Evaluation of two essential oils from medical plants for reduction of fungal growth and mycotoxin production.

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Abstract

The need for new antifungal substances and alternative antifungal treatments is becoming more and more obvious. One of the most promising and ecologically safe possibilities could be based on taking advantage of a plant's natural antifungal properties. The following study was conducted to investigate the antifungal effect of medicinal plants. Essential oil (EO) extracts of *Citrullus colocynthis* and *Linum usitatissimum* seeds were tested for their inhibitory action against *A. flavus and A. ochraceus* and mycotoxins formation on yeast extract broth and crushed yellow corn. Evaluation of radial growth on *Potatoes Dextrose Agar* (PDA) solid medium showed slight mycelial growth proportional to oil concentration added to the medium. Antifungal indices investigation allowed as to put our oils in the order of effectiveness: *C. colocynthis* (96.3 % &94.3) and *L. usitatissimum* (55.6%&54.3) against *A. flavus and A. ochraceus,* respectively. In the second experiment, yeast- extract sucrose medium (YES) was used as a basal medium to examine the mold growth and mycotoxin production by two pathogenic fungi *A. flavus and A. ochraceus.* The YES medium was supplemented with three different concentrations of *C. colocynthis and* , *L. usitatissimum* inoculated with 1mL of a spore suspension containing 10^5 conidia of each test mold and then incubated at 25º C for 14 days. After the incubation period, cultures were analyzed for mycelial dry weight and mycotoxin accumulation. . In the third experiment, crushed yellow corn was used as a basal medium and the same system of study was applied, the inoculated flasks incubated at 25 ^{0}C for 21 days. In this experiment, the level of 30µl/ml (3%) of the essential oil (EO) extracts of *Citrullus colocynthis* was effective in inhibition both mold growth and mycotoxin production for all tested molds.

Key words: *Citrullus colocynthis, Linum usitatissimum, essential oils, A.flavus, A.ochraceus, mycotoxins, aflatoxins, ochratoxin A.*

Introduction

Molds and mycotoxins contamination of feed and feed ingredients occurs worldwide and because of ubiquitous nature of these microorganisms they cannot be totally eliminated from feeds and ingredients **(Accensi *et al*., 2004)**. The presence of molds and mycotoxins in poultry feeds result from the raw materials used in their production. Molds and mycotoxins contamination of the raw materials occur during the pre-harvest and/or the post-harvest and the storage periods. During these periods, temperature and humidity, as well as processing and handling of animal feed play an important role in the growth of fungi and mycotoxins contamination **(Adebajo *et al*., 1994** and **Badripour, 2010)**

Many parasitic and saprophytic fungi infect growing crops and may continue to develop during post-harvest, processing and formulation of finished feeds **(Mabbett, 2003 and Vieira, 2003)**. Fungal growth causes direct losses in volume and quality of feed ingredients and subsequently feeds made from them, leaving behind some poisonous mycotoxins, which contaminate feed raw materials and finished feeds **(Okoli *et al*.,2006).**

Plant essential oils and their components have been known to exhibit biological activities, especially antimicrobial, since ancient time. With the growing interest of the use of either essential oils or plant extracts in the food and pharmaceutical industries, screening of plant extracts for these properties has become of increasing importance **(Amvam *et al.,* 1998).** Biologically, because of their natural origin,

they are biodegradable and do not leave toxic residues or by-products to contaminate the environment **(Yaouba *et al.,* 2010).**

The essential oils and extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have recently gained momentum in many pharmaceutical foods processing application **(Ahmet *et al.,* 2009).** The essential oils are important because of their antibacterial, antifungal, antioxidant and anti-carcinogenic properties **(Tzortzakis and Economakis, 2007).**

Citrullus colocynthis, also known as bitter apple is a desert plant of the family *Cucurbitaceae* naturally adapted to arid environments. Each plant produces 15-30 round fruits, about 3-4 inches in diameter, green with undulate yellow stripes, and becoming yellow all over when become dry. Seeds are small, smooth and brownish when ripe. The fruits are widely used medicinally, especially for stomach pains, because of its content of glucosides such as colocynthin; it is an effective cathartic and laxative **(Jayaraman et *al*., 2009).** Essential oils are complex compounds, and their chemical composition and concentrations of various compounds are variable. Essential oils basically consist of two classes of compounds, the terpenes and phenylpropenes, depending on the number of 5- carbon building blocks. Plant essential oils constitute about 1% of plant secondary metabolites and are mainly represented by terpenoids, phenylpropanoids or benzenoids, fatty acid derivatives and amino acid derivatives (**Lodhia *et al.,* 2009)**. Essential oils are potential sources of novel antimicrobial compounds especially against bacterial and fungal pathogens (**Doss *et al.,* 2011)**.

