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REVIEW ARTICLE

Melissopalynological studies in India: A review

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Abstract: Melissopalynological study was an age old technique of examining honey samples for their pollen content and was of high research interest in Europe and US for a long time. In the present review study an attempt has been made to accumulate the research studies of melissopalynology in India which was started in early fifties converging to the recent advancements. The different studies from different parts of India explore major bee foraging plants of the studied regions with their seasonal variations. Apart from the pollen investigations some physicochemical parameters were also identified in Indian honey, though not sufficient level to establish a good quality control. The present review is also trying to demarcate the present status and scope in this research area to enhance the knowledge for apicultural market.

Key words: Honey, melissopalynology, pollen, physicochemical.

Introduction

The use of honey has long and varied historical evidences in human civilization. In many cultures as well as in many countries, honey has associations that go beyond its use as a food (Codex Alimentarius Committee, 2001). Study of pollen grains within honey under microscope is referred as melissopalynology which was in long practice among botanists and by this study the botanical as well as geographical origin of honey could be ascertained. In India, the melissopalynological investigation was started in early fifties by eminent scientists (Deodikar and Thakar, 1953) which is continued through many years till date. India, being a vast country with diverse vegetation is a resourceful place for apicultural industry. Though a number of melissopalynological studies were done by many eminent scientists in this field, but the knowledge of potential bee foraging plants in many places of India is inadequate. In India, three species of honeybees are documented namely, *Apis cerena indica, Apis dorsata* and *Apis florae*, though *Apis cerena indica* is most common and utilized in bee keeping apiaries. In the present review work an attempt has been made to sum up the melissopalynological studies by different investigators in different places of India.

History of melissopalynological investigations

Pfister (1895) at the end of nineteenth century examined the pollen contents of various Swiss, French, and other European honeys. He discussed the possibility of determining the geographical origin of honey from the pollen and was able to identify pollen grains based on pollen morphology of European pollen types. Pollen is an essential tool for the analysis of honey. The major and minor nectar sources for a honey sample can be determined by the relative pollen frequency. The first scientific investigation of U.S. honey begins in the early 1900s by W. J. Young, who published a brief report on the analysis of domestic honey produced in the United States (Vaughn and Bryant, 2001). Young demonstrated that if honey is adulterated with sugar syrup, this could be detected by finding a reduction in contents of the pollen. However, the method he used to determine the pollen concentration values is not considered accurate by today's standards. Young (1908) determined pollen concentration ratios for 19 of 100 honey samples by extracting only one gram of honey from each sample, diluting it with water, and then counting a small portion. Using the pollen concentration value for each of his 19 samples as a basis, he determined that the range of pollen concentration values varied from a low of 123 pollen grains /g to a high of 5,410 grains /g of honey. Although Young's report focused mainly on the chemical aspects of honey and honeydew samples, he was one of the first to examine the pollen contents of U.S honey and focused on the importance of protecting honey samples from airborne contaminants. In 1911, Fehlman published his work on the pollen spectra found in various examples of Swiss honey (Maurizio 1951; Maurizio and Louveaux 1965; Lieux 1969). Fehlman was the first European to use pollen as a way to identify and differentiate honeydew from nectar honeys, and proved that pollen contents were the key to determining the nectar sources in honey samples.

In 1920s, Parker (1923) conducted a study on U.S honey, where he described 28 different kinds of pollen collected by honeybees and published photographs of the 12 most important ones. Parker demonstrated that the pollen content in honey is a valuable tool for identifying the foraging sources used to make it. Other research advancements in Melissopalynology during the 1920s were made by Betts and Allen, who worked separately on English honey. Allen (1928a) noted that some pollen grains remain on the surface of the honey, instead of becoming mixed with the honey, because they must be lighter and less dense than the honey. He was also the first to prove that pollen found mixed with nectar could come from sources other than the nectar plant's own anthers and pollen (Allen 1928a). Allen also reported that airborne pollen could easily contaminate honey during removal of hives and also during the subsequent honey extraction process. He published a series of articles in Bee World (Allen 1928a, 1928b, 1928c,

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1928d; 1929). Zander stands out as being the leader in melissopalynology research in Europe, during the 1930s and 1940s by publishing five-volume works over a span of nearly two decades (Zander 1935, 1937, 1941, 1949 and 1951). He also studied other types of materials in honey, such as fungal spores and hyphae. Because of his long and dedicated works in the field, Maurizio (1975), Maurizio and Louveaux (1965) referred Zander as the "Leader in Melissopalynology research in Europe".

