Research Article

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# Analysis of aflatoxins, caffeine, nicotine and heavy metals in Palestinian multifloral honey from different geographic regions

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## Abstract

**BACKGROUND: Honey is a healthy and nutritious natural product. However, it may contain some natural as well as anthropogenic contaminants that can affect consumer health. The present study was aimed at analysing the content of aflatoxins, nicotine, caffeine and heavy metals in Palestinian multifloral honey.**

**RESULTS: The results indicated the presence of variable amounts of aflatoxins (0.5–22 μg kg$^{-1}$, mean 12.1 μg kg$^{-1}$) in all samples analysed, with the highest levels in honey from humid hot semi-coastal regions. Caffeine was found in 80% of honey samples analysed at levels from 94 to 3583 μg kg$^{-1}$ (mean 1567 μg kg$^{-1}$). High levels were recorded in regions where citrus cultivation is common. Nicotine was detected in 67% of honey samples analysed at concentrations between 178 and 9389 μg kg$^{-1}$ (mean 1567 μg kg$^{-1}$). High levels were recorded in honey samples from the Northwest Plains where tobacco plantation is practised. Cd and Pb levels in all honey samples were below detection limits, while levels of other toxic metals were generally low.**

**CONCLUSIONS: All honey samples contained aflatoxins, mostly in health-threatening concentrations. Caffeine and nicotine were recorded in 80 and 67% of honey samples respectively. Heavy metal levels were generally low.**


**Keywords:** honey; aflatoxin; caffeine; nicotine; heavy metals

## INTRODUCTION

Honey is one of the oldest natural foods known to humans. It is a very nutritious bioproduct with antibacterial characteristics and can be used as an indicator of environmental pollution.[1,2] As a bioproduct, honey has always been considered a healthy food that can be used to cure many gastrointestinal diseases and wound infections.[3,4] Natural honey contains carbohydrates, organic acids, amino acids, proteins, enzymes, hydroxyamines, vitamins, ferments and minerals.[5,6] However, beekeepers use several antibiotics and pesticides to combat diseases of honeybees. In addition, honeybees are subjected to pesticides and other pollutants from the nectar they suck from flowers or from pollen grains.[7] In addition, pollen may form the original source of some microbes (bacteria and fungi) in honey.[8] Therefore it is important to investigate the potential presence of harmful residues in honey.

Mycotoxins are natural secondary metabolites produced by fungi on agricultural products in the field and during storage. Under favourable conditions of temperature and humidity, fungi grow on certain foods and feeds, resulting in the production of toxins. The optimal temperature for fungal growth is 36–38 °C, while maximum toxin production occurs at 25–27 °C; fungal growth in storage facilities is favoured by humidity above 85%. Mycotoxins are potent toxins that have a wide range of actions on animals and humans, e.g. cyto-, nephro- and neurotoxic, carcinogenic, mutagenic, immunosuppressive and oestrogenic effects.[9,10] Aflatoxins are among the most important mycotoxins; they are produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus* and cause a significant threat to human and animal health.[11]

Honey contains many elements that are essential for human health, such as P, Fe, Al, Mg, Cu, Mn, Si, Cl, Ca, K and Na.[6] Plants take in these elements from soil before transporting some of them to nectar, the raw material for honey. Therefore the elemental composition of honey is related to that of soil.[12] In addition, it is known that honey samples from industrial areas contain higher heavy metal (e.g. Cd, Pb, Hg, Zn, Cu, Ni and Cr) levels than those from relatively clean areas.[2,13] Toxic metals can bioaccumulate in the body and in the food chain, causing chronic toxicity. Therefore monitoring the elemental composition of honey is of great importance for consumer health.

Caffeine is a natural plant product that can be found in food products originating from plant materials such as honey. Caffeine is one of a group of compounds known collectively as purine alkaloids. It was discovered in tea (*Camellia sinensis*) and coffee (*Coffea arabica*) in the 1820s.

