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Full Length Research Paper

Chemopreventive potential of some plant essential oils against *Aspergillus flavas* and *Aspergillus ochraceus* growth and mycotoxin production

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In an effort to screen the essential oils of some Egyptian plants for novel aflatoxin (AFB1) and ochratoxin (OTA) inhibitors, celery, parsley, dill and coriander essential oils (EOs) were evaluated as a potent inhibitor of fungal growth of *Aspergillus flavus* 3 and *Aspergillus ochraceus* 2. Also the effect of EOs added to synthetic media and wheat grains on aflatoxin (AFB1) and ochratoxin (OTA) accumulation by *A. flavus* 3 and *A. ochraceus* 2 were tested at different concentrations (2, 4, 6 and 8 µl ml-^{1}). In addition, EOs were evaluated for their ability to detoxify aflatoxin (AFB1) and ochratoxin (OTA). Mycelia growth was significantly ($p < 0.01$) inhibited by the different concentrations of all essential oils. The EOs at all concentrations tested had an inhibitory effect against AFB1 and OTA formation by *A. flavus* 3 and *ochraceus* 2 in synthetic media and wheat grains. The percentage inhibition of AFB1 and OTA production showed an ascending pattern with the increase in the concentrations used. Higher content of OTA was detected at the concentration of 2 µl ml-^{1} as compared to AFB1 in both synthetic media and sterile wheat grains. There was a drastic reduction in the AFB1 and OTA content at the concentration of 4 µl ml-^{1} in both synthetic media and sterile wheat grains. The concentration 6 µl ml^{-1} completely checked AFB1 in synthetic media and sterile wheat grains, while the concentrations of 6 and 8 µl ml^{-1} significantly ($P < 0.05$) suppressed OTA formation in synthetic media and sterile wheat grains, respectively. EOs induce much greater suppression of fungal growth and AFB1 and OTA production in synthetic media compared to the sterile wheat grains. The inhibition of AFB1 and OTA biosynthesis by the celery and coriander essential oils were more than the parsley and dill essential oils. The test of AFB1 and OTA detoxification revealed that coriander and dill essential oils showed the maximum degradation of AFB1 and OTA up to 70 and 66%, respectively, while irradiated coriander (7 kGy) and dill (10 kGy) essential oils were more effective than the unirradiated essential oil capable of degrading >93 and 83% of the AFB1 and OTA, respectively. The celery, parsley, dill and coriander essential oils may provide a biologically safe method to protect wheat grains or cereal products and other agricultural commodities from aflatoxins and ochratoxin.

Key words: Essential oils, chemopreventions, antifungal, detoxification of aflatoxins, ochratoxins, irradiation.

INTRODUCTION

Aflatoxins, produced by *Aspergillus flavus*, *Aspergillus parasiticus, Aspergillus nomius, Aspergillus tamari, Aspergillus bombycis* and *Aspergillus pseudotamarii* are both acutely and chronically toxic to both humans and animals (Elshafie et al., 2011; El-Nagerabi et al., 1012). Some strains of *A. flavus* have been re-identified as *A. parasiticus* and *A. nomius* (Santacrose et al., 2008). Ochratoxin (OTA) is produced by *Aspergillus ochraceus* and Penicillium species and in particular by *Penicillium verrucosum*. OTA has been classified as a “possible human carcinogen” (Group 2B) (Lyon, 1993). Various agricultural commodities have been found to be contaminated with either aflatoxin producing fungi or aflatoxins. Although the presence of *Aspergillus* mould

does not necessarily indicate aflatoxin contamination, there is certainly an increased risk (Robertson, 2005). The foods at highest risk of aflatoxin contamination are corn, peanut and cotton seed (Speijers and Speijers 2004). Cereals and other crops are exposed to fungal attack in the field or during storage and this attack may result in mycotoxin contamination of the crop. The Food and Agriculture Organization (FAO) estimates that at least 25% of world cereal production is contaminated with mycotoxins (Villa and Markaki, 2009). Aflatoxin B1 has been detected in 80% of maize samples obtained from different locations in Southeast Nigeria (Aja-Nwachukwu and Emejuaiwe, 2006). Similarly, 92% of animal feed samples taken from commercial sources in Thailand were contaminated with aflatoxin B1 (Charoenpornsook and Kavisarasai, 2006). In at least three parts of the world, East Africa, the Philippines and Thailand, good epidemiological evidence has been collected showing a correlation between the incidence of liver cancer and exposure to aflatoxins (Villa and Markaki, 2009). Aflatoxins have also been identified as a potential biological weapon for food and water contamination (Smith, 2004). Ochratoxin A (OTA) and Aflatoxin B1 (AFB1) are among the most frequent observed combinations of mycotoxins in different plant products (Speijers and Speijers, 2004).

