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## Mycotoxins production by *Aspergillus ostianus* Wehmer and using phytochemicals as control agent

Saber SM<sup>1</sup>, Youssef MS<sup>1</sup>, Arafa RF<sup>2</sup>, Hassane AMA<sup>2</sup>

<sup>1</sup>Botany Department, Faculty of Science, Sohag University, Egypt

<sup>2</sup>Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut,

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**Abstract** *Aspergillus ostianus* Wehmer was a new record to the Mycological Laboratories in Egypt, isolated from cabbage seeds and tested for aflatoxins and ochratoxin A production. Aflatoxins B<sub>1</sub> and G<sub>1</sub> and ochratoxin A were demonstrated in the crude extracts of *Aspergillus ostianus* cultivated on different media for mycotoxins production. Both of essential oil and ethanol extract of clove were tested in order to control *Aspergillus ostianus*. The minimum inhibitory concentration (MIC) was 46.77 and 500 mg/ml for essential oil and ethanol extract, respectively. Also, qualitative phytochemical screening of the extracts and their chemical components using GC/MS analysis were determined.

**Keywords** Aflatoxins, ochratoxin A, *Aspergillus ostianus*, clove extracts, GC/MS analysis.

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### 1. Introduction

Mycotoxin contamination of various foodstuffs and agricultural commodities is a major problem in the tropics and sub-tropics, where climatic conditions and agricultural and storage practices are conducive to fungal growth and toxin production [1-5]. The term mycotoxin is derived from “mykes”, a Greek term for fungus and the Latin word “toxicum” meaning poison. It is usually reserved for a structurally diverse group of mostly low molecular weight (MW≤700) secondary metabolites formed mostly by saprophytic moulds growing on a variety of foodstuffs including that of animal feeds and also by many plant pathogens [6-9].

More than 500 different mycotoxins are known, which show a great diversity in different chemical, biological and physicochemical properties. Generally, mycotoxins are chemically and thermally stable compounds [7, 10-11]. The number of mycotoxins known to exert toxic effect on human and animal health is constantly increasing as well as the legislative provisions taken to control their presence in food and feed [12].

Mycotoxins are produced by toxigenic fungi or moulds of the *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and *Claviceps* genera, which under suitable temperature and humidity conditions, may develop on many agricultural commodities in the field or after harvest or during transportation or storage, causing significant reductions in crop yield and cause economic losses [2,3, 10, 13]. These compounds cause a diverse range of toxic effects (acute or chronic or both) for human and animal health through the ingestion of contaminated food products [14-16].

In several countries, many individuals are chronically exposed to high levels of mycotoxin in their diet [17]. Mycotoxicosis is the consequence or effect (disease or pathological abnormalities) resulting directly of ingesting toxin contaminated foods of plant origin by man and animals. It may also result indirectly from consumption of animal products such as milk from livestock exposed to contaminated feed [18-19].

Although currently more than 500 mycotoxins are known, scientific attention is focused mainly on those that have proven to be carcinogenic and/or toxic and several epidemiological studies have confirmed their ill effects [20-22]. These chemicals may affect many target organs and systems, notably the liver, kidney, nervous system,



endocrine system, immune system muscular, skin, respiratory organs, digestive tract, genital organs, etc. [23-25]. When present in foods in sufficiently high levels, these fungal metabolites can have toxic effects that range from acute (for example, diarrhoea, vomiting, liver or kidney deterioration), to chronic (for example, liver cancer), mutagenic, and teratogenic; and resulting symptoms range from skin irritation to immunosuppression, birth defects, neurotoxicity, and death [26-29]. The main toxic effects of these metabolites are carcinogenicity, genotoxicity, teratogenicity, nephrotoxicity, mutagenicity, neurotoxicity, hepatotoxicity and immunotoxicity [10, 12, 20-32].

*Aspergillus ostianus* Wehmer is belonged to *Aspergillus ochraceus* group according to the key of *Aspergillus* of Raper and Fennell [33], also, it belonged to section *Circumdati* according to the key of Frisvad and Samson [30]. Federico *et al.* reported that old species involved in ochratoxin A (OTA) production are *Aspergillus alutaceus*, *A. ostianus*, *A. quercins*, *A. sulphureum* and *Penicillium verrucosum* [34]. *A. melleus*, *A. ostianus*, *A. persii* and *A. petrakii* may produce trace amounts of OTA [35-37].