Nicotine is a naturally occurring alkaloid that is widely distributed in the plant kingdom but best known in the

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family Solanaceae, which includes many agricultural crops and tobacco.[14,15] Nicotine is highly toxic to most herbivores through its action on acetylcholine receptors, thus affecting various biological functions.[16–18]

Although small in geographic area, Palestine has rich biodiversity because of its geophysical characteristics and climate. Its flora consists of over 2780 flowering plant species. Besides, Palestine is well known for its citrus cultivation, especially in the western semi-coastal plains and the Jordan Valley. The floral biodiversity of the country is of great importance for honey production. In 2010 the number of honeybee hives was 51 698, producing some 500 tons of honey.[19] While most honey quality evaluation studies focus on some typical parameters such as sugar content, pH, moisture content and mineral content, the present study was aimed at evaluating the quality of Palestinian multifloral honey of different geographic origins in terms of aflatoxins, caffeine, nicotine and heavy metals (Cd, Pb, Cu, Zn, Ni and Cr).

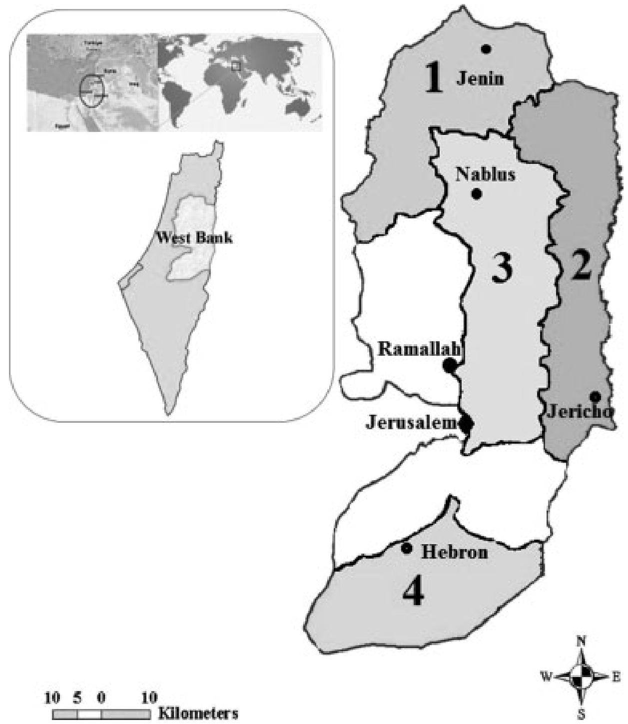


**Figure 1.** Sampling regions of the West Bank, Palestine: 1, Northwest Plains; 2, Jordan Valley; 3, Central Mountains; 4, Southern Semiarid Region.

## MATERIALS AND METHODS

Samples of fresh honey ($n = 21$) weighing 2 kg each were obtained from beekeepers in different regions of the West Bank in the period between April and August 2009. Samples were collected from the Northwest Plains (region 1), the Jordan Valley (region 2), the Central Mountains (region 3) and the Southern Semiarid Region (region 4) (Fig. 1) and kept in glass containers in the laboratory at room temperature for later analysis.

Total aflatoxin analysis was carried out in the Poison Control and Chemical/Biological Analysis Center at An-najah University, Nablus, Palestine. The analysis was done using the AflaTest® method (AOAC licence no. 940801) and a VICAM Series-4EX Fluorometer™ (BBI-Source Scientific, Irvine, CA, USA). The VICAM equipment was adjusted using standards provided by the manufacturer and all accepted recoveries were not less than 98% of the standard values. A 25 g sample of honey was placed in a blender jar with 5 g of salt (NaCl). Then 125 mL of methanol/water (60:40 v/v) was added and the mixture was blended for 1 min. The extract was filtered through a fluted filter paper and the filtrate was collected in a clean vessel. Then 20 mL of the filtrate was diluted with doubly distilled water, mixed and filtered as above. A 5 mL aliquot of the filtered diluted extract was passed through an AflaTest®-P affinity column at a rate of one to two drops per second until air came through the column. Thereafter, 10 mL of purified water was passed through the column at a rate of about two drops per second. The affinity column was eluted by passing 1 mL of high-performance liquid chromatography (HPLC)-grade methanol through it at a rate of one to two drops per second, collecting all of the sample eluate in a glass cuvette. Finally, 1 mL of AflaTest® Developer was added to the eluate in the cuvette, mixed well and placed in a calibrated fluorometer, then the aflatoxin concentration was read after 60 s.

