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# ANTIFUNGAL ACTIVITY OF METHANOLIC ROOT EXTRACT OF *WITHANIA SOMMNIFERA* AGAINST *FUSARIUM OXYSPORUM* F. SP. *CEPAE*

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## Abstract

**Background**: Basal rot of onion (*Allium cepa* L.) caused by *Fusarium oxysporum* f. sp. *cepae* is a common soil-borne disease that causes significant yield losses. Generally, synthetic fungicides are used to combat the menace which causes environmental pollution. The present study was carried out to assess the antifungal activity of *Withania somnifera* (L.), Dunal, a Solanaceous medicinal plant, against the pathogen of this disease.
**Materials and Methods:** Different concentrations (from 0.5 to 4%) of methanolic extract of root stem and fruit of the test plant species were prepared and their bioactivity was assessed against the target fungal pathogen. Methanolic extract of root was further fractionated with *n*-hexane, chloroform, ethyl acetate and *n*-butanol. A range of concentrations of these extracts viz. 200, 100… 3.125 mg $mL^{-1}$ were prepared and assessed for their antifungal activities.
**Results:** Methanolic root extract exhibited the best antifungal activity, causing up to 93% decrease in biomass of the fungal pathogen. *n*-hexane, chloroform and ethyl acetate fractions of methanolic root extract exhibited pronounced antifungal activity resulting in 46–79%, 40–73% and 35–76% reduction in fungal biomass respectively.
**Conclusion:** The present study concludes that root extract of *W. somnifera* possesses potent antifungal constituents which can be used for the control of *F. oxysporum* f. sp. *cepae.*

**Key words:** Antifungal activity, basal rot of onion, bioassays guided fractionation, *Fusarium oxysporum* f. sp. *cepae, Withania sommnifera.*

## Introduction

Onion (*Allium cepa* L.) is a horticultural crop of great economic importance. It is widely cultivated all over the world and probably was one of the first domesticated vegetables by man (Griffiths et al., 2002). Onion bulbs are commonly used in human diet across the world (Golovchenko et al., 2012). In addition to its value as a food crop, it also has medicinal importance (Virginia, 2006; Jenwitheesuk et al., 2012). Onion is susceptible to various fungal pathogens including *Fusarium oxysporum* f. sp. *cepae* that causes basal rot disease in many countries of the world (Bayraktar, 2010). The disease can result in serious crop failure and monetary losses in various regions of the world where onion is cultivated (Özer and Köycü, 2004). Although, there are numerous strategies to deal with fungal diseases and minimize yield losses but presently, use of chemical fungicides is considered the most suitable one (Than et al., 2008). Although, use of synthetic agro-chemicals are helpful in controlling soil-borne diseases but they also have bad effects on beneficial soil microorganisms and pollute the environment. Environmental pollution can be minimized by using natural compounds from plants (Javaid and Iqbal, 2014; Javaid and Rauf, 2015). In the recent years, there are many success stories regarding the management of fungal plant pathogens by using crude plant extracts (Iqbal and Javaid, 2012; Javaid and Saddique, 2012; Javaid and Samad, 2012; Synowiec et al., 2014), purified isolated compounds (Kanwal et al., 2010; Jabeen et al., 2011), and incorporation of plant materials in the soil (Riaz et al., 2010; Javaid and Saddique, 2011).

*Withania somnifera*, a perennial shrub of the family *Solanaceae*, is a plant of great medicinal importance being widely used as a home remedy for many diseases in various regions of the world, especially in Indian subcontinent (Alam et al., 2012). It helps the body to adapt against different types of stresses and particularly useful in stress related disorders like hypertension, diabetes and arthritis (Kaurav et al., 2012). Its medicinal properties are due to withanolides, the characteristic secondary metabolites of this plant species (Matsuda et al., 2001). Few studies also show that the plant has herbicidal (Javaid et al., 2010, 2011), and antifungal activates (Ghosh, 2009). However, studies regarding antifungal activity of *W. somnifera* against *F. oxysporum* f. sp. *cepae* are entirely lacking. This study was, therefore, carried out to assess the antifungal properties of methanolic extracts of various parts of *W. somnifera* for the management of *F. oxysporum* f. sp. *cepae*, isolated from onion suffering from basal rot disease.

## Materials and Methods

### Bioassays with Methanolic Extract

Fruits, stems and roots of mature *W. somnifera* were collected from Lahore, Pakistan, sun dried and ground to fine powders. One hundred and fifty grams of each powdered plant material was soaked in 1000 mL methanol separately for 5 days. The soaked materials were filtered first through muslin cloth and finally by filter papers. The filtrates were evaporated on rotary evaporator (Model ROTVAP, UTECH Products INC. Albani NY, USA) at 45 °C.

