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FUNGAL LOAD AND MYCOTOXINOGENESIS OF SOME EGYPTIAN MEDICINAL PLANTS

Abol-Ela, M. F.; Zeinab M. AbdeL-Ghany; M. A. Atwa and Kh. M. El- Melegy
Regional Center for Food and Feed-Agricultural Research Center – Giza.

ABSTRACT

This investigation was designed to evaluate the fungal load of five randomly selected plant samples. A total of 150 dry samples of Basil, Mint, Marjoram, Chamomile and Fennel were collected from some markets in Egypt. All samples were analyzed among moulds and yeasts, also the samples were analysis for mycotoxins status. Fungi were found in most of the collected samples. The highest count of moulds presents in Mint, Basil, Marjoram, Fennel, and Chamomile in descending order, respectively, while the highest count of yeasts was presented in Chamomile, Mint, Fennel, Basil, and Marjoram , respectively. *Aspergillus* spp*. Fusarium* spp*., Penicillium* spp*. Mucor* spp. *and Humicola spp*.were more frequently detected. Total aflatoxins, ochratoxin A, zearalenone, and fumonisins B1 and B2 were determined in all samples.The genus *Aspergillus* was the most dominant fungal, where it was recovered(3761 isolates)followed by *Fusarium* (1023 isolates) while genus of *Penicillium* was recovered(687 isolates). These three species were found in 87.42 5% of the samples. *Aspergillus . flavus* exhibited total aflatoxins of 3220 ppb. The direct use of the medicinal plants may be high risk due to their high contents of many species of moulds.

The effect of preparing method of the selected plants to drink was studied. Adding of sugar or honey as sweeters to medicinal plant drinks showed no effect on the fungal load status. No inhibition zones were seen against the investigated fungi.
Keywords: Fungal load; medicinal plants; mycotoxins; Egypt.

INTRODUCTION

In the past, medicinal plants were the first line of treatment known to man and traditional medicinal practice remain an important part of the primary healthcare delivery system in most of the developing world (Akerele, 1998). According to a World Health Organization (WHO,2003) survey, about 70-80% of the world's populations, particularly in the developing countries, rely on non-conventional medicines, mainly from herbal sources, for their primary healthcare (Akerele, 1993). Traditional and folklore medicines play an important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for healthcare (Kiran *et al.,* 2010).

An understanding of the market profile, social economic attributes influencing trade, species traded and impact of trade on plant pollution is critical for effective resources management (Botha *et al.*, 2004). The safety and quality of raw medicinal plant materials and finished products depend on factors that may be classified as intrinsic (genetic) or extrinsic (environment, collection methods, cultivation, harvest, post harvest, processing, transport and storage practice; WHO, 2003).

The global and national markets for medicinal herbs have been growing rapidly and significant economic gains are being realized with global sales of herbal products totaling an estimated US$ 62 million in 2000 (WHO, 2003). However, the current global market for herbal medicines stands at over US$ 62 billion. The sale of herbal medicine is expected to reach an annual average growth rate of 6.4% (Innamdar *et al.,* 2008and Aneesh *et al.,* 2009)

Over the past 20 years, there has been an increased interest in the investigation of natural materials as a source of new antifungal agents. Different extracts and essential oils from traditional medicinal plants have been tested to identify the source of therapeutic effects. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action (Kaur and Kaur, 2010).

Yeast and mould densities vary considerably with the individual spices but are usually quite low. They range from less than 10 per gram in the case of such species as Mace, Mustaro seed and Cloves to greater than 10,000 per gram for a variety of other species, mainly Basil, Black pepper, Capsicum, Celery and Cinnamon (Guarino, 1974 and Powers *et al*., 1975). However, spices are raw agricultural materials and if the moisture content is too high, toxigenic moulds, like *Aspergillus* spp., *penicillium* spp*. and Fusarium* spp*.,* may grow offering the opportunity for toxins production (Reddy *et al*., 2001).

