Materials and Methods
KARNATAKA MAP Showing study area viz KOLAR and TUMKUR DISTRICT
1. Topography and climatic condition of Kolar and Tumkur

I KOLAR

Kolar is one of the twenty seven districts in the state of Karnataka. It is also called “Land of Gold, Silk and Milk”, and is situated in the south eastern part of Karnataka state on National Highway No. 4 at a distance of about 72km from Bangalore. The district is situated between 12°46' and 13°58' north latitude and 77°21' and 78°35' east longitude. The district is situated halfway between eastern and western coast and is bound on the west by Bangalore and Tumkur districts, south by North Arcot and Dharmapuri districts of Tamilnadu, east and north by Chittor district of Andhra Pradesh. The district has an area of 8,233sq km and occupies 12th place in the state having 11 taluks viz., Bagepalli, Bangarpet, Chikaballapur, Chintamani, Gudibande, Gauribidanur, Kolar, Malur, Mulbagal, Siddlagatta and Srinivasapura (Maps 1 and 2).

a) Physiographic structure

Physiographically, Kolar district comprises of maidan/plain areas. The central and eastern parts of the district forming the valley of Palar river is undulating and well cultivated. The general ground level is between 850m at Kolar, 920m at Malur and 911m at the foot of the Ambajidurga considerable depression occurs in the valley of north Pinakini towards Gowribidnur taluk.

The Kolar district has a variety of topographical situations from plains to gentle slope and culminating in the spectacular heights of the Nandi hills in the Papagni hill range. The greater part of the district has 860m to 911m above mean sea level, in certain places however, the elevation reaches over 1,479m. The highest peak being at Nandi hills in the Papagni ranges. While, the lowest being 802m at Kolar. The district spreads about 137kms from north to south and 135kms from east to west.
The Kolar district is endowed with a number of hills with peaks of varying heights, particularly in the northern part. The principal chain of hills maintained in Nandidurga range. This range of hills constitutes the chief watershed and most of the rivers in the district have their origin. Parallel to this range about 50 to 60km the east runs a range of hills which are entering the Chitravathi and Papagni. A third range of low hills represented in places commencing near Srinivasapura and pressing the same ground directions passed to the east of Kolar and extends through the southern most parts of Bangarpet taluks to Kangondi Kuppam and Palamaner ghats in Andhra Pradesh.

b) Rainfall

The district comes under the influence of both the south-west and north-east monsoons as it is half way between eastern and western coasts. The average normal rainfall of the district was 760.5mm with 48 rainy days, in the Nandi hills region and it is around 1,195.8mm with 64 rainy days in the Kolar district. The greater portion of the rainfall is during the north-east monsoon from September to November.

The rainfall in the district is scanty and erratic with uneven distribution during monsoon. Larger variation in rainfall is noticed from year to year and amongst taluks it varies to a larger extent.

Modern agriculture requires precise information on rainfall, droughts, floods and agro-climatic conditions for different situations prevail in the district. The week-wise probability of rainfall has been worked out and this has helped in predicting the rainfall and to plan the agricultural operations. The climate of the district is basically tropical and determined largely by the geographic location with respect to the monsoons behaviour prevailed in the district. As the district enjoys two monsoons, it is called the land of two seasons because both south-west and north-east monsoons accounts for major
part of the rainfall. A small quantity of the rainfall is received during cold and hot weather seasons.

c) Climate

The district enjoys an agreeable climate during March to May. There will be continuous rise in temperature resulting in experiencing the highest temperature (35.3°C) during the month of May in a year, with mean temperature of 34.7°C at Kolar Gold Fields which can be taken as representative of the district in general.

With an advancement of south-west monsoon in early June, the temperature decreases, throughout the monsoon season, the weather remains pleasant. At the end of south-west monsoon and the beginning of cold season i.e., November, day and night temperature begins to reduce. With mean daily temperature of 25.8°C in the month of December.

d) Seasons

On the basis of climate the district enjoys four seasons in a year. They are:

1) **Dry season**: with clear bright weather from December to February.

2) **Hot season**: from March to May.

3) **South-west monsoon season**: from June to end of October.

4) **The retreating season**: November.

e) Soils

It is clearly observed in the district of Kolar that the soils of Kolar district as classified by large physical studies and by the survey of the National Commission on Agriculture State Soil Survey, Bangalore that these soils have been classified into three broad categories:

1) The red loam soil
2) The clay loam soil
3) The laterite type of soil

Soils are natural bodies which help the sustainable plant growth of largely agriculture oriented it becomes the major source of livelihood. Soil is the surface and adjoining horizons of parent materials, which have undergone more or less natural change under the influence of water, air and various species of organisms living or dead. This change is reflected to a certain degree in the composition, structure and colour of the products of weathering. Soils mainly being the resultant is matter of textures, colour and contents.

