FUNGAL LOAD AND MYCOTOXINOGENESIS OF SOME EGYPTIAN MEDICINAL PLANTS
Abol-Elia, 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% 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 (Akerel, 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 (Akerel, 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., 2008 and 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, Mustar 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 marjorana), 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°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 (25°C) without sugar or honey for 30 min.
2- Cooling at room temperature (25°C) with sugar (10 gm/ 150 ml plant drink) for 30 min.
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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 °C 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 44°C 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 spores/ 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 analyzed to measure levels of ochratoxin, zearalenone and fumonisins, 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 Kacanoiava 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 25°C and inhibition zones were measured after two days( Kacanoiava 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 $10^3$-$10^5$ cfu/g, whereas 36.7% of dry mint ranged between $10^2$-$10^3$ cfu/g. While 6.7% ranged between $10^2$-$10^5$ 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 $10^4$-$10^5$ 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 $10^2$-$10^5$ cfu/g moulds then 40% of samples ranged between $10^3$-$10^4$ cfu/g and only one sample (3.3%) was between $10^3$-$10^5$ 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 $10^2$-$10^5$ 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 $10^2$-$10^5$ cfu/g moulds, and 10% of samples ranged between $10^2$-$10^5$ 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 $10^5$ 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

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Basil</th>
<th>Mint</th>
<th>Marjoram</th>
<th>Chamomile</th>
<th>Fennel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts (cfu/g)</td>
<td>Mould</td>
<td>Yeast</td>
<td>Mould</td>
<td>Yeast</td>
<td>Mould</td>
</tr>
<tr>
<td>High</td>
<td>$2.4 \times 10^4$</td>
<td>10</td>
<td>$2.8 \times 10^4$</td>
<td>10</td>
<td>$1.2 \times 10^4$</td>
</tr>
<tr>
<td>Low</td>
<td>$10$</td>
<td>10</td>
<td>$10$</td>
<td>10</td>
<td>$10$</td>
</tr>
<tr>
<td>Mean</td>
<td>$7.6 \times 10^4$</td>
<td>10</td>
<td>$3.6 \times 10^4$</td>
<td>10</td>
<td>$4.0 \times 10^4$</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Basil</th>
<th>Mint</th>
<th>Marjoram</th>
<th>Chamomile</th>
<th>Fennel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts (cfu/g)</td>
<td>Mould</td>
<td>Yeast</td>
<td>Mould</td>
<td>Yeast</td>
<td>Mould</td>
</tr>
</tbody>
</table>
| $<10^2$ | 0/30 | 0% | 0/30 | 0% | 0/30 | 0% | 0/30 | 0% | 0/30 | 0%
| $10^2$-$10^3$ | 1/30 | 0.3% | 0/30 | 0% | 2/30 | 6.7% | 29/30 | 96.7% | 6/30 | 20%
| $10^3$-$10^4$ | 2/30 | 6.7% | 0/30 | 0% | 0/30 | 0% | 9/30 | 30% | 0/30 | 0%
| $10^4$-$10^5$ | 16/30 | 53.3% | 0/30 | 0% | 0/30 | 0% | 10/30 | 33.3% | 0/30 | 0%
| $10^5$-$10^6$ | 11/30 | 36.7% | 0/30 | 0% | 0/30 | 0% | 11/30 | 36.7% | 0/30 | 0%
| $>10^6$ | 0/30 | 0% | 0/30 | 0% | 0/30 | 0% | 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 Aspergillus 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. roseum were free of aflatoxins, (B1, B2, G1 and G2). A. ochratoxin, zearalenone and fumonisins (B1 and B2) respectively.

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

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

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. roseum showed no ability to produce fumonisins.

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

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Mycotoxins production (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ochratoxin</td>
</tr>
<tr>
<td>A. ochraceus</td>
<td>200.0</td>
</tr>
<tr>
<td>A. flavus</td>
<td>0.0</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>0.0</td>
</tr>
<tr>
<td>F. roseum</td>
<td>0.0</td>
</tr>
</tbody>
</table>

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 $5 \times 10^4$ 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:

