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PHYSICO-CHEMICAL AND MELISSOPALYNOLOGICAL CHARACTERISTICS OF NEPALESE HONEY

Thesis Submitted for the Degree of Doctor of Philosophy
to the University of Agricultural Sciences
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by

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Abstract

This thesis entitled ``Physico-chemical and Melissopalynological Characteristics of Nepalese Honey`` consists of four chapters. Chapter One provides an introduction to the topophysiography and agro-ecology of the country and highlights the importance of beekeeping in providing food, nutrition and ecological benefits. Chapter Two focuses on honeybee species, honey hunting practices, past and present status of *Apis cerana* beekeeping and the impact of introduced European honeybee, *Apis mellifera*. Chapter Three is the major part of the present study and deals mainly with honey; its kinds, composition, utilization, sources, and physico-chemical characteristics. The principal concern of this chapter was to identify the differences, if any, in the physico-chemical properties of honey samples collected from different bee species and from different ecozones of Nepal. This study involved four different types of honey samples, viz. (1) samples collected from *Apis dorsata, A. cerana* and *A. mellifera* colonies by cutting small piece of sealed honey comb at the same day and from the same floristic region of Chitwan district, central Nepal, (2) samples collected from *Apis cerana* and *A. mellifera* colonies during different months of the year in Kathmandu valley, Nepal, (3) *Apis cerana* honey samples collected from tarai, hills, and mountais and honeys from supermarket in Kathmandu, (4) *Apis laboriosa, A. florea, Trigona* and *Melipona* honey samples collected from different areas in Nepal. These samples were analysed for moisture content, pH, electrical conductivity, invertase activity, proline content and glucose oxidase by following the harmonized methods of European Honey Commission. The carbohydrate composition of honey and separated sugars (e.g. fructose, glucose, sucrose, maltose and other higher sugars) were measured by HPLC following the methods of German Institute for Norms. In general, the analytical results show that each honey has some of its own characteristic properties. Therefore, by carrying out the chemical analysis of honey it could be possible to distinguish *Apis mellifera* honey from *A. dorsata* and *A. cerana* honeys. Chapter Four narrows the focus on honey-pollen analysis. The pollen analysis was carried out for 311 honey samples in order to identify the important nectar sources for bees in different agro-ecozones of Nepal. In addition, this chapter describes the foraging preferences of different honeybee species within the same floristic areas and geographic indicators of honeys for different altitudinal zones with the illustration of pollen photomicrographs of 57 common bee plants.

CONTENTS

(847 species) of the birds and 4% (183 species) of mammals. Physiographically, Nepal can be divided into the five sub-divisions: Tarai (<500masl), Churia/Siwaliks (500-1000masl), Mid Hills (1000-3000masl), High Mountains (3000-5000masl), and High Himalayas (>5000masl). For the sake of convenience, respectively the second and third, and the fourth and fifth are often aggregated into the simpler classification of 'Hills' and 'Mountains'.

Tarai which is about 25-32 km wide occupies about 17% of the total land area of Nepal. It forms the northern part of the Indo-gangetic basin and is fertile with alluvial soil; which has good water holding capacity. The climate is hot and monsoonal, with annual rainfall ranging between 1,000 mm to 2,700 mm. The remaining 83% of the area belongs to the Hills and Mountains (NPC, 1993). At present, the Hills and Mountains account for 56% of the total population while they represent one third of the total arable land. In the Hills and Mountains as many as 90% of the farmers have small land holdings up to 0.5 to 2 hectares per household on average. Every year the land holdings is getting smaller, divided and sub divided as families grow larger. At present, the average household size is 5.5 persons and the population growth rate is 2.6% per annum. In real terms, this means that each year another 525,000 people are added to the population of the country. Nepal outnumbers many other countries not only in human population but also in livestock population per unit area. The average number of livestock per house hold is estimated to be 5.8. This situation clearly indicates high population pressure on the cultivable land

Agriculture is the main occupation and almost 90% of the population depends on subsistence farming. However, the land use data show that only 18% of land area is under cultivation. Only 13% land has irrigation facilities and on average 17 kg chemical fertilizers per hectare per annum has been used (Pant and Gautam, 1990). Due to the lack of environment specific techniques, highly fragmented land holdings and limited use of production inputs (e.g. irrigation, fertilizers and good quality seeds), the crop yield rate of Nepal is very disappointing. Where as the per capita consumption of food grains is very high. According to one survey, the per capita consumption of food grain is estimated at 293.39 kg; viz. 192.13 kg rice + 72.62 kg maize + 19.43 kg wheat +9.2 kg millet (c.f. Bhatt, 1977). The data on food grain yields reveal that the growth rate in Nepal is significantly lower than the world as a whole, and very substantially less than the Asian average (FAO Production Year Books 1971; 1992, c.f. Gill, 1996).

Despite their sheer industriousness and fortitude, Nepalese farmers are hardly managing two meals a day. The available data suggest that the actual rate of most cereals, in fact has either declined or remained stagnant (ICIMOD, 1990, Gill. 1996). A dangerous spiral of tragedy is now setting in-as the demand is growing, the labour per yield increasing, but total per capita yield is decreasing. It is quite obvious that farming alone could not sustain the livelihoods of the people unless environmentally sustainable activities were promoted.

Reports from various sectors suggest that the ecological balance is also deteriorating. The most familiar symptoms of the problems are; massive deforestation throughout the country; wide spread erosion on denuded slopes and frequent landslides in heavily abused areas; overuse or misuse of land by a large human population dependent on agriculture; an excessive number of domestic animals that are over grazing pastures and competing with a dwindling number of wild herbivores and so on (Cronin, 1979). The major factors related to deforestation are; expansion of land for growing agricultural and horticultural crops, the looping of trees to gather fodder, and cutting of trees for timber and fuel-wood. As a result of these factors, square kilo meters of land are being turned to desert. A serious environmental problem is now threatening not just the welfare of the animals and forests, not the mere economic development of the people, but the fate of the nation itself.

In this connection, the remark made by Edouard Saouma, Director General of FAO seems quite relevant `` Our real enemies are not only desertification, deforestation, land degradation and agrochemical pollution but also, above all, underdevelopment, poverty and inequalities of the rural world`` (Saouma, 1991). There is an intricate relationship between population growth, poverty and pollution (environmental degradation), and human being is a critical element in this equation of 3P.

For the poor people, deforestation is not a matter of right or wrong; it is a matter of priority. Their present demands are so high that they can't think about their future. As Arielle Renouf of the France-based Tourism for Development says, ``You can't talk to somebody about ecology if they are starving. The poverty got to be dealt with first`` (TIME 153(24) June-21. 1999). Therefore, to control the deforestation it is necessary to explore the location specific potentialities which can contribute to generate off-farm employment and income opportunities (Banskota, 1986; Mahat, 1987; Thapa and Weber, 1990, Partap, 1997).

Beekeeping is considered to be as one of such options which can help to alleviate the pressure on land, on the one hand, and improve the economic conditions of the people on the other. Beekeeping is an environment friendly food, nutrition and income generating activity which offers comparative advantages of using an unharnessed ecological niche; nectar and pollen of flowers. These plant products are, otherwise, unattainable for us. It is a flexible occupation and can create off-farm employment opportunities for many sectors including women and old men. Being relatively less time, capital and space consuming it can be operated at a small scale even by the poor farmers, landless labours and other disadvantaged groups of the society. It does not require heavy work, sophisticated education and high cost technology. Therefore, it suits very well to the mountain communities in Nepal (see Box 1). With its high-value, low-volume and low-perishability, honey and other bee products are excellent examples of mountain products, that should be promoted (Pelink, 1997). An equally important role of beekeeping is the increase in productivity of agricultural, horticultural, and forage crops. Many plants require the visits of insects (pollination) for the production of viable seeds. To attract the insects they secrete nectar and have some highly coloured blossom parts (corolla). Honeybees in their search for nectar and pollen, go over the flowers thoroughly and help to transfer the pollen grains from one flower to the stigmas of other flowers of the same species, where it germinates. The tendency of foragers to concentrate on one species at a particular time greatly increases the chances of pollination and fertilization of ovum. Honeybees, thus, provide free ecosystem services in the form of cross pollination and propagation of several cultivated and wild plant species, thereby maintaining biological diversity (Verma, 1990).

Pollination needs

Go to your fields & your gardens, and you shall learn that
it is the pleasure of the bee to gather honey of the flower,
But it is also the pleasure of the flower
to yield its honey to the bee
For the bee a flower is a fountain of life
And to the flower a bee is a messenger of love
And to both, bees & flower, the giving & receiving of pleasure is a need and an ecstasy.

- Khalil Gibran, the prophet

Why is beekeeping an appropriate activity for rural people in Nepal ?

-It is long established household activity integrated to the cultural heritage of rural people.

-It does not compete with other resource demanding components of farming systems and, thus, helps in the prevention of further increase in pressure on land, which otherwise deteriorates ecological balance.

-It does not require land needed for crops; hives can be placed on non agricultural field edges, forest and waste land. Thus, beekeeping can be done by small holders and landless peasants.

-It does not require large investment, high cost technology, heavy work and sophisticated education, so can be done by most people from poor farmer to the rich.

-It has positive impact on environment; i.e. honeybees pollinate the plants and ensure their regeneration and help in maintaining bio-diversity.

-As pollinator, bees improve the yields and quality of many crops, oil seeds, vegetables and fruits.

-It is flexible enough; can be operated as leisure time, part time or full time occupation by people of all ages.

-It is not time consuming. Bees do not need daily care and beekeeping can be done when other work allows.

-Its products are relatively easy to transport; less bulky and holds higher economic value, beeswax does not need container or packing materials and fetches a good price in the market.

-It is also appropriate in areas where agriculture is prevented by undulating physiography, cold and harsh climatic conditions, steep and rocky slopes, broken terrain and other inherent problems of the mountain areas,

-It is a source of income generation (i.e. sale of honey and beeswax).

-Honey can be used for multipurpose utility viz. health, nutrition, natural antibiotics, wound healing and cleansing, and sugar substitute, and wax is used in making candles, soaps, creams and so on.

-The whole family can become involved since the work can be done at home by old. young, men, women, older children.

(Source : compiled from multiple sources)

1.2 Why This Study ?

Despite its multipurpose utility, the amount of scientific information on Nepalese honey is very meagre. The earlier research in Nepal is mainly focussed on honeybees, traditional beekeeping, and honey hunting. Research on honey quality and plant sources has generally been neglected. There is very little scientific information on physico-chemical properties of Nepalese honeys. Physico-chemical properties are determined by chemical constituents that exist in it. Several of these constituents, viz. moisture percentage, sugar content, enzymes, proline content etc. are of great importance in honey industry as they influence its keeping quality, granulation, texture etc. The chemical composition of honey also determines nutritional and antibacterial efficiency of honey. Therefore, to get good quality honey for a better market price it is necessary to have information on its physico-chemical properties. Similarly, designating honey by geographical, topographical or botanical names has a huge influence on the retail price of the particular honey. In many countries, floral origin of a honey is important since premium prices are paid for honeys of specific floral origin. For example, in Austria *Brassica* (rape-seed) honey costs c.50 Shillings (US $4) where as the *Rhododendron* and *Castanea* honey cost more than 150 Schillings. In Morocco, thyme (*Thymus spp*) honey costs US $15-20 where as eucalyptus (*Eucalyptus diversicolor*) honey costs US $ 3-5. Honey from Ouneine valley, southern high atlas of Morocco costs 4-5 times as much as from other regions of the country. In Nepal, ``*Kattike Maha*`` (autumn honey) is more expensive than ``*Chaite Maha*`` (spring honey). Similarly, the price of *Apis laboriosa* honey (US $ 12) in Nepal is four times greater than the price of *Apis cerana* honey (US $ 3).

The botanical, geographical and topographical origin of honey can be determined by identifying the pollen in honey. Therefore, the microscopic analysis of pollen in honey (also known as melissopalynology) has proved of considerable economic value to apiarists and in the food industry (Cowan, 1988). Besides the biological interests in pollination and pollen foraging, the study of pollen in honey has become important in the protection of consumers from adulteration or mislabelling of honeys (Ricciardelli D`Albore, 1997). The botanical resources from which the bees make honey have profound effect on honey quality because the aroma, flavour, colour and many other properties of honey depend on them. The knowledge about the bee flora is also essential for the production of surplus honey and to manage healthy bee colonies for pollination (Partap, 1997). However, no systematic work on honey pollen analysis has so far been carried out in Nepal (Kerkvliet, 1994).

Based on their direct observation a number of investigators have reported more than 100 bee plants from the vicinity of Kathmandu valley (Kafle, 1984; 1992, Maskey, 1989; 1992, Partap and Verma,1996). With direct observation it is only possible to ascertain whether a species is more or less extensively visited by bees, but not the extent to which it contributes to honey production (Ricciardelli D`Albore, 1997). Therefore, it was considered necessary to analyse the physico-chemical and melissopalynological characteristics of Nepalese honey.

1.3 Objectives : The specific objectives of this study are as follows :

-to provide general information on bees and beekeeping in Nepal.

-to carry out physico-chemical anylysis of *Apis cerana* honeys collected from different agro-ecozones of Nepal in order to evaluate its quality.

-to analyse the honey samples collected from different bee species (*Apis cerana, A. mellifera, A. dorsata, A. laboriosa, A. florea, and Melipona/Trigona*) in order to identify the differences, if any, exist in their physico-chemical properties.

- to carry out melissopalynological studies in order to identify the most important bee plants for different agro-ecozones of Nepal.

CHAPTER TWO

BEES AND BEEKEEPING IN NEPAL

2.1 Introduction to Bees

There are more than 20,000 species of bees grouped as super family Apoidae in the insect order Hymenoptera. The wasps and ants too are members of the order Hymenoptera and have some close resemblance to bees. The most familiar bees are the social ones which provide man with honey and wax. The true honeybees (the genus *Apis*) are a biologically well defined within the family Apidae which comprises, besides the *Apis*, the Euglosini (orchid bees), Bombini (bumble bees), and Meliponini (stingless bees). The main characteristics which separate *Apis* from all other bees are listed as follows (Koeniger, 1995):

-the comb is constructed out of pure beeswax.
-the construction of hexagonal cells on either side of the combs and repeated use of these comb cells for brood rearing and storage of honey and pollen.
-the use of 9-oxo-2 decenoic acid as a main component of queen's pheromone and as a major component of queen's sexual attraction during mating.
-the use of isopentyl acetate as the main alarm pheromone.
-the dance communication to direct colony to newly found food sources.
-colony multiplication by swarming.

Honeybees exhibit the most complex but well organized social behaviour which has always fascinated man to draw comparison between his own social life and that of honeybee. For thousands of years honeybees have been regarded by mankind as symbol of `State`, `Good management`, `Peace`, `Fortune`, `Industriousness`, `Good character`, `Love affairs` and so on. There is widespread belief that nest of honeybees at a person's house bring luck (Dewan, 1995, Joshi, 1998). As a symbol of proud to his beloved state, Napoleon Bonaparte used to put the logo of honeybee in his `Royal Dress`. Bernard Shaw, a great poet and hobbyist beekeeper mentioned honeybee as a symbol of wisdom:

`Go to the bee, then poet,
consider her ways and be wise` - Bernard Shaw

Mahatma Gandhi mentioned honeybee as a symbol of industriousness:

`If we tap all our resources, I am quite sure, we can be again the richest country in the world..... All we need is to be industrious not like a machine but like the honeybee`

- Mahatma Gandhi

2.2 Honeybee Species

The genus *Apis* `true honeybees` has 9 species. Based on the mode of nesting these are divided into two sub units; the open-nesting honeybees; ***Apis florea* Fabricius, 1787** (dwarf honeybee), ***Apis andreniformis* Smith, 1858** (little honeybee of Thai-Malayan region), ***Apis dorsata* Fabricius, 1798** (giant honeybee) and ***Apis laboriosa* Smith, 1871** (Himalayan giant honeybee) and cavity-nesting species; ***Apis cerana* Fabricius, 1793** (Asian hive bee) and ***Apis mellifera* Linnaeus, 1758** (European honeybee), ***Apis koschevnikovi* Buttel-Reepen, 1906** (red hive bee of Borneo), ***Apis* nuluensis Tingek, Koeniger and Koeniger, 1996** (Sabah, Borneo, Malaysia) and ***Apis nigrocinata* Smith, 1861** (Sulawesi, Indonesia). The open-nesting species normally build only a single comb and nest in the open air on tall trees, on shrubs or on vertical cliffs. These species are migratory in habit; usually choose a shady nesting place during the hot summer and sunny place during the winter. The cavity-nesting bee species build several parallel combs under the ceiling of the cavity and attached to the cavity's wall.

A colony of honeybee consists of a single fertile female, who is the mother of the colony and is commonly known as `the queen`, several thousands under-developed and sterile females known as `workers` who nurse the brood, tend the hive, guard the entrance and forage for nectar and pollen. At particular times of the year up to several hundred males known as `drones` are present. These drones are usually fed by the workers and have as their main function mating and insemination of the queens. Honey is stored in the upper portion of the comb. In central and lower part of the comb, cells are used for brood rearing. Pollen storage cells are found between brood rearing area and honey cells. Queen cells are constructed at the lower edge of the comb.

Indigenous species of honeybees found in Nepal are *Apis cerana, Apis dorsata, Apis laboriosa* and *Apis florea* (*figure 1, 2, 3 & 4*). European honeybee, *Apis mellifera* (*figure 5*) has been introduced by human. Among the native honeybee species, *Apis dorsata, Apis florea* and *Apis laboriosa* can not be kept in hives, though more than 50 % of total honey comes from honey hunting of these species.

Apis florea is the smallest amongst the honeybee species and is, therefore, called the dwarf honeybee. It builds its comb around the branches of bushes or trees in such a way that the comb has an open convex surface on the top (*figure 4b*). This species is distributed in the plains and hills and found up to 1500masl. The honey yield of *Apis florea* is very low, varies between ½ to 2 kg per harvest. In Nepal, it has been considered as superior quality and used for medicinal purposes.

Apis dorsata also known as the giant honeybee or rockbee is found in lower warmer belt of the country up to an altitude of 1200masl. It constructs its comb under the water tank, bridges and the branches of tall tree, mainly *Bombax ceiba, Mangifera indica* and *Ficus religiosa.* Unlike in *A. florea*, the upper portion of its comb is never exposed (*figure 2b*). As many as 70 or more colonies may aggregate on a single tree (*figure 7*) or on a single nesting site.

Apis laboriosa is found at altitudes ranging from 1200 to 3500masl. Mostly it establishes its nest at 2500-3200masl. It nests beneath the rock overhangs of vertical cliff ledges (*figure 3b*). Like *A. dorsata*, 70 or more colonies can be found at a single cliff site. A single comb of *A. dorsata* and *A. laboriosa* can provide up to 20 kg honey in one harvest.

Besides the above five species of *Apis*, there exist a stingless bee of genus ***Trigona*** and ***Melipona*** (*figure 6*). This has occupied the same habitat as that of *Apis florea* and *Apis dorsata* in the lower warmer belt of the country. Some entrepreneurial farmers in Dadeldhura, Doti, Dang, Rolpa, Surkhet and Dhading districts have managed stingless bees in hollow logs, like those used for *Apis cerana.* This species makes its nest within cavities and stores honey in special honey cups kept separately from the brood cells. The honey yields ranges from 1 to 2 kg per colony per year. The honey is thinner in consistency and acidic in taste. The stingless bee is an important and efficient pollinator of crops, but its uses and management as a crop pollinator are largely unexplored (Crane, 1990).

Honeybee Species of Nepal (I)

Fig. 1a *Apis cerana*

Fig. 1b *Apis cerana*

Fig. 2a *Apis dorsata*

Fig. 2b *Apis dorsata*

Fig. 3a *Apis laboriosa*

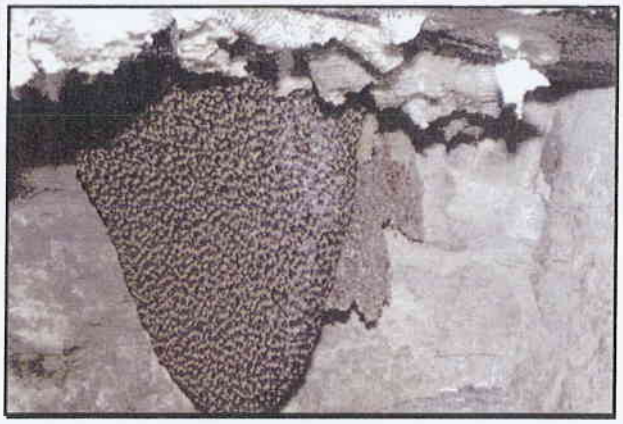

Fig. 3b *Apis laboriosa*

Honeybee Species of Nepal (II)

Fig. 4a *Apis florea*

Fig. 4b *Apis florea*

Fig. 5a *Apis mellifera*

Fig. 5b *Apis mellifera*

Fig. 6a Meliponinae

Fig. 6b Meliponinae

2.3 Honey Hunting in Nepal

Fig. 7: *Bombax ceiba*: favorite tree of *Apis dorsata*

Honey hunting from wild nests of *Apis laboriosa* and *Apis dorsata* is an important part of apiculture, integrated to the cultural and natural heritage of Nepalese people. Some ethnic groups, such as Raji, eke-out their existence mainly from the honey hunting (Valli, 1998). Raji people, like *Apis laboriosa* and *Apis dorsata* bees, are migratory in habit; usually they camped near by the forest where the bees are prevalent. Strickland (1982), Valli and Summers (1988), Summers (1990), Oppitz (1991), Claire (1997) and Valli (1998a; 1998b) have described the methods, and socio-economic and religious factors involved in honey hunting. Among them `Honey Hunters of Nepal` is the most outstanding pictorial reports. However, these all reports are mainly concerned with the honey hunting practised in central and mid-western Nepal. In western and far western Nepal, there also exist many communities such as Amlise in Bajura district, Dhameni in Bajhang district, who have long tradition of honey hunting and follow somewhat different methods. Similarly in some tarai districts there are some professional honey collector who take special care about bees. They use smoke to disorient the bees and cut the honey combs leaving some portions of honey and brood for bees (*figure 8*). But the others use fire on the colonies and kill a lot of bees and harvest brood as well. Therefore, there is a need to teach the honey hunters about appropriate honey harvesting techniques and the importance of bees as pollinator to increase the yields of their crops.

2.4 Beekeeping in Nepal

Rearing of honeybees in a hive usually for honey production, or wax production or crop pollination is called beekeeping. At present there are two species of hive honeybees are available in Nepal; indigenous *Apis cerana* and exotic *Apis mellifera.*

2.4.1 Beekeeping with Indigenous Hive Bee, *Apis cerana*

The cavity nesting honeybee, *Apis cerana* is native to southern and eastern Asia and is, therefore, called Asian hive bee. It is very similar to *Apis mellifera* in nesting and dancing behaviour and in building of parallel combs. However, they show several other distinct differences. *Apis cerana* is well adapted to the local climates, environment and native flora. It is widespread up to 3,000masl throughout the country. It is gentle in temperament, industrious, mite resistant and can be handled easily. The honey yield of *Apis cerana* varies between 10-12 kg/colony/year. However, this species has not become popular among the commercial beekeepers because of its low honey yield and frequent swarming, absconding and robbing behaviour. Three sub species of *Apis cerana* have so far been recognized in Nepal; *Apis cerana cerana, Apis cerana himalaya* and *Apis cerana indica* (Ruttner, 1987, Verma, 1990; 1992). Besides these, there may be several eco-types and geographic races. *Apis cerana cerana* of high mountain region (e.g. Jumla district) is larger in size, more productive and gentle in temperament (Verma, 1992, Partap and Verma, 1996).

Traditionally, bees are kept in several different types of hives made from hollowed out logs, wall recesses, wooden pitchers and wicker baskets (made of *Bauhinia vahlii* leaves and bamboo strips). These traditional bee hives are prepared out of the available local materials and cost almost `nothing`. The most common bee hives are log hives (*Mude ghar*) and wall hives (*khope ghar*).

Log hives are a simple structure without any frames nor separate brood or super chambers. These hives are made from hollowed out logs with one or two c.6mm entrance holes placed at the centre along their length and both ends closed with wooden stopper and plastered with cow-dung and mud. The log hives are of two types; Jumla type (*figure 9*) and Arun valley type (*figure 10*). In the latter type, the hollowed out log is vertically split into two parts, like in

top-bar log hives. Hence, in this type of hives, colony inspection and even colony dividing is possible. The size of these log hives vary greatly depending up on the size of tree trunk. On average, it measures 38.8±14.2 litre in size (Pechhacker et al., 1998).

Wall hives are either rectangular or square in shape but vary little in dimensions. A small round, triangular or rectangular entrance hole either at the bottom or centre is located on the outside of the house. Each recess or cavity in the wall is 40-60 cm (a measurement of length is one hand i.e. from elbow to finger tips) long and 25-30 cm or one `*bitta*' (the span of hand) deep. The side walls are plastered with mud and cow-dung, where as the floor and roof are provided with wooden planks. Bees generally build the combs on the wooden roof parallel to the entrance of the hives (*figure 11*). The backside of the recess is closed by a wooden plank which is temporarily fixed to the house walls with a mixture of mud and cow-dung. While harvesting honey from the wall hives, the back door is removed by scraping off the mud plaster.

Fig. 9 Jumla type of log hive

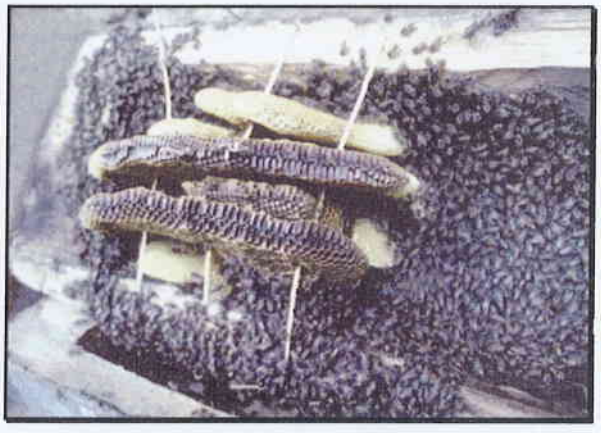

Fig. 10 Arun valley type of log hive

Fig. 11 Fixed-comb wall hive

Fig. 12 Movable frame-wall hive

In recent years, aid agencies have introduced several types of movable comb hives in Nepal. Ten different types of hives are currently in use (Allen, 1995). Under the technical aid programme of His Majesty's Government of Nepal (HMGN), UNICEF, UNDP, Swiss Association for Technical Assistance, FAO, GTZ and the American Peace Corps have made many efforts to develop beekeeping programme with *Apis cerana* in log top bar hives. Several of these development organizations have recommended the use of top bar log hives for *Apis cerana* beekeeping because these hives are simple, require low cost, and easy to make. However, top bar log hives have not become popular among the Nepalese beekeepers because in these hives, comb building can not be controlled and there are often cross combs or double combs on a single bar (Verma, 1990, Partap et al., 1997).

Taking this in view, many different types of movable frame hives, varying in dimension and designs have also been introduced in Nepal (Kafle,1992). The Newton B type of hives; (1) Khopasi and Lumle models -designed by Department of Agriculture, (2) Godavari bee hive - designed by Saubolle and Bachman (1979) and (3) BETRESP hive -designed by Beekeeping Development Programme of SNV/HMGN are more in use. The wood from tooni (*Cedrela toona*), champ (*Michelia champaca*) and alder (*Alnus nepalensis*) is generally used for constructing hive.

To diffuse the modern technology and movable frame hives, HMGN has established Bee Development Programme (BDP), which provides different kinds of training and distributes Newton B movable-frame hive in subsidised rate. Since 1993 ICIMOD Beekeeping Project with the financial support of Federal Chancellery of Austria, has also been engaged in beekeeping training, extension and research activities. Similarly, other institutions like, GTZ, USAID, JOCV and other NGOs and INGOs have also made efforts to introduce movable-frame hive beekeeping as a source of sustainable income to the rural communities. Cottage and Small Scale Industry Development Board, Small Farmer Development Programme and Agricultural Development Offices are also providing training and offering subsidised frame hives to the farmers.

Movable-frame hives and beekeeping equipment required for modern beekeeping such as honey extractor and honey processing equipment are very expensive for the Nepalese beekeepers. More over, the Newton B wooden hives practised in Nepal have poor insulation

and hence, wild swarms and migratory colonies prefer traditional log and wall hives). The occupacy rate of movable frame hive is extremely low, less than 50 per cent (Bishop, 1992). Keeping this in mind, ICIMOD, in collaboration with Austrian experts (H Pechhacker and E Hüttinger of Beekeeping Institute, Lunz am See) has designed movable-frame straw hive (*figure 13*), which is cheap and provides better insulation than wooden frame hives (Partap et al., 1997, Pechhacker et al., 1998). ICIMOD has also made similar efforts to adapt wall hives in such a way that movable frames can fit into them. ICIMOD has provided movable frames in 15 wall hives in Tarebhir village of Shivapuri Watershed area (*figure 12*). However, the acceptance rate of these hives by the beekeepers has not yet evaluated.

Fig 13a Eco-friendly movable-frame straw hive

Fig 13b Colony transfer from log hive to straw hive

Fig. 13c Straw Hive plastered with mud/cow dung

Fig 14 Log hives placed for baiting swarms

In Nepal, bees are considered as the `symbol of fortune`. Therefore, farmers rely more on luck than management techniques. Where traditional hives are used, only a few management practices are undertaken. Beekeepers generally handle the colony during honey harvest and for swarm trapping. Natural swarming is only the way of colony multiplication, feeding is not

practised by most of the beekeepers. Only a few beekeepers provide occasional feeding with pumpkin syrup or honey. Baiting of hives for the collection of wild swarms or migratory colonies is among the most important activities.

For collecting wild swarms and migrating colonies, log hives are placed for baiting on sunny, sheltered cliffs (*figure 14*). Before placing, the bait hives are fumigated with smoke from medicinal herbs (*Artemisia vulgaris*) and dehydrated butter and rubbed with bees wax. When bees enter and occupy the hive, it is brought and placed under the eaves of the houses.

Honey harvesting period differs from place to place, depending upon the climatic conditions and forage availability. Generally honey is harvested twice a year; i.e. during April/May and October/November. Honey is extracted by squeezing the whole combs (*figure 15*). Brood combs have been considered as highly nutritious food and fed to milch cattle or to the children. Only the small portion of harvested honey is used for family consumption. Most of it is bartered for grains, '*ghee*' (dehydrated butter) and so on or sold to earn cash. The honey is marketed in all sorts of ways; in narrow mouthed glass containers of alcoholic drinks, in wide mouthed glass bottles, in plastic gallons, and in tins. Such honey contains high moisture content and does not fetch a good price and ferments quickly. To prevent fermentation, some farmers cook their honey by direct heating. The resulting honey does not ferment but looses its nutritional and medicinal benefits. Therefore, there is a need of providing technical knowledge to farmers/beekeepers for harvesting good quality honey to get a better price in the market.

Fig. 15 Honey sqeezing

2.4.2 Beekeeping with the Exotic Bee Species, *Apis mellifera*

In recent years, the Italian strain of European honeybee, *Apis mellifera ligustica* has been imported to the country for commercial honey production. Because of the long period of

domestication and selective breeding, this species has less swarming and absconding tendencies and has good honey gathering qualities. The honey yields of this species varies between 20-30 kg/colony/year. The species has become popular among the commercial beekeepers of tarai region like Chitwan district and in Kathmandu valley. *Apis mellifera* beekeeping is practised in movable frame hives; mainly in Langstroth hive. The most of the colonies are well managed, with all necessary technical resources and medicines to combat the indigenous diseases and predators to which exotic bees are susceptible. Dabur India Ltd, the leading Indian Company has recently established the Dabur-Nepal Office and is making efforts to sell *Apis mellifera* colonies on a wide scale. Agricultural Development Bank is also offering loan to the farmers . In Chitwan district, beekeeping with *Apis mellifera* is likely to prove successful (*figure 16*). Large-scale, irrigated agriculture providing mono-cultures of mustard and buckwheat provide excellent forage sources for bees. However, the infestation rate of bee mites (i.e.*Varroa jacobsoni* and *Tropilaelaops clareae*) is increasing at an alarming rate. Beekeepers are using sulphur, formic acid, Chinese Vicks (mint) and other drugs to control mites (personal observation). According to Capt. D. B. Thapa, of Thapa Bee Cosultancy, he lost his 30 % of *Apis mellifera* colonies due to *Varroa* infestation during 1997.

Fig. 16 *Apis mellifera* beekeeping in Chitwan district, tarai Nepal

The performance of *Apis mellifera* in the hills and mountains seems very poor. In Gopghat, Doti district (c. 1800masl), for example, all the 10 colonies introduced by an local NGO in 1995 died within one year. Similarly in Jumla district (c. 2500masl) the *Apis mellifera* colonies introduced by UNICEF funded Appropriate Technology Development Centre in 1993 were collapsed within a year. The reason is still unknown (i.e. infestation of mites, mating competition, lack of forage, cooler climatic conditions ?). As reported by earlier

investigator, a species which has smaller number of drones at the congregation area fails in mating. For example, imported *Apis mellifera* did not produce mated queens in Asia (Akratanakul, 1976; Ahmad, 1984) and imported *Apis cerana* did not successfully mate in Germany (Ruttner et al., 1972). Therefore, large scale import and multiplication of exotic *Apis mellifera* is still a controversial subject in Nepal. Doubts are being expressed that this species may not adapt well in its new environment due to different climatic condition, flora, mating competition and parasites (Mattu and Verma, 1980; Verma, 1988; 1992).

2.4.3 Harmony or Conflict ? *Apis mellifera* Beekeeping in Nepal

The competition for forage, where colonies of both *Apis mellifera* and *Apis cerana* are located at the same place and transfer of pathogens from one species to another has become a major problem in Nepal. Examples of *Apis cerana* suffering from parasites of *Apis mellifera* are Thai Sac Brood Virus (Verma, 1985) and *Acarapis woodi* (Ahmad, 1984). Similarly, the examples of *Apis mellifera* suffering from parasites of *Apis cerana* is *Varroa jacobsoni* and parasites of *Apis dorsata* is *Tropilaelaps clareae*. The wild nests of *Apis dorsata* and *Apis laboriosa* were also found to be infested with the European Foul Brood (EFB) and American Foul Brood (AFB) diseases, which were also transferred from the parasites of *Apis mellifera*. *Melisococcus pluton* was detected from *A. laboriosa* colonies in Nepal (Allen et al., 1990). Similary, in Barabise, near Kodari-Highway, more than 50% of the brood in one *A. laboriosa* colony were found to be infested with EFB (Pechhacker, 1994, personal communication). Thai Sac Brood Virus disease killed more than 90% of *Apis cerana* colonies in Nepal in 1983 (Kafle, 1992). Recently, after the introduction of *A. mellifera* in 1993, more than 80% of *A. cerana* colonies were collapsed in Jumla district, one of the most remote area of Nepal.

At present, the existing long established and widespread farmers' *Apis cerana* beekeeping is threatened by the introduction of *Apis mellifera* (Crane, 1992, Verma, 1993, Pechhacker and Juntawong, 1994, Allen, 1995, Pechhacker et al, 1999). The large financial investment in equipment and the labour intensive techniques needed for *Apis mellifera* beekeeping make it unsuitable for rural farmers-beekeepers who comprise the majority of beekeepers in Nepal. The another problem with *Apis mellifera* beekeeping is pollination of native flora. During present study it is found that the exotic bee, *Apis mellifera* visits less number of native flora

than *Apis cerana* and *Apis dorsata*. Native crops and wild plants may need their co-evolved pollinators.

If we are concerned about the relatively under-privileged areas of the mountains where poverty is the rule and even two meals a day is very hard to manage, then we should rethink about the large scale import of exotic bee, *Apis mellifera*. The high level of inputs in terms of capital and management required for *Apis mellifera* beekeeping naturally bring in constraints for the poorer sections of the population, for whom these have been the very constraints (see Box).

Will *Apis mellifera* generate more income for the rural people in Nepal ?

`When we consider, instead of the bees, the people in mountain regions who might be or who might become beekeepers, we find different and even more significant forces at work. European honeybee, *Apis mellifera*, requires a higher capital investment and higher technological operation if it is to be effective. If the rural population is rich and educated enough, to satisfy these requirements, much good can arise. If not, the opportunity for a modest improvement in the standard of living of the present families will be lost.`

-Eva Crane, 1992

`Based on the data obtained from 23 *Apis cerana* beekeepers and 22 A*pis mellifera* colonies, *Apis cerana* was found to be more profitable; production cost per kg honey was US $ 0.20 compared with US $ 0.37 for *Apis mellifera*. The ratio of income to production costs were 2.56 and 0.38, respectively. If only a few colonies are kept, *Apis cerana* is still profitable where as *Apis mellifera* is not.`

-Nguyen Quang Tan & Pham Thanh Binh, 1994

`When the *Apis cerana* population is destroyed a native and well adapted pollinator for both native and agricultural plants will be lost....Therefore, all possible efforts should be made to improve the economic value of the native bee, *Apis cerana* rather than the importation of *Apis mellifera*`.

-H Pechhacker and N Juntawong, 1994

Àpis mellifera is not the biologically `dominant` species in Asia and it will remain there only in total dependence on humans. Even after beekeepers have destroyed many of its competitors, predators, and other natural `problems` it will not survive without constant care....`

-Nikolaus Koeniger, 1995

`The importation of *Apis mellifera* into other countries in Asia has resulted in the development of large-scale, high input beekeeping and the decline of beekeeping with indigenous *Apis cerana*. This may also occur in Nepal`.

-Mark F. Allen, 1995

`With appropriate management *Apis mellifera* can deliver higher yields of honey and beeswax than *Apis cerana*. The input costs will also be greater. This is because *Apis mellifera* is an exotic species from a temperate climate, and it requires more resources (time, treatment against endemic diseases and predators). It is already well known from other countries in Asia that beekeeping with *Apis mellifera* can only be more economical than *Apis cerana* when practised on a large scale.`

- Nicola Bradbear and Pratim Roy, 1998

` The higher honey yield of some races of *Apis mellifera* is only because of the long period of domestication and intensive selection and breeding. The other races of *Apis mellifera* which are completely unselected and kept in traditional fixed comb hives are even less productive than *Apis cerana*. For example, *Apis mellifera intermissa* of Ouneine valley, Morocco is less productive than *Apis cerana cerana* of Jumla, Nepal. Therefore, how productive the bees are is only the question of technical standards and selective breeding. To get higher honey yield, it would be the best to start selection and breeding programme with native bees rather than the importation of exotic bees`.

-H. Pechhacker, S. R. Joshi, and A. Chatt, 1999

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CHAPTER THREE

PHYSICO-CHEMICAL CHARACTERISTICS OF NEPALESE HONEY

3.1 Honey

Honey is the sweet viscous liquid prepared by bees from nectar or from honeydew. The Codex Alimentarius Commission defines honey as 'the natural sweet substance produced by honeybees from nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in the honey comb to ripen and mature'. While codifying the standards, the commission stressed that 'honey shall not have added to it any food ingredient, including food additives, or other substance foreign to honey, and shall not have any objectionable matter, flavour, aroma or taint absorbed from foreign matter during its processing and storage. Honey shall not be heated or processed to such an extent that its essential composition is changed and/or its quality is impaired. Chemical or biochemical treatments shall not be used to influence honey crystallization. Honey shall be free from heavy metals, pesticide residues, and any substance originating from micro-organisms and plants in amounts which may represent a hazard to health'.

3.1.1 Kinds of Honey

The most widely accepted criteria used for classification of honey is the plant sources of honey. Based on this criteria, honey is classified into two categories: **unifloral honey** and **multifloral honey**. The honey in which the major part of the nectar has been derived from a single plant species and has at least 45% of total pollen count in the honey from a single species is called unifloral honey. The honey which has mixed floral origin is called multifloral honey. The unifloral honey which can be identified by the prominent floral source in Nepal are; *Brassica*, *Litchi*, and sunflower honey in tarai, chiuri and *Citrus* honey in mid-hills, niger honey in high-hills. According to origin, honey is classified into two types; **blossom** or **nectar honey** and **honeydew honey**. Blossom or nectar honey is that which comes mainly from floral or extrafloral nectaries. Honeydew honey comes from secretions of or on living parts of the plants.

According to mode of processing honey is classified into three categories; **sealed comb honey, extracted honey** and **squeezed honey**. Sealed comb honey is honey stored by bees in the cells of the broodless comb, and sold in sealed whole combs or sections of such combs. Extracted honey is that which is obtained by centrifuging decapped broodless combs. Squeezed honey is obtained by squeezing the combs.

On the basis of its appearance (consistency) honey is classified into two groups; **liquid honey** and **crystallized honey**. Liquid honey is either thinner or thicker in consistency and is free of visible crystals and crystallized honey is that which is completely granular or solidified.

Nepalese honey may also be classified on the basis of bee species, harvesting seasons and geographical variations. The following terms are more widely used for the categorization of honey in Nepal:

1). `*Bheriko Maha*` wild bee honey and `*Ghareko Maha*` hive bee honey.

2). `*Kattike Maha*` autumn honey (Sep-Nov), `*Chaite Maha*` spring honey (Mar-Apr) and *Jethako Maha*` summer honey (May-Jun).

3). `*Taraiko Maha*` tarai honey, `*Parbatko Maha*` hills honey, `*Pahadko Maha*` mountains honey.

3.1.2 The Composition of Honey

The major component of honey are the sugars (about 80%) and water (17-21%) in which the sugars are dissolved. In addition, so far 181 different substances have been identified in honey. These include protein, amino acids, enzymes, vitamins, minerals, organic acids, pollen and other substances, and may include traces of fungi, algae, yeast and other solid particles resulting from the process of obtaining honey.

Moisture Content: The moisture content has profound influence on keeping quality and granulation of honey. It is a major factor which determines whether and when honey will ferment at a given temperature. If the water content in honey is 19 per cent or more, it undergoes the process of fermentation, looses flavour, granulates irregularly, separates into two layers during storage and deteriorates in quality (Dustmann, 1993). Therefore, the control

of the water content is an important requirement of the Codex Alimentarius Commission (CAC, 1989), which sets an upper limit for moisture content of 21 per cent for honey in general.

Sugar Content: The sugars of honey are responsible for its typical sweetness and wholesomeness. They also prevent or suppress the onset of fermentation if they are present in sufficiently high concentration. The percentage of water decreases as that of sugars increases. Sugars are classified according to the size and complexity of their molecules. The simple sugars (monosaccharides; fructose and glucose) are the building blocks of the more complex types and account for 85-95% of the honeybee honey sugars. The enzyme invertase, which is produced by the hypopharyngeal glands of bees, inverts sucrose into glucose and fructose. At the same time small amount of higher sugars e.g. erlose, maltose are synthesized by transglucosidase effect (Dustmann, 1993). Many of these sugars probably do not occur in nectar but formed during ripening and storage by effect of enzyme action (White, 1975, Wehling et al, 1997). A high sucrose content is generally associated with heavy sugar feeding. Therefore, the European Honey Commission has proposed upper limit of 5 g/100g apparent sucrose content for nectar honey, up to 15 g/100g for honeydew and up to 10g/100g for *Robinia, Lavandula, Hedysarum, Citrus, Medicago* honeys, and a minimum reducing sugar content of $\geq$65 g/100g for nectar honey and $\geq$ 60 g/100g for honeydew honey or blends of honeydew honey and blossom honey (Bogdanov et al., 1997).

Enzymes in Honey: The enzymes of honey are constituents of great interest and importance. Present only in merest traces, they play a vital role in the production of honey from its ultimate raw material, phloem sap of the plant. Enzymes are light and heat sensitive and are usually used as quality criteria for honey.

