UNDEREXPLORED RISKS OF BEEKEEPING PRODUCTS: PYRROLIZIDINE ALKALOIDS IN FLORAL AND HONEYDEW HONEYS FROM SANTA CATARINA

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RESUMO – Alcaloides pirrolizidínicos (AP) são metabólitos naturais de plantas que podem contaminar pólen e mel, representando riscos tóxicos após o metabolismo hepático humano. Este estudo analisou AP em 15 amostras de mel de Santa Catarina, incluindo 8 méis florais (MF) e 7 méis de melato da bracatinga (MMB). Foram avaliados o conteúdo de pólen e 21 PA internacionalmente regulamentados. AP foram detectados em 5 MMB e 2 MF, sendo os MMB provenientes do exsudato de *Mimosa scabrella* Bentham e os pólens dos MF predominantemente de *Escallonia* sp. e *Mimosa scabrella*. Os AP licopsamina e intermedina foram detectados em concentrações somadas variando de 11,69 a 179,83 µg/kg. A ingestão diária recomendada foi estimada em 7,9 a 108,5 g mel/dia, dependendo dos níveis de PA. Embora as amostras analisadas sejam seguras para consumo, os apicultores devem monitorar as plantas propensas a produção de AP, visando mitigar a contaminação dos méis.

ABSTRACT – Pyrrolizidine alkaloids (PA) are natural metabolites from plants, that can contaminate pollen and honey, posing toxic risks after human liver metabolism. This study analyzed PA in 15 honeys from Santa Catarina, including 8 floral honeys (FH) and 7 bracatinga honeydew honeys (BHH). Pollen content and 21 internationally regulated PA were assessed. PA were detected in 5 BHH and 2 FH, with BHH derived from *Mimosa scabrella* Bentham exudate and FH pollens predominantly from *Escallonia* sp. and *Mimosa scabrella*. The PA lycopsamine and intermedine were detected in summed concentrations ranging from 11.69 to 179.83 µg/kg. Recommended honey intake was 7.9 to 108.5 g/day, depending on PA levels. While samples are safe for consumption, the beekeepers must monitor the PA-prone plants, aiming to mitigate honey contamination with PA.

PALAVRAS-CHAVE: limites regulatórios; contaminantes naturais; metabólitos secundários; produtos da colmeia.

KEYWORDS: regulatory limits; natural contaminants; secondary metabolites; beehive products.

1. INTRODUCTION

Certain plant families naturally synthesize pyrrolizidine alkaloids (PA), the most commonly known sources being the genera *Senecio* and *Eupatorium* (Asteraceae family), *Echium* (Boraginaceae family), and *Crotalaria* (Fabaceae family). The 1,2-unsaturated PA can be bioactivated, mainly in the liver, by the action of cytochrome P450, forming reactive metabolites capable of alkylating cellular nucleophiles, including protein and deoxyribonucleic acid, inducing acute and chronic hepatotoxicity (BRUGNEROTTO et al., 2021).

Once present in nectar- and pollen-producing plants, bees transport PA to the hive (BRUGNEROTTO et al., 2021). Plants from the genera *Senecio*, *Echium*, and *Eupatorium* (CARPINELLI DE JESUS et al., 2019; FLADE et al., 2019) are frequently evaluated for the presence of PA, not only due to their botanical origin but also for their higher pollen contributions detected in honey. The Commission Regulation (EU) 2020/2040 has established amendments for Regulation (EC) 1881/2006 that set maximum concentration levels for PA in food. These limits vary, ranging from 1.0 to 1000 µg kg^{-1}, with a maximum limit of 500 µg kg^{-1} established for pollen (EUROPEAN COMMISSION, 2020). No specific limits were set for honey.

Although the number of studies on PA in honey has been increasing worldwide in the last few years, the data are still limited, especially considering Brazilian honeys. There is still a lack of knowledge about the scenario in the southern region of Brazil, especially in the state of Santa Catarina, which is the national leader in honey productivity with approximately 65 kg/km², 13 times higher than the national average (ABEMEL, 2022). Therefore, this study aimed to assess data on PA in floral- and bracatinga honeydew honeys from Santa Catarina, as well as to assess the botanical source of the floral hones through pollen analysis, to evaluate plants that can be related to PA production and effectively collaborate with the Santa Catarina beekeeping sector, seeking prevention strategies to reduce the presence of contaminants in honey.

2. MATERIAL AND METHODS

2.1 Samples

Fifteen (*n* = 15) honey samples were analyzed. Nine samples were collected from São Joaquim, SC and 1 one sample was collected from each one of the cities: União da Vitória - PR, Urupema - SC, Xaxim - SC, Joinville - SC, Lages - SC, and Luzerna - SC. Among these, 7 were bracatinga honeydew honeys (BHH), and 8 were floral honeys (FH) collected directly from the beekeepers in screw-capped polypropylene bottles and delivered at the laboratory and stored at room temperature (25 °C) until their use.

