

Received: 14 November, 2022
Accepted: 29 November, 2022
Published: 30 November, 2022

***Corresponding author:** Silvia Bartolacci, Marche Environmental Protection Agency, Italy, Tel: +393383787446;
E-mail: silvia.bartolacci@ambiente.marche.it

ORCID: <https://orcid.org/0000-0002-0434-4295>

Keywords: Melissopalynology; Pollen; Honey; Nectariferous plants; Allergenic potential; Taxa; Louveaux method

Copyright License: © 2022 Trobiani N, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

<https://www.peertechzpublications.com>



Short Communication

Melissopalynology: The Louveaux method on honey samples in the marche region and their allergenic potential

Nadia Trobiani and Silvia Bartolacci*

Marche Environmental Protection Agency, Italy

Abstract

The research arises from the interest in investigating the pollen contained in the various honey samples analyzed to identify its geographical and botanical origin and to evaluate its allergenic potential.

With the Louveaux method, the pollen content shall be observed under a microscope using the same procedure as the aerobiological analysis described in national and European standards.

From the microscopic observation of the pollen grains, it is possible to identify the plants that produced them, thus going back to a particular type of vegetation that distinguishes the production area.

The percentage values for pollen of nectariferous plants identified have been calculated to establish frequency classes and dominant species. Starting from an exploratory analysis, the results obtained with melissopalynology provide assessments that, combined with sensory and physical analysis, represent a useful control tool to address the growing trend of food fraud as an element of consumer choice. By comparing the percentages of the pollen taxa contained in the honey analyzed with those reported in the characterization cards, it was possible to verify the conformity to the declared botanical origin. The samples were analyzed in duplicate to reduce operator uncertainty and to ensure the identification of the largest number of species/families present and the recognition of botanical families. The results provided a clear picture of a first assessment of the conformity of the reference values; in general, most of the samples examined, both those taken on the market and those of local producers, are in line with the data obtained. Bibliographical research on cross allergies related to the presence of pollen in honey has established that allergic forms to honey are still a rare condition and the incidence does not exceed 0.001%. Studying the few reported cases worldwide, it is difficult to say that pollen proteins are the only and directly responsible for the allergic manifestations that follow the ingestion of honey; however, if we wanted to attribute the responsibility, we could refer in particular to Compositae pollens, such as mugwort, ragweed, and dandelion. This work, while identifying many possible triggers for allergic reactions, has detected a very low number of cases of ingestion of honey; therefore, the study can be a starting point to investigate the allergenic potential.

Introduction

This research starts from the idea of studying the pollen contained in the various honey samples analyzed in the laboratories of the Marche Environmental Protection Agency (ARPAM) [1-3]. Honey is a product closely linked to the production area thus the research has the purpose to explore the complexity of the microscopic nature of pollen and investigate

its composition characteristics since they derive mainly from the type of foraged flora.

Melissopalynology is a branch of palynology that studies pollen and other microscopic elements that make up the honey sediment; this analysis was created with the purpose of determining the botanical and geographical origin of honey, as the honey itself contains its certificate of origin [4-8].

The morphological pollen characteristics, contained in honey, act as a starting point for its origin taxa identification, useful to track it back to phylogenetic relationships between honey and plants.

While bees collect nectar from flowers, they unknowingly become vectors for pollen grains, which will end up in honey. The pollen thus arrives in honey transported by bees, which guarantees the cross-pollination of distant plants, thus favoring genetic mixing, which is essential for the survival and strengthening of the species. Pollen has another fundamental role for the bees: it constitutes the only protein source for nourishment of bees.

Honey's composition and organoleptic characteristics derive mainly from the type of flora being harvested; in addition to changes in vegetation, other elements related to the territory affect the characteristics of the product: the type of soil, the development of different human activities with the possible negative effects on the health of the product (pollution).

Materials and methods

The Louveaux method was used, extended in the context of the definition of the botanical origin because the pollen spectrum of the sample reflects the floristic situation of the place where it was produced and essentially involves the phases of adding water and centrifugation phases, which in the complex lead to the total dissolution and elimination of sugars. The pollen content was observed under a microscope using the same procedure as the aerobiological analysis described in national and European standards (UNI 11299 – 2008). Although this discipline is still in the attention of a few experts, in our case we tried to obtain data that could provide useful information to verify the respect of the botanical origin declared on the label for the honey taken on the market or declared by the local producers of our 24 samples subjected to analysis (23 of nectar honey and 1 of honeydew). Fundamental is the presence of a comparison Palinoteca, which is a collection of slides after the collection of fresh pollen, taken from plants of certain identification [9-11].

