Botanical origin and chemical composition of bee pollens collected from *Apis cerana* hives domesticated in Pauri Garhwal, Western Himalaya, India

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Abstract. The present investigation aims to determine the botanical origin and chemical composition of bee pollen samples (n =22) harvested from *Apis cerana* hives domesticated in Pauri Garhwal (Uttarakhand, India). The majority (95%) of the samples were unifloral in their botanical origin. All the identified pollens belonged to eighteen plant families, among which Rutaceae, Asteraceae and Brassicaceae were found dominant. The chemical parameters soluble sugars, starch, crude protein, amino acids and phenolic contents were analyzed calorimetrically and were found in the range from 0.2 to 26.09 mg/g, 0.22 to 11.04 mg/g, 13.40 to 191.41 mg/g, 2.01 to 6.48 mg/g, and 5.10 to 35.50 mg GAE/g, respectively. Statistically significant differences ($p<0.05$) were observed between the chemical contents of the analyzed samples and a moderate correlation ($r= 0.40$; $n=22$) was observed between total soluble sugars and crude protein. Bee pollens as a good source of nutrition, medicine and dietary supplement for both humans and bees, demonstrate the important need to define bee pollen from different regions of India in order to develop bee pollen quality standards.

Key words: Unifloral bee pollen, palynological analysis, soluble sugars, phenolic contents, Himalaya.

1. Introduction

Bees and plants are mutually interdependent on each other. Honey bees collect both nectar and pollen during their foraging activity and act as a potent pollinator for plants due to their morphological structure that facilitates pollen attachment, transfer and deposition (Suwannapong et al., 2012). Nectar is the primary source of carbohydrates while pollen acts as a rich source of protein which guarantees the survival and longevity of honeybees (Schmidt et al., 1987; Roulston & Buchmann, 2000). Bees during their visit to plants collect pollen, mix it with nectar and salivary secretions to constitute the pollen pellet (or pollen load), which is transported and stored in the hive by carrying the pellets in their pollen baskets (or corbiculae) present on their hind legs (Luz & Barth, 2001). There is about 10% nectar in a pollen load, which is necessary for packaging (Campos et al., 2008). Hence, bee pollen has peculiar characteristics that distinguishes it from hand-collected and wind-pollinated pollens (Villanueva et al., 2002).

Bee pollen is actually a nutritious and healthy source of energy, which is used by the honeybees to feed their young ones at different stages of development in the hive (Schmidt et al., 1987). It is composed of crude fibre (0.3–20%), lipids (1–13%), ash content (2–6%), proteins (10–40%) and carbohydrates 13–55% (Campos et al., 2008). It also contains all necessary fatty and amino acids, as well as minerals, vitamins (primarily B-complex), secondary metabolites such anthocyanins, phytosterols, carotenoids, and polyphenols (particularly flavonoids) and other nutrients (Thakur & Nanda, 2018).

This natural product (bee pollen) is being given more importance due to its high nutraceutical potential and positive relation to physiological or psychological health (Bobis et al., 2010). Bee pollen is also considered as 'the only perfectly complete food' (Kostic et al., 2015). It possesses a diverse range of significant benefits, such as antioxidant (free radical scavenging), anti-carcinogenic, anti-inflammatory, anti-ageing, cardioprotective, and anti-atherosclerosis properties (Kostic et al., 2015). Polyphenols or free radicals are the potent constituents responsible for the antioxidant activity in pollen (Bonvehi et al., 2001). Bee pollen is also used to treat some cases of benign prostatitis and for oral desensitization of children suffering from allergic problems (Schmidt, 1997).

Several researches related to bee pollen are available throughout the world from countries like Australia (Somerville & Nicol, 2006), Brazil (Carpes et al., 2009; Vasconcelos et al., 2017; Arruda et al., 2021), China (Yang et al., 2013), Italy (Domenici et al., 2015), Lithuania (Adaskeviciute et al., 2019), Morocco (Asmae et al., 2021), Portugal (Morais et al., 2011; Feas et al., 2012; De-Melo et al., 2018), Romania (Ilie et al., 2022), Saudi Arabia (Taha, 2015), Turkey (Mayda et al., 2020), etc. Some of these countries have been using bee pollen as a dietary supplement. However, only few reports (Ketkar et al., 2014; Thakur & Nanda, 2018, 2020) are available related to composition and beneficial aspects of bee pollen from Indian subcontinent.