Honey is mentioned in the Holy Quran. The use of honey as a remedy has been reported not only in folk medicine but also it is reborn in modern medicine. It has been demonstrated that honey possesses important biological activities and therapeutic properties. Its use in modern medicine being evaluated more and more. It has been used for treatment of respiratory diseases, ulcers, wounds, eczema, psoriasis, and dandruff (Nejabat *et al*., 2009 and Zghloul *et al*., 2001). Reportedly, honey has an inhibitory effect on aerobic and anaerobic bacteria, yeast, fungi and viruses (Al-Jabri *et al*., 2003; Al-Waili, 2004; Lusby *et al*., 2005 and Asadi Rooya *et al*., 2003). Numerous studies demonstrated that honey possesses antimicrobial activity (Dustmann, 1979; Molan, 1992). More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, gram-positive and gram-negatives (Molan, 1992), it destroys and/or inhibits the growth of some pathogeniysc vegetative micro-organisms (Chick and Shin, 2001). An antifungal action of honey has also been observed for some yeasts and species of *Aspergillus* and *Pencillium* (Quinn *et al*., 1994), as well as all the common dermatophytes (Brady *et al*., 1997).

The present study aimed to throw light on the fungal load of some medicinal plants for direct human use in Egypt. Also, ability of the isolated fungi for mycotoxin production will be considered. The preparing treatments to drink service of the selected medicinal plants was suggested.

MATERIALS AND METHODS

Collection of Samples:

A total of 150 dry samples of medicinal plants and spices, i.e. Basil (*Ocimum basilicum*), Mint (*Mentha crispata*), Marjoram *(Origanum marjoram),* Chamomile *(Matricaria recutita),* and Fennel *(Foeniculum* vulgare) (30 samples of each plant) were collected from local Egyptian markets. The samples were collected with sterile cloves into sterile bags for up to used.

Isolation and identification of isolated fungi

The samples were disinfested by immersing in 5% sodium hypochlorite for 2 min, washed thoroughly with sterilized water (Eisa, *et al.*, 1996). Fungal isolation and identification were made according to Kulwant, *et al*. (1991) in Regional Center for Food & Feed(RCFF) and confirmed by Plant Pathology Department, Agriculture Research Center(ARC).

Total count fungi and yeast:

Ten grams of each sample were added to 90 ml portion of sterile saline solution (0.85% NaCl) in 500 ml Erlenmeyers flask and homogenized thoroughly on an electric shaker at constant speed for 15 minutes. Ten fold serial dilutions were then prepared. One ml portion of suitable dilutions were used to inoculate Petri dishes containing 15 ml Rose Bengal Agar fortified by 0.5 mg chloromphnicol/ ml medium. Plates were inverted and incubated at 30 + 2 ^{O}C for 3 days. Colonies of each plate were counted after 3 days of incubation. The plates containing fewer than 150 colonies were retained (Raper and Fennel, 1977).

Determination of mycotoxins:

Total aflatoxins ,ochratoxin A, zearalenone and fumonisins B1&B2 were determined by HPLC as described in AOAC (2006).

Chemicals and reagents:

Methanol and acetonitrile were purchased from Riedel (Poole, Dorset, UK). Trifluoroacetic acid (TFA) was obtained from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals and solvents were purchased from merck (Darmstadt, Germany). For sample clean up, immunoaffinity columns were obtained from Vicam (Watertown, Ma, USA).

Standards:

Standard for mycotoxins were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Stock solutions and standards were prepared and assay according to (AOAC, 2006). The quantity of each toxin was measured as ppb.

Honey and sugar samples

The honey and sugar were obtained from local market in Cairo, Egypt. It was in closed sterile containers. The effects of cooling and heating with/without sugar and honey on total count of fungi and yeast of some medicinal plant drinks were studied. The treatments of medicinal plant drinks were as follows:

1- Cooling at room temperature (25ºC) without sugar or honey for 30 min.
2- Cooling at room temperature (25ºC) with sugar (10 gm/ 150 ml plant drink) for 30 min.

