

## Research Article

## Antifungal activity of *Brassica napus* water extract on some phytopathogenic fungi

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**Abstract:** Some plant extracts have been reported to exhibit antimicrobial properties. Plant based pesticides appear to be an alternative for the synthetic pesticides because of their less dangerous impacts. The objective of this study was to assess the inhibitory activity of *Brassica napus* water extract on mycelial growth of six phytopathogenic fungi *Rhizoctonia solani*, *Phytophthora drechseleri*, *Pythium aphanidermatum*, *Verticellium dahliae*, *Fusarium oxysporum*, and *Gaeumannomyces graminis*. Antifungal activity test was performed by disc diffusion method. Concentrations of 100 and 50 ppm had the highest and the lowest inhibitory effects on all studied species respectively. The shoot extract (SE) was significantly exerted higher antifungal activity than root extract (RE). At 100 ppm, *V. dahliae* (17.02% inhibition by RE) and *F. oxysporum* (50% inhibition by SE) were the most sensitive species however, *R. solani* (1.8 and 15%) was the most resistant fungus to both extracts. Active compounds of *B. napus* extract were determined by high performance liquid chromatography (HPLC). Phenolic compounds had the highest concentrations in extracts and are probably the main cause of the mycelial growth inhibition. Application of canola aqueous extract or incorporation of canola in crop rotation program can be considered as a method for management of some soil-borne phytopathogenic fungi.

**Keywords:** chromatography, disk diffusion, phenolic compound, plant extract, soil borne fungi.

### Introduction

Management of fungal disease in food crops and ornamental plants is economically important. Numerous methods including physical, chemical, cultural and biological are available to control various plant diseases. Various control methods of soil-borne diseases have been explored throughout the world, including fungicide (Asherand payne, 1989;

Heijbroek and Huijbregtes, 1995) biological control agents (Lewis and Papavizas, 1987), Seed priming (Obsurn and Schroth, 1989), Crop rotation (Rush and Winter, 1990), Fumigation (Harveson and Rush, 1994) and resistant plant cultivars (Goldman, 1996). Some of these means and methods are costly and time-consuming. Effective management of crop disease can generally be achieved using synthetic pesticides (Kiran *et al.*, 2006). Due to environmental risks of pesticides application, much attention is focused on alternative methods of pathogen control. The high cost of chemical fungicides, soil pollution, accumulation of residues in the water and air,

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development of resistant races, are noticed by scientists to look for the methods which are environmentally friendly and act specifically against pathogens or have fewer side effects. The continual use of fungicides has exposed human health to serious threats such as mutagenic, carcinogenic or teratogenic effects. Keeping in view the drawback of chemical management of plant diseases, the use of plant extracts in the management of plant diseases is considered as a safe and cost effective method. (Bankole, 1997; Gould, 1996). Natural plant extracts are of interest as a safer source or more effective substitutes for synthetically produced antimicrobial agents and may provide an alternative way to prevent food or feed from fungal contamination (Mahmoud, 1999; Mei-chin and Wen-shen, 1998; Thanaboripat, 2002). Therefore, alternative biodegradable compounds without creating environmental pollution should be discovered to replace synthetic pesticides. Natural plant extracts can play a role in protecting plants from fungal infections (Mei-chin and Wen-shen, 1998).

Effect of plants essential oils has been investigated on fungal activities previously. Bluma *et al.* (2008) analyzed extracts of shoot, root, seed and bulbs of 40 Argentinean native plants including, thyme, poleo, rosemary, eucalyptus etc. against *Aspergillus flavus*. The results showed of 22 plants as sources of essential oils, only clove, anise, mountain thyme, oregano, boldus and poleo were able to produce growth inhibition zone in all *Aspergillus* section *Flavi*, whereas peppermint, eucalyptus, peperina, Louis herb, chamomile, marjoram, rosemary and lavender could only inhibit their sporulation. Of 40 ethanolic extracts, clove, poleo, mountain thyme and eucalyptus extracts affected growth and sporulation. Rodriguez *et al.*, 2005 tested shoot extract of *Aloe vera* against *Rhizoctonia solani*, *Fusarium oxysporum* and *Colletotrichum gloeosporioides* and showed that the best inhibitory effect was on *R. solani*. It was reported that *Aloe vera* extract could be used in the management of fungal diseases, although formulation of the compounds in

large scale trials would be problematic (Rodriguez *et al.*, 2005).

