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Antifungal Activity of Nettle (*Urtica dioica L.*) and European Pennyroyal (*Mentha pulegium L.*) Extracts on *Alternaria alternata*

Mitra sayidi [1] and Sevil Nematollahi* [2]

1. Former MSc student, Department of Plant Protection, Malekan branch, Islamic Azad University, Malekan, Iran
2. Assistant Prof. Department of Plant Protection, Tabriz branch, Islamic Azad University, Tabriz, Iran

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ABSTRACT

Increasing public concerns about fungicide residues have prompted interests in biological control especially use of herb extracts derived from several medicinal plants as an alternative strategy for disease management. Recently tomato leaf spot disease caused by *Alternaria alternata* has become epidemic in almost areas of Iran. This study was conducted for the identification of the effect of antifungal activity of two medicinal plants extracts; *Mentha pulegium* L. and *Urtica dioica* L. against *A. alternata*. Both plant extracts exhibited antifungal potential against the pathogenic fungus. The Nettle extract showed a more degree of inhibition with 59.78% and 77.81% inhibition rate at concentration of 1000 and 1500 ppm, respectively. The inhibition of the mycelial growth was strongly reduced in the concentration of 1500 ppm of both plant extracts. The results of this study confirm that extracts of the selected medicinal plants can be used as an alternative fungicide in control of this pathogenic fungus.

1. Introduction

Various plant pathogens as fungi, viruses and bacteria reduce plant productivity and lead to huge losses (Tapwal *et al*. 2011). The *Alternaria* associated with a wide variety of substrates including seeds, plants, agricultural products, animals, soil and the atmosphere. *Alternaria* species are known as important plant pathogens which mostly cause diseases on aerial parts of many plants worldwide and causing major losses on a variety range of crops (Woudenberg, 2013). *A. alternata* usually reported as a opportunistic pathogen which attack vigorous plants and cause economic losses (Ellis, 1985). The *A. alternata* is a saprophytic pathogen of tomato causing postharvest losses (Akhtar, 1994).

One of the most efficient method for protecting the crops against fungal infection is the use of saprophytic pathogen of tomato causing postharvest losses fungicides (Akhtar, 1994).

However, these chemical compounds are toxic and easily transmit to non-target species even polluted the environment through air, water, soil etc. Its residual effects also persist in food chain for longer time (Tapwal, 2011). In recent years the increasing of resistance of pathogenic fungi towards the synthetic fungicides is one of great concern. Therefore, it has made necessary to use some biological approaches which have been advanced as potential alternatives to synthetic fungicides for the management of diseases (Wang, 2008).

*Corresponding authors: Dr. nematollahi sevi
E-mail address: nematollahi2001@yahoo.com

Traditionally, many plants oils and extracts have been reported with antimicrobial properties. It is important to investigation for new plant-derived natural substances that can obstruct the fungal pathogenicity and is considered as a safe alternate to synthetic fungicides in plant disease managements, due to their less negative impact on environment (Gujar, 2012; Hadizadeh, 2009). Nowadays, there has been a growing interest in the determination of the biological properties of herb extracts derived from several medicinal plants such as *Mentha* and *Urtica* species. *Mentha pulegium* L. and *Urtica dioica* L. are considered as medicinal plants because of its pharmacological and biological properties (Motamedi, 2014; Joshi, 2014).

The objective of this study was the exploring the *in vitro* antifungal potential of plant extracts for inhibition activity against *Alternaria alternata*.

2. Materials and Methods

2.1. Collection of plant materials

Mentha pulegium L. and *Urtica dioica* L. plants used in this study were collected from the surrounding area of Harbil, Tabriz, Iran during 2014, and brought to the laboratory. Tomato plants were collected from agricultural lands in East Azarbaijan province.

2.2. Isolation of the pathogen

Fungal pathogen was isolated from infected tomato that collected from Tabriz province of Iran, during 2014. Samples transferred to mycology lab of Azad University of Malekan and stored at 4°C. Plant materials were washed in running tap water for 10 min. A small piece of leaf tissue (5×5 mm) was taken from the margin of a lesion, surface disinfected with 1% sodium hypochlorite solution for 1 min and then rinsed with sterile water (Frisvad 1983). Sterile small pieces were placed in Petri dishes containing potato dextrose agar (PDA), then incubated at 24±1°C until the pure fungal colonies were appeared. Pure culture was established via single spore and maintained on PCA (20 g white potato, 20 g carrot, and 20 g agar per 1 L of distilled water). The cultured fungus identified as *A. alternata* using morphological characteristics, according to the description provided by Simmons, 2007.

