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ORIGINAL SCIENTIFIC PAPER

Honey-bearing potential of dendroflora in Bosnia and Herzegovina

Medonosni potencijal dendroflore u Bosni i Hercegovini

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ABSTRACT

The floristic composition and ecological characteristics of the area where honey grazing is carried out directly define the botanical origin as well as the physical and chemical properties of honey. The goal of this research was to determine the potential of woody and shrubby plant species in the apiflora from Bosnia and Herzegovina (B&H) based on the qualitative-quantitative palynological analysis of honey samples. In the research, 100 different types of honey samples from B&H were collected and analyzed. The melissopalinological preparations were prepared and analyzed in accordance with the Rulebook on methods for the control of honey and other bee products of B&H, as well as the methods proposed by ICBB. After the melissopalinological analysis, 25 plant families with a total of 30,000 pollen grains were identified, of which 16 were woody or shrubby plants with 18,126 pollen grains in the preparations. In the research, the most presented honey-bearing woody plants were: black locust (*Robinia pseudoacacia*), chestnut (*Castanea sativa*) and linden (*Tilia* sp.). Each analyzed palynological profile represented a unique combination of pollen from honey-bearing plants, as a specific biological imprint of the place of honey grazing.

Key words: *Melissopalynology, honey, pollen, woody apiflora*

INTRODUCTION – *Uvod*

The honey plants include all plant species whose natural products (pollen, nectar and honeydew) are the main source of food for honey bees (*Apis mellifera*) (Dujmović Prugar & Hulina, 2007; Ljevnaić-Mašić et al, 2019). In the diet of bees, nectar is the primary source of carbohydrates, while pollen is the main source of proteins, but lipids, vitamins, minerals, and polyphenols and flavonoids also occur (Stanimirović et al, 2000; Campos et al, 2008; Bogdanov, 2012 Ljevnaić-Mašić et al, 2019).

The quantitative and qualitative properties of nectar and pollen are defined by internal (systematic affiliation and physiological properties of the plant species) and external (abiotic environmental parameters: temperature, air humidity, light, soil and wind) factors (Pešić et al, 2004). The physiological processes in bees, as well as the amount of honey production, are directly affected by the distribution of honey-bearing plant species on which bees graze (Diniz Frias et al, 2016).

The floristic composition of honey pasture is determined by ecological, biogeographic and anthropogenic factors in the area (Chiș & Purcarea, 2011; Tomczyk et al, 2019). Therefore, the selection of honey pasture, based on its floristic composition, is crucial for the creation of botanical origin and physico-chemical properties of honey (Ball, 2007; Sari & Ayyildiz, 2013; Alibabić et al, 2017; Altay et al, 2018).

