Antifungal effects of assafoetida seed essential oil on in vitro growth of five species of plant pathogenic fungi

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ABSTRACT: Asafoetida is one of important medicinal plant of the Apiaceae family. Recently has shown that the use of plant extracts with fungicidal properties that do not have any adverse effects are as most important alternative to chemical fungicides. In this study the antifungal effect of asafoetida seed essential oil on some of plant pathogens fungi including: Bipolaris sorokiniana, Verticellium Sp, Fusarium graminearum, Fusarium solani and Aspergillus niger at concentrations of 0.15% and 0.3% in a factorial experiment based on completely randomized design and on in vitro were conducted. PDA medium was used as the substrate for growth fungal medium and without essential oil medium was used as control. Values of inhibition percentage of seed essential oil on the growth of fungal colonies were measured. The results showed that asafetida seed essential oil compared with controls significantly inhibits the growth of all fungal species were tested. Bipolaris sorokiniana growth in both essential oil concentrations completely inhibited, on the other species increased with increasing concentration of essential oil inhibiting effect, But only two fungal species consisting of Fusarium solani and Aspergillus niger have significant differences in the growth of fungus colonies under the influence of different concentrations of essential oils was observed.

Key words: biological control, Ferula assa-foerida, fungus colonies, inhibition percentage, seed extract.

INTRODUCTION

One of the major challenges in the agriculture is appearance of lesions in agricultural and horticultural crops caused by fungi and pests are attacking that perennial saw a lot of damage to human societies in addition to the loss of food due to health endangerment by production and consumption chemical fungicides and pesticides caused to eliminate these pests and prevent their destructive effects (Maskuki and Mortezavi, 2004). The global statistics of losses of crops in our country between 30 and 50 percentage have estimated that 15 percent its production process is caused by fungal attack, storage and processing of agricultural products to consumption (Iran Deportments of Agriculture, 1998). Fungal diseases in developing countries, the amount of damage before harvest to be about 12 percentage (Lee et al., 2001).

Fungal species of Aspergillus and Fusarium genera are the most species producer’s mycotoxins in food and in addition to diseases such as mildew, seeds corruption, stem rot, wilt and dwarf plants involved (Blat, 1969; Fandohan et al., 2003). Chemicals used in controlling plant diseases lead to environmental pollution and also the qualitative properties of plants are affected. To avoid of chemical hazards, use of natural compounds produced by some plants to control plant diseases is recommended (Rahber-Bhatti, 1986; Bowers and Locke, 2000; Momin and
Nair, 2001). Herbal products, including: gum, oil, resin, etc. are used as antifungal agents (Dwivedia et al., 1990; Daoud et al., 1990). Many research approaches indicate that plant extracts and its constituents are effective antifungal agents (Sridhar et al., 2003; Daferera et al., 2000).

Asafoetida is one of the valuable medicinal plants belonging to the apiaceae family that applied in the treatment of human diseases has been used extensively. In addition, some researches showed antifungal effects for it versus phytopathogenic fungi. Houghton et al. (2006) reported the antifungal effects of asafoetida in Microsporum gypseum and Trichophyton interditalis. Thyagaraja and Hosono (1996) reported the inhibitory effect of asafoetida on the fungus Rhizopus sporus, Mucor dimorphosphorous, Penicillium commune and Fusareum solani were studied. Sitara et al. (2008) reported that the seed oil of asafoetida at concentrations of 0.1% And 0.15% completely inhibitions from fungi growth Fusarium solani, F.moniliform, F.oxysporum, F. nivale, F. semitectum, Derecheslera hawaiensis and Alternaria alternate and inhibited the growth of Aspergillus flavus and Aspergillus niger has been resulted in a significant reduction compared with control.

The present study is attempts to use natural materials and products, and no negative effects, such as natural extracts. Using these materials, while maintaining environmental health and reduce the consumption of chemical fungicides, to avoid possess for production the effective formulation to control fungal diseases of producers dangerous toxins, warehouse and cold storage contaminants.

MATERIALS AND METHODS

This study was conducted in 2011 in the Laboratory of Plant Pathology, Faculty of Agriculture, University of Birjand. Asafoetida seeds were collected from Rshid Kooh in Jandagh region (located in the center of Iran) with a height of 1800 meters and longitude 54°, 49°, 15 east and latitude 21°, 57°, 33° north in July 2010. Cleverger apparatus used for seed essential oil extraction. Then the essential oil was maintained in freezer at -15 °C until the experiment. For evaluation the antifungal effects of essential oil used as factorial experiment based on compleetly randomized design with 10 treatments and 3 replications. Experimental factors, consisting species of phytopathogenic fungi in 5 levels of Bipolaris sorokiniana, Verticillium. Sp, Fusarium graminearum, Fusarium solani and Aspergillus niger and essential oil concentrations in 2 levels of 0.15% and 0.3% mixed with PDA culture medium. About 10 ml treated or untreated medium were poured into petri plates(70 mm diameter). Untreated medium was used as control. This study was conducted by a factorial experiment based on completely randomized design with three replications. Petri plates incubated at 27±1 °C for 10 days and then measured the diameter of fungal colonies by the Caliper with an accuracy of 0.05. Cm and the inhibition percentage of essential oils on the growth of fungal colonies was calculated by using the following equation:

\[ IP = \frac{(dc-dt)}{dc} \times 100 \]

\[ IP = \text{inhibition percentage} \]

\[ dc = \text{colony diameter in control} \]

\[ dt = \text{colony diameter in treatment} \]

For data normalization was used from angular transform (arcsin \( \sqrt{x/100} \)) . The analysis of variance was performed on normalized data. For statistical data analysis using SASS software version 8.2 and the drawing graphs was done by Excel 2007 software. Comparison test was conducted by FLSD test at 5% level.

