

## 1. INTRODUCTION

Honey is a unique product of the Nature. Its demand amongst human folk has remained consistently persistent since a time immemorial. This natural product has remained matchless till now, due to the complete absence of any synthetic product comparable to it. It is produced by honey bees as well as some other insects (Crane, 1990). It is the regurgitated floral nectar of honey bee. On account of diversity in nectar sources along with the diversity in the preferences of bee species for different plant species, countless varieties of honey are available worldwide. Bees store the regurgitated product in multi-chambered hive, the colonial abode of bees, after collection of nectar. Collected honey is kept by bees in wax made honeycomb (Crane et al, 1984; Crane, 1990). Honey has a long shelf life as it is not easily fermented (National Honey Board, 2010). Though uses of honey is mostly referred with its medicinal attributes, the product has much wider implications on being a food adjunct for the preparation of a variety of food-stuff as well as being used for various purposes in different religious and cultural practices (<https://en.wikipedia.org/wiki/Honey>). Any record of the earliest practice of collection and use of honey is lacking. However, the discovery of a cave painting by human being in Valencia, Spain, on the collection of honey by people, of a date almost of 6000 B.C. (Hunt and Atwater, 1915, Crane, 1983) proves the very early association of this natural product with human civilization. Collection of honey from the natural sources like the hives constructed by the wild bees on trees, rocks and even on the masonry structures in urban areas has always remained uncertain and an arduous job. So, with time people have learnt and devised the method for taming some species of bees and somehow captivating them in artificially constructed bee hives to have a constant supply of honey with minimum effort. This artificial method of bee keeping is named 'apiary' and widespread worldwide due to its simplicity, efficacy and easy accessibility. Apiary has had a long course of journey since a very early time and till now it serves as a means of economic sustenance for many people throughout the world. In the present time the artificial

hives are constructed with wooden box. There was a practice of beekeeping in the ancient Egypt, even prior to 2422 BCE, by constructing hives with moulded mud Kritsky (2017). In Latin the word 'apis' stands for bees and so the word apiary was derived from that ([www.merriam-webster.com](http://www.merriam-webster.com)) and its first practice is claimed to be in 1654 (Kritsky, 2017). It has been noticed to be a major livelihood of many economically marginalized families and communities of people. The success in establishment of a hive, the volume of honey collection and the quality thereof, in every case, are solely dependent on the vegetation in the surroundings in an area the culture is maintained. Plants are the main sources of both of nectar for preparing honey and the pollens, used as food by the honey bees. While in nature honey bees make hives of different sizes in inaccessible areas causing the collection of honey a harder job, taming and rearing honey bees in artificial hives ensures the collection of honey an easier job.

In this regard the European honey bee *Apis mellifera* has been proved to be the most efficient one for making apiary viable worldwide. However, this species is having at least 20 subspecies ([http://entnemdept.ufl.edu/creatures/MISC/BEES/euro\\_honey\\_bee.htm](http://entnemdept.ufl.edu/creatures/MISC/BEES/euro_honey_bee.htm)), made by intermating with others, in different countries for the purpose of increasing the yield and other attributes.

Cushman (1998) highlighted the reasons for the wider acceptance of *A. mellifera* as, low temperature flying, thrifty uses of the stored honey and pollen, cessation of brood rearing during the time of scarcity, early cessation of brood rearing in late summer and good wintering characteristics. He denied the undeserved notion about *Apis mellifera* for having bad temper or virulence and also put support for the selection of pure bred of it for docility an easy affair. Nineteen members team led by Sceiner (2013) presented a comprehensive review on standard methods for behavioural studies of this species. Sharma and Kumar (2010) have experimentally shown the changing behavior and biology of this species under the influence of cellophane radiation, which is often considered as a menace in influencing life processes of different

organisms. Abou-Shaara (2014) gave an illustrative account on the foraging behaviour of *A. mellifera*.