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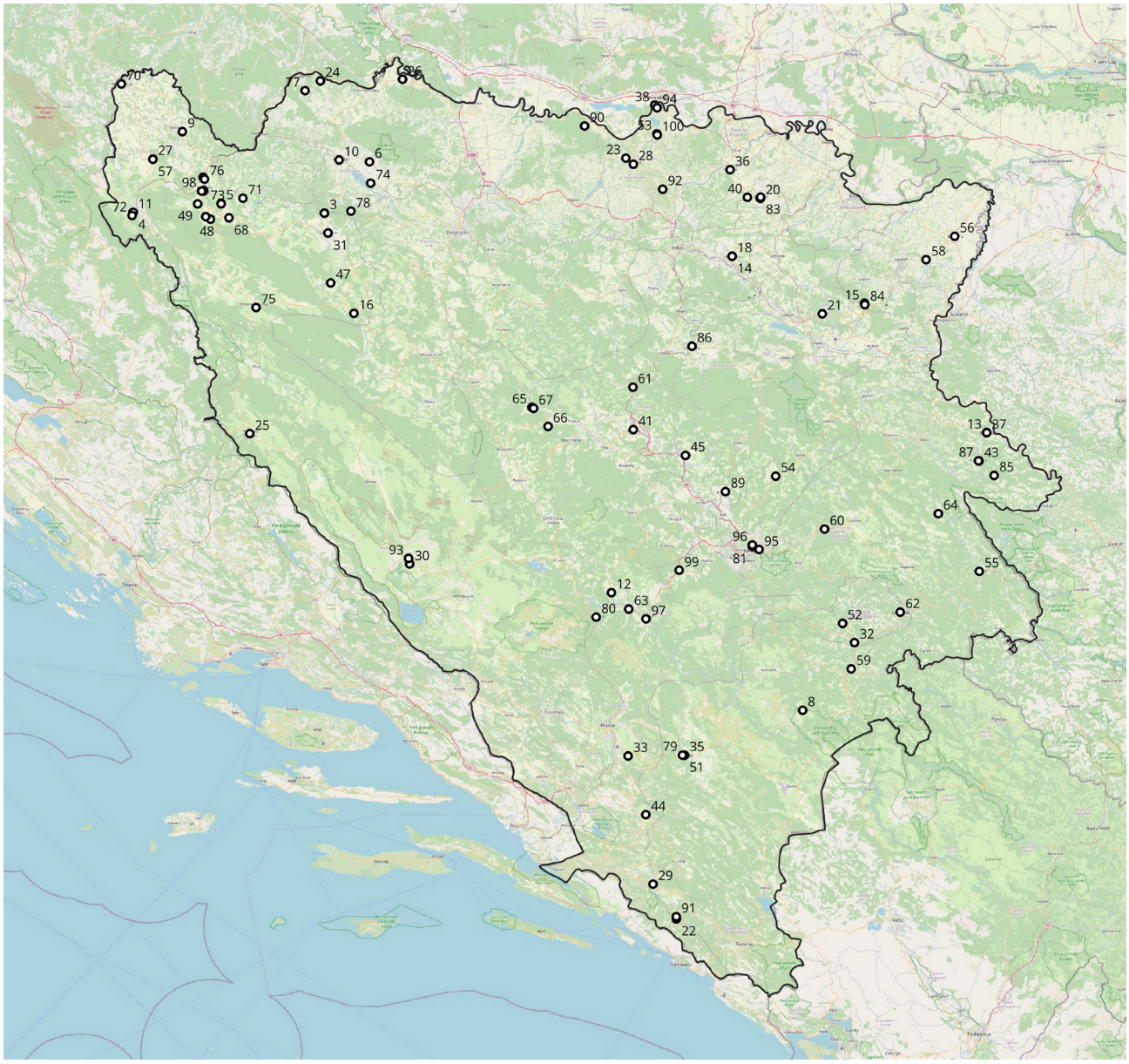


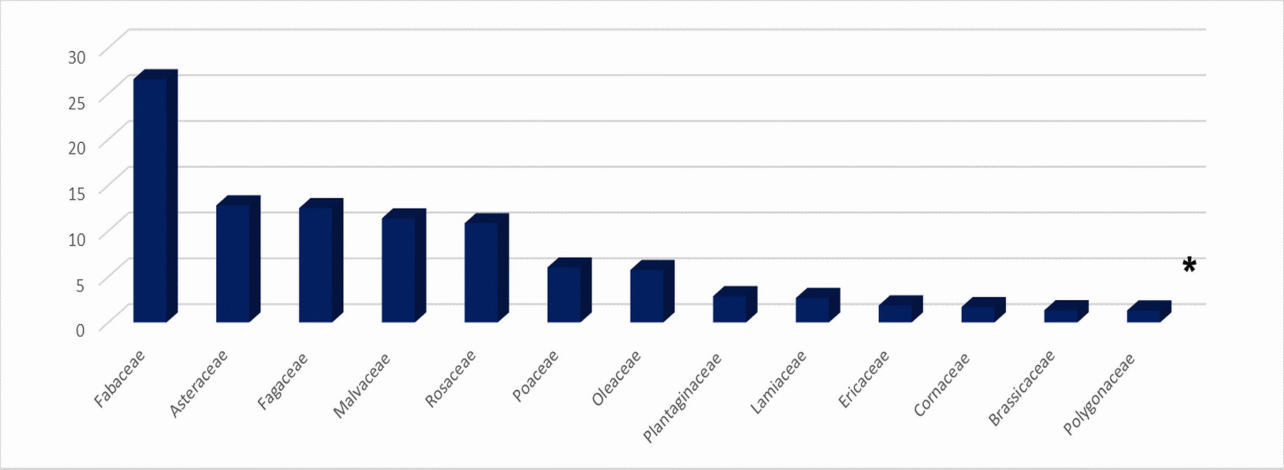
Figure 1. Map of the distribution of the analyzed samples