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Counts cfu/g</th>
<th>Total fungi</th>
<th>Fungi identification</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooling without sugar or honey</td>
<td>1 $\times$ 10^4</td>
<td>A. candidus</td>
<td>A. calavatus</td>
<td>Zero</td>
</tr>
<tr>
<td>Cooling with sugar</td>
<td>9.5 $\times$ 10^4</td>
<td>A.niger</td>
<td>A.flavus</td>
<td>Zero</td>
</tr>
<tr>
<td>Cooling with honey</td>
<td>5 $\times$ 10^4</td>
<td>A.flavus</td>
<td>A.niger</td>
<td>1.3 $\times$ 10^3</td>
</tr>
<tr>
<td>Heat without sugar or honey</td>
<td>2 $\times$ 10^2</td>
<td>A.niger</td>
<td>P. citrinum</td>
<td>Zero</td>
</tr>
<tr>
<td>Heat with sugar</td>
<td>Zero</td>
<td>A.niger</td>
<td>P. citrinum</td>
<td>Zero</td>
</tr>
<tr>
<td>Heat with honey</td>
<td>Zero</td>
<td>10 $\times$ 10^3</td>
<td>Zero</td>
<td>Zero</td>
</tr>
<tr>
<td>Boiling for 3 min</td>
<td>Zero</td>
<td></td>
<td></td>
<td>Zero</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Species of Aspergillus</th>
<th>Inhibition zones (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Honey 50%</td>
</tr>
<tr>
<td>A. niger</td>
<td>0</td>
</tr>
<tr>
<td>A. candidus</td>
<td>0</td>
</tr>
<tr>
<td>A. calavatus</td>
<td>0</td>
</tr>
<tr>
<td>A. flavus</td>
<td>0</td>
</tr>
</tbody>
</table>

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
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these products, monographs of the US pharmacopoeia for products that contain raw material of natural origin establish a maximum fungal contamination limit of $2 \times 10^2$ 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 genera 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 field conditions (Aziz et al., 1998). Bampton and Fusari (1963) reported that the presence of lipolytic mould such as $A. \text{niger}$ and $P. \text{sp.}$ is of great concern as they have been implicated in food poisoning.

Strain of $A. \text{niger}$ 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 $A. \text{flavus}$ 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 $A. \text{flavus}$, $A. \text{ochraceus}$, $F. \text{oxysporum}$, and $F. \text{roseum}$ were evaluated for their ability to produce aflatoxins, ochratoxin A, zearalenone, and fumonisins (B1 and B2). There isolates were found to produce mycotoxins. $A. \text{flavus}$ presented the ability to produce total aflatoxin at level of 3220 ppb; $A. \text{ochraceus}$ presented ability to produce ochratoxin at level of 200 ppb; and $F. \text{oxysporum}$ presented ability to produce zearalenone at level of 250 ppb. On contrary, $F. \text{roseum}$ presented no ability to produce fumonisins. 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 sweeteners 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 الفطري لبعض النباتات الطبية المصرية
محمد فتحي أبو العلا، زينب محمد عبد الغني، محمد عبد المطلب عطوة و خالد مصطفى الملكي

المركز الإقليمي للأغذية والأعلاف، مركز البحوث الزراعية، الجيزة

هَدَّت هذه الدراسة إلى قياس مدى الثبوت الفطري لبعض النباتات الطبية المصرية (البراقوق والريحان والنعناع والشمر والبايكونج) حيث تم اختبار 50 عينة من الأغذية المصغرة وتم العزل الفطري ودراسة الحمل الميكنوري لهذه النباتات وتقدير السوسم الفطري (الألافاتكسين والأوكراتكسين والزيروفان والميوكون).

أوضحت الدراسة أن عينات النعناع هي الأكثر ثباتًا بالفطريات وليبيا عينات الريحان والبراقوق والشمر والبايكونج على التوالي. كانت أمض الأدلة الفطري هي الإسرج والفوزراتون والميوكون والميوكون. وأوضحت النتائج أن عزلات الأسيرج كانت هي الأكثر عدًا (3761 عزلة) ثم عزلات البايكونج (3671 عزلة). وكانت 87.41% من العينات ملوأة بالثلاثة أجناس مماثلة للذكاء.

وتعد دراسة مدى عدد العلاجات الفطرية الملؤة المعروفة من هذه النباتات الطبية على إنتاج السوسم الفطري (الألافاتكسين والأوكراتكسين والزيروفان والميوكون) تحت ظروف المختبر. و أشارت النتائج إلى إمكانية عشر الأسيرج لإنتاج جزء 3220 جزيء في البايكونج من سوسم الألافاتكسينات.

وأوضح الدراسة أن هذه النباتات الطبية تعتبر طرقية ليست متواضعة نتيجة للحمل الميكروبي والسوسم الفطري الموجودة بها. و تعدد البكتيريا التي تحدث هذه المشروبات الطبية. أظهرت النتائج إلى أنه لم يكن لإضافة عسل النحل الخام أو السكر كمكملات طبيعية أي تأثير على الحمل الفطري لهذه المشروبات.

قام تحكم البحث

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

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