RESEARCH ARTICLE

A Change in Foraging Preference of Honey Bees and Plant Diversity during the Last Decade: A Case Study from Lucknow City, India

Sumaiyah Farooqui[1], Anjum Farooqui[2*]

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Abstract

The expansion of cities around its rural suburbs in recent years is changing the landscape very fast due to human activities planned for their benefit in varied ways. The economically important plants and agri-pharmacoculture have been in focus during recent years. Two decades back the study area (Chinhat, Lucknow city extension, India) was an open, marshy land with a large perennial ox-bow lake (Kathauta tal) of the river Gomti which now flows through the middle of Lucknow city. During the last decade, this extensive agricultural land has changed to a posh urban set-up. The honey samples were collected from beehive in Amity Institute campus and in adjoining areas of Kathauta tal, Chinhat to unravel the foraging pattern of honey bees with the help of its pollen content illustrating the plant resources in the vicinity and for evaluating its forensic application. The pollen spectra of honey reveals more than 50 plant taxa accounting to 60% of trees and the honey produced here is multifloral type. An agri-pharmaco industry was explored in the area with high percentage of *Cichorum intybus* and *Tinospora cordifolia* pollen in honey samples. We discuss here the potentials of melissopalynology as a quick, low-cost and highly reliable technique in understanding the pattern of reforestation and reclamation in urban areas, honey quality and its applicability in monitoring the activities of urban human.

Keywords: Anthropogenic activity, Melissopalynology, Plant diversity, Urban extension.

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Introduction

Bees are among the most beneficial insects since they produce honey and facilitate pollination in plants while foraging flowers for nectar. Several factors such as attractiveness towards plant species, climate and physical characteristics of the area make a suitable place for a bee-hive (Free, 1960). The flight range of honey bees ranges from 3 to 3.5 km from the bee-hive, although it can go even little distance beyond this estimated range. The pollen in honey is characterized by the diversity of plants and their preference for foraging within their flight range in the region. Subsequently, the honey produced in the bee-hive indicates the foraging preferences of honey bees. Primarily, the microscopic analysis of honey for pollen assemblage is used for defining its botanical and geographical origin. The concern for biodiversity and in particular, the plant diversity in urban areas is subjected to changes in varied ways by human beings for their sustenance. The ancient civilization used their cleverness to domesticate the animals and diverse plant species for their sustenance (Bedi, 1949; Dave, 1954). However, with the passage of about 7000 years from now the urbanization, industrialization, agriculture, agri-pharmacoculture, arboriculture, horticulture and various other modifications in biodiversity with the knowledge of highly developed technology, no doubt, human beings have excelled and paralleled with super power to genetically create new varieties of several plant species.

Yet, a grave concern about fast depleting biodiversity across the globe is mind boggling in the process of intentions to save the species richness of our mother earth while ensuring an eco-friendly livelihood for highly populated and under-developed countries based on agricultural economy. The advancement in varied anthropogenic activities in recent decades with focus on aesthetic and ornamental development of urban areas has replaced indigenous plant species with exotic and fast growing plants. This is a matter of concern for ecosystem in larger perspective and honey bees, in particular. Recently, a large variability has been observed in the diversity of the flora foraged by Honey Bees in time and space exhibiting the shift in foraging preferences (Ponnuchamy *et al.,* 2014; Chauhan *et al.,* 2017). The honey produced in nature illustrates the pollen assemblage depicting the change in plant diversity through melissopalynological studies.Studies from Lucknow and nearby areas have been done earlier (Chaturvedi and Sharma, 1973; Chauhan and Singh, 2010; Chauhan and Trivedi, 2011). Melissopalynological studies from other parts of India (Sahney *et al*., 2018) and from south Asia have been documented earlier (Noor *et al*., 2016). The natural adaptation of plants induced by climate change at decadal, centennial or long-term scale can also bring about changes. The veracity of honey is however doubtful and is a matter of concern for honey consumers especially those who are allergic to certain pollen protein. Melissopalynology

[1]Amity University, Noida-201313, Uttar Pradesh, INDIA

[2]Birbal Sahni Institute of Palaeosciences, 53, University Road, Lucknow-226007, Uttar Pradesh, INDIA

***Corresponding author:** Dr. Anjum Farooqui, Birbal Sahni Institute of Palaeosciences, 53, University Road, Lucknow-226007, Uttar Pradesh, India, Email: afarooqui_2000@yahoo.com

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can also be used as auxiliary method in identifying the possible frauds and adulteration in commercial honey (Durkee, 1971; Barth, 1989; Seijo *et al.,* 1992).