Invertase : Invertase (also known as α-glucosidase, saccharase, sucrase) inverts sucrose present in the nectar and honeydew, into fructose and glucose. Some invertase is still present in the finished honey. Invertase, in combination with other criteria is measured in order to find out if honey is preserved in its natural condition or damaged by heat and storage (Dustmann et al., 1985). The European Honey Commission has recommended a limit of enzyme invertase for fresh honey as $\geq$ 50 Siegenthaler scale (Bogdanov et al., 1997). The

invertase measurement has been widely used in some countries such as Germany, Austria, Switzerland, Italy as a freshness indicator of honey.

Diastase (Amylase) : It does not appear to be involved in chemical reactions that occur in the elaboration of honey. Its importance lies in its ease of measurement and its instability of heat. Generally, it is used as an indicator of overheating. The European Honey commission has proposed the limit of ≥8 Schade scale for diastase (Bogdanov et al., 1997). However, White (1994) strongly criticises the use of diastase as quality standard for honey: 'Where can one set the starting point from which to calculate a heat damage or so-called partial destruction? The entire approach is in error because the diastase activity varies from one type of honey to another depending up on the floral resources.'

Glucose Oxidase : The enzyme oxidises glucose, producing gluconic acid and H_2O_2 which is microbicidal. H_2O_2 quickly breaks down into H_2O and O_2 , and its production and decomposition are continuous while the nectar is being converted into honey. The H_2O_2 concentration remains constant under a given set of conditions of temperature, sugar concentration, etc. and is sufficiently high to give protection against harmful micro-organisms by a biochemical mechanism which disrupts their metabolism (Crane, 1990).

However, there are many factors which influence the enzyme content of honey. For instance, honeys from very heavy flows have lower enzyme level than from less profuse flows. Similarly, honeys from more concentrated nectar have lower invertase and diastase level than nectar with low sugar content (Crane, 1990). It also depends up on the period of time bees work on honeys; the longer the bees work on the honey the greater is the amount of enzyme in it (Von der Ohe, 1994). In order to distinguish between heat or light damage and natural weakness of enzyme, it is necessary to evaluate enzyme activity in combination with other chemical characteristics; eg. proline, HMF (Dustmann, 1993). HMF is produced by the degradation of fructose: it is virtually absent in fresh honey and increases more or less quickly, according to storage conditions and certain chemical properties of the honey such as acidity (Persano Oddo et al., 1999).

Amino Acids : Amino acids are breakdown products of proteins, and pollen is by far the most important source of them for bees. The amino acid content of honey has also been used

as a measure of honey purity. **Proline** is the major free amino acid which is contributed by the bees, and must originate in the pollen they consume early in life (Crane, 1990). The proline measurement used as a criterion of honey ripeness or in some cases sugar adulteration. In Germany, a honey with less than 180 ppm is considered as either non ripe or adulterated. Von der Ohe et al. (1991) have proposed 200 ppm as a lower limit of proline for honey.

Minerals : The minerals originate in the soil, and get into honey via plants and the materials bees collect. Minerals are among the many components that affect honey colour. Generally the dark honeys have higher mineral contents than light coloured honey. Honeydew honey is darker in colour and has higher content of minerals than nectar honey.

Besides the above mentioned components honey has many other minor constituents such as acetylcholin, antibacterial substance, vitamins and other plant constituents, i.e. aroma and flavour components (Dustmann, 1993).

3.1.3 Utilization of Honey

History of Uses of Honey: Since ancient times honey has been treasured by man for its nutritional and medicinal value. The ancient Greeks, Romans, Chinese and Egyptians used honey to heal wounds and cure diseases of the gut (Zumla and Lulat, 1989). In the Indian sub-continent honey has been referred as man's earliest food and it has been valued throughout successive civilization (Verma, 1990). In Hinduism it is considered as one of the five components of `*Panchamrit*` (milk + curd + dehydrated butter + sugar + honey). In Sanskrit literature this word refers as `nectar of immortality`. Honey has, therefore, been used in several religious functions including birth and death rites and festivals such as wedding ceremony, `*Pasni*` (the ceremony of feeding a baby with cereals for the first time) and so on. In `Vedas`; the sacred book of Hindu honey is mentioned as:

The winds waft sweets, the river pour sweets for the man who keeps the law;
So may the plants be sweet for us.....
may our plants be full of nectar for us and full of honey.....

-Yejur Veda-13: 27-29 (400 BC)

In the Koran it is mentioned as:

68, And thy Lord taught the bee
To build its cells in the hills,
on trees, and in men's habitations
69, Then to eat of all fruits and follow the ways of lord.../...there issues
from within their (the bees) bodies,
A drink of varying colours where as in healing for men.

- Surat 16. An Nahl

Honey is frequently mentioned in Bible. Salomon in his proverbs (24: 13) advises; `**My son, eat thou honey for it is good**`. The ancient Greeks called it `Meli` which means something better than others. Romans used the related word `Melior` and French `Meilleur`. These words are used to express something which are better than good (Dustmann, 1993). The word honey is often used to address a person or thing that is excellent or delightful. In Morocco, honey is considered as a symbol of love and offered to the guest to show the love, affection or respect.

Medicinal Uses of Honey : Honey has been used as medicine for thousands of years. The sacred scripture of Hinduism, thought to be about 5000 years old, mention the use of honey as medicine (Stomfay, 1960). In more recent years it has been scientifically proved that honey is very effective in rapidly cleaning up infection and promoting healing (Molan, 1992; 1999). A number of instances of successful treatment of severe burns and wounds with honey appear in the beekeeping literature (Molan, 1999). As an **antibiotic** or **antibacterial** substance it was found to be very effective for the treatment of ulcers and bad sores and other surface infections resulting from burns and wounds (Zumla and Lulat, 1989, Bogdanov, 1984). The antibacterial activity of honey is partially due to its osmotic effects (Molan, 1992). Honey is super saturated solution of sugars and is said to have osmotic properties (i.e. water withdrawing properties). Water molecule strongly reacts with the sugars in honey leaving little water available for micro-organisms. The bacteria that cause infection are unable to survive in honey because they become dehydrated. The production and decomposition of H_2O_2 also contributes to its antibiotic activity (White et al., 1963; Wakhle & Desai, 1991; Molan, 1992; Postmes, 1995). As H_2O_2 decomposes it generates highly reactive free radicals which react with and kill bacteria.

Subramanyam (1991) suggests that honey is effective for treatment of burn wounds because:

⇒ it inhibits the growth of both gram positive and gram negative bacteria and prevents infection

⇒ it provides a viscous barrier to fluid loss and wound invasion by bacteria thus preventing infection

⇒ it contains enzymes which may aid the healing process by promoting tissue formation

⇒ it absorbs oedema fluid (pus) thereby cleaning the wound

⇒ it reduces pain and irritation and eliminates offensive smell

The water solubility of honey allows easy removal and its mild non-corrosive properties prevent any additional harm to either damaged or healthy tissue. Therefore, it has been used for **dressing** the wounds, burns and cuts. Honey has been used with success in treating gastrointestinal disorders (Haffejje and Moosa, 1985). The honey is also used as stable palatable formulations of ferrous sulphate, triple sulpha and turpin hydrate, as well as several effective cough preparation (The Wealth of India, 1948). According to Crane (1990) at least 200 tonnes of honey are purchased annually world-wide for use in the manufacture of cough mixtures and sweets. Formulations containing the vitamins riboflavin and thiamine as well as riboflavin alone are sufficiently stable for commercial use.

In Nepal honey has traditionally been used to cure eye diseases, stomach-ache, diarrhoea, dysentery, wounds, cuts and to ease child birth (by taking honey during the birthing period). The honey is also found to be very effective against the snake bite and other poisons.

Food or Nutrition Value: Honey is a valuable high energy food. Its energy value is estimated about 3040 kcal per kg (White, 1975). It is primarily a carbohydrate food which is easily digestible and widely acceptable by all the people. Its main sugars; fructose and glucose are absorbed directly into the blood and supply a rapid source of energy (Zander and Maurizio, 1984). Because of its easy digestion, absorption and metabolism honey is the most valuable carbohydrate diet in infant feeding and for sick and older people also. It increases appetites and peristalsis and complements the iron deficiency in human. Honey has been used with beneficial results by athletes and active people. It has been used as a source of energy in

climbing Mt. Rainer, in crossing the Grand Canyon, and in the conquest of Mt. Everest by the New Zealand beekeeper Edmund Hillary. In addition to above mentioned utilities, honey has also been used in cosmetics, confectionery and fermented to make alcoholic drinks.

3.1.4 Sources of Honey

Honeybees produce honey mainly from the flowers' nectar and from honeydew. However, in some areas bees may also produce honey from extrafloral nectaries, or sap from sugarcane and some other plants. Even chocolate honey from a confectionery factory and coca cola honey from used tins have also been reported to occur in London (Crane, 1980).

Nectar: Nectar is the most important raw materials of honey. It is a secretion of nectar gland or nectary, an organ which secretes sugar sap, and is one of the characteristics of flowers pollinated by insects or birds. Nectaries can be found on any parts of a plant which are above ground, and they occur in ferns as well as in flowering plants. Based on their position, nectaries are categorised into two groups; **floral nectaries** and **extrafloral** nectaries. Floral nectaries are found on the axis of the flower, on sepals, or petals, on stamens or on carpels. Extrafloral nectaries are found on the vegetative aerial parts, e.g. on the trunk, on leaves, stipules, bracts, or on petioles, etc.. The well known species which have extrafloral nectaries are broad bean (*Vicia faba*), cotton (*Gossypium spp*), the castor oil plant (*Ricinus communis*), peach *(Prunus persica)* and other species of *Prunus* (Shuel, 1975). There is no basic difference between floral and extrafloral nectaries in either structure or function (Maurizio, 1975). Both floral and extrafloral nectaries may occur on the same plant, e.g. cotton and broad bean (Butler et al., 1972, Davis et al., 1988).

Origin of Nectar: Nectar originates basically from the phloem sap. The process of nectar secretion in the nectary includes the unloading of sap from the phloem sieve tubes to the vicinity of the nectaries via sub-glandular tissue and secretory cells by the transport system of the plants.

Composition of Nectar: Nectar is an aqueous, sugar containing secretion of plant glands. Other substances added to the sugar solution include protein, amino acids, organic acids, lipids, antioxidants, dextrins, minerals, volatile oils and enzymes (Baker and Baker, 1983).

The total sugar contents in nectar varies from 5-80% and there are great differences in the sugars present and their proportion. The sugar concentration in Indian butter tree is found higher (37.5 ± 4.3%) at 12.00 h than (27.83 ± 1.94 and 31.5 ± 4.92%, respectively) at 9.00 and 15.00 hours of a day. The amino acid content of nectar is 0.002 - 4.8 mg/100 mg total solids. The ash content is 0.23 - 0.45% and pH is 2.7 - 6.4 (Maurizio, 1975).

Factors Influencing Nectar Production: The nectar production of a plant is usually measured by the quantity (in mg or µl) and sugar concentration (%) of nectar secreted by one flower in 24 hours. The factors influencing the production of nectar, and of sugar, are classified into two categories; **internal factors** and **external factors**.

Internal Factors: The factors inherent in the plant itself are called internal factors which include genetic and physiological factors. Heredity may affect nectar production by way of regulation of the photosynthetic activity, the capacity of sugar conducting system, nectary size and the nectary enzyme component (Shuel, 1975). The secretory capacity of nectary and the quantity of sugar delivered to it also depends up on innervation; the veins which are connected to the phloem sieve tubes. The size of the flower and of the nectary surface, the age and maturity of the flower, the position of the flower on the plant, and the species, variety or cultivated race to which the plant belongs also have great influence on the nectar production.

Collison (1973) found that in cucumber maximum secretion occurred on the day of anthesis; most flowers secreted no nectar on the second day. On the same plant where male and female flowers are present, the nectar yield may differ. In banana (*Musa paradisica*), for example, the male flowers secrete 4-5 times as much nectar and sugar as female ones, and in willow (*Salix spp*) male flower produce more nectar than the female flowers (Fahn, 1949). While reverse is true with cucumber; the female flowers of cucumber produce 3-4 times as much nectar and sugar as male flowers (Beutler, 1953, Collison,1973).

Pollination and fertilization have also important effect on the secretion of nectar. Usually nectar secretion ceases after fertilization. If pollination and fertilization don't occur , nectar secretion may go on for quite a long time.

External Factors : The external factors that affect nectar production on the plants are; temperature, relative humidity, rainfall, soil, length of day and sunshine, etc. Secretion does not start below a certain temperature; minimum threshold temperature for nectar secretion varying according to plant species. Similarly, if the relative humidity is high, nectar is generally secreted in large amounts but contains little sugar, in dry air less is secreted, but the sugar concentration is high (Beutler, 1953, Fahn, 1949).

The nature of the soil, its moisture content, and the use of fertilizers, can affect nectar secretion in various ways. Poor aeration and lack of moisture in soil reduces nectar production. An increase in secretion was obtained with a soil saturation of 45-75%, with an optimum at 60% (Beutler, 1953). Soil temperature affects the number of flowers produced by the plant as well as the amount of nectar produced by individual flowers. Less nectar is produced at a soil temperature of 16 °C than at 20 °C and yield is also reduced in soil with a water content either above or below the optimum (Shuel, 1992).

In addition to above mentioned genetic, physiological and environmental factors, nectar secretion is frequently effected by man through his plant breeding manipulation and other activities which alter the normal process.

Honeydew: Some plant sucking insects belonging to the order Homoptera, excrete a sweet substance on the surface of the living parts of the plant which is called honeydew. These insects are adapted to puncture plant tissues by special feeding apparatus (i.e. four pricking bristles which can move against each other) through which they gain access to the plant sap. From this they extract the nutrients they require, while the remainder of the sap passes from their mouth parts to digestive tract and is deposited on leaves, twigs, or on other plant surface in small droplets and is known as honeydew (Maurizio, 1975, Crane, 1980; 1990). In its passage from mouth parts to the digestive tract of the insects, the plant sap has undergone considerable physico-chemical changes. So, the honeydew honey differs greatly from the original phloem sap

From left to right: Honeydew honey, blends of honeydew honey and nectar honey, nectar honey

and nectar honey. In honeydew honey some additional enzymes, minerals and free acids are added from the secretion of salivary glands and gut of plant sucking insects (Maurizio, 1965; 1975 and references therein). Honeydew honeys have (0.58%) a higher total content of minerals (ash) than nectar honeys (0.26%) and darker in colour (Crane, 1990). Honeydew honeys also contain higher amounts of trisaccharide sugars such as melezitose, erlose, and raffinose and smaller amount of reducing sugars; fructose and glucose.

The origin of honey (nectar or honeydew) can be determined by measuring its electrical conductivity. A lower limit (< 0.7 mS/cm) has been proposed for blossom than for honeydew honeys (> 0.7 mS/cm) although nectar honeys from *Tilia, Erica, Calluna, Arbutus, Gossypium, Lavandula, Eucalyptus* are some exceptions (Bogdanov et al., 1997). The presence of fungal spores, more air born pollen grains, hyphae of algae, and dust particles are the typical sign of honeydew honey.

Factors Influencing Honeydew Production: Honeydew production depends directly on the population trends of honeydew excreting insects and these vary greatly from one species to another (Pechhacker, 1976; Ricciardelli D'Albore, 1997; 1998). A large quantity of honeydew can only be produced when insect population density is very high. There is positive correlation between insect population density and honey flow (Pechhacker, 1976). Therefore, by counting the number of insects per unit surface, or by counting the drops of honeydew per surface unit per time, a forecast of honey flow can be predicted in a given area. However, the population trends of honeydew excreting insects depend on numerous biotic (e.g. population of ants, lice eater insects (coccidae), & other predators) and abiotic factors including rainfall, temperature, wind, etc. which also influence the honeydew flow of the particular area (Pechhacker, 1988). It has been shown repeatedly that the presence of a nest of ants, especially the red wood ant, *Formica polyctena* Forst, actively increases honeydew production in its immediate neighbourhood (Scheurer, 1965). The ants feed on honeydew and are reported to protect honeydew producing insects from predation.

Honeydew Plants of Nepal: Honeydew is the major source of honey in some areas of Nepal. In higher altitude areas such as Jumla, Langtang more than 50% of honey samples had more than 0.7 mS/cm electrical conductivity (Joshi et al., 1998), a limit proposed for honeydew honey by Europen Honey Commission (Bogdanov et al., 1997). There are many plants on

which honeydew is deposited are found in Nepal but the honeydew secreting insects are largely unexplored (Annex 1). Three honeydew excreting species have so far been identified; *Cinara eastopi* Pintera on *Pinus wallichiana* Jackson and *Cinara comater* Doncaster and *Cinara sp* on *Picea smithiana* Wallich. Bees collecting honeydew from *Sapium insigne* (Euphorbiaceae), *Ficus religiosa* (Moraceae), *Mangifera indica* (Anacardiacea) and Zea mays (Graminae) were also observed by some beekeepers in Dadeldhura district, Nepal (Personal communication).

Pinus wallichiana (Pinaceae) and *Ilex dipyrena* (Aquifoliaceae) have been reported as major sources of honeydew honey in Jumla district. The local beekeepers/farmers in Jumla say that the honeydew honey, especially `*salle maha*` and `*dalle maha*`, repectively from pine tree and *Ilex* tree, is bad for bees. They claim that bees died out in the forest after collecting the honeydew and also from bringing it back to store in the hives (Saville, 1999-personal communication). However, there are no any records about honeydew toxicity in the literature consulted (Von der Ohe, 1999-personal communication).

***Cinara pintera* on *Pinus wallichiana* in Jumla**

***Cinara comater on Picea smithiana* in Jumla**

Honeybees collect honeydew from *Vicia faba*

3.2 Physico-chemical Analysis of Honey

3.2.1 Why physico-chemical Analysis of Honey ?

Honey is the main product of most apiaries and has been an article of commerce for many thousands of years. The total world honey crop is nearly one million tonnes, worth about 1000 million US dollars (Crane, 1990, Dustmann, 1993). This huge amount of honey is made by billions of bees collecting nectar and/or honeydew in tiny amounts. It is interesting to note that, to make one kilogram of honey a single bee has to travel a distance equivalent to 6 orbit around the earth. In a single trip a bee carries no more than 50 milligrams of nectar, which is reduced in the hive by water evaporation to one third and finally sealed in comb. Honey sealed (capped) in the cells of the comb is always a good quality; resistant to spoilage by fermentation...and represents a high energy pack (Crane, 1990).

However, the botanical resources from which bees make honey have great influence on the sweetness, flavour, antibiotic efficiency and nutritional values of honey. The nectar or honeydew contains varying amounts of sugars (mainly sucrose), minerals, amino acids and so on. The enzyme invertase added by bees inverts the sucrose into fructose and glucose. Simultaneously, the small amount of di- and trisachharides are synthesized by transglucosidase effect (Dustmann, 1993). All above mentioned components (i.e. enzymes, moisture, sugars, etc; see also Section 3.1.2 on Composition of Honey) which involved and formed during converting nectar or honeydew to honey are influenced by various factors either natural, environmental or operational factors. The number and combination of these various components give honey a specific individual note. As Eva Crane (1980) says, 'each honey is unique'. All honeys are sweet but some honeys are more sweet or better flavoured than others. Therefore, honey is generally evaluated by carrying out physico-chemical analysis of these constituents. Several of these constituents are of great importance in honey industry as they influence the keeping quality, granulation, texture, flavour as well as the nutritional and medicinal quality of honey. Some constituents of honey are the subject of European Honey Directive (74/409/EC). European Honey Commission of APIMONDIA for future EU, which is also recognized by Codex Alimentarius Commission, has proposed some constituents as quality criteria of honey. These include; moisture content, electrical conductivity, reducing sugars, sum of fructose and glucose, sucrose content, individual sugars,

minerals, free acidity, diastase, HMF, invertase, proline, specific rotation (Bogdanov et al, 1997).

However, in Nepal, there is not any effective legislation and policy for the quality control of honeys. Honey has been used more as medicine and for religious purposes than as normal nutritional food. For a common man, it is still a luxury item which is scarcely available and highly priced; i.e. one kg honey is equivalent to 8 kg rice or 3 kg chicken or 3 days wage (Pechhacker et al., 1999). Honey is packed in an odd assortment of glass bottles, mostly in pre-used utensils of alcoholic drinks and plastic gallons. Some beekeepers and traders also sell overheated and adulterated honey (Kerkvliet et al., 1995; Shrestha, 1997; 1998). The price varied from honey to honey and place to place. At present, honey from commercial beekeepers produced by exotic *Apis mellifera* is sold at higher price than the squeezed honey harvested from native *Apis cerana* colonies. Though, honey from native bee colonies harvested in a 'correct way' or sealed comb honey is of excellent quality (i.e. without any pollutant or residuals of medicament/chemical drugs). In Japan, because of its well recognized medicinal value and better flavour, the honey from native hive bee, *Apis cerana japonica* fetches 5 times more price than honey from *Apis mellifera* (Sukai, 1989)

In the recent years, with the introduction of *Apis mellifera*, honey production is likely to increase tremendously and marketing aspect is getting more attention. There is a great demands for *Apis laboriosa* and *Apis florea* honeys. People from Korea, Japan and some western countries prefer specific quality honeys hunted from the nest of wild honeybees. Keeping this in view, it was felt necessary to evaluate the physico-chemical properties of honeys collected from different bee species and from different agro-ecozones of Nepal. The following were principal concern of this study;

- to identify the differences, if any, in the physico-chemical properties of honeys harvested from *Apis dorsata, A. cerana* and *A. mellifera* colonies in Chitwan district Nepal

- to identify the physico-chemical properties of *Apis cerana* honeys collected from tarai, hills, mountains and supermarket in Kathmandu (source is not known).

- to carry out the physico-chemical analysis of *Apis florea, A. laboriosa, Trigona* and *Melipona* honeys.

3.2.2 Review of the Literature on Physico-chemical Analysis of Asian Honeys

The amount of scientific information on Asiatic hive bee, *Apis cerana* in comparison to that on European honeybee, *Apis mellifera* is very meagre (Crane, 1993). However, Phadke (1962; 1967a; 1967b; 1968), Phadke et al. (1970; 1973) in India and Latif et al. (1956) in Pakistan made the extensive study on *Apis cerana* honeys. *Apis dorsata, Apis laboriosa* and *Apis florea* honeys are still largely unexplored in this respect. Some analytical studies made by different investigators in South East Asia are reviewed in this chapter.

Moisture Content : Shrestha (1997; 1998) measured moisture content of 300 honey samples collected from *Apis cerana* in Nepal, which ranged from 17 to 21% (average 18.57%). Kerkvliet et al. (1995) reported 17.2 and 22.5% moisture content for Neplaese honey. Olek et al (1987) reported water content of one *Apis cerana* honey sample from central Nepal as 17.3 % by hand refractometer and 15.8 % by vaccum oven. In Pakistan, the water content for *Apis cerana* honeys varied from 14.3-21.2 % with average of 16.4 % (Latif et al. (1956). Iwada et al.(1969) reported the moisture content of domestic honeys in Japan as 23 % and imported honeys as 21%. Aso et al.(1960) and Arai et al. (1960) reported moisture content in Japanese honeys as 20.5 % and 20.4 % respectively. In Taiwan honeys, the moisture content varied from 20-24% (Lin et al., 1977). A number of investigators in India have reported the moisture content in *A. cerana* honeys, as 20.9 $\pm$2 in samples from all over India (Phadke, 1967a), 17.2-19.1% from Mahabaleshwar (Phadke, 1967b), 16.2-22.1% (average 19.2%) in Madras honey (Giri, 1938), 25.2-28.4 % in Travancore honeys (Nair et al., 1950), 20.5 % in Calcutta honeys (Mitra and Mathew, 1968), 21.9-27.0 % (average 23.8%) in Assam honeys (Joshi and Sharma, 1998).

Olek et al., (1987) measured the moisture content of one *Apis laboriosa* honey sample from Nepal as 25.3 %. They suggested the higher water content in *Apis laboriosa* is due to the possible dilution by rain water. A number of investigators from India also reported the higher content of moisture in *Apis dorsata* honeys such as 19.0-27.1 % (average 23.5%) from Calcutta (Mitra and Mathew, 1968); 27.8 % from Travancore (Nair et al. 1950), and 18.9-24.2

% (average 20.9%) from all over the India (Phadke, 1968), 23.8-25.5% (average 24.6%) from Orang Wildlife Sanctury, Assam (Joshi and Sharma, 1998). Perti and Pandey (1967) reported average moisture content of summer and rainy season honeys from *Apis dorsata* colonies as 17.0 and 25.9 per cent. Jhansi et al. (1992) measured the moisture content of summer honey from tropical dry deciduous forest of Andhra Pradesh which ranged from 22-23 %. In Philippines, Laude et al. (1991) reported the significantly higher amount of moisture content in *A. dorsata* (23.1%) and *A. cerana* (22.0%) honeys than in *A. mellifera* (19.5%) honeys. Minh et al. (1971) measured the moisture content of 7 *A. dorsata* honey samples from Philippines, which ranged from 23.4-35.9 % with an average of 27.8 %. Latief et al. (1956) reported the average moisture content of 5 *A. dorsata* honey samples from Pakistan as 16.2 %. They also measured the water content of 5 *A. florea* honey samples as 17.4 %. While Nair et al. (1950) in India reported the moisture content of one *A florea* sample as 23.8 % and Phadke (1968) measured 5 honey samples which ranged from 16.0-17.5 %. with average of 16.5 %. Wakhle and Desai (1991) also measured water content of 8 *A. cerana* samples and 2 each from *A. dorsata, A. florea* and *Trigona* samples and reported maximum water content in *A. cerana* honeys (25.5 %) followed by *A. dorsata* (24 %), *A. florea* (23.5 %) and *Trigona* (22.8 %).

Electrical Conductivity and pH : Electrical conductivity measurement is the quick and easy method used to determine the origin of honey; whether nectar or honeydew honey. pH value is measured to check the acidity of honey. The electrical conductivity of Nepalese honey ranged between 0.36 mS/cm to 1.06 mS/cm and pH value varied from 4.17 to 5.5 (Shrestha, 1998). Kerkvliet et al. (1995) measured the pH and electrical conductivity of 8 honey samples from Nepal, which had 3.2-3.9 pH and 0.4-0.7mS/cm electrical conductivity. Olek et al. (1987) have also reported the pH value of 4.49 for *A. cerana* and 5.57 for *A. laboriosa*. Generally it was found that higher the electrical conductivity higher is the pH value (Joshi et al., 1998). Mahajan (1984) found significant positive correlation of electrical conductivity with most of the minerals such as potassium, magnesium, iron and manganese content. Laude et al. (1991) measured the electrical conductivity and pH for *A. dorsata, A. cerana* and *A. mellifera* honeys from Philippines, which averaged respectively, as 4.18, 3.98 and 4.28 pH and 1.02, 0.38 and 1.15 mS/cm electrical conductivity.

Sugar Spectrum : Sugars constitute by far the largest portion of dry matter in honey and are responsible for such physical properties as viscosity, hygroscopicity and granulation. Shrestha (1998) measured total reducing sugars of *A. cerana* honey samples in Beekeeping Shop, Kathmandu, which ranged between 58.1-74.18 per cent and sucrose content was below 5 per cent. However, she didn't mention the average value of reducing sugars and proportion of fructose and glucose. Kerkvliet et al. (1995) reported 23.1-41.3% glucose, 20.3-37.2% fructose and 0-32.7% sucrose for 8 honey samples; 4 each collected from tarai and Kathmandu valley. Olek et al. (1987) investigated two Nepalese honey samples; one each from *A. cerana* and *A. laboriosa*, which had sugar percentages as follows; 36.05% fructose, 28.65% glucose, 5.71% maltose, 3.34% sucrose and 0.41% higher sugars for *A. cerana* honey and 40.25% fructose, 34.01% glucose, 6.14% maltose, 2.51% sucrose and 0.36% higher sugars for *Apis laboriosa* honey.

Latif et al.(1956) investigated samples of Pakistan honeys, which had sugar percentages as: reducing sugars (69.8-76.9), fructose (39.0-53.9), glucose (27.1-34.2) and sucrose (1.09-2.75) for *A. cerana*; 69.2% reducing sugars, 42.2% fructose, 27.0% glucose and 1.43% sucrose for *A. dorsata*; and 62.9% reducing sugars, 40.4% fructose, 28.3% glucose and 1.8% sucrose for *A. florea*. Phadke (1967a) carried out such analyses on Indian honeys and reported that total reducing sugars averaged 70.2± 2.9, fructose 36.5±2.5, glucose 33.4± 2.8 for *A. cerana*; 34.6 to 39.9% fructose and 29.8 to 33.8% glucose for *A. dorsata*; and 36.9-41.3% fructose and 28.2-35.6% glucose for *A. florea*. Nair et al. (1950) reported the sugar spectrum of honeys from Travancore, India as; 28.9% fructose, 33.3% glucose and 5.98% sucrose for *A. cerana*; 62.0% reducing sugars, 26.7% fructose, 35.3% glucose and 2.41% sucrose for *A. dorsata*; and 67.9% reducig sugars, 33.6% fructose, 34.2% glucose and 0.6% sucrose for *A. florea* honey.

According to Mallick (1958) reducing sugars in Indian honeys varied from 53.9 to 78.4%, and 5 samples, possibly from sugar producing area, had more than 10% sucrose. Kalimi and Shonie (1964) confirmed the increase of higher sugars and decrease of monosaccharides during honey storage at 28 to 30°C for six to twelve months. These authors also reported that the total sugar content of unifloral honeys varied from 74.7-80% and reducing sugars from 72-78% of the total. Other authors also analysed honey samples for their sugar contents from Calcutta (Mitra and Mathew, 1968), Madras (Giri, 1938) and Mahabaleshwar (Phadke1962) and showed regional differences in sugar percentages. Laude et al. (1991) from Philippines

reported 31.4% fructose, 30.5% glucose and 3.59% sucrose for *A. dorsata* honeys, 26.9% fructose, 27.2% glucose and 9.51% sucrose for *A. cerana* honeys and 34.4% fructose, 28.7% glucose and 1.97% sucrose for *A. mellifera* honeys.

Enzymes: Enzymes play vital role in the conversion of nectar into honey. These are highly sensitive to heat and light and therefore, measured to detect the heat and storage damage of honey. Glucose oxidase and diastase value for Nepalese honey ranged between 0-25 µg/g/hr at 20°C and 5-22 respectively (Shrestha, 1998). Wakhle et al (1983) studied the enzyme activities of Indian honeys. Wakhle and Desai (1991) estimated the antibacterial activities of some Indian honeys. Laude et al. (1991) reported invertase activity as 45.8 U/kg for *A. dorsata*, 4.4 U/kg for *A. cerana* and 38.0 U/kg for *A. mellifera* honeys.

Mineral Contents (ash): Various characteristics of honey are influenced to some extent by its mineral composition. Ash represents the amount of dry matter present in honey. Latif et al. (1956) showed that the ash content in samples of Pakistan honey ranged from 0.11 to 0.32%. In Indian honeys, the ash contents averaged 0.19± 0.05 (Phadke, 1967a). According to Mahajan (1984), white or light coloured honey samples of *A. cerana* from Shimla hills showed lower ash content (0.06-0.9%), whereas, darker coloured honey samples were higher (0.33-0.76%). The total ash content in *A. dorsata* honeys from the Philippines, Pakistan and India averaged 0.17, 0.26 and 0.39%, respectively (Minh et al., 1971; Latif et al., 1956; and Phadke, 1968).

3.2.3 Materials and Methods

Honey Samples Details

Moisture content, pH and electrical conductivity were measured for 416 honey samples. Invertase, proline and glucose oxidase were measured for some 250 representative samples and sugar spectrum were measured only for 170 samples. The area of sample collection are shown in figure 1.

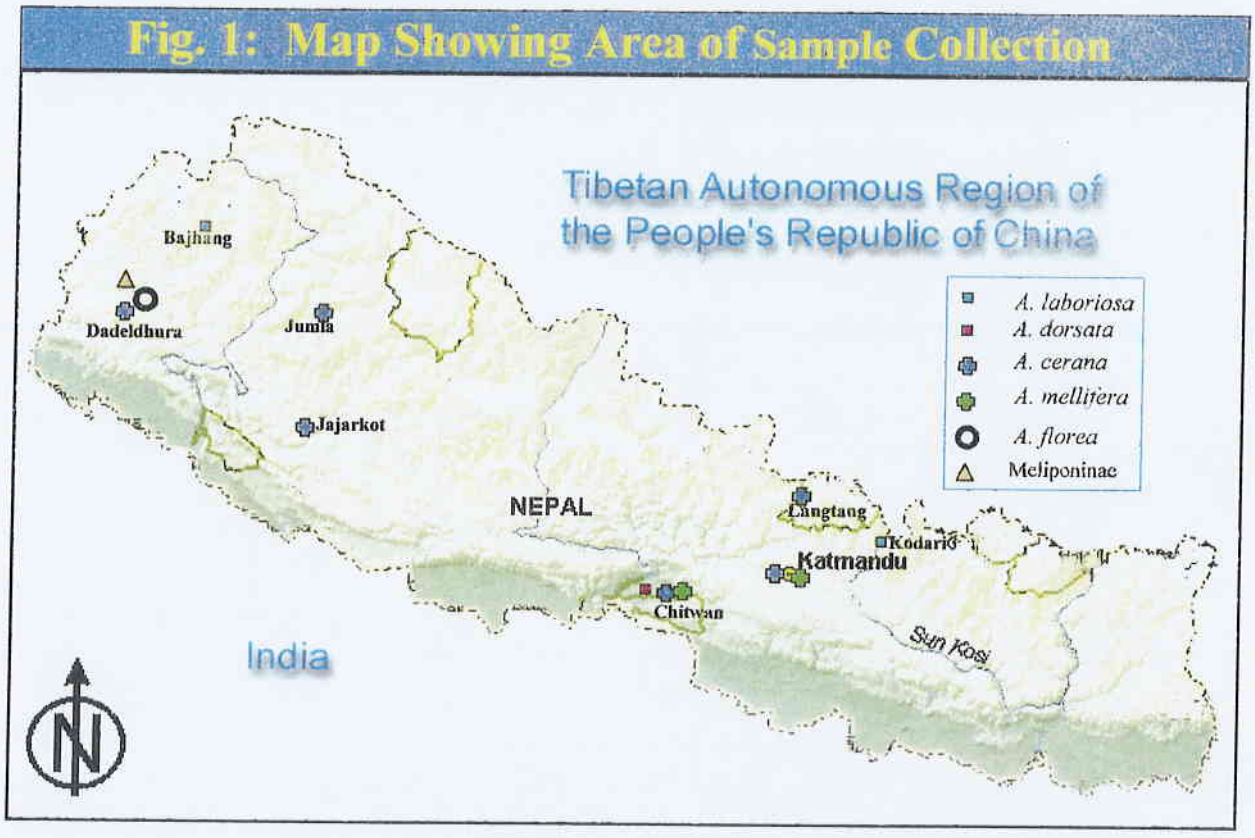


Fig. 1: Map Showing Area of Sample Collection

Based on the bee species and mode of honey harvesting , the samples are categorized into four classes;

a. Honey samples were collected from different bee species at the same day and from the same floristic region of Chitwan district, Nepal. Total 81 honey samples ; 28 samples from *Apis dorsata*, 26 from *Apis cerana* and 27 from *Apis mellifera* were collected directly from the colonies by cutting a small piece of combs and kept in deep freezer within a week. Therefore, there was minimal influence of operational factors and samples were quite

comparable. Samples were analysed at the same laboratory conditions in Institute fur Bienenkunde, Lunz am See and Beekeeping Institute, Celle, Germany.

b. *Apis cerana* honey samples were collected from the beekeepers of different agro-ecozones such as Chitwan, Jajarkot, Kathmandu, Dadeldhura, Jumla and Langtang areas during different months of the year. Based on their altitudinal ranges samples were aggregated in simpler classification of tarai, hills and mountains. The honey samples collected, from Chitwan district were grouped under tarai (<500masl), samples from Jajarkot, Dadeldhura and Kathmandu were aggrigated in hills (500-2000masl) and samples from Jumla and Langtang were placed under mountains (2000-3000masl). The fourth category comprised the honey samples collected from the supermarket in Kathmandu. In these samples the operational factors are unquantified.

c. *Apis florea* and *Trigona/Melipona* honey samples were collected from the lower warmer belt of the western Nepal. The altitudinal range of these samples is similar to that of category `a` but the honey harvesting time was not the same. These samples are also comparable with category `a`.

d. *Apis laboriosa* honey samples were collected from Bajhang district and Kodari Highway. These two areas have different floristic composition which may influence the composition of honey.

Determination of Moisture Content, pH and Electrical Conductivity

The moisture content, pH and electrical conductivity were measured according to the harmonized methods of European Honey Commission published by Bogdanov et al. (1997). The moisture content was measured by hand refractometer Atago 818117 especially designed for honey with necessary correlations and adjustments. The results of moisture content were expressed in percentage The pH was measured in solution containing 10g of honey in 75 ml of distilled water. WTW model 91 pH meter accurate to 0.01 units was used. Electrical conductivity was measured in solution containing 20% dry matter which was prepared by dissolving 10g of honey in 50 ml of distilled water. Where sample is not enough 1 in 5 w/v dilution was made (Vorwohl, 1964). All the measurements were taken by a radiometer conductimeter with a 1 centimetre radiometer electrode. Sample temperature was maintained at 20°C$\pm$ 0.1°C. The results were expressed in milli siemens per centimetre (mS/cm).

Determination of Invertase

Invertase activity was measured according to the original method of Siegenthaler (1977), also proposed by European Honey Commission (Bogdanov et al., 1997) and results were expressed in Siegenthaler unit, where one unit is defined as the number of micro molecules of substrate destroyed per minute. The pNPG (pNitrophenyl-α-D glucopyronoside) solution was used as a substrate for the determination of invertase. In principle, this pNPG is split into glucose and p-Nitrophenol by α-glucosidase (invertase). By adjusting the pH value to 9.5 the enzyme reaction is stopped and at the same time nitrophenol is transformed into the nitrophenolate anion, which corresponds to the amount of converted substrate and is determined photometrically.

Reagents :

The following reagents needed for the determination of invertase were prepared freshly;

1. Buffer Solution: prepared by dissolving 11.66g KH_2PO_4 (Potassium Hydrogen Phosphate) and 2.56g of Na_2HPO_4 .$2H_2O$ (Disodium Hydrogen Phosphate) in distilled water and diluted to 1 litre.
2. Substrate Solution: 6.0252g of pNPG (eg fluka) was dissolved in buffer solution by heating at 60°C and made up to 1 litre, it was then cooled and kept in a refrigerator in a dark bottle.
3. Reaction Terminating Solution: 363.42g of tris (hydroxymethyl) aminomethane was dissolved in water and diluted to 1 litre. pH was adjusted to 9.5 with 3m HCL.

Procedure

5g of honey was dissolved in buffer solution and diluted to 25 ml in a flask. 5 ml each of pNPG solution was pipette in two test tubes and kept in water bath for 5 minutes. Then 0.5 ml honey solution was added to a test tube, leaving one blank. The solution was briefly mixed in a mixer and incubated at 40°C. After exactly 20 minute, 0.5 ml of reaction terminating solution was added in both the tubes, and mixed thoroughly. The 0.5 ml honey solution was now added to the blank tube and then cooled at room temperature for 15 minutes. The

solution was measured in photometer. To confirm the results, one standard honey sample provided by Dr W. Von der Ohe of Beekeeping Institute, Celle was measured in each observation and compared with the reproducibility and repeatability proposed for invertase (Bogdanov et al., 1997).

Determination of Proline

The proline content was determined according to the methods of European Honey Commission (Bogdanov et al., 1997) based on the original method of Ough (1969). The results were expressed in ppm or mg per kg honey. The proline content is determined from the ratio. In principle, proline and ninhydrin form a complex. After adding 2-propanol, the extinction of the sample solution at a wavelength maximum is determined. The ratio between proline reference solution and honey solution is determined photometrically.

Reagents : The following reagents were needed for the determination of proline;

1. Formic acid HCOOH, 98-100%
2. Ninhydrin Solution: 1.5 gram ninhydrin dissolved in Ethylene Glycomonomethyl Ether and filled up to the mark of 50 ml in a volumetric flask.
3. Proline Reference Solution : 40 mg proline was dissolved in distilled water and filled up to 50 ml. Then 1 ml of this solution was pipette into 25 ml volumetric flask and filled with distilled water to the mark.
4. Iso propanol, 50% by volume in water.

Procedure

5 gram honey dissolved in distilled water and diluted to 100 ml. 0.5 ml of this solution was pipette in one tube, 0.5 ml of distilled water into a second tube (blank tube), and 0.5 ml of proline reference solution into another 5 tubes. Then 1 ml of formic acid and 1 ml of ninhydrin solution were added to each tube. Tubes were tightly capped and shook vigorously and placed in boiling water-bath at 100 °C, immersing the tubes below the level of solution. After exactly 15 minutes, the tubes were transferred to a another water-bath at 70°C for 10 minutes. 5ml of iso propanol water solution was added to each tube, mixed briefly and

capped immediately. The solution was left to cool for 45 minutes at room temperature and then measured the absorbance at 510 nm. In each observation one standard honey sample was also measured to cross check the results.

Determination of Glucose Oxidase

Glucose Oxidase was measured according to the methods of Shepartz and Subers (1964). The results were expressed as μg H_2O_2/ g/min.

Reagents

1. Phosphate Buffer 0.2 M; pH 6.1: 3.56g Na_2HPO_4 (Disodium Hydrogen Phosphate) and 2.72g KH_2PO_4 (Potassium Hydrogen Phosphate)were dissolved with distilled water in each flask and made volume to 100 ml each. The Na_2HPO_4 solution then added to the KH_2PO_4 solution till the pH reached at 6.1.
2. 3.5 M D (+)- glucose: 69.3 gram glucose monohydrate dissolved by slight heating in phosphate buffer solution (PBS) and filled to 100 ml
3. O-Dianisidin (3,3 Dimethoxy Benzine): 87.5 mg of O-Dianisidine dissolved in 95% Ethanol and filled to the mark of 25 ml flask
4. Peroxidase Reagent: 0.1 ml of peroxidase dissolved in PBS (0.2 M; pH 6.1) to 25 ml flask.
5. Reaction Terminating Solution: Concentrated HCL (Hydrochloric acid):

Procedure

1.5 ml glucose, 1.8 ml PBS solution, 0.1 ml O-Dianisidin and 0.1 ml peroxidase were added to each tube, stirred thoroughly to make homogenous solution and kept in water-bath for 5 minutes at 37°C. 0.1 ml of sample solution (prepared by dissolving 5 gram of honey in PBS solution to 25 ml flask) was added to one tube leaving another blank. The solution were mixed briefly in a mixer and placed in a water-bath. After exactly 15 minutes, reaction terminating solution was added to both tubes. Then the honey solution was also added to the blank tubes and left to cool at room temperature. After 15 minutes the samples were measured in a photometer.

Determination of Sugar spectrum

Carbohydrate composition of honey and separated sugars (fructose, glucose, sucrose, maltose, dikojibiose and other higher sugars) were measured following the methods of German Institute for Norms (DIN 10758). 5g of honey was dissolved in HPLC water, 25 ml of methanol was added and filled with HPLC water up to the mark of 100 ml volumetric flask. Part of this solution filtered in milipore filter. This solution was then analysed by HPLC (NH_2-column, eluent 80% acetonitrile and 20% water, R1 detector) at Beekeeping Institute, Celle, Germany.

