DNA BARCODING OF HONEY ASSOCIATED WITH POLLINIFEROUS PLANTS DIVERSITY

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I have great pleasure in forwarding the thesis entitled "**DNA Barcoding of honey associated with polliniferous plants diversity**" submitted by R. Lalhmangaihi for the degree of Ph.D degree of Mizoram University. R.Lalhmangaihi has put in the prescribed number of terms of research work under my supervision. The data incorporated in the thesis are based on her own independent observations.

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DECLARATION

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JULY 2016

I, **R.Lalhmangaihi** herby declare that the subject matter of this thesis in the record of work done by me, that the contents of this thesis did not form basis of the award of any previous degree to me or to do to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University/Institute.

This is being submitted to the Mizoram University for the degree of ***Doctor of Philosophy*** in Botany.

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ABBREVIATIONS

aacD = Acetyl-CoA Carboxylase D

atp F =Adenosine triphosphate synthetase sub unit B

atpH = Adenosine triphosphate synthetase sub unit B

BLAST = Basic local alignment search tool

CBOL = Consortium for the barcode of life

CTAB = Cetryl trimethylammonium bromide

DNA = Deoxyribose nucleic acid

DTT = Dithiothreitol

matK = Maturase K gene

NCBI = National Center for Biotechnology Information

OD = Optical density

PCR = Polymerase chain reaction

psbA = Photosystem II protein D1

psbI = Photosystem II protein reaction centre protein I

psbK = Photosystem II protein reaction centre protein K

rbcL = Ribulose-1,5- biphosphate carboxylase/oxygenase

rpoB = RNA polymerase subunit beta

rpoC1 = RNA polymerase sub unit gamma

SDS = Sodium dodecyle sulfate

trnH = trnH codes for tRNA ^{His}(GUG)

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CHAPTER 1

INTRODUCTION

1.1 Brief history of honey

1.1.1 Honey in the world

Exactly how long honey has been in existence is hard to say because it has been around since as far back as it was record. Cave paintings in Spain from 7000BC show the earliest records of beekeeping, however, fossils of honey bees date back about 150 million years! Its 'magical' properties and versatility has given honey a significant part in history. The use of honey has long, and varied historical evidences in human civilization, in many culture as well as in many countries, honey has associations that go beyond its use as a food (Codex Alimentarius Committee, 2001). The earliest record of keeping bees in hives was found in the sun temple erected in 2400BC near Cairo. The bee featured frequently in Egyptian hieroglyphs and, being favoured by the pharaohs, often symbolised royalty. The ancient Egyptians used honey as a sweetener, as a gift to their gods and even as an ingredient in embalming fluid. Honey cakes were baked by the Egyptians and used as an offering to placate the gods. The Romans also used honey as a gift to the gods and they used it extensively in cooking. The Greeks viewed honey as not only an important food, but also as a healing medicine.Once Christianity was established, honey and beeswax production increased greatly to meet the demand for church candles. The Greeks too, made honey cakes and offered them to the gods. Honey continued to be of importance in Europe until the Renaissance, when the arrival of sugar

from further field meant honey was used less. Bee keeping flourished throughout the Roman empire. By the seventeenth century sugar was being used regularly as a sweetener and honey was used even less.

1.1.2 Honey in India

India was known in history to be the "cradle of humanity". India also made claim to being the birthplace of the honeybee. However, this unproven claim was vehemently contested by Egypt, and Greece. Honey is easily produced in India. This due to the fertile soil, the abundance of sunny days, a good water supply. Beehives are often found six feet square, suspended from the highest trees, hanging rocks, and other inaccessible places to gain protection from man and beast. The combs are visible from a distance of miles. Special honey hunters approach the nests with ladders and ropes, usually at night time, to collect their plentiful harvest (Anon. 1948).

As mentioned honey widely used in India for food preparation, and as medicine. It is also used in the preparation of alcoholic beverages. The Hindus drank "madhuparka", during religious ceremonies. Madhuparka, is a mixture of honey and curds."I drink thee for luck, glory, power, and for the enjoyment of food…" Madhuparka plays a large part in the Hindu wedding ceremony. Flavor and color of honey depend on the flowers the bee gathered its nectar from India is a one of the top 5 honey rich countries, and is known to have a large floral diversity. Here are just a few types of honey that India has become popular for producing; Eucalyptus Honey Mustard (Rapeseed) Sunflower Honey Karanj pongamia honey Acacia Honey Wildflower Honey. Honey hunting from the wild bee colony was known in India since pre historic

times. Collection of honey from wild nests is even today an important economic activity of tribal and other population in the country, most of the honey in the Indian market is obtained from forests (Lakshmi and Suryanarayana, 1997).

1.2. Nature of honey

Honey is a complex natural substance produced by honeybees from nectar and from secretions of living plants (Baroni *et al*., 2009). It reflects the honeybees' diet and the local plant communities. Honey also shows different plant compositions in different geographical locations. Honey is a natural sweet substance produced by honeybees from the nectar of plants, from secretions of living parts of plants, or from excretions of plant-sucking insects. It is stored in the honeycombs and used as food source during winter (Schnell *et al*., 2010). Once collected, it is subsequently transformed by different substances produced by the honeybee, by dehydration and by maturation in honeycombs (Azzedine *et al*., 2007). It is mainly composed of carbohydrates, which represent 95% of honey dry weight, it also contains organic acids, proteins, amino acids, minerals, polyphenols, vitamins and aroma compounds (White, 1975). Bee honey originates from either nectar or other sugar containing deposits produced by living plants. Bees of the genus *Apis* are not the only bees which contribute to the World's supply of honey and wax. Some species of *Meliponinae* form very large colonies and store sufficient honey to make their exploitation worthwhile.

Honey, as it is found in the hive, is a truly remarkable material, elaborated by bees with floral nectar, and less often with honeydew. Nectar is a thin, easily spoiled sweet liquid that is changed ("ripened") by the honey bee to a stable, high-density, high-

energy food. Colors of honey form a continuous range from very pale yellow through ambers to a darkish red amber to nearly black. The variations are almost entirely due to the plant source of the honey, although climate may modify the color somewhat through the darkening action of heat. The flavor and aroma of honey vary even more than the color. Although there seems to be a characteristic "honey flavor," almost an infinite number of aroma and flavor variations can exist. As with color, the variations appear to be governed by the floral source (Abrol, 1997). Honey is primarily a high-energy carbohydrate food. Because its distinct flavors cannot be found elsewhere, it is an enjoyable treat. The honey sugars are largely the easily digestible "simple sugars," similar to those in many fruits. Honey can be regarded as a good food for both infants and adults and honey have been used for centuries for its nutritional and medicinal properties (Liberato *et al*., 2013).

1.3. Honey bee – good pollinator

It is generally known that bees are needed to pollinate our crops several times more than the value of the worldwide production of honey. It is estimated of one-third of what we eat and drink is produced through service supplied by pollinators**.** Bees of all kinds belong to the order of insects known as Hymenoptera, literally "membrane wings". This order, comprising some 100,000 species, also includes wasps, ants, ichneumons and sawflies. Of the 25,000 or more described species of bees (more are recognised every year) the majority are solitary bees most of which lay their eggs in tunnels, which they excavate themselves. These bees provide a supply of food (honey

and pollen) for the larvae, but there is no progressive feeding of the larvae by the adult bees (Abrol, 1997).

Honey bees belong to the family of social bees which includes bumble bees and the tropical stingless bees of the genus *Meliponinae*. Two attributes of honey bees which have been essential to their evolution and biology are their clustering behaviour and, particularly in the case of the cavity-nesting species, their ability to cool the nest by evaporation of water collected outside. These attributes enable the colonies to achieve a marked degree of temperature regulation within the nest irrespective of the external temperature. The genus *Apis* was thus enabled to colonise a wide variety of environments, ranging from tropical to cool temperate and the *Meliponinae* which lack this capability are confined to tropical regions. Another behavioural character of honey bees is the communication of information about food sources and the recruitment of foragers by "dance language". The accurate dissemination of information concerning direction and distance of forage areas leads to efficient exploitation of food sources (Kumar & Chaudhary, 1993). Insect pollination of agricultural crops is a critical ecosystem service. Honeybees pollinate 16% of flowering plant species in the world and nearly 400 species of agricultural plants (Crane & Walker, 1984). Fruits, vegetables or seed production from 87 of the 115 leading global food crops depends upon animal pollination (Klein *et al*., 2007). The value of insect pollination for worldwide agricultural production is estimated to be 153 billion, which represents 9.5% of the value of the world agricultural production used for human food in 2005 (Gallai *et al*., 2009).

Whereas representatives of most types of bee were indigenous to all the continents, bees belonging to the genus *Apis* were originally to be found only in the Old World, namely Asia, Africa and Europe. This suggests that the genus appeared much later than the other types. The genus comprises four species: *Apis florea*, the Little Honey bee; *Apis dorsata*, the Giant Honey bee; *Apis cerana*, the Eastern Honey bee; and*Apis mellifera*, the Western Honey bee. (Some authors include *Apis laboriosa* and *Apis andreniformis* as separate species, but it is likely that these are geographical subspecies of *Apis dorsata* and *Apis florea* respectively which show greater physical variations than the other subspecies and are possibly in a more advanced stage of speciation. Five species of honeybee has been observed in Mizoram, which are *Apis cerana* (Local name: Khawivah), *Apis andreniformis* (Ln: Khawifung), *Apis florae* (Ln: Khawifung), *Apis dorsata* (Khuai thingawn) and *Apis laboriosa* (Ln: Khamkhuai). *Apis mellifera* is a European bees so it absent in Mizoram. *Apis florea* and *Apis dorsata* build single comb nests in the open, *florea* in low bushes and *dorsata* in trees. Like other tropical honey bees they are prone to migrations, at times over considerable distances. These migrations may be seasonal or in some cases may be a defence against predators and parasites (Pratap, 1997). Honey bees are the only surviving group of bees from the Apini tribe, which is under the *Apis* genus. They are known for producing and storing honey, or liquefied sugar, as well as building impressively large nests using wax secreted by workers in a particular colony (Kohli, 1958; Mishra, 2002).

Production of honey and other product depends on availability of floral resources (Beeflora). The great majority of angiosperm plants depend on animals for their pollination (Pratap & Verma, 2000). In addition to the direct economic importance for

agricultural pollination, bees play an essential role as the major pollinator of natural eco-systems (Nair, 1985). It is important to know pollination and bee flora to obtain more knowledge about bee pollinator essentials to improve the production of traditional crops and those crops that have important perspectives (Amsalu *et al*., 2003).

1.4. Bee keepers and bee hive

Beekeeping is an integral part of mixed farming system in many parts of Jordan and is also practiced as a cottage industry, especially in rural areas (Shahera *et al*., 2003). Beekeeping is agro-horticultural and forest based industry and it is of great importance to farmers for pollination benefit. Beekeeping industry solely depends upon bee plant interaction. A flora favourable to bee rearing is one which has both nectar and pollen producing plants with successive flowering periods so as to keep the bee colonies strong in various season (Chaturvedi, 1983). The success of developing modern beekeeping in North Badia, Jordan depends not only on using better strains of bees but also on the abundance and richness of nectar and pollen around the apiary (Mattu *et al*., 1989). Sugden and Furgala (1982) found a positive correlation between honey production and foraging activity of the honey bee. Morover, pollen collection correlated significantly with honey production (Cale, 1967). Many people believe that honey bees originated in Africa and spread to northern Europe, eastern India, China and the Americas. However, because honey bees have been domesticated to produce honey for human consumption, they are now found all over the world in different habitats.

The sub-family *Apinae* or honey bees, comprises a single genus, *Apis*, which is characterised by the building of vertical combs of hexagonal cells constructed bilaterally

from a midrib, using only the wax secreted by the worker bees. The cells are multi functional, being used repeatedly for rearing the larvae and for the storage of honey and pollen. Progressive feeding of the larvae is carried out by young bees with food produced by glands in the head of the bee from honey and pollen.

Honey bees in temperate climates, such as European honey bees, store larger amounts of honey than other subspecies, as they need to maintain a certain temperature inside the nest to survive during winter African honey bees, do not experience long weeks of cold weather, they do not need to build large and well-insulated nests, produce thousands of workers and store large amounts of honey (Kremen *et al.*, 2002). For a honey bee in a tropical habitat, swarming depends largely on the abundance of food sources, rather than seasonal factors. However, regardless of living in tropical or temperate climates, honey bees maintain their hives with a constant temperature of 90° F to 95 ° F.

Worker honey bees make hives to store honey and feed themselves throughout winter when they cannot go outdoors to forage for food. Honey bee hives are made of six-sided tubes, which are the shapes for optimal honey production because they require less wax and can hold more honey. Some hives develop broods which become dark in color over time because of cocoon tracks and travel stains. Other honey bee hives remain light in color. Wild honey bees make hives in rock crevices, hollow trees and other areas that scout bees believe are appropriate for their colony. Similar to the habits of domesticated honey bees, they construct hives by chewing wax until it becomes soft, then bonding large quantities of wax into the cells of a honeycomb. When worker bees

crowd together within a hive, the hive remains at around 30°C to 35°C, the temperature necessary to control the texture of the wax. Although worker bees only live for approximately six weeks, they spend their lives performing tasks that benefit the survival of their colony. Around the time a worker bee turns 10 days old, she develops a unique wax-producing gland inside her abdomen. Workers forage for food and gather nectar from different flowering plants. When they carry nectar within their pollen pouch, it mixes with a specialized enzyme. After returning to the hive, the worker bee transfers the nectar from her tongue to another worker's tongue, where the liquid from the nectar evaporates and becomes honey. The glands of worker bees convert the sugar contents of honey into wax, which oozes through the bee's small pores to produce tiny flakes of wax on their abdomens. Workers chew these pieces of wax until they become soft and moldable, and then add the chewed wax to the honeycomb construction (Kearns *et al.*, 1998) . The hexagonal cells of the honeycomb are used to house larvae and other brood, as well as to store honey, nectar and pollen. When beekeepers extract honey from hives, the comb is easily left intact, though beekeepers sell honey comb as well.

Modern apicultural methods are inapplicable, but tribes of Central and South American Indians have kept such bees in "hives" for hundreds of years. (It should not be inferred however, that Stingless bees are necessarily gentle and easy to handle; they may carry out mass attacks on large intruders such as man, inflicting painful bites with their powerful mandibles. Some species inject a caustic venom which causes severe burns to the areas of skin affected). The native bee community is important in providing crop pollination services but the temporal fluctuations in bee populations are known to be highly variable across space and time (Kremen *et al.*, 2002).

1.5. Polliniferous plants

There is a growing interest among botanists, economists and ecologists to determine the food resources for bees in the tropics and also to understand the interrelationships between bees and the plants on which they forage. Some of the research in this areas involves only observations of bees visiting flowers. Another type of research involves analysis of the pollen that is present in bee nest or honey and pollen loads of foragers in order to determine which plants are visited. Nectars and pollens are the two of the reward that plants offer to pollinators, so according to this , entomophilous plants can be divided into three groups; (1) nectariferous, (2) polliniferous and (3) nectariferous–polliniferous. In this work the most important polliniferous plants of the Mizoram were determined. Levin and Konopacka (1963) and Winston (1987) found that a honeybee colony has a mean flight range of 1.7km, with most foraging occurring within 6km of the colony. In some cases, European and Africanizes honeybees have been observed foraging at the range of 10km from their colonies (Vischer and Seeley, 1982 and Roubik 1989).

Pollination of flowering plants is an essential ecosystem service (Kremen *et al*., 2007; Lonsdorf *et al*., 2009), key aspects of which hinge on the foraging behavior of pollinators (Schemske & Horvitz, 1984; Wilson & Thomson, 1991 and Dick *et al*., 2003). Honey bee produce honey, bee wax, propolis, royal jelly and they are known to play a vital role in the pollination of plants (Adjare, 1990 and Rahman, 2006).Traditional approaches to characterizing relationships between plants and pollinators rely on time intensive observation of individual interaction (Mitchell *et al*., 2009). Because observations of floral visitation may not reflect pollinator efficiency or

the species identity of collected pollen (Schemske and Horvitz, 1984; Muchhala *et al*., 2009), definitive description of plants pollinator interactions require the direct identification of pollen carried by pollinators.

Honeybees and flowering plants have been considered as an example for co-evolution and mutualism. Honeybees need flowering plants for nectar and pollen as source of food and flowering plants need honeybees for pollination. Beekeeping is entirely depending on the types of flowering plants available in any given area. There is a need to understand honeybee plant relationship to study food preferences of honeybees and pollination requirement. Pollen of various plants representing potential source of nectar and pollen for the honeybees is an important pre-requisite for the developing apiary (Kalpana and Ramanujam 1997).

Pollination is a pivotal, keystone process in almost all terrestrial ecosystem food web, it supports global and sustainable productivity in agriculture and forestry and maintains the biodiversity of plants and animal life. Bees are the most important pollinators but bee decline in abundances

1.6. Melissopalynology

Melissopalynology is the study of pollen in honey, with the purpose of identifying the source plants used by bees in the production of honey. This is important to honey producers because honey produced by pollen and nectar from certain plants as mesquite, buckwheat, tupelo or citrus trees demand a higher price on the market than that produced by other plant sources. Some plants may produce nectar and pollen that is harmful to human health (Brodschneider and Crailsheim, 2010). A careful monitoring of

the pollen types found in honey may identify these toxic sources and the honey produced may be kept out of the commercial market. Pollen is the sole source of protein and lipids in the diet of honeybees, and is crucial for their survival and development. Pollen loads were not same throughout the year, there were great monthly fluctuations in the average weight of the pollen loads carried by the workers (Shahera *et al*., 2003) aswell as the content of pollen in the honey. Pollen is the sole source of protein and lipids in the diet of honey bees, and is crucial for their survival and development (Stanley and Linskens, 1974; Schmidt and Buchmann, 1999). Characterisation of plant sources for bee honey would be useful across a range of research areas. Pollen from the different flowers has specific shape, size and ornamentation. Pollen study has significant application in recognition of bee plants. Pollen are essential tools in analyses of honey. Different type of pollens are used to indicate floral nectar sources utilized by bee to produce honey, relative pollen frequency was often used to verify and label a honey sample as to major and minor nectar sources (Sadia *et al*., 2008). Palynological studies for pollen was carried out to determine the pollen sources for honeybees in Mizoram.

1.7. Molecular study

Plant identification is challenging when no morphological assignable parts are available. Other than identifying whole plants, it is also sometimes useful to be able to identify species from material, such as roots, seeds, or pollen or in mixtures of plants sampled from the air, soil, or water, although this may be difficult or impossible using traditional morphological approaches. The fact that DNA from the plant(s) may be present in honey represents a useful, analytical tool to identify the host plants. The haploid microspores of seed plants (the pollen) are the male part in sexual reproduction

of flowers. Pollen grains have a very hard outer shell, called the exine, which is tough and can be found in fossil deposits, millions of years old. For DNA extraction from pollen grains, it is necessary to find an effective method to destroy the exine. DNA-based analytical methods are less dependent on the analyst and can be applied in different laboratories equipped with suitable instruments. It is possible to identify the plant species as well as microflora of honey from the isolated honey DNA (Bennet and Parducci, 2006; Matsuki *et al*., 2007). Honey rarely comes from a single plant species, even if it is attributed to a single species. Taking into account the health risk from plants producing toxins, it is crucial to assess correctly the identity of the plants from which pollen comes.

Traditionally, the determination of the floral composition of honey has been achieved by the melissopalynology method, which is based on the identification of pollen by light microscopy. However, it requires highly specialized researchers, and for this reason, there is the need for an alternative and sensitive method. From this point of view, the application of molecular methods to the floral analysis in honey offers the possibility to detect a much greater range of plant species in honey, overcoming the limitations of the morphological identification of plant pollen and spores (Matsuki *et al*., 2008). DNA based analytical method are less dependent on the analyst and can be applied in different laboratory equipped with suitable instrument therefore the search for a more efficient means of extracting DNA of both higher quality and yield has lead to the development of a variety of protocols, however the fundamentals of DNA extraction remains the same. The present study aims to develop a simple and efficient method for the extraction of PCR amplifiable chloroplast DNA (cpDNA and rbcL) with a reduced

sample amount of honey compared with previous studies of Cheng *et al*., (2007) and Laube *et al*., (2010). In order to purify DNA, insoluble particulates are removed through centrifugation while soluble proteins and other material are separated through mixing with chloroform and centrifugation. DNA must then be precipitated from the aqueous phase and washed thoroughly to remove contaminating salts. The purified DNA is then resuspended and stored in TE buffer or sterile distilled water. This method has been shown to give intact genomic DNA from plant tissue. As other honey DNA isolation methods are mostly described as kit based approaches, we developed an easy and efficient method for honey DNA isolation by conventional phenol-chloroform method. We additionally show that the extracted DNA is of sufficient quality and quantity to enable PCR amplification and sequencing of genetic markers routinely used for plant barcoding. Accordingly, the DNA can be used to investigate the plant source of origin of the honey sample. To check the quality of the extracted DNA, a sample is run on an agarose gel, stained with ethidium bromide, and visualised under UV light.

Objective :

1. Collection, identification, phenology and palynological study of polliniferous plants associated with apiculture.
2. DNA barcoding of honey to analyse plant diversity in honey.

CHAPTER 2

REVIEW OF LITERATURE

2.1 An overview

Honey is a natural sweet substance produced by honeybees from the nectar of plants from secretions of living parts of plants, or from excretions of plants sucking insects. Once collected, it is subsequently transformed by different substances produced by the honey bee, by dehydration and by maturation in honeycombs (FAO, 1981). It is mainly composed of carbohydrates which represents 95% of honey dry weight (White, 1974). It also contains organic acids, proteins, amino acids, minerals, polyphenols, vitamins and aroma compounds.

Honey is a complex natural substance produced when nectar or sweet secreation from plants surrounding the apiary are collected, modified, and stored in the honey combs by eusocial bees (Baroni, 2009). Honey can be classified as unifloral (when a single plants species is the source of nectar) or multifloral (when honey is a blend of nectar collected from more than one plant species) (Mendes, 2009). Several types of honey have been used for centuries for its nutritional and medicinal properties (Viuda-Martos *et al*., 2008; Ferreira *et al*., 2009; Socha *et al.,* 2009; Peterson; Dwyer, 1998; Liberto *et al*., 2013).The chemical constituent and biological properties of honey depends on the nectar source. As a result, honey from different geographical locations with different source of nectar has different nutritional and biological properties (Wang Li, 2011).

Honey used as food and medicine to humanity is well known since ancient times. Our ancient scriptures, The Vedas (3000-2000BC) contain many references to both honey and the bees. In Central India Rock paintings dating back to Mesolithic period 15,000-11,000 BC have also been found to depict honey hunting in the country. Despite this fact, its use as high energy carbohydrate food has not yet been realize in India. One kg of honey has been estimated to give about 3,150 calories which are equal to 8kg of plums or 12kg apples or 13 kg milk or 19kg of green peas or 65 eggs (Abrol, 1997). The per capita consumption of 8.4g honey per annum in India in comparison to 1.5kg in Europe is extremely low (Mishra, 2002).

Beekeeping has great potential for the upliftment of national economy. The Indian subcontinent is ethnically, floristically and agriculturally very diverse (Arora, 1990). At present China is the world's largest exporter of honey. In India, honeybees are estimatedto be availing about one-fourthof the available floral resources, therby having vast scope for extension of this enterprise. The states of Jammu and Kashmir and Himachal Pradesh which fall in the north-west Himalaya are not only rich in floristic composition and panoramic views but also have varied pasturage availability in different agroclimatic conditions for sustaining bee colonies for increased honey production (Kaul, 1997; Chowdhery & Wadhwa, 1984; Polunin & Stainton, 1984). For the Indian perspective, the literature on honey plants (bee flora) is not focused, except a very few relevant accounts of Kumar & Chowdhary (1993), Mishra & Kumar (1987) and Mishra & Kaushik (1992) who enlisted honey plants of the country from North Hill, Indo-Gangetic, North-Eastern and Peninsular regions. For the region of north-west Himalaya, notable contribution on honey flora are those of Singh & Singh (1971) and Saraf (1972)

on Kashmir; and Sharma & Raj (1985) Garg (1989); Sharma (1989); Sharma & Gupta (1993) on Kangra Shivaliks, Paonta valley, Himachal Pradesh and Solan, respectively, whereas Partap (1997) enlisted more than 200 promising honey plants of the Hindu Kush Himalaya covering mountainous areas of Afghanistan, Bangladesh, Bhutan, China, India, Myanmar, Nepal and Pakistan. Researchers believe that the original habitats of the honey bee are tropical climate and heavily forested areas. Honey bees can thrive in natural or domesticated environments, though they prefer to live in gardens, woodlands, orchards, meadows and other areas where flowering plants are abundant. Within their natural habitat, honey bees build nests inside tree cavities and under edges of objects to hide themselves from predators. The species of bees responsible for all the benefits to man are varied. However, the predominant honeybee species in Africa is the African honeybee *Apis mellifera adonsonii* and it is well adapted to the African ecological conditions and produces several honey crops a year. It gathers its own food freely throughout the year and there is little need to feed it (Adjare, 1990) especially in the savannah regions.

In order to collect data of interest for the preparation of an exhaustive database on honey plant resources (bee flora) of north-east Himalaya, the procedure delineated by Schulted (1962) and Jain (1964, 1967) was followed to scrutinized the old literature (herbals, books, monographs, ancient treaties, floras, compendia, Materia medica, research journals), and the resultant information was compiled and collected in a set format designed by modifying that of Saklani and Jain (1994). For other aspect of information on synonyms, vernacular names(s), distribution, flowering and fruiting, botanical description and active constituents, the works of Hooker (1892), Kirtikar &

Basu (1984), Chopra *et al*., (1968), Rastogi and Mehrotra (1995), Asolkar *et al*., (1992), Bhattacharjee (1998), Sood *et al*., (1992) and Sood & Thakur (2004) have been consulted and referred.

Apidae are pollinators of many wild and cultivated plant species and as such play an important role in the conservation of plants species and whole ecosystems (Corbet *et al*., 1991; Kevan, 1999; Kratochwil, 2003; Klein *et al*., 2007; Kremen *et al*., 2007). Bees are social animals and use pheromones for communication among members of the same species, for mating and locating food and the most favourable environment for their survival. Flowers are the mainstay of bee's life as honeybee are dependent upon a wide variety of wild/cultivated plants for their requirement of pollen and nectar, constituting raw materials of the bee keeping industry which as a profession means rearing honey bees for the production of honeys, hive products and for crops pollination (McGregor, 1976; Partap and Verma, 2000; Mishra and Kumar, 2002). The foraging activities of plant pollinators like honey bees is known to enhance the performance of a cross pollinated crop in a kind of mutualistic relationships and bees are the most important pollinator taxon (Greenleaf *et al.*, 2007; Gomez *et al*., 2007; Sahli and Conner, 2007).

In India beekeeping is considered a side business and each bee keeper often handles quite a low number of colonies as compared to his counterpart in other countries. Till 1962, beekeeping in India was solely practiced with indigenous honeybee, *Apis cerana*, thereafter exotic bee *A.mellifera* was successfully introduced in the country (Gupta & Dogra, 2002). The concept of efficient beekeeping comprises of managing honeybee colony in such away as to obtain maximum colony population to

coincide with the period of major honey flow in an area and utilize the population for honey production and pollination (Abrol,1997).

A beekeeper should know about the qualities of good flora. These are: 1) long flowering period; 2) high density of flowers per unit area of the plants; 3) good quality of nectar with high concentration of sugars; 4) easy accessibility of the nectarines to the honey bees and ease in collection of nectar; and 5) availability of honey plants in the close vicinity of the apiary so that extra energy is not spent for search and collection of food by the bees (Mishra & Kumar, 2002). The speed of foraging activity (rate at which bees visit flowers) depends upon the amount of nectar and pollen present, type of flowers, stage of development, climatic conditions (temperature, light intensity, radiation, time of the day, dew drops on flowers, wind velocity) and the number of competing insects. For foraging, the optimum temperature is between 13°C and 38°C, and the pollen collection trips are usually shorter than nectar collection one (Abrol, 1997).

Studies in developed countries carried out by Noeller and Koval (1973) and Nye and Anderson (1974) have shown that honeybee pollination increased fruit set by 10 to 25 percent and fruit yield by 18 to 100 percent depending upon the cultivator. This knowledge will be vital to prospective commercial bee keepers and policy makers willing to incorporate beekeeping in programmes aimed at reducing unemployment and poverty (Abdullahi *et al*., 2011). Thus identification of bee foraging flora including their abundance, distribution and phenological information is essential for good yield of honey (Shivaram, 1995). Insecticides, herbicides and cultural practices had been reduced or eliminated the wild population of insects (Kearns *et al.,* 1998)-until the point that are

not enough to pollinate the commercial crops. This is of economic importance, and farmers should therefore consider enhancement of bee populations as part of their field management (Ricketts *et al.,* 2004), this could be done by a reduced use of pesticides and by improving pollen and nectar availability for bees (Klein *et al*., 2003). Scented wild flowers (Dieringer and Cabrera 2002; Bernhardt *et al.,* 2003), ornamentals (Corbet *et al*., 2001) and blooming crops (Cane and Schiffhauer 2003) with a pollen and nectar reward act as a lure for several pollinator insects.

2.2. Melissopalynological study done abroad, India and North- East India

Pfister (1895) at the end of nineteenth century examine the pollen content of various Swiss, French and other European honeys. The first scientific investigation of U.S. honey begins in the early 1990's by W.J. Young who published a brief report on the analysis of domestic honey produced in the United States (Vaughn and Bryant, 2001). Parker (1923) conducted a study on U.S. honey, where he described 28 different kinds of polen collected by honey bees. Zander stands out as being the leader in melissopalynology research in Europe during 1930s and 1940s by publishing works over a span of nearly two decades (Zander, 1951). Maurizio and Louveaux (1965) referred Zander as the leader in melissopalynology research in Europe.

One of the earliest melissopalynological works in India was done by Deodikar and Thakur (1953), some works were done over honey analysis form Kumaon Himalayan region, Uttar Pradesh by Chatur bedi (1983); from Western Ghats by Suryanarayan *et al*., (1981); from Andra Pradesh and Tamil Nadu by Agashe and Mary (1995); Mukhopadhyay *et al.,* (2007) analyzed the pollen content of 52 honey samples

from the eastern Himalayan part of West Bengal; Sahney and Seth (2012) from Rewa district of Madhya Pradesh; Chakraborti and Bhattacharya (2014) from honey samples of West Bengal. Bera *et al*., (2007) carried out an analysis of honey samples collected from Kamrup reserve forest of Assam; Sharma & Saharia (2011) analyzed pollen from Kamrup District, Assam and Nagaland. The microscopic analysis of honey was taken up and developed in a wider scale in different countries of the world (Maurizio 1979; Varies *et al*., 1982; Parent *et al*., 1990) and (Louveaux and Abed 1984) in Africa.

Microscopical analysis of pollen of plants forged by bees is an established method to determine the source of honey in the area. Earlier several studies on pollen morphology been done worldwide (Raj, 1969; Sowunmi, 1973; Tomb *et al*., 1974, Nair and Kapoor, 1974; Gill and Chinnappa, 1982). Kral (1992) has made palynological investigation of forest trees in relation to forest history and natural mixture of tree species on the basis of their pollen profile. Noor *et al*., (2004) has done the palynological studies of cultivated plants of Rawalpindi, Pakistan. Adekanmbi and Ogundipe (2006) described the pollen morphological of 20 cultivated plants of Nigeria. Perveen and Qaiser (2010) conducted the pollen studies of the family Moringaceae and Berberidaceae. Several taxonomists identify the plant species on the basis of phenotypic character of plant. But now the pollen morphological studies can provide a basis for the identification of plant species. An interest in pollen morphology has increased its full application in systematic, paleobotany and allergy has been recognized Noor *et al*., (2004). Another reason that pollen analyses of honey are required is to identify geographical source of origin (Vaughn and Bryant, 2001). Western Ghats of Karnataka has a great diversity of flowering plants and has good potential for commercial

beekeeping (Shubharani *et al*., 2013). The pollen grains are also good indicators of the botanical and geographical origin of bee products (Borges *et al.,* 2006). This work aims to identify the botanical origin of the pollen loads collected by *Apis cerana* L. in Mizoram, and to give scientific support to local cooperatives of beekeepers by indicating important plants for the development of regional apiculture, through identification of pollen types.

2.3. Molecular work

Some methods have been proposed for the determination of botanical and geographical origin of honey. The traditional approach consists of the microscopic examination of pollen present in honey (Mellisopalynology) (Anklam, 1998). However this method is very tedious to implement, and requires a considerable amount of training. Several chemical methods have also been proposed, such as aroma compounds (Cuevas *et al*., 2007), free amino acids (Hermosin *et al*., 2003) or minerals and trace element (Fernandez *et al.,* 2005). Even if all this methods work well for identifying the geographic origins and for distinguishing honey with different botanical origins, they provide only limited information on the plants composition. With advances in molecular genetics, techniques based on DNA have become the method of choice since they tend to be quick, precise and reliable. Pollen and other plant derived component present in honey can serve as the source of DNA, which can be used for the botanical origin of honey using DNA technology. A recent study has demonstrated that a molecular genetics can be used for analyzing the composition and geographical origin of honey (Laube *et al*., 2010). It was studied that *trn*L approach is fast, simple to implement, does

not need special skills for the analysis and is more robust than classical methods (Taberlet *et al*., 2010). CTAB and SDS based methods are the two most widely used methods for extracting DNA from plants (Bruni *et al*., 2015).CTAB- based DNA extraction method by Diversity Array Technology (2011) was utilized for the extraction of DNA from honey.

CHAPTER 3

STUDY AREA

3.1. General introduction to Mizoram

Mizoram lies in the northeastern region of India with an area of 21,087 sq.km with 21° 58’ N Latitude and 28°35’N and longitude 92°16’E and 93°29’E.Longitude. The state is border by Assam and Manipur in the north, Chin hills in the west, Tripura in the west and international bordered by Myanmar in the east and Bangladesh in the west. The simple classification of forest in Mizoram falls under three categories Tropical wet evergreen forest, Tropical semi evergreen forest and Montane sub tropical forest.

The climatic condition in the state with well distributed rainfall of 1900mm to 3000mm spread over 8 to 10 months in the year and location in tropic zone with various soil types have contributed to the occurrence of a wide spectrum of rich and varied flora and fauna. These natural features and resources also offer opportunities for growing variety of horticultural crops. Agriculture is the main stay of the people of Mizoram, more than 70% of its population is engaged in agriculture. The age old practice of jhum cultivation is followed by a large number of people living in rural areas.

3.2. Introduction to Aizawl and Champhai district

The region is situated in the extreme end of the Himalayan ranges in the North eastern part of India. The region has predominantly mountaineous terrain of tertiary origin, mountain ranges run in North to South direction intercepted by narrow deep

valleys and in numerable small hillocks. The slope gradient are very steep, major river flow either in northerly or southerly direction. The geology of the region consist of sandstone and shales of tertiary age thrown into long folds, soils is young, immature and moderate to highly acidic. The region enjoy a moderate climate, tropical location and due to its high elevation it is neither very hot nor too cold throughout the year. The region falls under the direct influence of the Southwest monsoon with an annual average rainfall of 250cm. The region receives an adequate amount of rainfall by southwest monsoon, moderate climate, generally warm in summer, very cold in winter and humidity is relatively high nearly all the year. The area is under the influence of different forest types tropical wet evergreen, tropical semi evergreen and sub tropical hill forest. Agriculture is the main occupation of the region and more than 70% of the population lived in rural areas and practiced shifting cultivation.

