

# Pollen of allergenic plants in honey samples from Bosnia and Herzegovina

Original Article

## Abstract:

Honey plants are characterized by the specific chemical composition of nectar and pollen grains, which depends on the physiological characteristics of plant species, ecological conditions, and anthropogenic pressure. In total, 100 honey samples were analyzed according to the Rulebook on methods for the control of honey and other bee products of Bosnia and Herzegovina. The melissopalynological analysis included plant identification based on the micromorphological characters of pollen grains and the number of pollen grains for each identified plant. In total, 31,183 pollen grains were detected in melissopalynological profiles. Based on the micromorphological features, 48 plant species, including seven with allergenic potential, were identified. The proportion of pollen grains of allergenic plants was 18.01% (5,616 pollen grains). Both the quality and health of honey are reduced due to presence of allergenic pollen in it which can cause allergic reactions. Hence the choice of bee pasture concerning its floristic composition and its distance from the pollution source are crucial parameters for the honey quality.

## Key words:

allergy, honey, melissopalynology, pollen

## Apstrakt:

### Polen alergenih biljaka u uzorcima meda iz Bosne i Hercegovine

Medonosne biljke karakteriše specifičan hemijski sastav nektara i zrnaca polena, što zavisi od fizioloških karakteristika biljnih vrsta, ekoloških uslova i antropogenog pritiska. Analizirano je ukupno 100 uzoraka meda prema Pravilniku o metodama kontrole meda i drugih pčelinjih proizvoda Bosne i Hercegovine. Melisopalinološka analiza uključivala je identifikaciju biljaka na osnovu mikromorfoloških karakteristika zrnaca polena i broja zrnaca polena za svaku identifikovanu biljku. Ukupno je detektovano 31.183 zrnaca polena u melisopalinološkim profilima. Na osnovu mikromorfoloških karakteristika identifikovano je 48 biljnih vrsta, uključujući sedam sa alergenim potencijalom. Proporcija zrnaca polena alergenih biljaka bila je 18,01% (5.616 zrnaca polena). Kvalitet i zdravstvena bezbednost meda smanjuju se zbog prisustva polena koji može izazvati alergijske reakcije. Izbor pčelinje paše, u pogledu florističkog sastava i udaljenosti od izvora zagađenja, ključan je parametar za kvalitet meda.

## Ključne reči:

alergija, med, melisopalinologija, polen

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## Introduction

Honey plants' nectar, honeydew and pollen grains represent an obligatory source of food for bees and ensure their existence, work, and community development (Dujmović Prugar & Hulina, 2007; Zima, 2007). Plant pollen is the dominant source of protein and fat, but also of various vitamins and minerals for the honeybee, and its origin is crucial for the physiological processes of the bee colony (Diniz Frias et al., 2016; Ljevnaić-Mašić et al., 2019). Honey plants are characterized by a specific combination of

physical and chemical properties of pollen grains and nectar, which depend on the systematic affiliation of the species, physiological characteristics, but also the ecological parameters in which the species exists (Evelin et al., 2011). Precisely because of its chemical specificity, many authors state that the pollen grains of some types of plants can be one of the strongest natural allergens (D'Amato et al., 1991; Oswalt et al., 2008; Mansouritorghabeh et al., 2019). The allergenic potential of pollen depends on the type of water-soluble proteins and glycoproteins, whose molecular mass ranges from 5 to 70 kDa (Peternel,

