Melisopalynological potential of chestnut (*Castanea sativa* Mill.) in Bosnia and Herzegovina

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Abstract

Tame chestnut (*Castanea sativa* Mill.) is a woody plant species from the family Fagaceae. Chestnut belongs to the group of honey-bearing plants with a high production of pollen and nectar, and honey is characterized by specific chemical and organoleptic properties and a high content of minerals. Its biogeographical distribution is limited to only three areas on the territory of Bosnia and Herzegovina. The aim of the work is to determine the distribution and representation of the pollen of this honey plant in honey samples originating from Bosnia and Herzegovina. For research purposes, 100 honey samples were collected directly from the producer. Based on the micromorphological characteristics of the pollen grains, honey plants, and the exact number of their pollen grains were identified. A total of 4,520 chestnut pollen grains were identified in 40 analyzed melissopalynological profiles of honey. It was concluded, that the specific geographical and ecological distribution of chestnuts in the territory of Bosnia and Herzegovina directly affects the melissopalynological composition of the samples, as well as the botanical characteristics of the honey.

Keywords: honey plant, chestnut, melissopalynology, pollen



1. Introduction

Honey plants provide food for bees with their nectar, honeydew and pollen grains and enable the existence, work and development of their community (Jašmak, 1980; Dujmović Purgar & Hulina, 2007; Zima, 2007). Each sample of honey is characterized by a unique combination of pollen of honey plants, which is the result of the influence of ecological parameters and the melissopalynological potential of plants in the locality of honey grazing.

Tame chestnut (Castanea sativa Mill.) is an entomophilous, mesophilic, acidophilic woody plant species from the Fagaceae family (Šilić, 1990, 2005). The flowering of the honeydew honeysuckle lasts for about 10 days when the bees can collect an abundant amount of pollen and a small amount of nectar from the fronds (Stanimirović, Soldatović & Vučinić, 2000). Chestnut honey is rich in catalase, ascorbic acid, carotenoid derivatives, organic acids, proteins, phenolic compounds and flavonoids such as myricetin, quercetin, luteolin, etc. (Dağ, Sıralı & Tarakçı, 2017; Güneş, 2021). The share of minerals in chestnut honey is higher than in other types of honey, and characterized by a high concentration of calcium, potassium, magnesium, manganese and barium (Küçük et al, 2007; Gašić, 2017). Due to the high content of bioactive substances, chestnut honey has antimicrobial, antioxidant and antiinflammatory potential (Güneş, 2021; Alissandrakis et al, 2011; Avşar, Özler, Berber & Civek, 2016; Temizer, Güder, Temel & Cüce, 2018; Horčinová Sedláčková et al, 2021) as well as hepatoprotective properties (Yıldız et al, 2013). Due to its exceptional effects on human health, chestnut honey is recommended in phytotherapy and phytopharmacology (Horčinová Sedláčková et al, 2021). Unlike other monofloral types, chestnut honey requires the highest frequency of pollen grains of the typical honey plant (85%) in order to meet the legal criteria for declaring this type of honey (Službeni glasnik Bosne i Hercegovine 37/09, 2009).

The chestnut is originally from Asia Minor, and today it is widespread in Europe, Crimea, the Caucasus and North Africa (Šilić, 2005). Chestnut forests in Europe occupy an area of about 2 million ha, and the broadest distribution is in France and Italy, where as much as 80% of their total area is located (Pasquale et al, 2010). The distribution of chestnut forests in Bosnia and Herzegovina is conditioned by the geological and pedological peculiarities of the area, and is limited to only three areas: the Neretva area in Herzegovina (the vicinity of Jablanica, Konjic and Prozor), where it builds communities *Castanetum sativae hercegovinicum*; north-eastern Bosnian area (surroundings of Srebrenica, Bratunac, Zvornik and Tuzla) where it makes communities *Castaneo-Fagetum submontanum*; and it has the broadest distribution in the area of Bosnian Krajina (surroundings of Cazin, Kladuša, Bosanska Krupa, Novi Grad, Dubica, Kostajnica, Gradiška, Laktaš, etc.) where it forms communities of *Querco-Castanetum sativae* (Glišić, 1954; Wraber, 1958; Stupar, Šurlan, Travar & Cvjetičanin, 2014; Milanović, Brujić, Đug, Muratović & Lukuć Bilela, 2015).

