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Melissopalynological and physicochemical analysis of honey samples from Prayagraj District, Uttar Pradesh

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ABSTRACT. In melissopalynological and physicochemical analyses of 14 honey samples collected from different rural and urban localities of Prayagraj District, Uttar Pradesh, 43 pollen types were identified and categorized as predominant (above 45%), secondary (16–45%), important minor (3–15%) and minor (below 3%). Five of the 14 samples were classified as unifloral due to the presence of predominant pollen types (above 45%), and the other 9 as multifloral. *Brassica campestris* and *Coriandrum sativum* were the predominant pollen types in unifloral honey; 14 pollen types were documented in the secondary pollen types (16–45%) in multifloral honey. The analyzed physicochemical parameters included pH (mean 3.40±0.15 to 4.74±0.4), electrical conductivity (mean 0.13±0.03 to 1.39±0.17 mS/cm), total dissolved solids (120±1.23 to 1260±1 ppm), moisture content (12.17±1.39 to 24.78±1.54 mg/100 g) and ash content (0.15±.04 to 1.68±.27 mg/100g). The color of the honey ranged from water-white to dark amber. Among the minerals, magnesium was found to be most abundant (9±0 to 11.8±0 mg/kg), followed by iron (2.0±0 to 4.8±0 mg/kg) and zinc (0.39±0 to 0.63±0 mg/kg). The heavy metals cadmium, lead, copper and arsenic were below the limit of detection (<0.01 mg/kg) in all honey samples except in samples H6 (Cd 0.01 mg/kg, Pd 0.27 mg/kg), H11 (Cd 0.02 mg/kg, Pd 0.05 mg/kg), H13 (Pd 0.02 mg/kg) and H14 (Cd 0.01 mg/kg, Pd 0.04 mg/kg). The physicochemical parameters varied significantly ($p<0.05$) between samples. Findings of the present study indicate that the honey is of good quality and should be used in projects for commercialization of regional honey.

KEYWORDS: melissopalynology, multifloral honey, physicochemical analysis, unifloral honey

INTRODUCTION

Melissopalynology is a branch of palynology that deals with pollen analysis of honey. Pollen analysis provides relevant information about the plants preferred by honey bees as sources of pollen and nectar, and also aids in determining the geographical and botanical origin of the honey (Cotte et al., 2004; Ponnuchamy et al., 2014). This information is useful in developing apiaries and commercial honey production. Honey is a natural carbohydrate-rich product produced by honey bees from the nectar of plants. Honey possesses valuable nutritive, healing and prophylactic properties (Pereira et al., 1995). The botanical and geographical origin of honey is related to the floral sources, soil, environmental conditions, and mode of extraction and processing (El-Metwally, 2015).

Melissopalynological and physicochemical analyses of honey from India have been published by Khatija and Ramanujan (1993), Nanda et al. (2003), Cherian et al. (2011), Ramnath and Venkataramegowda (2012), Gairola et al. (2013) and Shobham et al. (2017), but a literature survey indicates that for honey samples from Prayagraj District there are only a few pollen analyses (Sahney and Rahi, 2015; Sahney et al., 2018). To help fill the gap, we determined melissopalynological and

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physicochemical parameters (pH, electrical conductivity, total dissolved solids, moisture content, ash content, color, mineral content) of honey produced by *Apis dorsata* bees from different rural and urban localities of Prayagraj District, Uttar Pradesh.

MATERIALS AND METHODS

PALYNOLOGICAL ANALYSIS

Prayagraj District lies in northwestern Uttar Pradesh, India (Fig. 1). Fourteen squeezed winter honey samples of *Apis dorsata* were collected from hives in rural localities (Kotwa, H1, H9; Phaphamau, H10; Phoolpur, H5, H12; Handia, H11) and urban localities (Company garden, H4, H8; Govindpur, H2, H13; Lukarganj, H7; Naini, H6; Teliyarganj, H3, H14) in Prayagraj District in 2017–2018 by professionals under our supervision, then stored in airtight plastic bottles and labeled. During field surveys of the plants growing around the bee hive sites, floral materials were collected for preparation of reference slides, which were made according to the method of Wodehouse (1935). Pollen grain analyses followed the method recommended by the International Commission of Bee Botany (Louveaux et al., 1978). Ten grams of honey were weighed and dissolved in 20 ml distilled water, the solution was centrifuged, the supernatant was decanted and the sediments were placed on a slide. After drying, the sediments were mounted with glycerin jelly, covered with a cover slip and examined under a light microscope. Four pollen slides were made from each honey sample, and 300 or more pollen grains were counted from each slide at random, for a total of 1200 or more pollen grains per sample. Pollen recovered from the honey samples was identified against reference pollen slides. Pollen counts were made at random, covering the maximum mounted area to avoid repetition. Once identified and counted, the pollen grains were assigned to frequency classes: predominant pollen type (above 45%), secondary pollen type (16–45%), important minor pollen type (3–15%) and minor pollen type (<3%) (Louveaux et al., 1978). Honey samples containing more than 45% of a single predominant pollen type were categorized as unifloral, and samples with no predominant pollen type as multifloral. Based on frequency of occurrence in total honey samples, pollen types were classified as very frequent (>50%), frequent (20–50%), infrequent (10–20%) and rare (<10%) (Feller-Demalsy et al., 1987). Absolute pollen counts (APCs) of honey samples (number of pollen grains per 10 g honey) were calculated using a haemocytometer (Suryanarayana et al., 1981). Following Louveaux et al. (1978), honey samples were assigned to various groups: Gp I (APC <20000), Gp II (APC 20000–100000), Gp III (APC 100000–500000), Gp IV (APC 500000–1000000) and Gp V (APC >1000000). These pollen count groups correspond to extremely poor, poor rich, very rich and extremely rich, respectively, as in Feller-Demalsy et al. (1989).