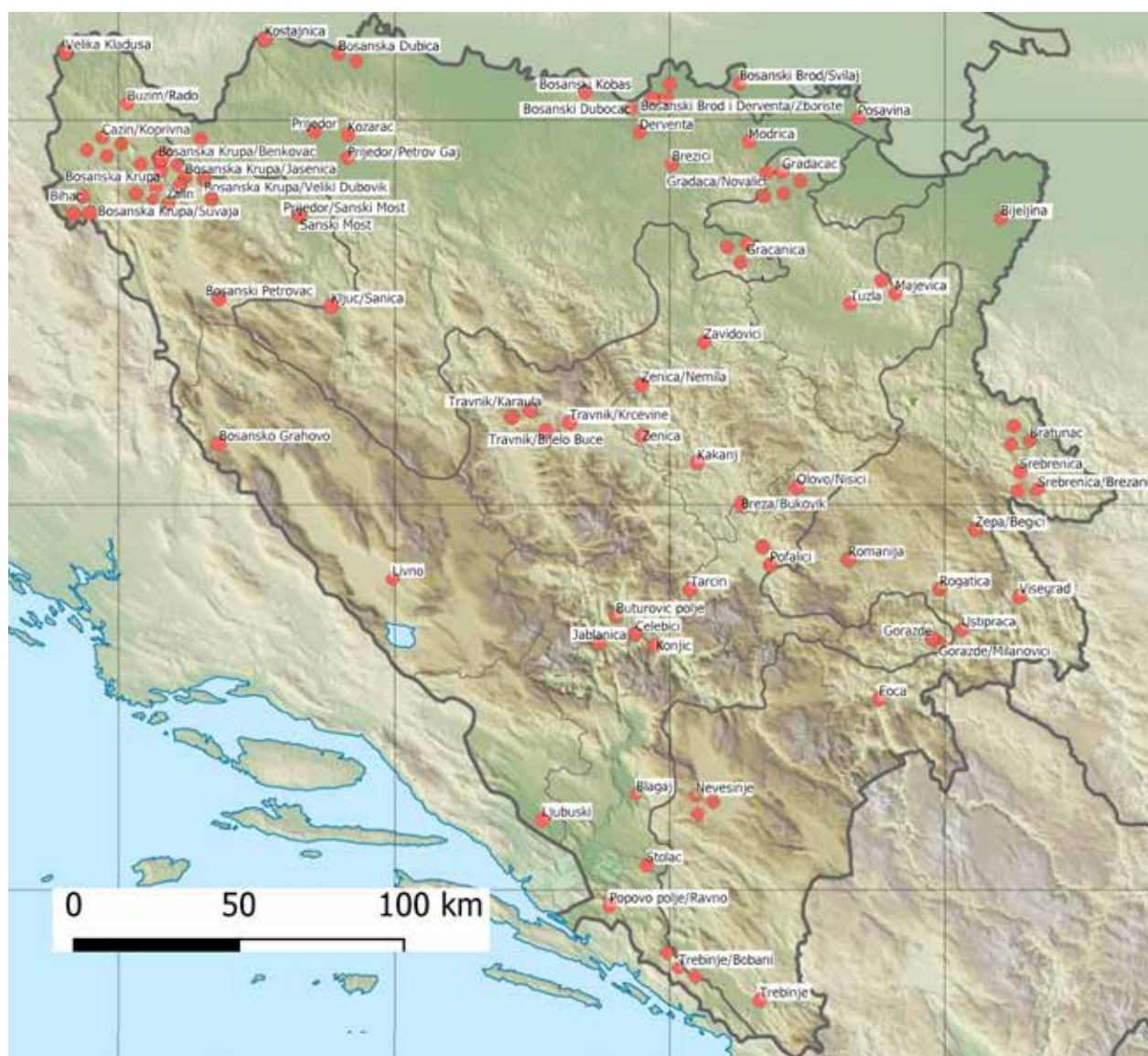


2011; Kostić, 2015). Numerous studies have shown that allergens, in just a few seconds, leave the pollen grain after contact with the moist mucous membrane (Chen et al., 2018). The apiflora of bee grazing is the result of environmental, biological, and anthropogenic factors. For these reasons, the selection of the floristic composition of the honey pasture, as well as its distance from various sources of pollution, is crucial in defining the botanical origin and the physicochemical properties of honey (Ball, 2007; Sari & Ayyildiz, 2013; Alibabić et al., 2017). This research aimed to determine a) whether there is and b) how much allergenic plant pollen is present in honey samples originating from Bosnia and Herzegovina.

## Materials and Methods

As part of the research, 100 samples of different types of honey from Bosnia and Herzegovina were collected and analyzed (**Fig. 1**).

