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Research Article
Anti-mycotic and Anti-mycotoxigenic Properties of Egyptian Dill

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Abstract

Background: Dill (*Anethum graveolens*) is considered as one of the medicinal herbal plants which were known from ancient Egyptian centaury. Dill had many benefits for healthcare and it could be used in some parts of the world as food additives. Gas chromatography of dill declared many major and minor components also phyto-constituents that may play an important role in the inhibition of toxigenic fungal growth and/or mycotoxin reductions. One of the major components that had importance was carvone also limonene and apiol otherwise terpenoids and tannins considered as minor ones. **Materials and Methods:** Effect of three types of dill (*Anethum graveolens*) products were studied to compare its ability to avoid fungal growth of six strains of toxigenic fungi, dill products were leaves and stems extract, roots extracts and seeds essential oil, meanwhile, the strains were *Aspergillus flavus* ITEM 8080, *Aspergillus parasiticus* ITEM 692, *Aspergillus ochraceus* ITEM 2456, *Penicillum verrucosum* ISPA 9618, *Fusarium graminearum* ISPA2020 and *Aspergillus niger* ITEM 2318. **Results:** Dills seeds essential oil appeared to be the most effective one could inhibit fungal growth of the toxigenic fungi at a concentration of 5 mg mL^{-1} of seeds essential oil the fungal growth of all toxigenic fungi under the study was avoided. Use of seeds essential oil as anti mycotoxigenic material against aflatoxin B_1 and aflatoxin G_1 was determined. **Conclusion:** By increasing the concentration of seeds essential oil in media, reduction effect increased either for aflatoxin B_1 or for aflatoxin G_1 toxins at a concentration of 5 mg mL^{-1} essential oil extract inhibition ratio reached 87.7% for aflatoxin B_1 and for aflatoxin G_1 it was 92.2% reducing of toxin.

Key words: Anti-mycotic, anti-mycotoxigenic, dill, aflatoxin B_1, aflatoxin G_1

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food materials and food products could be contaminating by mycotoxigenic fungi and mycotoxins that made a problem through post-harvest steps and production lines especially in equatorial and sub-equatorial regions. Throughout several mycotoxins, aflatoxins are the widespread contaminants of agriculture food materials within post-harvest processing. Harmful impacts of aflatoxins are exceedingly declaring in plants tissues as well as in animal's tissues. The plant tissues could be affected by aflatoxins presence in negative ways, which may appear in some biological operations such as a functioning of vital cellular enzymes, photo pigments and cut germination ability[1].

However, in human and animals, aflatoxins are known as a reason of many maladies like listlessness, lipogenesis, intoxication, anorexia, inhibition of protein synthesis and finally it realized as hepatotoxicity, mutagenic and carcinogenic factors[2,3]. Because of its thermo-stable characters, aflatoxins still keeping even food cooked. Furthermore, aflatoxins had a great role in free radicals generation which reflected in found of reactive oxygen species[4]. Inverse effect of reactive oxygen species in various biomolecules like proteins, lipids and carbohydrates also nucleic acids is widely recorded. According to Halliwell[5], mycotoxigenic fungi not only the main reason of quantitative losses but also had a great impact on qualitative losses of food and food materials. Proceeding on this fact, it is urgent of such monitoring measures, that are effective as anti-mycotic and anti-mycotoxigenic against fungal as well as toxin contamination, likewise as the antioxidant against the free radical oxidation to boost shelf life of food materials through post-harvest steps.

Shapat or dill (*Anethum graveolens*) is annually herbal plant with yellow teeny flowers pertinence to Umbelliferae family plant, the plant height around 1.5 m, it is also had medicinal usage in ancient and modern civilization. Dill arises from West Asia area and Mediterranean basin. It grows in many areas such as the Middle East, India, Russia, Iran and Egypt[6]. Seeds used as a flavoring agent in the food industry because of its intense spicy odor, leaves are used in salads and tea however, seeds commonly used in tea, bakery, soups, salad and canned foods[7]. Dill cultivated to utilize as a vegetable also to be source of essential oil. This volatile oil could have medicinal applications for example as carminative, antispasmodic, stomachic, diuretic and stimulant. Also, *A. graveolens* plant commonly used in the intestines and stomach aches, liver diseases, dyspepsia, bladder inflammation and cramps[8]. The volatile oil and plant extracts appeared much variation of ability to have antimicrobial actions. Various several studies have been done to point out on the chemical composition of the essential oil of dill seeds[9,10].

The main components of *A. graveolens* volatile oil which has light yellow color with the spice odor and acrid taste are a mix of carvone with limonene and paraffin hydrocarbon. Other essential oil components are triterpenes, α-phellandrene, flavonoids, eugenol, coumarins, phenolic acids and umbelliferones[11]. Previous studies declare that, there is some antimicrobial activity of shapat for example it had an impact against *Listeria monocytogenes* and *Saccharomyces cerevisia*[12]. Again, antioxidant effects for the aqueous extracts of *A. graveolens* was in compare with α-tocopherol, ascorbic acid and quercetin in *in vitro* systems[13]. Volatile oil that was produced from shapat seeds was recorded to have an inhibitory activity against vulvovaginal candidiasis in immunosuppressed rats[14]. Furthermore, dill essential oil has hypolipidemic activity and could have cardio-protective agent characters[15]. The chemical composition of shapat volatile oil and its quantity varies depending on the plant parts and the plant harvest time[16]. Phytochemical analysis demonstrated the presence of many phyto-components such as: Terpenes, tannin, saponin, glycoside, steroid and reducing sugar. Antimicrobial activity of shapat seed oil was applied on 8 multi-drug resistant clinical isolates of both Gram-positive and Gram-negative bacteria and 2 standard strains. It appeared broad antibacterial action against Gram-positive bacteria such as *Enterococcus* sp. and *Staphylococcus aureus*. Also, Gram-negative bacteria *Pseudomonas aeruginosa*, *E. coli* and *Klebsiella pneumonia*[17].

