International Journal of Scientific Research in Science and Technology
Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)
doi : https://doi.org/10.32628/IJSRST2293132

Pollen Analysis of Natural Honey Sample from the Chikhli taluka of Gujarat state India

Priya L. Dhumadiya[1], Dilipkumar D. Patel[2]
[1]Research Scholar, [2]Associate Professor
Department of Chemistry, B.K.M. Science College, Valsad, Gujarat, India

Article Info
Volume 9, Issue 3
Page Number : 628-637
Publication Issue
May-June-2022
Article History
Accepted : 03 June 2022
Published : 20 June 2022

ABSTRACT

Diverse kinds of pollen and nectar forage by honey bees generate numerous types of honey which can be explored for the establishment of beekeeping venture in an area . The taluka of chikhli is rich floristically but neither pollen analytical study. Considering this, the present work has been undertaken to develop melissopalynological database from the chikhli taluka and to evaluate the potential of the study area for apiculture. Pollen analysis of 6 natural honey samples collected from the six different village of chikhli taluka reveals that 2 are uniflorae, 4 multiflorae . plant species used for study are : Acacia nilotica , Sesamum indikum , Trachyspermum ammi , Anethum sowa, Brassica nigra, and mix type of honey sample. There were many bee plant , which provides pollen and nectar throughout the year. it was found that on a average Ajwain honey contained the highest moisture (19.9)followed by Mustard (18.7%) and mix honey (18.4%). The lowest moisture (17.4%) was found in Babool honey. Highest carbohydrates obtained in Babool (78.99) and Sowa honey (78.85%) respectively . the lowest carbohydrates was found in mustard (74.27) honey. Highest natural sugar was found in mustard (74.89%)honey followed by Dill (72.70%). The lowest natural sugar found in babool (70.63%)honey. The findings recommend that the competent authority, on the basis of this pollen analytical data, may initiate bee-keeping ventures in this economically backward area and the beekeepers may have the opportunity to share the profitable medicinal honey market. This would benefit the rural health care improving the socio-economy of the local inhabitants of this region.

Keywords : Honey Bees, Honey, Pollen Grains

I. INTRODUCTION

1.1 Historical context

Honey bees have been known to generate honey in nature since the late tertriary period (Culliney,2001). since prehistoric times,honey has been an important aspect of human life (Beck and smedly ,1944). prehistpric man may have painted several painting of hunting rock bee colonies as early as 15,000 to 11,000

BC (Mathpal 1978, 1984). Dams (1978) discovered the Honey is a viscous sweet edible fluid that contains numerous key nutrient and antipathogenic properties and has been used as a quick food and significant antibacterial agent by mankind since the dawn of civilization .honey bees collect nectar and pollen grains from a variety of plant typein order to make honey .because some plant are preferred nectar sources , pollen analysis of honey samples can assist in identifying the plants that bees prefer to visit for nectar. Bees use pollen grains to signify the floral nectar sources they use to make honey (lieux 1975; Moar 1985; Louveaux et al.1978; Sawyer 1998).

Honey was used in allof lifes major rituals,including birth ,marriage,and death. During the post vedic period, honey and bees were mentioned in mythological, poetical, philosophical, moral, and religious contexts (Beck abd smedley,1944).

Allchin as well as allchin built the neolithic shreds discovered in billa surgam caves is andhra pradesh ,india is thrown into a pot.in the arthasastra of kautilya in 400 B.C., chandragupta proposed a ley equal to one sixth of the cost of honey as means of increasing revenue.it demonstrates that honey was a common commodity in industry and commerce at that time in india.

Honey bee foraging behaviour was used in bhasa famous sanskrit drama swapnabasabdattam, a great dramatist of that time .honey bee imagery appeared in the works of kalidas, a great poet and dramatist.he frequently used it in his play such as abhigyana shakuntalam and kumarsambhavam.in the treatise on aryabhatya and lilavati of bhaskaracharya excelled in mathematics and astronomy. bees were used as examples in algebra and mathematics exercises in the 12th century.

Simultaneously, the technology of colony multiplication was devised. the ancient people knew how to harvest honey properly, leaving enough honey in the comb for brood rearing. honey bees were even utilised as a crop pollinator.

The use of honey as substantial antimicrobial agent can be traced back to ancient times. It was popular in India as food and in the preparation of alcoholic drinks. In India, renowned physicians Charaka and Susruta of post Vedic times (15th –13th B.C.) recorded available information in this regard in Samhitas (a famous treatise written in Sanskrit). Depending upon its source, method of collection and storage Charaka described the properties and medicinal uses of honey (Bedi, 1949; Joshi et al, 1983). Honey is a traditional remedy for dyspepsia and peptic ulcers (Al Somal et al, 1944). Jeddar (1985) found honey inhibitory to the growth of microorganisms at 40% dilution.