Melissopalynological preparations followed the Rulebook (Rulebook on methods for the control of honey and other bee products of Bosnia and Herzegovina, Official Gazette, 37/09, 2009). According to the Rulebook, making of the preparation included the following steps: a solution of 10 g honey and 20 ml distilled water was incubated in a water bath at a temperature of 45 °C (Julabo SW 22). Then the solution was centrifuged for 15 minutes at 3500 rpm. After draining off the supernatant, the sediment was transferred to a glass slide with a micropipette and embedded in glycerin-gelatin with fuchsin and the preparation was covered



**Fig. 1.** Map of the distribution of the analyzed samples

with a coverslip. For each sample of honey, two parallel preparations were prepared according to the instructions of the official protocol. The method of analysis of melissopalynological preparations acts by the Rulebook, as well as the methods proposed by ICBB (International Commission for Bee Botany) (Von Der Ohe et al., 2004; Rulebook on methods for the control of honey and other bee products of Bosnia and Herzegovina, Official Gazette, 37/09, 2009). All melissopalynological preparations were analyzed using a Wild M20 phase-contrast microscope. The plant species was determined according to the pollen grains' micromorphological characteristics (Hesse et al., 2009). The qualitative analysis included a list of determined honey plants, while the quantitative analysis involved recording the exact number of pollen grains in the sample. Samples were classified as mono- or polyfloral according to the aforementioned Rulebook. Based on the results of these analyses, melissopalynological profiles for each slide were created.

Marking of allergenic species in the palynological spectrum of honey samples was carried out based on the list of allergenic plants (Redžić & Mehić, 2009).

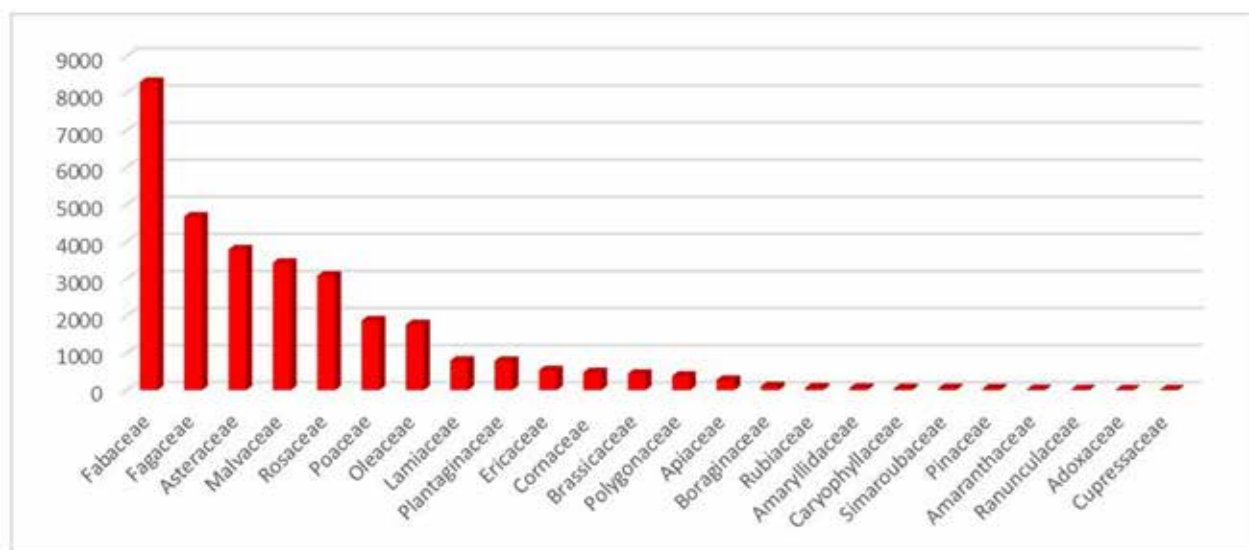
## Results and discussion

In the melissopalynological analysis of 100 honey samples, a total number of 31.183 pollen grains was counted. In the botanical sense, 24 plant families were identified, with the most numerous pollen grains for the families: Fabaceae (8.303), Fagaceae (4.683) and Asteraceae (3.792) (**Fig. 2**). Analyzed micromorphological features showed 48 different types of pollen grains. Based on the frequency of plant species in the palynological spectrum, 46 polyfloral and 54 monofloral types of honey were

detected.