Caffeine, nicotine and heavy metal analyses were done in Birzeit University Testing Laboratories (BZUTL). Caffeine and nicotine levels were determined using the standard addition and HPLC analysis method. The HPLC system was a Waters 2690 Alliance HPLC Separations Module equipped with a Waters 996 photodiode array detector and a reverse phase C18 XBridge (150 mm × 4.6 mm, 5 µm) column (Waters Corporation, Milford, MA, USA).

For metal analysis, honey samples were dried and ashed in a Bifatherm MS8 furnace (Bifa, Ramat Gan, Israel) at 600 °C according to AOAC method 920.181A. Residues were then dissolved in concentrated nitric acid and diluted to specific volumes. Finally, levels of heavy metals were determined against a multi-element standard by inductively coupled plasma optical emission spectroscopy (Optima 3000R, PerkinElmer, Waltham, MA, USA).

Statistical analysis was done using SYSTAT for Windows, Version 11.[20] Statistical differences were tested by analysis of variance (ANOVA) at $P < 0.05$. Where statistically significant differences were observed, Tukey's pairwise comparison test was applied.

## RESULTS AND DISCUSSION

### Aflatoxins

All samples of honey analysed were found to contain aflatoxins. The concentrations of aflatoxins ranged between 0.5 and 22 µg kg$^{-1}$, with a mean value of 12.1 µg kg$^{-1}$ (Table 1). Honey samples from different geographic regions contained significantly different ($P < 0.05$, ANOVA) aflatoxin concentrations, with the highest levels in samples from the Northwest Plains and the lowest levels in samples from the Southern Semiarid Region (Table 1, Fig. 2). This difference may be related to the differing climatic conditions (temperature and moisture) in the Northwest Plains and the Southern Semiarid Region. The Northwest Plains are hot and humid while the Southern Semiarid Region is hot and dry. Therefore the moisture and high temperatures of the Northwest Plains represent good conditions for fungal growth. According to EU regulations, the maximum permitted level of total aflatoxins in dried fruit intended for direct human consumption is 4 µg kg$^{-1}$.[21] The level of total aflatoxins above which the US FDA will take legal action to remove food products from the market is 20 µg kg$^{-1}$ (http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ChemicalContaminantsandPesticides/ucm077969.htm#afla). In the present study, only three honey samples out of 21 (14%) contained total aflatoxin levels



  

**Table 1.** Concentrations of total aflatoxins (μg $kg^{-1}$) in honey samples from different regions of the West Bank, Palestine. Samples ($n$ = 21) were collected in spring/summer 2009

| Region | Mean ± standard error | Median | Range |
|---|---|---|---|
| Northwest Plains | 16.6 ± 1.2a | 16.0 | 13.0–22.0 |
| Jordan Valley | 14.3 ± 3.7ab | 16.5 | 4.2–20.0 |
| Central Mountains | 10.9 ± 4.0ab | 11.0 | 0.5–21.0 |
| Southern Semiarid Region | 3.9 ± 0.8b | 4.2 | 1.7–5.6 |
| Overall | 12.1 ± 4.6 | 13.0 | 0.5–22.0 |

Different letters indicate statistically significant differences ($P < 0.05$) between geographic regions.

**Table 2.** Concentrations of caffeine (μg $kg^{-1}$) in honey samples from different regions of the West Bank, Palestine. Samples ($n$ = 21) were collected in spring/summer 2009

| Region | Mean ± standard error | Median | Range |
|---|---|---|---|
| Northwest Plains | 1577.4 ± 382.4 | 1392.0 | 346.0–3455.0 |
| Jordan Valley | 1660.0 ± 721.7 | 1481.0 | 94.0–3583.0 |
| Central Mountains | 387.8 ± 157.3 | 317.0 | ND–914.0 |
| Southern Semiarid Region | 955.5 ± 367.4 | 1078.5 | ND–1665.0 |
| Overall | 1567.0 ± 222.0 | 914.0 | ND–3583.0 |