The present study was undertaken to investigate the antifungal and anti-mycotoxigenic effects of the essential oil of the seeds of *A. graveolens* and it extract against some mycotoxigenic fungi and its toxins. The aim is to determine the activity of components in the extract to stop or degrade aflatoxin secretion.

MATERIALS AND METHODS

Plant material: The seeds of *Anethum graveolens* were purchased from local market of Arab republic of Egypt, all samples were purchased from herbal markets around Cairo and the seeds were deposited at the Herbarium, Department of Botany, National Research Center.

Extraction of the essential oil for chromatography: The seeds were thoroughly washed twice with distilled water, then it was dried carefully, ground and submitted to hydro-distillation using clevenger-type apparatus for 6 h. Essential oil was extracted using hexane and hydro-distillation. For hydro-distillation, 40 g of seeds was shredded superficially for 45 sec at low speed in water in a blender. The seed/water mixture was transferred to a custom-made hydro-distillation apparatus as described by Schild and Stahl[18] then, heated to 100°C. After 2 h, the essential oil was collected and the amount measured using a glass volumetric pipet. Distillates were diluted 150-fold with hexane before GC measurement. The extracted essential oil were dried over anhydrous sodium sulphate and stored in amber-colored bottles in a refrigerator at 4°C until the chromatographic step.

Gas chromatography/mass spectrometry analysis: The essential oil was analyzed on a Hewlett-Packard 6890NGC-MS system (Agilent technologies) coupled to a HP5973 mass-spectrometer. Separations were carried out using a DB-5 capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were maintained at 220 and 280°C, respectively. Column temperature was initially kept at 60°C for 2 min and then gradually increased to 240°C at the rate of 5°C min. Helium was used as carrier gas at a constant flow of 1.0 mL min^{-1} and an injection volume of 1 μL was employed. The MS scan parameters included electron impact ionization voltage of 70 eV, a mass range of 40-750 m/z and a scan interval of 0.5 sec. Samples diluted in n-hexane were injected manually in the split less mode. The identification of the components was based on comparison of their mass spectra with those of NIST3.0 Libraries provided with the computer-controlling GC-MS system as well as from the published literature.

Extracts and oil preparation

Preparation of dill seed extraction: Seeds of *Anethum graveolens* were surface sterilized, then washed by sterile distilled water and crushed using pestle and mortar. Hot water extracts of seeds were prepared by weighting 100 g of seeds that was extracted in a 100 mL of methanol/water (1:1, v:v) for 24 h to get the desired concentration (4 mg mL^{-1}), then each extracted material was filtered through Whatman filter paper No. 4, then centrifuged at 7200×g for 15 min and the supernatant was used for testing its antifungal activity.

Preparation of water extraction of dill plant: Dill was used after preparing from farms around Alexandria and approving by plant systematic scientist. In order to prepare the water extract, the leaves and stems of this plant were dried in the shade after washing and then dried leaf and stem were powdered in crusher. About 200 g of the results fine powdered of was quantitatively transfer to conical flask by warm water and the flasks covered well by using a para-film sheet, the conical flask shaking overnight in shaking incubator at around 50°C then, filtered resultant compound with Whatman filter paper No. 4, next condensed the filtrate of the first and second process with rotary machine at 60°C of temperature, rotation speed at 100 rpm up to one-fourth of the original volume. Obtained soluble poured into large vial and it was dried on laboratory oven by heating with indirect heat at temperatures around 50°C and under sterile conditions. The resulting powder was kept in a freezer until it used.

Preparation of fungal strains to test: A slant agar with 7 days growth of the six fungi strains was prepared to produce fresh colony of each one. Cover colonies with approximately 5 mL of sterile water supplemented with 0.1% tween 80. Then, the conidia are carefully rubbed with a sterile cotton swab and transferred to a sterile tube. The suspension was vortexes for 15 sec with a vortex mixer at 2,000 rpm. In general appropriate dilutions are made in order to attain the right concentration for counting in a haemocytometer chamber. Inoculum preparations should also be examined for the presence of hyphae or clumps. Transfer 5 mL of the suspension to a sterile syringe attached to a sterile filter with a pore diameter of 11 μm, filter and collect in a sterile tube. This step removes hyphae and yields a suspension composed of spores. The inoculum was shaken again in a vortex mixer for further 15 sec to remove any clumps detected. Adjust the suspension with sterile distilled water to 2×10^5 conidia mL^{-1} by counting the conidia in a haemocytometer chamber.

Determination of antifungal activity of dill extracts and essential oil

Determination of antifungal activity on fungal spores: Spores suspension of each fungus was mixed with graduated volumes of dill extracts, using a surfactant to enhance extract mixing characteristics and spores also (tween 80). Leave and stems extraction was test at concentrations of (1.0-5.0 mL), also it was (0.5-2.5 mg mL^{-1}) form seed and (0.5-5.0 mg mL^{-1}) for essential oil. In 15 mm diameter petri dishes of sabouraud dextrose agar loop of each spore/extract concentration was spread, positive control was done by using voriconazole 10 mg mL^{-1}, negative control was done by fungal strain suspend in autoclaved water with tween 80 as surfactant for spores. Petri dishes after inoculated aseptically on the surface of the media were incubated for 10 days at 25±2°C in incubator.

Determination of antifungal activity of essential oil of dill: Cut plug method recorded by Pridham *et al.*[19] with modification was employed to determine the antifungal activity of the chosen materials. Freshly prepared cells suspension of fungi (0.5 mL of about 2×10^5 cells mL^{-1}) was mixed well by vortex mixer for about 20 sec. Then, poured on sterile petri dishes with 10 mL of sterile potato dextrose medium after it's solidified. Regular wells were made in the inoculated agar plates by a sterile cork-borer with 1.0 mm diameter. Each well was aseptically filled up with 200 µL of essential oil with the concentration of 1% (v/v) that was prepared in 100 mL distilled waters containing 2% tween 80. Also, the well was filled with 200 µL of each plant extract with a concentration of (5 mg mL^{-1}).

