NDC E-BIOS (Online) Volume 3, pp 1-8 (2023)
ISSN: 2583-6447
DOI: https://doi.org/10.5281/zenodo.11116141

ORIGINAL ARTICLE

MELISSOPALYNOLOGICAL STUDY ON LITCHI HONEY COLLECTED BY *APIS MELLIFERA* FROM MALDA DISTRICT, WEST BENGAL

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Abstract: Malda district of West Bengal has global fame for producing delicious fruits like mango, litchi, etc. *Melissopalynological analyses of litchi honey samples (Apis mellifera) collected from four top litchi honey producing rural areas of Malda district were conducted in April-May, 2022. All the four honey samples (LH 1 - LH 4) were found to be unifloral in nature with 73-85% contribution of Litchi chinensis as the predominant pollen. In addition to Litchi chinensis pollen, 14 other pollen types were recorded with 1- 6% contribution to total palynoflora. These members were* Apiaceae, *Areca catechu*, Asteraceae, Brassicaceae, *Cassia* sp., Cheno-Amaranthaceae, *Cocos nucifera,* Fabaceae, Malvaceae, *Mangifera indica*, *Parthenium hysterophorus*, *Psidium guajava* and Solanaceae. The study indicates to potential of *Litchi chinensis* as the major nectar producing plant in its flowering season for *Apis mellifera* in Malda district of West Bengal.

Key words: Melissopalynological study, litchi honey, Malda district, West Bengal.

Communicated: 8.6.2023 Revised:10.7.2023 Accepted:14.7.2023

1. INTRODUCTION

Honey is a very useful and popular natural sweetener, with both nutritional and medicinal importance. It is produced by honey bees in their hives and basically made up of nectars of flowers, collected by them, in a particular geographic region. During foraging, in addition to sugary nectar, pollen grains are also collected by the bees. Due to phytogeographic variation, the pollen composition of honey differs from place to place, adding into the uniqueness of quality in honey samples. Study of the pollen grains found in honey or melissopalynology is an important tool to detect the adulteration and geographic origin of honey[1].

In the state of West Bengal, India, Malda district is very famous to produce fruits like mango, litchi, etc. Litchi (*Litchi chinensis* Sonn., Family: Sapindaceae) is a delicious juicy fruit of excellent quality. After China, India is the second largest producer of litchi in global market [2]. In India, the state of West Bengal

is the second largest producer of this delicious fruit, most of which come from the orchards Malda district [3-6].In this district, villages in the blocks of Kaliachak I, II, III and Ratua I are the producers of more than 60% yield of litchi fruits [6].

Based on pollen composition, honey may be of two types – unifloral (monofloral) and multifloral. In case of unifloral honey, majority of the pollen flora originates from a particular plant taxa. In case of multifloral honey, the sources of pollen grains are diversified. Regarding the economic value, unifloral honey are considered as exotic, which higher market value. Lichi/Lychee honey is very popular honey, with better market value (Figure 1).

In the present study, melissopalynological study was conducted to observe the pollen spectrum present in natural squeezed litchi honey samples collected from orchards of rural area of Malda district, at the end of litchi bloom.

Figure 1: Availability of litchi honey with wide range of market value as depicted in commercial website.

2. MATERIALS AND METHODS

Honey sample collection

Squeezed litchi honey samples (*Apis mellifera*) were collected from litchi orchard areas of the district of Malda (Figure 2) from the blocks of Kaliachak I (LH 1), Kaliachak II (LH 2), Kaliachak III (LH 3) and Ratua I (LH 4),when the flowering season of litchi (February-March) is over, in the advent of Summer (April-May) in 2022.

Melissopalynological Analysis

Microscopic analysis of the of pollen grains in the honey samples were carried out according to Louveaux *et al*. [7].For each sample, 5 ml honey was dissolved in double distilled water at the temperature below 40□. The suspension was centrifuged for 5 minutes at the speed of 5000 rpm. The pellet was kept in the tube after discarding the supernatant. Later, the pellet was suspended in double distilled water and

centrifuged for 5 minutes in similar manner. The process was repeated 2-4 times for the complete removal of the sugar traces in honey sample.

