Introduction
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Melittopalynology deals with the qualitative and quantitative analysis of palynoflora of honeys. Pollen analysis of honey samples provides information regarding plants visited by honey bees for nectar collection. Analysis of the pollen loads helps us to evaluate the pollen sources to the bees. Nectar is converted into honey by bees. Honey, a sugar syrup provides energy to the bees. Pollen load containing pollen, which provides proteins to the developing brood, is stored in some chambers of the hive.

Bees visit the plants, which are having highly sugar content nectar and highly protein content pollen. Flowering period of plants, foraging range of the bees, population of the bees in a hive and storage capacity, etc., play a vital role in honey production.

We have to know some general aspects of bees, bee hive, bee hive products with particular reference to the physical and chemical properties of honeys. Hence here the following information is given.

Honey bees, bee hives, bee hive Products

Honey bee live in colonies, and show social organisation. We can see division of labour and collective discipline among bees. Defense mechanism and inter communication system is also seen in bees.
Systematic position of honey bee

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<th>Phylum</th>
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<td>Class</td>
<td>Hexapoda or Insecta</td>
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<td>Order</td>
<td>Hymenoptera</td>
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The genus *Apis* consists of four species of honey bees namely *A. dorsata*, *A. cerana*, *A. florea*, and *A. mellifera*. The *Trigona* small stingless bee, belongs to the family Meliponidae. Except the European bee, *A. mellifera* all other types mentioned above are native to India.

*Apis dorsata*

This is known as gaint bee or rock bee. It is highly ferocious among four *Apis* species. It occurs in warmer regions i.e., Asia, Phillipines, Sumatra, Java and other Asiatic islands. It is not found out side Asia (FAO, Agricultural series bulletin No. 68, 1986). The gaint bee is a highly migrated one. Rock bee constructs a single huge comb upto 2.7 m high and 1.2 m width attached to protruding parts of rock, higher branches of tall trees, walls of high raised buildings and caves. These large exposed honey combs are semi-circular to U-shape. Due to ferocious nature of these bees all attempts to domesticate these bees have not been successful so far. They gathered honey from wide foraging range. *A. dorsata*
can fly up to 3 km long to the hive (Lindauer, 1957; Punchihewa et al., 1985). *A. dorsata* comb measures about 4 cms in thickness and 1 to 1.5 meters in diameter. The bee hunters drive off the bees from their combs particularly in the forest regions and the honey is generally collected by squeezing at the end of honey flow season. On an average a single comb of *Apis dorsata* yields more than 20 kg of honey and 2 kg of bee wax (Venkata Rao, 1946, Muttoo, 1947; Thakar and Tonapi, 1961). Over 60% of the total honey harvested in India is derived from *A. dorsata*. Therefore its contribution to honey production is very important.

Natural Hive (Comb) or colony nest

The honey bee colony consisting of a single comb as in *A. florea* and *A. dorsata* or a series of parallel combs as in *A. cerana indica* and *A. mellifera*. Worker bees secrete wax and build honey comb. The hive is heavily built at the point of attachment while its bottom hangs free. Each comb is composed of a double layer of hexagonal cells projecting in both directions from a central wax sheet. The cells open at their outer ends.

The honey comb is having three distinct zones. The upper zone is attached to the support and utilized for the storage of honey. Honey storing cells are three times deeper than brood cells and usually hexagonal to irregular in shape. Each cell filled with ripe honey is sealed with wax capping. The major portion of middle zone is the brood. Above the brood area and just below the honey storing portion, a small portion constitutes pollen storage region.
The lower part of the comb is used for rearing the drone brood and also for building the queen cell along the extreme periphery of the comb. The area where brood is present is compact, well defined and distinct in *A. florea* than *A. dorsata* hives, where the differentiation is rudimentary. The Drone brood area in *A. cerana indica* and *A. mellifera* hives are though compact. Often forms random clusters anywhere on their surface (Thakar and Tonapi, 1961, 1962).