1.2 Pollen analysis of honey

There are four natural resources required by honeybees for survival: water, resin, nectar, and pollen. Upon returning to their hive, the nectar is usually transferred to hive workers for processing into honey, although it can be fed directly to the brood or to the adults (Winston 1987). Pollen is the bee's major source of proteins, fatty substances, minerals, and vitamins and it is essential for the growth of larvae and young adult bees . Pollen can be incorporated into the honey produced in a beehive in a number of ways. When a honeybee visits a flower in search of nectar, some of the flower's pollen is dislodged and falls into the nectar that is sucked up by the bee and stored in her stomach. At the same time, other pollen grains often attach themselves to the hairs, legs, antenna, and even to the eyes of visiting bees. Later, some of the pollen that was sucked into her stomach with the nectar will be regurgitated with the collected nectar and deposited into open comb cells of the hive. Honey always includes numerous pollen grains from the plant species foraged by honey bees and honeydew elements (like was tubes, algae and fungal spores) that altogether provide a good fingerprint of the environment where the honey comes from and indeed the main critical point of melissopalynological analysis remain the correctness of pollen

identification and the subsequent interpretation of the results (Von Der Ohe et al. 2004).

Identifying the pollen in honey samples and quantifying relative pollen frequency are the best ways to determine and label the range of major and minor nectar sources used to produce a honey. Because of trade agreements, import tariffs, and legal trade restrictions, most of the leading honey producing nations of the world require accurate labeling of honey before it can be sold. The combination of taxa of pollen grains (palynotaxa) found in a honey sample often produces a pollen spectrum that is unique for a specific geographical region where it was produced. Pollen analysis of bee products proves useful in characterizing the composition of local and regional vegetation (Barth and Pinto da Luz, 2009). Pollen analysis is also indicative of potential pollinators in agricultural and natural ecosystems because during the course of their foraging activities, bees pollinate many economically important plants (Free 1993). This technique provides some important information about some kinds of adulteration and hygienic aspects such as contamination with mineral dust, soot, or starch grains and is of great importance for quality control of honey (Louveaux et a., 1978). Usually the beekeepers do not have information of all the important nectar plants contributing to honey production and thus pollen analysis proves to be a useful guide to beekeeping in a region (Song et al 2012).

Microscopic analysis of pollen grains of natural honeys therefore is the basis for identification of different nectar sources over the season and is thus useful to determine and control the geographical and botanical origin of honey. It is extremely useful for hive management and allows determination of likely periods of production of unifloral honey (honey from only one floral source), which has high commercial value (Oliveira et al, 2010, Costa et al, 2013) and may prove pivotal while considering apicultural venture in an area.

Chikhli is a midsize town in navsari district in the state of gujarat,india.the village of chikhli under area soldhara,rankuwa, sunthwad,tankal,pipalgabhan. the people of this place mostly are farmers, labour worker,and some people especially the local tribes.chikhli has a deciduous forest.chikhli has a tropical savanna climate with little to no rainfall from october to may.

II. BREIF REVIWE OF LITRATURE

2.1. Pollen analyis of honey

(Pfister,1895) the first work on the microscopy of honey dates back to the end of the 16th century.

(Allen,1929) was focusing on some of the problems of conducting accurate melissopalynology analyses. he was the first researcher to caution about some of the pitfalls and difficulties of pollen identification in melissopalynology.

(Woodhouse,1935) his identification of pollen grains was based on earlier studies of pollen morphology, structure, and identification of European pollen types by other botanists.

(K.H.Bhuyan, 2002)(K.H.Bhuiyan ,2002) according to him the honeybee and bee plant s are equally important to produce honey.

(Pradip hirapure,2014) according to him pollen grains is useful in identification of criminal investigation of forensic importance.

(Ashok jain ,2003) According to him all amino acids essential to human can be found in pollen with proline being the most abundant.

III. STUDY AREA

Chikhli Taluka is a Town in Chikhali Taluka in Navsari District of Gujarat State, India. It is located 30 KM towards South from District head quarters Navsari. It is a Taluka head quarter.Chikhli Taluka Pin code is 396521 and postal head office is Chikhli (Navsari).Thala (2 KM) , Malwada (2 KM) , Majigam (3 KM) , Samaroli (3 KM) , Talavchora (3

KM) are the nearby Villages to Chikhli Taluka. Chikhli Taluka is surrounded by Gandevi Taluka towards west , Valsad Taluka towards South , Navsari Taluka towards North , Mahuva Taluka towards North .Valsad , Navsari , Dharampur, India , Pardi are the nearby Cities to Chikhli Taluka. It is near to arabian sea. There is a chance of humidity in the weather.

Table.1. GPS Cordinates.

