

Pollen Analysis of *Apis* Honey, Karnataka, India.

Bhargava. H.R*, Jyothi. J.V.A., Bhushanam. M., and Surendra. N. S.

School of Chemical & Biotechnology,

SASTRA University,

Thanjavur, Tamilnadu, India. 613407

*E-mail: bhargavahr@rediffmail.com

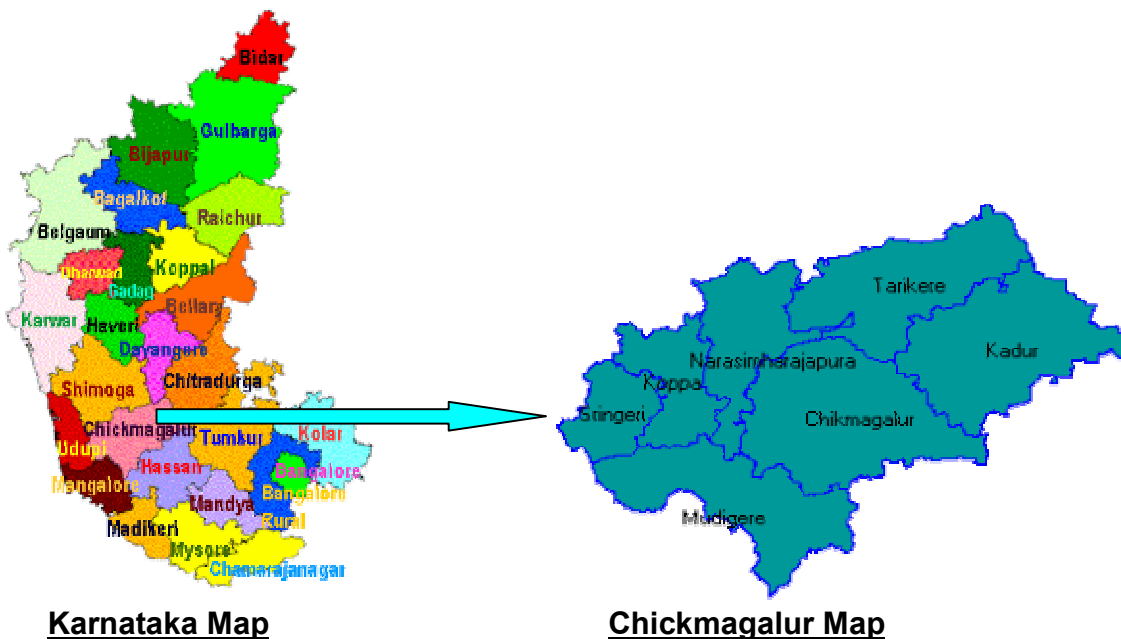
Abstract

Pollen analysis was carried out on ten honey samples from Western Ghats, Chickmagalur District, Karnataka collected during 2005. Sixteen morpho types of pollen were identified. The predominant pollen type was *Coffea sp.* and *Cocos nucifera*. The pollen count ranged from 4000 to 800000. The pollen morphotypes recorded were *Eucalyptus globulus*, *Coffea arabica*, *Cocos nucifera*, *Hevea brasiliensis*, *Coriandrum sativum*, *Areca catechu*, *Syzygium cumini*, *Nerium oleander*, Fabaceae and Poaceae. The data reflects the floral situation of the place where particular honey was produced and the identification of geographical origin based on the presence of a combination of pollen types of that particular area.

Key Words: Pollen, Honey, *Apis*, Western Ghats.

Introduction

Melittopalynology is an applied branch of Palynology dealing with the study of pollen grains in honey samples and its application in Apiculture. Plant produces nectar and pollen both of which are avidly sought after by the bees to provide nutrition to the colony. Melittopalynology is concerned with the identification of pollen in honeys. Evaluation of plants for their utility as sources of bee forage provides the information needed to assess the potential for beekeeping in an area [1,2]. Melittopalynological studies are thus helpful in bee management and in promoting the beekeeping development. Investigations of pollen analysis of honey samples in India are fragmentary. Significant work has been reported by [3,4,5,6,7].



