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Why not evaluate the allergenic potential of bee pollen with a skin testing method?

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ABSTRACT

Background: Bee pollen (BP) is a dangerous food for allergics because of allergenic pollens (AP). We present a method to evaluate allergenic potentials of AP in BP by using skin prick testing. **Method:** First, it is experimentally characterized relationship between mass of one AP ($Mass_{AP}$) content in BP and its allergenic potential measured by skin reactivity testing. $Mass_{AP}$ is identified in BP mass after having calculated the proportion of AP using the BP pollinic spectrum based on the European standard. Skin reactivity to AP is assessed by measuring wheal diameters (W_{AP}) from skin prick tests using three serial dilutions of BP on allergic patients to AP, in order to calculate equation of the relationship between $Mass_{AP}$ and skin reactivity. Relationship equations are calculated for several AP. Then, for a BP having several AP, allergenic potentials of AP (W_{AP}) are assessed by BP spectrum analysis, AP identifications, $Mass_{AP}$ calculations and W_{AP} calculations using equations. **Results:** Linear functions $Log(W_{AP}) = A(Log(Mass_{AP})) + B$ ($R^2 = 0.99$) were established using BP samples rich in only one genus of AP. For a polyfloral BP with different AP, W_{AP} of each AP is proportional to $Mass_{AP}$ included in a stated dose of BP. E.g., for a BP with 0.017 mg of artemisia (A) and 0.367 mg of forage grasses (FG) per mg, W_A and W_{FG} are 7.7 mm and 8.9 mm, respectively, for 1 g of BP and 12.5 mm and 13.1 mm for 5 g. **Conclusion:** This method could help to diagnose of anaphylactic accident after BP ingestion or to prevent anaphylactic risk for allergic consumers.

Introduction

Bee pollen (BP) is widely consumed by general public, and is also used as a functional food [1].

Flower pollen grains are assembled by the bee in loads. Each load has a weight of 5–10 mg [2] and has several hundred thousand grains of a single floral species. BP consists of various loads from different plant species.

Bees visit numerous plant flowers [3, 4] and some floral pollens are allergenic pollen (AP). BP could be a dangerous food for allergics. Anaphylactic accidents related to the use of BP are on the increase [5]. All published serious BP allergies have been among pollen allergics. Further, the prevalence of allergy is steadily increasing and half of the next generation in Europe could be allergic. Actual incidence of BP allergy is about 0.50% among Polish and the main BP allergic side effects are gastrointestinal symptoms and

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rashes [6]. But, the mild allergic accidents to beehive products could be common and could be in the matter under-reported and thus underestimated [7]. Currently the BP allergy impact is not estimated accurately, even for pollinosis patients.

Furthermore, allergy to BP is unconnected from other bee product allergies because of a lack of cross-reactivity between bee products [6]. BP has never appeared to contain *in fact* allergens of bee venom. Skin or serum sensitizations to bee venom were not referred by literature among allergic to BP [5]. The incidence of positive skin tests to bee proteins content in BP with no allergen pollen was zero among sensitive patients to pollens with no history of bee sting reactions and no food allergy [8]. The accident risks could be exacerbated because popular belief dissociates floral pollen collected by bee and airborne floral pollen (anemophilous) with the misconception that bees only use pollen from insect-pollinated plants (entomophilous) [9]. Allergy to BP is considered serious by the health authorities in some countries. The official Food Code is imposing BP statement to warn about potential allergic reactions in Australia and New Zealand, but there is no international standard [9].

In early spring and late summer, plants and trees (e.g., Oleaceae, Asteraceae, Graminaeae) represent allergenic pollen suppliers for honeybees at locations where the anemophilous trees and plants are an important pollen source [10]. Anemophilous pollens collected by bees are botanically closely related to common airborne AP or could cross-react with unrelated allergenic plants. These allergic cross-reactions are caused by proteins sharing important structural homologies with several plant families [11]. Allergenic proteins of AP appear to retain their allergenic properties in BP from the time they enter the beehive to the harvesting by the beekeeper and their use by the end consumer [12]. But sold BP is difficult to characterize or to standardize because it is highly variable.