Flax is native plant to the region extending from the eastern Mediterranean to India, and extensively cultivated since ancient Egypt. Flax *(Linum usitatissimum)* is an annual species of the *Linaceae* family, growing to a height of 0.3-1. m., which is cultivated for the production of textile fibre, seed and flaxseed (linseed) oil **(Gutiérrez *et al*., 2010).**

In recent years, flaxseed has become known as a functional food due to its nutritional composition. Likewise, antibacterial and fungistatic activity has been reported in oligosaccharides extracted from this seed **(Guilloux *et al,* 2009)**, which can control the growth of pathogens affecting the agricultural sector, such as *Alternia solani* and *Alternia alternata,* as well as the human pathogen *Candida albicans;* it can also control the deterioration of foodstuffs by the fungi *Penicillium chrysogenum, Fusarium graminearum* and *Aspergillus flavus* **(Xu *et al.,* 2008).**

Flaxseed oil, the principal component of this seed, is rich in alpha-linolenic, linoleic and oleic acids, and for years has been the focus of interest in this seed. After extraction of the oil a large quantity of pressed flaxseed cake remains, which is discarded and is still considered to be waste, or at best a sub-product of no value **(Figuerola *et al,* 2008)**. The flaxseed cake is mainly used as a cattle feed, although flaxseed meal is used as an additive in baking products **(Coskuner and Karababa, 2007).**

The aim of the present study was to assess the *in-vitro* antifungal properties of essential oils of *Citrullus colocynthis* and *Linum usitatissimum* against *A. flavus and A. ochraceus* and their mechanisms to control of aflatoxin production.

Materials and methods

1. Preparation of test microorganisms and cultures

The essential oil seeds of *Citrullus colocynthis* and *Linum usitatissimum* were assayed for antifungal activity against the fungal strains *A. flavus* and *A. ochraceus* obtained from Mycology Department at Animal Health Research Institute, Giza. Confirmation of *Aspergillus* genera were take place by subculture onto Malt Extract Agar and Czapek Dox agar **(Oxoid, 1990)**. Furthermore, confirmation of *A.flavus* and *A. ochraceus* species were carried out by Single Spore method using three cultures media: Malt Extract Agar (M.E.A) at 25°C, Glycerol Nitrate Agar (G25N) at 25°C and Czapek Dox Agar (C.D.A) at 5°C and 37°C. Using the identification keys of (**Pitt and Hocking,**

2009); observation has been made after the first and second week. These fungi were stored in tubes of PDA acidified at 4°C.

2. Determination of aflatoxins and Ochratoxin by TLC

A. flavus and *A. ochraceus* were inoculated into Yeast extract soluation containing 20% sucrose (YES) medium in case of *A. flavus* and Yeast extract solution containing 4% sucrose in case of *A. ochraceus*. After two weeks of incubation at 30 ± 2 °C, the biomass was removed by filtration of YES. The filtrate was added then to 180 ml of chloroform and stirred for 30min. After decantation, the organic layer was concentrated to 2ml. In case ochratoxin the filtrate was acidified with 0.1N hydrochloric acid, then ochratoxin was extracted by using chloroform. Aflatoxins were determined in each extract by spotting samples on thin layer chromatography plate. Plate was developed with a toluene/Ethyl acetate/ Formic acid (50:40:10, v/v/v) solvent system. Aflatoxin standard was spotted on the same plate as reference and aflatoxin spots were identified using 365 nm UV (**Munimbazi and Bullerman, 1998).** The presence of Aflatoxins was provided by appearance of blue fluorescence for AFB which has the same Rf as control, while in case ochratoxin A appears as a blue-green fluorescent under UV light.

