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Aflatoxigenic Fungi Occurrence in Some Processed Meat Products and their Control by Some Plant Extracts *in vitro*

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THE CURRENT research was focused on the isolation and identification of aflatoxigenic fungi and aflatoxins associated with some processed meat samples, i.e. basterma, beef burger, luncheon meat, and sausage samples and their control using some plant extracts as *Euphorbia cotinifolia* L., *E. tirucalli* L. and *Rhus coriaria* L. Randomly 48 samples of processed meat products (12 for each) were collected from different companies and analyzed for mycological examination and aflatoxins contamination. Data indicated that, Basterma samples had the highest mean total fungal count, (674 fungal colonies/10g). Identification of all isolated fungal genera were *Alternaria, Aspergillus*, *Cladosporium*, *Epicoccum, Geotrichum, Paecilomyces, Penicillium, Phoma* and *Trichoderma*. The highest aflatoxins contamination was found in basterma meat sample. Results also cleared that, all tested ethanolic plant extracts were found to significantly decrease the mycelium dry weight (g) and spore germination of *A. flavus* and *A. parasiticus* at all different concentrations. The most effective plant extract against tested fungi was *R. coriaria* L. extract.

Keywords: Processed meat, Fungal contamination, Aflatoxin, Plant extracts.

Introduction

Meat and meat products provide excellent growth media for an assortment of microflora, some of which are pathogens (Jay et al., 2005). Contamination of meat products with molds may occur at various stages at which the products are prepared it may occur during animal slaughtering under bad hygienic conditions using contaminated water, equipments and utensils or during processing by adding of contaminated meat additives with mold spores or during packing, handling, transportation and storage (Morshdy et al., 2015). Meat products contamination with various mold species is viewed as a genuine risk as it influences the quality of these meat products by raising the opportunity for its spoilage and deterioration. The most important side about the mold spoilage of food is, however, the formation of mycotoxins. The more common and the most hazardous types of mycotoxins are aflatoxins, which are the main toxic secondary metabolites of some *Aspergillus* species such as *A. flavus, A. parasiticus* and *A. nomius* (Alcaide-Molina et al., 2009 and Morshdy et al., 2015). Some fungi show less effect directly, but can affect by many indirect means. But *Aspergillus* is one such fungus that affects the human being by both of them. It not only causes diseases, but also poisoned the human diets. Some species such as *Aspergillus flavus* and *A. parasiticus* are toxigenic and can contaminate food and produce aflatoxins among others (Medeiros et al., 2011). More than 18 different types of aflatoxins were identified. Aflatoxins (AFs) B_1 B_2, G_1, G_2, M_1, and M_2 are the major members; all of them have the potency of toxicity, carcinogenicity, and mutagenicity. AFB_1 is the most toxic and is classified as a human carcinogen (Talebi et al., 2011 and Morshdy et al., 2015). Aflatoxins contamination of meat products could have originated either from the animal tissues previously fed on aflatoxin contaminated feed or by use of aflatoxin contaminated ingredient, e.g. cereals (Zohri et al., 1995). The

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risk of AFB_1 contamination of meat products was found to be minimal, generally due to the low rate of carryover of the examined mycotoxins to the edible tissues, given that the primary target of AFB_1 is the liver, only low levels of AFB_1 can be found, often below the detection limits of the methods used (Abramson et al.,1997; Beaver et al., 1990 and Bintvihok et al., 2002). Moreover, the use of spices contaminated with toxigenic mould strains may also represent a source of secondary mycotoxin contamination of the final products (Refai et al., 2003). Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and antioxidant properties (Bouamama et al., 2006). Numerous *Euphorbia* plants (family Euphorbiaceae) were found to have various biological effects, e.g. antimicrobial (Sudhakar et al., 2006), antiviral (Chaabi et al., 2007), antitumor (Lin et al., 2006), anti-inflammatory (Singh et al., 2006), antinociceptive **(**Ahmad et al., 2005), antidiarrhoeal (Atta & Mouneir, 2005), antioxidant (Lin et al., 2002), and immunosuppressive (Bani et al., 2005). Also, *Rhus coriaria* fruits (family Anacardiaceae) have strong antimicrobial and antioxidant activities (Bursal & Köksal, 2011; Fazeli et al., 2007; Kossah et al., 2009; McCune & Johns, 2002 and Rima et al., 2011). Since aflatoxins had hazardous effects on health and economics, therefore, this work was focused on the aflatoxigenic fungi and aflatoxins associated with processed meat as well as the use of some plant extracts to prevent the growth and suppress spore germination *in vitro*.

Materials and Methods

Samples

Randomly 48 samples belong to four uncooked processed meat products, i.e., basterma, beef burger; luncheon meat (sliced cold meats) and sausage (12 of each type) were collected into sterile polyethylene bags from different companies in Egypt.

Mycoflora analysis

Ten grams of each sample was added to 90ml of 1% peptone water into a sterile blender jar and blended for 2min and routinely diluted to 10^{-2} dilutions. One ml of the previously prepared serial dilutions were inoculated separately into sterilized Petri dishes, containing either sterilized PDA medium (containing 200g Potato slices, 20g Dextrose and 20g Agar) or SDA medium (containing 10gm Mycological peptone, 40gm Dextrose and 15gm Agar). Three replicates of each sample were prepared and incubated at 28 ±2°C for 3-5 days. All mold colonies were counted and recorded according to ICMSF (1978).

Identification

All colonies formed of molds were examined morphologically and microscopically then identified based on cultural and morphological characteristics according to Raper & Fennel (1965), Samson (1979), Domsch et al. (1980) and Pitt & Hocking (1997).

Aflatoxins production

All isolated aflatoxigenic fungi (*Aspergillus flavus* and *A. parasiticus*) were propagated as a pure culture in 100 ml yeast extract sucrose (YES) to be tested for aflatoxins production according to Munimbazi & Bullerman (1998). Each flask was inoculated with 0.1ml of spore suspension containing approximately 10^6 spores/ml. Cultures were incubated at 26±2°C for 14 days.