Determination of minimum inhibitory concentration of *A. graveolens* seed extract and its essentials oils against mycotoxigenic strains: The Minimal Inhibitory Concentration (MIC) of *A. graveolens* seed as well as leaf oils against the toxigenic strain of *Aspergillus flavus* ITEM 8080, *Aspergillus parasiticus* ITEM 692, *Aspergillus ochraceus* ITEM 2456, *Penicillum verrucosum* ISPA 9618, *Fusarium graminearum* ISPA2020 and *Aspergillus niger* ITEM 2318 were determined by poisoned food technique reported earlier by Singh *et al.*[20] with some modifications. A 5 mm diameter disk of paper was cut and sterile well by autoclaved in a petri dish, after that, fungi spore suspensions prepared in Eppendorf tube containing 1 mL of autoclaved water/tween 80, tubes were mixed vigorously by vortex to avoid the mycelial clotting. Paper disks were impeded in Eppendorf tube of each fungal tube to super saturated by spores, then inoculated aseptically on the center of the PDA petri dish amended with test extraction (0.5-2.5 mg mL^{-1}) form seed extract and (0.5-5.0 mg mL^{-1}) for essential oil. The PDA plates without essential oils served as negative controls and PDA plates with antimycotic served as positive control. Both the treatment and control sets were incubated for ten days at 25±2°C in incubator. The colony diameters of fungal isolate in treatments and control sets were measured. The percent inhibition of fungi in treatment sets at different doses of roots extracts and seeds essential oil was calculated. All tests were performed in triplicate and the antifungal activity was expressed as the mean of inhibition and least significant differences was calculated for each treatment and recorded under the table of treatment.

Aflatoxin determination by HPLC: The HPLC used for aflatoxin detection was water-alliance 2695 HPLC system instrument, the column temperature was 30°C. Mobile phase used as methanol:acetonitrile (v/v 50:50) with 0.1% formic acid for aflatoxins[21]. Injection volume 10 µL. Triple quadrupole HPLC and analytical column RP-18nd-capped, 150×4.6 mm, 5 µm particle size, flow rate 1.0 mL min^{-1}. Aflatoxins were quantified by fluorescence detection (exictation/emission equal to 365/455 nm for aflatoxins).

Brine shrimp lethality test: The brine shrimp (*Artemia salina*) lethality test was carried out using a modification of the procedure by McLaughlin[22]. For this experiment resting brine shrimp eggs were obtained from Ocean Star® International (OSI) Inc., made in USA, serial No. 342942387912. Eggs in desiccator were stored in a refrigerator (5°C). *Artemia salina* eggs were hatched in a sea salt solution (Instant Ocean®, 38 g L^{-1}) with an incandescent light bulb as the heat source. After 48 h, the newly hatched nauplii were counted using a micropipette and transferred to 20 mL vials. Nine vials each containing 10 *A. salina* nauplii in 10 mL of sea salt solution (same as the hatching solution) were prepared. Three vials were labeled as controls with first one containing no DMSO, another with 10 µL and the last one with 100 µL DMSO. Four replicate vials contained 10 µL of 1% extraction of seeds, extraction of plant (leaves and stems) or essential oil solution and the other four replicates were prepared by adding 100 µL of 1% extraction of seeds, extraction of plant (leaves and stems) or essential oil solution. Surviving *A. salina* was counted after 24 h.

Statistical analysis of data: Every experiment was performed in triplicate. The results were recorded as Mean±Standard Deviation. Statistical analysis was performed using one-way analysis of variance (one-way ANOVA) using SPSS statistical package (SPSS 16) and the statistical significance was determined at $p<0.05$.

RESULTS AND DISCUSSION

The secondary metabolites biosynthesis in botanical or medicinal plants is greatly influenced by environmental condition of plant growth. The volatile oil chemical composition of *A. graveolens* growing in varied geographical regions has been extensively studied[9,23]. Depending on the records of those studies, the major components of the *A. graveolens* oil are limonene, phellandrene, carvone, shapat ether, apiol, di-hydro carvone and myristicins[24,25]. One of the main reasons of this study was to determine if there is a different in chemical constituents as well as to evaluate the biological actions of *A. graveolens* plant growing in Egypt. A search of the literature has revealed that there is less

previous studies on the chemical composition or bioactivity of the volatile oil of *A. graveolens* for Egyptian plant. Essential oil composition of Egyptian seeds of *Anethum graveolens* was determined as in Table 1, this can help to explain why the dill essential oil had an effect on toxigenic fungal growth and/or mycotoxin production.

Phyto-chemical components of dill: The phyto-constituents for seeds, seed oil, roots and leaves with stems of *A. graveolens* was determined qualitatively for its appearance as natural components that may have antifungal effects (Table 2), many components such as terpenoids, flavonoids and tannins were found in all parts of the plant, otherwise many components like steroids, reducing sugar and glycosides were vacillated between appearance and disappearance in different plant parts of dill and its seeds. One of these components was anthraquinone, which appeared just in oil seeds of dill. Glycosides found to be disappear from leaves.

Vokk *et al*.[26] studied the phyto-chemical of dill (*A. graveolens*) they recorded that alkaloids, flavonoids, tannins and saponins were presence in the seeds of plant. They also quantitatively recorded their amounts, respectively as 2.8±0.1, 11.05±0.07, 19.71±0.28 and 0.55±0.04, otherwise cardiac glycosides were not detected.

Other study was done by Dahiya and Purkayastha[17] who tested the phyto-chemical components of shapat seed oil, results declared a lot of phyto-chemicals components were contained in seed volatile oil such as glycosides, tannins, steroids, saponins, reducing sugars and terpenoids. Also, Jana and Shekhawat[11] reported the appearance of flavonoids, terpenoids, steroids, glycosides, tannins and saponins in leaves, stems and roots of regenerated leaves and *in vitro* callus of *A. graveolens*.