The analysis of honey plants in the melissopalynological spectrum identified the pollen of seven allergenic plants. The identified plant species whose pollen has different allergic potentials are *Ambrosia artemisiifolia* L., *Artemisia vulgaris* L., *Ailanthus altissima* (Mill.), *Zea mays* L., *Tilia* sp., *Poa* sp., and the moderately allergen species *Medicago sativa* L. (Yakhlef et al., 2021) (**Tab. 1**). The total number of pollen grains with allergenic potential is 5.616, or 18.01% of the absolute number of pollen grains in the research. Greater number of allergenic plants was observed in the palynological profiles of polyfloral (seven species) types of honey compared to monofloral (six species).

In the melissopalynological profiles of polyfloral samples, pollen grains of *Poa* sp., *Ambrosia artemisiifolia* and *Artemisia vulgaris* represent the most important biological and health hazard. As part of the research, a total of 106 allergenic and non-native ragweed pollen grains were identified. *Ambrosia artemisiifolia* pollen grain has a very high allergic potential due to a combination of specific antigens (Peternel, 2011). Eleven different allergenic proteins found in pollen grains have been identified and named Amb a 1 to Amb a 11, whose molecular weights are in the range of 9 to 38 kDa, of which Amb a 1 is the strongest allergen (Chen et al., 2018). In addition to allergenic proteins, lactone compounds, flavonoids, monoterpenes, and bitter flavonoids were also detected in pollen grains (Kanter et al., 2013). *Ambrosia artemisiifolia* pollen grains were detected in the highest number in the palynological profiles from Bosanska Krupa (19.23%), Sanski Most (13.66%), and Bihać (6.25%). In this part of the country, this allergenic and invasive plant is marked



**Fig. 2.** Total number of pollen grains within families

**Table 1.** The percentage of pollen of allergenic plants in the investigated localities

Locality	Honey type	<i>Ambrosia artemisiifolia</i>	<i>Artemisia vulgaris</i>	<i>Tilia</i> sp.	<i>Poa</i> sp.	<i>Zea mays</i>	<i>Ailanthus altissima</i>	<i>Medicago sativa</i>
Drenova glavica	M			17%				
Cazin, Koprivna	M			1%	4%			
Cazin, Koprivna	M			1%				
Bihać	M			1%	1%			
Zalin	M			48%				
Kozarac	M				2%			
Bosanska Krupa	M			63%	4%			
Crno jezero	M			64%				
Bužim , Radoč	M			1%	2%			
Prijedor	M							
Bihać	P							
Buturović polje	M				1%			
Bratunac	P				35%			
Gračanica	P			23%	11%			
Majevica	M			22%	10%			
Bosanska Krupa	P	19%	27%		5%	3%		
Gračanica	M			41%	7%			
Gračanica	M				6%			
Trebinje, Bobani	M			12%	6%			
Gradačac	M				8%			
Tuzla	P			11%	19%			
Trebinje, Bobani	M							
Bosanski Dubačac	M			7%				
Bosanska Dubica, Babinac	M							
Bosansko Grahovo	M							
Bosanka dubica, Mededa	P			24%				
Cazin	M			9%				
Bosanski Brod i Derventa	M							
Popovo polje, Ravno	M							
Bratunac	M				2%			
Prijedor i Sanski Most	M				2%	3%		
Ljubuški	M							
Blagaj, Kamena	M					1%		
Gradačac	M			55%	4%			
Nevesinje	M				4%			
Modriča	M				3%			
Bratunac	M				8%			
Bosanski Brod, Svilaj	M				2%			