Champhai district lies in the eastern part of Mizoram between 93.21°E longtitude and 23.26°N latitude. The minimum and maximum temperature is 0°C to 20°C during winter and 15°C to 30°C during summer. It covers an area of 3185 Sq.km The average annual rainfall is 1814 mm. Whereas Aizawl is the largest district in the state, but still very remote. Aizawl is located at 3715 feet from the sea level, weather in summer maximum 30^{o}C and minimum 20^{o}C and in winter maximum 21^{o}C and minimum 11^{o}C with rainfall 3,000mm and altitude of 1132 metres/3715 feet. The district occupies an area of 3576 sq. km^2. Aizawl lies just north of Tropic of cancer between 92.71°E longtitude and 23.72°N latitude.

Table 1.1: Geographical condition of study sites.

Sites	Geographical area (sq.km)	Summer	Winter	GPS Map	
				Latitude	Longtitude
AIZAWL	3576	20°C-30°C	11°C-21°C	23.72°N	92.21°E
CHAMPHAI	3185	15°C-30°C	0°C-20°C	23.45°N	93.32°E

3.3 Site of collection in Aizawl and Champhai District

Table 1.2: Site for collection for polliniferous plants and honey sample in Champhai district.

District	Sample ID.	Sample Location	GPS MAP	
			Latitude	Longitude
CHAMPHAI	C1	Hmunhmeltha	23.4°	93.2°
	C2	N.Khawbung	23.54°	93.31°
	C3.	Ruantlang	23.44°	93.34°
	C4	Zote	23.49°	93.35°
	C5	Khawzawl	23.37°	93.12°
	C6	Champhai Vengsang	23.47°	93.31°
	C7	Chawngtlai	23.44°	93.19°
	C8	Tlangsam	23.46°	93.34°
	C9	N.Champhai	23.45°	93.32°
	C10	Mualkawi	23.41°	93.33°

Table 1.3: Site for collection of polliniferous plants and honey sample in Aizawl district.

District	Sample ID	Sample Location	GPS MAP	
			Latitude	Longitude
AIZAWL	A1	Falkland	23.73°	92.74°
	A2.	Thuampui	23.74°	92.73°
	A3	Tanhril	23.74°	92.67°
	A4	Durtlang	23.79°	92.72°
	A5	Sihphir	23.81°	92.73°
	A6	Hlimen	23.77°	92.66°
	A7	Melthum	23.69°	92.72°
	A8	Maubawk	23.72°	92.69°
	A9	Sairang	23.80°	92.65°
	A10	Sakawrtuichhun	23.76°	92.67°

CHAPTER 4

METHODOLOGY

4.1 Survey and selection of sites for collection of honey

i) Collection of information from literature

Information and necessary protocol for the research was started by collecting literature, published paper and books for getting the idea of steps to be taken which was available locally. This information compiled in the file and useful protocol were noted and marked which serve as an aid making it easy in the field investigation during the research.

ii) Collection of plant, honey and field observation

First step for field work is to search bee keeper in two study sites of Champhai and Aizawl District for collection of information regarding polliniferous flower visited by bees and availability of honey for sample collection in a year. Formal and informal interviewed with the bee keeper and villagers, in the study area information about honeybee plants was collected from local beekeeping farmers. The farmers were selected randomly among the farmers that do bee keeping. The plants were categorized simply from the information obtained from the respondents based on their experiences. The purpose of asking the respondents is obtain information about the honey plants expected to be found in the study area. Honey sample were collected directly from beekeeper soon after they extract without dilution.

A general survey of the area and listing of the flowering plants, collection of the plants found were conducted for the period of two years.The bee forage field observation were made on different kinds of plants, which are visited by bees in the study area.

iii) Identification

Identification of polliniferous flowers and pollen found in honey slides were done with the help of relevant floras and standard literature (Hooker, 1989, Kanjilal *et al*., 1982 and Kanjilal *et al*., 1982, Sawmliana, 2013). Polliniferous flowers visited by bees were identified and confirmed its presence by extracting DNA from honey samples and DNA Barcoding.

4.2 Phenology of polliniferous plants

Flowering phenology among species varies in timing, duration, habitat as well as in year was observe seasonally. List of all important polliniferous species in study area were marked and noted. Stratified random sampling procedure were used in which sampling was carried out on randomly chosen transect of 1,000 m in length of the area. The start and end of each transect marked with a red flag. On each study visit, observation for bee foraging plants and honey bees done on four transects at two different locations within the sites to form sampling units. Every two of this transects cross the other at the centre (500metre) perpendicularly. Plant seen with flowers within a 50metre radius of each transect visited and observed for the presence of honey bees. The honeybee foraging behavior documented.

The success of foraging attempt ascertain, the plant be scored as bee foraging species after atleast three honey bees visits the flower simultaneously within the observation period of 10 minutes. The honey bee time of visitation was observed from 6:30 to 9:00 a.m and 2:00 to 4:30p.m in the selected sites. If the success of foraging attempt is not certain (i.e.when it is apparent that bees flew off from flowers to continue the search for suitable once) the plant is regarded as a known as non-honey bee foraging species (Abdullahi *et al.,* 2011). Observation in its sites repeated every month ensuring as much as possible that previously surveyed areas will be avoided. If a plant is recorded as bee foraging species at a particular site and later encountered in subsequent survey on other site, it is only score for present (observation for bee foraging attempts are not repeated on them). Sample of plant that could not be identified in the field, a small twig or portion of a branch of the plant with the full complement of its leaves and flowers brought to the laboratory for the identification. Flowering period provide information that related the flowering phenology to the bee keeping cycle.

4.3. Melissopalynological study

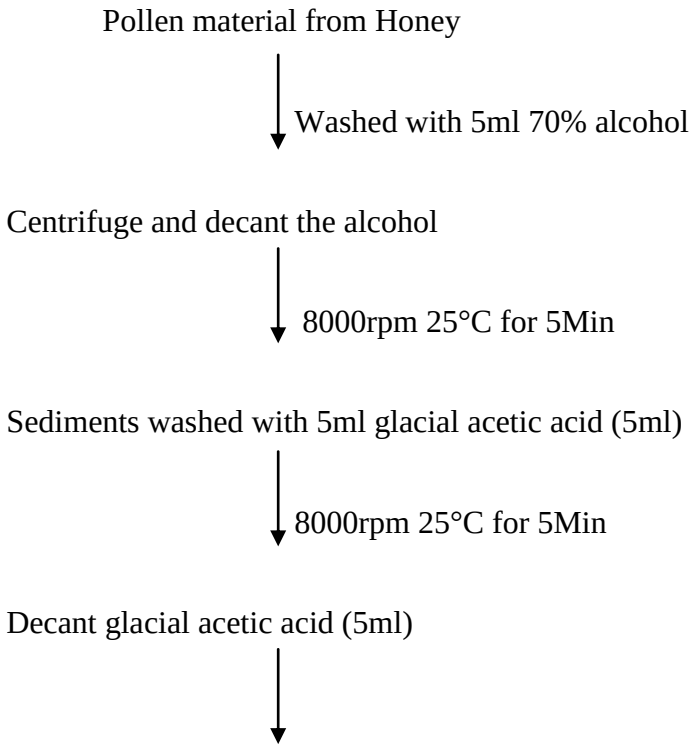
The preserved honey samples was prepared by acetolysis method according to Erdtman (1960) for light microscope which involve the introduction of acetolysis mixture comprising acetic anhydride mixed with concentrated sulphuric acid in the ratio 9:1. The tube were immersed in boiling water bath for 5 min, centrifuged and supernatant decanted. The residue was washed water and decanted, and about few drops of glycerin was added and mounted on slide (Subharani *et al*., 2013). Likewise fresh flower of known plant pollen slides was prepared according to same Acetolysis method as

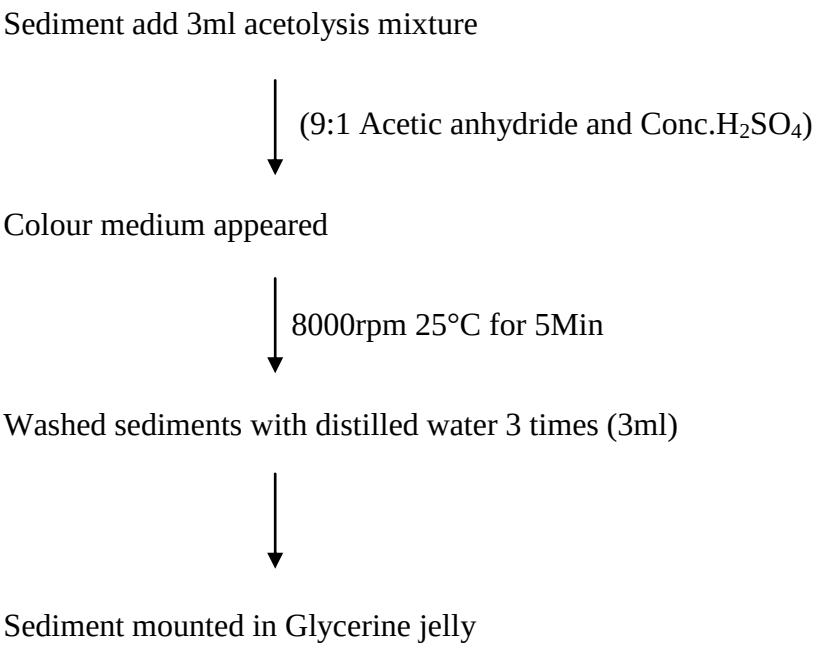
reference for identification (Azzedine *et al*., 2007).The prepared was studied under light microscope to identified pollen and photograph was taken to study pollen morphology.

4.3.1 Preparation of pollen slides from honey: Acetolysis method (Erdtmann, 1960)

1ml of honey sample was taken in a test tube and diluted to 10ml by hot distilled water of 40°C. The diluted honey is sieved through a mesh of 100µm. The suspension thus obtained was centrifuged at 3000rpm for 5minutes. The supernatant was decanted. The pellet of pollen sediment was subjected for acetolysis (Erdtmann, 1960). Pollen grains examined and identified under the microscope. Percentage occurrence of pollen was used to determine their frequencies following the system adopted by Louveax *et al.*, (1978) for determining the major and minor honeybee plants.

Acetolysis method is outlined below.





4.3.2 Preparation of pollen slides from flowers

For identification of pollen grains isolated from honey and bee pollen, pollen slides were prepared as reference slides. For this, anthers separated from filaments were put in distilled water and crushed with a glassrod. It is the sieved through a mesh of 100µm size. The pollen suspension was centrifuged at 3000rpm for 3min and decanted the supernatant. Pollen was subjected for acetolysis (Erdtmann, 1960). Five pollen slides were prepared for each honey sample and the pollen types were identified with the help of reference slides, examined and identified under the microscope. For quantification of pollen types, a total of 300 pollen grains were randomly counted all the five slides prepared from each sample.

4.3.3 Pollen spectrum study

After examining under microscope the pollen grains were identified using local floras and confirmed by comparing pollen types with reference pollen slides. Based on

the frequencies of pollen grain in various honey samples the pollen count and percentage of pollen types were calculated and pollen spectra were prepared. These pollen types were classified based on the recommendation of the International Commission for bee-Botany (Louveaux, 1978: Moore and Webb, 1978) i.e., “secondary pollen type (S)” (16-45%), “important minor pollen type (I)” (3-15%) and “minor pollen type (M)” (<3%).

4.4 Molecular approach

4.4.1 DNA isolation from honey samples

Honey (3ml; which might contain pollen cells derived from the plant DNA) was dissolved in 1ml sterile water and incubated at 65°C for 30 min, followed by centrifugation at 5000 rpm for 10 min. The supernatant was discarded, and the pellet was dried for 5 min at room temperature and dissolved in 500 µl extraction buffer (100 mM Tris-HCl, 50 mM EDTA, 50 mM NaCl, 10% SDS, pH 7.5). Sterilized glass beads 0.5 g (diameter 0.5–1 mm) were added, and the pellet was ground with a glass rod for 5–10 min. DTT (100 µl; 110 mM) and 10 µl proteinase K (10 mg/ml) were added, mixed by gentle inversion, and incubated at 56°C for 1h, followed by addition of 500 µl cetyltrimethyl ammonium bromide (CTAB) extraction buffer (20mM Tris-HCl, pH 8.0, 10mM EDTA, pH 8.0, 10% CTAB, 5% polyvinylpyrrolidone), 10 µl proteinase K, and 50 µl DTT and incubated at 65°C overnight in water bath. Phenol-chloroform-isoamyl alcohol (500 µl) was added and centrifuged at 10,000 rpm for 10 min. The supernatant was transferred to a 2 ml Eppendorf tube, followed by adding 500 µl isopropanol and 100 µl sodium acetate (3 mM) and kept in -20°C for 1 h for precipitation. The sample was centrifuged at 13,000 rpm for 10 min at 4°C, and the supernatant was mixed with

400 µl 70% ethanol and subsequently incubated at -20°C for 15 min. The sample was centrifuged at 13,000 rpm for 10 min at 4°C, the supernatant was poured off, and the pellet was dried in oven. Millipore water (30 µl) was added to the tube and mixed gently (Ralte *et al.*, 2014).

Procedure:

Step 1 :

3ml of honey + 1ml of water

⇩ Incubate 65°C for 30min

10,000rpm for 15min

⇩ Add Extraction Buffer – 1

Crush with glassrod add glassbeads for 5min

⇩ Add 100µL DTT +10µL Proteinase K

Incubate 56°C for 1hr

⇩ Add Extraction Buffer-11

CTAB (10µL)+PVP (10µL)+DTT (100µL)+10µL Proteinase K

⇩

Incubate 65°C overnight

Step 2 :

Take out from incubation overnight at 65°C

⇩ 12,000rpm,10min+PCI (500L)

Take clear upper layer in 2ml eppendorf tube

⇩ Isopropanol(500µL)+Sodium acetate1/10th vol.

-20°C for 1hr for precipitation

⇩ 13,000rpm, 10min, 4°C

Decant supernatant, add 400µL 70% ethanol

⇩ -20°C for 15min

13,000rpm,10min,4°C

⇩

Supernatant pour off and let it dried in oven

⇩

Elute with 30µL MilliQ water added and mixed well

DNA quality confirmation

Yield and DNA purity were checked by using a Cary 60 UV-Vis spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The quality of the extracted DNA was checked by using a 0.8% agarose gel in 1x Tris-acetic acid-EDTA buffer at 80 V for 30 min, stained with ethidium bromide. Images were obtained in a G:BOX gel documentation system (Syngene, Cambridge, UK). Confirm DNA quality, presence of a highly resolved high molecular weight band indicates good quality DNA and presence of a smeared band indicates DNA degradation.

Table 1.4.: Quantity and Quality of the DNA Ratio of absorbance at 260/280nm extracted from Honey Samples of A1–A10 and C1-C10

Sample	DNA yield ng/µl	DNA purity A260/A280	Sample	DNA yield ng/µl	DNA purity A260/A280
A1	20	1.82	C1	26	1.75
A2	23	1.79	C2	42	1.61
A3	43	1.67	C3	31	1.73
A4	45	1.62	C4	28	1.80
A5	27	1.79	C5	42	1.68
A6	29	1.71	C6	34	1.67
A7	35	1.72	C7	44	1.77
A8	36	1.68	C8	37	1.67
A9	32	1.84	C9	40	1.75
A10	25	1.60	C10	21	1.63

4.4.2 PCR amplification

PCR was performed with a set of primers amplifying a fragment of the plant cpDNA maturase K (*mat*K) and *rbc*L universal barcoding region (Table 1). The PCR products (10 µl) were subjected to electrophoresis using a 1.2% agarose gel, following the same conditions as above. PCR products were purified using a Qiagen gel extraction kit (QIAquick columns; Qiagen, Chatsworth, CA, USA) and stored at -20°C until sent for sequencing to SciGenom Labs (Cochin, India). The expected band for *mat*K and *rbc*L were 900bp and 700bp.

Table 1.5 : Primer used for amplification

Sl.No	Gene	Primer sequence	Reference
1.	*matK*	F- CGATCTATTCATTCAATATTC R- TCTAGCACACGAAAGTCGAAGT	Taberlet *et al*. 2007
2.	*rbcL*	F- CTGTATGGACCGATGGACTTAC R-CGGTGGATGTGAAGAAGTAGAC	Taberlet *et al*. 2007

PCR Amplification for *mat*K :

1. Initial denaturation : 95°C - 5mins
2. Denaturation : 95°C - 40 sec
3. Annealing temperature : 49.5°C- 40 sec
4. Extension : 72°C - 60 sec

} 35 cycles

5. Final extention : 72°C - 5min

PCR Amplification for *rbc*L :

1. Initial denaturation : 95°C - 5min
2. Denaturation : 94°C - 40 sec
3. Annealing temperature : 60°C - 40 sec
4. Extension : 72°C - 1min

} 35 cycles

5. Final extention : 72°C - 5min

Table 1.6 :PCR Reaction : *mat* K

Reaction	Stock Conc.	Working Conc.	Reaction volume(20µL)
Taq Buffer	10X	1X	2µL
MgCl2	25mM	1.5mM	1.2µL
dNTP	10mM	0.2mM	0.4µL
Taq DNA Polymerase	5U	1U	0.2µL
Forward Primer	10pmol.	0.25pmol.	0.5µL
Reverse Primer	10pmol.	0.25pmol.	0.5µL
Nuclease free water			Made upto 20µL

Table 1.7 : PCR Reaction : *rbc*L

Reaction	Stock Conc.	Working Conc.	Reaction volume(20µL)
Taq Buffer	10X	1X	2µL
MgCl2	25mM	1.5mM	1.2µL
dNTP	10mM	0.2mM	0.4µL
Taq DNA Polymerase	5U	1U	0.2µL
Forward Primer	10pmol.	0.25pmol.	0.5µL
Reverse Primer	10pmol.	0.25pmol.	0.5µL
Nuclease free water			Made upto 20µL

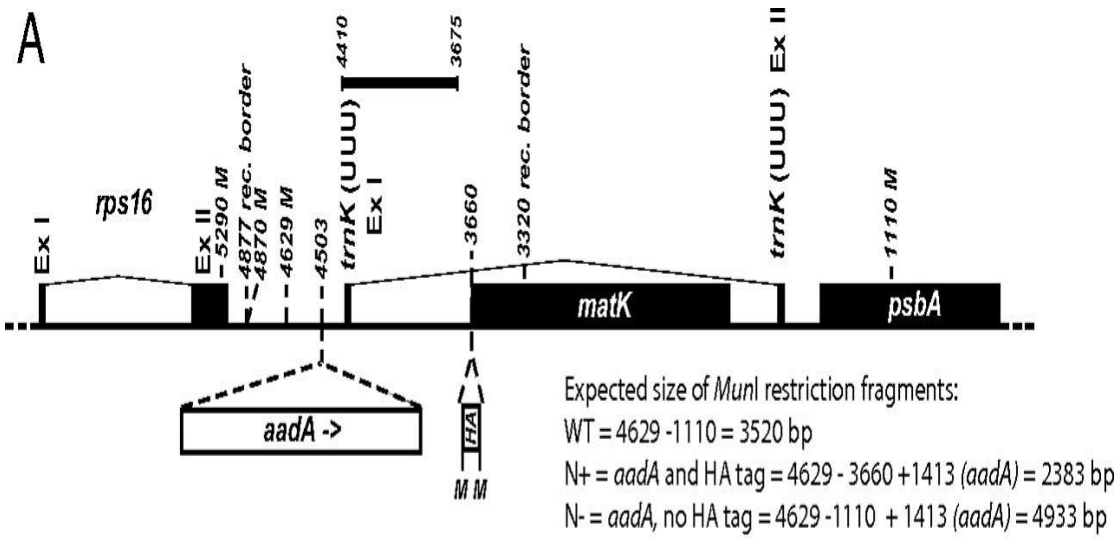


Figure 1.1 : Gene structure of *mat*K

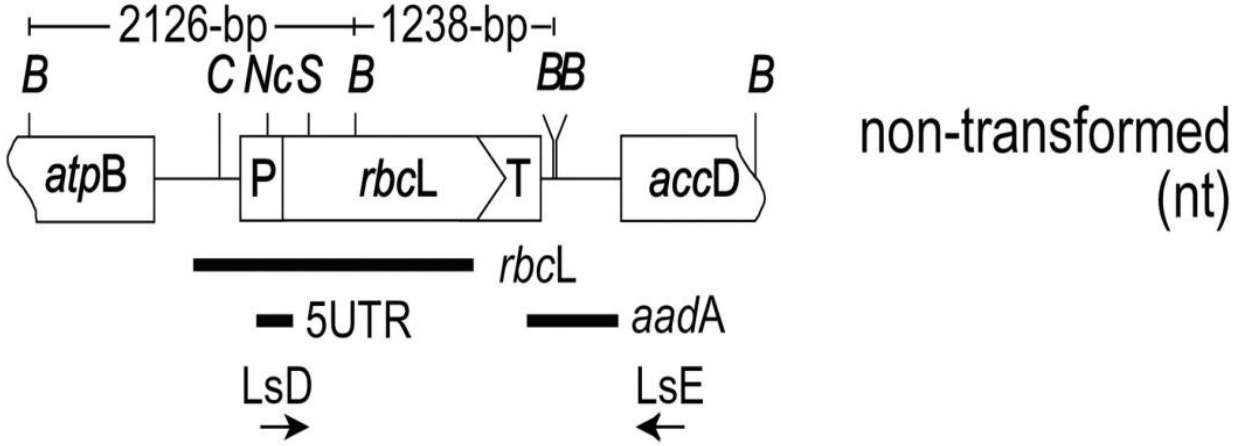


Figure 1.2: Gene structure of *rbcL*

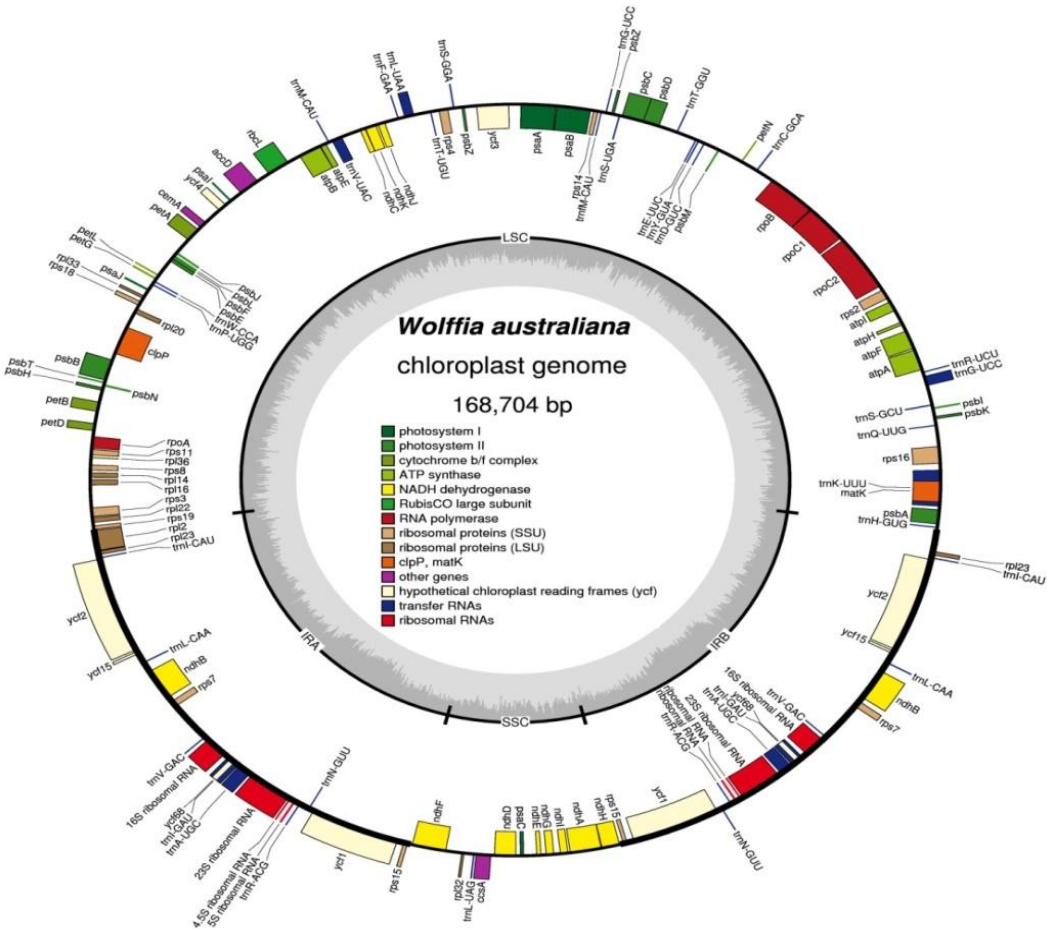


Figure 1.3 : Chloroplast genome - *mat*K, *aac*D, *atp* F-*atp*H, *nhd*J, *psb*K-*psb*I, *rbc*L, *rpo*B, *rpo*C1, *trn*H-*psb*A

4.4.3 Sequence analysis

The sequence file was annotated based on the chromatogram and BLAST results. Reference and query sequence alignments were simulated using a readily available pipeline in the National Center for Biotechnology Information (NCBI) BLAST program. Genetic distance was used to assign an identity to each query sequence, and the ID of the plant species was associated with the best BLAST hit and E-value < cutoff. This corresponds to choosing the top hit in the BLAST results. The

CHAPTER 5

RESULT

5.1 Polliniferous plants growing in the study area

Plants species visited by bees for foraging were recorded from the study area and 76 species belonging to 35 family useful to honey bee. Information on phenological studies of all the polliniferous plants described and represented with photo plates (Plate 3 to 28). The pollen morphology varies among different plant species occur in varying in shapes, forms and in symmetry cited in photo plate (Plate 3 to 28)

5.1.1 Phenological study of polliniferous plants

1. *Acacia pruinescens Kurz.*

Family - Fabaceae

English name - -

Local name - Khangpawl

Habit - Shrub

Flowering time -April-May

Colour of flower - White

Description - A large climber armed with recurved prichles, branchlets covered with a whity waxy bloom. Leaves pinnately compound arranged in pairs. Inflorescence head. Fruit is a legume.

Pollen morphology - Isopolar, syncolpate, 3-colporate, colpi broad and granula membrane, radial symmetry

2. *Ageratum conyzoides* Roxb.

Family - Asteraceae

English name - Goat weed, White weed

Local name - Vaihlenhlo

Habit - Herb

Flowering time - May to August

Colour of the flower - Violet

Description - A hispidly hairy annual herb. Leaves simple, alternate. Flowers is small, dense terminal corymbs, ray florets many, violet in colour. Fruit achenes black, pappus 5 awned.

Pollen morphology - Spinulous, prolate, echinate, radial symmetry

3. *Alnus nitida* Endl.

Family - Betulaceae

English name - Nepal alder

Local Name - Hriangpui

Habit - Tree

Flowering time - September to November

Colour of the flower - Pink

Description - A large deciduous tree upto 30m. Leaves alternate, simple, elliptic ovate with prominent veins. Inflorescence is catkins with the male and female flowers separate but produced on the same tree, the male flower are long and pendulus while the female flowers are erect.

Pollen morphology – Oblate, sherical, triangular, surface scabrate.

4. *Althaea rosea* L.

Family - Malvaceae

English name - Common hollyhock

Local Name - Anthur

Habit - Herb

Flowering time - January-December

Colour of the flower - Pink

Description - Annual or short lived perennial herbs. Leaves are simple, opposite and more or less deeply divided. Flowers are pink in coloured peduncled, axillary and in long terminal racemes. Seed solitary in each carpel.

Pollen morphology - Monocolpate, polyporate,

5. *Anthurium andreanum* Lindedn ex Andre

Family - Malvaceae

English name - Anthurium

Local Name - Anthurium

Habit - Herb

Flowering time - January to December

Colour of the flower –Red

Description - An evergreen perennial herb with heartshape leaves, leaves simple palmately lobed. Flower complete, bisexual, actinomorphic, fruit a capsule.

Pollen morphology - Oblate, colpate, prolate, oralolongate, brevicolpi, bilateral symmetry

6. *Antigonon leptopus* Hook. & Arn.

Family - Polygonaceae

English name - Maxican creeper or Coral vine

Local Name - Parsennote

Habit - Shrub

Flowering time - May to July

Colour of the flower – Pink

Description – Fast growing climber that holds with tendrils. It forms underground tubers and large rootstocks with prolific seed producer. It has cordate (heart shape) sometimes triangular leaves. Flower are borne in panicles, clusted along the rachis with pink flowers.

Pollen morphology - Striated surface, exine reticulate, oblate shape, bilateral symmetry

7. *Amaranthus sp.* L.

Family - Amaranthaceae

English name - -

Local Name - Zamzo

Habit - Herb

Flowering time - August

Colour of the flower - Pink

Description - Erect, glabrous annual herb. Leaves long petioled, lance shape, leaf axils with 5 spines. The plant is monoecious, with individual bearing

male and female flowers. The inflorescence is a large, dense cluster of flowers interspersed with spiny bracts. The fruit is capsule with black seed.

Pollen morphology - Sherical shape with polypantoporate

8. *Asclepias curassavica* L.

Family - Asclepiadaceae

English name - Milkweed

Local Name - Dingdi

Habit - Herb

Flowering time - July to October

Colour of the flower - Orange

Description - Perennial erect evergreen herbs. Leaves simple, alternate and linear oblong, pointed at both end, umbelliform cymes. Flowers small, complete, actinomorphic, corolla rotate, naked. Fruit follicle, seeds ovoid.

Pollen morphology – Decussate, tetrahedral, rhomboidal in shape, pores are round or oval.

9. *Averrhoa carambola* L.

Family - Oxalidaceae

English name - Star fruit

Local Name - Theiherawt

Habit - Tree

Flowering time - June

Colour of the flower – Orange

Description - Small tree, bushy evergreen with drooping branch. Leaflets are oval or ovate in shape. Flowers complete, hermaphrodite, actinomorphic, bears orange colour flowers. Fruit a capsule, yellow fruits and waxy.

Pollen morphology - Trizonocolpate, subprolate reticulate

10. *Bauhinia variegata* L.

Family - Fabaceae

English name - Orchid tree

Local Name - Vaube

Habit - Tree

Flowering time - February to April

Colour of the flower - White

Description - A moderate-sized deciduous tree. Leaves simple,alternate, inflorescence raceme, with white flowers, petals white, beautifully variegated. Fruit a pods, hard, flat, glabrous.

Pollen morphology - Tri-periporate prolate-spheroid, reticulate ora-lolongate, bilateral symmetry

11. *Bidens pilosa* L.

Family - Asteraceae

English name - Beggar's stick

Local Name - Vawkpuithal

Habit - Herb

Flowering time - June to July

Colour of the flower - White

Description - An erect wild herbs. Leaves simple, opposite. Inflorescence head on long stout peduncles, involucres bracts with broad scarious margins. Fruit achens, slender black.

Pollen morphology - Tricolporate with echinate ornament.

12. *Bombax ceiba* L.

Family - Bombacaceae

English name - Red cotton tree

Local Name - Phunchawng

Habit - Tree

Flowering time - January to March

Colour of the flower - Red

Description - Large decidous tall tree, Its trunk bear spikes to deter attacks by animals. Leaves are palmate with leaflets radiating from a central point with a long petiole. Flowers cub shaped, solitary or clustered, axillary or sub-terminal or near the ends of the branches and flower red in colour.

Pollen morphology - Colporate, prolate, per-oblate, bilateral symmetry

13. *Brassica camprestris* L.

Family - Brassicaceae

English name - Indian mustard

Local Name - Antam

Habit - Herb

Flowering time - February to June

Colour of the flower -Yellow

Description - An erect, stout, simple, branched, globrous, slightly hispid cultivated annual herb upto 1m high leaves simple, alternate with sessile or petiole, more or less pinnitifid, upper oblong or lanceolate. Inflorescence raceme, flower large, bright yellow. Fruit capsule, seeds small, smooth, pale or dark.

Pollen morphology – Sub prolate to prolate rarely prolate-spheroidal, ornamentation medium reticulate.

14. *Caesalpinia pulcherrima* L.

Family - Fabaceae

English name - Peacock flower/Dwarf gold mohur

Local Name - Aprilte

Habit - Shrub

Flowering time - March to July

Colour of the flower - Orange

Description - Shrub growing to 3m tall and is semi evergreen. The leaves are bipinnate bearing 3-10 pairs of pinnae, each with 6-10 pairs of leaflets. The flower are borne in racemes upto 20cm long, each flower with 5 yellow, orange petals. A fruit is a pod 6-12 cm long.

Pollen morphology - Isopolar, syncolpate, 3- colporate, colpi broad and granula membrane, radial symmetry

15. *Callicarpa arborea* L.

Family - Verbenaceae

English name - -

Local Name - Thingkha

Habit - Tree

Flowering time - May to July

Colour of the flower – Violet

Description - Tall tree evergreen, leaves simple, decussate, exstipulate, oblong elliptic, margin entire, opposite. Inflorescence and flowering stalk are densely velvette hairy. Purple flowers are borne in cyme. Fruit is a drupe.

Pollen morphology – Tricolporate , oblate, spheroid shaped with bilateral symmetry.

16. *Callistemon lanceolatus* Sweet.

Family - Myrtaceae

English name - Bottle brush

Local Name - Botol brush

Habit - Tree

Flowering time - February to June

Colour of the flower - Red

Description - A small evergreen tree having pendulous branches. Leaves alternate, linear lanceolate shaped. Inflorescence long spike with small crimson flowers having red coloured stamens with filaments projecting in varied direction. Fruit pod filled with small fine seeds.

Pollen morphology - Colporate, prolate, oblate-spheroid, obscure pattern, bilateral symmetry.

17. *Carica papaya* L.

Family - Caricaceae

English name - Papaya

Local Name - Thingfanghma

Habit - Tree

Flowering time - July to August

Colour of the flower – White

Description - Is a large tree like plant, with a single stem with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit are borne. The leaves are large, deeply palmately lobed. Flowers white, fruits large, black seeded, yellow or orange.

Pollen morphology - Tricolporate, finely reticulate.

18. *Cassia javanica* Roxb.

Family - Fabaceae

English name - Indian Laburnum, Purging cassia

Local Name - Makpazangkang

Habit - Tree

Flowering time - March to September

Colour of the flower – Pink

Description - Semi deciduous tree upto 25m in height, it has a straight trunk, the trunk frequently has many shoot. Leaves are peripinnate made upto 12 pairs of leaflets. Flowers are pale rose to crimson pink in colour and are found in open clusters. The fruit long cylindrical brown pods.

Pollen morphology - Isopolar, syncolpate, prolate sheroidal, radial symmetry

19. *Castanopsis tribuloides* (Sm.) A.DC.