Extraction of aflatoxins from the culture media

The extraction was performed according to the procedure offered by Kumar et al. (2010), with some modifications. The cultures were filtered and mycelial mats were collected. Aflatoxins were extracted from culture filtrates with chloroform. A certain volume of filtrate (25ml) was added to 10ml chloroform and was shaken for half an hour. The chloroform contained aflatoxins were separated by separating funnel which was allowed to stand for some time until the two layers appeared. The upper aqueous layer was re-extracted many times with chloroform for complete separation. The lower chloroform layer was filtered over anhydrous sodium sulfate in 250 ml beaker, evaporated in a water bath (70-80°C) near dryness and the residue was washed twice with chloroform (12ml) into a glass vial which evaporated till dryness (dry film). The dried extract was kept in the refrigerator at -5°C until analysis.

Thin layer chromatography (TLC) analysis

Aflatoxins were detected according to the method of Calvo et al. (2004). Extracts of fungal cultures grown on broth media from selected processed meat samples were screened for aflatoxins production. A volume of 20µl of each extract and aflatoxins standard were spotted on thin layer chromatography silica gel plate, then

transferred to a jar containing the following solvent system, toluene: ethyl acetate: formic acid (6:3:1). The solvent system was allowed to rise until it almost reached the top of the plate, which will give the maximum separation of the extract components. The plate was then observed under long wave Ultraviolet light for the presence of aflatoxins by their distinctive fluorescence properties. The blue fluorescence corresponding to aflatoxins B_1 and B_2 while the green fluorescence corresponding to G_1 and G_2. The intensity of fluorescence and the colors of the extracts were compared with aflatoxins standard. The sample extracts which were positive for the presence of aflatoxins were taken for HPLC analysis.

High-performance Liquid chromatography (HPLC) analysis

This procedure was performed according to A.O.A.C. (1995). The HPLC system used was a water 600-pump system equipped with a model 474-fluorescence detector (water) set at 360nm for excitation and 440nm for emission wavelength. Water Nova-pack C18 column (150×3.9) was used for aflatoxins separation. The mobile phase (water: Acetonitrile: Methanol, 65: 5: 30) was isocratically flowed at a rate of 1.0ml/ min. Data were collected and integrated with a waters Millennium 32 chromatography Manager software program.

Preparation of plants extract

Fresh plant material (1kg) of each *Euphorbia cotinifolia* L. (Caribbean copper plant) and *E. tirucalli* L. (Pencil-tree) plants and *Rhus coriaria* (Sicilian sumac) seeds were washed thoroughly under running tap water, shade dried and were separately ground into a coarse powder using a Thomas-Willey milling machine, then weight 300g of *E. cotinifolia* L.*, E. tirucalli* L. and *R. coriaria* seeds and separately subjected to successive extraction by a mixture solvent of absolute ethanol/water 80% (v/v), and left for 48h. The extracts were filtered using Whatman filter paper No. 1 (125mm). The ethanol filtrates were separately collected in the three different bakers and concentrated using a rotary evaporator at 40°C to obtain the crude extracts. The crude extracts were stored at -20°C to further analysis (Valadares et al., 2006; Fazeli et al., 2007 and Sultan et al., 2016).

Analysis of phenolic compounds

HPLC analysis of phenolic compounds was carried out using Agilent Technologies 1100 series liquid chromatograph equipped with an auto sampler and a diode-array detector. The analytical column was an Eclipse XDB-C_{18} (150X4.6µm; 5µm) with a C_{18} guard column (Phenomenex, Torrance, CA). The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The flow rate was kept at 0.8ml/min for a total run time of 70min and the gradient programme was as follows: 100% B to 85% B in 30min, 85% B to 50% B in 20min, 50% B to 0% B in 5min and 0% B to 100% B in 5min. The injection volume was 50µl and peaks were monitored simultaneously at 280 and 320nm for the benzoic acid and cinnamic acid derivatives, respectively. All samples were filtered through a 0.45µm Acro-disc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards (Kuyng et al., 2006).

Antifungal tests (in vitro)

Estimation of mycelia biomass

Potato dextrose broth (containing 4g Potato Infusion from 200g and 20g Dextrose) was prepared in sterilized flasks (250ml) with 1, 3 and 5% concentrations (v/v) of tested extracts were mixed separately *in vitro*. Potato dextrose broth free plant extracts were used as a control treatment. The flasks were inoculated with 5mm disc diam., of tested fungi as mentioned above and three replicates were used. All flasks were incubated at 28±2°C for 14 days. Thereafter, cultures were filtered through pre-weighed Whatman filter paper No. 1. Mycelial dry weight was calculated after drying at 70°C for 24h in oven drying (Giovanelli, 2008 and Özdemir et al., 2010).

Effect of plant extracts on spore germination

Viability of spores produced by tested fungi on agar plates of tested plant extracts (at 1, 3 and 5%) were studied. A disc of 0.5cm diameter of fungal culture on PDA of 7 days old was placed at the center of each Petri dish and incubated at 25±2ºC. To harvest the produced spores, 10ml of sterile water were flooded over the grown fungal mycelium and separated using a drawing brush, and then the spore suspension was filtered through muslin cloth. The concentration of collected spore suspension was adjusted to $1x10^2$ conidia/ml with the aid of haemocytometer slide. PDA medium free of plant extracts was inoculated with 0.1ml

of spore suspension (contain $1x10^2$ conidia/ml) then spread evenly over the plate. Each treatment had three replicate plates, then incubated at 25ºC. After 48h of incubation, Counts of germinated spores were calculated (by naked eyes) according to Meena & Mariappan (1993).

Statistical analysis

The data was performed according to SAS (1982).

