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SAFETY ASPECTS OF BEE POLLEN USE IN NUTRITION

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review paper

Summary

Bee pollen is popular nutraceutical and remedy used in traditional medicine since ancient times. Although it has indisputable beneficial action on human health, in recent years some issues regarding its safety have been raised. Mainly, they are a result of human actions, either indirectly (usage of pesticides, pollution with toxic trace elements) or directly (microbial contamination during handling). This review summarizes findings regarding safety aspects of bee pollen for human consumption.

Keywords: bee pollen, pesticides, toxic elements, bacteria, fungi

Introduction

Bee pollen is term referred to grains that honey bees (*Apis mellifera*) form in pollen baskets by compressing flower pollen mixed with secretion from the mouth (Mekki, 2019). It has long been recognised as a nutraceutical, functional food and remedy in alternative medicine. These attributes pollen owes to its chemical composition, which is providing virtually all essential compounds for human and animal nutrition (Kostić et al., 2020), although contents of specific compounds vary extensively, depending on botanical species, geographical origin and climate (Margaroan et al., 2019).

Major nutritive components in pollen are carbohydrates, proteins and fats. According to Li et al. (2018), carbohydrates take up 40 – 85 % of dry bee pollen, with fructose as a major carbohydrate, followed by glucose, sucrose, oligo- and polysaccharides. Total dietary fibre content ranges between 17.60 and 31.26 %, with cellulose and callose as main components (Thakur and Nanda, 2020).

On average, protein content varies from 10 to 40 g/100 g dry weight (Campos et al., 2008), although range 2.5 – 62 % has been reported (Nicolson, 2011). Although amino acid composition depends on botanical origin of pollen, bee pollen is considered as a valuable source of essential amino acids, among which leucine and lysine are often present in largest quantities (Thakur and Nanda, 2020), along with proline, glutamic and aspartic acid (Mekki, 2019).

Lipid content averages between 1 – 13 % of pollen dry weight, with significant contents of ω-3 fatty acids. Among lipids, Li et al. (2017) reported presence of 41 different phosphatidylcholines, 43 phosphatidylethanolamines, 9 phosphatidylglycerols, 10 phosphatidylserines, 12 lysophosphatidylcholines, 8 ceramides, 27 diglycerides, 137 triglycerides, and 47 fatty acids. Fatty acid profile is highly dependent not only on botanical source, but on geographical origin as well. Most prevalent saturated fatty acids are myristic, stearic and palmitic, and α-linolenic, linoleic and oleic acid are dominant unsaturated acids. Significant levels of arachidonic, behenic, capric, caproic, caprylic, 11-eicosenoic, elaidic, lauric, lignoceric, myristic, oleic and stearic acids have also been reported. On average, saturated fatty acids range from 4.29 – 71.47 %, monounsaturated from 1.29 – 53.24 % and polyunsaturated from 4.33 – 75.1 %. ω-3 fatty acids vary from 8.07 to 44.1 % and ω-6 fatty acids from 1.77 to 38.25 % (Thakur and Nanda, 2020). Kostić et al. (2020) designated pollen as a valuable source of polyunsaturated fatty acids (PUFA), basing their claim upon the fact that different Portuguese pollen samples contained app. 49 – 70 % PUFA, with UFA/SFA ratio 1.9 – 5.9, Serbian pollen samples had app. 22 – 54 % PUFA and Philippine stingless bee pollen samples app. 52% PUFA.

Bee pollen is a significant source of minerals, with 2.5 – 6.5 % of ash content. Over 25 minerals have been reported, among which Ca, Cu, Cr, Fe, K, Mg, Mn, Na, P and Zn are most abundant. However, exact composition and proportions of minerals are affected by soil, climate, geographical origin and botanical species (Thakur and Nanda, 2020). Kostić et al. (2020) accentuate the value of bee pollen as a selenium source, with app. content of 0.02%.