The comb is having three types of cells, queen, drone and worker cells. The size of cells varies for drone brood and worker brood. The major portion of the comb is occupied by the workers cells. These are hexagonal and more or less uniform in thickness. When compare to worker bee cells drone cells are larger in size. The drone cells in *A. dorsata* are randomly scattered and differentiated in height alone. In *A. florea, A. mellifera* the drone cells are distinguishable in both height and cross-section (Thakar and Tonapi, 1961). The queen cells are built at the lower border of the comb as pendent projections. Queen cells are the largest and in contrast to the horizontal alignment of the worker and drone cells, always hang downward. They are broader at the base and slightly taper at the free end. The number of queen cells varies from 2-10 in *A. florea* and 4-6 in *A. dorsata* (Thakar and Tonapi, 1961, 1962).

In a bee colony three types of bees are present. A single queen bee (the only fertile female) for the production of eggs, a few hundreds of drones (The fertile males). Their function is to mate with queen. The workers are thousands (20,000-30,000) in number (Sterile females) they are responsible for all the labour necessary for the maintenance of the colony.
Queen: The queens develop from fertilized eggs and their development takes place in queen cells. The queen is the largest among three types of bees. It is distinguished by long, tapering abdomen. Mating takes place only once during her life span of about five years. The queen collects sperms from male bee and stores them in her spermathecal sac for fertilizing the eggs, that will, depending upon the needs of her colony. Queen bee emits a chemical substance known as 'pheromone' or queen substance (with two substances a oxydecenoic acid and 9 hydroxydecenoic acid). Pheromone controls the multifarious activities of the bees in a colony. Queens are reared only under 3 conditions i.e., queenlessness supersedure and swarming.

Drones: They develop from unfertilized eggs in the large hexagonal cells. They are stingless males. Their task is restricted to mating with queen bee. They are larger and stouter than worker bees. The sole main function of drone is to copulate with queen and die soon after.

Worker bees: They develop from fertilized eggs laid by the queen in the smaller hexagonal cells. They are numerous in number. They possess special organs to do all the works of the colony viz., body covered with much branched hairs, long proboscis, pollen basket on the hind legs, salivary glands, 4 pairs of wax glands, hypopharyngeal glands and a well developed sting. On the basis of their function worker bees are divided into different groups viz. Nurse bees, Enraging bees, Scout bees and Guard bees.

The life span of the worker is very short (35 to 40 days). During the first half of their life time, they involve in indoor works such as secretion of royal jelly, catering to the needs of the queen, drones and brood, secreting bee's wax,
packing of pollen, ripening and storing of honey, cleaning, ventilating and guarding the hive. The second half of their life is spent in outdoor work involving collecting nectar, pollen, water and propolis.

When a scout bee discovers a nectar or pollen source, she comes back to the hive and communicates to the other worker bees by performing a particular type of dance. (i.e., tail wagging dance or round dance) according to the direction and distance of the source (Frisch, 1954). On the basis of information obtained, the foraging bees fix their preference to a particular plant species. The instinctive fixing of bees in favour of a particular floral species, preferred for a time being, is called "floral fidelity" which makes them the most efficient pollinators as compared to other insects (Deodikar and Suryanarayana, 1977).

Bee hive: By nature bees are wild. *A. dorsata* is a very ferocious bee and are not useful to tame, but *A. cerana* and *A. mellifera* are easily domesticated in manmade hives. On the basis of this specific character they are treated as important bees in apiculture. They are also called as "hive bees" *A. cerana* the Indian hive bee and *A. mellifera*, the European hive bee respectively are kept in wooden apiary boxes for production of honey and crop pollination.

Bee forage

Bees forage includes nectar and pollen. Nectar secreted by flower is the basic raw material by which bees make honey. Pollen is essential for the bees to the maintenance and establishment of the colony of the bees as they rich in proteins.
Pollen having rich in protein substances, amino acids, hormones and vitamins, is an essential food for the development of brood. Pollen grains collected by the bees mix with nectar and packed into corbiculae or pollen baskets present at the base of hind legs. The pollen loads or pollen pellets are then deposited into the cells of the comb one above the other in a superimposed manner. Water is required for bees for the elaboration of larval food. Nectar is a sweet substance secreted from the flower or extra floral glands of plants. It is made up of mainly sugars, water and small amount of nitrogen compounds, enzymes, minerals aromatic substances, pigments etc.