3- Cooling at room temperature (25°C) with honey (10 gm/ 150 ml plant drink) for 30 min.
4- Heat with boiling water without adding sugar or honey for 3 and 6 min.
5- Heat with boiling water with sugar (10 gm/ 150 ml plant drink) for 3 and 6 min.
6- Heat with boiling water with honey (10 gm/ 150 ml plant drink) for 3 and 6 min.
7- Boiling for 3 min at 100 ºC for 3 and 6 min.

Total fungal counts and fungal isolation were studied after the previous treatments according to Areerat *et al*.,(1992).

Moyctoxins production in medicinal plant samples:

Samples were disinfected by immersing in 5% sodium hypochlorite for 2 min, washed thoroughly with sterilized water and dried in hot-air oven at 44ºC for 42 hrs. (Osman ,1982). Samples were divided into four equal amount for (*Aspergillus flavus, A. ochraceus, Fusarium oxysporum and F. rosem*. Spore suspensions isolated from the medicinal plants were prepared from pure cultures (7 days old) grown on PDA plates (9.0 cm), these plates were flooded with 15 ml of sterilized distilled water and brushed thoroughly for 1-2 min. (Anon., 1962).The spore suspension was added to test samples to give final density of approximately 3000-3500 spors/ gram of plant samples as described by Eisa . *et al.* (1996). Moisture content of plant samples were adjusted to required moisture (18%) by adding calculated volumes of sterilized distilled water to the test quantity of samples, then stored for 21 days. Samples contaminated with *A. flavus* were analyzed to incidence of aflatoxins, while other samples contaminated with *A.ochraceus, Fusarium oxysporum,* and *F. rosem* were analysed to measure levels of ochratoxin, zearalenone and fumonisin, respectively.

Antifungal activity:

The concentration of the fungal inoculums was within the range of 10^6 cfu ml^{-1} determined by viable counts technique. The antimicrobial effect of the natural honey was tested using the agar well diffusion method as described by Kacaniova *et al* 2009.The test fungal cultures were used for surface inoculation of Petri dishes containing 15 ml of Sabouraud dextrose agar (SDA). Each Petri dish was aminded with 0.5 ml of strain inoculums streaked thoroughly all over the surface of the agar. Subsequently, four equidistant wells 9 mm in diameter each were punched in the inoculated medium with sterile glass Pasteur pipettes and were filled up with 250 µl of honey sugar syrup (100%or 50% diluted by sterile water)using a precise eppendorph. All plates were incubated at 25ºC and inhibition zones were measured after two days(Kacaniova *et al.*2009). All the experiments were repeated twice. After incubation the zones of inhibition of the growth of the fungi around the disks were measured.

RESULTS

A total of 150 samples of basil, mint, marjoram, chamomile and fennel (30 samples of each kind) were analyzed among moulds and yeasts, (Tables 1 and 2). 53.3% of basil samples showed 10^4 -<10^5 cfu/g for moulds and

36.7% ranged between 10^5-<10^6 cfu/g, whereas 36.7% of dry mint ranged between 10^5-<10^6 cfu/g. While 6.7% ranged between 10^2-<10^3 cfu/g. on the other hand 96.7% of dry mint samples ranged between 10^2-<10^3 cfu/g for yeast. However, 33.3% of marjoram ranged between 10^4-<10^5 cfu/g moulds, 30% of marjoram ranged 10^3-<10^4 cfu/g moulds, while 100% of samples were ranged between 10^2-<10^3 cfu/g for yeasts. For chamomile 56.7% of samples ranged between 10^2-<10^3 cfu/g moulds then 40% of samples ranged between 10^3-10^4 cfu/g and only one sample (3.3%) was between 10^4-<10^5 cfu/g moulds.