The microscopic analysis of honey was taken up and developed in a wider scale in different countries of the world (Maurizio 1936, 1951, 1975, 1979; Maurizio and Louveaux 1965, Vorwohl 1967, 1981, Varis *et al*., 1982, Louveaux *et al*., 1978, Kerkvliet and Beerlink 1991 etc., in Europe; Lieux 1972, Feller-Demalsy 1983, Feller-Demalsy *et al.,* 1987, Telleria 1988, Parent *et al*., 1990, etc., in America; Agwu and Akanbi 1985, Louveaux and Abed 1984 in Africa etc.).

The method of microscopical analysis was proposed and modified by various researchers from time to time (Erdtman 1960, Maurizio and Louveaux 1965, Vorwohl 1967, Louveaux *et al*., 1978, Lieux 1972, 1980).

Melissopalynological studies in India.

The major centers for melissopalynological investigations in India are:

(i) Botany Department, Osmania University, Hyderabad.
(ii) Central Bee Research Institute, Pune.
(iii) Birbal Sahni Institute of Palaeobotany, Lucknow.
(iv) Palynological Laboratory, National Botanical Research Institute, Lucknow.
(v) Division of Palynology and Environmental Biology, Bose Institute, Kolkata.
(vi) Botany Department, Calcutta University, Kolkata.
(vii) Botany Department, Bangalore University, Bangalore.

Floristic survey

One of the earliest melissopalynological works was done by Deodikar and Thakar (1953). Later some sporadic reports were published by Vishnu-Mittre (1958), Nair (1964). Some works were done over honey analysis from Kumaon Himalayan region, Uttar Pradesh by Sharma and Nair, 1965; Chaturbedi, 1973, 1983, 1989; from Western Ghats by Chaubal and Deodikar,1965; Deodikar,1965; Suryanarayan *et al*., 1981; from Andhra Pradesh and Tamil Nadu by Seethalaxmi and Percy, 1979, Seethalaxmi, 1980; Jhansi and Ramanujam, 1986, 1987, 1990; Kalpana and Ramanujam, 1989, 1991; Kalpana *et al*., 1990; Ramanujam and Kalpana 1993,1995; Jhansi *et al*., 1991, 1994; Agashe and Mary 1995; and from West Bengal by Sen and Banerjee 1956, Mondal and Mitra 1980, Ganguly and Chanda 1980, Chanda and Ganguly, 1981; Bhattacharya *et al*., 1983, Ganguly *et al*., 1984, Chakrabarti, 1987; Malakar and Chanda, 1995, Bera *et al*., 1997, Kumar 2000, etc.

Fatima and Ramanujam in 1989 studied two multifloral honey samples from Hyderabad showing no predominant pollen type. *Helianthus annus* and *Eugenia jambolana* are the two secondary pollen types. Significant pollen grains are *Cocos nucifera, Abutilon* sp., *Amaranthus* sp., etc. In 1990, Kalpana *et al.* worked on three honey samples, one of *Apis cerena* and two of *Apis florae*, along with 160 pollen loads to identify the bee foraging plants at Adikmet area of Karnataka.

Chanda and Ganguly (1981) analyzed honey samples collected from Kerala, Karnataka, Andhra Pradesh, Orissa and West Bengal. It has been found that most of the pollen grains were of entomophilous type, some were of anemophilus which may be due to (i) atmospheric contamination and/or (ii) deliberate collection of bees. By microscopical analysis supplemented by pollination ecology it has been possible to identify the biozones from where the samples originated. Sugar content was also measured in two samples predominantly containing sucrose.

Ganguly *et al.* (1984) analyzed two honey samples qualitatively and quantitatively from Balurghat and Jalpaiguri of West Bengal. Out of 22 pollen types recorded, some were of *Azadirachta indica, Ageratum* sp., *Blumea lacera, Bombax ceiba, Coriandrum sativum* etc. A comparative study of pollen types of two localities was made.