2.2 Botanical origin identification

The bracatinga honeydew honey is not produced by nectar, but from the exudate of scale insects on bracatinga tree. Therefore, the pollen analysis was carried out only on floral honey samples to identify the main botanical source. The analysis followed the methods described by Louveaux et al. (1978) and the overall procedure adopted by Antunes et al. (2024) for floral honeys. For the identification and counting of pollen grains, an Olympus BX50 optical microscope was used. The classification of pollen was based on the relative frequency of predominant pollen types in each sample, which exceeded 45%. Additionally, pollen was categorized as secondary (15–45%), important minor (3–15%), and minor (below 3%). The frequency of pollen occurrence in the samples was determined using the categories: rare (<10%), infrequent (10–20%), frequent (21–50%), and very frequent (>50%).

2.3 Pyrrolizidine alkaloids determination

The sample preparation for analysis of 21 PA regulated by the Commission Regulation (EU) 2020/2040 was performed according to Valese et al. (2021), and the quantification was adapted from the same study, using a 1290 Infinity HPLC system from Agilent Technologies Deutschland GmbH (Waldbronn, Germany) coupled to a hybrid quadrupole-linear ion trap mass spectrometer QTRAP® 5500 from AB Sciex LLC (Framingham, USA) equipped with an electrospray ionization source. A ZORBAX Eclipse Plus C18 column (3.5 µm, 3.0 × 100 mm; Agilent Technologies, Santa Clara, USA) at 30 °C was used for separation. The flow rate was 0.5 mL min^{-1}, the injection volume was 5 µL, and the gradient had the mobile phases A, water with 0.1% formic acid, and B, acetonitrile with 0.1% formic acid. A calibration curve (5 to 100 µg L^{-1}) was used, and the PA were expressed as µg per kg of honey. The assays were performed in three independent replicates, and the results were expressed as mean ± standard deviation.

3. RESULTS AND DISCUSSION

The pollen analysis (Table 1) demonstrated that the very frequent pollens in the samples were *Escallonia* sp., *Lamanonia* sp. and *Mimosa scabrella*, found in 100% of the samples; *Eucalyptus* sp., found in 88% of the samples; and *Vernonia* sp., found in 75% of the samples. *Baccharis* sp., *Schinus* sp., and *Ilex* sp., were very frequent, found in 21-50% of the samples. The pollen from *Escallonia* sp. was predominant in all the samples, while *Mimosa scabrella* was the main pollen in 4 samples and *Eucalyptus* sp. in 3 samples. In general (with some exceptions), honey is classified as monofloral when a single pollen type represents more than 45% of the total pollen spectrum; thus, only the sample 3FH could be considered monofloral.

Table 1 – Pollen types observed in floral honeys (botanical origin classification).

Honey	Main pollen types (>15%)	Minor pollen types
1FH	*Mimosa scabrella* (29.87%), *Escallonia* sp. (24.21%) and *Schinus* sp. (19.81%)	*Ilex* sp., *Baccharis* sp., *Bidens* sp., *Lamanonia* sp., *Machaerium* sp., *Aegiphila* sp., *Eucalyptus* sp., *Rubus* sp., *Coffea arabica* and *Zanthoxylum* sp.
2FH	*Escallonia* sp. (30.21%) and *Lamanonia* sp. (25.08%)	*Schinus* sp., *Ilex* sp., *Baccharis* sp., *Vernonia* sp., *Tabebuia alba*/*Handroanthus albus*, *Sapium* sp., *Machaerium* sp., *Mimosa scabrella*, *Eucalyptus* sp., *Ligustrum* sp., *Borreria*/*Galianthe* sp., *Zanthoxylum* sp. and *Matayba* sp.
3FH	*Escallonia* sp. (51.95%)	*Lamanonia* sp., *Sapium* sp., *Mimosa scabrella* and *Cupania vernalis*
4FH	*Eucalyptus* sp. (31.38%) and *Escallonia* sp. (17.59%)	*Schinus* sp., *Ilex* sp., *Baccharis* sp., *Vernonia* sp., *Ecchium* sp., *Lamanonia* sp., *Weinmannia* sp., *Mimosa scabrella* and *Zanthoxylum* sp.
5FH	*Mimosa scabrella* (21.52%), *Escallonia* sp. (20.18%). *Lamanonia* sp. (19.73%) and *Eucalyptus* sp. (16.59%)	*Ilex* sp., *Baccharis* sp., *Vernonia* sp., *Melothria* sp., *Zanthoxylum* sp. and *Allophylus* sp.
6FH	*Escallonia* sp. (41.93%) and *Mimosa scabrella* (17.70%)	*Vernonia* sp., *Lamanonia* sp., *Sapium* sp., *Crotalaria* sp., *Eucalyptus* sp. and *Zanthoxylum* sp.
7FH	*Eucalyptus* sp. (24.84%) and *Escallonia* sp. (21.12%)	*Vernonia* sp., *Lamanonia* sp., *Mimosa scabrella*, *Zanthoxylum* sp. and *Pouteria* sp.
8FH	*Escallonia* sp. (26.43%) and *Mimosa scabrella* (19.82%)	*Vernonia* sp., *Lamanonia* sp., *Sapium* sp., *Galactia* sp., *Eucalyptus* sp. and *Zanthoxylum* sp.