On the other hand one chamomile sample (3.3%) exceeds>- 10^6 cfu/g yeast. While 66.7% ranged between 10^2-<10^3 cfu/g yeast, one sample (3.3%) ranged between 10^3-<10^4 cfu/g. While 26.7% were relatively clean. Also 30 samples of fennel were analyzed among moulds and yeasts and it was clear that the count of yeasts and moulds not exceed 10^5 cfu/g (Table 2). 60% of fennel samples ranged between 10^2-<10^3 cfu/g moulds, and 10% of samples ranged between 10^4-<10^5 cfu/g for moulds while 16.7% of samples were less than 10^2 cfu/g. On the other hand for yeast 60%, 10%, 10% and 20% of fennel samples ranged between 10^2-<10^3 , 10^3-<10^4, 10^4-<10^5 and less than 10^2 cfu/g for yeast**.**The highest count of moulds presents in case of dry mint, basil, marjoram, fennel then chamomile respectively. (in descending order). While the highest count of yeasts present in chamomile, dry mint and fennel respectively. Basil and marjoram showed 10^2 cfu/g for yeasts

Table (1): High, low and mean counts (cfu/g) of mould and yeast detected in some Egyptian medicinal plants

Plant type	Basil		Mint		Marjoram		Chamomile		Fennel	
Counts (cfu/g)	Mould	Yeast	Mould	Yeast	Mould	Yeast	Mould	Yeast	Mould	Yeast
High	2.4×10^5	10^2	2.8×10^6	1.2×10^6	1.6×10^5	10^2	2.1×10^4	1.5×10^6	6.5×10^4	2.6×10^4
Low	10^2	10^2	10	10^2	10^2	10^2	10^2	10	10	10
Mean	7.4×10^4	10^2	3.6×10^4	4.0×10^4	3.0×10^4	10^2	1.7×10^3	5.0×10^4	4.6×10^3	2.8×10^3

Table (2): Percentage of fungi and yeast in positive infected medicinal plants

Plant type	Basil		Mint		Marjoram		Chamomile		Fennel	
Counts (cfu/g)	Mould	Yeast	Mould	Yeast	Mould	Yeast	Mould	Yeast	Mould	Yeast
<10^2	0/30 0%	30/30 100%	0/30 0%	0/30 0%	0/30 0%	0/30 0%	0/30 0%	8/30 26.7%	5/30 16.7%	6/30 20%
10^2 -< 10^3	1/30 3%	0/30 0%	2/30 6.7%	29/30 96.7%	8/30 26.7%	30/30 100%	17/30 56.7%	20/30 66.7%	18/30 60%	18/30 60%
10^3 -< 10^4	2/30 6.7%	0/30 0%	0/30 0%	0/30 0%	9/30 30%	0/30 0%	12/30 40%	1/30 3.3%	0/30 0%	3/30 10%
10^4 -< 10^5	16/30 53.3%	0/30 0%	0/30 0%	0/30 0%	10/30 33.3%	0/30 0%	1/30 3.3%	0/30 0%	3/30 10%	3/30 10%
10^5 -< 10^6	11/30 36.7%	0/30 0%	11/30 36.7%	0/30 0%	3/30 10%	0/30 0%	0/30 0%	0/30 0%	0/30 0%	0/30 0%
≥ 10^6	0/30 0%	0/30 0%	17/30 56.7%	0/30 0%	0/30 0%	0/30 0%	0/30 0%	1/30 3.3%	0/30 0%	0/30 0%

Fungal isolates and percentage of prevalence rate of isolation from medicinal plant samples shown in Table (3). Data revealed that the prevalence rate of fungal isolates showed that *Aspergillas niger* (32.38%) had the highest prevalence rate among fungi, in mint samples, respectively. While prevalence rate of *Fusarium oxysporum* were (41.6%), (29.11%), and (20.95%) in samples of fennel, marjoram and chamomile, respectively.The least frequently isolated fungi species were *A. flavus*, *A. candidus, A. ustus* and *Mucor* sp., with the least prevalence rate ranged between 0.0% and 9.36% in most medicinal plant samples under investigation. The predominant fungal species isolated from most samples were *Aspergillus niger, Aspergillus clavatus,* and *Fusarium oxysporum* .All samples of medicinal plants that had high levels of moulds contaminated by *A. flavus, A. ochraceus, F. oxysporum and F. rosem* were free of aflatoxins, (B1, B2, G1 and G2) A. ochratoxin, zearalenone and fumonisin (B1 and B2) respectively.

