Available online at www.scholarsresearchlibrary.com

Scholars Research Library

***J. Nat. Prod. Plant Resour.*, 2015, 5 (2):1-5**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

In vitro anti-fungal activity of garden thyme (*Thymus vulgaris* L.) extract against *Aspergillus flavus*, the major producer of aflatoxin*

Jafar Nikan[1] and Zahra Jafari[2]

[1] Research Assistant Professor at the Plant Protection Research Dept., Agricultural and Natural Resources Research Centre of Hamadan, Hamadan, Iran
[2] Former Student of the University of Applied Science and Technology of the Jihad Agriculture Organization of Hamadan, Hamadan, Iran

(This article is the results of the B.Sc research project of the second author presented to the University of Applied Science and Technology of the Jihad Agriculture Organization of Hamadan.)*

ABSTRACT

In this study the effect of garden time (Thymus vulgaris) extract on the growth of Aspergillus flavus, the major producer of aflatoxin, was evaluated in vitro. The assessment was done by conducting an experiment as a completely randomized design with six treatments and four replications. The treatments were four concentrations of garden time extract (150, 300, 450 and 600 mg/ml) and two controls (distilled water and fungicide solution). Each treatment was applied to the fungus by diffusing through a paper disc loaded with each treatment and placed in the center of a plate of PDA medium inoculated with spore suspension of the fungus. Measuring the average radius of A. flavus growth inhibition zone around the paper discs loaded with each of the treatments indicated that compared to the negative control, three concentrations of garden time (300, 450 and 600 mg/ml) could significantly inhibit mycelial growth of the fungus. After the treatment fungicide, the highest growth inhibition zone radiuses, 8mm and 6.5mm, were those of 600 mg/ml and 450 mg/ml of garden time extracts, respectively. Moreover, the rate of growth inhibition was concentration dependent. Considering these results it seems that garden time extract is potentially a natural alternative for controlling A. flavus mold.

Keywords: *Thymus vulgaris*, growth inhibition, natural fungicide

---

INTRODUCTION

A. flavus is an opportunistic fungal pathogen of various crops. It is a common soil fungus and has a broad host range. The major concern with this fungus in agriculture is that it produces highly carcinogenic toxins called aflatoxins which are health hazards to animals and human. *A. flavus* spores are found in the air and in the soil. These spores thrive on any organic substance with little moisture. Products with moisture levels above 16% are capable of supporting the growth of *A. flavus* [1]. In addition, their large number of enzymes enables them to utilize a variety of substances for food. Hence, they are often found on exposed food stuffs, and cause decay. *A. flavus* is one of the storage fungi that develops on a wide variety of stored grains such as wheat, peanuts, soybeans, corn, and so on [2, 3].

Under improper storage conditions, *A. flavus* is capable of growing and forming aflatoxin in almost any crop seed. It is also a pathogen of animals and human. In human it is predominantly an opportunistic pathogen of immune-suppressed patients that causes aspergillosis and it is the most common cause of superficial infection [4-6].

To prevent the fungus, a number of field control measures are being utilized or explored, including: modification of cultural practices; development of resistant crops; biological control, competitive exclusion using strains that do not produce aflatoxin; and the development of field treatments that would block aflatoxin production [7-10]. Chemical control of the fungus can be achieved by the application of synthetic fungicides [11]. Although efficient control of crop pests is generally achieved by the use of synthetic pesticides [12], due to increasing awareness on the risks associated with the use of pesticides including: the advent of pesticide resistant pests, ecological impact of pesticide application on the environment and particularly consumer concerns about pesticide residue in crops [13, 14], much attention is being focused on the alternative methods of pest control. Natural plant extracts have been recommended as suitable alternative choices to synthetic chemicals [15-21].

It has been shown that mycelial dry weight of *A. flavus*, grown on the medium with the extracts of lichens (*Ramalina farinacea*), was inhibited between 70% - 80% compared to unincorporated control medium [22]. The antifungal activity of the essential oil extracted from the bark of *Cinnamomum jensenianum* Hand.-Mazz was tested against *A. flavus*. It was found that the mycelial growth and spore germination of the fungus was inhibited by the essential oil in a dose-dependent manner [23].

The effect of *Zataria multiflora* essential oil on growth and spore production of *A. flavus* was studied. Growth of the fungus was significantly affected by all concentrations of the essential oil they used. Minimum inhibition concentration (MIC) and minimum fungistatic concentration (MFC) were 400 ppm and 1000 ppm, respectively. Moreover, scanning electron microscopy analysis showed excessive sporulation of the mold culture in the absence of essential oil while sporulation of culture containing essential oil was very sparse [24].