In India, the nectar collection is frequently carried out by *Apis dorsata, A. mellifera and A. cerana.* The source of nectar bearing plants and its economical and medicinal importance has been studied earlier (Louveaux *et al.*, 1978). In this work, we attempt to explore the foraging pattern of Honey Bee in the aesthetically designed urban extension of Lucknow city with introduction of plants as avenue trees, in entertainment parks, universities/ colleges, commercial establishments etc. In the process of study we recorded abundance of a medicinally important plant used for manufacturing drug for diabetic patients along with a large number of other pollens which was earlier never recorded from Lucknow city. Through the present study we explored i) variation in urban plant diversity during last two decades ii) honey bees were forced to change their preferences for foraging nectar from available plants and iii) honey produced by honey bees provide clue to human activities.

Study Site

Lucknow, the capital city of Uttar Pradesh state in India (Fig. 1) has expanded in recent years (Fig. 2) with large scale concrete establishments (Shukla and Jain, 2019). The study area is Chinhat, the urban extension of Lucknow city located in the northern part of India at Latitude 26.8508 and Longitude 81.049 (Fig. 1). A decade back the urban development started in this agricultural land with a large water body named Kathauta tal. A one meter sedimentary profile from this lake deposited since ~400 years from now revealed palynological results comprising pollen of *Brassica* and other crop plants along with aquatic plants (Chauhan *et al*., 1990). Climate of the study area, in general, is humid sub-tropical and largely influenced by southwest monsoon. The temperature in winter season from November to February ranges between 7.6 and 21°C. Occasionally it may go down to 0-1°C during cold months between December and January. April to June is the summer season characterized by dry winds. The temperature ranges between 27 and 32.5°C but may reach 46°C in the month of June. The rainy season is from July to middle of September and about 75% of the average rainfall (100-120 cm) is received through south-west monsoon. Most of the plant species flower during spring season between February to April and is the convenient season for honey bees.

Materials and Methods

Three honey samples were collected in 2019 from the beehives made by *Apis dorsata* (commonly known as Giant Honey bee) on the wall of building in the campus of Amity University, Lucknow Branch and in nearby areas. The honey samples were studied under light microscope (LM) and Scanning Electron Microscope (SEM) in BSIP, Lucknow by one of the authors SF, a student in Amity University who while pursuing her graduation in the campus observed the rigorous and patience activity of honey bees visiting plants and returning to bee-hive. The untiring efforts of little honey bees in producing highly nutritious honey for human beings was explored for the pollen content in honey for her short-term dissertation. The honey harvesting was done by local folks in the month of May, 2019 and was collected in glass bottles for the study. The honey samples were diluted in warm water before wet digestion in glacial acetic acid and centrifuged at 2000 rpm for ten minutes. Decantation of the supernatant and further acetolysis process was carried out following Erdtman (1969), Faegri and Iverson (1964). Finally, the acid was removed by washing in distilled water through decanting the supernatant after centrifuging. The pollen concentrate was then mounted in glycerin jelly on glass slides and observed under Olympus BX 51 Light Microscope (LM) in Birbal Sahni Institute of Palaeosciences, Lucknow. Micrographs of the pollen were taken with Olympus DP-26 digital camera attached to the microscope. The Scanning Electron Microscope (SEM) study of acetolysed pollen samples were carried out for authentic identification of medicinally important pollens.