Table 1: Essential oil composition ratio of seeds of *Anethum graveolens*

Essential oil components	Essential oil (%)	Note
Limonene	17.283	
Methoxy-4-propenyl benzene	1.764	
Terpinene	0.327	Good against fungi
2,6-dimethyl octatetraene	0.646	
m-cymene	0.211	Low component
Carvone	40.998	Main component
Myristicin	1.024	
Trans dehydro-carvone	10.014	
Apiol	27.733	

Table 2: Existent phyto-chemicals components in dill plant parts

Phyto-chemicals components	Seeds	Seeds oils	Roots	Leaves with stems
Terpenoids	+++	++	++	t
Flavonoids	++	+++	t	t
Steroids	-	+++	t	t
Reducing sugar	-	++	-	t
Anthraquinone	-	t	-	-
Saponins	+	t	t	-
Cardiac glycosides	t	t	t	-
Tannins	t	+++	+	t

-: Component not found, t: Trace, +: Low, ++: Moderate, +++: Highly found

Antifungal activities of dill extracts and seeds essential oils: In the present study, the anti-fungal effects of *Anethum graveolens* against six types of fungi were determined. The effect of the extracts and oil of dill were firstly examined on the sabouraud dextrose agar media. The extract of leaves+stems, also the extract of seeds were evaporated under vacuum in rotary evaporator until reached 1 mL, then it transferred into vial and dried under nitrogen. The dried extracts were resolved in 1 mL of tween/doubled distilled water to be ready for media additive. The spore suspension of each fungal type were prepared in 1 mL of water/tween solution, since the media reached 42°C, media pours into plates, the dill products (leaves and stems extract, seed extract and seeds oil) was added to compere its effect on the growth of fungi. Finally spore suspensions were spread on the plate's surface. In Table 3-5 the results showed that by adding

Table 3: Effect of leaves and stems extract against mycotoxiginic fungi growth

	Fungal strains and diameter (cm)																	
	Aspergillus flavus			*Aspergillus ochraceus*			*Fusarium graminearum*			*Pencillium verrucosum*			*Aspergillus parasiticus*			*Aspergillus niger*		
Treatments	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8
time (days)	(Days)																	
Negative control	1.5	2.7	5.5	1.2	2.8	4.6	0.5	1.3	2.1	1.3	2.9	5.2	1.1	3.2	5.9	1.7	4.3	7.2
1 mL	1.5	2.6	5.2	1.1	2.9	4.4	0.5	1.3	2.1	1.2	2.8	5.0	1.0	3.2	5.7	1.7	4.2	6.6
2 mL	1.5	2.3	4.9	1.2	2.7	4.4	0.6	1.2	2.5	1.2	2.7	4.8	0.9	2.8	5.0	1.5	4.1	6.3
3 mL	1.4	2.2	4.1	1.1	2.3	3.9	0.4	1.0	2.3	1.0	2.3	4.2	0.6	1.6	3.1	1.3	3.9	5.9
4 mL	1.2	1.8	3.9	0.9	2.2	3.6	0.5	1.3	2.2	0.6	2.1	4.2	0.4	0.9	1.7	1.2	3.8	5.8
5 mL	1.0	1.8	3.6	0.8	2.0	3.2	0.5	1.2	2.0	0.6	2.1	4.0	0.4	0.6	1.0	1.0	3.7	5.5

All value were determined in centimeter of fungal diameter, LSD: 0.399

a graduated amount from 1-5 mL of the extract to media, there was an effect on the fungal growth which determined in centimeter after 2, 4 and 8 days of fungi grew on the media, these effects were varying from fungi to the other one.

Figure 1 and 2 showed the effect of some extract and oil on *Aspergillus flavus* and *Aspergillus parasiticus* fungi. Figure 1a showed the effect of extract from leaves and stems of dill plant on the growth of *Aspergillus flavus*,

Table 4: Effect of roots extract against mycotoxiginic fungi growth

	Fungal strains																	
	Aspergillus flavus			*Aspergillus ochraceus*			*Fusarium graminearum*			*Pencillium verrucosum*			*Aspergillus parasiticus*			*Aspergillus niger*		
Treatments	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8
time (days)	(Days)																	
Control	1.2	2.5	5.2	1.1	2.6	4.9	0.7	1.5	2.7	1.5	2.7	4.8	1.1	3.5	5.7	1.9	4.1	6.5
0.5 mg mL^{-1}	1.1	2.5	5.2	1.1	2.4	4.8	0.5	1.4	2.7	1.4	2.6	4.8	1.1	3.1	5.3	1.8	4.1	6.6
1 mg mL^{-1}	1.0	2.1	4.6	0.9	2.2	4.6	0.6	1.4	2.4	1.3	2.3	4.5	0.7	2.4	4.6	1.4	3.8	6.1
1.5 mg mL^{-1}	1.0	1.9	4.0	0.8	1.9	3.8	0.4	1.0	2.1	1.0	1.9	4.1	0.0	1.1	2.2	1.1	3.3	5.6
2 mg mL^{-1}	0.6	1.4	3.4	0.6	1.6	3.5	0.5	0.8	2.0	0.6	1.8	3.8	0.0	0.0	0.3	0.8	3.4	5.1
2.5 mg mL^{-1}	0.5	1.5	3.1	0.6	1.6	3.3	0.5	0.6	2.0	0.3	1.5	3.5	0.0	0.0	0.0	0.8	3.0	4.7

All value were determined in centimeter of fungal diameter, LSD = 0.661

Table 5: Effect of seed essential oils against mycotoxiginic fungi growth

	Fungal strains																	
	Aspergillus flavus			*Aspergillus ochraceus*			*Fusarium graminearum*			*Pencillium verrucosum*			*Aspergillus parasiticus*			*Aspergillus niger*		
Treatments	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8	2	4	8
time (days)	(Days)																	
Control	1.3	2.5	5.1	1.4	3.4	4.8	0.4	1.1	1.7	0.7	3.1	5.6	1.2	3.1	6.3	1.5	3.9	6.7
0.5 mg mL^{-1}	0.6	1.7	3.2	1.1	2.3	4.4	0.3	0.65	1.7	0.4	1.8	3.5	1.1	2.9	6.0	1.5	3.8	5.6
1 mg mL^{-1}	0.5	1.2	2.6	1.0	1.9	2.9	0.1	0.5	0.8	0.3	1.45	3.2	1.1	2.5	3.5	1.1	3.2	5.3
2 mg mL^{-1}	0.2	0.8	1.9	0.7	1.0	2.2	0.0	0.0	0.2	0.2	0.8	2.0	0.9	1.8	2.7	0.7	2.5	4.8
3 mg mL^{-1}	0.0	0.2	1.1	0.4	0.55	0.7	0.0	0.0	0.0	0.0	0.75	1.3	0.0	0.4	1.3	0.0	1.4	3.5
4 mg mL^{-1}	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.1	0.0	0.0	0.0	0.0	0.0	0.8
5 mg mL^{-1}	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