Family - Fagaceae

English name - Chestnut

Local Name - Thingsia

Habit - Tree

Flowering time - August to November

Colour of the flower – White

Description - A middle sized, large evergreen tree. The leaves simple, tough lanceolate and entire. The flowers are unisexual, male flowers are borne in catkins, female flowers produced a single seed but are congregated in small clusters. The fruit is a calybium.

Pollen morphology – Radially symmetrical, iso polar, colporate.

20. *Citrus limon* (L.) Burm.f.

Family - Rutaceae

English name - Lemon

Local Name - Limbu

Habit - Tree

Flowering time - March to May

Colour of the flower – White

Description - A small tree. Leaves simple, alternate, entire and ovate. Young shoot purple with glabrous leaflets. Petiole winged. Inflorescence solitary

axillary, flower complete, actinomorphic. Fruits hesperidium, ovoid yellow in colour.

Pollen morphology - Radiosymmetric and isopolar

21. *Cocos nucifera* L.

Family - Arecaceae

English name - Coconut

Local Name - Coconut

Habit - Tree

Flowering time - January to December

Colour of the flower – White

Description - Palm to 27m tall trunk stout, rarely branched with fibrous root system. Leave large, entire, pinnate form a crown at the apex. Inflorescence spadix, produces the male and female flower in the same inflorescence and is monoecious. The female flower is much larger than the male flower, flowering occur continuously and is cross pollinated. The fruit is a drupe.

Pollen morphology - 1-colpate, elongate in shape, monad, reticulate, bilateral symmetry

22. *Coffee arabica* L.

Family - Rubiaceae

English name - Coffee

Local Name - Coffee

Habit - Shrub

Flowering time - September

Colour of the flower – White

Description - Evergreen globrous shrub or small tree upto 5m long. Leaves are opposite, simple, ovate to oblong, glossy dark green. The flowers are white and grow in axillary clusters. The seeds are present in a drupe contains two seeds.

Pollen morphology – 3-colporate, oblate, exine forming a ring, bilateral symmetry

23. *Coriandrum sativum* L.

Family - Apiaceae

English name - Dhania

Local Name - Dania

Habit - Herb

Flowering time - June to August

Colour of the flower – White

Description - Cultivated annual herbs. Leaves are simple, variable in shape, delicately branched. Inflorescence umbel compound, the white flower are tiny and borne in numerous umbel. Petals obovate, emarginate, white or purplish. Seed convexo-concave.

Pollen morphology - 3-Colporate, prolate, perprolate, bilateral symmetry.

24. *Croton jaufra* Roxb.

Family - Euphorbiaceae

English name - Rushfoil

Local Name - Valthi

Habit - Tree

Flowering time - February to March

Colour of the flower – White

Description - A small tree or shrubs with a few branch. Leaves simple, alternate, petiolate which are ovate, acuminate and serrate margin. Inflorescence terminal raceme, male flowers present in the upper region of the inflorescence, female flowers in the lower region of the inflorescence, straw colour petals. Fruit is a regma.

Pollen morphology – Inaperturate, retipilate, radial symmetry, clavateexine

25. *Cucumis sativus* L.

Family - Cucurbitaceae

English name - Cucumber

Local Name - Fanghma

Habit - Climber

Flowering time - May to July

Colour of the flower – Yellow

Description - It is a climbers that roots in the ground and grows with thin spiraling tendrils, stems angled and branched. Leaves simple, alternate, green, triangular, ovate and angled. Flower monoecious, pistillate flowers, axillary and solitary. Fruit fleshy pepo, yellowish- green and globrous. Seed flat, white, thick.

Pollen morphology - 3-zoniporate, subprolate, pores circular tenuimarginate

26. *Cucurbita pepo L.*

Family - Cucurbitaceae

English name - Pumpkin

Local Name - Mai

Habit - Climber

Flowering time - May to June

Colour of the flower – Yellow

Description - Climbing annual herb. Leaves simple, alternate, have 3-5 lobes with soft hairs, petiole often long as the blade. Inflorescence solitary axillary, monoecious, flowers yellow. Fruit a large fleshy pepo.

Pollen morphology - Porate, exine is reticulate, or retipilate, 8-10 radial symmetry

27. *Cyperus rotundus* L.

Family - Cyperaceae

English name - Nut grass

Local Name - Nubengchah

Habit - Herb

Flowering time - August to February

Colour of the flower – Green

Description - It is a perennial herbs with slender stolons hardening into wiry roots. Leaves narrowly linear, sheathing at the base, dark green above pale beneath. Inflorescence spikelets, flower is bisexual. The fruit is a three angled achenes, brown colour.

Pollen morphology - Aperture indistinct, pear shaped, 3-4 aperturoid areas, bilateral symmetry

28. *Cosmos sulphureus* Cav.

Family - Asteraceae

English name - Cosmos

Local Name - I love you par

Habit - Herb

Flowering time - November to December

Colour of the flower –Yellow

Distribution - Hardy annual herb. Leaves simple, opposite, pinnately divided. The flowers are produced in a capitulum in a ring of broad ray florets and a center of disc florets, hermaphrodites or monoecious. Fruit is a cypsela.

Pollen morphology – Rounded, triangular, prolate and tricorporate, aperture not very distinct.

29. *Derris robusta (D.C) Benth.*

Family - Fabaceae

English name - -

Local Name - Thingkha

Habit - Tree

Flowering time - April to June

Colour of the flower –White

Distribution \- A moderate sized deciduous tree with tall cylindrical bole and spreading crown. Leaves pinnately compound, leatlets 6-10 pairs. Flowers white in slender axillary pubescent racemes. Fruit a pod.

Pollen morphology – Triangular, smooth exine tricolporate.

30. *Elaeocarpus lanceifolius* Roxb.

Family - Elaeocarpaceae

English name - Himalayan olive

Local Name - Kharuan

Habit - Tree

Flowering time - May to June

Colour of the flower – White

Description - Evergreen tree upto 8m long, branchlet, globrous. Leaves simple, alternate spiral, cluster at twig ends. Inflorescence receme, flower petals white.

Pollen morphology - 3-colporate, prolate to prolate spheroid exine obscure, bilateral symmetry

31. *Emblica officinalis* Gaertn.

Family - Euphorbiaceae

English name - Emblica, Indian Gooseberry tree

Local Name - Sunhlu

Habit - Tree

Flowering time - March to April

Colour of the flower –Yellow

Distribution - A small to medium size deciduous tree. Leaves simple, alternate, globrous., stipulate, with small petiole. Inflorescence solitary, borne in axillary fascicles, male flowers are in the basal part of a branch and female flowers are in apical part. Fruit is called a capsule and 6 lobed globose.

Pollen morphology – Prolate, spheroidal or sub prolate and colporate.

32. *Euphorbia pulcherrima* Willd.

Family - Euphorbiaceae

English name - Christmas tree, Poinsettia

Local Name - X'mas par

Habit - Shrub

Flowering time - December to February

Colour of the flower – Red

Description - A shrub or small tree unarmed, soft wooden. Leaves simple, dark green dentate alternate, ovate, acute with soft red petiole, upper leaves clustered. Inflorescence cyathium with a single female flower and a number of male flowers, the bracts coloured, flowers is inconspicuous. Fruit a capsule.

Pollen morphology - 3-colporate, sheroid shape, furrow indistinct, reticulate, bilateral symmetry

33. *Eucalyptus tereticornis* Smith.

Family - Myrtaceae

English name - Blue gum, eucalyptus,forest red gum

Local Name - Nawalhthing

Habit - Tree

Flowering time - January to March

Colour of the flower – White

Description - Trees with large, erect trunk, smooth ash-coloured stem and long. Leaves simple, alternate, petiolate, falcate, lanceolate and green.

Inflorescence umbellate, clustered cyme, flowers occur axillary, pedicillate hermaphrodite. Fruit a capsule.

Pollen morphology - Colporate, prolate, oblate-sheroid, obscure pattern, parasyncoplate, bilateral symmetry

34. *Hibiscus rosa sinensis* L.

Family - Malvaceae

English name - China rose, shoe flower

Local Name - Mithipangpar

Habit - Shrub

Flowering time - January to December

Colour of the flower – Red

Description - Ornamental plants, bushy evergreen shrub or small tree, stem woody, branched, not prickly. Leaves simple, alternate, petiolate, glossy coarsely toothed at the apex and ovate in shape, margin is serrated. Inflorescence solitary axillary, flowers complete, actinomorphic, red in colour. Fruit is a capsule round and many seeded.

Pollen morphology - Pantaprolate, pores echinate, radial symmetry

35. *Holmskioldia sanguine* Retz.

Family - Lamiaceae

English name - Chinese hat plants/Lady's umbrella

Local Name - Sarawnte

Habit - Shrub

Flowering time - September to October

Colour of the flower – Red

Description - Erect shrub climber. Leaves simple, oval, decussate, opposite, petiolate. Inflorescence in racemes, bracts foliaceous, produce narrow trumpet shaped flowers with crimson petals and red sepals, broad circular calyx.

Pollen morphology - 3-colpate, oblate-sheroid shape, spilate surface, furrows are complex with 2 psedocolpi bilateral symmetry

36. *Ipomoea batatas* (L.)Lam.

Family - Malvaceae

English name - Sweet potato

Local Name - Kawlbahra

Habit - Climber

Flowering time - November to December

Colour of the flower –White

Description - Tuberous rooted perennial usually grown as an annual, stems, forming a running vine upto 4m, prostrate and slender. Leaves simple, alternate, lobed and stipulate. Inflorescence solitary axillary, flowers are white in colour, funnel shaped. Fruits is a pod, flattened and angular.

Pollen morphology - Pantoporate, pores, echinate, radial symmetry

37. *Ixora coccinea* L.

Family - Rubiaceae

English name - Jungle flame

Local Name - Mualhawihte

Habit - Shrub

Flowering time - April

Colour of the flower – Red

Description - Dense, multibranched evergreen shrubs. Leaves simple, sessile, glossy, oblong, with entire margin in opposite pairs or whorled on the stems.Inflorescence cymose, small tubular red flower, bisexual hermaphrodite in dense rounded cluster. Fruit a berry.

Pollen morphology - Colpate-prolate, oralalongate, radial symmetry

38. *Jatropha curcus* L.

Family - Rubiaceae

English name - Jatropha

Local Name - Kangdamdawi

Habit - Tree

Flowering time - March to April

Colour of the flower –White

Description - Large deciduous small evergreen or small tree, soft wooded plants. Leaves simple, alternate, broadly ovate, glabrous. Flowers male and female are produced in the same inflorescence. Flower in axillary cymes. Male flower elliptic, obtuse, imbricate. Female flower lobes ovate, acute. Ovary 3-celled. Fruit ovoid ,black.

Pollen morphology - Inaperturate, reticulate, gemmate exine,crotonoid pattern surface, radial symmetry

39. *Lagerstromia speciosa* (L.) Pers.

Family - Lythraceae

English name - Queen's flower

Local Name - Thlado

Habit - Tree

Flowering time - April to June

Colour of the flower –Violet

Description - Showy, large deciduous trees with globular crown and upto 17m tall. Leaves simple, alternate, broad elliptic, obtuse to long lanceolate. Flower scattered, petals margins erose-undulate, hardly fimbriate. Fruits large, seed light brown and angular.

Pollen morphology - Prolate punctitegillate ora circular

40. *Lantana camara* L.

Family - Verbenaceae

English name - Shillong tlangsam

Local Name - Wild sage, yellow sage

Habit - Shrub

Flowering time - March to September

Colour of the flower – Yellow

Description - A straggling aromatic shrub with recurved prickles on the branches. Leaves simple, ovate, opposite. Inflorescence umbellate cyme, flowers hermaphrodite, small tubular shaped, peduncled, calyx small, membranous. Fruit a drupe fleshy or nearly dry.

Pollen morphology – Tricolporate, reticulate, semi angular and sheroidal.

41. *Leucosceptrum canum* Sm.

Family - Lamiaceae

English name - Golden angel

Local Name - Koihthuang

Habit - Shrub

Flowering time - January to February

Colour of the flower – White

Description - A small evergreen trees. Leaves simple, elliptic lanceolate, papery, margin serrate and apex acuminate. Inflorescence verticillasters with white flower.

Pollen morphology – Porlate, sheroidal and tricolpate.

42. *Malvaviscus arboreus* Cav.

Family - Malvaceae

English name - Turkcap

Local Name - Palthing

Habit - Shrub

Flowering time - May to August

Colour of the flower –Red

Description - Freely branching shrubs. Leave simple, alternate, with slightly lobes downy green. Inflorescence solitary axillary and have showy flowers that have upright red petals, hermaphrodite, bisexual, petals overlapping to form a loose tube with the stamina column protruding. Fruit is indehisence schizocarp.

Pollen morphology - Pantoporate, echinate, sheriod, radial symmetry

43. *Mangifera indica* L.

Family - Anacardaceae

English name - Mango

Local Name - Theihai

Habit - Tree

Flowering time - March to April

Colour of the flower –White

Description - Tree is erect 30-100 feet with a broad rounded canopy. Leaves simple, spirally arranged on branch, linear oblong, lanceolate elliptical pointed at both end. Inflorescence occurs in panicles white colour flowers, flower hermaphrodite and bisexual. Fruit a drupe.

Pollen morphology - Colporate, psilateexine, radial symmetry

44. *Matricaria chomonulla* L.

Family - Asteraceae

English name - Scented mayweed

Local Name - Parvarte

Habit - Herb

Flowering time - April to May

Colour of the flower –White

Description - Annual herb, branched, erect and smooth stems. Leaves long narrow, bipinnate or tripinnate. Flowers are borne in capitula, ray florets are white and disc florets are yellow. Fruit cypsela.

Pollen morphology – Speroidal, porate, polyporporate and radial symmetry.

45. *Mikania micranta* Kunth.

Family - Asteraceae

English name - Bitter vine

Local Name - Japan hlo

Habit - Climber

Flowering time - August to December

Colour of the flower – White

Description - Climbing annual herb. Stem erect herbaceous. Leaves simple alternate, petiolate. Inflorescence racemose, head, ray florets and disc florets. Fruit cypsela.

Pollen morphology - Speroidal, porate, polyporporate and circular.

46. *Mimosa pudica* L.

Family - Fabaceae

English name - Mango

Local Name - Hlonuar

Habit - Herb

Flowering time - April to May

Colour of the flower –Purple

Description - Perennial herbs, stem erect, woody, solid, much branched, prickly. Leaves compound, bipinnate, very sensitive, stipulate, stipules lanceolate. Inflorescence head, flower small hermaphrodite, actinomorphic complete. Flowers purple colour. Fruit is pod, flat and pointed at apex.

Pollen morphology - Tetrad, tetragonal, psilate, radial symmetry

47. *Momordica charantia* L.

Family - Cucurbitaceae

English name - Bitter gourd, Carilla fruit

Local Name - Changkha

Habit - Climber

Flowering time - June to August

Colour of the flower –Yellow

Description - Herbaceous climber with simple tendrils, tendril are highly sensitive to contact. Leaves, simple, palmately notched, alternate, exstipulate. Flower solitary, unisexual, each plant bear separate yellow male and female flower, calyx lobes acute, ovate, petals yellow, female peduncle slender, bracteates, ovary fusiform, muricate. Fruit a fleshy pepo.

Pollen morphology - 3-zonicolporate, prolate, tenuimarginate, columnella very faint.

48. *Moringa oleifera* Lam.

Family - Moringaceae

English name - Ben tree, horse radish tree

Local Name - Thingbehlawi

Habit - Tree

Flowering time - January to May

Colour of the flower – White

Description - Deciduous trees with corky bark, soft wood. Leaves compound, pinnate, pinnate 4-6 pairs, leaflet opposite, gland linear. Flower bisexual, pedicelled, honey scented, sepals reflexed, petals spathulate and ovary hairy. Seeds winged.

Pollen morphology - Ptychotreme, psilateexine, oncus, periporate, radial symmetry

49. *Musa paradisiaca* L.

Family - Musaceae

English name - Banana

Local Name -Balhla

Habit - Herb

Flowering time - January to December

Colour of the flower – White

Description - Perennial herb with stem upto 7m above ground part, the plant is a false stem or pseudostem. Leaves simple, large and fused bases,leaf blades upto 2m, petiole 0.7m. Each pseudostem can produce a single flowering stem. Inflorescence a terminal spikes, flowers sessile, monoeceous, unisexual,unisexual, male flowers occur above female in the bracteates inflorescence. Fruit an elongated berry.

Pollen morphology – Polycolporate, psilate sculpturing ornamentation, spheroidal in shape.

50. *Nicotianum tobaccum* L.

Family - Solanaceae

English name - Tobacco

Local Name - Vaihlo

Habit - Herb

Flowering time - March-April

Colour of the flower – White

Description - Annually grown herbaceous plants, grows to height between 1-2metres with few branches. Leaves simple, lanceolate, alternate, spiraling around the stem. Inflorescence cymose, tubular flowers, white in colour, grow in a large, branching terminal clusters, pedicillate, hermaphrodite, actinomorphic. Fruit is oval to elliptical capsule.

Pollen morphology - Prolate, punctitegillate, furrow long.

51. *Oryza sativa* L.

Family - Poaceae

English name - Paddy

Local Name -Buh

Habit - Herb

Flowering time - September

Colour of the flower – Yellow

Description - Annual herb, stem erect, cylindrical hollow with nodes and internodes. Leaves long, narrow, one leaf at each node, alternate consisting of seed, hairy ligule and blade. Infloresence a terminal panicle, the panicle maybe compact or loose with spikelets. Flower is zygomorphic, hypogynous, irregular, incomplete and sessile. Fruit a cryopsis.

Pollen morphology – Spherical shape, radial symmetry.

52. *Parkia timoriana* (D.C.) Merr.

Family - Fabaceae

English name - Tree bean

Local Name -Zawngtah

Habit - Tree

Flowering time - October to November

Colour of the flower – Yellow

Description - Tall tree, leaves bipinnate devided into leaflets called pinna. The leaflet are linear oblong. Inflorescence is a head and contains several flowers with five lobed corollas. The fruit is a long flattened legume pod.

Pollen morphology – Circular, ornamentation pattern reticulate, isopolar.

53. *Phaseolus vulgaris* L.

Family - Fabaceae

English name - Beans

Local Name - Bean

Habit - Climber

Flowering time - May to June

Colour of the flower – White

Description - Herbaceous, climber, sub glabrous cultivated annual plant. Leaves alternate, simple, devided into three oval, smooth-edged leaflets. having racemes much shorter than the leaves. Flowers white. Bracteoles ovate or roundish, persistent. Pods glabrous, rostrate, turgid.

Pollen morphology - Oblate, spheroidal to prolate, colpus aperture, syncolpate, bilateral symmetry.

54. *Pisum sativum* L.

Family - Fabaceae

English name - Garden pea

Local Name - Chana

Habit - Climber

Flowering time - January to March

Colour of the flower – White

Description - A hairless glaucous climbing annual herb. Leaves alternate, compound, stipulate, the terminal leaflet is always a tendril. Inflorescence racemose, axillary flower pedicillate, zygomorphic, irregular and hermaphrodite. Fruit a legume.

Pollen morphology - Oblate, spheroidal to prolate, colpus aperture, syncolpate, radial symmetry.

55. *Prunus persica* L.

Family - Rosaceae

English name - Peach

Local Name - Theitehmul

Habit - Tree

Flowering time - February to May

Colour of the flower – Pink

Description - A large deciduous shrub or small tree. Leaves are lanceolate broad pinnately veined with pink flowers, solitary or paired with five petals and globurous twigs. The fruit is delicate and edible.

Pollen morphology – Prolate, spheroidal, sub prolate, isopolar and radial symmetry.

56. *Psidium guajava* L.

Family - Myrtaceae

English name - Guava tree

Local Name - Kawlthei

Habit - Shrub

Flowering time - June to August

Colour of the flower – White

Description - A small evergreen tree, pubescent on the young branches. Leaves opposite, simple, blade oblong to elliptic apex acuminate on very short petioles. Inflorescence cymose solitary axillary, flowers large, white, pedicillate, bracteates, complete hermaphrodite. Fruit an ovoid or pear shaped berry.

Pollen morphology - Triangular spore wall, smooth, prolate, oblate-spheroid, oralalongate, bilateral symmetry

57. *Punica granatum* L.

Family - Lythraceae

English name - Pomegranate

Local Name - Theibuhfai

Habit - Shrub

Flowering time - May to June

Colour of the flower – Orange

Description - Large deciduous shrubs or small tree with spinescent branchlets having smooth dark grey bark. Leaves simple, entire, lanceolate, opposite,

shinning. Inflorescence cymose, solitary, flowers orange in colour, actionmorphic, regular, bisexual and perigynous. Fruits a capsule, globose shaped.

Pollen morphology - Colpate, oblate, radial symmetry

58. *Raphanus sativus* L.

Family - Brassicaceae

English name - Raphanus

Local Name - Mula

Habit - Herb

Flowering time - March to April

Colour of the flower – White

Description - Biennial herbs having fleshy roots, stem usually tap stem is herbaceous becomes corm like thicken, eaten as vegetable. Leaves simple, exstipulate, arranged in a rosette, devided pinnately with an enlarged terminal lobed and smaller lateral lobes. Inflorescence racemose, flowers usually white in colour. Ebracteate, pedicillate, regular, hermaphrodite. Fruits is lomentum.

Pollen morphology - Prolate-sheroidal, 3-zonocolpate, granulate exine

59. *Ricinus communis* L.

Family - Euphorbiaceae

English name - Castor oil plant, Castor seed, Palma christi

Local Name - Mutih

Habit - Shrub

Flowering time - January to March

Colour of the flower – Red

Description - An evergreen perennial shrub having glaucous shoots and panicles. Leaves simple, long stalked, alternate serrated, palmate with deep lobes with gland. Inflorescence a sub-panicled raceme, flowers pedicillate, bracteates, unisexual, monoecious, the male flowers are yellowish green, stamens many present in the lower region and female flowers borne at the upper region with red stigma. Fruit a regma with spines.

Pollen morphology – 3-Colporate, prolate-sheroidal, finely reticulate, bilateral symmetry

60. *Rosa macrophylla* Lindl.

Family - Rosaceae

English name - Wild rose

Local Name - Rose

Habit - Shrub

Flowering time - January to December

Colour of the flower – Red

Description - It is a moderately prickly shrub with dark-brown peeled bark, prickles straight. Leaves simple, petiolate, stipulate. Inflorescences subterminal with solitary with red flower, flower pedicillate bracteates, complete, hermaphrodite, regular. Fruit etaerio of achenes.

Pollen morphology - 3-colporate, sherical shapeor prolate, sheroid, exine is intectate, surface psilate, bilateral symmetry

61. *Sechium edule* (Jacq.) Sw.

Family - Cucurbitaceae

English name - Chayote/Vegetable pear

Local Name - Iskut

Habit - Climber

Flowering time - July to August

Colour of the flower – White

Description - Perennial climber, the stem are angular-groove, glabrous and tendril on the stem. Leaves simple, heart shape, alternate, grooved petioles, are glabrous. Inflorescence cymose, the flower are unisexual bear male flowers in clusters and solitary female flowers with white flower. The fruit is pear shape light green, elongated with ridges length wise.

Pollen morphology – Isopolar, suboblate to prolate and spheroidal.

62. *Spilanthes acmella* L.

Family - Asteraceae

English name - Toothache plants/ paracress

Local Name - Ankasa

Habit - Herb

Flowering time - June to October

Colour of the flower – Yellow

Description - A small annual erect and ascending stout herbs. Leaves simple, opposite, petioleate, broadly ovate, narrow at base, acute or obtuse at the apex. Inflorescence in axillary and terminal panicles, involucral bracts two seriate ray florets few, inconspicuous, disc florets companulate. Fruit achenes dorsally compressed, black.

Pollen morphology - Porate, sheroidal shape,radial symmetry

63. *Solanum melongena* L.

Family - Solanaceae

English name - Brinjal, egg plant

Local Name - Bawkbawn

Habit - Shrub

Flowering time - June

Colour of the flower – Purple

Description - Perennial branch shrub, stem is often spiny. Leaves simple, alternate, each with a petiole, coarsely lobed leaves. Inflorescence cymose, flower hermaphrodite, purple in colour, long pedicel, actinomorphic, complete with five lobed corolla and yellow stamen. Fruit globous to oblong, fleshy berry.

Pollen morphology - Prolate, sub- spheroid, punctitegillate, furrow long, radial symmetry.

64. *Syzgium cumini* (L.)Skeel

Family - Myrtaceae

English name -Black plum, java plum

Local Name - Lenhmui

Habit - Tree

Flowering time - March to May

Colour of the flower – White

Description - Trees with crooked trunk. Stem erect branched woody. Leaves simple, smooth, shinning, entire exstipulate with short petiole. Inflorescence cymose type, flowers bracteates, actinomorphic, hermaphrodite, regular complete and sweet scented. Fruit a berry, bliquely oblong.

Pollen morphology - Sub triangular spore wall, smooth, psilateexine, colporate, prolate, oblate spheroid, obscure pattern,parasyncoplate, bilateral symmetry

65. *Syzygium jambos* L. Alston

Family - Myrtaceae

English name -Black plum, java plum

Local name - Rose apple

Habit - Tree

Flowering time - March to April

Colour of the flower – White

Description - Evergreen small tree, reaching a 25-40ft in height, wide spreading branched. Leaves simple, opposite, lanceolate or narrow elliptic have a aroma. Inflorescence short terminal or axillary corymbs, flower complete hermaphrodite, actinomorphic, white in colour. Fruit is a drupe oval shape.

Pollen morphology - Sub triangular spore wall, smooth, psilateexine, colporate, prolate, oblate- spheroid, obscure pattern, parasyncoplate, bilateral symmetry

66. *Tagetes erecta* L.

Family - Asteraceae

English name - Marigold

Local Name - Derhken

Habit - Herb

Flowering time - January to December

Colour of the flower – Orange

Description - Branched annual herbs upto 15-60cm tall with globrous angular stems. Leaves pinnately divided into oblong or lanceolate, serrared segments. Inflorescence capitulum with ray florets and disc florets, solitary, flower orange in colour, ligules numerous. Fruit achenes small, pappus and scaly.

Pollen morphology - Pantoporate, spinolous exine, radial symmetry

67. *Tamarindus indica* L.

Family - Fabaceae

English name -Tamarind tree

Local Name - Tengtere

Habit -Tree

Flowering time - June

Colour of the flower - White

Description - A large unarmed evergreen tree, stem woody, erect, cylindrical, branched solid. Leaves alternately arrange and pinnately compound, leaflets are green elliptical ovular. Inflorecence racemose, flowers are born in small racemes, bracteates, pedicillate, complete, zygomorphic, hermaphrodite. Fruit is a is legume.

Pollen morphology - 3-colporate, syncolpate, colpi long, isopolar,radially symmetry

68. *Tecoma stans* L.Juss.ex *Terminalia cr*Kunth.

Family - Bignoniaceae

English name -Tamarind tree

Local Name - Tawtawrawt par eng

Habit - Shrub

Flowering time - January to December

Colour of the flower - Yellow

Description - Small perennial shrubs. Leaves simple, opposite, hairy, borne on slender petiole. Inflorescence in racemose, flowers in terminal racemes, with short pedicels, bract yellow in colour, tubular in shape, zygomorphic, hermaphrodite and hypogynous. The corolla tube is long and has five rounded lobes. Fruit is large, linear, flattened, capsule.

Pollen morphology - Tri-zonocolpate, prolate-spheroid to sub prolate, bilateral symmetry

69. *Terminalia crenulata* Roth.

Family - Combretaceae

English name - Laurel

Local Name - Tualram

Habit - Tree

Flowering time - October to November

Colour of the flower – Yellow

Description - A deciduous tall tree, stem woody branched. Leaves simple, ovate, globrous, coriaceous, which are hairy beneath. Inflorescence racemose, flowers yellow in flowers and terminal axillary branch, complete, hermaphrodite. Fruits a drupe, globrous and oblong.

Pollen morphology - 3-colporate, prolate, sheroid shape, radial symmetry

70. *Terminalia bellirica* (Gaertn.) Roxb.

Family - Combretaceae

English name - Behda

Local Name - Thingvankawk

Habit - Tree

Flowering time - November to February

Colour of the flower - Yellow

Description - Wild medium-sized trees, stem solid, branched. Leave simple, alternate, having very long petiole. Inflorescence spike, solitary, axillary, flowers yellow and in simple axillary spikes, bracteate, actinomorphic, pentamerous, upper flowers of spikes are male and the lower ones hermaphrodite. Friuts globos and drupe.

Pollen morphology - 3-colporate, prolate, sheroid shape, radial symmetry

71. *Tetrameles nudiflora* R. Br.

Family - Datiscaceae

English name - False hemp tree

Local Name - Thingdawl

Habit - Tree

Flowering time - February to April

Colour of the flower – Yellow

Description - A very large deciduous tree with long clean bole and smooth, shiny brown bark, the trunk is buttressed. Leaves simple, alternate, exstipulate. Inflorescence racemose, flowers unisexual, colour yellow female flower sessile, in pendulous panicles, male flower subsessile, pubscent. Fruit a capsule.

Pollen morphology - Tricolporate, reticulate and circular

72. *Tithonia diversifolia* Hemsl.

Family - Asteraceae

English name - Tree marigold

Local Name - Bawngpu par

Habit - Shrub

Flowering time - September to November

Colour of the flower - Yellow

Description - Woody shrub. Leaves are simple, ovate, serrate and alternate. Inflorescence head, with ray florets and tubular disc florets and green bracts. The seed is achene with a ring of pappus.

Pollen morphology - Elliptic, porate, poly porporate and longer than wide.

73. *Tropaelum majus* L.

Family - Tropaeolaceae

English name - Nasturtium

Local Name - Serthlum rawng par

Habit - Herb

Flowering time - June to September

Colour of the flower - Orange

Description - Herbaceous annual plants. Leaves simple, opposite, large, nearly circular, hairless peltate, and entire palmately lobes. Blade round with large toothed or winding margins, globrous, juicy, the petiole long, flowers are bisexual, hermaphrodite, actinomorphic, solitary in axil and showy. Fruit a berry.

Pollen morphology – Tricolporate.

74. *Vitis vinifera* L.

Family - Vitaceae

English name - Grape

Local Name - Grape

Habit - Climber

Flowering time - April to May

Colour of the flower - Purple

Description - It is a perennial climber, shrub. Leaves simple, alternate palmately lobed and broad with stipules. Inflorescence racemose, the flowers small purple, bracteates, actinomorphic, bisexual, hypogynous, peduncle is flat and petals free near the base and united at the apex. The fruit is a berry and juicy.

Pollen morphology – Isopolar, prolate- spheroidal and sub prolate, triangular, radially symmetrical,

75. *Zea mays* L.

Family - Poaceae

English name - Maize, Corn

Local Name - Vaimim

Habit - Herb

Flowering time - July

Colour of the flower - White

Description - Cultivated annuals having solid stems showing well-developed nodes and internodes and protandrous flowers. Leave sessile, simple, alternate. Inflorescence monoecious, diclinous (stamen and pistil are borne in separate inflorescence) on the same individual plants, male inflorescence consists of a terminal panicle or tassel with a long central axis and female ones is a modified spike, the ear that develops from the axil of one of the largest leaves. Fruit a caryopsis.

Pollen morphology - 1-porate, spherical shape, radial symmetry.

76. *Zinnia elegans* Jacq.

Family - Asteraceae

English name - Common zinnia

Local Name - Sappangpar

Habit - Shrub

Flowering time - June to August

Colour of the flower - Red

Description - Annual upright shrub. Leaves simple, opposite, sessile, linear in shape. Inflorescence head, flowers hermaphrodite, monoecious pentamerous, actinomorphic and flower red in colour. Fruit is a cypsela.

Pollen morphology – Porate, spinolous, speroid shape radial symmetry.

5.1.2. Family wise distribution of polliniferous plants

In total of 35 families of polliniferous plants, out of which Asteraceae and Fabaceae family were dominated with increasing number of pollinifeous plant.

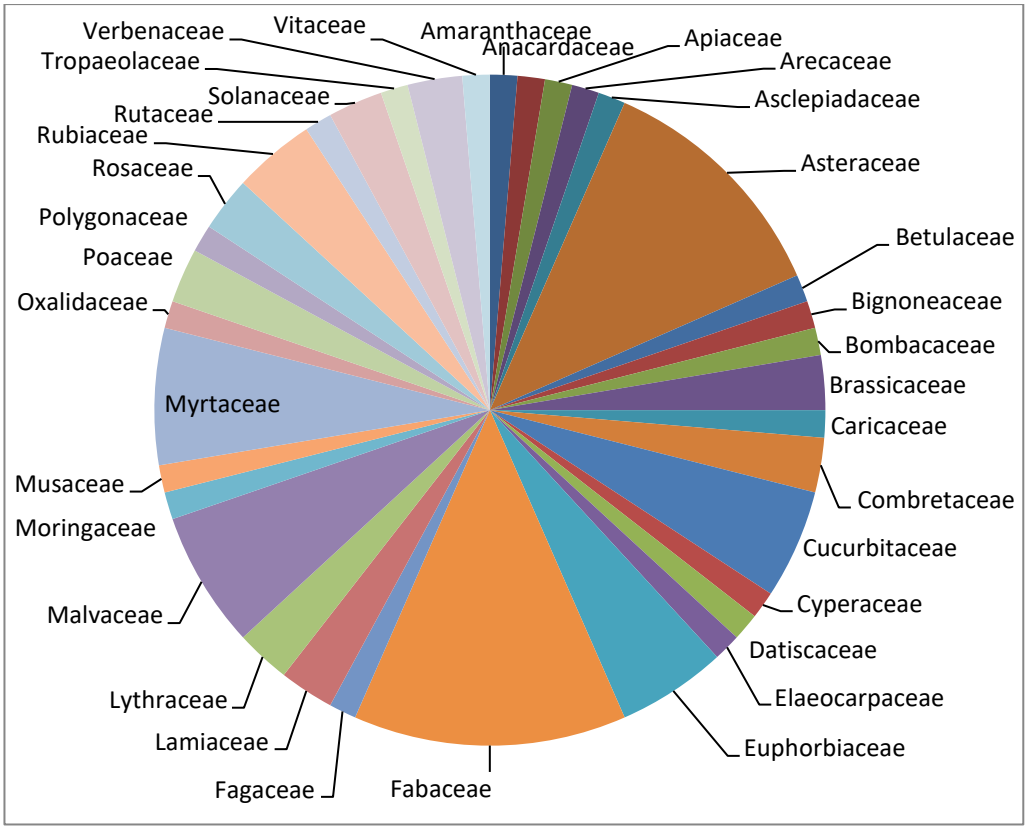


Figure 1.4 : Pie charts showing families of polliniferous plants in the study area.

5.1.3 Habitat of polliniferous plants

The bee forage plants includes lowest occurrence of habitat of polliniferous plants observes was 12% (9) climber followed by 25% (19) shrub, 26% (20) herb and 37 % (28) tree.