Methanolic extract (14.4 g) of each part was dissolved in 2 mL dimethyl sulphoxide (DMSO) and volume was raised to 18 mL by adding autoclaving distilled water to prepare stock solution. Seventy six milliliter malt extract was autoclaved in 250 mL flasks and cooled at room temperature. Eight concentrations viz. 0.5, 1, 1.5, ….4% were prepared by the addition of 0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 4 mL of stock solution to 3.5,

3, 2.5, 2, 1.5, 1, 0.5 and 0 mL of autoclaved distilled water respectively to raise the volume of the medium 80 mL in each flask. This amount of the medium was divided into four equal portions to serve as replicates. For control, 2 mL of DMSO was added to 16 mL of water, and 4 mL of this mixture was added to 76 mL malt extract. Mycelial discs of *F. oxysporum* f. sp. *cepae* were prepared from the tips of 7 days old fungal culture using a sterilized 2 mm diameter cork-borer and transferred to each 100-mL volume flasks. There was 20 mL of the medium in each flask. Flasks were incubated at 27±1°C for 7 days. After that, the fungal mycelium was filtered and dried in an electric oven at 60 °C (Iqbal and Javaid, 2012).

**Fractionation of Methanolic Root Extract**

One kilogram of dried and powdered roots was extracted with 3L of methanol at room temperature for one week. After that, material was filtered and the residues were again soaked in 3 L of methanol and filtered again after one week. Filtrates were evaporated on a rotary evaporator under vacuum. Extract obtained after completely evaporating methanol was dissolved in 200 mL distilled water and partitioned using *n*-hexane (500 × 4), chloroform (600 mL), ethyl acetate (400 mL) and *n*-butanol (400 mL) in separating funnels followed by evaporation under vacuum to yield 4.1 g *n*-hexane fraction, 2.7 g chloroform fraction, 3.0 g ethyl acetate fraction and 2.5 g *n*-butanol fraction. Finally, 3.2 g was obtained by evaporating materials in a similar way.

**Bioassay with Fractions of Methanolic Root Extract**

The various fractions obtained from methanolic root extract were evaluated for their antifungal activity against *F. oxysporum* f. sp. *cepae*. An amount of 1.2 g of each fraction of root extract was mixed in 1 mL DMSO. To this, malt extract broth was added to make total volume 6 mL. This medium of 200 mg $mL^{-1}$ concentration was continually diluted by adding growth medium and a range of concentrations viz. 100, 50, …, 3.125 mg $mL^{-1}$ were prepared. For control, 5 mL malt extract medium was mixed with 1 mL DMSO and successively diluted to prepare separate control treatments for different extract concentrations. Bioassays were carried out in 10-mL volume glass test tubes, each containing 1 mL of the medium. Inoculum of *F. oxysporum* f. sp. *cepae* was prepared at concentration of 1000 spores per mL by hemocytometer and 500 µl of this inoculum was added to each test tube. There were three replicates of each treatment. Incubation was done at room temperature. After 7 days, fungal mycelium was filtered, dried and weighed (Javaid and Samad, 2012).

**Statistical Analysis**

All the data were subjected to Analysis of Variance followed by application of Duncan's Multiple Range tests at $P \leq 0.05$ (Steel and Torrie, 1997).

## Results and Discussion

**Antifungal Activity of Methanolic Extracts**

The effect of 0.5–3.5% concentrations of the fruit extract was insignificant on fungal growth. The lower concentrations of 0.5–2.5% insignificantly reduced the fungal biomass by 6–15% while the higher concentrations stimulated the fungal growth. The highest concentration of 4% significantly enhanced fungal biomass by 50% over control. In general, there was a gradual increase in biomass of *F. oxysporum* f. sp. *cepae* as the concentration of the extract was increased (Fig. 1A, 2). The effect of lower concentrations of 0.5 to 2.0% of stem extract was inhibitory and significant where a decrease of 38–50% in fungal biomass was recorded. In contrast, the effect of higher concentrations of 2.5 to 4.0% was stimulatory and insignificant where generally fungal biomass was increased gradually with the increase of extract concentration from 0.5% to 4% (Fig. 1B, 2). Root extract played a very significant role in decreasing fungal growth as compared to control. The fungal biomass generally decreased as the concentration of extract increased from 0.5% to 4.0%. The effect of lower concentrations of 0.5 to 2.0% was insignificant where only 6 to 38% reduction in biomass of the fungus was noted. Conversely, the higher concentrations of 2.5 to 4.0% exhibited pronounced inhibitory effect on the fungal growth and significantly reduced the fungal biomass by 44 to 93% (Fig. 1C, 2). Earlier, Dhuley (1998) reported the antifungal activity of *W. somnifera* root against *Aspergillus fumigates*. In contrast to the present study, recently, Javaid and Munir (2012) reported that methanolic stem and fruit extracts of *W. somnifera* significantly declined growth of *Ascochyta rabiei* while methanolic root extract was not effective against this chickpea blight pathogen. It indicates that methanolic extracts of different parts of *W. somnifera* have specificity in their antifungal activity against different phytopathogens.