For some granules the genus has been determined, for others only the family to which they belong; the obtained data have been summarized in diagrams that allowed to follow the qualitative and quantitative development of the plant elements in each honey studied. From the microscopic observation of the pollen grains, it is possible to identify the plants that produced them, thus going back to a particular type of vegetation and, consequently, to the production area.

To determine the frequency classes, the percentage values of the pollen identified for nectar plants only have been calculated, since it is essentially their contribution that gives the honey a precise identity in terms of botanical origin. Bibliographical research has been carried out on the allergenic potential of honey as allergic manifestations have occurred after ingestion to assess whether the specific presence of pollen in honey is to be considered a risk factor for food allergies to honey for atopic subjects [12-14].

Results and Discussions

The contribution that the melissopalynological analysis could make to Italian honey is to know the national products more accurately than the remote image with which the flora of bees of other countries is represented. The work of several researchers has allowed mapping of the main national productions; this is essential to verify the veracity of voluntary territorial indications, but also to interpret the spectra of honey in which only the assessment of national origin is required. In fact, in the interpretation of a pollen spectrum, all available weapons are used, not only positive definitions but also those of exclusion. By comparing the percentages of the pollen taxa contained in the honey analyzed with those reported in the characterization cards, it was possible to verify the correspondence or not of the samples to their botanical origins. The samples were analyzed in duplicate to reduce operator uncertainty and to ensure the identification of the largest number of species/families present and the recognition of botanical families. The results provided a fairly clear picture of a first assessment of the conformity of the reference values.

In general, most of the samples examined, both those taken on the market and those of local producers, are in line with the data obtained. The pollens found in the samples reflect the floristic situation of the place where the honey is produced which is their geographical origin Figures 1,2.

Some bibliographical research on cross allergies related to the presence of pollen in honey has established that allergic forms to honey are still a rare condition and the incidence does not exceed 0.001%. Studying the few reported cases, it is difficult to say that pollen proteins are the only and directly responsible for the allergic manifestations that follow the ingestion of honey; however, if we wanted to attribute the responsibility, we could refer in particular to Compositae pollens, such as mugwort, ragweed, and dandelion.

Conclusion

This work, while identifying many possible triggers for allergic reactions, has detected a very low number of cases



Figure 1: Pollen of FABACEAE *Castanea sativa* in honey sediments -Leika optical microscope 40X.

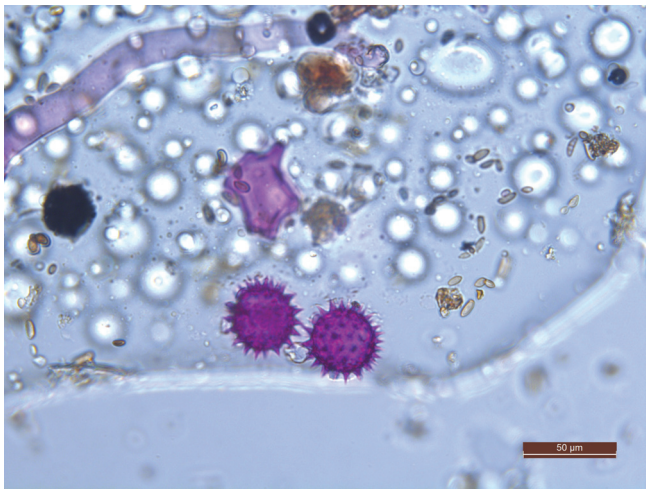


Figure 2: Pollen of ASTERACEAE *Helianthus annuus* in honey sediments -Leika optical microscope 40X.

of ingestion of honey; therefore, the study can be a starting point for a study focusing on the allergenic potential of the pollen contained in honey and the possible etiological role of Compositae pollen in patients sensitized to the same species although the nature of the antigen responsible for the allergic manifestations caused by honey is not yet clear. Based on the results obtained from this study, the research will continue by expanding the study area, in order to create a pollen spectrum typical of the regional territory and to promote the evaluation of the botanical origin of honey as a method for monitoring the agroecosystem over time. In this way, it will be possible to monitor incoming or lost species and finally to assess the geographical origin of honey as a guarantee for the consumer.

Since to date, it is not clear whether the allergens are in the saliva of the bees of the pharyngeal glands or in the pollens themselves this research will continue using the immunoblotting technique to isolate this allergen and clarify its actual location.