Furthermore, vitamins in pollen comprise up to 0.7% (Kostić et al., 2020), with high contents of B-complex (Thakur and Nanda, 2020) and carotenoids, vitamin A precursors, and polyphenols take up app. 1.6% of pollen (Kostić et al., 2020). Among polyphenols, flavonoids are dominant group (Kostić et al., 2020; Thakur and Nanda, 2020), but different geographical origin of pollen and different plant species result in large diversity of compounds and their contents reported in literature. Often, apigenin, epicatechin, hesperetin, isorhamnetin, catechin,

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kaempferol, luteolin, quercetin, naringenin, etc. and phenolic acids: chlorogenic acid, ferulic acid, caffeic acid, gallic acid, vanillic acid, syringic acid and *p*-coumaric acid are reported (Thakur and Nanda, 2020). The unique nutritional composition of pollen makes it valuable remedy in neurological disorders, from spinal cord injury to Alzheimer's and Parkinson's disease (El-Seedi et al., 2020), anti-inflammatory, anti-tumor and antimicrobial agent (Margaroan et al., 2019). On the other hand, although allergic reactions caused by ingestion of pollen with food are rare, Kostić et al. (2020) do not exclude pollen as a potential allergenic, it may contain pyrrolizidine alkaloids, toxic trace elements (such as arsenic and cadmium), mycotoxins and pathogenic microorganisms. Therefore, safety aspects of bee pollen use should not be disregarded in evaluation of its beneficial effects.

Pesticides

Pesticides used in plant protection have raised great concern among public due to great loss of pollinators, among which are honey bees, and presence of residues in food. As a response, scientists have been focusing on exposure routes and risks of pesticides for bees (reviewed by Zioga et al., 2020), and on different bee products as vectors of further transmission of contaminants to humans.

By definition, "pesticides are toxic chemicals used to kill or repel pests or to interrupt their reproduction, and are some of the most toxic, environmentally stable and mobile substances in the environment" (Andreo-Martinez, 2020). Bees are exposed to them through water, pollen nectar, dust-spray droplets collected on body hairs of bees, guttation drops, and even in the bee hive if beekeepers use them to control parasites. Since they do not have detoxifying enzymes, bees accumulate pesticides in pollen, brood, wax and honey. As a result, acute poisoning may manifest in a number of consequences, from reduced flying ability to increased mortality (Catalayud-Vernich et al., 2018).

Catalayud-Vernich et al. (2018) collected bee pollen from 39 locations in different parts of Spain and screened them for 63 pesticides and their degradation products. They found 14 different pesticides in pollen, 8 of which was for agricultural use and 6 was used in beekeeping. Although some samples were pesticide-free, an average count was 3 pesticides per sample and most commonly found were: coumaphos, fluvalinate and amitraz degradate DMF. Interestingly, there was no difference regarding the number of detected pesticides and their average count per sample between hives located in high- and low agricultural environment. Residues of coumaphos were found in pollen even though they were not applied in hives for months, indicating that, along with environmental contamination, bee pollen may be contaminated with pesticides present in the wax. Also, this proves that pesticides accumulate in hives over a long period of time, posing a risk to bees for prolonged period. Migdal et al. (2018) linked pesticide residues with colony collapse disorder, although clear cause-and-effect relationship is yet to be proven.

Chaimanee et al. (2019) analysed contents of pesticides in bee pollen collected at pollen traps at 16 non-agricultural and 20 agricultural sites in Northern Thailand. They also found no difference in contamination of pollen regarding the site type. They found 8 different pesticides (organophosphate chlorpyrifos, 2,4-dimethylphenyl formamide (DMPF), carbendazim, metalxyl, atrazine, imidacloprid, cypermethryn and fluvalinate) in agricultural sites and 4 (carbendazim, chlopyrifos, fluvalinate and DMPF) in non-agricultural sites. All detected pesticides are among most frequently used in Thailand.

Manning (2018) assessed bee pollen collected in area of canola farms as the only source of nectar. All pollen samples were contaminated with trifluralin. Atrazine was found in 61.5% of samples and chlorpyrifos in 30.8% of samples.

Ostiguy et al. (2018) monitored pesticide residues in bee pollen collected in USA over 4-year period. They reported that 79 pesticides and their metabolites were determined in pollen, with insecticides detected more frequently than other types. The most frequently detected fungicides were carbendazim, azoxystrobin, and propiconazole-1, the most frequent herbicide was atrazine and carbaryl was the most frequent insecticide. Although different pesticides in different concentrations were found depending on season, generally, pollen was more contaminated than wax. Similar conclusion was withdrawn by Raimets et al. (2020), who also found that bee pollen and beebread collected in southeastern Estonia were more frequently contaminated than honey or wax, and that pollen was most frequently contaminated by insecticides and fungicides.