**Antifungal Activity of Fractions of Methanolic Root Extract**

In general, all the organic solvent fractions of methanolic root extract exhibited antifungal activity against the target fungal pathogen. However, different fractions showed variable antifungal activity (Fig. 3). Different concentrations of *n*-hexane fraction decreased the fungal biomass significantly by 46-79% (Fig. 3A). Similarly, the adverse effect of all the concentrations of ethyl acetate, chloroform and *n*-butanol fractions was also significant. There was 40-73%, 35-76% and 40-67% reduction in biomass of the test fungus due to various concentrations of chloroform, ethyl acetate and *n*-butanol fractions, respectively, as compared to corresponding control treatments (Fig. 3B-D). Aqueous fraction, however, showed somewhat different behaviour than the organic solvent fractions. Higher concentrations of 12.5–200 mg $mL^{-1}$ significantly declined fungal biomass over corresponding control treatments. By contrast, in lower concentrations of 3.125–6.25 mg $mL^{-1}$ the fungal biomass increased from 4-24% as compared to control. Stimulatory effect of the lowermost concentration (3.125 mg $mL^{-1}$) was statistically significant as compared to corresponding control treatment (Fig. 3E). Since the four organic solvents used for fractionation of methanolic root extract had different polarity and all the organic solvent fractions exhibited antifungal activity, it seems probable that the root of *W. somnifera* possesses a verity of antifungal compounds for the management of *F. oxysporum* f. sp. *cepae*. Girish et al. (2006) identified a monomeric glycoprotein from roots of *W. somnifera* that demonstrated potent activity against bacteria and phytopathogenic fungi. Withanoldes are the chief chemical compounds reported in *W. somnifera*. Among these is

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withaferin A, the most important which may be the cause of antifungal activity (Kannan and Kulandaivelu, 2007). *W. somnifera* also contain flavonol glycosides, glycowithanoldes, phenolics and sterols (Kandil et al., 1994), which may be responsible for its antifungal activity.