II TUMKUR

Tumkur is the headquarters and town of the district, and the district is also called by the same name. But the original name of the place, according to the certain inscription of the tenth century, was Tumme gooru, which means the place of the tumme or tumbe, a common fragrant herb (*Leucas aspera*) found abundantly in the area.

Tumkur belongs to a group of districts called the maidan (plains) districts and is situated in the east-central part of the Karnataka state and to the south and south-east of Chitradurga district. It is situated between 12°45' and 14°20' north latitude and between 76°20' and 77°31' east longitude.

It is bounded on the north by the Anantapur district of Andhra Pradesh, on the east by the Kolar and Bangalore districts, on the south by the Mandya district and on the west by the districts of Chitradurga, Chikamagalur and Hassan. In area (10,500 sq km) and population it occupies the 8th place. The extreme length of the district from north to the south is 163 km and its greatest width from east to west is 107 km. There are 10 taluks in the district *viz.*, Chiknyakanahalli, Gubbi, Koratagere, Kunigal, Madhugiri, Pavagada, Sira, Tumkur, Turuvekere and Tiptur (Map 3).
Tumkur is land-locked district. It has no natural features like rivers or mountains dividing it from the other districts of the state. The district is generally an open tract except in the south of the Kunigal taluks, where the country is wooded and hilly, the other parts consisting mainly of undulating plains, interspersed with clumps of tall and well grown trees. To the east of Tumkur and north of Devarayanadurga, the appearance of the region presents the scenery of a hilly country intersected by the cultivated valleys. The open parts of the district maintain a generally even level above the sea, except Sira and Pavagada which are at a considerably lower level than the rest of the district.

a) Forest wealth

The forest region in the district is classified under the dry-belt zone, as distinct from the evergreen and the mixed belt, which are found in the extreme west of the old Mysore and are in the middle of the state, respectively. The dry belt zone lies to the east to the mixed-belt and includes in it the whole of the Tumkur area. In these dry area, the tree vegetation is very much inferior to that of the mixed belt, the change noticed being gradual and in some areas very marked. The exact boundary limit between the dry-belt zone and the mixed-belt zone is seen near the eastern slopes of the Bababudan hills. This line passes from north to south and the region laying east to the line is called the dry-belt in which Tumkur district is situated. Most of the tree vegetation found in the mixed-belt is also found in the dry zone, but in the latter, the growth is not so spectacular as is found in the mixed-belt.

The forest regions in the district are found, to a large extent, in the lower slopes of hill ranges viz., Devarayanadurga hills, hills around Koratagere, ranges near Madhugiri, chain of hills to the west of Kibbanahalli, the region around Bukkapatna, the area near Huliyurdurga, area around Kudurekanivi and Kepalpura. The total area of the state forest in the district is 548sq km.
b) Growth of Tree Vegetation

The forests in the districts are confined mostly to the lower slopes of the hill ranges and are spread over the entire district in small blocks. The forests were mostly open and consists of mixed species varying from dry deciduous to thorny bushes. Because of the scanty rainfall, which is about 685mm per year, the tree growth in the dry-belt zone never attains a height of more than 7.62m. The forest consists mostly of fuel trees, providing fuel throughout the year.

Characteristic of the zone to which the forest region belongs, the vegetation growth is of the dry deciduous type, typical of the maidan tracts. Classified technically according to the Champion method, the area of forests in the districts comes under the southern tropical thorn forest series. The northern half of the Bukkapatna state forest presents *Hardwickia* forest, which is an edaphic peculiarity. The growing stock is incoherent and consequently incapable of forming anything like a continuos forest canopy. The ground surface has no adequate soil or organic humus. Mineral or skeletal soil, therefore, lies exposed at the surface. The trees do not develop anything like a real 'bole' the stems being generally guarled, twisted, knotty and branchy. This is particularly so in the dry belts.

c) Climate

The climate of this district, excluding the northern most part, is similar to that of Bangalore district, and is generally agreeable. But the climate of the Pavagada region and the part of the district north of Sira, is like that of the Chitradurga district with a somewhat hotter summer. The year may be divided into four seasons;

1) The dry season, with clear bright weather, is from December to February.
2) The period from March to May constitutes the hot season.
3) The south-west monsoon season is from June to September.
4) October and November may be termed the post-monsoon season.
d) Rainfall

The average annual rainfall in the district is 687.9mm (27.08”). The rainfall increases from the north to the south generally, and in the western part of the district from the west to the east. Rainfall is mostly confined to the period from May to November. The rainfall during the south-west monsoon is only 50 per cent of the annual rainfall.

e) Temperature

The period from March to May is one of the continuous raise in temperatures. April is usually the hottest month. Maximum temperatures may sometimes reach 40 or 41°C with an advance of the south-west monsoon over the district in June, the temperature drops appreciably and throughout the monsoon season, the weather is pleasant. After October, temperature decreases steadily and the weather remains cool till February. December is generally the coolest month of the year. The daily minimum temperature in the cold season sometime goes down to 9 to 10°C.