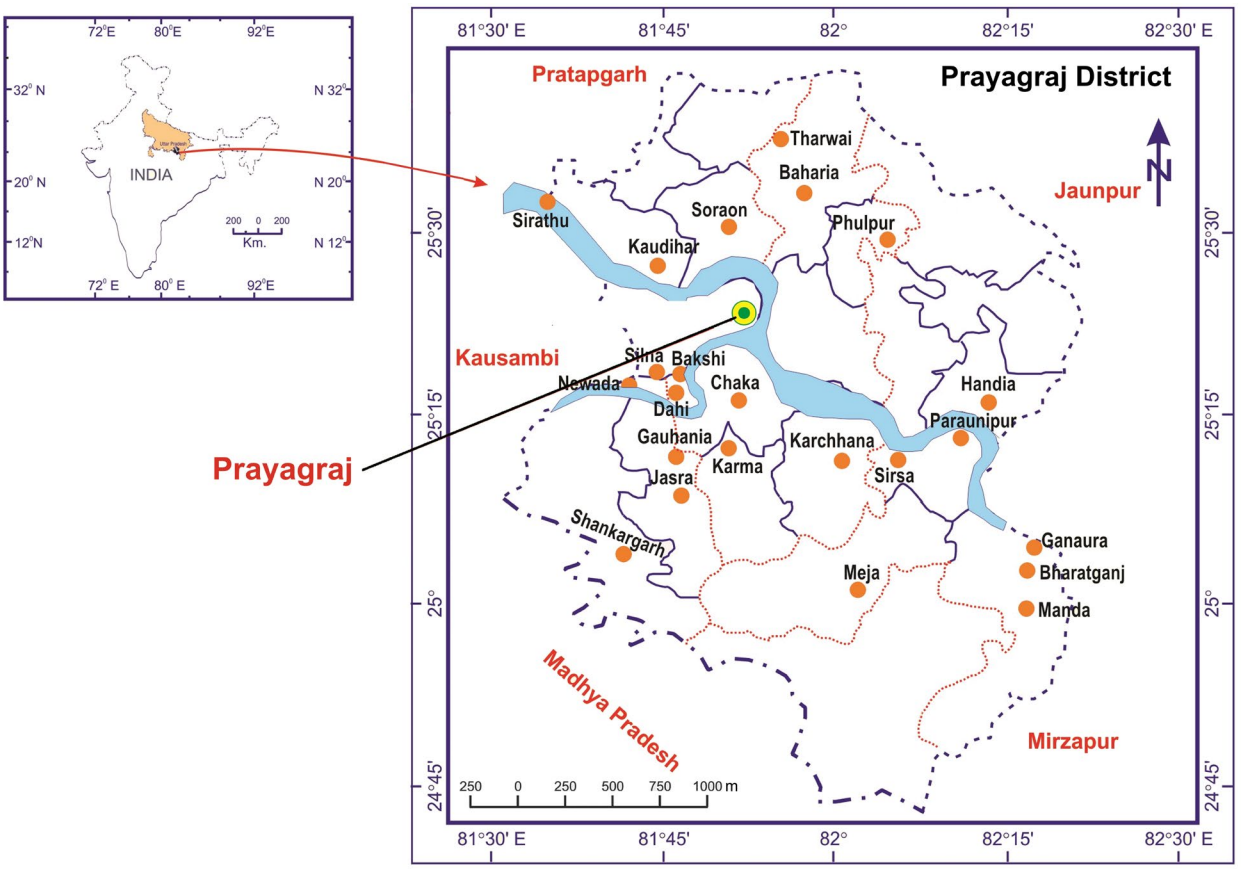

Fig. 1. Map showing the study area in Prayagraj District, Uttar Pradesh

PHYSICOCHEMICAL ANALYSIS

Physicochemical analyses of honey samples followed international recommendations (Bogdanov et al., 1997; AOAC 1999), using the following methods:

– Color was determined with a UV-VIS spectrophotometer (RIGOL – Ultra 3400, single-beam-based) by reading absorbance in aqueous solutions at 635 nm (10 g honey in 20 ml water). Table 2 shows honey colors and their mm Pfund values, obtained using the following algorithm (Bianchi, 1990): mm Pfund = –38.7 + 371.39 × absorbance.

– pH was determined with a digital pH meter (Labronics LT-49) from solutions prepared by dissolving 10 g honey in 75 ml CO_2-free distilled water.

– Electrical conductivity (EC) and total dissolved solids (TDS) were determined with a digital EC/TDS meter (Labronics LT-51) in solutions made by dissolving 20 g honey in 100 ml CO_2 free-distilled water.

– Ash content was determined according to AOAC (1999) using a muffle furnace (Thermotech Tic-4000). Three grams (g) of honey sample were weighed in a dry silica crucible. The weighed samples were gently heated in a muffle furnace until the sample became black and dry. Then the samples were ignited at 550°C for three to five hours. To ensure completion of ashing, it was reheated again in the furnace for half an hour (ash becomes white or grayish white). The ash was cooled in desiccators and weighed.

$$\text{Ash content} = \frac{w_1 - w_2}{w_0} \times 100$$

where,
w_0 = weight of honey taken
w_1 = weight of dish + ash
w_2 = weight of dish

– For moisture content, 3 g of a sample was placed in a pre-weighed flat-bottom dish and kept overnight in a hot-air oven at 100–110°C and weighed. The loss in weight was taken as the measure of moisture content, calculated by the following formula:

$$\text{Moisture (\%)} = \frac{w_1 - w_2}{w_1} \times 100$$

where,
w_1 = weight of fresh honey sample
w_2 = weight of dry honey sample

DETERMINATION OF MINERALS AND HEAVY METALS

Minerals (Mg, Fe, Zn) and heavy metals (Cd, Pb, Cu, As) were determined in a certified laboratory (University of Allahabad, Prayagraj) using an atomic absorption spectrometer (Perkin Elmer Analyst 700).

STATISTICAL ANALYSIS

All physicochemical analyses were done in triplicate, and the data presented as means and standard deviations. Statistical analyses employed SPSS ver. 16. Significance was calculated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($p < 0.05$).

RESULTS AND DISCUSSION

We identified 43 pollen types belonging to 26 families in the 14 honey samples collected from rural and urban localities in Prayagraj District, Uttar Pradesh, during the 2017–2018 winter season (Tab. 1, Fig. 2). Table 2 presents the obtained palynological data for the honey samples.

Thirty-five of the 43 pollen types were found to be from entomophilous plant taxa, two pollen types were found to be from amphiphilous taxa, and six pollen types were found to be from anemophilous taxa (Tab. 1). The 35 entomophilous taxa are *A. conyzoides*, *A. indica*, *A. leptopus*, *B. campestris*, *B. ceiba*, *Bougainvillea* sp., *C. cajan*, *Calliandra* sp., *Callistemon* sp., *C. fistula*, *Chrysanthemum* sp., *C. arietinum*, *Citrus* sp., *C. sativum*, *C. indicum*, *D. sisso*, *D. stramonium*, *Dianthus* sp., *H. rosa-sinensis*, *H. auriculata*, *J. simplex*, *Lathyrus* sp., *L. esculentum*, *M. oleifera*, *O. sanctum*, *P. hysterophorus*, *P. zeylanica*, *P. guajava*, *R. sativus*, *S. arvensis*, *Tagetes* sp., *T. stans*, *T. grandifolia*, *Verbena* sp. and *Vernonia* sp. The anemophilous taxa are *A. indica*, *Chenopodium* sp., *H. integrifolia*, *Solanum* sp., Cyperaceae and Poaceae; presumably they were incidentally transported by wind or were inadvertently transported by bees to the hives. The two amphiphilous taxa we identified are *M. alba* and *E. citriodora*.

The main nectariferous families we documented were Acanthaceae (3 pollen types), Apiaceae (1), Asteraceae (6), Bignoniaceae (1), Bombacaceae (1), Brassicaceae (2), Caryophyllaceae (1), Combretaceae (1), Fabaceae (6), Lamiaceae (1), Malvaceae (1), Meliaceae (1), Moraceae (1), Moringaceae (1), Myrtaceae (3), Nyctaginaceae (1), Plumbaginaceae (1), Polygonaceae (1), Rutaceae (1), Solanaceae (3) and Verbenaceae (1). We documented five nectarless plant families: Chenopodiaceae, Cyperaceae, Euphorbiaceae, Poaceae and Ulmaceae (Tab. 1; Figs 3, 4).

