An Unexpected Contaminant in Honey

Balda Beklenmedik Bir Kirletici

Research Article

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ABSTRACT

In this paper microscopic analyses of a honey sample from Black Sea Region of Turkey was done to determine its botanical origin. According to the results of microscopic analysis, the honey sample is evaluated as chesnut honey. Besides this an unexpected contaminant was observed in this honey sample. As well as pollen grains, Ustilago spores are also identified in the sample in quitely high values during the microscopic analysis. Members of Ustilago genus are plant pathogen fungus. Therefore, it is considered that these spores can be transported to the hive by honey bees while they are collecting pollen or nectar. Ustilago spores are reported as an allergen associated with bronchial asthma and with hypersensitivity pneumonitis. In the honey, amount of observed spores were found higher than pollen grains' and it is predicted that this honey could be hazardous to health. Consequently, our research exhibited the importance of microscopic analysis for honey quality and hygiene control.

Key Words
Fungal spore, pollen, honey, *Ustilago*

ÖZET

Bu makalede, Türkiye'nin Karadeniz Bölgesi'nden bir bal örneğinin botanik kaynağını saptamak amacı ile mikroskobik analizi yapılmıştır. Mikroskobik analizin sonucuna göre, bal örneği kestane balı olarak değerlendirilmiştir. Bunun yanında, bu bal örneğinde beklenmedik bir kirletici de gözlenmiştir. Mikroskobik analiz sırasında, polen tanelerinin yanında oldukça yüksek miktarda Ustilago sporlarına da rastlanmıştır. Ustilago cinsinin üyeleri bitki patojeni funguslardır. Bu nedenle, bu sporların bal arıları tarafından polen ve nektar topladıkları sırada kovana taşınmış olabilecekleri düşünülmüştür. Ustilago sporları, bronşiyal astım ve hipersensitivite pnömonisi ile ilişkili bir alerjen olarak bildirilmiştir. Balda, gözlenen spor miktarı polenlerinkinden daha fazladır ve bu balın sağlık açışından zararlı olabileceği öngörülmüştür. Sonuç olarak, yaptığımız araştırma balın kalitesi ve hijyeni açısından mikroskobik analizin önemini ortaya koymaktadır.

Anahtar Kelimeler
Fungus sporu, polen, bal, *Ustilago*

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INTRODUCTION

Honey is a natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature. Honey consists essentially of different sugars, predominantly fructose and glucose origin [1]. Besides these, it also contains certain minor constituents, proteins, enzymes (invertase, glucose oxidase, catalase, phosphatases), amino and organic acids (gluconic acid, acetic acid, etc.), lipids, vitamins (ascorbic acid, niacin, pyridoxine etc.), volatile chemicals, phenolic acids, flavonoids and caretenoid like substances and minerals. The colour of honey varies from nearly colourless to dark brown. The consistency can be fluid, viscous or partly to entirely cristallised. The composition of honey, the flavour and the aroma depends on the plant species visited by the honey bees and the environmental, processing and storage conditions [1,2].

Thus, the botanical origin of honey is very important for its quality. Many analytical methods (optical rotation, electrical conductivity etc.) are using to determine the botanical origin of honey. Microscopic analysis is able to detect the botanical origin much more exactly than other analytical methods. However it is difficult to correctly interpret results of melissopalynology and it needs a lot of experiences [3].

Another important point for quality of honey is also hygiene. According to the Codex Alimentarius [1] the products covered by the provisions of this Standard be prepared and handled in accordance with the appropriate sections of the Recommended International Code of Practise-General Principles of Food Hygiene recomended by the Codex Alimentarius Commission, and other relevant Codex texts such as Codes of Hygienic Practise and Codes of Practise. According to these codexes producers should as far as practicable implement measures to: control contamination from air, soil, water, feedstuffs, fertilizers (including natural fertilizers), pesticides, veterinary drugs or any other agent used in primary production; control plant and animal health so that it does not pose a threat to human health through food consumption, or adversely affect the suitability of the product; and protect food sources from faecal and other contamination [1].