No statistically significant differences ($P < 0.05$) were observed between samples from different geographic regions. ND, not detected

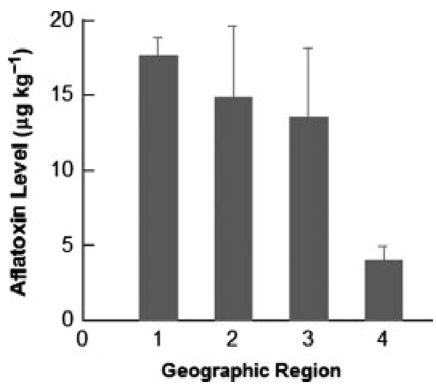


**Figure 2.** Aflatoxin levels (mean ± standard error) in honey samples from different geographic regions of the West Bank, Palestine: 1, Northwest Plains; 2, Jordan Valley; 3, Central Mountains; 4, Southern Semiarid Region.

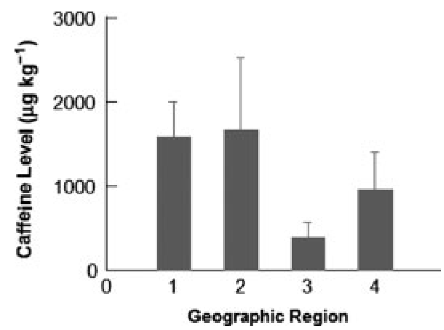


**Figure 4.** Caffeine levels (mean ± standard error) in honey samples from different geographic regions of the West Bank, Palestine: 1, Northwest Plains; 2, Jordan Valley; 3, Central Mountains; 4, Southern Semiarid Region.

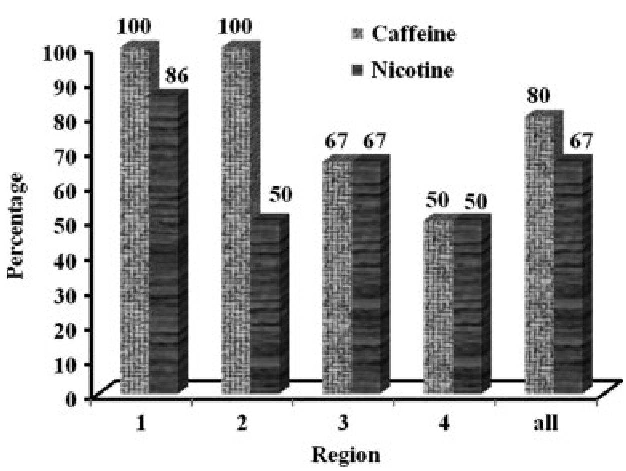


**Figure 3.** Percentages of honey samples from different geographic regions of the West Bank, Palestine that were found to contain caffeine and nicotine: 1, Northwest Plains; 2, Jordan Valley; 3, Central Mountains; 4, Southern Semiarid Region.

of less than 4 μg $kg^{-1}$. These samples were collected from the Southern Semiarid Region and the Central Mountains. In addition, four honey samples (19%) had total aflatoxin levels of more than 20 μg $kg^{-1}$. These samples originated from the Northwest Plains and the Central Mountains.

## Caffeine

Caffeine was found in 80% of honey samples (17/21) analysed (Fig. 3). Caffeine concentrations detected in these samples ranged between 94 and 3583 μg $kg^{-1}$, with a mean value of 1567 μg $kg^{-1}$ (Table 2). High concentrations were found in honey samples from the Northwest Plains and the Jordan Valley (Fig. 4). These two regions are well known for citrus fruit production. However, honey samples from different geographic regions did not show statistically significant differences in caffeine concentration. In the present study, most honey samples with caffeine levels above 1000 μg $kg^{-1}$ originated from the two regions rich in citrus trees. According to Detzel and Wink,[22] caffeine in nectar was found to act as a deterrent to insects, and its toxicity to honeybees was comparatively low under no-choice conditions. However, a study from the University of Haifa (Izhaki *et al.*, http://newmedia-eng.haifa.ac.il/?p=2425) suggested that honeybees clearly prefer nectar containing nicotine and caffeine over 'clean' nectar. The preferred nicotine concentration was 1 mg $L^{-1}$, similar to that found in nature. Based on the results obtained, they proposed that the plants that survive natural selection are those that develop 'correct' levels of these addictive substances, enabling them to attract and not repel bees, thereby giving them a significant advantage over other plants. The presence of caffeine in honey was accidentally discovered during the determination of xenobiotics in honey by a multiresidue method.[23] Further investigations showed that caffeine is present in citrus honey at about 1–10 mg $kg^{-1}$ and that its presence is of natural origin. In the literature, few studies have reported the presence of marked caffeine levels in honey. Trova *et al.*[23] reported, for the first time, the presence