Five of the 14 honey samples were classed as unifloral, due to the presence of predominant *B. campestris* (samples H1, H5, H8, H10) and *C. sativum* (H9). Nine honey samples were classed as multifloral (H2, H4, H6, H7, H8, H11, H13, H14). Fourteen secondary pollen types were recorded in multifloral honey samples: *A. conyzoides*, *A. leptopus*, *A. indica*, *B. ceiba*, *B. campestris*, *C. cajan*, *C. fistula*, *Callistemon* sp., *Chenopodium* sp., *C. sativum*, *E. citriodora*, *M. oleifera*, *P. hysterophorus* and *P. guajava*.

Table 1. Vernacular name, habit, flowering period, mode of pollination and sources of plant species. AM – Amphiphilous taxa; AN – Anemophilous taxa; EN – Entomophilous taxa; N – Nectariferous; P – Polliniferous

Plant species	Vernacular/Common name	Habit	Flowering period	Mode of pollination	Sources
Acanthaceae					
Hygrophila auriculata	Marsh barbel	Herb	Sep–Feb	EN	NP
Justicia simplex	Common justicia	Herb	Aug–Mar	EN	NP
Thunbergia grandifolia	Neel lata/Bengal trumpet vine	Liana	Sep–Jan	EN	NP
Apiaceae					
Coriandrum sativum	Coriander	Herb	Jan–Apr	EN	NP
Asteraceae					
Ageratum conyzoides	Tropical whiteweed	Herb	Jan–Dec	EN	NP
Chrysanthemum sp.	Guldaudi	Herb	Oct–Jan	EN	NP
Parthenium hysterophorus	Gajar ghas/Carrot grass	Herb	Jan–Dec	EN	NP
Sonchus arvensis	Milk thistle	Herb	Sep–Mar	EN	NP
Tagetes sp.	Marigold	Herb	Dec–Feb	EN	NP
Vernonia sp.	Sadodi	Herb	Mar–Apr	EN	NP
Bignoniaceae					
Tecoma stans	Yellow trumpetbush	Tree	Jan–Dec	EN	NP
Bombacaceae					
Bombax ceiba	Semul	Tree	Feb–Mar	EN	NP
Brassicaceae					
Brassica campestris	Mustard	Herb	Nov–Apr	EN	NP
Raphnus sativus	Radish	Herb	Nov–Apr	EN	NP
Caryophyllaceae					
Dianthus sp.	Indian pink	Herb	Jan–Apr	EN	NP
Chenopodiaceae					
Chenopodium sp.	Bathuwa	Herb	Jan–Dec	AN	P
Combretaceae					
Combretum indicum	Madhu malati	Liana	Oct–Mar	EN	NP
Cyperaceae	Cyperaceae	Herb	Jan–Dec	AN	P
Euphorbiaceae					
Acalypha indica	Kuppi	Herb	Sep–May	AN	P
Fabaceae					
Cajanus cajan	Tuvar/Pigeon pea	Herb	Oct–Mar	EN	NP
Calliandra sp.	Red powder puff	Shrub	Jan–Dec	EN	NP
Cassia fistula	Amaltas	Tree	Jan–Oct	EN	NP
Cicer arietinum	Chickpea	Herb	Oct–Mar	EN	NP
Dalbergia sisso	Shisham	Tree	Oct–Mar	EN	NP
Lathyrus sp.	Pulse	Herb	Jan–Apr	EN	NP
Lamiaceae					
Ocimum sanctum	Tulsi	Herb	Mar–Apr	EN	N
Malvaceae					
Hibiscus rosa-sinensis	Gurhal	Shrub	Jan–Dec	EN	NP
Meliaceae					
Azadirachta indica	Neem	Tree	Jan–Mar	EN	N
Moraceae					
Morus alba	Shahtoot/White mulberry	Tree	Feb–Jun	AM	NP
Moringaceae					
Moringa oleifera	Sajina	Tree	Feb–Apr	EN	N
Myrtaceae					
Eucalyptus citriodora	Eucalyptus	Tree	Dec–Apr	AM	NP
Psidium guajava	Guava	Tree	Aug–Sep	EN	NP
Callistemon sp.	Bottle brush	Shrub	Jan–Dec	EN	NP
Nyctaginaceae					
Bougainvillea sp.	Booganbel	Shrub	Jan–Dec	EN	NP
Plumbaginaceae					
Plumbago zeylanica	Chitrak	Herb	Jan–Apr	EN	NP
Poaceae	Grass family	Herb	Jan–Dec	AN	P
Polygonaceae					
Antigonon leptopus	Coral vine	Liana	Aug–Mar	AM	NP
Rutaceae					
Citrus sp.	Lemon	Shrub	Feb–May	EN	N
Solanaceae					
Datura stramonium	Dhatura	Herb	Nov–May	EN	NP
Lycopersicon esculentum	Tomato	Herb	Jan–Apr	EN	NP
Solanum sp.	Solanum	Herb	Jun–Dec	EN	NP
Ulmaceae					
Holoptelea integrifolia	Chilbil	Tree	Jan–Mar	AN	P
Verbenaceae					
Verbena sp.	Barbena	Herb	Oct–Apr	EN	NP

Table 2. Honey types, color, frequency classes, total pollen types and absolute pollen types in honey samples collected from Prayagraj District, Uttar Pradesh