Slika 1. Karta distribucije analiziranih uzoraka

The aim of this research was to determine: (i) which types of woody and shrubby plants are most frequent in honey samples collected throughout Bosnia and Herzegovina (B&H), (ii) frequency and importance of woody and shrubby plants in the apiflora of B&H.

MATERIALS AND METHODS – *Materijal i metode*

As part of the research, 100 samples of different types of honey from Bosnia and Herzegovina were collected (Figure 1). The collecting of honey samples covered the entire territory of Bosnia and Herzegovina, but the availability of samples was conditioned by the distribution of apiaries and the willingness of honey producers to cooperate. The analysis included 56 monofloral and 44 polyfloral samples that were continuously collected in the period from 2015 to 2021. All analyzed samples were collected directly from honey producers with stationary apiaries. The melisopalinological preparations were made in accordance with the Rulebook (Regulation on methods for the control of honey and other bee products of B&H, Official Gazette, 37/09, 2009). The method of analysis of melissopalinological preparations was applied according to the Rulebook as well as the methods proposed by the ICBB (International Commi-



Graph 1. Families with a percentage share of pollen grains above 1% in the total sample

* Families with a percentage share of pollen grains under 1% in the total sample: Apiaceae, Boraginacea, Pinaceae, Rubiaceae, Juglandaceae, Caryophyllaceae, Viburnaceae, Simaroubaceae, Chenopodiaceae, Cupressaceae i Ranunculaceae

Grafikon 1. Porodice sa procentualnim udjelom polenovih zrna iznad 1% u ukupom uzorku

** Porodice sa procentualnim udjelom polenovih zrna ispod 1% u ukupom uzorku*: Apiaceae, Boraginacea, Pinaceae, Rubiaceae, Juglandaceae, Caryophyllaceae, Viburnaceae, Simaroubaceae, Chenopodiaceae, Cupressaceae i Ranunculaceae

ssion for Bee Botany) (Von Der Ohe et al, 2004; Rulebook on methods for the control of honey and other bee products of B&H, Official Gazette, 37/09, 2009). The melissopalynological preparations were analyzed using the Wild M20 phase-contrast microscope. The identification of plant species was based on the micromorphological elements of pollen grains (Hesse et al, 2009; Erdtman, 1943, 1952), which was followed by the qualitative-quantitative analysis of preparations. The qualitative analysis related to the inventory of honey-bearing plants identified, while quantitative analysis determined the exact number of pollen grains in the preparations. Based on the qualitative-quantitative analysis results, the melissopalynological profiles of honey samples were created.