  

**Table 3.** Concentrations of nicotine (μg kg$^{-1}$) in honey samples from different regions of the West Bank, Palestine. Samples ($n$ = 21) were collected in spring/summer 2009

| Region | Mean ± standard error | Median | Range |
|---|---|---|---|
| Northwest Plains | 3169.0 ± 1232.6 | 2401.0 | ND–9389.0 |
| Jordan Valley | 423.5 ± 246.5 | 385.5 | ND–923.0 |
| Central Mountains | 784.0 ± 305.4 | 720.0 | ND–1875.0 |
| Southern Semiarid Region | 1081.0 ± 636.4 | 929.5 | ND–2466.0 |
| Overall | 1567.0 ± 487.9 | 771.0 | ND–9389.0 |

No statistically significant differences ($P < 0.05$) were observed between samples from different geographic regions. ND, not detected.

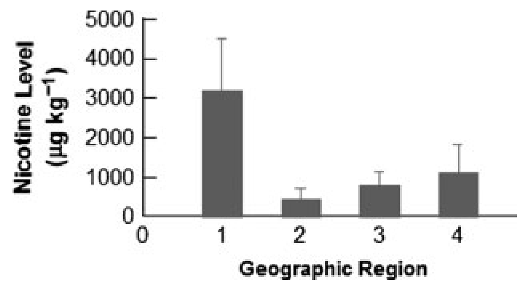


**Figure 5.** Nicotine levels (mean ± standard error) in honey samples from different geographic regions of the West Bank, Palestine: 1, Northwest Plains; 2, Jordan Valley; 3, Central Mountains; 4, Southern Semiarid Region.

of caffeine in citrus honey in the range 500–10 000 μg kg$^{-1}$ (2.6–52 nmol g$^{-1}$). Later, similar findings regarding the presence of caffeine in citrus honey, flowers and nectar were reported by Vacca and Fenu[24] and Vacca *et al.*[25] Vacca and Fenu[24] surveyed caffeine concentrations in Sardinian honeys of 11 different botanical origins. The results showed the presence of caffeine only in citrus and orange honeys, with average concentrations of 1790 and 4930 μg kg$^{-1}$ respectively. Kretschmar and Baumann[26] reported caffeine concentrations in citrus honey of between 39 and 6000 μg kg$^{-1}$. They found that the nectar of citrus fruits contains caffeine in the range 90–487 nmol mL$^{-1}$. According to Izhaki *et al.* (http://newmedia-eng.haifa.ac.il/?p=2425), the caffeine concentration in the nectar of citrus flowers varies between 11 000 and 17 500 μg L$^{-1}$. However, in the nectar of grapefruit flowers, caffeine is present in much higher concentrations, reaching 94 200 μg L$^{-1}$. It is worth mentioning that levels of caffeine in honey are usually much less than those in nectar. Some studies have reported that caffeine in nectar undergoes up to 90% reduction in fresh honey samples as compared with the nectar.[26] However, the actual mechanisms whereby bees reduce caffeine concentrations in honey are still unknown.[27]