Finally, the pellet was collected and suspended in centrifuge tube containing the mixture of acetic anhydride and concentrated sulphuric acid in the ratio of 9:1for three minutes in hot water bath at 100°C for acetolysis [8].

When, there is no acetolysis, to the pellet of honey sample,2 ml concentrated sulphuric acid was poured slowly. The resultant suspension was kept in a water bath for 5 minutes at 70°C. After centrifugation for 5 minutes at 5000 rpm, the sediment was collected after decantation and 2 ml of 20% potassium hydroxide was added to it. This suspension was again placed in 70°C water bath for 5 minutes and centrifuged in similar manner. The pellets were then suspended with double distilled water and undergone centrifugation for 5 minutes. To get better clarification, the whole procedure was repeated. Finally, the sediments were collected in 50% aqueous glycerin solution to make a suspension, which was preserved for analyses under microscope.

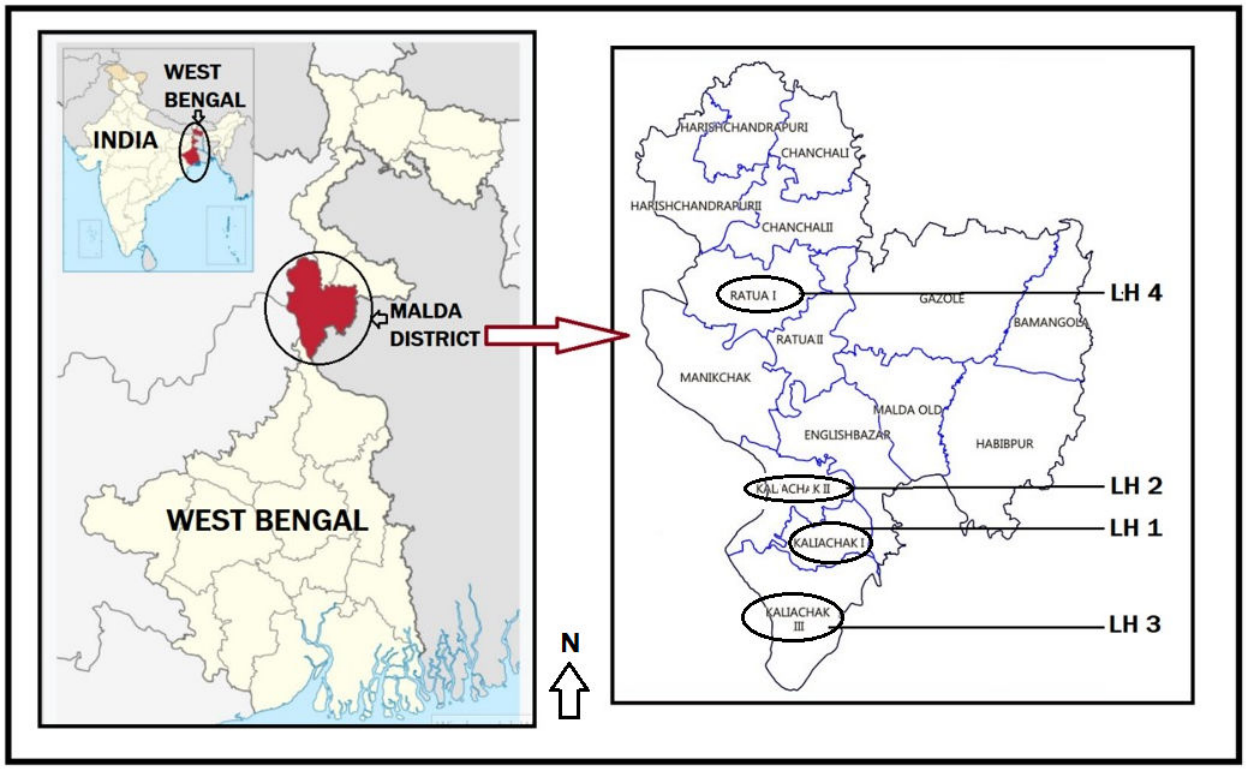

Figure 2. Geographical location of the collection sites of litchi honey samples of Malda district, West Bengal, India.