Sr.No	Locality	Latitude & Longitude
1	Pipal gabhan	20.7181⁰N,73.0916⁰E
2	Tankal	20.8529⁰N,73.1320⁰E
3	Rankuwa	20.8091⁰N,73.1556⁰E
4	Soldhara	20.7032⁰N,73.0789⁰E
5	Samroli	20.7694⁰N,73.0453⁰E
6	Sunthwad	20.8308⁰N,73.0848⁰E

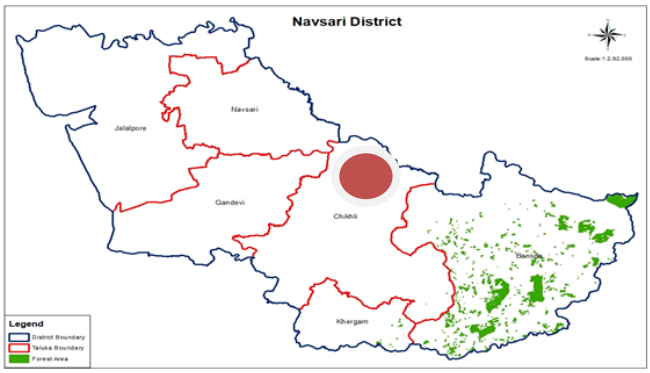


Fig.1. map of study area

Climate

Chikhli Taluka has a tropical savanna climate (Aw) with little to no rainfall from October to May and extremely heavy rainfall from June to September. The rainfall is much heavier than nearby Valsad due to the orographic lift of the Western Ghats.

Flora of chikhli

A total of 72 species were recorded which were represented by 40 families and 67 genera. Out of 72 species ,36 tree species,34 herb and 2 orchids .The maximum IVI recorded for Tectona grandis (76.385) and followed by Adina cordifolia (21.978) , Terminalia tomentosa (19.682), Syzygium cumini (14.929), and Oroxylum indicum (13.293), respectively. Among the herbs species ,the highest Shannon –wiener index recorded for Commelina beghalensis, Curculi goorchioides, Phaseoluss pecies , Sonchus oleraceus (0.162) and followed by Blainvillea acmella, Blumea wightii, Canscor adiffusa ,Curcuma odorata, Desmodium gangeticum, Hibiscus lobatus , and Tridax procumbens (0.124), respectivel.

IV. METHOD AND MATERIAL

4.1 Material

Six natural honey samples may i collected from 6 different areas of 6 village of Chikhli taluka during November to February in 2021 – 2022.The honey samples are freshly squeezed samples from naturally occurring beehives in local /rular location, harvested for family consumption as well as from apiaries and acquired directly from the honey bearing section of the beehive.

Apis indica were used as much as possible. All samples are carefully numbered, labeled, recorded into the sample registration dasabase, catalogued and stored in the repository.

all of the samples are kept in glass or plastic containers. Prior to testing ,containers were kept at room temperature in the dark and honey dilutions were made. Prior to testing , start over every day . The honey samples are mostly analyzed and tested immediately after collection.

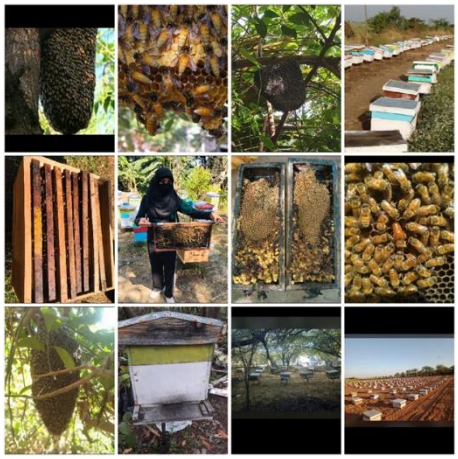


Fig .2. Bee foraging and collection of honey sample

4.2 METHODS

4.2.1. Microscopic analysis of honey

Honey samples were processed using Erdtman's (1960) acetolysis procedure with certain changes proposed by the International Melissopalynogical Association for qualitative melissopalynogical analysis. Botany Commission for Bees (Louveaux et al 1978, Von der Ohe et. Al., 2004). Ten grams of sugar Each honey sample was placed in a 50 mL pointed glass centrifuge tube and swirled. Dissolved in 20 ml distilled water (20-40 °C) and centrifuged for 10 minutes using a 1 ml pipette A minimum of 2000 g The supernatant was removed and decanted. The procedure was carried out twice more. The By inserting the tube in the decanter, all but the last drop of supernatant liquid was extracted. To allow the remaining extra liquid to be taken upside down at a 45° any remaining liquid to seep into absorbent paper. The acetolysis combination (acetic anhydride: conc. sulphuric acid = 9:1 v/v) was used to treat the silt. The acetolyzed pollen sediment centrifuge tube was placed in a 70°C oven. 10 minutes in a °C water bath The tube was centrifuged for 5 minutes after incubation and then decanted. into a dry beaker with care A drop of a powerful chemical was added to the distilled water in the tube. detergent added, firmly shaken, and centrifuged for another 5 minutes The liquid in the supernatant was taken away. On three slides, the entire silt was dispersed more or less evenly. spread out over a 20 X 20 mm surface area. On the affected area, a drop of glycerin jelly was administered. Another drop was poured on a cover glass, which was placed on the Sediment-glycerin jelly mixture. To analyze the pollen content three pollen slides were prepared In this way from each honey sample studied and photographed under a compound microscope. Dried acetolysed pollen sediments were also photographed using a compound Microscope . The pollen grains were identified with the Help of reference slides prepared from a collection of the local flora (Repository of spore-pollen. A final drop was poured into a cover glass, which was then placed on top of the sediment-glycerin jelly concoction. Three pollen slides were made in this manner from each honey sample analysed and photographed under a compound microscope to assess the pollen concentration. A camera was used to photograph dried acetolyzed pollen seen. With the help of a microscope, the pollen grains were identified. The majority of the pollen Types are identified at the species level and those types that could not be identified are mentioned unidentified. For qualification of pollen grain types 20 pollen grains are randomly counted by each sample.

