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# RADISH EXTRACTS AS NATURAL FUNGICIDES FOR MANAGEMENT OF *FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI*, THE CAUSE OF TOMATO WILT

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**Abstracts**

*Fusarium oxysporum* f. sp. *lycopersici*, the cause of tomato (*Solanum lycopersicum* L.) wilt disease, is responsible for significant yield losses in this crop. Generally, chemical fungicides are used to combat the menace. However, due to adverse effects of synthetic agrochemicals on health and environment, scientists are in search of eco-friendly alternatives for management of phytopathogens. The present study was conducted for the control of *F. oxysporum* f. sp. *lycopersici* using methanolic extracts of radish (*Raphanus sativus* L.). In a laboratory screening bioassay, antifungal activity of different concentrations (1-6%) of methanolic root, leaf and fruit extracts of radish was studied against *F. oxysporum* f. sp. *lycopersici*. Malt extract broth was used as growth medium. Root and leaf extracts showed higher antifungal activity than the fruit extract. Various concentrations of methanolic root, leaf and fruit extracts of radish reduced fungal biomass by 30–39%, 39–52% and 20–35, respectively, over control. Methanolic extracts of leaves and roots were further partitioned using *n*-hexane, chloroform, ethyl acetate and *n*-butanol. Antifungal activity of seven concentrations (ranging from 3.125 to 200 mg $mL^{-1}$) of each of the sub-fraction of methanolic extracts was evaluated against the pathogen. Among various sub-fractions of leaf extract, chloroform sub-fraction showed the best antifungal activity causing 52–64% reduction in fungal biomass. Likewise, ethyl acetate and *n*-butanol sub-fractions of root extract exhibited the best antifungal activity causing 52–96% and 62–95% reduction in fungal biomass over corresponding control treatments, respectively. The present study concludes that ethyl acetate and *n*-butanol sub-fractions of root extract of radish can be exploited for management of *F. oxysporum* f. sp. *lycopersici*.

**Key words:** Antifungal, *Fusarium oxysporum* f. sp. *Lycopersici*, Fungicides, Radish, *Raphanus sativus*, Tomato wilt.

## Introduction

Tomato is among the most popular and economically important vegetable crops grown throughout the world (Anitha & Rabeeth, 2009). Fusarium wilt caused by *F. oxysporum* f. sp. *lycopersici* is a destructive disease of tomato globally (Castano *et al.,* 2013). Prochloraz and carbendazim fungicides are very effective against this fungal pathogen (Song *et al.,* 2004). However, extensive use of agrochemicals can cause a number of negative effects including environmental pollution and development of resistance in the pathogen against the repeatedly applied chemicals (Oruc, 2010). Due to increasing public concerns on environmental problems, scientists are now in search of alternative eco-friendly disease management strategies based on natural resources (Jabeen *et al.,* 2011; Afridi & Khan 2014; Afridi *et al.,* 2014; Javaid & Rauf, 2015; Javaid *et al.,* 2015).

Radish belongs to the Brassicaceae family that is grown for its fleshy edible roots. It is a source of medicinally important compounds isothiocyanates and peroxidases (Curtis, 2003). Many cultivated species of Brassicaceae especially belonging to genus *Brassica* and some weeds such as *Coronopus didymus* are known to possess substantial antifungal potential (Fahey *et al.,* 2001; Javaid & Iqbal, 2014). Few reports also showed that radish exhibit antifungal activity (Truta *et al.*, 2011). However, studies regarding use of radish extracts for management of *F. oxysporum* f. sp. *lycopersici* causing Fusarium wilt of tomato are entirely missing. The present research work was, therefore, carried out to assess antifungal activity of methanolic extracts of different parts of radish and their sub-fractions against *F. oxysporum* f. sp. *lycopersici*.

## Materials and Methods

**Screening bioassays:** The dried fruit, leaf and root materials were ground into fine powder and 200g of each were soaked in 1000mL methanol separately for 14 days. The soaked materials were filtered through cheese cloth and then filter papers. The filtrates were evaporated on rotary evaporator at 45°C to yield 9.45, 13.28 and 15.5g of crude methanolic extract of leaves, roots and fruits of radish, respectively.