Sample	Honey type	Color (mm Pfund)	Predominant pollen (> 45%)	Secondary pollen (16–45%)	Important minor pollen (3–15%)	Minor pollen (<3%)	Total pollen types	Absolute pollen type (APC)
H1	Unifloral	Water white (2.1529)	*Brassica campestris* (73)	–	*Ageratum conyzoides* (14), Poaceae (5)	*Azadirachta indica*, *Datura stramonium*, *Cajanus cajan*, *Parthenium hysterophorus*, *Ocimum sanctum*	8	II
H2	Multifloral	Light amber (82.1744)	–	*Bombax ceiba* (27), *Ageratum conyzoides* (29), *Chenopodium* sp. (16)	*Pathenium hysterophorus* (8), *Coriandrum sativum* (5), *Acalypha indica* (3), *Brassica campestris* (3)	*Antigonon leptopus*, *Calliandra* sp., *Tecoma stans*, *Eucalyptus citriodora*, *Hibiscus rosa-sinensis*, *Justicia simplex*, *Lathyrus* sp. *Combretum indicum*, *Tagetes* sp.	16	III
H3	Multifloral	Amber (87.5726)	–	*Bombax ceiba* (36), *Cassia fistula* (19), *Antigonon leptopus* (16)	Poaceae (7), *Brassica campestris* (5), *Chrysanthemum* sp., (7)	*Combretum indicum*, *Dianthus* sp., *Lathyrus* sp., *Ocimum sanctum*, *Datura stramonium*, *Tagetes* sp., *Thunbergia grandifolia*	13	III
H4	Multifloral	Extra amber (13.2946)	–	*Eucalyptus citriodora* (23), *Psidium guavaja* (27), *Callistemon* sp. (16)	*Bombax ceiba* (11), *Antigonon leptopus* (7), *Citrus* sp. (5), *Brassica campestris* (3)	*Bougainvillea* sp., *Lathyrus* sp., *Combretum indicum*, *Justicia simplex*, *Verbena* sp., *Tagetes* sp., *Solanum* sp., Poaceae	15	III
H5	Unifloral	Water-white (2.1529)	*Brassica campestris* (79)	–	*Cajanus cajan* (9), *Dalbergia sisso* (3), *Holoptelea integrifolia* (3),	*Hygrophila auriculata*, *Tecoma stans*, *Datura stramonium*, *Lathyrus* sp.	8	III
H6	Multifloral	White (27.77)	–	*Moringa oleifera* (26), *Brassica campestris* (19), *Azadirachta indica* (19)	*Ageratum conyzoides* (11), *Antigonon leptopus* (5), Cyperaceae (3), *Holoptelea integrifolia* (7),	*Coriandrum sativum*, *Hibiscus rosa-sinensis*, *Ocimum sanctum*, *Raphnus sativus*, *Sonchus arvensis*, *Solanum* sp., *Tagetes* sp.	14	IV
H7	Multifloral	Light amber (64.1750)	–	*Ageratum conyzoides* (29), *Azadirachta indica* (25), *Parthenium hysterophorus* (16)	*Acalypha indica* (5), *Callistemon* sp. (6), *Tagetes* sp. (5), *Brassica campestris* (5)	*Antigonon leptopus*, *Bougainvillea* sp., *Calliandra* sp., Cyperaceae, *Psidium guajava*, *Justicia simplex*	13	III
H8	Unifloral	Amber (98.7143)	*Brassica campestris* (67)	–	*Azadirachta indica* (11), *Cicer arietinum* (6), *Cajanus cajan* (6),	*Moringa oleifera*, *Morus alba*, *Parthenium hysterophorus*, *Plumbago zeylanica*, Poaceae, *Psidium guajava*	10	II
H9	Unifloral	Dark amber (115.4268)	*Coriandrum sativum* (58)	–	*Brassica campestris* (13), *Psidium guajava* (13), *Dalbergia sisso* (6), *Cicer aritenium* (3)	Cyperaceae, *Parthenium hysterophorus*, *Ageratum conyzoides*, *Sonchus arvensis*	9	III
H10	Unifloral	Light amber (53.4047)	*Brassica campestris* (67)	–	*Eucalyptus citriodora* (13), *Citrus* sp. (11), Poaceae (4)	*Acalypha indica*, *Tecoma stans*, *Parthenium hysterophorus*	7	II
H11	Multifloral	Extra white (14.03738)	–	*Ageratum conyzoides* (33), *Brassica campestris* (21), *Coriandrum sativum* (16)	*Cicer arietinum* (11), *Citrus* sp. (3), Cyperaceae (3)	*Acalypha indica*, *Azadirachta indica*, *Chrysanthemum* sp., *Morus alba*, *Lathyrus* sp., *Raphnus sativus*, *Sonchus arvensis*, Poaceae	14	IV
H12	Multifloral	Water-white (2.5129)	–	*Brassica campestris* (27), *Antigonon leptopus* (21), *Moringa oleifera* (18)	*Psidium guajava* (13), *Combretum indicum* (5), *Ocimum sanctum* (3)	*Citrus* sp., Cyperaceae, *Datura stramonium*, *Holoptelea integrifolia*, *Lycopersicon esculentum*, *Morus alba*, *Parthenium hysterophorus*, *Solanum* sp.	14	III
H13	Multifloral	Light amber (54.1475)	–	*Brassica campestris* (43), *Cajanus cajan* (16), *Coriandrum sativum* (19)	Poaceae (7), *Psidium guajava* (3), *Moringa oleifera* (3)	*Tecoma stans*, *Datura stramonium*, *Holoptelea integrifolia*, *Morus alba*, *Raphnus sativus*, *Sonchus arvensis*, *Verbena* sp.	13	III
H14	Multifloral	Water-white (5.8668)	–	*Bombax ceiba* (33), *Cassia fistula* (17), *Moringa oleifera* (17)	*Brassica campestris* (11), *Morus alba* (7), Cyperaceae (3), *Holoptelea integrifolia* (3)	*Citrus* sp., *Lathyrus* sp., *Parthenium hysterophorus*, *Sonchus arvensis*, *Tagetes* sp., *Thunbergia grandifolia*, *Vernonia* sp.	14	II

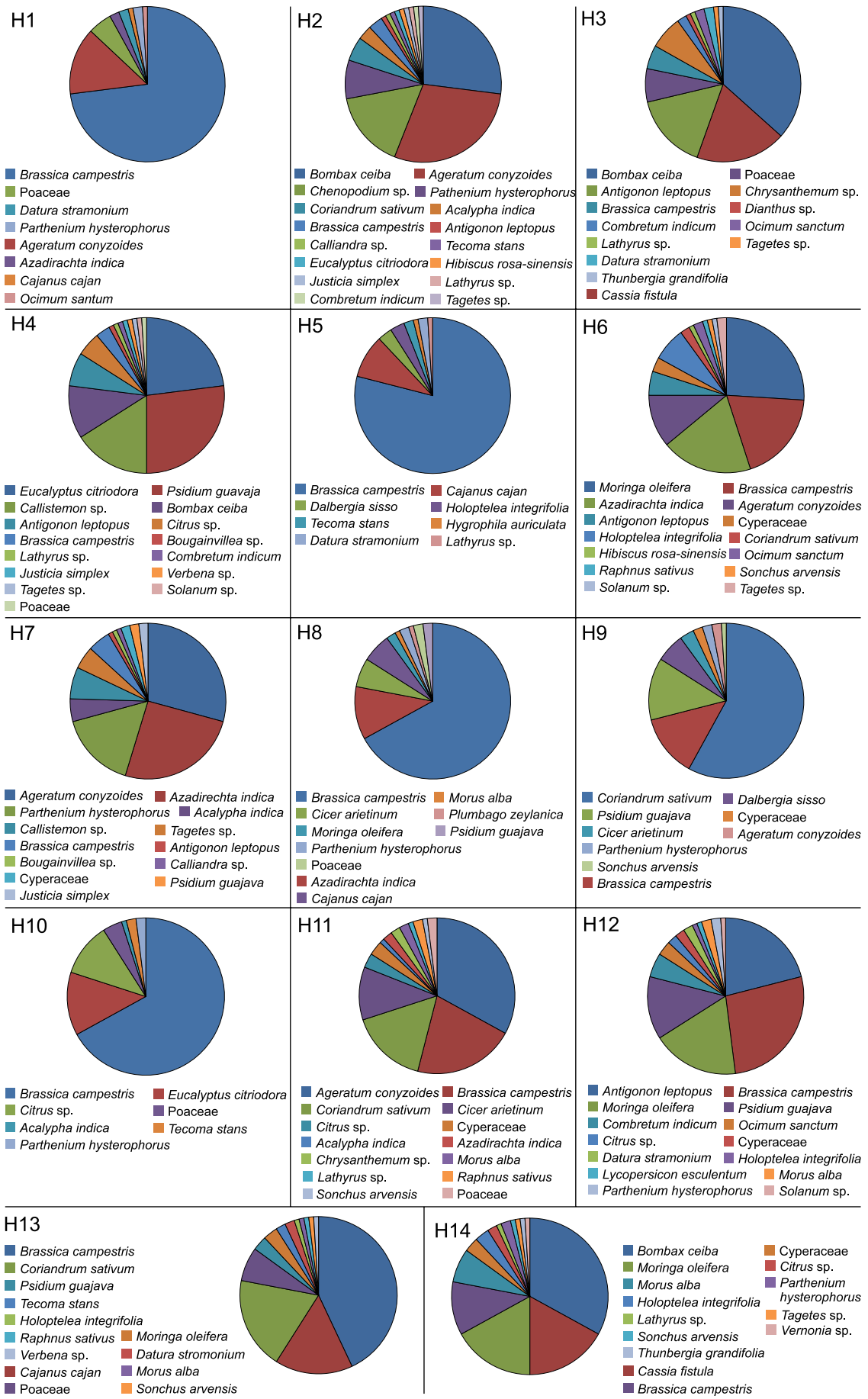

Fig. 2. Pollen spectra of each honey sample collected from different localities in Prayagraj District, Uttar Pradesh