Statistical Analyses

The statistical analyses were carried out with the Computer Software programme 6.12; SAS (Statistical Analysis System) at the Department of Livestock Sciences, University of Agricultural Sciences, Vienna. Significance test was carried out by using Bouferroni-Holm Test. Pearsons correlation coefficients and t-test were also employed for some honey groups.

3.2.4 RESULTS

Physico-chemical Properties of Chitwan Honeys

Mean results and basic statistics obtained from the physico-chemical analysis of honey samples are summarized in table 3.1.

Moisture Content, pH and Electrical Conductivity

Moisture content was found to be significantly higher in *Apis dorsata* honeys than in *Apis cerana* and *Apis mellifera* honeys. The percentage of samples having more than 21% moisture content were recorded as 57% in *A. dorsata*, 34.62 % in *A. cerana* and 7.4% in *A mellifera (figure 2)*. The pH value was found to be higher in *A. dorsata* honeys but not significantly different among the three groups of honeys *(figure 3)*.

The electrical conductivity values were significantly different between all the three different groups of honeys. The highest amount of electrical conductivity was obtained in *A. dorsata* honeys which was followed by *A. cerana* and *A. mellifera* honeys, respectively. The percentage of samples with more than 0.7 mS/cm electrical conductivity were recorded as 39.3% in *A. dorsata*, 30.8% in *A. cerana* and 3.7% in *A. mellifera* honeys (*figure 4*).

Figure 2 Moisture Content of Chitwan Honeys

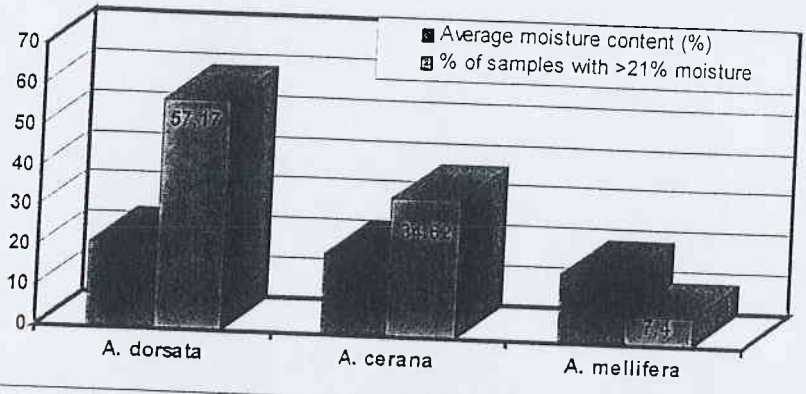


Figure 3 pH in Chitwan Honeys

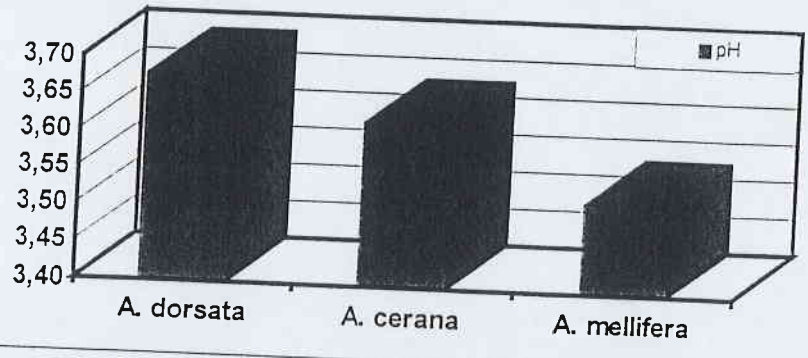
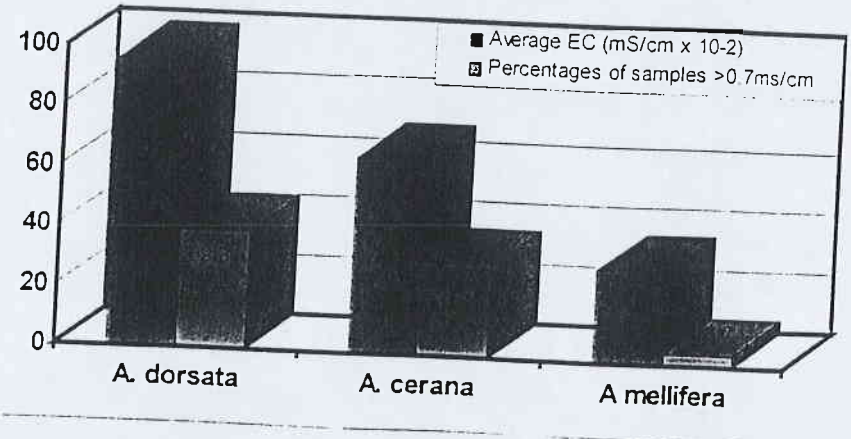


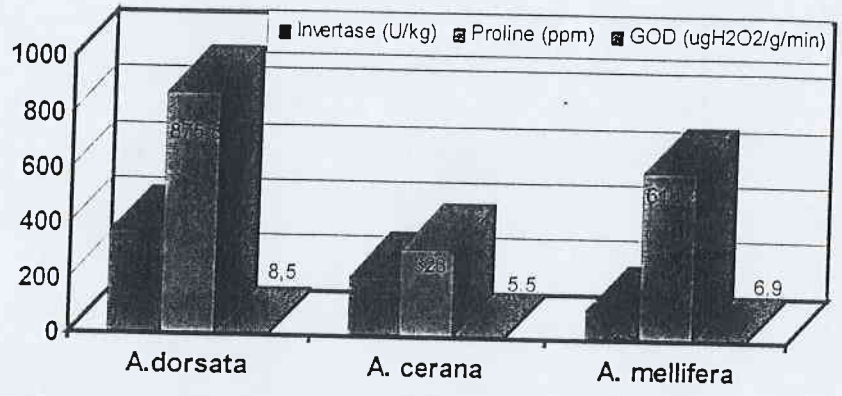
Figure 4 Electrical Conductivity of Chitwan honeys



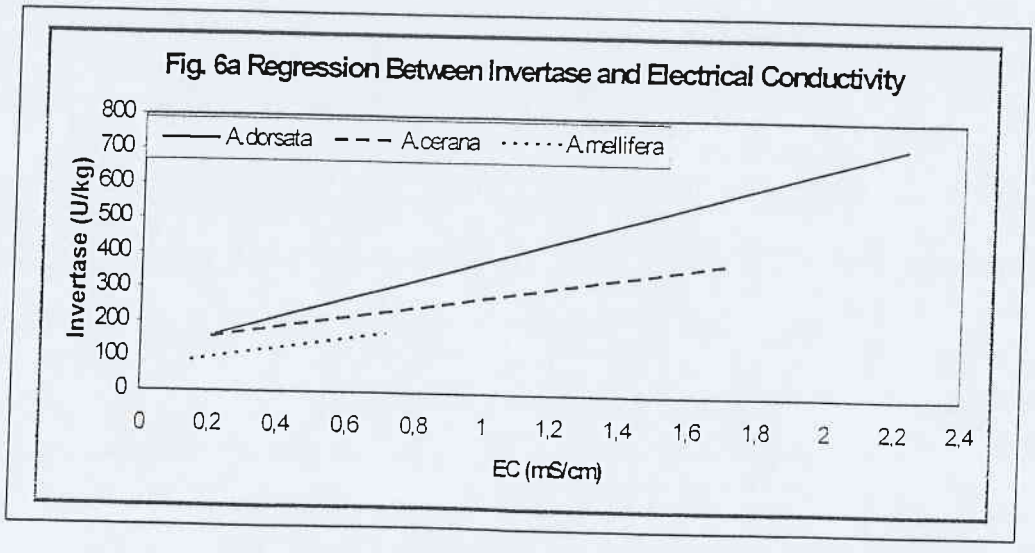
Invertase, Proline and Glucose Oxidase

Invertase and proline were significantly higher in *Apis dorsata* honeys than in *Apis cerana* and *Apis mellifera* honeys (*figure 5*). Proline content was significantly higher in *Apis mellifera* honeys than in *Apis cerana* honeys, where as reverse is true with invertase activity which was significantly higher in *Apis cerana* honeys than for *Apis mellifera* honeys. Glucose oxidase was found to be slightly higher in *Apis dorsata* honeys but not significantly different among other honeys (*figure 5*).

Figure 5 Invertase Activity, Proline and Glucose oxidase



There is a positive correlation between electrical conductivity, invertase activity, proline content and glucose oxidase (*table 3.2*). The higher the electrical conductivity higher is the enzyme invertase, proline and glucose oxidase (*figure 6 a,b,c & d*).



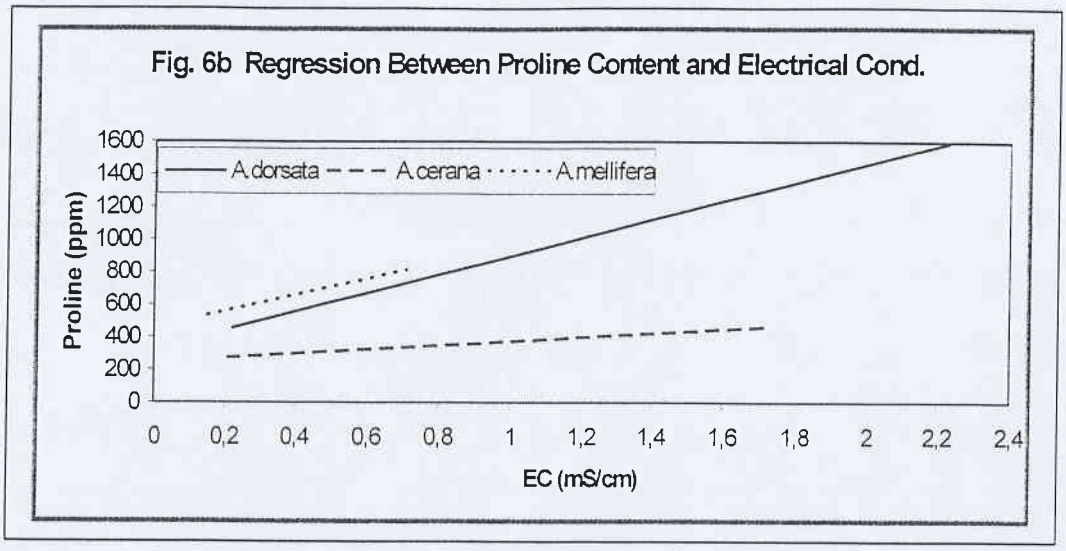


Fig. 6b Regression Between Proline Content and Electrical Cond.
A.dorsata
A.cerana
A.mellifera
Proline (ppm)
1600
1400
1200
1000
800
600
400
200
0
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
1,8
2
2,2
2,4
EC (mS/cm)

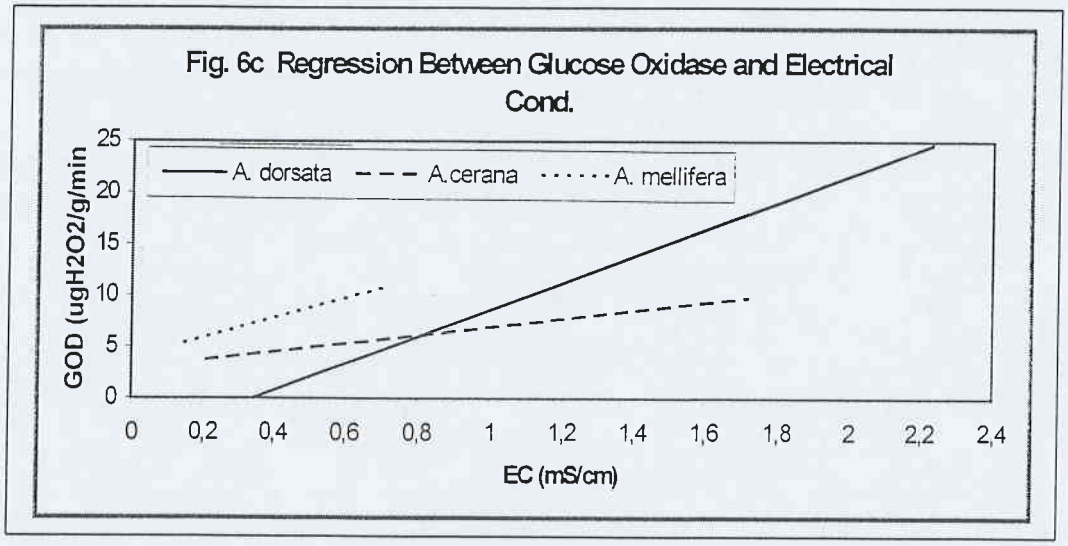


Fig. 6c Regression Between Glucose Oxidase and Electrical Cond.
A. dorsata
A.cerana
A. mellifera
GOD (ugH2O2/g/min
25
20
15
10
5
0
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
1,8
2
2,2
2,4
EC (mS/cm)

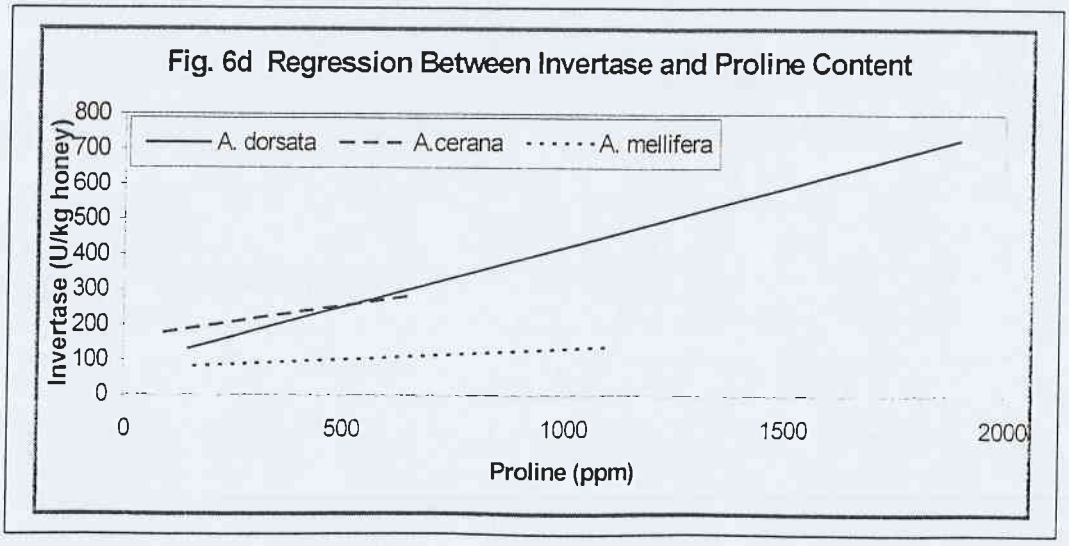


Fig. 6d Regression Between Invertase and Proline Content
A. dorsata
A.cerana
A. mellifera
Invertase (U/kg honey)
800
700
600
500
400
300
200
100
0
0
500
1000
1500
2000
Proline (ppm)

Sugar Spectrum

The statistical analysis of sugar spectrum of *A dorsata, A cerana* and *A mellifera* honeys shows the significant difference between various sugars of each honey group except fructose, glucose, and melezitose (*table 3.1, figure 7 & 8*). Total amount of sugars (sum of all identified sugars), sucrose and maltose are significantly higher in *Apis mellifera* than *Apis cerana* and *Apis dorsata* honeys. Oligosaccharides L_2 is significantly higher in *Apis dorsata* than *A. cerana* and *A. mellifera*. Maltotetraose was only recorded in *Apis dorsata* honeys where as melibiose was recorded in *A. cerana* and *A. mellifera* but not in *A dorsata*. There is positive correlatin between electrical conductivity and honeydew specific sugars such as trehalose, melezitose, raffinose, oligosaccharides L_2 (*table 3.3*). The regressions between electrical conductivity and oligosaccharide-L_2, a honeydew specific sugar is presented in figure 9.

Figure 7 Sum of Identified Sugars and % of Fructose & glucose

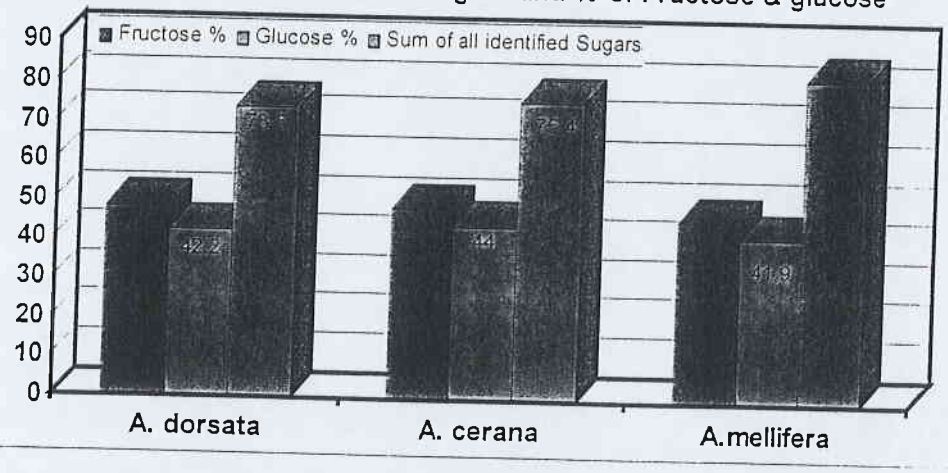
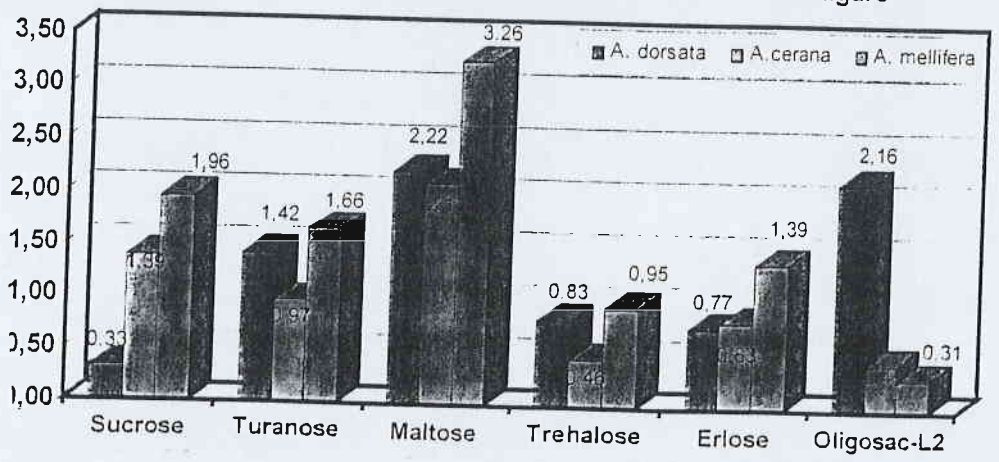
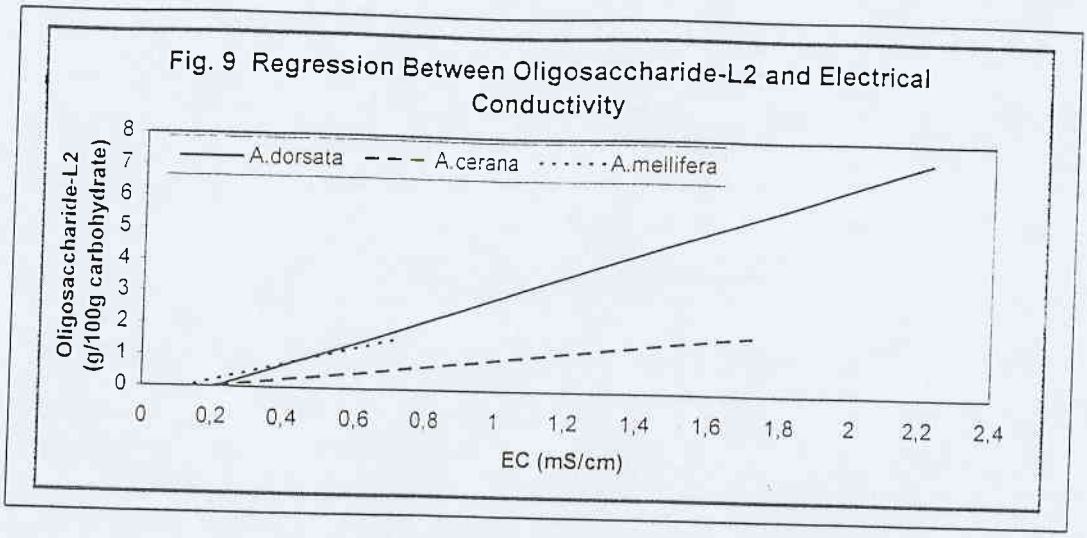


Figure 8 Perecenteges of Some Di- and Trisaccharide Sugars





The physico-chemical properties of honeys harvested in different years (1996, 1997, 1998) were also evaluated. The data for *A. dorsata, A. cerana* and *A. mellifera* honeys are presented respectively in table 3.1.1, 3.1.2 and 3.1.3. The analytical values obtained for *Apis dorsata* honey samples collected at the same time (23rd & 24th of March, 1997) but from two different nesting sites (trees) which were less than 500 m far from each other are presented in table 3.1.4.

Table 3.1 Comparison between physico-chemical properties of *Apis dorsata*, *A. cerana* and *A. mellifera* honeys collected from the same floristic region of Chitwan district, Nepal

Bee species ⇒	*Apis dorsata* (n = 28)		*Apis cerana* (n = 26)		*Apis mellifera* (n = 27)	
Parameters ⇓	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Moisture (%)	21.51^{a}	2.38	20.12^{b}	2.66	17.14^{c}	2.56
PH	3.68^{a}	0.36	3.62^{a}	0.4	3.52^{a}	0.32
EC (mS/cm)	0.96^{a}	0.75	0.65^{b}	0.45	0.0.31^{c}	0.14
Invertase (Siegenthaler U)	373.37^{a}	269.64	218.23^{b}	135.34	110.93^{c}	58.27
Proline (mg/kg)	875.82^{a}	497.07	323.0^{b}	169.52	610.19^{c}	220.11
Glucose Oxidase (μgH2O2/g/min)	8.51^{a}	10.96	5.51^{a}	4.78	6.92^{a}	3.58
Fructose	48.01^{a}	2.35	48.25^{a}	1.62	45.93^{b}	1.8
Glucose	42.23^{a}	4.94	44.02^{a}	4.54	41.95^{a}	2.53
Sucrose	0.33^{a}	0.29	1.39^{b}	1.71	1.96^{b}	1.93
Turanose	1.42^{a}	0.49	0.97^{b}	0.7	1.66^{a}	0.5
Maltose	2.22^{a}	0.73	2.09^{a}	0.86	3.26^{b}	0.61
Koji or Dikojibiose	1.11^{a}	0.47	0.81^{b}	0.52	1.48^{c}	0.41
Trehalose	0.83^{a}	0.39	0.46^{b}	0.44	0.95^{a}	0.27
Isomaltose	0.40^{a}	0.18	0.26^{b}	0.36	0.46^{a}	0.22
Melibiose	0.00^{a}	0.00	0.05^{ab}	0.15	0.12^{b}	0.19
Erlose	0.77^{a}	0.66	0.83^{a}	0.74	1.39^{b}	0.96
Melezitose	0.31^{a}	0.33	0.23^{a}	0.31	0.47^{a}	0.86
Maltotriose	0.01^{a}	0.02	0.16^{b}	0.31	0.04^{ab}	0.12
Raffinose	0.21^{a}	0.36	0.02^{b}	0.08	0.02^{b}	0.07
Oligosaccharide L_2	2.16^{a}	3.29	0.45^{b}	0.89	0.31^{b}	0.75
Maltotetraose	0.4^{a}	0.91	0.0^{b}	0.0	0.0^{b}	0.0
Total sugar g/100g honey	73.46^{a}	3.87	75.42^{a}	6.58	82.0^{b}	4.22
Fructose-Glucose ratio	1.15^{a}	0.13	1.11^{a}	0.13	1.1^{a}	0.05

Bouferroni-Holm Test, the different letters: a,b,c super-scribed after the means in a row denote the statistical significance between the analytical values obtained for three different honey groups (multiple $\alpha < 0.05\%$)

Table 3.2 Pearsons correlation coefficients between electrical conductivity, invertase activity, proline and glucose oxidase

(Total number of samples (n = 81); n=28 for *A. dorsata*, n=26 for *A. cerana* & n=27 for *A. mellifera*)

Variables	Invertase (Siegenthaler U/kg)				Proline (ppm)				Glucose Oxidase ($\mu gH_2O_2/g/min$)			
Bee Species	*A dor*	*A cer*	*A mel*	Over all	*A dor*	*A cer*	*A mel*	Over all	*A dor*	*A cer*	*A mel*	Over all
Electrical cond (mS/cm)	0.80 p=0.0001	0.50 p=0.0212	0.36 p=0.0660	0.80 p= 0.0001	0.85 p=0.0001	0.34 p=0.1449	0.32 p=0.1026	0.67 p=0.0001	0.87 p=0.0001	0.43 p=0.0782	0.38 p=0.0534	0.71 p=0.0001
Invertase (SiegenthalerU/kg)					0.64 p=0.0004	0.24 p=0.3115	0.22 p=0.2683	0.55 p=0.0001	0.76 p=0.0001	0.60 p=0.0104	0.67 p=0.0002	0.69 p=0.0001
Proline (ppm)									0.72 p=0.0001	0.59 p=0.0169	0.35 p=0.0780	0.64 p =0.0001

Table 3.3 Pearson correlations coefficients between electrical conductivity and honeydew specific sugars (ie trehalose, melezitose, and oligosaccharide L2) (Total number of samples (n = 81); n=28 for *A. dorsata*, n=26 for *A. cerana* & n=27 for *A. mellifera*)

Variables	Trehalose				Melezitose				Oligosaccharide L2			
Bee species	*A dor*	*A cer*	*A mel*	Over all	*A dor*	*A cer*	*A mel*	Over all	*A dor*	*A cer*	*A mel*	Over all
EC	0.88 p=0.0001	0.74 p=0.0002	0.27 p=0.1887	52 p=0.0001	0.78 p=0.0001	0.76 p=0.0001	0.26 p=0.2011	0.28 p=0.0158	0.94 p=0.0001	0.61 p=0.0041	0.61 p=0.0011	0.87 p=0.0001
Trehalose					0.77 p=0.0001	0.63 p=0.0032	0.43 p=0.0301	0.48 p=0.0001	0.84 p=0.0001	0.50 p=0.0264	0.19 p=0.3722	0.53 p=0.0001
Melezitose									0.78 p=0.0001	0.73 p=0.0027	0.57 p=0.0027	0.36 p=0.0017

A dor = Apis dorsata, A cer = Apis cerana, A mel= Apis mellifera, Over all =all 81 samples

Table 3.1.1 Comparison between physico-chemical properties of *Apis dorsata* honeys harvested in different year (i.e. 1996, 1997 and 1998) from the same floristic region of Chitwan district, Nepal

Year ⇒	1996 (n = 2)		1997 (n =20)		1998 (n =6)	
Parameters ⇓	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Moisture (%)	19.8 ^{a}	1.13	20.74 ^{a}	1.88	24.67 ^{b}	1.21
pH	3.26 ^{a}	0.37	3.84 ^{b}	0.29	3.31 ^{a}	0.05
EC (mS/cm)	0.48 ^{ab}	0.27	1.21 ^{b}	0.76	0.31 ^{a}	0.05
Invertase (Siegenthaler U)	113.83 ^{a}	44.23	403.2 ^{a}	299.0	370.37 ^{a}	170.28
Proline (mg/kg)	740.95 ^{ab}	743.66	1043.3 ^{a}	449.8	362.65 ^{b}	139.08
Glucose Oxidase (µgH2O2/g/min)	7.6 ^{a}	1.98	10.91 ^{a}	12.05	0.78 ^{a}	0.68
Fructose (g/100g CH_2O)	51.65 ^{a}	0.14	47.36 ^{b}	1.95	48.96 ^{ab}	2.68
Glucose ,,	45.38 ^{ab}	0.54	40.62 ^{a}	4.83	46.57 ^{b}	2.51
Sucrose ,,	0.04 ^{a}	0.06	0.4 ^{a}	0.3	0.20 ^{a}	0.18
Turanose ,,	0.66 ^{a}	0.19	1.66 ^{b}	0.23	0.89 ^{a}	0.55
Maltose ,,	1.11 ^{a}	0.07	2.50 ^{b}	0.58	1.65 ^{a}	0.62
Dikojibiose ,,	0.54 ^{a}	0.04	1.30 ^{b}	0.37	0.67 ^{a}	0.4
Trehalose ,,	0.37 ^{a}	0.03	1.00 ^{b}	0.31	0.43 ^{a}	0.25
Isomaltose ,,	0.17 ^{a}	0.03	0.46 ^{b}	0.15	0.26 ^{a}	0.16
Erlose ,,	0.10 ^{ab}	0.13	0.98 ^{a}	0.66	0.28 ^{b}	0.19
Melezitose ,,	0.00 ^{a}		0.41 ^{a}	0.34	0.09 ^{a}	0.15
Maltotriose ,,	0.00 ^{a}		0.01 ^{a}	0.03	0.00 ^{a}	
Raffinose ,,	0.00 ^{a}		0.29 ^{a}	0.4	0.00 ^{a}	
Oligosaccharide L_2 ,,	0.00 ^{a}		3.03 ^{a}	3.55	0.00 ^{a}	
Maltotetraose ,,	0.00 ^{a}		0.56 ^{a}	1.04	0.00 ^{a}	
Total sugar g/100g honey	71.46 ^{a}	8.06	73.54 ^{a}	3.97	74.64 ^{a}	5.79
Fructose-Glucose ratio	1.14 ^{a}	0.01	1.17 ^{a}	0.13	1.06 ^{a}	0.11

Bouferroni-Holm Test; the different letters; a.b.c super-scribed after the means in a row denote the statistical significance between the analytical values obtained for *Apis dorsata* honeys collected in year 1996, 1997 and 1998 (multiple $\alpha < 0.05$)

Table 3.1.2 Comparison between physico-chemical properties of *Apis cerana* honeys harvested in different year (i.e. 1996, 1997 and 1998) but from the same floristic region of Chitwan district, Nepal

Year ⇒	1996 (n = 2)		1997 (n = 14)		1998 (n = 10)	
Parameters ⇓	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Moisture (%)	17.5[a]	0.14	19.41[a]	1.99	21.65[a]	3.01
pH	3.35[a]	0.08	3.8[a]	0.34	3.42[a]	0.41
EC (mS/cm)	0.23[a]	0.03	0.78[a]	0.45	0.54[a]	0.43
Invertase (Siegenthaler U)	139.11[a]	0.86	254.62[a]	185.65	201.3[a]	83.88
Proline (mg/kg)	147.15[a]	39.53	316.11[a]	151.4	363.67[a]	184.16
Glucose Oxidase (μgH2O2/g/min)	1.47[a]	1.67	6.2[a]	4.37	5.78[a]	5.7
Fructose	46.49[a]	2.78	48.43[a]	1.73	48.47[a]	1.18
Glucose	49.12[a]	4.82	41.31[a]	3.92	45.59[a]	3.64
Sucrose	0.1[a]	0.14	1.77[a]	2.15	1.29[a]	1.32
Turanose	1.14[a]	0.23	1.26[a]	0.5	0.64[a]	0.83
Maltose	1.62[a]	0.86	2.59[a]	0.62	1.69[a]	0.87
Dikojibiose	0.73[a]	0.28	1.04[a]	0.24	0.60[a]	0.69
Trehalose	0.47[a]	0.21	0.59[a]	0.17	0.34[a]	0.62
Isomaltose	0.19[a]	0.08	0.28[a]	0.16	0.27[a]	0.52
Melibiose	0.00		0.04[a]	0.07	0.07[a]	0.22
Erlose	0.16[a]	0.23	1.26[a]	0.86	0.56[a]	0.39
Melezitose	0.00		0.43[a]	0.33	0.09[b]	0.21
Maltotriose	0.00		0.20[a]	0.25	0.15[a]	0.40
Raffinose	0.00		0.00		0.04[a]	0.13
Oligosaccharide L_2	0.00		0.79[a]	1.13	0.20[a]	0.61
Total sugar g/100g honey	79.76[a]	3.83	77.4[a]	5.62	72.47[a]	7.12
Fructose-Glucose ratio	0.96[a] 0.15		1.18[a]	0.13	1.07[a]	0.09

Bouferroni-Holm Test: the different letters; a,b,c super-scribed after the means in a row denote the statistical significance between the values of three honey groups (multiple $\alpha < 0.05$)

Table 3.1.3 Comparison between physico-chemical properties of *Apis mellifera* honeys harvested in different year (i.e. 1996, 1997 and 1998) but from the same floristic region of Chitwan district, Nepal

Year ⇒	1996 (n = 7)		1997 (n = 9)		1998 (n = 11)	
Parameters ⇓	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Moisture (%)	15.07[a]	2.63	15.64[a]	1.23	18.40[b]	2.82
pH	3.66[a]	0.26	3.68[a]	0.33	3.30[b]	0.22
EC (mS/cm)	0.31[a]	0.20	0.33[a]	0.16	0.29[a]	0.07
Invertase (Siegenthaler U)	87.21[ab]	43.57	85.76[a]	53.04	146.62[b]	55.43
Proline (mg/kg)	512.83[a]	153.05	755.37[a]	206.55	553.37[a]	220.51
Glucose Oxidase (μgH2O2/g/min)	4.13[a]	1.74	7.74[a]	3.75	8.14[a]	3.56
Fructose	47.04[a]	2.34	45.19[a]	1.29	45.81[a]	1.50
Glucose	42.25[ab]	1.2	40.14[a]	2.11	43.51[b]	2.67
Sucrose	1.05[a]	1.06	3.31[b]	2.44	1.33[a]	1.11
Turanose	1.98[a]	0.47	1.83[a]	0.35	1.24[b]	0.37
Maltose	3.2[a]	1.01	3.48[a]	0.37	3.10[a]	0.37
Dikojibiose	1.64[a]	0.33	1.46[a]	0.27	1.37[a]	0.56
Trehalose	0.97[a]	0.24	0.91[a]	0.17	0.98[a]	0.39
Isomaltose	0.52[a]	0.22	0.49[a]	0.17	0.40[a]	0.27
Melibiose	0.14[a]	0.17	0.17[a]	0.26	0.05[a]	0.08
Erlose	1.10[a]	0.85	0.18[b]	0.81	0.83[a]	0.65
Melezitose	0.14[a]	0.20	0.22[a]	0.18	0.99[a]	1.29
Maltotriose	0.00		0.09[a]	0.17	0.03[a]	0.08
Raffinose	0.00		0.06[a]	0.11	0.00	
Oligosaccharide L_2	0.00		0.49[a]	0.97	0.38[a]	0.79
Total sugar g/100g honey	84.41[a]	5.83	84.43[a]	1.70	81.26[a]	4.91
Fructose-Glucose ratio	1.11[a]	0.04	1.13[a]	0.03	1.05[b]	0.04

Bouferroni-Holm Test; the different letters; a,b,c super-scribed after the means in a row denote the statistical significance between the analytical values of three different honey groups (multiple $\alpha < 0.05$)

Table 3.1.4 Comparison between physico-chemical properties of *Apis dorsata* honeys harvested from two different nesting site (trees) at the same day and from the same locality of Chitwan, Nepal (Sugars are in gram per 100 gram carbohydrate)

Nesting site⇒	I Bombax tree (n =11)		II Narayani Hotel (n = 9)		Significance test
Parameters ⇓	Mean	SD (±)	Mean	SD (±)	Probability (pr=)
Moisture (%)	21.53[a]	1.59	19.78[b]	1.83	0.0344
PH	4.06[a]	0.19	3.57[b]	0.08	0.0001
EC (mS/cm)	1.79[a]	0.49	0.49[b]	0.09	0.0001
Invertase (Siegenthaler U)	678.16[a]	131.4	128.25[b]	51.85	0.0001
Proline (mg/kg)	1382[a]	303.9	628.58[b]	115.43	0.0001
Glucose Oxidase (μgH2O2/g/min)	18.4[a]	11.8	1.79[b]	1.04	0.0006
Fructose	47.11[a]	2.55	47.65[a]	0.82	0.5533
Glucose	36.87[a]	2.99	45.20[b]	1.16	0.0001
Sucrose	0.48[a]	0.32	0.29 a	0.25	0.1594
Isomaltose	0.55[a]	0.15	0.36[b]	0.08	0.0039
Other disaccharides	5.92[a]	0.43	4.91[a]	0.27	0.0797
Trehalose	1.21[a]	0.26	0.74[b]	0.07	0.0001
Erlose	1.27[a]	0.75	0.62[b]	0.26	0.0219
Melezitose	0.61[a]	0.32	0.15[b]	0.11	0.0007
Raffinose	0.52[a]	0.42	0.02[b]	0.05	0.0023
Oligosaccharide L_2	5.45[a]	3.09	0.06[b]	0.18	0.0001
Maltotetraose	1.01	1.25	0.00	0.00	0.0266
Total sugar g/100g honey	71.02[a]	3.16	76.63[b]	2.31	0.0003
Fructose-Glucose ratio	1.27[a]	0.08	1.05[b]	0.04	0.0001

t-test (the different letters; a and b super-scribed after the means in a row denote the statistical significance between the analytical values of two different honey groups (probability is in 3rd column, $P < 0.05$)

Physico-chemical Properties of *Apis cerana* and *Apis mellifera* Honeys Collected from Kathmandu

Comparative data for the physico-chemical properties of *Apis cerana* and *A. mellifera* honeys are given in table 3.4. The moisture content, pH, and electrical conductivity values were significantly higher in *A. cerana* honeys than in *A. mellifera* honeys (*figure 10*). The electrical conductivity value of honeys collected in the months of April, May and June was found to be significantly higher, respectively than July, August, September, October and November (*figure 11*). Proline content was significantly higher in *A. mellifera* than in *A. cerana*. Whereas the enzymes invertase and glucose oxidase were not significant between these two honey groups (*figure 12*).

Figure 10 Moisture Content, pH and Electrical Conductivity in *Apis cerana* and *A.mellifera* Honeys in Kathmandu, Nepal

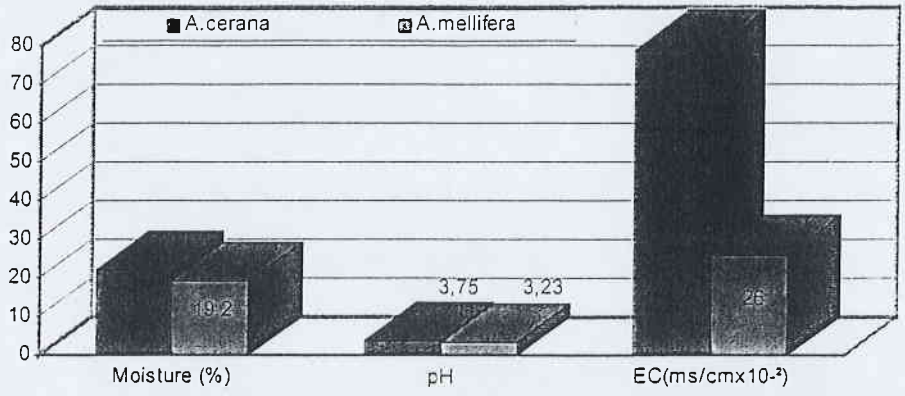
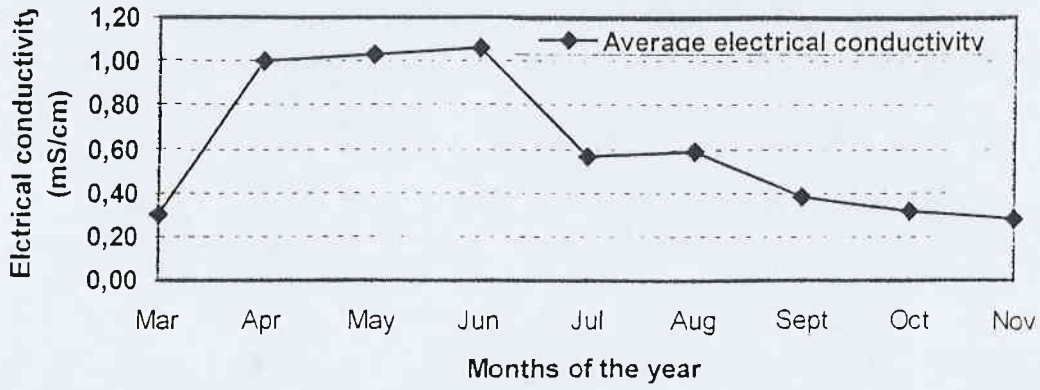


Figure 11 Electrical Conductivity of Honeys Harvested During Different Months of the Year in Kathmandu Valley



Percentages of fructose, glucose, sucrose and sum of all remaining di- and trisaccharides are given in figure 13. Except trehalose, melezitose and koji/dikojibiose, there was not significant difference between two groups of honeys (*table 3.4*).