Pesticide residues present in bee pollen and other bee products do not pose risk only to honey bees. Through consummation of bee products, humans are also exposed to these residues. There is evidence that numerous pesticides affect non-target species as well, including humans. For example, atrazine is nowadays banned in the EU due to demasculinizing and defeminizing effect on reptiles, birds, mammals and other species (Vandenberg et al., 2020). However, it is still being used in the USA, China and Australia as a herbicide, particularly in corn production.

Clorpyriphos, widely used insecticide both in agricultural and domestic use, has been linked to neurotoxicity, cytotoxicity, oxidative stress, mutagenicity etc., where humans are esp. sensitive after oral administration (Ubaidurrahman et al., 2020). Carbendazim has been reported to cause embryotoxicity, infertility, hepatocellular dysfunction and other disorders in different mammalian species, including human (Wang et al., 2020).

Toxic trace elements

Toxic trace elements may be found in bee pollen due to man-caused pollution of air, water and soil and uptake of these elements by plants. Uptake of the elements by plants depends on plant species and genotypes, soil type and pH (Radanović and Antić-Mladenović, 2012). Although flower pollen is mixed with nectar, saliva and honey to produce bee pollen and therefore concentration of trace elements is always lower in bee pollen than in flower pollen, bee pollen may contain significant amounts of toxic elements (Silva et al., 2012).

Among them, lead and cadmium are often found, because of industrial pollution and pesticide application. Altunatmaz et al. (2017) reported ranges of 0.006 – 0.181 μg/g for Cd and 0.000 – 0.479 μg/g for Pb in bee pollen collected in different regions of Turkey and Silva et al. (2012) reported 13.98 – 18.19 μg/mL for Pb in bee pollen collected in Teresina region of Brazil. Range of 0.003-0.233 mg/kg was reported for Cd contents in bee pollen collected in south-eastern (Morgano et al., 2010) and 0.0026 – 0.0244 mg/100 g for southern part (Rio Grande do Sul State) of Brazil (Sattler et al., 2016).

Arsenic was also found, mainly due to air and water pollution (Altunatmaz et al. 2017), but through pesticide application as well (Ratnaike, 2003). Altunatmaz et al. (2017) reported 0.006 – 1.035 μg/g of As in Turkish bee pollen and Morgano et al. (2010) reported <0.01-1.38 mg/kg for bee pollen collected in south-eastern Brazil, while Maragou et al. (2017) reported levels below 0.2 μg/g in bee pollen collected in northern and western parts of Greece.

Traces of mercury were also found in bee pollen. Namely, <0.0004-0.0068 mg/kg for Hg was reported for area of south-eastern Brazil (Morgano et al., 2010), 0.0036 – 0.0066 mg/kg for Poland (Roman, 2009) and bee pollen of Greek origin contained < 0.06 μg/g (Maragou et al., 2016). Although very toxic, these levels of mercury are not of concern for safety of bee pollen for human consumption, but they do show that bee pollen may serve as an indicator of environmental pollution with mercury.

However, Cd and Pb levels could pose concern. Long-term exposure to Cd causes damaging of cardiovascular, nervous, respiratory, urinary, skeletal and/or reproductive system and cancer. It has very long biological half-life (10 – 30 years), and can accumulate in body for a very long time (Rahimzadeh et al., 2017) which makes it especially concerning. Lead is non-biodegradable and highly toxic to virtually all organs. The most affected is nervous system and children are especially sensitive. Long-time exposure to Pb may cause behavioural problems, lowered IQ and learning disorders in children, and decreased cognitive performance in adults, and chronic exposure leads to its accumulation in bones and kidneys (Wani et al., 2015).

Arsenic is also highly poisonous. In small amounts it causes gastrointestinal problems, but chronic exposure leads to wide range of symptoms, since it is deposited in liver, kidney, heart, spleen, lungs, nails, hair and skin. Hyperpigmentation, diabetes mellitus, respiratory diseases, malignant changes of all organs are some examples of chronic arsenic poisoning (Ratnaike, 2003).