Canola (*Brassica napus* L.) is cultivated as a crop to produce oil, as a bio-diesel feedstock and also has been used as green manure to increase soil fertility (Copeland *et al.*, 1993, Griffin and Hesterman, 1991; Rinbott *et al.*, 2004, Shrestha *et al.*, 2007). There are also some reports indicating a considerable antimicrobial activity of canola extract against some soil-borne diseases. Larkin and Griffin (2007) observed antifungal activity of *B. napus* extract on soil-borne potato diseases including *Phytophthora erythroseptica*, *Pythium ultimum*, *Sclerotinia sclerotiorum*, *R. solani* and *F. oxysporum* with a range of inhibitory activity between 80-100%. An antifungal activity was also observed when Canola extract was applied against *R. solani* and *P. ultimum* (Charron and Sams, 1999). A number of antifungal compounds of diverse skeletal patterns have been found in plants, these compounds belong mainly to six broad chemical groups such as phenol and phenolic acids, coumarins and pyrones, flavonoids, isoflavonoids, steroids, alkaloids, and miscellaneous compounds (Mitra *et al.*, 1984).

Phenolic compounds or polyphenols are one of the most important groups of compounds occurring in plants, where they are widely distributed, comprising at least 8000 different known structures (Bravo, 1998). They are also products of the secondary metabolism of plants. They have other biological activities, such as antioxidant (Halliwell and Gutteridge, 2006), anti-inflammatory (Kupeli *et al.*, 2007), antibacterial (Rodriguez *et al.*, 2007) and assumed cancer-preventive effects (King and Young, 1999).

The analysis of phenolic compounds is very challenging due to the great variety and reactivity of these compounds (Bronze and Boas, 1998). There are various methods for analysis using modern separation and detection, such as hyphenated techniques of high performance liquid chromatography (HPLC) with mass spectrometry (MS), ultraviolet-visible light (UV/Vis), or nuclear magnetic

resonance (NMR) spectroscopy. The objectives of this study were (1) to test the *in vitro* inhibitory effect of *B. napus* extract on the mycelial growth of six soil-borne fungal plant pathogens that cause economic damage to commercially important crops such as potato, sugar beet, sunflower and wheat, (2) To determine the optimum concentration of *B. napus* extract that will reduce fungal mycelial growth and (3) to determine compounds present in its water extract by HPLC.

## Materials and Methods

### Plant materials

The plant materials (root and shoot) were collected from three months old plants which were grown in field condition at experimental field of Vali-e-Asr University of Rafsanjan, Iran during July 2011. Plant materials were washed and air dried in shade for two weeks.

### Fungal isolates

The fungal isolates used in this study included *Fusarium oxysporum*, *Rhizoctonia solani* (AG-4), *Pythium aphanidermatum*, *Phytophthora drechsleri*, *Verticillium dahliae* and *Gaeumannomyces graminis* and were obtained from fungal collection of Plant Protection Department of Vali-e-Asr University of Rafsanjan, Iran.

### Plant extraction

Dried plant materials were separately pulverized in a mill to a fine powder. Fifteen grams of dry powder were mixed with 100 ml distilled water and boiled for 1 h. The mixture was filtered using Whatman filter paper No 1 and then oven dried at 45 °C. Dried extract was weighted and resuspended in sterile distilled water to make concentrations of 0, 50, 75 and 100 ppm. The mixture was sterilized by syringe filtration (0.22 µm Millipore) and stored at -20 °C for further experiments.

### Antifungal activity

Potato dextrose agar (PDA) medium was used for culture maintenance and antifungal activity assessment. The extracts from either root or shoot were screened for their antifungal activity using standard disc paper diffusion according to

Bauer *et al.*, (1996) A small disc from the growing margin of a fungal colony on PDA was placed at the center of the plate and incubated for 24 h at 28 °C and then Paper disks were punched from Whatman filter paper (NO. 1) and were treated with different mentioned concentrations of the water extract and were placed in equidistant in each plate. Sterilized distilled water was used as a negative control. Inhibition zone was measured in mm after incubation period. The experiment was conducted in a completely randomized design with factorial arrangement of two factors (including fungus species and plant water extract) and three replications. Therefore, treatments consisted of combination of six fungus species and four concentrations (0, 50, 75 and 100 ppm) of plant water extract.

Mycelial growth of treatments was compared with the control. The percentages of inhibition of the extracts were calculated according to the following equation (Kasporowic *et al.*, 2010).

$$\text{Percentage of inhibition} = (T - C) / C \times 100$$

Where T and C are average mycelial growth in treatment and control respectively.

### Isolation of compounds using semi preparative HPLC

The phenolic composition of *B. napus* was determined by HPLC (Knauer, Germany), a method described by Gomez-Alonso *et al.*, (2002) with some modifications. Samples were filtered through a 0.45 µm membrane Millipore chromatographic filter before injection (injected volume, 50 µL). The separation was performed using C18 column (250 × 4.6 mm, Knauer, Germany). In the semi preparative HPLC analysis the mobile phases were acetic acid (0.5%) (Phase A) and acetonitrile (phase B), and the solvent gradient changed according to the following conditions: from 0 to 30 min, 95% A: 5% B. The injection volume for the isolation of the reference compounds was 100 µl of extract obtained from water extract.