On the other hand, in a study carried out by Varga *et al.* (1996), *Aspergillus ostianus* was tested for ochratoxin A production by the immunochemical test [38]. The result showed that OTA content not detected. Concerning aflatoxins biosynthesis, Wilson *et al.* (1968) was not detected them by *Aspergillus ostianus*. While, Scott *et al.* (1967) reported aflatoxins B<sub>1</sub> and G<sub>1</sub> production by *A. ostianus* [39-40].

A number of surveys and monitoring programs have been carried out in several countries attempting to obtain a general pattern of mycotoxins contamination in food [15,41-42].

Great success has been achieved to reduce mycotoxigenic fungi and mycotoxins in foods using plant products such as plant extracts and plant essential oils [4]. In addition, many natural compounds found in dietary plants, such as extracts of herbs and fruit extracts, possess antimicrobial activities against *Aspergillus parasiticus* [43-44]. Many spices and herbs, such as cloves, anise and star anise seeds [45], basil, cinnamon, marigold and spearmint [43], garlic and onion [46], thyme [44], cassia and sweet basil [47] have been reported to inhibit toxigenic and foodborne moulds.

Many investigators used essential oils such as cinnamon, peppermint, basil and thyme to protect maize kernels against *A. flavus* infection, without affecting germination and corn growth [48]. Strategies for the prevention of mycotoxigenic fungus growth and the further production of mycotoxins in agricultural produce and food are the use of fungicide and chemical preservatives or heat treatment to inactivate spores [49].

## **2. Materials and Methods**

### **2.1: Isolation and Identification of *Aspergillus ostianus*.**

This was made by using "Dilution-Plate Method" as described by Moubasher *et al.* [50]. Identification (purely morphologically; based on macro- and microscopic characteristics) was done using keys and references of identification.

### **2.2: Production and Detection of Mycotoxins**

#### **2.2.1: Coconut based medium test**

Coconut milk agar (CMA) medium was prepared [51-52]. The plate centre was inoculated with 5 µl of *Aspergillus ostianus* spore suspension and incubated in the dark at 28°C. The presence or absence of a fluorescence ring in the agar surrounding the colonies under UV light after 7 days incubation was noted and the results were scored as positive or negative.

#### **2.2.2: Ammonia vapour test**

*Aspergillus ostianus* isolate was grown on YES agar (2% yeast extract, 15% sucrose and 1.5% agar) as single colonies in the centre of plate and incubated in the dark at 28°C [53-54]. After 3 days, a set of plates were inverted over 2 ml of ammonium hydroxide. This was repeated with another set after 7 days. A change in colour of the culture medium was used to determine the toxicity or otherwise of isolates. After ten minutes, the undersides of aflatoxin producing isolates turned into pink to red color.

#### **2.2.3: Cultivation of *Aspergillus ostianus* for toxin production:**

One isolate of *Aspergillus ostianus* was grown in 250 ml flasks each containing 100 ml Czapek's yeast extract broth [39, 55-56] and 50 g of polished rice (medium grain) and 20 ml of tap water were autoclaved for 15 min at



121 °C [57-58] for aflatoxins and ochratoxin A production. Potato dextrose broth (PDB) [38], yeast extract-sucrose broth (YES; 2% yeast extract, 15% sucrose) [59] and malt peptone (MP) broth using 10 % (w/v) of malt extract and 0.1% bacto peptone (w/v) [60] were used for ochratoxin A production. Potato dextrose (PD) broth (hot water (50 ml) extract of potato (20 g), 1 g dextrose, 50 ml natural sea water) [61] for penicillic acid production. The cultures were incubated at 30°C for 10 days.

After incubation, the contents of each flask (medium + mycelium) were homogenized for five minutes in a high speed blender (1600 rpm) with 100 ml chloroform except PD with natural sea water broth which was homogenized with ethyl acetate. Extraction procedures were repeated three times and the chloroform or ethyl acetate extracts were combined, dried over anhydrous sodium sulphate, filtered then concentrated under vacuum to near dryness [62].