Geographical distribution of ordinal numbers: 1-Drenova glavica, 2-Cazin, Koprivna, 3-Cazin, Koprivna, 4-Bihać, 5-Zalin, 6-Kozarac, 7-Bosanska Krupa, 8-Crno jezero, 9-Bužim, Radoč, 10-Prijedor, 11-Bihać, 12-Buturović polje, 13-Bratunac, 14-Gračanica, 15-Majevica, 16-Ključ, 17-Gračanica, 18-Gračanica, 19-Trebinje, Bobani, 20-Gradačac, 21-Tuzla, 22-Trebinje, Bobani, 23-Bosanski Dubačac, 24-Bosanska Dubica, Babinac, 25-Bosansko Grahovo, 26-Bosanka Dubica, Međeđa, 27-Cazin, 28-Bosanski Brod i Derventa, 29-Popovo polje, Ravno, 30-Livno, 31-Prijedor i Sanski Most, 32-Ustikolina, 33-Blagaj, Kamena, 34-Gradačac, 35-Nevesinje, 36-Modriča, 37-Bratunac, 38-Bosanski Brod, Svilaj, 39-Gradačac, 40-Gradačac, Novalići, 41-Zenica, 42-Bosanska Krupa, 43-Srebrenica, 44-Stolac, 45-Kakanj, 46-Sanski most, 47-Ključ, Sanica, 48-Bosanska Krupa, Suvaja, 49-Bosanska Krupa, Vranjska, 50-Bosanksa Krupa, Benkovac, 51-Nevesinje, 52-Goražde, Milanovići, 53-Derventa, Zborišta i Bosanski Brod, 54-Olovo, 55-Višegrad, 56-Bijeljina, 57-Cazin, 58-Ustiprača, Radič, 59-Foča, 60-Romanija, 61-Zenica, Nemila, 62-Goražde, 63-Čelebići, 64-Žepa, Begići, 65-Travnik, Karaula, 66-Travnik, Bijelo Buče, 67-Travnik, Karaula, Krčevine, 68-Bosanska Krupa, Jasenica, 69-Bosanskaja Krupa, Suvaja, 70-Velika Kladuša, 71-Bosanska Krupa, Veliki dubovik, 72-Bihać, 73-Bosanska Krupa, Zalin, 74-Prijedor, Petrov gaj, 75-Bosanski Petrovac, 76-Bosanska Krupa, Halkići, 77-Kostajnica, 78-Bosanska Krupa, Velika Jasenica, 79-Nevesinje, 80-Jablanica, 81-Pofalići, 82-Bosanska Krupa, 83-Gradačac, 84-Majevica, 85-Srebrenica, Brežani, 86-Zavidovići, 87-Srebrenica, Osmanovići, 88-Bosanska Krupa, 89-Breza, Bukovik, 90-Bosanski Kobaš, 91-Trebinje, Bobani, 92-Brezići, 93-Livno, 94-Bosanski Brod, 95-Sarajevo, 96-Pofalići, 97-Konjic, 98-Bosanska Krupa, 99-Tarčin i 100-Derventa i Bosanski Brod, Zborišta

RESULTS AND DISCUSSION – *Rezultati i diskusija*

After the melissopalinological analysis, a total of 30,000 pollen grains were identified. In the botanical sense, a total of 25 plant families were determined, whereby the largest number of pollen grains originated from Fabaceae (7967), Asteraceae (3832) and Fagaceae (3741) (Graph 1). By analyzing micromorphological features, 48 different types of pollen grains were identified. The ave-

Table 1. Identification of honey-bearing woody and shrubby plants in the study

Tabela 1. Identifikovne medonosne drvenaste i žbunaste biljke u istraživanju

Latin name of the species	The family	Total number of pollen grains	The number of samples in which they were detected	C*	N*	P*
Ailanthus altisima (Mill.) Swingle	Simaroubaceae	45	2	5-6	good	good
Amorpha fruticosa L.	Fabaceae	252	2	5-6	very good	very good
Calluna vulgaris (L.) Hull	Ericaceae	376	5	7-9	good	weak
Castanea sativa Mill.	Fagaceae	3659	35	6	good	excellent
Cornus mas L.	Cornaceae	500	14	2-3	weak	good
Fraxinus ornus L.	Oleaceae	422	11	4-5	weak	good
Juglans regia L.	Juglandaceae	62	3	3-5	there is none	excellent
Pinus sp.	Pinaceae	115	11			
Prunus sp.	Rosaceae	2026	49			
Quercus sp.	Fagaceae	82	8			
Robinia pseudoacacia L.	Fabaceae	6140	67	5-6	excellent	weak
Rubus sp.	Rosaceae	564	10			
Salvia officinalis L.	Lamiaceae	70	1	5-6	excellent	weak
Sambucus sp.	Viburnaceae	53	3	5-6		
Satureja sp.	Lamiaceae	354	4	7-10	excellent	good
Tilia sp.	Malvaceae	3406	53	5-7	excellent	good