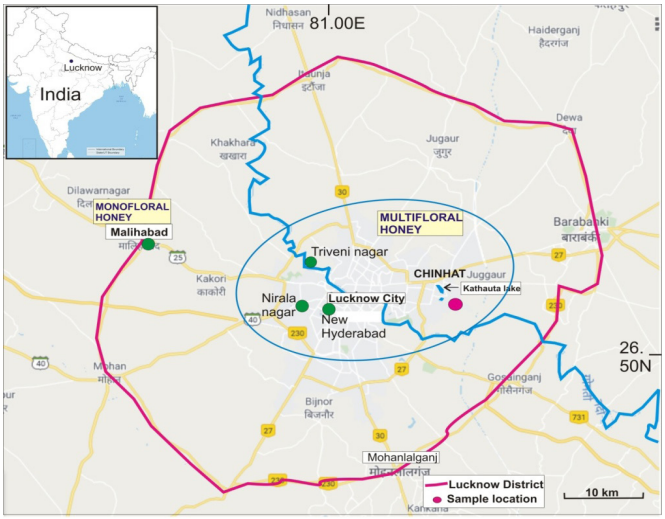

Fig. 1: The location map of honey sampling in Lucknow district and the city area

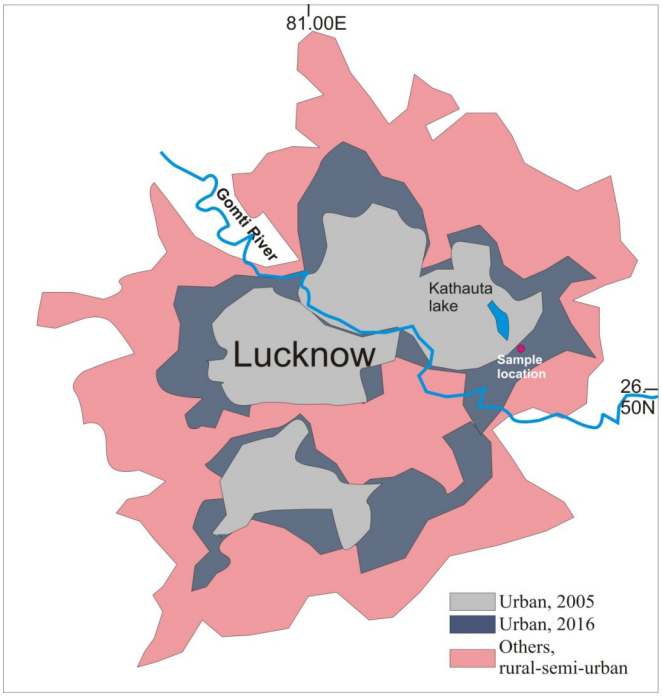

Fig. 2: Map showing urban extension from 2005 to 2016 in Lucknow district and Kathauta lake, Chinhat

The acetolysed honey sample was dehydrated in a series of alcohol and was mounted on a smooth glass piece fixed on aluminum stubs. The samples were coated with Palladium-platinum for observation in SEM (Farooqui *et al*., 2019). Standard literatures were used for identifying pollen (Chauhan and Bera, 1990; Nayar, 1990). The result is an average of three samples from the vicinity of Chinhat area. A comparative account of plant diversity recorded from honey in two decades from now in the Lucknow city, its extension and suburbs has been reviewed in order to analyze the change in foraging preferences of honey bees (Fig. 3). The methodology for classifying the frequency of pollen in Honey was followed (Louveaux *et al*., 1978; Wingenroth, 2001). Pollen greater than 45% are known as Predominant pollen. Similarly, the Secondary Pollen constitutes between 16-45% and the Important Minor Pollen constitute 3-15%. The other very Minor pollen in Honey make up less than 3% of the total pollen assemblage. The mutifloral honey is produced by bees foraging a wide range of plants in the locality with < 16% shares of pollen from more than three plant species (Wingenroth, 2001). The palynological spectrum (Fig. 3) is made in Tilia software using CONISS (Grimm, 1997) to differentiate the pollen percentage in urban area during 2018-19 and from earlier records

Results

The study of pollen composition in honey facilitated in unraveling the botanical origin i.e. the various plants visited by honeybees to glean the nectar and pollen from the urban extension of the city. The studied honey constitutes Important Minor Pollen (IMP: 3-15%) and Minor Pollen (MP:<3%).The relative abundance of different pollen types and the preferred plant sources of Honey Bees for nectar and pollen (Fig. 3) shows aesthetical, economical and medicinally important strategic plantations in the study area (Fig. 2). A comparative account of plant resources foraged by Honey Bees in the city and its extension (outskirts/suburb) during the last two decades has been discussed in relation to the present study (Fig. 3).