## Nicotine

Nicotine was detected in 67% (14/21) of honey samples analysed (Fig. 3). The concentrations of nicotine recorded were between 178 and 9389 μg kg$^{-1}$, with a mean value of 1567 μg kg$^{-1}$ (Table 3). High concentrations (mean 3169 μg kg$^{-1}$) were found in samples from the Northwest Plains, well known for tobacco plantation, while low concentrations (mean 423.5 μg kg$^{-1}$) were present in samples from the Jordan Valley (Fig. 5). However, statistical analysis did not show any significant differences in nicotine concentration between honey samples from different geographic regions. Nicotine is a natural plant product that is found in many plant species, especially tobacco. Honeybees encounter nicotine at trace concentrations in the floral nectar of a few *Nicotiana* spp. and *Tilia cordata*.[22,28] Nicotine is known as a feeding deterrent owing to its bitter taste, and pollinators may encounter nicotine in both nectar and pollen.[22] It may keep pollinators moving between plants and thus enhance cross-pollination.[29] In a caged experiment

**Table 4.** Concentrations of metals (μg g$^{-1}$) in honey samples from different regions of the West Bank, Palestine. Samples ($n$ = 21) were collected in spring/summer 2009

| Region | Cu | Zn | Cr | Ni |
|---|---|---|---|---|
| Northwest Plains | 0.76 ± 0.24 (ND–1.52) | 2.43 ± 1.13 (ND–9.00) | 0.15 ± 0.08 (ND–0.49) | 0.06 ± 0.04 (ND–0.24) |
| Jordan Valley | 0.37 ± 0.13 (ND–0.63) | 2.06 ± 0.81 (0.35–4.20) | 0.10 ± 0.06 (ND–0.27) | 0.03 ± 0.08 (ND–0.35) |
| Central Mountains | 0.28 ± 0.13 (ND–0.86) | 8.36 ± 3.65 (2.0–19.90) | 0.35 ± 0.13 (ND–0.74) | 0.13 ± 0.08 (ND–0.51) |
| Southern Semiarid Region | ND | 2.82 ± 1.49 (1.01–7.28) | ND | ND |
| Overall | 0.41 ± 0.11 (ND–1.52) | 4.13 ± 1.23 (ND–19.90) | 0.17 ± 0.05 (ND–0.74) | 0.09 ± 0.03 (ND–0.51) |

Values are expressed as mean ± standard error, with range of values in parentheses. No statistically significant differences ($P < 0.05$) were observed between samples from different geographic regions. ND, not detected.

**Table 5.** Metal levels (μg g$^{-1}$) in honey samples from the West Bank, Palestine and from various other countries

| Authors/country | Cu | Zn | Pb | Cd | Ni | Cr |
|---|---|---|---|---|---|---|
| Mohammad and Babiker[30]/Sudan | 2.94–58.12 | 4.86–961.00 | <0.45 | 0.000–0.100 | 0.00–4.06 | <0.10 |
| Frías *et al.*[2]/Spain | 1.28 | 2.83 | 0.04 | 0.040 | | |
| Staniškienė *et al.*[1]/Lithuania | 0.12–0.34 | 0.51–5.64 | 0.01–0.02 | 0.004–0.015 | | |
| Yarsan *et al.*[31]/Turkey | 0.55–0.96 | 6.24–11.50 | | | 0.24–1.63 | 0.17–0.37 |
| Atrouse *et al.*[32]/Jordan | 3.37–34.56 | 8.00–13.25 | 0.85–1.23 | 0.003–5.780 | | |
| Present study | 0.41 | 4.13 | ND | ND | 0.09 | 0.17 |

ND, not detected.

where honeybees had no alternative nectar source but only the experimental test solutions, Singaravelan *et al*.[27] noted a drastic reduction (>90%) in the concentration of nicotine in honey as compared with the consumed experimental solutions.

### Heavy metals

Concentrations of Cd and Pb were below detection limits in all honey samples analysed. Zn, Cu, Cr and Ni concentrations were below detection limits in 5, 38, 52 and 67% of all honey samples. Their concentrations were found to be in the order Zn > Cu > Cr > Ni (Table 4). Concentrations of metals in Palestinian honey seem to be comparable to (Cu, Zn and Cr) or less than (Cd, Pb and Ni) those reported in honey from other countries (Table 5).[1,2,30–32] The results indicate that, in terms of heavy metal concentrations, Palestinian honey can be considered as a good-quality product. Low levels of toxic metals in honey might be a reflection of a clean environment, as some studies suggest that honey can be used as an indicator of environmental contamination by metals.[1,33]

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