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RESEARCH ARTICLE

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In vitro inhibitory effect of plant extracts on growth of seed-borne *Alternaria alternata* fungus

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ABSTRACT

Antifungal substances of plant origin could be used as an inhibitor to control fungal seed diseases growth and development. The purpose of this study was to evaluate the influence of alcoholic and aqueous extracts of few medicinal plants (Fennel, Cichorium, Asafoetida, Echinacea, Balangu (Lady's mantle and Olender) on growth and development of *Alternaria alternata* fungi. The experimental treatments were arranged as factorial and the data were analyzed based on a completely randomized design with three replications. The effect of seed and plant extracts, plant type (plant), and their interactions were significant on the fungal growth and development. The strong antifungal properties of aqueous and alcoholic extracts of balangu and oleander showed a broad-spectrum natural antifungal resistance in those plants. However, the results indicated a high resistance for *A. alternata* against the aqueous extract of fennel. This study concluded a high potential for the alcoholic extract of balangu and oleander (100% inhibition) in the biological control of the *A. alternata* fungus.

Key words: *Alternaria*, biological seed treatment, extract, medicinal plant, seed born

Introduction

Production of safe and sufficient food is necessary for sustainable agricultural development. One of the major factors for reducing crop yield is plant diseases (Rodriguez et., 2005). The fungus Alternaria (*Alternaria alternata*) is one of the most important seed- borne plant pathogenic fungi which is greatly damage agricultural crops. This fungus can live in soil, on plant debris and weeds. It is usually spread out by wind, water, insects, farm workers, equipments, and especially the by infected seeds. Spores of the fungus can enter the leaves, stems, and fruit (Tsuge et al., 2013). Alternaria alternata infection on tomato leads to post harvest damages and puts product safety at risk in the farm and barn (Prasad & Upadhyay, 2010).

The consumers and environmental concerns are demanding less use of synthetic chemicals in agricultural production. Studies have shown the potential of secondary metabolites of plants which can be used to control plant pathogens (Ribera1 & Zuniga, 2012) and play an important ecological roles in plant defense reactions. In this regard, many researchers have studied anti-bacterial, anti-fungal, and insecticide effects of essential oils and plant extracts. Numertous aromatic compounds produced in plants could be sprayed on crops as metabolic products to reduce pathogenic adverse effects, it seems that anti-fungal and anti-microbial effects are the results of intensified activities of many compounds (Sokovic et al., 2007; Hadizadeh et al., 2009).

Medicinal plants are considered as one of the most important natural sources to produce beneficial secondary metabolites. Balangu produces seeds with high mucilage content. Mucilages are among the best polysaccharide hydrocolloids of herbal extracts (Abdulrasool et al., 2011). Antibacterial properties of natural extracts obtained from Balangu seed have been proved by many researchers (Mahmood et al., 2013). *Oleander* Sp. produces oleandrin as well as Nerienin and Neriin metabolites. Oleandrin as one of the most important cardiac glycoside is obtained from the leaves of oleander (Zibbu & Batra, 2010). The anti-fungal activity of sesquiterpens in Echinacea sp. seeds have been reported by other researchers. Phenolic compounds also have anti-fungal properties; so that most studies have focused on the effect of phenolic compounds which inhibit the growth of fungi deseases (Gulluce et al., 2007).

Aqueous and alcoholic extracts of Cichorium sp. are very effective as defense compounds (El-Shafey & Abdelgawad, 2012). The chemical components of Cichorium species include the lipophilic parts like alkamide (Jancic et al., 2016). Alkamides are the lipophilic and synthetic derivative of acid caffeic which have a significant contribution to defense compounds (Holzbach & Lopes, 2010). Effectively, the extracts of four medicinal plants including *Artemisia absinthium*, *Rosmarinus datura*, *Datura stramonium*, and *Xanthium strumarium* showed an inhibiting effect on the growth of *A. alternata* fungus as a seed-borne disease (Rodino et al., 2014).

The report showed the great inhibition effects of alcoholic extracts against the fungus *A. alternata*. The pathogen was inhibited by the extract of all five plants; however antifungal activities of *Terminalia alata* extract and *Terminalia arjuna* were more than other plants (Shinde et al., 2010). As there were secondary metabolites of mono and sesquiterpene categories such as carvacrol, thymol, γ- terpinene, and Paracemenu in Satureja khuzestanica (Ghorbanpour & Hadian, 2015) and also the presence of cineole and eucalyptol in eucalyptus which forms more than 80% of essential oil, has strong antiseptic properties that can be effective on inhibiting the growth of fungal pathogen (Sadlon & Lamson, 2010).

