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Inhibitory Effect of Cinnamon Oil on Aflatoxin Produced by *Aspergillus flavus* Isolated from Shelled Hazelnuts

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Detection of Aflatoxins produced by 23 isolates *Aspergillus* flavus under UV light were isolated from eight samples of hazelnuts . twelve isolates of *A. flavus* were positive for aflatoxin production, while eleven isolates were negative for fluorescence. Extraction of aflatoxin from shelled hazelnuts was then performed through high-performance liquid chromatography (HPLC), nine isolates were capable of producing different levels of aflatoxin B1 and B2 but two isolates failed to produce any amount of aflatoxin B1 or B2. The anti-aflatoxigenic effect of Cinnamon oil at 4% was examined. The highest levels of inhibition ranging from 68.9 to 81.6% with isolate no H16 and H 10 respectively. five isolates were capable of producing aflatoxin G1 and G2, four isolates produced G2 only while three isolates failed to produce G1 or G2. Cinnamon oil at a concentration of 4% led to the highest levels of inhibition which ranged from 72.7 to 93.1%

Key words: Antiaflatogenic, Cinnamon, *Aspergillus flavus,* Hazelnuts**.**

Mycotoxins are natural compounds, secondary metabolites produced by fungi which mostly belong to the *Aspergillus, Penicillium* and *Fusarium* species. The most important aflatoxins (AFS, B1, B2, G1 & G2) are can cause both acute and chronic toxicity in animals. Effects such as acute liver damage, liver cirrhosis, induction of tumours and teratogenic and other genetic effects are well (Kuiper-Goodman, 2004). hazelnuts (*Corylus avellana*) belong to family Betulaceae. are one of the most important oilseed crops and excellent source of nutrition due to their high protein content. Hazelnut plays role in health and nutrition of human because it contains fat (60%), rich in unsaturated fatty acids, proteins, carbohydrate, minerals, vitamins , antioxidants andphenolics (Beyhan *et al*., 2010). Turkey has a share about 70% in the world's hazelnut production. Turkey is followed by Italy and USA. EU countries have a share of %16 (INC, 2012). According to last 8 years' data, production of our country is 589 thousand tonnes (70%). On the other hand production of other countries is about 253 thousand tonnes (30%). (FAO, 2011).Several investigations have listed a large number of fungi which could be isolated from hazelnuts during storage (Ozay *et al*., 2006). *Aspergillus flavus* is the dominant storage fungus colonizing hazelnuts (Beyhan *et al*. 2011; Baltaci *et al*.,2012) Essantial oils obtained from higher plant parts have been shown to contain antitoxigenic properties such as phenylpropanoids, terpenoids and alkaloids derived (Kumar *et al.*, 2008; Holmes *et al.*, 2008; Al-Othman *et al*., 2013b). In the present study the effects of Cinnamon essantial oil on aflatoxin production by *Aspergillus flavus* have been studied

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with a view to find out a cheaper method for preventing aflatoxin contamination in hazelnuts in king Saud Arabia.

MATERIALS AND METHODS

Collection of samples

Eight samples from hazelnuts were collected randomly from different markets in Riyadh location from Saudi Arabia during 2013 for this experimental work. The samples were stored at 2 °C until used (Czerwiecki *et al*., 2002).

Isolation of *Aspergillus flavus* associated with shelled hazelnuts

samples were surface sterilized with 5% sodium hypochlorite solution for one minute, then rinsed three times with sterilized distilled water. three pieces were placed on the surface of Petri dishes 9 cm diam. containing potato dextrose agar (PDA), and each entry replicated three times. Petri dishes were incubated at 25º±2 C. Isolates were purified either by single spore methods and then transferred to PDA slants. The identification of isolates were confirmed by Regional Center of the Fungi and their Applications, Al-Azhar university, Cairo, Egypt.

Extraction of aflatoxin from shelled hazelnuts samples

For extraction aflatoxin, 20 g of each tested nuts was mixed with 100 ml of 4% acetonitrile aqueous solution of potassium chloride (9:1). Extraction was followed by shaking for 20 min and filtered through Whatman No.4 filter paper under vacuum condition. For purification, 100 ml of n-hexane were added to the filtrate and shaken for 10 min. After separating, the upper phase (n hexane) was discarded. To the lower phase, 50 ml deionized water and 50 ml chloroform were added and this solution was shaken for 10 min. After separation, the lower phase was collected and the upper phase was re-extracted twice with 25 ml of chloroform by using the above conditions. Then the chloroform was evaporator in a 40°C water bath at low speed. Methanol at the rate of 2 ml was added and the solution filtered through a 0.45 µl filter . (Zaboli *et al*, 2011).