Methanolic extracts (9.45 g) of each of the selected part of radish were dissolved in 6mL dimethyl sulphoxide (DMSO) and final volume (21 mL) was raised by adding autoclaved distilled water to prepare a stock solution. Similarly, a control solution was prepared by dissolving 6mL DMSO in 15mL distilled water. Thirty nine milliliters of malt extract were autoclaved in 250mL flasks and cooled at room temperature. Six concentrations were prepared by adding 1, 2, 3, 4, 5 and 6mL of stock solution with 5, 4, 3, 2, 1 and 0mL of control solution, respectively, to raise the volume of the medium 45mL in each flask, which was divided into three portions of 15 mL each in 100mL flasks. For control, 6mL of control solution was added to 39mL of autoclaved malt extract broth and divided into 3 equal parts. Mycelial discs of *F. oxysporum* f. sp. *lycopersici* were made using a sterilized 5 mm diameter cork borer from the tips of 7 days old fungal culture and transferred to each flask. Flasks were incubated at 27±1°C for 7 days. Thereafter, the fungal biomass in each flask was filtered and dried in an electric oven at 60 °C and weighed (Iqbal & Javaid, 2012).

**Fractionation of methanolic leaf and root extracts of radish:** Two kilograms of thoroughly crushed dried leaves and 1.5 kg of roots were soaked in 5L methanol for

one week. Thereafter, materials were filtered through cheese cloth and the residues were re-extracted with methanol. Methanolic extract was filtered through filter paper and evaporated. Evaporation of the solvent was done on a rotary evaporator.

To crude methanolic leaf extract, 200mL water was added and *n*-hexane soluble compounds were separated by repeatedly mixing the aqueous phase with *n*-hexane and separating using a separating funnel of 1000mL volume. Then the remaining extract was successively extracted with chloroform (300 mL), ethyl acetate (300 mL), and *n*-butanol (2 × 300 mL), respectively. All the organic solvent fractions and the remaining aqueous fraction were evaporated on a rotary evaporator to yield 9.1 g of *n*-hexane, 24.3 g of chloroform, 1.9 g of ethyl acetate, 6.2 g of *n*-butanol and 19.9 g of aqueous fraction.

To crude methanolic root extract, 200 mL water was added and fractionated by successive solvent extractions with *n*-hexane, chloroform, ethyl acetate and *n*-butanol, and after evaporation under reduced pressure on a rotary evaporator 4.1, 2.7, 3.0, 2.5 and 3.2 g of each fraction, respectively, were obtained.

**Bioassays with fractions of methanolic extracts:** The various sub-fractions from methanolic leaf and root extracts obtained were evaluated *in vitro* for their antifungal activity. An amount of 1.2 g of each sub-fraction of methanolic leaf and root extracts was dissolved in 1 mL DMSO. These dissolved materials were mixed in 5 mL malt extract broth to prepare 6 mL of stock solution having a concentration of 200 mg $mL^{-1}$. Half the medium was used for bioassays while the rest half was serially double diluted to prepare lower concentrations up to 3.125 mg $mL^{-1}$. For control, 1 mL of DMSO and 5 mL of malt extract broth were mixed and serially double diluted for preparation of control treatments with same concentrations of DMSO as were present in different extract treatments. Bioassays were conducted in triplicate in test tubes of 10 mL volume. Each test tube contained 1 mL of the growth medium. Inoculum of *F. oxysporum* f. sp. *lycopersici* was prepared in distilled water and 20µL of this inoculum was added to each test tube and incubated at 26°C. After 7 days, fungal biomass from each treatment was filtered on pre-weighed filter papers. Fungal biomass along with filter papers was dried at 60°C and weighed. Fungal biomass was calculated by subtracting filter paper weight from total weight (Javaid & Iqbal, 2014).