3. Plant material and essential oil preparation

The essential oil seeds of *Citrullus colocynthis* and *Linum usitatissimum* were obtained friendly from the pharmacognosy department, Faculty of Pharmacy, Cairo University,

4. Growth radial technique on solid medium

The determination of percent mycelial inhibition by growth radial technique on solid medium approved by the **(Singh *et al*., 2008)** with the following modification a final concentration of 0.5% (v/v) Tween 20 (sigma) was incorporated into the PDA after autoclaving to enhance oil solubility. Selected concentration of 20µl/ml, 25µl/ml and 30µl/ml (2 , 2.5 and 3 %) of seed oil extracts of *Citrullus colocynthis and Linum usitatissimum* were transferred into Petri plates .Plates were dried at 35 ^{0}C for 30 min., Puncture inoculation of *A.flavus* and *A.ochraceus* carried out by a point with deposition of the inoculum in the center of the plate. Growth was measured from the third day of incubation and compared to the control medium which was incubated for 7days .**(Suárez-Jimenez, 2007 and Kra *et al*., 2009).** The inhibition percentage of mycelial growth of each oil was calculated using the following formula: (PIg = ((DT –D)/DT) x 100), where DT is mean diameter of mycelial growth in control and D is mean diameter of mycelial growth in treatment (**Singh *et al.,* 2008).**

5. Determination of inhibition of growth and aflatoxin production of *A.flavus* and *A. ochraceu* by essential oils on yeast extract broth (YEB)

Fifteen ml of YES medium was put in a 250-ml flask and then autoclaved at 120°C for 15 min. Inoculation was carried out by adding 1 ml of a suspension of spores (10^5 spores) of a toxigenic *A. flavus* and *A. ochraceu* strains with 20 µl, 25 µl and 30 µl (2, 2.5 and 3 %) of one of the inhibitory essential oils (*Citrullus colocynthis* and *Linum usitatissimum*). YES without any essential oil added served as control. The flasks were incubated in the dark for 14 days at 25°C. After the incubation period, the growth of the mycotoxigenic fungi *A.flavus* and *A. ochraceus* in all flasks was visually examined. On the other hand, Aflatoxins were extracted and determined according to the **(AOAC methods, 1980)**. Ochratoxin A (OA) was extracted and determined according to the method of **(Scott *et al.,* 1971)**. The mycelia of each flask containing YES medium were harvested by filtration through preweighed filter paper and then washed twice with distilled water. The weight of mycelia was determined. The mycelia were allowed to dry at 70 ^{0}C for 12 h. The mycelium dry weight was then determined. Percent reduction of mycotoxin production was calculated by the following equation:

Percent reduction = 100 – {(A1 / A0) *100}

Where: A1 = the amount obtained by treatment.

A0 = the amount obtained by control.

6. Antifungal effect of essential oil (*Citrullus colocynthis* and *Linum usitatissimum*) on crushed yellow corn.

Crushed yellow corn used in this study was obtained commercially from Al-ahram Company of poultry ration. The crushed yellow corn was examined macroscopically for detection of fungal contamination, then subjected to the isolation of fungal contaminant and mycotoxins detection to ensure that the crushed corn is completely free from fungal contamination and its metabolites (mycotoxins). The crushed corn was quantized into conical flasks, each one contained 100 g. of the corn. These flasks were subjected to autoclaving at 121^0 C for 15 min. for 3 successive days (to ensure the complete absence of fungi). One ml. of spore suspension 10^5/ml. **(APHA, 1992)** was added to each flask. After that adjusted amount of each oil (*Citrullus colocynthis* and *Linum usitatissimum*) was added to obtain 20, 25 and 30µl/gm. (2.0, 2.5 and 3.0 %), as well as a flask of crushed yellow corn was kept without essential oils as control. The inoculated flasks were incubated at 25 ^{0}C for 21 days. Fungal growth of all tested molds were visually assessed as, no growth, very little growth, moderate and heavy growth covered the surface of the crushed yellow corn. Then crushed yellow corn was examined for the presence of aflatoxin production as described by the **AOAC TLC methods (1980).** For ochratoxin A, cultures were extracted, and OA levels were determined as described by **(Scott *et al*., 1971).**

Result and Discussion

Table (1). Effect of essential oils (EOs) of *Citrullus colocynthis* and *Linum usitatissimu* on mycelial growth of target fungi on PDA.