Pollen analysis of honey or mellisopalynology was introduced at the beginning of 20th century. The pollen analysis method has been described by the International Commission for Bee Botany and improved recently. This method can be used for the microscopic determination of the pollen grains, contained in honey. Pollen analysis of honey is used to identify the sources of the nectar flow of the honey bees and plants pollinated by bees during a flower visit. Knowledge of the pollen composition of honey helps discriminate multifloral honeys from "specific" types of honeys. Specific types of honeys are more attractive and more eagerly purchased by consumers [4].

Honey and bee products have the image of being natural, healthy and clean. However, today bee products are produced in an environment, polluted by different sources of contamination. The most recent example is the news about antibiotic-contaminated honey. Such messages will damage the good image of honey. The contamination sources can be roughly divided into environmental (pesticides, heavy metals, bacteria, GMO, radioactivity) and apicultural ones (acarasides for Varroa control, antibiotics against AFB and EFB, pesticides for wax moth control etc.). Contaminants can reach the raw materials of bee products (nectar, honeydew, pollen, plant exudates) by air, water, plants and soil and then be transported into the bee hive by the bees [5].

Contamination of honey with any microbial spore is about non-hygienic conditions. In this research we investigated a honey sample according to its pollen content under microscope. So it is investigated for any other contaminant or alduration that we can see by microscope (starch grains, spores, etc.).

Table 1. The pollen and spores identified in the honey sample

Plant Family	Plant Taxa	Identified pollen and spore numbers
Apiaceae		3
Asteraceae		1
Berberidaceae	*Berberis* spp.	76
Boraginaceae		4
	Heliotropium spp.	1
	Myosotis spp.	15
Brassicaceae		1
Campanulaceae		7
Cistaceae		5
Ericaceae		2
Fabaceae		50
	Vicia spp.	6
Fagaceae	*Castaneae sativa*	315
Lamiaceae		37
Poaceae	*Zea mays*	7
Rosaceae		11
Total pollen		*541*
Spores		
Ustilago maydis		16681

Figure 1. *Ustilago maydis* spores observed in pollen slide of honey (X40)

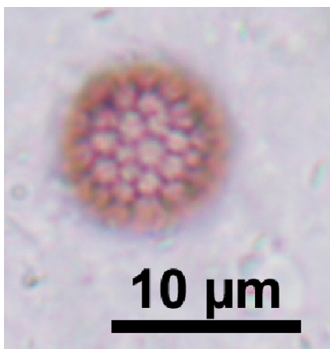

Figure 2. *Ustilago maydis* spore

MATERIALS AND METHODS

Preparation of honey sample for microscopic analysis

500 grams of stock honey was well stirred with a sterile glass stick and 10 grams of it was seperated. Then 20 ml distilled water was added and mixture was placed in a tube. To melt down the honey, tube was left for 10-15 minutes in a water bath of 45°C. After the honey melted, the material was centrifuged in 3500 rpm for 45 minutes. After centrifugation, the supernatant was poured and from the residual sediment slides were prepared by using glycerine gelatine with basic fuchsin [6]. After preparation of pollen slides of honey sample, it is investigated under microscope.

RESULTS

In this research we counted total 541 pollen. We observed pollen belong to the taxa of Apiaceae, Asteraceae, Berberidaceae, Boraginaceae, Brassicaceae, Campanulaceae, Cistaceae, Ericaceae, Fabaceae, Fagaceae, Lamiaceae, Poaceae and Rosaceae families. 315 of total 541 pollen were identified as Castanea sativa (chesnut) pollen. So the honey sample was

Figure 3. *Zea mays* pollen identified in pollen slide of honey sample (X100)

evaulated as chesnut honey. When scanned whole slide we counted 16681 Ustilago maydis spores (Figures 1,2). The observed pollen and spores in the pollen slides are given in Table 1.