Results and Discussion

Total fungal count of the tested processed meat using two different media

Mycological examination of four processed meat samples, i.e. Basterma, Beef Burger, Luncheon meat and Sausage (12 companies for each) on two different media (PDA and SDA medium) resulted that, no significant differences ($P < 0.05$) in between the two different media as shown in Table 1. On the other hand, significant differences ($P < 0.05$) in total fungal counts were recorded between all types of different processed meats with two different media used. Basterma samples had the highest mean total fungal count, which recorded 674 fungal colonies/10g of the examined 12 samples (660 and 688 fungal colonies on PDA and SDA media, respectively), followed by luncheon meat samples which recorded 302 fungal colonies/10g (272 and 332, respectively). The mean total fungal count of sausage samples was 111 fungal colonies/10g (106 and 116, respectively). Beef burger samples had the least mean fungal count, which recorded 39 fungal colonies/10g (31 and 47, respectively). Similar results were obtained by many investigators, El-Kady & Zohri (2000) who found that the fungal population ranged from l64 to 528 colonies/g from 20 beef burger samples collected from Assiut, Giza and Cairo Governorates. Zohri et al. (2014) analyzed forty samples of beef burger and sausage (20 for each) for their microbiological quality results showed that, the total number of fungal species on DRBC was higher than that on DG18 (31 species in case of beef burger and 33 in sausage while the total number of fungal colonies per sample ranged from 21-290 colonies/g of sausage on DRBC medium and ranged from 12.6-266 colonies/g of sausage On DG18 medium. Omorodion & Odu (2014) reported that the total fungal count of the meat samples ranged from $6.0x10^4$CFU/g to $4.4x10^5$CFU/g. While The variations in the fungal counts may be due to the geographical location of producing companies, duration of storage and the hygienic status of persons dealing with these products. These findings are in accordance with Zohri et al. (2014), who said that the techniques used for food analysis, types of media, incubation temperature are factors affecting the fungal counts.

Percentage of fungal frequency association

All mycoflora isolated from tested processed meat samples were identified and presented in Table 2. Results in this table presented that, nine fungal genera were identified and recorded. These are *Alternaria, Aspergillus*, *Cladosporium*, *Epicoccum, Geotrichum, Paecilomyces, Penicillium, Phoma* and *Trichoderma* (9 genera including 11 species in case of PDA medium and 8 genera including 12 species in case of SDA medium). Also data show that, on PDA medium, *Penicillium* genus was higher fungal frequency associated processed meat samples which record 89.71% followed by *Aspergillus* 4.50% (*A. flavus* 2.34%, *A. niger* 1.22% and *A. parasitcus* 0.94%), *Geotrichum* sp. 2.91%, *Cladosporium* sp. 1.50 %, *Phoma* sp. 0.56%, *Paecilomyces* sp. 0.47% and *Epicoccum* sp. 0.19 %. *Alternaria* sp. and *Trichoderma* sp. gave less fungal frequency 0.09%. On SDA medium, *Penicillium* genus was higher fungal frequency associated processed meat samples which recorded 89.10 % followed by *Aspergillus* 5.24% (*A. parasitcus* 2.20%, *A. flavus* 1.69%, *A. niger* 0.93% and *A. clavatus* 0.34 %, *A. terreus* 0.08%), *Geotrichum* sp. 3.38%, each of *Cladosporium* sp. and *Paecilomyces* sp. 0.76%, *Phoma* sp. 0.59%. Each of *Epicoccum* sp. and *Trichoderma* sp. gave less fungal frequency 0.08%. These findings are nearly similar to those obtained by Ismail et al. (2013), who reported that 7 mold genera could be identified from meat products. The identified mold genera were *Aspergillus, Pencillium, Eupencillium, Eurotium, Mucor, Cladosporium* and *Byssochlamys nivea*. The frequencies of isolated mold genera in examined luncheon samples were; *A. niger* 10 (26.3%), *A. flavus* 7 (18.4%), *P. corylophilum* 7 (18.4%), *Mucor* 5 (13.1%), *P. simplicissimum*, *P. digitatum*, *Eupencillium* species and *Cladosporium* species 2 (5.3%) and *Byssochlamys nivea* 1 (2.6%). For basterma samples the frequencies were; 7 (24.2%), 5(17.3%), 5 (16.3%), 3(10.4%), 2 (6.9%), 2 (6.9%), 1 (3.4%),1 (3.4%), 1 (3.4%), 1 (3.4%) and 1 (3.4%) for; *A. flavus*, *A. niger*, *Mucor, Cladosporium* species, *A. ochraceus A. gluacus, P. simplicissimum, P. chrysogenum P. raistrickii,*

P. rugulosum and *P. olsonii,* respectively. Morshdy et al. (2015) identified nine mold genera from the beef burger, sliced luncheon, Kofta, oriental sausage, and basterma. Genus *Aspergillus* had the highest incidence rate 49 (49%) followed by *Penicillium* 34 (34%), each of *Cladosporium* and *Alternaria* 15 (15%), *Acremonium* 12 (12), *Rhizopus* 10 (10%), *Rhizomucor* 8 (8%), *Absidia* 3 (3%) and *Chrysosporium* 2 (2%). Five species of *Aspergillus* could be isolated form examined meat products samples. *A. niger* had the highest incidence rate 22 (22%) followed by *A. flavus* 16 (16), *A. fumigatus* 12 (12%), *A. parasitcus* 2 (2%) and at least *A. ochraceus* 1 (1%). This variation in fungal species is may be due to using different types of media and storage temperature. Filtenborg et al. (2002) and Frisvad & Samson (2004) reported that, the frequent occurrence of *Penicillium* spp. on meat products is probably due to their common ability to tolerate low water activity (0.78-0.83) and to grow well at low to moderate temperatures and on protein-rich substrates.

Detection of aflatoxins contamination in meat products

Detection of aflatoxins in processed meat products, i.e., Basterma, Beef Burger, Luncheon meat, and Sausage were recorded in Table 3, while aflatoxin concentrations were tabulated in Table 4. Data indicated that higher aflatoxins contamination was found in basterma companies than other samples. Aflatoxins were produced by aflatoxigenic fungi isolated from seven basterma companies No. 1, 4, 5,7,8,9 and 10 followed by each of beef burger and sausage samples in which aflatoxins were produced by aflatoxigenic fungi isolated from six companies No.1, 5, 7, 8, 11 and 12 of beef burger samples and from six companies No. 2, 3, 6, 8, 9 and 12 of sausage samples. Less aflatoxins contamination was recorded in luncheon meat companies, in which aflatoxins were produced by aflatoxigenic fungi isolated from four companies of luncheon meat samples No. 2, 5, 8 and 11. These findings are in accordance with many researchers such as El- Maraghy & Zohri (1988), they reported the production of aflatoxins by two out of four tested isolates of *A. flavus* var. *columnaris* from sausage samples. Aziz & Youssef (1991) found that *Aspergillus flavus* (24 isolates), and *Aspergillus parasiticus* (16 isolates) were the predominant aflatoxin-producing molds isolated from processed meat products. Cvetnik & Pepeljnjak (1995) reported a 20% average occurrence of *Aspergillus flavus* and *Aspergillus parasiticus* in smoked meat products, pork salami and sausage, bacon and ham, which are potentially toxicogenic. Morshdy et al. (2015) reported that Aflatoxins B_1, B_2, G_1, and G_2 were detected in the examined beef burger, sausage, kofta, basterma and sliced luncheon samples. Contamination of meat with aflatoxins may be attributed to using toxigenic mold strains contaminated ingredients such as cereals and spices and the storage conditions. These findings are similar to Zohri et al. (1995), who reported that contamination of meat products by aflatoxins could have originated either from the animal tissues previously fed on aflatoxin contaminated feed or by use of mold contaminated ingredient, e.g. cereals and spices. Refai et al. (2003) stated that the use of spices contaminated with toxigenic mold strains may also represent a source of secondary mycotoxin contamination of the final product.