The aim of the work is to determine the melissopalynological importance of chestnut based on its distribution and its pollen representation in samples originating from Bosnia and Herzegovina.

2. Material and Methods

For research purposes, 100 honey samples were collected from different biogeographical and ecological areas of Bosnia and Herzegovina (Figure 1.).



Figure 1. Biogeographical distribution of analyzed honey samples

For all samples, melissopalynological preparations were made following the Rulebook (Službeni glasnik Bosne i Hercegovine 37/09, 2009). The applied method of analysis of melissopalynological preparations is harmonized with the Rulebook and the methods proposed by the ICBB (International Commission for

Bee Botany) (Službeni glasnik Bosne i Hercegovine 37/09, 2009; Von Der Ohe, Persano Oddo, Piana, Morlot & Martin, 2004). All melissopalynological preparations were analyzed using a Wild M20 phase-contrast microscope. The identification of honey-bearing plant species in the samples, was made based on the micromorphological elements of the pollen grains (Hesse et al, 2009). After the qualitative-quantitative analysis of the preparation, melissopalynological profiles were created for each analyzed sample.

3. Results

A total of 29,707 pollen grains were identified by melissopalynological analysis. In the botanical sense, grains from 23 plant families were detected, and the largest number of species from the families Fabaceae, Fagaceae and Asteraceae (Figure 2).

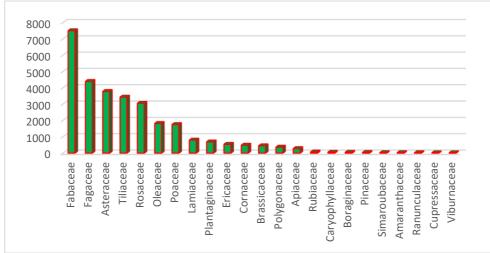


Figure 2. Total number of pollen grains for identified plant families

A total of 4,520 chestnut pollen grains were identified, which is 15% of the total number of grains. Considering the total number of pollen grains, chestnut ranks second in the research. The presence of its pollen grains was detected in 40 analyzed melissopalynological profiles of honey. It achieved the highest representation as a dominant species in 14 samples of typical, monofloral honey, while in 36 samples of polyfloral honey it appeared as an accessory species. The research results showed a correlation between the geographical

distribution of chestnuts and the melissopalynological profiles of honey samples. Thus, the largest number of monofloral samples, with the dominance of typical honey plants, was found in the area of Cazin, Bužim, Bosanska Krupa and Buturović Polje, where the largest natural stands of chestnut forests are located. As an accessory species in polyfloral samples, it has the highest frequency in samples from the localities of Ustiprača, Srebrenica, Žepa and Konjic.

Considering its biogeographical distribution, the chestnut had the greatest honey-bearing potential in the Continental region (28 samples), followed by the Alpine region (10), and the lowest in the Mediterranean biogeographic region (2).

4. Discussion

Chestnut honey is one of the fifteen typical monofloral honeys of the European continent (Persano Oddo et al, 2004). Analyzing the results of the research, we can see that monofloral honeys with a high percentage (from 85% to 99%) of pollen are typical honey-bearing species (Figure 3).

The high proportion of chestnut pollen ensures the melissopalynological and botanical originality of the samples, which is not the case with other monofloral types, such as honey sage, heather, or linden. In comparison with the results of other research, there are evident matches, so the samples from the area of Croatia (Sabo, Potočnjak, Banjari & Petrović, 2011), Albania (Pupuleku, Kapidani, Naqellari & Gjeta, 2016), Spain (Ramos, Pérez & Ferreras, 2002) and Turkey (Temizer et al, 2018) are characterized by a high proportion of typical honey plants. A significantly high proportion of chestnut pollen grains is used as a botanical marker of the region, which is also applicable to our research. The research showed a specific distribution of monofloral samples that corresponds with the ecological and geographical distribution of chestnut forests in Bosnia and Herzegovina (samples from Cazin, Bosanska Krupa, Buturović Polje, etc.).