Physical, chemical and biological methods have been investigated in order to prevent the growth of aflatoxin and ochratoxin producing fungi and to eliminate or reduce the levels of mycotoxin or to degrade or detoxify aflatoxins in foods and feeds (Thanaboripat, 2002). One of the most effective ways to control the problems caused by aflatoxins is to prevent the growth of fungi in the substrate, for example by the use of chemical inhibitors to suppress the spore germination of the fungi, as well as the development of the fungal mycelium, in the substrate susceptible to contamination by these toxins (Moreno-Martinez et al., 2000). Because of aflatoxins' effects on health and economics, the search for antifungal agents is extensive and natural plant extracts may provide an alternative way to prevent fungal contamination of food or feed (Sandosskumar et al., 2007). Control by naturally produced agents is becoming increasingly important because of consumers' mistrust of food and feed treatments that involve using synthetic xenobiotic substances. Natural plant compounds have been used traditionally to preserve foods in countries like Japan, India and Russia (García-Cela et al., 2012). Extracts and powders of various spices, herbs and essential oils have been reported to have antimicrobial activity against aflatoxin producing fungi and some of them also inhibit aflatoxin formation (Krishnamurthy and Shashikala, 2006; Thanaboripat, 2003; Thanaboripat et al., 2004). Many essential oils have also been reported as effective inhibitors of fungal growth and aflatoxin production (Razzaghi-Abyaneh et al., 2008; Sandosskumar et al., 2007). Great success has been achieved to reduce mycotoxigenic fungi and mycotoxins in foods using plant products such as plant extracts and plant essential oils (Reddy et al., 2010). Therefore, there has been increased interest in the research in using natural antimicrobial or antifungal substances, which may replace synthetic pesticides or contribute to the development of new pest control agents. During the past 22 years, essential oils (EO) have been shown to possess a broad spectrum of antifungal activity (Bluma and Etcheverry, 2008).

Chemopreventions have a long-standing history in Egypt. Current study investigated the antimycotoxins potentialities of celery, parsley, dill and coriander essential oils which are widely used in folk medicine by Egyptian housewives. However, there is a little information available for degrading AFB1 and OTA-contamination by plants. This research was therefore undertaken by the main objectives: (1) to find the best EOs of four Egyptian plants for reducing AFB1-content, (2) to determine their AFB1-degradation activity as well as anti-AFB1-biosynthesis activity, and (3) to introduce a new botanical source to degrade AFB1-contamination. These natural plant products may successfully replace synthetic chemicals and provide an alternative method to protect wheat grains as well as other agricultural commodities of nutritional significance from toxigenic fungi such as *A. flavus* and *A. ochraceus*.

MATERIALS AND METHODS

Plant materials

Four plant species were used in this work (Celery, Parsley, Dill and Coriander); these were selected in terms of commercial availability, common traditional use and zonal production in Egypt. Commercially available supplies of the plant materials used throughout the study were purchased from the local market. The EOs of Celery, parsley, dill and coriander were extracted from the entire plant (stems, leaves and flowers) by hydro-distillation assisted by microwaves using a Clevenger-type apparatus for 30 min. The oily layer obtained on top of the aqueous distillate was separated and dried for 30 min. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulphate (0.5 g). The extracted EOs was kept in sealed air-tight glass vials and covered with aluminium foil at -18°C until further analysis (Alves-Silva et al., 2012; Prakash et al., 2010). The volatile oils were analyzed by Gas Liquid Chromatography (GLC) (Adams, 2007).

Detection of aflatoxigenic and ochratoxigenic isolates

Randomly selected 20 isolates of *Aspergillus flavus* and *Aspergillus ochraceus* from contaminated cereals products were screened for their aflatoxin B1 (AFB1) and ochratoxin (OTA) producing potential detected by thin

layer chromatography (TLC) following the study of Kumar et al. (2007). *A. flavus* and *A. ochraceus* isolates were aseptically inoculated in 25 ml SMKY medium (Sucrose 200 g; MgSO4·7H2O, 0.5 g; KNO3, 0.3 g and yeast extract, 7 g; 1 L distilled water) and incubated for 10 days (27±2°C). The content of each flask was filtered and extracted with 20 ml chloroform. The extract was evaporated to dryness on water bath and redissolved in 1 ml chloroform. Fifty microliters of chloroform extract was spotted on TLC plates and developed in toluene:isoamylalcohol: methanol (90:32:2; v/v/v). The plate was air dried and AFB1 and ATO were observed in UV-transilluminator (360 and 333 nm, respectively).