4.2.2. Chemical analysis of honey

Six different types of honey were taken for study i.e., *Brassica nigra (mustard), Trachyspermumammi(ajwain),Sesamumindicum(sesame),Glossocardia bosvallia(Suva),Acacia nilotia* (babool) honey. the names of the honey followed the corresponding plant source from which nectar was collected by the Apis mellifera. during analysis of honey the following chemical parameters were taken into consideration: 1) moisture content 2) carbohydrates content 3) natural sugar content

The above parameters were determined using the method of Rangana, method of Anthrone , method of Lane and Eynon.

1] Moisture: Five grams of the honey was taken in porcelain crucibles in tetrad and oven dried at 100 C until the weight became constant.

2] Carbohydrates : weight 100 mg the sample into a boiling tube. hydrolyze by keeping it in a boiling water bath for three hours with 5ml of 2.5 n HCL and cool to room temperature .Neutralize it with solid sodium carbonate until the effervescence ceases. make up the volume to 50ml and centrifuge. collect the supernatant and take 0.5 and 1 ml aliqu ots for analysis. prepare the standards by taking 0,50,100,150,200 ul of the glucose standard.0 serves as blank. make up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water and add 4 ml of anthrone reagent. Heat for 8 minutes in a boiling water bath. cool rapidly and read the green to dark green colour at 630 nm.

3] Natural sugar : take known amount of the sample in 250 ml flask and add 100 ml of water .add 1 drop of indicator phenolphthalein .neutralize with 40% lead acetate and keep for 30 min. add 5 ml potassium oxalate. make volume 250 ml and filter it. take 100 ml filtrate in flask and add 5-20 ml 50% HCL. keep overnight for the inversion of sugars add 1 drop of indicator phenolphthalein and neutralize drop by drop with 40% NaOH .take it in burette .take the 10 ml fehling mixture (5 ml fehling – A+5 ml fehling - B)in flask .add 1-2 drop of methylene blue indicator. Titre (Keep boiling continue fehling mixture)till end point (blue to brick red colour) note the end point.

V. RESULT

5.1. Pollen analysis of honey

A total of 60 pollen grain found in 6 different types of honey sample. all pollen grain found in different types of families. families are: Fabaceae, Apiaceae, Pedaliaceae, Brassicaceae.

Table.2. plant species use for study.

Sr.no	Scientific name of plant	Family
1	*Sesamum indicum*	*Pedaliaceae*
2	*Anethum sowa*	*Apiaceae*
3	*Trachyspermum ammi*	*Apiaceae*
4	*Acacia nilotica*	*Fabaceae*
5	*Brassica nigra*	*Brassicaceae*

Table.3. Pollen grain content in each honey sample

Sr.no	Scientific name	Pollen grain content
1	*Sesamum indicum*	40%
2	*Anethum sowa*	48%
3	*Trachyspermum ammi*	25%
4	*Acacia nilotica*	70%
5	*Brassica nigra*	60%
6	*MIX*	33%

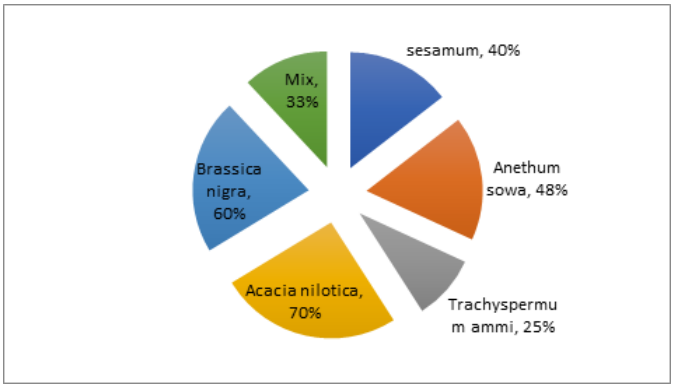
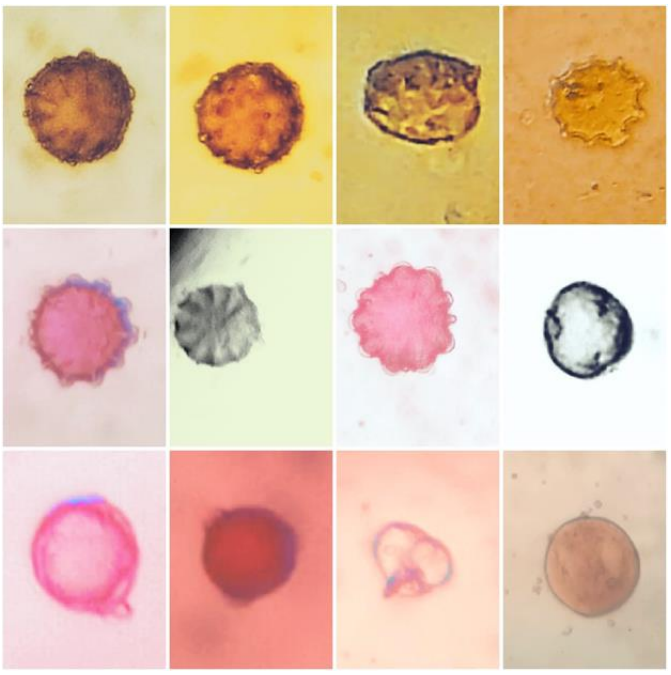