In the western part of the Indian Himalayan Region, the state of Uttarakhand serves as a dynamic site for beekeeping due to its rich and floristically diverse vegetation. Though some melissopalynological investigations (Gaur & Tiwari, 2001; Tiwari & Tiwari, 2009; Tiwari et al., 2010) have been carried out from this region, but no work has been conducted on the study of the chemical composition of bee pollen. In view of the above, the present study was undertaken to evaluate the botanical origin and chemical composition (total soluble sugars, starch, crude protein, amino acids and phenolic contents) of bee pollen samples collected from Pauri Garhwal, Uttarakhand, India.

2. Materials and methods

2.1. Study area

The present study was conducted at Srinagar (560 m asl; 30°13' N & 78°46' E) and village Jakh (1400 m asl; 30°10' N & 78°49' E) of district Pauri Garhwal, of state Uttarakhand. The district lies between 29°45' to 30°15' N latitude and 78°24' to 79°23' E longitude with an elevation from 560 m asl to 1765 m asl, and encompasses an area of ca. 5230 km^2 (Fig. 1). The region has a sub-montane to montane climate that remains pleasant throughout the year. The district has a unique and rich vegetation, possessing approximately 61.34% forest cover (Sati & Bandooni, 2018). The common bee forage species in the area include *Acacia catechu*, *Bauhinia variegata*, *Citrus* spp., *Lannea coromandelica*, *Lyonia ovalifolia*, *Myrica esculenta*, *Phyllanthus emblica*, *Prunus* spp., *Pyrus* spp., *Rhododendron arboreum*, *Toona ciliata*, *Berberis asiatica*, *Justicia adhatoda*, *Brassica* spp., *Zea mays*, etc. (Gaur & Tiwari, 2001; Tiwari & Tiwari, 2009; Tiwari et al., 2010).

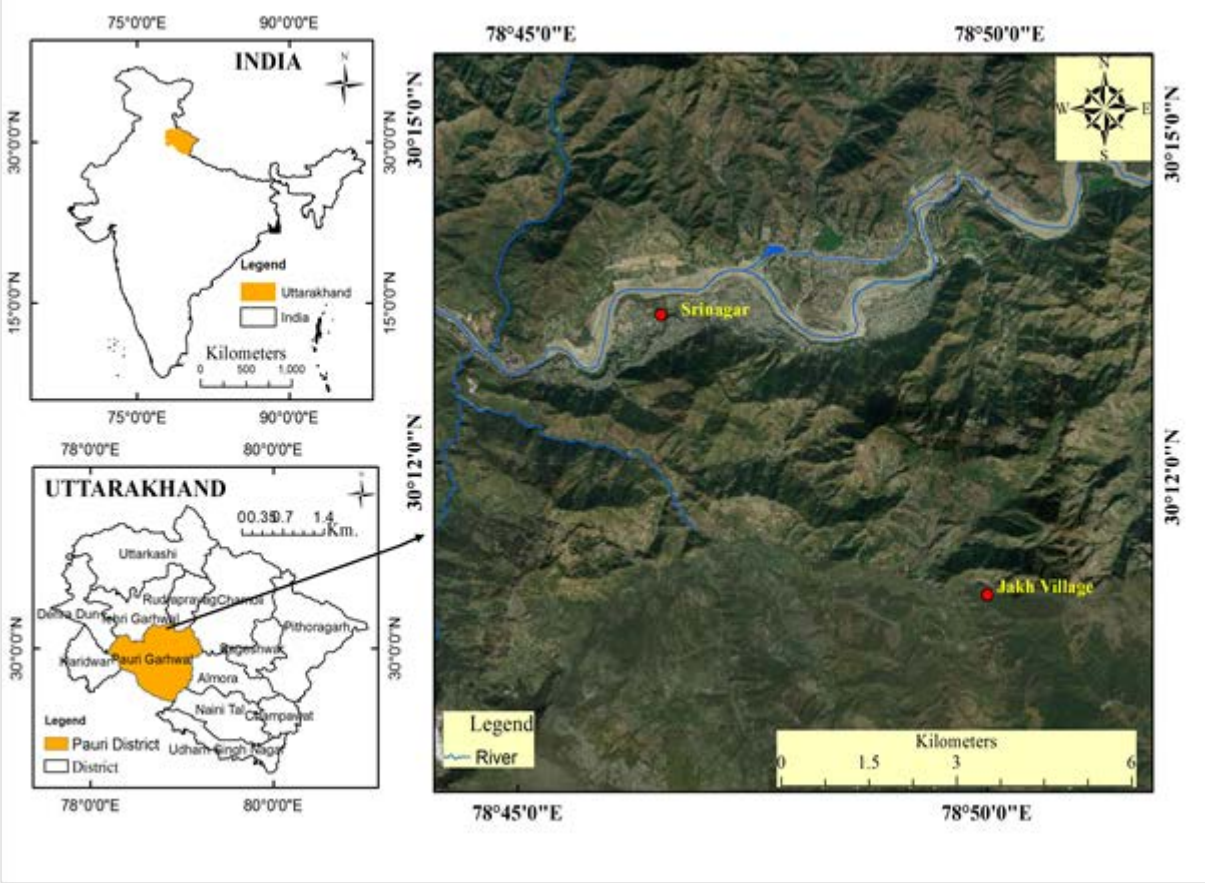

Figure 1. Location map of Pauri Garhwal showing the sampling sites i.e., Srinagar and Jakh village.

