



## Antifungal activity of *Artemisia haussknechtii* against *Fusarium oxysporum* and *Bipolaris sorokiniana*\*

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A very large number of fungi such as *Bipolaris sorokiniana* and *Fusarium oxysporum* are responsible for major plant diseases, glume spot, tombstone, head blight, root rot, leaf brown spot (Agrios 2005). Disease control relies mainly on the extensive use of fungicides with the risk of development of resistant pathogen strains. To obtain green food, the replacement of fungicides by effective and environment-friendly methods is the major way forward (Meliss *et al.* 2005, Zhang *et al.* 2008). Using extracts from plants containing natural antifungal compounds for plant disease control is considered to be one of the desirable methods for plant protection in agriculture (Kim *et al.* 2004).

*Artemisia haussknechtii* is an endemic plant in Asteraceae family since long time ago have been applied as insects and snakes repellents as the flavone sources (Regnault and Hamraoui 1994, Khanahmadi *et al.* 2009). Earlier studies revealed that the plants in the genus *Artemisia* sp. contain volatile compounds such as acetylenic, monoterpene and sesquiterpene materials which have antifeeding and antifungal effects (Negahban and Karamipour 2002, Wenqiang and Shuten 2006). Although so far there has been no study on fungicidal or pesticidal effects of *A. haussknechtii*, but there are many records on the other plant effects in *Artemisia* spp and many active compounds containing  $\alpha$ -pinene, cineol,  $\gamma$ -terpinen camphor, camphor, 1-phenyl 2-4 pentadiene, p-methyleugenol, p-cymene and  $\beta$ -pinene have been identified (Ahmad and Misra 1994, Dobab *et al.* 2005, Soliman 2007).

The main goal of this report was studying on antifungal

effect of *A. haussknechtii* essential oil and extract on mycelial growth of two plant pathogenic fungi, *F. oxysporum* and *B. sorokiniana*.

*A. haussknechtii* plants were gathered from Oramanat country in Kermanshah province during July 2010 sliced and air dried with active ventilation at ambient temperature. Cultures of *F. oxysporum* and *B. sorokiniana* were obtained from College of Agricultural, Razi university, Kermanshah.

Extraction and essential oil of this plant was prepared according to conventional procedures (Khanahmadi *et al.* 2009). Air-dried aerial parts of *A. haussknechtii* (100g) were subjected to hydro distillation in a Clevenger type apparatus for 4 hr period. After decanting and drying of the oils on anhydrous sodium sulfate, the oil was kept refrigerated until analysis.

In order to extraction, about 50g of powdered plant were treated with 100 ml ethanol at room temperature with stirring. This procedure was repeated five times until the extraction solvent became colorless. The obtained extract was filtered over Whatman No.1. Paper filter and the filtrates were collected, and then ethanol was removed in vacuum at temperature not exceeding 40°C.

Fungal bioassay was performed as described by Abdolmaleki *et al.* (2010). In this research, antifungal activity of ethanolic extract and essential oil of *A. haussknechtii* was investigated based on two methods: paper disc method and agar diffusion method. In paper disc method, each disc was loaded with 1, 2, 3, 4 and 5 mg/paper disc of the crude extract and 10, 20, 30, 40 and 50  $\mu$ l/paper disc of essential oil.

The discs were dried well after loading to remove the solvent. Loaded paper discs were placed on the plate. Plates were incubated at 25°C and the radius zone of inhibition (distance between the centre of the paper disc and margin of the inhibited mycelium) was recorded. The disc loaded by solvent was considered as a control (Bahraminejad *et al.* 2008). The experiments were performed in four replicates.

