2. REVIEW OF LITERATURES

Bee keeping or apiary has a long history since time immemorial, initiated by the pre-historic men. There are many apocryphal claims regarding its earliest record. Science daily (2007) reported the discovery of ancient bee hive at Tel Rehov in Israel which dates from the 10th to early 9th centuries B.C.E. (https:// www.sciencedaily.com/releases/ 2007/09/070904114558 .htm). Barnsley Beekeepers Association of South Yorkshire claimed that the earliest fossil evidence of bees was noted in a piece of amber obtained from a mine in the northern part of Burma. It is assumed to be from almost 100 million years back, at a time when bees and wasps split into two different lineages. The same report demands that the earliest evidence of fossilized *Apis* bee was from Europe nearly of 35 milion years before, though the common notion is that this bee originated at Africa. The fact of utmost significance is the taming of wild bee in captivity, a Mesolithic illustration inside the Cueva de la Arana, near Bicorp, Spain tells about its timing as 2400 BC.

The knowledge of keeping bees at a convenient place within an artificial enclosure near human habitation was a remarkable achievement and provided easy accessibility and harvest of honey; however, the productivity of honey and even sustainability of the endeavour was beyond the reach till the pioneering work of Pfister (1895) which showed the significance of studying pollen in honey. Melissopalynology, the science of studying pollens in honey, has been extensively used since then for the determination of purity of honey, geographical and floral origin of the product (Walter, 1915; Nair 1964; Maurizio, 1975; Moar, 1985, Ramanujam et al, 1992; Jones and Bryant, 1992; Bryant and Jones, 2001; Sajwani et al, 2007 and Song et al 2012). On accepting melissopalynology as an important aspect of apiary proper identification of pollens in respect of the plant source species becomes the most essential job. Plenty of works from different corners of the world help in proper identification of pollens. Researchers like Erdtman (1954), Nair (1970), Sowunmi (1976), Agwu and Akanbi (1985), Gupta and Sharma

(1986), Adeonipekun (1989, 2010, 2012) and Gosling *et al*. (2013) contributed a lot in this regard.

The success of apiary and honey production is dependent on a couple of factors, most important of which are the sources of nectar and pollen. While the selection of site for natural hives is instinctively done by bees amidst vegetation rich with source plants, a fair knowledge regarding such preferred plants is a prerequisite for establishing apiary artificially. Empowered with this knowledge the beekeepers can take proper decision regarding the placement of the hive boxes at right place and thus it holds much significance (Howes 1953). To trace the identity of nectariferous and polliniferous plants a variety of measures have been adopted so far, amongst which melissopalynology provides pollen as an effective index. In consideration of the significance of melissopalynological study due heed has been paid worldwide to it to get commercial success out of apiary business.

Barth (1989), out of his working experience, opined that in course of visiting flowers for the collection either of nectar or pollen pollens get inadvertently clung to the body hairs of bees. These pollens not only provide information about the botanical origin of honey, but also the geographical source of that.

Bees forage different plants; thus, honey is always a mixture of several sources. Differences in their composition also mean differences in the organoleptic and nutritional properties of these honeys. Anklam, (1998) highlighted that variations in nectar content, together with other factors, such as climatic conditions, soil type and beekeeper activities, contribute to the existence of different types of honeys.

While countless workers through their survey, research work and writings have unanimously accepted the utility of melissopalynology, many other works confronted with it putting caution in conducting proper study of it and in drawing inference on that. These studies have also been

concerned with temperature and rainfall of an area at any point of time in a year (Herrera, 1995; Jens, 2008; Rands and Whiteney, 2008; Thomas *et al*, 2009; Baldock *et al*, 2011; Singh *et al* 2011; Nascimento and Nascimento, 2012), which have much bearing on bee, a potent pollinator, pollination network and the pollination process. Louveaux (1970) provided a method for counting absolute number of pollens and also for determining frequency classes and frequency of Distribution (Louveaux, 1978). Moreover, ordination of melissopalynological data has been carried out with the aid of a variety of statistical analyses by different authors (Herrero *et al*, 2002; Corbella and Cozzolino, 2008; Aronne and Demicco, 2010), for the purpose of affirming the geographical and plant source of honey. Since only the pollen count cannot always ascertain the prime source of honey either on account of the source plant being a weak producer of pollen or underrepresentation of the species in the pollen population retrieved from the sample honey, many researchers attempted to devise various methods for rectifying the pollen data adopting pollen coefficient values. In many attempts even the considerations of pollen coefficient values could not be proved flawless and reliable. Bryant and Jones (2001) reviewed many such attempts and devised a new method for deducing this value. Punnuchamy *et al* (2014) computed similarities between pollen spectra using binary Bray-Curtis' index and Bray-Curtis' index and used multivariate analysis of variance for judging the impact of independent variables like month, year and location of source on pollen spectra.

Instances of melissopalynological works are plenty and reports on it abound both from India as well as abroad. Melissopalynology has been considered as a reliable method for determining the plant source of honey and geographical location thereof by many researchers.