Fig.3. Pollen grain content in each sample

Table no.2. defined the pollen grain content in each sample.the highest content in Acacia nilotica ,and second highest pollen grain content in Brassica nigra .the lowest pollen grain content in Trachysprrmum ammi .if honey contains 50% or more pollen of one species,it is called uniflorae.if less than 50% then called Multiflorae.

Sesamum indicum

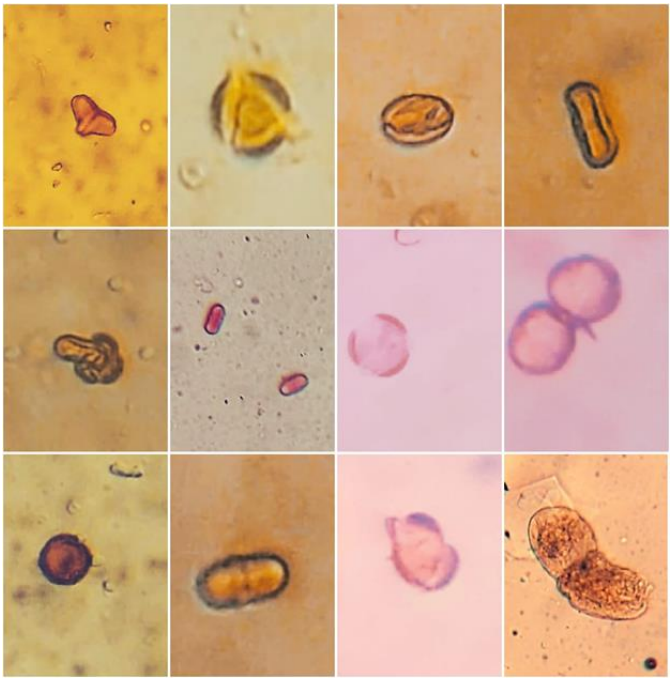
PLATE:1



Pollen grain of Sesamum indicum observed in under the microscope

Anethum sowa

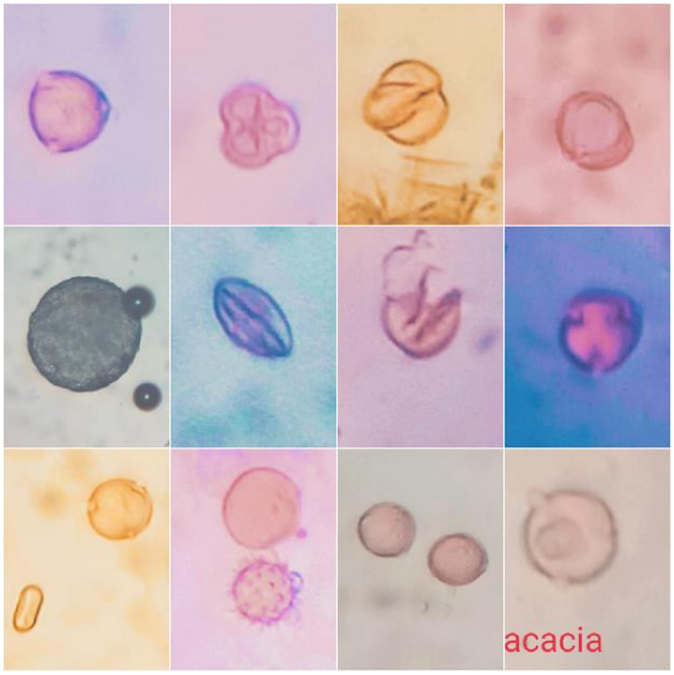
PLATE : 2



Pollen grain of Anethum sowa observed in under the microscope

Acacia nilotica

PLATE:4



Pollen grain of Acacia nilotica observed in under the microscope.