Figure 12 Invertase, Proline and Glucose Oxidase in *Apis cerana* and *A. mellifera* Honeys

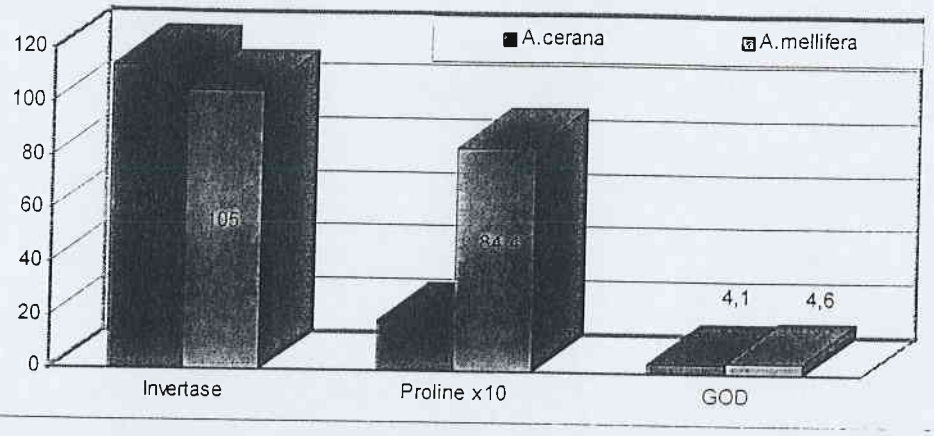


Figure 13 Percentages of Fructose, Glucose, Sucrose and other Sugars

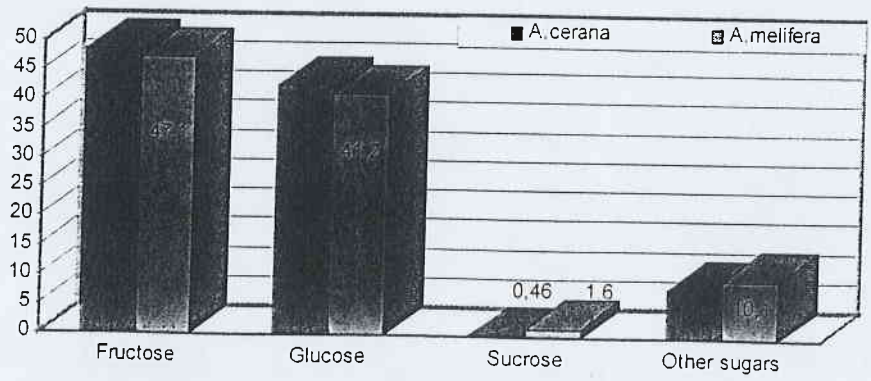


Table 3.4 Comparison between physico-chemical properties of *Apis cerana* and *Apis mellifera* honeys harvested from Kathmandu valley, Nepal (Sugars are in gram per 100 gram carbohydrate)

Bee Species ⇒	Apis cerana (n = 50)		Apis mellifera (n = 31)		Significance Test
Parameters ⇓	Mean	SD (±)	Mean	SD (±)	Probability (pr)
Moisture (%)	22.23 [a]	2.0	19.25 [b]	2.35	0.0001
PH	3.75 [a]	0.79	3.23 [b]	0.16	0.0005
EC (mS/cm)	0.79 [a]	0.54	0.26 [b]	0.06	0.0001
Invertase (Siegenthaler U)	114.08 [a]	68.33	105.23 [a]	77.31	0.6028
Proline (mg/kg)	189.97 [a]	136.45	843.89 [b]	266.28	0.0001
Glucose Oxidase (μgH2O2/g/min)	4.11 [a]	4.77	4.59 [a]	5.85	0.8156
Fructose	48.53 [a]	2.72	47.14 [a]	1.99	0.1005
Glucose	42.51 [a]	3.43	41.17 [a]	4.1	0.3202
Sucrose	0.46 [a]	0.69	1.59 [a]	2.5	0.1011
Turanose	1.65 [a]	0.63	2.07 [a]	0.86	0.1322
Maltose	2.41 [a]	0.95	3.1 [a]	1.2	0.0813
Dikojibiose	1.12 [a]	0.44	1.7 [b]	0.78	0.0164
Trehalose	0.79 [a]	0.35	1.27 [b]	0.7	0.0216
Isomaltose	0.72 [a]	0.53	0.88 [a]	0.72	0.4675
Melibiose	0.07 [a]	0.15	0.0 [a]		0.0676
Erlose	1.29 [a]	1.51	1.05 [a]	1.22	0.6148
Melezitose	0.29 [a]	0.45	0.04 [b]	0.11	0.0282
Raffinose	0.15	0.57	0.0		0.2802
Total sugar g/100g honey	77.45 [a]	4.22	73.89 [a]	2.46	0.0862
Fructose-Glucose ratio	1.15 [a]	0.11	1.15 [a]	0.12	0.8594

t-test (the different letters; a & b super-scribed after the means in a row denote the statistical significance between the values of two honey groups ($P < 0.05$)

Physicochemical Properties of *Apis florea*, *A. laboriosa* *Trigona* and *Melipona* honeys

Table 3.5 shows the analytical data (mean values ± SD) for physico-chemical properties of *Apis florea*, *A. laboriosa*, *Trigona* and *Melipona* honeys. *Trigona* and *Melipona* bees could not be distinguished by the farmers during honey harvesting. The honey samples of these two

species were distinguished after sugar analysis. The sugar spectrum of four of their honey samples contains extremely high amount of disaccharides and, hence, these samples kept under *Trigona spp* and other as *Melipona sp.* Moisture content in all the samples exceeded the maximum permissible level (21%) of European Honey commission. Significant test was not carried out for these samples because the number of samples were not enough and the honey operational factors (way of honey treatment) were not the same. However, pH and electrical conductivity showed some characteristic differences (*figure 14*). The comparison for invertase, proline and glucose oxidase was made between different bee species of genus *Apis* (*figure 15*). The data for *A. dorsata, A. cerana and A. mellifera* were drawn from Chitwan honeys. Sugar spectra stand as major criteria to distinguish *Melipona* and *Trigona* honeys (*figure 16*).

Figure 14 pH and EC in *Apis florea, A.laboriosa, Melipona* & *Trigona* Honeys

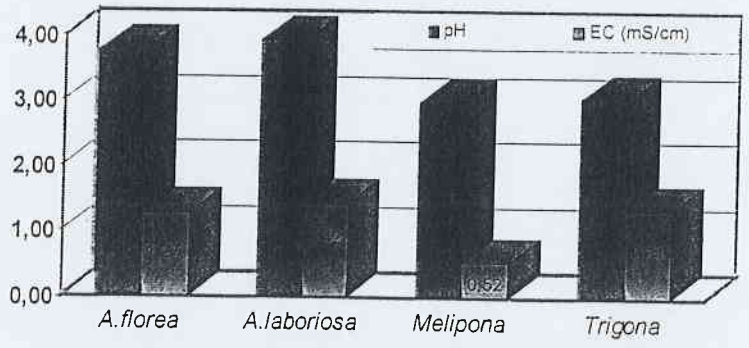


Figure 15 Invertase, Proline and God in Honeys from Different Bee Species of Genus Apis

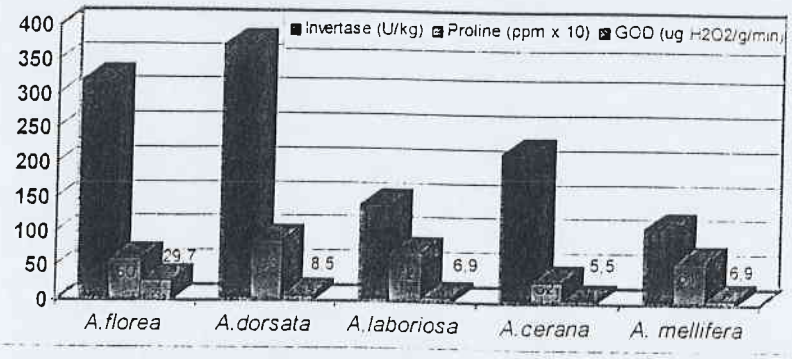
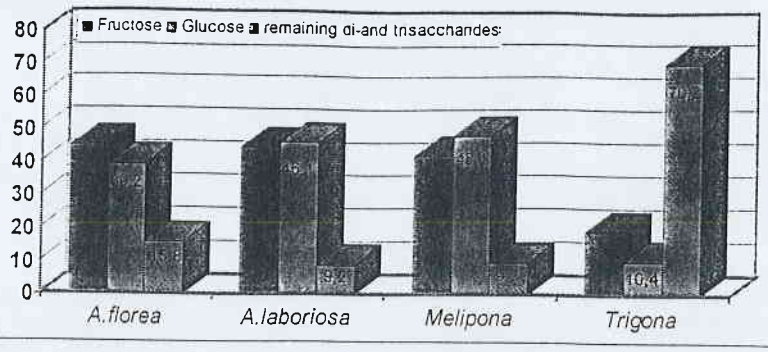


Table 3.5 Physico-chemical properties of *Apis florea, Melipona, Trigona* and *A. laboriosa* honeys collected from different places in Nepal

Bee Species ⇒	Apis florea (n=10)		Melipona (n=1)		Trigona sp (n=4)		A. laboriosa (n=2)	
Parameters ⇓	Mean	SD (±)	Mean	SD (±)	Mean (±)	SD	Mean	SD (±)
Moisture (%)	23.42	2.74	>26		>26		24.0	2.26
pH	3.78	0.2	2.98		3.05	0.09	3.93	0.38
EC (mS/cm)	1.27	0.51	0.52		1.34	0.03	1.42	0.47
Invertase (Siegenthaler U)	320.56	155.39	-		-		140.7	125.16
Proline (mg/kg)	596.0	189.48	-		-		720.0	578.41
Glucose Oxidase (µgH2O2/g/min)	29.67	16.9	-				6.87	9.67
Fructose	45.03	9.64	42.21		19.44	3.2	44.74	8.28
Glucose	39.19	9.54	48.1		10.4	1.96	46.09	4.49
Sucrose	0.01	0.04	0.0		0.08	0.16	0.0	
Turanose	2.06	0.67	0.0		1.3	0.66	1.75	0.81
Maltose	2.59[a]	0.69	0.0		2.07	0.64	2.9	1.17
Dikojibiose	6.02	13.03	9.05		62.75	2.91	1.83	0.95
Trehalose	1.14	0.66	0		0.14	0.28	1.18	0.44
Isomaltose	1.38	1.71	0.64		2.28	2.1	1.17	0.94
Melibiose	0.03	0.08	0.0		0.0		0.05	0.07
Erlose	0.04	0.13	0.0		1.12	1.05	0.22	0.30
Melezitose	2.02	4.08	0.0		0.18	0.21	0.1	0.13
Raffinose	0.07	0.15	0.0		0.18	0.22	0.0	
Oligosaccharide L_2	0.52	0.72	0.0		0.0		0.0	
Sum of these sugar (g/100g honey)	70.19	10.44	67.72		73.13	3.13	68.44	3.09
Fructose-Glucose ratio	1.17	0.13	0.88		1.87	0.10	0.99	0.28

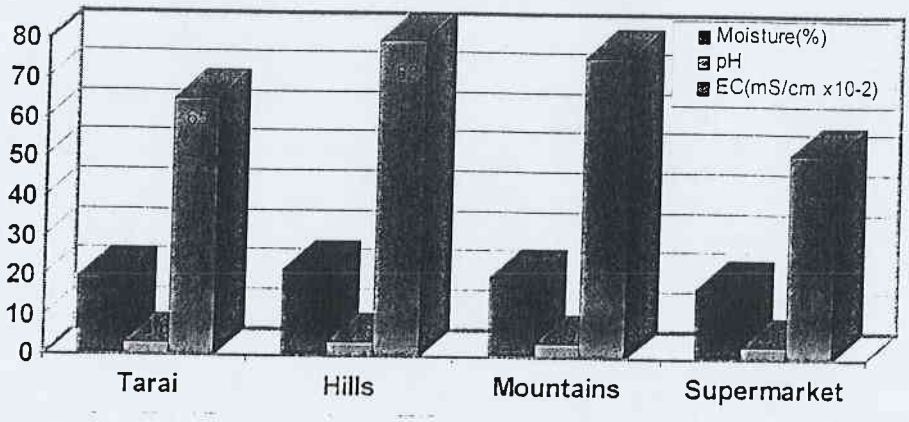
Figure 16 Percentages of Fructose, Glucose and Other Sugars in *Apis florea, A.laboriosa, Melipona* and *Trigona* Honeys



Physico-chemical Properties of *Apis cerana* Honeys Harvested from Different Ecozones and Commercial Beekeepers' Honeys from supermarket in Kathmandu, Nepal

Means and basic statistics obtained for *Apis cerana* honeys collected from different altitudes and commercial beekeepers' honeys from Kathmandu supermarket are presented in table 3.6. The moisture content was significantly higher in honeys collected from hills, respectively than from the mountains, tarai and supermarket (*figure 17*). More than 50% honey samples from hills and mountains exceeded the maximum allowable level of moisture content. In tarai, the percentage of honey samples with more than 21% moisture content were 34.62%. Whereas all the honey samples from Kathmandu supermarket were within a range of maximum permissible level of moisture content (21%). The pH value and electrical conductivity were not significantly different among the different groups of honeys (*table 3.6, figure 17*).

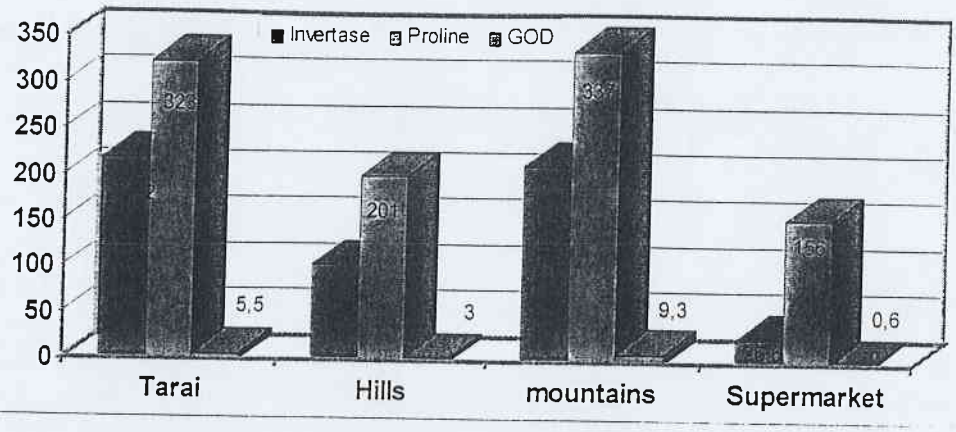
Figure 17 Moisture, pH and EC in *Apis cerana* Honeys Collected from Different Altitudes and Supermarket



The enzyme invertase and proline were significantly lower in commercial beekeepers honeys than in other three group of honeys (*figure 18*). Glucose oxidase was significantly higher in

mounatains, respectively than in tarai, hills, and supermarket honeys (*figure 18*). The HMF was measured in four representative samples collected from supermarket which was found to be (9.9, 30.1, 84.1 and 800 mg/kg honey).

Figure 18 Invertase, Proline and GOD in *Apis cerana* Honeys Collected from Different Altitudes and Supermarket



The percentage of fructose, glucose, sucrose and sum of other di-and trisaccharides are presented in figure 19. Fructose-glucose ratio and sugar isomaltose were significantly higher and sucrose content was significantly lower in hills and mountain than tarai and supermarket honeys. Some other sugars are also significantly different among different honey groups (*table 3.6*).

Figure 19 Percentages of Fructose, Glucose, Sucrose and Other Di- and Trisaccharides in *Apis cerana* Honeys

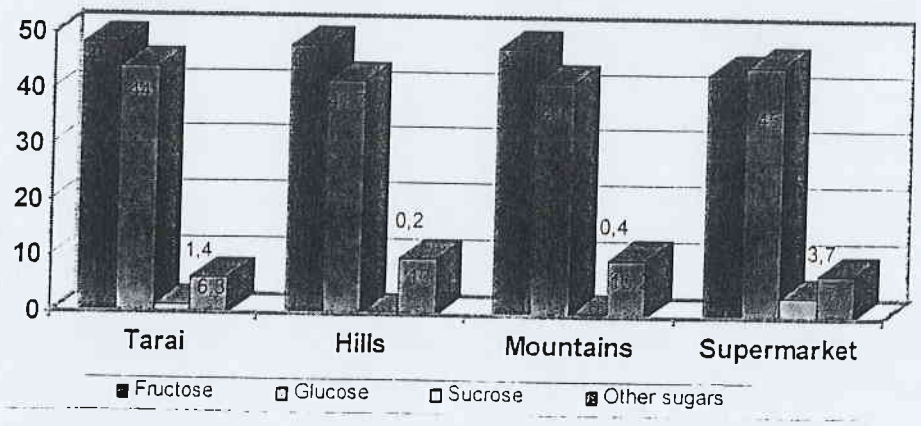


Table 3.6 Comparison between physico-chemical properties of *Apis cerana* honeys collected from the different agro-ecozones and from super market of Nepal (sugars are in gram per 100 gram carbohydrate)

Agro-ecozones (altitudes)	Tarai (n = 26)* (<500 masl)		Hills (n = 85)* (500-2000masl)		Mountains (n = 123)* (2000-3000masl)		Super market (n = 11)* (not mentioned)	
Parameters	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)	Mean	SD (±)
Moisture (%)	20.12^{ac}	2.66	22.19^{b}	2.53	20.35^{c}	2.54	18.13^{a}	2.04
pH	3.62^{a}	0.4	3.79^{a}	0.76	3.93^{a}	0.55	3.59^{a}	0.40
EC (mS/cm)	0.65^{a}	0.45	0.80^{a}	0.46	0.76^{a}	0.30	0.52^{a}	0.27
Invertase (Siegenthaler U)	218.23^{a}	135.34	103.4^{b}	70.67	213.14^{a}	101.86	26.59^{c}	31.01
Proline (mg/kg)	323.0^{a}	169.52	201.1^{bc}	119.26	336.79^{a}	154.52	155.81^{c}	98.2
Glucose Oxidase µg H2O2 /g/min	5.51^{a}	4.78	2.99^{a}	4.47	9.3^{b}	6.6	0.55^{a}	0.99
Fructose	48.25^{a}	1.62	48.07^{a}	2.28	47.71^{a}	1.8	43.62^{b}	3.94
Glucose	44.02^{a}	4.54	41.72^{a}	4.81	41.69^{a}	2.81	44.97^{a}	5.83
Sucrose	1.39^{ab}	1.71	0.23^{a}	0.52	0.37^{a}	1.05	3.66^{b}	8.28
Turanose	0.97^{a}	0.7	2.05^{a}	0.96	1.89^{a}	0.55	1.58^{a}	1.00
Maltose	2.09^{a}	0.86	3.02^{a}	1.47	2.48^{a}	1.03	2.02^{a}	1.37
Dikojibiose	0.81^{a}	0.52	1.60^{b}	0.99	1.89^{b}	0.96	1.16^{ab}	0.92
Trehalose	0.46^{a}	0.44	1.05^{a}	0.65	1.44^{a}	0.78	1.09^{a}	0.52
Isomaltose	0.26^{a}	0.36	1.14^{b}	1.18	1.46^{b}	0.93	0.87^{ab}	0.66
Melibiose	0.05^{a}	0.15	0.05^{a}	0.11	0.16^{a}	0.47	0.21^{a}	0.33
Erlose	0.83^{a}	0.74	0.79^{a}	1.17	0.52^{a}	0.89	0.38^{a}	0.49
Melezitose	0.23^{a}	0.31	0.21^{a}	0.33	0.13^{a}	0.36	0.31^{a}	0.49
Maltotriose	0.16^{a}	0.31	0.01^{a}	0.04	0.22^{a}	0.5	0.04^{a}	0.09
Raffinose	0.02^{a}	0.08	0.07^{a}	0.39	0.02^{a}	0.11	0.12^{a}	0.18
OligosaccharideL_2	0.45^{a}	0.89	0.0^{b}		0.02^{b}	0.09	0.0^{ab}	
Total sugar (g/100g honey)	75.42^{a}	6.58	74.31^{a}	5.00	78.65^{a}	7.27	81.17^{a}	2.62
F-G ratio	1.11^{ab}	0.13	1.17^{b}	0.14	1.15^{b}	0.07	0.98^{a}	0.13

Bouferroni-Holm Test (the different letters; a,b,c super-scribed after the means in a row denote the statistical significance between the analytical values of four different honey groups (multiple $\alpha < 0.05$)

* see section 3.2.3 for number of samples.

3.2.5 DISCUSSION

Apis dorsata, Apis cerana and *Apis mellifera* honeys from Chitwan district

Most of the analytical data obtained for *Apis dorsata, Apis cerana* and *Apis mellifera* honeys are within the well ranges of quality criteria proposed by European Honey Commission (Bogdanov et al, 1997). However, the moisture content in the majority of *A. dorsata* honey samples (*figure 1*) exceeded the maximum permissible level (21%) for honey. A number of investigators from India have also reported the higher moisture content for *A. dorsata* honey; as 20.9 (18.9-24.2) per cent by Phadke (1967), 25.9% for rainy season honeys by Perti and Pandey (1967), 22-23% by Jhansi et al. (1992), 21.0% by Malakar (1997). Laude et al. (1991) from Philippines also reported 23.1% moisture content for *A. dorsata* honeys. The presence of higher moisture content in *A. dorsata* suggest the possible dilution of honey by rain water or atmospheric humidity as the honey samples were collected directly from the combs. Latif et al.(1954), however, reported lower moisture content (16.2%) for *A. dorsata* honeys from Pakistan.

The moisture content in *A. cerana* honeys was also found to be significantly higher than *A. mellifera*. Aso et al (1960), Arae et al (1960) and Iwada et al (1969) in Japan, Phadke (1967), Nair et al (1950), Mitra and Mathew (1968) in India, Linh et al (1977) in Taiwan, Laude et al. (1991) in Philippines also reported more than 20% (on average) moisture content for *A. cerana* honeys. It is generally considered that the samples having more than 21% moisture content are not safe from the fermentation, but the presence of very high invertase activity seems to be sufficient to prevent the fermentation in *A. dorsata* and *A. cerana* honeys. It is already proved that an enzyme secreted by the bees keeps the raw materials safe while they are being converted into honey in the nest or hive (Crane, 1990).

The pH values did not show significant differences between *Apis dorsata, Apis cerana* and *Apis mellifera* honeys. The values obtained for Chitwan honeys agree with the results obtained by Malakar (1997) for the same type of honeys in West Bengal and are slightly lower than the ones obtained by Shrestha (1998) and Olek et al.(1987) for *A. cerana* honeys.

The electrical conductivity was found to be significantly higher in *A. dorsata* honeys, respectively than *A. cerana* and *A. mellifera* honeys. The data obtained for electrical conductivity (also sugars and pollen spectrum) suggest that *A. dorsata* and *A. cerana* bees collect more honeydew than *A. mellifera* bees. About 40% of *A. dorsata* and 30% of *A. cerana* honey samples had more than 0.7mS/cm electrical conductivity; the limit of electrical conductivity which has been proposed for honeydew honey than (<0.7mS/cm) for nectar or blossom honey (Bogdanov et al , 1997). The honey samples collected from the same nesting site or from the same apiary but in different years (1996, 1997 and 1998) did not have the same moisture content, pH and conductivity values which suggest that the different climatic conditions and differences in honey flow prevailing in three different years also have influence in these analytical values. The presence of higher amount of electrical conductivity in 1997 suggest the better honeydew flow of the year.

Invertase and proline were significantly higher in *Apis dorsata* honeys than for *Apis cerana* and *Apis mellifera* honeys. Whereas, proline content was significantly lower but the invertase activity was significantly higher in *Apis cerana* honeys than for *Apis mellifera* honeys. Glucose oxidase was found to be slightly higher in *Apis dorsata* honeys but not significantly different among other honeys. The enzyme invertase was found to be extremely high (1430 Siegenthaler Unit/kg honey) in one *A. dorsata* honey sample which was mixed with brood extract. This sample, however, excluded for statistical analysis suggest that the enzyme invertase can be increased by adding brood extract in honeys.

Five honey samples from *Apis cerana* were found to contain proline near to the 140-200 ppm; the indicator level for honey adulteration (White & Rudyj, 1978; Talpay, 1985; Von der Ohe et al, 1991; Dustmann, 1993). Since samples were collected directly from the combs and sugar spectrum did not show any adulteration or sugar feeding (i. e. the sucrose content was <5%), the indicator level of proline content for *A. cerana* honey should be lower than *A. mellifera* honey. Whereas the proline content of all *A. mellifera* honey samples was above the indicator level, ranged between 343-1118 (average 610) ppm. White and Rudyj (1978) reported the mean for 740 *A. mellifera* honey samples as 503 ppm. Thrasyvoulou & Manikis (1995) also reported the proline content of 80 Greek honey samples as 526 ppm, ranged between 264 to 1205 ppm.

However, Laude et al (1991) found no significant differences in the physicochemical properties of *Apis dorsata*, *Apis cerana* and *Apis mellifera* honeys from Philippines. They suggested that the electrical conductivity, invertase activity and other properties of honeys are influenced by different methods of beekeeping or treatment of honey by beekeepers, and not due to the different usage of honey sources by bee species. In the present study, samples were collected directly from the colonies by cutting a small piece of sealed comb at the same time and from the same floristic region. The operational factors or methods of treatment of honey were also the same. Therefore, the differences in their physico-chemical properties found in the present study were mainly attributed to the different usage of honey sources by honeybee species (either because of their different foraging preferences or varying amount of glandular secretion). It is already reported that the dance language to communicate food location varies genetically among honeybee population and species (Punchihewa et al., 1985; Rinderer and Beamann, 1995)

As reported by various investigators all of the glucose oxidase, and the major part of the proline and invertase in honey originate in secretion from the hypopharyngeal glands of the honeybees (Arnold & Delage-Darchen, 1978; Dustmann, 1967; Edelhauser & Bergner, 1987; Von der Ohe et al 1991; Von der Ohe, 1994). According to White (1967; 1975; 1978), the origin of invertase is mainly attributed to the bee. The nectar or/and honeydew collected is mixed with secretions from the salivary and hypopharyngeal glands of the bees. The amount of added enzymes depends on various factors such as age, diet and physiological stage of the bees, strength of the colony, pollen consumption, temperature, etc (Brouwers, 1982; 1983, Simpson, 1968, Persano Oddo et al., 1999). The concentration of the enzymes and proline also depends up on the period of time; how long and how intensively bees work in making honey (Von der Ohe, 1996). The quantity of glandular secretion may also vary from species to species. Being bigger in size, within a same period of time *A. dorsata* may add more glandular secretion in their honeys. It is also possible that the *A. dorsata* honey may possesses some other enzymes e.g. phosphatase which could not be detected by the substrate used. Though, the Siegenthaler method used for the determination of invertase activity is widely accepted and recommended by the European Honey Commission, it is not able to detect some other enzymes from invertase (Von der Ohe, 1999 personal communication). Honey may also contain enzymes of plant origin from nectar or honeydew, and possibly from pollen (Crane, 1990). Persano Oddo et al. (1999) suggested that all honeys with a high enzyme content are

produced in summer when brood rearing is less intensive and foragers are predominant; especially, honeydew contributes various enzymes, mainly invertase, from the gut and saliva of the insect producing it (Maurizio, 1975).

Interestingly, the *Apis dorsata* honey samples collected from the two different nesting site (trees) but at the same time (23 and 24 April, 1997) in Bharatpur, Chitwan district which are less than 500m far from each other, had significantly different amount of electrical conductivity and enzyme activity. Samples collected from *Bombax* tree nearby Narayani Hotel had lower enzyme activity than the samples collected from another nesting site. On the other hand the enzyme activity, electrical conductivity and other analytical values obtained in honey samples collected from the same tree but from different nests are more or less in the same range. Whether there exist a special communication system between different colonies nesting in the same tree is the subject of future investigation.

Within each bee species there was a positive correlation between electrical conductivity, proline content and enzyme activity. The higher the electrical conductivity higher is the enzyme invertase, proline and glucose oxidase (*figure 4a,b,c*). Therefore, virtually the honeydew honey has higher content of proline and enzyme activity than nectar or blossom honey. As suggested by earlier investigators proline serves to regulate the secretion of enzyme invertase into nectar during its conversion to honey (Crane, 1990). The positive correlation between proline and invertase found during present investigation supports their theory. Von der Ohe et al.(1991) also found strong positive correlation ($r = 0.96$) between invertase and proline. However, neither of these correlation of coefficient values were found for *A. dorsata* and *A. cerana* honeys in the literature consulted.

Results of sugar analysis were similar in glucose and fructose content to those obtained by Phadke (1967) for *Apis dorsata* and *Apis cerana* honeys in India (*table 3.5*). The proportion of fructose and glucose and sum of reducing sugars were also found to be the same as those compiled by Crane (1990) for *A. dorsata*, *A. cerana* and *A. mellifera* honeys (*table 3.5*). Some di- and trisaccharides are characteristically different from species to species. For example, oligosaccharide-L_2 was found to be significantly higher in *A. dorsata* honeys. This oligosaccharide-L_2 was recorded only in the samples which had more than 0.7 mS/cm electrical conductivity. That means this sugar must be originated from the honeydew (Von

der Ohe & Von der Ohe, 1996). The statistical analysis of honeydew specific sugars such as trehalose, raffinose, oligosaccharides-L_2 showed a positive correlation with electrical conductivity (*table 3.6*). The coefficient of correlation (r) with electrical conductivity were found as 0.52 for trehalose, 0.72 for raffinose and 0.88 for oligosaccharide-L_2. Pechhacker (1990) found significant positive correlation (r=0.97) between electrical conductivity and sugar content originating from honeydew in *A. mellifera*. In the present study oigosaccharide-L_2 stands as honeydew originating sugars (*figure 7*).

Table 3.5a Comparison between analytical data obtained for *Apis dorsata* honeys & earlier report (average sugar value g/100 g honey)

Author and Country	No. of Samples	Moisture	Total reducing sugars (TRS)	Fructose	Glucose	Sucrose	F/G
Minh et al (1971) Philippines	7	27.8	59.6	30.7	29.0	1.5	1.06
Laude et al (1991) Philippines	5	23.1±2.3	-	31.4±5.3	30.5±2.7	3.6±3.9	1.02
Latif et al. (1956) Pakistan	5	16.2	69.2	42.2	27.0	1.43	1.56
Mitra & Mathew (1968) Calcutta, India	14	23.5 (19.0-27.1)	69.9 (65.7-75.9)	35.0 (30.5-37.5)	35.0 (31.7-39.2)	0.27	1.0
Nair et al. (1950) Travancore, India	1	27.8	62.0	26.7	35.3	2.41	0.76
Phadke (1968) India	20	20.9 (18.9-24.2)	69.5	37.4 (34.6-39.9)	32.1 (29.8-33.8)	-	1.17
Malakar (1997) West Bengal, India	1	21.0	79.0 *(TS)	35.0	36.0	-	0.97
Present study	**28**	**21.5±2.38**	**73.46±3.8*(TS)**	**35.3±2.35**	**31.02±4.9**	**0.24**	**1.15**

* (TS) = Total Sugars, sugars are in gram per 100 gram honey

Table 3.5b Comparison between analytical data obtained for *Apis cerana* honeys & earlier report (average sugar value g/100 g honey)

Author and Country	No. of Samples	Moisture	Total reducing sugars (TRS)	Fructose	Glucose	Sucrose	F/G
Laude et al (1991) Philippines	9	22.0±3.7	-	26.9±3.9	27.2±3.1	9.5±4.1	0.99
Latif et al. (1956) Pakistan	5	16.4	73.5	42.8	32.5	2.12	1.32
Mitra & Mathew (1968) Calcutta, India	30	20.5 (17.5-24.0)	72.3 (66.9-77.4)	38.9 (33.3-42.9)	33.9 (28.8-37.7)	1.3	1.15
Nair et al. (1950) Travancore, India	1	25.2	-	28.9	33.3	5.98	0.87
Phadke (1967a), India	80	20.9±2.0	70.2±2.9	36.5±2.5	33.4±2.8	-	1.1
Phadke (1967b) Mahabaleshwor, India	8	17.2-19.1		35.2-43.3	31.2-38.3	-	1.13
Malakar (1997), West Bengal, India	1	20.0	78.0 (TS)	37.04	35.6	-	1.03
Olek et al.(1987), Nepal	1	17.3	83.27 (TS)	40.25	34.01	-	1.24
Present study	**26**	**20.1±2.66**	**75.4±6.58* (TS)**	**36.39±1.62**	**33.2±4.5**	**2.51**	**1.11**

* (TS) = Total Sugars, sugars are in gram per 100 gram honey

Table 3.5c Comparison between analytical data obtained for *A. mellifera* honeys & earlier report (average sugar value g/100 g carbohydrate)

Author/Country	S. N.	Moisture	TRS or F+G	Fructose	Glucose	Sucrose	Maltose Isomalt	Rest	F/G
A. mellifera									
White et al , 1962 USA	490	17.2	87.3 F+G	48.0	39.3	1.3	9.2	1.9	1.22
Maurizio, 1959 Swizerland	74	-	97.3 F+G	50.8	46.5	-	-	-	1.09
Tourn et al., 1980 Italy	1	-	67.4 F+G	42.9	24.5	1.6	-	-	1.75
Austin, 1958 Canada	39	-	89.4 F+G	47.8	41.6	7.6	-	1.6	1.15
Minh et al (1971) Philippines	1	20.7	64.7 TRS	29.4 *	35.3*	1.27	-	-	0.83
Laude et al (1991) Philippines	27	19.5±1.6	-	34.4±3.7*	28.7±4*	1.97±2	-	-	1.2
Malakar (1997) India	1	21.0	76.0 TRS	32.0*	34.0*	-	-	-	0.94
Present study	**27**	**17.1±2.6**	**87.9 F+G**	**46±1.8**	**42±2.5**	**1.96±2**	**5.3**	**4.4**	**1.1**

*Sugars in gram per 100 gram honey, TRS = total reducing sugars, F+G = sum of fructose and glucose, rest sugars = Erlose,Turanose, Oligosaccharides

Apis cerana and *Apis mellifera* honeys from Kathmandu valley

Except moisture content for *A. cerana* honeys, the analytical data obtained for honey samples from Kathmandu valley are all within the ranges of quality criteria of European Honey Commission (Bogdanov et al., 1997). *A. cerana* honeys contained higher moisture content than the maximum allowable level of moisture content (21%) for honeys (Bogdanov et al, 1997, CAC, 1989). Nair et al.(1950) and Mallick (1958) reported moisture content in Indian honeys as 25.2-28.4 and 16.6-26.4 per cent, respectively. Lin et al.(1977) reported 20-24 per cent moisture content for Taiwan honeys. Aso et al (1960) and Arae et al.(1960) reported moisture content of *A. cerana* honeys in Japan as 20.5 and 20.5% , respectively. These results also suggest that *A. cerana* honey naturally contain more moisture content than *Apis mellifera* honeys.

The pH and electrical conductivity were significantly higher in *Apis cerana* honey than in *Apis mellifera* honeys which agree with the results obtained by Shrestha (1998) for the same types of honeys. As in Chitwan district, in Kathmandu valley also *A. cerana* bees collect more honeydew than *Apis mellifera* bees. The electrical conductivity measurement of honeys harvested from different months of the year show that the honeydew flow in Kathmandu begins in April and lasts in July. The electrical conductivity of honey was found to be increasing from April to June and decreasing from July onwards (*figure 9*).

Enzyme invertase was found to be relatively higher in *A. cerana* honeys than *A. mellifera* honeys but not significant unlike in Chitwan honeys. Since *A. cerana* honey samples were collected in 1995 and exposed to light and slight heat (40-50°C) while preparing honey pollen microscopic slides in Kathmandu, they might have lost some enzyme activity. The proline content in *A. cerana* honey was significantly lower than *A. mellifera* honey which reconfirm the findings from Chitwan honeys. *A. cerana* are naturally poor in proline content than *A. mellifera* and, therefore, there is a possibility to distinguish *A. cerana* honeys from *A. mellifera* honeys by determining the invertase activity and proline content of honeys.

Glucose oxidase, like in Chitwan honeys, showed no significant differences in *A. cerana* and *A. mellifera* honeys. Sugar spectrum are more or less the same with those mentioned in table 5. Some honeydew specific sugars such as melezitose and raffinose were significantly higher in *A. cerana* honeys than *A. mellifera*. Erlose was also relatively high in *A. cerana* but not significant. However, other honeydew specific sugar such as oligosaccharide-L_2 was not detected in both the honey types.

Apis florea, Apis laboriosa, Trigona and *Melipona* honeys

The majority of *Apis florea, A. laboriosa, Trigona* and *Melipona* honey samples exceeded the maximum permissible level of moisture content for honeys. The moisture content reported by earlier investigators for same types of honeys were also spreaded in a large range (*table 3.6*). However, unlike in *A. dorsata, A. cerana* and *A mellifera* honeys, the present values recorded for *A. florea, A. laboriosa, Trigona* and *Melipona* honeys could not represent the moisture content of honey in natural stage. Because the number of samples were not adequate and the operational factors were not the same for all honeys.

Apis florea honey

Apis florea honey samples collected from the lower warmer belt (<500masl) of the country are quite comparable with those samples collected from Chitwan district. Therefore, the Bouferroni-Holm test was also carried out between various parameters of *A. dorsata, A. cerana, A. mellifera* and *A.florea* honeys which showed that the concentration of enzyme glucose oxidase is significantly higher in *A. florea* honeys than other three honey groups. In Nepal, *A. florea* honey has traditionally been used to cure several diseases and considered as superior quality. The presence of higher amount of glucose oxidase (29.67ugH2O2/g/min), which is responsible for the antibiotic activity of honey (White et al., 1963; Wachle & Desai, 1991; Molan, 1992; Postmes, 1995), supports the traditional concept concerning its high medicinal properties. The pH values did not show significant differences between *Apis dorsata, Apis cerana, Apis mellifera* and *A. florea* honeys. The electrical conductivity was found to be significantly higher in *A. florea* honeys, respectively than *A. dorsata, A. cerana* and *A. mellifera* honeys. The presence of higher amount of di-and trisaccharides including oligosaccharide-L_2 suggest that *A. florea* also collect a lot of honeydew.

Sugar analysis of *A. florea* honey gave results that were similar to those obtained by earlier investigators (*table 3.6*). It was found that the *A. florea* honey contained 84.2% monosaccharides (fructose+glucose) and the remaining (15.78%) comprised turanose, maltose, dikojibiose and other higher sugars.

Apis laboriosa honey

Analytical data obtained for *A. laboriosa* honey show more or less similar characters with those obtained for *A. dorsata* honeys (*table 3.5 & 3.6*). Sugar spectrum are slightly different from those obtained by Olek et al. (1987) for one *A. laboriosa* honey sample (*table 3.6*). In the present study, the sum of all identified sugars was found to be slightly lower and percentage of monosaccharide (90.83% of total sugars) is slightly higher than the values obtained by Olek et al. (1987). However, it would be unwise to deduce any conclusions with one or two honey samples.

Trigona and *Melipona* honey

Analytical data obtained for *Trigona* and *Melipona* honey samples are characteristically different from each other (*table 3.6*). The conductivity of the *Trigona* honeys (1.34mS/cm) was very high compared to *Melipona* honey (0.52 mS/cm). Bogdanov et al. (1996) found significantly higher ($P < 0.01$) conductivity for *Trigona* or non *Melipona* honeys (1.04-1.07 mS/cm) than that of the *Melipona* honeys (0.32-0.44 mS/cm). *Melipona* honeys had fructose and glucose as predominating sugars and account for 90.31% of total carbohydrates, the remainder is dikojibiose and isomaltose. Which are similar to those of honeybee honey sugars. In honeybee honey sugar, the monosaccharide fructose and glucose are the dominant fraction and account for 85-95% of the total sugars, the remaining being represented by a number of different di- and trisaccharides (Doner, 1977; Bogdanov and Baumann, 1988, see *table 5 & 6*). On the other hand, the *Trigona* honey samples had a completely different sugar spectrum (Bogdanov et al., 1996). There the main sugar is a disaccharide dikojibiose which accounts for 62.7% of total carbohydrates. Fructose and glucose represent only 30.3% of total carbohydrate. Turanose, maltose, erlose, melezitose and raffinose were detected only from *Trigona* honeys. Bogdanov et al. (1996) also reported significantly higher amount ($P<0.01$) of maltose, turanose, trehalose and erlose in *Trigona* honeys than that of *Melipona* honeys. The presence of disaccharides as a principal sugar in honey, thus, should be the identity of *Trigona* honeys.

Whether the high electrical conductivity and higher percentages of di- and trisaccharide sugars in *Trigona* honeys is due to the plant origin of honeys (may be some honeydew ?) or/and some specific enzymatic transformation of nectar by the *Trigona* species, should be the subject of future investigation (Bogdanov et al., 1996). There could be more transglucosidase or fructosidase effect by the enzymes of their saliva which can build the di- and trisaccharides (Von der Ohe-personal communication).

Table 3.6 Comparison between present analytical data and earlier report (average sugar values in g/100 honey)

Author & Country	No.	Moisture	Sum of sugars	Fructose	Glucose	Sucrose	Others!	F/G
A. florea								
Latif et al. (1956) Pakistan	5	17.4	62.9 ⊖	40.4	28.3	1.8	-	1.42
Nair et al. (1950) Taravancoe, India	1	23.8	67.9 ⊖	33.6	34.2	0.6	-	0.99
Phadke (1968) India	5	16.5	71.2 ⊕	38.9	32.3	-	-	1.21
Present study	**10**	**23.4±2.7**	**70.19**	**31.61**	**27.51**	**0.0**	**11.1**	**1.17**
A. laboriosa								
Olek et al. (1987)	1	25.3	74.15	36.05	28.65	3.34	6.12	1.26
Present study	**2**	**24**	**68.44**	**30.49**	**31.61**	**0.0**	**6.33**	**0.96**
Melipona								
Crane, 1990 (compiled f 1975)	-	-	-	49	44-46	-	-	1.08
Bogdanov et al., 1996, Venezuela*	14	-	74.7	34.8	36.9	0.1	2.9	0.94
Present study	**1**	**>26**	**67.72**	**28.59**	**32.57**	**0.0**	**6.53**	**0.88**
Trigona								
Nair et al. (1950) Taravancoe, India	1	29.1	-	28.5	33.3	2.37	-	0.86
Phadke (1968) India	5	24.1	-	32.3	20.1	-	-	1.58
Crane, 1990 (compiled f 1975)	-	-	-	28-35	14-33	0-2	-	1.1-2
Bogdanov et al., 1996 Venezuela*	8	-	76.0	24.4	18.1	0.2	33.4	1.35
Present study	**4**	**>26**	**74.12**	**15.09**	**8.05**	**0.18**	**50.8**	**1.87**

* Values for *Melipona favosa* and *Trigona* (Frieseomelitta aff varia) extracted from Bogdanov et al.(1996)
⊖ = TRS (Total reducing sugars)
! = sum of remaining di-and trisaccharide sugars

Apis cerana honeys from different altitudes

Moisture content of *Apis cerana* honeys varied from sample to sample and spread in a very large range from 14% to 26%. A number of investigators from other parts of the south east Asia also reported a very high range of moisture content for *A. cerana* honeys (Aso et al., 1960; Arae et al., 1960; Iwada et al., 1969; Phadke, 1967, Nair et al., 1950; Mitra and

Mathew, 1968; Linh et al., 1977). The moisture content of commercial beekeepers honeys is within the range (<21%) of European Honey Commission (Bogdanov et al., 1997). More than 50% honey samples collected from hills exceeded the maximum allowable limit of moisture content for honeys. Moisture content of tarai and mountain honeys lies in between hills and commercial beekeepers honeys. The pH and conductivity values are relatively higher in honeys collected from hills and mountains than tarai and commercial beekeepers honeys but not significantly different. Because the number of samples measured for conductivity were very irregular; 123 samples from mountains, 85 from hills, 26 from tarai and 11 from commercial beekeepers. Generally, it is found that the higher the altitude higher is the electrical conductivity of honeys (Joshi et al.,1998).

Enzyme invertase and proline content of honeys were found to be significantly higher in tarai and mountains than for hills and commercial beekeepers honeys. Invertase activity of honeys collected from hills was significantly higher than commercial beekeepers but interestingly, proline was not significantly different among these two honey groups. Except two, all the honey samples collected from commercial beekeepers did not meet the minimum level of invertase (50 Siegenthaler Unit) proposed for good quality honey (Dustmann, 1993). Some of these samples were also found to be completely overheated (i.e. HMF varied from 9.93 to 800mg/kg). Kerkvliet et al. (1995) also reported the HMF content of 8 Nepalese honey samples as 151, 183, 283, 401, 555, 582, 1110 and 1258 mg/kg. which exceeded the maximum limit of HMF (40 mg/kg) for honey. Since HMF is produced by the degradation of fructose, it is virtually absent in fresh honey and increases more or less quickly, according to storage condition and certain chemical properties (Persano Oddo et al., 1999). Therefore, it was not considered necessary to measure HMF for the other honeys which were collected directly from the combs and kept in deep freezer. Invertase is much more influenced by heat, light and other operational factors than proline (Von der Ohe et al., 1991). Therefore, the commercial beekeepers honeys might have lost their enzyme activity during processing and heating the honey. Like in Chitwan and Kathmandu valley, the proline content of some of the *A. cerana* honey samples collected from Jumla, Langtang, Dadeldhura areas was also much less (even below 100ppm) than the indicator level (140-200ppm) of honey adulteration (Von der Ohe et al., 1991; Dustmann, 1993).

Glucose oxidase is significantly higher in mountains than those of other groups of honeys. Since the per oxidase activity (i.e. the ultimate result of glucose oxidase activity) is responsible for the antibacterial activity of honeys (White et al., 1963; Wachle & Desai, 1991; Postmes, 1995), it can be said that the honeys from Mountains had more antibacterial efficiency than hills and tarai. Plachy (1944) also reported that the samples of honey produced at altitudes over 1000 meter had at least twice the bactericidal activity of samples from lower areas. However, the samples of honey collected from Hills, Tarai and commercial beekeepers did not show significant differences in their glucose oxidase. This could be because the samples from Hills were collected 1-2 year earlier than Tarai samples and, hence, they might have lost some enzyme activity. As already mentioned, the enzyme activity decreases in old or heated samples (Persano Oddo et al., 1999).