EO extract	% Inhibition of target fungi	
	A.flavus	*A.ochraceus*
Citrullus colocynthis		
2.0%	24.1 ± 0.1	20.0 ± 0.1
2.5%	55.6 ± 0.2	45.7 ± 0.1
3.0%	96.3 ± 0.1	94.3 ± 0.1
Linum usitatissimum		
2.0%	13.0 ± 0.2	17.1 ± 0.1
2.5%	31.5 ± 0.2	37.1 ± 0.3
3.0%	55.6 ± 0.2	54.3 ± 0.2

Several investigations have confirmed the antimicrobial action of essential oils (EOs) in model food systems and in real food. Evidence that EOs are more strongly antimicrobial than is accounted for by the additive effect of their major antimicrobial components; minor components appear, therefore, to play a significant role (**Gourama and Bullerman, 1995)** . Since EOs are considered as generally regarded as safe **(Kabara, 1991),** the possibility of reinforcing their natural antimicrobial effects by the addition of small amounts of other natural preservatives may be a way to attain a balance between fitness for consumption and antimicrobial efficacy.

Mycotoxins are secondary metabolites produced by specific filamentous fungi that contaminate agricultural commodities. They are toxic to humans and animals, causing significant reductions in crop yield and cause economic losses **(Gqaleni, *et al.* 1996)**. Their occurrence in various countries has been well documented. When the fungi invade and grow in commodities such as corn and other media the resulting contamination with aflatoxins often makes the commodities unfit for consumption **(Vardon, 2003)**.The inhibition of *Aspergillus flavus* and *A.ochraceus* growth by essen-

tial oils has already been previously reported (**Dikbas *et al*., 2008)**. In our study, results indicated antifungal efficacy of the two tested essential oils. The extent of inhibition of fungal growth and aflatoxin B_1 production was dependent on the concentration of essential oils used.

Data presented in Table (1) clearly indicated the results of growth radial technique which revealed that the two oils tested exhibited different degrees of antifungal activity against *Aspergillus flavus* and *A.ochraceus*. The percent of inhibition of essential oil of *C.colocynthis* was (96.3 and 94.3 %) compared to *L.usitatissimum* (55.6 and 54.3 %) at a percent of 3% of added essential oils. **Schafferman *et al.*, (1998)** suggested that the reduction of the growth of fungal strain may be correlated to linoleic acid which is present in more in *C.colocynthis* seeds oil. **Yingying *et al.*, (2008)** reported the antifungal activity of seeds oil of *L.usitatissimum* (seeds powder at 6% concentration inhibit completely (100%) the development of *A. flavus*. On the other hand**, Doss *et al*., (2011)** demonstrated that essential oil and methanol extracts of *C.colocynthis have* strong activity against both Gram positive and Gram negative bacteria and fungi.

Table (2), showed the comparison of biomass weight of fungi to selected concentrations of essential oils. *C.colocynthis* oil reduced biomass weights of fungi under examination at concentration of 3.0% (94.2 – 90 %) while *Linum usitatissimum* seed oil reduced biomass weights to (58.8 – 52.1 %). This reduction effect may be due to linolenic and linoleic acids abundant in these oils. This assumption has been confirmed by **Dale *et al.* (2004)** who reported that reduction of biomass weight at 1.0 % concentration. **Doss *et al*. (2011)** recorded that the Minimum Inhibitory concentration of *C.colocynthis* oil is 1.0 mg/ml. It could also be concluded that *A. ochraceus* was more sensitive one for the essential oil of *Citrullus colocynthis* than the *A.flavus*. The inhibitory effect of the two different essential oils on mycelial growth according to the mold type may be rankled as follow: *A.ochraceus* > *A.flavus.* These differences in the inhibitory effect may be mainly due to some interfering factors: the mold type, incubation temperature and type of extract and subsequently the differences in the chemical composition for each essential oil. In this respect, many publications indicated that the compositions and concentrations of compounds within the distinct types of oil preparations would differ and play an important role in its antifungal activity action. **(Abdel-Fattah and Abdel-Salam, 2004).**

