

Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market

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Abstract

Pesticide residues, heavy metal contents and aflatoxins were estimated in five medicinal plants frequently used by both infants and adults (peppermint, chamomile, anise, caraway and tilio). Samples were collected from different sources in the Egyptian market. Results showed that malathion, dimethoate and profenofos predominated in most of the analysed samples. On the other hand, the lowest mean levels were detected with aldrin, dieldrin, chlordane and lindane. Chlorpyrifos, parathion, diazinon and endosulfan were not detectable in most of the samples under investigation. The results indicated that some of the collected samples contained some types of organophosphorus and organochlorine pesticide residues, within the limits of The Egyptian Organization for Standardization and Quality Control (EOS) maximum limits for pesticide residues on medicinal aromatic plants (1991) and Pharmeuropo (1993). Heavy metal contents in the collected samples, i.e. Fe, Cu, Mn, Zn, Pb, Cd, Cr, Co, Sn and Ni, were found at different levels. The highest mean levels of Pb, Zn, Cu, and Fe were found in chamomile flower samples, while those of Cd, Cr and Mn, were detected in peppermint and of Ni, Co and Sn in caraway samples. The results also showed that the most frequently isolated fungi were *penicillium* sp., *A. niger* and *Fusarium* sp. Nevertheless, the finding of natural mycotoxin contamination was negative in all samples. © 1999 Published by Elsevier Science Ltd. All rights reserved.

Keywords: Pesticide; Heavy metal; Aflatoxin; Medicinal plants

1. Introduction

Medicinal plants are widely consumed as home remedies and raw materials for the pharmaceutical industries. The past decade has seen a significant increase in the use of herbal medicine. Environment in developing countries, pollution in irrigation water, atmosphere, soil, sterilization methods and storage conditions all play an important role in contamination of medicinal plants by pesticides, heavy metals and aflatoxins.

Pesticides represent a very serious health and environmental problem; preventing their eventual adverse effects is much more difficult than is the case with other substances used in industry (Illes, Mestres, Tourte,

Campo & Illes, 1976). Analytical publications on pesticide residues in crude herbal materials indicate that the presence of chlorinated pesticides is quite common. Other potentially contaminating pesticides include organophosphates, carbamate insecticides and herbicides, dithiocarbamate fungicides and triazine herbicides (Sovljanski, Lazic, Macko & Obradovic, 1990).

The sources of environmental pollution with toxic metals are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients, such as cadmium-containing dung, organic mercury fungicides, and the insecticide lead arsenate (Schilcher, Peters & Wank, 1987). Heavy metals may contaminate different plants causing serious health hazards such as injury of kidney, symptoms of chronic toxicity, renal failure and liver damage. Several investigators have performed studies on residual levels

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of toxic metals in medicinal herbs (Gravel, Yakoulev, Petrrov, Stuloukii & Listov, 1994).

Contamination of herbal medicine raw materials with aflatoxins can cause potential carcinogenic effects if absorbed even in small amounts (Schilcher et al., 1987). There have been few reports on aflatoxin levels in herbs and spices. Several authors, for the determination of aflatoxins, (Wang, Tan & Wee, 1993) have studied medicinal plants.

The medicinal plant raw materials normally carry a great number of molds, often from soil. Current practices of harvesting, handling and production, cause additional contamination and microbial growth.

Economical value of the medicinal plant exportation, as well as frequent use of some herbal preparations in infant, elderly and even normal persons evoked the authors to plan this work.

The aim of the present research is to study some medicinal plant contaminants to assure safety and quality.

2. Materials and methods

2.1. Sample collection

Twenty samples (ten for both packed and non-packed) each of peppermint (*Mentha piperita* L.), chamomile flowers (*Matericaria chamomilla* L.), anise (*Pimpinella anisum* L.), caraway (*Carum cravi* L.), and tilio (*Lindin blossom* L.), were collected randomly from the Egyptian market.

2.2. Standards

Pesticide standards were purchased from Chem. Service, Inc. (West Chester, PA). Merck (Darmstadt, Germany) provided heavy metal standard solutions. Aflatoxin standards (B₁, B₂, G₁, and G₂) were provided by Sigma.