Effect of Cinnamon oil on aflatoxin produced by *Aspergillus flavus*.

Three different concentration (1,2 and 4%) of Cinnamon oil used in this experiment were obtained as follow: 4mL of essential oil and 0.4mL of Tween 80 were taken in sterile tubes and the volume was completed to 5mL using distilled sterile water. This mixture was shaken for 5 minutes using Vortex and serial dilutions were made to obtain solutions with final concentrations of and 1,2 and 4%. Control was carried out with Tween 80 (Evandro *et al*., 2005). Antiaflatoxigenic efficacy values of each tested oil were determined using SMKY liquid medium (sucrose, 20 g; magnesium sulfate, 0.5 g; potassium nitrate, 3 g; yeast extract, 7 g and distilled water, 1000 ml) . Three different concentrations of oils (1,2 and 4 %) were prepared and added to flasks then inoculated with discs of 6 mm diameter of the toxigenic *Aspergillus flavus* at 25 ± 2°C for 7 days (Paranagama *et al.*, 2003) and the control set was kept parallel to the treatment without oil. After incubation, content of each flask was filtered (Whatman, No. 1). The filtrates of each flask were treated three times with 50 ml of chloroform in a separating funnel. The chloroform extract was separated and dehydrated with anhydrous sodium sulfate and evaporated till dryness on water bath at 50°C under vacuum. The residues were dissolved in 10 ml methanol. (Mostafa *et al*. 2011).

High -performance liquid chromatography (HPLC)

The aflatoxins were measured using high-performance liquid chromatography (HPLC) (model PerkinElmer series 200 UV/VIS) with a C18 column with an internal diameter of 300 mm x 3.9 mm. The HPLC was equipped with an UV detector and fluorescence with 365 nm excitation and 430 emission wavelengths. The mobile phase consisting of methanol: acetic acid: water (20:20:60 v/v/v). The total run time for the separation was approximately 30 min at a flow rate of 1 ml/min. (Christian, 1990) The aflatoxin inhibition was calculated according to Mostafa *et al*. (2011) as follows: percentage of inhibition toxin = [A – a / A] x 100, where" A" is the concentration of aflatoxin in the treated sample and "a" is the concentration of aflatoxin in the control.

RESULTS AND DISCUSSION

Isolation of *Aspergillus flavus* associated with shelled hazelnuts

The data presented in Table 1 show that detection of Aflatoxins produced by 23 isolates

Aspergillus flavus under UV light from eight samples of hazelnuts . The presence or absence of fluorescence in the agar surrounding the assayed colonies was noticed using UV radiation (365 nm) and was expressed as positive or negative. The data presented in Table 1 show that non-aflatoxigenic aspergilli did not display fluorescence, whereas the aflatoxigenic strains were positive for fluorescence. This table also shows that twelve isolates of *A. flavus* were positive for aflatoxin production, while eleven isolates were negative for fluorescence. These results agree with those of Fente *et al.* (2001) and Al-Gahtani *et al*.(2013).

Table 1. Detection of Aflatoxins produced by *Aspergillus* flavus isolates under UV light

Isolates number	Detection under UV light
H1	+
H2	+
H3	+
H4	-
H5	+
H6	-
H7	-
H8	-
H9	+
H10	+
H11	-
H12	+
H13	-
H 14	-
H15	-
H16	+
H17	+
H18	+
H19	+
H20	-
H21	-
H22	-
H23	+

Effect of Cinnamon oil at 4% on aflatoxin B (µg/ml) produced by *A. flavus* were isolated from shelled hazelnuts:

The data presented in Table 2 indicate that nine isolates were capable of producing different levels of aflatoxin B1 and B2, isolate (H16) just produced B1 but two isolates (H17 and H23) failed to produce any amount of aflatoxin B1 or B2. The anti-aflatoxigenic effect of Cinnamon oil at 4% was examined. The highest levels of inhibition ranging from 68.9 to 81.6% with isolate no H16 and H 10 respectively.