\*Short note

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In agar diffusion method, for preparing of 1 000, 2 000, 3 000, 4 000 and 5 000 ppm concentrations initially enough amount of essential oil and extract were solubilized in 1.5 ml of ethanol solvent and were shaken well by a vortex. The potato dextrose agar medium (PDA) was sterilized at 121°C for 20 min. and 1 atmosphere pressure. The prepared extract was added to culture medium when the temperature of the medium decreased to about 40 °C. The culture media loaded by 1.5 ml of ethanol was considered as a control. The culture media immediately was poured into plates. A 6 mm diameter plug of 7 day fungal colonies were placed at the centre of the plates. Plates were incubated at 25 °C ± 4 and diameter of colony was measured until the control plates was covered by the fungus mycelia completely. The experiments were performed in four replicates. Percentage of inhibition of growth for each fungus was calculated based on conventional formula (Hadian *et al.* 2006, Sarkar *et al.* 2003).

The data were analyzed using MSTAT-C software and comparison of means was analyzed by Duncan's multiple ranges test.

The results of the antifungal activity of the extract of *A. haussknechtii* are given in Table 1. The inhibitory data was obtained using two different methods. The inhibitory effect on the mycelia growth of *F. oxysporum* using paper disc method showed that there is significant differences among extract concentrations, so that all treatments can be classified as five groups and 5mg/paper was the most active concentration. Similarly, five concentrations were classified

in five significant groups when *B. sorokiniana* exposed to the extracts with this method. The radius zone of inhibition was increased harmoniously with increasing in the amount of the extract. The agar diffusion method was also used to evaluate the inhibitory effect of different concentrations of *A. haussknechtii*. The results indicated that the growth of *F. oxysporum* completely inhibited when the fungus was exposed to 4 000 ppm of the extract. Whereas, the mycelia growth of *B. sorokiniana* was completely suppressed by 2 000 ppm of the extract. The results obtained from both methods showed that *B. sorokiniana* is more sensitive to the extract than *F.oxysporum*.

The results of the antifungal activity of essential oil obtained from *A. haussknechtii* are given in Table 2. As can be seen from this table, both fungi were inhibited by the essential oil of the tested plant species. Significant differences were observed among the concentrations of the essential oil when both fungi were exposed to the essential oil loaded paper discs. The mycelia growth inhibition of both fungi was increased as the concentration of essential oil was increased. Paper disc method indicated that *F. oxysporum* is more sensitive than *B. sorokiniana*. Whereas, agar diffusion method showed that *B. sorokiniana* is more sensitive. The difference between the two tested methods representing the difference between the solubility of the essential oil in the medium.

Non inhibitory effect on mycelial growth of *F. oxysporum* and *B. sorokiniana* was seen in test control.

In control of the test complete growth of *F. oxysporum*

Table 1 Inhibitory effect percentage of *A. haussknechtii* extract on *F. oxysporum* and *B. sorokiniana*

Conc. (mg/paper disc)	Paper disc		Conc.(ppm)	Agar diffusion	
	<i>F. oxysporum</i>	<i>B. sorokiniana</i>		<i>F. oxysporum</i>	<i>B. sorokiniana</i>
1	3.33 e ±0.13*	4.70 e ±0.16	1 000	63.00 d ±0.91	91.70 b ±0.30
2	4.40 d ±0.11	6.23 d ±0.11	2 000	77.50 c ±1.19	100a
3	6.78 c ±0.11	8.20 c ±0.15	3 000	90.25 b ±0.52	100a
4	10.30 b ±0.18	12.30 b ±0.17	4 000	100a	100a
5	14.80 a ±0.15	19.08 a ±0.23	5 000	100a	100a

\*, The numbers represent mean of radius inhibition zone (mm)±standard error, n= 4; Means followed by the same letters in each column are not significantly different at P=0.05, when Duncan's multiple ranges test was used

Table 2 Inhibitory effect percentage of *A. haussknechtii* essential oil on *F. oxysporum* and *B. sorokiniana*