#### **2.2.4: Detection of Mycotoxins:**

##### **2.2.4.1: Thin layer chromatography (TLC):**

The analysis of the extracts on precoated silica gel type 60, F<sub>254</sub> (MERCK) plates for detection in the presence of different mycotoxins was performed according to the standard procedures [63-64].

##### **2.2.4.2: TLC conditions:**

The solvent systems was applied as in Samson *et al.*, 1995 [64] and used including the percentages as following: Toluene : chloroform : acetone (15 : 75 : 10, v/v) for aflatoxins [65].

Toluene : ethyl acetate : formic acid (6 : 3 : 1, v/v) for ochratoxin A according to the method of [66].

##### **2.2.4.3: Detection:**

After developing and air drying, the TLC plates were examined visually under long wave UV light (365 nm) and short wave UV light (254 nm). Mycotoxins were identified by comparison with appropriate reference standards.

#### **2.3: Bioassay Methods for Mycotoxins:**

##### **2.3.1: Brine shrimp test:**

The immature stage (nauplii) of brine shrimp (*Artemia salina* L.) was used for mycotoxins bioassay. Larvae of shrimp are suitable for rapid assay of extremely small quantities of mycotoxins [67-68].

##### **2.3.2: Bacterial test:**

*Bacillus cereus* was used as test organism according to Sleight and Timburg [69] for the assay of toxin production by *Aspergillus ostianus*.

#### **2.4: Preparation of Clove Extracts:**

Sequential extraction method was employed to extract the plant powders using n-hexane and ethanol 95% [70].

#### **2.5: Antimycotic Activities of Essential Oils and Ethanol Extracts of Clove**

##### **2.5.1: Agar disc diffusion method:**

The antimicrobial activities of different plant extracts were carried out by agar disc diffusion method [71]. Disc diameter was 5 mm. Nystatine was used as positive control, while n-hexane, Dimethylsulfoxide (DMSO) and ethanol were used as negative control. 1% glucose-Czapek's plates previously inoculated with a spore suspension of and moulds was used for antimycotic activity.

##### **2.6: Determination of Minimum Inhibitory Concentration (MIC):**

The diameter of the inhibition zone around the disc, measured in millimeter, is used as positive bioactivity. MIC was determined according to Lamikanra [72] and employed by Ayoola *et al.* [73-74].

##### **2.7: Qualitative Phytochemical Analysis:**

Phytochemical screening for the presence of glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids and coumarins was undertaken using standard qualitative methods as described [75-80]

##### **2.8: Gas Chromatography-Mass Spectrometry (GC/MS) Analysis:**

The chromatographic procedure was carried out at Special Unit for Scientific Services and Technology, City for Scientific Research and Technology Application, Universities and Research Center District, New Borg El-Arab, Alexandria, Egypt. The essential oils and ethanol extract of basil were analyzed using GC/MS [81].



### 3. Results

#### 3.1: Toxins production and detection by *Aspergillus ostianus* Wehmer isolate:

*Aspergillus ostianus* was isolated from cabbage seeds for the first time in Egypt and tested for aflatoxins, ochratoxin A. It was cultivated on six media for detection of mycotoxin production especially aflatoxin B<sub>1</sub>, G<sub>1</sub> and ochratoxin A. *Aspergillus ostianus* isolate was determined to be aflatoxigenic by presence of blue fluorescence on the reverse side of colonies after exposure to UV light on CAM. The color response to ammonia vapor was in 100% agreement with CAM and TLC detection of the toxicity of *A. ostianus* isolate.

Crude extracts of the fungus on different media were assayed using *Artemia salina* L. and *Bacillus cereus*. The results in table (1) indicated that the fungus is toxic. Hence, thin-layer chromatographic technique was used to identify mycotoxin type. The data revealed that the fungus produced ochratoxin A on all media but with different degrees. The remarkable media stimulated ochratoxins production were malt peptone followed by potato dextrose, Czapek's yeast extract, polished rice and yeast extract-sucrose. While, aflatoxins were produced on Czapek's yeast extract and yeast extract-sucrose (Table, 1 & Figure, 3). Also, the growth of this fungus on Czapek's-glucose media and microscopic characters were shown in figures (1 & 2).