2.1. MELISSOPALYNOLOGICAL RESEARCHES IN INDIA

In India, investigations on pollen analysis of honey samples are available from various parts of the country. Quite an early report from Maharashtra was done by Deodiker and Thaker (1953) and Deodiker *et al*., (1958). Nair (1964) also made analytical study of pollen from Indian honey. Garg and Nair's work (1974) used pollen as bioindicator for understanding bee pasturage in Bhimtal area of Western Himalayas. Suryanarayana *et al*. (1981) suggested absolute pollen count (APC) of honey sample using haemocytometer for the purpose of accuracy. Bhattacharya (1983) analyzed pollens in honey sample from Salt Lake City, Calcutta. Jhansi *et al* (1991) presented similar work for the honey from rock bee in Andhra Pradesh. Ramanujam and Kalpana (1991) analysed pollens in the honey of *Prosopis juliflora* from Ranga Reddy district of Andhra Pradesh in an attempt of reviewing its relevance in apiculture. Ramanujan and Khatiza (1992) worked out the summer pollen sources in Andhra Pradesh. A survey of bee foraged plants based on melissopalynology from north eastern hill region was carried out by Sing (1999). Working with 21 honey samples during summer and early winter from 10 localities they identified *Adhatoda* sp., *Ageratum* sp., *Brassica* sp., *Clematis* sp. etc. as major ones and *Acer* sp., *Bauhinia* sp., *Caesalpinia* sp. etc. Kumar (2003) studied the pollen and nectar sources of *Apis mellifera* L. honey bees at Kadasikadau, Idukki in Kerala. Lakshmi and Suryanarayana (2004) conducted melissopalynological investigation of the honeys collected from Cuddaph forest of Andhra Pradesh. Bhusari *et al* (2005) worked on melissopalynology from Maharashtra. Daga *et al* (2006) conducted a consorted characterization of avocado honey from *Persea americana* in terms of physico-chemical and palynological characterization. Bera *et al* (2007) made pollen analysis of Kamrup forest honey of Assam. Bilisik *et al* (2008) recorded seasonal variation in pollen loads of *Apis mellifera*. Reddy and Reddy (2008) identified pollens of medicinal plants in the honey collected in Andhra Pradesh. Bhargava *et al* (2009) made similar approach with pollen analysis of Karnataka. Chakraborti and Bhattachrya (2011) presented floristic composition obtained in

honey samples collected from West Bengal. Shubharani *et al* (2012) assessed plant sources for honey in Coorg honey from the Karnataka state of India, based on the melissopalynological evidences. In their study altogether 91 pollen species from 42 families were recorded. Predominant pollen species were found as *Coffea* sp., *Cocos nucifera*, *Aster* sp., etc. Ramakrishna and Swathi (2013) reported on pollen diversity in honeys from Adilabad district of Andhra Pradesh. Sahney and Seth (2013) worked out pollen population in winter honeys of Rewa district of Madhya Pradesh. An account of comprehensive work on the melissopalynology related to Indian honey bee of southern Kerala has been presented in the Ph. D. thesis of Aswini (2013). Rehel and Padmavati (2014) presented melittopalynological studies from Nilgiri, Tamil Nadu. The elaborate work of Punnuchamy *et al* (2014) demonstrated the complexity of environmental factors in the ultimate outcome of foraging by bees in a heterogeneous and complex landscape, while they were carrying out the work with a notion that honey collected from different sites, during a certain year and month, would be much less similar than samples procured from a particular site and in a month during different years. Diversity in pollen population in Trigona honey from Pederu forest of Visakhapatnam was revealed in the work of Devender and Ramakrisna (2015). In course of analysing the recorded data Sahney and Rahi (2015) classified the pollen sources as of entomophilous, amphiophilous and anemophilous. In the honey sample collected during March they found the airborne pollens of anemophilous *Holoptelia integrifolia* to be present in abundance. This finding was corroborated with the inference drawn by an earlier work of Sahney and Chaurasia (2008), where the work on airborne pollens throughout the year showed the preponderance of *Holoptelia integrifolia* during February – March. Based on such finding Sahaney and Rahi (2015) inferred that there is every reason to get airborne pollens of the plants clung on the bee body surface and mixed with honey, though the plant neither represents aspolliniferous nor nectariferous. Kamble *et al* (2015) carried out melissopalynological studies of honey from

Sunderban of West Bengal. Sahney *et al* (2016) analysed pollens in six honey samples from Varanasi district, two of which were unifloral and four were multifloral in nature. In total 37 pollen species could be identified. In unifloral honeys the predominant pollen types were *Brassica campestris* and *Ageratum conyzoides*. In multifloral honey *Ageratum conyzoides, Brassica campestris, Callistemon citrinus, Holoptelea integrifolia, Lathyrus aphaca* and *Parthenium hysterophorus* were the secondary pollen types. For most obvious reason the majority (54.05%) of pollen grains were of entomophilous nature, 32.48% of amphiphilous nature and 13.15% pollen were of anemophilous taxa. Dama *et al* (2016) treated melissopalynology as a tool for studying diversity of pollen constituents in the crude honey collected from south Solapur of Maharashtra. Saharia and Sarma (2016) investigated on the pollen populations in the honey samples collected throughout the year in the Darrang district of Assam of India and recorded 42 species belonging to 41 genera and 27 families. They also identified 19 species as wild and 17 as cultivated ones, while six species as both cultivated and wild. Regarding the habit of plants 13 species were trees, nine species of shrubs, 15 species of herbs and the rest five were climbers. Tripathi *et al* (2017) scrutinized eighteen honey samples from nine locations of Bongaigaon district in Assam. They could determine 12 samples to be of monofloral or unifloral origin and 6 multifloral. Amongst monofloral ones five samples were of *Brassica campestris*, two from *Elaeocarpus rugosus* and one each from Meliaceae, *Mimosa pudica*, *Salmalia malabaricum*, *Syzygium cumini* and *Xanthium strumarium*. Major members in multifloral honey samples were *Brassica, Coriandrum*, *Mimosa*, *Salmalia* and *Ziziphus mauritiana*. Like many other's works they also recorded a host of species as the minor contributors. The diversity of pollens recorded in their work indicated that bees travel a long distance to collect nectar and pollen. Their work also enabled in understanding the allergenicity of some unifloral honey of *Eupatorium* and *Xanthium*. Kaur and Mattu (2016) on working with the honey collected by *Apis cerena* from Shiwalik hills determined the pollen spectrum.

Dhawan *et al* (2018) have analyzed pollen grains from different honey samples collected from Newasa tehsil of Maharashtra and have recoded pollens of the members from Amaranthaceae, Asteraceae, Cactaceae, Convolvulaceae, Fabaceae, Moringaceae and Solanaceae, characteristic of that region.