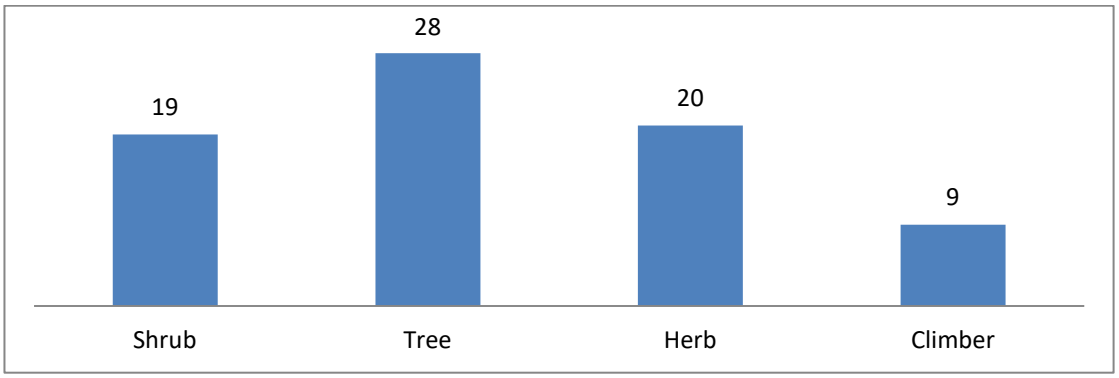


Figure 1.5 : Bar graph showing habitat of polliniferous plants.

5.1.4 Flowering months of polliniferous plants

The increase number of flowers during March to June enhance the bee to collect more pollen during this period whereas low pollen percentage was found in the month of November to February (Figure 1.8), which is characterized by low plant flower density

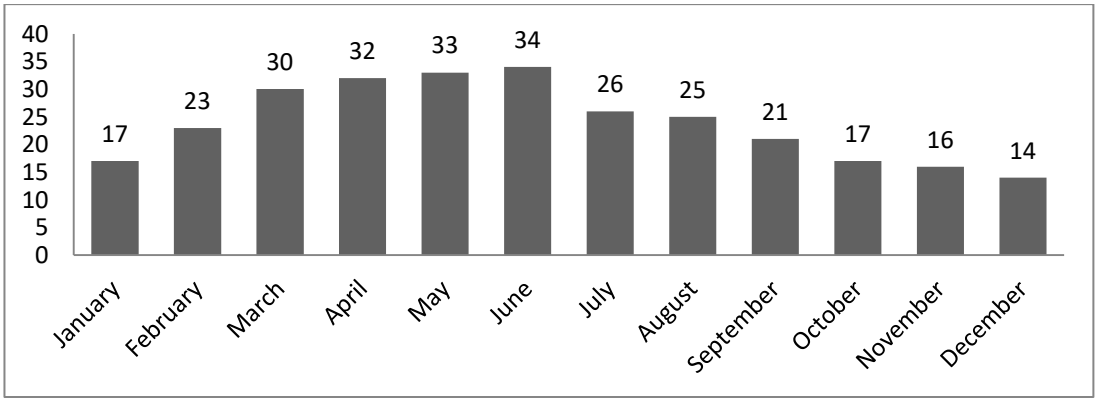


Figure 1.6 : Bar graph showing flowering month of polliniferous plants.

5.1.5 Colour of polliniferous plants

Indeed, violet or blue flowers are often the most rewarding flower colors in many habitats.

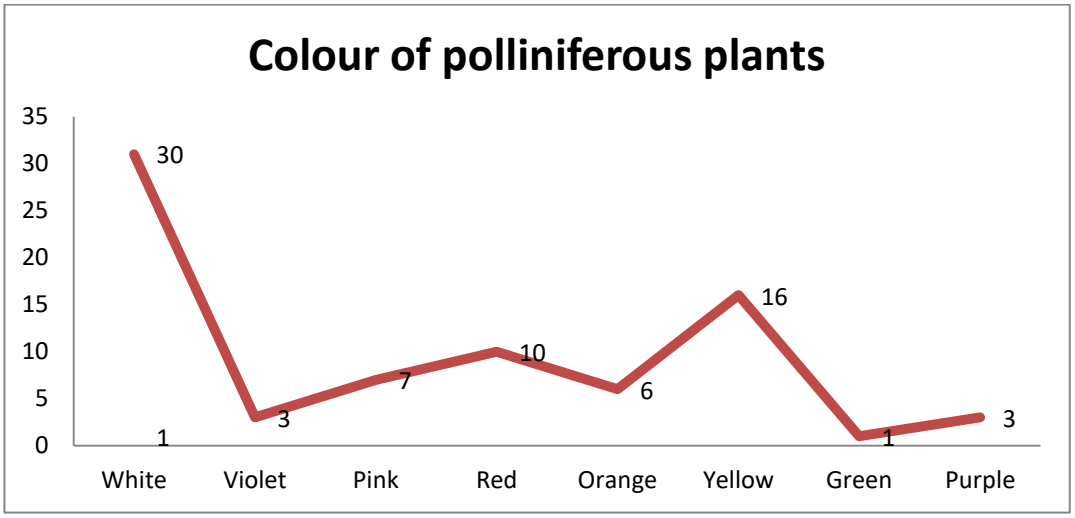


Figure1.7 : Line graph showing colour of polliniferous plants

5.1.6 Vegetational classification of polliniferous plants

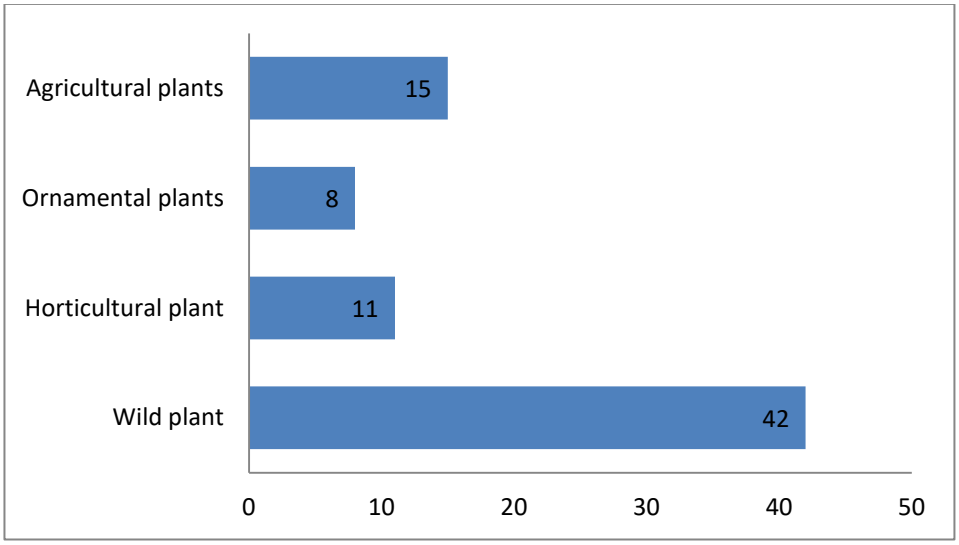


Figure1.8 : Vegetation pattern of the two studied district.

Table 1.8 : Pollen spectrum identified in honey samples A1 to C10 (Vg- Vegetation; WP-Wild plant; HP- Horticultural plant; OP- Ornamental plant; AP- Agricultural plant, I- Important minor pollen; M- Minor pollen; S- Secondary dominant pollen)

Sl.No	Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
	Amaranthaceae																					
1.	*Amaranthus sps.*	WP	-	I	-	-	-	-	M	-	-	-	-	I	I	I	-	-	-	-	-	M
	Anacardaceae																					
2.	*Mangifera indica*	HP	-	M	-	I	-	-	-	-	-	-	M	-	-	I	I	-	-	M	-	
	Apiaceae																					
3.	*Coriandrum sativum*AP	M	-	-	-	S	-	-	I	-	-	I	-	-	-	S	S	I	-	-	S	
	Arecaceae																					
4.	*Cocos nucifera*	HP	M	S	M	-	I	-	-	I	-	M	-	I	M	S	-	-	-	-	I	-
	Asclepiadaceae																					
5.	*Asclepias curassavica*	OP	-	-	-	I	-	-	-	I	-	-	M	-	-	-	-	-	M	-	-	I

Sl.No	Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
	Asteraceae																					
6.	*Ageratum conyzoides*	WP	-	S	-	M	-	S	-	I	-	I	-	-	S	M	-	-	-	I	-	M
7.	*Bidens pilosa*	WP	-	-	M	-	-	-	M	-	-	-	I	-	M	-	-	-	-	I	I	I
8.	*Cosmos sulphureus*	WP	-	-	-	I	M	-	-	-	M	-	I	-	-	M	I	-	-	-	I	-
9.	*Matricaria chomonulla*	WP	-	-	-	-	-	M	-	-	-	-	-	-	-	-	-	M	-	-	-	-
10.	*Mikania micranta*	WP	S	-	S	-	-	M	-	-	S	S	-	S	-	-	M	-	-	M	-	I
11.	*Spilanthes acmella*	WP	-	-	S	-	-	I	I	-	-	-	I	-	-	-	-	-	S	M	-	-
12.	*Tagetes erecta*	WP	M	-	I	-	I	-	M	-	M	-	-	M	-	-	-	-	M	-	-	I
13.	*Tithonia diversifolia*	WP	-	M	-	M	-	I	-	M	-	I	-	M	-	M	-	-	I	-	I	-
14.	*Zinnia elegans*	OP	M	-	I	-	I	-	M	-	M	-	-	M	-	-	-	-	M	-	-	I
	Betulaceae																					
15.	*Alnus nitida*	WP	I	-	-	I	M	I	-	M	-	S	-	-	-	-	I	I	-	-	S	S
	Bignoniaceae																					
16.	*Tecoma stans*	WP	-	-	-	-	M	-	-	-	-	I	-	-	I	-	I	-	-	M	-	-

Sl.No	Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
	Bombacaceae																					
17.	*Bombax ceiba*	WP	-	-	S	-	I	M	-	-	S	S	-	-	M	-	-	-	S	I	-	M
	Brassicaceae																					
18.	*Brassica camprestris*	AP	I	-	-	S	-	-	S	S	-	-	S	-	-	I	-	M	I	-	M	M
19.	*Raphanus sativus*	AP	-	-	I	I	-	-	-	-	-	-	-	-	-	-	M	I	-	-	-	-
	Caricaceae																					
20.	*Carica papaya*	HP	M	I	-	M	-	-	-	S	-	-	-	-	-	-	S	-	M	-	-	S
	Combretaceae																					
21.	*Terminalia crenulata*	WP	-	-	-	-	-	-	-	M	-	-	I	-	-	-	-	-	-	-	-	-
22.	*Terminalia bellirica*	WP	S	I	-	S	-	I	M	-	M	-	S	M	M	I	S	-	I	S	M	I
	Cucurbitaceae																					
23.	*Cucumis sativus*	AP	M	M	I	-	S	M	I	S	-	M	M	-	I	-	-	S	S	-	-	-
24.	*Cucurbita pepo*	AP	-	-	M	-	-	I	-	-	-	-	M	-	-	-	M	-	-	-	-	I

Sl.No Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
25. *Momordica charantia*	AP	M	-	-	-	I	-	M	-	-	I	M	-	I	-	-	M	-	-	-	I
26. *Sechium edule*	AP	-	-	I	M	-	-	-	M	-	-	-	M	M	-	-	-	I	-	-	M
Cyperaceae																					
27. *Cyperus rotundus*	WP	M	-	-	-	M	-	S	-	I	M	-	M	-	M	-	I	-	-	M	-
Datiscaceae																					
28. *Tetrameles nudiflora*	WP	-	M	-	-	-	-	S	-	-	-	-	-	M	-	-	M	S	M	I	S
Elaeocarpaceae																					
29. *Elaeocarpus lanceifolius*	WP	-	-	M	-	-	I	-	-	-	I	-	-	-	-	M	-	-	M	-	-
Euphorbiaceae																					
30. *Croton jaufra*	WP	I	-	I	M	-	M	-	-	-	-	-	M	M	-	-	M	-	M	-	-
31. *Emblica officinalis*	WP	-	-	S	-	-	I	-	I	M	-	-	-	-	M	I	-	S	-	-	I
32. *Euphorbia pulcherrima*	OP	-	-	-	-	M	-	-	-	-	M	I	-	-	-	-	-	-	-	I	-
33. *Riccinus communis*	WP	M	-	-	I	-	-	I	-	-	M	-	M	-	-	I	M	-	-	M	-

Sl.No Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Fabaceae																					
34. *Acacia pruinescens*	WP	M	-	M	-	S	M	-	S	M	M	-	M	M	M	-	-	-	I	I	M
35. *Bauhinia variegate*	WP	-	M	-	M	-	-	I	-	-	I	-	-	I	-	M	-	-	I	-	-
36. *Caesalpinia pulcherrima*	WP	M	-	-	-	-	-	M	-	-	-	I	-	-	I	-	-	I	M	M	-
37. *Cassia javanica*	WP	-	-	I	-	-	I	-	-	I	-	-	-	I	-	-	-	I	-	-	M
38. *Derris robusta*	WP	-	-	-	-	M	-	-	M	-	I	I	M	-	I	-	M	-	-	-	I
39. *Mimosa pudica*	WP	-	M	-	-	M	-	-	-	M	-	-	M	-	M	-	I	-	-	M	M
40. *Parkia timoriana*	WP	-	S	-	-	-	M	-	M	-	-	M	-	-	I	-	-	-	I	S	-
41. *Phaseolus vulgaris*	AP	-	-	-	M	-	-	M	-	-	-	-	-	M	-	I	-	M	-	I	-
42. *Pisum sativum*	AP	M	-	-	-	S	S	-	M	-	M	-	I	-	S	-	-	M	-	I	-
43. *Tamarindus indica*	WP	M	-	I	I	I	-	I	-	M	S	-	-	S	I	-	M	-	-	M	-
Fagaceae																					
44. *Castanopsis tribuloides*	WP	M	-	-	I	-	-	-	M	-	-	-	M	-	-	-	-	M	-	-	-

Sl.No Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Lamiaceae																					
45. *Holmskioldia sanguine*	WP	-	-	M	-	-	-	-	-	M	-	M	M	-	I	-	-	-	M	-	-
46. *Leucosceptrum canum*	WP	-	M	-	-	I	-	-	-	M	-	M	-	M	-	I	-	-	M	-	I
Lythraceae																					
47. *Lagerstromia speciosa*	WP	-	S	-	-	-	S	-	-	M	-	-	M	M	-	M	-	M	-	-	M
48. *Punica granatum*	HP	-	-	-	M	-	-	M	-	-	I	-	-	-	-	-	-	-	M	M	I
Malvaceae																					
49. *Althaea rosea*	WP	-	M	-	M	-	-	-	M	-	M	-	-	-	I	-	I	M	-	M	-
50. *Anthurium andreanum*	OP	M	M	-	I	-	-	M	-	I	-	M	-	-	-	M	-	M	-	M	-
51. *Hibiscus rosa sinensis*	OP	-	-	M	-	-	M	-	-	-	I	-	-	I	-	M	-	M	M	-	M
52. *Ipomoea batatas*	AP	-	M	-	-	I	-	-	I	-	-	M	-	-	S	-	I	-	-	-	I
53. *Malvaviscus arboreus*	WP	-	-	-	M	-	-	-	-	-	-	-	-	-	M	I	M	-	-	-	M

Sl.No Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Moringaceae																					
54. *Moringa oleifera*	AP	-	S	-	-	-	-	-	M	M	M	S	-	-	S	M	-	-	M	-	I
Musaceae																					
55. *Musa paradisiaca*	HP	M	-	M	I	-	-	M	M	-	-	-	S	M	-	-	I	-	M	-	M
Myrtaceae																					
56. *Callistemon lanceolatus*	WP	-	-	-	-	-	-	I	-	-	-	M	-	-	I	-	-	M	-	-	-
57. *Eucalyptus tereticornis*	WP	-	S	-	-	M	-	I	-	-	-	-	-	S	-	-	M	S	-	S	M
58. *Psidium guajava*	HP	S	-	I	-	I	M	M	-	I	M	S	-	M	-	M	M	-	M	-	-
59. *Syzygium cumini*	HP	S	-	-	M	M	-	I	-	I	-	I	-	-	M	S	-	I	-	I	M
60. *Syzygium jambos*	HP	-	-	-	-	-	M	-	-	-	-	-	I	-	-	-	M	-	I	M	-
Oxalidaceae																					
61. *Averrhoa carambola*	WP	I	-	I	-	-	-	M	-	-	-	-	-	I	-	-	M	-	-	M	I

Sl.No Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Poaceae																					
62. *Oryza sativa*	AP	M	M	-	S	M	-	S	-	-	S	-	M	-	M	-	S	I	S	M	M
63. *Zea mays*	AP	-	I	S	-	S	M	-	S	I	-	M	-	S	-	M	-	I	-	-	S
Polygonaceae																					
64. *Antigonon leptopus*	WP	-	-	M	-	-	M	-	I	M	M	-	-	M	-	-	M	-	I	-	M
Rosaceae																					
65. *Prunus persica*	*WP*	-	-	M	-	I	-	M	-	-	M	-	M	-	-	-	M	-	-	-	M
66. *Rosa macrophylla*	OP	M	-	I	-	M	-	-	M	-	M	-	M	-	I	-	-	M	-	M	-
Rubiaceae																					
67. *Coffee Arabica*	HP	-	-	-	S	-	M	S	-	S	S	S	-	M	-	I	-	-	I	-	I
68. *Ixora coccinea*	OP	M	-	-	-	-	M	-	-	-	-	-	-	-	M	-	M	-	I	-	-
69. *Jatropha curcus*	WP	-	M	-	-	M	-	-	-	M	-	-	-	I	-	I	-	-	M	I	M

Sl.No Taxa	Vg	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
Rutaceae																					
70. *Citrus limon*	HP	M	-	I	-	M	I	-	-	M	-	-	M	-	I	-	M	M	-	M	-
Solanaceae																					
71. *Nicotianum tobaccum*	WP	-	M	-	-	M	M	I	-	I	M	M	I	-	-	-	M	-	-	M	M
72. *Solanum melongena*	AP	-	-	-	M	-	I	-	M	-	M	-	M	-	M	M	-	M	I	M	-
Tropaeolaceae																					
73. *Tropaelum majus*	WP	-	-	-	-	-	-	-	M	-	-	M	-	M	M	-	I	-	-	-	M
Verbenaceae																					
74. *Lantana camara*	WP	M	-	-	M	M	-	M	-	-	-	-	-	-	M	-	-	I	-	M	-
75. *Callicarpa arborea*	WP	-	-	M	-	-	M	-	I	-	-	-	-	M	-	-	M	-	I	-	-
Vitaceae																					
76. *Vitis vinifera*	HP	-	-	M	-	-	M	-	-	-	-	-	M	M	-	M	M	-	I	-	M

Pollen spectrum analysis of honey samples -

The pollen count derived that some species were more frequent in the sample because some plant species readily produces nectar and their flowering period is longer if compared with other species, some flowering plants maybe having good quality of nectar. This analysis showed that bee collect the nectar of that plants which are available in that area.

Sample A1: The analytical data show that 29 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 4), important minor pollen (4 different types) and minor pollen (21types).

Sample A2 : The analytical data show that 22 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 5), important minor pollen (4 different types) and minor pollen (13types).

Sample A3 : The analytical data show that 29numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 5), important minor pollen (12 different types) and minor pollen (12types).

Sample A4 : The analytical data show that 25 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 4), important minor pollen (8 different types) and minor pollen (13types).

Sample A5: The analytical data show that 31 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 5), important minor pollen (11 different types) and minor pollen (15types).

Sample A6 : The analytical data show that 29numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 2), important minor pollen (10 different types) and minor pollen (17types).

Sample A7 : The analytical data show that 31 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 6), important minor pollen (9 different types) and minor pollen (16types).

Sample A8 : The analytical data show that 27 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 5), important minor pollen (8 different types) and minor pollen (14types).

Sample A9: The analytical data show that 25 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 3), important minor pollen (7 different types) and minor pollen (15types).

Sample A10 : The analytical data show that 30 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 6), important minor pollen (9 different types) and minor pollen (15types).

Sample C1 : The analytical data show that 28 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 9), important minor pollen (5 different types) and minor pollen (14types).

Sample C2 : The analytical data show that 28 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 3), important minor pollen (4 different types) and minor pollen (21types).

Sample C3 : The analytical data show that 29 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 4), important minor pollen (12 different types) and minor pollen (13types).

Sample C4 : The analytical data show that 30 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 4), important minor pollen (12 different types) and minor pollen (14types).

Sample C5 : The analytical data show that 28 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 4), important minor pollen (11 different types) and minor pollen (13types).

Sample C6 : The analytical data show that 32 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 3), important minor pollen (9 different types) and minor pollen (20types).

Sample C7: The analytical data show that 31 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 6), important minor pollen (10 different types) and minor pollen (15types).

Sample C8 : The analytical data show that 30 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 2), important minor pollen (13 different types) and minor pollen (15types).

Sample C9 : The analytical data show that32 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 2), important minor pollen (11 different types) and minor pollen (19 types).

Sample C10 : The analytical data show that 40 numbers of pollen types are identified and all are classified as secondary dominant (no's of pollen 5), important minor pollen (16 different types) and minor pollen (19types).

5.2 Molecular work

5.2.1 Genomic DNA of honey sample of Aizawl and Champhai

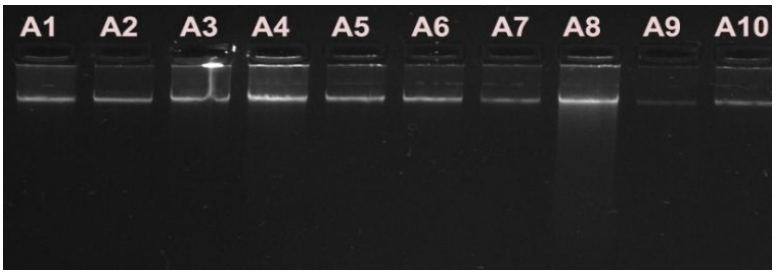


Figure 1.9: Extracted genomic DNA of honey sample from Aizawl

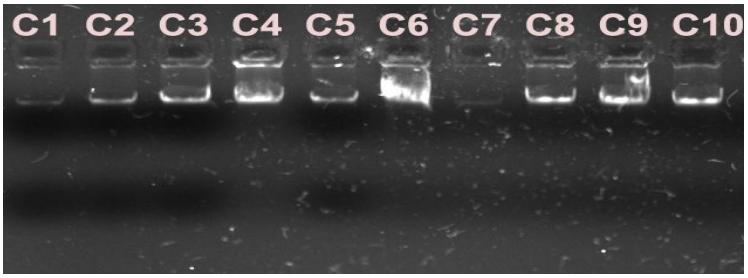
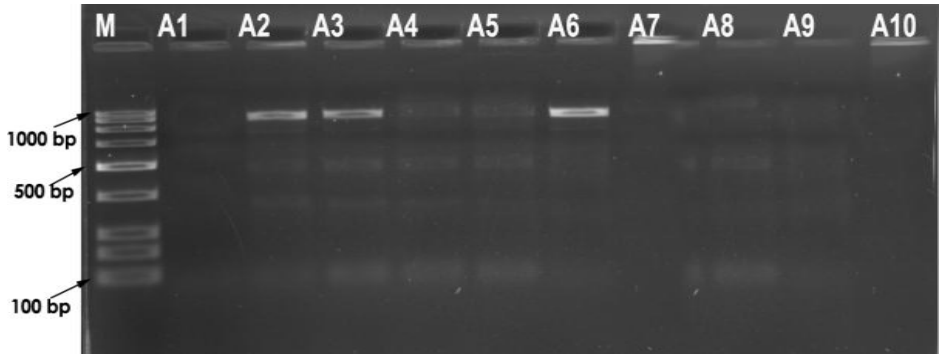


Figure 2.0: Extracted genomic DNA of honey sample from Champhai

5.2.2 Polymerase Chain reaction (PCR) Gel picture of *mat*K region



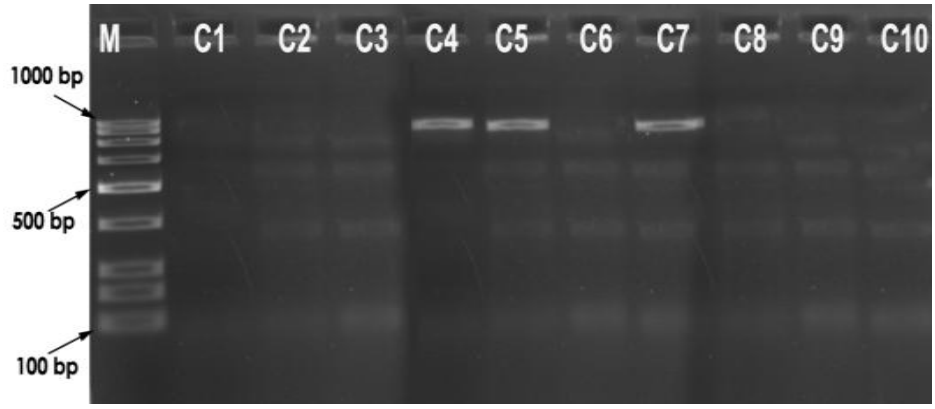


Figure 2.1 : PCR amplification of *matK* region

5.2.3 Polymerase Chain reaction (PCR) Gel picture of *rbc*L region

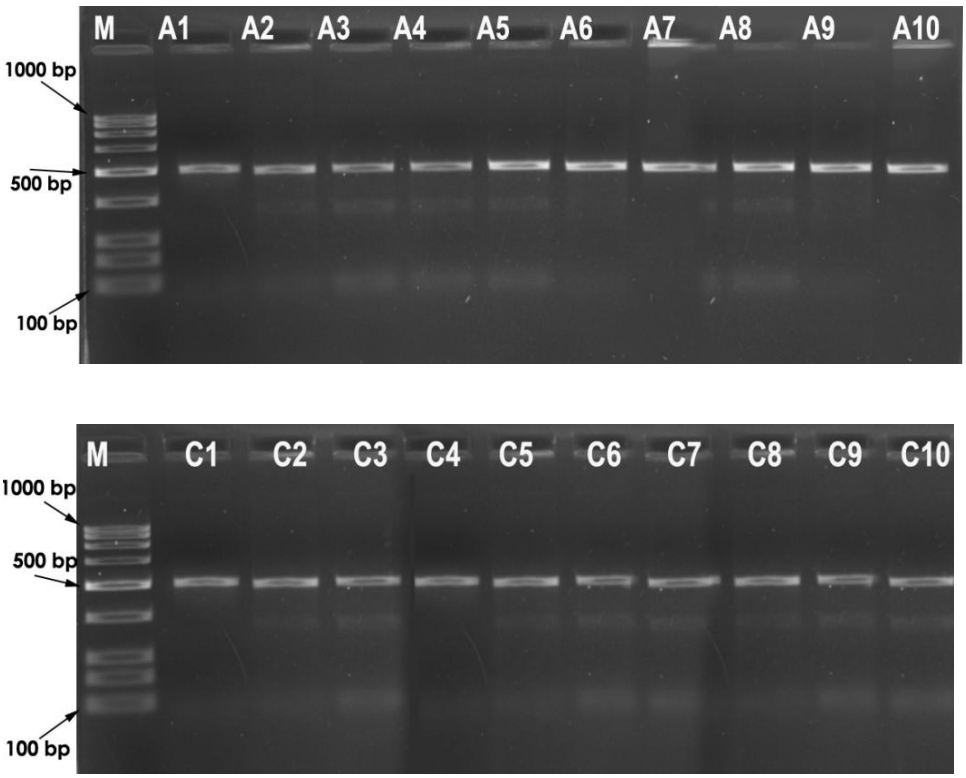
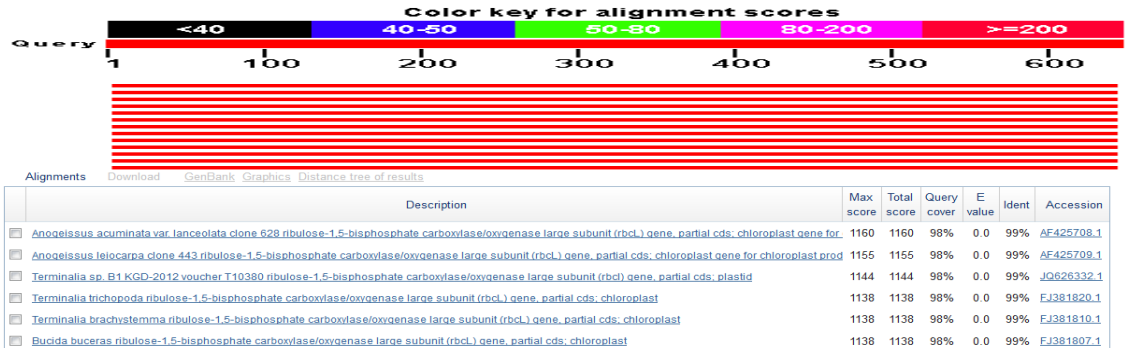


Figure 2.2 : PCR amplification of *rbc*L region

Table 1.9 : Molecular identification of pollen from Aizawl and Champhai.

DISTRICT	SAMPLE SITE	SAMPLE ID	*rbc*L	*mat*K
AIZAWL	Falkland	A1	*Terminalia Sps.*	
	Thuampui	A2	*Ageratum conyzoides*	*Mikania sps.*
	Tanhril	A3	*Alnus nepalensis*	*Prunus trichantha*
	Durtlang	A4	*Nicotiana tobaccum*	
	Sihphir	A5	*Mikania scandens*	
	Hlimen	A6	*Riccinus communis*	*Alnus nepalensis*
	Melthum	A7	*Pisum sativum*	
	Maubawk	A8	*Eucalyptus erythrocarys*	
	Sairang	A9	*Mikania micrantha*	
	Sakawrtuichhun	A10	*Juglans cineria*	
CHAMPHAI	Hmunhmeltha	C1	*Riccinus communis*	
	N.Khawbung	C2	*Alnus nepalensis*	
	Ruantlang	C3	*Alnus incana*	
	Zote	C4	*Alnus nitida*	*Tetrameles nudiflora*
	Khawzawl	C5	*Alnus nepalensis*	*Tetrameles nudiflora*
	Champhai vengsang	C6	*Riccinus communis*	
	Chawngtlai	C7	*Phylanthus emblica*	*Mikania scandens*
	Tlangsam	C8	*Phylanthus emblica*	
	N.Champhai	C9	*Parkia* sps.	
	Mualkawi	C10	*Pisum sativum*	

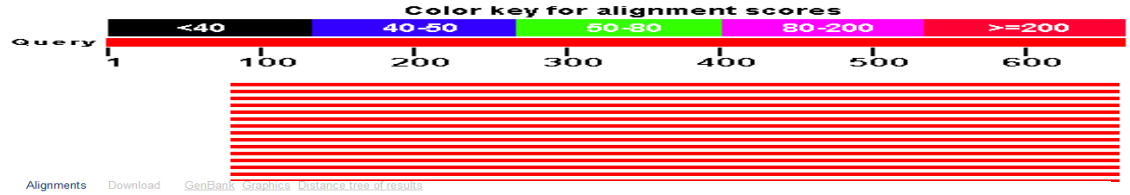
5.2.4 Blast result reported for the two barcode region of sample (*rbc*L and *mat*K gene)



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Anogeissus acuminata var. lanceolata clone 628 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for	1160	1160	98%	0.0	99%	AF425708.1
Anogeissus leiocarpa clone 443 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast prod	1155	1155	98%	0.0	99%	AF425709.1
Terminalia sp. B1 KGD-2012 voucher T10380 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; plastid	1144	1144	98%	0.0	99%	JQ626332.1
Terminalia trichopoda ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1138	1138	98%	0.0	99%	FJ381820.1
Terminalia brachystemma ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1138	1138	98%	0.0	99%	FJ381810.1
Bucida buceras ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1138	1138	98%	0.0	99%	FJ381807.1

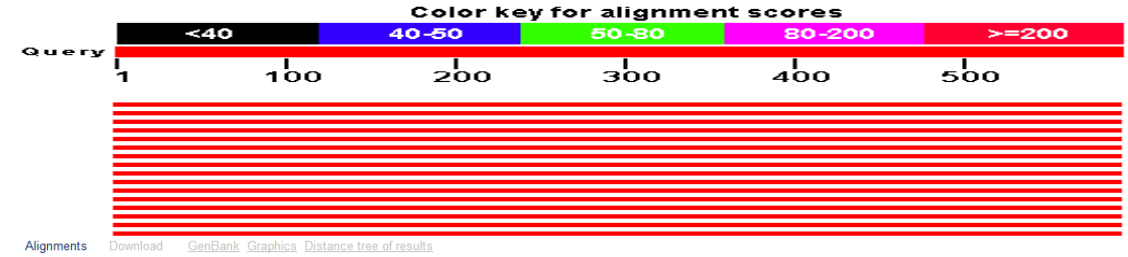
Figure2.3: Blast analysis of A1 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mikania scandens ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	793	793	87%	0.0	91%	KJ773685.1
Eupatorium sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T310	793	793	87%	0.0	91%	AB586220.1
Mikania cordifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	787	787	87%	0.0	91%	KJ773684.1
Zexmenia serrata ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	765	765	87%	0.0	91%	AY215199.1
Steiractinia sodiroi ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	760	760	87%	0.0	90%	AY215181.1
Rensonia salvadorica ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	760	760	87%	0.0	90%	AY215171.1

Figure 2.4: Blast analysis of A2 sample



Alignments Download GenBank Graphics Distance tree of results

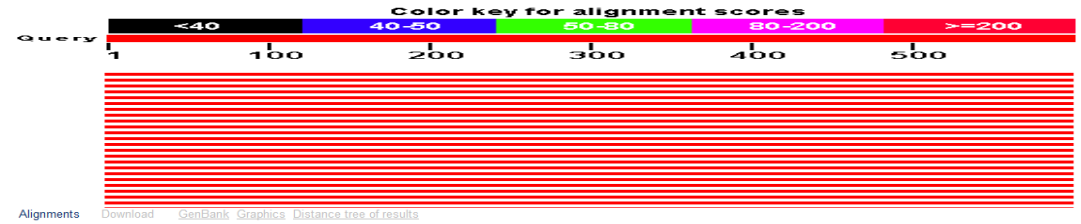
Description	Max score	Total score	Query cover	E value	Ident	Accession
Alnus glutinosa ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid	1092	1092	100%	0.0	99%	KM360625.1
Alnus rubra ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1092	1092	100%	0.0	99%	KF418933.1
Alnus nitida ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1092	1092	100%	0.0	99%	KF418931.1
Alnus nepalensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1092	1092	100%	0.0	99%	KF418930.1
Alnus glutinosa ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1092	1092	100%	0.0	99%	KF418927.1
Alnus formosana ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1092	1092	100%	0.0	99%	KF418926.1

Figure 2.5: Blast analysis of A3 sample.