2.3. Determination of pesticide residues, heavy metals and aflatoxin

Residues of pesticide, as well as heavy metal contents, in medicinal plant samples were determined according to the method of WHO/PHARM (1992). Aflatoxins were determined according to the method of the American Spice Trade Association (ASTA, 1976).

2.4. Isolation and identification of molds

Fungi associated with packed and non-packed medicinal plants (peppermint, chamomile, anise, caraway and tilio) were isolated and identified according to the method of Louw and Webely (1959), Allen (1961), Gil-

man (1957), Barnett and Hunter (1972) and Nelson, Toussoun and Marasas (1983).

3. Results and discussion

Table 1 shows the distribution of some types of organophosphorus and organochlorine pesticide residues in the samples under investigation. Results indicate that malathion, dimethoate and profenofos predominated in most samples. The highest mean levels of malathion (0.694) and chlordane (0.212) mg/kg were detected in chamomile II, while the anise II samples showed the highest levels of profenofos (0.611), followed by total DDT and its derivatives (0.575), total lindane and aldrin (0.255) and endrin (0.091) mg/kg. However, the highest levels of pirimiphos-methyl (0.121), dimethoate (1.141) and HCB (0.608) mg/kg were recorded in peppermint, tilio II and chamomile I, respectively. The percentage of contaminated samples with the previous pesticides ranged from 70 to 100%.

From the monitoring data, the results obtained indicated that the collected samples contained various types of organophosphorus and organochlorine pesticide residues. Organophosphorus pesticides are widely used in agriculture for the control of various insects. These compounds have higher acute toxicity than other chlorinated pesticides and they have the advantage of being more rapidly degraded in the environment. Moreover, organochlorine pesticides are highly persistent insecticides which were used in Egypt, more than 20 years ago. Most of them have been banned, yet their residues still appear as pollutants in food as well as in the environment. Aldrin and dieldrin were expected to appear in different types of plants.

It was realized from the monitoring data in Table 1 concerning DDT and its derivatives, that the residues detected in medicinal plants were at decreased levels and low occurrences. It can be concluded that DDT was one of the most important and probably the most common insecticide in the world as well as in Egypt, where it was extensively used for insect control. The decreased levels with low occurrences of DDT and its derivatives in the current study may be due to the Agricultural Ministry of Egypt's advice to the farmers not to use DDT. However, significantly elevated levels of this insecticide were detected in samples under investigation. Probably these insecticides are still being applied, despite the Ministry's recommendation. Continuous contamination with DDT might be due to low concentrations in the irrigation water, mainly from the River Nile (El-Mekkwawi, 1994), which passes through other developing countries in which organochlorines are still in use.

It can be concluded that the levels of organophosphorus pesticides under investigation in different plants,

Table 1
The mean levels (mg/kg) of pesticide residues detected in the studied samples of medicinal plants collected from Egyptian markets