### Statistical analysis

Statistical analysis was performed using SPSS (version 16.0) and data were subjected to analysis of variance (ANOVA). Means

were separated by Duncan's multiple range test at  $P < 0.05$ .

**Results**

Results of analysis of variance showed that the main effects of fungal species, extract concentration and incubation time as well as all double and triple interactions were significant (Table 1).

**Effect of root extract on mycelial growth**

The results showed that mycelium growth decreased with increasing time and concentration of root extract. Inhibition effect after 24, 48 and 72 h of incubation showed no significant differences between control and 50 ppm concentration (except for *F. oxysporum*). Application of 75 and 100 ppm of root water extract in all 3 incubation times significantly reduced mycelial growth compared to 0 and 50 ppm concentrations (except for *R. solani*) (Tables 2 and 3, Fig. 1-A). The highest inhibitory effects at 100 ppm after 72 h incubation were 17, 16.6, 15, 10, 9.2 and 1.8 percent for *V. dahliae*, *F. oxysporum*, *G. graminis*, *P. aphanidermatum*, *Ph. Drechseleri* and *R. solani*, respectively (Tables 2 and 3).

**Table 1** Summarized results of analysis of variance of the mycelial growth (cm) inhibition of shoot and root extracts on plant pathogen.

| Source of Variation | df  | F-value       |              |
|---------------------|-----|---------------|--------------|
|                     |     | Shoot extract | Root extract |
| Fungi (F)           | 5   | 26.004**      | 21.050**     |
| Concentration (C)   | 3   | 4.829**       | 0.108**      |
| Time (T)            | 3   | 460.161**     | 709.354**    |
| F × C               | 15  | 0.34 **       | 0.047**      |
| F × T               | 15  | 4.653**       | 3.290**      |
| C × T               | 9   | 0.545**       | 0.015 *      |
| F × C × T           | 45  | 0.053**       | 0.007ns      |
| Error               | 192 | 0.008         | 0.008        |
| CV                  |     | 2.730         | 2.730        |

ns, \*, \*\*: non-significant, significant at 0.05 and 0.01 probability levels respectively.  
CV: Coefficient of variation.

**Table 2** Radial growth of phytopathogenic fungi exposed to *Brassica napus* root extract.

| Fungus species           | Incubation time (h) | Radial growth (mm) |        |        |         |
|--------------------------|---------------------|--------------------|--------|--------|---------|
|                          |                     | 0 ppm              | 50 ppm | 75 ppm | 100 ppm |
| <i>F. oxysporum</i>      | 0                   | 0                  | 0      | 0      | 0       |
|                          | 24                  | 3.5                | 3.5    | 3.5    | 3.3     |
|                          | 48                  | 4.5                | 4.5    | 4.4    | 4.3     |
| <i>R. solani</i>         | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 4.0                | 4.0    | 3.9    | 3.8     |
|                          | 48                  | 8.3                | 8.3    | 8.3    | 8.2     |
| <i>G. graminis</i>       | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 1.5                | 1.5    | 1.5    | 1.4     |
|                          | 48                  | 3.5                | 3.5    | 3.5    | 3.4     |
| <i>P. aphanidermatum</i> | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 1.6                | 1.6    | 1.6    | 1.5     |
|                          | 48                  | 2.5                | 2.5    | 2.4    | 2.4     |
| <i>Ph. derchseleri</i>   | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 2.0                | 2.0    | 2.0    | 1.9     |
|                          | 48                  | 4.0                | 4.0    | 3.8    | 3.7     |
| <i>V. dahliae</i>        | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 1.2                | 1.2    | 1.2    | 1.2     |
|                          | 48                  | 2.5                | 2.5    | 2.5    | 2.4     |
|                          | 72                  | 4.7                | 4.7    | 4.5    | 3.9     |

**Table 3** Growth inhibitory effect of *Brassica napus* root extract on phytopathogenic fungi at different concentrations and incubation times.