Melissopalynological analyses provide the most useful information to get an insight about the foraging behaviour of bees. The use of palynology in microscopical analysis of honey was propounded by Pfister (1895). Zander's contribution through his long time involvement in the field help construct the scientific basis of analytical technique of melissopalynology. International Commission for Bee Botany (ICBB) elaborated and proposed a method of melissopalynology (Louveaux et al, 1978). The work of Ohe et al (2004) provided a comprehensive account on the basic facts of melissopalynology and methodology of it, also corroborating with other physicochemical analyses. Significance of such work in identifying and establishing the geographical and more particularly the botanical origin of honey was also highlighted in the paper. Moreover, they rightly pointed out the essentiality of the correctness in identification of pollens and in drawing interpretation of the findings. Melissopalynology enables understanding of some basics about honey extraction, filtration and fermentation (Russmann, 1998). It also provides information on adulteration (Kerkvliet et al, 1995) and also about some contaminations like mineral dust, soot, starch grain etc (Louveaux et al, 1978). Behm et al (1996) emphasized that at least 300 pollens are to be considered in any count to decide on the relative frequency of the pollen types, while more than 500 pollens to determine the relative frequency of species in the sample. Reference pollens, worked out from the flowers of known plants as well as photographic atlas, have always been claimed to be most helpful in identifying the pollens (Maurizio and Louveaux, 1965; Sawyer, 1988; Ricciardelli d'Albore, 1997, 1998). ICBB method has been widely used in European laboratories and has been considered by Persano Oddo and Piro, (2004), though many other workers (Lutier and Vaissière, 1993; Jones and Bryant, 2001a, b) solicit for superiority of recently developed methods. Dinkov, (2015) propounded a new method of quantitative estimation of pollen grains in honey to serve the melissopalynological studies in a better way. Burker

chamber, which is generally used for white blood cell counting, was used in this study for the purpose. The author presented in this paper the account of the development of the method and its algorithm. He devised an equation to enumerate pollen grain after studying with the aid of Burker chamber and microscope. He also presented density of pollen studied from honey samples of different origins and their multifloral or unifloral nature, even mentioning the source plants in unifloral ones. The author showed the points in favour of his proposed method by highlighting the reliability of it, proved in the light of relative standard deviation (RSD) or coefficient of variation (CV), and also due to the merit of requiring minimum instrumental facility and least time in carrying out the study. Low et al, (1989) emphasized the need of proper centrifugation for collecting complete pollen population from a honey sample and acetolysis of pollens as a mandatory measure to get rid of any ambiguity in pollen identity. Tamic et al (2016) devised a method for rapidly counting pollens from harvested honey samples to quickly arrive at a conclusion about the plant source of the collected honey.

Understanding the immense significance of melissopalynology countless investigations in the same line have been carried out throughout the world by innumerable workers. Though in India people are still less concerned about the source of honey, quality thereof and toxicity factor, western world has always remained much concerned about these factors since quite an earlier period. In many countries abroad every honey product is provided with a declaration of the geographical origin of that, like many other agricultural products. The work of Maurizio (1951) provides a comprehensive account of the earliest works on melissopalynology in different countries, which shows intense works during first half of twentieth century in the western countries like Denmark, Germany, Finland, Italy, Sweden etc. The importance of honeydew from green algae, spores of sooty moulds, living organisms like yeast and impurities from airborne materials like soot particle, insect hairs, chitin fragments, starch grains, fibres etc. in determining the geographical origin of honey has been emphasized. Sajwani et al, (2007) examined 48 honey

samples during three consecutive years throughout the year from 14 locations of Oman, of which 32 samples were unifloral and 16 were multifloral. Altogether 122 pollen types belonging to 50 families were identified. Different regimes of plants are represented through pollens in winter and summer. *Ziziphus spina-christi*, *Prosopis juliflora*, *Prosopis cineraria* were noted to be major species during winter and during summer the species were *Acacia tortilis*, *Citrus* sp., *Maerua crassifolia*, *Phoenix dactylifera*. Using the melissopalynological information 89 samples collected from 5 geographical zones were classified into four classes on the basis of pollen availability. Vegetations of two classes were noted to be basophilous and other two acidophilous. Cluster analysis was used to discriminate the classes of honey based on botanical and geographical origins (Herrero, 2001). Dongock (2016) recorded the occurrence of altogether 52 melliferous plants in Chad. These species belonged to 16 families of which Mimosaceae being the abundant one and Tiliaceae being the least in number. Amongst them majority was tree and polliniferous plants were noted to be quite more than nectariferous plants. Nascimento (2015) carried out melissopalynological analyses in Brazil. Therefrom 112 pollen species were identified. Honeys were noted to be multifloral. Plants under Mimosaceae were noted to be predominantly foraged species. Sajwani et al (2014) studied on 249 pollen pellets from 22 honeycombs of *Apis florea* and *A. mellifera* in the locations of Muscat and Al Batinah. They designated individual loads as unifloral, bifloral and multifloral ones and also distinguished individual plants as nectariferous or polliniferous or the source of both of pollen and nectar. Their study revealed that amongst of 94 pollen types worked out, 67 types were from 39 families and interestingly all of them were obtained from both of honey and pollen loads. Twenty pollen types being available only from honey, source plants of them seem to serve as the contributors. Major foraged species of the locality were identified.