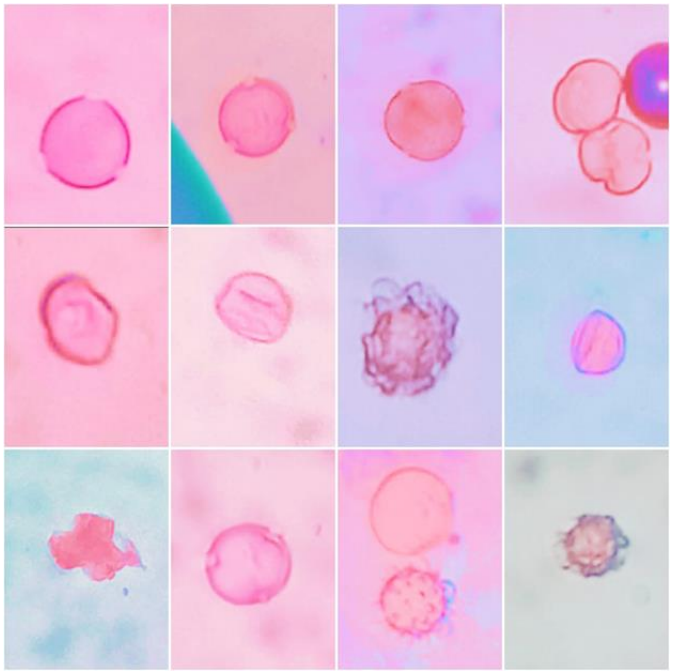
Trachyspermum ammi

PLATE :3

Pollen grain of Trachyspermum ammi observed in under the micro.

