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Evaluation of Antifungal Properties of *Zataria multiflora* L. and *Mentha piperita* L. Extracts on *Aspergillus flavus*

[1]Mehdi Mohamadian Sarcheshmeh, [3]Mmarjan Nikan, [2]Abolfazl Haseli and [4]Sahar Omidpanah
[1]Department of Plant Pathology, Islamic Azad University, Abarkouh Branch,
[2]Head of Veterinary Hospital, Abarkouh, Yazd,
[3]Medicinal Plants Research Center, Tehran University of Medical Sciences,
[4]Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran

Abstract: Pest control is one of the important challenges of the agriculture and food commodity. The contamination of pistachio with a flatoxin produced by *Aspergillus flavus* is the main problem in the export of this product in recent years. *Aspergillus flavus* is one of the fungus which has a wide range of hosting in seed, air and soilalong within animal foods. In the present research, the effect of aqueous extracts of zataria (*Zataria multiflora* Boiss) and peppermint (*Mentha piperita* L.) aerial parts were examined against *A. flavus* isolated from contaminated pistachio using agar dilution method. The extracts with concentration of 100-600 ppm were added to Potato Dextrose Agar (PDA) medium and all tests were done at least in triplicate. In order to eliminate the effect of osmotic potential of the extracts, Poly Ethylene Glycol (PEG) was used with similar osmosis pressure. The fungus growth diameter were calculated and expressed as mean±Standard Deviation (SD).

Key words: *Aspergillus flavus*, aflatoxin, *Zataria mulriflora*, *Mentha piperita*, Iran

INTRODUCTION

In the recent years, Iran have been recognizing as the biggest and the most important producer and exporter of pistachio in the world. The contamination of pistachio with *A. flavus* and therefore, aflatoxin are the main problems in the export of this product in recent years. Contamination of pistachio nut with Aspergillus species was indicatedin previous study (Cotty, 1994; Mojtahedi *et al*., 1979) and this have beenvalued as the main problem in production, consumption and export of this product. Several environmental factors are known to influence aflatoxin production but temperature and relative humidity are considered to be the most critical ones. In several studies, it has been reported that extracts and powders from various herbs and oils have antifungal activity and some of them are even inhibit the production of aflatoxin (Rosca-Casian *et al*., 2007; Krishnamurthy and Shashikala, 2006; Thanaboripat *et al*., 2004). This fungus can be isolated from soil, air-borne dust, plants and insects. Corn, peanuts, tree nuts and cottonseed are often contaminated with aflatoxins, making them unfit for human and animal consumption (Abbas *et al*., 2004). Medicinal plants are a rich source of antimicrobial agents and normally produce bioactive secondary meolites and many of them exhibit activity against microorganisms, hence they could be used as antimicrobial agents (Mahesh and Satish, 2008; Ibrahim, 1997). For instance, antimicrobial effects of essential oils of *Z. multiflora* toward yeast, gram-positive and negative bacteria, as well as *A. flavus* and *A. parasiticus* has been well documented in the previous studies (Gandomi *et al*., 2009; Rasooli *et al*., 2009; Zomorodian *et al*., 2011). In addition, inhibitory effect of *M. piperitaoil* was examined toward *A. fumigatus*, *A. flavus* and *A. ochraceus* during 10 days indicateing inhibitory effect of the oil against all tested fungus (Skrinjar *et al*., 2009). Chemical pesticides have adverse effects on human health and environment, therefore there is a necessity for finding acceple substitute for them. In the present study, aqueous extracts of zataria (*Z. multiflora*) and peppermint (*M. piperita*) belonging to Lamiaceae family examined against *A. flavus* isolated from contaminated pistachio.

MATERIALS AND METHODS

Plant materials: Fresh leaves of *Z. mulriflora* and *M. piperita* were collected during flowering in April, 2011 from Abarkoh (Yazd Province) in Iran. Samples were dried at room temperature.

Corresponding Author: Sahar Omidpanah, Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran

Antifungal assay: The antifungal properties of the extracts were examined using agar dilution method in the culture medium (Viuda-Martos *et al*., 2007). The fungus species was obtained from Pistachio Research Center of Rafsanjan, Kerman Province (ASTCP: 1684 FERDOSIEH). The extracts were filtered using Millipore membrane filter (0.22 μm) and culture medium mixed with each extracts to obtain concerned concentrations. The blank disks (6 mm) were impregnated in the fungus suspension (1×106 CFU mL^{-1}) and placed in the center of mediums with different concentrations of the extracts (100-600 ppm). In order to eliminating osmotic effects of the extracts on the fungus growth, PEG with equal osmotic potential of plant extracts were added to PDA medium as a negative control. The tests were performed at least in triplicate and the mean diameters of fungus growth were calculated as well. All mediums were kept at 25°C and growth diameter of fungi were measured at the end of 7 days.