Effect of Cinnamon oil at 4% on aflatoxin G (µg/ml) produced by *A. flavus* isolated from shelled hazelnuts

The data shown in Table 3 indicate that five isolates were capable of producing aflatoxin G1 and G2, four isolates produced G2 only while three isolates failed to produce G1 or G2. Cinnamon oil at a concentration of 4% led to the highest levels of inhibition which ranged from 72.7 to 93.1% . These results indicate that the examined toxigenic fungi are sensitive to cinnamon oil. These results have been confirmed by the findings of many

Table 2. Effect of Cinnamon oil at 4% on aflatoxin B (µg/ml) produced by *A. flavus* isolated from Hazelnuts

Isolates number	Control		Cinnamon %		Inhibition
	B1	B2	B1	B2	
H1	35.4	11.1	11.3	2.6	70.1
H2	52.1	16.3	20.6	0.0	69.8
H3	64.3	22.0	18.3	6.2	71.6
H5	27.1	9.1	9.2	3.1	66.4
H9	40.2	14.9	11.0	1.6	77.1
H10	51.1	10.7	16.3	2.9	68.9
H12	31.5	21.3	8.6	4.5	75.2
H16	28.3	0.0	5.2	0.0	81.6
H17	0.0	0.0	0.0	0.0	-
H18	39.4	13.8	11.1	1.8	75.8
H19	27.6	14.5	6.0	2.2	80.5
H23	0.0	0.0	0.0	0.0	-

Table 3. Effect of Cinnamon oil at 4% on aflatoxin G (µg/ml) produced by *A. flavus* isolated from hazelnuts

Isolates	Control		Cinnamon		%
	G1	G2	G1	G2	Inhibition
H1	0.0	0.0	0.0	0.0	-
H2	23.2	0	2.7	0.0	88.3
H3	16.8	15.4	1.2	1.8	90.6
H5	27.1	0.0	6.3	0.0	76.7
H9	18.2	12.9	6.1	1.6	75.2
H10	36.2	13.4	11.4	2.1	72.7
H12	33.1	0.0	3.7	0.0	88.8
H16	0.0	13.2	0.0	0.91	93.1
H17	26.2	0.0	4.6	0.0	82.4
H18	0.0	0.0	0.0	0.0	-
H19	35.4	17.1	12.3	1.09	74.4
H23	0.0	0.0	0.0	0.0	-

researchers (Sinha *et al*.,1993; Thanaboripat *et al.,* 2004*;*Thanaboripat *et al*.,2007; Eweis *et al.,* 2012; Abd El-Aziz *et al.,* 2012; Al-Othman *et al*., 2013a).

Effectiveness of essential oils on fungal growth and mycotoxin production are dependent on the concentration of the essential oils (Soliman and Badeaa, 2002).

Effect of essential oils due to the presence more compounds like terpenes cinnamaldehyde (Toda *et al.,* 1989; Ebana *et al.,* 1991). which are penetrating the interior of the cell and interacting with critical intracellular sites (Cristani *et al*., 2007). Antimicrobial activity of essential oil due to phenols cause lysis of hyphae and spores of the toxigenic fungi are characteristic of the aflatoxin deactivation process (Knobloch *et al.,* 1988; Namazi *et al.,* 2002). And leading to various components exiting the cytoplasm, causing loss of rigidity of cell wall. (Nicola *et al.,* 2005; Sharma and Tripathi, 2008).

Table 4. Determination of aflatoxin (µg/kg) from hazelnuts

Sample	Aflatoxin (µg/kg)			
number	B1	B2	G1	G2
1	46.2	0	13.2	17.3
2	36.2	15.1	35.2	12.3
3	30.7	0.0	12.5	13.1
4	77.5	32.2	46.1	15.0
5	47.1	14.5	22.1	20.7
6	0.0	0.0	0.0	0.0
7	28.2	0.0	0.0	0.0
8	0.0	0.0	0.0	0.0

Determaination of aflatoxins from shelled hazelnuts samples

Data in Table 4 obtained that concentrations of aflatoxins (B1, B2, G1 and G2) ranged from 28.2 to 77.5, 14.5 to 32.2, 12.5 to 46.1 and 12.3 ton 20.7 µg/kg, respectively. The highest contamination levels of aflatoxins were found in samples No. 4 . Three samples (no 2,4,5) produced all four aflatoxins (B1, B2, G1 and G2) in varying amounts, one sample (no 7) failed to produced B1, two samples (no 1,3) failed to produced aflatoxins B2 whereas sample no (6 and8) failed to produced any aflatoxins.

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