Table (3): Fungal Isolates and the percentage of prevalence rate of isolation from medicinal plant samples

Isolates fungi	Medicinal plants				
	Basil	Mint	Marjoram	Chamomile	Fennel
Aspergillus niger	32.38	31.19	31.64	24.66	5.86
A. ochraceus	1.27	0.92	2.15	1.45	9.59
A. flavus	4.65	7.79	3.18	2.65	3.95
A. candidus	0.75	0.04	3.08	0.26	0.27
A. ustus	0.75	0.84	9.36	0.66	1.36
Fusarium oxysporum	1.87	0.0	29.11	20.95	41.6
F. rosem	15.55	0.0	1.59	0.0	0.0
Penicillium citrinum	0.90	9.1	1.49	9.94	17.46
Penicillium citreonigrum	0.79	0.84	1.0	13.26	13.64
Mucor spp.	0.0	0.120	4.58	4.11	0.0
Humicola spp..	0.0	29.8	0.0	0.0	0.0

Results in Table (4) showed that *A. flavus* had the ability to produce aflatoxin at level of 3220 ppb. A. ochracious produced ochratoxin A at level of 200 ppb, while *F. oxysporum* showed zearalenone at level of 250 ppb. On contrary, *F. rosem* showed no ability to produce fumonisin.

Table (4): Total aflatoxins, ochratoxin A, zearalenone and fumonisins B1&B2 (ppb) levels produced by spores isolates from the medicinal plants .

Isolates	Mycotoxins production (ppb)			
	Ochratoxin	Total aflatoxins	Zearalenone	Fumonisins B1&B2
A. ochraceus	200.0	0.0	0.0	0.0
A. flavus	0.0	3220.0	0.0	0.0
Fusarium oxysporum	0.0	0.0	250.0	0.0
F. rosem	0.0	0.0	0.0	0.0

The effect of adding honey and/or sugar to some medicinal plants drinks with/without heat on the count of fungi and yeast are shown in Table (5). Data showed that the adding of honey or sugar at room temperature had

no effect on total fungi and yeast counts. Mould count was 5 x 10^4 cfu/g by adding honey to medicinal plants drink. The dominant strains of fungal isolates were *A. flavus, A. niger, P. citrinum, , F.pallidorosem, A. calavatus, F. proliferatum and F. oxysporum*. Using heat and boiling treatments with honey or sugar was more effective on fungal and yeast incidence.

Table (5): The effect of cooling, heating, adding sugar and honey on the counts of fungi and yeast of some medicinal plants:

Counts cfu/g / Treatments	Total fungi	Fungi identification	Yeast
Cooling without sugar or honey 30 min	1×10^4	*A. candidus* *A. calavatus* *F. pallidoroseum*	Zero
Cooling with sugar 30 min	9.5×10^4	*A.niger* *A.flavus* *F.oxsysporum* *F. proliferatum*	Zero
Cooling with honey 30 min	5×10^4	*A.flavus* *A.niger* *P. citrinum*	1.3×10^3
Heat without sugar or honey 3 min 6 min	2×10^2 Zero	*A.niger* *P. citrinum*	Zero Zero
Heat with sugar 3 min 6 min	Zero Zero	-- --	Zero Zero
Heat with honey 3 min 6 min	Zero Zero	-- --	10×10^3 Zero
Boiling for 3 min	Zero	--	Zero

Antifungal activities of honey and sugar(50 % and 100%) against fungi *A. niger, A. flavus, A. calavatus and A. candidus* strains are presented in Table (6). The antimicrobial activity of honey and sugar was assessed by the diameter (mm) of the obtained sterile zones around the disks. Data showed that no inhibition zones were seen against the investigated fungi.

Table (6): Inhibition zone diameters (mm) of honey and sugar against some tested fungi.