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Berberidopsis corallina ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, complete cds; chloroplast	778	778	100%	0.0	98%	EU002274.1
Rhodoleia championii voucher BOP017796 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	767	767	100%	0.0	98%	KC737406.1
Miconia punctata voucher LV109480 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; plastid	767	767	100%	0.0	98%	JQ625848.1
Davidia involucrata ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit protein (rbcL) gene, partial cds; chloroplast	767	767	100%	0.0	98%	HM100376.1
Ternstroemia peduncularis voucher Clase&Delpreter 3059 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplas	767	767	100%	0.0	98%	HQ437969.1
Ternstroemia stahlii voucher Axelrod&Fritsch 12569 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	767	767	100%	0.0	98%	HQ437968.1
Ternstroemia sp. WH-2010 voucher Kelly 1372 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	767	767	100%	0.0	98%	HQ437963.1
Cassinopsis sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T931	767	767	100%	0.0	98%	AB586342.1

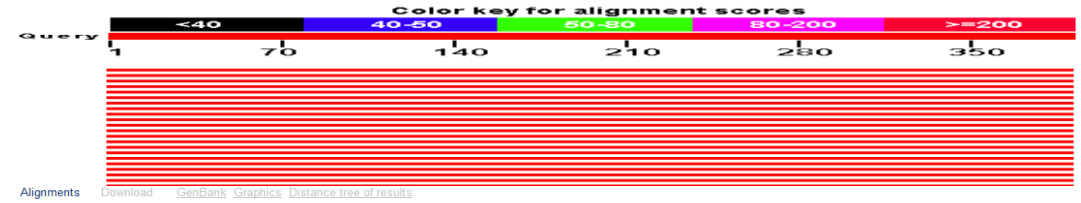
Figure 2.6: Blast analysis of A4 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mikania scandens ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1083	1083	100%	0.0	99%	KJ773685.1
Eupatorium sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T310	1083	1083	100%	0.0	99%	AB586220.1
Mikania cordifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1066	1066	100%	0.0	99%	KJ773684.1
Zexmenia serrata ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	1038	1038	100%	0.0	98%	AY215199.1
Wedelia tegetis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	1038	1038	100%	0.0	98%	AY215196.1
Steiractinia sodiroi ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	1038	1038	100%	0.0	98%	AY215181.1
Rensonia salvadorica ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	1038	1038	100%	0.0	98%	AY215171.1

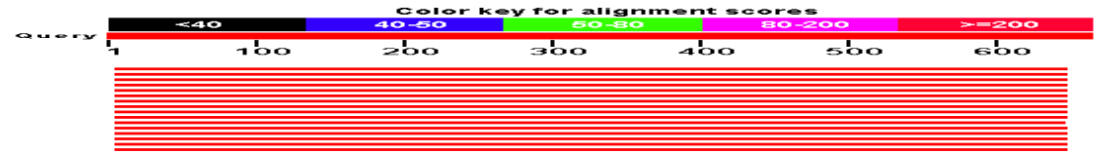
Figure 2.7: Blast analysis of A5 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Macaranga denticulata ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	725	725	100%	0.0	100%	KP752375.1
Macaranga aleuritoides ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	719	719	100%	0.0	99%	FJ976147.1
Macaranga aleuritoides chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	719	719	100%	0.0	99%	AB267922.1
Macaranga pustulata ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	708	708	100%	0.0	99%	KP752384.1
Macaranga tanarius chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	708	708	100%	0.0	99%	AB233866.1
Macaranga sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T202	702	702	100%	0.0	99%	AB586298.1
Macaranga grandifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit-like (rbcL) gene, partial sequence; chloroplast	702	702	100%	0.0	99%	AY794935.1
Mallotus barbatus ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	697	697	100%	0.0	99%	KP752381.1

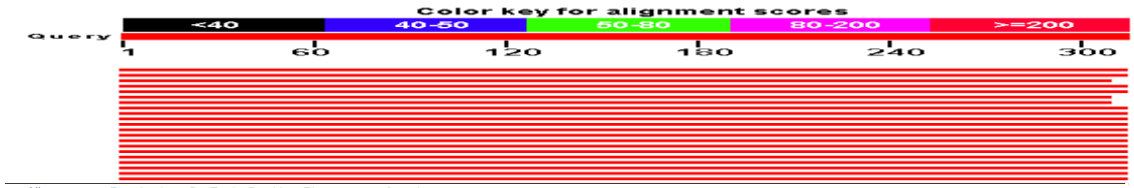
Figure 2.8: Blast analysis of A6 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pisum sativum subsp. elatius, bio-material JI1794, chloroplast complete genome	1166	1166	96%	0.0	99%	HG966675.1
Pisum sativum subsp. sativum, bio-material WL1238, chloroplast complete genome	1166	1166	96%	0.0	99%	HG966674.1
Pisum sativum chloroplast, complete genome	1166	1166	96%	0.0	99%	KJ806203.1
Pisum sativum subsp. sativum isolate HS878 ribulose 1,5-bisphosphate carboxylase (rbcL) gene, partial cds; chloroplast	1166	1166	96%	0.0	99%	JN661190.1
Pisum sativum cultivar Feltham First chloroplast, complete genome	1166	1166	96%	0.0	99%	HM029370.1
Pea chloroplast gene for ribulose 1,5-bisphosphate carboxylase (rbcl)	1166	1166	96%	0.0	99%	X03853.1

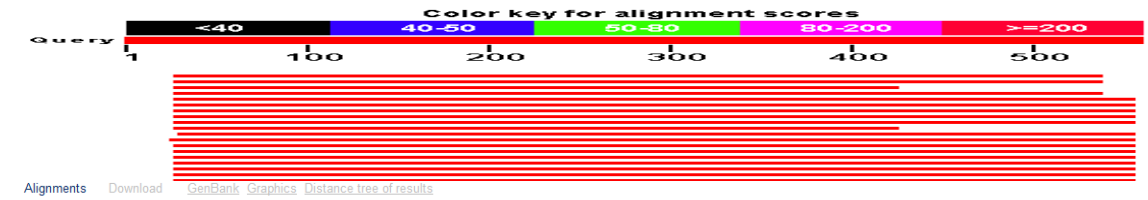
Figure 2.9: Blast analysis of A7 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Tapiscia sinensis ribulose bisphosphate carboxylase gene, partial cds; chloroplast gene for chloroplast product	575	575	100%	2e-160	99%	AF447750.1
Dipentodon sinicus chloroplast partial rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit	553	553	100%	8e-154	98%	AJ428890.1
Lacistema aggregatum chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	551	551	98%	3e-153	99%	AB233894.1
Gerrardina foliosa ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	547	547	100%	4e-152	98%	AY757086.1
Dipentodon sinicus ribulose-1,5-bisphosphate carboxylase oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	547	547	100%	4e-152	98%	AF375609.1
Lacistema aggregatum isolate 27 ribulose 1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid	545	545	98%	1e-151	98%	AY935746.1
Lacistema aggregatum	545	545	98%	1e-151	98%	AF206787.1
Eucalyptus cladocalyx chloroplast, complete genome	542	542	100%	2e-150	98%	KC180786.1

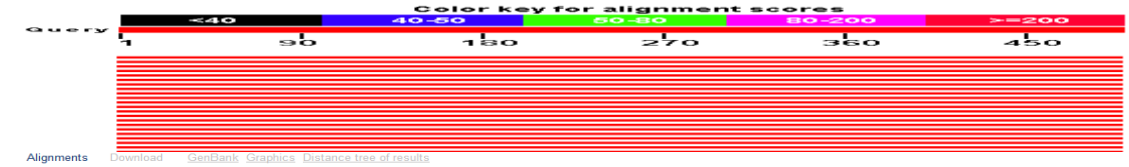
Figure 3.0: Blast analysis of A8 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mikania scandens ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	630	630	91%	7e-177	89%	KJ773685.1
Eupatorium sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T310	630	630	91%	7e-177	89%	AB586220.1
Mikania micrantha voucher C.A. Pendry et al., DNEP2 A16, (E) ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plasti	617	617	71%	5e-173	95%	JQ933409.1
Mikania cordifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	614	614	91%	7e-172	89%	KJ773684.1
Eutetras palmeri ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	614	614	94%	7e-172	88%	AY215116.1
Pericome caudata ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	614	614	94%	7e-172	88%	AY215156.1
Venegasia carpesioides ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	608	608	94%	3e-170	88%	AY215192.1

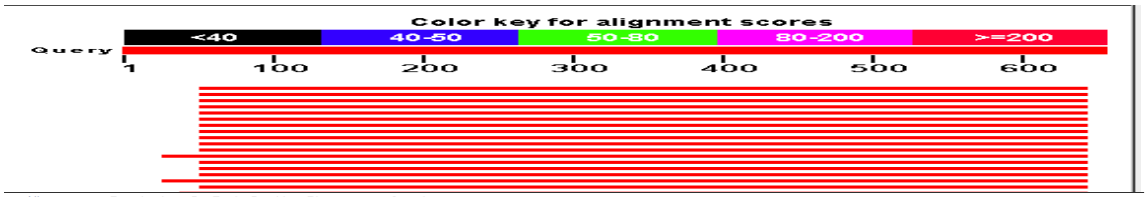
Figure 3.1: Blast analysis of A9 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Juglans cinerea ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	845	845	100%	0.0	97%	KF418919.1
Engelhardia serrata ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	845	845	100%	0.0	97%	KF418914.1
Annamocarya sinensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product	845	845	100%	0.0	97%	AY263935.1
Carya floridana ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	839	839	100%	0.0	97%	KJ773367.1
Carya aquatica ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	839	839	100%	0.0	97%	KJ773366.1
Carya alba ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	839	839	100%	0.0	97%	KJ773365.1
Pterocarya fraxinifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid	839	839	100%	0.0	97%	KM360945.1
Juglans cathayensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	839	839	100%	0.0	97%	KF418918.1

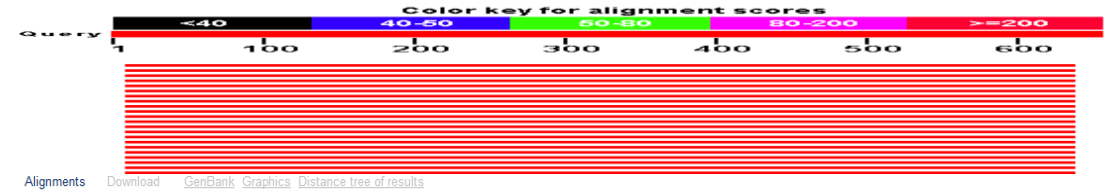
Figure 3.2: Blast analysis of A10 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mallotus sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T364	1018	1018	90%	0.0	98%	AB586303.1
Macaranga aleuritoides ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1018	1018	90%	0.0	98%	FJ976147.1
Macaranga aleuritoides chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1018	1018	90%	0.0	98%	AB267922.1
Mallotus japonicus chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1007	1007	90%	0.0	97%	AB267923.1
Macaranga tanarius chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1007	1007	90%	0.0	97%	AB233866.1
Mallotus japonicus ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit-like (rbcL) gene, partial sequence; chloroplast	1007	1007	90%	0.0	97%	AY794934.1
Macaranga sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T202	1002	1002	90%	0.0	97%	AB586298.1
Macaranga grandifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit-like (rbcL) gene, partial sequence; chloroplast	1002	1002	90%	0.0	97%	AY794935.1

Figure 3.3: Blast analysis of C1 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Alnus glutinosa ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; plastid	1160	1160	95%	0.0	100%	KM360625.1
Alnus rubra ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1160	1160	95%	0.0	100%	KF418933.1
Alnus nitida ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1160	1160	95%	0.0	100%	KF418931.1
Alnus nepalensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1160	1160	95%	0.0	100%	KF418930.1
Alnus glutinosa ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1160	1160	95%	0.0	100%	KF418927.1
Alnus formosana ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1160	1160	95%	0.0	100%	KF418926.1
Alnus x mayrii voucher MBG990061 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1160	1160	95%	0.0	100%	FJ844589.1

Figure 3.4: Blast analysis of C2 sample.

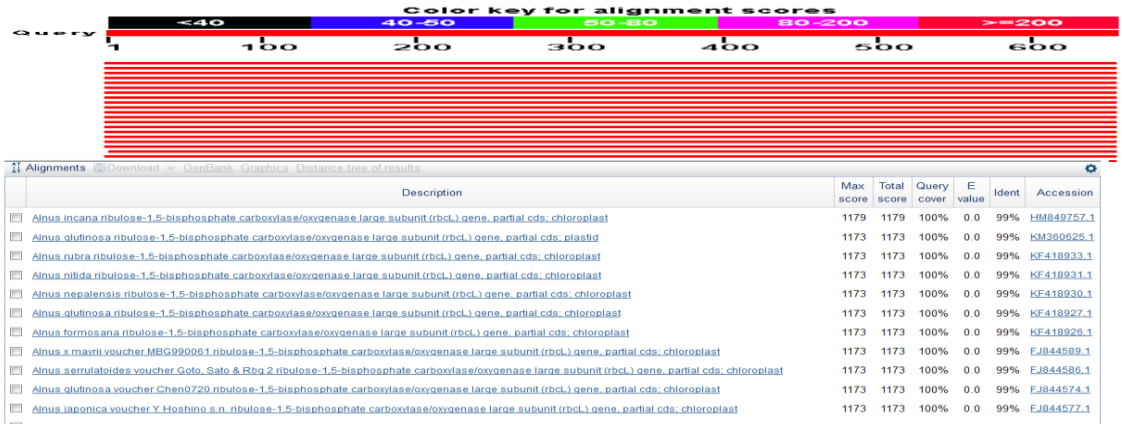


Figure 3.5 : BLAST analysis of C3 sample.

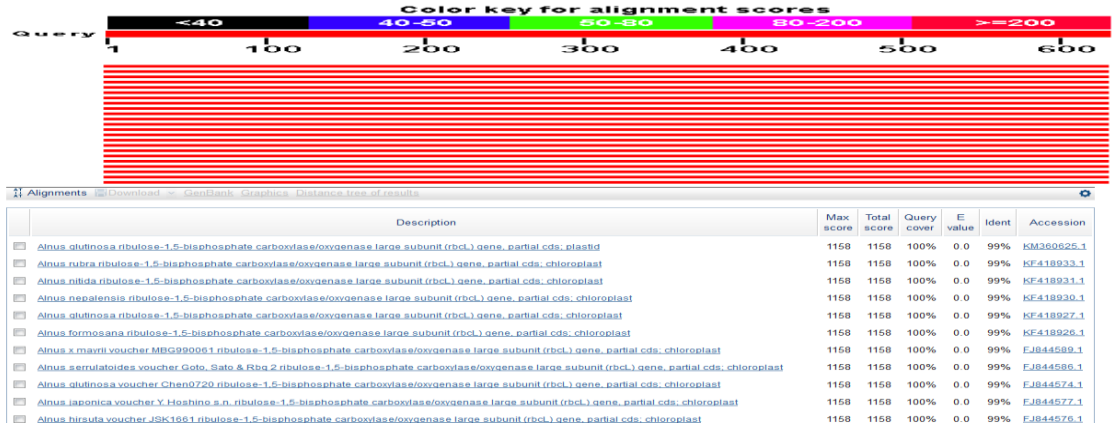


Figure 3.6: BLAST analysis of C4 sample.

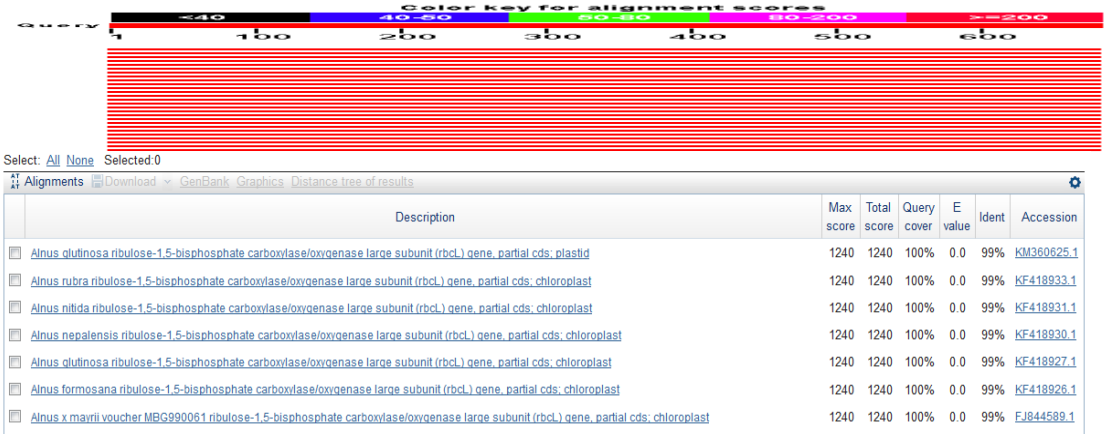
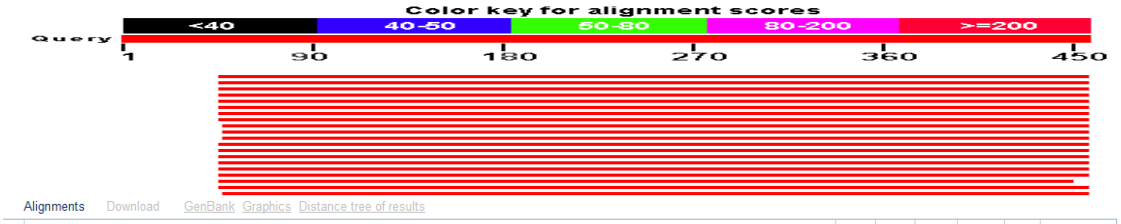


Figure 3.7: BLAST analysis of C5 sample.

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Macaranga aleuritoides ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1131	1131	99%	0.0	99%	FJ976147.1
Macaranga aleuritoides chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1131	1131	99%	0.0	99%	AB267922.1
Macaranga tanarius chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1120	1120	99%	0.0	98%	AB233866.1
Macaranga sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T202	1114	1114	99%	0.0	98%	AB586298.1
Macaranga grandifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit-like (rbcL) gene, partial sequence; chloroplast	1114	1114	99%	0.0	98%	AY794935.1
Mallotus sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T364	1086	1086	99%	0.0	97%	AB586303.1
Mallotus japonicus chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1086	1086	99%	0.0	97%	AB267923.1
Mallotus japonicus ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit-like (rbcL) gene, partial sequence; chloroplast	1086	1086	99%	0.0	97%	AY794934.1
Trewia nudiflora ribulose 1,5-bisphosphate carboxylase large subunit (rbcL) gene, partial cds; chloroplast	1070	1070	99%	0.0	97%	AY663648.1

Figure 3.8: Blast analysis of C6 sample.



Alignments Download GenBank Graphics Distance tree of results

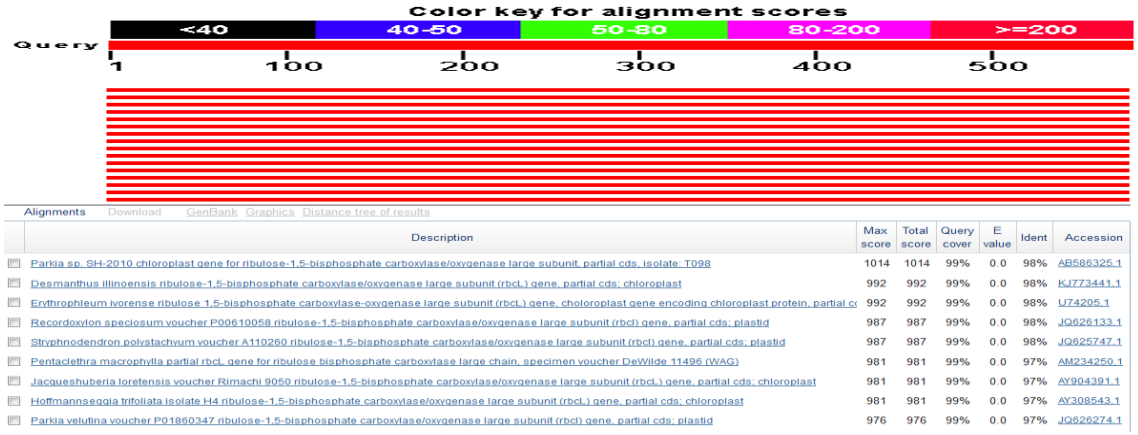
Description	Max score	Total score	Query cover	E value	Ident	Accession
Pnivea chloroplast rbcL gene for rubisco (large subunit) (partial)	610	610	89%	7e-171	94%	Z37430.1
Prostanthera calycina ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	599	599	89%	2e-167	93%	HQ384867.1
Prostanthera rotundifolia chloroplast ribulosebisphosphate carboxylase large subunit (rbcL) gene, partial cds	595	595	89%	2e-166	93%	L14008.1
Bourreria cassinifolia ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	593	593	89%	7e-166	93%	KJ773317.1
Cordia saccellia isolate 815 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	593	593	89%	7e-166	93%	KF158129.1
Bourreria moaensis isolate 450 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	593	593	89%	7e-166	93%	KF158116.1
Cordia sp. MAG-2009 voucher NL110292 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	593	593	89%	7e-166	93%	FJ037970.1

Figure 3.9: Blast analysis of C7 sample.

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Breynia cernua chloroplast rbcL gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds	1037	1037	99%	0.0	99%	AB233918.1
Phyllanthus emblica ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	1037	1037	99%	0.0	99%	AY765269.1
Breynia sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T866	1031	1031	99%	0.0	99%	AB586484.1
Breynia sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T186	1031	1031	99%	0.0	99%	AB586483.1
Sauropus racemosus ribulose 1,5-bisphosphate carboxylase large subunit (rbcL) gene, partial cds; chloroplast	1031	1031	99%	0.0	99%	AY663617.1
Phyllanthus polyphyllus ribulose 1,5-bisphosphate carboxylase large subunit (rbcL) gene, partial cds; chloroplast	1031	1031	99%	0.0	99%	AY663611.1

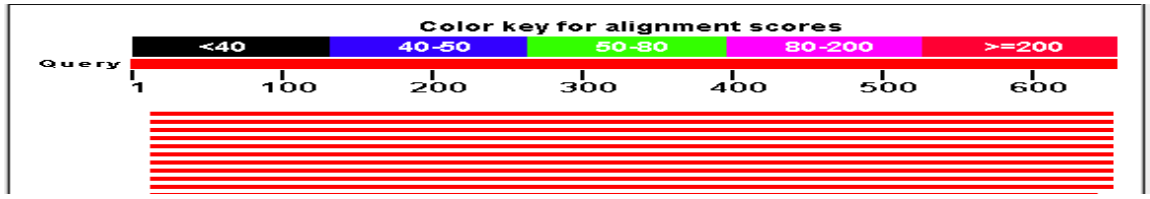
Figure 4.0: Blast analysis of C8 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Parkia sp. SH-2010 chloroplast gene for ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial cds, isolate: T098	1014	1014	99%	0.0	98%	AB586325.1
Desmanthus illinoensis ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	992	992	99%	0.0	98%	KJ773441.1
Erythrophleum ivorense ribulose 1,5-bisphosphate carboxylase-oxygenase large subunit (rbcL) gene, choloroplast gene encoding chloroplast protein, partial c	992	992	99%	0.0	98%	U74205.1
Recordoxylon speciosum voucher P00610058 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; plastid	987	987	99%	0.0	98%	JQ626133.1
Stryphnodendron polystachyum voucher A110260 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; plastid	987	987	99%	0.0	98%	JQ625747.1
Pentaclethra macrophylla partial rbcL gene for ribulose bisphosphate carboxylase large chain, specimen voucher DeWilde 11496 (WAG)	981	981	99%	0.0	97%	AM234250.1
Jacqueshuberia loretensis voucher Rimachi 9050 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	981	981	99%	0.0	97%	AY904391.1
Hoffmannseggia trifoliata isolate H4 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; chloroplast	981	981	99%	0.0	97%	AY308543.1
Parkia velutina voucher P01860347 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcl) gene, partial cds; plastid	976	976	99%	0.0	97%	JQ626274.1

Figure 4.1: Blast analysis of C9 sample.

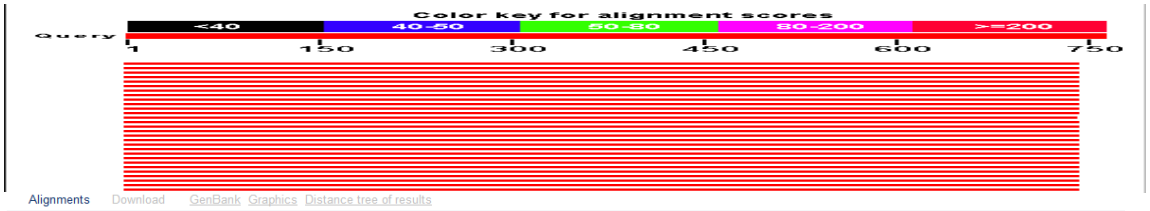


Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Pisum sativum subsp. elatius, bio-material JI1794, chloroplast complete genome	1166	1166	97%	0.0	99%	HG966675.1
Pisum sativum subsp. sativum, bio-material WL1238, chloroplast complete genome	1166	1166	97%	0.0	99%	HG966674.1
Pisum sativum chloroplast, complete genome	1166	1166	97%	0.0	99%	KJ806203.1
Pisum sativum subsp. sativum isolate HS878 ribulose 1,5-bisphosphate carboxylase (rbcL) gene, partial cds; chloroplast	1166	1166	97%	0.0	99%	JN661190.1
Pisum sativum cultivar Feltham First chloroplast, complete genome	1166	1166	97%	0.0	99%	HM029370.1
Pea chloroplast gene for ribulose 1,5-bisphosphate carboxylase (rbcl)	1166	1166	97%	0.0	99%	X03853.1
Pisum sativum subsp. elatius, bio-material L100, chloroplast complete genome	1160	1160	97%	0.0	99%	HG966676.1
Pisum sativum subsp. elatius, bio-material 721, chloroplast complete genome	1160	1160	97%	0.0	99%	HG966673.1

Figure 4.2: Blast analysis of C10 sample.

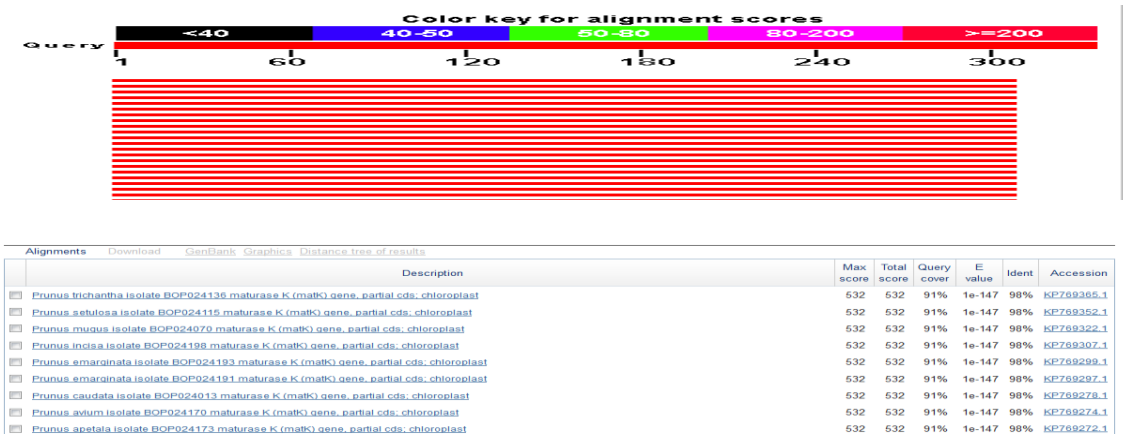
5.2.5 Blast result of sample (*mat*K gene)



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Mikania glomerata voucher A.C.Rabonato 11 (RB) maturase K (matK) gene, partial cds; chloroplast	1323	1323	97%	0.0	99%	JF826294.1
Dyscritothamnus mirandae maturase K (matK) gene, complete cds; chloroplast	1301	1301	97%	0.0	98%	AY215786.1
Eutetras palmeri maturase K (matK) gene, complete cds; chloroplast	1290	1290	97%	0.0	98%	AY215797.1
Pseudoclappia arenaria maturase K (matK) gene, complete cds; chloroplast	1284	1284	97%	0.0	98%	AY215849.1
Podanthus mitiqui maturase K (matK) gene, partial cds; chloroplast	1284	1284	97%	0.0	98%	AY215846.1
Peucephyllum schottii maturase K (matK) gene, complete cds; chloroplast	1284	1284	97%	0.0	98%	AY215842.1
Pericome caudata maturase K (matK) gene, complete cds; chloroplast	1284	1284	97%	0.0	98%	AY215838.1

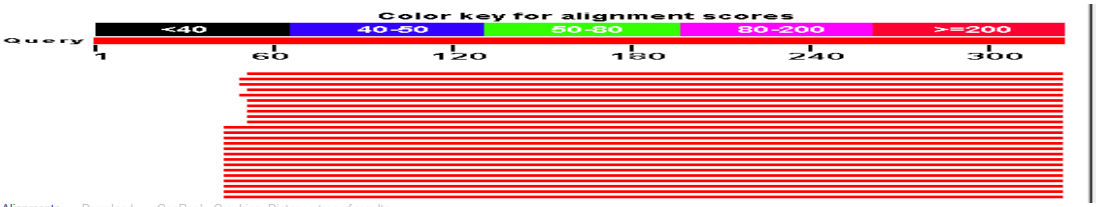
Figure 4.3: Blast analysis of A2 sample.



Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Prunus trichantha isolate BOP024136 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769365.1
Prunus setulosa isolate BOP024115 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769352.1
Prunus mugus isolate BOP024070 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769322.1
Prunus incisa isolate BOP024198 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769307.1
Prunus emarginata isolate BOP024193 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769299.1
Prunus emarginata isolate BOP024191 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769297.1
Prunus caudata isolate BOP024013 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769278.1
Prunus avium isolate BOP024170 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769274.1
Prunus apetala isolate BOP024173 maturase K (matK) gene, partial cds; chloroplast	532	532	91%	1e-147	98%	KP769272.1

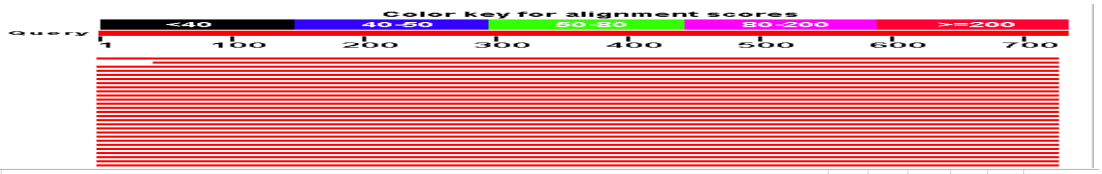
Figure 4.4: Blast analysis of A3 sample.



Alignments Download GenBank Graphics Distance tree of results

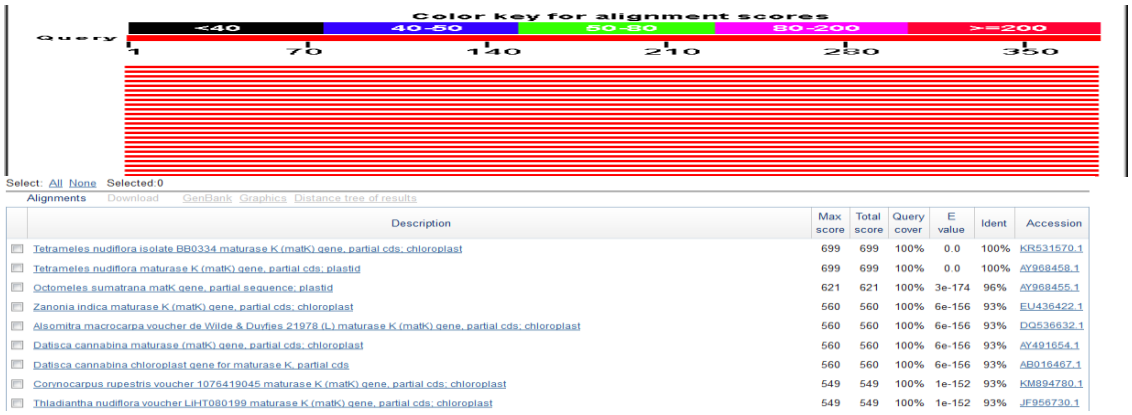
Description	Max score	Total score	Query cover	E value	Ident	Accession
Alnus nepalensis voucher Tibet2213 maturaseK (matK) gene, partial cds; chloroplast	219	219	83%	4e-53	81%	FJ011819.1
Alnus serrulata maturase K (matK) gene, partial cds; chloroplast	213	213	84%	2e-51	81%	KJ772532.1
Alnus incana voucher WAB_0132469439 maturase K (matK) gene, partial cds; chloroplast	213	213	84%	2e-51	81%	KJ592842.1
Alnus rubra maturase K (matK) gene, partial cds; chloroplast	213	213	83%	2e-51	81%	KF419031.1
Alnus maritima maturase K (matK) gene, partial cds; chloroplast	213	213	84%	2e-51	81%	KF419027.1
Alnus alnobetula subsp. crispa voucher 09PROBE-05302 maturase K (matK) gene, partial cds; chloroplast	213	213	83%	2e-51	81%	JN966067.1
Alnus rubra voucher Nathan004-BCA29 maturase K (matK) gene, partial cds; chloroplast	213	213	83%	2e-51	81%	JF953074.1

Figure 4.5: Blast analysis of A6 sample.



Description	Max score	Total score	Query cover	E value	Ident	Accession
Tetrameles nudiflora maturase K (matK) gene, partial cds; plastid	1230	1230	99%	0.0	97%	AY968458.1
Tetrameles nudiflora isolate BB0334 maturase K (matK) gene, partial cds; chloroplast	1157	1157	93%	0.0	97%	KR531570.1
Octomeles sumatrana matK gene, partial sequence; plastid	1125	1125	99%	0.0	95%	AY968455.1
Corynocarpus laevigata chloroplast, complete genome	998	998	99%	0.0	91%	HQ207704.1
Hillebrandia sandwicensis voucher Montreal Botanic Garden 296057 tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; chlo	998	998	99%	0.0	91%	GU397085.1
Corynocarpus laevigatus maturase (matK) gene, partial cds; chloroplast	998	998	99%	0.0	91%	AY491652.1
Hillebrandia sandwicensis maturase K (matK) gene, partial cds; plastid	998	998	99%	0.0	91%	AY968452.1
Corynocarpus laevigata maturase K (matK) gene, partial cds; plastid	998	998	99%	0.0	91%	AY968448.1
Begonia poculifera voucher Royal Botanic Garden Edinburgh 19923143 tRNA-Lys (trnK) gene, partial sequence; and maturase K (matK) gene, complete cds; cl	987	987	99%	0.0	91%	GU397108.1

Figure 4.6: Blast analysis of C4 sample.

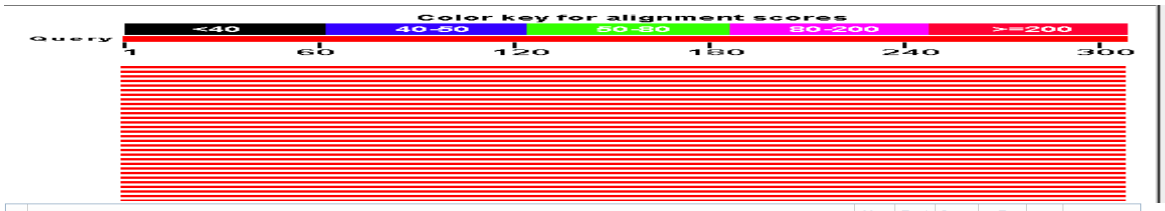


Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Tetrameles nudiflora isolate BB0334 maturase K (matK) gene, partial cds; chloroplast	699	699	100%	0.0	100%	KR531570.1
Tetrameles nudiflora maturase K (matK) gene, partial cds; plastid	699	699	100%	0.0	100%	AY968458.1
Octomeles sumatrana matK gene, partial sequence; plastid	621	621	100%	3e-174	96%	AY968455.1
Zanonia indica maturase K (matK) gene, partial cds; chloroplast	560	560	100%	6e-156	93%	EU436422.1
Alsomitra macrocarpa voucher de Wilde & Duyfjes 21978 (L) maturase K (matK) gene, partial cds; chloroplast	560	560	100%	6e-156	93%	DQ536632.1
Datisca cannabina maturase (matK) gene, partial cds; chloroplast	560	560	100%	6e-156	93%	AY491654.1
Datisca cannabina chloroplast gene for maturase K, partial cds	560	560	100%	6e-156	93%	AB016467.1
Corynocarpus rupestris voucher 1076419045 maturase K (matK) gene, partial cds; chloroplast	549	549	100%	1e-152	93%	KM894780.1
Thladiantha nudiflora voucher LiHT080199 maturase K (matK) gene, partial cds; chloroplast	549	549	100%	1e-152	93%	JF956730.1

Figure 4.7: Blast analysis of C5 sample.