C* - flowering period, *N - nectar production, *P - pollen production (Umeljić, 2013, 2015; Šilić, 1990, 2005)

rage number of honey plants per sample was seven, whereas the maximum number of 16 honey species was identified in the palynological profile from Livno.

By the analysis of melissopalynological profiles, 16 woody or shrubby plant species were identified in samples with a total of 18,126 pollen grains (Table 1). The pollen of woody or shrubby plants, in different percentages (5-100%), was identified in as many as 98 (98%) melissopalynological profiles (Graph 2). In botanical terms, a total of 11 families of woody or shrubby plants were identified (Table 1).

The woody honeydew plant with the greatest melissopalynological significance was black locust (*Robinia pseudoacacia*) with 6140 pollen grains, while the tree of heaven (*Ailanthus altisima*) with 45 pollen grains was the species with the smallest share in this research.

As part of the pollen research, black locust grains were identified in 67% of the palynological profiles as a dominant or accessory species (Graph 2). Black locust is one of the most important honey-bearing plants in Europe (Dujmović Prugar & Hulina, 2007) and is characterized by high nectar production so that the daily intake on pasture can be up to 15 kg of nectar (Umeljić, 2015). These honey-bearing properties make black locust one of the most desirable honey-bearing plants in B&H as well as in the region. So that black locust honey in Croatia (Uršulin-Trstenjak et al, 2014; Denžić Lugomer et al, 2017) and Serbia (Lazarević et al, 2012) is among the most abundant type of monofloral type, and it is also on the list of the 15 most important monofloral types of honey in Europe (Persano Oddo et al, 2004).

However, this plant species is marked as invasive in B&H and we must be extremely careful in introducing new individuals and controlling existing populations of this invasive species (Djug et al, 2019; EPPO, 2023). Given that Luigi et al (2023) determined the extremely negative effects of black locust on the growth and development of chestnut individuals, we must pay great

attention to the protection of our native species of honey plants (Luigi et al, 2023). The negative influence of black locust on the distribution of chestnuts was confirmed in the area of S Switzerland and N Italy, where it suppressed complete chestnut forests (Sabo, 2000; Sitzia et al, 2012). Black locust is characterized as a dangerous invasive species due to its invasiveness in native forest communities, and its distribution is strictly controlled in many Asian and European countries (Lazzaro et al, 2018; Lazzaro et al, 2020; Luigi et al, 2023; De Marco et al, 2023)