Jhansi *et al*., (1991) palynologically analyzed six squeezed honey samples collected from rock bee *(Apis dorsata)* combs during the period 1982-1984. All the samples represented summer honeys from tropical dry deciduous forest tracts of the Prakasam district of Andhra Pradesh, India. The samples were all of multifloral in nature, contained a total of 57 pollen types. The results indicated that *Cassia fistula*, *Terminalia alata*, *Bauhinia racemosa*, *Feronia elephantum*, *Lagerstroemia parviflora*, *Strychnos potatorum*, *Zizyphus xylopyrus*, *Phyllanthus* sp., *Soymida febrifuga*, *Syzygium cumini*, *Dalbergia latifolia* and *Caesalpinia bonduc* constitute fairly reliable nectar sources for honey bees in this area during the summer.

Garg (1996) had done melissopalynological investigations of *Apis cerana indica* pollen loads for autumn season (September-October) from Bhimtal in the Kumaon Himalaya. All the loads were of unifloral types representing 15 families and 18 taxa. Pollen grains of Asateraceae were most dominant followed by Poaceae, Lamiaceae and Fabaceae.

Bera *et al*., (1997) worked on seven honey samples from Sikkim, Darjeeling and West Dinajpur during the summer. Three samples were of unifloral type, while rests were multifloral. Some significant pollen grains were *Trifolium repens*, *Calendula officinalis*, *Brassica campestris*, *Clematis montana*, *Moringa oleifera* etc.

Jana *et al*., (2002) palynologically analyzed 25 apiary honey samples and 6 squeezed honey samples from Murshidabad district. Majority of them were unifloral types with *Brassica nigra*, *Coriandrum sativum*, *Zizyphus* sp. etc., indicating the important bee foraging source during winter.

Mukhopadhyay *et al*., (2003) assessed seven honey samples from Sub-Himalayan West Bengal, of which two were of unifloral type and rest were multifloral in nature. Important pollen grains were *Aristolochia* sp., *Rosa* sp. *Brassica* sp., *Bidens pilosa*, *Camellia* sp., etc. Jana and Bera (2004) worked over nine honey samples of Sundarbans during summer. Eight were found to be unifloral and one was multifloral.

Sonneratia apetala was predominant in seven samples reflecting as major nectar source during mid-summer days in Sunderbans.

Pollen analyses of 26 apiary honey samples along with 4 squeezed honey samples of winter season from Bankura district were done by Bandopadhyay *et al.* (2005). 50% of the honey sample showed unifloral nature. The major sources of nectar during winter were indicated. Bandopadhyay and Bera (2005) assessed eight honey samples of *Apis cerana indica* from Bankura district of West Bengal. The result showed that five samples were of unifloral type, while rests were multifloral in nature. The data explained the importance of *Barringtonia acutangula* as a major bee foraging plant.

Mukhopadhyay *et al*., (2007) analyzed the pollen contents of 51 honey samples from the Eastern Himalayan part of West Bengal, 48 honey samples were from *Apis cerana indica* and two from *Apis florae*. 18 honey samples were unifloral. The predominant pollen types were recognized depending upon different seasons of that area and three honey flow periods (HFP-I-III) were recognized. HFP-I (March-June) is the principal flow period, while HFP-II (July-October) and HFP-III (November-February) are secondary. The study explored the suitability for establishing commercial bee keeping in the remote areas of the Eastern Himalayan part of West Bengal. Datta *et al*., (2008) analyzed 51 honey samples from Uttarakhand and Uttar Pradesh, among which 8 pollen types of Uttarakhand and 7 of Uttar Pradesh were considered as the predominant pollen types. In addition to already known bee forage-*Brassica*, *Coriandrum*, lichi and some other species including *Eucalyptus*, *Callistemon* are also heavily utilized by bees as forage reveled by the investigation. Flora like *Myrica*, *Rumex*, *Erigeron* are also utilized and therefore are important for apiculture in these regions.

Sixteen pollen grains were identified by analyzing ten honey samples of Western Ghats by Bhargav *et al*., 2009. Predominant pollen types were *Coffea* sp. and *Coccos nucifera*. Other recorded types were *Eucalyptus globulus*, *Hevea brasiliensis*, *Coriandrum sativum*, *Areca catechu* etc. The study reflects the floral diversity of the places of origin of honey samples.