Description	Max score	Total score	Query cover	E value	Ident	Accession
Hymenopappus tenuifolius voucher Steele 1325 plastid sequence	555	555	100%	2e-154	99%	KP126891.1
Hymenopappus filifolius var. filifolius maturase K (matK) gene, complete cds; chloroplast	555	555	100%	2e-154	99%	AY215808.1
Mikania scandens maturase K (matK) gene, partial cds; chloroplast	549	549	100%	1e-152	99%	KJ772944.1
Venegasia carpesioides maturase K (matK) gene, complete cds; chloroplast	549	549	100%	1e-152	99%	AY215876.1
Peucephyllum schottii maturase K (matK) gene, complete cds; chloroplast	549	549	100%	1e-152	99%	AY215842.1
Oteiza scandens maturase K (matK) gene, complete cds; chloroplast	549	549	100%	1e-152	99%	AY215833.1
Engelmannia peristenia maturase K (matK) gene, complete cds; chloroplast	549	549	100%	1e-152	99%	AY215791.1
Dyscritothamnus mirandae maturase K (matK) gene, complete cds; chloroplast	549	549	100%	1e-152	99%	AY215786.1
Desmanthodium fruticosum maturase K (matK) gene, complete cds; chloroplast	549	549	100%	1e-152	99%	AY215782.1

Figure 4.8: Blast analysis of C7 sample.

5.2.6 Sequence analysis

There is a critical factor that need scrupulous attention when a PCR- based method is applied to the analysis of honey DNA sample, honey consist of atleast 80% of sugar and this may act as an inhibitory factor for the PCR. As a consequence, the DNA extraction protocol has to be optimized to ensure a sufficient amount of DNA, free of PCR-sinhibiting substances. We found that a preliminary extensive 65°C incubation for 1hour and glass beads grinding of the sample were very important to minimized the effect of high concentration of polysaccharides and the lysis of the pollen exine was done in an Eppendorf tube. As a pilot study, the amount and purity of DNA extracted from the sample were determined using spectrophotometry and the total DNA yields of each honey sample range from 20 to 45ng/µl. However, the 260/280 optical density (OD's), a measure of extract purity, varied from 1.60 to 1.84 (Table 1.4).

As shown in Figure 1.9 and Figure 2.0 the extracted DNA was intact, the method provided positive result in all the genomic DNA samples and it shows a high molecular weight, PCR amplified band (900bd for *rbc*L and *mat*K 700bp) in the gel (Fig. 2.1 and Fig.2.2). DNA sequences were subjected to the BLAST program of *rbc*L and *mat*K in NCBI. Number A1 sample matches - 99% with species *Terminalia* within the family Combretaceae; A2 sample matches - 91% with species *Mikania* scandens within the family Asteraceae and *Mikania* sps. family Asteraceae; A3 sample matches - 99% with species *Alnus nepalensis* within the family Betulaceae and *Prunus tichantha* under Rosaceae family; A4 sample matches - 98% with species *Berberidopsis corallina* within family Berberidopsidaceae; A5 sample matches - 99% with species *Mikania scandens* within family Asteraceae; A6 sample matches - 100% with species *Macaranga*

denticulata within family Euphorbiaceae and *Alnus nepalensis* under family Betulaceae; A7 sample matches - 99% with species *Pisum sativum* within family Fabaceae; A8 sample matches - 97% with species *Ecalyptus erythocarys* within family Myrtaceae; A9 sample matches - 95% with species *Mikania micrantha* within family Asteraceae; A10 sample matches - 95% with species *Juglans cineria* within family Juglandaceae; C1 sample matches - 98% with species *Mallotus sps.* within family Euphorbiaceae; C2 sample matches - 100% with species *Alnus nepalensis* within family Betulaceae; C3 sample matches - 99% with species *Alnus incana* within family Betulaceae; C4 sample matches - 99% with species *Alnus nitida* within family Betulaceae and *Tetrameles nudiflora* within family Datiscaceae; C5 sample matches - 99% with species *Alnus nepalensis* within family Betulaceae and *Terameles nudiflora* within family Datiscaceae; C6 sample matches - 98% with species *Macaranga aleuritoides* within family Euphorbiaceae; C7 sample matches - 94% with species *Prostanthera nivea* within family Meliaceae and *Mikania scandens* within family Asteraceae; C8 sample matches – 99% with species *Phylanthus emlica* within family Euphorbiaceae; C9 sample matches - 98% with species *Parkia* within family Fabaceae and C10 sample matches - 99% with species *Pisum sativum* family Fabaceae (Table 1.9). The BLAST method, which is to accept the top hit as the species identification and perform the best.

CHAPTER 6

DISCUSSION

The result reveals that 76 plant species belonging to 35 families were useful to honey bees which are well distributed and commonly found in the study area, it reflected the vegetational characteristic feature of the region. The information on various families, genera, species of polliniferous plants of the region used as honey plants, habit wise categorization, plants along with botanical name, local name and phenological features. As they provide food in the form of pollen and nectar during different month of the year. The study area has mixed vegetation, and consist of family Fabaceae consist of 10 species which is highest; followed by Asteraceae 9 species; Malvaceae and Myrtaceae 5 species; Cucurbitaceae and Euphorbiaceae 4 species; Rubiaceae (3 species); Brassicaceae, Combretaceae, Lamiaceae, Lythraceae, Poaceae, Rosaceae, Solanaceae and Verbenaceae (2 species each); and the remaining families Amaranthaceae, Anacardiaceae, Apiaceae, Arecaceae, Asclepediaceae, Betuliaceae, Bignoniaceae, Bombacaceae, Caricaceae, Cyperaceae, Datiscaceae, Eleaeocarpaceae, Fagaceae, Moringaceae, Musaceae, Oxalidaceae, Polygonaceae, Rutaceae, Tropaeolaceae and Vitaceae were represented by single species each (Figure 1.4)

Study area exhibit diversified flora and habit, the herb include the family Asteraceae (3 species); Brassicaceae (3 species); Bombacaceae, Malvaceae, Poaceae (2 species each) and the remaining family Amaranthaceae, Asclepediaceae, Cyperaceae, Fabaceae, Musaceae, Rosaceae, Solanaceae, Tropaeolaceae were represented by single species; the shrub Asteraceae, Euphorbiaceae, Fabaceae, Lamiaceae, Malvaceae,

Rubiaceae (2 species each), and the remaining families Arecaceae, Bignoniaceae, Rosaceae, Verbenaceae were represented by single species each. The tree habit consist of 5 species which is highest by Fabaceae and Myrtaceae; Combretaceae, Euphorbiaceae, Lythraceae with 2 species each. And the remaining families Anacardiaceae, Asteraceae, Betulaceae, Bombacaceae, Caricaceae, Datiscaceae, Elaeocarpaceae, Fagaceae, Moringaceae, Oxalidaceae, Rosaceae, Rubiaceae, Rutaceae and Verbenaceae were represented by single species each. Among the climbers, the dominant family Cucurbitaceae consist of 4 species; Asteraceae and Fabaceae 2 species each, and the remaining families Malvaceae, Polygonaceae, Vitaceae were represented by single species each (Figure 1.5).

The colour prefered by honey plants for bee forage are important. The families Amaranthaceae, Betulaceae, Fagaceae, Polygonaceae and Rosaceae consist of one species with pink flower. The white flowers were found to be in the family Fabaceae consist of five species; Myrtaceae four species; Asteraceae three species; Rubiaceae two species and the remaining families, Anacardiaceae, Apiaceae, Arecaceae, Brassicaceae, Caricaceae, Eleaocarpaceae, Euphorbiaceae, Fagaceae, Lamiaceae, Malvaceae, Moringaceae, Musaceae, Poaceae, Rutaceae, Solanaceae were represented by single species each; the orange colour flower were seen Asclepidiaceae, Asteraceae, Fabaceae, Lythraceae, Oxalidaceae and Tropaeolaceae were represented by single species each; The violet flower colour were present in the family Asteraceae and Lythraceae each were single species; the yellow colour flower were represented by Cucurbitaceae two species; Asteraceae and Combretaceae two species; and the remaining families Bignoniaceae, Brassicaceae, Datiscaceae, Euphorbiaceae, Fabaceae, Poaceae, Rosaceae

and Verbenaceae were represented by single species each. The red colour flower seen in the family Malvaceae three species; Euphorbiaceae two species, and the ramaining families Asteraceae, Lamiaceae, Myrtaceae, Rubiaceae were represented by single species each; the purple flower colour in the family Fabaceae two species; Solanaceae, Verbenaceae and Vitaceae were represented by single species, the green colour flower the family Cyperaceae represented by single species (Figure1.7)

The flowering months referred to the diversity of the flora in the region, flowering prevail the whole year to January Malvaceae three species and the remaining family Asteraceae, Bignoniaceae, Bombacaceae, Combretaceae, Cyperaceae, Fabaceae, Lamiaceae, Moringaceae, Musaceae, Myrtaceae, Rosaceae were represented by single species each. In the month of February the family consist of Euphorbiaceae and Malvaceae with three species each, Fabaceae, Myrtaceae and Rosaceae with two species and the remaining families Arecaceae, Asteraceae, Bignoniaceae, Bombacaceae, Brassicaceae, Combretaceae, Cyperaceae, Datiscaceae, Lamiaceae, Moringaceae, Musaceae were represented by single species. In March, the family Fabaceae and Myrtaceae with four species each, Malvaceae with three species, Brassicaceae and Rosaceae with two species each, and the remaining families Anacardiaceae, Arecaceae, Bignoniaceae, Bombacaceae, Datiscaceae, Moringaceae, Musaceae, Rubiaceae, Rutaceae, Solanaceae, Verbenaceae were represented by single species each. In the month of April, Fabaceae with six species, Malvaceae and Myrtaceae with three species each, Asteraceae, Rosaceae and Rubiaceae with two species each, and the remaining families Anacardiaceae, Arecaceae, Bignoniaceae, Datiscaceae, Lythraceae, Moringaceae, Musaceae, Solanaceae, Verbenaceae, Vitaceae were represented by single

species each. In the month of May, the family Fabaceae consist of seven species, followed by Malvaceae with four species, Asteraceae with three species, Cucurbitaceae, Lythraceae, Rosaceae with two species each and the remaining families Arecaceae, Bignoniaceae, Brassicaceae, Elaeocarpaceae, Moringaceae, Musaceae, Polygonaceae, Rutaceae, Verbenaceae, Vitaceae were represented by single species each. In the month of June, the family Asteraceae and Fabaceae consist of five species each followed by Malvaceae with four species, Lythraceae, and Myrtaceae with two species each and the remaining families Apiaceae, Arecaceae, Bignoniaceae, Elaeocarpaceae, Musaceae, Oxalidaceae, Polygonaceae, Rosaceae, Solanaceae, Tropaeolaceae and Verbenaceae were represented by single species each. In the month of July, the family Asteraceae consist of five species, Malvaceae with four species, Cucurbitaceae with three species, Fabaceae with two species and the remaining families Apiaceae, Arecaceae, Asclepidiaceae, Bignoniaceae, Caricaceae, Musaceae, Myrtaceae, Poaceae, Polygonaceae, Rosaceae, Tropaeolaceae, Verbenaceae were represented by single species each. In the month of August, the family Asteraceae consist of five species which is highest followed by Malvaceae with four species, Cucurbitaceae with two species and the remaining families Amaranthaceae, Apiaceae, Arecaceae, Asclepidiaceae, Betuliaceae, Caricaceae, Cyperaceae, Fabaceae, Fagaceae, Moringaceae, Myrtaceae, Rosaceae, Tropaeolaceae and Verbenaceae were represented by single species each. In the month of September, the family Asteraceae consist of four species which is highest followed by Malvaceae with three species, and the remaining families Arecaceae, Asclepidiaceae, Betulaceae, Bignoniaceae, Cyperaceae, Fabaceae, Fagaceae, Lamiaceae, Musaceae, Poaceae, Rosaceae, Rubiaceae, Tropaeolaceae,

Verbenaceae were represented by single species each. In the month of October, the family Asteraceae consist of four species which is highest, followed by Malvaceae with three species and the remaining families Arecaceae, Asclepidiaceae, Betulaceae, Bignoniaceae, Combretaceae, Cyperaceae, Fabaceae, Lamiaceae, Musaceae and Rosaceae were represented by single species each. In the month of November, the family Asteraceae and Malvaceae with four species each, followed by Combretaceae with two species, and the remaining families Arecaceae, Bignoniaceae, Cyperaceae, Fabaceae, Musaceae and Rosaceae were represented by single species each. In the month of December, the family Malvaceae consist of four species which is highest, followed by Asteraceae with three species, and the remaining families Arecaceae, Bignoniaceae, Combretaceae, Cyperaceae, Euphorbiaceae, Musaceae and Rosaceae were represented by single species each.(Figure 1.6). Seasonal fluctuation of pollen collection by honey bees with two well separated peaks over the study periods, no pollen collection percentage was recorded in the winter months which is characterized by low plant flowering density (Cope and EL-Eisawi, 1998) and unfavourable environmental condition with cool nights and low day time temperature. The study shows here that increase number of flowers during March to June enhance the bees to collects more pollen of pollen foraging during this period.

From the region of Norhwest Himalaya notable contribution on honey flora are those of Sharma (2005), Partap and Verma (2007) enlisted more than 200 promising honey plants of the Hindu kush Himalaya covering mountaineous areas of Afghanistan, Bangladesh, Bhutan, China, India, Myanmar, Nepal and Pakistan. Diversity of polliniferous of Western Ghats, India revealed that 52 plants species were useful to

honey bees by Waykar *et al*., (2014). Flowers are the main stay of the bees life however flowering plants of several plants family are blossoming at different time interval of the year (Free, 1970) depending upon the soil type, climatic factors and the habitat of the vegetation, the time of the blooming may change for even the same nectar plant (Rodinov & Shabanshov, 1986). Sound information on duration of flowering and blooming time is essential for proper bee keeping management (Kumar *et al*., 2013). Pollen from the different flower has specic shape, size and ornamentation. Microscopical analysis of pollen of plants forged by bees is an established method to determine the source of honey in the area. Several studies on pollen morphology have been done worldwide (Kral 1992, Adekanmbi and Ogundipe 2006, Perveen and Qaiser 2010). Pollen morphological study can provide a basis for the identification of plant species and has significant application in recognition of bee plants (Shubharani *et al.*, 2013) and diversity of forage plants in Mizoram, Northeast India reported (Laha and Ralte, 2014 and Laha and Ralte, 2015)

The study of polliniferous plant in the sites were classified under different class of vegetation like, wild plants, horticultural plants, ornamental plants and agricultural plants. Under wild plants, 42 plants species were identified among which Fabaceae family had eight species followed by Asteraceae family seven species, three species under Euphorbiaceae family, Malvaceae, Myrtaceae, Lamiaceae, Rosaceae, Combretaceae and Verbenaceae have two species each and one species each by a family of Betulaceae, Polygonaceae, Amaranthaceae, Oxalidaceae, Bombacaceae, Fagaceae, Rubiaceae, Cyperaceae, Elaeocarpaceae, Lythraceae, Solanaceae and Bignoniaceae. Horticultural plants consist of 11 plants species in which highest number of three species

obtained by Myrtaceae family followed by Caricaceae, Rutaceae, Arecaceae, Rubiaceae, Lythraceae, Anacardaceae, Musaceae and Vitaceae each by one species. Ornamental plants consist of least number of plant species that is 8 plants in which polliniferous plants fall under two plant species in Malvaceae family followed by one species each form Asteraceae, Rubiaceae, Euphorbiaceae and Rosaceae family. Agricultural plants consist of 15 polliniferous plants in which families of Cucurbitaceae contain highest number of plants of four species, followed by two species obtained from families of Brassicaceae and Poaceae family. Lastly, one species of plant species is found each in families of Malvaceae, Apiaceae, Moringaceae, Solanaceae and Vitaceae (Table 1.8 and figure 1.8)

The study area has mixed vegetation, the present study confirmed that all the honey samples were found to be multifloral. The family Fabaceae consist of 10 species which is highest; followed by Asteraceae 9 species; Malvaceae and Myrtaceae (5 species); Cucurbitaceae and Euphorbiaceae (4 species); Rubiaceae consist of 3 species; Brassicaceae, Combretaceae, Lamiaceae, Lythraceae, Poaceae, Rosaceae, Solanaceae and Verbenaceae (2 species each); and the remaining families Amaranthaceae, Anacardaceae, Apiaceae, Arecaceae, Asclepediaceae, Betulaceae, Bignoniaceae, Bombacaceae, Caricaceae, Cyperaceae, Datiscaceae, Eloeocarpaceae, Fagaceae, Moringaceae, Musaceae, Oxalidaceae, Polygonaceae, Rutaceae, Tropaeolaceae and Vitaceae were represented by single species each (Table 1.8).

The pollen count derived that some species were more frequent in the sample because some plant species readily produces nectar and their flowering period is longer if compared with other species, some flowering plants maybe having good quality of

nectar. This analysis showed that bee collect the nectar of that plants which are available in that area. During the study period the secondary pollen type (16-45%) were dominated by the families Fabaceae consist of four species (*Acacia pruinescence, Parkia timoriana, Pisum sativum, Tamarindus indica*); Asteraceae consist of 3 species (*Ageratum conyzoides, Mikania micrantha, Spilanthes acmella*); Myrtaceae consist of 3 species *Eucalytus tereticornis, Psidium guaiava, Syzygium cumini*); Poaceae consisit of 2 species (*Oryza sativa, Zea mays*) and the remaining families, Apiaceae (*Coriandrum sativum*); Arecaceae (*Cocos nucifera*); Betulaceae (*Alnus nitida*); Brassicaceae (*Brassica campestris*); Caricaceae (*Carica papaya*); Combretaceae (*Terminalia bellerica*); Cucurbitaceae (*Cucumis sativas*); Cyperaceae (*Cyperus rotundus*); Datiscaceae (*Tetrameles nudiflora*); Euphorbiaceae (*Emblica officinalis*); Lythraceae (*Lagerstromia speciosa*); Malvaceae (*Ipomea batatas*); Moringaceae (*Moringa oleifera*); Musaceae (*Musa paradisiacal*) and Rubiaceae (*Coffee arabica*) were represented by single species. Other important minor pollen types (3-15%) confirmed in all the honey samples consist of the family Fabaceae consist of 10 species (*Acacia pruinescens, Bauhinia variegate, Caesalpinia pulcherrima, Cassia javanica, Derris robusta, Mimosa pudica, Parkia timoriana, Phaseolus vulgaris, Pisum sativum* and *Tamarindus indica*) followed by Asteraceae consist of 8 species (*Ageratum conyzoides, Bidens pilosa, Cosmos sulphureus, Mikania micranta, Spilenthes acmella, Tagetes erecta, Tithonia diversifolia* and *Zinnia elegans*); Malvaceae (*Althaea rosea, Anthuriom andreanum, Hibiscus rosa sinensis, Ipomoea batatas* and *Malvaviscus arboreus*) and Myrtaceae (*Callistemin lanceolatus, Eucalyptus tereticornis, Psidium guajava, Syzygium cumini, Syzygium jambos*) 5 species each; Cucurbitaceae (*Cucumis sativus*, Cucurbita pepo,

Momordica charantia, and *Sechium edule*) and Euphorbiaceae (*Croton jaufra*, *Emblica officinalis, Euphorbia pulcherrima, Riccinus communis*) 4 species each; Rubiaceae consist of 3 species (*Coffee arabica*, *Ixora coccinea*, *Jatropha curcus*); Brassicaceae (*Brassica campestris and Raphanus sativus*), Combretaceae (*Terminalia crenulata and Terminalia bellerica*), Lamiaceae (*Homskioldia sanguine*, *Leucosceptrum canum*), Poaceae (*Oryza sativa* and *Zea mays*), Rosaceae(*Prunus persica* and *Rosa macrophylla*), Solanaceae (*Nicotiana tobaccum* and *Solanum melongena*) and Verbenaceae (*Lantana camara* and *Callicarpa arborea*) by 2 species each; Amaranthaceae (*Amaranthus* sps), Anacardiaceae (*Mangifera indica*), Apiaceae (*Coriandrum sativum*), Arecaceae (*Cocos nucifera),* Asclepediaceae (Asclepias currasavica), Betulaceae (*Alnus nitida*), Bignoniaceae (*Tecoma stans*), Bombacaceae (*Bombax ceiba*), Caricaceae (*Carica papaya*), Cyperaceae (*Cyperus rotundus*), Datiscaceae (*Tetrameles nudiflora*) Eleaocarpaceae (*Eleaocarpus lenceifolius*), Fagaceae (*Castanopsis tribuloides*), Lythraceae (*Punica granatum*), Moringaceae (*Moringa oleifera*), Musaceae (*Musa paradisiacal),* Oxalidaceae *(Averhoa carambola*), Polygonaceae (*Antigonon leptopus*), Rutaceae (*Citrus limon*), Tropaelaceae (*Tropaelum majus*), Vitaceae (*Vitis vinifera*) were represented by single species. Minor pollen types (<3%) confirmed in all the honey samples consist of the family Fabaceae consist of 10 species (*Acacia pruinescens*, *Bauhinia variegate*, *Caesalpinia pulcherrima*, *Cassia javanica, Derris robusta, Mimosa pudica*, *Parkia timoriana*, *Phaseolus vulgaris*, *Pisum sativum* and *Tamarindus indica*) followed by Asteraceae consist of 9 species (*Ageratum conyzoides, Bidens pilosa, Cosmos sulphureus*, *Matricaria chomonulla*, *Mikania micranta*, *Spilenthes acmella*, *Tagetes erecta*, *Tithonia diversifolia* and *Zinnia elegans*);

Malvaceae (*Althaea rosea*, *Anthuriom andreanum*, *Hibiscus rosa sinensis*, *Ipomoea batatas* and *Malvaviscus arboreus*) and Myrtaceae (*Callistemin lanceolatus*, *Eucalyptus tereticornis*, *Psidium guajava*, *Syzygium cumini*, *Syzygium jambos*) 5 species each; Cucurbitaceae (*Cucumis sativus*, *Cucurbita pepo*, *Momordica charantia*, and *Sechium edule*) and Euphorbiaceae (*Croton jaufra*, *Emblica officinalis*, *Euphorbia pulcherrima*, *Riccinus communis*) 4 species each; Rubiaceae consist of 3 species (*Coffee arabica*, *Ixora coccinea*, *Jatropha curcus*); Brassicaceae (*Brassica campestris* and *Raphanus sativus*), Combretaceae (*Terminalia crenulata* and *Terminalia bellerica*), Lamiaceae (*Homskioldia sanguine*, *Leucosceptrum canum*); Lythraceae (*Lagerstromia speciosa*, *Punica granatum*), Poaceae (*Oryza sativa* and *Zea mays*), Rosaceae(*Prunus persica* and *Rosa macrophylla*), Solanaceae (*Nicotiana tobaccum* and *Solanum melongena*) and Verbenaceae (*Lantana camara* and *Callicarpa arborea*) by 2 species each; Amaranthaceae (*Amaranthus sps*.), Anacardiaceae (*Mangifera indica*), Apiaceae (*Coriandrum sativum*), Arecaceae (*Cocos nucifera*), Asclepediaceae (*Asclepias currasavica*), Betulaceae (*Alnus nitida*), Bignoniaceae (*Tecoma stans*), Bombacaceae (*Bombax ceiba*), Caricaceae (*Carica papaya*), Cyperaceae (*Cyperus rotundus)*, Datiscaceae (*Tetrameles nudiflora*) Eleaocarpaceae (Eleaocarpus lenceifolius), Fagaceae (*Castanopsis tribuloides*), Moringaceae (*Moringa oleifera*), Musaceae (*Musa paradisiaca*), Oxalidaceae (*Averrhoa carambola*), Polygonaceae (*Antigonon leptopus*), Rutaceae (*Citrus limon*), Tropaelaceae (*Tropaelum majus*), Vitaceae (*Vitis vinifera*) were represented by single species (Table 1.8).

Honey spectrum analysis of pollen from Karnataka has good potential for sustaining bee keeping venture because of the diversity of nectar, pollen taxa in the

families Asteraceae, Poaceae, Euphorbiaceae, Rutaceae and Fabaceae (Bhargava *et al*., 2009). Coorg is a very important district of Karnataka in honey production, the investigation of 20 honey samples revealed that 91 plants belonging to 42 families are useful for honey bees and all the honey samples were found to be multifloral (Shubharani *et al*., 2012). The result of pollen analysis of Hizan district of Bitlis province, eastern region of Turkey, pollen content of 20 honey samples were analysed and 9 botanical families were identified Fabaceae, Asteraceae, Boraginaceae, Brassicaceae, Rosaceae, Apiaceae, Chenopodiaceae, Lamiaceae and Arecaceae (Omer *et al*., 2016). The study revealed pollen analysis of honey of Kumaon region, honey samples were unifloral and multifloral dominated with families Asteraceae, Brassicaceae, Labiatae, Rosaceae, Euphorbiaceae, Acanthaceae, Rutaceae and Poaceae (Mithilesh, 1983). Pollen analysis of honey sample from Western ghat, Tamil nadu, India, polliniferous plants belong to 22 taxa belonging to 32 families (Mahendran *et al*., 2015). Mellissopalynology of forest honey from Chintapalli Hills, Andra Pradesh contain both natural and cultivated vegetation type with crops and plantation (Lakshmi and Suryanarayana, 1997).

According to Sowunmi (1976), most Nigerian honey comes from the Savannah Regiion (Mosaic a lowland rain forest and secondary grassland). Earlier investigation from different parts of the world (Mourizio, 1951) has shown that the geographical origin of honey can be established through the pollen content. The dominance of *Anacardium occidentale, Lannea acida* reflects the vegetation of lowland rainforest and guinea savanna (White, 1975). The occurrence of *Elaeis guineensis, Nauclea latifolia, Lannea acida andanacardium Occidentale* characterized farmland and homesteads. The

occurrence of all the above listed pollen types in different proportions confirms their geographical origin as reflecting Guinea savanna. On the basis of ethno cultural knowledge, information and market survey results, most of the Nigerian honey is produced during the season of little or no rainfall: September to April. This season coincides with the flowering period of the most important honey plants.

Our results showed that the pollen spectra were equally comparable between location and also between month and year, the importance of this result, is that it helped to demonstrate the complexity of ecological/ environmental phenomena involved in the process of foraging by bees is the heterogeneous and complex landscape. This shows that single, random samples of honey are likely to provide reliable replicates of the pollen spectra. Furthermore, samples and taxa groups were well delineated based on the three factors considered, the importance of this result is that we now have a tool to classify additional pollen spectra, even when there is a low overall replicability.

The honey pollen content reflected the vegetation characterized by Tropical dry evergreen forest (TDEF) species typical of Coromandel Coast (Jhansi and Ramanujam, 2004): markers were both predominant (e.g., *Lannea, Dodonaea* and *Mollugo*) and less represented (e.g., Melastomataceae and *Borassus* have been reported to be forages by the same bee species in other parts of south India (Padmavathy and Rehel, 2014). The present study, one of the first comprehensive melissopalynological contribution in the context of plant pollinator interaction, documented over time and space, difference in the pollen contents of honey, even within the confined landscape.

The result demonstrate that the DNA isolation method is successful, even in farm samples, where the sugar content will be high. DTT was used as a reducing agent for thiolated DNA. The terminal surfur atoms atoms of thiolated DNA have a tendency to form dimers in solution, especially in the presence of oxygen (Lexa *et al*., 2008), and honey contains lots of different biomolecules which form crosslink with the DNA. DTT was used for isolation of thiolated DNA, as it would facilitate protein digestion and assist in releasing DNA into the solution. The most important part of the DNA isolation mehod is the time duration involved in grinding sample by glassbeads, which determined the pellet formation of the centrifugation. The excess sugar contain in honey sample is also a main problem for good DNA yield. In the presence study 3 samples had OD value of 1.6 which indicate the presence of saccharides. Hence, removal of sugar from the honey sample will be important during the DNA extraction process. On the other hand, the high carbohydrate concentration in the honey help DNA preservation (Taberlet *et al*., 2010) as sugar stabilized nucleic acids (Schill *et al*., 2009), and honey provide an airtight seal that prevent oxygen from entering and thus preserving DNA from being degraded (Taberlet *et al*., 2010). The presence of polyphenolic content make the isolation of high quality nucleic acid problematic in addition, residual polyphenolic interfere in enzymetic reaction such as PCR and endonuclease restriction digestion (Michiels *et al*., 2003). In this study, initial incubation of the sample dissolved in water resulted in removal of sugar and polyphenols as supernatants after the centrifugation process.

In the present study, the r*bc*L gene and some sample with chloroplast *mat*K gene was amplified successfully from the DNA isolated from honey samples. It is difficult to

amplified the cpDNA of different plants from a single honey samples, as PCR primers need perfect conditions to track the exact position for amplification of degraded DNA (Taberlet *et al*., 2007), as remains in decomposing plants (King *et al*., 2008). The informal identification of an unknown plant specimen from honey samples using BLAST, to search large public databases, may be as reliable a method as any other. Furthermore, the application of a decision criterion that some the "weight of evidence" which intergrates across top ranking BLAST hits, is no more reliable than simply using the best hits (Ross *et al*., 2008). However the reliability of BLAST is mainly dependent on the comprehensiveness of the taxon representation in the database. The altering of the E-value cutoff to more or less restrictive values will tune down or up the probability of BLAST incorrectly making a positive identification. The E-value the probability of a random match having rhe observed quality is a proportional to the sized of the sequence search space so that increasing either the number or length of sequences in the reference database will reduce the E-value of the given match.

It is noteworthy that Asteraceae, Berberidopsidaceae, Betulaceae, Combretaceae, Datiscaceae, Euphorbiaceae, Fabaceae, Juglandaceae, Meliaceae, Myrtaceae, Rosaceae families are important in the bee foraging as they include many nectariferous and polliniferous species. The identification of these families reflects the abundance of the flora and the richness of vegetation surrounding the sample collection site.

Molecular based techniques and analysis of *rbc*L and *mat*K region was successful in identifying plants species, from a technical point of view this study aimed to assess the effectiveness of DNA Barcoding to identify species from pollen collected

by honey bees and characterized a list of plants pollinated by insects including rare and endemic taxa at the molecular level. The list a reference data based of DNA Barcoding sequences for taxonomic identification of pollen samples. Morover, beginning with extensive knowledge of plant phenology in the study area, it was evaluated the effects of local floral biodiversity in different periods and localities.

CONCLUSION

Honey is a well known natural sweetener, used for centuries and has been proved to have many therapeutic properties. Hence, understanding the honey is very important, as the honey bee speices and its associated plants determined its chemical composition. Bee obtained pollen, nectar from flower which are important from honey bee life. Plant types and their flowering duration differ from place to place due to variation in topography, climate, cultural and farming practice. The knowledge of bee flora in the region is essential for successful bee keeping, so that the beekeeper can harvest a quality honey and other bee products. Each and every region has its own bee flora, effective pollinator and to enhanced a crop yield, knowledge on bee flora would be in the effective management of bee colonies. Bees are the most important pollinator taxon, therefore understanding the scale at which they flourish will have an important ecological implications and conservation application. Melissopalynology study the microscopic analysis of pollen contenet of the honey from the locality, with field study involving phenology provide reliable information regarding the floral types which serve as the pollen sources for the honey bees. Pollen found in honey is used to determine the honey types, quality control and to ascertain whether honey is adulterate or not (Villaneuva, 1999). Pollen spectra of the local honey samples varied according to the vegetation type utilized by the bees within the floristically diverse region. From the pollen spectra it was observed that the two district include both naturalized flora as well as cultivated crops. It also give a wider knowledge of bee preferences in local floral. The microscopical analysis of honey is immensed in establishing the seasonal pollen spectra of honeys from various climatic and geographical areas, in evaluation of honey

originated from different physiographic region (Chaturvedi, 1983). The conventional approach, used of microscopic observation of pollen present in honey (Melissopalynology) which is very tedious and time consuming process. The other common method is analysis of chemical components which need sophisticated and expensive instruments. The study employed in the work has demonstrated that the molecular genetic can be used for analyzing the composition and geographical origin of honey using gene markers ie. DNA Barcoding technique, when short region of DNA is used to distinguished the individual species based on the differences in the nucleotide bases called as DNA barcodes. The DNA extraction, PCR amplification used in the DNA Barcode primers and the DNA sequencing carried out to identify the flora. DNA Barcoding provide rapid, accurate and automatable species identification using a variation in standard DNA region (*mat*K and *rbc*L) used for the study.

The outcome of the research depict the interaction between honey bees and its foraging plants species, honeybee foraging plant diversity using a DNA Barcoding approach. It will be of immense value for the development of bee keeping industry for the studied area and for the entire region and this information could be used to selectively grow native plants that are important for the honey bees. Identification of bee flora helps in providing and improved the efficiency of bee keeping industry and honey production. This study helps beekeepers to formulate bee management for migrating of bee colonies to different floral sources. These studies will be helpful for identifying different floral sources used by honey bees and improved the conservation of economically viable plants. The study of pollen morphology is helpful to identify various species in different families. The present information can help in establishing an

apicultural calendar for the region. Generally, entomorphilous plants were numerous in the pollen spectrum of each honey sample studied and the honeys from the source localities were fairly rich in pollen types**.** The traditional quality of honey can be maintain which is the primary criteria for its medicinal properties. This studies will be useful for identifyingthe flora used by honeybees and improved the conservation status of economically important plants. The success of beekeeping in the country depends not only on using better strains of bees but also on the abundance and richness of nectar and pollen around an apiary.

References:

Abdullahi G., Sule H., Chimoya I.A. and Isah M.D. (2011). Diversity and relative distribution of honeybees foraging plants in some selected reserve in Mubi region, Sudan savannah ecological zone of Nigeria. *Adv.Applied Sci. Res.* 2(5): 388-395.

Abrol D.P. (1997). *Bee and beekeeping in India*. Kalyani Publ. Ludhiana.

Adekanmbi O.H. and Ogundipe O. (2006). Pollen grains of some cultivated plants in Nigeria. *J. Sci. Res. Dev.* 10:101-110.

Adjare S.O. (1990). Beekeeping in Africa. *FAO Agriculture service bulletin* 68/6. Food and Agricultural Organisation of the United Nations, Rome.