| Fungus species           | Incubation time (h) | Growth inhibition (%) |        |        |         |
|--------------------------|---------------------|-----------------------|--------|--------|---------|
|                          |                     | 0 ppm                 | 50 ppm | 75 ppm | 100 ppm |
| <i>F. oxysporum</i>      | 0                   | 0c                    | 0c     | 4.2b   | 17.0a   |
|                          | 24                  | 0c                    | 0c     | 2.0b   | 3.2a    |
|                          | 48                  | 0c                    | 0c     | 1.6b   | 2.5a    |
|                          | 72                  | 0c                    | 0c     | 3.1b   | 9.2a    |
| <i>R. solani</i>         | 0                   | 0c                    | 0c     | 5.0b   | 7.0a    |
|                          | 24                  | 0c                    | 0c     | 2.5b   | 6.5a    |
|                          | 48                  | 0c                    | 0c     | 5.0b   | 10.0a   |
|                          | 72                  | 0c                    | 0c     | 3.2b   | 4.0a    |
| <i>G. graminis</i>       | 0                   | 0c                    | 0c     | 3.1b   | 6.2a    |
|                          | 24                  | 0c                    | 0c     | 3.3b   | 15.0a   |
|                          | 48                  | 0c                    | 0c     | 1.4b   | 4.2a    |
|                          | 72                  | 0b                    | 0b     | 0b     | 5.3a    |
| <i>P. aphanidermatum</i> | 0                   | 0a                    | 0a     | 0a     | 0a      |
|                          | 24                  | 0b                    | 0b     | 0b     | 1.8a    |
|                          | 48                  | 0c                    | 0c     | 2.5b   | 5.0a    |
|                          | 72                  | 0d                    | 1.6c   | 3.3b   | 16.7a   |
| <i>Ph. derchseleri</i>   | 0                   | 0d                    | 1.1c   | 3.3b   | 5.5a    |
|                          | 24                  | 0c                    | 0c     | 1.4b   | 5.7a    |
|                          | 48                  | 0c                    | 0c     | 4.2b   | 17.0a   |
|                          | 72                  | 0c                    | 0c     | 2.0b   | 3.2a    |
| <i>V. dahliae</i>        | 0                   | 0c                    | 0c     | 1.6b   | 2.5a    |
|                          | 24                  | 0c                    | 0c     | 3.1b   | 9.2a    |
|                          | 48                  | 0c                    | 0c     | 5.0b   | 7.0a    |
|                          | 72                  | 0c                    | 0c     | 2.5b   | 6.5a    |

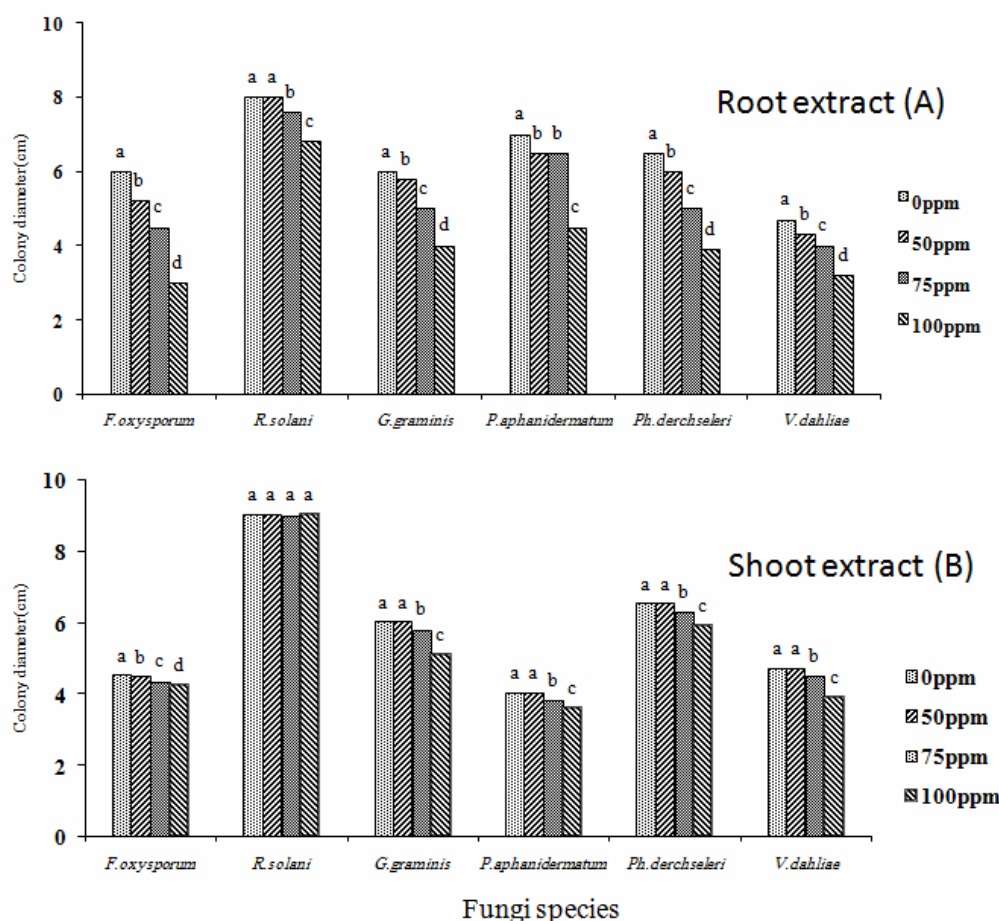
Means followed by the same letters in each row are not significantly different (Duncan's multiple range test,  $P < 0.05$ ).