Organophosphorus	Peppermint		Chamomile		Anise		Caraway		Tilio	
	I ^a	II ^b	I	II	I	II	I	II	I	II
Organophosphorus										
1-Malathion	0.211	0.316	0.406	0.694	ND ^c	ND	0.094	0.141	0.009	0.061
2-Dimethoate	0.201	0.349	0.096	1.09	0.121	0.114	0.214	0.64	0.006	1.141
3-Profenofos	0.200	0.386	ND	ND	ND	0.611	ND	0.096	0.008	ND
4-Primiphos Me.	ND	0.121	ND	0.116	ND	0.016	ND	ND	ND	0.031
5-Chlorpyrifos	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6-Parathion	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7-Diazinon	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Organochlorines										
1-O.P-DDT	0.006	0.114	0.011	0.024	ND	0.311	ND	0.048	0.004	0.043
2-P.P-DDT	ND	ND	ND	ND	ND	ND	ND	0.095	0.006	ND
3-O.P-DDD	0.091	0.116	ND	ND	0.006	ND	ND	0.048	ND	0.064
4-P.P-DDD	ND	0.078	ND	2.81	ND	0.058	ND	0.084	ND	0.031
5-O.P-DDE	ND	0.060	0.018	0.018	ND	0.206	0.034	0.076	ND	0.103
6-P.P DDE	0.014	0.016	0.018	0.810	0.008	ND	0.021	ND	0.061	ND
Total	0.111	0.384	0.029	0.039	0.014	0.575	0.055	0.352	0.071	0.241
7-Lindane	0.036	0.028	0.201	0.501	ND	0.346	0.061	0.311	ND	0.081
8-Aldrin	ND	0.061	ND	0.006	ND	0.164	0.011	0.196	ND	0.048
9-Dieldrin	ND	0.011	ND	ND	ND	0.091	ND	ND	ND	ND
Total	ND	0.072	ND	0.006	ND	0.255	0.011	0.196	ND	0.048
10-Heptachlor	ND	0.168	0.071	0.062	ND	0.361	0.031	0.491	ND	0.036
11-Hep. Epoxide	ND	ND	ND	ND	ND	ND	0.003	ND	ND	ND
Total		0.168	0.071	0.062	ND	0.361	0.034	0.491	ND	0.036
12-Chlordane	0.064	0.176	ND	0.212	ND	0.11	ND	0.091	ND	0.053
13-HCB	ND	0.118	0.608	0.416	ND	0.121	ND	0.361	ND	0.262
14-Endrin	ND	0.068	ND	0.089	ND	0.091	ND	0.016	ND	0.015
15-Endosulfan	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

^a I = Packed samples.

^b II-non-packed samples.

^c ND-non detectable.

except dimethoate in non-packed chamomile flowers, caraway and tilio samples, were below the maximum residue levels (MRLs) proposed by the Egyptian Organization for Standardization and Quality Control (EOS, 1991) and Pharmedropa, (1993). It can also be seen that levels of organochlorine pesticides in the present study were below the MRLs, except for aldrin and dieldrin (0.05), heptachlor and its epoxide (0.02), chlordane (0.10), HCB (0.10) and endrin (0.01) (ppm) in some samples.

Considering these results, it could be recommended that the advantages of the application of pesticides in agriculture for producing better plants must be weighed, against the possible health hazard arising from the toxic pesticide residues in food. Pesticides should be applied correctly according to good agricultural practice, such as using only the required amounts, following label directions and avoiding spraying before rain or spraying in windy conditions. Spray tank rinse water and empty pesticide containers should also be disposed of properly.

4. Monitoring of heavy metal contents in medicinal plants

Heavy metal contents in the samples under investigation are shown in Table 2. Results reveal that the highest mean levels of Pb (0.242 and 0.308), Zn (116 and 122), Cu (8.9 and 10.4) and Fe (105 and 125) mg/kg were found in chamomile flower samples. However, peppermint samples showed the highest mean levels of Cd (0.294 and 0.306), Cr (0.088 and 0.096), and Mn (36.9 and 38.8) mg/kg. The caraway samples had the highest mean levels of Ni, Co, and Sn (0.711 and 0.814), (0.091 and 0.162) and (0.06 and 0.04) mg/kg, respectively. On the other hand, the lowest mean levels of Pb and Zn were observed in peppermint, being (0.046 and 0.081) and (34.6 and 38.8) mg/kg, respectively. It is clear from the same table that the lowest levels of Cd (0.054 and 0.081), Ni (0.114 and 0.106), Co (0.061 and 0.042) and Fe (44.4 and 36.6) mg/kg were detected in anise samples. Data also indicated that caraway samples had the lowest mean levels of Cr and Cu, which showed

Table 2
The levels (mg/kg) of heavy metals in medicinal plants collected from Egyptian markets