Mycotoxins are harmful substances produced by fungi in various foods and are estimated to affect as much as 25% of the world's crop each year. The most commonly encountered mycotoxins in feedstuffs and foods are aflatoxins and ochratoxins. Aflatoxins are common in humid climatic conditions like those existing in Asian and African countries and certain parts of Australia. Mycotoxins are regularly found in feed ingredients such as maize, sorghum, barely, wheat, rice meal, cotton seed meal, groundnuts and other legumes The problem of mycotoxins does not just end in animal feed or reduce animal performance, many become concentrated in meat, eggs and milk of animal and can pose a threat to human health **(Akande *et al*., 2006).** Aflatoxins are the most notorious of the mycotoxins causing acute and chronic toxicoses in foodstuff **(CAST, 2003).**

Antimicrobial activity of essential oils depends not only on their components but also on the chemical structure of these components **(Farag *et al.*, 1989).** The seed oil of *Citrullus colocynthis* contains linoleic acid, as a major fatty acid in 62%. The seeds could be extracted for oil and furthermore used for edible purposes, and the meal could be used as a meat substitute, and also for animal and poultry feed or protein production. The fatty acid profiles of the seed oil showed an unsaturated fatty acid content of 77.4% and the high content of 63.2% of PUFA. The predominant fatty acid was linoleic (18:2) acid in 62.2%. The presence of other fatty acids ranged in 10-14% for oleic (18:1), stearic (18:0) and palmitic (16:0) acids, respectively, **Milovanović and Jovanović (2005).** The fungal membrane has the fundamental role of

maintaining cell order and integrity and hence antifungal treatment mostly target the fungal membrane **(Avis, 2007)**. **Avis and Bélanger (2001)** determined the general mechanism with which antifungal fatty acids directly interacts with the fungal cell membrane. The antifungal fatty acids naturally insert themselves into the lipid bi-layer of the fungal membranes and physically disturb the membrane, resulting in increased fluidity of the membrane. These elevations in membrane fluidity will cause a generalized disorganization of the cell membrane that leads to conformational changes in membrane proteins, the release of intracellular components, cytoplasmic disorder and eventually cell disintegration.

Table (2). Mold growth (mg/50 mL media) and percent reduction of the target molds as affected by the two essential oil on YES broth media for 14 days at 25 ° C. (n=3).

EO extract (%)	Mold growth and percent of reduction for different toxigenic strains			
	A.flavus		A.ochraceus	
	*Mould growth	% reduction	*Mould growth	% reduction
Control	323.3± 14.4	0	507±7.9	0
Citrullus colocynthis				
2.00%	205.0 ± 2.9	36.6	388.3 ± 0.8	23.5
2.50%	101.0 ± 0.9	68.7	198.3 ± 3.3	60.9
3.00%	32.7± 4.5	90	29.7 ± 0.9	94.2
Linum usitatissimum				
2.00%	235.0 ± 7.6	27.3	450.0 ± 5.8	11.3
2.50%	167.3 ± 3.7	48.3	303.0 ± 0.8	40.2
3.00%	168.3 ± 4.4	52.1	209.0 ± 2.1	58.8

* Mould growth: weight of mycelial mat (Biomass).

Several studies have focused on the potential use of essential oil applications in biological control of aflatoxin producing fungi and insect pests. Certain essential oils can be applied as mold inhibitors in order to prevent the growth of aflatoxigenic fungi in stored food. However, the appropriate application of essential oils should further be investigated **(Thanaboripat, 2011).**

Data represented in Tables (3and 4) clearly indicate that oil extract of *Citrullus colocynthis*, completely inhibited mycelial growth and mycotoxin production of *A. flavus and A. ochraceus* at level 30µl/ml (3.0%) in YES broth medium and crushed yellow corn. In this respect**, Sayda *et al.* (2012)** reported that *Citrullus colocynthis* seed meal may be used up to 4% of broiler chicken without adverse effect on their performance. Furthermore, they mentioned that an improvement in the following parameters were observed: Reduced feed intake, Enhanced growth rate and higher live body weight, improved feed efficiency in terms of lower feed conversion ratio and reduced abdominal fat pad.