Sugar profiles of honeys showed similar characters with those obtained for *A. cerana* honeys by Phadke (1967), Latif et al (1956) and other investigators ((*table 3.5*). Monosaccharides fructose and glucose were detected as the principal sugars of honeys and account for more than 88% of total carbohydrates. The sum of total sugars, fructose, glucose, turanose, maltose, trehalose, melibiose, erlose, melezitose, maltotriose and raffinose did not show significant differences in different groups of the honeys. Sugar feeding is not practised with traditional log and wall hive beekeeping which is more common in hills and mountains. Therefore, virtually sucrose content was significantly lower in honeys collected from these areas than those of tarai and commercial beekeepers honeys. Similarly, the sucrose content of the honeys collected from supermarket in Kathmandu valley is significantly higher than the sucrose content of hills and mountains. It ranged from 0-20% (average 3.7%). Kerkvliet et al. (1995) also reported a very high amount of sucrose (up to 32.7%). They suggested that the presence of such a high level of sucrose is due to the adulteration of honey with acid-hydrolyzed cane sugar syrup. Honeys from tarai, hills, mountains and from supermarket also showed some specific characters in the fructose-glucose ratio and in the content of koji or dikojibiose, isomaltose and oligosaccharide- L_2 (*table 3.6*).

CHAPTER FOUR

MELISSOPALYNOLOGICAL STUDIES OF SOME NEPALESE HONEYS

4.1 Introduction

Honey normally contains a few grains of pollen from the flowers the bees have visited in collecting nectar. Like finger prints, the pollen grains from different types of plants have a distinctive shape that allows the family, genus or often the individual species of plant that produced the pollen to be identified. The microscopical examination of these pollen grains in the honey is known as melissopalynology or honey-pollen analysis. It is an accepted technique for identifying botanical and geographical origin of specific types of honey. Botanical origin of honey is determined by comparing the pollen in honey with reference pollen slides of known flowering plants. Melissopalynological studies are helpful in both quantitative and qualitative pollen analysis of honey (Louveaux et al., 1978).

Identification of bee plants from diverse geographic areas and floristic communities helps immensely in apiculture (Cowan, 1988). Identifying the pollen in honey helps not only to identify the plant sources of honey but also to protect the consumers from possible frauds, mislabelling and adulteration of honeys. It is highly advantageous to a beekeeper who might wish to know the plant origin of his honey because it has an exceptionally good flavour and colour and demands a high price. If he knows the floral source, he can attempt to grow that plant or migrate his colonies to that place and can produce more honey of such quality. Similarly, this can also be important when poor tasting or undesired honey has been produced and beekeeper wishes to avoid the responsible plants. Melissopalynology also enables us to identify the plant sources of toxic honey and to determine the relative preferences of honeybees for individual plants that flowers simultaneously. In addition, melissopalynological study provides relevant information about bee ethology, the bee-plant relationship, bee forage and bee's role in pollination of crops (Ricciardelli D'Albore & Persano Oddo, 1978; Ricciardelli D'Albore, 1997). As a measure of environmental pollution, melissopalynology is used to see whether there are substances toxic to man as well as for bees in any given phyto-sociologial environment. Heavy metals and other elements causing

environmental pollution have been recorded by honey analysis (Tong et al., 1975, Bogdanov et al., 1986, Bunzl et al., 1988, Balesta et al., 1992, Molzan and Assmann- Werthmuller, 1993).

Melissopalynological studies help to trace the geographical origin of a particular type of honey; since its pollen spectrum reflects the floral situation of the place where that particular honey was produced (Ricciardelli D'Albore, 1997). Different geographical areas have their own characteristic plant associations that are reflected in the pollen spectrum of local honeys (Pfister,1895, Maurizio, 1975, Nair, 1985, Ricciardelli D'Albore, 1997). The pollen spectrum of tropical honeys are quite different from that of temperate honeys. Particularly in Nepal, due to great variety of climates and topographical variation, the floristic composition varies markedly within a short distance. Accordingly, pollen spectrum of honeys are greatly different from one place to another. For example, the Indian butter tree (*Aesandra butyracea*) is widely distributed in western Nepal but not found in eastern part. Sal tree (*Shorea robusta*) distributed up to 934masl in western Nepal while in eastern Nepal it is found only below 500masl. Within Kathmandu valley, the pollen composition of honeys harvested from Royal apiary, Gokarna are markedly different from that of Himalayan Bee Concern, Kirtipur.

However, the pollen grains in honey does not give unequivocal results for determining the botanical origin of honeys (Molan, 1998). There are several important factors to consider relying on pollen identification to determine honey sources (Maurizio, 1975, Bambara, 1991, Ramalho et al., 1991, Dustmann, 1993). The first is the morphological characteristics of the flowers and pollen. Some flowers yield much pollen, and their nectar is exposed (like *Castanea*). Others are very small (like *Myosotis*). The nectar of these flowers receive a much dusting with pollen than the nectar of flower with wide calyx and which yield much nectar (like *Robinia*), or flowers whose nectar is separated from the anthers by special device e.g. *Salvia* and other Lamiaceae (Maurizio, 1975). Both the shape and the position of the flower can facilitate or limit the amount of pollen in the nectar. Some important bee plants may be a source of unifloral honey, but their pollen grains never reach the dominant category because they are under-represented (Ramalho et al., 1991). For example, the pollen granules which are bigger in size are consumed by bees or filtered by their pro-ventricular valves and are less likely to be found in the nectar. Similarly the grains with short spines on the surface such as *Aster spp* are better attached on hairs of the bees than those with smooth surface like *Trifolium*

spp. Pollen content can also be limited by the presence of extra-floral nectaries and the lack of synchronisation between anther dehiscence and the moment of maximum nectar secretion (Ricciardelli D'Albore, 1997). Honey produced from extra-floral nectaries (e.g. cotton plants, castor oil plants, field beans and rubber plants) and the honeydew will contain more air borne pollen grains that become trapped and which will not necessarily be from the plant that was the source of the secretion. Some plants produce very little pollen in their flowers and female flowers produce not at all. The pollen of such plants are under represented in the honey.

Second is related to the honey extraction operations. When the beekeepers extract honey, pollen reserve stored in the honey comb and honey from different hives mix together. Particularly in Nepal, the honey traders collect honeys from different agro-ecozones and mix together for processing. These honey samples typically hold several different pollen up on examination. Third, there is a risk that the heavier pollen may be removed from the honey more easily during centrifugation, yielding an additionally biased sample. Fourth is the period of time when the bees working on the gathered nectar. The larger the nectar remains in the crop, the lesser is the amount of pollen found in honeys (Von der Ohe, 1994). Even the amount of pollen content depends up on the foraging distance. The farther the nectar sources, lesser is the amount of pollen (Maurizio, 1975). Fifth; the mechanical action of insects, winds, etc. may also contaminate the honey from one floral source with pollen from other flowers (Molan, 1998). For example, one *Citrus* honey has been reported to have 18% of its pollen content from kiwifruit, which does not produce nectar (Moar, 1985). Sixth is the difficulties in the identification of pollen grains. There are many pollen types which look similar in size and structure under light microscope. For example, pollen grains from *Salix* (willow) and *Brassica* (rapeseed, canola) appear to be identical when examined without acetolysis. Both pollen types are tricolporate with reticulate exine sculpture (Low et al., 1989). Similarly, the pollen grains of *Rubus spp* and *Acer spp* look quite similar under light microscope. In some cases, such as when a grain is in a bad condition (crumpled, obscured or eroded) or if it is a member of difficult morphological group (e.g. Rosaceae, Asteraceae) the identification to the species or even to the genus level may prove impossible without type grains for comparison (Moore et al, 1991).

Thus, the results of melissopalynological analysis are reliable but may not guarantee the precision (Bambara, 1991, Molan,1998). The percentage of plants in the honey sediment do

not correspond exactly to the proportions of the nectar contributed to the resultant honey by the individual plant species. Therefore, it is necessary to combine other factors such as honey colour, flavour, physico-chemical and organoleptic characteristics. Von der Ohe (1994) found negative correlation between the quantity of pollen grains and the both enzymatic activity and amount of proline. To estimate the ratio of pollen grains or to ascertain botanical origin of honey, it is necessary to carry out chemical analysis of specific unifloral honey with absolute pollen count (Von der Ohe, 1994).

In the present study, the melissopalynological analysis was carried out with the following objectives;

-to identify the botanical and geographical origin of honeys collected from different bee species and from different altitudes,

-to evaluate the role of individual plants in honey production.

-to determine the foraging preferences of different honeybees within the same floristic communities

-to study the structural features of pollen grains that occurred in honey samples.

4.2 What is Pollen ?

Pollen is the male spore plasma of the plant, produced on the flowers' anthers, each of which is at the outer end of a stamen. Pollen appear as very fine grains which, under microscopic examination, show very characteristic forms and sizes for each species of plants. The pollen grains are spread by the wind, water, gravity, animal or insects and germinate when they reach to the receptive stigma. Flowers produce many more pollen gains than are actually needed for pollination. Besides pollination, pollen has two other apicultural interests; as the chief source of protein, fats and minerals in the honeybee diet, and as a possible surplus product of the apiary. It plays fundamental role in feeding the colony, mainly the larvae and the young bees. It contributes to body growth in general and is a determining factor in the development of certain organs such as the hypopharyngeal glands, ovaries and the adipose body (Ricciardelli D'Albore, 1997).

4.2.1 Composition of Pollen : Chemical analysis of pollen have shown it to be rich in lipids, and to contain free amino acids, carbohydrates, minerals (Ca, Mg, P, Fe, Na, K, Al, Mn, S and Cu), vitamins such as pantothenic acid, nicotinic acid, thiamine, riboflavin, ascorbic acid and small amounts of carotene (Lunden, 1956). Fresh pollen contains from 3.91 to 17.14 per cent water, 3 to 10 per cent fat, 10 to 15 percent sugars and 7.02 to 35.5 per cent protein (Standifer, 1967).

4.2.2 Uses of Pollen : Pollen is an indicator which enables researchers to study the phytogeography of the past, plant evolution, climates, rock and soil characteristics, air pollution levels, plant-insect relationships and the botanical and geographical origin of bee products, etc. (Moore et al., 1991; Ricciardelli D'Albore, 1997). In many developed countries pollen is used in controlled orchards for pollination. It is applied by brush, duster gun, blower or by honeybees. Even in Himachal Pradesh of India and in China bee collected pollen or the small branches of male flowers are used in apple orchard for pollination (Partap, 1998; Partap and Partap, 1999). Pollen is also used as protein supplement in the form of patties inside the hive to stimulate egg laying. The use of pollen as a human dietary supplement is also recommended by many investigators. Traub (1973) suggested the use of pollen for maintaining prostrate gland in man. Date palm pollen has been used for treating human sterility caused by the presence of gonadotropic hormones (Ridi et al., 1960). Bee pollen acts as an immunizing agent for allergies by building resistance with gradual intake (Wade, 1978). According to Hanssen (1979), bee pollen is also very helpful for sufferers from rheumatism and arthritis. Yoirish (1977) studied the pollen and made remark as;

'At present we can scarcely imagine how wide will be the use of biotic preparations which man will make from pollen in the near future. The time will come when thousands and millions of tons of pollen which is now lost to us will be used in the pharmaceutics industry.'

However, the pollen that has begun to ferment may cause harmful impact in human health. Pollen from some species of butter cup (Ranunculaceae) family and *Rhododendron spp* are reported to be toxic. Similarly, pollen produced in agricultural areas where pesticides are sprayed may be unfit for consumption.

4.2.3 Structural Features of Pollen Grain

Size: The sizes of the pollen grains vary greatly. For the sake of convenience these are divided into different categories. Ricciardelli D'Albore (1997) divided pollen sizes into 6 categories, where as Sawyer (1981) used only 5 size classes. Their classification based on the diameter of the pollen. However, not all pollen grains are round. Many are either elliptical or triangular, even elongated. Vorwohl (1968) categorized pollen into 9 classes and suggested the use of length and breadth instead of diameter for defining the sizes of the pollen (*table 4.1*). Since the size varies greatly between grains of the same species, and between grains given different treatments in the extraction process, Moore et al (1991) have suggested to avoid this criterion for pollen identification.

Aperture: Pollen grains normally have apertures on the surfaces, but in some cases they do not have aperture (nonaperturate). The code number can vary from 1 to 9; 1 is attributed to pollens with one aperture; 8 is attributed to pollen with more than 7 apertures and 9 is used for nonaperturate or unclear pollen (*table 4.1*).

Aperture type: The types of the apertures are also very important for the identification of pollen. The following five types were used in the present study;

1. Pore: Equitorial or more or less isodiametric round aperture; e.g. Gramineae
2. Colpa (Furrow): Equitorial, longitudinal aperture, usually tapering towards the end; e.g., *Allium*
3. Colporate: Pores with colpa; e.g., *Symphytum*
4. Syncolpate: Apertures are formed as colpi or colporate where the ends of the colpi meet at the pole field; e.g., *Berberis* spp
5. Heterocolpate: Both pore and colpi occur separately on the same pollen grain; e.g. *Lythrum* spp.

Exine Sculpture: Exine is the outermost layer of the pollen which is composed of 4 layers: base, rods or column, tectum, and ornamentation. Exine sculpture means the ornamental elements modelling the pollen surface, such as granules, various kinds of spines, reticulum, window or finger like projections. The ornamentation of the exine has a major affect on the

surface of the pollen and is very helpful in pollen identification. The forms of sculpture were categorized into 7 classes (*table 4.1*):

1. Psilate, Faveolate, Fossulate : When surface of the exines are smooth or without any particular ornamentation , they are known as psilate; e.g., *Betula, Viola*. Ornamentation with little pits or more or less rounded depressions is called faveolate; e.g., *Tilia spp* and exine sculpture with relatively larger pits or elongated depressions is called fossulate; e.g., *Gentiana spp, Berberis spp*.

2. Scabrate, Verrucate, Gennuate : Scabrate sculpture characterized by irregular elements which are < 1 μm in diameter; e.g., Quercus spp. Ornamentation is rough and with little warts, which are >1 μm in diameter is called verrucate; e.g., *Erica spp*. Gennuate is an exine sculpture with round warts which are narrower at the base, e.g. *Ilex spp*.

3. Echinate : Pollen surface with series of more or less uniformly distributed spine shaped ornament is called echinate exine sculpture, e.g., Asteraceae, Malvaceae

4. Clavate, Bacculate : Exines with rods which have thicker ends are clavate and those with stick like rods, whose height is roughly twice to their base are bacculate, e.g., *Viscum spp* .

5. Rugulate, Striate : Exine sculpture with furrow shaped ornaments are rugulate, e.g., *Acer spp, Rubus spp* and sculupture with parallel rugulate type elements are striate, e.g., *Prunus spp*.

6. Reticulate : In this type of sculupture, the ornaments are arranged in the form of reticulum or net like structure, e.g., *Ligustrum spp, Hedera helix*.

7. Fenestrate : Exines in which there are window-like holes between the ribs belongs to this category, e.g., *Taraxacum spp*.

Aggregation : In some cases pollen grains are aggregated in a compound form. For example, the pollen grains of family Ericaceae form a tetrad (i.e. 4 pollen grains are aggregated in a compound form), where as in the Mimosae, 8, 12 or 16 pollen grains are aggregated to form a polyad. But, more frequently the pollens are monads (not aggregated).

Besides above mentioned structural features some pollen grains possess other distinguished characteristics. For example, saccate (two large hollow projections from the main body of the grain) in *Pinus spp,* vestibulum (two layers of exine become separated in the vicinity of the apertures) in *Alnus* and *Betula spp*. Beekeeping Institute of Celle, Germany has developed a key for such characteristics (*table 4.2*) .

4.3 Review of the Literature on Melissopalynology

The earliest research on the pollen analysis of honey was undertaken by Pfister in 1895. Since then various other researchers have devoted themselves to this subject (Fehlman, 1911, Armbruster, 1929; 1934; 1935, Griebel, 1930-31, Zander, 1935; 1937; 1941; 1949; 1951, Maurizio, 1936; 1979; 1984, Vorwohl, 1981, Ricciardelli D'Albore, 1997; 1998). However, melissopalynological studies in Asia have been very few (Suryanarayan et al., 1992). Some analytical studies made by different investigators in south-east Asia are reviewed as follows :

In Nepal, no systematic work on this subject has so far been carried out (Kerkvliet, 1994). Nevertheless, a few years ago in collaboration with Beekeeping Institute, Lunz am See, melissopalynological studies were started by ICIMOD. They built a computer assisted pollen data bank of more than 1000 reference honey-pollen microscopic slides and analysed some *Apis cerana* honey samples collected from Jumla district and Kathmandu valley (ICIMOD, 1996, Partap, 1997)). Shrestha et al. (1994) sampled some bee plants through field survey and carried out the pollen morphological studies of 84 bee plants belonging to 38 families with some 163 photomicrographs, which could be the important reference material for honey-pollen analysis.

In India, melissopalynological studies were initiated by Deodikar and Thakar (1953) who conducted the pollen analytical study of major honey yielding plants of Mahabaleshwar hills, Maharashtra. Sen and Benerjee (1956) analysed the honey samples from Calcutta and observed an over-abundance of one anemophilous sporomorph. Deodikar (1965) made systematic investigation of the pollen sources on the Mahabaleshwar. Routine analysis to evaluate the sources of pollen have been made in Central Bee Research and Training Institute, Pune by different investigators (Suryanarayana, 1975;1978, Chaudhary, 1977, Sing et al., 1987, Suryanarayana and Singh, 1989). Singh (1989) made honey pollen analysis of honeys from north-east India and reported 16 plants as important sources of honey. Singh et al (1994) analysed 21 samples from 10 areas of the north east Himalayas and reported 83 bee forage plants belonging to 46 families. Their results showed that the most of the honeys were unifloral type and dominant pollens came from several plants including *Brassica spp, Wendlandia spp, Solanum spp, Ageratum spp, Clematis spp, Adhatoda spp, Mussaenda spp and Helianthus spp.* Sharma (1983; 1989) made an extensive melissopalynological study and

botanical survey of the honey plant of Himachal Pradesh. Malakar (1997) analysed 50 honey samples from four bioclimatic zones of West Bengal and reported 35 unifloral honey types, 7 multifloral type and remainig samples had a very few number of pollen grains. Pollen grains in a number of honeys from UP, India have been studied by Gaur and Nanwari (1989) and Chaturvedi (1983; 1989). Mattu et al. (1994) have analysed honey samples from 10 different localities of Kashmir, of which 5 samples are found to be unifloral and 5 multifloral. They recorded total 37 pollen types with *Plectranthes, Salix, Rumex, Brassica, Salvia, Artemisia* as major pollen type. A number of investigators made pollen analysis of honeys from Andhra Pradesh, India (Nair,1964, Seethalaxmi, 1980, Jhansi and Ramanujam, 1987; 1990). Kalpana and Ramanujam (1991) and Kalpana et al. (1990) also made quantitative and qualitative analyses of *Apis cerana* and *Apis florea* honeys from Rangareddy and Hyderabad districts of Andhra Pradesh.

Pasha et al. (1991) made melissopalynological investigations of Sunderban honey in Bangladesh. Chen et al. (1984) examined 88 honey samples from Taiwan and identified 53 taxa belonging to 35 families. They reported 78 samples as unifloral honeys, of which 72 from *Eucalyptus spp*, 3 from *Psidium gaujava*, 2 from *Litchi chinensis* and one Compositae.

4.4 Materials and Methods

Methods of melissopalynology were proposed and modified in different times by Erdman (1935; 1960), Louveaux and Maurizio (1963), Maurizio and Louveaux (1967), Vorwohl (1967), Louveaux et al.(1970 ; 1978), Lieux (1980), Low (1989). In the present study, the melissopalynological analysis was carried out in accordance with the methodology established by the International Commission of Bee Botany (ICBB; now the International Commission for Plant-Bee Relationship (ICPBR) of the International Union of Biological Science (IUBS), as described by Louveaux et al., 1978.

4.4.1 Honey Samples

Three hundred and eleven honey samples were analysed for the melissopalynological studies. Of these, 16 honey samples had only a few grains of pollen and, therefore, excluded from the

comparative analysis. The areas of sample collection are shown in the map of Nepal (see Chapter Three .3.3.1).

4.4.2 Preparation of Honey-pollen Microscopic Slides

The pollen grains suspended in honey were extracted by mixing 10 g of honey with 20 ml of warm (below 40°C) distilled water. After a thorough mixing the solution was centrifuged at 2500 rpm for 10 minutes. The surface proteins and carbohydrates were removed by decanting the supernatant liquid, and the sediment was dispersed again with distilled water and re-centrifuged for 5 minutes. The supernatant liquid was again decanted, and the sediment was transferred to a microscope slide using a fine pipette, spread over a suitable area, and dried by slight heating (below 40°C). From each honey sample 2 slides were prepared; one was mounted with stained (fuchsin) glycerine gelatine and another with unstained clear glycerine jelly.

Preparation of Mounting and Staining Media : Of the various mounting media available, glycerine jelly was used for this study because it is easy to handle (i.e. it is being molten when placed in boiling water-bath, yet solid at room temperature). Glycerine jelly was prepared by dissolving 7 g of gelatine in 42 ml of cold distilled water. 50 ml of glycerine was added, warmed gently and stirred until it was dissolved; 0.5 g of phenol was then added to prevent the growth of mould. To prepare the stained glycerine jelly, 0.1 g of basic fuchsin was dissolved in 10 ml of alcohol (methylated sprit). This stain was mixed thoroughly to produce a uniform suspension of red stained solids and then added drop by drop to the glycerine jelly until a clear pink colour was produced.

Sealing of Slides : The cover slip of both-honey pollen microscopic slides were sealed along the edges with clear nail varnish. Though, Hill (1983) considers it unsatisfactory, it worked well in the temperate climate of Lunz am See, Austria.

4.4.3 Identification of Pollen in Honey

Identification of pollen recorded in honey was made by comparing pollen grains with reference pollen slides. The comparison of pollen grains was made with the help of

Laboratory Colour Image Analysis (LUCIA), computer soft-ware programme digitalized from the microscope. The computer assisted pollen data bank installed in DV system was also implied to simplify the work in sorting out of the similar kind of pollen from reference material. It means when we enter the data from the unidentified pollen into the computer, it displays the names and pictures of the pollen which are of similar kind. By which we can save our time and compare more easily. In a number of cases, the photomicrographs of Moore et al (1991), Partap (1997), Ricciardelli D`Albore (1997; 1998) were also used for the pollen identification. The keys developed by Vorwohl (1968) on the basis of certain pollen grain features are used to distinguish pollen (*table 4.1*). As per the suggestion of Katharina Von der Ohe, expert of melissopalynology in Beekeeping Institute, Celle, Germany, some special criteria were also taken into consideration for the pollen identification (*table 4.2*).

4.4.4 Qualitative Analysis of Honey

Qualitative analysis of honey was carried out to determine the botanical and geographical origin of different honeys. Most of the pollen grains were identified to the genus or species level. In some cases, where it was not possible to ascertain exact species and sometimes even genus, general categories such as type, group or form were used. For example, *Ligustrum* type (a tri-colporate reticulate medium sized pollen grain). To evaluate the contribution of a particular plant in honey production the pollen percentages of each type was estimated. To determine the frequency classes at least 1000-1200 pollen grains were counted. However, in some cases where number of pollen were not enough, only 200-300 or even less number of pollen were counted. The following terms were used for frequency classes;

- `Predominant` >45%
- `Secondary Pollen` 16-45%
- `Important minor` 3-15
- `Minor` <3%

The following nomenclature were used when determining the frequency of the pollen grains.

- Very frequent >45%
- Frequent 16-45%
- Rare 3-15%
- Sporadic <3%

Table 4.1 Pollen grain features (Keys) used for pollen identification

S. N.	Parameters	Code	Pollen Grain Features
1	Length and breadth (μm)	1	<10
		2	10-15
		3	15-20
		4	20-25
		5	25-30
		6	30-35
		7	35-40
		8	40-45
		9	>50
2	Aperture Number	1	1
		2	2
		3	3
		4	4
		5	5
		6	6
		7	7
		8	>7
		9	Absent or not clear
3	Aperture Type	1	Pore
		2	Colpa
		3	Colporate
		4	Syncolpate
		5	Heterocolpate
4	Exine Sculpture	1	Psilate, Faveolate, Fossulate
		2	Scabrate, Verrucate, Gennuate
		3	Echinate
		4	Clavate, Bacculate
		5	Rugulate, Striate
		6	Reticulate
		7	Fenestrate
5	Aggregation	1	1
		2	2
		3	3
		4	4
		5	5
		6	6
		7	7
		8	>7

Source : Vorwohl (1968).

Table 4.2 Pollen grain features and some special notes required for pollen identification

Outline	Dimension µm	aperture type	Aperture number	Exine sculpturing types	Specials exine, aperture apertures border intine, plasma	inner	Plant family
◊-monade ◊-oval ◊-circular ◊-semiangular ◊-inter semiangular ◊-angular ◊-interangular ◊-lobate ◊-inter lobate ◊-bilateral ◊-heteropolar ◊-irregular ◊-dyade ◊-tetrade ◊-polyade ◊-vesiculate	◊ <10 ◊ <15 ◊ <25 ◊ <50 ◊ <100 ◊ <200 ◊ >200	◊ colpate ◊ pericolpate ◊ syncolpate ◊-trichotomo colpate ◊ porate ◊ stephano porate ◊ colporate ◊ peri colporate ◊ hetero colporate ◊ inaperturate	◊ 1 ◊ 2 ◊ 3 ◊ 3-4 ◊ 4 ◊ 4-5 ◊ 5 ◊ 5-6 ◊ 6 ◊ <10 ◊ >10	◊ scabrate ◊ verrucate gemmate ◊ baculate clavate ◊ echinate ◊ striate ◊ rugulate ◊ reticulate foveolate ◊ psilate	◊ margo ◊ anulus costa ◊ vestibulum ◊ operculum ◊	◊ oslo longata ◊ osla longata ◊ aperture membrane granular ◊ oncus ◊ intine extr. thick ◊ plasma grained ◊ ◊	◊

comment outline :
aperture specials :
exine specials:
intine:
plasma:

Source : Katharina Von der Ohe, 1998 (personal communication)

4.5 RESULTS

4.5.1 Pollen Spectrum of *Apis dorsata, A. cerana* and *A. mellifera* Honey from Chitwan District.

Most of the pollen retained in *Apis dorsata, Apis cerana* and *Apis mellifera* honeys collected from Chitwan district were identified to the species level (to the closest possible taxon) or to the family level. Some pollen grains which were not possible (due to lack of reference material) to identify even to the family level are represented by using code (*table 4.1*). For example, 40x39.3321 (i.e. pollen that is 40μm in length , 39μm in breadth, tricolporate and granular in exine sculpture). The word type used to indicate all the genera or species represented by the same morphological type of pollen.. However, the family Graminae excludes the *Zea mays* and Asteraceae excludes the *Eupatorium odoratum, Helinathus annus, Taraxacum officinale* . On the other hand, *Eucalyptus spp* may also represents *Callistemone* and *Citrus spp* includes all Rutaceae pollen having four apertures.

The extent to which the particular plant contribute to the honey production and percentage of honey samples having each type of pollen are presented in table 4.3. Of 83 honey samples analysed 3 samples (one *A .cerana* and two *A. mellifera* honey samples) had only a few grains of pollen and, therefore, excluded for the comparative studies of honey. Total 51 morphological types of pollen were identified in Chitwan honeys (*table 4.3*). The pollen types which occurred very frequently in honeys from one or another bee species are given in figure 4.1. The number of pollen types per honey sample was significantly higher in *Apis dorsata* and *A. cerana* than in *A. mellifera*. On average, it came out as 11 (ranging from 6-15) for *Apis dorsata*, 10 (ranging from 5-18) for *Apis cerana*, and 8 (ranging from 5-13) for *A. mellifera* honeys. The fuchsin stained photomicrographs of some major pollen grains retained from Chitwan honeys are given in plates I-X. The pollen spectrum of honey showing geographic indication of Chitwan are presented in figure 61(Plate XI).

Table 4.3 Pollen types recorded in Chitwan honeys
(Total number of honey samples 80; n=30 for A. dorsata, n=25 for A. cerana and n=25 for A. mellifera)

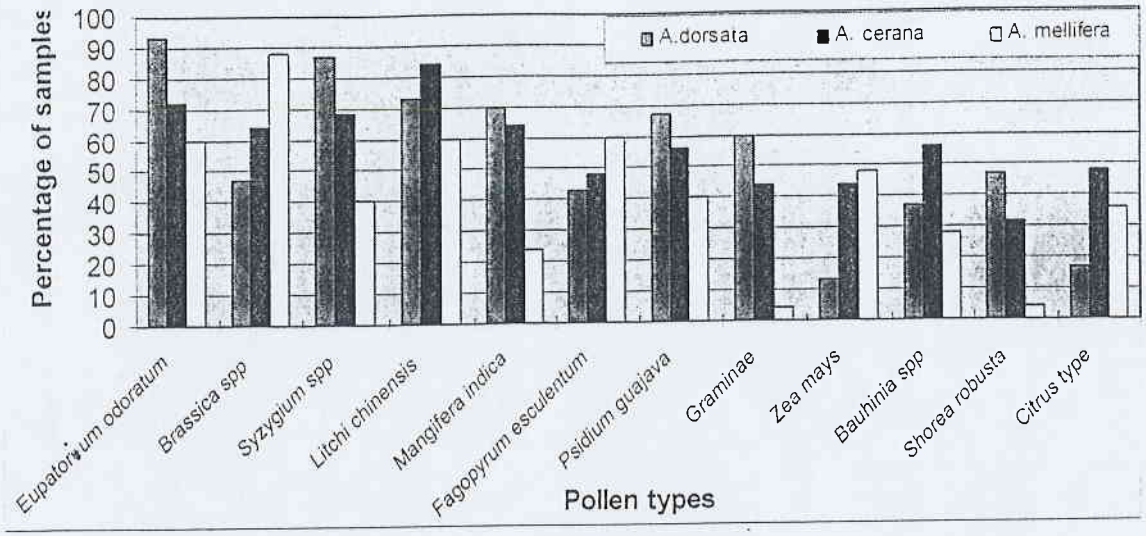
Frequency class ⇒	Predominant (>45%)			Secondary (16-44%)			Important Minor (3-15%)			Minor (<3%)			Percentage of honey sample having each pollen type* (Frequency)		
Pollen Type ⇓	Ad	Ac	Am	Ad	Ac	Am	Ad	Ac	Am	Ad	Ac	Am	Ad	Ac	Am
Eupatorium odoratum	6	-	-	15	2	1	3	6	4	4	10	10	93% (28)	72% (18)	60% (15)
Brassica spp	1	4	9	4	3	10	4	3	2	8	6	1	47% (14)	64% (16)	88% (22)
Syzygium spp	1	2	-	14	3	2	4	6	4	7	6	4	87% (26)	68% (17)	40% (10)
Litchi chinensis	-	-	2	1	9	3	8	4	4	13	8	6	73% (22)	84% (21)	60% (15)
Mangifera indica	-	3	-	5	3	2	5	2	3	11	8	1	70% (21)	64% (16)	24% (6)
Fagopyrum esculentum	-	1	-	1	3	2	2	3	6	10	5	7	43% (13)	48% (12)	60% (15)
Psidium guajava	-	-	-	5	5	-	6	6	2	9	3	3	67% (20)	56% (14)	40% (10)
Graminae type	-	-	-	2	4	-	7	2	-	8	5	1	60% (18)	44% (11)	4% (1)
Bauhinia spp	-	-	-	-	-	-	-	1	3	11	13	4	37% (11)	56% (14)	28% (7)
Zea mays	-	-	-	-	2	-	-	1	2	4	8	10	13% (4)	44% (11)	48% (12)
Citrus spp	-	-	-	-	4	-	1	-	1	4	8	2	17% (5)	48% (12)	36% (9)
Shorea robusta	1	-	-	1	-	-	2	3	-	10	5	1	47% (14)	32% (8)	4% (1)
Melia azedarach	-	-	2	-	2	-	-	1	1	7	1	-	23% (7)	16% (4)	16% (4)
Coriandrum type	-	-	-	1	-	1	2	1	3	7	9	7	33% (10)	40% (10)	20% (5)
Ligustrum type	-	-	-	-	-	1	1	-	4	4	5	6	17% (5)	20% (5)	44% (11)
Bombax ceiba	-	-	-	-	-	-	-	1	-	6	4	2	20% (6)	20% (5)	12% (3)
Eucalyptus spp	-	-	-	-	-	-	1	1	-	10	2	1	33% (10)	12% (3)	4% (1)
Helianthus annuus	-	-	-	1	-	-	1	-	2	7	2	4	30% (9)	8% (2)	24% (6)
Acanthaceae	-	-	-	-	-	-	-	-	-	6	2	-	20% (6)	8% (2)	-
Campsis type	-	-	-	-	-	-	1	-	-	4	-	-	17% (5)	-	-
Albizia type	-	-	-	-	-	-	-	-	-	5	-	1	17% (5)	-	4% (1)
Vitex negundo	-	-	-	-	-	-	-	-	-	6	4	-	20% (6)	16% (4)	-
Phoenix type	-	-	-	-	-	-	-	-	-	5	2	-	17% (5)	8% (2)	-
Lagerstroemia type	-	-	-	-	-	-	1	-	-	10	7	1	37% (11)	28% (7)	4% (1)
Vicia faba	-	-	-	-	-	-	-	-	-	2	-	-	10% (3)	-	-
Amaranthus spp	-	-	1	-	-	-	-	-	1	3	5	3	10% (3)	24% (6)	12% (3)
Medicago type	-	-	-	-	-	-	-	-	-	1	-	2	10% (3)	-	12% (3)
Allium spp	-	-	-	1	1	2	-	-	-	4	-	1	17% (5)	-	4% (1)
Cassia type	-	-	-	-	-	1	-	-	-	2	-	-	10% (3)	-	-

Continuation of Table 4.3

Frequency class ⇒	Predominant (>45%)			Secondary (16-44%)			Important Minor (3-15%)			Minor (<3%)			Percentage of honey sample having each pollen type* (Frequency)		
Pollen Type ⇓	Ad	Ac	Am	Ad	Ac	Am	Ad	Ac	Am	Ad	Ac	Am	Ad	Ac	Am
Raphanus sativus	-	-	-	-	-	-	-	-	-	2	5	4	7% (2)	20% (5)	16% (4)
Lamiaceae	-	-	-	-	-	-	-	-	-	2	2	2	7% (2)	8% (2)	8% (2)
Dellonix regia	-	-	-	-	-	-	-	-	-	-	2	-	-	8% (2)	-
Salix type	-	-	-	-	1	-	-	1	1	-	2	2	-	16% (4)	12% (3)
Gravilea robusta	-	-	-	-	-	-	-	-	-	-	-	1	-	-	4% (1)
Leucaena leucocephala	-	-	-	-	-	-	-	-	-	1	1	1	3,3% (1)	4% (1)	4% (1)
Taraxacum officinale	-	-	-	-	-	-	-	-	-	1	2	-	3,3% (1)	8% (2)	-
Liliaceae	-	-	-	-	-	-	-	-	-	3	-	-	10% (3)	-	-
Asteraceae	-	-	-	-	-	-	1	-	-	-	-	1	3,3% (1)	-	4% (1)
UK1 19x18,5.3341	-	-	-	-	-	-	-	-	1	-	-	1	-	-	8% (2)
UK2 38x26. 3211	-	-	-	-	-	-	-	-	-	2	-	-	6,6% (2)	-	12% (3)
Melilotus spp	-	-	-	-	-	1	-	-	-	-	-	1	-	-	8% (2)
Lauraceae	-	-	-	-	-	-	-	-	-	-	2	-	-	8% (2)	-
Ericaceae	-	-	-	-	-	-	-	-	-	-	1	-	-	4% (1)	-
Prunus type	-	-	-	-	-	-	-	-	-	-	1	-	-	4% (1)	-
Corylus type	-	-	-	-	1	-	-	-	-	-	1	-	-	4% (1)	-
Jatropha curcas	-	-	-	-	-	-	-	-	-	-	-	-	-	4% (1)	-
Imaptiens spp	-	-	-	-	-	-	-	-	-	-	-	1	-	-	4% (1)
Juglens type	-	-	-	-	-	-	-	-	-	1	-	-	3,3% (1)	-	-
Pinus roxburghii	-	-	-	-	-	-	-	-	-	1	-	-	3,3% (1)	-	-
Reinwardtia spp	-	-	-	-	-	-	-	-	-	1	-	-	3,3% (1)	-	-

* >45% Very frequent, 16-44% frequent, 3-15% rare, <3% sporadic (Pollen type recorded in total number of honey samples with every type such a pollen type)
Ad = *Apis dorsata*, Ac = *Apis cerana*, Am = *Apis mellifera*

Figure 4.1 Pollen Types Occurred Very Frequently in Chitwan Honeys



Pollen Spectrum of *Apis cerana* and *Apis mellifera* Honeys from Kathmandu.

Unlike in Chitwan district, the honey samples collected from Kathmandu valley are not well comparable. Because *A. cerana* honey samples were collected from different localities; i.e. (i) Himalyan Bee Concern, Chovar (ii) ICIMOD's Apiary, Godavari (some samples were also collected from ICIMOD office, Jawalakhel, center of the town, and iii) Royal Apiary, Gokarna. Where as *A. mellifera* honey samples were collected only from Himalayan Bee Concern, Chovar. These three localities suppose to have different floristic composition. Therefore, the degree of similarity in the botanical origin of honeys collected from 3 different localities in Kathmandu; viz. (i) Himalyan Bee Concern, Chovar (ii) ICIMOD's Apiary, Godavari (some samples from ICIMOD office, Jawalakhel, center of the town, and iii) Royal Apiary, Gokarna was calculated between the possible 5 pairs of honey samples (*table 4.4*) by using the formula **Similarity Index = 2c/a+b**, where C= number of pollen types common between the two pairs and a and b represent the total number of pollen types in each of the two pairs (Kalpana and Ramanujam, 1991). The extent to which the particular plant contribute to the honey production and frequency of occurrence of each pollen type are presented in table 4.5. The pollen which were present very frequently in one or another honey samples are shown in figure 4.2. Photomicrographs of some of the pollen types are presented in Plates I–X. The pollen spectrum of representative honey sample, showing geogrphic indication of Kathmandu valley are presented in figure 62. Total 50 different types of pollen

were identified. The number of pollen types per honey sample ranged between 4 to 12 (8.5 on average) in *A. mellifera* and 4-19 (9 on average) in *A. cerana* honey.

Fig. 4.2 Pollen Types Occurred Very Frequently in Kathmandu Honeys

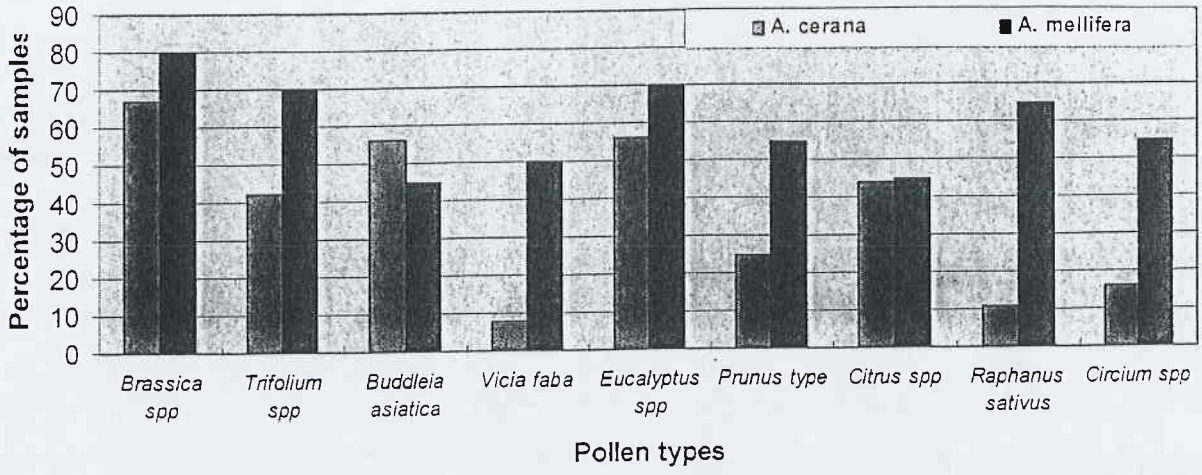


Table 4. 4 Similarity index between different pairs of honey samples

Pairs of samples*	Types of pollen recorded (A = a+b)	Pollen common in both (C)	Similarity Index I = 2C/a+b
Apis mellifera (n=20) *Apis cerana* (n=36)	a= 27 b= 48	(*Brassica, Trifolium, Buddleia, Rubus, Vicia, Circium, Viburnum, Allium, Eucalyptus, Salix, Prunus, Helianthus, Pyrus, Melilotus, Ageratum type, Lagerstroemia, Raphnus, Taraxacum, Oenothera, Robinia, Jasminum, Citrus*, Gramnae, Asteraceae, Lamiaceae) = **25**	2x25/27+48 = 0.66
A. cerana HBC (n=10) *A. mellifera* HBC (n=20)	a= 29 b= 25	(*Brassica, Trifolium, Buddleia, Rubus, Vicia, Circium, Viburnum, Eucalyptus, Salix, Prunus, Citrus, Pyrus, Melilotus, Lagerstroemia, Taraxacum, Oenothera, Robinia*, Gramineae, Lamiaceae, Asteraceae) = **20**	2x20/29+25 = 0.74
A cerana HBC (n=10) *A cerana*Gokarna (n=8)	a= 29 b= 28	(*Brassica, Buddleia, Rubus, Viburnum, Eucalyptus, Salix, Prunus, Zea mays, Fagopyrum, Citrus, Amaranthus*, Asteraceae, Lamiaceae, Gramineae) = **14**	2x14/29+28 = 0.49
A. cerana HBC (n=10) *A.cerana* ICIMOD (n=18)	a= 29 b= 35	(*Brassica, Trifolium, Buddleia, Rubus, Vicia, Circium, Viburnum, Eucalyptus, Salix, Prunus, Citrus, Pyrus, Melilotus, Robinia, Amaranthus, Adhatoda, Allium, Vitex*, Asteraceae, Lamiaceae, Gramineae, Acanthaceae) = **22**	2x22/29+35 = 0.68
A.cerana Gokarna (n=8) *A. cerana* ICIMOD (n=18)	a= 28 b= 35	(*Brassica, Buddleia, Helianthus, Viburnum, Eucalyptus, Salix, Prunus, Citrus, Myrica, Raphnus, Taraxacum, Amaranthus, Lonicera, Ziziphus, Rubus*, Lauraceae, Lamiaceae, Asteraceae, Gramineae) = **19**	2x19/28+35 = 0.60

*number of honey samples are not regular which also have some influences on similarity indices

Table 4.5 Pollen types recorded in *Apis cerana* and *A. mellifera* honeys collected from Kathmandu (n=36 for *A. cerana*, n= 20 for *A. mellifera*)

Frequency class⇒	Predominant (> 45%)		Secondary (16-44%)		Important minor (3-15%)		Minor (<3%)		Frequency of occurrence*	
Pollen type* ⇓	A. cer	A. mel	A. cer	A.mel	A. cer	A. mel	A. cer	A. mel	A. cer	A. mel
Brassica spp	3	7	6	7	5	2	10	-	67% (24)	80% (16)
Buddleia asiatica	3	-	7	1	7	-	3	8	56% (20)	45% (9)
Rubus spp	3	-	3	-	1	-	4	2	31% (11)	10% (2)
Trifolium spp	2	-	4	3	3	2	6	9	42% (15)	70% (14)
Vicia faba	-	2	-	3	-	2	3	3	8% (3)	50% (10)
Circium spp	-	2	-	1	-	-	6	8	16% (6)	55% (11)
Viburnum spp	2	-	1	-	1	-	-	1	11% (4)	5% (1)
Eucalyptus spp	1	-	2	-	2	3	15	11	56% (20)	70% (14)
Gramineae	1	-	-	1	5	-	6	7	33% (12)	40% (8)
Ziziphus spp	1	-	-	-	3	-	3	-	19% (7)	-
Salix type	-	-	3	-	3	-	-	1	16% (6)	5% (1)
Prunus type	-	-	-	3	2	1	7	7	25% (9)	55% (11)
Citrus spp	-	-	3	-	2	3	11	6	44% (16)	45% (9)
Myrica esculenta	-	-	2	-	2	-	3	-	19% (7)	-
Helianthus annuus	-	-	1	1	-	2	7	4	22% (8)	35% (7)
Robinia type	-	-	2	-	-	5	3	2	14% (5)	35% (7)
Melia azedarach	-	-	1	-	-	-	1	-	5% (2)	-
Amaranthus spp	-	-	-	-	2	-	9	-	31% (11)	-
Lamiaceae	-	-	-	-	1	1	5	-	16% (6)	5% (1)
Asteraceae	-	-	-	-	1	-	9	2	28% (10)	10% (2)
Fagopyrum esculentum	-	-	-	-	1	-	5	-	16% (6)	-
Albizia type	-	-	-	-	1	-	1	-	5% (2)	-
Campsis type	-	-	-	-	1	-	-	-	2.7% (1)	-
Raphanus sativus	-	-	-	-	-	1	4	12	11% (4)	65% (13)
Melilotus spp	-	-	-	-	-	1	2	5	5% (2)	30% (6)
Ageratum type	-	-	-	-	-	1	2	2	5% (2)	15% (3)
Allium spp	-	-	-	-	-	-	3	6	8% (3)	30% (6)
Lagerstroemia spp	-	-	-	-	-	-	1	5	2.7% (1)	25% (5)
Zea mays	-	-	-	-	-	-	5	-	14% (5)	-
Taraxacum officinale	-	-	-	-	-	-	5	1	14% (5)	5% (1)
Pyrus spp	-	-	-	-	-	-	3	1	8% (3)	5% (1)
Castnopsis spp	-	-	-	-	-	-	3	-	8% (3)	-
Cuphea micrantha	-	-	-	-	-	-	3	-	8% (3)	-
Oenothera spp	-	-	-	-	-	-	3	1	8% (3)	5% (1)
Vitex negundo	-	-	-	-	-	-	2	-	5% (2)	-
Acanthaceae	-	-	-	-	-	-	2	-	5% (2)	-
Adhatoda vasica	-	-	-	-	-	-	2	-	5% (2)	-
Lonicera spp	-	-	-	-	-	-	2	-	5% (2)	-
Lauraceae	-	-	-	-	-	-	2	-	5% (2)	-
Coriandrum sativum	-	-	-	-	-	-	2	-	5% (2)	-
Impatiens spp	-	-	-	-	-	-	-	2	-	10% (2)
Jasminum type	-	-	-	-	-	-	1	1	2.7% (1)	5% (1)
Ranunculaceae	-	-	-	-	-	-	1	-	2.7% (1)	-
Bauhinia spp	-	-	-	-	-	-	1	-	2.7% (1)	-
Quercus spp	-	-	-	-	-	-	1	-	2.7% (1)	-
Cassia spp	-	-	-	-	-	-	1	-	2.7% (1)	-
Gravillea robusta	-	-	-	-	-	-	1	-	2.7% (1)	-
Phoenix spp	-	-	-	-	-	-	1	-	2.7% (1)	-
Plectranthes spp	-	-	-	-	-	-	1	-	2.7% (1)	-
Pinus spp	-	-	-	-	-	-	-	1	-	5% (1)

*The number of honey samples having each pollen type are in bracket. Pollen types are arranged in descending order on the basis of their contribution to the honey production not on the basis of frequency of occurrence.