Metal	Peppermint		Chamomile		Anise		Caraway		Tilio	
	I	II	I	II	I	II	I	II	I	II
Lead	0.046	0.081	0.242	0.308	0.211	0.224	0.103	0.214	0.096	0.121
Cadmium	0.294	0.306	0.094	0.211	0.054	0.081	0.211	0.226	0.088	0.141
Chromium	0.088	0.096	0.084	0.069	0.064	0.088	0.036	0.050	0.064	0.196
Nickel	ND	ND	ND	ND	0.114	0.106	0.711	0.814	ND	ND
Cobalt	ND	ND	ND	ND	0.061	0.042	0.091	0.162	0.049	0.064
Zinc	34.6	38.8	116	122	50.5	61.8	40.4	38.0	42.4	60.4
Manganese	36.9	38.8	25.40	28.80	36.4	36.6	21.6	28.4	33.1	36.4
Copper	6.10	6.90	8.90	10.40	3.60	4.10	1.04	1.38	4.4	4.90
Iron	76.4	84.8	104.8	125	44.4	36.6	62.6	66.4	46.9	49.4
Tin	ND	ND	ND	ND	ND	ND	0.060	0.040	ND	ND

(0.036 and 0.050) and (1.04 and 1.38) mg/kg, respectively. It could be observed from the results, that peppermint and chamomile did not contain Ni, Co and Sn. However, Ni and Sn were not detectable in tilio samples, while Sn was not detectable in anise samples.

There are two major reasons to monitor levels of toxic metals in medicinal plants. First, contamination of the general environment with toxic metals has increased. The sources of this environmental pollution are quite varied, ranging from industrial and traffic emissions to the use of purification mud and agricultural expedients, such as cadmium-containing dung, organic mercury fungicides, and the insecticide lead arsenate (Gosslim, Smith, Hodge & Braddock, 1984; Schilcher et al., 1987). Second, exotic herbal remedies, particularly those of Asian origin, have been repeatedly reported to have toxic levels of heavy metals and/or arsenic. As these two types of contamination are quite different, both in their nature and in their health risks, they will be treated separately.

The relatively high concentrations of Pb and Cd found in different samples are certainly due to irrigation with contaminated water, as well as the addition of some fertilizers and herbicides. In addition, it was reported that the contamination of plants with lead depends on several factors, e.g. traffic densities and distance from the road (Bosque, Schuhmacher, Domino & Lobet, 1990). Pb accumulated in plants is more pronounced at locations close to the emission source of Pb vapour and fine particles. On the other hand, heavy metal contents of medicinal plants depend on the plant species and climatic factors (Sovljanski et al., 1990). It is notable that there were no significant correlations between heavy metal contents in soil and cultivated medicinal plants (Yoo-Sung & Song-Kyung-Sik, 1991). If we compare the results with the data of Kim, Kim, Lee and Yoo (1994), which investigated the contents of heavy metals in 291 samples of medicinal plants, grown on unpolluted sites, data coincided with the results obtained in our samples in most cases. They reported

that the contents of Cd, Cu, Pb, Zn, Cr, Ni and As in the plants were 0.386, 6.636, 0.817, 27.776, 1.448, 0.729 and 0.277 mg/kg, in that order. They reported also that the average contents of Cd, Cu, Pb, Zn, Cr, Ni and As in the soil were 0.195, 3.391, 2.236, 1.930, 0.967, 3.993 and 0.588 mg/kg, respectively. On the other hand, Gravel et al. (1994) and Januz et al. (1994) determined the content of heavy metals in industrialized regions. They reported that the plants growing in an industrialized region had higher contents of heavy metals than plants growing in a second less industrialized region.

Some types of metal, such as Cu, Mn and Zn, are the natural essential components of enzymes and coenzymes and are important for growth, photosynthesis and respiration. Although other metals, such as Pb and Cd, have no biochemical or physiological importance, so they are considered as very toxic pollutants (Sovljanski et al., 1990).

The current results show that the levels of various metals coincided with those given by Listow and Petrov (1990). On the other hand, the levels of various metals were lower than those obtained from Saudi Arabia, Bahrain, and Iraq by Al-Kathiri and Al-Attar (1997), Al-Saleh and Chudasama (1994) and Jawad, Al-Khafaji and Ali (1986), respectively.