Antifungal assay

Effect of Celery Essential Oil (CEO), Parsley Essential Oil (PEO), Dill Essential Oil (DEO) and coriander Essential Oil (CoEO), on radial growth of mycelium was assayed using an agar dilution method (Soliman and Badeaa, 2002). The EOs concentrations including 2, 4, 6 and 8 µl ml^{-1} were prepared by adding appropriate volumes of EOs to the sterilized molten Potato dextrose agar (PDA) medium and then poured into sterilized plates. A 5 mm diameter Whatman No. 1 filter paper disc was placed at the center of each plate and inoculated with 10 µl. ml^{-1} of spore suspension (10^6 spore's ml^{-1}) of *A. flavus* 3 and *A. ochraceus* 2. Plates were incubated for 10 days at 26 ± 1°C in the darkness and the average of two perpendicular diameters of colony was daily calculated. After the incubation period, discs showing no growth were transferred to PDA plates without EOs and incubated as previously described, to determine the fungistatic or fungicidal effect. The lowest concentration which inhibited the growth of the fungus (fungus revived on untreated PDA) was considered as minimum inhibitory concentration (MIC) and the lowest concentration of EOs which killed the test fungus (no growth was observed on fresh medium) was taken as minimum fungicidal concentration (MFC). All treatments were carried out in triplicate at least on three separate occasions. The antifungal effect was expressed as percent inhibition of radial growth by the following formula:

$$\text{Inhibition of growth (\%)} = \frac{Dc - Ds}{Dc} \times 100$$

where Dc is the diameter of colony in the control sample, and Ds is the diameter of colony in the treated sample.

Efficacy of tested EOs in checking AFB1 and OTA production by the toxigenic isolates of A. flavus 3 and A. ochraceus 2

Requisite amounts of the tested CEO, PEO, DEO and CoEO were dissolved separately in 0.5 ml acetone and added to 24.5 ml SMKY to achieve the various concentrations (2, 4, 6 and 8 µl. ml^{-1}). The medium was inoculated separately with 1 ml spore suspension (10^6 spore's ml^{-1}l) of toxigenic strains *A. flavus* 3 and *A. ochraceus* 2, then the incubation was done for ten days at (27±2°C). The medium was filtered and mycelium was dried at 80°C (12 h). AFB1 and OTA were detected by thin layer chromatography as mentioned in the text. The spots on TLC plate were scratched, dissolved in methanol (5 ml) and centrifuged at 3000 rpm (5 min). Absorbance of the supernatant was recorded at 360 and 333 nm, and AFB1 and OTA, respectively were calculated as follows:

$$\text{Toxin content (µg.ml-}^{1}\text{)} = \frac{D \times M}{E \times L} \times 1000$$

D = absorbance, M = molecular weight (312) of AFB1 and 403.8 of OTA, E = molar extinction coefficient (21800) of AFB1 and 5550 of OTA, L = path length (1 cm).

Efficacy of tested EOs in checking AFB1 and OTA production by the toxigenic isolates of A. flavus and A. ochraceus in wheat grains

Wheat grains were irradiated with 12 kGy of gamma irradiation and checked for sterility and absence of AFB1 and OTA. EOs were added at different concentrations (2, 4, 6 and 8 µl. ml^{-1}) of wheat grain by spraying, after that, flasks were inoculated separately with 1 ml spore suspension (10^6 spore's ml^{-1}) of toxigenic strains of *A. flavus* and *A. ochraceus* and were incubated for ten days at (27±2°C). Calculation of aflatoxin and ochratoxin production was determined as described:

$$\text{Toxin content (µg.g-}^{1}\text{)} = \frac{D \times M}{E \times L} \times 1000$$

Tests for AFB1 and OTA detoxification

Aflatoxins B1 (AFB1) and ochratoxin (OTA) purified from *A. flavus* and *A. ochrice* were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions were prepared in methanol at 1 mg/ml level and stored at 4°C.

Test for aflatoxin and ochratoxin detoxification by EOs

Five hundred microlitre of Parsley, Celery, Dill and coriander essential oils were mixed with 50 or 100 ng of both aflatoxin and ochratoxin and were incubated at 37°C for 48 h. After incubation, the aflatoxin and ochratoxin in

the mixture was extracted with equal volume of chloroform. The chloroform fraction was evaporated on a heat block at 60°C and the residue was dissolved in 10 µl of methanol and analyzed by TLC. Ten microlitre of the chloroform extract was separated on 0.25 mm silica gel G TLC plate (Merck). The plate was developed using the solvent chloroform and acetone (9:1, v/v). The chromatogram was viewed under UV light (365 and 333 nm) according to Velazhahan et al. (2010).

Effect of γ irradiation and heat on aflatoxin and ochratoxin detoxification properties of coriander and dill essential oils

Parsley, Celery, Dill and Coriander essential oils samples were exposed to gamma irradiation at doses of 2 to 10 kGy using an experimental ^{60}Co Russian gamma chamber (dose rate 2.5 kGy/h), in the National Center for Radiation Research and Technology, NCRRT (Nasr City, Cairo, Egypt). The aflatoxin and ochratoxin detoxification activity of the essential oils were tested as described earlier. In order to study the effect of heat on aflatoxin and ochratoxin detoxification properties of essential oils, 1 ml of essential oils in a 1.5 ml Eppendorf tube was placed in a boiling water bath for 10 min, cooled to room temperature and then assayed for aflatoxin and ochratoxin detoxification activity according to Velazhahan et al. (2010).

