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Microbiological Sanitary Aspects of Pollen

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ABSTRACT

Medicinal plants have been used for centuries as remedies against human diseases because they contain components of therapeutic value. Bee-Pollen was used for thousands of years as functional food and medicinal plant product. Various beneficial effects were attributed to it and its consumption was increased years. The positive effects of a functional food can be either maintaining a state of wellbeing and health or reducing the risk of pathologic consequences. Gastrointestinal functions, redox and antioxidant systems, and metabolism of macronutrients are the most promising targets for functional food science. Negative and undesirable side effects of food are important threads for human health, especially, unprocessed functional foods. In this work, sanitary microbiological analysis of unprocessed pollen collected from different Algerian regions was examined. Results obtained indicate that pollen obtained from Blida is more contaminated than samples obtained from Sétif and Biskra. Total aerobic count was 3,0 ; 5,80 ; and 5,67 Log_{10} cfu/g for Blida, sétif and Biskra respectively. However, the occurrence of *Staphylococcus aureus* was observed only with pollen obtained from Sétif region. Total coliforms count increases respectively with the increase of environmental humidity and temperature (3,0; 4,27 ; and 5,47 Log_{10} cfu/g for Biskra, sétif and Blida respectively). This phenomenon was also noticed for total moulds. From this primary microbiological analysis, consumption of unprocessed pollen can present a challenge for human and animal health, as it could be a carrier for numerous microorganisms, which may present a detrimental effects on body health.

***Key words*:** bee-pollen, medicinal plants, functional foods, microorganisms, microbial quality, mycotoxins, endotoxin.

Introduction

Stamens are the male reproductive organs of flowering plants. They consist of an anther, the site of pollen development, and in most species a stalk-like filament, which transmits water and nutrients to the anther and positions it to aid pollen dispersal. Within the anther, male sporogenous cells differentiate and undergo meiosis to produce microspores, which give rise to pollen grains, whereas other cell types contribute to pollen maturation, protection, or dispersion [1]. Faegri and Van der Pijl [2], defined two major types of pollen dispersal: *Biotic pollination* in which the pollen dispersal agent is an animal (i.e., either an invertebrate or a vertebrate); and *abiotic pollination* where pollen is dispersed by an inanimate physical agent, such as wind or water. Mutually beneficial ecological relationships have been established between bees and plants. There are about 250 thousand species of flowering plants on earth, many of which have amazingly complex relationships to bees and other pollinators including flies, beetles, moths, butterflies, birds and bats [3]. Honeybees, mainly *Apis mellifera*, remain the most economically valuable pollinators of crop monocultures worldwide and yields of some fruit, seed and nut crops decrease by more than 90% without these pollinators [4]. Honeybees remove pollen from an anther by using their tongue and mandibles mixed it with salivary secretions from her mouth, and transfer it to the corbicula, or "pollen basket", on her posterior pair of legs. To prevent bacterial growth and delay pollen germination various enzymes produced by worker bees are added to the pollen as it is packed into broad-free combs [5]. Historically, bee-collected pollen has been used as a food and medicine by various civilizations, as different nutritional and medicinal traits were attributed to it [6]. Nowadays, tons of pollen, either processed or unprocessed, are

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sold for human and animal consumption. Pollen is a riche plant product, containing carbohydrates, proteins, enzymes, fatty acids, minerals, and vitamins. However, unlike honey, from the anther dehiscence to comb cells, pollen is exposed for microbial contamination. This contamination can be attributed to various factors and sources, honey bees, weather, plant materials, insects and animals, humans and their agricultural devices. Numerous studies were revealed the occurrence of fungal and bacterial species in pollen, but little is known about its microbiological quality. For this reason, pollen quality control is of utmost importance, particularly if the pollen is intended for human consumption. The aim of this preliminary investigation is to examine microbial quality of tow pollens collected by honeybees and one hand collected pollen from Algeria.

Materials And Methods

Sampling and storage:

Pollen samples were collected from different regions in Algeria during flowering season (from March to May 2010). Pollen sample 1 (PS1) was collected from Sétif (North east of Algeria), pollen sample 2 (PS2) was from Biskra (North Algerian Sahara), whereas pollen sample 3 (PS3) was from Blida (North of Algeria). Samples were obtained from clean ecological regions situated in agricultural lands. PS1 and PS 3 were trapped from honey bees workers, whereas, PS2 was collected from date palm trees by hands. All the samples were transported to the laboratory in sterile glass vials and conserved at 4°C until use.