In India, investigations on pollen analysis of honey samples are available from various parts of the country viz. Maharashtra (Deodiker and Thaker 1953; Deodiker *et al*., 1958), Andhra Pradesh (Ramanujam and Kalpana, 1991; Lakshmi and Suryanarayana, 2004; Ramakrisna and Swati, 2013; Devender *et al*., 2015), in Uttar Pradesh from Lucknow (Sharma and Nair, 1965, Chaturvedi and Sharma, 1973), Shahjahanpur of Uttar Pradesh (Chandra and Sharma, 2011), Himanchal Pradesh (Sharma, 1970; Sharma and Raj, 1985), Uttarakhand (Garg and Nayer,1974), West Bengal (Bhattacharya *et al*.,1983; Chakraborti and Bhattacharya, 2011; Kamble *et al*., 2015), Ramanujam and Kalpana, 1991; Bihar (Suryanarayan *et al*., 1992), Lakshmi and Suryanarayana, 2004; Ramakrisnaand Swati, 2013; Devender *et al*., 2015), Karnataka (Agashe and Rangaswami, 1997; Chauhan and Murthy 2010; Shubharani et al., 2012; Raghunathan *et al*., 2013), Orissa (Upadhyay and Bera, 2008, 2012, 2014) and Madhya Pradesh (Chauhan and Quamar, 2010; Sahney and Seth, 2013), and Allahabad (Sahney and Rahi, 2015) only.

2.2. MELISSOPALYNOLOGICAL RESEARCHES IN ABROAD

Study of pollens retrieved from honey or honey comb made its beginning with the work of Pfister (1895). After a long gap Young (1908) from USA reported on the study of pollen from honey. Thereafter Fehlmann (1911) made significant observations in discriminating honeydew and floral honey in course of studying pollen spectrum of Swiss honeys. Further development of microscopic examination of honey was made by Armbruster *et al* (1929, 1934, 1935), Griebel (1930, 1931) and Zander (1935). Afterwards reports were made from Denmark by

Hammer *et al*, (1948), Mikkelsen (1948), from Germany by Evenius, (1932, 1933a, b), Gassner, (1931), Koch, (1933), Zander, (1932, 1937a, b), from Britain by Yate Allen, (1937), Deans, (1939), Melville, (1944, 1945), from Finland by Martimo, (1945), in Holland by de Boer, (1933), from Italy by Grandi, (1934), from Portugal by de Mendia, (1939), from Sweden by Lunder, (1945), from Switzerland by Maurizio, (1936a, b, 1938, 1939, 1940, 1941a, b, 1946, 1947, 1949a, b), from Spain by Vieitez, (1948), from Czechoslovakia by Niethammer, (1928, 1929, 1931). Akpo (2017) made a comparative analysis of pollen between the honey samples collected from apiary and open market in Nigeria and Benin Republic.

Melissopalynology, the identification of pollen collected by bees, has proven to be an indispensable tool in the fields like, pollination biology (Kearns and Inouye, 1993 ; Cusser and Goodell, 2013), pollinator foraging behaviour (Louveaux, 1959; Wilson *et al*., 2010; Baum *et al*., 2011), sourcing and authentication of apicultural products (Louveaux *et al*., 1978; Jones and Bryant, 1992; Dimou *et al*., 2007), and honey bee (*Apis mellifera* L.) nutritional biology (Severson and Parry, 1981 ; Forcone *et al*., 2011 ; Girard *et al*., 2012). Traditional melissopalynology involves the careful preparation of pollen samples for microscopic inspection followed by the identification of individual pollen grains by comparison to a similarly prepared reference collection of pollen from local taxa (Erdtman, 1943; Kearns and Inouye, 1993).

The word ‘pollen’ is derived from Latin, meaning fine flour or dust (Jarzen and Nichols 1996). Pollens, collected from a locality, give a picture of the regional vegetation (Janssen 1984). This information is useful in paleoecological interpretation and in biostratigraphic studies (Jarzen and Nichols 1996). Identification of a specific pollen or collection of pollens found associated with insect is used to determine the insect’s feeding and migratory activities (Hendrix and Showers 1992; Gregg *et al*. 1993; Lingren *et al*. 1993, 1994; Berkhousen and Shapiro 1994; Loublier *et al*. 1994). In case some plants grow only in certain ecological environment or

geographic locations, the presence of pollens of those plant species can help locate the geographical origin of the insect. Pollen has been used to determine geographic origins since 1895, when it was demonstrated that the geographical origin of honey could be determined from identification of the pollen within the honey (Lieux 1969). Parker (1923) opined that by scrutinizing the stomach contents of honeybees the foraged plant species and their geographical location can be ascertained. Pollen analysis of a honey sample provides information about the plants visited by bees giving relevant information about the nectar and pollen sources of an area and helps to determine geographical and botanical origin of honey (Louveaux *et al*., 1998; Von Der Ohe *et al*., 2004; Barth, 2004).

The collection of pollen by the honeybee has been discussed in detail by Free (1970) and Butler (1972). The pollen spectrum of any honey sample gives the picture of qualitative and quantitative natures of pollen constituents in it. Barth (1989) expressed that such spectrum gives information on the involvement of nectareriferous plants, contaminations, tainted honey and any other kind of mixtures in the honey under test. Barth (1989) also recounted that such qualitative analysis of honey can also provide evidences in regard of its geographic origin, nectar producing plants, the season of collection etc. Quantitative analysis of pollens within a honey sample give idea about the plant source(s) of nectar. Such usefulness of pollens as indices for ascertaining the plant sources of nectar and pollen in a honey has been affirmed from the works of many workers, only a few of them to mention are, Santos (1963, 1978), Iwama and Melhem (1979), Barth (1969, 1970 a, b, c, 1971a, 1989, 1990, 2004), Cortopassi-Laurino and Gelli (1991), B. M. Freitas (personal communication), Aires and Freitas (2001), Sodré *et al*. (2001), Persano-Oddo *et al*. (2004) and Arruda *et al*. (2005).

Among multiple factors for the success of honey production subsistence and presence of bees in sufficient number as well as accessibility of nectar source plants during their flowering seasons have been identified as important ones by different authors and publications (Ayalew

1994, Crane 1999 and EARO 2000). Louveaux *et al* (1978) reviewed the method of melissopalynology.