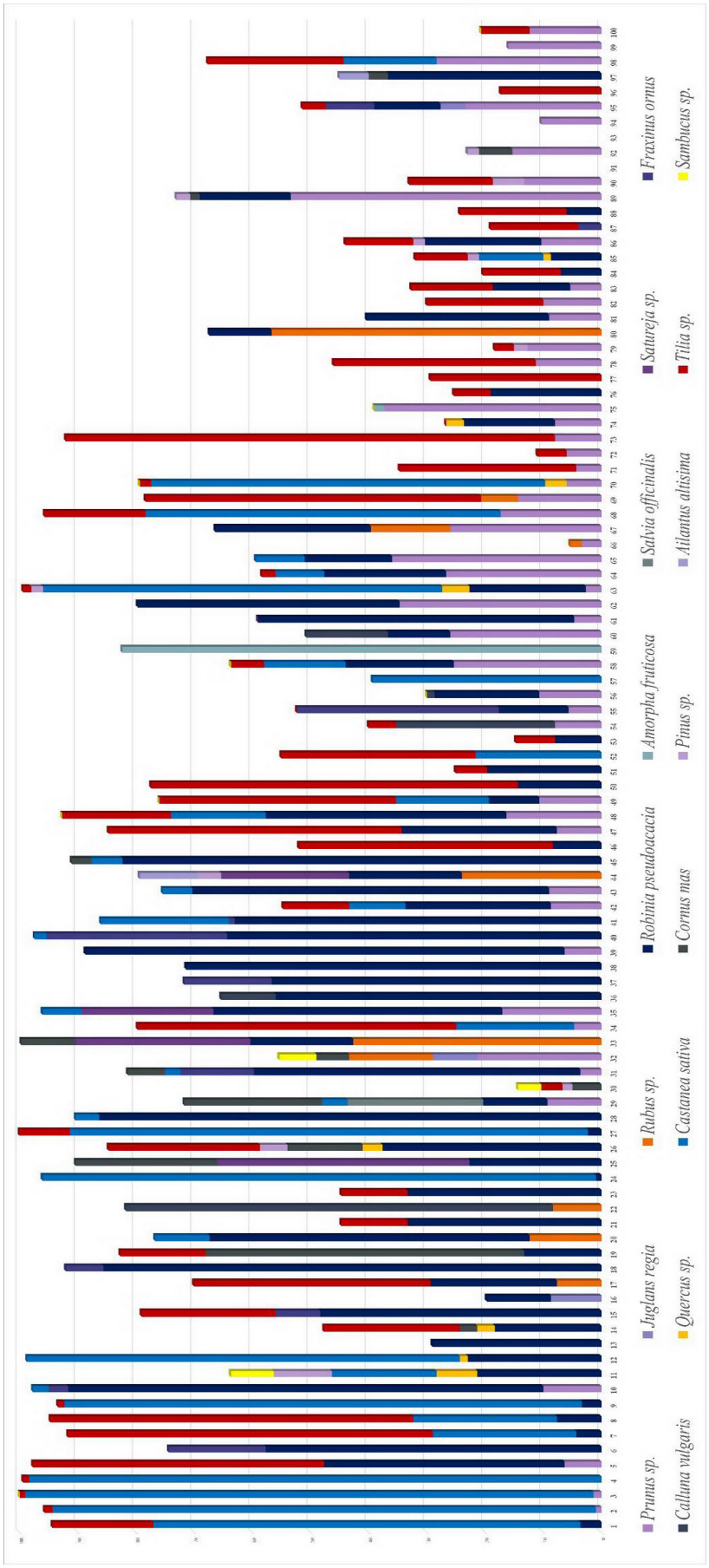
A total of 3,659 chestnut (*Castanea sativa*) pollen grains were identified within this research. Pollen grains of this honey plant were identified in 35% of the palynological profiles (Graph 2). Chestnut gives copious amounts of nectar and is specific because it gives much more pollen than any other honey plant (Umeljić, 2015). The research established that the ecological distribution of the species (Stupar et al, 2014; Milanović et al, 2015) is directly reflected through palynological profiles. Thus, the samples with the highest percentage share of this honey plant were identified in the localities where the largest natural stands of chestnut forests are located: Cazin (97%), Bihać (96%), Bužim (89%), Bosanska Krupa (75%), Buturović polje (74%), Čelebići (68%). Apart from certain regions of B&H, a significantly high proportion of chestnut pollen grains was detected in Croatia (Sabo et al, 2011), Albania (Pupuleku et al, 2016), Spain (Ramos et al, 2002) and Turkey (Temizer et al, 2018). Chestnut honey is characterized by its dark color and bitter taste (Umeljić, 2015), and due to its distinct antibacterial, antioxidant and anti-inflammatory properties (Alissandrakis et al, 2011; Avşar et al, 2016; Temizer et al, 2018; Horčinová Sedláčková et al, 2021; Güneş, 2021) is recognized as a very important monofloral type of honey in Europe (Persano oddo et al, 2004; European Commission DG Agriculture and Rural Development, 2013).