Given the importance of seed and reduction of diseases caused by seed-borne pathogens like *A. alternata*, it is necessary to identify the potentials of the native plants as biological control resources. Hence the aim of this study was to investigate the inhibitory effect of plant extracts of Asafoetida, Olender, Cichorium, Balangu, Fennel, and Echinacea on the seed-borne desease caused by *A. alternata* fungi at in-vitro conditions.

Materials and Methods

Fungal strains and growth conditions

Fungal isolates of *Alternaria alternata* was prepared by Iranian Research Institute of Plant Protection. To prepare a new culture a disc of fungus was placed on potato dextrose agar plates. The plates were incubated at 20ºC for 5 to 7 days. The seed plants used in this study were provided from Pakan Bazr Co., Isfahan, Iran (Table 1).

Preparation of aqueous extraction

Seeds of Echinacea, Asafoetida, Balangu, Fennel, Chicory and leaves of Oleander were washed with tap water, and shade-dried in greenhouse. 10 grams of powdered plant material of each plant was dissolved in 100 ml deionized water and heated at 85 ºC for 24 h then kept undisturbed at room temperature. Subsequently, it was filtered through whatman No.1 filter paper. Then prepared extracts were stored until use at 4 °C.

Preparation of alcoholic extract

Seeds of Echinacea, Asafoetida, Balangu, Fennel, Chicory and Leaves of Oleander were ground in an electric grinder. Subsequently, half a gram (0.5 g) of ground dry material was extracted with 10 ml of 80% (v/v) ethanol, and the mixture was vigorously shaken and centrifuged for 5 min at 5000 g. Again, the same amount of solvent was added to the crude extract and the process was repeated three times (Maspi et al., 2010).

Antifungal assay

Antifungal activity of plant material was determined by radial growth technique. The effect of alcoholic and aqueous extracts of the plants on the radial growth of fungal mycelium A. alternata was studied. The purpose of this study was to evaluate the influence of alcoholic and aqueous extracts of few medicinal plants (Fennel, Cichorium, Asafoetida, Echinacea, balangu and Olender) on growth and development of *Alternaria alternate* fungi. The experimental treatments were arranged as factorial and the data was analyzed based on a completely randomized design with three replications. The first factor was the type of extract: Alcohol and aqueous and second factor included six plants, Echinacea, asafoetida, Balangu, Fennel, Chicory, and Oleander plus control (without inhibitor). Standard extracts (1 ml) was mixed with 6 ml of sterilized PDA medium and transferred equally into two sterilized Petri plates. An agar plug of fungal inoculums (6 mm diameter) was removed from the fungal strains tested, and placed in the center of the Petri dishes. All dishes were incubated at 20°C for 7 days and radial growth of colony was measured. Petri dishes containing PDA medium (without plant extracts) served as control. The results were compared with control. Inhibition % was calculated by using the following formula:

$$GI\% = \frac{(dc - dt)}{dc} \times 100 ,$$

where dc = average mycelial growth in control, dt = average mycelial growth in treatment.

After recording the data required, analysis of the variance was performed using the software SAS, and Duncan's

Table 1: *Characteristics of plants used in the experiment*

Name	Family name	Scientific name	Family name	Name	Scientific name
Balangu (Lady's mantle)	*Lamiaceae*	*Lallementia royleana*	*Apocynaceae*	Oleander	*Nerium oleander*
Echinacea	*Asteraceae*	*Echinacea angustifolia*	*Asteracea*	Chicory	*Cichorium intybus*
Asafoetida	*Apiaceae*	*Ferula assa feotidai*	*Apiaceae*	Fennel	*Foeniculum vulgare*

Table 2: *Analysis of variance of the effect of aqueous extract and plant on growth inhibition of Alternaria alternate.*

	Mean square	
Sources of variation	Degree of freedom	Inhibition
Extract	1	20848.2**
Plant	6	5985.8**
Extract × Plant	6	853.4**
Error	28	30.1
Coefficient of Variation (%)		2.06

** Significant at 1% probability level

multiple range test at the level of five percent probability.

Results

The Analysis of variance showed that the effect of extracts types, plants types, and their interaction on the percentage of radial growth of *A. alternata* was significant at the 1% level (Table 2). The mean comparison indicated the growth inhibition was 72.4% in whereas aqueous extract was 27.9%; Therefore, alcoholic extracts had the higher he inhibition effect than aqueous extract (Figure 1).