Karnataka state extends from 11° 51' N to 19° N latitudes and from 74° E to 78° E longitudes. It lies in Deccan plateau with three major physical divisions viz Coast, Malnad and Maidan. The

total geographical area of the state is 1,91,781 sq Kms of which 54.70% as net cultivated area, 16.14% forests, 10.66% not available for cultivation, 9.54% uncultivated land and 8.96% fallow land. Chickmagalur district lies in the west of Karnataka state with Chickmagalur as District head quarters between $12^{\circ}51'$ & $13^{\circ}54'N$ latitude and between $75^{\circ}51'$ & $76^{\circ}22'E$ longitude. The main purpose of the district is compared by the most mountain regions included within the limits of Karnataka, bordered on the west by the Western Ghats. Chickmagalur district is situated in the Malnad (high level) region enjoys a good climate throughout the year and affords a pleasant retreat during the hot months (March & May). The hottest month is March with a mean maximum temperature of $36^{\circ}C$. The vegetation of this area abounds in many valuable medicinally important plants such as *Acacia snuata*, *Artiemia nilagiria*, *Asparagus recemosus*, *Piper nigrum*, *Murraya paniculata*, *Syzygium cumini*, etc. The major cereals are *Euleusine coracana*, *Geartn*, *Oryza sativum*, hybrids of *Sorghum moench* and *Zea*. Among the pulses are *Arachis hypogea*, *Cajanus cajan*, and *Vigna munga*. Some of the oil yielding plants are *Carthamus tinctorius*, *Ricinus communis*, and *Sesasum orientale*. The chief vegetables grown are *Allium sativum*, *Raphanus sativus*, *Solanum melongena* and *Tuberosum*. Other economically important plants are *Areca catechu*, *Cocus nucifera*, *Coffea arabica*, *Eletteria cardamom*, *Maton*, *Mangifera indica*, *Nicotiana tobacum*, etc. The areca orchids occupy most of the moist valleys and produce the nuts [8]. *Cinchona* (rubber) and *Mulberry* are the other important plants cultivated for large-scale trials by the state forest department.

Karnataka is one of the leading states in India for beekeeping development. There were 29,302 beekeepers in 1990-91 who kept 143,182 *Apis cerana indica* bee colonies. The state stood third in the country in honey production [9]. The ecological and vegetation features of the Kodagu district, including their honeys and melittopalynology on Jamun (*Syzygium cumini*) honeys, have been studied [10]. The two honey samples from Bhagamandala and Puttur (*Schefflera* sp. and *Sapindus emarginatus vahl*) were considered major sources of honey by Seethalakshmi [11]. The analysis of a few honey samples collected in and around Bangardka, Dakshina Kannada district, Karnataka was conducted by Sheshagri [12]. Melittopalynological studies have been undertaken by the CBRI (Central Bee Research Institute) on honeys collected at different fields in India. However, not much information is available related to pollen analysis in these areas. Thus the present work was carried out to determine the critical analysis of different honey samples and to identify the pollen types in honey samples of Chickmagalur District, Karnataka.

Materials & Methods

For the present study ten honey samples were collected from various Apiaries of *Apis cerana indica* colonies located in Kottigehara (CHK-KT-SH), Gutti (CHK-GU-SH), Mudigere (CHK-MU-EH), Horanadu (CHK-HR-EH), Kundur (CHK-KU-SH), Sringeri (CHK-SR-EH), Banur (CHK-BN-SH), Bhyrapura (CHK-BY-SH), Koppa (CHK-KO-EH) and Kumbadi (CHK-KB-EH) of Western Ghats of Chickmagalur District during the period of March – May 2005. Honey samples were procured from domesticated hive bees as well as from the beekeepers. All honey samples were raw and unprocessed. The honey samples were collected in sterilized polythene bottles from the place of honey extraction. The honey was filtered through single thickness fine cloth to remove suspended particles like dirt, beeswax, and other impurities. Later it was stored in airtight container at room temperature.