Acacia nilotica

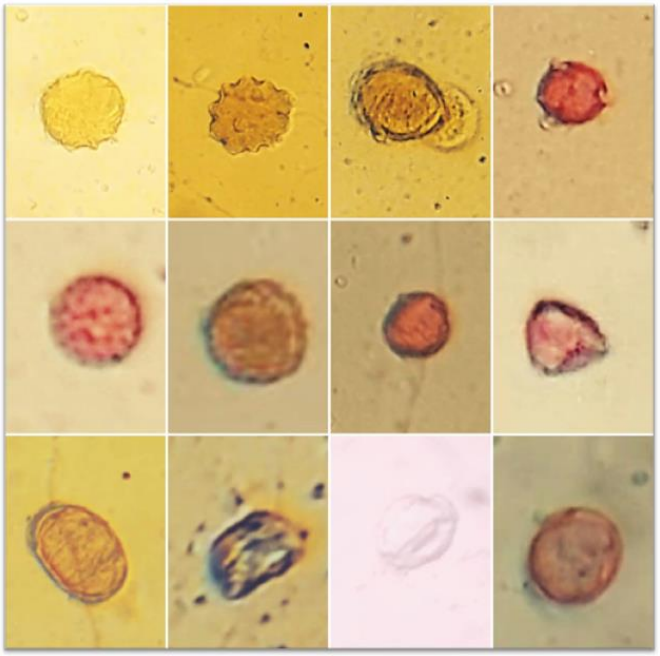
PLATE:5



Pollen grain of Acacia nilotica observed in under the microscope

Brassica nigra

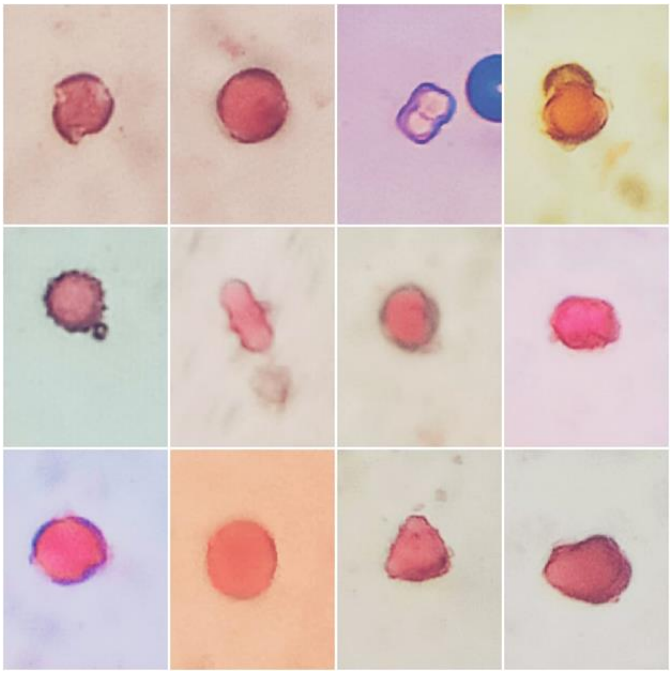
PLATE:6



Pollen grain of Brassica nigra observed in under the microscope

Brassica nigra

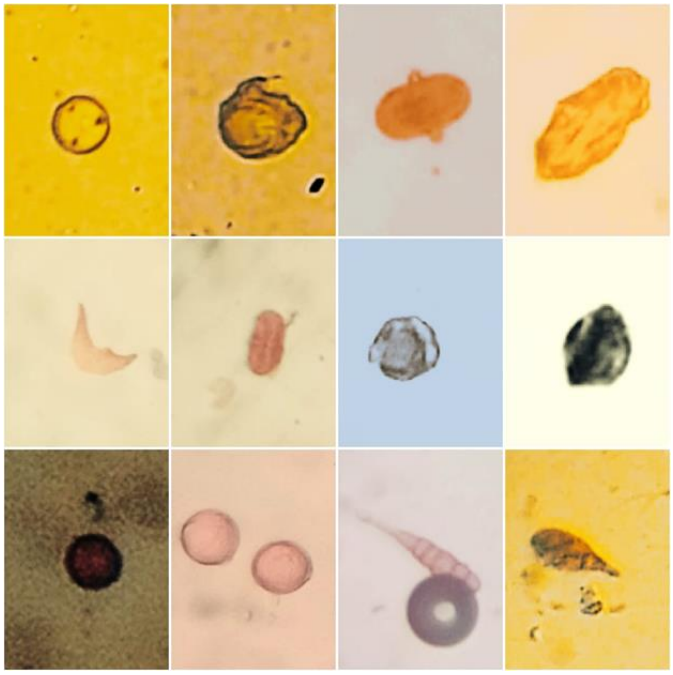
PLATE:7



Pollen grain of Brassica nigra observed in under the microscope

Mix

PLATE :8



Pollen grain of Mix honey sample observed in under the microscope

5.2. Analysis of honey

After analysis of honey it was found that on an average Ajwain honey contain the highest moisture (19.9%) Followed by mustard (18.7%) and mix honey(18.4%).The lowest moisture (17.4%) was found in Babool honey. Highest carbohydrates obtained in Babool (78.99%) and Dill honey (78.85%) respectively. the lowest carbohydrates was found in mustard (74.27%) honey. Highest natural sugar was found in mustard(74.89%)honey followed by Dill (72.70%). The lowest natural sugar found in babool (70.63%)honey.

Table .4 pollen composition of honey collected from diffrent source.

Sr.no	Local name	Scientific name	moisture	carbohydrates	Natural sugar
1	Babool	*Acacia nilotica*	17.4%	78.99%	70.63%

2	Ajwain	*Trachyspermum ammi*	19.9%	76.35 %	71.66%
3	Dill	*Anethum graveolens*	17.7%	78.85 %	72.70%
4	Sesame	*Sesamum indicum*	18.0%	77.99 %	71.45%
5	Mustard	*Brassica nigra*	18.7%	74.27 %	74.89%
6	Mix	-	18.4%	76.66 %	72.20%

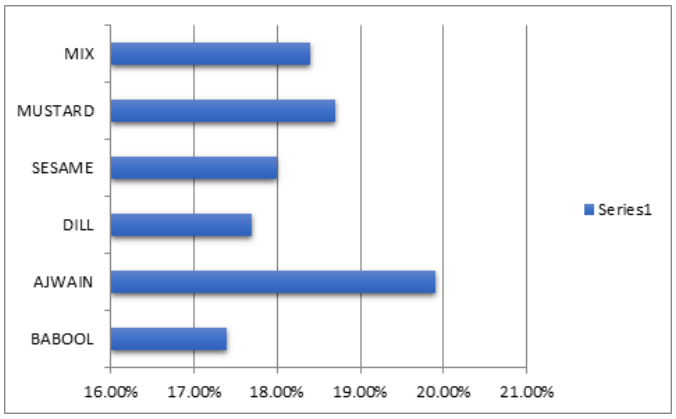


Fig.4. moisture content in honey sample

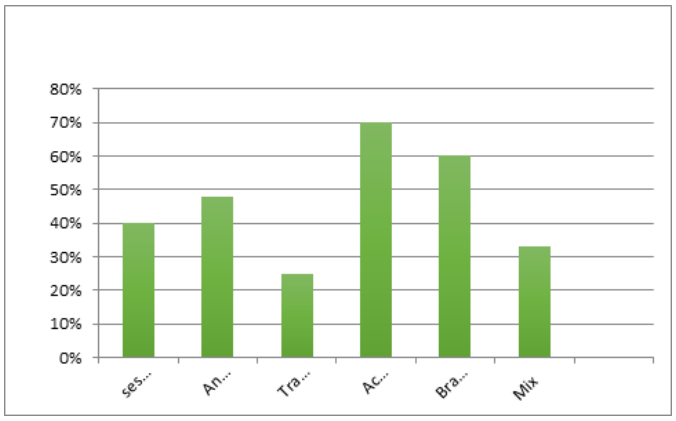


Fig.5. Carbohydrates content in honey sample

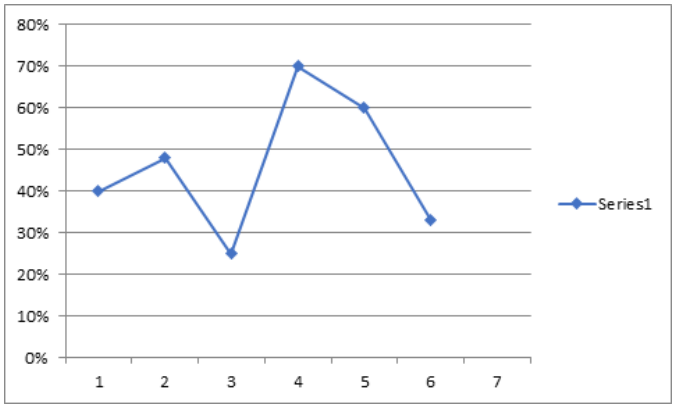


Fig.6. natural sugar content in honey sample

VI. CONCLUSION

The comparative results indicated that the mustard honey contained more natural sugar and was therefore, the most preferable honey. but babool honey could be stored for a longer period because of the presence of less amount of moisture in it. Successful bee keeping and honey production was entirely depend on the availability of bee plant. its interesting to note that the relationship between honey bees and bee plants is on give and takes basis .many plants require the visits of honey bees for cross pollination. Every minutes the bees remove pollen from their bodies with their pollen brushes and collect surplus pollen in their buskets. Whereas, honeybee help to bring the male and female parts of flowers together and thus arrange fertilization of the ovum. The blossoms give nectar and pollen for the bees to eat and make honey from nectar only and not from pollen. Thus the honeybee and bee plants are equally important to produce honey.