Agashe S.N. and Mary S.J.D. (1995). Pollen spectrum of pollen loads collected from Thally Block, Dharampuri district, Tamil nadu. *J. Palynol*. 31:207-212.

Amsalu B., Nuru A., Sarah E. and Radloff H. (2003). Multivariate morphometric analysis of honey bees in the Ethopian region. *Apidolgie*. 35: 71-81.

Anklam E. (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chemistry*. 63: 549–562.

Anonymous (1984-1976). *The wealth of India- Raw materials*. Vol. 1. ICMR, New Delhi.

Arora R.K. (1990). Native food plants of the northeastern tribals. *Glimses of Indian ethnobotany.* Oxford and IBH Publishing Co., New Delhi.

Asolkar L.V., Kakkar K.K. and Chakre O.J. (1992). *Second supplement to glossary of Indian medicinal plants with active principles*. Part-I (A-K) CSIR, New Delhi.

Azzedine C., Jose B.M., Yasmina A.K., Salima B. and Ali T. (2007). Mellisopalynologic and analysis of some north-east Algerian honeys. *Eur. J. Sci. Res*. 389-401.

Baroni M.V. (2009). Composition of honey from Cordoba (Argentina) assessment of North/South provenance by chemomatrics. *Food chem*. 114(2):727-733.

Bennet K.D. and Parducci L. (2006). DNA from pollen; principles and potential. *The Holocene*. 16:1031-1034.

Bera S., Mukhopadhyay S.K. and Das A.P. (2007). Detection of honey samples form Kamrup reserve forest of Assam. *J. Palynol*. 33:209-211.

Bernhardt P., Sage T., Weston P., Azuma H., Lam M., Thien L. B. and Bruhl J. (2003). The pollination of *Trimenia moorei* (Trimeniaceae): floral volatiles, insect/wind pollen vectors and stigmatic self-incompatibility in a basal angiosperm. *Annals of Bot*. 92: 445-458.

Bhargava H.R., Jyothi J.V.A., Bhushanam M. and Surendra N.S. (2009). Pollen analysis of Apis honey, Karnataka, India. *APIACTA*. 44:14-19.
Bhattacharjee S.K. (1998). *Handbook of medicinal Plants.* Pointer publishers, Jaipur, India.

Borges R.L.B., Lima L.C.L., Oliveira P.P., Silva F.H.M., Novais J.S., Dórea M.C. and Santos F.A.R. (2006). O pólen no mel do Semi-Árido brasileiro. In: Santos, F.A.R. (ed.). *Apium plantae*. Recife, *IMSEAR*. Pp.103-118.

Brodschneider R. andCrailsheim K. (2010). Nutrition and health in honey bees. *Apidologie*. 41:278-294.

Bruni I., Galimberti A., Caridi L., Mattia F. and Casriaghi M. (2015). A DNA Barcoding approach to identify plants species in multiflower honey. Food Chem. 170:308-315.

Cale G.H. (1967). Pollen gathering relationship to honey collection and laying in honeybees. *Proc. Int. Apiculture Cong*. Apimondia Publishing,Bucharest, Romania. 230-232.

Cane J. H. and Schiffhauer D. **(**2003). Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon* [Ericaceae]). *Amer. J. Bot*. 90: 1425-1432.

Chakraborti T. and Bhattacharya K. (2014). Vegetational impact over bee forage in relation to seasonal variation and observation in some honey samples of west Bengal. *J. Palynol.* 47:69-76.

Chaturvedi M. (1983). Pollen analysisi of autumn honeys of Kumaon region. *Proc. Indian Natn. Sci. Acad*. 2:125-144.

Chaudhuri H.J. and Wadhwa B.M. (1984). Flora of Himachal Pradesh. *Bot. Surv. India,* Calcutta.Vol. 1-3

Cheng H., Jin W. and Wu H. (2007). Isolation and PCR detection of foreign DNA sequences in honey bee raised on genetically modified Bt cotton. *Food Bioproc. Proc.* 85:141-145.

Chopra R.N., Nayar S.L. and Chopra I.C. (1968). *Glossary of Indian medicinal plants.* CSIR, New Delhi.

Chowdhery H.J. and Wadhwa B.M. (1984). *Flora of Himachal Pradesh*. Vol 1-3. Bot.Surv. India, Culcutta.

Codex Alimentarius Committee on sugars. (2001). Codex standard 12, Revised Codex Standard for honey. Standards and standards Methods 11: 1-7.

Cope T.A. and El- Eishaw D. (1998). Checklist of the flora. *Arid Land Res. And their Manag*. Jordan Desert Margin. Kegan Paul International. Limited, U.K.

Corbet S. A., Bee J., Dasmahapatra K., Gale S., Gorringe E., La Ferla B., Moorhouse T., Trevail A., Van Bergen Y and Vorontsova M. **(**2001). Native or exotic. Double or single. Evaluating plants for pollinator-friendly gardens. *Annals of Bot.* 87: 219-232.

Corbet S.A., Williams I.H. and Osborne J.L. (1991). Bees and the pollination of crops and wild flower in the European community. *Bee world*. 72:47-59.

Crane E. and Walker P. (1984). Pollination directory for world corps. *Inter. Bee Res. Ass*. London.

Cuevas-Glory L.F., Pino J.A., Santiago L.S. and Sauri-Duch, E. (2007). A review of volatile analytical methods for determining the botanical origin of honey. *Food Chem. 103*: 1032-1043.

Deodikar G.B. and Thakur P.N. (1953). A pollen study of major honey yielding plants of Mahabaleswar hills. *Apicultural Laboratory.Bull*. No.1.

Dick C.W., Etchelecu G. and Austerlitz F. (2003). Pollen dispersal of tropical trees (Dinizia excels: Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Mol. Ecology*. 12:753-764.

Dieringer G. and Cabrera R. L. (2002). The interaction between pollinator size and the bristle staminode of *Penstemon digitalis* (Scrophulariaceae). *Amer. J.Bot.* 89: 991-997.

Diversity array Technology Pvt. Ltd. (2011). *Plant extraction protocol for DarT*. Disponivel em :<http://www.diversityarrays.com/>.

Erdtman G. (1960). *The acetolysis method- A revised description*. In Svensk. Bot. Tidskr. 54:561-564.

FAO, Standard for Honey (1981). In *Codex Alimentarius: Sugars, Cocoa Productsand Chocolate and Miscellaneous Products*. Rome, Italy. Vol. 11.

Fernandez-Torres R., Perez-Bernal J.L., Bello-Lopez M.A., Callejon-Mochon M., Jimenez- Sanchez J.C. and Guiraum-Perez A. (2005). Mineral content and botanical origin of Spanish honeys. *Talanta*. *65*: 686-691.

Ferreira I.C.F.R., Aires E., Barreira J.C.M. and Estevinho L.M. (2009). Antioxidant activity of Portuguese honey samples: Different contributions of the entire honey and phenolic extract. *Food Chem*. *114*: 1438-1443.

Free J.B (1970). *Insect pollination of crops*. Academic press, London. 544.

Gallai N., Salles J.M., Settele J. and Vaissiere B.E. (2009). Economic valuation of the vulnerabilityof world agriculture confronted with pollinator decline. *Ecol. Economics.* 68(3):810-821.

Garg R. (1989). Bee flora of Paonta valley. *Indian Bee J*. 51: 113-114.

Gill L.S. and Chinnappa C.C. (1982). Pollen morphology of the West Himalayan Labiatae. *Bangladesh J. Bot*. 11:107-123.

Gomez J.M., Bosch J., Perfectii F., Fernandez J. and Abdelaziz M. (2007.) Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalisation. *Oecologia* (Berl.) 153:597-606.

Greenleaf S.S., Williams N.M., Winfree R. and Kreman C. (2007). Bee foraging ranges and their relationship to body size. *Oecologia.* 153:589-596.

Gupta J.K. and Dogra G.S. (2002). Constraints and thrust areas for the development of agriculture in India. *Perspective in Indian apiculture.* Agrobios, India, Jodhpur.

Hermosin I., Chicon R.M. and Cabezudo M.D. (2003). Free amino acid composition and botanical origin of honey. *Food Chem. 83*: 263-268.
Hooker J.D. (1989). *The flora of Bristish India*.Vol I-VIIL. Reeve & Co., London.

Jain S.K. (1964). Wild food plants of the tribals of Bastar (M.P). *Proc.National Inst. Sci.* India 30B:56-80.

Jain S.K. (1967). Ethnobotany- Its scope and study. *Indian Muss. Bull.* 2:39-43.

Jhansi P. and Ramanujam C.G.K. (2004). Pollen analysis of honey samples from Coromandel coast. *Asian J. Plant Sc.* 2:19-26.

Kalpana T.P. and Ramanujam C.G.K. (1997). Melissopalynology, bee plants and beekeeping potential in some coastal district of Andra Pradesh, India. *Indian Bee J*.59:1-8.

Kanjilal U.N., Kanjilal P.C. and Das A. (1982). *A Flora of Assam*. Vol.I–II. Avon Book Company, Delhi-6.

Kanjilal U.N., Kanjilal P.C., De R.N. and Das A. (1982). *A Flora of Assam*. Vol.III & IV. Avon Book Company, Delhi–6.

Kaul M.K. (1997). *Medicinal plants of Kashmir and Ladakh, temperate and cold arid Himalaya.* Indus Publishing company, New Delhi.

Kearns C. A., Inouye D. W. and Waser N. (1998). Endangered mutualism: The conservation of plant pollinator interactions. *Annual Rev. Ecol. Syst.* 29: 83-106.
Kevan P.G. (1991). Pollinators as bioindicators of the state of the environment, species activity and diversity. *Agr. Ecosyst. Environ*. 74:373-393.

King R., Read D., Traugott M., Symondson W. (2008). Molecular analysis of predation: a review of best practice for DNA based approaches. *Mol. Ecol*. 17:947-963.

Kirtikar K.R. and Basu B.D. (1984). *Indian medicinal plants*. Vol I-IV. Bishan Singh Mahendra Pal Singh, Dehradun.

Klein A. M., Stefaffan-Dewenter I. and Tscharntke T. (2003). Bee pollination and fruit set of *Coffea arabica* and *C. canephora* (Rubiaceae). *Amer.J. Bot.* 90: 153-157.

Klein A.M., Vaissiere B.E., Cane J.H., Steffan Dewenter I., Cunningham S.A., Kremen C. and Tscharntke T. (2007). Importance of pollinators in changing landscapes for wild crops. *Proc. R. Soc. Lond.B*. 274:303-313.

Kohli N. (1958). Bee flora of Northern India. *Indian Bee J.* 40:14-19.

Kral F. (1992). Forest history and the natural mixture of tree species in Vienna wood. Palynological investigation. *Central blatl-fur-das-gesamte-forestwesen*. 3:163-183.

Kremen C., William N.M., Aizen M.A., Gemmill Herren B., Lebuhn G., Minckley R., Packer L., Potts S.G.,Roulston T., Steffan Dewenter I., Vazques D.P., Winfree R., Adams L., Crone E.E., Greenleaf S.S.,Keitt T.h., Klein A.M., Rogetz J. and Ricketts T.H. (2007). Pollination and other ecosystemservices produced by mobile organisms, a conceptual framework for the effects of land use change. *Ecol. Lett*. 10:299-314.

Kremen C., Williams N. M. and Thorp R. W. (2002). Crop pollination from native bees at risk from agricultural intensification. *Proc. Nat. Acad. Sci.* (U S A). 99: 16812-16816.

Krotochwil A. (2003). Bees (Hymenoptera, Apidae) as keystone species, specific of resource and requisite utilization in different habitat types. Ber. Reinh. Tuxen.Ges. 15:59-77.

Kumar R. and Chaudhary O.P. (1993). Bee plants in India. *Khadi Gramodyog*. 39:844-854.

Kumar R., Rajput G.S., Mishra R.C. and Agrawal O.P. (2013). A study on assessment of duration of dearth period for honey bees in Haryana, India. *Munis Entomology & Zoology*. 8(I):434-437.

Laha R.C. and Ralte L. (2014). Study on honeybee (*Apis cerana*) forage plants in Mizoram, Northeast India. *IJPAES*. 197-204.

Laha R.C. and Ralte L. (2015). Polliniferous plants associated with honey bee (*Apis cerana*) in Aizawl district, Mizoram, Northeast India.*Inter. J. Sci. Res.* 4:36-40.

Lakshmi K. and Suryanarayana M.C. (1997). Melissopalynology of forest honeys from Chintapalli Hills, Andra Pradesh, India. Proc. *Indian Natn. Sci. Acad.* B63 No. 6.581-596.

Laube I., Hird H., Brodmann P., Ullmann S., Schone-Michling M., Chisholm J. and Broll H. (2010). Development of primer and probe sets for the detection of plant species in honey. *Food Chem*. *118*: 979-986.

Levin M.D. and Konopacka G. (1963). Responses of foraging honeybees in alfalfa to increasing completion from other colonies. *J. Apicult. Res*. 2:33-42.

Lexa G.M., Francis N.W., Miraim G.K., Anne W.T. and Titus K.M. (2008). Leaf storage conditions and genomic DNA isolation efficiency in *Ocimum gratissimum* L.from Kenya. *Afri. J. Biotech*. 7:557-564.

Lonsdorf E., Kremen C. and Rickett T. (2009). Modelling pollination services across agriculture landscapes. *Annals of Botany*. 103: 1589-1600.

Louveaux J. and Abed I. (1984). North African honeys and their pollen spectrum. *Apidologie.* 15(2):146-170.

Louveaux J., Maurizio A. and Vorwhol G. (1978). Methods of mellisopalynology. *Beeworld.* 59:1139-1157.

Mahendran S., Kumarasamy D. and Saravanakumar K. (2015). Pollen analysis of honey samples from Western Ghats, Tamil Nadu, India. *Intern. J. Currnt. Res. Dev.* 3(I): 29-35.

Matsuki Y., Isagi Y. and Suyama Y. (2007). The determination of multiple microsatellite genotypes and DNA sequences from a single pollen grain. *Mol. Ecol. Notes*. 7:194-198.

Matsuki Y., Tateno R., Shibata M. and Isagi Y. (2008). Pollination efficiencies of flower visiting insects as determined by direct genetic analysis of pollen origin. *Am. J. Bot*. 95:925-930.

Mattu V.K., Mattu N., Verma L.R. and Lakhanpal N. (1989). Pollen spectrum of honeys from *A.cerena* colonies in Himachal Pradesh, India. *Proc. Of the fourth Int. Conf. on Apicultre in Trop. Climates,* Cairo. 146-53.

Maurizio A. (1951). Pollen analysis of honeys .*Bee world*. 32: 1-5.

Maurizio A. (1979). The pollen spectrum of some honeys from Norway. *Apidologie*. 10(4): 359-394.

Maurizio A. and Louveaux J. (1965). *Pollen de plantes melloferes d'Erope.* Union group. Fr. Paris.

McGregor, S.E. (1976). Insect pollination of cultivated crop plants. *Agric. Hanb. U.S. Dep. Agric*. No. 496:411.

Mendes C.G. (2009). As analyses de mel: revisao. *Revista caatinga.* 22(2):7-14.

Michiels A., Vanden- Ende W., Tucker M., Van- Riet L. and Van-Laere A. (2003). Extraction of high-quality genomic DNA from latex containing plants. *Anal Biochem*. 315:83-89.

Mishra R.C. and Kaushik H.D. (1992). Bee flora and beekeeping maps of India. *Changing village.*11:88-107.

Mishra R.C. and Kumar J.(1987). Importance of beekeeping in social forestry. *Social forestry for rural development.* Indian Soc. of tree scientist, Solan. 189-206.

Mishra R.C. and Kumar R. (2002). Bee flora and beekeeping map of India. *Perspective in Indian agriculture.* Agrobios Jodhpur.

Mitchell R.J., Irwin R.E., Flanagan R.J. and Karron J.D. (2009). Ecology and evolution of plant pollinator interactions. *Annals of Botany*. 103: 1355-1363.

Mithilesh C. (1983). Pollen analysis of Autumn honeys of Kumaon region. *Indian Natn. Sc. Acad.* 2:125-133.

Moeller F.E. and Koval C.F. (1973). *Honeybee Pollination of Strawberries in Wisconsin.* Resource Report, Co-operative Extension, University of Wisconsin No. A 2549.

Moore P.D. and Webb J.A. (1978). *An illustrated guide to pollen analysis*. 1st ed., Hodder and Stoughtonn, London. Pp133.

Muchhala N., Caiza A., Vizuete J.C.and Thomson J.D. (2009). A generalized pollination system in tropics, bats, birds and *Aphelandra acanthus*. *Annals of Botany*. 103:1481-1487.

Mukhopadhyay S.K., Gupta S., Das A.P. and Bera S. (2007). The beekeeping potential of Sub-Himalayan West Bengal, India. A palynological assessment of honey. *J. of Apiculture Research and bee world*. 46:165-180.

Nair P.K.K. (1985). *Melissopalynology.*In: *Essentials of palynology*. Today and tomorrow's publication, New dDelhi. 59-64.

Nair P.K.K. and Kapoor S.K. (1974). Pollen morphology of Indian vegetable crops. *Glimpses Pl. Res*.2: 106-201.

Noor M.J., Ahmad M., Asghar R., Kanwal A. and Pervais S. (2004). Palynological studies of cultivated plant species at University of Arid agriculture. Rawalpindi, Pakistan. *Asian J. Plant Sc.* 3(4): 476-479.

Nye W.P. and Anderson J.L. (1974). Insect pollinators frequenting strawberry blossoms and the effect of honeybees on yield and fruit quality. *J.Amer. Soc.Hor. Sci.* 99**:**40-44.

Omer K., Mehmet A.K. and Fethi A.O. (2016). Pollen analysis of honey from the Hizan district of Bitlis province, eastern region of Turkey. *IJPAES*.6(I): 19-26.

Parent J., Feller M.J. and Richard P.J.H. (1990). Pollen and nectar sources near Rimouski Quebeck, Canada. *Apidologie.* 21(5):431-446.

Parker R.L. (1923). Some pollen gathered by bees. *American bee J*. 63:16-19.

Partap U. (1997). *Bee flora of the hindu kush Himalayas. Inventory and management.* International Centre for intergrated mountain development, Kathmandu, Nepal.

Partap U. and Verma L.R. (2000). Honey plants. *Natural resources and development in Himalaya*, New Delhi, India. 386-401.

Parveen A. and Qaiser M. (2010). Pollen flora of Pakistan- LXV. Berberidaceae. *Pak. J. Bot*. 42:1-6.

Patmavathy S. and Rehel S.M. (2014). Bee plants of *Apis dorsata* from Nilgiris, South India. *J. Acad Indust. Res.* 2(10):570-572.

Peterson J. and Dwyer J. (1998). Flavonoids dietery occurrence and biochemical activity. *Nutrition Res*.18(12):1995-2018.

Pfister R. (1895). Versuch einer Mikroskopie des honing. Forschber. Lebensmitt. U. ihre Bez. Z. Hygiene, Forens. *Chem., Pharmakogn*. 2:29-35.

Polunin O. and Stainton A. (1984). *Flowers of the Himalaya*. Oxford University Press, New Delhi.

Pratap U. and Verma L.R. (2007). Bee flora and bee keeping maps of Hindu kush Himalaya. *Indian Bee J*. 45:58-65.

Rahman A., Kumar A.T. and Pradhan S. (2006). Handbook of Agriculture. *Indian Council of Agri. Res.*, New Delhi. 509-529.

Raj B. (1969). Pollen morphology of some medical and aromatic plants. *J.Osmania Univ*. 5:17-25.

Ralte L., Souvik G., Laha R.C., Guruswami G. and Kumar N.S.(2014). Protocol for optimal quality and quantity pollen DNA isolation from honey samples. *J Biomol. Tech.* 25:92-95.

Rastogi R.P. and Mehrotra B.N. (1995). *Compendium of Indian medicinal plants*. Vol. V. 1990-1994. CDRI, Lucknow , Publication and Information Directorate, New Delhi.

Ricketts T. H., Daily G. C., Ehrlich P. R.and Michener C. D. (2004). Economic value of tropical forest to coffee production. *Proc. Nat.Acad. Sci.* (USA). Proc Nat Acad Sci. 101: 12759-12582.

Rodinov V.V. and Shabanshov (1986). *The fascinating world of bees.* Mir publishers, Moscow (Russia).

Ross H.A., Murugans S. and Li W.L. (2008). Testing the reliability of genetic method of species identification via simulation. *Sys. Biol*.57:216-230.

Roubik D.W. (1989). *Ecology and natural history of tropical bees*. Cambridge University, Cambridge, UK. p.514.

Sadia B., Syed Z.H. and Riffat N.M. (2008). Pollen analysis and Heavy metals detection in honey samples from seven selected countries. *Pakistan Journal of Botany*. 507-516.

Sahli H.F. and Conner J.K. (2007). Visitation, effectiveness and efficiency of 15 Genera of visitors to wild Radish, Raphanus raphanistrum (Brassicaceae). *American Journal of Botany*. 94: 203-209.

Sahney M. and Seth H.K. (2012). Medicinally important plants as bee forage in winter honeys of Rewa district, M.P. India. *Proc.Nat.Sem. on medicinal plants in India.*

Saklani A. and Jain S.K. (1994). *Cross cultural ethnobotany of Northeast India*. Deep publication, New Delhi.

Saraf S.K. (1972). Bee flora of Kashmir. *Indian Bee J*.34:1-10.

Sawmliana M. (2013). *The Book of Mizoram Plants*. 2nd Ed. Aizawl, Mizoram.

Schemske D.W. and Horvitz C.C. (1984).Variation among floral visitors in pollination ability - a precondition for mutualism specialization. *Science*. 225:519-521.

Schill R.O., Mali B., Dandekar T.,Schnolzer M., Reuter D. and Frohme M.(2009). Molecular mechanisms of tolerance in tardigrades; new perspectives of preservation andstailization of biological material. *Biotechnol. Adv*.27:348-352.

Schmidt J.O. and Buchmann S.L. (1999). The hive and the honeybee. Dadant, Hamilton, IL, USA. *Other products of the hive.* 927-88.

Schnell I.B., Fraser M., Willerslev E. and Thomas M. (2010). Characterisation of insect and plant origins using DNA extracted from small volumes of bee honey. *Arthropod plant interactions*. 107-116.

Schultes R.E. (1962). The role of the ethnobotanist in the search for new medicinal plants. *Lloydia*. 25:257-266.

Shahera T.Z. and Guenther V. (2003). Major pollen plant species in relation to honeybees activity in the Jordanian desert area. *Inter. J. Agri. Biol.*

Sharma G.C. and Saharia D. (2011). Important winter bee plant of Sonapur area, Kamrup District, Assam. *Ind. J. of Fund. and Appl. Lif. Sc.* Vol I (3): 166-171.

Sharma H.K and Gupta J.K. (1993). Diversity and density of bee flora of solan region of Himachal Pradesh (India). *Indian bee J*. 55:9-20.

Sharma N. (1989). *Melissopalynology and survey of honey plants in Himachal Pradessh.* Ph.D thesis, H.P. University, Shimla, India.

Sharma O.P. and Raj D. (1985). Diversity of bee florain Shivaliks and its impact on beekeeping. *Indian Bee J.* 47:21-24.

Sharma S. (2005). Diversity of bee flora in Northwest Himalaya and its impact on bee keeping. *Indian Bee J*. 47:21-24.

ShivaramV. (1995). *Bee flora, honey flow and beekeeping in the plains of Karnataka,* Ph.D thesis, Bangalore University, Bangalore.

Shubharani R., Roopa P. and Sivaram V. (2013). Pollen morphology of selected bee forage plants. *Global J. Bio Sc. Biot.* Vol 2:82-90.

Shubharani R., Sivaram V. and Roopa (2012). Assessment of honey plant resources through pollen analysis in Coorg honeys of Karnataka State. *Intern. J. Plant Repr. Bio.* 4(I):31-39.

Singh G. and Singh G. (1971). *Plectranthus rugosus* Wall.The major honey plants in Kashmir valley. *Indian Bee J*. 33:58-59.

Socha R., Juszczak L., Pietrzyk S. and Fortuna T. (2009). Antioxidant activity and phenolic composition of herb honeys. *Food Chem*. *113*:568-574.
Sood S.K. and Thakur S. (2004). *Ethnobotany of Rewalsar Himalaya (Distt. Mandi, Himachal Pradesh, India)*. Deep publication, New Delhi.

Sood S.K., Nath R. and Kalia D.C. (2001). *Ethnobotany of cold desert tribes of Lahoul-Spiti* (N.W. Himalaya). Deep publication, New Delhi.

Sowunmi M.A. (1973). Pollen grains of Nigerian plants. Woody species. *Grana.* 13: 145-186.

Stanley R.G. and Linskens H.F. (1974). *Pollen, biology, biochemistry and management.* Springer, Berlin, Germany. p. 462.

Sugden M.A. and Furgala B. (1982). Evaluation of six commercial honeybee (*Apis mellifera* L.) stocks used in Minnesota. Part-III Productivity. *American Bee J*. 122:283-

Suryanarayana M.C., Seethalakshmi T.S. and Phadke R.P. (1981). Pollen analysis of Indian honey from Litchi (Nephelium litchi) and jamun (Syzygium cumini). *IV Int. Polynol. Conf.* Lucknow. 491-498.

Taberlet P., Coissac E., PompanonF., Geilly L. and Miquel C. (2007). Power and limitations of the chloroplast trnL (UAA) intron for plant DNA Barcoding. *Nuc.l Acid.Res*.35:14-21.

Taberlet P., Valentini A. and Miquel C. (2010). DNA Barcoding of honeu biodiversity. *Diversity*. 2:610-617.

Varies A.I., Helenine J. and Koivulehto K. (1982). Pollen spectrum of finnish honey. *J. Scient. Agri. Soc. Finland*. 54:403-420.

Vaughn M. and Bryant Jr. (2001). Palynology Laboratory, Texas A&M University College station. USA *CAP Newsletter*. 10-24.

Villaneuva G.R. (1999). Pollen sources of European and Africanized honeybees in the eastern Yucatan Peninsula, Mexico. *J. Apicult. Res.* 38: 105-111.

Visscher P. K. and Seeley T.D. (1982). Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology.* 63: 1790-1801.

Viuda-Martos M., Ruiz-Navajas Y., Fernàndez-Lòpez J. and Pérez-Alvarez J.A. (2008). Functional properties of honey, propolis, and royal jelly. *J. Food Sci. 73*:117-124.

Wang J. Li (2011). Chemical composition, characterization and differentiation of honey, botanical and geographical origin. *Advances Food and Nutri. Res*. 62:89-137.

Waykar B., Baviskar R.K. and Nikam T.B. (2014). Diversity of nectariferous and polliniferous bee flora of Anjaneri and Dugarwadi hills of Wstern Ghats of Nasik district (M.S.), India. *J. Entomo.* 2(IV):244-249.

White J. (1975). Composition of honey. In *Honey: A Comprehensive Survey*; Crane, E., Ed. Heinemann, London, UK. pp. 175-206.

Wilson P. and Thomson J.D. (1991). Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology*. 72:1503-1507.

Winston M. (1987). *The biology of the honey bee*. Harvard University, Cambridge, Mass., USA. p. 281

Young W.J. (1908). A microscopical study of honey pollen. *United states Bereau of chemistry, Bulettin*, Washington D.C. 110:93.

Zander E.V. (1951). Letzte Nachtrage zur pollengestaltung und herkunftsbestimmung bei blutenhonig, liedloff, loth & Michaelis, Leipzig.

Plate 1: Bee box, honey frame and extractor, honey collection and container.

(a) Outside view of beebox

(b) Inside view of beebox

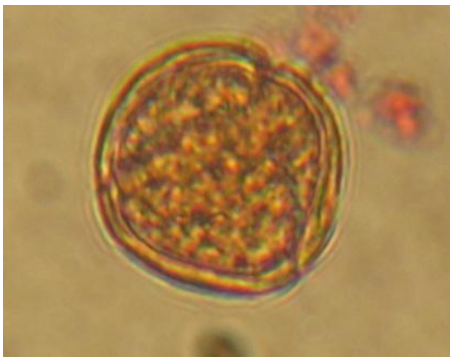
(c) Honey frame

(d) Honey extractor

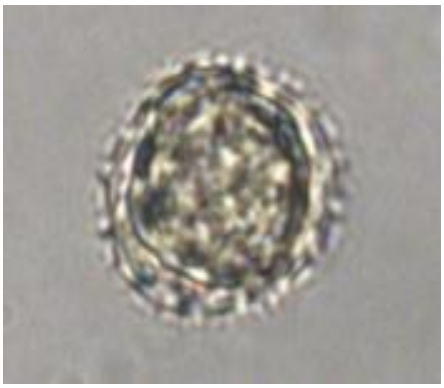
(e) Honey collection

(f) Honey collected in container

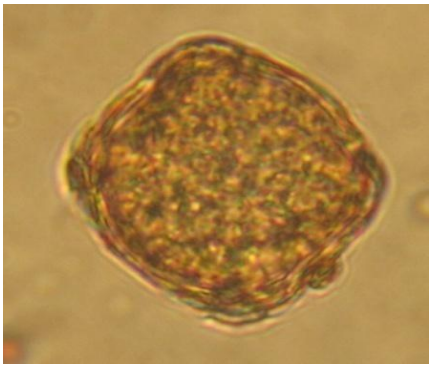
Plate 2– Polliniferous plants of *Acacia pruinescens*, *Ageratum conyzoides*, and .*Alnus nitida.*



Acacia pruinescens Kurz. and pollen

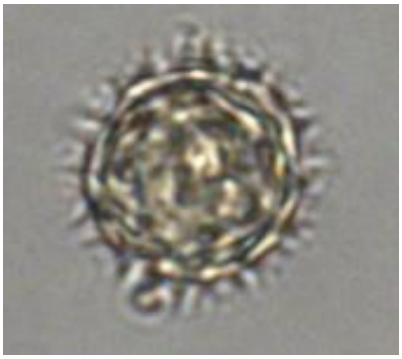


Ageratum conyzoides Roxb. and pollen

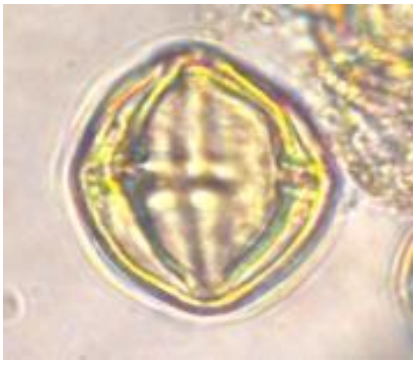


Alnus nitida Endl. and pollen

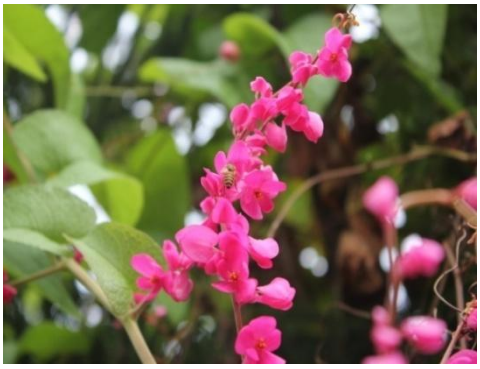
Plate 3- Polliniferous plants of *Althaea rosea* , *Anthurium andreanum* , *Antigonon leptopus* .