The pollen assemblage in all the three honey samples from Chinhat indicate that the honey bees foraged a variety of plant species. About seven species were foraged frequently in the study area. These are *Cassia fistula, Eucalyptus* sp., *Cichorum intybus, Bombax ceiba, Prosopis juliflora, Tinospora cordifolia* and *Ageratum conyzoides* which constitutes 11 to 7 % (IMP) in descending order of the total pollen species recovered in honey (Fig. 3). The honey produced was of multifloral type. These are dominated by avenue trees and two highly medicinally important herbs (*Cichorum intybus* and *Tinospora cordifolia,* Fig. 4) used for manufacturing hepatoprotective drug. The others (IMP) in a range of average 2-8% comprise of mostly fruit bearing trees, avenue ornamental trees and crop plant (*Brassica*) along with seasonal herbs. These are *Syzigium cuminii*, *Ceiba pentandra, Delonix regia, Asteraceae, Cassia siamia,* Meliaceae, *Brassica campestris, Psidium guajava, Phyllanthus, Alangium salvifolium* , Anacardiaceae, Rutaceae. The other 31 plant species (MP) foraged by bees are provided in Fig. 3. The LM and SEM photographs of pollen assemblage (Fig. 4) displays multifloral honey. The medicinally important plants such as *Cichorum intybus* L. var. *sativum* (Bisch.) Janch. was identified with the help of SEM images. The pollens are monad, spheroidal, lophate and range in size between 24-26 µm. The aperture is tricolporous and have rimmed pore. The exine ornamentation is echinate and perforate. The pollen of *Tinospora cordifolia* (Thunb.) Miers are prolate, spheroidal, measuring average size 15 x18 µm. Pollen grains are prolate, spheroidal, planaperturate, tri-zonocolporoidate, colpi operculate and syncolpate, lumina polygonal with fine reticulate sculpturing, small size (17.0 × 15.0 µm). The amb is triangular with rounded angles. While *T. cordifolia* is indigenous, *C. intybus* is adapted to Indian soil and are widely cultivated now in different parts of India for its use as ingredients in medicine and substitute food products.

Comparative account of pollen in honey prior to the extension of Lucknow city (Fig. 2-3) reveals indigenous avenue trees (*Syzygium cuminii*) along with other ornamental trees and exotic weed *Ageratum conyzoides* (Chauhan *et al.,* 2017). *Prosopis juliflora*, *Eucalyptus, Syzygium* etc. were observed in high percentage with very low per cent of medicinally important *Tinospora* pollen in Malihabad, the suburb of Lucknow city. *Ageratum conyzoides* shows highest per cent here indicating the preference of honey bees and the availability of this exotic weed proliferating in fallow land in Malihabad. The details of pollen per cent recorded from the city and urban extension reveals that honey produced in the region is mostly multifloral type but in semi-urban outskirts of Lucknow it is monofloral type (Fig. 3).