**Table 1:** Analysis of mycotoxins production by *Aspergillus ostianus* grown on different types of media using thin layer chromatographic technique (TLC) and toxicity to *Artemia salina* (total number assayed, 100) and *Bacillus cereus* assay.

No.	Type of medium	Mycotoxins detected	<i>Artemia salina</i> (%mortality)	<i>Bacillus cereus</i> assay
1	Czapek's yeast extract broth	Ochratoxin A and aflatoxins B <sub>1</sub>	100	17
2	Malt peptone (MP) broth	Ochratoxin A	80	23
3	Potato dextrose broth (PDB)	Ochratoxin A	60	15
4	Potato dextrose broth (PDB) + natural seawater	Ochratoxin A	35	18
5	Polished rice (medium grain)	Ochratoxin A	60	21
6	Yeast extract-sucrose broth (YES)	Ochratoxin A and aflatoxins B <sub>1</sub> and G <sub>1</sub>	100	15

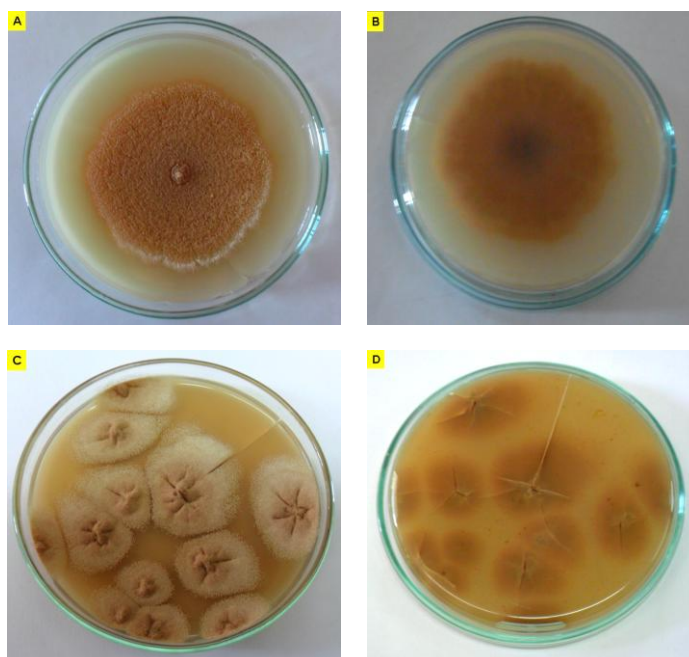


Figure 1: Growth of *Aspergillus ostianus* on: (A) glucose-Czapek's agar; (B) reverse; (C) potato dextrose agar; (D) reverse.





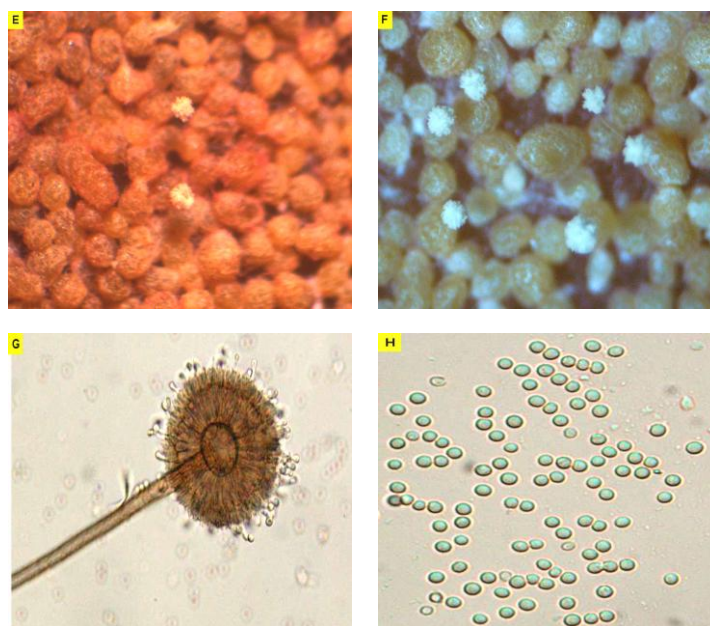


Figure 2: *Aspergillus ostianus* under microscope: (E) with sclerotia on glucose-Czapek's agar; (F) with sclerotia on potato dextrose agar; (G) conidial head with strigmata; (H) conidia.