In contrast to monofloral samples, polyfloral samples are represented in transitional areas of natural stands of chestnut forests. The share of chestnut pollen grains in polyfloral honeys is significantly lower and varies between 2% and 32% (Figure 3).

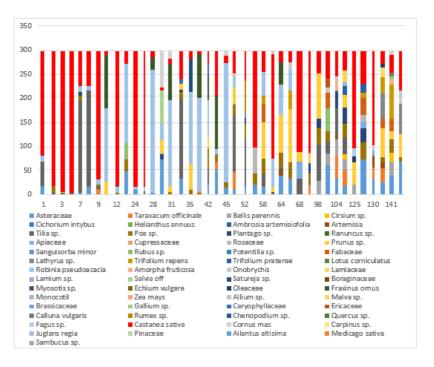


Figure 3. Melisopalynological profiles of samples in which chestnut pollen grains were identified

By comparing palynological profiles of polyfloral samples with other research (Ramos et al, 2002; Sabo et al, 2011; Pupuleku et al, 2016; Rašić, Štefanić, Antunović, Jović & Kristek, 2018; Temizer et al, 2018) similarities are observed in terms of varying the share of chestnut as a honey plant, in the interval from 3 to 40%. So far, chestnut research in Bosnia and Herzegovina has mainly focused on its ecological and synecological differentiation (Sučić, 1953a, 1953b; Glišić, 1954; Wraber, 1958; Macanović, 2011; Stupar, Šurlan, Travar & Cvjetičanin, 2014), morphological characteristics (Mujić et al, 2010; Mujagić-Pašić & Ballian, 2011), chemical composition (Mujić, Alibabić, Ibrahimpašić, Jahić & Muslimović, 2006) and diseases (Treštić, Dautbašić & Mujezinović, 2009). This paper represents original research in the context of defining the potential of chestnut as a honey-bearing plant in the flora of Bosnia and Herzegovina.

5. Conclusions

Based on the results of the analysis of honey samples, it was concluded that the specific biogeographical distribution of chestnuts in the territory of Bosnia and Herzegovina directly affects the melissopalynological composition of the samples, as well as the botanical characteristics of the honey. As part of the research, 4,520 chestnut pollen grains were identified in 40% of the analyzed honey samples. The research showed that the chestnut, as a honey-bearing plant, has great honey-making potential in the analyzed seasons. Given that this honey plant is characterized by a limited geographical distribution, high honey potential, natural enemies (chestnut wasp and fungi), but also high anthropogenic pressure, natural chestnut forests must be protected and marked in the cadastre of bee grazing in Bosnia and Herzegovina.

6. References

- Alissandrakis, E., Petros, A., Tarantilis, P.A., Pappas, C., Harizanis, P.S., Moshos Polissiou, M. (2011). Investigation of organic extractives from unifloral chestnut (*Castanea sativa* L.) and eucalyptus (*Eucalyptus globulus* Labill.) honeys and flowers to identificationof botanical marker compound. LWT -*Food Science and Technology*, 44(4), 1042-1051.
- Avşar, C., Özler, H., Berber, I., Civek, S. (2016). Phenolic composition, antimicrobial and antioxidant activity of *Castanea sativa* Mill. pollen grains from the Black Sea region of Turkey. *International Food Research Journal*, 23(4), 1711-1716.
- Dağ, B., Sıralı, R., Tarakçı, Z. (2017). Investigation of Some Properties of Chestnut Honey Produced in Black Sea Region of Turkey. *Batman University Journal of Life Sciences*, 7(2/2), 118-123.
- Dujmović Purgar, D., Hulina, N. (2007). The honey plants of Plešivica hills (NW Croatia). *Agronomski glasnik*, 69(1), 3-33.
- Gašić U. (2017). *Fitohemijski marker botaničkog i geografskog porekla meda*. Beograd: Hemijski Fakultet, Univerzitet u Beogradu. Doktorska disertacija.
- Glišić, M. (1954). Prilog poznavanju fitocenoza pitomog kestena i bukve u Bosni. *Šumarstvo, 7*(3), 162-174.
- Güneş, ME. (2021). Chestnut Honey as a Complementary Medicine: Determination of Antibacterial Activity, Heavy Metal Residue and Health Risk