Honey bees collect the nectar and convert into honey through chemical and physical changes. The changes makes honey more digestible and also to eliminate surplus of water to store and free of microorganisms. The nectar largely consists of water and sucrose, or sugar with 12 carbon atoms. First, bees add an enzyme called invertase which converts the sucrose into two six-carbon sugars, glucose and fructose. Then the second enzyme, glucose oxidase, converts a very small amount of glucose into gluconic acid, which makes honey as acidic as wine or vinegar. The very low pH protects the honey from bacteria. Both these enzymes are secreted by the hypopharangel glands of the worker bees. The moisture content of nectar is reduced generally to 18 to 20% by collective fanning. Due to reduction of water osmotic pressure increases and it keeps honey free of bacteria and yeasts.

Honey : Honey is a sweet liquid gathered by honey bees from nectar or other secretions of plants which they transform by addition of enzymes and evaporation
of water in it and is stored in their combs as food. It consists of reducing sugars, levulose and dextrose. Levulose being more predominant.

According to the definition given by world wide standards (1982), honey is the unfermented, sweet substance produced by honey bees from the floral or extra floral nectar of plants, which they collect, transform and combine with specific substances and store in honey combs.

Chemical composition of honey

Honey is the only substance which is matured naturally occurring sweet. Honey is composed of various substances i.e., levulose 35-40% dextrose 30-35%, moisture 18-20%, maltose and other sugars 4% and other substances 3%.

Sugars : Sweetness of honey depends upon sugars which are its major components (70-80%). Sugars are the sources of metabolic energy that are essential for life processes. The sugars include mainly, glucose and fructose almost in equal proportion. Those are easily digestible and give instant energy. In this levulose and dextrose are predominant and sucrose and maltose are in small amount. One molecule of sucrose and one molecule of water react in the presence of enzyme invertase and hydrolyzed to form one molecule of dextrose and one molecule of levulose. This chemical reaction occurs when bees ripen honey. (Chaudhary, 1994).

Levulose (fructose) and dextrose (glucose) are monosaccharides or hexoses, \( (C_6H_{12}O_6) \) or \( C_6(H_2O)_6 \). Generally levulose is the more abundant of the sugars in
honey. It is water soluble and can readily decompose than dextrose. This partial loss of levulose slowly converts the honey into granular.

Proteins and Amino Acids: They are mainly of plant origin and their content varies in different honeys. Proteins and all essential amino acids required by the human body for growth are present in honey. Honey from Karvi (Carvia callosa) contains 1.2% proteins, perhaps the highest among Indian honeys.

About eighteen free amino acids are known to occur in honey (Chaudhary, 1994). All these are essential amino acids required by the honey bees, but they are in too small amounts to be of nutritional significance to man. The most abundant among the amino acids is proline.

Vitamins: Honey contains several vitamins i.e., Vitamin A, vitamin-B1 or thiamine, vitamin-B2 or riboflavin, Niacin or nicotinic acid, vitamin-B6 or pyridoxal, pantothenic acid and vitamin-C or ascorbic acid.

Enzymes: Several enzymes are present in the honey viz., Invertase converts sucrose to dextrose and levulose, diastase converts starch to dextrin and maltose, inulase converts inulin to levulose, catalase decomposes hydrogen peroxide and Glucose oxidase produces gluconic acid.

Pigments: The most important pigments present in honey are carotenes, xanthophyll, anthocyanin, tannin, and colloidal particles.
Essential Oils: Terpenes, aldehydes, methyl anthranilate are some essential oils present in the honey.

Mineral elements: Generally minerals are referred to as 'Ash' about 11 minerals and 17 trace elements are identified in normal honeys (Chaudhary, 1994). Mineral contents vary greatly. Darker honey generally contains more minerals than lighter ones. The minerals present in honey are potassium, calcium, magnesium, iron, copper, manganese, phosphorus, sulphur, chlorine and traces of chromium, nickel, tin, silver, gold etc.

Acids: Acetic, buteric, citric, formic, gluconic malic, lactic, succinic, tartaric acids etc., are the important acids present in the honey.

Kinds of honeys:

Two types of honeys are identified based on the mode of extraction. viz., extracted or apiary honey and squeezed honey.

Extracted honey: It is extracted by centrifugation of the honey frames of the super portion of the hive of A. cerana and A. mellifera.