Linden (*Tilia* sp.) pollen grains were identified in 53% of the palynological profiles, a total of 3406 pollen grains of this honey-bearing plant were determined (Graph 2). Linden is one of the important honey-bearing plants of the temperate climate area of the northern hemisphere (Gašić et al, 2014) because it produces abundant amounts of nectar and pollen, so bees visit it very often (Umeljić, 2015). The honeydew pasture of linden can also carry corresponding deficiencies, mannose (Argoti, 2016), phenols, alkaloid nicotine were detected in the nectar. Linden honey pasture can also carry corresponding deficiencies, mannose (Argoti, 2016), phenols, alkaloid nicotine (Singaravelan et al, 2006; Tiedeken et al, 2014; Baracchi et al, 2015) and volatile secondary metabolites (Jacquemart et al, 2018) were detected in the nectar which can be toxic to bees after heavy consumption. However, linden honey, thanks to its specific chemical properties (Gašić et al, 2014), has strong probiotic, anticancer (Celebioglu et al, 2021) and antioxidant (Ćetković et al, 2014) effects, and is extremely important in human nutrition.

Pollen grains of the genus *Quercus* sp. were identified in the palynological spectra and *Pinus* sp. (Table 1, Chart 2). These honey-bearing species are extremely important for beekeeping, because they provide certain amounts of pollen, and after flowering, copious amounts of honeydew. Honey can be given up to four times a year, depending on the honey-bearing species on which the pasture is collected (Stanimirović et al, 2000; Dujmović Prugar & Hulina, 2007). The antibacterial effect of the honeydew of some species from the Pinaceae family has been proven, such as fir honeydew (*Abies alba* L.) inhibits the growth and adhesion of the infectious bacterium *Campylobacter jejuni* (Ramić et al, 2023).

Representatives of the Lamiaceae family are extremely important in apiflora due to the presence of essential oils, iridiodes, flavones, flavonoids, etc. (Frezza et al, 2019; Kačániová et al, 2021). As part of the research, *Salvia officinalis* pollen was identified in the paleontological profile from the locality Ravno (70 pollen grains, 23%), while the pollen of species from the *Satureja* genus was identified in the largest number at the localities Bosansko Grahovo (130, 43%), Blagaj (90, 30%) and Nevesinje (68, 22%) (Chart 2). Due to their specific characteristics, honey-bearing species from the Lamiaceae family are of great importance in honey-bearing grazing not only in the Mediterranean and sub-Mediterranean regions of Bosnia and Herzegovina, but also in regions in Croatia (Britvec, 2013), Turkey (Topal et al, 2023) and Romania (Ion et al, 2018).

As part of the research, non-native and invasive species *Amorpha fruticose* and *Ailanthus altisima* were identified (Djug et al, 2019; EPPO, 2023). Tree of heaven (*Ailanthus altisima*) and indigo bush (*Amorpha fruticosa*) have a negative allelopathic effect that can have very harmful consequences on the biodiversity of native apiflora (Gómez-Aparicio & Canham, 2008; Novak et al, 2018). Pollen of the tree of heaven was identified in the melissopalynological profiles of honey from Stolac and Konjic, where it spreads intensively in the Mediterranean and sub-Mediterranean area (Boškailo et al, 2017). While indigo bush pollen grains were detected in honey samples from the vicinity of Foča and Bosanski Petrovac. Both species are characterized by high production of nectar and pollen and low ecological requirements towards the environment (Umeljić, 2015), which makes



Graph 2. The percentage share of the researched honey-bearing species in the analyzed palynological profiles (geographical distribution of ordinal numbers of localities is indicated in Figure 1)

Grafikon 2. Procentualni udio istraživanih medonosnih vrsta u analiziranim palinološkim profilima (geografska distribucija rednih brojeva lokaliteta je označen na slici 1)

them extremely desirable in the apiflora of B&H and the region. Thus, pollen grains of these invasive species were identified in honey samples originating from Croatia (Rašić et al, 2018; Zima et al, 2018), Serbia (Nešović et al, 2020), Bulgaria (Atanassova et al, 2009; Tashev et al, 2015) and Hungary (Bodó et al, 2021).