Puusepp and Koff (2014) presented the results of pollen analysis from honey samples procured from Estonia for a period 2000 to 2011. Estonian honey is typically polyfloral. Altogether 120 types of pollens could be recorded. The dominant species of pollens were of Brassicaceae, Salix, Trifolium and Rosaceae. Pollens of *Fagopyrum esculentum*, *Frangula Alnus*, *Calluna*, Apiaceae, Fabaceae, Asteraceae and Poaceae, represented in more than 25% of the samples. Atanassova *et al* (2004) work with honey samples from three villages of West Bulgaria for two successive years identified wild and cultivated nectariferous plants available in those regions. Stawiarz and Wroblewska (2010) carried out melissopalynlogical works on multifloral honey from Poland. Sabo *et al* (2011) analyzed pollens in the honey samples collected from Varaždin county of Croatia. Out of 8 total number of honey samples collected they recorded 20 different types of pollen grains and identified different predominant species from different samples. The species were *Castanea sativa*, *Brassica napus* and *Trifolium pratense*. Moreover, six collections were noted to be multifloral and two collections as unifloral. Kayode and Oyeyemi (2014) analyzed pollen varieties from the honey samples collected by *Apis mellifera*. They scored pollens of 85 species belonging to 33 families of which they identified some members like, *Spondias mombin*, *Alchornea cordifolia*, *Elaeis guineensis*, *Pavetta* sp, *Oldenlandia corymbosa* etc. as predominant ones. Erdoğan and Erdoğan (2014) conducted palynological analyses of the honey samples from Coruh valley of Turkey. While working with 32 honey samples they obtained 69 pollen species representing 33 families. The study revealed the species from Asteraceae, Fabaceae and Poaceae as the predominant ones. Hamid *et al* (2015) worked on the pollen analysis of honey of four selected floral origins in Malayasia and found Tualang honey to be multifloral, though named after Koompassiaexcelsa (Tualang), while other three honeys Gelam (*Melaleuca cajuputi*), Acacia (*Robinia pseudoacacia*) and Nenas

(*Anana scomosus*) as unifloral. Rosdi *et al* (2016) conducted melissopalynological analyses of honey obtained from north Malaysian forest. Amongst three collections one was unifloral of *Mimosa scabrella,* while other two were multifloral. Arriaga *et al* (2011) made pollen analysis of Mexican honeys. Vargas-Sánchez et al (2016) worked on pollen profiles of propolis from Sonoran desert of Mexico. Out of eight total samples they found six to be bifloral in nature and two as multifloral. *Mimosa distachya* and *Prosopis velutina* were detected to be characteristic pollen types.

Diafat *et al* (2017) reported pollen analysis of Algerian honey. They analysed 25 samples of honey and found all to be multifloral. Noor *et al* (2009) carried out palynological analysis of pollen loads in Islamabad, Pakistan. Khan *et al* (2016) surveyed and determined the areas in Karak and Kohat of Pakistan having different potentials for the honey production by *Apis mellifera*. They recorded different locations like Terawal Banda, Hassan Banda, Darmalok etc. to have different potentials. Ahmed *et al* (2016) made a review on the pollen populations available in south Asian honeys to illustrate the flora ideal for the harvest by bees in that region. They identified 750 plant species as the sources consulting 124 research papers. Information has been supposed to succour well in the sustenance of apiculture industry in the region. Nessa (1980) worked on the pollen collection pattern of honey bees in Bangladesh. Hossain and Sharif (1988) provided a comprehensive report on the honey and pollen sources in northern Chittagong region of Bangladesh for the purpose of prospecting the potential in Bangladesh. Pasa *et al* (1991) carried out melissopalynological study of honey from Sundarbans in Bangladesh. Moar (1985) made pollen analysis of New Zealand honey. Lieux (1988) analyzed honey samples of USA from Mississippi on the basis of pollen, moisture and colour. Puusepp and Koff (2014) made pollen analysis of the honey from Baltic region. Barth's (2004) work presented a comprehensive account of pollens in honey, propolis and pollen load in Brazil. This paper reviews current knowledge on the occurrence of several types of pollen grains in

the sediments of honey samples, propolis and bee loads of Apiinae and Meliponinae in Brazil. After a short historical introduction about research activities in Melissopalynology using Brazilian samples, bee products were analyzed in respect to the greater Brazilian regions (South, Southeast, Northeast and North), emphasizing monofloral honeys and the green propolis. Numerous bibliographic references and a short glossary of the technical terms used is presented. Pioneer, traditional and standard studies in Melissopalynology (Louveaux *et al.* 1970; 1978) consolidated the development of reports on the occurrence of pollen grains in honeys, propolis and bee loads in Europe (France, Germany and Switzerland). The trophic resources for *Apis* in the state of Roraima were investigated by Silva (1998) and Silva and Absy (2000), and in the state of Rondônia, by Marques-Souza *et al.* (1993). Moar (1985) provided a 30-page comprehensive account of pollen taxa available in New Zealand honey. Gikungu (2009) listed around 200 plants as bee plants in Africa while presenting a comprehensive account on the resources for beekeeping and honeybee races of that continent. Morimoto (2009) recounted the traditional beekeeping practices in Kitui-Kamba district of eastern province of Kenya and identified many plants as the sources for harvest. Belay *et al.* (2014) examined samples of forest honey of Ethiopia with respect to their botanical origin using pollen and sensory analysis and the quantification of crystallization and colour. A total of 16 samples of honey were collected from two typical localities (Chiri and Wabero). The botanical origin of the samples was examined via qualitative pollen analysis by counting 500 pollen grains using a harmonised method. Granulation, colour, and sensory properties of the honey were determined by visual inspection. The samples were analysed for tetracycline. It is clear from the results that honey from the Wabero locality comes mainly from *Syzygium guineense*, whereas honey from the Chiri locality was of a multi-flower origin. The honey samples were amber in colour, no tetracycline residues were detected and the formation of granules was slow.