Regarding the PA, only lycopsamine and intermedine were found in the analyzed samples (Table 2). Intermedine was detected in 7 out of the 15 analyzed samples, 2 from FH and 5 from BHH, ranging from 11.7 ± 1.55 µg/kg (sample 11BHH, honeydew from *Mimosa scabrella*) to 102.23 ± 9.95 µg/kg (sample 3FH, predominant pollen from *Escallonia* sp.). Lycopsamine was detected in 4 samples, 1 from FH and 3 from BHH, ranging from 15.18 ± 8.61 µg/kg (sample 25BHH, honeydew from *Mimosa scabrella*) to 77.62 ± 2.54 µg/kg (sample 3FH, predominant pollen from *Escallonia* sp.). The sum of the PA concentrations in the samples ranged from 11.69 to 179.83 µg/kg.

Samples with higher PA content, 3FH and 9BHH, had predominant botanical origin from *Escallonia* sp. nectar and honeydew from *Mimosa scabrella*, respectively. This does not mean that all samples from these plants are classified as PA-contaminated. For instance, bracatinga honeydew honeys, despite sharing the same botanical origin, exhibit varying PA concentration profiles. Notably, no PA was detected in samples 13 and 14BHH. Since PA are secondary metabolites produced by plants, their production can fluctuate due to different biotic and abiotic factors. Therefore, beekeepers should remain vigilant about the risk of contamination, regularly monitoring honey from each harvest to identify any high PA levels.

Table 2 – Pyrrolizidine alkaloids (µg/kg) in the honeys analyzed.

Honey	Intermedine	Lycopsamine	Sum	EDI*
1FH	nd	nd	-	-
2FH	nd	nd	-	-
3FH	102.23 ± 9.95	77.62 ± 2.54	179.83	7.90
4FH	nd	nd	-	-
5FH	nd	nd	-	-
6FH	13.08 ± 5.78	nd	13.08	108.58
7FH	nd	nd	-	-
8FH	nd	nd	-	-
9BHH	71.71 ± 1.00	43.16 ± 3.55	114.86	12.36
10BHH	49.5 ± 8.92	15.18 ± 8.61	64.67	21.96
11BHH	11.7 ± 1.55	nd	11.69	121.45
12BHH	60.23 ± 1.91	12.64 ± 4.99	72.85	19.49
13BHH	nd	nd	nd	-
14BHH	nd	nd	nd	-
15BHH	37.35 ± 0.89	nd	37.35	38.02

Data expressed as mean ± standard deviation. EDI, estimated daily intake; FH, floral honey; BHH, bracatinga honeydew honey; nd, not detected. *Maximum amount of honey per day (g/day) for a person weighing 60 kg, considering the recommended daily intake of 1.42 µg of APs.

The recommended daily intake was calculated as the maximum amount of honey per day (g of honey/day) for a person weighing 60 kg, considering the European Food Safety Authority's recommendation of a daily intake of 1.42 µg of APs (EFSA et al., 2017). The values ranged from 7.90 to 108.50 g of honey/day, considering that the more PA a sample contains, the lower its consumption should be (Table 2).

4. CONCLUSION

The analysis of predominant pollens in FH samples revealed that PA-contaminated samples were primarily associated with *Escallonia* sp. and *Mimosa scabrella*. *Mimosa scabrella* is also the botanical source of the bracatinga honeydew honeys. Among the 21 PA regulated by the European Commission, only intermedine and lycopsamine were detected, present in 47% of the samples. While no regulatory limit exists for PA in honey, the total PA content did not exceed the maximum limit established for pollen (500 µg/kg). These findings provide valuable insights for beekeepers, enabling them to strategically manage hive locations to minimize PA contamination in honey. Furthermore, these data enhance knowledge about honey from Santa Catarina and support the future establishment of regulatory limits for PA presence in honey, facilitating the export.

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