Assessment. Journal of Advances in VetBio Science and Techniques, 6(2), 82-89. doi.org/10.31797/vetbio.931144

- Hesse, M., Halbritter, H., Zetter, R., Weber, M., Büchner, R., Frosch-Radivo, A., Ulrich, S. (2009). *Pollen Terminology An illustrated handbook*. Wien New York: Springer.
- Horčinová Sedláčková, V., Grygorieva, O., Fatrcová Šramková, K., Shelepova, O., Goncharovska, I., Mňahončáková, E. (2021). The chemical composition of pollen, staminate catkins, and honey of *Castanea sativa* Mill. *Potravinarstvo Slovak Journal of Food Sciences*, *15*, 433-444.
- Jašmak, K. (1980). Medonosno bilje. Beograd: Nolit.
- Küçük, M., Kolayh, S., Karaoğlu, S., Ulusoy, E., Baltaci, C., Candon, F. (2007). Biological activities and chemical composition of three honeys of different types from Anatolia. *Food Chemistry*, 100(2), 526-534.
- Macanović, A. (2011). Ekološko-sintaksonomska analiza šuma pitomog kestena (*Castanea sativa* Mil.) na području BIH. Struktura i dinamika ekosistema Dinarida stanje, mogućnosti i perspektive. *Akademija nauka i umjetnosti Bosne i Hercegovine*.
- Milanović, Đ., Brujić, J., Đug, S., Muratović, E., Lukuć Bilela, L. (2015). *Vodič kroz tipove staništa BiH prema Direktivi o staništima EU*. Belgium: Prospect CiSs.a. Rue du Price Royal 83, 1050 Brussels.
- Mujagić-Pašić, A., Ballian, D. (2011). Systematical position of sweet chestnut (*Castanea sativa*Mill.) within the genus *Castanea* in Bosnia and Herzegovina. *Naše šume. Časopis za unapređenje šumarstva, hortikulture i očuvanja okoline, 24-25*, 17-23.
- Mujić, I., Alibabić, A., Živković, J., Jahić, S., Jokić, S., Prgomet, Ž., Tuzlak, Z. (2010). Morphological characteristics of chestnut *Castanea sativa* from the area Una-Sana Canton. *Journal Central European Agriculture*, 11(2), 185-190.
- Mujić, I., Alibabić, V., Ibrahimpašić, J., Jahić, S., Muslimović, D. (2006). Characteristics of the chestnuts from Una Sana Canton in comparison to other chestnut varieties and the influence of different preservation techniqueson nutritive values. *Acta Horticulturae*, *768*, 359-366.