TABLE 1. Total fungal count of tested processed meat using two different media.

Type of processed meat	The number of fungal counts (per ten grams meat)		Mean	P value	LSD 5% between samples
	PDA	SDA			
	T.C	T.C			
Basterma	660 ± 3.21 d	688 ± 4.73 d	674 ± 3.97	0.012	15.889 D
Beef Burger	31 ± 2.08 a	47 ± 1.53 a	39 ± 1.81	0.003	7.178 A
Luncheon meat	272 ± 1.73 c	332 ± 6.00 c	302 ± 3.87	0.001	17.361 C
Sausage	106 ± 2.65 b	116 ± 1.15 b	111 ± 1.90	0.002	8.025 B

-Significant in between different alphabetical, non-significant in between the same alphabetical
-PDA = Potato dextrose agar, SDA = Saboraud dextrose agar, T.C = Total count

TABLE 2. Percentage of fungal frequencies associated processed meat samples.

Fungal species		Percentage of fungal frequencies									
		PDA					SDA				
		Basterma	Beef Burger	Luncheon	Sausage	T.C	Basterma	Beef Burger	Luncheon	Sausage	T.C
Alternaria sp.	T.C	NF	NF	1	NF	1	NF	NF	NF	NF	NF
	%	-	-	0.37	-	0.09	-	-	-	-	-
Aspergillus clavatus	T.C	NF	NF	NF	NF	NF	NF	4	NF	NF	4
	%	-	-	-	-	-	-	8.51	-	-	0.34
Aspergillus flavus	T.C	18	NF	6	1	25	9	3	8	NF	20
	%	2.73	-	2.21	0.94	2.34	1.31	6.38	2.41	-	1.69
Aspergillus niger	T.C	3	6	1	3	13	2	7	1	1	11
	%	0.45	19.35	0.37	2.83	1.22	0.29	14.89	0.30	0.86	0.93
Aspergillus parasiticus	T.C	6	1	3	NF	10	11	5	4	6	26
	%	0.91	3.23	1.1	-	0.94	1.60	10.64	1.20	5.17	2.20
Aspergillus terreus	T.C	NF	NF	NF	NF	NF	NF	1	NF	NF	1
	%	-	-	-	-	-	-	2.13	-	-	0.08
Cladosporium sp.	T.C	10	6	NF	NF	16	4	5	NF	NF	9
	%	1.52	19.35	-	-	1.50	0.58	10.64	-	-	0.76
Epicoccum sp.	T.C	NF	1	1	NF	2	NF	NF	1	NF	1
	%	-	3.23	0.37	-	0.19	-	-	0.30	-	0.08
Geotrichum sp.	T.C	6	NF	NF	25	31	9	NF	NF	31	40
	%	0.91	-	-	23.58	2.91	1.31	-	-	26.72	3.38
Paecilomyces sp.	T.C	NF	NF	NF	5	5	NF	NF	NF	9	9
	%	-	-	-	4.72	0.47	-	-	-	7.76	0.76
Penicillium spp.	T.C	617	12	259	71	959	653	17	316	68	1054
	%	93.48	38.71	95.22	66.98	89.71	94.91	36.17	95.18	58.62	89.10
Phoma sp.	T.C	NF	5	NF	1	6	NF	5	1	1	7
	%	-	16.13	-	0.94	0.56	-	10.64	0.30	0.86	0.59
Trichoderma sp.	T.C	NF	NF	1	NF	1	NF	NF	1	NF	1
	%	-	-	0.37	-	0.09	-	-1	0.30	-	0.08
Total	T.C	660	31	272	106	1069	688	47	332	116	1183

PDA = Potato dextrose agar, SDA = Saboraud dextrose agar, NF= Fungi not found, T.C = Total count

TABLE 3. Aflatoxins contamination of different processed meat products.

Samples	Aflatoxins contamination											
	1	2	3	4	5	6	7	8	9	10	11	12
Basterma	+	ND	ND	+	+	ND	+	+	+	+	ND	ND
Beef Burger	+	ND	ND	ND	+	ND	+	+	ND	ND	+	+
Luncheon	ND	+	ND	ND	+	ND	ND	+	ND	ND	+	ND
Sausage	ND	+	+	ND	ND	+	ND	+	+	ND	ND	+

ND = Aflatoxins not detected, + = Positive reaction

Determination of aflatoxins conc.