Squeezed honey: This is obtained by pressing and squeezing the honey storing portion of wild comb. When compared to extracted sample the pollen contents found in squeezed honey always appear in high percentage. In this present work squeezed honeys of A dorsata are studied.
Honeydew honey

Honeydew honey is from sugary secretions of various plant sucking insects (aphids). Dextrin content is more in honeydew. It consists of trisaccharide melezitose and very active enzyme (Maurizio, 1962). Pollen grains are very poor in honeydew honey when compare to fungal elements (spores of rusts, uredinaceae, and smuts, and hyphal shreds) and algal elements like Myxophyceae (Louveau et al., 1978). It has a more pronounced flavour and darker in colour. For the production of honeydew honeys coniferous forests present in Northern Europe are one of the main areas and in tropics and subtropics little or nothing is known of the honeydew honey. (FAO, Agricultural series bulletin No. 68, 1986).

Pollen in Honey: Honey is having different types of pollen. Those can be studied by extraction method. Critical microscopic examination helps to identify the pollen types which gives clues its identity and mixing up of different honeys. The pollen grains in honey help us to identify the plant species visited by the bees for nectar collection.

Colloids in honey

Honeys generally contains colloids also. They are gummy, non-crystalline substances consisting largely of nitrogenous compounds (proteins), highly emulsified wax particles, with pentosans and inorganic constituents.
Viscosity of honey

Honey viscosity is controlled by its gravity and the lower the water content, the greater would be the viscosity. Presence of colloidal material in honey increases viscosity.

Colour, Aroma and Flavour of honeys

The colour and flavour of honeys are due to presence of various chemical compounds in the original nectar. The colour of the honey is often an index of its flavour and mineral content. Honey shows various colours such as pale yellow, light amber, dark amber, amber, dark brown, greenish yellow and golden brown depending upon the plant source from where the nectar is collected. Light coloured honeys are generally mild in flavour than the dark coloured honeys, due to various pigments viz., tannins, chlorophyll derivatives and colloidal particles. The delightful aroma of honey is traceable to the essential oils viz., terpenes or aromatic aldehydes.

Thixotropic honey

Thixotropy is the property exhibited by some gels with a tendency to become fluid when shaken. This is a reversible process. The classical example of thixotropic honey from India is the "Karvi honey from Carvia callosa". Pryce-Jones (1936) attributed high protein content and presence of pectins as a probable cause
for the thixotropic property of honey, but the Karvi honey, however, does not contain high protein content but exhibits thixotropic property (Deodikar et al., 1957). This may be due to its high content of non-reducing sugar dextrin.

**Granulation**

There is a misconception among the common consumers, that granulated honey is an adulterated honey, having common sugar. In fact, granulation is a natural process of crystallization of the glucose content in honey and also it depends upon the concentration of its sugars in relation to its water content and relative proportion of dextrose and levulose. The rate of granulation varies from honey to honey depending upon the source of nectar and its composition. However if the L/D ratio is less than 1 the honey samples tends to granulate fast. Some honey with high L/D ratio don’t granulate even after long period ex. Jamun honey (*Syzygium cumini*) contrary to this there are others which granulate even in the comb itself ex. mustard (*Brassica nigra*) honey (see CBRI technical bulletin no. 24, 1987), rape honeys (Maurizio, 1959; White et al., 1962).

**Fermentation**

In honey, fermentation is brought about by sugar tolerant yeasts. In ripe honey sugar tolerant yeasts are not able to grow because of high sugar concentration which keeps them in dormant stage while in unripe honey (with more than 20% moisture) provide favourable medium for the growth of yeasts and their ac-
tion on dextrose and levulose, results in the formation of alcohol, carbon dioxide, acetic acid and water. Fermentation results in enhanced acidity and honey becomes sour. Prevention of granulation or destruction of the yeasts by heating the honey at 145°C for 30 minutes controls fermentation.

Bee hive products

Honey, pollen, bees wax, bee bread, propolis and royal jelly represent the bee hive products.

Honey: Honey, the liquid gold of nature, is a natural product incomparable to anything else in terms of its nourishment and healing properties. The importance of honey in Ayurvedic and Unani systems of medicine is a well established fact. It is mildly laxative and known for antiseptic properties. Antibiotic activity of honey is mainly responsible for its use since ancient times in healing the wounds due to cuts, burns etc. The hygroscopic nature of honey enables the wound to dry quickly, thus also helps in protecting the tenderness of the skin and cleaning of eyes. Honey is used internally in treatments of cough, cold, hay fever, gastrointestinal disorders etc. It has been recognized as an important energy giving food (a table spoon of honey provides 75 calories of energy). Honey sources as a general tonic for all ages.