After the chemical treatment, a drop of the sediment suspension was placed on a clean glass slide and a cover slip was mounted over it. It was then wax sealed. In this way, for every sample five slides were prepared, for microscopic observation using compound light microscope (Olympus). Observed pollen grains were identified using pollen identification keys for consultation [9-11]. For preparation of identification key, in addition to palynological standard references, reference pollen slides of local vegetation were prepared on the basis of ecofloristic survey.

A total of 500 pollen grains were counted for each honey sample for calculation of percentage frequency of each pollen type, following the formula:

$$\% \text{ Frequency} = \frac{\text{Number of particular pollen type counted}}{\text{Total number of pollen grains counted (500)}} \times 100$$

The recorded pollen grains were categorized into different frequency classes according to International Commission for Bee Botany [7] as following:

1. Pollen grain with >45% of occurrence - predominant type,
2. Pollen type with 16-45% occurrence -secondary pollen type
3. Pollen with 3-15% of frequency - important minor pollen
4. Pollen with <3% frequency - minor pollen type

The pollen grain types with at least 1% frequency contribution were considered [12].

When there was the presence of a particular pollen type with more than 45% frequency, the pollen type is predominant type. Then the honey sample was designated as unifloral or monofloral honey. On the other hand, when there was no predominant pollen type observed, the honey sample was designated as multifloral in nature [13].

3. RESULTS AND DISCUSSION

In the present study, all the four samples of litchi honey from Malda district of West Bengal were found to be unifloral in nature (Figures 3 and 4).Including the pollen grains of *Litchi chinensis*, a total of 14 other pollen types were recorded from Apiaceae, *Areca catechu*, Asteraceae, Brassicaceae, *Cassia* sp.,Cheno-Amaranthaceae,*Cocosnucifera,*Fabaceae,Malvaceae,*Mangifera indica*, *Parthenium hysterophorus*, *Psidium guajava* and Solanaceae. Percentage of *Litchi chinensis* pollen in four unifloral litchi honey samples ranged between 73-85%. In this regard, litchi honey is not a very well-studied type in palynological aspect. There are reports on antioxidant [14] along with antibacterial properties [15] from the mango honey samples of Malda district. A melissopalynological study from Taiwan in 1984 [16]with 88 honey samples, 76 were reported as unifloral type with two samples predominated by litchi pollen (75-86%), with the presence of 10 other pollen types namely *Euphoria longana*, Asteraceae, *Trema* sp., *Thea* sp., Poaceae, *Rumex* sp., *Saplum* sp, Moraceae, *Psidium guajava* and *Macaranga* sp.