In Nigeria, Sowunmi (1976), Agwu and Akanbi (1985), Adeonipekun (1989), Ayodele *et al*. (2006), Njokuocha and Ekweozor (2007), Adekanmbi and Ogundipe (2009), Adeonipekun (2010, 2012), Ige and Modupe (2010), Aina and Owonibi (2011), Aina *et al*. (2015) are the major works on melissopalynology. These workers have supplied the available information about the botanical and geographical origins and studied the biochemistry of honey, as well as its quality determination across the country. Ebenezer and Olugbenga (2010) identified 20 honey samples from North-Central Nigeria as unifloral and multifloral ones and determined the dominant species. Adeonipekun (2012) performed palynological study from honeycomb as well as honey samples of apiary in Lagos of Southwest Nigeria. Honeycomb was chosen to refute the chances of allegation of adulteration. The findings of Agwu *et al* (2013) recorded the pollens of the species in the honey samples of Dekhina area under Kogi state of Nigeria to designate the source species as phytoecological indicators and recorded the predominant species as *Acanthus* spp., *Alchornea cordifolia*, *Anacardium occidentale*, *Cassia mimosoides*, *Elaeis guineensis*, *Hymenocardia acida*, *Phyllanthus niruri*, *Mangifera indica*, Tridaxprocumbens, and *Zea mays*. Ige and Obasanmi (2014) carried out palynological analysis of honey samples from Delta state of Nigeria. While carrying out palynological study with 25 honey samples they recorded 72 pollen morphotypes belonging to 28 families. Pollens from the members of Combretaceae/Melastomataceae, *Diospyros* sp., *Lennae* sp., Elaeisguineenses, Rubiaceae, *Syzigivm* sp. Myrtaceae, Sterculiaceae and *Hymenocardia acida* were found to dominate. The occurrence of these species affirmed the geographical source of honey as freshwater swamp forest vegetation of Niger delta of Nigeria. Adeonipekun *et al* (2016) made proximate and elemental study of honey from three regions of Nigeria. Estevinho *et al* (2012) carried out melissopalynological works along with physicochremical characterization of honey from Lima valley of Portugal and found 6 pollen types of Fabaceae and 2 pollen types of Rosaceae. Pollens of Erica were most preponderant, so that two samples were listed as *Ereica*

monofloral honey. Dobre *et al* (2013) carried out palynological analysis of Romanian honey samples. Yu-jia *et al* (2013) thorough work on comparative analysis of the performances of *Apis cerana ceraca* and *Apis mellifera ligustica* in respect of absolute pollen concentration, number of species the nectar being collected from, trophic niche breadth, *A. cerana cerana* was found to be superior and the competition between two species to be quite fierce due to common foraging species. A melissopalynological study of 54 honey samples collected during two consecutive harvest seasons from different parts of Romania, carried out by Dobre and co-workers (2013) registered 77 types of pollens from 35 botanical families. Pollens of *Brassica napus*, *Castanea sativa*, *Helianthus annuus* etc. were recorded as the major pollen species. Azmi *et al* (2015) worked on delving in foraging activities of stingless bees by studying melissopalynology from the honey samples from apiary of Besut in Terengganu. Pound *et al* (2018) could arrive at a consideration of *Brassica napus* as a vital species in supporting honey production in either of suburban or rural area, through melissopalynological studies of honey from Ponteland, UK. Delphine and Joseph (2015) on analysing pollens in honey from Sudano-Guinean zone of Cameroon identified 41 species of 25 families. Representing members were mostly from Asteraceae and Myrtaceae. Major species were *Nymphaea maculata, Terminalia avicennioides* and *Syzygium guineense*, of which the last one was present in all analyzed honey samples. Dongock Nguemo (2016) worked for determining the pollen composition in the honey collections of different seasons in the Soudano-guinean highland zone of Cameroon. Ozler (2015) determined all honey samples collected from 21 different localities of Sinop, Turkey as multifloral through melissopalynologiocal study. Major members of pollen species were *Castanea sativa* and of Fabaceae. Altogether 61 taxa from 19 families were identified. Silici and Gokceoglu (2007) carried out pollen analysis of 25 samples of honey from different localities of Anatolia in the Mediterranean. Eleven samples were detected as monofloral, 3 of which were of Apiaceae, 2 of *Pimpinella anisum*, 2 of *Raphanus raphanistrum* etc. Song and

co-workers (2012) examined pollens from natural honeys of Central Region of Shanxi, North China. Nineteen Chinese honeys were classified by botanical origin to determine their floral sources. A wide spectrum consisting of 61 pollen taxabelonging to 37 families was noted, of which fourteen samples were unifloral and the remaining samples as multifloral. Ceksteryte *et al* (2013) depicted pollen diversity in honey available in Lithuania's protected landscape. Melissopalynology helped in Botanical origin of honey was determined by the method. Monofloral lime honey was specific for the south (Dzūkija National Park) and east (Armona Geological Reserve) of Lithuania, where pollen of *Tilia cordata* Mill. made up79.0% and 53.9%, respectively. Monofloral caraway honey was found in the Salantai Regional Park close to Žemaitija National Park. The first investigations in Brazil were made by Santos (1961a; 1961b; 1963; 1964) on pollen grains of bee plants and honeys collected in the region of Piracicaba, SP, followed by studies of Barth (1969; 1970a; 1970b; 1970c; 1971a; 1971b; 1973; 1996) in different regions of the country. The position of Palynology in Brazil was presented during the First Latin American Congress of Botany by Barth (1972), including extensive references and all the data of Melissopalynology available at the time. In the state of São Paulo, Carvalho and Marchini (1999), Carvalho *et al*. (2001), Marchini *et al*. (2000) and Moreti *et al*. (2000; 2002) analyzed honeys of *Apis* and *Meliponinae*, as well as pollen loads and the corresponding flowering for bees. Barth (2004) recounted different types of pollen grains in the honey samples, propolis and pollen loads in Brazil. Sodre *et al* (2007) made pollen analysis of honey samples from two regions of north east Brazil. In studying with 58 samples, 38 samples from the State of Piaui and 20 from the State of Ceara they found pollens of the species characteristic for the two places. Sodre *et al* (2007) contributed the account of pollen populations available in the honey produced in two main honey producing areas northwest Brazil. The dominant species in Cearáwere noted to be of *Mimosa caesalpiniaefolia, M. verrucosa, Borreria verticillata, Serjania* sp. etc. and in Piauí *Piptadenia* sp., *M.*