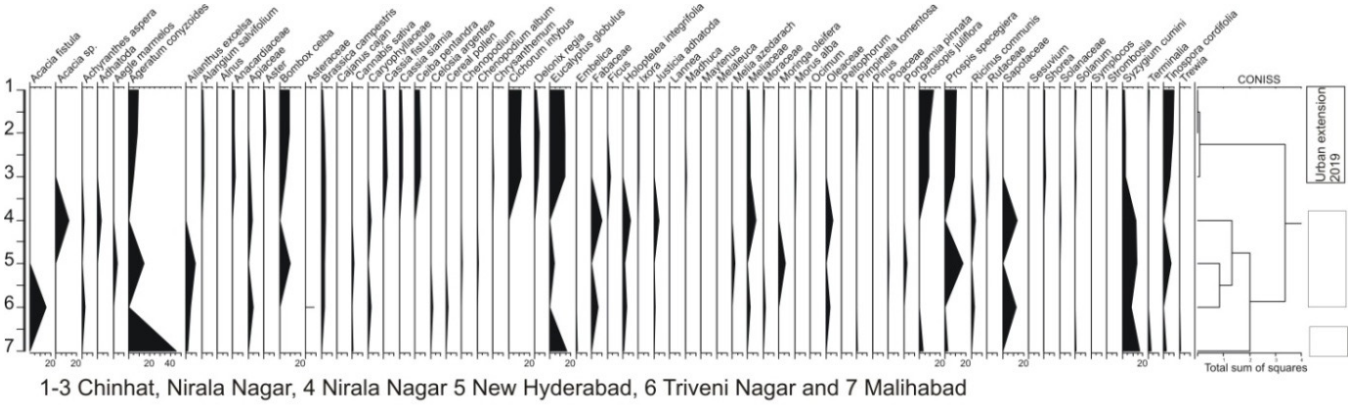

Fig. 3: The Honey flora of Lucknow city and its recent extension in the last 2 decades from Chinhat (recent urban extension), Nirala Nagar, New Hyderabad, Triveni Nagar (Lucknow city) and Malihabad (outskirts, semi-urban, Lucknow)