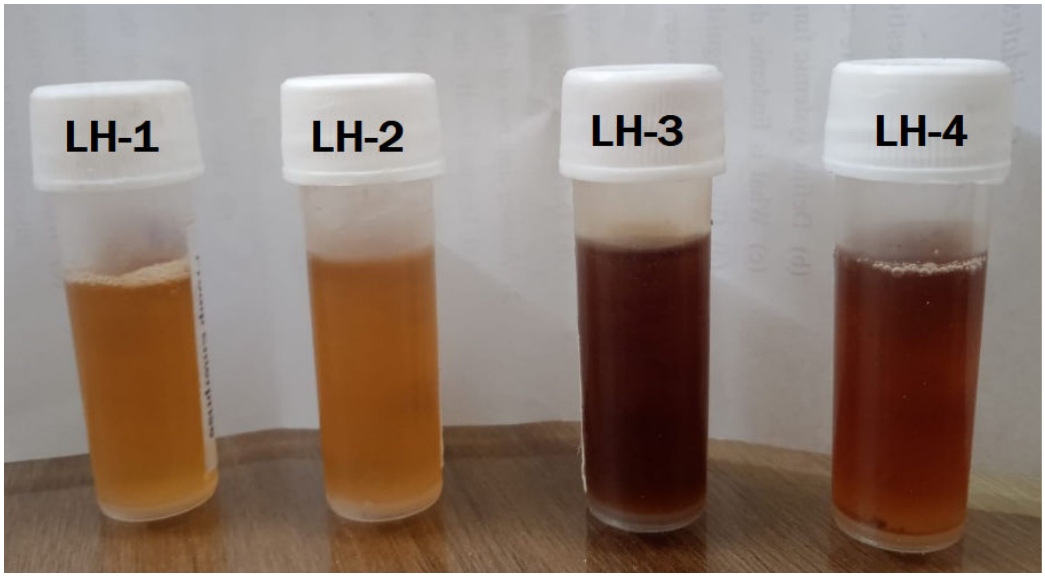

Figure 3. Litchi honey samples collected from Malda district for melissopalynological study.

In Indian context, the pollen spectrum of litchi honeys from the area near Pune, Maharashtra, depicted the pollen types of *Datura* sp., *Lagerstroemia* sp. and *Amaranthus* sp. [17]. Litchi was found as a predominant pollen source in Himachal Pradesh in a study with 30 different honey samples collected in 1989 [18].

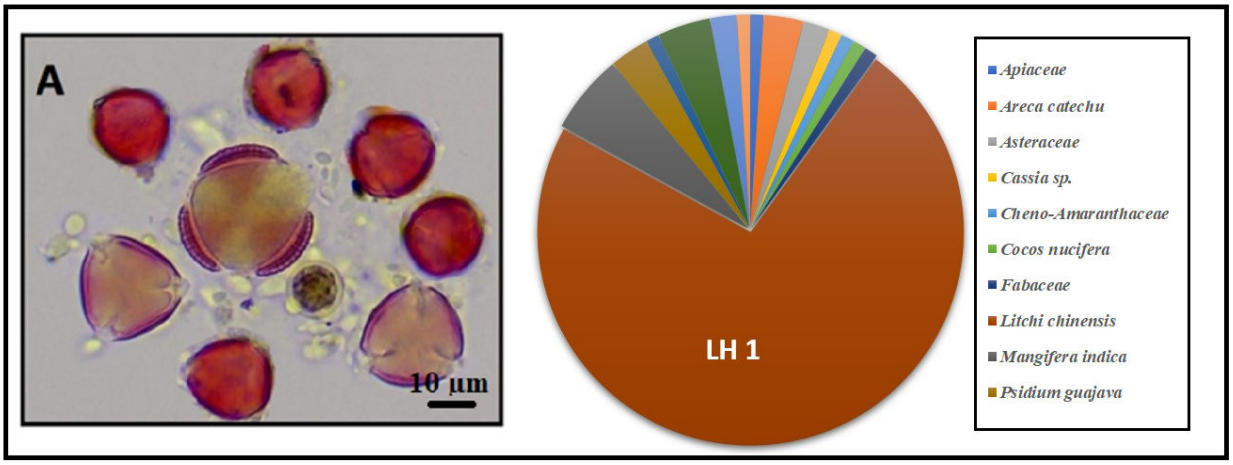

Figure 4. (A) Pollen grains present in the litchi honey sample LH 1 of Malda district of West Bengal, as observed under compound microscope. (B) Percentage contribution of pollen grains present in LH 1.

In case of LH 1, 73% of pollen grains were recorded from *Litchi chinensis*, indicating the predominance of litchi pollen in this honey sample (Figure 4). The other pollen members were – *Mangifera indica* (6%), Brassicaceae (4%), *Psidium guajava* and *Areca catechu* (3% each), Asteraceae and Malvaceae (2% each), Cassia sp., Cheno-Amaranthaceae, *Cocos nucifera*, Fabaceae, *Parthenium hysterophorus* and Solanaceae (1% each).