Conclusions

It can be concluded that the oil extracts of the plants used showed prominent antifungal activity against *A. flavus* and *A.ochraceus*. This potency may be attributable to fatty acids especially linoleic and linolenic acids or synergic effect of their constituents. Also we recommended that essential oil of *Citrullus colocynthis* seed may be used up to 3% of broiler and animal feeds. The essential oils can be applied as mold inhibitors in order to prevent the growth of aflatoxigenic and ochratoxigenic fungi in stored feed. Further investigations on their mechanisms can provide a great potential for the control of AFB production.

Table (3). Mycotoxin production and percent reduction of the target molds as affected the two essential oil on YES broth media for 14 days at 25 ° C. (n=3).

EO extract	Mycotoxins production (µg/ 50ml of YEB)			
	A.flavus		*A.ochraceus*	
	Aflatoxin B_1	% reduction	Ochratoxin A	% reduction
Control	295.0 ± 2.9	0	175.3 ± 2.5	0
Citrullus colocynthis				
2.00%	205.3 ± 2.9	30.5	135.0 ± 2.9	54.2
2.50%	70.0 ± 11.5	76.3	63.3 ± 8.8	78.5
3.00%	0	0	0	0
Linum usitatissimum				
2.00%	230.0 ± 11.5	22	158.3± 1.7	10.7
2.50%	80.0 ± 11.5	72.9	73.3 ± 8.8	58.2
3.00%	0	0	0	0

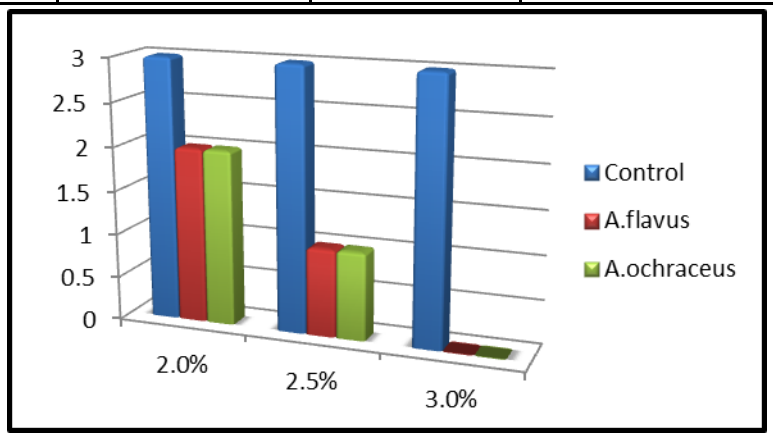

Figure (2). Effect of various concentrations of seed oil of *Linum usitatissimum* on molds growth on crushed yellow corn incubated for 21 days at 25 ºC.

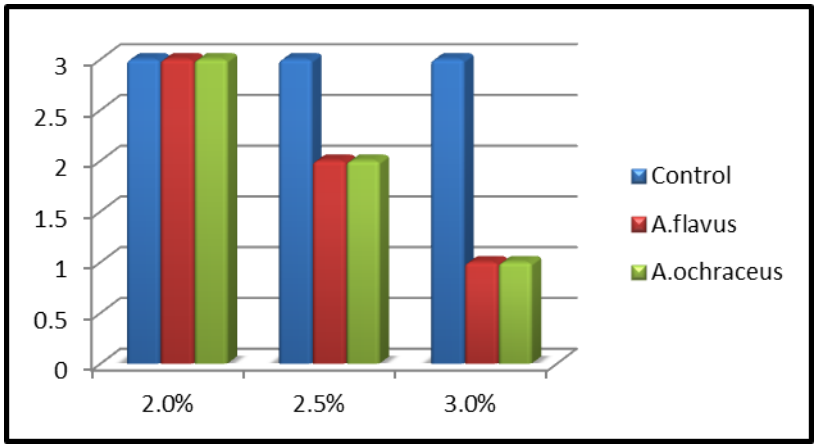

Figure (2). Effect of various concentrations of seed oil of *Linum usitatissimum* on molds growth on crushed yellow corn incubated for 21 days at 25 ºC.

(0) = No growth, (1) = very little growth, (2) = Moderate and (3) = corn surface covered with mycelia.