4.5.3 Pollen Spectrum of Honeys Collected from Supermarket in Kathmandu

The extent to which the particular plant contribute to the honey production and frequency of occurrence of each pollen type are presented in table 4.5. The pollen types that occurred frequently or very frequently in one or another honey samples are presented in figure 4.3. Total 34 different types of pollen were identified. The number of pollen types per honey sample ranged between 3 to 16, averaging 9.6. The photomicrographs of some major pollen grains retained in honeys are given in plates I-X.

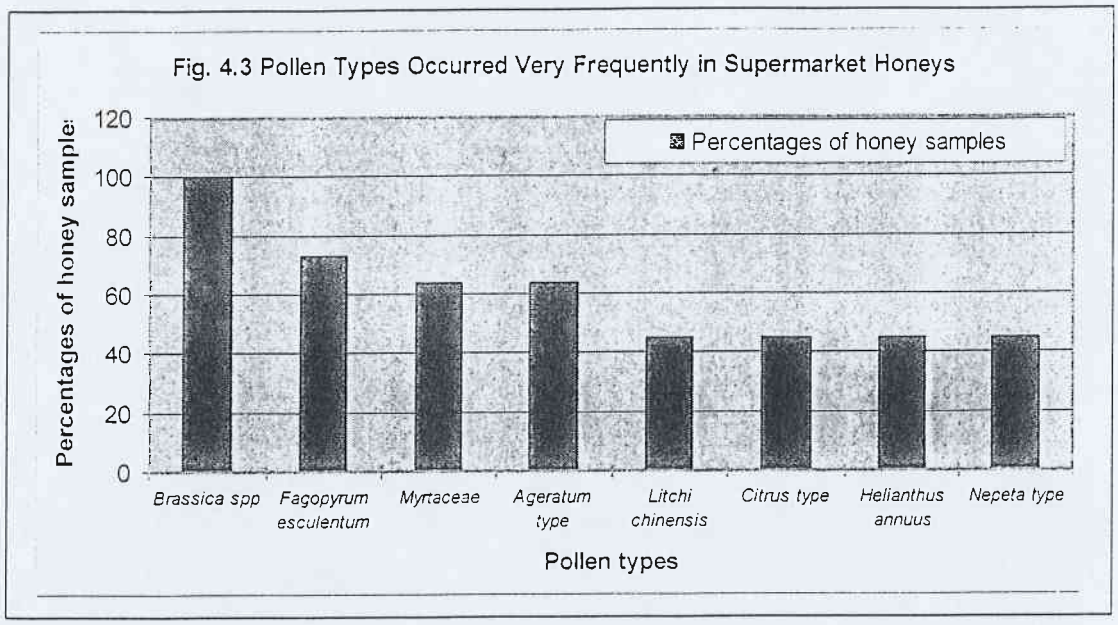


Table 4.6 Pollen types recorded in honeys collected from super market in Kathmandu (n=11)

Frequency class⇒ Pollen type* ⇓	Predominant (>45%)	Secondary (16-44%)	Important minor (3-15%)	minor (<3%)	Frequency of the each pollen type
Brassica spp	8	3	-	-	100% (11)
Fagopyrum esculentum	-	-	5	3	73% (8)
Myrtaceae	-	-	1	6	64% (7)
Ageratum type	-	-	-	7	64% (7)
Citrus spp	-	2	1	2	45% (5)
Helianthus annuus	-	-	3	2	45% (5)
Litchi chinensis	-	-	-	5	45% (5)
Nepeta spp	-	-	-	5	45% (5)
Graminae	-	-	-	4	36% (4)
Melia azedarach	-	1	1	1	27% (3)
Trifolim spp	-	-	1	2	27% (3)
Psidium guajava	-	-	1	2	27% (3)
Raphanus sativus	-	-	-	3	27% (3)
Elscholtzia type	-	-	-	3	27% (3)
Bombax ceiba	-	-	-	3	27% (3)
Strobilanthes spp	-	-	-	3	27% (3)
Syzygium cumini	-	-	-	3	27% (3)
Prunus type	-	-	-	2	18% (2)
Pyrus spp	-	-	-	2	18% (2)
Coriandrum sativum	-	-	-	2	18% (2)
Alnus nepalensis	-	-	-	2	18% (2)
Bauhinia spp	-	-	-	2	18% (2)
Buddleia asiatica	-	-	-	2	18% (2)
circium spp	-	-	1	1	18% (2)
Lauraceae	-	-	-	1	9% (1)
Ocimim basilicum	-	-	-	1	9% (1)
Rubus spp	-	-	-	1	9% (1)
Zea mays	-	-	-	1	9% (1)
Vitex negundo	-	-	-	1	9% (1)
Salix type	-	-	-	1	9% (1)
Impatiens spp	-	-	-	1	9% (1)
Loranthus spp	-	-	-	1	9% (1)
Taraxacum offcinalis	-	-	-	1	9% (1)
Asteraceae				1	9% (1)

*Pollen types are arranged in descending order on the basis of frequency of their occurrence

4.5.4 Pollen Spectrum of Three Jajarkot Honey Samples

The details of the pollen analysis of three *Apis cerana* honey samples provided by Jajarkot Permacultural Program is presented in table 4.7. The photomicrographs of some major pollen grains retained in honeys are given in plates I-X. All the three honey samples were of mixed floral in origin (i.e. they did not show any predominant pollen type). Total 16 different types of pollen were identified. The number of pollen types per honey sample was recorded as 15 in Sample No.175 and 14 each in other two honey samples.

Table 47 Pollen types recorded in Jajarkot honey

S. N.	Predominant (> 45%)	Secondary (16-44%)	Important minor (3-15%)	minor (<3%)
174	-	*Brassica spp, Citrus spp*	*Strobilanthes spp, Nepeta* type, *Aesandra butyracea*	*Fagopyrum esculentum, Pyrus spp, Helianthus annuus, Loranthus spp, Prunus* type, Graminae, *Ageratum* type, Myrtaceae, *Elscholtzia* type
175	-	*Brassica spp, Citrus spp*	*Aesandra butyracea*	*Fagopyrum esculentum, Pyrus spp, Helianthus annuus, Loranthus spp, Prunus* type, Graminae, *Ageratum* type, *Strobilanthes spp, Nepeta* type, Myrtaceae, *Elscholtzia* type, *Buddleia asiatica*
176	-	*Brassica spp, Citrus spp*	*Aesandra butyracea*	*Pyrus spp, Helianthus annuus, Loranthus spp, Prunus* type, *Ageratum* type, *Strobilanthes spp, Nepeta* type, *Buddleia asiatica, Elscholtzia* type, Myrtaceae, Graminae

Table 4.8 Pollen types recorded in Dadeldhura honeys (n = 50)

Frequency class⇒ Pollen type* ⇓	predominant (>45%)	Secondary (16-44%)	Important minor (3-15%)	Minor (<3%)	Frequency of the each pollen type
Brassica spp	9	11	11	6	74% (37)
Citrus spp	8	7	10	11	72% (36)
Rubus type	4	9	9	4	52% (26)
Asteraceae	-	-	1	21	44% (22)
Lamiaceae	-	-	8	10	36% (18)
Aesandra butyracea	1	3	8	5	34% (17)
Myrica esculenta	-	1	1	13	30% (15)
Prunus type	1	5	3	5	28% (14)
Rumex spp	-	7	-	7	28% (14)
Acanthaceae	-	-	1	10	22% (11)
Graminae	-	-	-	11	22% (11)
Coriandrum sativum	-	-	-	11	22% (11)
Eupatoreum type	1	-	4	5	20% (10)
Castanopsis type	-	-	-	10	20% (10)
Woodfordia type	-	6	-	3	18% (9)
Caryophyllaceae	-	-	2	7	18% (9)
Fagopyrum esculentum	-	-	1	7	16% (8)
Zygophyllaceae	-	1	1	5	14% (7)
Cedrela toona form	1	2	1	2	12% (6)
Melilotus type	-	2	1	3	12% (6)
Bauhinia spp	-	-	-	6	12% (6)
Raphanus sativus	-	-	-	6	12% (6)
Myrtaceae	-	4	1	-	10% (5)
Circium spp	-	-	1	4	10% (5)
Pinus spp	-	-	-	5	10% (5)
Corylus spp	-	-	-	4	8% (4)
Lauraceae	-	1	-	2	6% (3)
Zea mays	-	-	2	1	6% (3)
Vicia faba	-	-	1	2	6% (3)
Helianthus annus	-	-	1	2	6% (3)
Loranthus spp	-	-	-	3	6% (3)
Taraxacum officinale	-	-	-	3	6% (3)
Oenothera spp	-	-	-	3	6% (3)
Myosotis spp	-	-	-	3	6% (3)
Malvaceae	-	-	-	3	6% (3)
Berberis spp	-	-	-	3	6% (3)
Allium spp	-	-	1	1	4% (2)
Trifolium type	-	-	-	2	4% (2)
Rhododendron spp	-	-	1	-	2% (1)
Bombax ceiba	-	-	-	1	2% (1)
Sapindus type	-	-	-	1	2% (1)
Vitex negundo	-	-	-	1	2% (1)
Pyrus type	-	-	-	1	2% (1)
Sesamum indicum	-	-	-	1	2% (1)
Lagerstroemia type	-	-	-	1	2% (1)
Agave americana	-	-	-	1	2% (1)
Impatiens spp	-	-	-	1	2% (1)
Buddleia spp	-	-	-	1	2% (1)
Viola spp	-	-	-	1	2% (1)
Tilia spp	-	-	-	1	2% (1)
Cucurbitaceae	-	-	-	1	2% (1)

*Pollen types are arranged in descending order on the basis of frequency of their occurrence

Table 4.9 Pollen types recorded in Jumla honeys (n = 85)

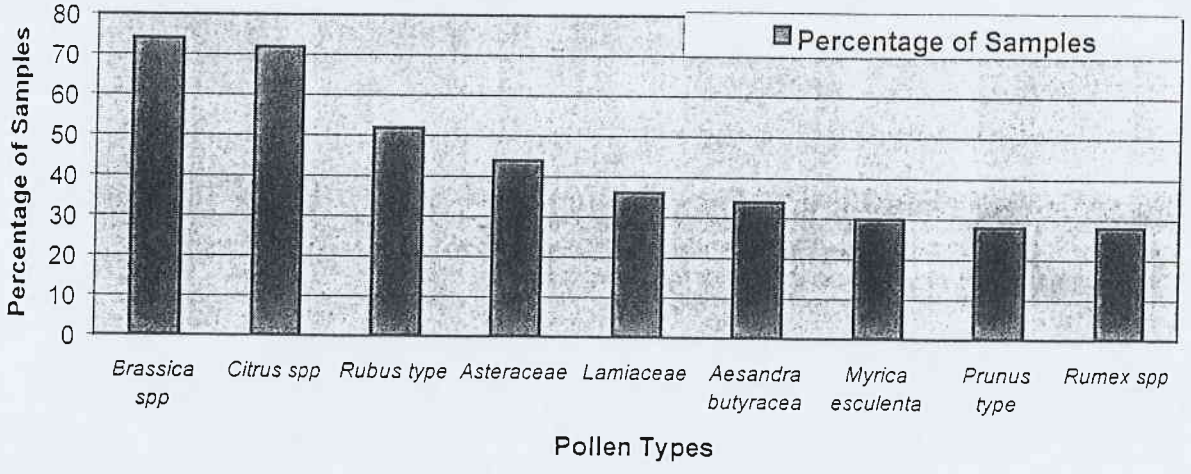
Frequency class⇒ Pollen type* ⇓	Predominant (>45%)	Secondary (16-44%)	Important minor (3-15%)	Minor (<3%)	Frequency of the each pollen type
Malus type	18	39	5	1	74% (63)
Lamiaceae	10	28	28	15	95% (81)
Rubus type	5	14	22	13	64% (54)
Brassica spp	4	15	22	15	66% (56)
Impatiens spp	1	3	3	11	21% (18)
Acanthaceae	1	-	2	16	22% (19)
Prunus type	-	9	6	12	32% (27)
Asteraceae	-	6	8	21	41% (35)
Spiraea type	-	4	2	5	13% (11)
Salix type	-	-	8	9	20% (17)
Taraxacum officinali	-	-	5	23	33% (28)
Caryophyllaceae	-	-	4	4	9% (8)
Viburnum spp	-	-	4	3	8% (7)
Unknown 11x11.3311	-	-	3	3	7% (6)
Allium spp	-	-	2	2	5% (4)
Primula spp	-	-	2	2	5% (4)
Graminae	-	-	1	8	11% (9)
Coriandrum sativum	-	-	1	7	9% (8)
Pinus type	-	-	-	37	44% (37)
Cucurbitaceae	-	-	-	33	39% (33)
Zygophyllaceae	-	-	-	33	39% (33)
Circium spp	-	-	-	14	16% (14)
Zea mays	-	-	-	12	14% (12)
Loranthus spp	-	-	-	11	13% (11)
Rhododendron spp	-	-	-	9	11% (9)
Fagopyrum esculentum	-	-	-	7	8% (7)
Agave americana	-	-	-	7	8% (7)
Tilia spp	-	-	-	5	6% (5)
Amaranthus spp	-	-	-	4	5% (4)
Lonicera spp	-	-	-	4	5% (4)
Berberis spp	-	-	-	4	5% (4)
Trifolium spp	-	-	-	3	4% (3)
Rumex spp	-	-	-	3	4% (3)
Medicago type	-	-	-	2	2% (2)
Alnus spp	-	-	-	1	1.2% (1)
Raphanus sativus	-	-	-	1	1.2% (1)
Castanopsis spp	-	-	-	1	1.2% (1)
Ericaceae	-	-	-	1	1.2% (1)
Viola spp	-	-	-	1	1.2% (1)
Mrica esculenta	-	-	-	1	1.2% (1)
Juglans regia	-	-	-	1	1.2% (1)
8 furrows(Lamiaceae ?)	-	-	-	(Lamiaceae)	-
Epilobium spp	-	-	-	1	1.2% (1)

* Pollen types are arranged in descending order on the basis of their contribution to the honey production not on the basis of frequency of occurrence.

4.5.5 Pollen Spectrum of Dadeldhura Honeys

The extent to which the particular plant contribute to the honey production and percentage of honey samples having each type of pollen are presented in table 4.8. Of the 50 honey samples analysed, 25 samples showed predominant pollen type. *Brassica spp, Citrus spp, Rubus* type, *Aesandra butyracea, Prunus* type, *Eupatoreum* type and *Cedrela toona* were found as predominant pollen type (*table 4.8*). The pollen that occurred very frequently in honey samples are presented in figure 4.4. The photomicrographs of some major pollen grains retained in honeys are given in plates I-X. The pollen present as geographic indicators of Dadeldhura honeys are given in figure 63.

Figure 4.4 Major Pollen Types Recorded in Dadeldhura Honey Samples



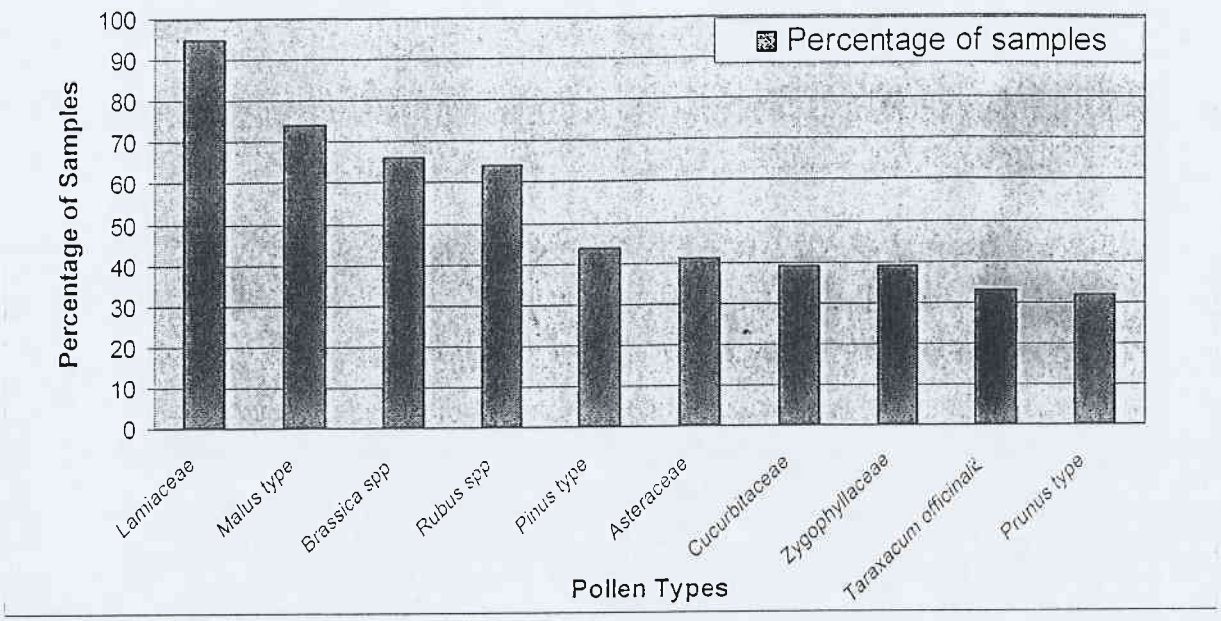
A total of 49 morphological types of pollen were identified in Dadeldhura district. Of which *Brassica spp, Citrus spp*, and *Rubus type* occurred as very frequent pollen type. The number of pollen types per honey sample averaged at 8 ranging from 3 to 16.

4.5.6 Pollen Spectrum of Jumla Honeys

The extent to which the particular plant contribute to the honey production and percentage of honey samples having each type of pollen are presented in table 4.9. Of the 96 honey samples analysed, 11 samples had only a few grains of pollen, less than 100, and hence excluded from the study. Out of remaining 85 honey samples 39 samples showed predominant pollen type. *Malus* type, Lamiaceae, *Brassica spp, Rubus spp, Impatiens spp* and Acanthaceae were occurred as predominant pollen type (*table 4.9*). The pollen that occurred very frequently in

honey samples are presented in figure 4.5. The photomicrographs of some major pollen grains retained in honeys are given in plates I-X. The pollen present as geographic indicators of Jumla honeys are given in figure 64.

Figure 4.5 Major Pollen Types Retained in Jumla Honeys



A total of 43 distinct morphological types of pollen were identified in Jumla district. The number of pollen types per honey sample averaged at 8 ranging from 4 to 15.

4.5.7 Pollen Spectrum of Langtang honeys

The extent to which the particular plant contribute to the honey production and percentage of honey samples having each type of pollen are presented in table 4.10. Of the 9 honey samples analysed, only one sample had *Guizotia abyssinica* as predominant pollen type, all other sample had mixed floral origin.

Total 25 different types of pollen were identified in Langtang honeys. Of which *Brassica, Malus type, Rubus type, Raphnus sativus, Taraxacum officiale* and Asteraceae were occurred as very frequent pollen type (*figure 4.6*). The number of pollen types per honey sample was recorded as 7 varying from 5 to 10. The photomicrographs of some major pollen grains retained in honeys are given in plates I-X. The pollen spectrum of a honey sample are given in figure 65 (Plate XI).

Figure 4.6 Major Pollen Types in Langtang Honey

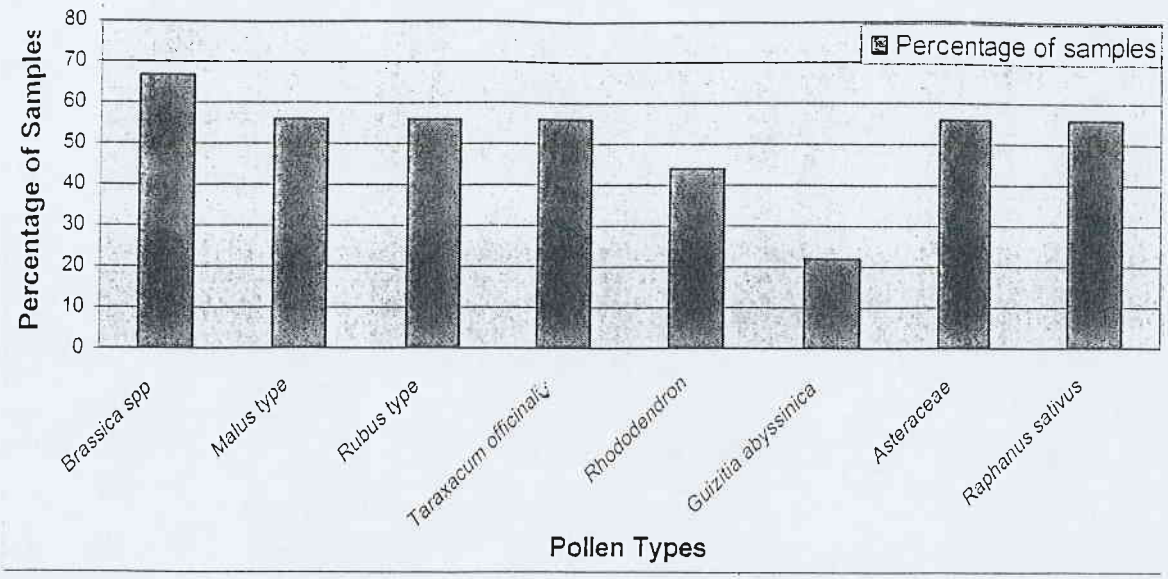


Table 4.10 Pollen types recorded in Langtang honeys (n = 9)

Frequency class⇒ Pollen type ⇓	predominant (>45%)	Secondary (16-44%)	Important minor (3-15%)	minor (<3%)	Frequency of the each pollen type
Guizotia abyssinica	1	1	-	-	22% (2)
Brassica spp	-	5	-	1	67% (6)
Malus type	-	5	-	-	56% (5)
Rubus type	-	-	5	-	56% (5)
Raphanus sativus	-	-	-	5	56% (5)
Taraxacum officinale	-	-	1	4	56% (5)
Asteraceae	-	-	-	5	56% (5)
Rhododendron arboreum	-	-	4	-	44% (49
R. campanulatum	-	-	-	4	44% (4)
Prunus type	-	2	-	1	33% (3)
Helianthus annus	-	-	1	1	22% (2)
Ageratum conzoides	-	-	1	1	22% (2)
Quercus spp	-	-	-	2	22% (2)
Alnus nepalensis	-	-	2	-	22% (2)
Myrica esculenta	-	1	-	-	11% (1)
Lauraceae	-	1	-	-	11% (1)
Caryopteris spp	-	-	-	1	11% (1)
Amaranthus spp	-	-	-	1	11% (1)
Rumex spp	-	-	-	1	11% (1)
Berberis spp	-	-	-	1	11% (1)
Spiraea type	-	-	-	1	11% (1)
Ligustrum spp	-	-	-	1	11% (1)
Papaveraceae	-	-	-	1	11% (1)
Lamiaceae	-	-	-	1	11% (1)
Graminae	-	-	-	1	11% (1)

4.4 DISCUSSION

4.4.1 Pollen Spectrum of *Apis dorsata, A. cerana* and *A. mellifera* Honey from Chitwan District, Nepal :

Chitwan district is very rich in floral resources and yields a rich crop of honey for at least 3 species of honeybees; *Apis dorsata, A. cerana* and *A. mellifera*. The period from March to May and September to October are the major nectarflow (honeyflow) seasons of this area. However, for the present study the honey samples were collected only during March and April. Therefore, the pollen spectra reflected in honey mostly represent the flora that are blooming during that period not all through the year. Some important nectariferous plants which bloom in later months (from May to September) such as *Ziziphus spp, Tamarindus indica, Cucurbita spp* were not recorded in honey.

Honeybees are very specific in their choices for nectar and pollen collection. They visit some plants very frequentlly, and others they do not visit at all. The pollen spectrum of honey, in general, showed the preference of bees for collection of nectar and pollen from selected plants of their choices. This study shows that in addition to nectar sources such as *Brassica spp, Litchi chinensis, Syzygium spp, Fagopyrum esculentum* that are well known to beekeepers, a number of other sources such as *Eupatorium odoratum, Mangifera indica, Shorea robusta* and honeydew also available as a source of honey for bees in Chitwan district, Nepal.

Eupatoreum odoratum recorded as the most frequent pollen type in Chitwan honey. The percentage of samples having *Eupatorium odoratum* pollen was highest in *A. dorsata,* followed by *A. cerana* and *A. mellifera (figure 5.1)*. Thapa and Wongsiri (1997) reported *E. odoratum* as the second most important source of honey in Thailand (the first major source is longan). They observed that this plant is eagerly visited by *A. dorsata, A. cerana, A. mellifera, A. florea* and stingless bees. Barth (1990) reported this plant as a source of monofloral honeys in Brazil. Kafle (1992), and Partap (1997) already highlighted the apicultural importance of *E. odoratum* in Nepal. Kerkvliet et al (1995) also found its pollen in honeys from tarai Nepal.

Litchi chinensis pollen was present in 72.5% of honey samples which points to its key role in honey production in Chitwan district and its importance as a geographic indicator of Tarai honey. This species is already listed in the Directory of important world honey sources (Crane et al, 1984). The honey potential of this plant is very high; 500-1000 kg/ha (Joshi,1992). The honey yield from this plant with *Apis mellifera* is reported to be about 20 kg colony/season in Myanmar (Zmarlicki, 1984) and 27 kg/colony/15 days in Bihar, India (Nair, 1981). Based on their field observation, Kafle (1984;1992), Shrestha et al.(1994), Allen (1995) and Partap (1997) reported this plant as major honey source of Nepal. Melissopalynological analysis of honey from Taiwan (Chen et al., 1984) and Himachal Pradesh, India (Singh, 1983, Sharma, 1989) revealed it as major source of honey. Nair (1964; 1981), Seethalaxmi (1980), Suryanarayana et al. (1992), Sharma and Raj (1985) also reported *Litchi chinensis* as major honey source in India.

The pollen grains of *Brassica spp* were among the very frequent pollen type and predominantly occurred in one *A. dorsata*, 4 *A. cerana* and 9 *A. mellifera* honey samples (*table 4.3*). The presence of relatively high percentage of *Brassica* pollen in *A. mellifera* could be because of the migration of colonies in the area where this crop is cultivated extensively for its oil yielding seeds. It is already reported that bees often chose to visit the closest flower (Marden and Keith, 1981). The apicultural value of *Brassica* has been recognized by different authors in other honeys (Sharma, 1989 from Himachal Pradesh, Chaturvedi,1989; 1983 from Kumaon and Garhwal, Mattu et al., 1994 from Kashmir and Malakar, 1997 from West Bengal, India, Shahid and Qayyum,1977 and Ahmad,1988 from Pakistan, Kafle, 1984; 1992, Allen, 1995 and Kerkvliet, 1995 from, Nepal, Partap, 1997 from HKH region, Shawer, 1987 from Egypt, Feller-Demalsy et al, 1987a; 1987b; 1989, respectively from Saskathewan, Alberta and Manitoba, Canada). However, the different species and varieties of *Brassica* have different honey potential. Joshi (1992) estimated the honey potential of *Brassica campestris* as 50-100 kg/ha and *Brassica napus* as 300-500 kg /ha. Through honey pollen analysis, though, it was not possible to distinguish the pollen type of different species and varieties of *Brassica*. All the *Brassica* species are visited by bees for both pollen and nectar. Generally, the pollen grains of *Brassica spp* are over-represented in honey (Maurizio, 1958, Ricciardeli D`Albore, 1997) .

Syzygium spp were occurred as very frequent pollen type in *Apis dorsata* and *A. cerana* but not very frequent in *A. mellifera;* predominating one *A. dorsata* and two *A. cerana* honey samples. This plant is among the worlds important honey sources (Crane et al., 1984). Kafle (1984;1992), Kerkvliet et al.(1996) and Partap (1997) reported *Syzygium* as major source of honey in Nepal. Sharma (1989) from Himanchal Pradesh, Jhansi et al (1991, 1994) from Andhra Pradesh, Malakar (1997) from West Bengal, and Seethalaxmi (1980) from various parts of India also reported *Syzygium cumini* as a predominant pollen type in their honey samples. Chen et al. (1984) reported *Syzygium* as frequently occurring pollen type in Taiwan honeys. Kerkvliet and Beerlink (1991) found *Syzygium cumini* pollen in 36% of samples, comprising up to 74% of the pollen in the sample. Vit and Ricciardelli D'Albore (1994) reported this plant as secondary source of honey for stingless bees in Venezuela. Joshi (1992) estimated the honey potential of this plant as 200-300 kg/ha. Based on his field survey, Ahmad (1988) also reported it as major honey source in Pakistan.

Similarly, the pollen grains of *Mangifera indica* were very frequently occured in *A. dorsata* and *A. cerana* but not very frequent in *A. mellifera* (*figure 4.1*). Based on their field observation, Kafle (1984; 1992), Shrestha et al (1994), and Allen (1995) reported this plant as major source of honey in Nepal. This plant is also reported as major source of nectar and pollen in India (Nair; 1985, Sharma and Raj; 1985; Suryanarayana et al.; 1992) and in Mediterranean countries (Ricciardelli D'Albore, 1998). Kalpana et al.(1990) reported this plant as a predominant pollen type, having 68.7% of the pollen in *Apis cerana* honey from Hyderabad, India. Jhanshi and Ramanujam (1987), however, found only 25% *Mangifera* pollen in the same type of honeys from Hyderabad, India. Kalpana and Ramanujam (1994) reported this plant as significant pollen type for *A. florea* and *A. dorsata* bees. Honey production from *Mangifera indica* is reported as 30 kg/colony/season (Lovell, 1961). Honeybees are reported to forage on leaves either for extra-floral nectar or for honeydew (Singh, 1962) and also collect juice from damaged fruits(Crane et al., 1984, Partap, 1997). *A. dorsata* colonies also build their nests in this tree.

Presence of *Psidium guajava* pollen in 67% of *A. dorsata*, 56% of *A. cerana* and 40%, of *A. mellifera* honey samples indicates the significance of this plant as an important source of nectar or/and pollen for bees. Kafle (1984;1992), Allen (1995) and Partap (1997) also highlighted its role in the honey production of Nepal. Malakar (1997) from West Bengal,

India reported it as a predominant pollen type in *Apis dorsata*, *Apis cerana* and *A. mellifera* honeys. It is also reported as a major source of honey in Bihar (Suryanarayana et al., 1981) and Andhra Pradesh (Nair, 1964, Kalpana and Ramanujam, 1989) of India, the North West Frontier Province of Pakistan (PARC, 1977; Sahid and Qayyum, 1977), in Taiwan (Chen et al., 1984) and in Spain (Kempff Mercado, 1980). Honeybees also collect juice from the damaged fruits (Kempff Mercado, 1971). But, through honey-pollen analysis it was not possible to detect whether it was produced from damaged fruits or mainly from nectar.

Fagopyrum esculentum pollen were very frequently occurred in honey samples. The reflection of its pollen in honey is quite reasonable because Chitwan is only the tarai district where *Fagopyrum esculentum* is extensively cultivated (Gill, 1996). Based on their field survey, Kafle (1984,1992) from Nepal, Saraf (1972) from Kashmir, India, Ma (1981) from China reported *Fagopyrum esculentum* as major honey source. Its pollen were also retained in Jumla honey (ICIMOD, 1996). Sharma (1989) recorded *Fagopyrum* pollen as a secondary pollen type in rainy season honey and as important minor pollen type in summer and autumn honey in Himachal Pradesh, India. Chaudhary (1977) reported it as a major source of nectar and pollen in Punjab. This plant is also listed as an important world honey sources (Crane et al., 1984). Its pollen show great variation in size and are generally over-represented in honey (Stanley, 1974). Similarly, the pollen grains of *Bauhinia,* though found as minor or important minor type, presented in 56% of the *A. cerana* honey samples, 37% of the *A. dorsata* honey samples and only 8% of the *A. mellifera* honey samples. Jhansi et al.(1991, 1995) reported this plant as chief honey source for *A. dorsata* in Andhra Pradesh, India. Based on their field survey, Sharma and Raj (1985) reported *Bauhinia variegata* as an important bee plant of Shivalik Hills in Himachal Pradesh, India. However, Kafle (1992) and Partap (1997) reported *Bauhinia* as a minor source of honey in Nepal. The low numbers of pollen grains of *Bombax ceiba* may be a consequence of the scarcity of these trees in the area of Chitwan district. In some parts of India, this tree is reported as a major (Naim and Phadke, 1967) to medium (Chaubal and Kotmire, 1980) source of honey for bees. *Leucaena leucocephala* also provides nectar and pollen for bees (Partap, 1997). It is very frequently occurred in Malaysian honeys and, therefore, used as a marker to distinguish Malaysian honeys from foreign honeys (MBRDT, 1987). However, due to the scarcity of this tree in Chitwan area, its pollen were occurred only in 3 samples (less than 5% of total samples).

Shorea robusta pollen were very frequent in *A. dorsata*, frequent in *A. cerana* but rare in *A. mellifera*. This plant is widely distributed in Tarai districts including Chitwan and reported as a major source of honey in Nepal (Kafle, 1984; 1992), Uttar Pradesh of India (Singh, 1983 and Singh et al., 1983) and Bangaladesh (Alam and Zannat). Similarly, *Lagerstroemia* pollen also frequently occurred in *Apis dorsata* and *A. cerana* but rare in *A. mellifera* honey. In India, this plant is reported as chief honey source for *Apis dorsata* (Jhansi et al.,1991; 1995) and for *A. cerana* (Seetalaxmi,1980). Ahmad (1988) also reported *Lagerstroemia* as major honey source in Pakistan. Similarly, *Vitex negundo* pollen frequently occurred in *A. dorsata* and *A. cerana* but not at all in *A. mellifera*. It is also reported as a major source of honey in areas where it occurs in abundance (Ahmad;1984; 1988, MBRDT;1987, Partap, 1997). *Phoenix* pollen also frequently occured in *A. dorsata*, rare in *A. cerana* and absent in *A. mellifera*. Malakar (1997) and Ramanujam and Kalpana (1995) also found *Phoenix* pollen in their honeys. Crane et al. (1984) and Suryanarayana et al. (1990) have also reported *Phoenix* as a fairly important source of nectar. The pollen grains of these native plants; *Shorea robusta, Lagerstroemia spp, Vitex negudo* and *Phoenix spp* were very rare or absent in *A. mellifera* which could be because of the lack of synchronisation between exotic bee, *A. mellifera* and the flowering of these plants. Nagamitsu and Inoue (1999) also found the diffrences in the foraging traits between Japanese honeybee, *A. cerana japonica* and European honeybee, *A. mellifera*. They suggested that both resource location and visual attractiveness of pollen sources differed between the honeybee species.

Pollen types from flowers that do not produce any nectar were found in the honey samples. This is the case with species like *Zea mays* (occurred in 34% samples) and other Graminae(recorded in 37.5% samples). This supports the observation of Roubik (1989), that the pollen in honey does not simply represent nectar sources, but all pollen used by the colony whether from nectariferous flowers or those producing pollen only. Maurizio and Schaper (1994) suggested that the pollen grains of grasses were collected by bees as food reserve. The pollen grains of Graminae were also reported in the honey produced by European and Africanized honeybee (Villanueva-G, 1994). Kerkvliet and Beerlink (1991) reported the percentages of honey samples having *Zea mays* pollen grains as 3% and other Graminae as 69% from Surinam. Shahid and Qayyum (1977) and Ahmad (1988) mentioned *Zea mays* as major crops for migratory beekeeping in Pakistan. Honeydew honey from Zea mays is also reported in USSR (Glukhov, 1955) and in Mediterranean countries (Ricciardelli D'Albore,

1998). MBRDT (1987) reported this plant as major source of pollen in Malaysia. Bees are also reported to collect sap from its split stems (Pellett, 1976). Chen et al (1984) reported Graminae pollen in Taiwan honeys. Jhansi et al (1991) from Andhra Pradesh, India reported Graminae pollen grains in *Apis dorsata* honey samples. They suggested that the presence of Graminae pollen grains in honey is due to the contamination by wind, as the combs of *Apis dorsata* are found in exposed areas. Malakar(1997) also reported Graminae pollen in *A. dorsata, A. cerana* and *A. mellifera* honeys from West Bengal, India. She found many honeydew elements and more dust particles in honeys which had more Graminae pollen grains. Like in West Bengal honeys, in the present study the honey samples which had more Graminae pollen grains also had more honeydew elements and dust particles. This suggests that these plants could have been visited by bees for honeydew. As additional evidence these honey samples had higher electrical conductivity and honeydew originating sugars.

Citrus pollen were very frequent in *A. cerana* and frequent in *A. dorsata* and *A. mellifera* honey samples. The exact identity of that plant is not known and here, in Chitwan honeys, all the Rutaceae pollen having four apertures were represented by *Citrus* type. The role of various species of *Citrus* in the production of honey in different countries is highlighted by many authors (Nair, 1964, Crane et al., 1984, Ramalho et al., 1991, Partap, 1997). Among the various species of *Citrus*, Ma (1981) and PAK (1977) reported *Citrus reticulata* as major honey source in China and Pakistan, respectively. The pollen grains of *Callistemone* and some species of *Eucalyptus* are quite similar. Since, their pollen were present only in a few samples, both types were grouped under *Eucalyptus spp*. *Eucalyptus* is of great apicultural value in other parts of the world (Serra, 1989, Crane et al., 1984, Ramalho, 1991, Partap, 1997). But as a consequence of the scarcity of these trees in Chitwan area, its pollen were retained only in a few samples.

Despite their great abundance and the strong preference of the bees for their flowers, the pollen grains of *Coriandrum sativum* and *Allium spp* appeared in relatively low percentages. Similarly, pollen grains of *Carica papaya, Woodfordia fruticosa, Dalbergia sisso* and *Pogostemone spp (rudilo)* were not retained in honeys. However, these four plant species were also considered as major sources of nectar in many areas (Partap, 1997, and references there in). The reason for this might be the relative preferences of honeybees in selecting botanical resources; nectar or/and honeydew or/and pollen from diverse floristic communities

of the area. Honeybees can detect fairly small difference, when given a choice, they have a preference mainly for those that are nearest to the hive, those providing a better reward (that is, the largest amount of pollen or nectar or both), those having more concentrated nectar, and those that have not been foraged on previously by other bees (Baker, 1978, Winston, 1987). The preference of bees for the collection of nectar might also be influenced by several other factors such as structure of the flowers, colour, fragrance, flowering period, weather condition during blooming time and strength of the colonies

Interestingly, the pollen spectrum of *Apis dorsata* honey samples collected from two different trees (nesting site) which are less than 500 m far from each other were markedly different. But the pollen spectrum of honey collected from different colonies nesting in the same tree were more or less similar. In all the honey samples collected from *Bombax* tree near Bharatpur, *Eupatoreum odoratum* and honeydew occurred as major source of honey. Moreover, all the samples collected from that tree had more than 0.7 mS/cm electrical conductivity and contained, to lesser or greater extent, oligosaccharide L_2 (see *table 3.1.4*) . Whereas, the honey samples collected from another nesting site nearby Narayani Hotel had less than 0.7 mS/cm electrical conductivity and *Syzygium*, *Mangifera, Brassica* and *Shorea robusta* as major pollen types. Whether there exist any special communication system between different colonies nesting in the same tree, at least for searching botanical resources, should be the subject of future investigation.