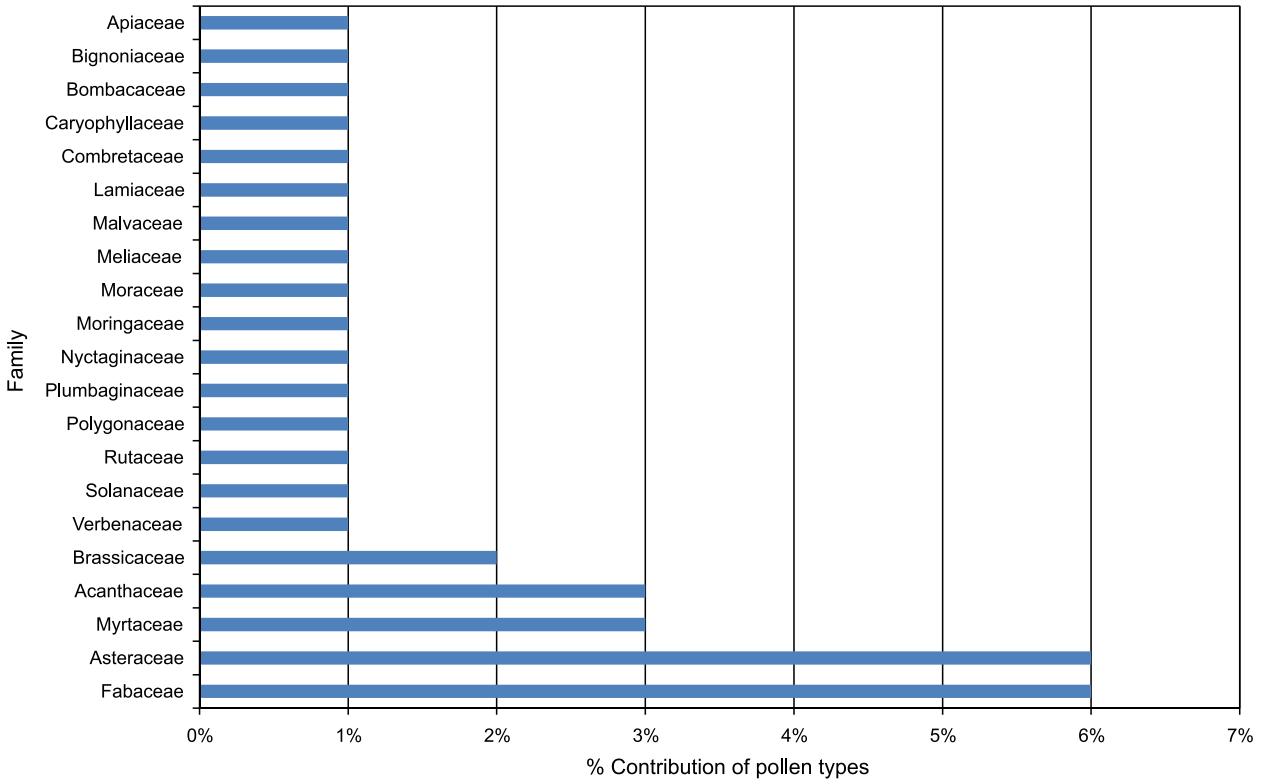

Fig. 3. Contribution per family of nectariferous pollen types

Twenty-four pollen types were recorded as important minor types of the pollen content: *A. indica*, *A. conyzoides*, *A. leptopus*, *A. indica*, *B. campestris*, *B. ceiba*, *C. cajan*, *Callistemon* sp., *C. fistula*, *Chenopodium* sp., *Chrysanthemum* sp., *C. arietinum*, *Citrus* sp., *C. indicum*, *C. sativum*, Cyperaceae, *D. sisso*, *E. citriodora*, *H. integrifolia*, *M. oleifera*, *M. alba*, *O. sanctum*, *P. hysterophorus*, *P. guajava* and Poaceae.

Thirty-four pollen types were recorded as minor components: *A. indica*, *A. leptopus*, *A. indica*, *Bougainvillea* sp., *C. cajan*, *Calliandra* sp., *T. stans*, *Chrysanthemum* sp., *Citrus* sp., *C. indicum*, Cyperaceae, *D. stramonium*, *Dianthus* sp., *E. citriodora*, *H. rosa-sinensis*, *H. integrifolia*, *H. auriculata*, *J. simplex*, *Lathyrus* sp., *L. esculentum*, *M. oleifera*, *M. alba*, *O. sanctum*, *P. hysterophorus*, *P. zeylanica*, Poaceae, *P. guajava*, *R. sativus*, *S. arvensis*, *Solanum* sp., *Tagetes* sp., *T. grandifolia*, *Vernonia* sp. and *Verbena* sp. (Tab. 2).

Brassica campestris and *C. sativum* were very frequent, 16 pollen types were recorded as frequent, and the remaining 25 were infrequent in the total honey sample (Fig. 5). *B. campestris* was predominant in four samples