The relative humidities are high during the south-west monsoon period and are generally moderate in the rest of the year. The humidities in the summer afternoons are comparatively lower.

2. Plant survey and collection

Plant survey of the place under investigation is prerequisite for melissopalynological or melittopalynological studies. The knowledge of the flowering plants around the bee colony is imperative to study the bee pasturage or bee forage of the place. Frequent field (botanical) survey of 10 places selected for the study was carried out, during which the duration of the flowering period and the nectar yield or pollen production or both were observed. In addition to the above, anemophilous or entomophilous nature of the flower was noted. List of plants, flowering during different seasons of the
year was prepared with reference to bee forage. Fresh flowers were collected for preparing reference slides and they are preserved for future reference.

The plant survey is useful in preparing a bee floral calendar for the place, identifying the nectar or pollen producing plants or both, poisonous plants, medicinal plants, plants flowering during dearth period and accessory bee foraging plants.

The survey helps to confirm the data obtained as a result of microscopic analysis of honey samples collected from the comb cells. A proper knowledge plants of bee preference is necessary to improve the bee keeping industry and to assess the bee keeping potentiality of the place. To introduce better bee plants, which will adopt to the local conditions, which in-turn will boost up the honey production.

Fresh plant specimens collected during the field trips were identified by referring the Gamble's Flora of Presidency of Madras (1915-1935), Flora of Bangalore District by Ramaswamy and Razi (1973), Flora of Karnataka by Cecil. J. Saldanha (1984) and Flora of Coorg (Kodagu), by Keshavamurthy and Yoganarasimhan (1990).

3. Source of honey samples

Kolar is known as land of gold, silk and milk on account of luxuriant growth of different types of plants in wild and cultivated. Trees flowering one after the other throughout the year is responsible for a favourable growth of apiary industry in Kolar and Tumkur.

Ten centres were selected for the present investigation (Maps 2 and 3). Honey samples were collected and stored in clean, dry, coloured glass bottles and labelled indicating the date, place of collection and colour (Table 1). The honey samples were given code ADH (Apis Dorsata Honey) and ACH (Apis Cerana Indica Honey).
4. Preparation of Reference Slides

The slides prepared from the honey samples collected from the honeybees and comb cells were scanned microscopically to identify pollen by comparing with reference slide. Slide collection maintained in the Paleobotany and Palynology Laboratory, Botany Department, Bangalore University, Bangalore. A collection of more than 400 pollen reference slides of flowering plants in and around Kolar and Tumkur district have been maintained. For identification of the pollen grains, we bank on the works of Erdtman (1952 and 1969). Our observation on pollen morphology of various pollen grains (major and minor bee plants) that are encountered in the honey samples 1 to 50 is presented. Various technical terms used in this study have been followed from the works of Wodehouse (1935), Erdtman (1952, 1954, 1956, 1958), Faegri (1950) and Hyde (1958).

5. Melittopalynological methods used for the analysis of honey sample

The pollen grain present in the honey samples is the only basis of identification of the plant source. The microscopic analysis of the honey samples reveals their botanical origin. The method proposed by International Commission for Bee Botany (ICBB, 1962; Louveaux, Anna Maurizio and Vorwohl, 1970) was followed.

a) Acetolysis method Erdtman (1952)

Acetolysis method is used in the study of pollen morphology. The pollen grains subjected to acetolysis became transparent revealing the structural pattern for correct identification.