caesalpiniaefolia, M. verrucosa, Croton urucurana etc. The review work is a trove of numerous bibliographic references as well as technical terms. Oliveira, along with co-workers (2010) did the similar job with the honey taken from Caatinga vegetation in Bahia of Brazil and a total of 73 types of pollen were recorded, which belonged to 30 families, 64 genera and 30 species. The major representing families in the total pollens collected were Mimosaceae, Caesalpiniaceae, Rubiaceae and Fabaceae. Predominant pollen types were: *Mimosa arenosa* in four samples. de Novais *et al* (2010) worked with bee pollen loads to deduce flowering nature in Catingaa region of Brazil during two years' span of study. Thirty-six plant families were found to contribute to the composition of the pollen spectrum of the samples, with 85 different pollen types. Fabaceae was the most represented family and *Mimosa filipes* was the single most frequently observed pollen type. Costa Dórea *et al* (2010) studied the pollen profile obtained from the southern coastal region of Bahia, Brazil. Out of thirtyfive bee-pollen types scrutinized, *Elaeis*, *Mimosa pudica* and *Cecropia* were the most prevalent among the samples. Da Luz *et al* (2010) made study on the comparative pollen preferences by *Apis mellifera* in Pará de Minas, Minas Gerais of Brazil. Totally 56 pollen species, belonging to 43 genera and 32 families were observed. Major richness of pollen types was of the families Mimosaceae, Asteraceae, Fabaceae and Arecaceae. In the same line of work de Novais *et al* (2010) studied bee pollen loads availed at Caatinga region of Brazil to enumerate pollen types available in them. Eightyfive different species of pollens could be obtained from 62 bee pollen samples. Members of Fabaceae were noticed to be most dominating. D'Apolito and co-workers (2010) studied pollen harvest by mellifera bees in the Duorados area of Mato Grosso do Sul state, Brazil. The most significant representation was of the families namely, Myrtaceae, Asteraceae, Euphorbiaceae, Brassicaceae and Poaceae. Among the genera or species showing majority were of *Eucalyptus* (19%) followed by *Raphanus raphanistrum* (13%) and others. Boff *et al* (2011) could identify plants species foraged by Africanized honeybees in southern Pantanal

Brazil, based on their melissopalynological studies. Altogether 28 species of 15 plant families were designated as potential sources of pollen for *A. mellifera*, and out of them 24 species of 13 families were registered to have made direct visit. Pinto da Luz and Barth (2012) analyzed pollens from honey and beebread obtained from mangroves of Brazil. *Laguncularia racemose* dominated there. It was identified both as a polliniferous and nectariferous species. de Freitas *et al* (2015) also made a melissopalynological study on the pollen loads of mellifera bees in southern Brazilian macro- region. de Jesus *et al* (2015) made thorough study of pollens in the light honeys from Piaui state of Brazil. A total of 151 pollen types were identified in samples of light honeys, representing 41 botanical families, three of which are noteworthy: Euphorbiaceae, Fabaceae and Rubiaceae, especially the genera Mimosa and Pityrocarpa, contribute greatly to the production of light honeys in Piaui State, Brazil. Omran and his co-authors enumerated (2017) the major pollen sources for honeybees in desert area of Toshka, Egypt. Eighteen plant sources belonging to 11 families were noted and amongst them family Cucurbitaceae represented by four species was most prepondering. Abou-Shaara (2015) in the publication presented a long list of potential bee-plants available in Egypt and knowledge in that respect has been considered to be useful to bee keepers. Herrero *et al* (2002) attempted characterization of honeys by melissopalynology and statistical analysis. They analyzed pollen from 89 honey samples, collected in León and Palenciaprovinces of NW Spain. According to their pollen spectra, 46 were considered monofloral. The most abundant monofloral honeys were *Erica* types followed by *Castanea*, *Centaurea*, *Reseda* and *Helianthus*. One hundred and forty-two different pollen types were recorded, belonging to 47 families. Sekine *et al* (2013) recorded melliferous flora by characterizing pollens from honey samples procured from apiaries of Ubiratã and Nova Aurora, PR. A very rich flora with 208 species of 66 families was identified in having potential of being foraged. Major pollen species hailed from Asteraceae, Myrtaceae and Solanaceae. Carpes along with co-workers (2009) characterized the pollens

chosen by *Apis mellifera* in consideration of the nutraceutical significance of pollens. Thirtysix bee pollen samples collected from southern Brazil were analyzed for the purpose. Statistical analyses refuted any difference in the elementary constituents of pollen species in the studied area. Novais and coworkers (2009) analyzed pollens harvested by mellifera bees and the influence of climatic factors on pollen samples. They focused on 46 pollen species recorded in their study and the family Leguminosae of 10 pollen types was noted to be the most dominating group. Some species like, *Diodia radula*, *Rhaphiodon echinus*, and *Mimosa misera* were found to occur most consistently in the collections. Dórea *et al* (2010) worked out botanical profile of bee pollens collected by *Apis mellifera*. The list of polliniferous plant species from the Atlantic Forest biome is considered to provide information supporting development of apiary in the region. Species like *Elaeis, Mimosa pudica, Cecropia* were the most abundant in the collected honey. Sniderman *et al* (2018) provided a comprehensive account on the pollen analysis of honey procured from Australia. Amongst the studied 173 unblended honey samples the most dominating member was found to be characteristically of *Eucalyptus*, as well as other member *Corymbia* of the family Myrtaceae. Bryant expressed that beekeepers are mostly ignorant about the floral sources of honey and the products carry wrong labels. He also pointed out that more than 60% of field identification of pollens are incorrect and that may happen due to any one of a number of reasons, for example, the honey bees swarm around blooming flowers of many species or, species growing nearby the hive and blooming does not necessarily ensures major source of nectar, moreover, bees can eliminate some of the ingested pollens selectively before returning hive and difference is also created as all bees are not equally efficient in removing pollens. Removal of pollens is also dependent on the size and shapes of pollens and larger pollens are removed more efficiently. Such removal of pollen was also attested by the experiment of Todd and Vansell (1943). They found only 1/30th of the total ingested pollen to

remain in the honey stored in hive. Such an incident, most obviously, has an impact on the accuracy of the information obtained from melissopalynological studies.