However, skin reactivity was always called to witness of the allergenic potential of a “cocktail” of allergenic proteins extracted from an allergen raw material such as floral pollen. The *in vivo* skin tests to allergens provide information about the biological potency of the allergenic raw material, but not on its specific composition [13].

To the best of our knowledge, however, there is currently no technical definition of the allergenic potential of AP in BP. Therefore, it is important to have a technical evaluation of allergenicity potential of floral allergenic pollen of BP among allergics, to give a better information to BP consumers and to better prevent accident risks. The purpose of this paper is to present a method to evaluate the allergenic potential of AP in bee pollen among allergics to airborne pollen by using *in vivo* skin prick testing.

Materials and methods

1) Analysis of bee pollen spectrum:

A pollen analysis of bee products is usually performed by a specialist laboratory by analysing the beehive products. In our case, we used Honey Expertise Laboratory – Naturalim France Miel, 39330 Port-Lesney, France.

Such an analysis defines the type and frequency of each botanical genus or family floral pollen and determines the total mass of floral pollen.

BP is thoroughly cleaned when intended for human consumption. Cleaning the pollen separates pollen dust balls, bee body fragments, wax or propolis.

Melissopalynology is based on the European standard without acetolysis, as recommended by the International Commission for Bee Botany [14].

Counting and identifying floral pollen grains are made by examination under a microscope. Identification of pollen types is based on the laboratory pollen reference collection for local plants or on guides specialized in the pollen morphology of floral species.

Ten grams of well-homogenized BP were dissolved and washed in distilled water, centrifuged, then prepared using aqueous glycerine and paraffin for smear preparations. Each smear was studied under a microscope to identify 500 floral pollen grains in order to determine the percentage of each type of floral pollen.

For each of the 1 to n types of floral pollen observed in the BP analysis, $\%_{pn}$ is the percentage of floral pollen pn.

With BP, the floral pollen mass is equated with the BP mass ($Mass_{BP}$) because it is accepted that the BP pellets only contain kneaded floral pollen grains.

Indeterminate fractions are ignored.

2) Calculation of each AP mass $Mass_{AP}$ in BP:

2.1) Calculate the volume V_{pn} of each of the 1 to n types of floral pollen from the BP spectrum.

It is used the formula $V_{pn} = 4/3\pi r^3$ if the pollen grain is spherical or the formula $V_{pn} = 4/3\pi e^2 l$ if the floral pollen has an ellipsoidal shape.

The values of the radius r and of the mid-equatorial and longitudinal axes e and l are obtained from the literature from observations made on bee product pollen, including BP.

It is important to take into account changes in volumes of floral pollen due to orthodox or recalcitrant pollen qualities when pollen grains come into contact with aqueous bee fluids.

2.2) Calculate the proportion of the volume P_{AP} of each AP

$$P_{AP} = \frac{V_{AP} \times \%_{AP}}{(V_{AP} \times \%_{AP}) + (V_{p2} \times \%_{p2}) + \cdots + (V_{pn} \times \%_{pn})}$$

($\%_{pn}$ is the percentage of floral pollen pn in BP analysis).

2.3) Calculate the mass of each AP $Mass_{AP}$

$$Mass_{AP} = P_{AP} \times Mass_{BP}$$

3) Calculation of the equations defining the allergenic potential of the AP existing in the BP:

Before calculating the equation defining the allergenic potential of one AP, it is necessary to:

- Use a BP with only one AP,
- Calculate the mass of AP as indicated above,
- Use a BP without any AP as a “BP negative control” to eliminate a skin sensitization to bee specific allergens.

3.1) Preparation of BP extracts:

Samples were prepared with the two types of BP defined above. Five grams of fresh or frozen BP was well homogenized on a glass plate and 450 mg of BP was diluted in 4.5 ml