Irkin & Korukluoglu [25] investigated the antifungal activity of garlic (*Allium sativum* L.), onion (*Allium cepa* L.) and leek (*Allium porrum* L.) against *A. niger*. It was found that the ethanolic extract of onion (275 mg/ml) and aqueous extracts of garlic (325 mg/ml) and leek (900 mg/ml) were most inhibitory in controlling the growth of the fungus.

The essential oil and methanol extract of *Satureja hortensis* could control *A. flavus in vitro* and *in vivo* [26]. Spray of methyleugenole (0.5%), a flavoring agent naturally occurring in many fruits and foods, on peanut pod and kernels prevent colonization of *A. flavus* and aflatoxin production [27]. Garden time (*Thymus vulgaris* L.) belongs to the plant family Lamiaceae. It is thought that the origin of this plant is Mediterranean region, north of America, and some parts of Asia [28]. Thyme is a perennial, short woody plant that has C_3 metabolism system, the plant is grey in color and will grow up to 30-50cm high depending on the climate [29-31]. At present time, this plant is cultivated in large scale in Iran. Evidently, thyme continues to have an important place in the expanding medicinal plants world market. It has been reported that the Thyme volatile phenolic oil to be among the top 10 essential oils showing antibacterial, antifungal, antioxidative, natural food preservative, and mammalian age delaying properties [32, 33]. In this study the effect of alcoholic extract of garden time on inhibiting the mycelia growth of *A. flavus*, the main producer of aflatoxin was investigated *in vitro*.

MATERIALS AND METHODS

The plant materials

The medicinal plant used in the experiment was garden time (*Thymus vulgaris*) collected from the experimental fields of the Agricultural and Natural Resources Research Centre of Hamadan (ANRRCH), Hamadan, Iran. The collected plants were then air dried in the shade.

Preparation of plant extracts

The alcoholic plant extract of garden time was prepared using maceration method described previously [34]. The extraction was done briefly as follows: some dried garden time (300 g) was ground and 1000 ml 70% ethanol was added to the resulting powder and mixed well. The mixture was stirred every day for three days. The resulting extract was clarified with filter paper. The clarified extract was then allowed to dry at 37°C in an incubator.

The isolate of the fungus

The fungus was an isolate of *A .flavus* that kindly provided by the dept. of plant protection, college of agriculture, Bu-Ali Sina University, Hamadan, Iran. The fungus was then sub-cultured on potato dextrose agar (PDA) medium.

Preparation of spore suspension of the fungus

Two weeks old purified cultures of the *A. flavus* (when mycelia had produced spores) were used for preparing spore suspension. The spore suspension was prepared by adding some sterilized distilled water (~ 1 ml) to each petri dish

containing the fungus culture to allow the spores to be suspended in the water. The spore suspension was then collected and using a spore counter, its concentration was adjusted to 1×10^6 spore/ml.

Assessment of antifungal activity of the extract

Using the paper disc diffusion method [35], the assessment was done by measuring the radius of the growth inhibition zone of the fungus on PDA medium. Different concentrations (150, 300, 450 and 600 mg/ml) of garden time extract were prepared by dissolving the required amounts of dried extract into one milliliter distilled water. To remove any microbial contamination, the extracts were filtrated by using two-micrometer filters (Millipore filter 2). Then some autoclaved paper discs (6 mm in diameter) were submerged in any of the prepared concentrations of garden time extract or in sterilized distilled water (as negative control) and/or in 0.2 percent fungicide carbendazim+iprodion 52%WP solution (as positive control). The discs were allowed to be saturated with the solutions. The submerged paper discs were then air dried. Petri dishes containing PDA medium were inoculated with *A. flavus* by transferring half a milliliter of spore suspension of the fungus (1×10^6 spore/ml) and evenly spreading it on the surface of the medium. Then one paper disc impregnated with each solution was placed in the center of each *A. flavus* inoculated petri dish. The experiment was conducted as a completely randomized design with six treatments and four replicates. The treatments were four concentrations of garden time extract, as mentioned above and two controls (sterilized distilled water as negative control and fungicide solution as positive control). The treated plates were then incubated at 26°C in the dark. The average radius of the mycelia growth inhibition zone around the paper discs impregnated with each treatment was measured seven days post inoculation (until the plates were completely covered with mycelia of the fungus).

Statistical analysis of data

The statistical analysis of the data and comparison of the means was done using the SAS software and the Duncan's multiple range test, respectively.