Microbial contamination

Unlike pesticides and trace elements, microorganisms can contaminate pollen in different stages of collecting and handling. Beev et al. (2018) state that pollen may be contaminated from its natural habitat, bee activities (foraging and transport), human activities (handling during colleting, drying, packaging) and environmental factors (wind, rain-splash, dew or fog drip etc.). Lopez et al. (2020) differentiate primary sources of bacterial contamination of pollen: digestive tracts of honeybees, dust, air, earth and nectar, and post-harvest sources: humans, equipment, containers, pests and water. Viruses, bacteria and fungi have all been detected on pollen, showing that pollen is favourable environment for microbial development. This is due to favourable chemical composition (carbohydrates, proteins and lipids), discussed above.

Beev et al. (2018) analysed 13 fresh and 19 dried pollen samples collected in different areas of Bulgaria. Along with significant difference in water activity (a_w) (0.717 in fresh compared to 0.359 in dried samples) and pH (4.23 in fresh and 5.21 in dried samples), they reported significantly higher total viable count (182153.8 CFU/g compared to 30352.6 CFU/g in dried samples) and fungal load in fresh bee pollen (mean value 10512.3 CFU/g compared to 2418.4 CFU/g). Apparently, difference in pH of samples was not so remarkable to show an effect on microorganisms' growth like water activity. There was no significant difference in *Enterobacteriaceae*, *Staphylococcus* spp. and lactic acid bacteria count. Dinkov (2016, 2018a,b) also analysed fresh and dried pollen collected in different regions of Bulgaria, also showing contamination with *Enterobacteriaeae* in fresh and dried pollen over period of years. Belhadj et al. (2014) collected fresh bee pollen samples in the public market in Algeria. Along with different parts of Algeria, Egyptian and Chinese pollen sold at the market were also sampled. Considering total aerobic mesophilic count and total yeast and mold count, all samples were satisfactory, however, *Enterobacteriaceae* were detected in majority of samples, including *Salmonella* and *Listeria* ssp., indicating non-hygienic handling of pollen by bee keepers. Additionally, only one sample was free of *Staphylococcus aureus.*

Among fungi, Beev et. al. (2018) reported that most often *Penicillium* and *Fusarium* species were isolated, indicating that mycotoxin presence could also pose a problem. In research of Belhadj et al. (2014) *Aspergillus, Penicillium, Alternaria* and *Mucor* were frequently isolated, all of which are mycotoxin producing. Mycotoxin-producing moulds were found in Portuguese (Estevinho et al., 2011), Lithuanian (Sinkevičiene et al., 2019) and Slovakian (Kačaniova et al., 2009) pollen as well, namely *Aspergillus, Penicillium* and *Fusarium*. Among mycotoxins, fumonisins, ochratoxins, deoxynivalenol (DON) and zearalenone were found in pollen (Kačaniova et al., 2011; Rodriguez-Carasco et al., 2013).

Dried pollen has better microbial quality, as shown by da Silva et al. (2019) and DeMelo et al. (2015) who did not detect *Salmonella, E. coli* and *S. aureus* in dried pollen collected in Brasil. De Arruda et al. (2017) reported presence of *E. coli* in 11% and *S. aureus* in 30% of analysed dehydrated pollen samples collected in Brazil, and Dinkov (2016) found *Enterobacteriacease* in dried pollen collected in Bulgaria, but the incidence and number of present microorganisms are still lower than in the case of above mentioned results for fresh pollen.

Conclusions and Future Remarks

The nutritional and pharmaceutical values of bee pollen are unquestionable, however, safety aspects and quality of pollen must be standardised. There is no standard of bee pollen quality and safety on EU or IHC level, although some countries do have legislation regarding these issues.

Beekeepers should be constantly educated regarding good hygiene practices in apiaries, and during processing and packaging of bee pollen. Namely, high incidence of presence of different *Enterobacteriaceae* species indicates poor hygienic practices. Identification of *Listeria* in some researches is of special concern. This bacterium is capable of reproducing in large temperature range (4 – 45 °C) and is very difficult to eradicate once it establishes in facilities.

In addition, awareness of beekeepers regarding environmental pollution and choice of pasture areas for bees further from industrial and agricultural pollution should be raised. At the same time, crop growers need to be educated regarding integrated pest control, which enables usage of lower quantities of pesticides.

Additional research is needed regarding assessment of bee pollen as a vector of dietary intake of harmful substances.

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