### 3.2: Antimycotic activity of clove extracts against *A. ostianus*.

Results in the present research showed that clove essential oil and ethanol extract had highly antifungal activity against *A. ostianus* with inhibition zones 40 mm and 25mm, respectively. The MICs of clove essential oil and ethanol extract on the growth of *A. ostianus* were 46.77 and 500 mg/ml, respectively (Table, 2).

Qualitative analysis was carried out for screening the presence of major phytochemical constituents such as glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids and coumarins in the highly active extracts. Data of phytochemical analysis of clove essential oil showed the presence of flavonoids, terpenoids, coumarins and glycosides. On the other hand, clove ethanol extract had flavonoids, glycosides, terpenoids and tannins, while alkaloids and coumarins and saponins were absent (Table, 3).

Four compounds including Eugenol (Phenol, 2-methoxy-4-(2-propenyl) [4-Allyl-2-methoxyphenol]) 47.69%, Aromadendrene,  $\alpha$ -Caryophyllene (Humulene) and Phenol 2-methoxy-4-(2-propenyl)- acetate (eugenol acetate) 16.45% were identified by GC/MS analysis of essential oil of clove (Table, 4).

Three compounds including Phenol, 2-methoxy-4-(2-propenyl)- acetate (eugenol acetate) 71.7%, Cis- $\alpha$ -Bisabolene and Eugenol (Phenol, 2-methoxy-4-(2-propenyl) [4-Allyl-2-methoxyphenol]) 17.35% were identified by GC/MS analysis of ethanol extract of clove (Table, 5).

**Table 2:** Antimycotic activities and Minimum Inhibitory Concentration (MIC mg/ml) of clove essential oil and ethanol crude extract against *Aspergillus ostianus* isolate [After 72 h]. Inhibition zone diameter in millimeter

Extract	Inhibition zone (mm)	MIC mg/ml
essential oil	40	46.77
ethanol extract	25	500
<b>Nystatine</b>	NI	NI
<b>n-hexane, ethanol, DMSO</b>	NI	NI