VII. REFERENCES

[1]. Allchin FR ,Allchin B (1962) A Neolithic pot from Andhra Pradesh. Antiquity 302-303.

[2]. Al Somal N, Coley KE, Molan PC, Hancock BM (1944) Susceptibility of Helicobacter pyroli to The Antimicrobial activity of Manuka Honey. Journal of Royal Society of Medicine 87: 9-12.

[3]. Beck BF, Smedley D (1944) Honey and your health (Revised edn.) Dodd, Mead and Company Publisher, New York.
[4]. Barth OM, Pinto Da Luz CF (2009) Palynological analysis of Brazilian red propolis samples. Journal of Apicultural Research and Bee World 48 (3): 181-188.
[5]. Beck BF, Smedley D (1944) Honey and your health (Revised edn.) Dodd, Mead and Company Publisher, New York.
[6]. Bedi R (1949) Honey (in Hindi). Himalaya Herbal Institute, Gurukul Kangdi, Hardwar.
[7]. Bhuyan KH (2002),Identification of bee plants and analysis of honey collected from different plant sources.Pakistan journal of biological sciences 5 (11):1199-1201.
[8]. Culliney TW (1983) Origin and evolutionary history of the honey bees Apis. Bee world 64: 29-38.
[9]. Costa MC, Vergara-Roig VA, Kivatinitz SC (2013) Amelissopalynological study of artisanal Honey produced in Catamarca (Argentina). Grana 52 (3): 229-237.
[10]. Erdtman G (1960) The acetolysis method: A revised description. Svensk Botanisk Tidskrift 54: 561-564.
[11]. Dams LR (1978) Bees and honey-hunting scenes in the Mesolithic rock art of eastern Spain. Bee World 59: 45-53.
[12]. Free J.B. 1993.Insect pollination of crops.Academic press. London,684 pp
[13]. Hirapure P (2014)Morphological study of pollen as an Aid in criminal investigation.Scholars Academic journal of biosciences 2 (3): 187-192.
[14]. Jeddar A, Kharsan YA, Ramsaroop UG, Bhamjee A, Hafejee E, Moosa A (1985) The Antibacterial action of Honey: An vitro study. South Africal Medical Journal 67.
[15]. Joshi CG, Madhukanta A, Divan VV, Suryanaryana MC (1983) Bees and honey in ancient India.
[16]. Jain AK,(2003) School of studies in botany jiwanji university gwalior.journal of Melissopalynology,M.P.
[17]. Bhuyan KH (2002),Identification of bee plants and analysis of honey collected from different plant sources.Pakistan journal of biological sciences 5 (11):1199-1201.
[18]. Louveaux J, Maurizio A, and Vorwohl G (1978) Methods of melissopalynology. Bee World 51: 125-131.
[19]. Lieux MH (1975) Dominant pollen type recorded from commercial Louisiona honey. Economic Botany 29: 87-96.
[20]. Mathpal Y (1978) Prehistoric paintings of Bhimbetka, Central India. Ph. D Thesis. Dept. Archeology, University of Poona, Pune.
[21]. Moar NT (1985) Pollen analysis of New Zealand honey. New Zealand. Journal of Agricultural Research 28: 39-70.
[22]. Oliveira PP, van den Berg C, Santos Fdeard (2010) Pollen analysis of honeys from Caatinga Vegetation of the state of Bahia, Brazil. Grana 49(1): 66-75 .
[23]. Pfister,R (1895) Vensucheiner Mikroskopie des honigs.Forschber.Lebensitt.u. ihreBez.z. Hygiene,Forens . Chem.,Pharmakogen (2) (1/2):1-9,29-35.
[24]. Sawyer R (1988) Honey Identification. Cardiff Academic Press, Cardiff, Wales, UK.
[25]. Song XY, Yao YF, Yang WD (2012) Pollen Analysis of Natural Honeys from the Central Region of Shanxi, North China.
[26]. Von der Ohe W, PersanoOddo L, Piana L, Morlot M., Martin P (2004) Harmonized methods of Melissopalynology. Apidologie 35: 18-25.
[27]. Wodehouse RP (1935) pollen grains ,New york-London: McGraw Hill.
[28]. Winston M L (1987) The Biology of the Honey Bee. Harvard University Press, London.

Cite this Article

Priya L. Dhumadiya, Dilipkumar D. Patel, "Pollen Analysis of Natural Honey Sample from the Chikhli taluka of Gujarat state India", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 9 Issue 3, pp. 628-637, May-June 2022. Available at doi :
https://doi.org/10.32628/IJSRST2293132
Journal URL : https://ijsrst.com/IJSRST2293132