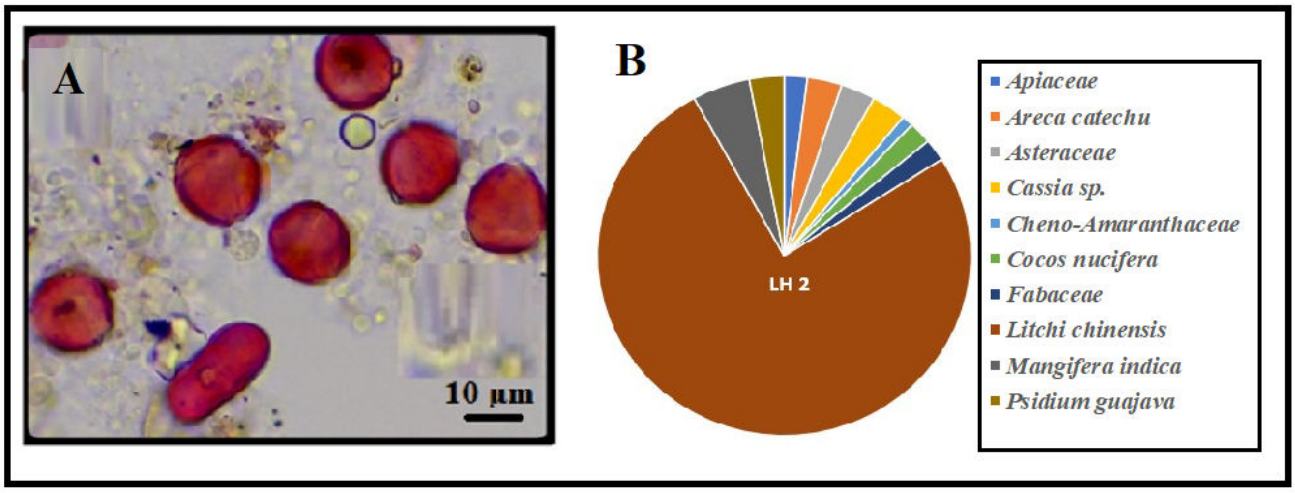

Figure 5. (A) Pollen grains present in the litchi honey sample LH 2 of Malda district of West Bengal, as observed under compound microscope. (B) Percentage contribution of pollen grains present in LH 2.

For the honey sample LH 2, 76% contribution of the pollen spectrum in honey has the source from litchi plants, followed by *Mangifera indica* (5%), *Areca catechu*, Asteraceae, *Cassia* sp., and other pollen types (Figure 5).

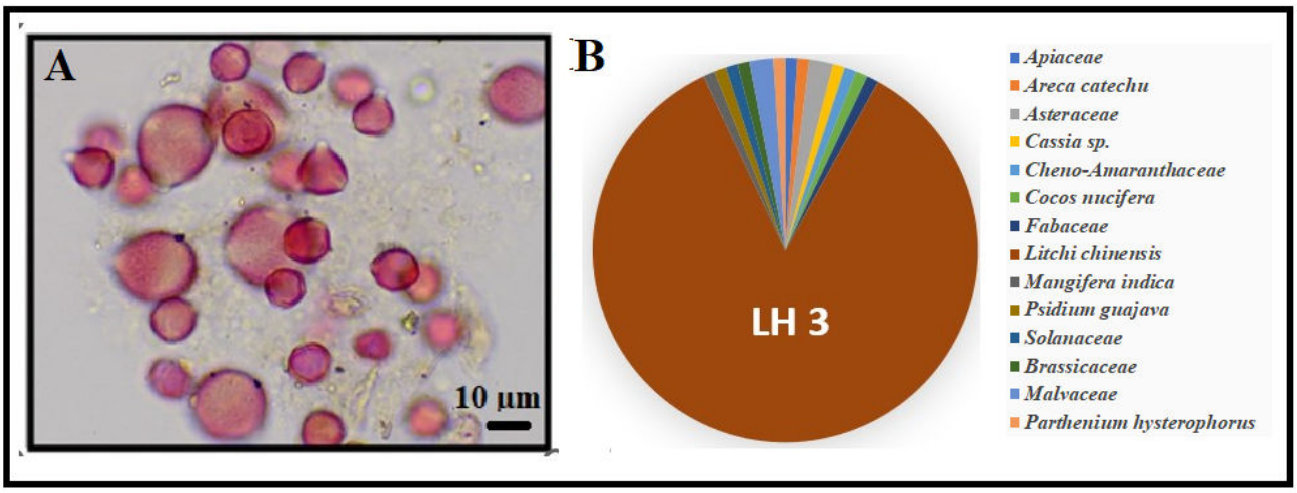

Figure 6. (A) Pollen grains present in the litchi honey sample LH 3 of Malda district of West Bengal, as observed under compound microscope. (B) Percentage contribution of pollen grains present in LH 3.