2.2. Sample collection

A total of twenty-two bee pollen samples (n =22) were collected between January and July 2021 from the selected hives of *Apis cerana* by installing bee pollen traps. Samples obtained were dried, sieved and stored in the refrigerator for further analysis.

2.3. Palynological analysis

To ascertain the floral origin of samples, 1000–1200 pollen grains were counted and assessed following the acetolysis method (Erdtman, 1960). Pollen types were identified by preparing reference slides, consulting pollen atlas and published literature. The frequency classes of pollen types present in studied samples as well as their botanical origin were determined as per Louveaux et al. (1978) i.e., predominant pollen (PDP) more than 45% of pollen grains counted, secondary pollen (SP) 16–45%, important minor pollen (IMP) 3–15% and minor pollen (MP) 1–3%; samples having single pollen type more than 45% were categorized as 'unifloral samples' while those with several pollen types in considerable percentages less than 45% were considered as 'multifloral samples' (Table 1).

Some pollen grains present in the samples could be identified up to generic level hence, the term spp. has been used e.g., *Brassica* spp., (pollens which are identical to *Brassica* in shape and morphological characteristics, but may belong to different species like *B. campestris*, *B. rapa*, *B. napus*, etc.). In few cases where details (pollen atlas and literature) were not available to proper identification, such pollen grains were associated to larger groups (forms or types) e.g., Acanthaceous type (prolate, 3-colpate grains), Asteraceous type (3–4 colporate grains with spines; *Calendula*, *Tagetes*, *Coreopsis*, *Bidens*, *Tridax*, etc.) and Rosaceous type (3-colporate grain with striate ornamentation; *Pyrus*, *Prunus*, etc.) (Louveaux et al., 1978).

2.4. Chemical analysis

Total soluble sugars and starch content

Anthrone method described by Yemm and Willis (1954) was followed to determine the total soluble sugars and starch content. For soluble sugar estimation, 100 µl of ethanolic bee pollen extract diluted with 900 µl of double distilled water, followed by addition of 4 ml anthrone reagent (0.2%) was recorded spectrophotometrically (Spectrophotometer CE; Model No. 2371, S.No. UEB1409007) at 620 nm against the reagent blank. Similarly, for starch estimation, samples were treated with 80% (v/v) ethanol to remove sugars, and then starch was extracted with perchloric acid (52% v/v). Glucose was used for constructing the standard calibration

curve (0.001, 0.002, 0.003, 0.004, 0.005, 0.006 mg/ml). Total soluble sugars and starch content were evaluated from the equation ($y = 0.1131x + 0.2185$; $R^2 = 0.9967$) and the results were expressed as mg/g sample.

Total crude protein

Protein content was estimated by following Bradford (1976). 50 mg of sample homogenized with tris buffer (pH 7.5) was centrifuged at 10,000 rpm for 10 minutes. Then, 100 µl of supernatant mixed with 4.9 ml of Bradford reagent was recorded spectrophotometrically at 595nm against the reagent blank. Bovine Serum Albumin (BSA) was used as a standard to calibrate the curve (0.02, 0.04, 0.06, 0.08, 1 mg/ml). Total protein content was expressed as mg/g sample evaluated from the equation ($y = 0.0047x + 0.0835$; $R^2 = 0.9934$).

Total amino acid content

Ninhydrin method as per Sadasivam and Manickam (1992) was followed to determine the amino acid content. 100 µl of ethanolic extract diluted with 900 µl of double distilled water was followed by the addition of 1ml ninhydrin reagent. After vortexing, the tubes were placed in the water bath at 70–80ºC for 20 minutes followed by their cooling. Then, 5 ml diluent (50% ethanol and distilled water v/v) was added to the tubes and the absorbance was read at 570 nm against the reagent blank. Glycine was used as a standard to calibrate the curve (0.02, 0.03, 0.04, 0.05, 0.06 mg/ml). Total amino acid content was expressed as mg/g sample evaluated from the equation ($y = 0.0132x + 0.0065$; $R^2 = 0.9952$).