- Pasquale, G. Di, Allevato, E., Russo Ermolli, E., Coubray, S., Lubritto, C., Marzaioli, F., Yoneda M., Takeuchi, K., Kano, Y., Matsuyama, S., De Simone, G. F. (2010). Reworking the idea of chestnut (*Castanea sativa* Mill.) cultivation in Roman times: New data from ancient Campania. *Plant Biosystems*, 144(4), 865-873.
- Persano Oddo, L., Piro, R., Bruneau, E., Guyot-Declerck, C., Ivanov, T., Piškulová, J., Flamini, C., Lheritier, J., Morlot, M., Russmann, H., Von Der Ohe, W., Von Der Ohe, K., Gotsiou, P., Karabournioti, S., Kefalas, P., Passaloglou-Katrali, M., Thrasyvoulou, A., Tsigouri, A., Marcazzan, G. L., Piana, M. L., Piazza, M. G., Sabatini, A. G., Kerkvliet, J., Godinho, J., Bentabol, A., Ortiz Valbuena, A., Bogdanov, S., Ruoff, K. (2004). Main European unifloral honeys: descriptive sheets. *Apidologie 35*, 38-S81.
- Pupuleku, B., Kapidani, G., Naqellari, P., Gjeta, E. (2016). Melissopaynological Study of Albania's Honey. Academic Journal of Interdisciplinary Studies, MCSER Publishing, 5, 261-268.
- Ramos, I.L.S., Pérez, B.M., Ferreras, C.G., (2002). Pollen spectra of different unifloral honeys from La Palma (Canary Islands, Spain). *Grana*, *41*, 48-57.
- Rašić, S., Štefanić, E., Antunović, S., Jović, J., Kristek, S. (2018). Pollen analysis of honey from north-eastern Croatia. *Poljoprivreda/Agriculture, Faculty of Agrobiotechnical Sciences Osijek, Agricultural Institute Osijek, 24*, 43-49.
- Sabo, M., Potočnjak, M., Banjari, I., Petrović D. (2011). Pollen analysis of honeys from Varaždin County, Croatia. *Turkish Journal of Botany*, *35*(5), 581-587.
- Šilić, Č. (1990). Atlas drveća i grmlja. Sarajevo: Svjetlost, Zavod za udžbenike i nastavna sredstava.
- Šilić, Č. (2005). Atlas dendroflore (drveće i grmlje) Bosne i Hercegovine. Matica hrvatska Čitluk: Franjevačka kuća Masna Luka.
- Službeni glasnik Bosne i Hercegovine, broj 37/09 (2009). Pravilnik o metodama za kontrolu meda i drugih pčelinjih proizvoda Bosne i Hercegovine.
- Stanimirović, Z., Soldatović, B., Vučinić, M. (2000). *Biologija pčela. Medonosna pčela*. Beograd: Medicinska knjiga.

- Stupar, V., Šurlan, M., Travar, J., Cvjetičanin, R. (2014). Fitocenološka analiza mezofilnih šuma pitomog kestena (*Castanea sativa* Mill.) u okolini Kostajnice (Bosna i Hercegovina). *Glasnik Šumarskog fakulteta Univerziteta u Banjoj Luci, 21*, 25-43.
- Sučić, J. (1953a). O arealu pitomog kestena (*Castanea sativa* Mill.) na području Srebrenice sa kratkim osvrtom na ostala nalazišta kestena u NR BiH. *Institut za naučna istraživanja u Sarajevu, 2*(4).
- Sučić, J. (1953b). Rasprostranjenost pitomog kestena na području Bosne i Hercegovine. *Narodni šumar, 7*(9-10), 361-378.
- Temizer, I. K., Güder, A., Temel, F. A., Cüce, H. (2018). Antioxidant activities and heavy metal contents of *Castanea sativa* honey. *Global NEST Journal, 20*(3), 541-550.
- Treštić, T., Dautbašić, M., Mujezinović, O. (2009). Štetni organizmi ploda pitomog kestena. Naše šume. *Časopis za unapređenje šumarstva, hortikulture i očuvanja okoline, 14-15*, 3-11.
- Von Der Ohe, W., PersanoOddo, L., Piana, M. L., Morlot, M., Martin, P. (2004). Harmonized methods of melissopalynology. *Apidologie*, *35*, 18-S25.
- Wraber, M. (1958). Biljno sociološki prikaz kestenovih šuma Bosne i Hercegovine. *Godišnjak Biološkog instituta Univerziteta u Sarajevu, 9*(1-2), 139-182.
- Yıldız, O., Can, Z., Saral, Ö., Yuluğ, E., Öztürk, F., Aliyazıcıoğlu, R., Kolaylı, S. (2013). Hepatoprotective potential of chestnut bee pollen on carbon tetrachlorideinduced hepatic damages in rats. *Evidence-Based Complementary and Alternative Medicine, 2013,* 1-9. doi.org/10.1155/2013/461478
- Zima, D. (2007). Prilog poznavanju medonosnog bilja. *Agronomski glasnik,* 69(2), 147-160.