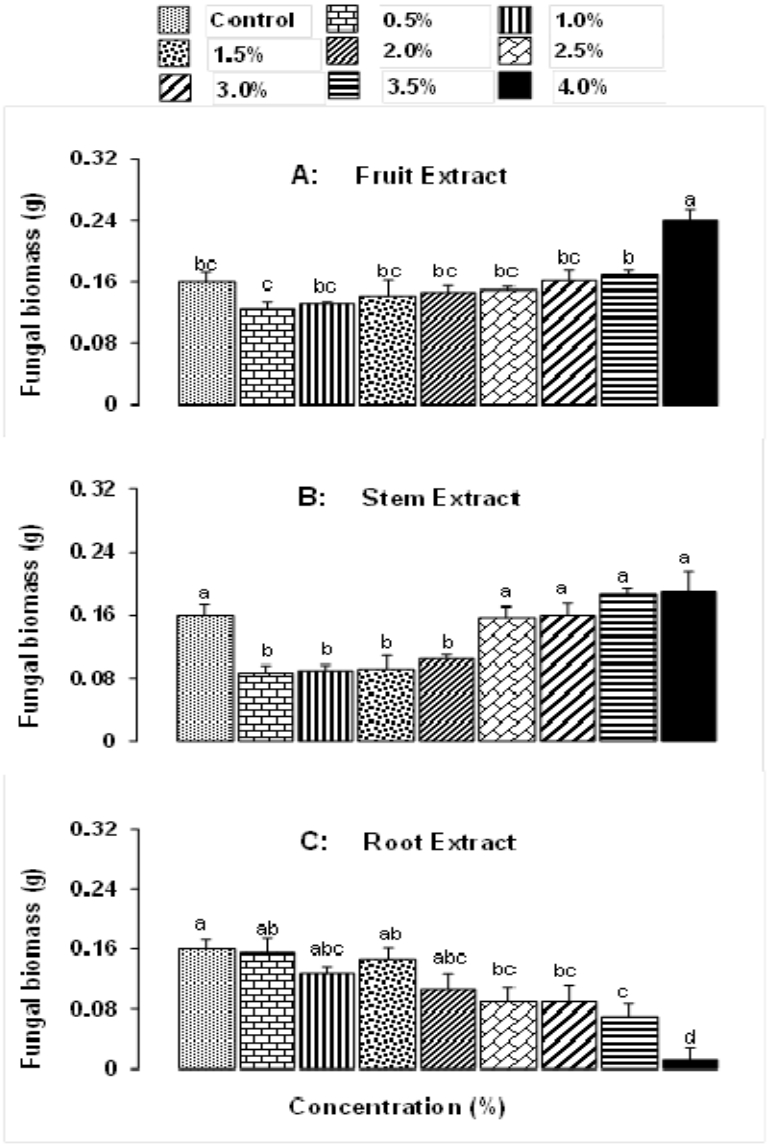


**Figure 1:** Effect of different concentrations of methanol fruit, stem and root extracts of *Withania somnifera* on biomass of *Fusarium oxysporum* f.sp. *cepae.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

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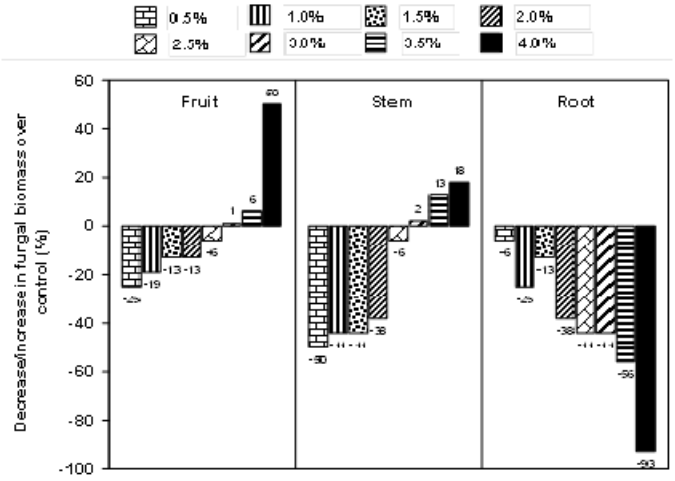


**Figure 2:** Percentage increase/decrease in biomass of *Fusarium oxysporum* f. sp. *cepae* due to different concentrations of methanolic fruit, stem and root extracts of *Withania somnifera* over control

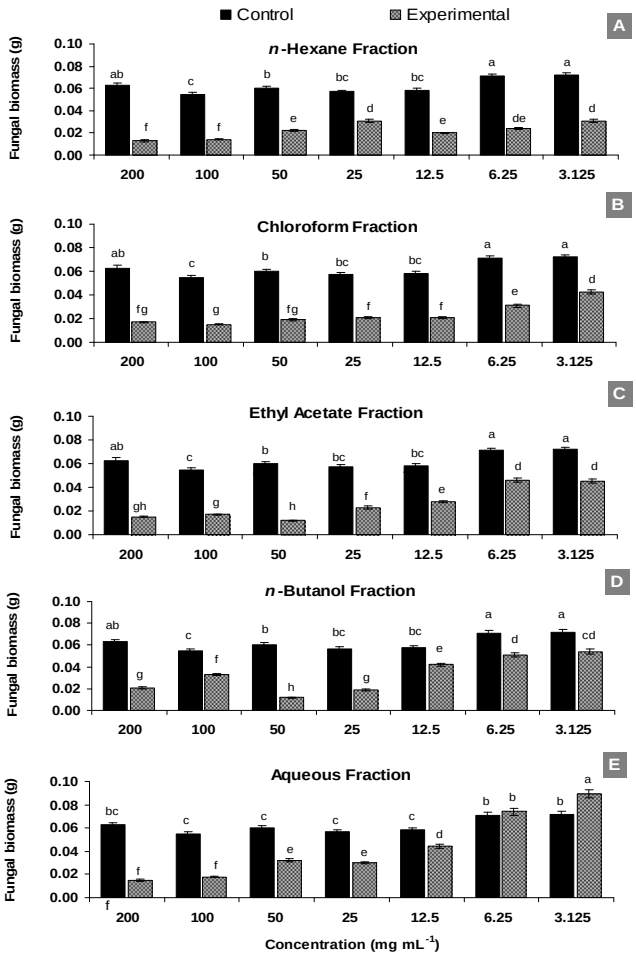


**Figure 3:** Effect of different fractions of methanolic root extract of *Withania somnifera* on growth of *Fusarium oxysporum* f. sp. *cepae*. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

**Conclusion**

The present study concludes that methanolic root extract of *W. somnifera* and its different organic fractions especially n-hexane fraction can be used for the control of *F. oxysporum* f. sp. *cepae*.

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