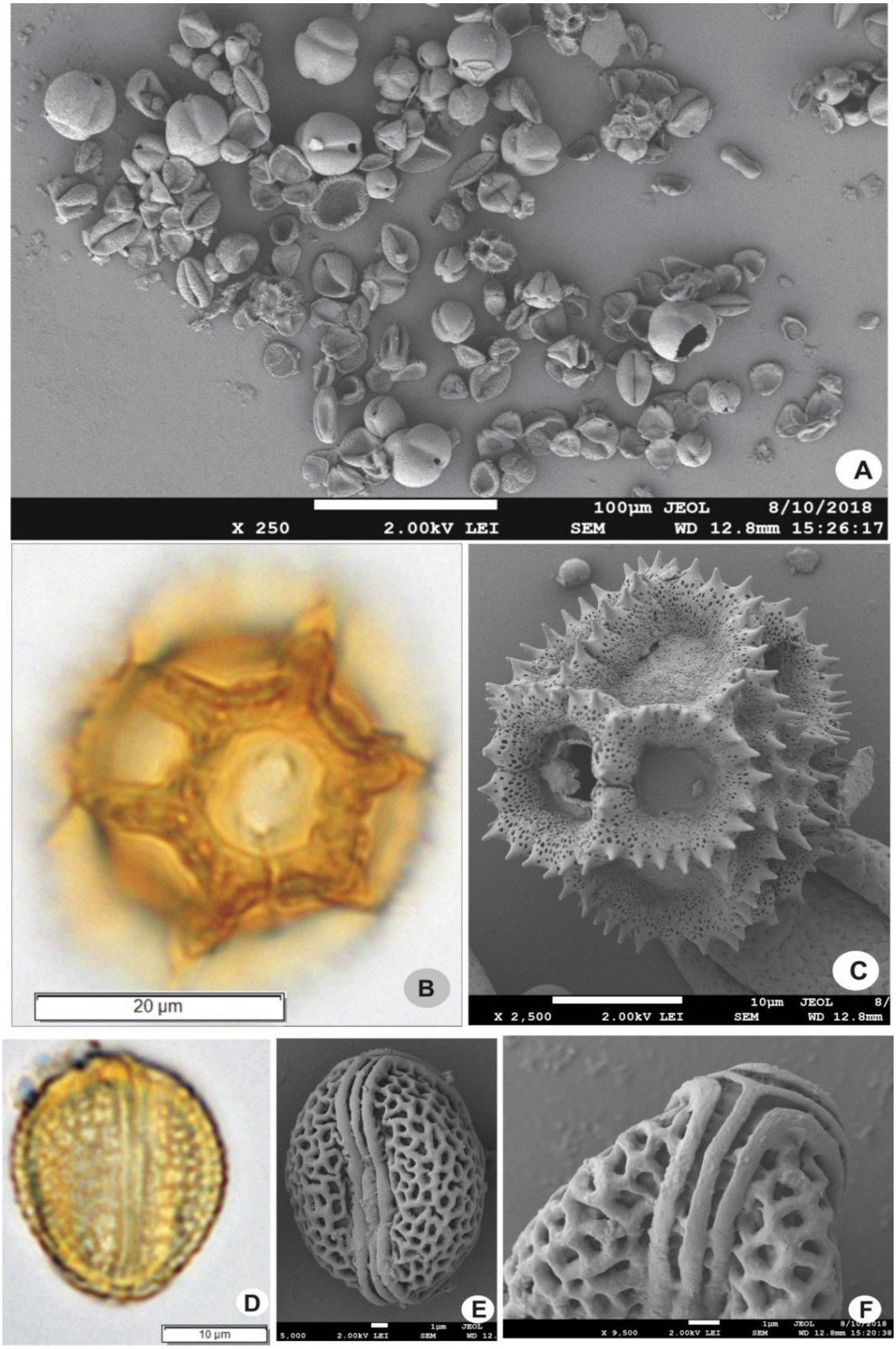

Fig. 4: Scanning Electron Microscopic (SEM) and Light microscopic (LM) photographs of pollen in honey from Chinhat. **A:** Variety of pollen (SEM) assemblage in honey; **B:** *Cichorum intybus* pollen (LM); **C:** A detailed morphology of *C. intybus* (SEM); **D:** *Tinospora cordifolia* pollen (LM); **E-F:** Pollen image of *T. cordifolia* (SEM) showing syncolpate character

Discussion

Two decades back, Chinhat area was an open agricultural land (Chauhan *et al*., 1990). The development of this area has now reduced the expanse of Kathauta lake and agricultural area in the vicinity due to concrete buildings and parks (Kumar *et al.,* 2014; Shukla and Jain 2019). The present study shows production of multifloral honey by bees gathering pollen evidences of about 50 plant species occurring in the area. Interestingly, the pollen abundance of *Cichorum intybus* (commonly known as Chicory) was observed in honey. Therefore, we explored the source of this plant gleaned by honey bees in the vicinity. It was interesting to note that in the vicinity of the honey collection there existed an agri-pharmaco industry where *C. intybus* was grown for its medicinal use in manufacturing/ exporting the raw material for a hepatoprotective drug made from its root. Thus, melissopalynology provided clue to human activities in the area as these are grown for its medicinal value and as a substitute in various food products. Another highly foraged plant was *Tinospora cordifolia* which is again grown and used for manufacturing drugs. The other minor plants foraged by honey bees here are generally those which flower during the spring season between February to April where honey bees get more access and thereby, enrichment of their pollen in honey is observed. Thus, the preferred favorable time for bee hive formation in natural conditions depends on the flowering season and its availability within the flight range of bees. Due to extreme high temperature during summers the honey production in regions with high seasonality hinders the activities of honey bees and wax retention in hives.

In the past, the melissopalynological records reveal that the fruit bearing tree such as *Syzygium cuminii* was highly preferred by Honey Bees in the absence of exotic or other introduced exotic plants. However, the gleaning of nectar from *Ageratum conyzoides* weed in the residential areas of Lucknow city was significantly high during last decade suggesting its preference by honey bees as primary or secondary plant for foraging (Chauhan *et al*., 2017). Due to scarce vegetation in the city within the flight range of honey bees, they were forced to visit the available vegetation and developed their preference for exotic *A. conyzoides* along with the other ornamental and fast growing avenue trees like *Eucalyptus*, *Cassia, Prosopis, Holoptelea,* etc. to produce multifloral type of honey in Lucknow city.

The pollen assemblage in honey from rural outskirts of the city (Malihabad) shows more than 18 plant species, but instead of visiting indigenous plant species the honey bees foraged *A. conyzoides* for nectar suggesting the proliferation of this exotic weed overpowering the other indigenous species in the vicinity. Abundance of *P. spicigera* and *P. juliflora* pollen in honey is due to its large scale plantation for reclamation of sodic soil in the region. It also has its utility for fodder and fuel used by local folks. The rest of the area is arable land used for crop cultivation. Another fast growing plant is *Eucalyptus* which is commercially planted for its use in timber industry. Honey bees have therefore, adapted to forage nectar frequently from introduced plants or exotic weeds that are in abundance in the region due to the need and demand of humans and the available fallow land (Chauhan *et al*., 2017). Hence, the change in foraging pattern through time in the city is reflected by the pollen assemblage in honey and the changing plant diversity in the vicinity (Chauhan and Singh, 2010; Chauhan and Trivedi, 2011).