The honey samples were subjected to Qualitative and Quantitative Palynological analysis. For Qualitative and Quantitative analysis, the methodology recommended by the International Commission for Bee Botany (ICBB) was followed [13]. Slides were prepared by following the acetolysis adopted by Erdtman [14]. Five ml of honey per sample was dissolved in 10 ml of distilled water and centrifuged for 10 minutes at 2500 rpm and the supernatant liquid was drawn out. The sediment was treated with 5 ml of glacial acetic acid and centrifuged at 2500 rpm for 10 minutes. After decanting acetic acid, the sediment was acetolysed [14]. One part of Concentrated Sulphuric acid was added drop by drop to 9 parts of Acetic Anhydride and warmed in a water bath

till the liquid turned chestnut brown colour. After cooling it was again centrifuged at 2500 rpm for 10 minutes. The supernatant liquid decanted off. The sediment then treated with glacial Acetic acid, later centrifuged at 2500 rpm for 10 min. followed by two or three rinsing with distilled water. After each rinsing, again centrifuged at 2500 rpm for 10 minutes. The 50% aqueous glycerin prepared in distilled water was added and centrifuged for 10 minutes at 3500 rpm. The supernatant liquid was decanted off. The pollen sediment was taken on a pallet of glycerin jelly and transferred to the centre of the slides of 75X25 mm size having 0.8 mm thickness. After being warmed slightly, the melted jelly with pollen sediment was covered by cover glass (22 mm). The cover glass was later sealed with paraffin wax [15]. Pollen grains were identified with the help of standard slides prepared from the local flora. The pollen grains were placed into one of the following four pollen frequency classes: Predominant (more than 45%), Secondary (45-16%), Important Minor (3-16%) and Minor pollen types (Less than 3%) and the data was graphically represented in pie charts [13].

Results

The results of the pollen analysis of *Apis cerana indica* honey of Chickmagalur region were found to be Unifloral (Table 1).

Sample Number	Date of Collection	Type of Honey	Absolute Pollen Count (APC)	Pollen Type			
				Predominant Pollen (> 45%)	Secondary Pollen (16-45%)	Important Minor Pollen (3- 15%)	Minor Pollen (<3%)
CHK-KT-SH	06-03-05	U	4000	<i>Cocus nucifera</i> (52%)	Asteraceae (17%), Poaceae (16%)	<i>Eucalyptus globulus</i> (14.5%)	<i>Areca catechu</i> , Poaceae
CHK-GU-SH	08-03-05	U	800000	<i>Coffea arabica</i> (62.16%)	<i>Cocus nucifera</i> (24.32%)	<i>Areca catechu</i> (10.8%)	Fabaceae, <i>Croton sp.</i> , <i>Syzygium cumini</i>
CHK-MU-EH	12-04-05	U	21000	<i>Coffea arabica</i> (47%)	<i>Syzygium cumini</i> (38%)	<i>Areca catechu</i> (14.2%)	<i>Coriandrum sativum</i> , Poaceae, Asteraceae.
CHK-HR-EH	12-04-05	U	11000	<i>Cocus nucifera</i> (54.5%)	Poaceae (36.36%)	Asteraceae (9%)	<i>Eucalyptus globulus</i> , <i>Delonix regia</i> .
CHK-KU-SH	20-04-05	U	14000	<i>Coffea arabica</i> (64.12%)	Asteraceae (21.4%)	<i>Cocus nucifera</i>	<i>Areca catechu</i> , <i>Ricinus communis</i> , <i>Eucalyptus globulus</i> .
CHK-SR-EH	08-05-05	U	32000	<i>Coffea arabica</i> (59.3%)	<i>Areca catechu</i> (25.11%)	<i>Eucalyptus globulus</i> (12.5%)	Poaceae etc
CHK-BN-SH	08-05-05	U	41000	<i>Coffea arabica</i> (51.2%)	<i>Syzygium cumini</i> (39.02%)	<i>Ricinus communis</i> (9.7%)	Asteraceae, Fabaceae, Poaceae.
CHK-BY-SH	17-05-05	U	45000	<i>Ludevigia sp.</i> (62.2%)	<i>Croton sp.</i> (31.1%)	<i>Terminalia chebula</i> (4.4%)	<i>Cassia sp.</i>
CHK-KO-EH	17-05-05	U	28000	<i>Cocus nucifera</i> (50.1%)	<i>Syzygium cumini</i> (35.72%)	Asteraceae (10.7%)	Poaceae, Euphorbiaceae
CHK-KB-EH	23-05-05	U	63000	<i>Coffea arabica</i> (53.9%)	<i>Sapindus sp.</i> (31.7%)	<i>Areca catechu</i> (9.5%)	Asteraceae, Rutaceae

Table 1: Pollen frequency class in *Apis cerana indica* Honey.