Procedure

Fresh flower buds which are about to bloom were selected and the anthers were removed with the help of forceps. The anthers were taken into a
centrifuge tube containing 70% alcohol. The material was crushed with the help of glass rod. The pollen grains released from the anthers were filtered through a sieve. The pollen grains free from the fats and oils were collected by placing a centrifuge tube beneath the sieve. The solution was centrifuged and the superantant portion was decanted. To the sediment, glacial acetic acid was added and centrifuged. The supernatant was discarded and to the sediment 5cc of freshly prepared acetolysis mixture (9:1 acetic anhydride and concentrated sulphuric acid) was added. The centrifuge tube with the content was placed in a water bath, when the colour of the solution changes to golden brown, the centrifuge tube was removed from the water bath, cooled and centrifuged. The supernatant was decanted into a separate bottle and to the sediment, glacial acetic acid was added, centrifuged and the supernatant was decanted. After washing the sediment several time with water (by centrifuging the solution), the sediment at the bottom of the centrifuge tube was taken on a pellet of glycerine jelly using a clean needle and placed on a glass slide. The slide was gently heated over the flames of the spirit lamp. The molten jelly was spread evenly using a clean needle and a cover slip was placed over it. The sides of the coverslip were sealed using wax to avoid contamination. The slide was numbered and labelled.

b) Wodehouse Technique (1935)

The anther of the flower bud which is about to bloom was placed on a clean glass slide. The pollen grains were released by teasing out the anther wall. After removing the debris, the pollen material was treated with a drop of 70% alcohol to remove fats and oil present in the pollen grains. After a few seconds a rig was formed around the pollen grains. The area around the pollen grains was cleaned using a cotton bud. A drop of melted glycerine jelly was added to the fat free pollen grain and a clean cover slip was placed over it. The edges of the coverslip were sealed with paraffin wax.
For staining pollen grains, a small piece of glycerine jelly prestained with saffarin was placed over the fat free pollen material. The slide was gently heated over the flames of a spirit lamp to melt the jelly and a clean cover glass was placed over the material. The edges of the cover glass were sealed with wax. The permanent slides thus prepared were labelled, numbered and deposited in the Pollen Herbarium of the Paleobotany and Palynology Laboratory, Bangalore University, Bangalore.

c) Microscopic examination of honey samples

The microscopic examination of the slides prepared from the honey sediments helps to determine the botanical origin of honey and an attempt was made to identify the pollen up to species level.

The pollen grains counted by scanning the whole slide or slides prepared (depending on the quantity of the sediment) are expressed in percentage frequencies represented in Table 2 (pollen spectrum of honey samples).

"Predominant pollen" means constituting pollen count of more than ... 45%

Secondary pollen .............................................................................................................. 16-45%

Important minor pollen ........................................................................................... 3-15%

Minor pollen ................................................................................................................. <3%

The honey sample is classified as unifloral honey, if the honey contains pollen, mainly from a certain one plant species, in other words, if the pollen of that species is predominant i.e., above 45%. In case of multifloral honey, the individual species pollen count is less than 45%.

d) Qualitative analysis

10 grams of honey sample was dissolved in 20 ml of hot water (not above 40°C). The solution was centrifuged for 10-15 minutes and the supernatant
liquid was decanted. For the better removal of the honey sugars the sediments was dispersed again in 10 ml of distilled water and the solution was centrifuged for 10-15 minutes. The supernatant liquid was decanted and the sediment was taken on to a clean glass slide (as completely as possible) with the help of a piece of glycerine jelly. The jelly was cut into a small pieces and placed on the glass slides. Stained and unstained preparation of the sediment was made on the same slide. The slides were numbered and preserved for microscopic examination.

e) Quantitative analysis

A drop of solution was placed in 1 mm square of the Haemocytometer using 0.1 ml pipette and covered with cover slip. The pollen types and their number present in the central square were counted. Twenty readings were taken for each honey samples and the average was calculated. The absolute pollen count in 10 grams of honey samples was calculated by multiplying the average by 1,000. The absolute pollen count of honey sample and the amount of the sediment determined from the graduated centrifuge tube provides the information about the method of honey extracted (squeezed or machine extracted).

6. Methods used for chemical analysis of unifloral honey samples

Honey is a complex substance and each content varies according to its botanical and geographical origin and climatic conditions.

The chemical analysis of the honey samples was carried out in the laboratory at Central Bee Research and Training Institute, Pune. The method given in the ISI handbook of food analysis, Indian Standard Institutions, New Delhi, 1984, was followed for the chemical analysis of the honey samples.
7. **Determination of optical density of honey samples**

About 2gms of honey was dissolved in distilled water to make 10ml of the solution. The calibrator was adjusted to 0, absorbance 100% and transmittance of 660µm. The honey sample was taken in a cuvet and the absorbance was read directly or as the percentage transmittance at the same wave length (Table I).

8. **Photography**

For the photography, FM-2 Nikon camera with Konika 100 ASC film were used for outdoor as well as photomicrography and were printed at New Vishnu Studio, Bangalore using Fuji paper. The observations have been provided in the next chapter.