All the data were analyzed by analysis of variance. Means were separated by LSD method at 5% level of significance using computer software Statistics 8.1.

## Results and Discussion

Analysis of variance showed that the effect of methanolic extract of various parts of the test plant (P) was significant for fungal biomass. In a similar way, the effect of extract concentration (C) as well as the effect of P × C was also significant for the studied parameter (Table 1). The highest fungal biomass (230 mg) was recorded in control. Methanolic leaf extract showed significant antifungal activity. Fungal biomass in different concentrations of leaf extract ranged from 140–160 mg. All the leaf extract concentrations significantly reduced fungal biomass by 30–39% over control. Similar to that of leaf extract, root extract also proved highly effective in suppressing fungal growth. There was 110–140 mg fugal biomass in different root extract treatments as compared to 230 mg in control. Different concentrations of root extract declined fungal biomass by 39–52% as compared to control. All the concentrations of fruit extract also suppressed fungal biomass significantly as compared to control. However, in general fruit extract proved comparatively less effective than leaf and root extracts in suppressing the fungal growth. Various fruit extract concentrations reduced fungal biomass by 20–35% as compared to control (Fig. 1). There are some earlier reports where methanolic extracts of Brassicaceous plant species such as *Coronopus didymus* were used to control *Sclerotium rolfsii* and *Fusarium oxysporum* f. sp. *gladioli*, and very encouraging results were obtained (Riaz *et al.*, 2010; Iqbal & Javaid, 2012).

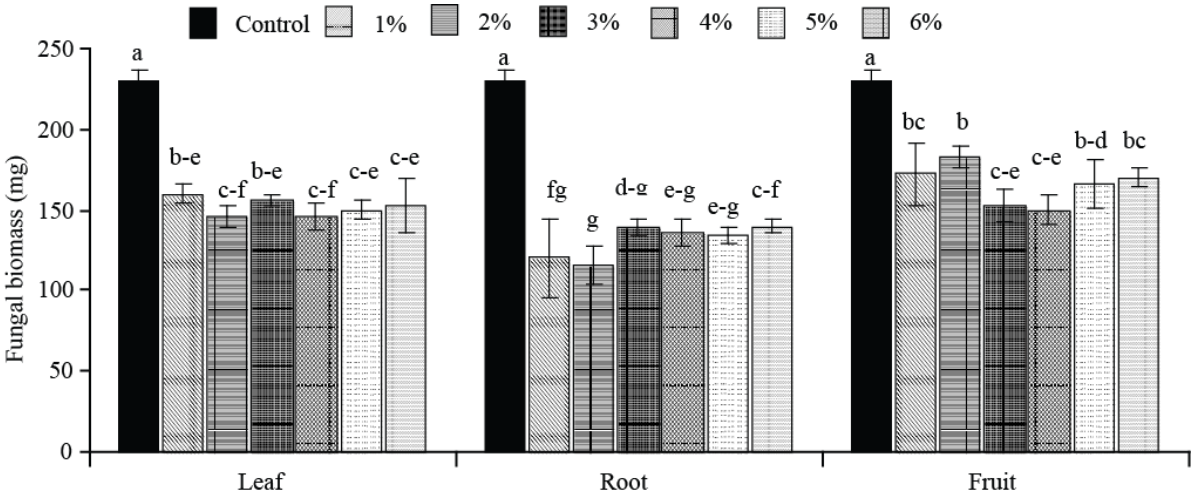


Fig. 1. Effect of different concentrations (1-6%) of methanol leaf, root and fruit extracts of *Raphanus sativus* on biomass of *Fusarium oxysporum* f. sp. *lycopersici.* Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD method.