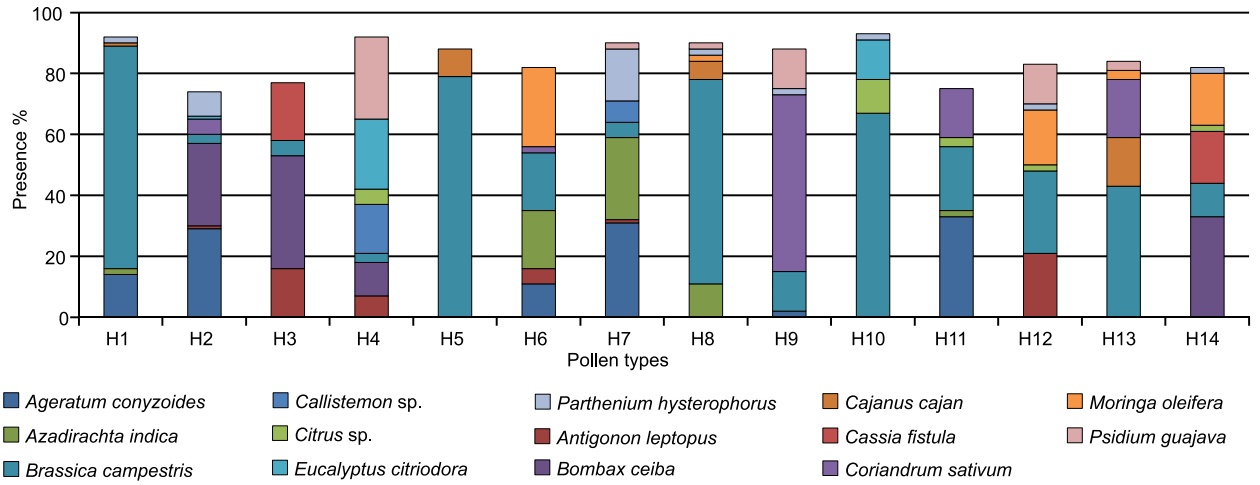

Fig. 4. Pollen types of main nectariferous plants of each honey sample

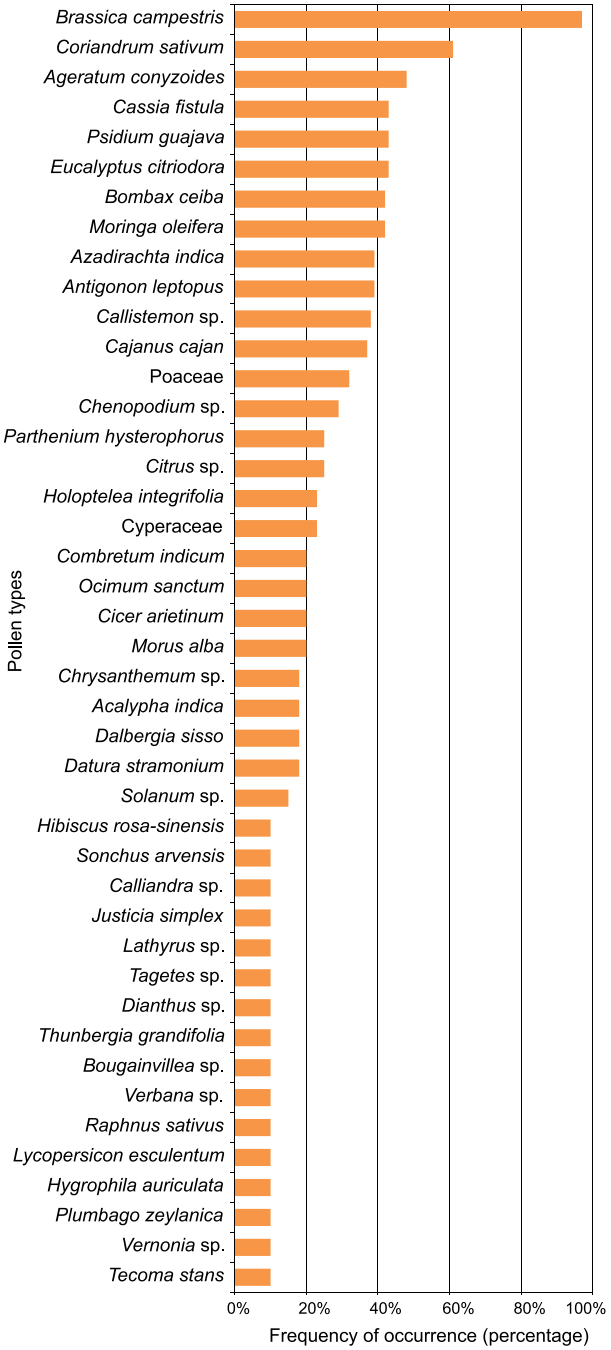

Fig. 5. Frequency of occurrence of pollen types recovered from the total honey samples collected from Prayagraj District, Uttar Pradesh

(H1, H5, H8, H10), secondary in four samples (H6, H9, H11, H12, H14) and an important minor pollen type in five samples (H2, H3, H4, H7, H13). *B. campestris* was documented as the main bee-forage plant. Similar observations have been published from different regions of India (Chauhan and Trivedi, 2011; Sahney and Rahi, 2015; Sahney et al., 2018). *C. sativum* was predominant in one sample (H9), secondary in two samples (H12, H13) and an important minor pollen type in one sample (H2).

Among the frequent types in Prayagraj District, *A. conyzoides*, *A. indica*, *B. ceiba*, *Callistemon* sp., *Citrus* sp. and *E. citriodora* are major nectariferous/ polleniferous plant taxa documented in different states of India (Datta et al., 2008; Pal and Karmarkar, 2013; Ramakrishna and Swathi, 2013; Chauhan et al., 2017; Sahney et al., 2018).

The pollen spectra of the 14 honey samples collected from different localities in Prayagraj District showed a diversity of pollen types, ranging in number from 7 to 16 (Fig. 2) and reaching maximum in multifloral honey sample H2 (16 pollen types), followed by H4 (15), H6 (14), H11 (14), H12 (14), H14 (14), H3 (13), H7 (13) and H13 (13). Minimum pollen diversity was recorded in unifloral sample H10 (7 pollen types), followed by H1 (8), H5 (8), H9 (8) and H8 (9).

With respect to absolute pollen count, four samples belonged to Gp II (poor: H1, H8, H10, H14), eight samples to Gp III (rich: H2, H3, H4, H5, H7, H9, H12, H13) and two samples to Gp IV (very rich: H6, H11).

The investigated region is mainly tropical, where the flowering of herbs, shrubs, climbers and trees is abundant during the winter season. These plants are profusely distributed along the extensive cultivated land in rural areas; in urban areas, tree avenues are common, as are herb and shrub plantings. In the present study we found that herbs (*A. conyzoides*, *B. campestris*, *C. sativum*, *Chenopodium* sp., *J. simplex*, *P. hysterophorus*), shrubs (*Citrus* sp., *Callistemon* sp., *T. stans*), climbers (*A. leptopus*, *C. indicum*, *T. grandifolia*) and trees (*B. ceiba*, *E. citriodora*, *M. alba*, *P. guajava*) were the main forage plants of *Apis dorsata* in Prayagraj District.

PHYSICOCHEMICAL ANALYSIS

Table 3 gives the results of physicochemical analyses for pH, electrical conductivity, total dissolved solids, moisture content, ash content and mineral content. Results for honey color are given in Table 2. The values of these parameters are summarized below.

The pH of all honey samples ranged from 3.40±0.15 to 4.74±0.4 (Tab. 3). The low pH inhibits the growth of microorganism and influences the texture and stability of honey (Terrab et al., 2003). Low pH (3.49–4.70) of honey samples has also been reported from different regions of India, Argentina, Algeria and Estonia

Table 3. Physicochemical analysis of honey samples collected from Prayagraj District, Uttar Pradesh