Table (4). Effect of various concentrations of the two essential oils on mycotoxin production on crushed yellow corn for 21 days at 25 °C. (n=3)

EO extract	Mycotoxins production (µg/ 100gm of corn)			
	A.flavus		*A.ochraceus*	
	Aflatoxin B_1	% reduction	Ochratoxin A	% reduction
Control 0.0%	123.7 ±14.3	100	108 ± 8.5	100
Citrullus colocynthis				
2.0%	73.3 ± 8.8	40.7	89.3 ± 0.6	17.3
2.5%	0.0	0.0	0.0	0.0
3.0%	0.0	0.0	0.0	0.0
Linum usitatissimum				
2.0%	103.7 ± 3.3	16.2	83.0 ± 3,5	32.1
2.5%	84.0 ± 2.6	32.1	73.7 ± 1.9	31.7
3.0%	ND	ND	ND	ND

ND= amount not detected by TLC.

Photo (1). Flask on right showing no to faint growth of *A.flavus* on YES supplemented by seed oil of Citrullus colocynthis while flask on the left without seed oils showing normal heavy growth *of A.flavus*.

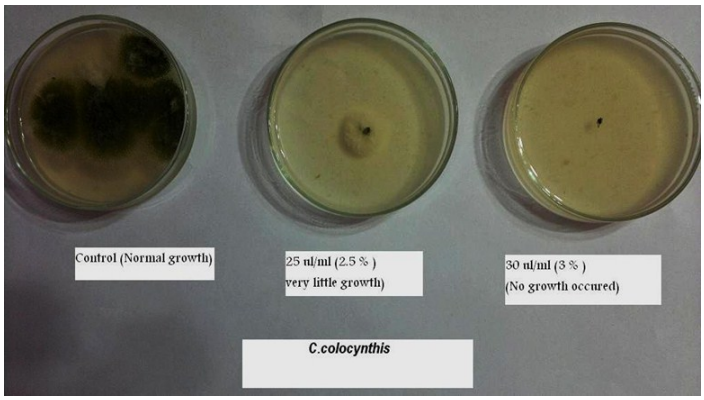

Photo (2**).** Normal growth of *A.flavus* on PDA (on the left without essential oils). Very little growth and no growth (middle and right plates) of *A.flavus* on PDA supplemented with essential oils

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تقييم بعض الزيوت الأساسية من النباتات الطبية للحد من نمو الفطريات وإنتاج السموم

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الملخص العربي

لقد هدفت هذه الدراسة الى معرفة التأثير المضاد للفطريات لمستخلصات الزيوت الأساسية للنباتات الطبية لكل من بذور نبات الحنظل والكتان . فقد تم دراسة التأثير المضاد لنموالفطريات لهذه الزيوت على كل من فطرى الأسبرجيليس فلافس والأسبرجيليس أوكراشيس وكذلك التأثير المضاد لتكوين السموم الفطرية لتلك الفطريات المختبرة على كل من مستخلص الخميرة السائل والذرة الصفراء المجروش.

وقد تم تقييم تأثير تلك الزيوت على نمو الفطريات باستخدام مستنبت البطاطس دكستروز أجار المضاف اليه تلك الزيوت حيث أوضحت النتائج وجود اعاقة لنمو تلك الفطريات وكان معدل اعاقة النمو يتناسب طرديا مع معدل اضافة تلك الزيوت. وكانت تلك الزيوت مؤثرة على نمو الفطريات وكان تأثير زيت بدور الحنظل بنسبة 96.3 % , 94.3 % على فطر الأسبرجيليس فلافس والأسبرجيليس أوكراشيس على الترتيب . وجد أن تأثير زيت بذرة الكتان فكان التأثير المضاد لنفس النوع من الفطريات بمعدل أقل 55.6 % , 55.3 % .

وعلى الجانب الأخر فقد تم دراسة تأثير تلك الزيوت على نمو الفطريات المفرزة للسموم وبالتالى قدرتها على افراز السموم الفطرية باستخدام مستخلص الخميرة السائل والذرة الصفراء المجروشة. أثبتت النتائج أن زيت بذرة الحنضل عند معدل 3 % له القدرة على ايقاف نمو فطر الأسبرجيليس فلافس والأسبرجيليس أوكراشيس وبالتالى منع تكوين السموم الفطرية لتلك الفطريات . وقد تم مناقشة تلك النتائج .