Microbial and weather parameters analysis:

Twenty-five grams of each sample of pollen were diluted in 225 mL of peptone saline solution (1 g/L peptone and 8.5 g/L NaCl) and homogenized for 10 min by shaking in an orbitrary shaker (200 rpm). After 30 min at room temperature, serial dilutions of suspension were made in a saline solution (8.5 g/L NaCl) and analyzed. The formed colonies on the plates were counted and expressed as log colony forming units/g (log cfu/g). Another 25 g were diluted in 225 mL of buffered peptone water for the isolation of *Salmonella spp*.

Total Aerobic microflora was determined by spreading 0.1 ml from the appropriate dilution onto sterile disposable Petri dishes to which Plate count Agar (Merck, 5463) was poured. Plates were incubated aerobically at 30° C and counted after 3 days.

Total Anaerobic microflora: The plat count agar was used to enumerate anaerobic microflora by spreading 100 µl from the appropriate dilution on plate count agar. The plates were incubated at 30°C for 3 days under anaerobic conditions using candle jars.

Total Mold and Yeasts From appropriate dilutions. 100 µl were surface plating onto sterile disposable Petri dishes to which was added Potatoes Dextrose agar (SIGMA, P2181). Plates were incubated at 28°C and counted after 7 days.

Total Coliforms: A 100 µl aliquot of the appropriate dilution were transferred into Petri plates and poured with Violet Red Bile Glucose agar (VRBG Agar). Solidified plates were overlaid with 5 ml of VRBG agar. Plates were incubated at 35°C and typical colonies (dark red, 0.5 mm. or more in diameter) were counted after 24 hr.

Staphylococcus aureus was determined by spreading of 100 µl from the appropriate dilutions on Baird-Parker agar (Merck, 5406) and incubated at 37 °C for 48 h.

Salmonella spp.: Salmonella spp. were determined Pre-enrichment was conducted from samples diluted in 225 ml buffered peptone water incubated at 37°C for 18 hours. Secondary selective enrichment was performed in Rappaport-Vassiliadis peptone broth (41°C for 24 h) and Muller-Kaufmann tetrathionate broth (37°C for 24 h), and plating on Brilliant Green Agar and XLD agar incubated at 37°C for 24 h.

Weather parameters (temperature, relative humidity and precipitation amounts) as well as topographic indicators (Latitude, Longitude, and Altitude) data were obtained from three weather stations [Blida, 603900 (DAAG); Biskra, 605250, (DAUB); and Sétif, 604450 (DAAS)] (Table 1 and Figure 1).

Table1 : Topographic data of the nearest weather stations for sampling sites

Sampling site	Weather station	Latitude	Longitude	Altitude
Blida	603900 (DAAG)	36.68	3.25	25 m
Biskra	605250 (DAUB)	34.8	5.73	87 m
Sétif	604450 (DAAS)	36.18	5.41	1038 m

Results:

Microbial content of three pollen samples is presented in Table 2. It should be emphasized that microbial enumeration varies considerably. Statistical analysis of the data showed that total aerobic counts were significantly higher in pollens from Sétif and Blida (620 x 10^3 and 470 x 10^3 cfu/g respectively), significantly lower in pollen from Biskra (10^3 cfu/g). Similarly, total mould counts were 400 x 10^3/g for pollen from Sétif, 50 x 10^3/g for pollen from Biskra and more than 200 x 10^3/g for pollen from Blida. *Salmonella* was not detected in all samples, whereas, staphylococci were recoded only

in pollen from Blida (27.3 x 10^3/g). Like total aerobic counts and mould counts, higher levels of coliforms were observed in pollen from Sétif (300 x 10^3/g), 19x 10^3/g and 10^3/g for Blida and Biskra respectively. Statistical analysis shows that enumeration of the different microbial groups within each sample has extremely significant differences (P< 0.0001), either for normal set or transformed data. Also, from region to region or from sample to another, microbial content differs greatly (P = 0, 0037; < 0.05). Considering now the weather conditions (Fig. 1), Significant differences in relative humidity and precipitation amount (P <0.05) were recorded between the regions Sétif/Biskra and Blida/Biskra, whereas, there was no statistical differences between Sétif and Blida. In addition, temperature does not differs between all area sampling (P >0.05). Analysis of correlation between the organisms enumerated showed that total aerobic plat counts correlated (r = 0.34) only with total coliforms and relative humidity (RH) (r = 0.12) in pollen from Sétif and correlated (r = 0.31) with total coliforms and (r= 0.69 and r= 0.69) with RH and precipitation amount respectively in pollen from Biskra. In Blida's pollen total aerobic plat counts correlated (r = 025) with staphylococci and both (r = 0.74 and r = 0.47) with relative humidity and precipitation amount. In addition, total molds and yeast counts correlated (r = 0.58) with temperature in pollen from Biskra, and in pollen from Blida correlated (r = 0.37) with relative humidity.