**Table 1. Analysis of variance (ANOVA) for the effect of different concentrations of methanolic leaf root and fruit extracts of radish on biomass of *Fusarium oxysporum* f. sp. *lycopersici*.**

| Sources of variation | df | SS | MS | F values |
|---|---|---|---|---|
| Treatments | 20 | 64276 | 3214 | 11.3* |
| Plant parts (P) | 2 | 8429 | 4214 | 14.8* |
| Concentration (C) | 6 | 50055 | 8343 | 29.3* |
| P × C | 12 | 5792 | 483 | 1.7NS |
| Error | 42 | 11978 | 285 | |
| Total | 62 | 76254 | | |

*, Significant at P≤0.001.,NS: Non-significant

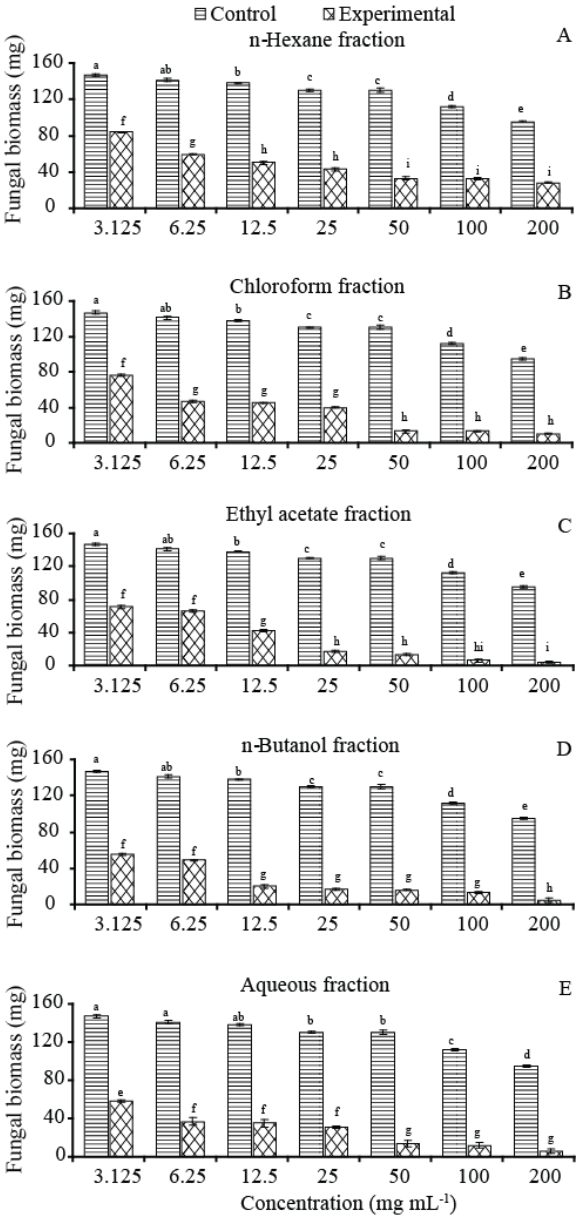


Fig. 2. Effect of different concentrations of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction of methanolic leaf extract of *Raphanus sativus* on growth of *Fusarium oxysporum* f. sp. *lycopersici.* Values with different letters at their top show significant difference (P≤0.05) as determined by LSD method.