A comparative account of melissopalynological study reveals that the indigenous tree *S. cuminii* constitutes about 16 and 14 per cent in honey collected from semi-urban areas and in the Lucknow city respectively in the past. However, the present study indicates only 2-3 per cent of its composition in honey suggesting the neglect of planting this economically and medicinally important plant as it is slow growing. The pollen protein in honey and dried powdered seeds of *S. cuminii* are often used for curing diabetic patients amongst local folks and also used in patented medicines (Maiti *et al*., 2013). However, due to sparse *S. cuminii* in the area, the honey bees preferred exotic *A. conyzoides* weed in Malihabad. As a result, the highly beneficial pollen protein from *S.cuminii* was replaced by pollen protein of *A. conyzoides* in honey. Previous mellissopalynological studies (Chauhan *et al*., 2017) from Lucknow city in the last decade revealed a high percentage of Sapotaceae pollen (*Madhuca indica*) which was earlier common in the city but the present study does not show pollen of this tree in honey. Instead, *Acacia* and *Prosopis* species were foraged by honey bees which were planted as avenue trees in the city for its ornamental and fast growing characteristics. It is inferred that honey bees are highly adaptive and quickly change the preference for plants depending on the easy access to whichever plant they come across in the region within their flight range.

Nectar is the source of proteins to the broods and the pollen collected along with it provides energy to the entire colony in beehives (Freitas and Silva, 2006). Honey bees are insensitive to pollen protein quality but the broods are sensitive to the quality of pollen in the bee-hive (Pernal and Currie, 2001). The quantitative and qualitative assemblage of pollen in honey shows its richness in proteins useful for the strong growth and population of broods (Liolios *et al.,* 2016). Therefore, the knowledge of the local flora significantly contributes in ascertaining the quality of protein in honey which is useful for broods and for sensitive consumers who are allergic to a particular type of pollen protein. Across the world, about more than 30% of allergenic reactions in humans are commonly caused by pollen protein (Singh and Mathur, 2012). The allergenic properties of *A. conyzoides* (long flowering time from October to June) and other members of family Asteraceae has been studied since long (Jaggi and Gangal, 1987). Approximately, 60-75% of seasonal rhinosinusitis, asthma and dermatitis are attributed to weed pollen mostly from members of Asteraceae and Gramineae including *A. conyzoides* (Ghosh *et al.,* 2017; Singh, 2017). Pollen retains its allergenic properties in honey and is likely to cause allergic sensitivity (Stanley and Linskens, 1974; Fernández *et al*., 1993). The knowledge of pollen assemblage in honey is yet another important means for quickly understanding hazardous allergenic reactions in honey consumers caused by the protein in it. Medicinally important or allergenic pollen protein information available for honey consumers is a valuable record which can be used for branding and marketing particular types of honey (Chauhan *et al*., 2017) relevant for awareness in persons sensitive to pollen protein. The allergens present in pollen protein are water-soluble glycoproteins. These make them respond to IgE antibody-mediated allergic reaction within

seconds. Therefore, the type of honey with pollen assemblage data is essentially important to verify allergic medical problems in patients of a particular region consuming honey or honey products.