Time course of aflatoxin and ochratoxin detoxification by coriander and dill essential oils

To study the time course of aflatoxin and ochratoxin detoxification, 500 µl of essential oils separately was mixed with 50 ng both AFG1 and OTA and incubated at 37°C for 3, 6, 12 and 24 h. After incubation, the aflatoxin and ochratoxin content in the reaction mixture was determined as described earlier.

RESULTS AND DISCUSSION

GLC of essential oils

Among Egyptian plants, species belonging to the Parsley, Celery, Dill and Coriander fruits gained increasing interest because their essential oils are composed of different bioactive chemicals. Compositional analysis of these essential oils revealed that limonene (58.5) and Sedanolide (17.3) are the main components present in celery, Carvacol (36.9) and γ-terpinene (37.4) are present in parsley, D-limonene (46.3) and D-carvone (49.5) are the main phytochemicals of dill, and Linalool (55.4) and geranol (20.69) are the main components of coriander. Aboul-Enein et al. (2012) reported that Egypt includes wide areas of desert and tropical regions which encourage the growing of wild plants resistant for those hard conditions. Therefore, these plants might contain different secondary metabolites with high biological value which can be used as antimicrobial, antioxidant and anticancer.

Aflatoxigenic and ochratoxigenic isolates

Amongst the 20 isolated *A. flavus* and *A. ochraceus* tested for their aflatoxigenic and ochratoxigenic potency, 9 strains were found toxigenic (6 isolated of *A. flavus* and 3 *A. ochraceus*) (Untabulated data). The toxigenic strains of *A. flavus* 3 and *A. ochraceus* 2 producing maximum toxigenic were used for antiaflatoxigenic and antiochratoxigenic assay for further course of investigations.

Antifungitoxicants activity

The efficacies of CEO, PEO, DEO and CoEO on the mycelia growth of *A. flavus* 3 and *A. ochraceus* 2 are shown in Table 1. The four EOs analyzed have been shown to influence mycelia growth; their efficacy depended mainly on the concentration. EOs at 4 and 6 µl. ml^{-1} showed significantly higher inhibition than at lower concentrations 2 µl. ml^{-1}. Similarly, Tian et al. (2012) reported that the Cinnamomum jensenianum essential oil had a little effect on the antifungal activity of *A. flavus* at the concentration of 2 µl. ml^{-1}. However, the spore germination was greatly inhibited at the concentration of 6 and 8 µl. ml^{-1}.

In this study, the PEO and CoEO completely inhibited (100%) the mycelia growth at 4 and 6 µl. ml^{-1} for *A. flavus* 3 and*. ochraceus* 2, respectively, while fungal growth was completely inhibited by both CEO and DEO at the concentration of 6 and 8 µl. ml^{-1}, respectively. In general, PEO and CoEO exhibited stronger antifungal activity than the CEO and DEO to prevent mycelial growth of tested fungi. Among tested plants, EOs of species belonging to the genus celery and coriander gained increasing interest because they are composed of different bioactive chemicals such as oxygen containing monoterpenes (carvacrol, Linalool and geranol) and sesquiterpenes, with a small amount of monoterpene hydrocarbons (Hadian et al., 2012). Also the data in this study revealed that *ochraceus* 2 was the most resistance than *A. flavus* against all tested essential oils. Gorran et al. (2013) reported that Thymus daenensis and S. khozistanica Eos at 375 mg/l as well as their ethanol extracts at 4000 mg/l could completely inhibit *Aspergillus flavus* growth. Antifungal activities of EOs may result in damage in lipids, proteins, cell wall, cell membrane and cellular organelles (Bakkali et al., 2008).

To determine fungistatic or fungicidal effect, the disks showing no growth were transferred to the medium without EOs and indicated that concentrations of ≥ 4 and 6 µl. ml^{-1} for *A. flavus* and *A. ochraceus* respectively had reversible inhibitory effect while EOs at ≥ 6 and 8 µl^{-1} was found to be fungicidal and no growth observed on PDA

Table 1a. Antifungal activity of some essential oils against *Aspergillus flavas* 3 and *Aspergillus ochraceus* 2.