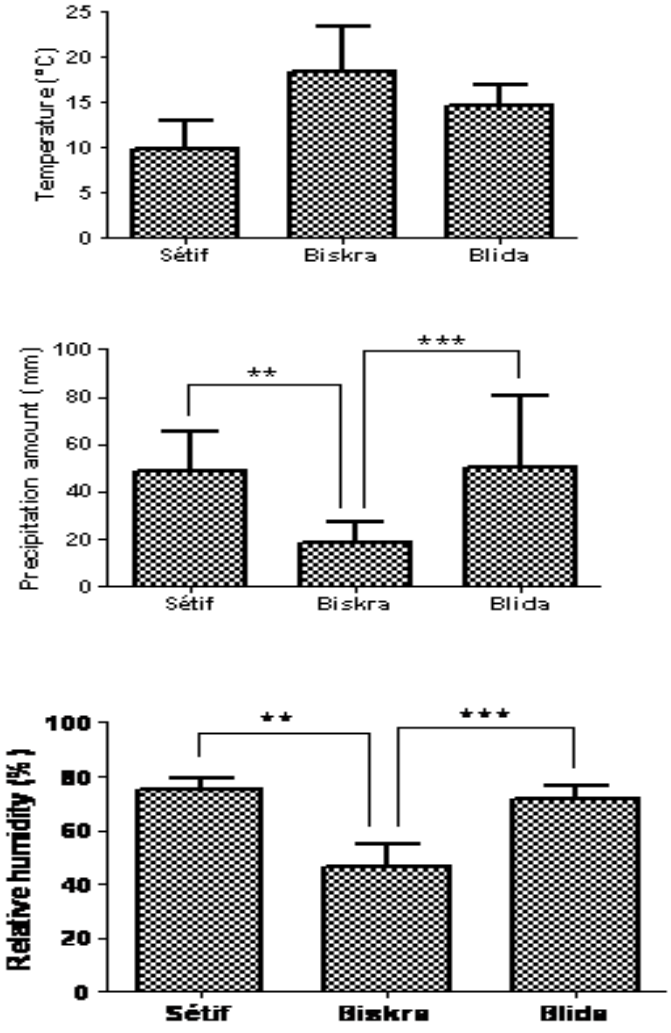

Fig. 1: Recorded temperature (°C), precipitation amount (mm) and relative humidity (%), at the sampling sites (sétif, Biskra, and Blida), columns represent means of five values, upper bares indicate SD (standard deviation), (***) and (**) indicates that P was <0.01 between represented data.

Table 2: Microbial enumeration of three pollen samples collected in Algeria from March to May 2010. (Results were expressed as cfu/g or Log_{10} cfu/g of wet pollen).

	Origin of Pollen Samples					
	PSa (Sétif)		PSb (Biskra)		PSc (Blida)	
	10^3 cfu/g	Log_{10} cfu/g	10^3 cfu/g	Log_{10} cfu/g	10^3 cfu/g	Log_{10} cfu/g
Total aerobic counts	620	5,80	01	3,00	470	5,67
Total anaerobic counts	600	5,77	00	0,00	82	4,91
Moulds and Yeasts	400	5,60	50	4,69	230	5,36
Staphylococcus aureus	00	0,00	00	0,00	27.3	4,43
Salmenella	00	0,00	00	0,00	00	0,00
Total coliforms	300	5,47	01	3,00	19	4,27

Discussion:

Microbial contamination of herbs and/or products may results from improper handling during production, collection and packaging [7]. World Health Organization [8] contaminant guidelines propose that contamination should be avoided and controlled through quality assurance measures such as good agricultural and collection practices (GACP) for medicinal plants, and good manufacturing practices (GMP) for herbal medicines. In recent years, only a small percentage of medicinal plants are collected from the wild, and there are too few data to compare biological contamination between wild and cultivated medicinal herbs. Guidelines such as the GACP and GMP aim at reducing the overall risk of contamination, not only biological [7]. According to Campos et al. [9], pollens should have the following microbial aspects: absence of *Salmonella*/10g; absence of *Staphylococcus* and *Escherichia coli* /01 g; TAPC could not exceed than 10^5 cfu /g; TMYC should be less than 5. 10^4 cfu/g; and the maximum of enterobacteria is 100 cfu/g. Comparing, however, these recommendations with our results reveals clearly that bee-collected pollens (Sétif and Blida) have a poor microbial quality, in contrast to hand collected pollen (Biskra) which have an acceptable criteria. In Algeria, peoples consume pollens. Great populations purchase it from beekeepers and use it as dietary supplement, mainly with dairy products such as milk. However, numerous studies have been conducted to determine microorganisms associated with pollen. Gilliam et al. [10] identified more than six yeast genera (*Cryptococcus, Kloeckera, Candida, Rhodotorula, Torulopsis, Hansenela*) from almond pollen. Later [11] *Bacillus subtilis, B. megaterium, B. licheniformis, and B. circulans* were isolated from the same pollen of almonds. In addition, the majority of molds identified from pollen by Gilliam et al. [12] were penicillia, mucorals and aspergilli. Inside anther pollen is sterile, thus its microbial contamination can be attributed to plant materials, environment, insects (eg, Honeybees), and humans and their agricultural devices. Naturally, pollen grains contain antimicrobial substances (flavonoids, phenolic acids, and other phytochemicals) [13,14] as well as microbial spore germination inhibitors [15]. As reviewed by Gonzâlez et al. [16], growth and sporulation of fungi, both on standing crops and in stored grains, are largely dependent on environmental factors. The most important determinants are probably a_w and temperature. On this fact, results of this work point out that differences of microbial content between and within pollen samples were related to relative humidity and precipitation amount, as temperature does not differs greatly between regions. World wide, pollen processing includes harvesting, drying, cleaning, packaging and storage [17]. Gonzalez et al. [16] concluded in their work that the most critical stage is pollen collection from traps. The longer period of collection the highly contaminated pollen was obtained. In contrast, based on the fact that corbicular pollen is mixture of pollen, nectar and salivary secretions of honeybees [18]. Furthermore, Gilliam et al. [19] reported the occurrence of both Gram negative and Gram positive bacilli as well as Gram positive cocci in nectar. In addition, fungi are found in association with honey bee colonies where they persist on nectar, pollen, in colony debris, and inside bees themselves [20]. And from the results of this work, it seems that honeybees are the main source and/or carrier for pollen contamination. The high incidence of fungi in pollen grains studied in this work point out that raw pollen in not ready-to-eat directly without processing (drying). In Algeria, pollen is dried naturally by exposing it to sunlight or oven heated at a low temperature (less than 45°C). Serra and Alegret [21] recommend the avoidance of natural pollen drying, because at low temperatures, fungal growth and mycotoxin production might occur. If raw pollen was stored without processing, fungi can flourish under appropriate conditions (humidity and temperature) as pollen is a suitable plant product either for fungal growth or mycotoxin production. However, mycotoxins production, such as ochratoxin A (OTA) and aflatoxins B_1, B_2, G_1, and G_2 by pollen associated fungi is known [16, 22]. Aflatoxins and ochratoxin A are thermostable and carcinogenic molecules [23, 22], thus, pollen dried naturally or not may present a threat for human and animal health. Considering now bacterial content, however, the incidence of staphylococci in pollen from Blida make it undesirable dietary supplement. *Staphylococcus aureus* remains a versatile and dangerous pathogen in humans. The microoorganism produce numerous toxins that are responsible for diverse syndromes and life-threatening diseases, as the staphylococci are well known for their multiresistance to antimicrobial drugs [24, 25]. It is

truly that analyzed pollen samples in this work contain high levels of enterobacteria and exceed recommended values. *Enterobacteriaceae* are a group of bacteria that can be found in many environments. They can be found in the intestinal tract of humans and animals. They can also be found in soil, vegetable matter and marine environments. The group includes both pathogenic and non-pathogenic bacteria. As they can be found in raw foods, their detection may not be an indication of fecal contamination and is inappropriate to test ready-to-eat foods containing raw components (particularly fresh fruits and vegetables) [26, 27, 28]. But taking in mind the fact that enterobacterial cell membrane contains an antigenic, pyrogenic and thermostable molecule, the endotoxin or lipopolysaccharide (LPS). However, the bacterial endotoxin is released during bacterial multiplication or death and triggers a series of important biological events that lead to an inflammatory response and bone resorption [26]. Indeed, either raw or dried pollen may be considered as a potentially hazardous medicinal plant product, as it is exposed for contamination by toxigenic fungi and pathogenic bacteria such as aspergilli, penicillia and staphylococci. This contaminated pollen present a suitable environment and/or a suitable delivery system for mycotoxins and/or bacterial endotoxins. Peoples, especially in developing countries, continue to use pollen without the existence of international microbiological quality guidelines and reglementations for pollen, as well as the ineffectiveness of pollen sanitization methods designed for human consumption, more studies are needed to understand the microbial content of pollen and to establish international microbiological quality parameters and standard processing protocols.

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