Cichorium intybus var. *sativum* (blue flowers) commonly called as Chicory belong to the Asteraceae family (Compositae) and its roots are used to produce inulin and roasted to produce a substitute for coffee. The allergenic properties of protein found in chicory causes asthma and other oral and cutaneous manifestations which was first reported in 1989 (Cadot *et al.,* 1996; Morita *et al.,* 2007). The 17-kDa chicory protein was responsible for sensitizing allergy symptoms through the respiratory route causing asthma and rhinoconjuctivitis (Pirson *et al.,* 2009). Thus, the agri-pharmaco industry is likely to pose an occupational hazard in urbanized residential areas and requires awareness among the people. In the absence of medicinally important indigenous plants containing useful pollen protein, the honey bees are nowadays forced to forage allergenic weeds like *Ageratum conyzoides* (Chauhan *et al.*, 2017). *C intybus* is a high nectar producing plant and therefore preferred by honey bees (Leonora, 2017). The honey produced may bear allergenic pollen proteins that can create problems among protein sensitive honey consumers. Through melissopalynology, we explored in Lucknow extension, a large scale manufacturer, distributer and exporter of Chicory, in addition to other organic products marketed for nutritive value. This is located in the vicinity (~1 km) of the honey sample collected from Chinhat. Another medicinally important pollen recorded in honey was of *Tinospora cordifolia* which is native to India and is used as an ingredient for treating several common physiological disbalances in human body such as diabetes, allergic rhinitis, stomach upset, lymphoma, hay fever, gonorrhea, syphilis and also to boost immune system. Until now, the allergenic reactions of its pollen protein in honey is not known. Until 2008, the pollen of *C. intybus* and *T. cordifolia* was not recorded in about 51 samples of honey studied from the entire state of Uttar Pradesh of which Lucknow is the capital city (Datta *et al.,* 2008). Neither of these two pollen were recorded in honey from the Lucknow city too, but in 2019 we observe that the honey bees have preferred to forage these two medicinally important plants which are now frequently grown and harvested for their medicinal use. The honey thus produced in urban areas have potential for analyzing anthropogenic activities in the vicinity. The role of pollen assemblages in honey has been widely used in forensic science across the globe (Bryant and Jones, 2006). Thus, the melissopalynology data of the region has potentials in identifying the region- specific human activities or any other nefarious activities related to plants and plant products. The quality of honey patented with its pollen content can enhance its commercial utility by creating awareness in honey consumers facilitating identification of allergenic reactions if any.

Conclusions

The honey collected from the urban extension of Lucknow city revealed 50 pollen taxa that were planted by human beings for aesthetic, ornamental, economic and medicinal reasons. About 10 years back the area was an agricultural land and a big Kathauta lake existed which is an ox-bow lake of the Gomti river. The honey bees have preferred to glean nectar from several invasive exotic weeds (*Ageratum conyzoides*), exotic fast growing avenue trees (*Cassia, Prospis, Eucalyptus* species) and medicinally important plants (*Cichorum intybus, Tinospora cordifolia*). In the process of urbanization and increase in concrete buildings many indigenous plant species (*Syzygium cuminii, Madhuca indica*) have declined during the last two decades, thereby, the pollen protein has changed in the honey produced which needs further investigation for health of broods in bee-hive and for honey consumers as well. Through melissopalynology we detected an agri-pharmaco industry in the vicinity of the honey collection that grew *Cichorum intybus* and *Tinospora cordifolia* for harvesting ingredients used in manufacturing drugs related to hepatoprotection and arthritis which is nowadays a widespread health problem in urban areas. The pollen protein in these plants as well as in *A. conyzoides* is known to induce allergenic sensitivity which can instantly cause sickness in honey consumers. In general, the selection of plants is random in the process of reforestation, reclamation and urbanization and main focus is either on aesthetic/economic or medicinal value. The earnest efforts of honey bees to serve mankind by producing honey as an important food product demands the wiser selection of plants for reforestation and reclamation of land which should be useful for the sustenance of broods and the quality of honey. Therefore, awareness for strategic planning during plantation is essential for the health of honey bees and ecosystem sustainability. The advancement in biotechnology and awareness for the importance of plants in urban settlements facilitated the increase in plant diversity in the studied area which was otherwise an open land in the past. Despite the shrinking of lake and reduction in agricultural land, the study area enhanced the utility of land for various benefits rather than impairing the ecosystem. Hence, urban expansion has increased the plant diversity to 50 taxa in this case study which was good for gleaning nectar by honey bees as compared to a lesser number of plants foraged by honey bees earlier in the Lucknow city and in suburbs. However, in the process of urbanization, the type/quality of honey with regard to pollen protein has changed in recent years which is a necessary information provided to honey consumers in case they are allergic to a certain pollen protein.

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