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14
pH	3.68±0.15^{b}	3.78±0.25^{c}	3.66±.13^{b}	3.64±.01^{b}	3.69±.13^{b}	4.16±.14^{c}	3.66±.13^{b}	4.31±.04^{b}	4.74±.04^{b}	3.4±.15^{b}	4.28±.03^{b}	3.43±.17^{c}	3.92±.13^{d}	3.58±.10^{c}
EC	0.13±0.03^{a}	0.67±.10^{a}	0.31±.06^{a}	0.33±.13^{a}	0.3±.06^{a}	1.29±.14^{a}	1.16±.14^{a}	1.06±.17^{a}	1.39±.17^{a}	0.5±.41^{a}	1.16±.15^{a}	0.66±.26^{ab}	1.02±.10^{b}	0.3±.15^{a}
TDS	128±1.73^{f}	376±1.52^{f}	184±.95^{e}	125±1.15^{e}	222±.57^{f}	667±1.73^{f}	713±2.20	634±1^{e}	1260±1^{e}	350±1.56^{e}	153±1.96^{e}	207±2.05^{f}	120±1.23^{g}	368±.69^{f}
moisture content	13.02±.96^{d}	17.4±.56^{e}	14.52±1.13^{d}	12.55±1.41^{d}	14.77±.83^{e}	18.99±.95^{e}	18.87±1.64	18.51±1.54^{d}	24.78±1.54^{d}	12.17±1.39^{d}	16.18±1.02^{d}	19.14±.89^{e}	12.66±.58^{f}	17.02±1^{e}
Ash content	0.42±.41^{a}	0.15±.04^{a}	0.25±.09^{a}	0.4±.43^{a}	0.43±.09^{a}	0.21±.092^{a}	1.07±.08^{a}	0.83±.12^{a}	1.37±.12^{a}	0.23±.06^{a}	1.24±.23^{a}	1.68±.27^{b}	0.55±.21^{ab}	0.47±.17^{a}
Color (absorbance)	0.11±.004^{a}	0.247±.008^{a}	0.34±.002^{a}	0.14±.004^{a}	0.11±.002^{a}	0.179±.005^{a}	0.277±.003^{a}	0.37±.008^{a}	0.415±.008^{a}	0.248±.007^{a}	0.142±.005^{a}	0.11±.006^{a}	0.12±.004^{a}	0.25±.007^{a}
Mg (mg/kg)	11.5±0^{c}	10.5±0^{d}	11±0^{c}	10±0^{c}	11±0^{d}	11±0^{d}	10.8±0	10±0^{c}	11±0^{c}	11.3±0^{d}	9.8±0^{c}	9±0^{d}	11±0^{e}	11.8±0^{d}
Fe (mg/kg)	4.65±0^{b}	2.5±0^{b}	2.85±0^{b}	3.5±0^{b}	4.5±0^{c}	2±0^{b}	3.2±0^{b}	3.68±0^{b}	4.8±0^{b}	5.9±0^{c}	3.5±0^{b}	4.6±0^{c}	2.45±0^{c}	2±0^{b}
Zn (mg/kg)	0.5±0^{a}	0.6±0^{a}	0.4±0^{a}	0.55±0^{a}	0.49±0^{a}	0.63±0^{a}	0.45±0^{a}	0.5±0^{a}	0.39±0^{a}	0.42±0^{a}	0.57±0^{a}	0.45±0^{ab}	0.5±0^{ab}	0.55±0^{a}
Cd (mg/kg)	BLD	BLD	BLD	BLD	BLD	0.01	BLD	BLD	BLD	BLD	0.02	BLD	BLD	0.01
Pd (mg/kg)	BLD	BLD	BLD	BLD	BLD	0.27	BLD	BLD	BLD	BLD	0.05	BLD	0.02	0.04
Cu (mg/kg)	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD
As (mg/kg)	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD	BLD

Values are mean ±S.D.; n=3; BLD= below the limit of detection (0.01 ppm).
Means are compared by using One way ANOVA-Post hoc multiple comparisons in each row.
Means that do not share a small alphabet letter (after standard deviation – SD) within the same row indicate significant differences ($p < 0.05$).

(Ouchemoukh et al., 2007; Cantarelli et al., 2008; Saxena et al., 2010; Kirs et al., 2011).

Electrical conductivity is a good criterion of the botanical origin of honey. Blossom honey and mixtures of blossom and honeydew honey should have conductivity of less than 0.8 mS/cm (Codex Alimentarius 2001). All the honey samples had electrical conductivity values ranging from 0.13±0.03 mS/cm to 1.39±0.17 mS/cm.

Total dissolved solids (TDS) is a measure of the combined content of all inorganic and organic substances in honey in molecular, ionized or micro-granular (colloidal solution) suspended forms. All the honey samples had total dissolved solid values ranging from 120±1.23 ppm to 1260±1 ppm.

The moisture content of all honey samples ranged from 12.17±1.39 mg/100g to 19.14±.89 mg/100g, except for sample H9, which measured 24.78±1.54 mg/100g (Tab. 3). The recommended limit for moisture content of honey samples is 20 mg/100g according to international quality regulations (Codex Alimentarius, 2001). Low moisture content prevents fermentation of honey and attack by microorganisms. It helps in its preservation and storage and increases the shelf life of honey (Buba et al., 2013; El-Metwally, 2015). Higher moisture content can lead to undesirable fermentation of honey during storage (Saxena et al., 2010). Moisture content depends on environmental factors during honey production, such as weather and hive humidity. It also depends on nectar conditions and on the treatment of honey during extraction and storage.

The ash content of the all honey samples varied between 0.15±.04 and 1.68±.27 mg/100g, which is an acceptable range (Codex Alimentarius 2001). These results reflect the abundance of pollen sources in the vicinity of the bee hive sites during honey production. Similar observations have been made by various workers from different regions of India (Nanda et al., 2003; Sahney and Kumar, 2017), Turkey (Unal and Kuplulu, 2006), Argentina (Cantarelli et al., 2008) and Estonia (Kirs et al., 2011).

The color of the studied honey ranged from water-white to dark amber (Tab. 2). One honey sample from Prayagraj District was darker amber in color and had the highest Pfund value (H9). The other honey samples were water-white to amber in color: water-white in samples H1, H5, H12 and H13 (2.5129 mm Pfund), extra white in H14 and

H11 (13.2946 and 14.0373 mm Pfund), white in sample H6 (27.77 mm Pfund), light amber in H14 (54.1475 mm Pfund), light amber in H7 (64.1750 mm Pfund), amber in H3 and H8 (87.5726 and 98.7143 mm Pfund) and dark amber in H9 (115.4268 mm Pfund). The color of honey depends on its ash content, temperature and storage time (De Silva et al., 2016).

Among the minerals, magnesium content was the highest, ranging from 9.0±0.0 to 11.8±0 mg/kg, followed by iron (2.0±0.0 to 5.9±0.0 mg/kg) and zinc (0.39±0.0 to 0.6±0.0 mg/kg). The heavy metals cadmium (Cd), lead (Pd), copper (Cu) and arsenic (As) were below the limit of detection (<0.01 mg/kg) in all samples except in H6 (Cd 0.01 mg/kg, Pd 0.27 mg/kg), H11 (Cd 0.02 mg/kg, Pd 0.05 mg/kg), H13 (Pd 0.02 mg/kg) and H14 (Cd 0.01 mg/kg, Pd 0.04 mg/kg).

CONCLUSION

In this study we conducted melissopalynological and physicochemical analyses of honey samples collected from rural and urban localities of Prayagraj District, Uttar Pradesh. We found unifloral honey mostly in rural areas, while multifloral honey was found in urban areas. Cultivated rural land is dominated by *Brassica* sp. crops, with sparse trees, while urban areas have tree avenues along with herb and shrub plantings. Our work provides information related to the pattern of vegetation of Prayagraj District. The studied honey can be considered as of good quality, as all samples were found to meet the guidelines of Codex Alimentarius (2001) and the European Commission (2002) for all physicochemical parameters (pH, EC, TDS, moisture content, ash content and color).

Our analyses indicate that Prayagraj District has a rich flora conducive to the production of good-quality honey. The study should be of use in promoting the commercialization of regional honey and in establishing the apiary industry in Prayagraj District, Uttar Pradesh.

