 1.998, 2.664, 3.33 and 3.99 ml stock solution and 3.33, 2.668, 2.002, 1.336, 0.67 and 0.01 ml distilled autoclaved water to each flask to make total volume of the medium 80 ml. The 80 ml of each treatment was divided into four equal portions in 100 ml conical flasks to serve as replicates. Control treatment was prepared by adding 4 ml of distilled autoclaved water to 76 ml of ME broth.

Mycelial discs of 5 mm diameter were removed from the edges of 7 days old actively growing culture of *M. phaseolina* using a sterilized cork borer of 5 mm diameter put in each conical flask. Flasks were incubated for 10 days in an incubator at 20 ± 2°C.

Fungal harvest was taken by filtering the fungal mat through pre weighed Watman No. 1 filter papers, followed by oven drying to gain dry biomass from each flask and then, filter papers with biomass were weighed again. Fungal growth was measured by subtracting the weight of the filter paper from the weight of fungal mass plus filter paper. Percentage of growth inhibition of the fungal biomass was calculated by applying the following formula:

\[
\text{Growth inhibition} (\%) = \left( \frac{\text{Growth in control} – \text{Growth in treatment}}{\text{Growth in control}} \right) \times 100
\]

**Statistical analysis**

All the data were analyzed by analysis of variance (ANOVA) followed by Duncan’s Multiple Range Test to delineate the treatment means (Steel and Torrie, 1980), using computer software SPSS.

**RESULTS AND DISCUSSION**

Analysis of variance regarding the effect of different concentrations of methanolic shoot, root and inflorescence extracts of *S. halepense* is presented in Table 1. The effect of different plant parts (P), the extract concentration (C) as well as the P × C was significant (P≤0.001) for fungal biomass.

**Antifungal activity of shoot extract**

Data regarding the effect of methanolic shoot extract of *S. halepense* on the biomass of *M. phaseolina* is illustrated in Figures 1A and 2. Among the various parts of the test grass species, shoot extract exhibited the highest antifungal activity where all the extract concentrations significantly reduced the fungal biomass. There was a gradual reduction in fungal biomass with increasing extract concentration. Different concentrations of the shoot extract reduce fungal biomass by 14 to 61%.

This concentration dependent activity of the plant is in
agreement with the finding of earlier workers, where higher concentration of plant extracts exhibited greater antifungal activity than the lower ones (Abd-El-Khair and Haggag, 2007; Aslam et al., 2010). Recently, Yanar et al. (2011) evaluated 26 plant extracts including S. halepense for the control of Phytophthora infestans using radial growth technique. They found out that shoot extract of S. halepense was effective and reduced the fungal growth significantly as compared to the control. Antifungal action of S. halepense could be attributed to a number of phenolics and flavonoids like p-hydroxybenzadehye, tricin, p-hydroxybenzonic acid, p-hydroxycinnamic, luteolin, apigenin and salcolon present in aerial part of S. halepense (Huang et al., 2011). Antifungal activity of phenolic compounds of plants has also been reported by other workers (Okwu, 2007; Esekhiagbe et al., 2009). Huang and Chaung (2003) described possible mechanism for antifungal activity of phenolics includes swelling of hyphal tip followed by seeping and leaking of plasma due to distortion of cell wall resulting in abnormal branching, fusion and wrinkling of hyphae. In addition, Kanwal et al. (2010) observed significant reduction in the growth of five plant pathogenic fungi including M. phaseolina due to the action of flavonoid isolated from mango leaves. The major groups of flavonoids in sorghum are the flavans, flavinols and anthocyanidins (Waniska, 2000), which may be responsible for its antifungal activity. Results of other studies also provide evidence that plant flavonoids inhibited spore germination and growth of fungal pathogens like Phomopsis longicolla, Colletotrichum truncatum, Verticillium albaatrum, Trichophyton
mentagrophytes and Cryptococcus neoformans (Svetaz et al., 2004; Galeotti et al., 2008; Sathiamoorthy et al., 2007).

Antifungal activity of root extract

Data concerning the effect of methanolic root extract of S. halepense on the biomass of M. phaseolina is presented in Figures 1B and 2. The antifungal effect of methanolic root extract against M. phaseolina was less pronounced as compared to the shoot extract. The effect of the lowermost concentration (0.5%) was insignificant, while the rest of the concentrations significantly suppressed the fungal biomass. There was 9 to 48% reduction in fungal biomass over control due to various concentrations of the methanolic root extract. Liu et al. (2011), identified ethyl p-hydroxybenzoate and p-hydroxybenzaldehyde as active phytoxins in subterranean part of Johnson grass, which may be responsible for antifungal activity against M. phaseolina.

Antifungal activity of inflorescence extract

Data about the effect of methanolic inflorescence extract on biomass of M. phaseolina is as shown in Figures 1C and 2. Although, all the concentrations of methanolic inflorescence extract exhibited adverse effect on the growth of M. phaseolina; however, inflorescence extract was found to be the least effective against the target fungal pathogen. The effect of lower concentrations of 0.5 to 1.5% was insignificant, while higher concentrations exhibited significant effect. The overall reduction in fungal biomass was 4 to 39% due to different concentrations of inflorescence extract. The present study concludes that methanolic extracts of different parts of S. halepense contain antifungal constituents. The highest antifungal activity was, however, exhibited by shoot extract. Further studies are required to isolate and identify the effective antifungal constituents from methanolic shoot extract.

REFERENCES


Table 1. Analysis of variance for the effect of different concentrations of methanol shoot, inflorescence and root extracts of S. halepense on in vitro growth of M. phaseolina.

<table>
<thead>
<tr>
<th>Sources of variation</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F values</th>
</tr>
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<tbody>
<tr>
<td>Treatments</td>
<td>20</td>
<td>0.138</td>
<td>0.00689</td>
<td>28.6*</td>
</tr>
<tr>
<td>Plant parts (P)</td>
<td>2</td>
<td>0.044</td>
<td>0.02290</td>
<td>92.4*</td>
</tr>
<tr>
<td>Conc. (C)</td>
<td>6</td>
<td>0.083</td>
<td>0.01393</td>
<td>57.7*</td>
</tr>
<tr>
<td>P × C</td>
<td>12</td>
<td>0.010</td>
<td>0.00081</td>
<td>3.4*</td>
</tr>
<tr>
<td>Error</td>
<td>63</td>
<td>0.015</td>
<td>0.00024</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>2.78</td>
<td>-</td>
<td>-</td>
</tr>
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*Significant at P ≤ 0.001.