Determination of aflatoxins content in basterma companies resulted that higher aflatoxin quantity was produced by *Aspergillus parasiticus* (isolate No 12) isolated from basterma company No. 8 which recorded 8.620ng/g, followed by *A. parasiticus* (isolate No 4) isolated from company No. 4 which produced 2.280ng/g, *A. parasiticus* (isolate No 5) from company No. 5 recorded 1.89ng/g, *A. parasiticus* (isolate No 1) from company No. 1 produced 1.880ng/g, *A. parasiticus* (isolate No 9) from company No. 7 recorded 1.530ng/g and *A. parasiticus* (isolate No 14) from company No. 10 recorded 0.870ng/g. Isolate No 13 of *A. parasiticus* from company No. 9 produced less aflatoxins quantity which recorded 0.730ng/g, while none of the isolates from basterma companies No (2, 3, 6, 11&12) was aflatoxins producer. Determination of aflatoxins content in beef burger companies resulted that, isolate No 5 of *A. parasiticus* isolated from beef burger company No.11 produced the highest aflatoxin quantity which recorded 1.060ng/g, followed by *A. parasiticus* (isolate No 2) from company No. 5 which produced 0.740ng/g, *A. parasiticus* (isolate No 3) from company No. 7 recorded 0.580ng/g, *A. parasiticus* (isolate No 6) from company No.12 recorded 0.340ng/g and *A. parasiticus* (isolate No 4) from company No. 8 produced 0.100ng/g. *A. flavus* (isolate No 1) from company No. 1 produced the least aflatoxins quantity (0.060ng/g) while none of the isolates from beef burger companies No (2, 3, 4, 6, 9 and10) was aflatoxins producer. On the other hand, determination of aflatoxins in luncheon meat companies resulted that, *Aspergillus parasiticus* (isolate No 7) isolated from luncheon company No (11) produced the highest aflatoxin quantity which recorded 3.810ng/g, followed by *A. parasiticus* (isolate No 5) from company No. 8 which produced 0.380ng/g, *A. parasiticus* (isolate No 1) from company No. 2 recorded 0.140ng/g. Isolate No 2 of *A. flavus* from company No. 5 produced less aflatoxins quantity which recorded 0.080ng/g, while none of the isolates from luncheon meat companies No (1, 3, 4, 6, 7, 9, 10 and 12) was aflatoxins producer. Determination of aflatoxins content in sausage companies resulted that, isolate No 5 of *Aspergillus parasiticus* isolated from sausage company No. 9 produced the highest aflatoxin quantity which recorded 0.240ng/g, followed by *A. parasiticus* (isolate No 3) from company No. 6 which produced 0.230ng/g, *A. flavus* (isolate No 1) from company No. 2 recorded 0.200ng/g, *A. parasiticus* (isolate No 4) from company No. 8 produced 0.180ng/g and *A. parasiticus* (isolate No 1) from company No. 3 recorded 0.150ng/g. Isolate No 6 of *A. parasiticus* from company No. 12 produced less aflatoxins quantity 0.100ng/g, while none of the isolates from sausage companies No (1, 4, 5, 7, 10 and 11) was aflatoxins producer as shown in Table 4. Similar results were obtained by Ismail et al. (2013), who reported that 5 (20%) samples of luncheon contain aflatoxin B_1 minimum 1.3, maximum 24.5 and average ± SE 10.4± 5.1ppb, while the value of aflatoxin B_2 in Basterma samples minimum 1.2, 2.5 and average ± SE 2.3±o.4 ppb. Shaltout et al. (2014) determined the aflatoxins concentration in hundred samples of different meat products represented by (kofta, sausage, luncheon and basterma) and found that the average concentration of aflatoxin B_1 (µg/kg) in kofta, sausage, luncheon and basterma were 13.38±1.52, 9.03±1.17, 8.8±0.95 and 4.53±0.61, respectively. The average concentration of B_2 (µg/kg) in kofta, sausage, luncheon and basterma were 8.50±0.7, 5.20±0.69, 5.57±0.72and 2.33±0.15, respectively, the average concentration of aflatoxin G_1(µg/kg) in kofta, sausage, luncheon and basterma were 4.76±0.83, 3.35±0.49, 3.84±0.58 and 1.85±0.22, respectively. The average concentration of aflatoxin G_2 (µg/kg) in kofta, sausage, luncheon and basterma were 3.18±0.52, 2.33±0.29 and 2.50±0.03, respectively. Morshdy et al. (2015) examined Aflatoxins B_1, B_2, G_1 and G_2 in examined beef burger samples with mean

values of 2.14±0.35, 2.88±1.08, 0.48±0.15 and 0.17±0.05 ppb, respectively, In examined oriental sausage samples with mean values of 0.69± 0.22, 0.31±0.15, 0.62 ±0.28 and 0.16±0.05 ppb, respectively. In examined kofta samples the mean values were 0.42±0.11, 1.60±0.39, 0.67±0.30 and 0.57±0.14 ppb, respectively. In examined basterma samples the mean values were 0.43±0.17, 0.68±0.25, 0.62±0.13 and 0.23±0.09ppb, respectively. In sliced luncheon samples the aflatoxins detected with mean values of 0.17±0. 07, 0.64±0.14 and 0.27±0.14ppb, for B_1, B_2 and G_1, respectively but G_2 cannot be detected. These different mean values of aflatoxins residues in samples may be related to the amount of additives contaminated with toxigenic mold strains used in the processing.

Determination of phenolic acid quantity

High-performance liquid chromatography (HPLC) analysis was used for the identification and quantification of important phenolic acids in *Euphorbia cotinifolia* L., *E. tirucalli* L. and *Rhus coriaria* L. extracts. Determination of important phenolic acids contents were recorded in the Table 5. Data showed that, higher quantity of gallic acid were recorded with *R. coriaria* L. extract, which recorded 439.63µg/g of plant material, followed by *E. tirucalli* L. extract (287.68µg/g), while *E. cotinifolia* L. extract had less quantity and recorded 160.44µg/g. Protocatechuic acid was highly recorded in *E.tirucalli* L. extract (64.95µg/g), followed by *E. cotinifolia* L. extract which recorded 18.56µg/g, while *R. coriaria* L. extract gave 6.21µg/g. Higher chlorogenic acid was recorded with *R. coriaria* L. extract which gave 47.91µg/g, followed by *E. cotinifolia* L. extract gave 7.36µg/g, while *E. tirucalli* L. extract was free. Higher caffeic acid, syringic acid acid, ferulic acid, sinapic acid, coumarin and cinnamic acid were recorded with *E. cotinifolia* L. as 83.38, 8.51, 70.10, 3.36, 3.01 and 0.83µg/g, respectively followed by *E. tirucalli* L. extracts which recorded 20.34, 2.90, 13.53, 1.30, 1.48 and 0.41µg/g, respectively, while not found in *R. coriaria* L. extract. Vanillic acid was highly recorded in *E. tirucalli* L. extract (14.57µg/g), followed by *E. cotinifolia* L. extract which recorded 6.41µg/g, but it not recorded in *R. coriaria* L. extract. Higher rosmarinic acid was recorded with *E. tirucalli* extract (9.20µg/g), followed by *R.coriaria* L. extract which recorded 1.38µg/g, while not found in *E. cotinifolia* L. extract. Similar results were obtained by Hossain et al. (2014), who found that, the phenolic acids and flavonoid contents of ethanol extract of *E. cotinifolia* L. leaves were in the range 328.7±3.01mg of ascorbic acid/g, 64.64±2.14mg/g of gallic acid, and 81.72±6.05mg/g of quercetin equivalent, respectively. Polyphenolic compounds like catechin, vanillic acid, p-coumaric acid, rutin hydrate and quercetin were obtained in the extract by reverse-phase HPLC and varied within the range of 80.14±1.75, 3.09±0.12, 1.22±0.05, 27.09±1.09 and 1.73±0.03mg/100g extract, respectively. Abu-Reidah et al. (2015) reported that, phenol compounds such as (gallic acid, protocatechuic acid, syringic acid, caffeoylquinic acid, coumarin, were detected in sumac (*R. coriaria* L.) fruits. Machado et al. (2016) found that, the concentrations of the phenolic compounds present in the crude extract of *E. tirucalli* L. as gallic acid, caffeic acid, chlorogenic acid, condensed tannins, quercetin, kaempferol, rutin and 12-O-tetradecanoyl phorbol-13-acetate (TPA) were 30.52±1.19, 15.61±3.12, 12.37±2.69, 689.50±24.64, 1.47±0.11, 3.45±0.11, 0.49±0.07 and 3.12±2.87 (µg/ml).