DISCUSSION

Spores run across in the honey *Ustilago* is a plant pathogen fungus. There are several plant pathogen species of the genus all around the world, such as *U. maydis, U. tritici, U. nuda, U. violacea*, ect. *U. maydis* is the casual agent of common smut in corn (*Zea mays*) [7]. In addition, fungal spores are usually known as wind-spread. However, Jennersten [8] reported that *U. violacea* is an insect-dispersed fungus.

As seen in the Table 1. the mostly observed pollen belongs to the *Castanea sativa* taxon. Thereby we can characterize this honey as chesnut honey. After *Castanea sativa* pollen we observed the pollen of *Berberis* spp. mostly. Besides pollens, *Ustilago maydis* spores observed much more from counted pollens. It is quite well known that pollinating and flower-visiting insects can carry some plant diseases and are themselves infected by diseases while foraging at flowers. Despite the most observed pollen belong to *Castanea sativa* taxon, there is no data about fungal diseases of *Castanea sativa* that its agents are transformed to the hives. It is considered that these spores are transported by bees when they collect pollen from the plants of Poaceae family. In the pollen content of honey sample we observed the pollens of Zea mays, that is the host of *Ustilago mayadis*, in minor ratio. Nevertheless seven *Zea mays* pollen (Figure 3) were observed, the number of spores sourced from Poaceae plants were quitely high.

Members of the kingdom fungi comprise numerous plant pathogens, including the causal agents of many agriculturally relevant plant diseases such as rust, powdery mildew, rice blast and cereal head blight [9].

Ustilaginales constitute a group of important plant pathogens which produce a variety of diseases in monocotyledons worldwide. These pathogens have the potential of causing severe disease outbreaks despite chemical treatment of the seeds and the plants, the existence of partially resistant cultivars, and the different plough methods employed. Of all Ustilaginales, *Ustilago maydis* is the best known member of the group. *U.maydis* is a pathogen specific for corn (*Zea mays*) and teozinte (*Zea mexicana*), which is considered to be the ancestor of cultivated corn. *U.maydis* is the agent responsible for corn smut, a worldwide distributed disease, which under some conditions may cause severe economical losses in the agriculture [10]. Corn smut appears when the basidiomycete *Ustilago maydis* (DC) Corda infects maize (*Zea mays* L.), and is characterized by the formation of galls or tumours on aerial parts of the plant, including stalk, leaves, tassels and ears [11].

Fungal spores are usually wind-dispersed. There are, however, exceptions where insects are responsible for their transport. It is stated that insects visiting infected flowers in search of nectar may become covered with spores of the smut and disperse them to other plants [8]. The pollen capacity of *Zea mays* is 4 over 4 and nectar capacity is lack [12]. Although *Zea mays* has no nectar flow capacity, honey bees can visit it to colect pollen and so can tranport the spores to the hive. Then from the hive it can be mixed with the honey. Maize pollen is not uncommon in honey but would not be the major pollen type. Bees may pick up maize pollen whilst out foraging for a better source in perhaps a hedgerow. Some researchers stated that,"Although corn is wind pollinated it produces such copious amounts of pollen that it is highly attractive to bees, both honey bees and a wide variety of solitary bees" [13].

Eventually, by our results it is showed that bees visit wind pollinated *Zea mays* to collect pollen as mentioned previous studies. Besides this, bees can transport dissease effects from one flower to another and also to hive, so can contaminate bee products by this dissease agents. In our study we observed that honey bee tranport *Ustilago maydis* spores from *Zea mays* to honey. Singh and Kumar [14] reported *Ustilago* spores as among major allergenic fungi. Thereby it is important to determined these spores in honey sample before put it to market. Due to its high spore concentration it would be harmfull for human health especially sensitive persons to allergic reactions. As a result fungal spore contamination in honey is about non-hygienic conditions. Also it is understood from scanning literatures, it is the first data for observing of *Ustilago maydis* in honey.

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