As concrete threshold limits could not be found in the European Pharmacopoeia or the German Pharmacopoeia we took limit values of the so-called Zentrale Erfassungs und Bewertungsstelle für Umweltchemikalien (ZEBS) as a point of reference. The national ZEBS regulation offers maximum allowable values for herb-like food products, such as grains and vegetables (Table 3). It should be noted that the limits usually refer to the fresh weight of foodstuffs destined for consumption, whereas medicinal plants are generally tested in their dried form (Schilcher et al., 1987). Comparing the results of this investigation with the ZEBS limits, medicinal plants were found to exceed with Cd but were lower with Pb. Failure to meet those limits does not

Table 3
Maximum allowed levels of heavy metal in foodstuffs, according to ZEBs regulation (Schilcher et al., 1987)

Metal	Year or ZEBs	Maximum allowed level per type of food stuff (mg/kg)				
		Grain	Rye/rice	Fruit/root vegetables	Leavy vegetables	Sprout vegetables
Lead	1979	0.5	ND	0.5	1.2	1.2
	1986	ND	0.4	0.25	0.8	0.5
Cadmium	1979	0.1	ND	0.1	0.1	0.1
	1986	ND	0.1	0.1	0.1	0.1

imply, however, that a real health risk is involved. Comparison of test results with the ZEBs values should merely be considered as a tool of quality assurance for the timely detection of potentially undesirable contamination (Schilcher et al., 1987).

5. Aflatoxinogenesis and residues in medicinal plants

5.1. Fungal contamination of medicinal plants

Thirteen isolates belonging to different genera were isolated and identified from packed and non-packed samples of medicinal plants (peppermint, chamomile, anise, caraway, and tilio).

The results presented in Table 4 showed that the *Asperigillus* and *Penicillium* genera were more frequently detected and in greater abundance in the packed (I) and non-packed (II) samples than other genera of fungi. Flannigan and Hu (1976) found similar results.

Concerning *Asperigillus*, it was found that, *A. ochraceus* was detected in only two kinds of medicinal samples, i.e. peppermint and caraway in 16.7 and 33.3%, respectively. *A. ochraceus* was not detectable in other types of medicinal plants under investigation. *A. flavus*, was

detected in all kinds of samples in different degrees, while it was not present in packed tilio samples. Table 5 shows the frequency of occurrence of fungi in the tested medicinal plants. *Penicillium* spp is the predominant fungus in all kinds of these samples. The highest percentage of *penicillium* spp infection was recorded in packed samples, especially caraway (56%), followed by non-packed tilio (76.9%). While a lower percentage of infection was recorded in non-packed anise samples (18.7%).

For the *A. flavus* infection, the results showed that all tested medicinal plants were infected with the exception of packed tilio. The highest percentage of infection was in peppermint (15.8%) followed by non-packed tilio (15.4%), as well as non-packed caraway (13.5%). The other tested medicinal plants showed a low percentage of *A. flavus* infection.

In some kinds of the tested medicinal plants, the percentage of infection in packed samples was higher than those non-packed and this may be due to other factors, such as, rise of humidity inside the pack and also unsuitable methods of keeping and storing of the pack. Generally, bad circumstances cause high infection by fungi. Many investigators recorded the presence of mold in medicinal plants, drugs and herbs (Chourasia, 1995; Roy & Chourasia, 1989; Yamazaki et al., 1980).

5.2. Monitoring of aflatoxin in medicinal plant samples

The previous results in Tables (4 and 5) show the presence of molds in some medicinal plants. Muralimohan and Reddy (1995) and Chourasia (1995) reported that *A. flavus*, *A. ochraceus* and many other types including *penicillin*, are known for their mycotoxin-producing ability and potential hazards to human and animal health. Shotwell (1977) found that *A. parasiticus* produces aflatoxins.

In the present study *A. flavus* was predominant in most samples with the highest level in peppermint.

