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Antifungal activity of essential oils on *Aspergillus parasiticus* isolated from peanuts

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Abstract: *Aspergillus parasiticus* is one of the most common fungi which contaminates peanuts by destroying peanut shells before they are harvested and the fungus produces aflatoxins. The aim of this study was to evaluate the antifungal activities of seventeen essential oils on the growth of the aflatoxigenic form of *A. parasiticus* in contaminated peanuts from commercial outlets in Georgia. The agar dilution method was used to test the antifungal activity of essential oils against this form of *A. parasiticus* at various concentrations: 500; 1,000; 1,500; 2,000; 2,500 ppm. Among the seventeen essential oils tested, the antifungal effect of cinnamon, lemongrass, clove and thyme resulted in complete inhibition of mycelial growth. Cinnamon oil inhibited mycelial growth at ≥ 1,000 ppm, lemongrass and clove oils at ≥ 1,500 ppm and thyme at 2,500 ppm. However, cedar wood, citronella, cumin and peppermint oils showed partial inhibition of mycelial growth. Eucalyptus oil, on the other hand, had less antifungal properties against growth of *A. parasiticus*, irrespective of its concentration. Our results indicate that the aflatoxigenic form of *A. parasiticus* is sensitive to selected essential oils, especially cinnamon. These findings clearly indicate that essential oils may find a practical application in controlling the growth of *A. parasiticus* in stored peanuts.

Key words: agar dilution method, antifungal agents, essential oils, mycelial growth inhibition, peanuts

Introduction

Aspergillus species is a very common fungus in the environment, and can be a problem in stored grains. *Aspergillus parasiticus* and *A. flavus* synthesize aflatoxins when they grow on a variety of susceptible food and feed crops. These fungi have been reported as one of the serious contaminants of different plants and plant products such as maize, peanuts, rice, cotton seeds, and spices, in addition to milk products (El-Nagerabi *et al*. 2012). Aflatoxins are among the most carcinogenic naturally occurring compounds known and they pose significant health risks to humans and animals (Elshafie *et al*. 2010). The state of Georgia has suffered a setback in peanut production due to *A. flavus* contamination. Currently, there's no direct action peanut farmers can take to control fungi that produce aflatoxin (Achar *et al*. 2009).

Over the past few years, much effort has been put into research on new antifungal agents to control the growth of *Aspergillus* species in peanuts intended for human and animal consumption. Chemical preservatives have been used to control the growth of or to exclude the *Aspergillus* species (Daferera *et al*. 2003). However, many disadvantages are associated with the use of chemical preservatives as antifungal agents. Extensive use of these substances may produce several side effects to consumers (Basilico and Basilico 1999).

It is a well-established fact that, some plant based essential oils contain compounds that are able to inhibit fungal growth. There is considerable interest in these essential oils from aromatic plants with antimicrobial properties to control pathogens and toxin-producing moulds (Soliman and Badeea 2002; Tepe *et al*. 2005). The growth and aflatoxin production by *A. flavus* and *A. parasiticus* were found to be sensitive to essential oils extracted from some medicinal plants like *Mentha spicata* L., *Thymus vulgaris* L., *Majorana hortensis* Moench., *Mentha longifolia* L. (Mahfouz *et al*. 1995). Although the majority of the essential oils are classified as Generally Recognized As Safe (GRAS), their use in foods as preservatives is often limited due to flavor considerations (Lambert *et al*. 2001). There is an increasing demand for accurate knowledge about the minimum inhibitory concentrations (MIC) of essential oils to enable a balance between sensory acceptability and antifungal efficacy. Therefore, in the present investigation, essential oils from different plant origins were evaluated for their efficacy, as antifungal agents against *A. parasiticus* from contaminated peanuts in Georgia.

Materials and Methods

Aspergillus parasiticus strains

In the present study, the strains of *A. parasiticus*, isolated from contaminated peanuts obtained from commercial outlets in Georgia, were selected. Peanuts were directly

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plated on Potato Dextrose Agar (PDA) medium. After the incubation period of 12 h darkness and 12 h of light at 28+2°C for 7 days, the fungal colonies were observed under a light microscope (Leica, M13595) and representative isolates of fungal colonies were transferred onto fresh PDA plates to study their macro- and micromorphological characteristics such as color of the colony, conidial heads, vesicle, phialides and conidia. The isolates were identified using the taxonomic key prepared by using fungal keys and manuals (Klich 2002; Samson *et al.* 2004).

Mycotoxicological analysis of *Aspergillus parasiticus* species

Yeast Extract Sucrose (YES) agar was used in this study, as a medium for aflatoxin production, since YES has been reported to favor the production of high concentrations of aflatoxin (Gqaleni *et al*. 1997). All the isolated *Aspergillus* species were inoculated onto YES agar medium plates, sealed with parafilm and incubated at 27°C in a CO_2 incubator (Fischer Scientific, Isotemp) for 10–15 days. After the incubation period, plates were observed under ultraviolet (light Spectroline CC-80) to detect the presence of aflatoxin production (Fente Fete *et al*. 2001). If the mould fluoresced under UV light it was considered to be aflatoxin positive and confirmed as an aflatoxigenic form of *A. parasiticus.*

Sources of essential oils

Seventeen, pure and natural essential oils such as cinnamon, clove, lemongrass, thyme, cedar wood, myrrh, cumin, citronella, spearmint, peppermint, tea tree oils, lavender, ginger, cardamom, black pepper, orange and eucalyptus were purchased (Sigma Aldrich, USA).