N = 10

Kottigehara (CHK-KT-SH), Gutti (CHK-GU-SH), Mudigere (CHK-MU-EH), Horanadu (CHK-HR-EH), Kundur (CHK-KU-SH), Sringeri (CHK-SR-EH), Banur (CHK-BN-SH), Bhyrapura (CHK-BY-SH), Koppa (CHK-KO-EH) and Kumbadi (CHK-KB-EH).

U – Unifloral.

The samples Gutti (CHK-GU-SH), Mudigere (CHK-MU-EH), Kundur (CHK-KU-SH), Sringeri (CHK-SR-EH), Banur (CHK-BN-SH) and Kumbadi (CHK-KB-EH) were of *Coffea arabica* with pollen ranging from (47% to 62.16%). The samples Kottigehara (CHK-KT-SH), Horanadu (CHK-HR-EH), Koppa (CHK-KO-EH) were of *Cocos nucifera* (Fig.1) with pollen ranging from (50.1% to 54.5%) and (CHK-BY-SH) contained 62.2% of pollen belonging to *Ludevigia sp.* were the predominant pollen type among the ten samples of honey. The importance of *Syzygium carophyllatum* and *Psidium guajava* as major source of forage for honeybees and according to their studies the *Cocos nucifera* is the only pollen source available through out the year where as, *Terminalia sp.* serves as a good pollen and nectar source [16].