Pollen: Large quantities of pollen are collected using pollen traps and recovered from the bees or directly from the hives. The recovered pollen is used in cosmetic and pharmaceutical industries.
Bees wax: Bees wax is secreted by the wax glands of honey bees for constructing their combs. It is a yellowish to grey brown solid with a mild odour. In India, the main source of bees wax are the combs of wild bees of *A. dorsata* (Singh, 1962). It is used in cosmetic, pharmaceutical, aeronautical industries and for making honey comb foundations.

Bee-bread: Bee-bread (Bee-meat) used by the bees for their protein requirements consists of loads of pollen grains mixed with glandular secretions. Bee bread is recovered from combs and used extensively in cosmetic and pharmaceutical industries.

Propolis (Bee glue): It is a resinous, sticky, aromatic substance chestnut red to light brown in colour. Propolis is collected by bees from the buds of certain trees such as species of *Calophyllum, Ipomoea, Bursera, Clusia, Cocos* etc., (FAO Agricultural series bulletin No. 68, 1986). Bees use this for minor building work, to fill the cracks reduce openings and smoothen the inner surface of the nest. Propolis is used in cosmetics, violin varnish and as an antiseptic. Recently it is used in anticancer treatment.

Royal Jelly: A balanced and highly nourishing food product used to feed the larvae of workers and drones in the initial stages, and the larvae of the queen throughout their developmental period. Young workers generally fed with nectar and pollen. Nurse bees secrete royal jelly from their hypopharyngeal and mandibular glands.
Royal Jelly, harvested from 3-days old brood cells is the most highly prized of all the bee products, as it is supposed to add years of healthy life to normal people and cure many ailments (Brown, 1989; Karsali et al., 1988).

Bees as crop pollinators

The importance of bees in pollination exceeds their value in the production of honey and beeswax. Honey bees are considered as best pollinators due to their floral fidelity an inherent character. Bees fix their preference on the basis of the nutritive value of nectar and pollen. The tendency of honey bees to visit consistently a single species of flowers, increases their value as pollinators. Suryanarayana (1986, 1987) highlighted the relevance and importance of melittopalynological studies to apiary industry and enhanced crop production.

Bee botany melittopalynology

Nectar and pollen are the essential food ingredients of honey bees. Nectar serve as the principal energy source because of its highest sugar content, where as pollen grains are the chief source of protein requirements of the bees which are useful for building their body tissues during brood stage. The pollen grains which are present in honeys and pollen loads are the only source of recognition of bee forage plants. Melittopalynology is a special branch of palynology involved in the study of pollen contents of honeys and its application in the field of apiculture. It deals with the critical study involving qualitative and quantitative analysis of pollen.
contents of honeys and pollen loads of bees from diverse floristic and geographical regions. It also involves the study of honey dew elements composed of fungal spores, fungal hyphae, algae and wax elements of the honeys.

The honey bees serve as main pollination agents while their collection of pollen and nectar from different flowers. This type relationship between honey bees and flowers exhibit a type of symbiosis called as synagonism (Deodikar, 1961).

The bee plants are generally classified into three major types.

I  Nectar sources - visited by the bees for nectar alone
II Pollen sources - visited for pollen alone
III Nectar and pollen sources - visited for both nectar and pollen.

Pollen analysis of honey samples and pollen loads revealed that the preferential plants visited by the bees for nectar and pollen or both. The pollen contents of honey samples and pollen loads reflects the characteristic local flora and vegetational types and their deference with respect to geographical origin and collection period of honeys. This pollen analysis helps in detection of blends of honey samples from different regions and also adulteration of honeys.

This type of melittopalynological studies provides information in the identification of unifloral and multifloral honeys. This type of study also helps in the determination of honey flow season and dearth period of an area. Thus
Melittopalynological studies help to recognize the key bee plants of different floristic and geographical regions and their abundance in different seasons which help for maintaining high potential commercial honey production.