As stated above, the knowledge of phenophases of flowering for species is most important in order to maximize its melissopalynological potential (Topal et al, 2023). Analyzing the flowering period of the identified species, we can observe that the largest number of plants express their honey-bearing potential in the period from May to July (seven species). The species that start the phenophase of flowering the earliest are dogwood (*Cornus mas*), walnut (*Juglans regia*) and species of the genus *Prunus*, the abundant production of these honey-bearing species accelerates the development of the brood and stimulates greater bee activity, which is a key phase during the spring development of the colony (Perišić et al, 2004). At the latest, during September and October, species of the genus *Satureja* and *Calluna vulgaris* bloom, which represent a very important autumn honey pasture, which provides a sufficient amount of nutrients for the unfavorable (winter) period of the year (Momirovski & Šimić, 1953; Stanimirović et al, 2000).

CONCLUSIONS – *Zaključak*

In the research, 100 melissopalynological profiles were analyzed and 30,000 pollen grains identified. A total of 48 pollen types were identified, 16 of which belong to woody and shrubby honey-bearing species. Of the total number of pollen grains identified, 60.42% (18126) belong to woody apiflora. Three species belonging to the group of invasive species were identified within the honey-bearing dendroflora. With the introduction of woody invasive species that have greater melissopalynological potential and greater economic benefit for beekeepers, we have to be careful because they may pose a biological threat to the native apiflora (Ion et al, 2018).

Based on the results, we can conclude that woody or shrubby forms of honey-bearing plants are of great importance in honey production. Also, these results point to the importance of forest ecosystems as irreplaceable grazing grounds for honey bees and the need for their conservation. Such studies aim to investigate the potential and biodiversity of apiflora as a important component of the resource potential of B&H.

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SAŽETAK

Medonosne biljke podrazumjevaju sve biljne vrste čiji su prirodni produkti (polen, nektar i medljika) glavni izvor hrane za medonosne pčele (*Apis mellifera*). U okviru istraživanja prikupljeno je i analizirano 100 uzoraka različitih tipova meda iz BiH (Slika 1). Melisopalinološki preparati izrađeni su i analizirani u skladu sa Pravilnik o metodama za kontrolu meda i drugih pčelinjih proizvoda Bosne i Hercegovine, kao i metodama koje predlaže ICBB. Nakon melisopalinologe analize 100 uzoraka meda identifikovano je ukupno 30000 polenovih zrna. U botaničkom smislu, determinisano je ukupno 25 biljnih porodica, a najveći broj polenovih zrna je konstatovan za porodice: Fabaceae (7967), Asteraceae (3832) i Fagaceae (3741) (Grafikon 1). Analizom mikromorfoloških osobenosti, identifikovano je 48 različitih tipova polena.

Analizom melisopalinoloških profila identifikovan je polen 16 drvenastih i žbunastih vrsta biljaka sa ukupno je 18126 (60,42%) polenovih zrna (Tabela 1.). Polen drvenastih i žbunastih biljaka, u različitom procentualnom udjelu (5-100%), identifikovan je u čak 98% melisopalinoloških profila (Grafikon 2). U botaničkom smislu ukupno je identifikovano 11 porodica drvenastih i žbunastih biljaka. Na osnovu rezultata možemo zaključiti da su drvenaste i žbunaste forme medonosnih biljaka imale veliki značaj u proizvodnji meda u analiziranom periodu. Takođe, ovi rezultati upućuju na značaj šumskih ekosistema kao nezamjenjive ispaše medonosnih pčela i potrebe za njihovom očuvanjem. Ovakve studije imaju za cilj proučavanje potencijala i biodiverziteta apiflore kao jednoj vrlo važnoj komponenti resursnog potencijala Bosne i Hercegovine.

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