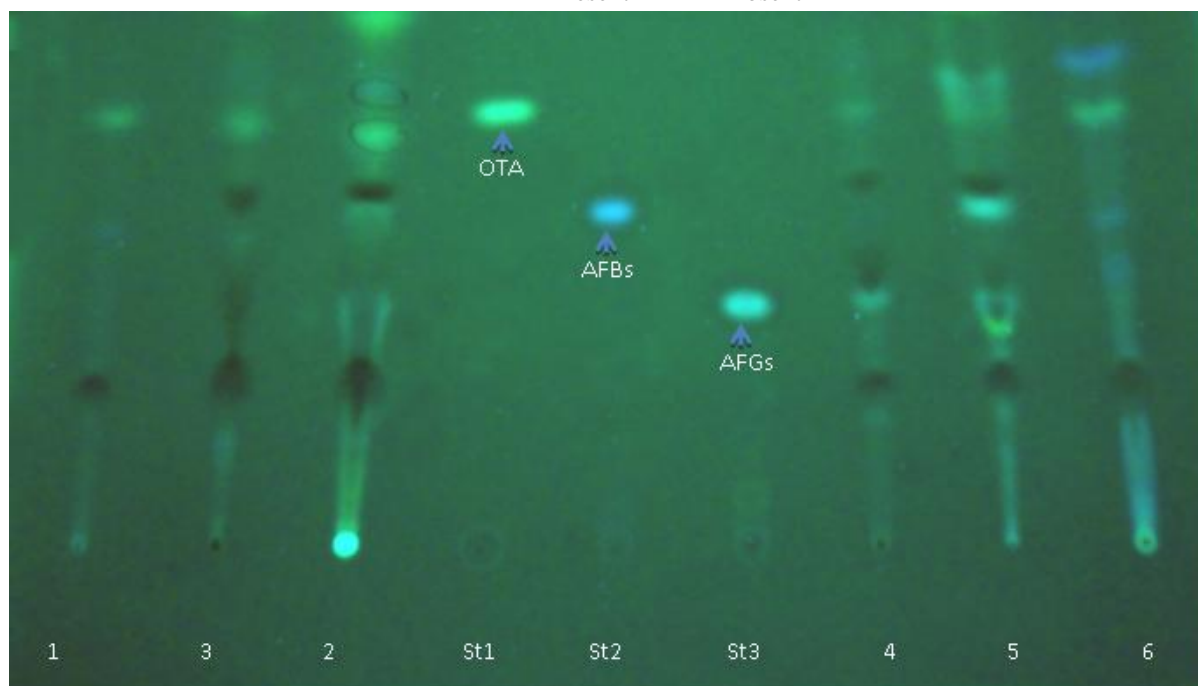
NI = No Inhibition



**Table 3:** Phytochemical screening of the bioactive compounds in wide spectrum highly active antimycotic extracts.

Type of extract	Medicinal plant	Flavonoids	Alkaloids	Terpenoids	Glycosides	Coumarins	Tannins	Saponins
n-hexane (essential oils)	Clove	+	-	+	+	+	-	-
Ethanol 95%	Clove	+	-	+	+	-	+	-

+ = Present      - = Absent

**Figure 3:** Mycotoxins produced by *Aspergillus ostianus* on different types of media: 1, 2, 3, 4, 5 & 6 as listed in table (27).**Table 4:** GC/MS Analysis of essential oil of clove.

No.	Component Name	Retention time	Percentage %
1	Eugenol	24.522	47.69
2	Aromadendrene	26.646	12.1
3	$\alpha$ - Caryophyllene	27.711	1.6
4	Phenol, 2-methoxy-4-(2-propenyl)acetate (Eugenol acetate)	29.762	16.45

**Table 5:** GC/MS Analysis of ethanol extract of clove.

No.	Component Name	Retention time	Percentage %
1	Phenol, 2-methoxy-4-(2-propenyl)-, acetate (Eugenol acetate)	24.429	71.7
2	Cis- $\alpha$ -Bisabolene	26.617	6.62
3	Eugenol	29.708	17.35



#### 4. Discussion

*Aspergillus ostianus* was isolated from cabbage sample No. 11 for the first time from medicinal plants in Egypt as a new record and tested for aflatoxins, ochratoxin A production. *Aspergillus ostianus* Wehmer is belonged to *Aspergillus ochraceus* group according to the key of *Aspergillus* of Raper and Fennell [33], also, it belonged to section *Circumdati* according to the key of [30]. It was cultivated on six media for 10 days in order to assay its ability in mycotoxin production especially aflatoxins B<sub>1</sub>, G<sub>1</sub> and ochratoxin A. The results indicated that the fungus is toxic. Hence, thin-layer chromatographic technique was used to identify mycotoxin types. The data revealed that the fungus had the ability to produce aflatoxins B<sub>1</sub>, G<sub>1</sub> and ochratoxin A.

Ochratoxin is a mycotoxin with nephrotoxic, nephrocarcinogenic, teratogenic and immunosuppressive properties, and has received growing interest in the scientific community and food committees in the last few years [82-83]. Only species belonging to the genera *Aspergillus* and *Penicillium* have been reported as capable of producing ochratoxins. They were initially described by Scott [40] in *Aspergillus ochraceus*, but have also been found in other species of the section *Circumdati*: *A. alliaceus*, *A. melleus*, *A. ostianus*, *A. petrakii*, *A. sclerotiorum*, *A. sulphureus* [36], *A. albertensis* and *A. auricomus* [38]; as well as in the black aspergilli of section *Nigri*: *A. niger* var. *niger* and *A. carbonarius* [84]. Species of the *Circumdati* section (*A. ochraceus*, *A. melleus*, *A. alliaceus*, *A. petrakii*, *A. ostianus*, *A. sclerotiorum*, *A. sulphureus*, *A. auricomus* and *A. albertenses*) as well as *Penicillium verrucosum* are able to produce ochratoxin A (OTA) [58]. Federico *et al.* (2009) reported that old species involved in ochratoxin A (OTA) production are *Aspergillus alutaceus*, *A. ostianus*, *A. quercinus*, *A. sulphureum* and *Penicillium verrucosum* [34].