Conc. (µl /paper disc)	Paper disc		Conc.(ppm)	Agar diffusion	
	<i>F. oxysporum</i>	<i>B. sorokiniana</i>		<i>F. oxysporum</i>	<i>B. sorokiniana</i>
10	5.97 e ±0.27*	4.33e ±0.13	1 000	73.50 d ±0.95	92.87 b ±0.06
20	7.16 d ±0.20	6.40 d ±0.11	2 000	80.25 c ±0.48	100a
30	9.65 c ±0.07	8.53 c ±0.19	3 000	85.37 b ±0.24	100a
40	15.50 b ±0.10	12.70 b ±0.22	4 000	100 a	100a
50	19.33 a ±0.14	15.27 a ±0.16	5 000	100a	100a

\*, The numbers represent mean of radius inhibition zone (mm)±standard error, n= 4; Means followed by the same letters in each column are not significantly different at P=0.05, when Duncan's multiple ranges test was used

and *B. sorokiniana* was seen.

Application of the extract at concentration of 1000 ppm inhibits mycelial growth of *F. oxysporum* and *B. sorokiniana* with 63 % and 91.70 % inhibition, respectively. It increased to 73.5% and 92.87% when essential oil was applied. The inhibitory effect of essential oil was higher than the extract when the data in both tables were compared.

In this experiments we found that inhibitory effect of *A. haussknechtii* not only depends on kind of extracted material (extract or essential oil) but also the sensitivity of the pathogen is critical *F.oxyspoum* in the presence of 1 000 ppm of the essential oil showed more sensitivity than *B. sorokiniana* but at the same concentration of the extract *B. sorokiniana* was more sensitive than *F.oxysporum*.

This experiment based on paper discs revealed that *B. sorokiniana* was inhibited more by the extract than *F.oxysporum*, while *F.oxysporum* was inhibited more than *B. sorokiniana* when essential oil was used. It could be due to the type or the amount of ingredients in the essential oil or the extract. However, geographical conditions can be effective on the amount and even the kind of metabolites in the essential oil or the extract and their quality depends on environmental factors (Liu and Ho 1999, Ramezania *et al.* 2004).

The data indicated that the concentration of 4 000 ppm of the plant extract had the best inhibitory effect on the mycelial growth of both fungi. Experiments of other researchers have approved this issue (Cowan 1999, Mahmoud and soliman 2007, Ramezania *et al.* 2004).

Extraction of plants ingredients in different regions may lead to producing of compounds with different properties, such as methyleugenol, p-cymene and  $\alpha$ -pinene in *A. campestris* (Dobab *et al.* 2005) and  $\gamma$ -terpinen, caoilene, 1-Pheny 2,4- pentadiyene for *A. absinthium* (Orav *et al.* 2006). Similarly liu *et al.* (2004) suggested that *A. judaica* contains high levels of antioxidant and flavonoid compounds. Quality and quantity of cineol are different in various climates. With this consideration cineol is the prevalent factor in secondary metabolites of the essential oil of this plant and flavonoids and other compounds also have lower rates in the extract but because of antimicrobial function of flavonoids it is possible to introduce these compounds singly or with each other as effective factor in this plant against fungi (Dobab *et al.* 2005, Liu and Ho 1999, Ramezania *et al.* 2004).

#### SUMMARY

Since long time ago, *Artemisia* species have been used as the flavone sources and as insects and snakes repellants. In this research, antifungal activity of ethanolic extract and essential oil of *Artemisia haussknechtii* was investigated based on two methods: Agar diffusion method and paper disc method. Inhibitory effects of these samples on mycelial growth of two fungi *Fusarium oxysporum*, *Bipolaris sorokiniana* were determined. Results showed that in paper

disc method the essential oil of *A. haussknechtii* had remarkable inhibitory effect against *F. oxysporum* with 19.33 mm inhibitory radius zone in 50  $\mu$ l /paper disc. The most inhibition on *B. sorokiniana* was belonging to *A. hauskenechti* extract with 19.08 mm inhibitory radius zone in 5 mg/paper disc. Via agar diffusion method both *A. haussknechtii* extract and essential oil inhibit mycelial growth of *F. oxysporum* and *B. sorokiniana* with 100% inhibition in 4 000, 5 000 ppm.

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