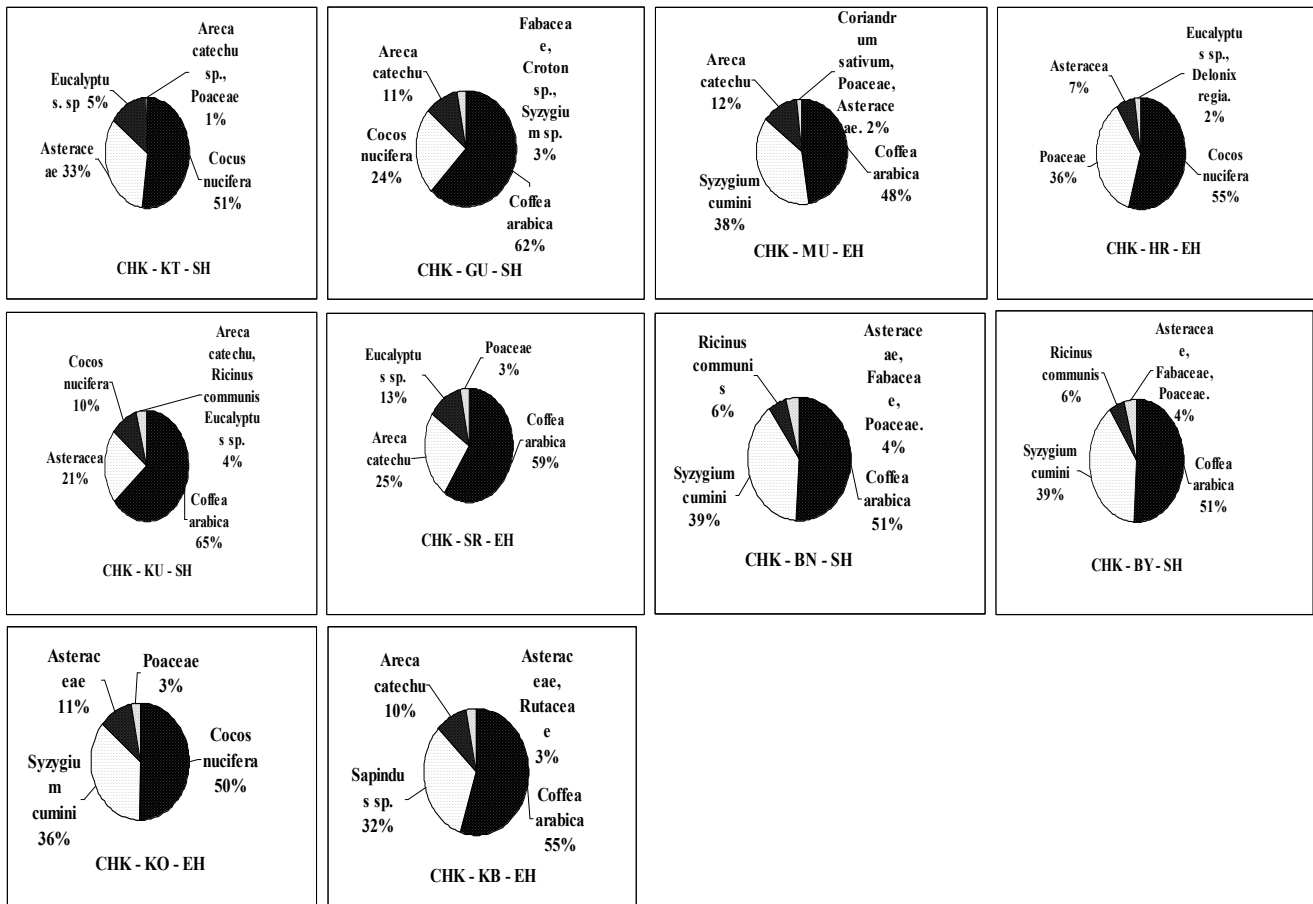


Fig 1. Pie charts showing pollen spectra of *Apis cerana indica* honey samples.

The pollen types identified are (Fig.2) *Eucalyptus globulus* (12.5% to 14.5%), *Croton sp.* (31.1%), *Terminalia chebula* (4.4%), *Cassia sp.*, *Ricinus communis*, *Areca catechu* (9.5% to 25.11%), *Coriandrum sativum*, *Syzygium cumini* (35.72% to 39.02%), *Hevea brasiliensis*, members of Asteraceae (10.7%), Poaceae (36.36%), Euphorbiaceae, Rutaceae and Fabaceae family. The economically important plants constitute major part of the flora of this area. There is potential to produce considerable quantity of honey from these sources.

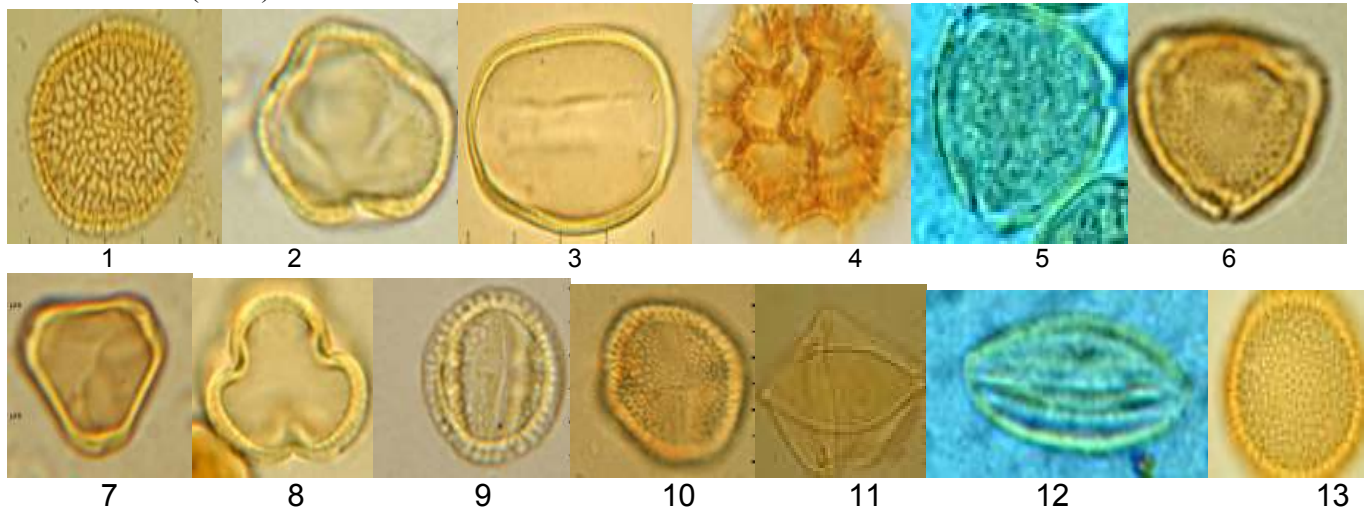


Fig 2. Photo Micrographs of Pollen types identified from *Apis cerana* honey samples of Chickmagalur, Karnataka. All figures X1000.

1. *Areca catechu*, 2. *Coffea arabica*, 3. *Arecaceae*, 4. *Asteraceae*, 5. *Ludevigia sp.*
6. *Eucalyptus globulus* 7. *Syzygium communis*, 8. *Fabaceae*, 9. *Rutaceae*, 10. *Euphorbiaceae*,
11. *Poaceae*, 12. *Cocus nucifera*, 13. *Croton sp.*

Discussion

Honey analysis indicates a good potential for the development of bee colonies in this locality. Bees used pollen for brood rearing, growth in colony strength, and nectar for their carbohydrate requirement. The identification of pollen and nectar sources in honey would help beekeepers in maintaining their colonies [17].