**Effect of shoot extract on mycelia growth**

The minimum and maximum mycelial growth inhibition was measured after 24, 48 h and 72 h of incubation (Table 4). The results showed that incubation time and shoot extract decreased mycelium growth. Shoot extract had significant effect on mycelial growth inhibition after 24, 48 and 72 h incubation compared to control, except for *G. graminis* at 50 ppm and 48 and 72 h. At 100 ppm significant inhibition of mycelial growth were observed for *F. oxysporum* (50%), *P. aphanidermatum* (35.71%), *G. graminis* (33.33%), *Ph. drechseleri* (40%), *V. dahliae* (31.91%) and *R. solani* (15%) (Tables 4 and 5, Fig. 1-B).

**Phenolic compound detected by HPLC**

HPLC detected and separated a wide range of phenolic compounds. Phenolic contents of shoot and root of *B. napus* (mg L<sup>-1</sup>) are shown in Table 6. Levels of phenolic compounds were significantly influenced shoot and root antifungal activity of *B. napus*. The greatest quantity of phenolic substances detected in shoot extract were Gallic acid, *p*-Coumaric acid, Quercetin, Ferulic acid, Vanillin and *o*-Coumaric acid and those in root extract were catechin, Ferulic acid, Trans cinamic acid and Caffeic acid.



**Figure 1** Effect of root extract (A) and shoots extract (B) of *Brassica napus* on mycelial growth of phytopathogenic fungi on PDA medium after 72 h incubation.

**Table 4** Radial growth of phytopathogenic fungi exposed to *Brassica napus* shoot extract.

| Fungus species           | Incubation time (h) | Radial growth (mm) |        |        |         |
|--------------------------|---------------------|--------------------|--------|--------|---------|
|                          |                     | 0 ppm              | 50 ppm | 75 ppm | 100 ppm |
| <i>F. oxysporum</i>      | 0                   | 0                  | 0      | 0      | 0       |
|                          | 24                  | 3.5                | 3.0    | 2.5    | 2.0     |
|                          | 48                  | 4.5                | 4.1    | 3.8    | 3.0     |
|                          | 72                  | 6.0                | 5.2    | 4.5    | 3.0     |
| <i>R. solani</i>         | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 4.0                | 3.9    | 3.7    | 3.5     |
|                          | 48                  | 6.0                | 5.9    | 5.8    | 5.2     |
|                          | 72                  | 8.0                | 8.0    | 7.6    | 6.8     |
| <i>G. graminis</i>       | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 3.0                | 2.7    | 2.4    | 2.3     |
|                          | 48                  | 4.5                | 4.2    | 3.8    | 3.3     |
|                          | 72                  | 6.0                | 5.8    | 5.0    | 4.0     |
| <i>P. aphanidermatum</i> | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 3.0                | 2.9    | 2.7    | 2.4     |
|                          | 48                  | 4.5                | 4.2    | 4.0    | 3.8     |
|                          | 72                  | 7.0                | 6.5    | 6.5    | 4.5     |
| <i>Ph. derchseleri</i>   | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 2.0                | 1.8    | 1.6    | 1.5     |
|                          | 48                  | 4.2                | 4.1    | 3.2    | 3.0     |
|                          | 72                  | 6.5                | 6.0    | 5.0    | 3.9     |
| <i>V. dahliae</i>        | 0                   | 0.0                | 0.0    | 0.0    | 0.0     |
|                          | 24                  | 2.0                | 1.9    | 1.7    | 1.5     |
|                          | 48                  | 3.5                | 3.3    | 3.0    | 2.6     |
|                          | 72                  | 4.7                | 4.3    | 4.0    | 3.2     |

## Discussion

This research provides information about antifungal activity of root and shoot extracts of canola on six soil-borne pathogens. The extracts from both root and shoot were able to inhibit fungal growth *in vitro*. It is evident from the results that all canola extracts significantly inhibited radial growth of the selected fungi. The inhibition capability depends on plant extract concentration, type of extracts and fungus species. More inhibition of fungal growth was observed at higher concentrations of the crude water extract. The results showed that shoot extract as well as root extract could inhibit the mycelial growth. According to results of the percentage of growth inhibition, *F. oxysporum* and *V. dahliae* were determined as susceptible and *R. solani* as resistant to canola extracts. The results of present study agree with Larkin and Griffin (2007) who

showed the inhibitory effect of Brassicaceae extract on soil borne phytopathogenic fungi. Furthermore Potter *et al.*, 1998, in laboratory experiments, showed that tissue amendments from a variety of wild and cultivated *Brassica* spp. could kill the root lesion nematode (*Pratylenchus neglectus*) in soil. Soil amended with leaf tissues had high nematicidal activity, killing 56.2–95.2% of exposed nematodes but amending soil with root tissues was less effective, causing 0–48.3% mortality.