2.3. Preparation of plant extracts

Leaves of selected plants were thoroughly washed in running water and kept in shade to dry. Dry materials then grinded using a cutting mill. Pure methanol (96%) was used as solvent. Four hundred milliliter of solvent were added to 40 g of dry powder materials of each plant and homogenized for 1 h with a magnet. After two days' mixtures were passed through 0.2 µ size Whatmann filter paper then shaken at 160 rpm and 40 C to obtain clear extracts. The methanol was completely removed from clear solutions using a rotary evaporator. Exact extract concentrations (500, 1000 and 1500 ppm) were prepared in methanol solvent (Zaker, 2010).

2.4. Antifungal evaluation of plant extracts on mycelial growth of A. alternata in vitro

In this experiment, the extracts were mixed with sterile molten PDA to obtain final concentrations 500, 1000 and 1500 ppm, and in the case of control extract free PDA is used. The PDA was poured into 80 mm Petri dishes and then inoculated with 4 mm plugs from 7-day-old culture of *A. alternata*. The fungal pathogen was placed in the center. Petri dishes were incubated at 25°C. The measurements of the mycelial growth dynamic of the fungus were recorded on a daily basis, beginning with 24 hours after inoculation. Four replicates were used per treatment and the screening procedure repeated twice. Percentage inhabitation of mycelia growth was calculated according to the formula (Mohana, 2007):

Percentage inhibition= (C-T)/C×100

Where C, average diameter of the fungal colony of the control plate and T, average diameter of the fungal colony treated with the treatment plate.

2.5. Statistical analysis

Statistical analysis of the data obtained in the present study was carried out in a completely randomized design layout using SPSS ver. 19, where the comparison of means of different treatment was performed using factorial design.

3. Results

In the present investigation, various concentrations of plant extracts significantly showed antifungal potential against *A. alternaria*, which is demonstrated by inhibition of fungal mycelia growth (Table 1). Table 2 shows means comparison of the effect of different concentrations the plant extracts on mycelial growth. The results revealed that the antifungal activity of the extracts being positively influenced by increasing the concentration of the extracts in the growth media. The plant extracts at 1500 ppm showed potent and inhibitory effect on the radial growth of *A. alternata*. The final measurements revealed that the extracts obtained from *Mentha pulegium* had highest inhibitory activity, while *Urtica dioica* presented lower inhibition activity against tested pathogen mycelium growth.

Based on the results, all extracts concentrations had significant inhibition on the growth of *A. alternate* (Fig1). ranging from 67.87 and 77.81% at 1500 ppm of *Urtica dioica* and *Mentha pulegium* extracts, respectively. The lowest antifungal activity of plant extracts results was related to the concentration of 500ppm which had 40% inhibition of growth approximately in both extracts.

Table 1. Variance analysis of effects of plant extract for control of *A. alternata.*

Source of variation	Degree of freedom	Means square	F
Plant extracts	1	2.195	92.727**
Concentration	3	86.055	36.353**
Daily records	5	50.613	21.383**
Plant extracts × concentration	3	0.459	19.375 **
Plant extracts × daily records	5	0.051	2.172 ns
Concentration × daily records	15	4.056	171.329 **
Plants extracts × concentration × daily records	15	0.037	1.557 ns
Error Coefficient of variation	143	0.024	

**Significant at 1% probability level.

Table 2. Mean comparison of effects of plant extracts concentration on mycelial growth of *A. alternata.*

Plants name	Mean mycelial growth			
	0 ppm	500 ppm	1000 ppm	1500 ppm
Mentha pulegium	4.45 [a]	2.59 [b]	1.82 [c]	1.16 [d]
Urtica dioica	4.45 [a]	2.70 [b]	2.12 [c]	1.55 [d]

Means within each column followed by same letter are not significantly different at 1% probability level.

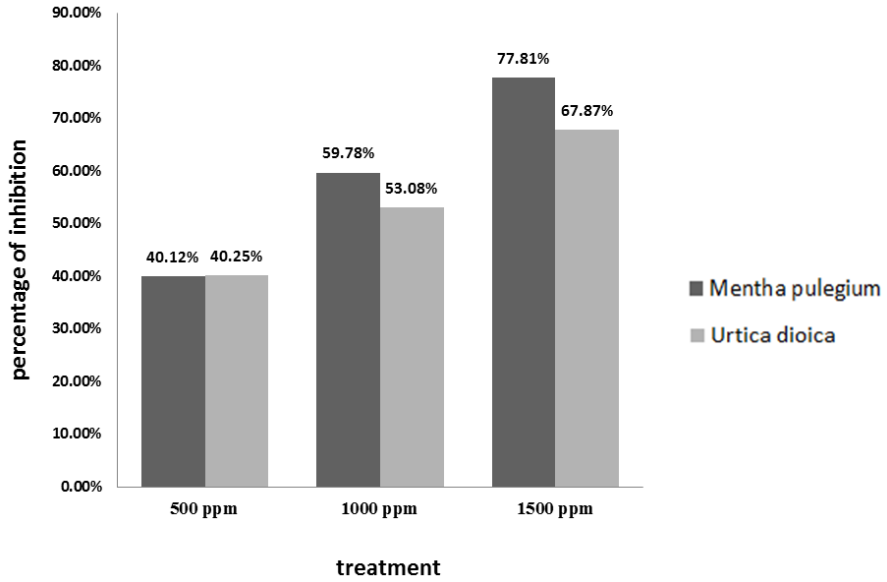

Fig. 1. Mean comparison of effect of plant extracts concentrations on growth inhabitation of *A. alternata*.