Most of the pollen types found in honey samples were common in all three bee species but with different representation (i.e. they showed variations in their frequencies). The number of pollen types per honey sample was significantly higher in *A. dorsata* and *A. cerana* than that of *A. mellifera*. Total 51 types of pollen (morphological types) were identified in 80 honey samples collected during March-April from Chitwan district which shows the great diversity of bee flora. Chen et al (1984) recorded 53 taxa in 88 honey samples from Taiwan and Chaturvedi (1989) found total 62 pollen types with the number of pollen varying 1-31 in honeys from Garwal and Kumaow. These authors, however, collected honey samples all through the year.

Pollen grains of *Mangifera indica, Litchi chinensis, Psidium gaujava, Shorea robusta* stand out as a geographic indicators of honey from Chitwan district (tarai), Nepal. The other

melissopalynological characteristics of honeys from the present studies can be summarized as: (1) the presence of *Eupatorium odoratum, Syzygium spp, Brassica spp, Litchi chinensis, Mangifera indica, Fagopyrum esculentum, Psidium guajava*, Graminae as very frequent pollen type; (2) high pollen diversification (i.e. 51 types of pollen in one season); (3) a high percentage of *Eupatorium odoratum* pollen, which plant is recently naturalised in the area and less known to the beekeepers as a source of honey; (4) the different bee species have different choices; the most important nectar or/and honeydew sources for native bee species; *Apis dorsata* and *A. cerana* were wild herbs (e.g. *Eupatorium odoratum*, Graminae) and trees (e.g. *Syzygium spp, Shorea robusta, Mangifera indica, Litchi chinensis* and *Psidium gaujava*), where as the most important nectar source for exotic bee species, *Apis mellifera* were cultivated herbs (*Brassica, Fagopyrum esculentum*, and *Zea mays*) and trees like *Litchi chinensis* and *Psidium guajava*.

4.4.2 Pollen Spectrum of *A. cerana* and *A. mellifera* Honey Samples from Kathmandu valley, Nepal

The pollen composition of honeys studied from Kathmandu valley showed 10 predominant pollen types: *Brassica, Buddleia, Rubus, Trifolium, Vicia, Circium, Viburnum, Eucalyptus, Ziziphus* and Gramineae. The last three taxa were found to be predominant in each one sample collected from Royal apiary, Gokarna. *Eucalyptus* and *Ziziphus* are well known sources of nectar for bees and already listed in the Directory of Important World Honey Sources (Crane et al., 1984). However, these plants are not very abundant in Kathmandu valley. The sample which had Graminae pollen (two different sizes 35x35 and 27x26 μm) as predominant pollen type, had high electrical conductivity (0.76 mS/cm), suggesting the possible origin of honey from honeydew. *Circium* and *Viburnum*, though occurred as predominant pollen type in each of the 2 *A. mellifera* honey samples, are of little importance in terms of the total honey crop of Kathmandu valley. *Brassica, Buddleia, Rubus, Trifolium* and *Vicia* are well known and definitely the very significant nectar plants. The role of *Brassica* species in the production of honey is already highlighted in previous section (see 4.5.1). *Buddleia asiatica* blooms during February-March and produces small, white flowers which are eagerly visited by *A. cerana* and *A. mellifera* for their nectar (Partap, 1997). This species contributes to spring forage which helps to facilitate brood rearing and also honey production. Its pollen are very small in size and possibly over-represented in honey. The

apicultural value of *Rubus* has been recognized by different authors in others honeys (Maurizio and Grafl,1982, Crane et al., 1984, Jato et al., 1991, Von der ohe, 1994, Ricciardelli D` Albore, 1998). Unifloral honeys from *Rubus* species have been reported in many Mediterranean countries (Spain, Italy, Yugoslavia, etc.). In Europe, the honey potential of *Rubus spp* has been estimated at 5-26 kg/ha (Maurizio and Grafl, 1982). Based on their field observation, Maskey (1989; 1992), Kafle (1984; 1992) and Partap (1997) reported *Rubus* as one of the major sources of nectar in Kathmandu valley. Similarly, the role of various species of *Trifolium* in the production of honey has been highlighted by different investigators in other countries (Pellet, 1977, PARC, 1977, Howes, 1979, Maurizio and Grafl, 1982, Feller-Demalsy, 1987, Joshi, 1992, Bangyu et al., 1996, Ricciardelli D`Albore, 1998). The honey potential of *Trifolium* has been estimated as 65 kg/ha from *T. alexandrinum* (PARC, 1977), 28.9 kg/ha from *T. pratense* (Petkov, 1977), 32.2 kg/ha from *T. repens* and 70-100 kg/ from *T. resupinatum* (c.f. Partap, 1997). The pollen of *Trifolium*, in the present study, probably comes from *T. repens*. Because this taxon is more widely distributed in Kathmandu valley. *Vicia faba* occurred as predominant pollen type in 2 of 20 *A. mellifera* honey samples (*table 4.5*). This plant is considered as major source of honey in Belgium (Grandjean, 1965) and UK (Howes, 1979). Sugar concentration of its nectar is estimated as 28% and honey potential as 30-60 kg/ha (Cirnu, 1980). Honeybees are also found to collect a lot of honeydew from this plant in Morocco (Personal observation) and in Europe (Kloft et al., 1965, Maurizio and Grafl, 1982). However, Partap (1997) reported it as a minor source of honey in Kathmandu valley.

Of the 36 *Apis cerana* honey samples analysed, 16 samples were unifloral, that is, containing more than 45% of pollen of one type. The remaining 20 samples could be regarded as `mixed floral` honey. Present as secondary pollen in these were *Brassica, Buddleia, Rubus, Trifolium, Viburnum, Eucalyptus, Salix* type, *Citrus, Myrica, Helianthus, Robinia* and *Melia azedarach*. Among them *Brassica spp, Eucylyptus* type and *Buddleia asiatica* pollen occurred as very frequent pollen type (*figure 4.2*).

Similarly, out of 20 *A. mellifera* honey samples, 11 samples were of `unifloral` type. There the 7 samples were predominated by *Brassica* pollen and 2 each by *Circium* and *Viburnum* pollen. The secondary pollen type in remaining `mixed floral` honeys were *Brassica, Buddleia, Trifolium, Vicia, Circium, Prunus, Helianthus* and Gramineae. Like in Chitwan

honeys, the percentage of *Brassica* pollen is higher in *A. mellifera* honey than in *A. cerana* (*table 4.4*).

In *A. mellifera* honey, *Brassica, Buddleia, Trifolium, Eucalyptus, Vicia, Circium, Prunus, Citrus* and *Raphanus sativus* occurred as very frequent pollen type (*figure 4.2*). *Trifolium* and *Eucalyptus* pollen were present in 70% of honey samples but qualified, respectively as secondary and important minor pollen type. Similarly, *Raphanus sativus* pollen, though present in 65% (13 of 20) of honey samples, occurred as minor and important minor pollen type. *Vicia faba* and *Prunus* pollen were occurred, respectively in 50% and 55% of *A. mellifera* honey samples (*table 4.4*). Where as in *A. cerana* honey samples, these pollen types were occurred respectively in 8 % and 25% of samples. This could be because the most of the *A. cerana* honey samples were collected from Gokarna and Godavari area where these plants are less extensively cultivated. It is already reported that the plants that are not fairly abundant in an area may not be of much importance to honeybees (Partap, 1997). *Citrus* pollen occurred in 44% of A. cerana and 45 % of *A. mellifera* honey samples (*figure 4.2*). Three types of *Citrus* pollen (more probably from *C. grandis, C reticulata and C. aurantifolia*) were recorded in honeys but the exact identity is not known. *Citrus spp* are reported as the major source of nectar in Kathmandu valley (Kafle, 1984; 1992, Maskey, 1989; 1992, Partap and Verma, 1996, Partap, 1997). However, in the present study their pollen never reached to the predominant level. This could be because of its under-representativity in honey which is already accentuated by many investigators (Ricciardelli D`Albore, 1998).

Buddleia, Citrus, Rubus and *Prunus* pollen stand as geographic indicators of honeys from Kathmandu valley. Some plants, especially Cucurbitaceae that flower during rainy season, and are reported as good nectariferous plants, were not represented by the pollen in honey. This could be because their nectar is poor in pollen and bees tend to filter its pollen (bigger in size) through their zander valve. There might have an influence of climate; during winter and spring seasons, the activity of bees decreases because of relatively low temperature and frequent rains. In contrast, during summer and autumn bees accumulate the greater part of the honey crop of the year. Hence, plants that flower during spring and winter are probably poorly represented by the pollen in honey (Jato et al., 1992). Particular flower morphology might also have some influences on pollen representativity (Ricciardelli D`Albore, 1998).

This is the case with species like *Borago* (upside-down flowers) and *Paulownia* (very large corollas).. These two exotic species are recently grown nearby ICIMOD apiary and provide abundant nectar for bees (Partap, 1997) but their pollen did not occur in honeys.

As mentioned earlier, *A. cerana* honey samples were collected from three different apiaries: HBC apiary (Kirtipur), Royal apiary (Gokarna) and ICIMOD apiary (Jawalakhel, Godavari). Where as *A. mellifera* honey samples were collected only from HBC apiary (Kirtipur). Royal apiary is located near the forest in Gokarna, HBC apiary is situated in between the Kirtipur town and horticultural farm, where as ICIMOD apiary is located in between the Botanical Garden and Godavari forest (here some samples collected from ICIMOD Office, Jawalakhel; in the centre of the town, were also included). These three apiaries supposed to have different floristic composition. However, the degree of similarity calculated between 5 different pairs of honey samples showed that more than 50% of pollen types in honeys were from the same botanical origin. Except in the pollen spectra of honeys collected from Royal apiary and HBC apiary, for all other pairs the similarity index was found to be above 0.5 (*table 5.5*). The maximum similarity index of 0.74 is found between *A. cerana* and *A. mellifera* honey samples which were collected from the same place (HBC apiary), indicating the foraging competition for available food resources between two bee species. And the minimum similarity index of 0.49 is found between the honey samples collected from HBC apiary and Royal apiary (*table 4.5*). However, the number of honey samples for different pairs of honeys were not the same which might have some influences in the pollen composition of honey. Therefore, further studies with homogenous honey samples are needed in order to evaluate the foraging preferences of different bee species and to discover the significant forage plants of all through the year.

4.4.3 Pollen Spectrum of Honeys Collected from Supermarket in Kathmandu

Pollen spectrum of honeys produced by commercial beekeepers in Kathmandu showed high pollen diversification. Some samples contained pollen from subtropical plants, like *Litchi chinensis, Psidium guajava*, to temperate plants such as *Alnus nepalensis, Prunus spp, Pyrus spp*. This suggest that the commercial beekeepers collect honey from different altitudinal zones and mix together for processing and marketing of it. It is also possible that the pollen from different altitude retained in honeys because of migratory beekeeping. Even the same

honey samples contained pollen from the plants that bloom during different months of the year such as *Brassica, Litchi chinensis, Bombax ceiba* (February-March). *Nepeta spp* (May-August). *Fagopyrum esculentum, Helianthus annuus* (August-September). Therefore, except for a few samples which had pollen mainly from subtropical plants that bloom during February-March, it was not possible to ascertain the geographical origin of commercial beekeepers honeys.

Brassica spp found in all honey samples predominating 8 of 11 samples. In remaining 3 honey samples there was not any predominant pollen type. There the combination of *Brassica- Citrus, Brassica-Helianthus annuus* and *Brassica-Citrus-Melia azedarach* occurred as secondary pollen type. *Fagopyrum esculentum, Citrus spp, Trifolium spp, Psidium gaujava, Circium spp* and Myrtaceae pollen were occurred as important minor pollen type. Sharma (1989) also found the similar type of association- *Fagopyrum spp, Helianthus spp* and *Trifolium spp* in her honey samples in Himachal Pradesh of India. The apicultural importance of all these plants in the honey production of Nepal is already highlighted by Kafle (1984, 1992) and Partap (1997).

Brassica spp, Fagopyrum esculentum, Elscholtzia spp and Myrtaceae pollen were recorded in more than 60% of honey samples. The first two genera are already discussed in Section 4.5.1. *Elscholtzia* and various species of Myrtaceae (eg *Callistemone, Eucalyptus*) are well known to the beekeepers and provide abundant source of nectar for bees. The other very frequent pollen types which were present in more than 45% of honey samples were *Citrus spp, Helianthus annuus, Nepeta spp* and *Litchi chinensis* (*figure 4.3*). Total 34 types of pollen were recorded in 11 honey samples analysed. The number of pollen type per honey sample ranged between 3 to 16 (average 9.6). The sample which had only 3 types of pollen grains had very high sucrose content (25 g/100 g carbohydrate) suggesting the possible adulteration of honey with table sugar.

4.4.4 Pollen Spectrum of *A. cerana* Honey Samples from Jajarkot District

Jajarkot honey samples were taken from three different tins collected by Jajarkot permaculture programme. There is not any predominant pollen type. *Brassica* and *Citrus* occurred as secondary pollen type in all honey samples. *Aesandra butyracea* which is the most important

geographic indicator of autumn and winter honey produced in Jajarkot district, occurred in all honey samples as important minor type. However, this plant is an important honey source of the area and provide abundant nectar for bees (Kafle, 1984; 1992, Sharma, 1995, Partap, 1997, Joshi, 1998, Joshi and Pechhacker, 1999). This plant blooms from September to middle of the February. After that there begins the honey flow of *Brassica* and *Citrus*. Since the honey samples for the present study were collected in April, the presence of relatively low percentage of *Aesandra butyracea* and high percentages of *Brassica-Citrus* pollen spectrum seems quite reasonable.

Strobilanthes and *Nepeta* pollen were present as important minor pollen type in one sample but in other two samples it occurred as minor pollen type. The other pollen which were present as minor source of nectar are *Fagopyrum esculentum, Helianthus annus, Prunus spp, Pyrus spp, Loranthus spp, Ageratum* type, *Buddleia asiatica, Elscholtzia* type, Myrtaceae and Gramineae. Except *Loranthus* these all pollen types were already mentioned as major, medium to minor source of honey in Nepal (Partap, 1997).

The number of pollen types per honey sample is relatively very high in Jajarkot honey. This could be because in other areas samples were collected directly from the colonies but here the samples were collected from the tins which might be collected from many colonies and mixed together for marketing purposes.

4.4.5 Pollen Spectrum of *A. cerana* Honey Samples from Dadeldhura District, West Nepal

The pollen composition of honeys studied from Dadeldhura district showed 7 predominant pollen types: *Brassica spp, Citrus spp, Rubus spp, Aesandra butyracea, Eupatoreum type, Cedrela toona,* and *Prunus* type. Among these *Brassica spp, Citrus spp, Rubus spp* occurred very frequently (respectively in 74%, 72% and 52%) in the honey samples (*table 4.9, figure 4.3*). The apicultural importance of these three plant species is already highlighted in previous section (see 4.5.1). The presence of *Brassica* and *Citrus* pollen in more than 70% of honey samples, predominating 9 and 8 of 50 honey samples, respectively, indicate their high significance in the honey production of Dadeldhura district. Among the various species of *Citrus, C. aurantifolia* and *C. reticulata* are widely cultivated in Dadeldhura district. One

beekeeper. Chandra Bahadur Karki of Belapur VDC, who provided 12 honey samples for the present study is a commercial orange grower. Therefore, the reflection of *Citrus* pollen in Dadeldhura honey is quite expected.

Aesadra butyracea (Indian butter tree) is the most important honey source of Dadeldhura district (Joshi, 1998; Joshi and Pechhacker, 1999). But, interestingly its pollen were not very frequently occurred in the honey samples. This could be because after the honey-flow of Indian butter tree there begins a spring honey flow from various species of *Brassica, Rubus, Prunus*, etc. Therefore, during the end of January or middle of the February, when flowering of Indian butter tree is over, the beekeepers cut all the combs and, hence, there is no or minimal chance to have its pollen in honeys harvested from February to September.

The pollen morphological structure of *Eupatoreum* type looked quite similar to that of *Eupatoreum odoratum* recorded in Chitwan honeys, both pollen types are small (15 μm) in size, tricolporate and echinate in exine sculpture but the spines in the latter type are thinner than the earlier one. Except predominating one sample, its pollen occurred as important minor or minor pollen type (*table 4.9*). Without knowing exact identity of that plant it would be unwise to say how important this plant is in the production of honey in Dadeldhura district.

Cedrela toona has very short flowering period and is confined only in some areas. Therefore, its pollen though occurred as predominant and secondary pollen type but found only in 6 of 50 honey samples. Similarly, *Prunus* type predominantly occurred in 1 sample and found as secondary pollen type in 5 samples, important minor type in 3 samples and minor in 5 samples. The role of different species of *Prunus* in production of honey is evaluated in section 4.4.7.

The pollen that do not produce any nectar also found frequently in honey samples. For example, *Rumex* pollen were found to be present in 28% of honey samples. This supports the explanation of Vorwohl (1994) that the pollen grains of nectarless flower occurred in honeys because of an admixture of pollen grains during harvesting by bees and extraction by centrifugation or pressing the combs. Similarly, some air borne pollen grains such as *Pinus* were found in 10% of honey samples. These samples had very high electrical conductivity (> 0.7mS/cm). Therefore, it can be assumed that bees might have visited these trees for collecting honeydew.

Myrica esculenta. Asteraceae and Lamiaceae pollen though occurred frequently in honey samples never reach to the predominant level. Their representativity in honey, whether under-represented or over-represented is not found in the literature consulted.

The number of pollen types varied from sample to sample. On average, it came out as 8.2 (ranged from 3-16) per honey sample. Total 53 morphological types of pollen were identified. Of which 25 taxa occurred as minor source of honey.

4.4.6 Pollen Spectrum of Jumla Honeys

The pollen composition of honeys studied from Jumla district (>2000masl) showed 6 predominant pollen types: *Malus* type, Lamiaceae, *Rubus* type, *Brassica spp, Impatiens spp,* and Acanthaceae. Among them *Malus* type, Lamiaceae, *Brassica spp, Rubus spp* occurred in more than 60% of the honey samples (*table 4.9, figure 4.6*). As the pollen grains of many species of Rosaceae show similar structural features, it was not possible to identify its pollen to the genus or species level. It is already accentuated that the identification of Rosaceae pollen is impossible without type grains of comparison (Moore et al., 1991). However, in the present study, for the sake of convenience the pollen having striate exine sculpture and are 25-35 μm in size are grouped under *Prunus* type (*figure41*, Plate7). This type, in addition of *Prunus avium, Prunus persica, Prunus domestica*, may include other similar type of pollen grains. Similarly, *Pyrus pashia, Prinsepia utilis, Pyracantha crenulata, Malus domestica* and other Rosaceae pollen which have smooth exine sculpture and 25-35 μm diameter are represented by *Malus* type (*figure34* ; Plate VI) . And, the pollen having smooth (sometimes rugulate in high magnification) exine and 15-25 μm in diameter are aggregated under *Rubus* type (*figure 44* ; Plate VIII). Other Rosaceae pollen having granular exine and less than 15 μm diameter are included in *Spiraea spp*.

Malus domestica (apple) is the most important fruits cultivated in Jumla district. *Prinsepia utilis* (Dhatelo) is also widely grown as hedgerow plant for its oil yielding seeds. The apicultural importance of all above mentioned species of Rosaceae is already highlighted by different investigators (Crane et al., 1984, Partap, 1997, and references there in). Sharma (1989) reported Rosaceae pollen as predominant pollen type in *A. cerana* honey samples collected during early winter and late winter season in Himachal Pradesh, India. Rao and

Surayanarayana (1983), Singh (1983), Singh et al.(1983) from Uttar Pradesh, and Saraf (1972) from Kashmir valley reported *Pyrus spp, Prunus spp*, as major source of honey in India. *Rubus spp* are reported as important honey source in Pakistan (PARC, 1977). Some species of family Rosaceae also produce extra floral nectar and honeydew (Kloft et al., 1965). The presence of very high electrical conductivity is possibly due to the honeydew origin of these honey samples. The honey potential of different species of Rosaceae show great variation. For example, it is estimated as 30-42 kg/ha from *Malus domestica*, 30-50 kg/ ha from *Rubus fruticosus* (Cirnu, 1980) and 100-300 kg/ha from each *Prunus cerasoides* and *Prunus armeniaca* (Joshi, 1992).

Lamiaceae pollen occurred in 95% of honey samples. Due to lack of reference materials its pollen could not be distinguished to the genus level. All the pollen types having 6 apertures (pores or furrows) and even pollen with 8 furrows (*figure 59*; Plate X) are included in this type. However, the most frequently occurring pollen types were quite similar to that of *Salvia* pollen (*figure 29 ;* Plate V) and *Mentha* pollen (*figure 30 ;* Plate V).

Brassica pollen occurred as very frequent (66%) pollen type. Out of 85 honey samples it occurred as predominant pollen type in 4 honey samples and as secondary pollen type in 15 honey sample. In remaining samples it occurred as important minor or minor pollen type. The apicultural importance of *Brassica* pollen is already highlighted in previous section (see section 4.4.1).

Three types of *Pinus* pollen (most probably from *Pinus wallichiana, Picea smithiana* and *Cedrus deodara*) were found in Jumla honey. Though they present as minor pollen type, occurred frequently (44%) in honey samples. These air borne pollen grains could have mixed with honey while collecting honeydew by bees from these tree. Bees collecting honeydew from *Pinus wallichiana* and *Picea smithiana* have already reported in Jumla district (Joshi et al., 1998).

Impatiens spp and Acanthaceae pollen types though reached to the predominant level, did not occur very frequently (<25%) in honey samples. The other pollen types which occurred in more than 25% of honey samples are : Asteraceae, *Taraxacum officinale*, Cucurbitaceae and Zygophyllaceae.

Pollen grains stand out as geographic indicators of honey from Jumla district: *Rhododendron, Prunus, Malus, Pinus*, Lamiaceae (*figure 64* ; Plate XI). The number of pollen types per honey sample ranged from 4 to 15 (8 on average). Total 43 distinct morphological types of pollen were identified. However, this number did not represent all individual plant species contributing in honey production.

4.4.7 Pollen Spectrum of *A. cerana* Honeys from Langtang Area

Seven honey samples collected from Syabru basin and two honey samples; one each from Tistung and Daman were grouped under Langtang honeys. However, Tistung and Daman areas are located at 1800masl and 2300masl, respectively, whereas Syabru basin area is located at c.2500masl. Therefore, the pollen spectrum of Tistung and Daman areas were markedly different from those of Syabru basin. In Tistung honey *Guizotia abyssinica* occurred as predominant pollen type. This plant is extensively cultivated in that area and considered as a major source of nectar and pollen for bees. The sugar concentration of its nectar is reported as 29% (Zmarlicki, 1980) and pollen productivity is 4,300 grains/anther (Kalpana and Ramanujam, 1991), which explains its reasonably high percentage in honey sample. This plant is eagerly visited by both *Apis cerana* and *A. mellifera* (Partap, 1997). Kalpana and Ramanujam (1991) reported it as major source of nectar and pollen for *A. florea* bees. In Myanmar, the honey yield of this crop with *A. mellifera* bees is reported as 30kg/colony/season (Zmarlicki, 1980).

In Daman honey, *Guizotia abyssinica* in combination with *Prunus* type occured as secondary pollen type. This association could be because of the more abundance of *Prunus* species (eg *P. cerasoides, P. armeniaca, P. domestica, P. persica*) and less abundance of *Guizotia* plant in Daman area. *Prunus* type and *Alnus* type were common in both honeys, which could be useful for designating geographical origin of these honey. The other pollen types recorded in Tistung honey are *Taraxacum officinalis, Ageratum type, Brassica spp, Amaranthus* spp and Gramineae. And, in Daman are *Myrica esculenta, Caryopteris spp, Raphnus sativus, Quercus spp* and Papaveraceae. Among them *Brassica spp, Taraxacum officinalis, Ageratum type* and *Myrica esculenta* are considered as major to medium source of honey for bees (Crane et al., 1984, Partap, 1997).

In Syabru basin area there is not any predominant pollen type. The pollen grains of various species of Rosaceae occurred in all the honey samples. Like in Jumla honeys, these pollen types were grouped under four categories ; *Prunus* type, *Malus* type, *Rubus* type and *Spiraea* type. Among them *Prunus* type, *Malus* type and *Rubus* type of pollen occurred very frequently in honey samples. The apicultural importance of various species of Rosaceae is already highlighted in previous section (4.5.6).

Brassica pollen occurred as most frequent (66%) pollen type. Out of 9 honey samples it occurred in 5 honey samples as secondary pollen type and in one honey sample as minor pollen type. The apicultural importance of *Brassica* pollen is already highlighted in previous section (see section 4.4.1). *Myrica esculenta* occurred as secondary pollen type in one honey sample but absent in other samples. Lauraceae pollen also occurred as secondary pollen type in one sample but did not retain from other samples.

Interestingly, 4 honey samples from Syabru basin area had exactly similar type of pollen spectrum. There the combination is *Malus-Brassica* as secondary pollen type, *Rhododendron-Rubus* as important minor pollen type and *Taraxacum officinalis, Raphanus sativus*, Asteraceae and *Rhododendron campanulatum* as minor pollen type. Therefore, doubts can be expressed whether these four samples were collected from the same colony and put into four different glasses or it happened by a coincidence. In all other honey samples pollen spectrum were markedly different from each other.

Pollen grains of flowering plants stand out as geographic indicators of honey types from Langtang area : *Rhododendron, Prunus, Malus, Myrica, Berberis spp.*

Epilogue

The principal concern of the present study was to evaluate the quality of honey harvested from different bee species and to identify the important honey plant sources for bees in different eco-zones of Nepal. Chapter One provides an introduction to the country and highlights the importance of beekeeping in providing food, nutrition and ecological benefits. Chapter Two focuses on honeybee species, honey hunting and beekeeping practises. Chapter Three deals with the physico-chemical properties of honeys. Chapter Four concerns mainly with the honey-pollen analyses. The main conclusions derived from each of these chapters are as follows:

Beekeeping as a sustainable source of income for mountain communities

Nepal is predominantly a mountainous country where almost 90% of the population depends upon subsistence farming. However, the cultivable land in Nepal is very scarce and the land holdings is getting smaller, divided and subdivided as the families grow larger. At present, the Nepalese people are facing a near crisis situation; as the demand is growing (i.e. population is increasing at the rate of 2.6% per annum), the labour per yield increasing, but the total per capita yield is decreasing. To fulfil the immediate food requirement of rapidly increasing human population, people are destroying the forest for growing rice, wheat and other crops, which resulted the lack of forage, loss of habitat as well as erosion, land slides and flood. This situation has created a need to explore off-farm based and environmentally sustainable activities which can contribute to generate food, nutrition and cash income. Beekeeping is one of such options which provides not only nutritious food and cash incomes, but also assists the increased agricultural production of various crops through pollination.

Nepal has great potential for beekeeping/apiculture development

Nepal has at least 4 indigenous honeybee species, viz. of *Apis cerana, A. laboriosa, A. dorsata* and *A. florea*. Honey hunting from wild nests of *A. laboriosa, A. dorsata* and *A. florea* and beekeeping with *A. cerana* form an integral part of cultural heritage in Nepal. *A. cerana*, like European honeybee, *A. mellifera*, can be kept inside hives and managed for commercial honey production and pollination. It is better adapted to the local environments

and can survive well up to 3000masl. Unlike exotic bee *A. mellifera*, it is capable of fighting various predators (*Varroa* mites, wasps) and needs less chemical treatments. However, this species has not become popular among the commercial beekeepers because of its frequent swarming and absconding behaviour. In recent years, some beekeepers have imported a large number of *A. mellifera* colonies to the country. So far, this species is doing well in some tarai districts, like Chitwan, but in the hills and mountains the performance of *A. mellifera* seems very poor.

Because of the diseases and parasitic mites that introduced with exotic honeybees, *A. mellifera,* the population of indigenous honeybee species has been rapidly declining. Evidence from other regions of Asia suggest that *A. mellifera* and *A. cerana* can not coexist in the same ecological habitat. Therefore, zonation of areas for apiculture, with these exotic and native species of honeybee species, is essential if beekeeping with both the species is to be promoted. Otherwise, it would be the best to improve the economic characters of native bee *A. cerana* by selection and breeding programme rather than the importation of exotic *A. mellifera.*

Why and how physico-chemical and melissopalynological analysis of honey

The quality of honey is generally evaluated by determining the chemical constituents that exist in it. Several of these constituents, viz. moisture percentage, sugar content, enzymes, proline content etc. are of great importance in honey industry as they influence its keeping quality, granulation, texture as well as its nutritional and antibacterial efficiency. Therefore, to get good quality honey for a better market price it is necessary to have information on its physico-chemical properties. Similarly, designating honey by geographical, topographical or botanical names has a huge influence on the retail price of the particular honey. In many countries, floral origin of a honey is important since premium prices are paid for honeys of specific floral origin. The knowledge about the bee flora is also essential for the production of surplus honey and to manage healthy bee colonies for pollination. However, no systematic work on honey pollen analysis has so far been carried out in Nepal (Kerkvliet, 1994).

The present study was carried out to evaluate the physico-chemical and melissopalynological properties of honey samples collected from different bee species and from different eco-zones

of Nepal. The samples were analysed for moisture content, pH, electrical conductivity, invertase activity, proline content and glucose oxidase in accordance with the harmonized methods of the European Honey Commission (Bogdanov et al., 1997). Sugar spectrum were analysed by HPLC based on German Institutes for Norms (DIN 10758) and the pollen analysis was carried out without acetolysis (Louveaux et al., 1978).

Each honey has some of its own characteristic properties

The physico-chemical analyses of honeys collected from *A. dorsata, A. cerana* and *A. mellifera* colonies in Chitwan district, Nepal showed some characteristic differences in their properties. For example, *A. dorsata* honey samples possess significantly higher ($P<0.05$) amount of electrical conductivity, enzyme activity and proline content than in *A. cerana* and *A. mellifera* honeys. Similarly, in *A. cerana* honey samples the electrical conductivity and invertase activity are significantly higher than *A. mellifera* but the proline content is very low. Therefore, by measuring enzyme activity and proline content it could be possible to distinguish *A. dorsata, A. cerana* and *A. mellifera* honey.

Some di- and trisaccharide sugars are also characteristically different from species to species. The oligosaccharide-L_2 was only recorded in the samples which had more than 0.7 mS/cm electrical conductivity; the limit of electrical conductivity which has been proposed for honeydew honey than (<0.7mS/cm) for nectar honey. Therefore, this sugar could be useful to confirm the origin of honey, whether floral or honeydew.

The analytical values obtained for *A. cerana* and *A. mellifera* honeys collected from Kathmandu valley also agree with the results obtained for Chitwan honeys. *A. cerana* honeys collected from different altitudes (viz. tarai, hills, mountains) and from supermarket in Kathmandu (source is not known) also showed some of their own characteristic properties. Honey from supermarket produced by commercial beekeepers did not meet some of the quality criteria of Codex Alimentarius Commission; most of the samples had very low enzyme activity, and lower amount of proline. Some of their honey samples contained very high amount of sucrose content (up to 25g/100g sugars) and HMF (up to 800ppm) which suggest the possible adulteration of honey with table sugar and overheating. The enzyme

glucose oxidase which is known to be responsible for the antibiotic activity of honey is found to be significantly higher in honeys collected from Jumla and Langtang (mountainous areas).

A. florea, A. laboriosa, Melipona and *Trigona* honeys also showed some specific characteristics in their honey. The moisture content in their honeys exceeded the maximum permissible level (21%) for honeys by the Codex Alimentarius Commission. Generally, it is considered that the honeys having more than 21% moisture content are not safe from fermentation, but the presence of very high enzyme activity, which keeps the raw materials (nectar or/and honeydew) safe while they are being converted into honey (Crane, 1990), seems to be sufficient to prevent fermentation. In Nepal, *A. florea* honey has traditionally been used to cure several diseases and considered as superior quality. The presence of very high amount of glucose oxidase (29.6 $\mu gH_2O_2/g/min$ on average) supports this concept. In all species of genus *Apis* and *Melipona,* monosaccharides; fructose and glucose occurred as a predominating sugars and comprises more than 80% of total sugars. Whereas, in *Trigona* honeys disaccharides occurred as principal sugar and comprises 62% of total carbohydrate. Therefore, it could be possible to distinguish *Trigona* and *Melipona* honeys by carrying out sugar analysis of their honeys.

The pollen spectrum of *A. dorsata, A. cerana* and *A. mellifera* honeys studied from Chitwan district showed 7 predominant pollen types: *Eupatorium odoratum, Brassica spp, Litchi chinensis, Syzygium spp, Mangifera indica, Fagopyrum esculentum* and *Shorea robusta.* Of these, the first three taxa were present as very frequent pollen types in honeys from all bee species. The number of pollen types per honey sample was significantly higher ($p<0.005$) in *A. dorsata* and *A. cerana* compared to *A. mellifera.* It was also found that native bee species *A. dorsata* and *A. cerana* visit more wild and native plants, whereas introduced European honeybee, *A. mellifera* visits more exotic plants and cultivated crops.

The pollen composition of honeys from Kathmandu valley showed 10 predominant pollen type: *Brassica, Buddleia, Rubus, Trifolium, Vicia, Viburnum, Eucalyptus, Ziziphus* and Graminae. Of these *Brassica, Buddleia, Rubus, Trifolium* and *Vicia* are well known to the beekeepers and definitely a good source of nectar for bees. The samples which had Graminae pollen as predominant pollen type, had high electrical conductivity, suggesting the possible origin of this honey from honeydew. The pollen spectrum of Jajarkot honeys did not show

any predominant pollen type. There *Brassica, Citrus, Aesandra butyracea, Strobilanthes* and *Nepeta* formed major part of honey. Honey samples collected from Dadeldhura district revealed 7 predominant pollen types; *Brassica, Citrus, Rubus type, Aesandra butyracea, Prunus* type, *Eupatorium* type and *Cedrela toona.*

In Jumla honeys *Malus* type of Rosaceae pollen and Lamiaceae pollen occurred in more than 70% of samples predominating 18 & 10 samples, respectively. *Rubus* type and *Brassica* pollen were also occurred very frequently in honeys and predominated 5 and 4 honey samples, respectively. *Impatiens* and Acanthaceae pollen though present in less than 25% of honey samples, reached to the predominant level in each of one honey sample. Similarly, the pollen spectrum of Langtang honeys showed only one predominant pollen type: *Guizotia abyssinica.* Present as secondary pollen in these were *Guizotia, Prunus, Malus, Brassica, Myrica, Rubus* and Lauraceae.

The results of the present study can be summarised as; (1) Honey from native bee species have higher invertase activity than exotic bee, *A. mellifera,* (2) Native bees visit more wild and native plants, whereas, exotic bee visit more cultivated herbs and exotic plants, (3) Honey samples from *A. dorsata* colonies nesting in the same tree have more or less same analytical values, suggesting the possible inter-colonial communication for searching botanical resources, (4) Some honey samples from commercial beekeepers did not meet the quality criteria of Codex Alimentarius Commission, (5) *A. cerana* honey samples collected from different ecozones also show some differences in their properties, (6) Honey samples from *A. laboriosa, A. florea, Trigona* and *Melipona* have more than 21% moisture content (7) Honey samples collected from different ecozones have some of their own characteristic pollen types which could be useful for designating geographic origin of honeys.

Annexes

Annex 1 Honeydew Plants of Nepal*

Common name	Species/Family	Distribution	Economic importance	Honeydew excreting insects	Literature/Authors (cf)
1. Spruce	*Picea smithiana* (Pinaceae)	Temperate (2200-3200masl)	Timber, fuel wood	*Cinara comater* Doncaster*, *Cinara costata* Zetter & *Cinara piceae* Panz.	*Joshi et al, 1998 Ricciardelli D'Albore, 1997; 1998
2. Blue pine	*Pinus wallichiana* (Pinaceae)	Temperate (2100-3600masl)	Timber, fuel wood, turpentine, resin	*Cinara eastopi* Pintera & other species of *Cinara*	Joshi et al., 1998
3. Poplar	*Populus spp* (Salicaceae)	Sub trop-Temperate (1500-2500masl)	Timber, pulp wood	*Chaitophorus populeti* Panzer & *C. tremulae* Kock	Ricciardelli D'Albore, 1998
4. Willow tree	*Salix spp* (Salicaceae)	Sub tropical-Temperate	Timber, fuelwood	*Tuberolachnus salignus* Gmelin	Ricciardelli D'Albore, 1998
5.Sugarcane	*Saccharum officinarum* (Gramineae)	Tropical-Sub tropical	Sugar, cane sucked as sweet meat & molasses	*Melanaphis sacchari* Zehntner & *Perkinsiella saccharicida* kirkaldy	Crane et al., 1984, Partap, 1997
6. Oak tree	*Quercus spp* (Fagaceae)	Subtropical- Temperate (1500-2500masl)	Timber, fodder	*Tuberculatus annulatus* Hartig, *T. borealis* Krezywiecz* *Kermes quercus* L., *Lachnus iliciphilus* Del Guercio, *L. roboris* L., *Thelaxes dryophila* Schrk.	Crane et al., 1984 ; Kloft et al., 1965, Partap, 1997 * Ricciardelli D'Albore, 1998
7. Eucalyptus	*Eucalyptus spp* (Myrtaceae)	Tropical-Sub tropical	Timber, fuel wood, gum, oil	not specified	Crane et al., 1984
8. Pears	*Pyrus communis* (Rosaceae)	Subtropical-Temperate	Edible fruits	*Cacopsylla pyricola* Foerster	Kloft et. al., 1965
9. Broad-bean	*Vicia faba* (Leguminosae)	Tropical-Sub tropical	Protein rich food	*Megoura viciae* Buckt	Kloft et. al., 1965
10. Potatoes	*Solanum tuberosum* (Solanaceae)	Tropical-Alpine	Staple food, vegetable	*Planococcus citri* Risso	Kloft et. al., 1965

Annex 1 Honeydew Plants of Nepal*

Common name	Species/Family	Distribution	Economic importance	Honeydew excreting insects	Authors (cf)
11. Roses	*Rosa spp* (Rosaceae)	Tropical-Temperate	Ornamental; cultivated or wild	*Macrosiphum rosae* L.	Kloft et. al., 1965
12. Chestnut	*Castanea sativa* (Fagaceae	Sub tropical	Nuts, timber	*Lachnus roboris* L.* *Myzocallis castanicola* Baker	*Fossel, 1958 Crane et al., 1984
13. Wheat	*Triticum aestivum* (Gramineae)	Tropical-Temperate	Staple food	*Sitobin avenae* F.	Ricciardelli D'Albore, 1998
14. Mays	*Zea mays* (Gramineae)	Tropical-Temperate	Staple food	not specified	Crane et al., 1984, Partap, 1997
15. Barley	*Hordeum vulgare* (Gramineae)	Subtrop-Temperate	Staple food	not specified	Personal observation
16. Mango	*Mangifera indica* (Anacrdiaceae)	Tropical	Edible fruits	not specified	Crane et al., 1984, Partap, 1997
17. Birch	*Betula spp* (Betulaceae)	Sub trop -Temperate	Timber, fuel wood	*Betulaphis spp*	Ricciardelli D'Albore, 1997
18. Walnut	*Jugles regia.* (Juglandaceae)	Temperate	Edible fruits, hard wood for furniture	*Callphis juglendis* Goeze	Ricciardelli D'Albore, 1997
19.Falseacacia	*Robinia pseudoacacia* (Leguminosae)	Subtropical-Temperate (1600-2200)	Fuel wood, fodder	*Aphis craccivora* Kock*, *Parthenolecanium corni* Bouch	*Ricciardelli D'Albore, 1997 Kloft et al., 1965
20. Juniper	*Juniperus communis* (Cupressaceae)	Sub tropical-Temperate	Timber, fuel wood	*Cinara cupressi* Buchton	Ricciardelli D'Albore, 1997
21. Elm	*Ulmus spp* (Ulmaceae)	Sub tropical-Temperate	Fuel wood	*Erisoma ulmi* L.	Ricciardelli D'Albore, 1997

Annex 1 Honeydew Plants of Nepal*

Common name	Species/Family	Distribution	Economic importance	Honeydew excreting insects	Authors (cf)
22. KHIRRO	*Sapium insigne* (Euphorbiaceae)	Sub tropical	Hedgerow plant, medicinal	not specified	Personal observation
23. Lime tree	*Tilia spp* (Tiliaceae)	Subtropical- Temperate	Fuel wood	*Eucalypterus tiliae* L *Pulvinaria vitis* L.	Ricciardelli D'Albore, 1997
24. Fig tree	*Ficus religiosa* (Moraceae)	Tropical-Temperate	Sacred tree of Hindu	*Homotoma ficus* L.	Ricciardelli D'Albore, 1998
25. Maple	*Acer spp* (Aceraceae)	Temperate	Road side plant	*Peryphyllus acericola* Walker	Ricciardelli D'Albore, 1998
26.Citrus	*Citrus spp* (Rutaceae)	Tropical-Temperate	Edible fruits	*Aleurothrixus, Planococcus, Icerya purchasi Mask, Coccus pseudomagnoliarum* Kuw, *C. hesperidum* L.	Ricciardelli D'Albore, 1998 Kloft et al., 1965
27. Embelica	*Embelica officinalis*	Sub tropical (<1200masl)	Edible fruits, tannin, medicine, fodder	not specified	Personal observation
28. Alfa-alfa	*Medicago sativa* (Leguminosae)	Tropical	Fodder, Lucerne meal	*Therioaphis trifolli*	Crane et al., 1984
29. Wildcherry	*Prunus cerassoides* (Rosaceae)	Subtropical-Temperate	Timber, fuel wood, religious	not specified	personal observation
30. Wildclover	*Trifolium alexandrinum* (Leguminosae)	Tropical-Sub tropical	Fodder	not specified	personal observation
31. Oleander	*Nerium oleander* (Apocynaceae)	Tropical-Sub tropical	Medicinal & Ornamental	not specified	personal observation

* The above listed plants which are reported as the major source of honeydew in other parts of the world are also widely distributed in Nepal. But except from *Pinus walliciana* Jackson and *Picea smithiana* Wallich , no authentic reports are available about the honeydew excretion from these plant