**Table 5** Growth inhibitory effect of *Brassica napus* shoot extract on phytopathogenic fungi at different concentrations and times.

| Fungus species           | Incubation time (h) | Growth inhibition (%) |        |        |         |
|--------------------------|---------------------|-----------------------|--------|--------|---------|
|                          |                     | 0 ppm                 | 50 ppm | 75 ppm | 100 ppm |
| <i>F. oxysporum</i>      | 0                   | 0d                    | 8.5c   | 14.0b  | 31.9a   |
|                          | 24                  | 0d                    | 5.7c   | 14.0b  | 28.0a   |
|                          | 48                  | 0d                    | 5.0c   | 15.0b  | 25.0a   |
|                          | 72                  | 0d                    | 7.6c   | 23.1b  | 40.0a   |
| <i>R. solani</i>         | 0                   | 0d                    | 8.8c   | 23.0b  | 40.0a   |
|                          | 24                  | 0d                    | 10.0c  | 20.0b  | 25.0a   |
|                          | 48                  | 0d                    | 7.1c   | 7.1b   | 35.7a   |
|                          | 72                  | 0d                    | 6.6c   | 12.5b  | 22.0a   |
| <i>G. graminis</i>       | 0                   | 0c                    | 3.3c   | 10.0b  | 20.0a   |
|                          | 24                  | 0c                    | 3.3c   | 16.0b  | 33.3a   |
|                          | 48                  | 0c                    | 6.0c   | 15.0b  | 26.0a   |
|                          | 72                  | 0d                    | 10.0c  | 20.0b  | 23.0a   |
| <i>P. aphanidermatum</i> | 0                   | 0c                    | 0c     | 2.0b   | 15.0a   |
|                          | 24                  | 0d                    | 1.6c   | 3.3b   | 13.0a   |
|                          | 48                  | 0d                    | 2.5c   | 6.6b   | 12.5a   |
|                          | 72                  | 0d                    | 13.0c  | 25.0b  | 50.0a   |
| <i>Ph. derchseleri</i>   | 0                   | 0d                    | 8.8c   | 16.0b  | 33.0a   |
|                          | 24                  | 0d                    | 1.4c   | 2.8b   | 4.2a    |
|                          | 48                  | 0d                    | 8.5c   | 14.0b  | 31.9a   |
|                          | 72                  | 0d                    | 5.7c   | 14.0b  | 28.0a   |
| <i>V. dahliae</i>        | 0                   | 0d                    | 5.0c   | 15.0b  | 25.0a   |
|                          | 24                  | 0d                    | 7.6c   | 23.1b  | 40.0a   |
|                          | 48                  | 0d                    | 8.8c   | 23.0b  | 40.0a   |
|                          | 72                  | 0d                    | 10.0c  | 20.0b  | 25.0a   |

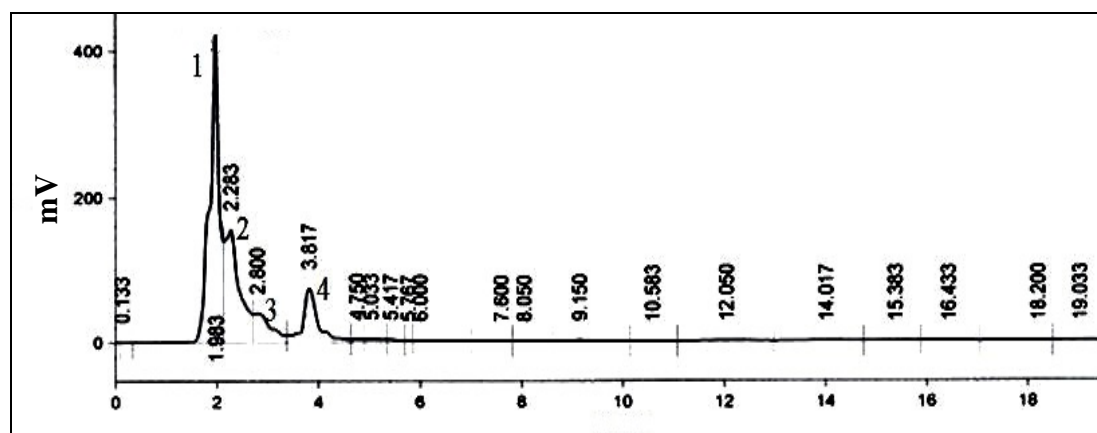
Means followed by the same letters in each row are not significantly different (Duncan's multiple range test,  $P < 0.05$ ).