RESULTS AND DISCUSSION

Statistical analysis of the data indicated that the growth inhibition rates for *A. flavus* treated with the various treatments were significantly different (table1). Comparison of the means of growth inhibition zone radius of the fungus around the discs impregnated with different treatments and their grouping have been shown in table 2.

The highest growth inhibition radius (9.2 mm) was that of fungicide treatment and the lowest ones were those of sterilized distilled water and 150 mg/ml of garden time extract. Among various concentrations of garden time extract, the highest growth inhibition radius of *A. flavus* (8 mm) was that of the concentration 600 mg/ml.

Moreover, the effect of garden time extract on the growth inhibition of *A. flavus* was concentration dependent, i.e. the higher the concentration the more growth inhibition of the fungus. However, no significant differences regarding to the fungus growth inhibition rates were seen between the treatments 450 mg/ml and 600 mg/ml of garden time extracts and the fungicide.

Table 1.Statistical analysis of the data of growth inhibition rate of *A. flavus* by different concentrations of garden thyme extract

Sources of variance	Degrees of freedom	Sam of squares	Mean of squares	F-value
Treatment	5	317.07	63.41	13.44*
Error	18	84.92	4.71	
Total	23	401.99		

** p<.001*
cv: 4.63

Table 2. Comparison of the means[*] of growth inhibition zone radius of *A. flavus* treated with different concentrations of garden thyme extract using Duncan's multiple range test

Treatment	Growth inhibition zone radius mean (mm)
Garden thyme extract 600mg/ml	8^{ab}
Garden thyme extract 450mg/ml	6.5^{ab}
Garden thyme extract 300mg/ml	5^{b}
Garden thyme extract 150mg/ml	0^{c}
Fungicide	9.2^{a}
Sterilized distilled water	0^{c}

[*]*The means followed by different superscript letters are significantly different*

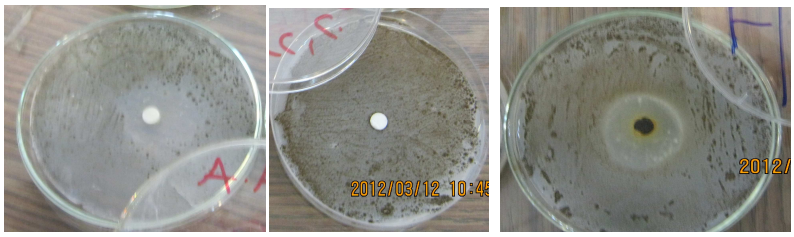

Fig.1 *In vitro* growth Inhibition zone of *A. flavus* on PDA medium resulting from treatment of the fungus with 600 mg/ml of garden thyme extract (left), negative control, distilled water (middle) and positive control, fungicide (right)

This report indicates that the extract of garden thyme has anti-pathogenic, in particular antifungal activity, the fact that has been reported previously [36, 37]. We found that the growth of *A. flavus* can be inhibited by the application of garden time extract as was found by using the extract of lichens (*Ramalina farinacea*) [22], or by using the essential oil extracted from the bark of *Cinnamomum jensenianum* [23] and by the application *Zataria multiflora* essential oil [24]. This preliminary study proved that the garden time extract could be used as an alternative for the control of *A. flavus*, *in vitro*. However, it needs to be tested under virtual conditions.

CONCLUSION

It was proved that the alcoholic extract of garden thyme was capable of bringing about the desired antifungal effect against A .flavus in a concentration dependent manner.

Acknowledgement
The authors would like to thank to dr. K. Razaghi (former staff of ANRRCH) for providing the required plant extract and to A. Moradi-Payam (an inviting lecturer at UAST-JAOH) for the statistical analyses of the data.