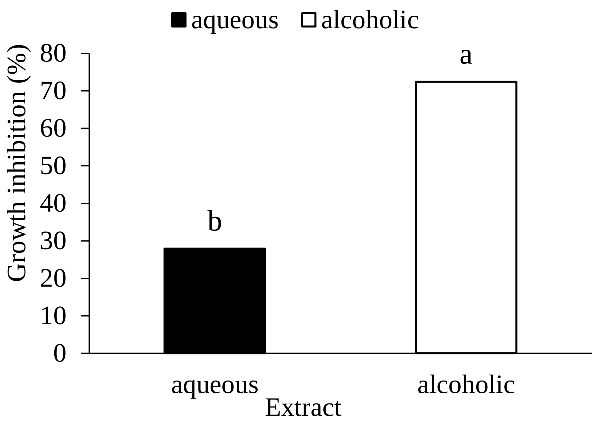

Figure 1. *Mean comparison of the effect of extract on growth inhibition percentage of Alternaria alternate. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. The black and white bars are aqueous and alcoholic extract respectively.*

The results indicated that inhibition effect in Oleander and Balangu was 82.5 and 82.7%, respectively; such that, extracts of Oleander and Balangu had the highest growth inhibition compared to other extracts of plants and control (Figure 2). In the present experiment, interaction effects of plant extracts and plant types revealed that across all plants, the alcoholic extracts showed higher inhibition effect compared to aqueous extract. The results indicated that growth inhibition effect alcoholic extracts of Oleander and Balangu were 100%, respectively, Such that, inhibition affect this extracts of Oleander and Balangu were higher than the aqueous and alcoholic extract of plants. Furthermore, observed that inhibition effect in control and aqueous extracts of Fennel reached to point 0.00% (Figure 3).

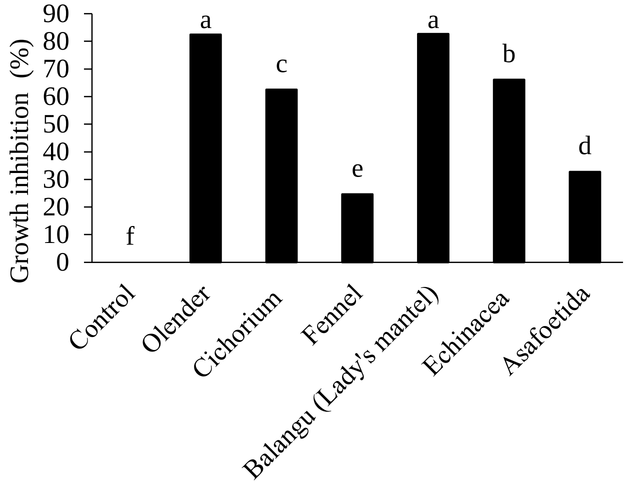

Figure 2. *Mean comparison of the effect of different plants on growth inhibition percentage of Alternaria alternate.Means with similar letters in each column are not significantly different at 5% level according to Duncan test. The black bars are plant extracts.*

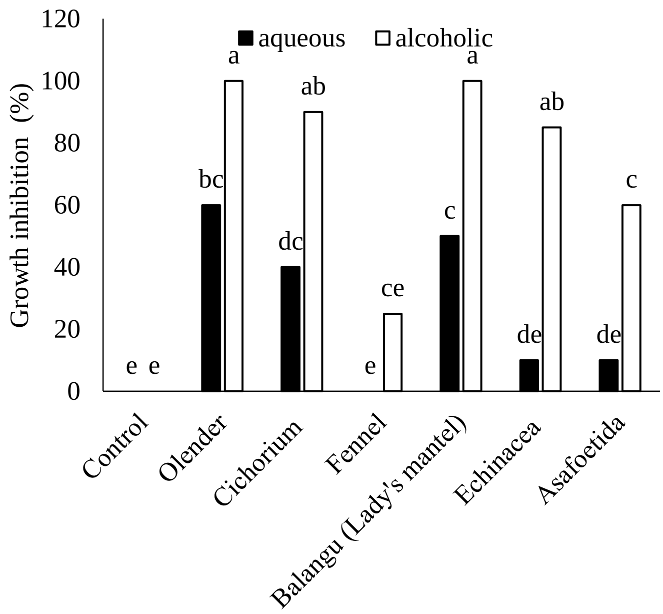

Figure 3. *Mean comparison of the interactions of different plant and extracts on growth inhibition percentage of Alternaria alternate. Means with similar letters in each column are not significantly different at 5% level according to Duncan test. The black and white bars are aqueous and alcoholic extract respectively.*

Discussion

The extract of the oleander showed 20% decline that this decrement was about 16.4% more than the inhibition effect of Echinacea and Cichorium (Hadizadeh et al., 2009). This result was consistent with the results of other researchers (Shinde et al., 2010; Hadizadeh et al., 2009). The of this experiment also supports the findings of other researcher working with alcoholic and aqueous extracts of lemongrass, oleander, basil and olive (Bokhari, 2009). According to the results, the alcoholic extract of Balangu, Cichorium, and Oleander showed 100% inhibition while no inhibitory effects of aqueous extract of fennel and Asafoetida were observed (Figure 2).