Table 4
The percentage of the infected samples with fungi of the studied medicinal plants collected from the Egyptian market

Isolated mold	% infected sample							
	Peppermint		Anise		Caraway		Tilio	
	I	II	I	II	I	II	I	II
<i>Alternaria</i>	33.3	50.0	100.0	50.0	0.0	0.0	50.0	0.0
<i>A. crothecium</i>	0.0	0.0	0.0	0.0	0.0	33.3	100.0	0.0
<i>A. condius</i>	0.0	16.7	0.0	0.0	0.0	0.0	0.0	0.0
<i>A. flavus</i>	33.3	33.3	50.0	50.0	50.0	33.3	0.0	100.0
<i>A. niger</i>	33.3	100.0	0.0	100.0	100.0	33.3	0.0	100.0
<i>A. ochraceus</i>	0.0	16.7	0.0	0.0	0.0	33.3	0.0	0.0
<i>A. terreus</i>	33.3	50.0	0.0	0.0	50.0	0.0	0.0	0.0
<i>Fusarium</i> spp	0.0	33.3	50.0	50.0	0.0	33.3	0.0	0.0
<i>Penicillium</i> spp	100.0	83.3	100.0	100.0	100.0	100.0	100.0	100.0
<i>Rhizoctonia</i>	0.0	0.0	0.0	0.0	0.0	33.3	0.0	0.0
<i>Trichoderma</i>	0.0	16.7	50.0	0.0	0.0	0.0	0.0	0.0

Table 5
The frequency (%) occurrence of fungi associated with some medicinal plants

	Tilio	Caraway	Anise	Peppermint	Fungi
I	20.0	0.0	55.0	25.0	<i>Alternaria</i>
II	0.0	0.0	10.0	5.3	
II	33.0	0.0	0.0	0.0	<i>A. crothecium</i>
I	0.0	0.0	0.0	0.0	
I	0.0	0.0	0.0	0.0	<i>A. condius</i>
II	0.0	0.0	0.0	1.3	
I	0.0	12.8	6.2	15.8	<i>A. flavus</i>
II	15.4	13.5	5.0	15.8	
I	0.0	18.7	0.0	15.8	<i>A. niger</i>
II	7.7	5.0	30.0	18.4	
I	0.0	0.0	0.0	0.0	<i>A. ochraceus</i>
II	0.0	0.0	0.0	2.6	
I	0.0	12.5	0.0	5.3	<i>A. terreus</i>
II	0.0	0.0	0.0	5.3	
I	0.0	0.0	20.0	0.0	<i>Fusarium</i> spp
II	0.0	5.0	20.0	3.9	
I	47.0	56.0	18.7	36.9	<i>Penicillium</i> spp
II	76.9	45.0	35.0	47.3	
I	0.0	0.0	0.0	0.0	<i>Rhizotonia</i>
II	0.0	31.5	0.0	0.0	
I	0.0	0.0	0.0	1.3	<i>Trichoderma</i>
II	0.0	0.0	0.0	0.0	

However, this result is considered as relatively high, and mycotoxin contamination was not detected.

Results show that medicinal plants are not ideal substrates for aflatoxin formation, due to their essential oils, which may inhibit the aflatoxin production. This agrees with Lwellyn, Mooney, Cheatle, and Flannigan (1992) and is indicative of a causal contamination after harvesting and drying. Eppley (1968) found that mycotoxin-producing molds are quite ubiquitous and frequently contaminate food and agricultural commodities. However, the presence of toxigenic mold in food does not automatically mean the presence of mycotoxin and vice versa.

Several researchers reported that, no aflatoxins were present in different crude herbal drugs, even though these samples were relatively heavily contaminated with *Aspergillus* (Hitokoto, Morozumi, Wauke, Sakai, & Kurata, 1978; Hila, Sayed, Mahmoud, & El-Kirsh, 1986; 1987; Leimbeck, Soliman, Mahmoud, & El-Kirsh, 1987). On the other hand, Kumari, Chourasia, and Roy, (1989) and Salem and Slim (1994) recorded that, low concentrations of aflatoxins occurred in a few samples of herbs and medicinal plants.

It should be mentioned that the isolated *A. flavus* was grown on corn substrate and produced aflatoxins with different concentration.

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