Data collection: The average diameter of inhibition zone of *A. flavus* growth were measured after 7 days.

Statistical analysis: Experiment was carried out using factorial analysis with randomized complete design, on each of the 3 repetitions. The extract at concentrations of 10-600 ppm and PEG as the control were tested. For analysis of variance using SAS statistic system and LSD test was used for comparison of means. Also, graph drawing was done with Excel software.

RESULTS AND DISCUSSION

Fungus growth diameter of *A. flavus* isolated from contaminated pistachio in the presence of aqueous extracts of two medicinal plants were successfully investigated in this study in PDA medium using disk diffusion method. The results of this study revealed that the plants extracts were significantly inhibited *A. flavus* growth in comparison with each other and with negative control (PEG) (Table 1). Based on the results, it is clear that the plants extracts decreased the fungus growth dose dependently. Among them, the aqueous extracts of zataria (*Z. multiflora*) with concentration of 600 ppm rather arrested the fungus growth. They also effectively prohibited the fungus even at lower concentrations (200-500 ppm) in comparison with peppermint extract and negative control. Inhibition effect zataria extract with concentration of 100 ppm on the fungus growth were similar to peppermint (*M. piperita*) with concentrations of 600 ppm. The growth of *A. flavus* have been effectively inhibited by zataria extract at high concentrations (600 ppm) with fungus growth diameter of 8.0±1.0 mm but the extract of peppermint, exhibited lower activity against the fungus (600 ppm) with fungus growth diameter of 19.0±1.0. In addition, MIC values of both plants appeared to be >600 ppm. The fungus growth diameter in the presence of PEG was calculated as 57.6±0.5, showed that the plants extracts effectively prohibited the fungus growth in comparison with control group. The results of a previous investigation suggested that aqueous extracts of thyme and coriander were mostly inhibited the isolated strain of *A. flavus* followed by dill and rose extracts (Yahya Abadi *et al*., 2011). Previous study mostly examined antifungal activity of plants oils, for example essential oils of *T. vulgaris*, *Z. multiflora*, *M. piperita*, *M. pulegiumand*, *O. basilicum* demonstrated inhibitory activity against growth of *A. flavus* and other microorganisms (Montes-Belmont and Carvajal, 1998; Mahboubi and Haghi, 2008; Gandomi *et al*., 2009; Rasooli *et al*., 2009; Zomorodian *et al*., 2011). However, in this investigation aqueous extracts of *Z. multiflora* mostly arrested growth of the fungus attribute to their polar chemical constituents which are water soluble. On the basis of the aforementioned studies, the antifungal activity of the plants attributed to the various kind of secondary meolites like flavonoids, alkaloids, phenolic acids and the essential oils constituents (Bais *et al*., 2002; Owoyale *et al*., 2005). Based on the results of this study, it could be proposed that aqueous extract zataria and peppermint effectively inhibited *A. flavus* growth attributed to their polar secondary meolites and they are suile, as natural antifungal agents to prevent the fungus activity. Evaluation of synergistic activity of the examined plants in prevention of the fungus growth for longer periods of time followed by organoleptic properties, such as taste and smell of the processed food with those active extracts followed by isolation and identification of their active compounds are recommended for the further studies.

Table 1: Effects of the plants extracts on *A. flavus* growth at different concentrations expressed as fungus growth diameters (±SD)

	Fungus growth diameter (mm)						
Conc. (ppm)	100	200	300	400	500	600	MIC
Z. multiflora	20.6±0.5	15.3±0.5	14.6±0.5	10.6±0.5	8.0±0.0	8.0±1.0	>600
M. piperita	34.6±0.5	30.3±0.5	29.3±0.5	21.3±1.5	20.0±1.0	19.0±1.0	>600
Control	60.0±0.0	59.3±0.5	58.6±0.5	58.3±0.5	58.3±0.5	57.6±0.5	

CONCLUSION

The results indicated that all concentrations of the extracts significantly inhibited the fungi growth comparing with each other and also with control (PEG). The aqueous extract of zataria was more active against the fungus growth in comparison with extract of peppermint. The Minimum Inhibition Concentration (MIC) values of both plants calculated as >600 ppm.

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