Annex 2

Plate I: Photomicrographs of some Pollen Grains

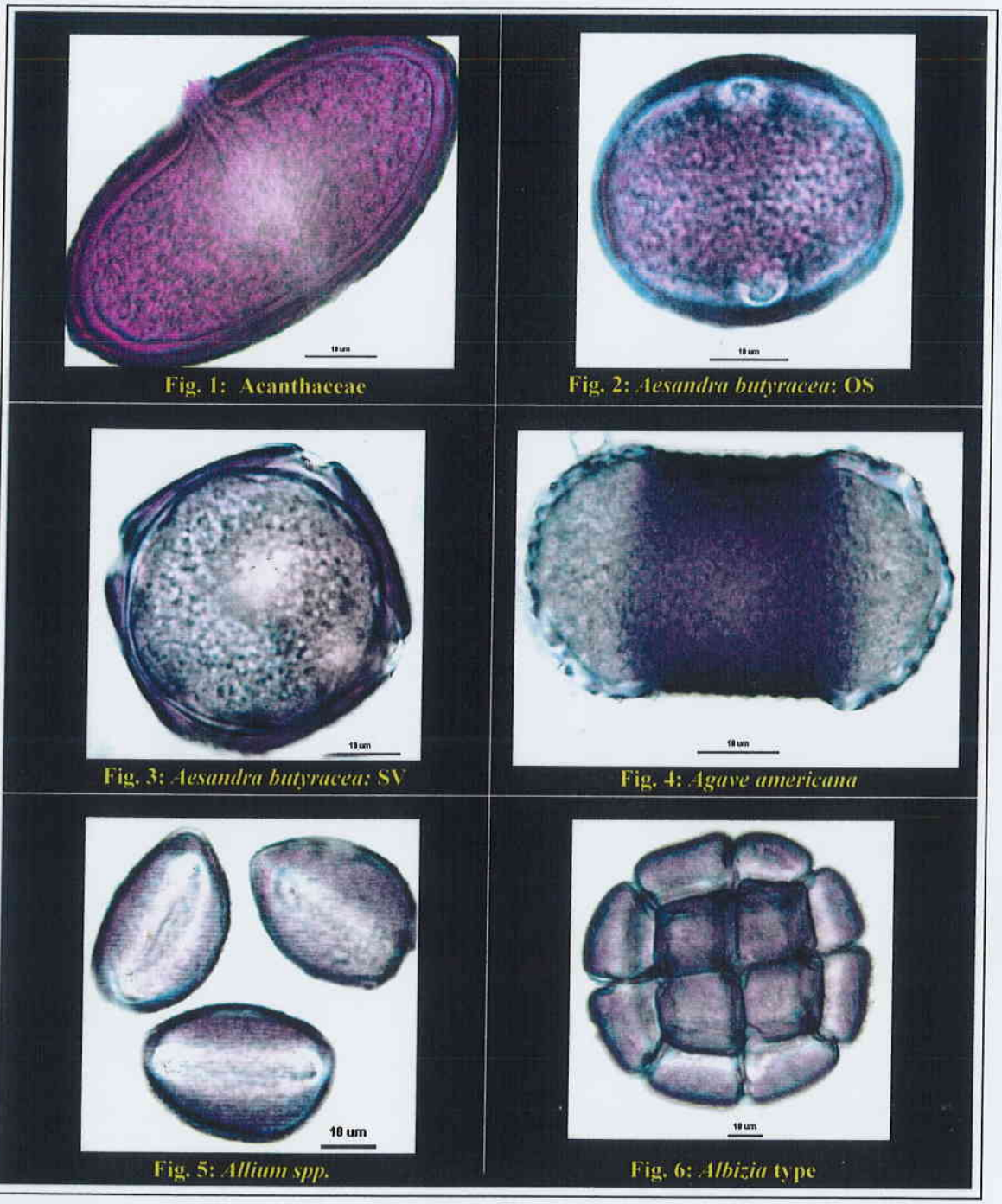


Fig. 1: Acanthaceae

Fig. 2: *Aesandra butyracea*: OS

Fig. 3: *Aesandra butyracea*: SV

Fig. 4: *Agave americana*

Fig. 5: *Allium spp.*

Fig. 6: *Albizia* type

Annex 2

Plate II:Photomicrographs of some Pollen Grains

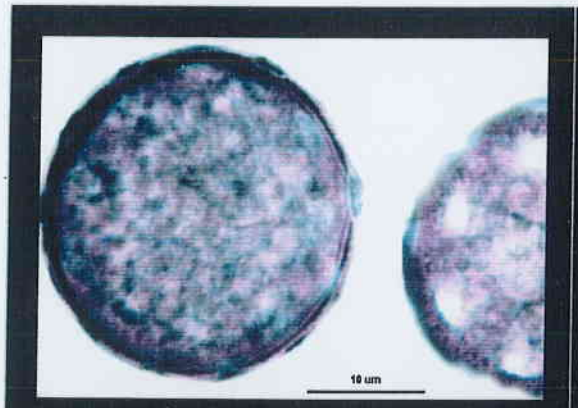


Fig 7 *Amaranthus spp*

Fig 8 *Bauhinia spp*

Fig 9 *Berberis spp*

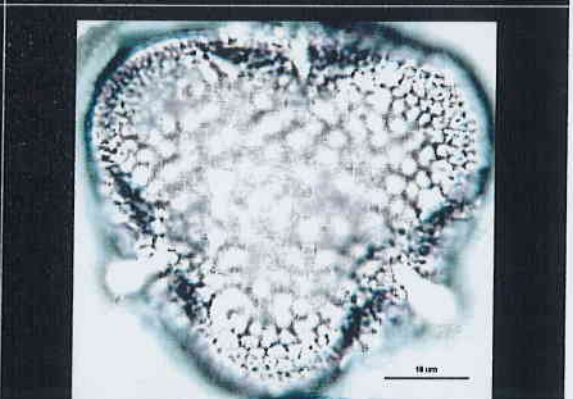


Fig 10 *Bombax ceiba*

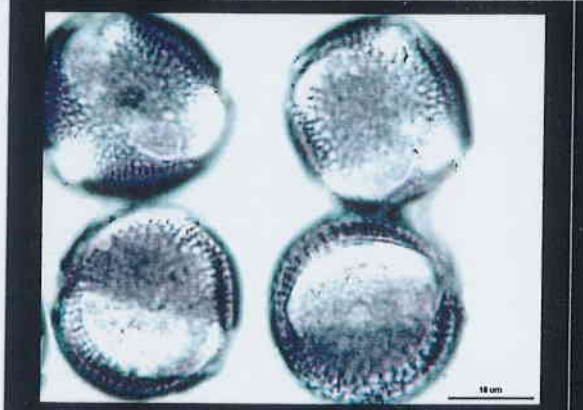


Fig 11 *Brassica spp*

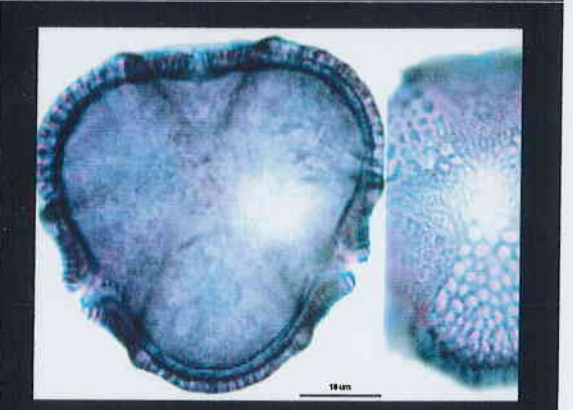


Fig 12 *Caesalpinia spp*

Annex 2

Plate III: Photomicrographs of some PollenGrains

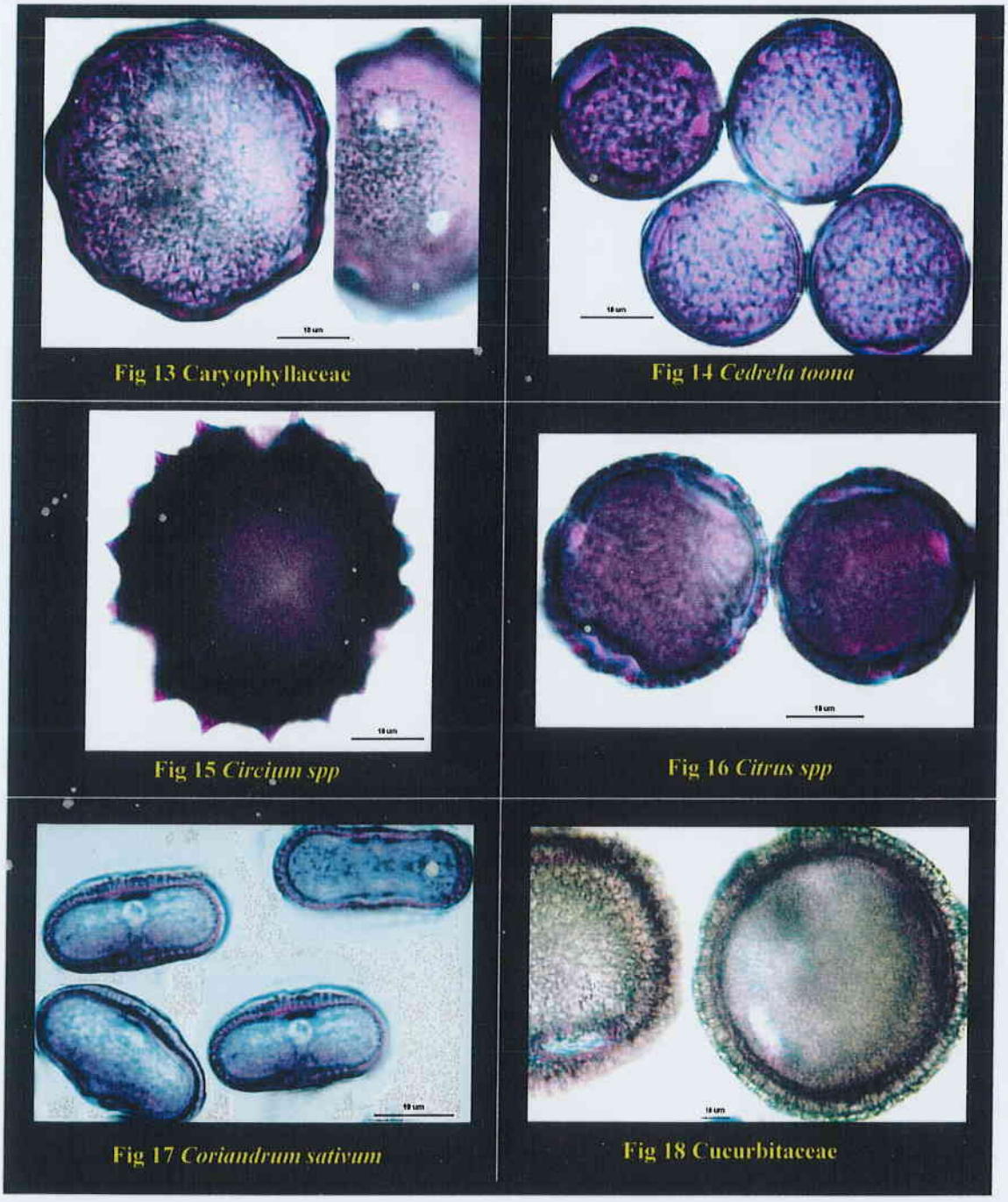


Fig 13 Caryophyllaceae

Fig 14 *Cedrela toona*

Fig 15 *Circium spp*

Fig 16 *Citrus spp*

Fig 17 *Coriandrum sativum*

Fig 18 Cucurbitaceae

Annex 2

Plate IV: Photomicrographs of some Pollen Grains

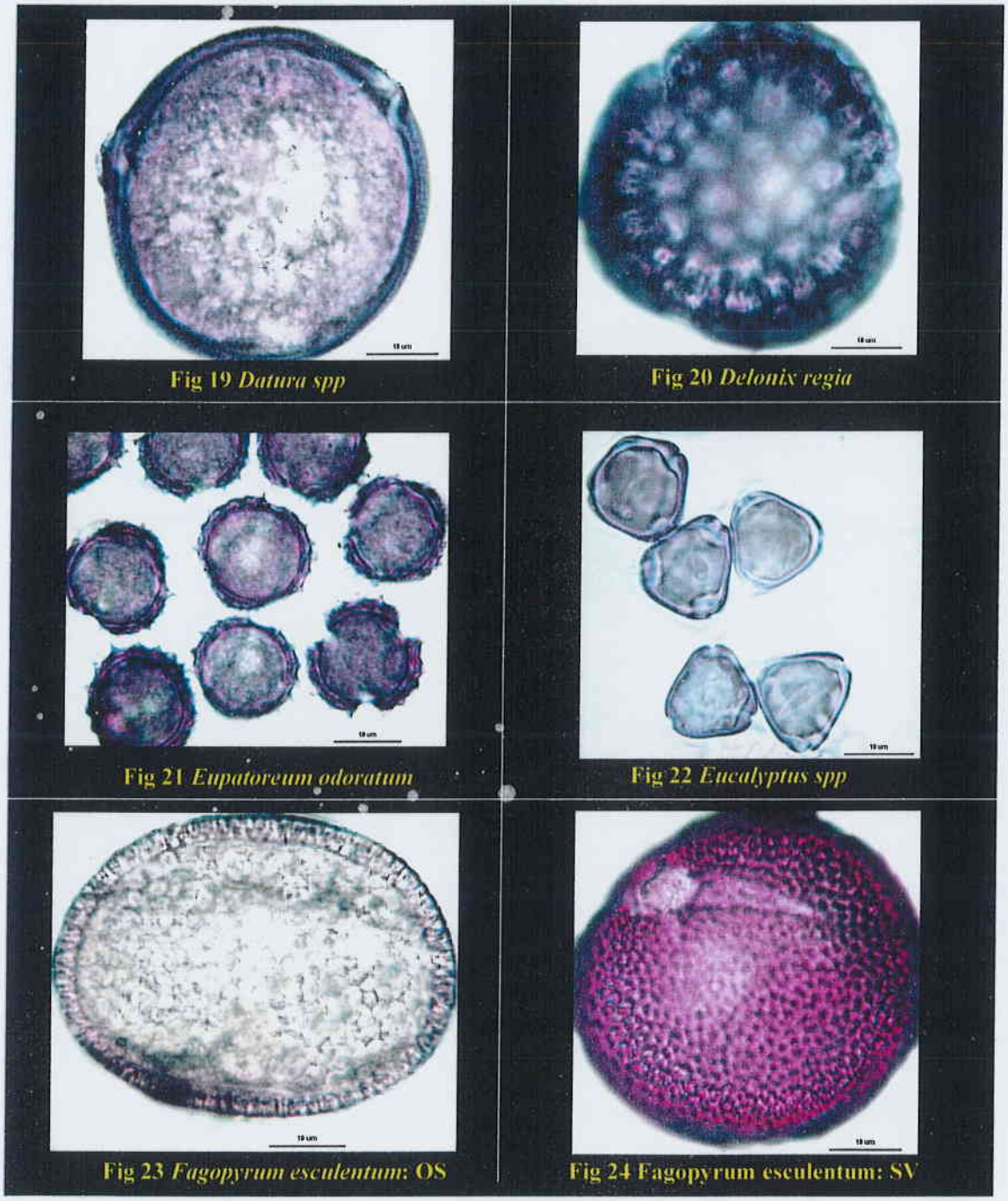


Fig 19 *Datura spp*

Fig 20 *Delonix regia*

Fig 21 *Eupatoreum odoratum*

Fig 22 *Eucalyptus spp*

Fig 23 *Fagopyrum esculentum*: OS

Fig 24 Fagopyrum esculentum: SV

Annex 2

Plate V: Photomicrographs of some Pollen Grains

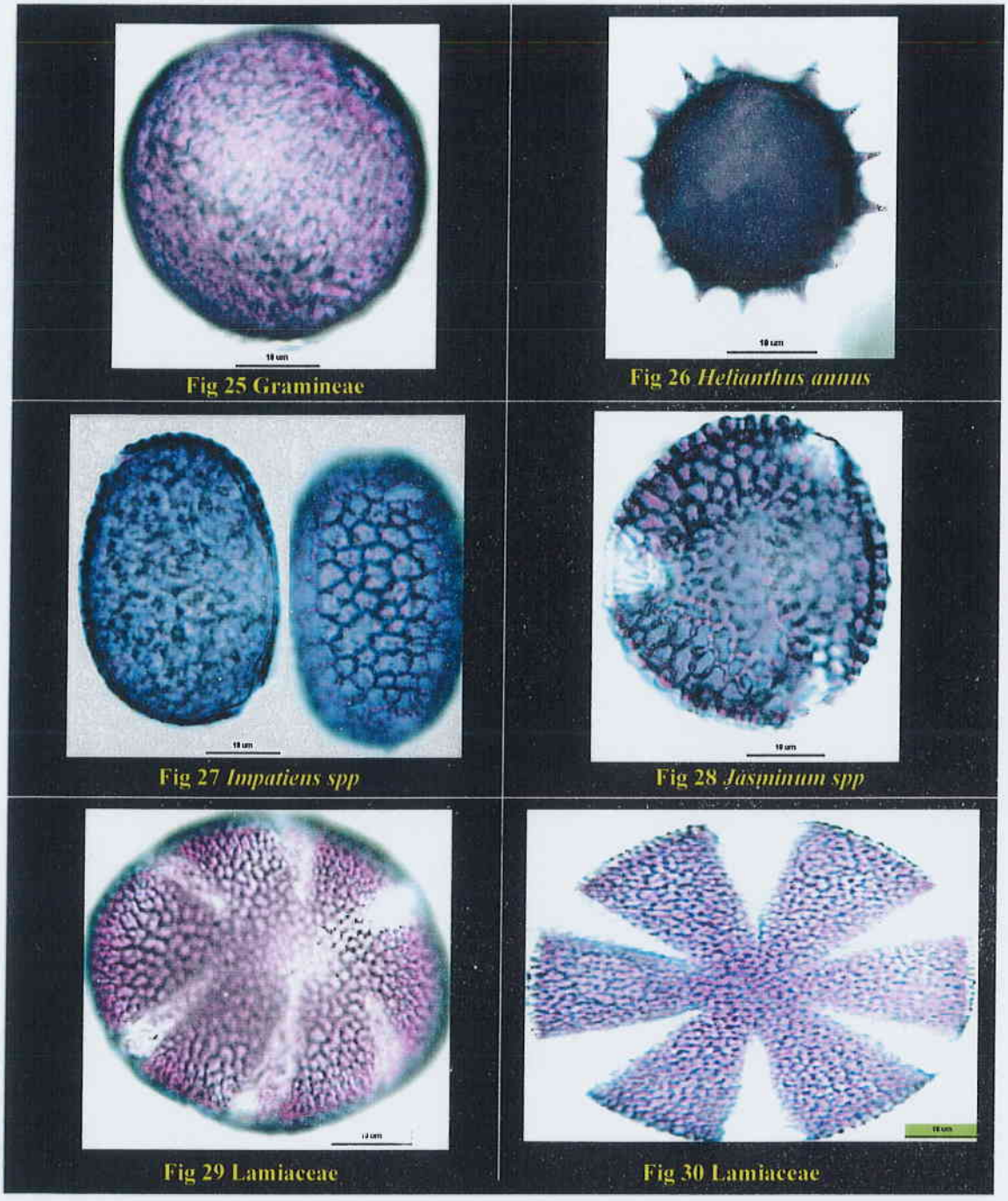


Fig 25 Gramineae

Fig 26 *Helianthus annus*

Fig 27 *Impatiens spp*

Fig 28 *Jasminum spp*

Fig 29 Lamiaceae

Fig 30 Lamiaceae

Annex 2

Plate VI: Photomicrographs of some Pollen Grains

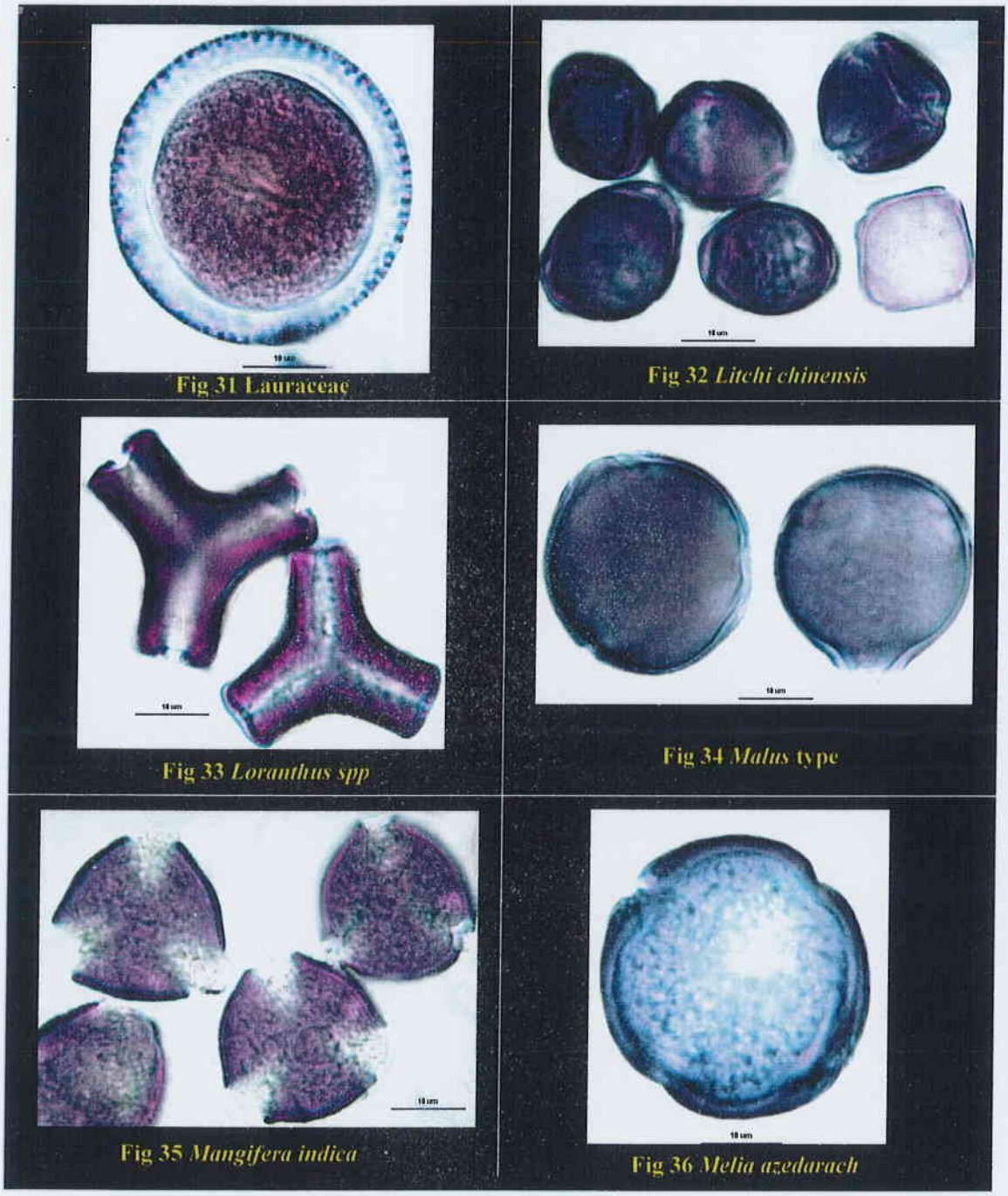


Fig 31 Lauraceae

Fig 32 *Litchi chinensis*

Fig 33 *Loranthus spp*

Fig 34 *Malus* type

Fig 35 *Mangifera indica*

Fig 36 *Melia azedarach*

Annex 2

Plate VII: Photomicrographs of some Pollen Grains

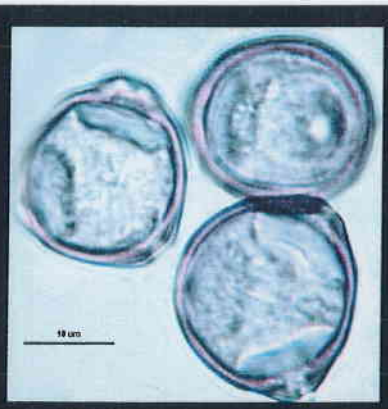


Fig 37 *Myrica esculenta*

Fig 38 *Oenothera spp*

Fig 39 *Emblica officinalis*

Fig 40 *Pinus* type

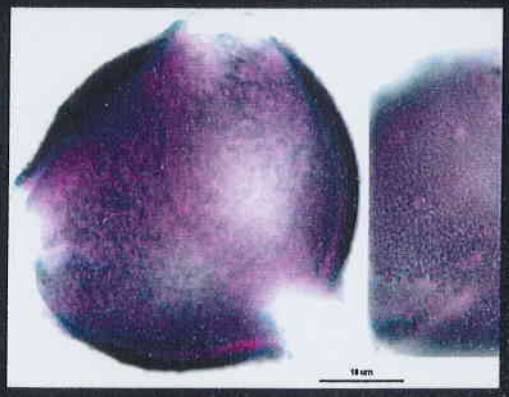


Fig 41 *Prunus* type

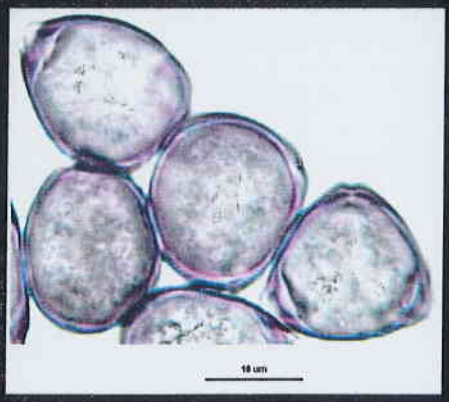


Fig 42 *Psidium guajava*

Annex 2

Plate VIII: Photomicrographs of some Pollen Grains

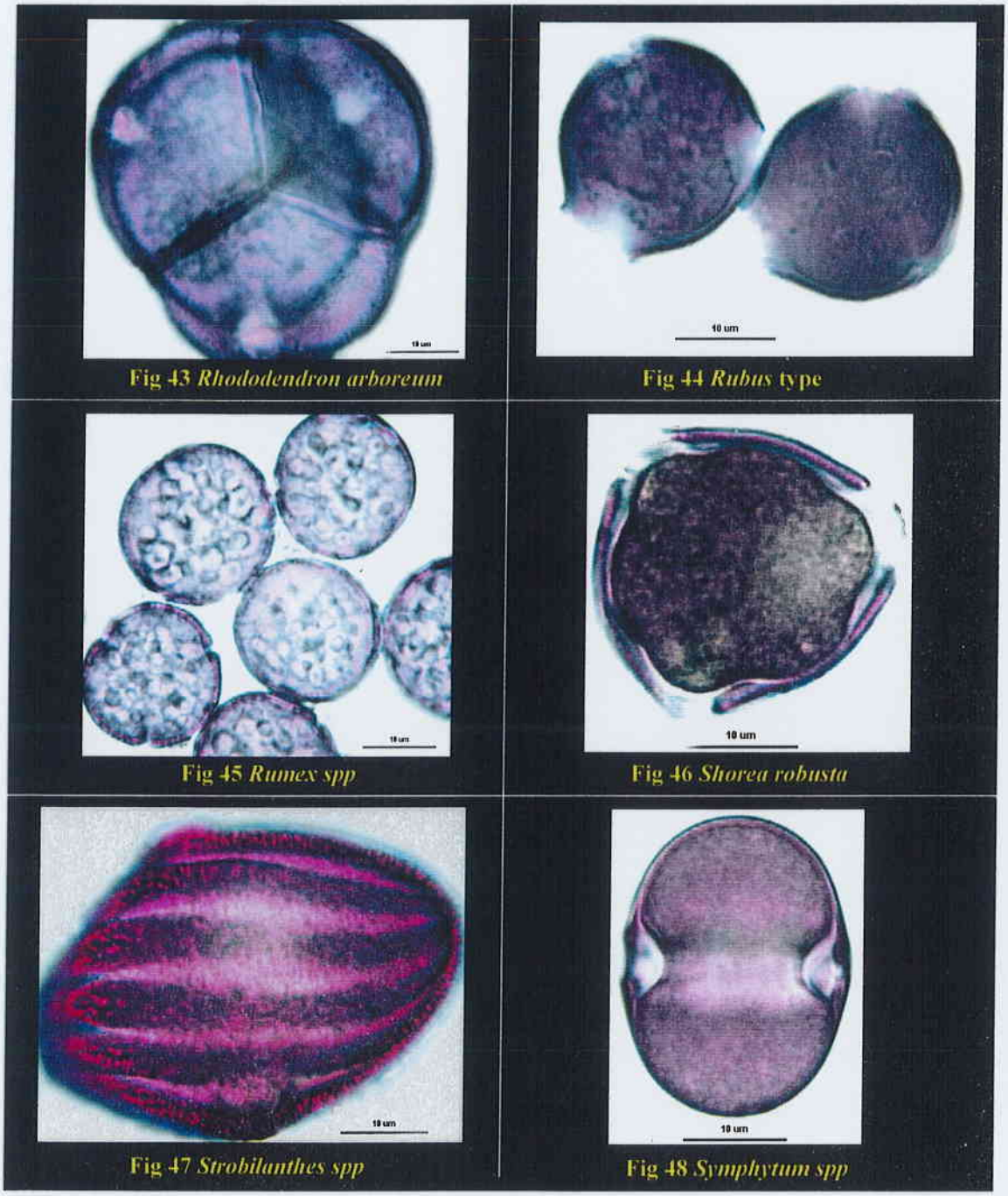


Fig 43 *Rhododendron arboreum*

Fig 44 *Rubus* type

Fig 45 *Rumex spp*

Fig 46 *Shorea robusta*

Fig 47 *Strobilanthes spp*

Fig 48 *Symphytum spp*

Annex 2

Plate IX: Photomicrographs of some Pollen Grains

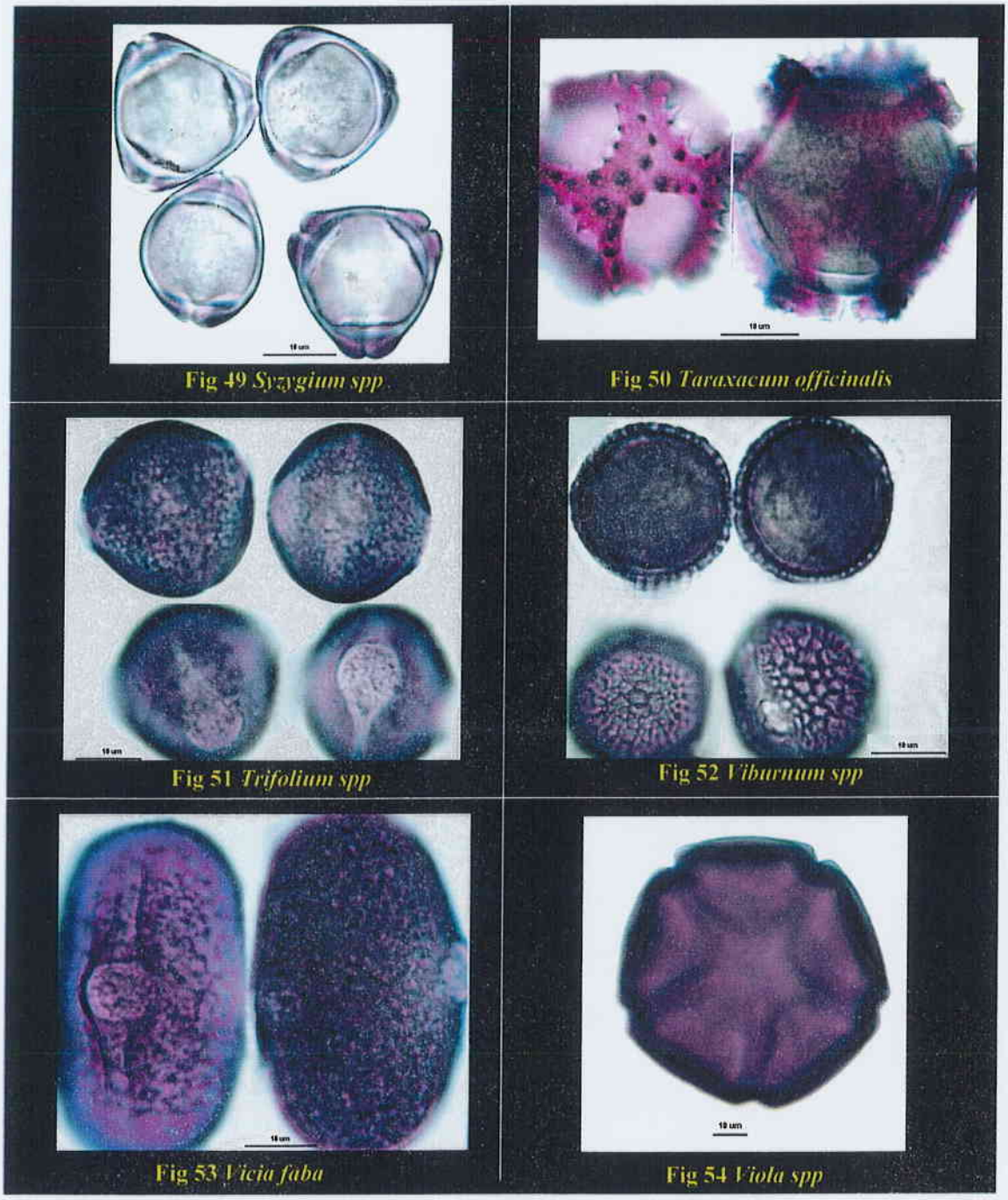


Fig 49 *Syzygium spp*

Fig 50 *Taraxacum officinalis*

Fig 51 *Trifolium spp*

Fig 52 *Viburnum spp*

Fig 53 *Vicia faba*

Fig 54 *Viola spp*

Annex 2

Plate X: Photomicrographs of some Pollen Grains and Spores

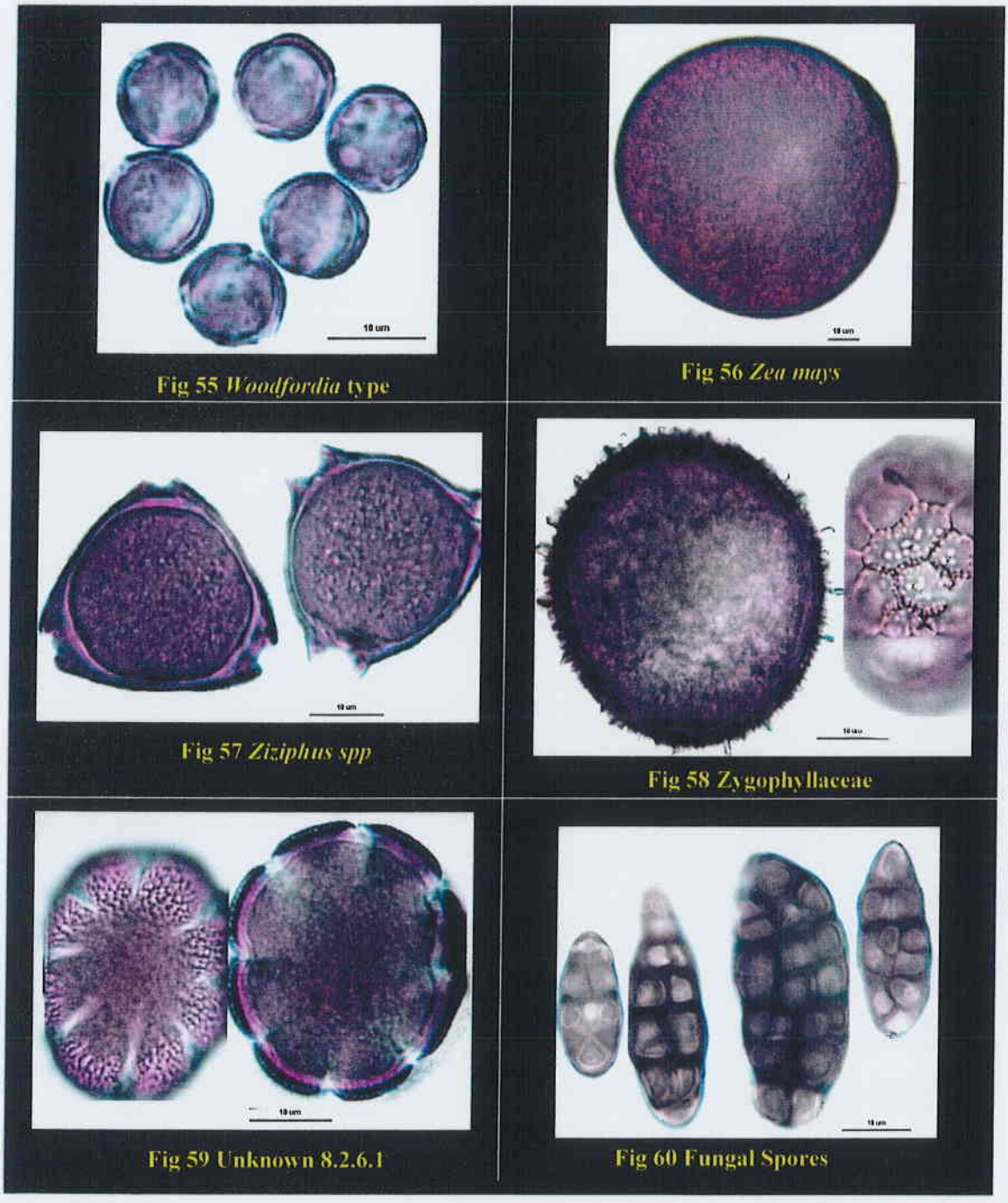


Fig 55 *Woodfordia* type

Fig 56 *Zea mays*

Fig 57 *Ziziphus spp*

Fig 58 Zygophyllaceae

Fig 59 Unknown 8.2.6.1

Fig 60 Fungal Spores

Annex 2

Plate XI: Pollen in Honeys from Different Ecozones

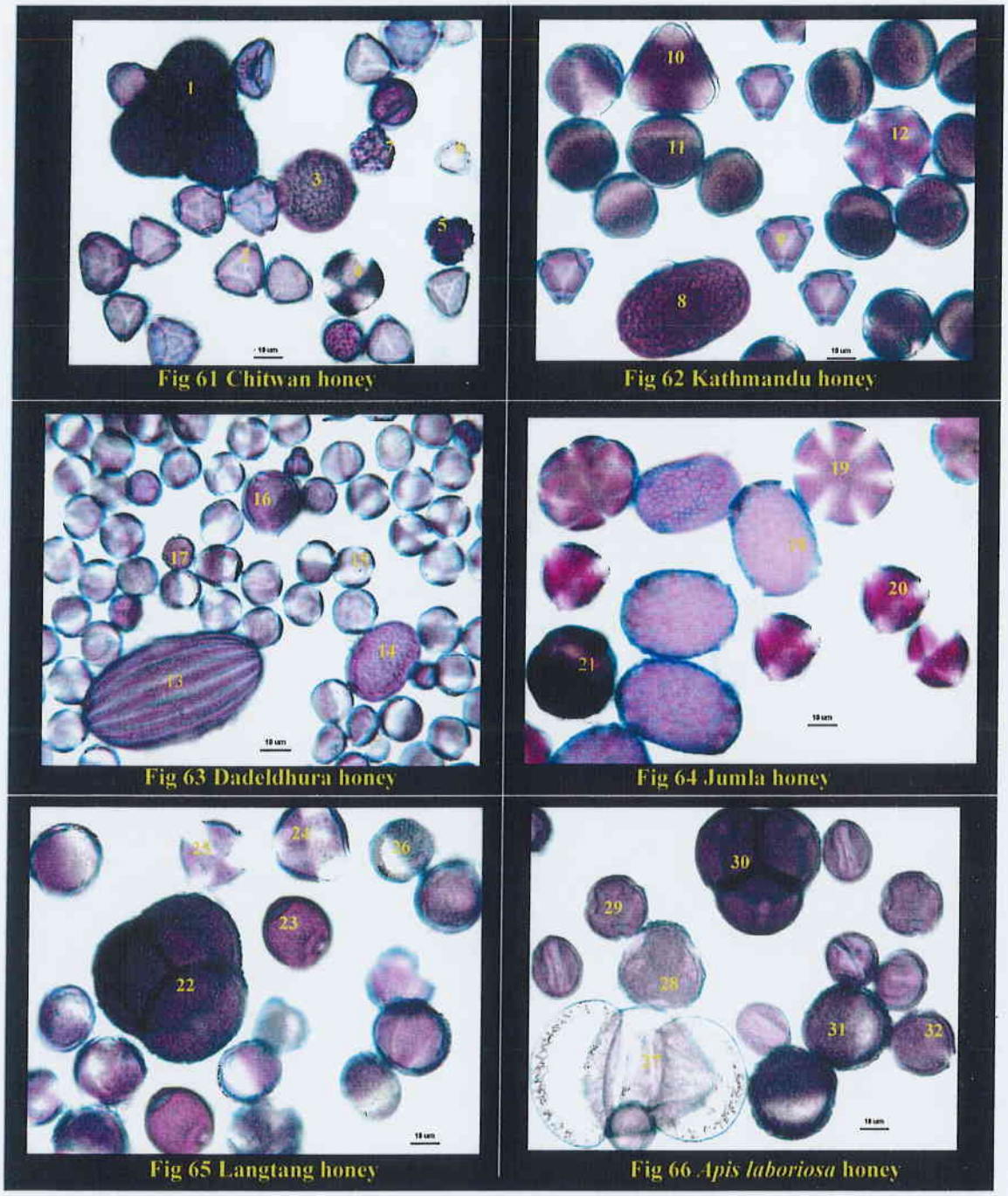


Fig 61 Chitwan honey

Fig 62 Kathmandu honey

Fig 63 Dadeldhura honey

Fig 64 Jumla honey

Fig 65 Langtang honey

Fig 66 *Apis laboriosa* honey

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Zusammenfassung : In der vorliegenden Arbeit wurden die physikalisch-chemischen Parameter von Honigen untersucht. Die Honigproben stammten von verschiedenen Bienenarten aus einer Region und von *Apis cerana* aus verschiedenen Trachtgebieten Nepals. Gemessen wurden Wassergehalt, pH-Wert, elektrische Leitfähigkeit, Invertaseaktivität, Prolingehalt, Glocoxydase und das Zuckerspektrum. Die Ergebnisse zeigten, daß die Honige der einzelnen Bienenarten in bestimmten Merkmalen signifikant unterschiedlich sind. So haben Honige aus dem gleichen Trachtgebiet und zur gleichen Zeit geerntet von *Apis dorsata* und *Apis cerana* eine signifikant höhere elektrische Leitfähigkeit und Invertaseaktivität als die Honige von *Apis mellifera*.

Die Pollenanalyse zeigte verschiedenes Sammelverhalten der einzelnen Bienenarten. Die heimischen Bienen besuchen mehr heimische Bäume beziehungsweise krautige Pflanzen. Die exotische Biene *Apis mellifera* dagegen besucht vorwiegend exotische und kultivierte Pflanzen. Die heimischen Bienen *Apis dorsata* und *Apis cerana* besuchen auch signifikant mehr Pflanzenarten als *Apis mellifera*. Die Pollenspektren der Honige gesammelt aus Tarai, Mittelgebirge, den Bergen und von Supermärkten sind deutlich verschieden. Jede Ökozone weist ganz typische Pollenformen auf, die als geographische Indikatoren benutzt werden können.

Summary: The present study was carried out to analyse the physico-chemical properties of honeys collected from different bee species and to identify the important honey plant sources for bees in different ecozones of Nepal. The samples were analysed for moisture content, pH, electrical conductivity, invertase activity, proline content, glucose oxidase and sugar spectrum. The results showed that the honey from each bee species has some of its own characteristic properties. Within the same floristic area, honey from native bee species, *Apis dorsata* and *A. cerana* have significantly higher amount of electrical conductivity and invertase activity than that of exotic, *A. mellifera*.

The pollen analysis of honey showed that the foraging preferences of each bee species is different. Native bees visit more native trees and wild plants. Whereas, exotic bee species, *A. mellifera* visits more cultivated crops and exotic plants. The number of pollen types per honey sample were found to be significantly higher in *A. dorsata* and *A. cerana* than in *A. mellifera*. The pollen spectra of honeys collected from tarai, hills, mountains and supermarket are markedly different. Each ecozones has some of its own pollen types which could be regarded as the geographic indicators of honey.

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Biographical Sketch

Surendra Raj joshi was born on April 24, 1968 in Bajhang, one of the most remote districts of Nepal. After having School Leaving Certificate (SLC) from Satyavadi Secondary School in 1984, he attended the Tribhuvan University where he obtained his Bachelor of Science degree (Biological Sciences) in 1989. In 1990, he enrolled in Central Department of Botany of Tribhuvan University and obtained his Master of Science degree in 1992. As a private student of Tribhuvan University, he also studied Law and obtained his Bachelor of Law degree in 1994. In August, 1994 he joined Beekeeping Project at ICIMOD where he engaged in melissopalynological research, collected honey samples, prepared herbariums, established demonstration apiaries at Dadeldhura district, far west Nepal, provided training to the beekeepers and collected indigenous information about bees and beekeeping. From October, 1997, he has conducted his PhD studies in Austria sponsored by Austrian Academic Exchange Service (ÖAD).