The fungus inhibition rate was significantly affected by plant type which indicates the different secondary compounds produced by different plants. The types of herbal metabolites produced by different plants in extracts have different effects on plant disease control (Cerqueira Sales et al., 2016). The plant extracts are more effective for inhibition of fungal agent in the alcoholic extract while these results suggest that the pure solvent was more effective in the extraction of active antifungal ingredients (Singh et al., 2013). However, in an experiment, the alcoholic extraction resulted in complete inhibition of fungal growth, whereas the aqueous extract did not inhibit fungus activity (Singh et al., 2013). Some antioxidant containing compounds reported in Oleander extract demonstrate multifunctional effects like anti-mutagenic effects, as well as anti-fungal activities (an immune system) which make them an important ingredient in pesticides.

These compounds as structural subunits of many natural products are very toxic encountered in fungi, bacteria and mammals. Oleandrin was detected as bioactive compound against fungus. This has an antibacterial activity against endophytic fungus and a wide variety of deterrence against it. Some of the secondary metabolites of flavonoids available in Oleander extract are fungus growth inhibitors which efficiently control fungus activities (Huang et al., 2007). In a study, researchers found that flavonoids, phenols, steroids, and terpenoids are antifungal alkaloid agents. Studying the secondary metabolites of oleander against fungus revealed that these compounds have biological toxicity activity against fungal growth and development (RaviKumar & RajKumar, 2013). The researchers expressed that phytochemical compounds react with the soluble proteins in the walls of fungi or bacteria cells and impair microbial and fungal cell membranes (Bokhari, 2009).

The adverse effect of Balangu extract on growth and development of *A. alternata* in both aqueous and alcoholic forms showed that this plant has herbal anti-fungal compounds. It was further demonstrated that the alcoholic solvent is more efficient than the aqueous solvent. According to the results of this study, the antibacterial effects of ethanol, methanol, and chloroform extracts of Balangu against gram positive and gram negative bacteria was more efficient than aqueous extracts (Mahmood et al., 2013). This would suggest that this plant has a range of active compounds that could be used against bacteria and fungi. This plant has been used in traditional medicine and has showed capabilities in skin inflammation and gastrointestinal irritation treatments (Hayat et al., 2008).

Results of previous studies on *A. alternata* control have shown that aqueous extracts of thyme and nettle, alcoholic extracts of neem and olive (Ahmad & Abdelgaleil, 2005; Shafique et al., 2007), as well as garlic, Espand and Datura (Hadizadeh et al., 2009) extracts had the most inhibitory effect on *A. alternata*. However, in this study, it was revealed that in addition to the pathogen control by aqueous and alcoholic extracts of oleander leaves, balangu, chicory, cichorium, and asafoetida seed the highest inhibition as 100% in the application of the alcoholic extract of oleander and balangu (Figure 2).

Conclusions

Extract application for post-harvest disease control is a safe method against the fungal pathogens in numerous plants. According to our information, no report has been registered in radial growth inhibitory effect of Balangu extract on *A. alternata* fungus. This research has documented the inhibitory effects of alcoholic and aqueous extracts of Balangu as well as few other medicinal plants against *A. alternata* fungus (seed-borne pathogen). The results indicated that the aqueous and alcoholic extracts of oleander leaf and Balangu seed will reduce the radial growth of *A. alternata* fungus by 50% and 100%, respectively. The other plants such as Echinacea and Cichorium also have significant inhibitory effect on growth of the fungus *A. alternata*. The present research confirmed the results of other studies in the field of anti-fungal properties of oleander. Since Balangu is cultivated as a low-cost medicinal plant and has good production potential in unfavorable conditions, it seems feasible if it could be considered in the context of biological control for fungal diseases. The results of this study warrant further research to identify the inhibitory mechanism of balangu seed extract on growth and development of fungus *A. alternata*. Popular tendency to reduce the use of pesticides leads researchers to search to achieve environmentally friendly and natural compounds for pest and diseases control. Compounds should be disintegrated rapidly and remain low residuals in food and while guarantee efficacy of toxicity.

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