Inhibition of the isolated fungi in vitro

Effect of different plant extracts on mycelial dry weight

Effect of *E. cotinifolia* L., *E. tirucalli* L. and *R. coriaria* L. extracts on the mycelial dry weight were tabulated in Table 6. Data presented that, all plant extracts were found to reduce significantly ($P < 0.05$) the mycelial dry weight of the tested aflatoxigenic fungi, i. e., *A. flavus* and *A. parasiticus* with the three concentrations used comparing with untreated control. Also, data revealed that, by increasing the concentration of the plant extract, the reduction percentage of the mycelial dry weight increases, this means that the inhibitory effect of the plant extract increases. No significant differences between the three tested plant extract. *Rhus coriaria* L. extract at 5% conc., was enhanced which reduce significantly ($P < 0.05$) the mycelial dry weight of *A. flavus* and *A. parasiticus* from 0.59 and 0.63g with untreated (control) to 0.32 and 0.29g with 45.76 and 53.67% reduction, respectively, followed by 3% conc. which decrease significantly ($P < 0.05$) the mycelia dry weight of *A. flavus* and *A. parasiticus* to 0.37 and 0.35g with 37.85% and 44.44% reduction, respectively. *Rhus coriaria*

L. extract at 1% conc., was less effective which decrease significantly ($P < 0.05$) the mycelia dry weight to 0.45 and 0.46g with 24.29% and 26.98% reduction, respectively. *Euphorbia tirucalli* L. extract reduces significantly ($P < 0.05$) the mycelia dry weight of the tested fungi from 0.59 and 0.63g with untreated (control) to 0.34 and 0.33g with 42.37 and 47.62% reduction, respectively at 5% conc., while 3% conc. gave 0.41g for both *A. flavus* and *A. parasiticus* with 31.07 and 34.92% reduction, respectively. Less reduction of the mycelial dry weight was recorded with 1% of *E. tirucalli* L. extract which records 0.51and 0.48g of the mycelial dry weight with 13.56 and 23.81% reduction, respectively. *Euphorbia cotinifolia* L. extract reduces significantly ($P < 0.05$) the dry weight of mycelia from 0.59 and 0.63g with untreated (control) to 0.36 and 0.40g with 39.55 and 35.45% reduction, respectively at 5% conc., and to 0.46 and 0.44g with 22.03 and 30.69% reduction, respectively when treated with 3% conc. Less reduction of the mycelial dry weight of the same tested fungi was recorded with *E. cotinifolia* L. extract at 1% conc. It was found to reduce significantly ($P < 0.05$) the mycelial dry weight to 0.52g for both the tested fungi with 11.86 and 17.99% reduction, respectively. This is the first time for the study of the effect of *Euphorbia cotinifolia* L. on the mycelial dry weight of *A. flavus* and *A. parasiticus* till now. Mohana & Raveesha (2007) reported that the percentage of inhibition of mycelial dry weight of *F. solani and A. flavus* in Richard's medium *with Euphorbia tirucalli* extract was 12.6%, 2.3%, respectively. Reddy et al. (2011) found that the petroleum ether fractions of *Euphorbia microphylla* reduced the dry weight of *A. flavus***.** from 10.37mg/ml in control sample to 9.90mg/ml, acetone fractions to 8.53mg/ml, benzene fractions of *E.microphylla* to 7.24mg/ml, chloroform fractions to 4.41mg/ml , while methanol fractions to 2.94mg/ml.

TABLE 4. Screening of different aflatoxigenic fungi in different processed meat sources.

Type of sample	Sources No	Aflatoxin producer fungi	Isolate No	AFG_1 (ng/g)	AFB_1 (ng/g)	AFG_2 (ng/g)	AFB_2 (ng/g)	Total (ng/g)
Basterma	1	*A. parasiticus*	No 1	ND	ND	1.880	ND	1.880
	4	*A. parasiticus*	No 4	2.190	0.040	ND	0.050	2.280
	5	*A. parasiticus*	No 5	ND	ND	1.890	ND	1.890
	7	*A. parasiticus*	No 9	1.530	ND	ND	ND	1.530
	8	*A. parasiticus*	No 12	6.740	0.090	1.790	ND	8.620
	9	*A. parasiticus*	No 13	ND	0.250	0.480	ND	0.730
	10	*A. parasiticus*	No 14	0.400	0.010	0.460	ND	0.870
Beef Burger	1	*A. flavus*	No 1	ND	ND	ND	0.060	0.060
	5	*A. parasiticus*	No 2	0.140	0.340	0.120	0.150	0.740
	7	*A. parasiticus*	No 3	0.570	ND	0.010	ND	0.580
	8	*A. parasiticus*	No 4	0.090	0.010	ND	ND	0.100
	11	*A. parasiticus*	No 5	0.940	ND	0.100	0.020	1.060
	12	*A. parasiticus*	No 6	ND	ND	0.220	0.120	0.340
Luncheon meat	2	*A. parasiticus*	No 1	ND	0.030	0.110	ND	0.140
	5	*A. flavus*	No 2	ND	0.060	ND	0.020	0.080
	8	*A. parasiticus*	No 5	ND	0.010	0.330	0.030	0.380
	11	*A. parasiticus*	No 7	ND	0.020	3.580	0.210	3.810
Sausage	2	*A. flavus*	No 1	ND	0.010	ND	0.190	0.200
	3	*A. parasiticus*	No 1	0.130	ND	ND	0.020	0.150
	6	*A. parasiticus*	No 3	0.170	0.020	0.040	ND	0.230
	8	*A. parasiticus*	No 4	0.080	ND	0.100	ND	0.180
	9	*A. parasiticus*	No 5	0.230	ND	ND	0.010	0.240
	12	*A. parasiticus*	No 6	0.080	ND	0.020	ND	0.100

ND = Aflatoxins not detected

TABLE 5. HPLC analysis of phenolic compounds contents.