All value were determined in centimeter of fungal diameter, LSD: 0.443

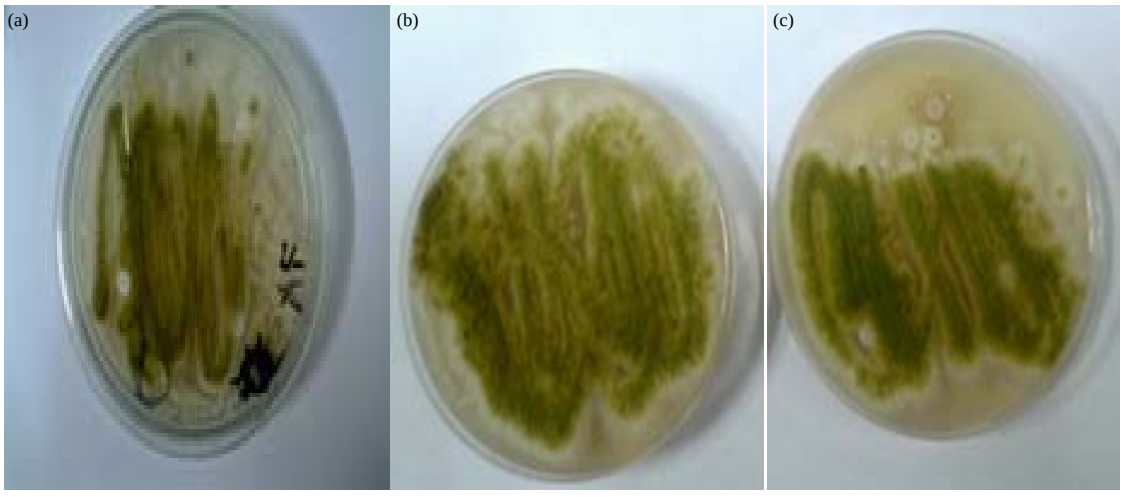

Fig. 1(a-c): Leaves/stems extract effect on *Aspergillus flavus*, (a) Effect of dill extracts against *Aspergillus flavus*, (b) Control of *Aspergillus flavus* growth and (c) Seeds extract effect on *Aspergillus flavus* growth rate

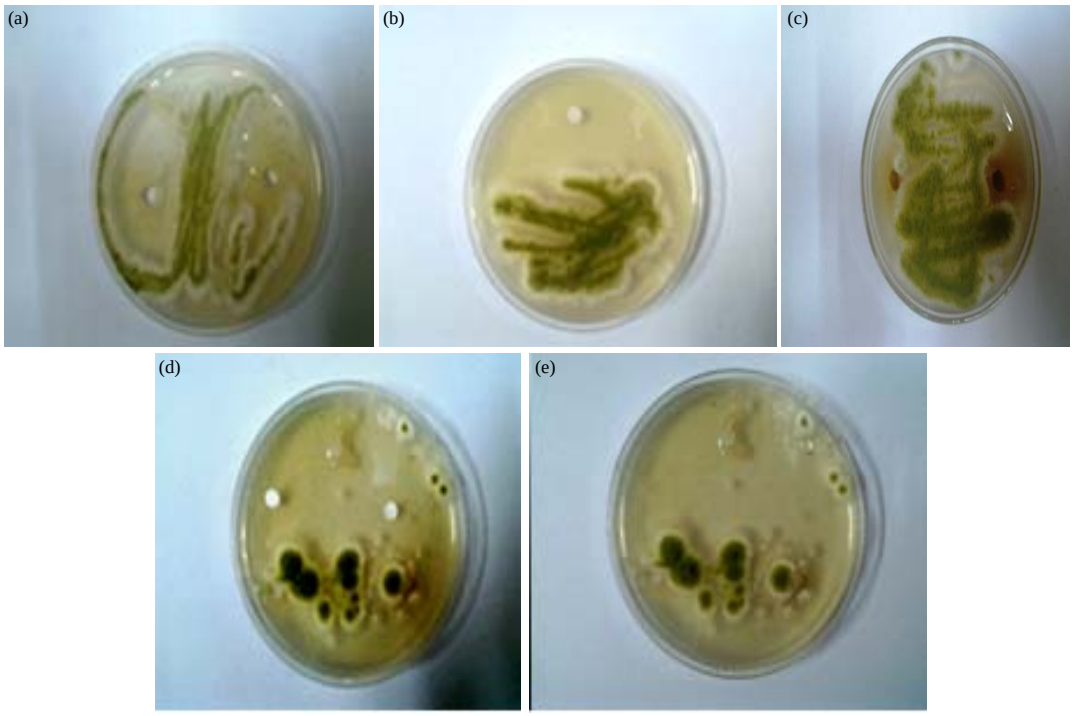

Fig. 2(a-e): Effect of dill extracts and essential oil on *Aspergillus parasiticus* essential oil effect on (a) *Aspergillus flavus*, (b) *Aspergillus parasiticus*, (c) Leaves/stem extracts effect on *Aspergillus parasiticus* and (d, e) Roots extract impact on *Aspergillus parasiticu* growth rate

Fig. 1b was the control of fungi, Fig. 1a and 2a showed the effect of roots extract and seeds essential oils respectively on *Aspergillus flavus.* Figure 2b-e showed dill products effect on *Aspergillus parasiticus* fungi.