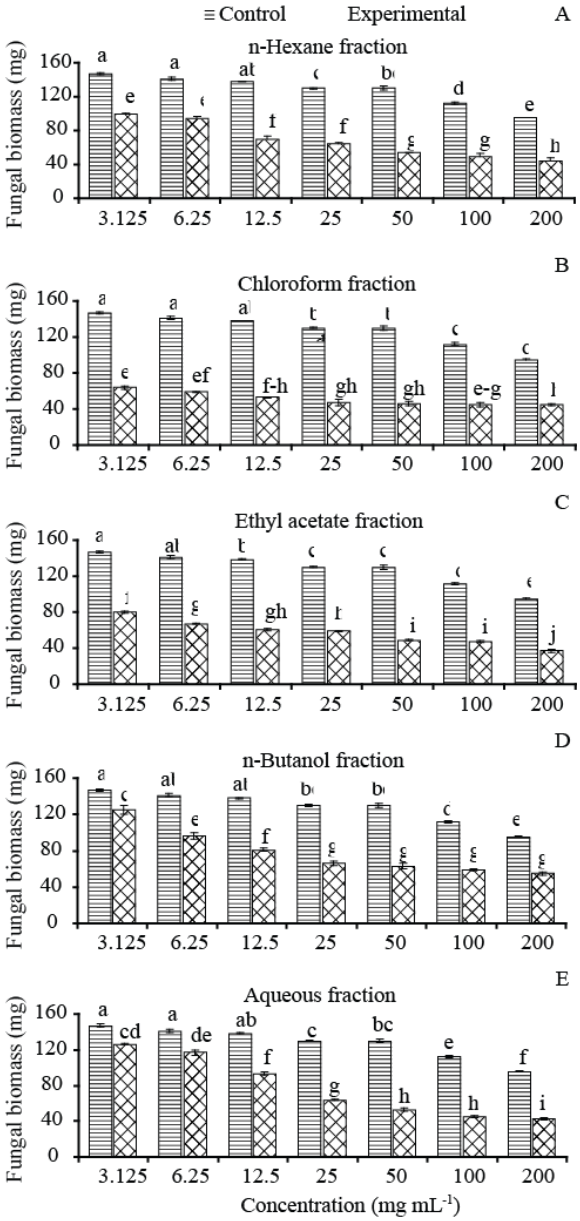


Fig. 3. Effect of different concentrations of *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fraction of methanolic root extract of *Raphanus sativus* on growth of *Fusarium oxysporum* f. sp. *lycopersici.* Values with different letters at their top show significant difference (P≤0.05) as determined by LSD method.

Various sub-fractions of methanolic leaf extract showed highly variable antifungal activity against *F. oxysporum* f. sp. *lycopersici*. Chloroform sub-fraction exhibited the highest antifungal activity followed by ethyl acetate sub-fraction resulting in 52–64% and 45–62% reduction in fungal biomass, respectively, over corresponding control treatments. *n*-hexane, *n*-butanol and aqueous sub-fractions proved comparatively less effective resulting in 32–58%, 15–51% and 14–60% reduction in fungal biomass, respectively, over respective control treatments (Fig. 2).

In general, the antifungal effect of different sub-fractions of methanolic root extract against *F. oxysporum* f. sp. *lycopersici* was more pronounced than different sub-fractions of methanolic leaf extract. The highest antifungal activity was exhibited by *n*-butanol followed by ethyl acetate sub-fraction resulting in 62–95% and 52–96% reduction in fungal biomass, respectively, over control. Chloroform, *n*-hexane and aqueous sub-fractions also exhibited marked antifungal activity causing 48–90%, 43–75% and 60–76% reduction in fungal growth, respectively, over control (Fig. 3). Major chemical constituents of radish include isothiocyanates and glucosinolates (Papi *et al.,* 2008; Hanlon & Barmes, 2011), phenolic acids (Sgherri *et al.,* 2003) and anthocyanins (Wang *et al.,* 2010). Antifungal effects of Brassicaceous species are generally due to the degradation products of glucosinolates. Over twenty different aromatic and aliphatic isothiocyanates have been identified among degradation products of glucosinolates from different species of Brassicaceae (Brown & Morra, 1995, 1996). Isothiocyanates are known to inhibit mycelial growth of many fungal species including *Leptosphaeria maculans* (Mithen & Lewis, 1986), *Gaeumannomyces graminis* (Angus, 1994), *Fusarium graminearum*, *Rhizoctonia solani*, *Pythium irregulare* and *Bipolaris sorokiniana* (Sarwar *et al.,* 1998). In addition, radish also contains phenolic acids which are well known for their antifungal activity (Reddy *et al.,* 2012). Raphanin is another radish component with antibacterial and antifungal properties (Truta *et al.*, 2011).

It is concluded that ethyl acetate and *n*-butanol fractions of methanolic root extracts possess pronounced antifungal potential against *F. oxysporum* f. sp. *lycopercisi* and can be used for management of this pathogen.

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