Antifungal index (% mycelial growth)				
Essential oils				
Concentrations (µl. ml^{-1})				
	2	**4**	**6**	**8**
CEO	$49.12^{c}_{c} \pm 0.08$	$78.30^{b}_{b} \pm 0.01$	$100^{a}_{a} \pm 0.00$	$100^{a}_{a} \pm 0.06$
PEO	$65.47^{b}_{b} \pm 0.02$	$100^{a}_{a} \pm 0.04$	$100^{a}_{a} \pm 0.03$	$100^{a}_{a} \pm 0.08$
DEO	$34.10^{c}_{d} \pm 0.06$	$61.39^{b}_{c} \pm 0.01$	$100^{a}_{a} \pm 0.05$	$100^{a}_{a} \pm 0.01$
CoEO	$69.87^{b}_{a} \pm 0.08$	$100^{a}_{a} \pm 0.04$	$100^{a}_{a} \pm 0.01$	$100^{a}_{a} \pm 0.00$

Antifungal index (% mycelial growth)				
Essential oils				
Concentrations (µl. ml^{-1})				
	2	**4** [1]	**6**	**8**
CEO	$36.33^{d}_{b} \pm 0.03$	$65.14^{c}_{b} \pm 0.05$	$82.26^{b}_{b} \pm 0.00$	$100^{a}_{a} \pm 0.02$
PEO	$53.96^{c}_{a} \pm 0.00$	$83.10^{b}_{a} \pm 0.03$	$100^{a}_{a} \pm 0.01$	$100^{a}_{a} \pm 0.00$
DEO	$26.72^{d}_{c} \pm 0.01$	$48.13^{c}_{c} \pm 0.08$	$78.14^{b}_{b} \pm 0.07$	$100^{a}_{a} \pm 0.01$
CoEO	$55.12^{d}_{a} \pm 0.02$	$76.14^{c}_{a} \pm 0.04$	$100^{b}_{a} \pm 0.08$	$100^{a}_{a} \pm 0.04$

* Mean values followed by different superscripts (within columns) are significantly different at the 5% level.

without EOs of *A. flavus* 3 and *ochraceus* 2, respectively.

Efficacy of Parsley, Celery, Dill and Coriander essential oils on the aflatoxins (AFB1) and ochratoxins (OTA) accumulation

Cereals are very rich substrates for mycotoxins production (Murphy et al., 2006; Reddy and Muralidharan, 2009). Figure 1 (a, b, c and d) shows the effects of CEO, PEO, DEO and CoEO on the aflatoxin B1 (AFB1) and ochratoxin (OTA) production in synthetic medium and sterile wheat grains as a food model. The EOs at all concentrations tested, had an inhibitory effect against AFB1 and OTA formation by *A. flavus* 3 and *A. ochraceus* 2 in synthetic media and wheat grains. At low concentration of EOs (2 µl ml^{-1}), toxigenic strain of *A. flavus* and *A. ochraceus* can produce AFB1 and OTA in both synthetic media and sterile wheat grains. It shows that the low fungicide doses create some stress condition which was responsible for the production of more secondary metabolites as a defense mechanism by the fungus. Some earlier workers have also reported that low fungicide doses stimulate the toxin production (Bluma and Etcheverry, 2008; Prakash et al., 2010). In this study, there was a drastic reduction in the AFB1 and OTA content at the concentration of 4 µl. ml-^{1} in both the synthetic media and sterile wheat grains. The concentration of 6 µl ml^{-1} completely checked AFB1 in synthetic media and sterile wheat grains, while the concentrations of 6 and 8 µl ml^{-1} significantly ($P < 0.05$) suppressed OTA formation in synthetic media and sterile wheat grains, respectively. Gorran et al. (2013) reported that Thymus daenensis and Satureja khozistanica EOs at 375 mg/l inhibited (51-87%) AFB1-production and exhibited stronger anti-AFB1-biosyntesis activity.

In this study, both AFB1 and OTA showed the same pattern. Higher content of OTA was detected at the concentration of 2 µl ml-^{1} as compared to AFB1 in both synthetic media and sterile wheat grains.

The inhibition of AFB1 and OTA biosynthesis by the parsley and coriander essential oils were more than the parsley and dill essential oils. These results agree with the data of fungal growth (Table 1).

Comparing Figure 1a and b with Figure 1c and d, it may appear that EOs induce much greater suppression of fungal growth and AFB1 and OTA production in synthetic media compared to the sterile wheat grains. It has been reported that higher levels of essential oils are usually necessary to inhibit microbial growth in foods compared to synthetic media (Bagamboula et al., 2004) because of interaction between phenolic compounds and proteins in foods (Ultee et al., 1998). These data are in agreement with those of Gandomi et al. (2009) who found that the effect of Zataria multiflora Boiss essential oil on the reduction of AFB1 accumulation in cheese was more than that in culture media.