Determining antifungal activity

Antifungal activity of each essential oil was tested using the agar dilution technique as described by Viuda-Martos *et al*. (2007) with minor modification. The essential oils were incorporated into the PDA medium with 0.5% Tween 20 as an emulsifying agent to final concentrations of essential oils: 500; 1,000; 1,500; 2,000 and 2,500 ppm, respectively. The agar plates were dried at room temperature for 30 min prior to the inoculation. To these plates, 100 µl of spore suspension taken from 4–6 day old *A. parasiticus* culture were inoculated at the center of the plate, using micropipettes. The plates were incubated for alternating periods of 12 h darkness and 12 h light at 28+2°C for 7 days. Three replicates were maintained for each treatment. The control consisted of only an agar plate mixed with 0.5% Tween 20 (v/v) without essential oil. The efficacy of essential oils as antifungal agents was determined by measuring the fungal colony diameter using a centimeter scale.

Statistical analysis

Statistical analysis was carried out using the statistical software SPSS for Windows version 10.0.1 to calculate the means, standard errors and standard deviations. One-way analysis of variance (ANOVA) was applied to the data to determine differences. To check significant differences between the levels of the mean factor, Tukey's multiple comparison tests was applied with 5% significance.

Results and Discussion

Peanuts are ranked the thirteenth most important food crop in the world, the third most important source of vegetable protein and the world's fourth most important source of edible oil (Gang yao 2004). Production of peanuts contributes to more than four billion dollars to the United States economy annually. The state of Georgia alone produces approximately 40% of the peanuts in the United States, and peanuts are its largest cash crop (Achar and Sanchez 2006). In the United States, nearly 26 million dollars are lost per year due to the contamination of peanuts by *Aspergillus* species and aflatoxins according to the United States Department of Agriculture (USDA) and more than $1 billion are spent on infection prevention (Abbas *et al*. 2005). A number of field control measures are being utilized or explored, including modification of cultural practices, development of resistant cultivars through molecular and proteomic techniques, competitive exclusion using strains that do not produce aflatoxin, and development of field treatments that would block aflatoxin production (Klich 2007). In view of this, the present investigation aimed at screening plant based essential oils as antifungal agents against *A. parasiticus* in contaminated peanuts. The main reasons for considering the essential oils as antifungal agents include their natural origin and the very low risk of pathogens developing resistance.

The antifungal activity of seventeen essential oils against mycelial growth of *A. parasiticus* including statistical analysis (mean, standard deviation and standard error) is presented (Table 1). Tukey's multiple comparison test ($p = 0.05$) showed that all essential oils tested, had antifungal activity against *A. parasiticus*, with the exception of eucalyptus oil. However, cinnamon, lemongrass, clove and thyme oils showed complete inhibition of mycelial growth at various concentrations. It was also observed that as concentrations of oil increased, the inhibitory effect also increased. Several previous reports elucidate the antimicrobial activity of essential oils including lemongrass, citronella, clove, peppermint, thyme and oregano oils (Viuda-Martos *et al*. 2007) against different fungal species. Sokmen *et al*. (2004) demonstrated the ability of thyme at 10 ml to inhibit the growth of moulds such as *Alternaria* spp., *A. flavus*, *Fusarium* spp. and *Penicillium* spp. The antifungal property of thyme has also been demonstrated by Montes and Carvajall (1998); Basilico and Basilico (1999) against fungi such as *A. flavus*, *A. parasiticus*, *A. ochraceus*, *A. fumigatus* and *Fusarium* spp. Other authors including Inouye *et al*. (2000) also confirmed these results.

In our study, the agar dilution method revealed MIC with 100% inhibition for cinnamon oil against *A. parasiticus* at 1,000 ppm and higher concentrations. While lemongrass and clove oils resulted in complete inhibition of mycelial growth at ≥ 1,500 ppm, thyme resulted in com-

Table 1. Effect of essential oils on the growth of *Aspergillus parasiticus* in contaminated peanuts from commercial outlets