Species of Aspergillus	Inhibition zones (mm)			
	Honey		Sugar	
	50%	100%	50%	100%
A. niger	NI	NI	NI	NI
A. candidus	NI	NI	NI	NI
A. calavatus	NI	NI	NI	NI
A. flavus	NI	NI	NI	NI

NI: No inhibition zone.

DISCUSSION

The risk of the presence of microorganisms in a pharmaceutical product depends on this finality of the use, its nature and its potential damage that may be caused to the consumers. Considering natural flora, current production conditions and the need to warrant the quality and the safety of

these products, monographs of the US pharmacopoeia for products that contain raw material of natural origin establish a maximum fungal contamination limit of 2 x 10^2 CFU/g of the product. The results showed that 96.6% of the samples exceeded the limit determined by the US Pharmacopoeia (Anon 2005) and these results are in agreement with those of previous studies (Reif and Metzger, 1995; Roy and Chourasia, 1989

Roy and Kumari, 1991; and Tassaneeyakul *et al*., 2004). The genus *Aspergillus* was the most dominant genus recovered (3761 isolates) followed by *Fusarium* (1023 isolates), while genus *Penicillium* recovered (687 isolates). These three genus were found in 87.42 of the samples analyzed. These results are in agreement with that reported by others (Abou-Arab *et al*., 1999; Aziz *et al*., 1998; Halt, 1998; Mandeel, 2005 and Roy *et al*., 1988). Other fungal genera isolated, such as *Cladosporium, Curvularia, Fusarium and Helminthosporium,* have been reported previously to be the most common resident fungi isolated from medicinal plants under filed conditions (Aziz *et al*., 1998). Bampton and Fusari (1963) reported that the presence of lipolytic mould such as *A. niger* and *Penicillium* sp. is of great concern as they have been implicated in food poisoning.

Strain of *Aspergillus niger* was the most dominant in the collected samples, and these results are in line with that reported by Aziz *et al.* (1998), Abou-Arab *et al.* (1998), and Mandeel (2005), while other reports revealed that strain *A. flavus* particularly, was the main contaminant of different herbal and spices samples (Martins *et al*., 1999). The contamination with fungal species resulted from contamination by dust following storage with humid conditions (Domsch *et al.*, 1981).The isolates of *A. flavus, A. ochruceous, F. oxysporum, and F. rosem* were evaluated for their ability to produce aflatoxins, ochratoxin A, zeralenone, and fumonisin (B1 and B2). There isolates were found to produce mycotoxins. *A. flavus* presented the ability to produce total aflatoxin at level of 3220 ppb; A. ochraceus presented ability to produce ochratoxin at level of 200 ppb; and *F. oxysporum* presented ability to produce zearalenone at level of 250 ppb. On contrary, *F. rosem* presented no ability to produce fumonisin. Bugno *et al.* (2006), demonstrated that the identified moulds have the ability to produce mycotoxins. Contamination by fungal is influenced by the environment, improper handling and storage of medicinal plants.

Frajier and Westhoff (2003) reported that one of the major short comings of herbal preparations in developing countries is the unhygienic condition under which they are produced.

All samples of medicinal plants that contaminated by fungal species were found free mycotoxins. This result agrees with Abou Donia (2008) who reported that different samples of spices and medicinal plants were analyzed for aflatoxins and it was found free of aflatoxins, and this could be explained in light of that spices and medicinal plants are not ideal substrate for aflatoxin formation, due to the presence of essential oils with anti-mycotoxin effects, may inhibit the production of aflatoxin or reduce fungal infestation and/or subsequent aflatoxins production. Mandeel (2005), reported that although high fungal loads may be accepted due to the natural origin of those products, they indicate the potential for spoilage and mycotoxigenesis.