Table 1 and Figure 1 (a, b, c and d) revealed that the inhibition of AFB1 and OTA required the same EOs dose for inhibition of fungal growth. These results agree with those obtained by Murthy et al. (2009) who found that growth and OTA production decreased progressively with the increase of Ajowan ethanolic extract (AEE) concentrations: at the dose of 250 µL/g, complete inhibition of fungal growth and OTA production

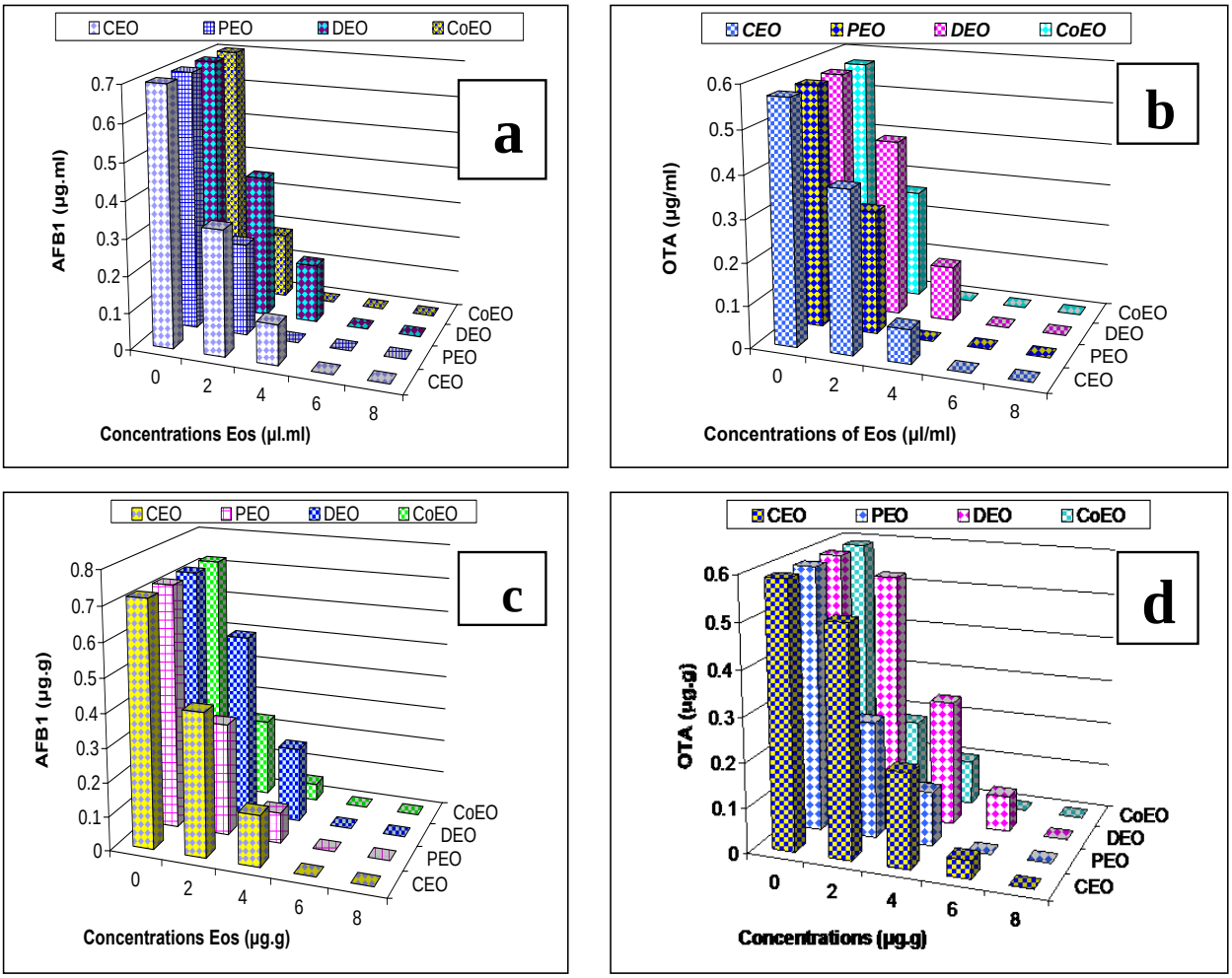

Figure 1. Effect of different concentration of celery, parsley, dill and coriander essential oils on Aflatoxin B1 and ochratoxin accumulation in synthetic media and sterile wheat grains at 25°C during 10 days of incubation period. a = AFB1 in synthetic media; b = OTA in synthetic media; c = AFB1 in wheat grains; and d = OTA in wheat grains.

was possible. The inverse correlation was reported by Pereira et al. (2006) who observed that clove EO completely inhibited the mycelial growth of *A. ochraceus*, but did not have a pronounced antiochratoxigenic action.

The inhibitory effects of some plants' EOs against AFB1 and ochratoxin- biosynthesis by *A. flavus* and *A. ochraceus* were reported in previous studies (Kumar et al., 2010; Tian et al., 2012; Gorran et al., 2013). The antifungal activity of essential oils components, particularly aliphatic aldehydes, might be due to their ability to form charge transfer complexes with electron donors in the fungus cell (Rasooli et al., 2005). The action of the oils on the integrity of nuclear membrane has not been ruled out. Changes in ultrastructure of the aflatoxin producing fungi treated with neem leaf extracts showed that the mycelia membrane is very susceptible to this treatment (Allameh et al., 2001). The antiaflatoxigenic actions of essential oil may be related to inhibition of the ternary steps of aflatoxin biosynthesis involving lipid peroxidation and oxygenation (Alpsoy, 2010). It is clear that phenolic compounds inhibited one or more early rather than late steps in the aflatoxin B1 biosynthesis pathway.