*A. melleus*, *A. ostianus*, *A. persii* and *A. petrakii* may produce trace amounts of OTA [35-37]. *Aspergillus ostianus* NRRL 5225 has previously been reported to produce trace amounts of ochratoxin A [36]. More *Aspergillus* species have been found to produce OTA, for example, *Aspergillus glaucus*, *A. ostianus* and *A. petrakii* [85-86]. *Aspergillus ostianus* is a member of section *Circumdati* has been reported to produce ochratoxin A [35, 87-88].

On the other hand, in a study carried out by Varga *et al.*, [38], *Aspergillus ostianus* was tested for ochratoxin A production by the immunochemical test. The result showed that OTA content not detected. Concerning aflatoxins biosynthesis, Wilson *et al.*, [39] did not detect them by *Aspergillus ostianus*. While, Scott *et al.*, [40] reported aflatoxins B<sub>1</sub> and G<sub>1</sub> production by *A. ostianus*.

A trace amount of ochratoxin A was produced by *Aspergillus ostianus* and confirmed using HPLC with diode array detection and comparison to authentic standard in addition to penicillic acid, xanthomegnins and mullein (a polyketide extrolite apparently not directly linked to ochratoxin, penicillic acid or xanthomegnin) [60]. A fungal rice culture of *A. ostianus* was prepared as previously described by Carlton and Tuite [89] produced 1,600 ppm ochratoxin A by spectrophotofluorometric analysis [90].

It has been for some time that certain herbal drugs and spices contain substances with antimycotic activity [91-93]. Natural plant extracts are of interest as a source of safer or more effective substitutes for synthetically produced antimicrobial agents and may provide an alternative way to prevent fungal contamination of food and some also to inhibit aflatoxin formation [93-97]. Different crude extracts of spices, herbs and other plant materials are becoming increasingly important in food industries because of their antifungal and bioregulatory properties [98-101]. Volatile substances from different aromatic plants have proven to be efficient antifungal against food spoiling moulds [102-103].

Results in the present research showed that clove essential oil and ethanol extract had highly antifungal activity against *A. ostianus*. Essential oil and ethanol extracts were active against *A. ostianus* with inhibition zones 40 mm, respectively for essential oils and 25mm, respectively for the ethanol extract.

Clove, thyme and eucalyptus essential oils were found to be among the most effective in controlling aflatoxigenic strains [104]. With exposure to 5 µl, eugenol and clove oils gave clear zones but with residual growth inside them (less for eugenol). Eucalyptus 60 µl did not give any inhibition. Mahmoud (1994) tested the antifungal effect of some essential oils on growth of moulds and aflatoxin production; clove had highly antifungal activity [105]. It was found that cinnamon, cloves or both inhibited both growth (50%) and Aflatoxin G<sub>1</sub> production (100%) of *A. flavus* [106]. Clove essential oil at conc. 1500 µl/l completely inhibited growth of *A. flavus* and *A. parasiticus* [107]. Clove strongly inhibited growth of toxigenic *A. flavus*, *A. versicolor* and *P.*



*citrinum* [106]. Many spices and herbs, such as cloves [45], basil and cinnamon [108], garlic [46], thyme [109], cassia and sweet basil [47] have been reported to inhibit toxigenic and food-borne moulds.

The effects of clove essential oil and its principal component, eugenol, on growth and mycotoxin production by some toxigenic fungal genera such as *Aspergillus* spp., *Penicillium* spp., and *Fusarium* spp. have been reported [110-114]. This component was able to inhibit both growth and/or mycotoxin production [107].

Eugenol is a phenolic compound. Phenols are known to have antiseptic properties [115], which is consistent with the antimicrobial data obtained for these compounds. Caryophyllene has also been shown to possess antimicrobial properties, though not as potent as eugenol [116]. The spectrum of fungitoxicity of eugenol was determined at 0.2 µl/ml against toxigenic isolate *Aspergillus flavus* and 12 fungal species viz. *Aspergillus candidus*, *A. fumigatus*, *A. niger*, *A. paradoxus*, *A. terreus*, *A. versicolor*, *Cladosporium cladosporioides*, *Curvularia lunata*, *A. alternata*, *F. nivale*, *F. oxysporum* and *Penicillium* sp. Nearly, 100% growth inhibition was achieved [117].