Adding of honey or sugar to medicinal plants drinks as a sweeters at room temperature had no effective on the fungal load. The results were different from the preliminary reported by Obaseiki-Ebror *et al*.(1983) and (1984) who showed that honey provided the antifungal activity against *Aspergillus* spp and *Penicillium* spp. Hamid and Saeed (1991) reported that three major system are responsible for the antimicrobial activity of honey i.e. inhibition, high osmotic pressure and acidity. On contrary, our results are in agreement with that suggested by Areerat *et al*. (1992), who reported that honey samples has no antifungal effect on A. niger and 2 yeasts: *S. cerevisiae* and *C. albicans*. No inhibitory effect was noted on *E. coli, S. avreus, A. niger* and *C. albicans* (Muli *et al*., 2008). However, there was only a few reports referred to the antifungal activity of honey.

Conclusion

In the present study, 96.6% of the medicinal plants samples did not comply with the maximum acceptable limit for fungal contamination. Among fungal isolates the presence of the genera *Aspergillus, Fusarium,* and *Penicillium* was greater than other genera. All medicinal plants samples under investigation were free of mycotoxins. Fungal isolates presented the ability to produce mycotoxins, such as aflatoxins, ochratoxins, and zearalenone.

Adding of honey or sugar to medicinal plants drinks at room temperature has no antifungal effect. High thermal treatment was recommended before use these drinks. It was also concluded that medicinal plants may be high risk products as it contained many species of moulds.

Considering the worldwide increased use of herbal products as alternative medicines and the risk of purchase and use of natural products contaminated with moulds and mycotoxins, it is necessary setting appropriate standards for toxigenic moulds and mycotoxins in crude herbal drugs and medicinal plants in order to reduce the risks for consumers' health.

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دراسة الحمل الميكروبي والسمية الفطرية لبعض النباتات الطبية المصرية

محمد فتحي أبو العلا، زينب محمد عبد الغني، محمد عبد المطلع عطوة و خالد مصطفى المليجي

المركز الإقليمي للأغذية والأعلاف، مركز البحوث الزراعية، الجيزة

هدفت هذه الدراسة الي قياس مدى التلوث الفطري لبعض النباتات الطبية المصرية (البرداقوش والريحان والنعناع والشمر والبابونج) حيث تم اختيار ١٥٠عينةعشوائيا من الأسواق المصرية ،وتم العزل الفطرى ودراسة الحمل الميكروبى لهذة النباتات و تقديرالسموم الفطرية (الأفلاتوكسين والأوكراتوكسين والزيراليون والفيومينزين).

أوضحت الدراسة أن عينات النعناع هي الأكثر تلوثا بالفطريات ويليها عينات الريحان والبراداقوش والشمر والبابونج على التوالي. كانت أهم الأجناس الفطرية هي الإسبرجلس والفيوزاريوم والبنسيليوم والميوكر والهيوميكولا. وأوضحت النتائج أن عزلات الأسبرجلس كانت هي الأكثر عددا (٣٧٦١ عزلة) ثم عزلات البنسيليوم (٦٨٧ عزلة). وكانت ٨٧.٤١% من العينات ملوثة بالثلاثة أجناس سابقة الذكر.

وتم دراسة مدى قدرة العزلات الفطرية الملوثة المعزولة من هذة النباتات الطبية علي إنتاج السموم الفطرية (الأفلاتوكسين والأوكراتوكسين والزيراليون والفيومينزين) تحت الظروف المثلى . وأشارت النتائج الي إمكانية فطر الاسبرجلس فلافيس علي إنتاج ٣٢٢٠ جزء في البليون من سموم الأفلاتوكسينات.

وأوضحت الدراسة أن طريقة النقع لهذه النباتات الطبية تعتبر طريقة ليست مثالية نتيجة للحمل الميكروبى والسموم الفطرية الموجود بها و الافضل استخدام الحرارة العالية.

وتم دراسة تأثير اضافة السكر او العسل عند تقديم هذه المشروبات الطبية. أشارت النتائج إلى انه لم يكن لإضافة عسل النحل الخام أو السكر كمحليات طبيعية أي تأثير على الحمل الفطري لتلك المشروبات.

قام بتحكيم البحث

أ.د / محمد منصور قاسم	**كلية الزراعة – جامعة المنصورة**
أ.د / الشحات محمد رمضان	**كلية الزراعة – جامعة عين شمس**