Aflatoxin and ochratoxin detoxification by tested essential oils

There is no information available in the literature for structural degradation and detoxification of AFB1 and OTA by the action of the plant essential oils. In the present study, the PEO, CEO, CoEO and DEO were evaluated for their ability to detoxify the AFB1 and OTA by TLC. The coriander essential oil (CoEO) showed the maximum degradation of AFB1 while the dill essential oil (DEO) was more degradation for OTA and their recorded degradation of up to 70.8 and 66.2%, respectively (Figure

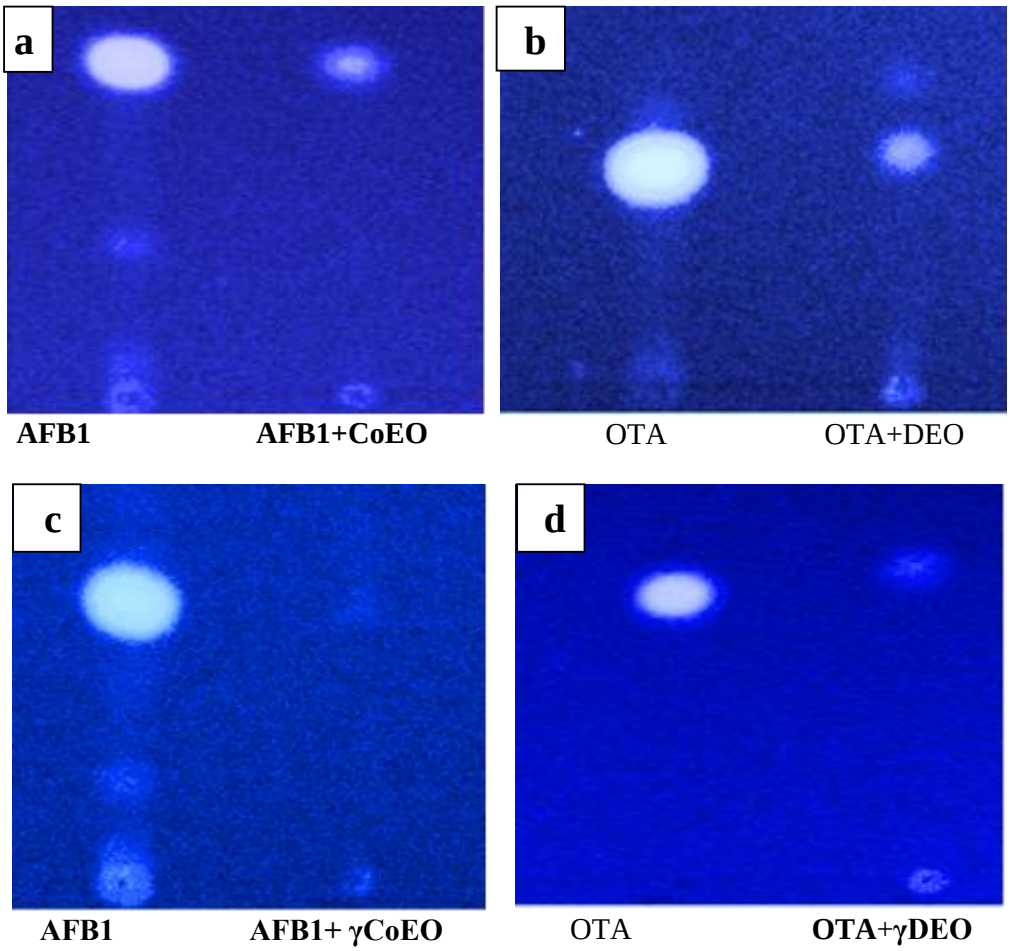

Figure 2. Effect of corindar essential oils (CoEO) and dill essential oil (DEO) on AFB1 and OTA detoxification, and effect of irradiated corindar essential oils (CoEO+γ) and irradiated dill essential oil (DEO+γ) on AFB1 and OTA detoxification. a = AFB1+ corindar essential oils; b = OTA+ dill essential oil; c = AFB1+ γ coriander essential oil at 7 kGy; d = OTA+ γ dill essential oil at 10 kGy.

2a and b and Table 2). Hence, further studies were performed with CoEO for AFB1 and DEO for OTA. The results of this study agree with those obtained by Velazhahan et al. (2010) who reported that seed aqueous extracted by Ajowan (Trachyspermum ammi (L.) Sprague ex Turrill) showed significant levels of AFB1-degradation activity (61%) after 24 h incubation. The volatile oils contain a large number of phytochemicals (phenylpropanoids, terpenoids and alkaloids) that can react with the aflatoxins and ochratoxin structure, and convert them to less toxic and mutagenic compounds.