2.3. MELISSOPALYNOLOGICAL WORKS FOR VARIOUS PURPOSES

Jones and Jones (2001) made a vivid attempt in analyzing the implications of pollens on entomology. Not only for identifying the nectariferous or polliniferous plants but also to have a knowledge about the plants involved in the formation of propolis the usefulness of melissopalynological study has been considered with due importance in the works of Santos (2011) and Siva *et al* (2013). A very early attempt of microscopic analysis of propolis was done by Warakomska and Maciejewicz (1992). Arvanitoyannis *et al* (2005) made approach on novel quality control for the purpose of detecting honey authenticity in respect of chemo-metrics. Atanassova and Kondova (2004) scrutinized pollen and chemical – physical characteristics of Bulgerian unifloral honey. Honey, propolis and pollen loads of bees of Brazil have been analyzed with pollen analysis by Barth (2004). Baum *et al* (2011) determined the diurnal patterns of feral honey bees in southern Texas. Devillers *et al* (2004) attempted to classify monofloral honey based on specific data. Dimou and Thrasyvoulou (2009) reported on the analysis of pollen, obtained from the rectum of bees to get an idea about the bee flora.

2.4. COUNTING METHODS & STATISTICS IN USE

Simple counting of the populations of pollen taxa in honey, often, does not provide an actual representation of the fact. Statistics, in that situation, succour well. Statistical analyses were carried out to characterize monofloral honeys (Battesti and Goeury, 1992; Aira *et al*. 1998; Seijo and Jato, 1998). Davis and Faegri (1967) translated the work of Lennart von Post (1916) where Post expressed that relative pollen count did not give precise information regarding the vegetation they represented. In accordance with such perception Davis (1963) proposed the concept of ‘R-value’. Revelation of such fact led Bryant and Jones (2001) considering pollen

coefficient in the context of R values of honey. Bryant (2013) showed that even the presence of 75% pollen of rapeseed in honey does not make it unifloral, as it does not provide the real picture. So, in contrast with the conventional consideration of the presence of more than 45% of pollen of any species to designate any honey as unifloral for that species they solicited for the R value in terms of pollen coefficient. Corbella and Cozzolino (2008) considered multivariate analysis along with pollen count for the purpose of classifying pollens of different botanical origins. Aronne and De Micco (2010) worked on the traditional melissopalynology in conjunction with multivariate analysis and sampling method for the improvement of honey in respect of botanical and geographical identity. Principal component analysis (PCA) and linear discriminant analysis (LDA) combined with pollen identification proved useful in characterizing honey samples from different botanical origins.

2.5. DNA INFORMATION AS AN AID IN MELISSOPALYNOLOGY

DNA barcoding is a sophisticated method for precise identification of any organism. In recent years DNA analysis of pollen from honey has also been launched with a purpose of authenticating its botanical and geographical origin and to identify any attempt of adulteration. Valentini *et al* (2010) applied DNA barcoding for determining honey diversity. Only a few methods of extracting DNA from honey are available, and they are time-consuming and laborious. Guertler *et al*. (2014) developed an automated method of extracting DNA from pollen in honey using the Maxwell 16 and Maxwell 16 FFS instruments. The optimized method included modifications of several parameters. The automated extraction was faster and the DNA purity and yield were higher. The results from real-time PCR using DNA extracted by the automated method are comparable to those obtained using manually extracted DNA. No inhibition of PCR was detected. The utility of this method was verified on several different common honey samples. Hawkins *et al* (2015), too, used DNA metabarcoding for floral identification of honey. They used both of melissopalynology as well as DNA metabarcoding

with rbcL DNA barcode marker and 454-pyrosequencing. Metabarcoding showed more accuracy over the only use of palynology. Similar works with ITS2 metabarcoding was done by Richardson et al (2015). Bell *et al* (2016) reviewed the pollen DNA metabarcoding for the purpose of more accuracy in identifying the plant source of honey products. Prosser and Hebert (2017) proposed DNA metabarcoding as the rapid identification of plant and entomological sources of honey. They assured that DNA metabarcoding of three genes, ITS2, rbcLa, and COI provided information on the plant sources and entomological origins of honey. However, they identified that the honey samples with polyphenols or that subjected to crystallization remain unresponsive.

2.6. PROTEINS AND OTHER CHEMICAL ANALYSES

Collection purely of pollens, only of some chosen plants by honey bees on their corbicules as pollen loads certainly holds significance. Howells, (1969) pointed out that without the proteins the honeybee cannot develop and grow. Pollen is considered as the only source of protein for honey bees. It also helps brood rearing and glandular development of the younger bees (Ohe *et al*, 2004). Besides the protein constituents, many other elements from pollen have been studied by different authors for having a thorough understanding about the status of nutritional supplements for bees. Winston, (1987) rightly identified pollen as the main source of amino acids, lipids, vitamins, minerals and sterols act as food supplement in the diet of honey bee. The agricultural development has been claimed to cause displacement of such bee-foraged plants, leading to the malnutrition of bees and ultimate decline in honey production by many researchers (Naug, 2009; van Engelsdorp and Meixner, 2010; Huang, 2012). The chemical composition of pollen obtained from different kinds of flowers varies enormously. Analysis of pollen from thirtytwo different plant species carried out by Todd and Bretherick (1942) revealed that pollen contained 21% proteins, 11% water, 30% carbohydrates, about 5% fats, oils and waxes and mineral elements such as potassium, phosphorous, calcium, magnesium

and iron were also shown to be present. The presence of other constituents such as amino acids, organic acids, sterols, nucleic acids and pigments, and those listed above have been reviewed in detail by Stanley and Linskens (1974).