Meanwhile, in Fig. 3 and 4 showed the effect of dill extracts and dill essential oils on *Aspergillus ochraceus*, *Fusarium graminearum* and *Penicillum verrucosum* fungi were observed, the Fig. 3a-d showed the inhibition effect of dill on *Aspergillus ochraceus* fungi, finally Fig. 4a-c showed the effect of dill on *Fusarium graminearum* fungi and Fig. 4d showed the effect of dill seeds essential oils on *Penicillum verrucosum* fungi.

Many types of essential oils obtained from the plants were recorded to have antimicrobial activity against a various types of microorganisms including the Gram-negative, Gram-positive bacterial and fungal strains. Nevertheless, the variances in the antimicrobial effects may illustrated by predisposition, testing circumstances, physicochemical advantage of volatile oils, also dissimilarity between different strains. The antimicrobial impacts for essential oil were reported by Singh *et al.*[20]. Previous study by Delaquis *et al.*[12] on volatile oil of *A. graveolens* recorded that: Against *Listeria monocytogenes* and *Saccharomyces cerevisiae*, volatile oil and its components had good antibacterial activity.

Badar *et al.*[27] studied the effect of dill essential oils as antibacterial material on some kind of bacteria, they used a local Pakistani isolates of *E. coli*, *Salmonella typhi*, *Bacillus subtilis* and *Staphylococcus aureus.* They use the suitable standard antibiotic for each strain as a positive control (penicillin, chloramphenicol, gentamicin and oxy-tetracycline) and the negative control was not added neither antibiotic nor essential oils. They found that, essential oils of *A. graveolens* had approximately the same effects as antibiotic on strain of *E. coli*, but that effect was decreased (not far from) in the other bacterial strains. They also recorded that; the original essential oil and the first dilution of oil (1:10) had the same effects on bacterial strains inhibition, at the last dilution of the dill essential oil (1:200) antibacterial characteristics of oil were disappeared.

Three product types of *Anethum graveolens* were prepared, the extracts were recorded as leaves and stems extract, roots extract and seeds oil. The effect of each product was varied on the fungal growth and toxin production. First of all the effect of leaves and stems extract was studied to determined its effects on fungal growth all species of fungi used in this study, result showed that at 5 mL extraction of leaves and stems the effect on fungal growth recorded as the highest ratio. The highest inhibition effect found on the

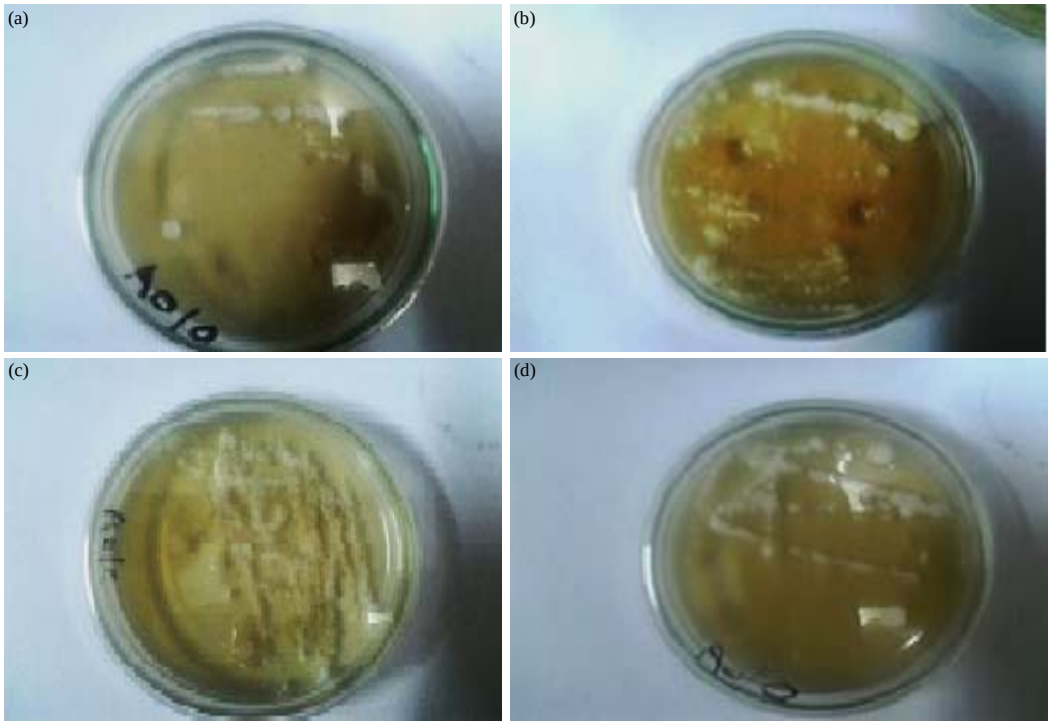

Fig. 3(a-d): Effect of dill extracts against *Aspergillus ochraceus*, (a, b) Essential oil, (c) Leaves/stems extract and (d) Roots extract