In case of LH 3 honey sample, *Litchi chinensis* had the contribution of 85% of total palynoflora (Figure 6), followed by Asteraceae (2%), Arecaceae (2%), Malvaceae (2%), Apiaceae, *Areca catechu,* etc.

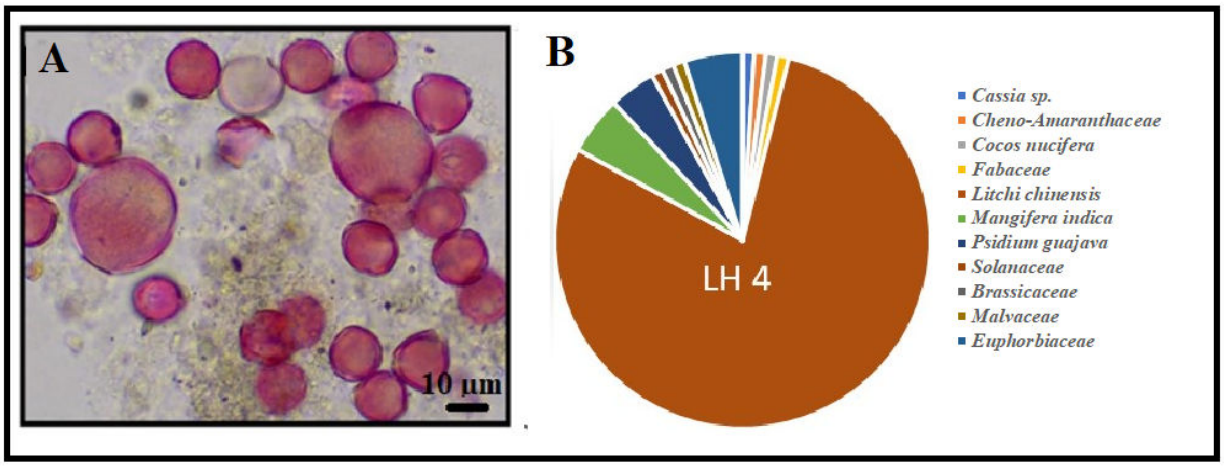

Figure 7. (A) Pollen grains present in the litchi honey sample LH 4 of Malda district of West Bengal, as observed under compound microscope. (B) Percentage contribution of pollen grains present in LH 4.

Lichi chinensis pollen contributed 79% of total palynoflora for LH 4 honey sample, followed by *Mangifera indica* (5%), Euphorbiaceae (5%), *Psidium guajava* (4%), *Cassia* sp. (1%), Cheno-Amaranthaceae (1%) and others.

When different reports are considered, *Litchi chinensis* was further reported as a predominant pollen source in 14 *Apis mellifera* honey samples and as secondary pollen type in three samples among various Indian honey samples [19]. Litchi chinensis was also found also as a major source of nectar and pollen to honeybees in North-west Himalayan region (Sood et al., 2006).

Present investigation highlights the unifloral nature of palynoflora of the litchi honey samples collected in early summer from Malda district of West Bengal, which is due to the homogenous nature of flowering vegetation in the study area.

4. CONCLUSION

The present study indicates *Litchi chinensis* is the important nectar producing plant in its flowering season for *Apis mellifera* honey bees in Malda district of West Bengal. For honey, the nectar source is the sole determinant of its taste, flavor and therapeutic application. Hence, melissopalynological analyses of litchi honey is helpful to authenticate its exotic unifloral nature to assure its commercial value.

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