Gradačac	M			6%	
Gradačac, Novalići	P			1%	
Zenica	M				
Bosanska Krupa	M		11%	12%	
Srebrenica	M			8%	
Stolac	M			9%	10%
Kakanj	M			4%	
Sanski Most	M	14%	1%	44%	
Ključ, Sanica	M			50%	4%
Bosanska Krupa, Suvaja	P			19%	
Bosanska Krupa, Vranjska	M			41%	6%
Bosanska Krupa, Benkovac	M			63%	
Nevesinje	P			5%	25%
Goražde, Milanovići	M			33%	10%
Derventa, Zborišta i Bosanski Brod	P		18%	7%	6%
Olovo	P			5%	25%
Višegrad	P			5%	
Bijeljina	P		6%		9%
Cazin	P				6%
Ustiprača, Radič	P		1%	6%	11%
Foča	M			3%	
Romanija	P			4%	
Zenica, Nemila	M			12%	4%
Goražde	P				
Čelebići	M			1%	
Žepa, Begići	P		4%	2%	10%
Travnik, Karaula	P				10%
Travnik, Bijelo Buče	M			2%	
Travnik, Karaula, Krčevine	P			8%	
Bosanska Krupa, Jasenica	M			17%	
Bosanska Krupa	M			58%	2%
Velika Kladuša	M			2%	4% 1%
Bosanska Krupa, Veliki dubovik	M			30%	2%
Bihać	P			5%	6%
Bosanska Krupa, Zalin	M			84%	5%
Prijedor, Petrov gaj	P			3%	11%
Bosanski Petrovac	P			3%	
Bihać	P	6%		6%	1%
Kostajnica	M			29%	
Bosanska Krupa	M			35%	5%
Nevesinje	P		1%	3%	11%
Jablanica	P				

Pofalići	P		7%	7%
Bosanska Krupa	P	20%	31%	
Gradačac	P	14%	15%	
Majevica	P	13%		
Srebrenica, Brežani	P	10%	9%	
Zavidovići	P	12%	11%	
Srebrenica, Osmanovići	P	1%	15%	11%
Bosanska Krupa	P	18%	6%	
Breza, Bukovik	P		5%	
Bosanski Kobaš	P	14%		6%
Trebinje, Bobani	P			
Brezići	P		7%	
Bosanski brod	P	6%	10%	
Livno	P		20%	
Sarajevo	P	4%	3%	8%
Pofalići	P	17%		7%
Konjic, Mostar	P		10%	8%
Bosanska Krupa	P	23%	11%	3%
Tarčin	P		8%	
Derventa i Bosanski Brod, Zborišta	P	8%	8%	3%

\*M– monofloral, \*P– polyfloral

with code A2 (Djug et al., 2019; EPPO, 2023). The spread of ragweed represents an important biological problem. Its aggressive spreading on honey pastures suppresses native apiflora and reduces diversity or prevents the development of competitively weaker native honey species (Šoljan & Muratović, 2004; Bašić et al., 2017). The pollen of this allergenic plant species, apart from samples from Bosnia and Herzegovina, was confirmed in numerous honey samples from Croatia (Štefanić et al., 2013; Rašić et al., 2018), Albania (Pupuleku et al., 2016) and Serbia (Nedić et al., 2022) which, according to the European Food Safety Agency (EFSA, 2010), represents a serious health problem. A total of 178 mugwort (*A. vulgaris*) pollen grains were also determined. Mugwort pollen has a high allergenic potential and causes the development of various allergic symptoms. According to Gao et al. (2019), seven allergen components were identified. The most important are Art v 1 and Art v 3 located in the wall of the pollen grain. Allergenic Art v 2 is present in the cytoplasm or the pollen coat, while Art v 7 is located around the nucleus (Gao et al., 2019). The allergenic protein Art v 6 shows approximately 58% similarity with the mentioned Amb a 1, so there is a cross-reaction between ragweed and mugwort allergens (Celenk, 2019). Mugwort pollen was

detected in ten polyfloral honey samples, with the largest number in samples from Bosanska Krupa (27.35%), Derventa (18.33%) and Bosanski Brod (6%). It is specific that in the palynological spectrum of the samples, in addition to mugwort pollen, ragweed was also identified as an accessory species, which is an indicator that honey grazing was carried out in places with intense anthropogenic pressure.