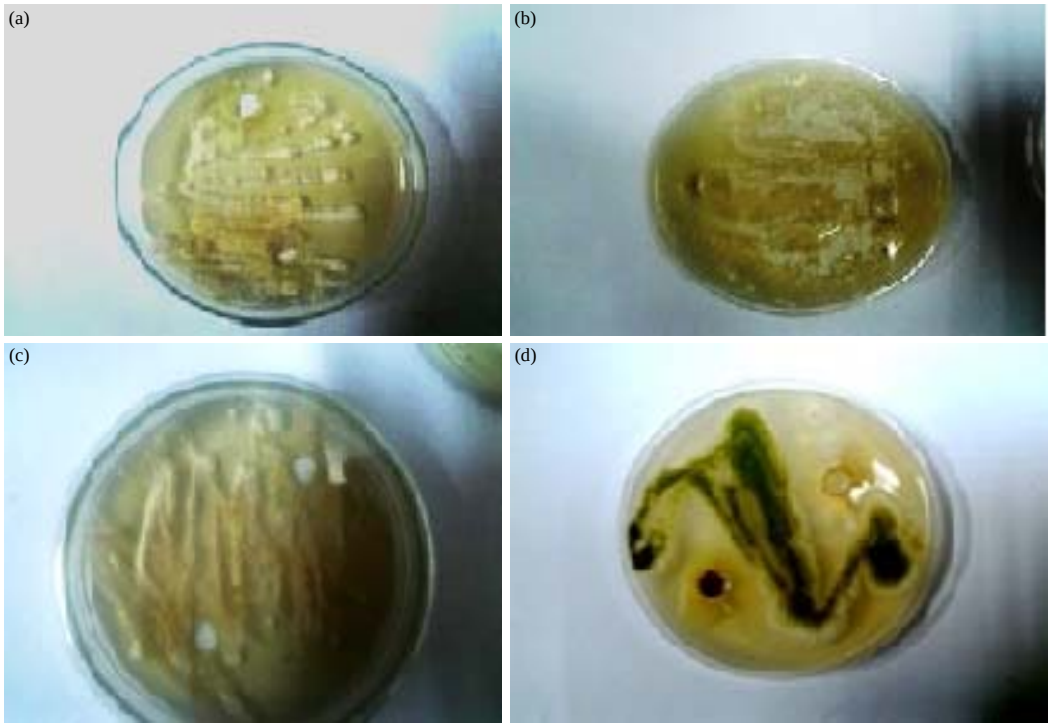

Fig. 4(a-d): Effect of dill extracts against *Fusarium graminearum* on (a) Leaves/stems extract, (b) Seeds oil impact, (c) Roots extract and (d) Seeds oil against *Penicillum verrucosum*

Aspergillus parasiticus fungi followed by *Fusarium graminearum*, the third one was *Aspergillus ochraceus*, then *Aspergillus flavus* came as the fourth one in inhibition values, after that *Penicillum verrucosum*, finally *Aspergillus niger* was the lowest fungi that inhibited by using the extract, in Table 3-5 the means of triplicates values were recorded as for 2, 4 and 8 days of fungal growth.

The results showed that, the antifungal compound which used as positive control effected on the six types of fungi under experimental conditions, it's stopped fungal growth except for *Fusarium graminearum*, which had a very small growths after 4 days of growth (0.2 cm) and after 8 days of growth (0.6 cm) on the plate media (Table 5). For *Aspergillus flavus*, the effect of leaves and stems extracts of the plant was significantly appeared after addition of 3 mL of the extract to media (Table 3). The extracts was prepared and save at Frigidaire until media autoclaved, at 42°C and just before media pours into dishes, the extracts were added in graduated values to media under aseptic conditions. The spores suspensions in tween 80 were prepared, a disk of paper with 0.5 cm diameter were supersaturated by the spore suspension after added to the center of the dish media. *Aspergillus niger* recorded as the lowest fungi in inhibition by extraction of dill plant.

The effect of *Anethum graveolens* roots extract on fungal strains under experimental conditions clearly demonstrates that, roots extract did not have a great effect on fungal growth except in case of *A. parasiticus*, the extract prevent *A. parasiticus* even six days on media at extract concentration 2 mg mL^{-1} and the growth was completely stopped at extract concentration 2.5 mg mL^{-1} (Table 4). Volatiles compounds from herbal plants often obtained by using hydro distill or steam handling that was first progressing by the Arabs scientists in the middle centuries. Herbal plants known to have antiseptic also medical characteristics such as bactericidal, fungicidal, antiviral and essence also it may use as preservation for foods, it has been used as antimicrobial, painkiller, calmative, anti-inflammation, spasmolytic and local anesthetized for diseases. Volatiles oil of medicinal plants may contain about 20-60 components in quality different concentrations.

Bakkali *et al.*[28] elucidated the foundation of volatiles components in plants, they found that these components are recognized by 2 or 3 master components at fairly high concentrations (20-70%) in compare with others present which presence in trace amounts. As yet, numerous researchers have revealed that *Aspergillus* growth was completely inhibited by plentiful plants volatiles components. In a review by Alpsoy[29], recorded that by the examination of the effects of essential oils of 58 plant species which distributed in 18 family on *A. flavus* and/or *A. parasiticus* evolution. In these experiment of essential oils were reformatted from leaf, stem and flower, these plants have been taken up from domestic market. He recorded that, used of variances concentration from essential oils had been found to make varying inhibition degrees in development of *Aspergillus* species.

In Table 5, results showed that the oil extracted from the seeds of the plant dill (*Anethum graveolens*) was more effective impact on fungal growth from the previous extracts whether the plant's leaves extract or roots extracts (Table 3, 4). Inhibitory effect of essential oils of seeds was a clear impact on *Aspergillus* fungi (*A. parasiticus* followed by *A. ochraceus*, then *A. niger* and finally *A. flavus*), the results also appeared that, most fungal strains had stopped in its growth by using a concentration of 4 mg mL^{-1} of seed oil, the growth of all fungi under experiment was prevented after addition concentration of dill oil seeds more than 4 mg mL^{-1}. By using dill essential oil at concentration of 3 mg mL^{-1} most of fungi were late in growth more than 2 days, in *F. graminearum* at 3 mg mL^{-1} of seeds oil the growth of fungi were completely stopped, *A. parasiticus*, *P. verrucosum* and *A. flavus* were weaken in growth after 3 days of inoculation, *A. niger* were later growth until 2 days of inoculation but after that it grow normally and *A. ochraceus* had weak growth but fungi mycelial appeared at two days of inoculation.

In comparing of *A. graveolens* extracts and essential oil effects on toxigenic fungal growth of six strains results showed that, essential oil was the best one for inhibition the growth of mycelia, the reducing rate reached 100% by using concentration equal to 4-5 mg mL^{-1} essential oil. However, other two extracts had variety effects on fungal growth, the results appeared better effect in case of root extract, it cuts fungal growth by around 40% for *A. flavus* and 100% for *A. paraciticus*, leaves and stem extract had inhibition effect closed to that was given by root extract except on *Fusarium* fungi.