4. Discussion

Plant extract include ethanol extracts and its fractions, resin and essential oils have been reported to have antifungal activity (Gahukar, 2012). Most investigation of plant extracts have been shown effective inhibitory against a wide range of pathogens *in vitro* but these researches almost failed to control diseases under field conditions (Benner, 1993; Chang, 2008). However, *in vitro* screening of plant extracts is an important first step in identifying plants with potential application (Tegegne, 2007).

Previously, Tegegne (2008) showed that different natural substances, such as essential oils and extracts of different species have varying degree of growth inhibitory effects against some *Alternaria*, *Fusarium*, *Botrytis* and *Rhizoctonia*. Methanolic extracts of six selected plant leaves screened for their antifungal activity against *A. alternata* at 5, 10 and 20% concentration *in vitro*. Phytochemical analysis of leaf extracts determined the presence of some chemical compounds including Alkaloids, Terpenoids, Phenols, Saponins and Tannins at various concentrations (Singh, 2014).

Rodino (2014), showed *in vitro* effects of antifungal properties of ethanolic and aqueous extracts of traditional medicinal plants including; *Xanthium strumarium*, *Artemisia absinthium*, *Rosmarinus officinalis* and *Datura stramonium*, against plant pathogenic fungus *Alternaria alternata*. Also recently Buch and Arya (2017) showed that three main pathogenic fungal diseases in Cotton (*Gossypium herbaceum*) can be successfully controlled using selected plant extract and have demonstrated that plant extracts have potential capabilities for use as chemical fungicides replacement. Similar to this research, it has been reported that three plant extracts have significant inhibition effects on *Alternatia solani* (Tomazoni et al., 2017)

Although mechanism of action of plant extracts and essential oils is unclear, several studies have been conducted to understand their function. Several researches attributed this function to the phenolic compounds and some investigations have been proposed to possible action mechanisms by which mycelial growth may be reduced or totally inhibited. Researches showed that it is the toxic effects of essential oils components and extracts on the functionality and structure of the cell membrane (Sikkema, 1995; Veldhuizen, 2006). Essential oils components and extracts might act on the hyphae of the mycelium and resulting in collapse and death of the mycelium, the loss of rigidity and integrity of the hypha cell wall, by providing exit of components from the cytoplasm (Sharma, 2006).

Over the past two decades' extensive investigations in an attempt to reduce the use of synthetic fungicides have been made into the possible exploitation of plant compounds as

natural commercial products that are safe for humans and the environment, and also they are being easily accessible and relatively cost effective (Daayf, 1995; Rodino, 2014).

In this study, the ability of ethanolic extracts derived from selected plants to inhibit the mycelial growth of *A. alternata* was evaluated *in vitro* contact assays. The active extract was *Mentha pulegium* followed by *Urtica dioica*. Antifungal screening indicated that *Mentha pulegium* and *Urtica dioica* extracts at a concentration of 1500 ppm markedly inhibited the mycelial growth of test pathogen.

In conclusion, examination of various concentrations of *Mentha pulegium* and *Urtica dioica* on *A. alternata* in this study showed promising prospects for the utilization of natural plants extracts in postharvest disease control. So organic extract of selected medicinal plants could be applied as alternative industrial products to synthetic fungicides for using in agro-industries and also to screen and develop such novel types of selective and natural fungicides in the biocontrol of many agricultural plant pathogens causing drastic losses to crop.

Refereces

Akhtar, K. P., Matin, M., Mirza, J. H., et al. (1994). Some studies on the postharvest diseases of tomato fruits and their chemical control. Pak. J. Phytopathol. 6: 125-129.

Benner, J. P. (1993). Pesticidal compounds from higher plants. Pest Management Science. 39(2): 95-102.

Buch, H., Arya, A. (2017). Antifungal activity of selected plant extracts against three pathogenic fungi of Gossypium herbaceum. Current Research in Environmental & Applied Mycology, 7(2), 103-108.

Chang, H. T., Cheng, Y. H., Wu, C. L., et al. (2008). Antifungal activity of essential oil and its constituents from Calocedrus macrolepis var. formosana Florin leaf against plant pathogenic fungi. Bioresource technology. 99(14): 6266-6270.