Phenolic acids	*E. cotinifolia* L. (ug/g)	*E.tirucalli* L. (ug/g)	*R.coriaria* L. (ug/g)
Gallic acid	160.44	287.68	439.63
Protocatechuic acid	18.56	64.95	6.21
Chlorogenic acid	7.37	ND	47.91
Caffeic acid	83.38	20.34	ND
Syringic acid	8.51	2.90	ND
Vanillic acid	6.41	14.57	ND
Ferulic acid	70.10	13.53	ND
Sinapic acid	3.38	1.30	ND
Coumarin	3.01	1.48	ND
Rosmarinic acid	ND	9.20	1.38
Cinnamic acid	0.83	0.41	ND

ND = Phenolic acid not detected

TABLE 6. Effect of different plant extracts on the mycelial dry weight of aflatoxigenic fungi.

Plant extracts	Conc.	*A. flavus*		*A. parasiticus*		P-value	LSD 5% between plant extract
		Mycelia dry weight (g)	R %	Mycelia dry weight (g)	R %		
	1%	0.51 ± 0.01 e	13.56	0.52 ± 0.03 f	17.99	0.847	
E. cotinifolia	3%	0.46 ± 0.01 d	22.03	0.41 ± 0.01 c	35.45	0.027	0.055 A
	5%	0.36 ± 0.01 b	39.55	0.33 ± 0.01 b	47.62	0.065	
	1%	0.52 ± 0.01 e	11.86	0.46 ± 0.01 de	26.98	0.010	
E. tirucalli	3%	0.41 ± 0.01 c	31.07	0.44 ± 0.01 cd	30.69	0.074	0.055 A
	5%	0.34 ± 0.02 ab	42.37	0.41 ± 0.00 c	34.92	0.016	
	1%	0.45 ± 0.00 d	24.29	0.48 ± 0.01 ef	23.81	0.007	
R.coriaria	3%	0.37 ± 0.01 b	37.85	0.35 ± 0.01 b	44.44	0.189	0.000 A
	5%	0.32 ± 0.01 a	45.76	0.29 ± 0.01 a	53.97	0.081	
Control		0.59 ± 0.01 f		0.63 ± 0.00 g		0.005	0.000 B
P value		0.001		0.001			
LSD 5% between fungi		0.000 A		0.000 A			
LSD 5% between Conc.		1%=0.055 C		3%=0.055 B		5%=0.055 A	

-R= Reduction percent
-Significant in between different alphabetical, non-significant in between the same alphabetical

Effect of different plant extracts on spore germination (viability)

Effect of *Euphorbia cotinifolia* L., *Euphorbia tirucalli* L. and *Rhus coriaria* L. on spore germination (viability) of *A. flavus* and *A. parasiticus* was studied. Data in Table 7 presented that, all tested plant extracts were found to reduce significantly ($P < 0.05$) spore germination (viability) as well as significantly increase ($P < 0.05$) reduction percent of the tested aflatoxigenic fungi compared with untreated control. The data also revealed that, by increasing the concentration of the plant extract, the reduction percent of spore viability increases, this means that the inhibitory effect of the plant extract increases. No significant differences between the three tested plant extract. *R. coriaria* L. was found to decrease significantly ($P < 0.05$) spore viability of *A. flavus* and *A. parasiticus* from 28 and 120 germinated spores with untreated control to 10 and $25x10^2$ spores with 64.29% and 79.17% reduction, respectively when treated with 1% conc., while the germinated spores were 7 and 18 with 75 and 85% reduction at 3% conc. and record $4x10^2$ germinated spores for the two tested fungi with 85.71 and a 96.67% reduction, respectively at 5% conc. *Euphorbia tirucalli* L. extract was found to decrease significantly ($P < 0.05$) the spore germination (viability) of *A. flavus* and *A. parasiticus* from 28 and $120x10^2$ germinated spores with untreated control to 13 and $26x10^2$ spore viability with 53.57 and 78.33% reduction, respectively when treated with 1% conc. of extract. Also, decrease significantly ($P < 0.05$) the germinated spores to 9 and $19x10^2$ spores with 67.86 and 84.17% reduction at 3% conc., respectively. Higher concentration of *E. tirucalli*