According to Baydar *et al.*[30] substantial components can representing around 85% from volatile components of any plant oils, whereas, other components were found as a trace. Other studies explained the relationship between compounds chemical structures which found in essential oil and antimicrobial effects for this oil. Constituent's analysis of essential oils showed that compounds such as carvacrol, r-cimene, a-terpinolene, anethol and eugenol were the main components present in the different essential oils studied. Phenolic components, such as eugenol, anethole and carvacrol, its precursors r-cimene and g-terpinolene and its

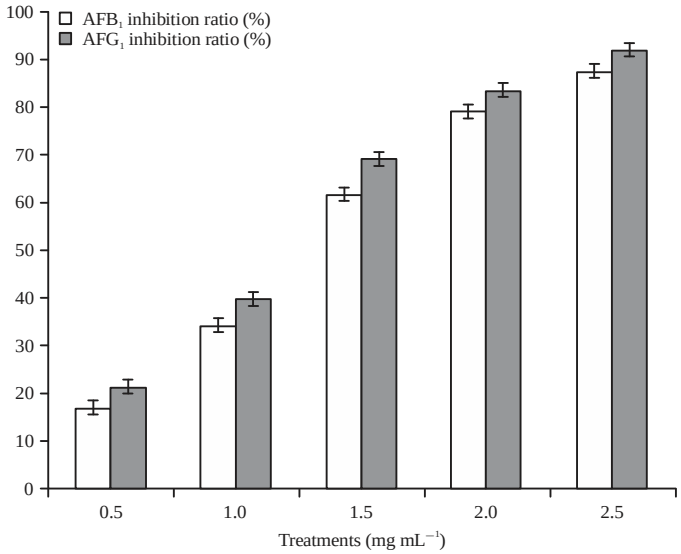

Fig. 5: Effect of seed essential oils against aflatoxin B_1 and aflatoxin G_1

isomers thymol are chiefly responsible for the antimicrobial properties of essential oils. Also, the physical nature of essential oils, that is low molecular weight combined with pronounced lipophilic tendencies allow them to penetrate cell membrane more quickly than other substances[31]. However, there is an evidence for say that; minor components have a critical part to play in antimicrobial activity, possibly by producing a synergic effect between other components[32].

Anti-microbial effects for essential oils of plants were elucidated by several suggested theories: (1) The effect may depended on major and/or minor components that could be resulted in the form of damage to the enzymatic cell system, including those associated with energy production and synthesis of structural compounds[33,34], otherwise reason, (2) The active or volatiles oils component could made denaturation for enzymes responsible in spore germination or it may interference with the amino acids that involved in germination[35], (3) It may cause irreversible damage in cell membrane, cell wall and cellular organelles when *A. parasiticus* and *A. flavus* were exposed to different essential oils[36,37].

Essential oils of dill (*Anethum graveolens*) and its components was studied in this experiment for its ability to reducing fungal excretion of toxin, a strain of *A. flavus* ITEM 8080 which had ability to produce toxin in media used as a control for study reduction effect of dill essential oils on aflatoxin B_1 and aflatoxin G_1, the results showed that by increasing the amount of seeds essential oil that added to the ingredients of Chloramphenicol Potato Dextrose Agar (CPDA) media the reduction ratio of aflatoxin B_1 and aflatoxin G_1 were increased (Fig. 5). The maximum inhibition ratio was recorded ad 87.7% for aflatoxin B_1 and it was recorded as 92.2% inhibition ratio for aflatoxin G_1 in media.

Alpsoy[29] also recorded that, it is possible to use essential oils from plants to degrade the growth of *A. flavus* and *A. parasiticus* along with reduce aflatoxin production. Chemical structure of volatile oil might appoint to antimicrobial efficacy for herbal plants. Such consequence might elucidate by the great content of those substances in the herbal volatile oil. Numerous studies referring to components of volatile oil and its rules to reduce growth rate of *A. flavus* and *A. parasiticus*. In a study of Razzaghi-Abyaneh *et al.*[38] who isolate dill apiole, this component was phenyl propanoid compound, they isolate it from leaf volatiles oil of shapat and classified it as specific compound to reduce aflatoxin G_1 production from *A. parasiticus,* the inhibition ratio may reach 50% of its concentration without any apparent impact on fungal growth or aflatoxin B_1 synthesis. Other apiole, isolated from seed oil of *P. crispum* appeared similar effects to dillapiole for AFG_1 reducing. It is suggest that those componds perhaps inhibit AFG_1 biosynthesis through inhibition of CypA whose one of the P450 monooxygenases enzymes that are involved in the formation of aflatoxin G_1.

In a comparison between uses of plant parts extracts (leaves and stems, roots and seeds essential oil), the results elucidated that, seeds essential oil had the best results for fungal inhibition on six strains mycotoxigenic fungi under experiment. By using antimycotic solution as positive control, *F. graminearum* was the most fungi which had great

inhibition, by using more than 2 mg mL^{-1} of essential oil extract. Nevertheless, *P. verrucosum* and *A. niger* could resist the effect and there were still some mycelial growth until concentration of essential oil in media reached 4 mg mL^{-1}. At concentration of 5 mg mL^{-1} dill essential oil extract the growth of all six strains of mycotoxigenic fungi was stopped. Using of seeds oil against aflatoxin B_1 and G_1 could reduce their amounts to 87.7 and 92.2% for aflatoxin B_1 and G_1, respectively. This inhibition ratio happened at 5 mg mL^{-1} of dill seeds essential oil in toxin producing fungi media.

CONCLUSION

Dill is an herb plant natively found in the Mediterranean area and Western Africa. Shapat or dill was known as a medicinal herbal for ancient Indian and Egyptian as far as 3000 BC. Many secondary metabolites were found in dill seeds oil, such as carvone, apiol and limonene, those components considered as major components. Otherwise, cymene, terpinene and myristicin were considered as minor components, major and minor components in dill could be one of the most reasons that caused inhibition for fungal growth and toxin production in toxigenic fungi. Phyto-chemical components that were found in the dill plant parts could play also an important role in fungal inhibition and/or mycotoxin production, these components were varied and distributed between plant parts.

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