Crane *et al* (1984) listed the colour, grain, yield and chemical composition of pollens for 467 plants, which are known as major sources of honey produced in the world. Nielsen *et al* (1955) reported the presence of a-aminobutyric acid and hydroxyproline in *Zea mays* pollen. Virtanen and Kari (1955) noted the presence of hydroxyproline and pipecolic acid in the pollen of six wind pollinated plants. Britikov and Musatova (1964) reported an extraordinarily high amount of proline in the pollen from sixtyfour plant families they examined. Shellard and Jolliffe (1968) could find no difference in the amino acid content of eleven grass pollens which they examined. Gilliam *et al* (1980) showed aspartic acid and glutamic acid to be predominant amino acids in the pollens from citrus cultivar flowers. Most interestingly they also noted the stored pollens to have higher amounts of proline than the pollens taken right from flowers.

Kauffeld (1980) recorded nineteen different amino acids, namely, a- and 0-alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine, in the pollens collected by *Apis mellifera*. Moreover, he noted the amino acids glycine, lysine, phenylalanine and proline to show considerable variation throughout a year. Ceausescu and-Mosarie (1981) identified ten amino acids by the technique of paper chromatography in the monofloral pollens collected by *Apis mellifera carpatica*. These research workers reported that Compositae pollen contained alanine, aminobutyric acid, arginine, glutamic acid, hydroxyproline, lysine, methionine, norvaline, proline and serine; Ranunclaceae pollen contained alanine, aminobutyric acid, glutamine, glycine, hydroxyproline, leucine, lysine, phenylalanine, proline and valine; and Jaglandaceae pollen contained arginine, cysteine, hydroxyproline, leucine, methionine, proline, serine, threonine, tryptophan and tyrosine. Zhu

and Jiang (1982) observed that the free amino acid content of pollen showed species variation, in that, pollen of *Pinus elliottii* contained arginine and valine, whereas, pollen of *Ginkgo biloba* contained serine and tyrosine. Nadezhdin *et al* (1983) reported that the free amino acid constituted approximately 43% of the total pollen amino acid. These researchers noted that the Siberian larch pollen contained more free amino acids than the pollen of the other conifers. Baruch and Sharma (1984) observed wide variation in the number and type of amino acids in the pollen from nineteen plants they examined.

Naumkin (1984) showed the presence of alanine, arginine, asparagine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine methionine, phenylalanine, proline, serine, threonine, tyrosine and valine in the pollen of buckwheat (*Fagopyrum* sp.), raddish (*Raphanus sativus*), cornflower (*Centaura* sp.), red clover (*Trifolium pratense*), sow-thistle (*Sonchus* sp.), codlins-and-cream (*Epilobium hirsutum*), burdock (*Archium* sp.) and musk-thistle (*Carduus* sp.). Raddish pollen was reported to be the richest in amino acid content, whereas the buckwheat and musk-thistle to be the poorest. Wang *et al* (1985) found that the free amino acid content of linden pollen was high compared to that of rape pollen. Feo *et al* (1985) reported that proline was found in large amounts in both the pollens of *Cunninghamia Zanceo Zata* and *Cephalotaxus drupacea* and ý-alanine was found in trace amounts in the former pollen.

Rayner and Langridge (1985) examined the amino acid content of pollen, collected by bees, from 10 indigenous and 16 Australian plants. They realized that the amino acid contents of pollen from all plant sources, recorded by them, were quite higher than the bees' requirement. However, low concentration of tryptophan was found to be rate limiting for the honeybees. Katgaye and Kalkar (2015) reported on the pollen analysis and protein estimation of pollen loads. Depending on the species they registered considerable variation in the protein content of pollen load. Bifloral load of *Careya arborea* and *Butea monosperma* yielded greatest amount of protein.

Jantakee and Tragoolpua, (2015) mentioned about the general emphasis laid on the nectar of foraged plants; meanwhile, Stanley and Linskens, (1974) drew attention to the fact that pollen grains also contain a good number of minerals, vitamins and proteins, as well as lipids. These make them excellent for bees' diet. De Groot (1953) observed that addition of pollen to bees' diet greatly increased their longevity, while Kropacova et al. (1968) observed that the development of the ovaries of bees was favoured by pollen in their diets, as cited by Stanley and Linskens (1974). Taylor (1974) reported that bees feed their young ones and the larval queen with pollen in the form of ordinary jelly, while royal jelly – a secretion from the pharyngeal and mandibular glands of worker bees is fed to the queen. The adult bees use pollen directly as food to get essential elements, proteins and lipids.

2.7. AUTHENTICITY OF HONEY SOURCE

The progressive increase in the market of imported honey, with lower prices and inferior quality, has recently led to a growing need to assess the authenticity of local, specially monofloral honeys (Andrade *et al*., 1999; Azeredo *et al*., 2003; Pires *et al*., 2009; Silva *et al*., 2009; Gomes *et al*., 2010; Feás *et al*., 2010a). However, the full characterization of honey is not abundant and there is a lack of information about the characteristics of honey certified as organic (Estevinho *et al*., 2012). Even though the beekeepers themselves declared honey as monofloral lavender, all the samples were subjected to pollen analysis as per the acetolysis method of Erdtman and reported previously in detail (Feás *et al*., 2010a).

Monofloral status generally refers to the presence of a single pollen type in quantities greater than 45% of the total pollen content in the spectrum. For honey samples having under-represented pollen grains, botanical classification may be achieved with a minor pollen frequency percentage, as for example, lavender honey needs 15% of *Lavandula* sp. pollen to

be monofloral. The analysed samples had always *Lavandula* sp. (16–45%) and can be classified as monofloral lavender.

Estevinho (2013) worked out the organic *Lavandula stoechas* honey from Portugal in respect of palynological, physicochemical, and microbiological attributes. Drawing correlation between palynological, physicochemical and microbiological characters is necessary for establishing the authenticity, quality and sanitation of honey. Castro-Vázquez *et al*. (2014) analysed the indicators useful for identification of the botanical origin of lavender honey. For the confirmation of lavender honey in addition to verifying the samples chemically and sensorically, pollen analysis was undertaken. Twentysix taxa of pollens, 14 physico-chemical properties, 13 sensory properties, and 80 volatile substances were recorded in the studied lavender honey. The results identified lavender honey as a single-origin honey with respect to its botanical origin, on the basis of the analysis of a sufficient number of substances to enable such identification.