Daayf, F., Schmitt, A., Belanger, R. R. (1995). The effects of plant extracts of Reynoutria sachalinensis on powdery mildew development and leaf physiology of long English cucumber. Plant Disease. 79(6): 577-580.

Ellis, M. B., Ellis, J. P. (1985). Microfungi on land plants, Biddles Ltd. Guildford and King's Lynn, Great Britin, 818 pp.

Frisvad, J. C. (1983). A selective and indicative medium for groups of Penicillium viridicatum producing different mycotoxins in cereals. Journal of Applied Microbiology. 54(3): 409-416.

Gahukar, R. T. (2012). Evaluation of plant-derived products against pests and diseases of medicinal plants: A review. Crop Protection. 42: 202-209.

Gujar, J., Talwankar, D. (2012). Antifungal potential of crude plant extract on some pathogenic fungi. World J Sci Tech. 2(6): 58-62.

Hadizadeh, I., Peivastegan, B., Kolahi, M. (2009). Antifungal activity of nettle (Urtica dioica L.), colocynth (Citrullus colocynthis L. Schrad), oleander (Nerium oleander L.) and konar (Ziziphus spina-christi L.) extracts on plants pathogenic fungi. Pakistan journal of biological sciences: PJBS. 12(1): 58-63.

Joshi, B., Mukhija, M., Kalia, A. (2014). Pharmacognostical review of Urtica dioica L. International Journal of Green Pharmacy. 8(4): 201.

Mohana, D. C., Raveesha, K. A. (2007). Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. Journal of Agricultural Technology. 4(1): 119-137.

Motamedi, H., Seyyednejad, M., Dehghani, F., Hasannejad, Z. (2014). Investigation of Antibacterial Activity of Ethanolic and Methanolic Extracts of Mentha pulegium L. Zahedan Journal of Research in Medical Sciences. 16(10): 55-59.

Rodino, S., Butu, M., Petrache, P., et al. (2014). Antifungal activity of four plants against Alternaria alternata. Scientific Bulletin. Series F. Biotechnologies. 18: 60-65.

Schmitz, H. (1930). Poisoned food technique. Industry of Engineering Chemical. London, USA, 333-361.

Sharma, N., Tripathi, A. (2006). Fungitoxicity of the essential oil of Citrus sinensis on post-harvest pathogens. World Journal of Microbiology and Biotechnology. 22(6): 587-593.

Sikkema, J., De Bont, J. A., Poolman, B. (1995). Mechanisms of membrane toxicity of hydrocarbons. Microbiological reviews. 59(2): 201-222.

Simmons, E.G. (2007) Alternaria: an identification manual. CBS, Utrecht, 775 pp.

Singh, G., Gupta, S., Sharma, N. (2014). In vitro screening of selected plant extracts against Alternaria alternate. Journal of Experimental Biology. 2(3): 344-351.

Tapwal, A., Garg, S., Gautam, N., Kumar, R. (2011). In vitro antifungal potency of plant extracts against five phytopathogens. Brazilian archives of biology and technology. 54(6): 1093-1098.

Tegegne, G., Pretorius, J. C. (2007). In vitro and in vivo antifungal activity of crude extracts and powdered dry material from Ethiopian wild plants against economically important plant pathogens. BioControl. 52(6): 877-888.

Tegegne, G., Pretorius, J. C., Swart, W. J. (2008). Antifungal properties of Agapanthus africanus L. extracts against plant pathogens. Crop Protection. 27(7): 1052-1060.

Tomazoni, E. Z., Pauletti, G. F., da Silva Ribeiro, R. T., Moura, S., Schwambach, J. (2017). In vitro and in vivo activity of essential oils extracted from Eucalyptus staigeriana, Eucalyptus globulus and Cinnamomum camphora against Alternaria solani causing early blight in tomato. Scientia Horticulturae, 223, 72-77.

Veldhuizen, E. J., Tjeerdsma-van Bokhoven, J. L., et al. (2006). Structural requirements for the antimicrobial activity of carvacrol. Journal of agricultural and Food Chemistry. 54(5): 1874-1879.

Wang, Y., Bao, Y., Shen, D., et al. (2008). Biocontrol of Alternaria alternata on cherry tomato fruit by use of marine yeast Rhodosporidium paludigenum Fell & Tallman. International journal of food microbiology. 123(3): 234-239.

Woudenberg, J. H. C., Groenewald, J. Z., Binder, M., Crous, P. W. (2013). Alternaria redefined. Studies in Mycology. 75: 171-212.

Zaker, M., Mosallanejad, H. (2010). Antifungal activity of some plant extracts on Alternaria alternata, the causal agent of Alternaria leaf spot of potato. Pakistan journal of biological sciences: PJBS. 13(21): 1023-1029.