Clove and basil chemical constituents in essential oils are linalool eugenol, tannins and flavonoids [118-119]. Phytochemical screening of clove ethanol and aqueous extracts indicated the presence of alkaloids, reducing sugars, flavonoids, tannins, steroids and saponins in both extracts, except steroids in the ethanol extract [120].

Results of phytochemical screening of powdered clove for the existence of anthraquinones, alkaloids, saponins, tannins and cardiac glycosides indicated the presence of saponins, tannins and cardiac glycosides [121]. Another phytochemical screening of clove ethanol extract showed the presence of alkaloids, glycosides, tannins and reducing sugar, and the absence of terpenoids, steroids and flavonoids [122].

To determine the active antibacterial and antimycotic ingredients in the most potent inhibitory extracts, we used gas chromatography coupled with mass spectrometry (GC/MS) analysis. The data of MIC test and phytochemical analysis proved that the essential oil and ethanol extract of each of basil and clove were the highest antibacterial and antimycotic agents, respectively. By using gas chromatography coupled with mass spectrometry (GC/MS), the major components and their retention times were recorded. Most of the identified compounds in different analyzed extracts were known for their interesting antimicrobial activity.

Four compounds including Eugenol (Phenol, 2-methoxy-4-(2-propenyl) [4-Allyl-2-methoxyphenol]) 47.69%, Aromadendrene,  $\alpha$ -Caryophyllene (Humulene) and Phenol 2-methoxy-4-(2-propenyl)- acetate (eugenol acetate) 16.45% were identified by GC/MS analysis of essential oil of clove. Three compounds including Phenol, 2-methoxy-4-(2-propenyl)- acetate (eugenol acetate) 71.7%, Cis- $\alpha$ -Bisabolene and Eugenol (Phenol, 2-methoxy-4-(2-propenyl) [4-Allyl-2-methoxyphenol]) 17.35% were identified by GC/MS analysis of ethanol extract of clove.

Antimicrobial and antioxidant activities of plant extracts correlated well with their phenolic fractions [123]. The mechanism of the antimicrobial effects involves the inhibition of various cellular processes, followed by an increase in plasma membrane permeability and finally ion leakage from the cells [124-125]. Additionally, after penetration into the cells, they can interact with intracellular sites and cause death of the cell, e.g., by alteration of protein structures [126]. The essential oil fractions sensitize the cell membrane, causing an increase in permeability and leakage of vital intracellular constituents, as well as the impairment of bacterial enzyme system and cell respiration [127-128]. Phenolic components of essential oils sensitize the phospholipid bilayer of the cell membrane, causing an increase of permeability and leakage of vital intracellular constituents or impairment of bacterial enzyme systems [127].

Compositional analysis carried out previously on clove oil obtained by different methods revealed that the main components were eugenol (48.82–99.16%), eugenol acetate (3.89–21.75%), caryophyllene (0.3–36.94%) and  $\alpha$ -humulene (1.9–4.41%) [74, 129-134]

Low-molecular-weight phenolic compounds are among the most typical components of essential oils with antimicrobial activity [135]. They could have an activating or inhibiting effect on microbial growth according to their structure and concentration [136-137]. Eugenol has been reported to be one of the most active antimicrobials in essential oils, being 95% of the clove essential oil [135]. It was indicated that the type of phenolic structure is more important than the concentration [32]. Hydroxyl groups assigned to the phenolic compounds may form hydrogen bridge bonds with active enzymes, resulting in their deactivation and a consequent effect on the development of the fungal biomass and the production of mycotoxins [138]. Previous





studies of eugenol and cinnamaldehyde have presented evidence indicating possible roles for membrane interactions and the inhibition of specific cellular processes or enzymes [125-139-140].

## 5. Conclusion

Isolation, identification and mycotoxins production by *Aspergillus ostianus* were recorded for the first time in Egypt. Medicinal plants can be used as alternative antimicrobial drugs such as basil and clove with regard to their quality and storage under hygienic conditions to avoid problems of fungal and mycotoxins contamination.

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