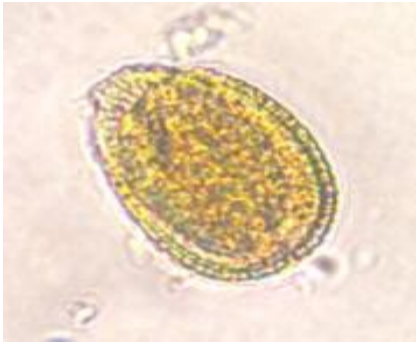


Althaea rosea L. and pollen



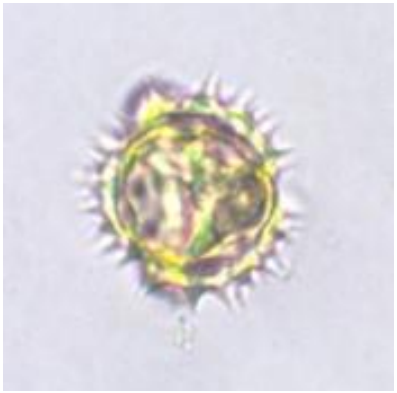
Anthurium andreanum Lindedn ex Andre and pollen



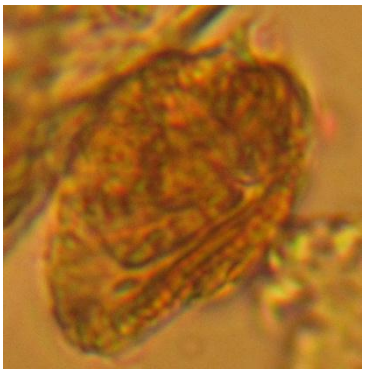


Antigonon leptopus Hook. & Arn. and pollen

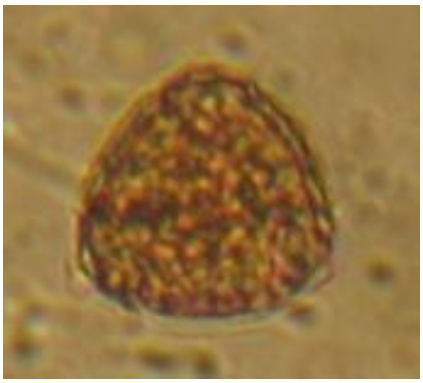
Plate 4- Polliniferous plants of *Amaranthus sp.*, *Asclepias curassavica* and *Averrhoa carambola*



Amaranthus sp. L. and pollen

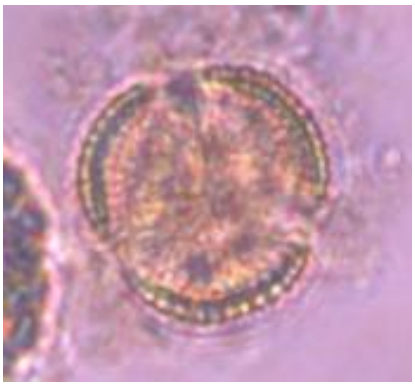


Asclepias curassavica L. and pollen

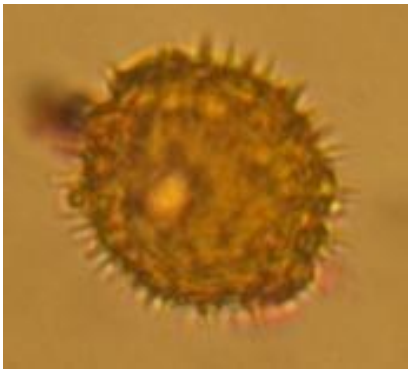


Averrhoa carambola L. and pollen

Plate 5- Polliniferous plants of *Bauhinia variegate,, Bidens pilosa* and *Bombax ceiba.*



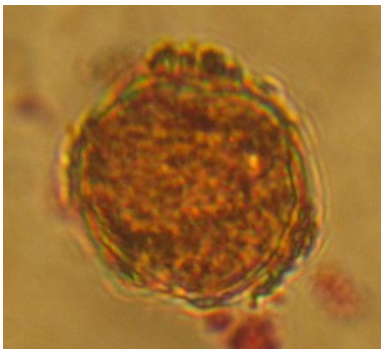
Bauhinia variegata L. and pollen



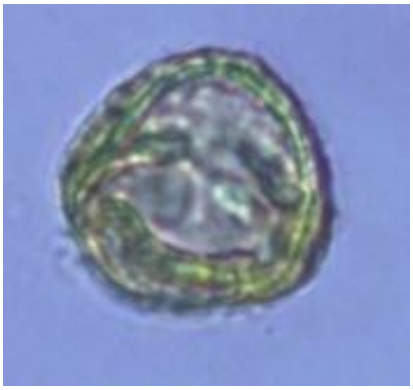
Bidens pilosa L. and pollen

Bombax ceiba L. and pollen

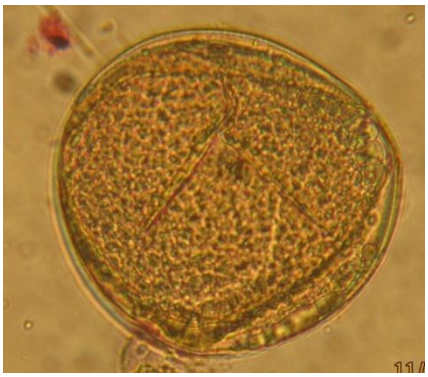
Plate 6- Polliniferous plants of *Brassica camprestris* , *Caesalpinia pulcherrima* and *Callicarpa arborea.*



Brassica camprestris L. and pollen

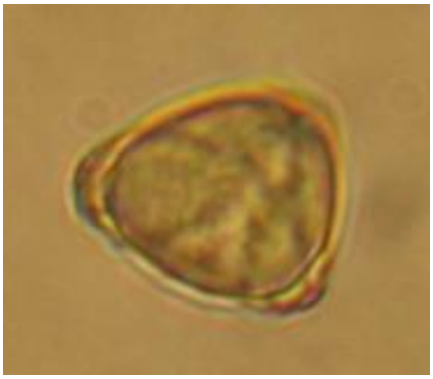


Caesalpinia pulcherrima L. and pollen

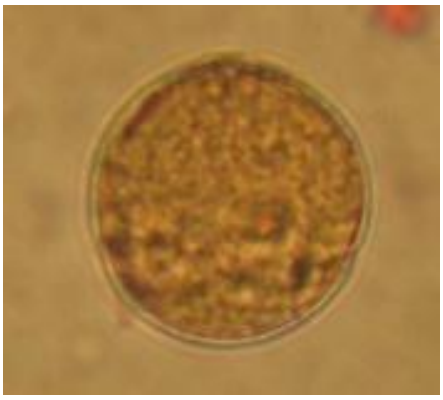


Callicarpa arborea L. and pollen

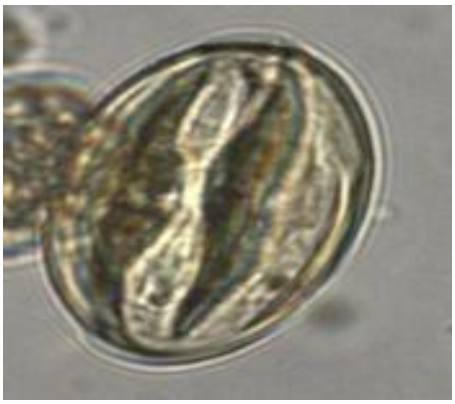
Plate 7- Polliniferous plants of *Callistemon lanceolatus*, *Carica papaya* and *Cassia javanica.*



Callistemon lanceolatus Sweet. and pollen

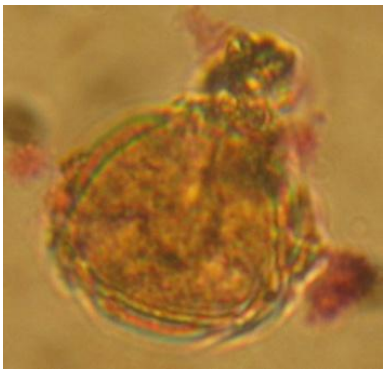


Carica papaya L. and pollen

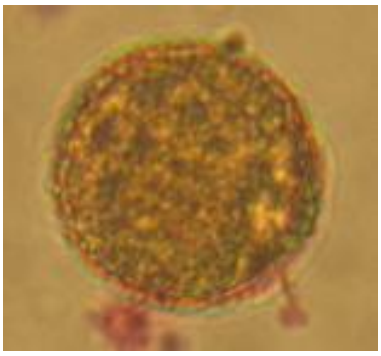


Cassia javanica Roxb. and pollen

Plate 8- Polliniferous plants of *Castanopsis tribuloides*, *Citrus limon* and *Cocos nucifera*



Castanopsis tribuloides (Sm.) A.DC and pollen

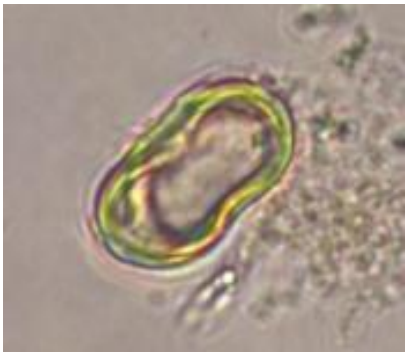


Citrus limon (L.) Burm.f. and pollen

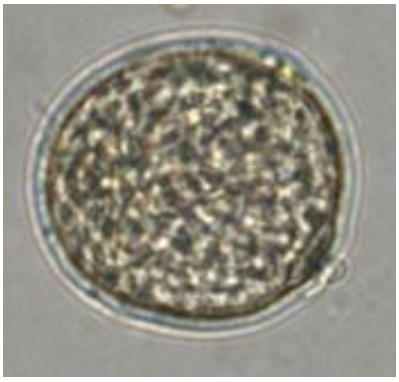
Cocos nucifera L. and pollen

Plate 9- Polliniferous plants of *Coffee arabica* , *Coriandrum sativum* and *Croton jaufra*

Coffee arabica L. and pollen

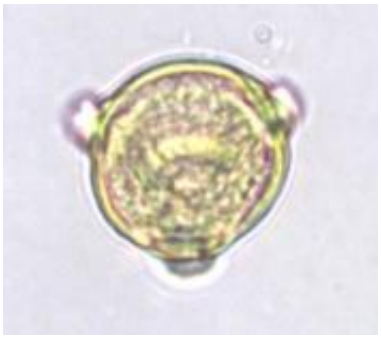


Coriandrum sativum L. and pollen

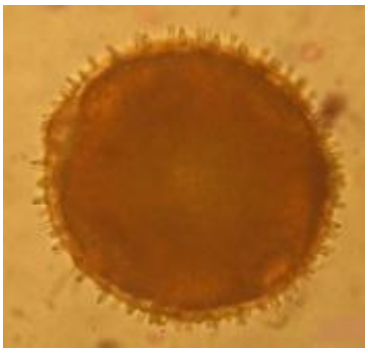


Croton jaufra Roxb. and pollen

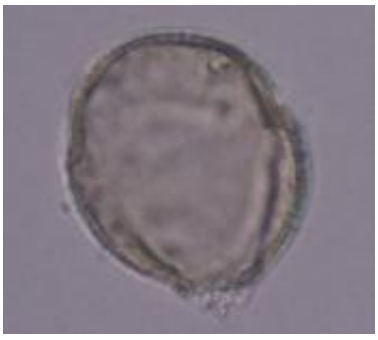
Plate 10- Polliniferous plants of *Cucumis sativus*, *Cucurbita pepo* and *Cyperus rotundus*



Cucumis sativus L. and pollen

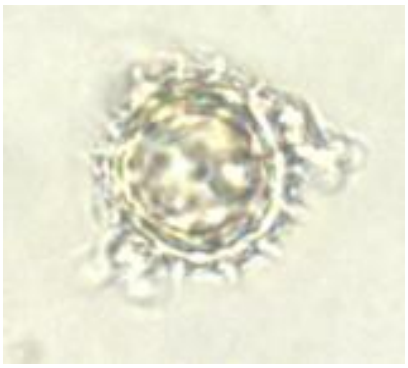


Cucurbita pepo L. and pollen

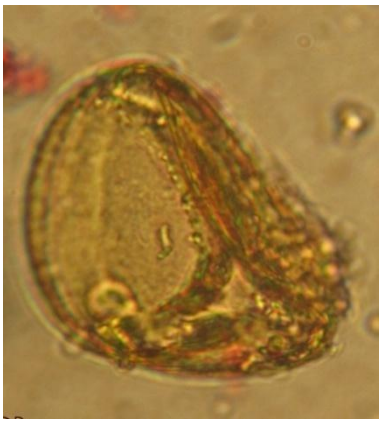


Cyperus rotundus L. and pollen

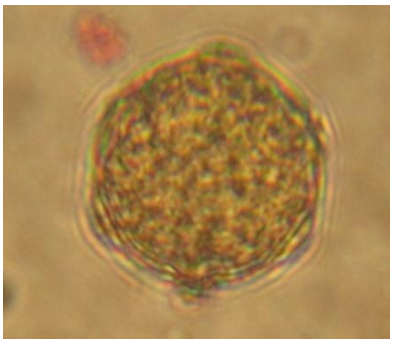
Plate 11- Polliniferous plants of *Cosmos sulphureus*, *Derris robusta* and *Emblica officinalis*



Cosmos sulphureus Cav. and pollen

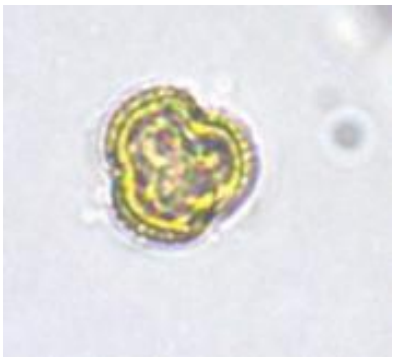


Derris robusta (D.C) Benth. and pollen

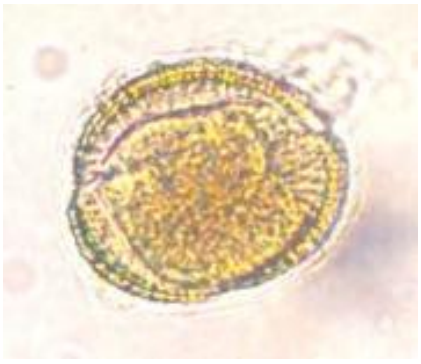


Emblica officinalis Gaertn. and pollen

Plate 12- Polliniferous plants of *Elaeocarpus lanceifolius*, *Eucalyptus tereticornis* and *Eucalyptus tereticornis*



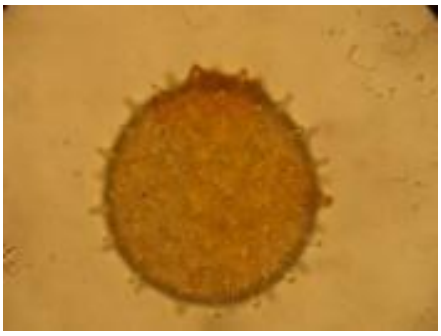
Elaeocarpus lanceifolius Roxb. and pollen



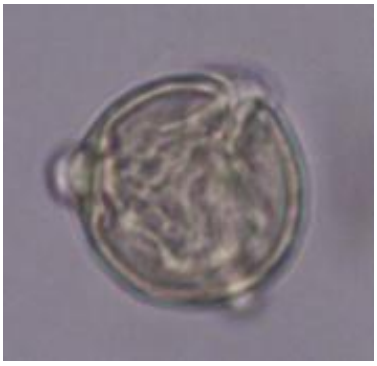
Eucalyptus tereticornis Smith. and pollen

Eucalyptus tereticornis Smith. and pollen

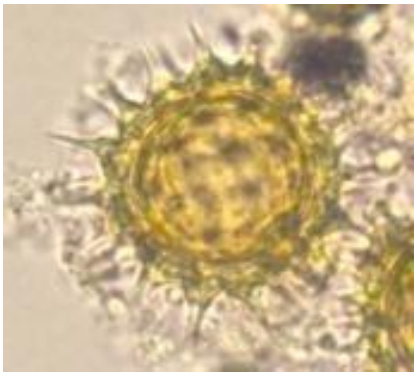
Plate 13- Polliniferous plants of *Hibiscus rosa sinensis*, *Holmskioldia sanguine* and *Ipomoea batatas*



Hibiscus rosa sinensis L. and pollen



Holmskioldia sanguine Retz. and pollen

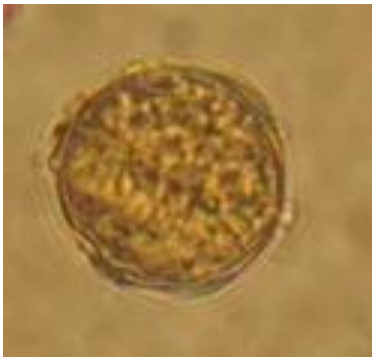


Ipomoea batatas (L.) Lam. and pollen

Plate 14- Polliniferous plants of *Ixora coccinea*, *Jatropha curcus* and *Lagerstromia speciosa*

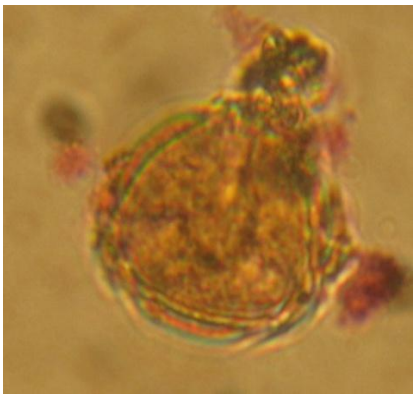
Ixora coccinea L. and pollen

Jatropha curcus L. and pollen

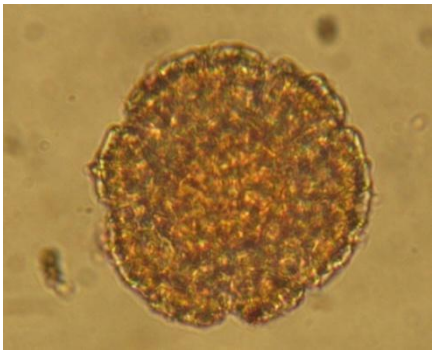


Lagerstromia speciosa (L.) Pers. and pollen

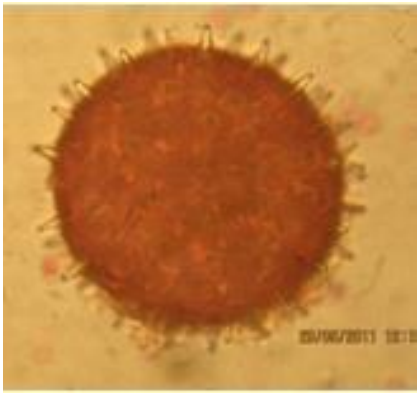
Plate 15- Polliniferous plants of *Lantana camara, Leucosceptrum canum* and *Malvaviscus arboreus*



Lantana camara L. and pollen

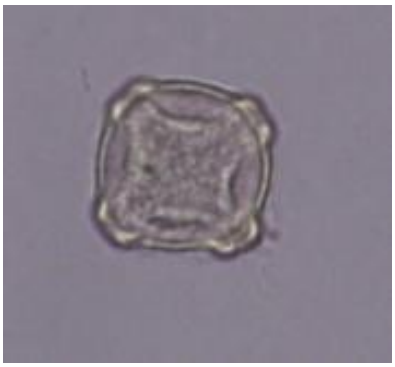


Leucosceptrum canum Sm. and pollen

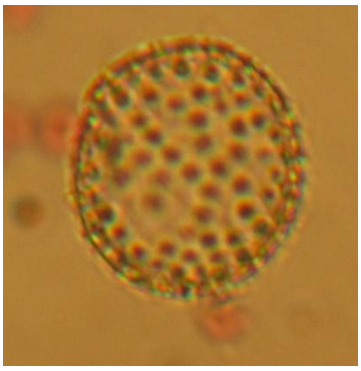


Malvaviscus arboreus Cav. and pollen

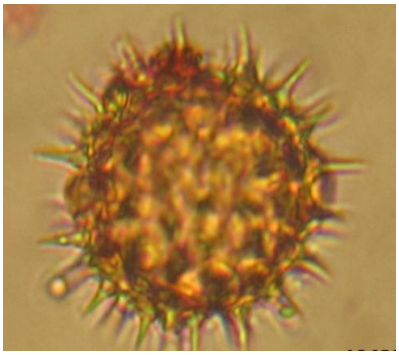
Plate 16- Polliniferous plants of *Mangifera indica*, *Matricaria chomonulla* and *Mikania micranta*



Mangifera indica L. and pollen

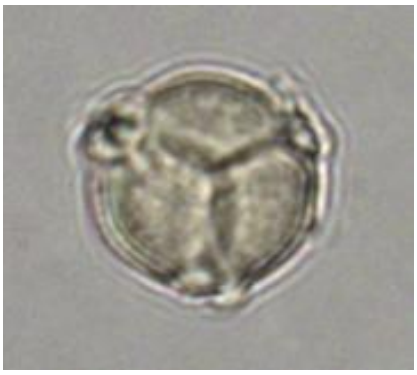


Matricaria chomonulla L. and pollen

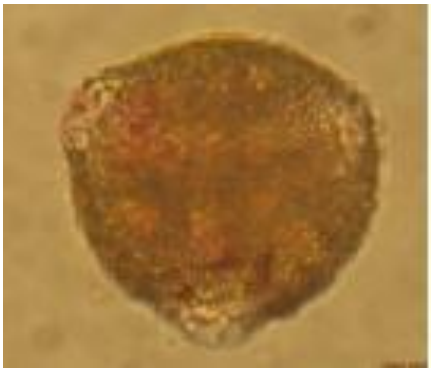


Mikania micranta Kunth. and pollen

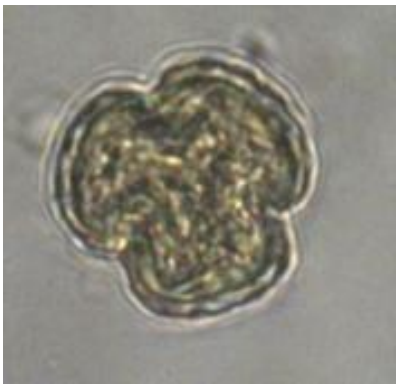
Plate 17- Polliniferous plants of *Mimosa pudica, Momordica charantia* and *Moringa oleifera*



Mimosa pudica L. and pollen

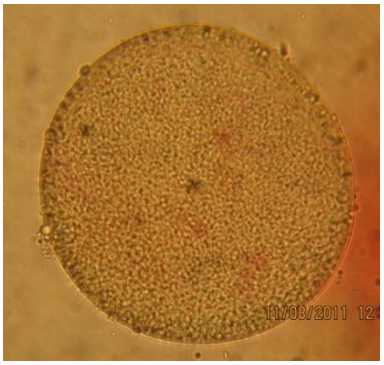


Momordica charantia L. and pollen

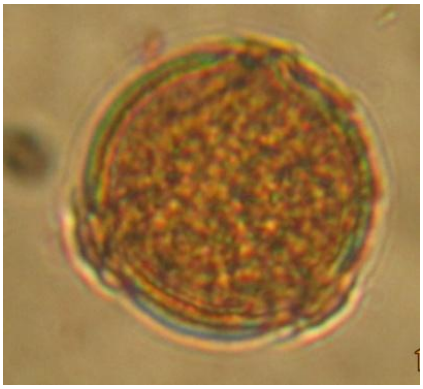


Moringa oleifera Lam. and pollen

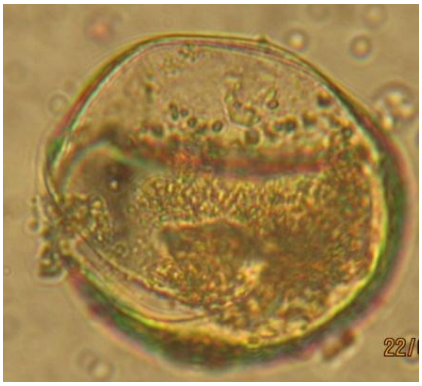
Plate 18- Polliniferous plants of *Musa paradisiacal*, *Nicotianum tobaccum* and *Oryza sativa*



Musa paradisiaca L. and pollen

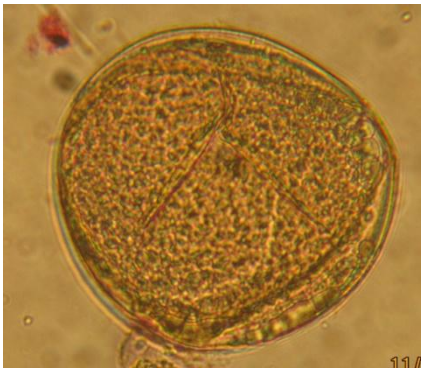


Nicotianum tobaccum L. and pollen

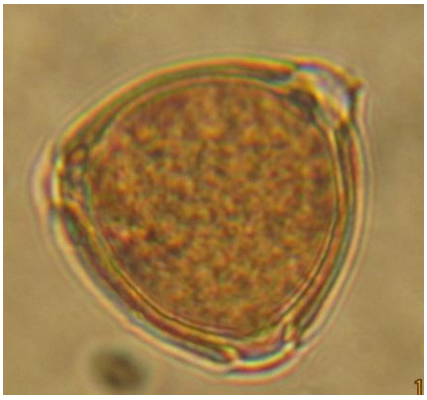


Oryza sativa L. and pollen

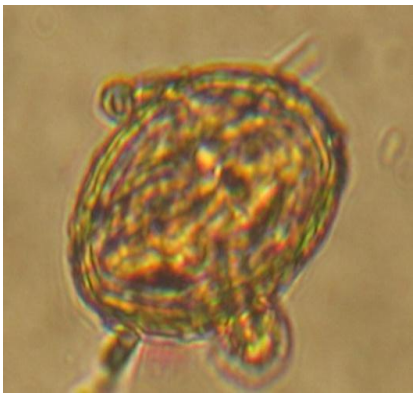
Plate 19-Polliniferous plants of *Parkia timoriana* ,*Phaseolus vulgaris* and *Pisum sativum*



Parkia timoriana (D.C.) Merr. and pollen

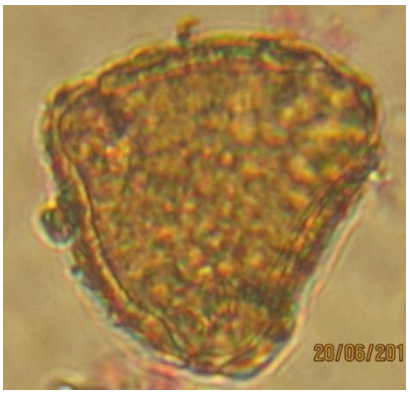


Phaseolus vulgaris L. and pollen



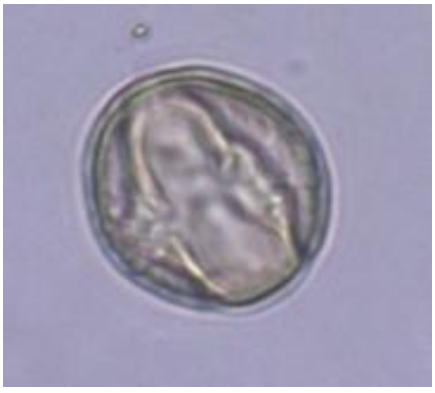
Pisum sativum L. and pollen

Plate 20- Polliniferous plants of *Prunus persica*, *Psidium guajava* and *Punica granatum*



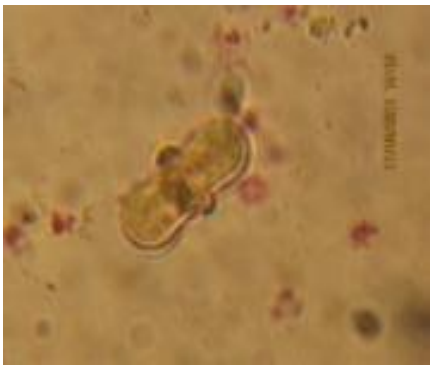
Prunus persica L. and pollen

Psidium guajava L. and pollen

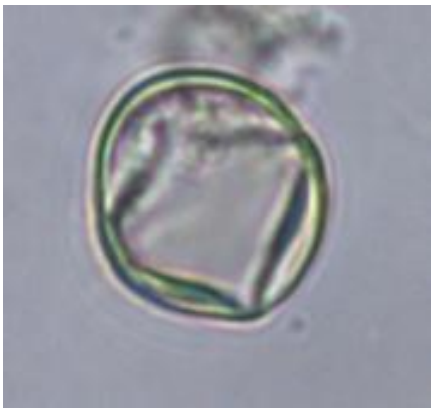


Punica granatum L. and pollen

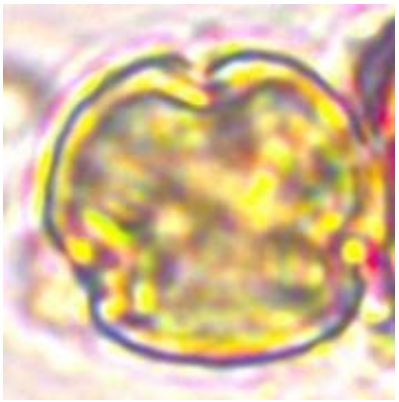
Plate 21- Polliniferous plants of *Raphanus sativus*, *Ricinus communis* and *Rosa macrophylla*



Raphanus sativus L. and pollen

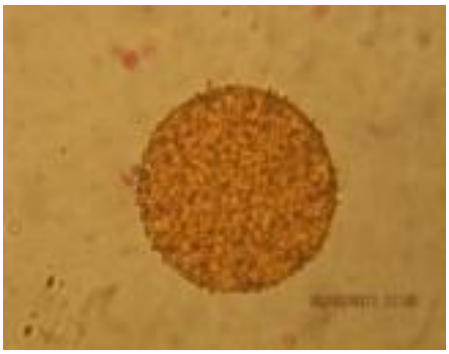


Ricinus communis L. and pollen

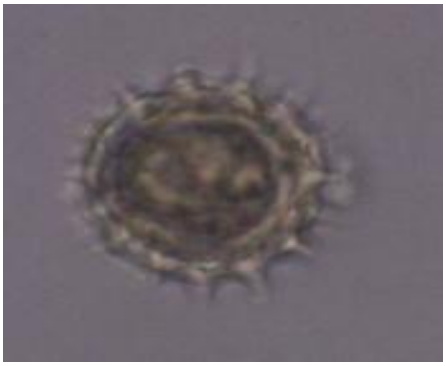


Rosa macrophylla Lindl. and pollen

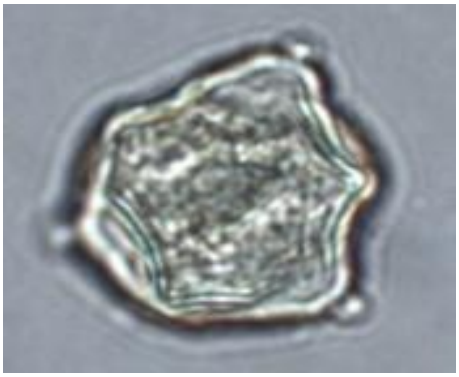
Plate 22- Polliniferous plants of *Sechium edule*, *Spilanthes acmella* and *Solanum melongena*



Sechium edule (Jacq.) Sw. and pollen



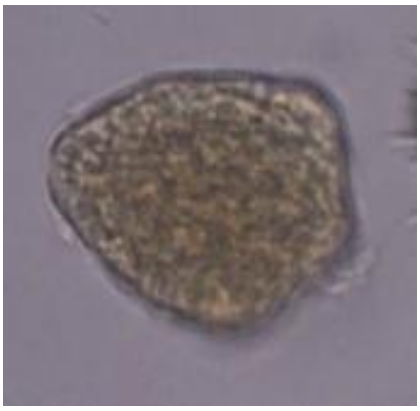
Spilanthes acmella L. and pollen



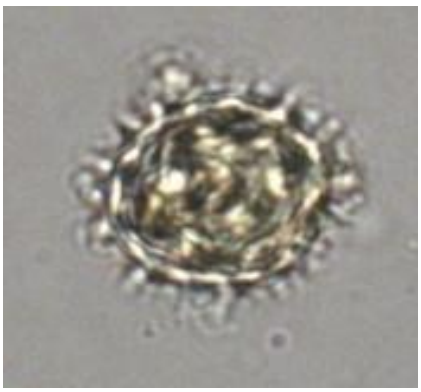
Solanum melongena L. and pollen

Plate 23- Polliniferous plants of *Syzgium cumini*, *Syzygium jambos* and *Tagetes erecta*

Syzgium cumini (L.)Skeel and pollen

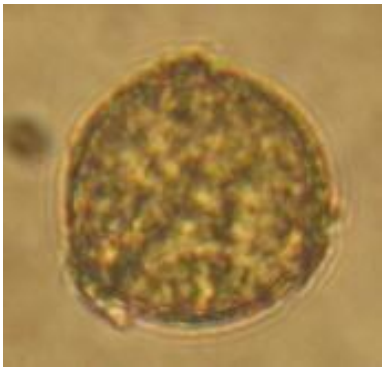


Syzygium jambos L. Alston and pollen

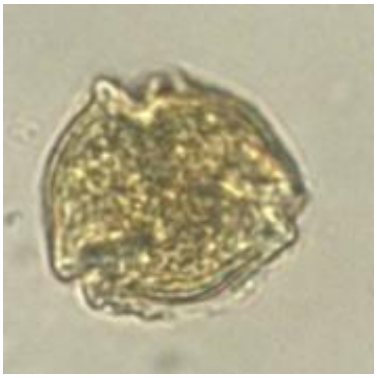


Tagetes erecta L. and pollen

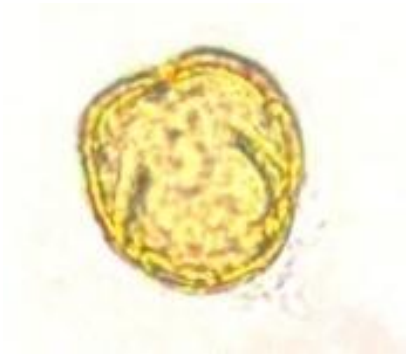
Plate 24- Polliniferous plants of *Tamarindus indica*, *Tecoma stans* and *Terminalia crenulata*



Tamarindus indica L. and pollen

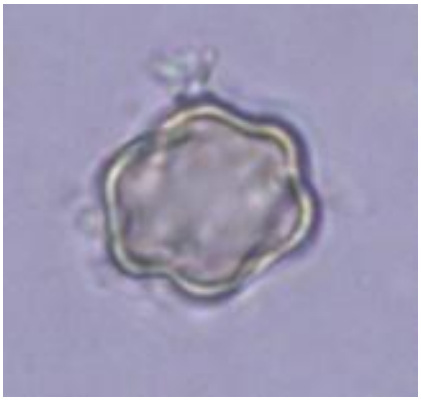


Tecoma stans L.Juss.ex *Terminalia cr*Kunth. and pollen

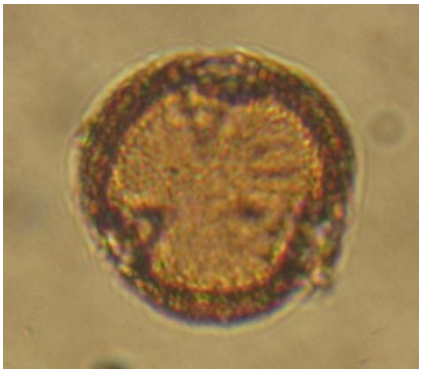


Terminalia crenulata Roth. and pollen

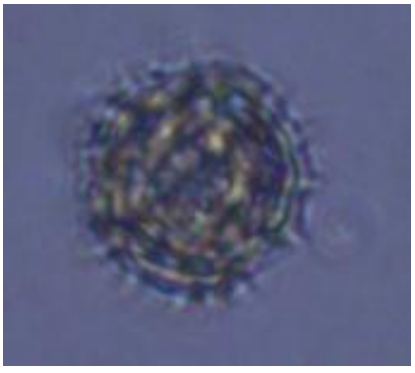
Plate 25- Polliniferous plants of *Terminalia bellirica*, *Tetrameles nudiflora* and *Tithonia diversifolia*



Terminalia bellirica (Gaertn.) Roxb and pollen



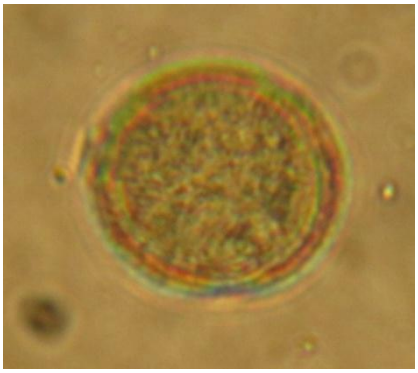
Tetrameles nudiflora R. Br. and pollen



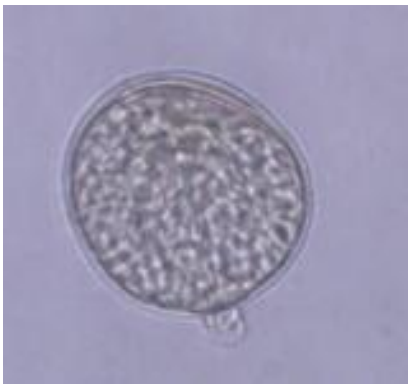
Tithonia diversifolia Hemsl. and pollen

Plate 26- Polliniferous plants of *Tropaelum majus*, *Vitis vinifera* and *Zea mays*

Tropaelum majus L. and pollen

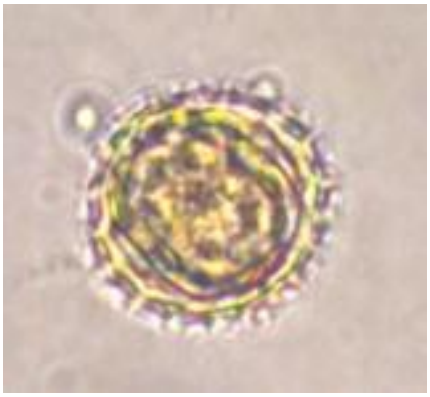


Vitis vinifera L. and pollen



Zea mays L. and pollen

Plate 27- Polliniferous plant of *Zinnia elegans*



Zinnia elegans Jacq. and pollen

List of publications

1. Ralte L., Souvik G., Laha R.C., Guruswami G. and Kumar N.S.(2014). Protocol for optimal quality and quantity pollen DNA isolation from honey samples. *Journal of BIomolecular Technique.* 25:92-95.

2. Laha R.C. and Ralte L. (2014). Study on honeybee (*Apis cerana*) forage plants in Mizoram, Northeast India. *International Journal of Plant, Animal and Environmental Sciences*. 197-204.

3. Laha R.C. and Ralte L. (2015). Polliniferous plants associated with honey bee (*Apis cerana*) in Aizawl district, Mizoram, Northeast India. *International Journal of Scientific Research.* 4:36-40.

4. Ralte L. and Laha R.C. (2014).Foraging plants associated with honey bee (apis cerana) in champhai district, mizoram, northeast India. *Indian Journal of tropical diversity.*

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HSLC	2003	MBSE	II	59.2%
HSSLC	2005	MBSE	II	57.6%
B.Sc.	2008	MZU	I	64.75%
M.Sc.	2010	MZU	1	73.33%