Melissopalynological studies of Rewa district of Madhya Pradesh, India were carried out by Seth (2009), Sahney and Seth (2007, 2010 and 2012). The pollen grains retrieved from the honey samples reflect the local vegetation. The results explained the importance of many taxa as major bee foraging plants.

Chakraborti and Bhattacharya (2011a) analyzed ten different honey samples of West Bengal for their pollen content as well as some physicochemical parameters like pH, free acidity, moisture content and electrical conductivity. Some important pollen grains like *Phoenix paludosa*, *Sonneratia apetala*, *Ceriops* sp., *Bruguiera* sp., etc were found from Sundarbans honey samples. Other important pollen grains were *Borassus*, *Sesemum*, *Coriandrum*, *Brassica*, *Peltophorum*, etc.

Eight different honey samples from Orissa and West Bengal were also studied by Chakraborti and Bhattacharya (2011b) to evaluate their floristic composition and there vegetational impact over the bee forage. They have identified 25 different pollen types.

Chemical analysis of honey

In 1995, Malakar *et al*., studied three honey samples from West Bengal for their pollen profile, protein and lipid contents as well. The range of protein contents were varied between 0.022% - 0.875%. Some major protein bands 50 to 37.5 kDa were also identified through SDS-PAGE and 37.5 kDa band was found to be common in all the samples. The lipid range was 0.012% to 0.024% and palmitic acid was found to be a major fatty acid. Chattopadhyay *et al.* (1996) identified some mangrove pollen grains from an index honey sample of Sundarbans and assessed lipid constituents and fatty acids of the sample by thin layer and gas liquid chromatography. There were saturated, normal and branched chain hydrocarbons in lipid profile mostly C19 to C40 with odd and even carbon numbers. Fatty acids were composed of wax esters, sterol esters and triglycerols with carbon chain length between C15 to C23. The percentage of saturated fatty acid was greater than that of unsaturated fatty acids. Among the all lipids, palmitic acid was the principal saturated fatty acid and oleic acid was the major unsaturated fatty acid. Carbon chain length ranged from C15 to C34 in the alcohols of the wax esters.

Bera *et al.,* (2003) tested three natural honey samples from sub-Himalayan West Bengal for heavy metals such as Cu, Zn, Mn, Cd and Pb by atomic absorption spectrophotometry. The result showed exceeds level of Cu and Zn in respect to standardized average amount because of pesticides used in tea gardens and crop fields. Kaur *et al*., (2010) determined the quality of honey by evaluating physicochemical properties of different honey samples collected from local market using chemometrics. Along with honey samples, cane sugar, fructose, glucose and their mixtures were also taken as samples and were used for building and calibrating the model. Eight out of 33 samples were used for prediction. The coefficient of determination (R2) and standard error of prediction (SEP) were 0.9958 and 0.0159 respectively. The results demonstrated that computation technique along with instrumentation may be used as a simple method for quality assessment of honey.

Gairola *et al*., (2013) determined physicochemical properties viz. viscosity, specific gravity, moisture, HMF content, total reducing sugars, fructose, glucose and sucrose of 11 honey samples of *Apis cerana indica* from Uttarakhand. Manzoor *et al.* (2013) analyzed moisture content, viscosity, surface tension, ash content, density and pH of honey samples of Jammu and Kashmir.

Chakraborti and Bhattacharya (2014) analyzed 21 honey samples for their pH, free acidity, moisture content, electrical conductivity, HMF (Hydroximethyl furfural) content and invertase activity depending on their storage durations. 16 honey samples out of 21 showed higher values of HMF content than the recommended standard limit. Product moment correlation between HMF content and invertase activity showed strong negative correlation and

one-way ANOVA suggests these two parameters are related to the storage duration of honeys. It was concluded that these high range of HMF values (642.31 mg/kg) with low value of invertase activity may be due to the heat stress in a tropical country like India.

Present status & scope

Pollen analyses of honeys in different states of India were done throughout a long time by many scientists and thus, various bee foraging plants were identified from season to season which enhances the knowledge of apiculture. So far the knowledge of physicochemical components of honey is concerned, it is not adequately worked out. Various important chemical parameters those are internationally recommended with their maximum admissible limits could be well analyzed as a crucial step to build up a proper quality control for honey market in India.

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