No.	Name of the essential oils tested	Diameter of mycelial growth [cms]						
		concentration of the essential oils tested [ppm]						
		control	500	1,000	1,500	2,000	2,500	overall
1	Cinnamon	8.00±0.00	2.97±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.82±0.03 a
2	Clove	8.00±0.00	4.23±0.01	1.43±0.02	0.00±0.00	0.00±0.00	0.00±0.00	2.28±0.03 b
3	Lemongrass	8.00±0.00	5.06±0.01	2.06±0.01	0.00±0.00	0.00±0.00	0.00±0.00	2.52±0.03 c
4	Thyme	8.00±0.00	3.37±0.06	2.50±0.02	2.06±0.01	1.50±0.01	0.00±0.00	2.90±0.02 d
5	Cedar wood	8.00±0.00	5.20±0.02	4.77±0.01	3.43±0.01	2.43±0.01	2.43±0.02	4.38±0.02 e*
6	Myrrh	8.00±0.00	5.73±0.01	4.80±0.02	3.50±0.01	3.47±0.02	3.33±0.01	4.80±0.01 f
7	Cumin	8.00±0.00	6.03±0.04	4.73±0.01	3.63±0.02	3.60±0.02	2.80±0.04	4.80±0.02 f
8	Citronella	8.00±0.00	7.90±0.02	4.63±0.02	3.90±0.03	3.10±0.02	1.40±0.02	4.82±0.02 f
9	Spearmint	8.00±0.00	6.47±0.01	4.53±0.03	4.27±0.01	3.63±0.05	3.27±0.04	5.03±0.01 f
10	Peppermint	8.00±0.00	7.40±0.00	6.80±0.02	5.70±0.03	4.43±0.01	2.20±0.01	5.76±0.02 g
11	Tea tree	8.00±0.00	6.40±0.02	6.40±0.03	5.73±0.02	4.57±0.04	3.57±0.03	5.78±0.01 g
12	Lavender	8.00±0.00	7.70±0.03	7.63±0.05	5.50±0.02	5.67±0.02	5.00±0.05	6.58±0.01 h
13	Ginger	8.00±0.00	8.00±0.00	7.00±0.02	6.43±0.01	5.63±0.01	5.57±0.01	6.77±0.01 hi
14	Cardamom	8.00±0.00	7.83±0.01	7.00±0.04	6.60±0.00	6.30±0.04	5.47±0.02	6.87±0.09 i
15	Black pepper	8.00±0.00	7.80±0.00	7.73±0.01	6.77±0.01	6.03±0.02	5.57±0.03	6.98±0.09 i
16	Orange	8.00±0.00	7.73±0.03	7.83±0.01	7.87±0.02	7.73±0.05	7.70±0.05	7.81±0.02 j
17	Eucalyptus	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00 j

*figures followed by superscript of same letter in a column are not statistically significant (p = 0.05) according to Turkey's multiple-range test

Values given are mean±standard error (n = 3)

plete inhibition at ≥ 2,500 ppm. For all other combinations of oils MIC could not be determined as none resulted in 100% inhibition at any tested concentrations. *Aspergillus parasiticus* was found to be inhibited more than 50% by cedar wood, citronella, cumin and peppermint oils. Of all the oils tested, eucalyptus oil was the least efficient in inhibiting mycelial growth, irrespective of its concentrations. Essential oils are natural products extracted from vegetative parts of plants. In general, the levels of essential oils and their compounds necessary to inhibit microbial growth are higher in foods than in culture media. This could be due to interactions between phenolic compounds and the food matrix (Nuchas and Tassou 2000) and should be considered for commercial applications (Tzortzakis *et al*. 2007). Numerous studies have been conducted on the antifungal properties of essential oils and their mode of action (Sharma and Tripathi 2006). Lis-Balchin and Deans (1997) reported that antimicrobial activity could be correlated with essential oils containing a high percentage of monoterpenes, eugenol, cinnamic aldehyde and thymol. Davidson (2001) reported that the major constituent of essential oils is terpenes and phenolic compounds, which are responsible for possible modes of action of essential oils. Corner and Beuchat (1984) suggested that the possible antimicrobial activity of the essential oils could be due to its ability to damage the enzymatic cell systems, including those associated with energy production and synthesis of structural compounds. Prindle and Wright (1977) mentioned that, the effect of essential oils (phenolic compounds) is concentration dependent and at low concentrations, phenolic compounds affect enzyme activity but at greater concentrations, they cause protein denaturation.

In the present study, cinnamon oil showed more inhibitory effect than the other oils tested. In traditional medicine, cinnamon has been used for digestive ailments such as indigestion, gas and bloating, stomach upset, and diarrhea (herbwisdom.com). More recently, modern medical research has researched cinnamon with some intriguing results such as its mild anti-inflammatory properties. Furthermore, it slows food, spoilage and is antifungal (Khan *et al*. 2003; Valero and Salmeron 2003; Anderson *et al*. 2007). From our results we can conclude that since cinnamon oil showed antifungal properties, this essential oil has great potential as a biological control agent against *A. parasiticus* in peanuts. Based on the present study, the active ingredients in cinnamon with antifungal properties can be further tested and recommended as a biological control agent against *A. parasiticus* and *A. flavus* which is also a common producer of aflatoxin in peanuts and other edible nuts. Therefore, cinnamon, lemongrass, clove and thyme oils may be further explored as preservative materials for peanut based food products.

Conclusions

The present study clearly indicates that essential oils such as cinnamon, lemongrass, clove and thyme have broad-spectrum antifungal properties and are effective against *A. parasiticus*. These findings show definitely that essential oils should find a practical application in the control of and the growth of *A. parasiticus* in peanuts. They are

suitable because of their natural origin, which consumers find comforting. Furthermore, they are eco-friendly, which is beneficial for the environment. These natural plants based essential oils and their active components may successfully replace synthetic chemicals and provide an alternative method of preservation.

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