Likewise, many researchers contributed immensely, so far, to the field of melissopalynological works in India and many workers are still continuing it. Chakraborti and Bhattacharya (2015) recounted a comprehensive review of works in India. Deodikar and Thakar, (1953) made a pioneering approach in India in this field. Afterwards Chanda and Ganguly (1981) gave an effort in analyzing pollens in honey samples collected from different states of India, including West Bengal. A comparative analysis between the pollen constituents in honey samples from Balurghat and Jalpaiguri was carried out by Ganguly et al (1984). Many other workers contributed during that later part of twentieth century were Vishnu-Mittre (1958), Nair (1964), Sharma and Nair, (1965); Chaturbedi, (1973, 1983, 1989); Chaubal and Deodikar,(1965); Deodikar,(1965); Suryanarayan *et al.*, (1981); 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); 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. Garg (1996) worked with the samples from Kumaon Himalaya, Bera *et al* (1997) and Jana (2002) worked on different parts of West Bengal. Mukhopadhyay et al (2003) analyzed samples from sub-Himalayan West Bengal. Fatima and Ramanujam (1989) gave report on two multifloral samples from Hyderabad. Bhargav *et al.*, (2009) presented an account of 16 pollen species from the study of 10 samples from Western Ghats. Pal and Karmakar (2013) worked on foraging behaviour of *Apis mellifera* in Gangetic West Bengal. In 2018, Layek and Karmakar worked out palynological investigation of *Apis dorsata* honeys from Bankura District, West Bengal. These examples are only a very fragmentary picture of the entire works on melissopalynology in India.

Even being a very rich place of plant diversity and amicable environment apiary is not practiced in large scale West Bengal; however, the state holds much potential in this regard. Still then whatsoever the number of apiary is working in this state they use *Apis mellifera* for the purpose. Success of apiary with *Apis mellifera* in this state proves that the species must have been accustomed with the physical environment of this place and also in regard of the foraged plants to harvest nectar and pollen. An enumeration of the plants species foraged by this bee, in an area, provides an insight to the foraging behavior of it, which is also essential for the bee to thrive in the locality. After a cursory survey a couple of families were found to be engaged in apiary in some parts of North 24 Paraganas district of West Bengal. In consideration of proximity of the place with respect to the laboratory at university and other conveniences this location was chosen for the study. Apiaries at different sites of this area were accessed through a key person having association with it for years and bearing profound knowledge about the practice, as well as having contact with many people engaged in the same business.

In addition to the most glaring contribution of producing honey, *Apis mellifera* is considered as one of the most important pollinators of agricultural species, in particular, and absence of that may lead to 90% decline in the yield of some fruits, seeds and nut-crops (Klein et al., 2007). While wild populations of *A. mellifera* are scarce for the success of pollination of such agricultural crops availability of tamed folks of this bee species in the vicinity of the land under cultivation of different agricultural species seems to be essential (Aizen and Harder, 2009).

However, spraying or other means of application of pesticide on agricultural plants mostly pose serious threat to the devastation of honey bee colony. Neonicotinoid class of compounds are often held responsible for such loss (Sánchez-Bayo, 2014; Goulson et al., 2015; Pisa et al., 2015).

### **1.1. OBJECTIVE OF THE STUDY**

1. To scrutinize the forage behaviour of *Apis mellifera* in the district of North 24 Paraganas of West Bengal.
2. To record the species from which specifically pollens are being harvested and which other species are being visited for nectar.
3. To register the change of visited plant species with the change of seasons.
4. To characterize morphologically the pollens obtained from *Apis mellifera*.
5. To review whether the foraged species are otherwise important to humankind or not. Basically the agri-horticultural species, if foraged, are also in turn benefited with better yield, due to a better pollination.