HPLC with direct injection was used for separation of phenolic compounds. Although normal phase chromatography has been used for the separation of phenolic compounds, there was an agreement between normal phase chromatography and HPLC in the separation of a variety of phenolic compounds (Liu *et al.*,

2012). The separation of phenolic compounds is shown in Figs. 2 and 3. Phenolic contents of canola are shown in Table 6. Phenolic compounds of shoot significantly inhibited phytopathogens in comparison to root extracts. It can be attributed to the type of phenolic material and higher amount of the phenolic compounds within these organs (Cherif *et al.*, 2007). Galic acid (38.1%), *p*-Coumaric acid (20.88%) and Quercetin (24.03%) accounted for the most abundant components of shoot extract respectively. It may be deduced that these compounds act as fungal growth inhibitors. Ferulic acid was detected in root and shoot extract at different concentrations. The activity of these phenolic compounds is dependent on the size of the added alkyl or alkenyl groups, where the larger the size of the alkyl or alkenyl groups, the stronger the antimicrobial activity (Kurita *et al.*, 1981, Knobloch *et al.*, 1989; Pelczar *et al.*, 1993). Gallic acid has been extracted from several plants and found to have strong antifungal activity against several phytopathogenic fungi (Ahn *et al.*, 2005; Shukla *et al.*, 1999). Gallic acid caused inhibition of spore germination and appressorium formation of rice blast fungus of *Magnaporthe grisea* (Ahn *et al.*, 2005). Thus, the phenolic based compound was suggested as a natural substance for the development of target-site specific biofungicide against fungicide-resistant phytopathogenic fungi which is consistent with our study. It was also reported that 3, 4-dihydroxybenzoic acid isolated from *Cananga odorata* showed antifungal activity (Rahman *et al.*, 2005). Catechin has been found to be ineffective against several yeast, gram-positive and gram-negative bacteria compared to coumarins and gallic acid derivatives (Hirasawa and Takada, 2004, Kayser and Kolodziej, 1997).

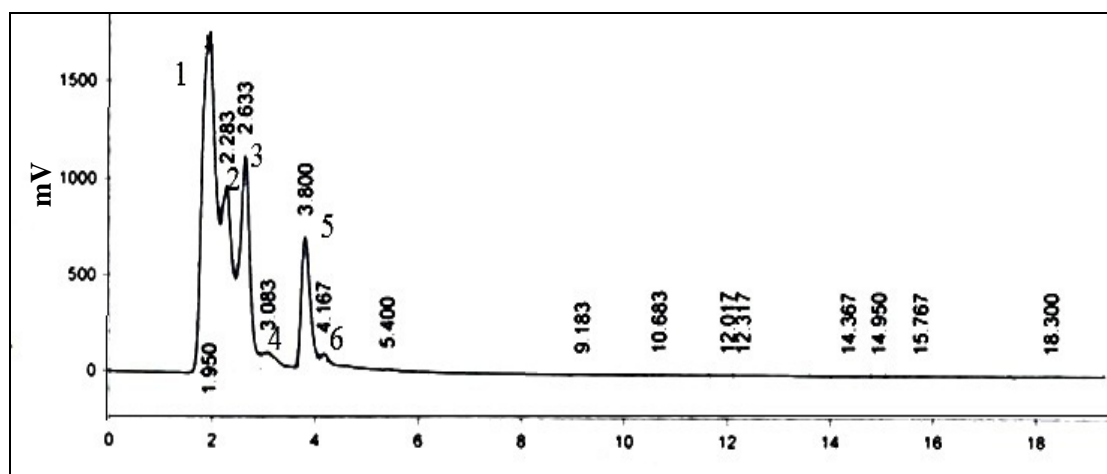
Lopez-Malo *et al.* (2006) found that antimicrobial activities of phenolic compounds are concentration-dependent, at lower concentration these compounds affected enzymes associated with energy production, whereas at higher concentrations