REFERENCES

[1] CJ Alexopoulos**.** Introductory Mycology. 2nd Edition. John Wiley and Sons, Inc., New York. London, **1962**, 271**.**
[2] CM Christensen. *CRC Critical Reviews in Environmental Contamination*, **1971**, 2, 57- 80.
[3] M Houshiyarfard; H Rouhani; M Falahati-Rastegar; E Mahdikhani Moghaddam; S Malekzadeh. *Journal of Nuts*, **2012,** 3(3), 13-20.
[4] AM Saad; AM Abdel Gadir; MO Moss**.** *Food additives and contaminants*, **1995**, 12 (2), 255-261.
[5] MT Hedayati; AC Pasqualotto; PA Warn; P Bowyer; DW Denning. *Microbiology*, **2007**, 153 (6), 1677-1692.
[6] A Ghosh; A Raj; V Mohanasrinivasan**.** *Bulletin of Environment, Pharmacology & Life Sciences*, **2012**, 1(2), 30 - 32.
[7] MA Klich. *Mol. Plant Pathology*, **2007**, 8(6), 713-22.
[8] Y Yan-ni; Y Lei-yan; J Jin-hua; M Zhong-hua**.** *Journal of Zhejiang University of Science B*, **2008**, 9(10): 787–792.
[9] S Amaike; NP Keller. *Annual Review of Phytopathology*, **2011**, 49, 107-133.
[10] KC Ehrlich. *Frontiers in Microbiology*, **2014**, 5, 1-9.
[11] S Formenti; N Magan; A Pietri; P Battilani. *Phytopathologia Mediterranea*, 2012, 51(1), 247−256.
[12] K Kiran; S Linguraju; S Adiver. *Journal of Mycology and Plant Pathology*, **2006**, 36, 77-79.
[13] E Viana; JC Malto; G Font. *Journal of Chromatography*, **1996**, 754, 437-444.
[14] J Nikan; M Morowati. Final Report of the research project coded 04-83-16-89102, Agricultural and Natural Resources Research Organization, **2013**, 44300.
[15] KL Suhr; PV Nielsen. *Journal of Applied Microbiology*, **2003**, 94, 665–674.
[16] A Ownagh; A Hasani; K Mardani; S Ebrahimzadeh. *Veterinary Research Forum*, **2010**, 1(2), 99 – 105.
[17] S Bahraminejad; M Maerefatzadah Khamenah; S Abbasi; A ZareKhafari. *Modern Technology in Agriculture*, **2010**, 2, 1-11.
[18] S Yahyaabadi; E Zibanejad; M Doudi. *Journal of herbal drugs*, **2011**, 2 (1), 69-81.
[19] M Jafarpour; AR Golparvar; A Lotfi. *Journal of Herbal Drugs*, **2013**, 4(3), 115-118.
[20] A Babaei; H Tavafi; M Manafi; A Fahimifar. Feyz, *Journal of Kashan University of Medical Sciences*, **2014**,17(6), 537-544.
[21] J Nikan; H Khavari. *Journal of herbal drugs*, **2014**, 5(1), 19-24.
[22] H Suberu. African *Journal of Biotechnology*, **2004**, 3(9), 468-472**.**

[23] T Jun; H Bo; L Xiuli; Z Hong; B Xiaoquan; H Jingsheng; W Youwei. *Food Chemistry*, **2012**, 130(3, 1), 520–527.
[24] H Gandomi Nasrabadi; A Misaghi; A Akhondzadeh basti; A Khosravi; S Bokaei; A Abbasifar. **2008**, *Journal of Medicinal Plants*, 3(27), 45-51.
[25] R Irkin; M Korukluoglu. *African Journal of Biotechnology*, **2007**, 6(4), 384-387.
[26] NDikbas; R Kotan; F Dadasoglu; F Sahin. *International Journal of Food Microbiology*, **2008**, 124(2), 179–182.
[27] P Sudhakar; P Latha; Y Sreenivasulu; BV Bhaskar Reddy; TM Hemalatha; M Balakrishma; K Raja Reddy. *Indian Journal of Experimental Biology*, **2009**, 47, 63-67.
[28] E Stahl-Biskup; F Saez. Thyme, **2002**, London: Taylor and Francis, 20-42.
[29] E Stahl-Biskup. *Journal of Essential Oil Research*, **1991**, 3, 61-82.
[30] M Ozguven; S Tansi. *Turkish Journal of Agriculture and Forestry*, **1998**, 22, 537-542.
[31] AR Golparvar. *International Journal of Life Science and Medical Research*, **2011**, 1(1), 1-4.
[32] W Letchamo; A Gosselin. *Journal of Horticultural Science and Biotechnology*, **1996**, 71, 123 – 134.
[33] SAL Jackson; RKM Hay. *Annals of Horticultural Science*, **1994**, 69, 275-281.
[34] NE Durling; O J Catchpole; JB Grey; RF Weebby; KA Mitchell; LY Foo; NB Perry**.** *Food Chemistry*, **2007**, 101, 1417-1424.
[35] WH Himratul-Aznita; N Mohd-Al-Faisal; AR Fathilah. *Journal of Medicinal Plants Research*, **2011**, 5(6), 878-884.
[36] DJ Daferera; BN Ziogas; MG Polissiou. *Journal of Agriculture and Food chemistry*, **2000**, 48(6), 2576 -81.
[37] A D Zambonelli; AZ Aurelio; A Bianchi ; A Albasini. *Journal of Phytopathology*, **1996**,144, 491-494.