References

1. Louveaux J, Maurizio A, Vorwohl G. Methods of Melissopalynology. *Bee World*. 1978; 59: 139-154. <http://dx.doi.org/10.1080/0005772X.1978.11097714>

2. Louveaux J. Recherches sur la recolte du pollen par les abeilles (*Apis Mellifica* L). *Ann Abeille*. 1958; 197-221.
3. Aguiar R, Duarte FC, Mendes A, Bartolomé B, Barbosa MP. Anaphylaxis caused by honey: a case report. *Asia Pac Allergy*. 2017 Jan;7(1):48-50. doi: 10.5415/apallergy.2017.7.1.48. Epub 2017 Jan 26. PMID: 28154806; PMCID: PMC5287071.
4. Bauer L, Kohlich A, Hirschwehr R, Siemann U, Ebner H, Scheiner O, Kraft D, Ebner C. Food allergy to honey: pollen or bee products? Characterization of allergenic proteins in honey by means of immunoblotting. *J Allergy Clin Immunol*. 1996 Jan;97(1 Pt 1):65-73. doi: 10.1016/s0091-6749(96)70284-1. PMID: 8568139.
5. Helbling A, Peter C, Berchtold E, Bogdanov S, Müller U. Allergy to honey: relation to pollen and honey bee allergy. *Allergy*. 1992 Feb;47(1):41-9. doi: 10.1111/j.1398-9995.1992.tb02248.x. PMID: 1590566.
6. Zhang Q, Chen X, Chen S, Ye Y, Luo J, Li J, Yu S, Liu H, Liu Z. FATAL HONEY POISONING IN SOUTHWEST CHINA: A CASE SERIES OF 31 CASES. *Southeast Asian J Trop Med Public Health*. 2017 Jan;48(1):189-96. PMID: 29644839.
7. Mukhopadhyay SK, Gupta S, Das AP, Bera S. The beekeeping potential of Sub-Himalayan West Bengal, India: A palynological assessment of honey. *Journal of Apicultural Research and Bee World*. 46(3): 165–180.
8. Popescu FD. Cross-reactivity between aeroallergens and food allergens. *World J Methodol*. 2015 Jun 26;5(2):31-50. doi: 10.5662/wjm.v5.i2.31. PMID: 26140270; PMCID: PMC4482820.
9. Von Der Ohe W, Persano Oddo L, Piana ML, Morlot M, Martin P. Harmonized methods of melissopalynology. *Apidologie*. 2004; 35:S18–S25.
10. Persano Oddo L, Piana ML, Ricciardelli D'Albore G. I mieli regionali italiani - Caratterizzazione melissopalynologica. Ministero delle Politiche Agricole Alimentari e Forestali C.R.A. - Istituto Sperimentale per la Zoologia Agraria, Sezione di Apicoltura, Roma. 2006.
11. Pucci N, Asero R, Calvani M, Indirli MC, La Grutta S. La diagnosi delle allergie alle profiline. *Rivista di Immunologia e Allergologia Pediatrica*. 2011.
12. Travaglini A, Brighetti MA, Vinciguerra F. Atlante del polline delle principali specie allergeniche in Italia. Il raggio verde Edizioni. COD. 2014.
13. UNI – 11299 :2008 Analisi microscopica o Melissopalynologica. 2008.
14. ISPRA. Linee guida per il monitoraggio aerobiologico. Manuali e Linee Guida 151/2017 ISBN 978-88-448-0820-4 – Delibera del Consiglio Federale. Seduta del 03.11.2015.Doc.n.61/15-CF.

Discover a bigger Impact and Visibility of your article publication with Peertechz Publications

Highlights

- ❖ Signatory publisher of ORCID
- ❖ Signatory Publisher of DORA (San Francisco Declaration on Research Assessment)
- ❖ Articles archived in worlds' renowned service providers such as Portico, CNKI, AGRIS, TDNet, Base (Bielefeld University Library), CrossRef, Scilit, J-Gate etc.
- ❖ Journals indexed in ICMJE, SHERPA/ROMEO, Google Scholar etc.
- ❖ OAI-PMH (Open Archives Initiative Protocol for Metadata Harvesting)
- ❖ Dedicated Editorial Board for every journal
- ❖ Accurate and rapid peer-review process
- ❖ Increased citations of published articles through promotions
- ❖ Reduced timeline for article publication

Submit your articles and experience a new surge in publication services (<https://www.peertechz.com/submission>).

Peertechz journals wishes everlasting success in your every endeavours.