they caused protein denaturation. Also phenolic compounds could affect the enzymes responsible for spore germination and interfere with amino acids that are necessary in germination processes (Nychas, 1995). The effect of essential oils extracted from sweet basil, fennel, summer savory and thyme plants were investigated both on mycelial growth of *Botrytis cinerea* under *in vitro* condition and fungal decay and quality sensors of table grape under *in vivo* conditions. Results showed that essential oils especially those containing more phenolic compounds had a great antifungal activity and could be used as a beneficial and safe tool for preservation of table grape (Abdolahi *et al.*, 2010). Brassicaceae seed extracts have been shown to have activities against various bacterial pathogens, as well as herbicidal, insecticidal, nematocidal and fungicidal effects (Tenore *et al.*, 2012). Bai *et al.* (2012) showed that aqueous extract of Brassicaceae has higher antifungal activity than the methanolic extract. According to results of Bai *et al.* (2012) and because of better environmental compatibility of aqueous extracts, this type of extract was used in our study. According to previous research aqueous extract at high concentration had high antifungal activity (Ademe *et al.*, 2013). In this study Gallic acid and catechin had high concentration in shoot and root extracts respectively. Both of these compounds have high antifungal activity. These results agree with other researches (Kayser and Kolodziej, 1997, Ahn *et al.*, 2005; Shukla *et al.*, 1999). The use of *B. napus* extract to control some soil borne plant pathogens will be preferred safe alternative to chemical control. This formulation may be considered suitable for seed and foliar treatments. The results of present study can be further exploited for integrated management of some soil borne fungi by application of Canola in rotation with susceptible hosts. studies on formulation of extracted essential oils can be successfully devised and carried out using a simple process with minimum instrumentation.



Retention time

**Figure 2** The chromatogram of the active compounds in root extract of *Brassica napus* by UV detection (1- Catechin, 2- Ferulic acid, 3- Trans cinamic acid, 4- Caffeic acid).



Retention time

**Figure 3** The chromatogram of the active compounds of shoot extracts of *Brassica napus* by UV detection (1- Gallic acid, 2-*p*-Coumaric acid, 3-Quercetin, 4-Ferulic acid, 5-Vanillin, 6-*o*-Coumaric acid).

**Table 6** Major phenolic compounds of canola root and shoot extract identified by HPLC.

| Root compounds     | Area (%) | Shoot compounds         | Area (%) |
|--------------------|----------|-------------------------|----------|
| catechin           | 45.92    | Gallic acid             | 44.91    |
| Ferulic acid       | 26.76    | <i>p</i> -Coumaric acid | 19.52    |
| Trans cinamic acid | 7.88     | Quercetin               | 22.19    |
| Caffeic acid       | 12.81    | Ferulic acid            | 2.34     |
| -                  | -        | Vanillin                | 10.41    |
| -                  | -        | <i>o</i> -Coumaric acid | 0.34     |

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## خاصیت ضدقارچی عصاره آبی گیاه کلزا (*Brassica napus*) بر برخی قارچ‌های بیمارگر گیاهی

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**چکیده:** عصاره برخی از گیاهان دارای خاصیت ضد میکروبی می‌باشند. آفت‌کش‌های گیاه پایه جایگزین‌های مناسبی برای آفت‌کش‌های شیمیایی محسوب می‌شوند زیرا اثرات مخرب زیست‌محیطی کم‌تری دارند. هدف از این پژوهش بررسی اثر بازدارندگی عصاره آبی کلزا روی رشد میسلیمی شش قارچ بیمارگر گیاهی شامل: *Verticellium dahliae*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophthora derchseleri* و *Pythium aphanidermatum* می‌باشد. خاصیت ضدقارچی با استفاده از روش دیسک انتشار مورد بررسی قرار گرفت. نتایج نشان داد که غلظت‌های ۱۰۰ و ۵۰ پی‌پی‌ام به‌ترتیب دارای بیش‌ترین و کم‌ترین میزان تأثیر بر روی تمام گونه‌های قارچی بودند. بیش‌ترین و کم‌ترین خاصیت ضدقارچی به‌ترتیب توسط عصاره اندام هوایی گیاه و عصاره ریشه ایجاد شد. نتایج بازدارندگی رشد میسلیمی در غلظت ۱۰۰ پی‌پی‌ام از عصاره‌های ریشه و قسمت‌های هوایی نشان داد که به‌ترتیب *V. dahliae* (۰.۱۷/۰.۲) و *F. oxysporum* (۰.۵۰) حساس‌ترین گونه‌ها و *R. solani* (۱/۸ و ۰.۱۵) مقاوم‌ترین گونه نسبت به هر دو عصاره بود. ترکیبات فعال در گیاه کلزا با استفاده از روش کروماتوگرافی مایع با کارایی بالا تعیین شد. نتایج نشان داد که ترکیبات فنلی بیش‌ترین غلظت را داشته و احتمالاً عامل اصلی بازدارندگی از رشد میسلیمی می‌باشند. استفاده از عصاره آبی کلزا یا به‌کار بردن کلزا در تناوب، می‌تواند به‌عنوان یک روش در مدیریت قارچ‌های بیمارگر گیاهی خاکزی موردنظر قرار گیرد.

**واژگان کلیدی:** کروماتوگرافی، دیسک انتشار، ترکیبات فنلی، عصاره گیاهی، قارچ‌های خاکزی