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REFERENCES

AOAC, 1999. Official methods of analysis of AOAC international (P. Cunniff, ed.) 16th ed. Revision. Vol. II. Association of Analytical Chemists. USA.

Bianchi, E.M., 1990. Quality control of honey and wax. UN for Agriculture and Food. FAO Agricultural Service Bulletin 68/3. Rome. p. 69.

Bogdanov, S., Martin, P., Lüllmann, C., 1997. Harmonised methods of the European honey commission. Apidologie, 1–59.

Buba, F., Gidado, A., Shugaba, A., 2013. Analysis of biochemical composition of honey samples from North-East Nigeria. Biochemistry and Analytical Biochemistry 2(3), 139.

Cantarelli, M.A., Pellerano, R.G., Marchevsky, E.J., Camiña, J.M., 2008. Quality of honey from Argentina: Study of chemical composition and trace elements. Journal of the Argentine Chemical Society 96(1–2), 33–41.

Chauhan, M.S., Trivedi, A., 2011. Pollen analysis of honey from Lucknow district, Uttar Pradesh. Journal of Applied Biosciences 37(1), 48–51.

Chauhan, M.S., Farooqui, A., Trivedi, A., 2017. Plant foraged by bees for honey production in northern India: The diverse flora of India and its implications for apiculture. Acta Palaeobotanica 57(1), 119–132.

Cherian, K.J., Bhowal, M., Godghate, S.D., 2011. Pollen and physicochemical analysis of honey produced by *Apis cerana indica* of nagpur, Maharashtra (India). Journal of Environmental Research And Development 5(3), 542–550.

Codex Alimentations, 2001. Draft revised standard for standard for honey (at step 10 of the Codex procedure). Alinorm. 01(25), 19–26.

Cotte, J.F., Casabianca, H., Chardon, S., Lheritier, J., Grenier-Loustalot, M.F., 2004. Chromatographic analysis of sugars applied to the characterisation of monofloral honey. Analytical and Bioanalytical Chemistry 380, 698–705.

Datta, K., Verma, P.C., Chatterjee, A., 2008. Mapping of key bee flora of upper Gangetic region of India – a palynological assessment through regional honeys. Grana 47, 159–170.

De Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O., 2016. Honey: Chemical composition, stability and authenticity. Food Chemistry 196, 309–323.

El-Metwally, A.A.E., 2015. Factors Affecting the Physical and Chemical Characteristics of Egyptian Beehoney. Ph. D. Thesis, Fac. Agric. Cairo Univ., 320 p.

European Commission. 2002. Council Directive 2001/110/EC of 20th, December 2001 relating to honey. Official Journal of the European Communities 10, 47–52.

Feller-Demalsy, M.-J., Parent, J., Strachan, A.A., 1987. Microscopic analysis of honeys from Alberta. Canada. Journal of Apicultural Research 26(2), 123–132.

Feller-Demalsy, M.-J., Parent, J., Strachan, A.A, 1989. Microscopic analysis of honey from Manitoba, Canada. Journal of Apicultural Research 28, 41–49.

Gairola, A., Tiwari, P., Tiwari, J.K., 2013. Physicochemical properties of *Apis cerana-indica* f. honey from Uttarkashi district of Uttarakhand, India. Journal of Global Biosciences 2(1), 20–25.

Khatija, F., Ramanujan, C.G.K., 1993. Pollen and chemical characterization of *Apis cerana* and *A. florae* honeys from Hyderabad urban complex. Journal of Palynology 29, 59–67.

Kirs, E., Pall, R., Martverk, K., Laos, K., 2011. Physicochemical and melissopalynological characterization of Estonian summer honeys. Procedia Food Science 1, 616–624. doi: 10.1016/j.profoo.2011.09.093

Louveaux, J., Maurizio, A., Vorwohl, G., 1978. Methods of melissopalynology. Bee World 59, 139–157.

Nanda, V., Sarkara, B.C., Sharma, H.K., Bawa, A.S.V., 2003. Physicochemical properties and estimation of mineral content in honey produced from different plants in Northern India. Journal of Food Composition and Analysis 16, 613–619.

Ouchemoukh, S., Louaileche, H., Schweitzer, P., 2007. Physicochemical characteristics and pollen spectrum of some Algerian honeys. Food Control 18, 52–58.

Pal, P.K., Karmarkar, K., 2013. Pollen analysis in understanding the foraging behaviour of *Apis mellifera* in Gangetic West Bengal. Geophytology 42, 93–114.

Pereira, P.C.M., Barraviera, B., Burini, R.C., Soares A.M.V.C., Bertani, M.A., 1995. Use of honey as nutritional and therapeutic supplement in the treatment of infectious diseases. Journal of Venomous Animals and Toxins. Preliminary Report 1, 1–2.

Ponnuchamy, R., Bonhomme, V., Prasad, S., Das, L., Patel, P., 2014. Honey pollen: using melissopalynology to understand foraging preferences of bees in tropical South India. PLoS ONE 9(7), 1–11.

Ramakrishna, H., Swathi, S., 2013. Pollen diversity in some *Apis* florae honeys from Adilabad District. Andhra Pradesh, India. Geophytology 42, 11–20.

Ramnath, S., Venkataramegowda, S., 2012. Physicochemical and pollen analysis of Western Ghats honey of Karnataka, South India. International Journal of Sciences and Nature 3(4), 831–835.

Sahney, M., Kumar, A., 2017. Physiochemical and mineral analysis of honey samples from Varanasi district. International Journal of Pharma and Bio Sciences 8(2), 160–166

Sahney, M., Rahi, S., 2015. Pollen analysis of honey samples from Allahabad district, Uttar Pradesh, India. Geophytology 45(1), 21–30.

Sahney, M., Rahi, S., Kumar, A., Jaiswal, R., 2018. Melissopalynological studies on winter honeys from Allahabad, Uttar Pradesh. Palynology 42(4), 540–552.

Saxena, S., Gautam, S., Sharma, A., 2010. Physical, biochemical and antioxidant properties of some Indian honeys. Food Chemistry 118, 391–397.

Shobham, Chitluri, K.K., Nayar, J., 2017. Physico-Chemical Analysis of Some Commercial Honey Samples from Telangana. Indian Journal of Nutrition 4(1), 150–153.

Suryanarayana, M.C., Seethalakshmi, T.S., Phadke, R.P., 1981. Pollen analysis of Indian honeys 1. Honeys from Litchi (*Nephelium litchi*) and Jamun (*Syzygium cumini*). Proc. IV Int. Palynol. Conf. Luc. (1976–77) 3, 491–498.

Terrab, A., Diez, M.J., Heredia, F.J., 2003. Palynological, physicochemical and color characterization of Moroccan honeys: I. River red gum (*Eucalyptus camaldulensis* Dehnh) honey. International Journal of Food Science & Technology 38, 379–386.

Unal, C., Kuplulu, O., 2006. Chemical quality of strained honey consumed in Ankara. Ankara Universitesi Veteriner Fakultesi Dergisi 53, 1–4.

Wodehouse, R.P., 1935. Pollen grains, their structure, identification and significance in science and medicine. New York and London: McGraw Hill Book Company, Inc.