Advancements in a Molecular Approach for Determining the Botanical Origin of Sri Lankan Bee Honey

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In the pursuit of sustainable agricultural systems, the botanical origin of bee honey plays a crucial role in its market value and consumer preference. The objectives of this study were to determine the association between pollen density and geographical origin, as well as to evaluate different pollen DNA extraction methods. Ten bee honey samples were subjected to pollen extraction using the Waiblinger protocol followed by four DNA extraction methods (DNeasy® Plant Mini Kit and three CTAB-based procedures), both with and without modifications. Significant variations in pollen density were observed quantitatively across geographical locations: Nuwara Eliya (290950), Gannoruwa (26086), Bowalawatta (39060), Ogastawatta (35055), Pilimathalawa (15400), Welimada (7395), Uva-Paranagama (1960), and Buttala (425) (pollen grains/10 g), and this can be attributed to different floral sources. Awissawella sample originated from *Hevea brasiliensis*, as honeybees were found to collect nectar from the extra-floral nectaries of its leaf petioles, indicating the absence of rubber pollen. Conversely, the Badulla sample contained a minute amount of pollen not visible to the naked eye. While no single method demonstrated superiority for pollen DNA extraction, an optimized CTAB protocol (125 mM Tris.HCl, 50 mM EDTA, 50 mM NaCl, 3.5% CTAB, pH 7.8, 4% β-mercaptoethanol) with an overnight incubation at 65°C, as measured using UV-VIS spectrophotometer, yielded the highest DNA purity for all samples (Nuwara Eliya: 1.06, Bowalawatta: 0.88, Ogastawatta: 0.91, Pilimathalawa: 0.90, Welimada: 0.95, Uva-Paranagama: 0.77, Buttala: 0.79). Buffer components of the optimized protocol were sufficiently effective in breaking down the outer cell wall of pollen grains. Pretreatment techniques prior to pollen extraction and incorporating additional modifications, including the use of higher concentrations of buffer components and longer incubation periods, resulted in higher DNA purity. This study emphasizes that DNA barcoding techniques enhance transparency, authenticity, and eco-friendly practices in honey production, benefiting sustainable agriculture.

Keywords: Authenticity, Botanical origin, Melissopalynology, Multifloral, PCR

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