The region selected for the present study has good potential for sustaining beekeeping ventures because of the diversity of nectar and pollen taxa. Since *Coffea arabica*, *Cocus nucifera* and *Ludevigia sp.*, are major sources of forage for honey bees, efforts should be made to increase their cultivation as well as plants in the families *Asteraceae*, *Poaceae*, *Euphorbiaceae*, *Rutaceae* and *Fabaceae* in these areas.

To improve the beekeeping industry, a proper understanding and mutualism between bees and available plant taxa in the region and in a particular season is necessary [7]. The identified taxons were not only the economic crops but also play an important role in the development of beekeeping in these areas. These data reflects the floral situation of the place where particular honey was produced and the identification of geographical origin based on the presence of a combination of pollen types of that particular area.

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References

- [1] Moses, T. S., Singh, Joshi, Madhukanta, A., Suryanarayana, M. C., Evaluation of sources of pollen to Honey Bees at Vijayarai (Andhra Pradesh), Proc. 5th All India Symp. Palynol, October 7–9, 1987, Dept. Bot., Inst. Sci, Nagpur, India. 1987, pp. 65–71.
- [2] Ramanujam, C. G. K., Khatija, F., Melittopalynology of the agricultural tracts in Guntur District, Andhra Pradesh. J. Indian Inst. Sci. 71 (1991), 25–34.

- [3] Sen, J., Banerjee, D., A pollen analysis of Indian honey. *Bee World*. 37 (1956), 52-54.
- [4] Phadke, R. P., Physico chemical composition of major unifloral honeys from Mahabaleshwar (Western Ghats). *Ind. Bee J.* 24 (1962), 59-65.
- [5] Nair, P. K. K., A pollen analytical study of Indian honeys. *J. Ind. Bot. Soc.* 43 (1964), 179-191.
- [6] Seethalakshmi, T. S., Melittopalynological investigations on some Indian Honeys, In : *Proc 2nd Int. Conf. Apic. Trop. Climates, New Delhi, (1980) (ed org. Comm.), Indian Agric. Res. Inst., New Delhi. 1983, pp. 609–621.*
- [7] Bhusari, N. V., Matte, D. M., Mahde, K. H., Pollen of Apis Honey from Maharashtra, *Grana*. 44 (2005), 216–224.
- [8] Yoganarasimhan, S. N., Subramanyan, K., Razi, B. A., *Flora of Chickmagalur district, Karnataka, India. 1982, pp. 4-15.*
- [9] Anonymous., *Annual report for the year 1990-1991. Directorate of Beekeeping. Khadi and Village Industries Commission. Bombay 1991.*
- [10] Suryanarayana, M. C., *Studies on the Flora of Coorg with emphasis on bee forage plants, I. Ecological and Botanical Features. Indian Bee. J. 28 (1966), 59–75.*
- [11] Seethalakshmi, T. S., *Melittopalynological Investigations on some Indian honeys. Proc. 2nd Int. Conf. Apic. Trop. Climates, New Delhi, India. 1980, pp. 609-620.*
- [12] Sheshagri, T. N., *Melittopalynological studies of Bangalore and its Environs, M.Phil Thesis, Bangalore University, Karnataka. (1985).*
- [13] Louveaux, J., Maurizio, A., Vorwhol, G., *Methods of Melissopalynology, Bee World. 59 (1978), 139–1157.*
- [14] Erdtman, G., *The Acetolysis method. A revised description sv, Bot. Tidskr. 54 (1960), 561–564.*
- [15] Nair, P. K. K., *A modification in the method of pollen preparation, J. Sci. Industri. 19C (1960), 54–53.*
- [16] Shripad N. Agashe., Rangaswamy, B. E., *Melittopalynological studies of Honey samples from Bangardka, Dakshin Kannada District, Karnataka, Indian Bee J. 59 (1), (1997), 8–11.*
- [17] Rakesh kumar, Jagtap, S. D., *Melittopalynological studies on Rock bee honey samples during Winter season in Purander area of Pune district in Maharashtra. J. of Palynol. 34 (1988), 157-162.*