As part of the research, 1.767 grass (*Poa* sp.) pollen grains were identified. Grass pollen has a high allergenic potential due to the presence of 13 different groups of allergens. Due to the amount and existence of pollen grains, groups 1 and 5 are considered immunodominant among grass pollen allergens. Besides them, there are allergens from group 12 (profilins) and 7 (polcalcin), which belong to the group of proteins responsible for cross-allergic reactions between grass, tree, and other weed pollens (Andersson & Lidholm, 2003; Garcia-Mozo, 2017). Grass pollen grains appeared in 62 samples, predominantly in polyfloral (76%), but also in some monofloral (24%) honeys. Given that the honey-bearing grazing of polyfloral honey is most desirable in meadows where the grass family generally has its ecological optimum, they are often accessory species in the palynological profiles of this type of honey. The presence of grass family

pollen has been established in numerous studies of honey (Silici et al., 2007; Maćukanović-Jocić & Jarić, 2015; Pupuleku et al., 2016; Alty et al., 2018; Rašić et al., 2018), but in individuals with pollen allergies it can trigger allergic or even anaphylactic reactions (Martín-Muñoz et al., 2010; Aguiar et al., 2017). In contrast to polyfloral, monofloral honey was dominated by linden (*Tilia* sp.) and tree of heaven (*A. altissima*) pollen. A total of 3.433 linden (*Tilia* sp.) pollen grains were identified. It was dominantly represented as an allergenic plant in the research. Linden pollen grains condition the development of moderate to strong allergic reactions in humans, and there is also a cross-reaction between linden and known allergenic plants such as birch and olive (Ognjenović et al., 2012; Ognjenović, 2013). In 2001, the presence of three protein allergens with a molecular weight of 10,23 and 50 kDa was confirmed for pollen grains of linden (Mur et al., 2001), and in 2021, two more (molecular weight 40 and 80 kDa) that can cause sensitization in humans were discovered (Tivsiz et al., 2021). Linden is one of the most honey-bearing woody plants, it provides generous amounts of pollen and nectar, so bees visit it very often (Umeljić, 2015). Argoti (2016) detected mannose in the nectar, together with phenols and the alkaloid nicotine, which can have a very toxic, and in some cases lethal, effect on bees (Singaravelan et al., 2006; Tiedeken et al., 2014; Baracchi et al., 2015). However, Jacquemart et al. (2018) state that concentrations of mannose and nicotine in linden nectar may not be lethal to bees and indicate that other volatile secondary metabolites may be dangerous. Linden pollen, as a primary and accessory honey-bearing species, was found in a significant number of honey samples, but due to its chemical characteristics, has to be consumed with precaution. The pollen of the tree of heaven (*A. altissima*) was not recognized as dangerous for provoking allergic reactions in recent years. However, several studies have shown that over 125 of its proteins, of which the most active are enolase and pectate lyase, can cause allergic reactions (Mousavi et al., 2017; Mousavi et al., 2019). Considering that it is an invasive plant species, in addition to the allergenic potential of pollen in honey, it represents a great danger for native flora (Djug et al., 2019). The presence of pollen from this allergenic, and invasive species, was detected in the melissopalynological profiles of honey from the area of Mostar, Konjic (8.33%) and Stolac (10%). According to Boškailo et al. (2017), it is intensively spreading throughout the Mediterranean and sub-Mediterranean areas.

The pollen of the tree of heaven is also detected in honey samples originating from Croatia (Rašić et al., 2018; Zima et al., 2018) and Bulgaria (Atanassova

et al., 2009; Tashev et al., 2015). Thanks to its eurivalence and allelopathy, the tree of heaven aggressively and quickly conquers new areas and suppresses honey-bearing plant species from their natural habitats, thus reducing the biodiversity of the autochthonous flora of BiH (Gómez-Aparicio et al., 2008).

## Conclusion

After the analysis of 100 melissopalynological profiles, a total of 5.616 (18.02%) pollen grains of allergenic plants were found in the honey samples. Out of the total number of analyzed samples, pollen from seven allergenic plant species was identified in 88 (88%) palynological profiles. The detected pollen grains of ragweed, mugwort, and the tree of heaven were bioindicators of the area burdened by anthropogenic pressures. Honey-bearing grazing in localities with such floristic composition reduces the biological quality of honey due to the presence of allergenic pollen. The selection of the floral composition in honey pastures is crucial to guarantee a botanical origin suitable for the honey we consume.

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