RESULTS AND DISCUSSION

Analysis of data in this study showed that all three factors including species of fungi, concentration of seed essential oil and interactions could affect the growth of fungi colonies(\( P < 0.01 \)) significantly ( tabel 1). Inhibitory effect of various concentrations of asafoetida seed essential oil on the growth of colonies are shown in Table 2. Based on results obtained in this study, both the concentration of asafoetida essential oil in 0.15 and 0.3% completely inhibited than growth Bipolaris sorokiniana. The mycelium growth of Aspergillus niger at 0.15% concentration, decrease about 81.24 percentage compared to controls and increase essential oil concentration in culture medium to 0.3 % completely prevented its growth. Also the use of essential oils, greatly inhibited from growth of the fungus Fusarium graminearum, and the inhibition percentage caused by the use of essential oils with concentrations of 0.15 And 0.3% in these species was 86.81 and 92.6 percentage respectively. However, the impressibility of Verticillium .Sp and Fusarium solani form asafoetida essential oil utilization was lowers than other species, So that for Verticillium .Sp at conentration of 0.15 and 0.3 % of the amount of essential oil respectively 47.12 and 55.56 percentage, and in Fusarium solani respectively 37.28 and 51.15 percentage inhibition was recorded.
Table 1. Anova table showing antifungal effects of asafoetida seed essential oil on the growth of fungal pathogen.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Mean of square</th>
<th>F value</th>
</tr>
</thead>
<tbody>
<tr>
<td>fungus</td>
<td>0.794 **</td>
<td>147.11</td>
</tr>
<tr>
<td>concentration</td>
<td>0.172 **</td>
<td>31.39</td>
</tr>
<tr>
<td>interaction</td>
<td>0.044</td>
<td>8.27</td>
</tr>
<tr>
<td>residual</td>
<td>0.005</td>
<td></td>
</tr>
</tbody>
</table>

** Indicates significant at 1% level. C.v = 6.44%

Table 2. Interactions between asafoetida seed essential oil concentration and fungi species on the average inhibition percentage.

<table>
<thead>
<tr>
<th>fungi species</th>
<th>essential oil concentration (%)</th>
<th>Inhibition percentage ± Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bipolaris sorokiniana</td>
<td>0.15 100±0 a</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 100±0 a</td>
<td></td>
</tr>
<tr>
<td>Verticillium .sp</td>
<td>0.15 47.12±8.42 de</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 55.65±2.46 d</td>
<td></td>
</tr>
<tr>
<td>Fusarium graminearum</td>
<td>0.15 86.81±5.83 bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 92.6±1.15 ab</td>
<td></td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>0.15 37.28±4.05 e</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 51.15±1.47 d</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>0.15 81.24±2.01 c</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3 100±0 a</td>
<td></td>
</tr>
<tr>
<td>LSD (P&lt;5%)</td>
<td></td>
<td>12.5</td>
</tr>
</tbody>
</table>

Non-identical letters indicating significant difference.

Figure 1. Effect of essential oil concentration on the inhibition percentage of growth of fungal colonies.

Figure 2. Effect of Fungi species on inhibition percentage of the asafoetida seed essential oil.
Results showed that the concentration of essential oil used in the culture medium has statistically significant effect on the inhibition of mycelia growth of studied fungi, and with increasing concentrations of essential oil from 0.15 to 0.3 %, the rate of inhibition increased from 70.49 percentages to 79.88 percentages (Figure 1). Mohana and Raveesha (2007) showed that increasing in concentration of essential oils of different plant species increas inhibition percentage of mycelial growth of pathogenic fungi. Results of in this study suggests that the intensity of antifungal effects of asafoetida essential oil is different in various fungi species. Comparison of data showed that, between reaction of different species to the antifungal activity of essential oil there was significant differences in amount of inhibition percentage of essential oil (Figure 2). There for the resistance of different species against asafoetida essential oil antifungal activity was different and this causes that essential oil has a different performance in contrast with fungal pathogens. This should be considered in biological control. Most essential oil inhibition among species examined in this study were 100 percentage in species of Bipolaris sorokiniana. Inhibition percentage of colony growth in Aspergillus niger, Fusarium graminearum, Verticellium. Sp and Fusarium solani, was 90.82, 89.7, 51.38 and 44.22 percentage respectively. Sitara et al. (2008) showed that the reaction of different species of fungal pathogens to antifungal activity of essential oils of various plant species is different. The antifungal activity of asafoetida extract of the fungus Aspergillus niger and Aspergillus flavus by Siddiqui et al. (1996) has been reported.

Results showed that asafoetida essential oil could be a natural fungicides with suitable potential for biological control of different pathogens, including the seed born fungi and also to protect the fruit in storage and food during the maintenance period be considered. Also based on the results (Figure 1) are expected to use higher concentrations of plant essential oils have create deterrence potential in a wider range of plant pathogens.

REFERENCES