In this study, the effect of heat treatment and γ-irradiation at doses 2, 5, 7 and 10 kGy on AFB1 and OTA detoxification potential of CoEO and DEO, respectively was tested. It was found that the aflatoxin and ochratoxin detoxifying activity of the CoEO and DEO were drastically decreased upon boiling at 100°C for 10 min and their recorded degradation was 55.4 and 49.2%, respectively (Table 2). The obtained results were in agreement with those obtained by Velazhahan et al. (2010) who reported that the heat treatment of Trachyspermum ammi extract caused a decrease in AFG1 degradation (44.8%) compared with crude extract (64.5%). Heating induced aroma volatilization was often recorded and can be quantified due to essential oil content for spices and herbs, though the losses of volatile oils are up to 20% (Fine and Gervais, 2005).

Table 2 and Figure 2c and d revealed that the irradiated CoEO and DEO at doses 7 and 10 kGy, respectively were more effective than the crude tested essential oils, degrading 93.2 and 83.4% of the AFB1 and OTA, respectively. γ-irradiation of coriander and dill essential oils at doses 7 and 10 kGy may induce changes in the volatile oil composition. These changes could presumably be due to the sensitivity of the components of the volatile oil, while the changes in molecules conformation may be due to irradiation. The changes resulted from the oxidation and hydroxylation of the

Table 2. Effect of γ-irradiation and heat treatment on AFB1 and OTA detoxification potential of coriander (CoEO) and dill essential oils (DEO).

Treatment	AFB1 recovered (ng)*	Degradation (%)	Treatment	OTA recovered (ng)*	Degradation (%)
Untreated CoEO	14.6	70.8[b]	Untreated DEO	16.9	66.2[c]
Boiling (100°C for 10 min) CoEO	22.3	55.4[c]	Boiling (100°C for 10 min) DEO	25.4	49.2[b]
Irradiated CoEO (7 kGy)	3.40	93.2[a]	Irradiated DEO (10 kGy)	8.32	83.4[a]

* Means within the column followed by the same letter are not significantly different (P = 0.05). Mean of eight replications.

Table 3. Time course of AFB1 and OTA detoxification by irradiated coriander and dill essential oils.

Time intervals (h)	AFG1 recovered (ng)*	Degradation (%)	OTA recovered (ng)*	Degradation (%)
3	36.7 [d]	26.6	42.5 [d]	15.0
6	29.8 [c]	40.4	38.4[c]	23.2
12	11.7 [b]	76.6	24.2 [b]	51.6
24	3.40 [a]	93.5	8.5 [a]	83.0

* Means within the column followed by the same letter are not significantly different (P = 0.05). Mean of eight replications.

aromatic rings of terpenes and the possible degradation of some essential oil constituents during γ-irradiation as well as the radiolytic effect and the possible production of free radicals which might react with AFB1 and OTA and induce modulate changes in their structure, and convert them to new or another compound or structural alterations in aflatoxin and ochratoxin molecules after detoxification. Mass spectral analysis of the degradation products of AFG1 revealed that the most abundant molecular ion peak in positive-ion mode was at m/z 288.29. MS/MS analysis of precursor ion at m/z 288.29 showed fragment transition to m/z 270.16 corresponding to the loss of 18 D. Mass spectral analyses of the degradation products of AFG1 suggest the modification of lactone ring structure (Velazhahan et al., 2010).

Time course study of AFB1 and OTA detoxification by irradiated CoEO and DEO, respectively showed that approximately 76 and 51% degradation occurred within 12 h and 93 and 83% degradation occurred 24 h after incubation of AFB1 and OTA, respectively (Table 3). Time course study of aflatoxin G1 detoxification by dialyzed Trachyspermum ammi extract showed that more than 78% degradation occurred within 6 h and 91% degradation occurred 24 h after incubation (Velazhahan et al., 2010).

Conclusion

According to the best of our knowledge, this is the first report of considerable inhibitory effect of celery, parsley, dill and coriander essential oils against AFB1 and OTA-biosynthesis by *A. flavus* and *A. ochraceus*. The important reduction of AFB1 and OTA accumulation produced by celery, parsley, dill and coriander essential oils, native species of our country, indicates that these essential oils could be an important source of chemopreventive compounds with antifungal activity to control the presence of this toxic metabolite in stored wheat grains and agricultural commodities. In addition, irradiated essential oils of dill and coriander destruct AFB1 and OTA-structure and introduce a new source to degrade them. However, celery, parsley, dill and coriander are one of the endemic species to Egypt which are widely used for various purposes such as food, pharmaceutical, cosmetic and perfume industries applications. Therefore, essential oils compounds of celery, parsley, dill and coriander, responsible for the degradation of AFB1 and OTA, have probably non-toxic property and can be used in foods and feeds process for reducing and detoxification of AFB1 and OTA - contaminated agricultural commodities.

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