L. extract (5%) was found to reduce significantly ($P < 0.05$) the germinated spores from 28 and $120x10^2$ with untreated control to 4 and $8x10^2$ with 85.71 and 93.33% reduction with *A. flavus* and *A. parasiticus,* respectively. *Euphorbia cotinifolia* L. extract was found to reduce significantly ($P < 0.05$) the germinated spores of the tested fungi (*A. flavus* and *A. parasiticus*) with all concentration used. The most effective concentration was recorded at 5%. It was found to decrease significantly ($P < 0.05$) spore viability to 5 and $15x10^2$ germinated spores with 82.14 and 87.50% reduction for the two tested fungi, while 3% conc. significantly reduce ($P < 0.05$) the spore viability to 11 and $22x10^2$ with 60.71 and 81.67% reduction, respectively. The Less reduction percent was recorded with 1% conc., which gave 16 and $28x10^2$ viable spores with 42.86 and 42.86% reduction for the two tested fungi. This is the first time for study the effect of *Euphorbia cotinifolia* L. on spore viability of *A. flavus* and *A. parasiticus* till now. Mohana & Raveesha (2007) reported that the aqueous extracts of *E. tirucalli* had a significant percent inhibition of spore germination of *A. flavus*. Murugan et al. (2007) reported inhibition of spore formation and thus 100% inhibition of the growth of *Aspergillus flavus* and *A. parasiticus* by using the extracts of *Euphorbia* spp. Radmehr & Abdolrahimzade (2009) reported that ethanol extract of sumac was found to be effective in count-decreasing of the total microbial count in the minced meat, in which a significant antimicrobial potential was shown for the ethanol extract compared to controls. The antifungal activities of the tested plant extracts are due to the phenolic acids (vanillic and caffeic acids) and flavonoids (coumarins). The variations in their antifungal activities may be due to the variations in their phytochemical compositions. These findings are in accordance with Scalbert (1991), Murugan et al. (2007), Roshan et al. (2012) and Hussaini et al. (2014), who reported that the antifungal activity may be attributed to presence of some phenolic acids and flavonoids (coumarins). Wahdan (1998) found that, phenolic acids (vanillic and caffeic acids) completely inhibited the growth of *Aspergillus flavus* and *A. parasiticus*. Ansari et al. (2013) reported that, phenolic compounds are emerging as reasonably effective and efficient antifungal agents that could be used as fungicides. Peeler et al. (1989) mentioned that, phenolic compounds such as caffeic, chlorogenic and sinapic acids show antifungal activity by inhibiting ergosterol which is the component of fungal cell membrane. Brul & Klis (1999) said that**,** the structures of the phenolic compounds is such that they can diffuse through the microbial membrane and can penetrate into the cell, where they can interfere in the metabolic pathways by interfering with the synthesis of ergosterol, glucan, chitin, proteins and glucosamine in fungi. Meschini et al. (2003) reported that, some of the phenolic compounds like phenolic acids, flavonoids and catechins being lipophilic in nature are able to inhibit the activity of ABC transporters that makes the fungal pathogens resistant to the drug administered. Mariita et al. (2011) said that, the inhibition mechanism of phenolic compounds occurred via suppression of extracellular fungal enzymes (cellulase, pectinase, laccase, xylanase, etc.) actions and cellular membrane functions of pathogenic fungi**.**

TABLE 7. Effect of different plant extracts on spore viability of aflatoxigenic fungi.

Plant extracts	Conc.	*A. flavus* Viable spores $x10^2$	R %	*A. parasiticus* Viable spores $x10^2$	R %	P value	LSD 5% between plant extract
	1%	16.00 ± 0.58 e	42.86	25.00 ± 1.15 de	79.17	0.002	
E. cotinifolia	3%	11.00 ± 0.58 cd	60.71	19.00 ± 0.58 bc	84.17	0.001	8.817 A
	5%	5.00 ± 0.58 ab	82.14	15.00 ± 0.58 b	87.50	0.001	
	1%	10.00 ± 0.58 cd	64.29	28.00 ± 0.58 e	76.67	0.001	
E. tirucalli	3%	9.00 ± 0.58 bcd	67.86	22.00 ± 1.15 cd	81.67	0.001	12.553 A
	5%	4.00 ± 0.58 a	85.71	8.00 ± 0.58 a	93.33	0.008	
	1%	13.00 ± 0.58 de	53.57	26.00 ± 2.31 de	78.33	0.005	
R.coriaria	3%	7.00 ± 0.00 abc	75.00	18.00 ± 1.15 bc	85.00	0.001	9.910 A
	5%	4.00 ± 1.15 a	85.71	4.00 ± 0.58 a	96.67	1.000	
Control		28.00 ± 4.04 f		120.00 ± 2.89 f		0.001	13.807 B
P value		0.001		0.001			
LSD 5% between fungi		4.180 A		3.283 B			
LSD 5% between Conc.		1%=13.538 C		3%=10.560 B		5%=6.222 A	

R= Reduction percent,

-Significant in between different alphabetical, non-significant in between the same alphabetical

Conclusion

Most of the examined meat product samples were contaminated with different types of mold genera which considered as a major cause in the spoilage of meat products, leading to great economic losses and constitute a public health hazard by the production of a wide variety of mycotoxins. One of the most effective ways to control the problems caused by aflatoxins is to prevent the growth of fungi in the substrate. Data shows that some tested processed meat samples, i.e. basterma, beef burger, Luncheon meat and sausage were found to contaminant with one or more aflatoxin(s). Data presented that, all tested plant extracts were found to suppress the mycelium dry weight (g) and spore viability of the aflatoxigenic fungi at all different concentrations used. *Rhus coriaria* L. extract was enhanced than others.

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ظهور الفطريات المنتجة لسموم الأفلاتوكسين في بعض منتجات اللحوم المصنعة ومقاومتها باستخدام بعض المستخلصات النباتية فى المعمل

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يستهدف هذا البحث التركيزعلى عزل وتعريف الفطريات المنتجه لسموم الافلاتوكسين و سمومها المصاحبة لبعض عينات اللحوم المصنعة مثل البسطرمة، البيف برجر ، اللانشون، وعينات السجق ومقاومتها باستخدام بعض المستخلصات النباتية مثل نباتات الإيفوربيا الحمراء(*E.cotinifolia* L.) ، ونبات صبار أم اللبن (*E. tirucalli* L.) ، ونبات السماق (*R.coriaria* L.). تم جمع 48 عينة عشوائية من منتجات اللحوم المصنعة سالفة الذكر بواقع 12 لكل صنف وذلك من شركات مختلفة.

أظهرت نتائج العزل أن عينات البسطرمة بها أعلى عدد من الفطريات وكان متوسط عدد المستعمرات التى تم عزلها 674 مستعمرة فطرية/10 جم. تم تعريف الأجناس الفطرية المعزولة وهى ألترناريا، أسبراجيلس، كلادسبوريوم، إيبيكوكم، جيوتريكم، بسيليوميسيس، بنسيليوم، فوما و ترايكودرما. أوضحت النتائج أن البسطرمة كان بها أعلى نسبه تلوث بالأفلاتوكسين. أظهرت النتائج أيضا أن المستخلص الكحولى لجميع النباتات المستخدمة تحت الدراسة قد خفضت بشكل معنوي الوزن الجاف لميسليوم فطر أسبراجيلس فلافيس وأسبراجيلس بارازيتيكس كما خفضت من إنبات الجراثيم مع كل التركيزات المستخدمة. كما أوضحت النتائج أن مستخلص نبات السماق(R. coriaria L.) كان أكثرهم كفاءة.