Total phenolic content

Folin-Ciocalteau method given by Singleton et al. (1999) was used for the estimation of phenolic content in samples. 100 µl of methanolic bee pollen extract diluted with 7.9 ml of double distilled water was mixed with 500 µl of Folin-Ciocalteau's reagent (2N) and incubated for 10 minutes. After incubation, 1.5 ml of sodium carbonate (20% w/v) solution was added and the tubes were heated at 40ºC for 20 minutes in a water bath. Absorbance was read at 760 nm against the reagent blank. Gallic acid was used as a standard to calibrate the curve (0.01, 0.02, 0.03, 0.04, 0.05, 0.06 mg/ml). Total phenolic content was expressed as mg GAE/g dry samples from the standard equation ($y = 0.0104x + 0.0042$; $R^2 = 0.9944$).

2.5. Statistical analysis

Samples were analyzed in triplicates, and the results were expressed as mean ± standard deviation (SD). ANOVA and Duncan's multiple range test was performed to the results with the help of SPSS software. *p*-value <0.05 was considered as statistically significant. Pearson's

correlation coefficient (r) was used to find the correlations between the results while Hierarchal Cluster Analysis (HCA) was done using software Past 4.03.

3. Results and discussion

3.1. Palynological identification

A total of 22 pollen taxa belonging to 18 families were found in the analyzed samples (Table 1, Figs 2 and 3). Rutaceae, Asteraceae and Brassicaceae were found the most dominant families. None of the family was represented in all the samples as botanical origin of the bee pollen pellets depends on the availability of surrounding flora as well as on the climatic conditions for flowering (Luz et al., 2010). Foraging usually occurs near hives and an average bee forages within a radius of ca. 1 to 3 km; *Apis cerana* normally forages within a range of ca. 1 to 1.5 km while *Apis mellifera* extends its foraging up to 3 to 14 km (Abrol, 2011). All the analyzed samples were found unifloral except one sample (S19), which was multifloral in nature due to its different pollen types i.e., *Tropaeolum majus* (37%), Asteraceous type (34%) and *Verbascum thapsus* (29%). The predominant pollen type (>45%) observed was S1: *Justicia adhatoda* (Acanthaceae), S2 and S12: *Brassica* spp. (Brassicaceae), S3: *Bombax ceiba* (Malvaceae), S4: *Melia azedarach* (Meliaceae), S5: *Melaleuca viminalis* (Myrtaceae), S6: *Phyllanthus emblica* (Phyllanthaceae), S7: *Dalbergia sissoo* (Fabaceae), S8: *Verbascum thapsus* (Scrophulariaceae), S9: *Lannea coromandelica* (Anacardiaceae), S10: *Murraya koenigii* (Rutaceae), S11: *Amaranthus* spp. (Amaranthaceae), S13: *Coriandrum sativum* (Apiaceae), S14: *Mangifera indica* (Anacardiaceae), S15 and S18: *Juglans regia* (Juglandaceae), S16: *Sonchus* spp. (Asteraceae), S17: *Citrus* spp. (Rutaceae), S20: *Ageratum conyzoides* (Asteraceae), S21: Asteraceous type and S22: *Zea mays* (Poaceae). The secondary pollen type (16–45%) in samples was S12: *Prunus-Pyrus* type (Rosaceae), S15 and S18: *Citrus* spp. (Rutaceae), S19: *Tropaeolum majus* (Tropaeolaceae) and *Verbascum thapsus* (Scrophulariaceae). The important minor pollen (3–15%) were identified as *Coriandrum sativum* and *Grevillea robusta* while *Brassica* spp., *Citrus* spp. and *Justicia* were also present as minor pollen in some samples (Table 1, Figs 2 and 3). Carpes et al. (2009) stated that bees are attracted to a particular floral source because of the nutritional quality of pollen grains as unifloral pollen represents the uniform organoleptic and biochemical characteristics as that of the original plant, while multifloral pollen exhibits varying properties.

Table 1. Details of bee pollen samples (n= 22) collected from *Apis cerana* hives domesticated in district Pauri Garhwal (Uttarakhand, Western Himalaya).

Sample No.	Month of collection	Pollen types and frequency
Srinagar (560m asl)		
S1	January	*__PDP__: *Justicia adhatoda* L. (100%)
S2	January	***PDP**: *Brassica* spp. (99%); **MP**: *Coriandrum sativum* L., *Justicia adhatoda* L. (1%)
S3	February	*__PDP__: *Bombax ceiba* L. (98%); **MP**: *Brassica* spp. (2%)
S4	March	*__PDP__: *Melia azedarach* L. (100%)
S5	March	*__PDP__: *Melaleuca viminalis* (Sol. ex Gaertn.) Byrnes (98%); **MP**: *Citrus* spp. (<2%)
S6	March	*__PDP__: *Phyllanthus emblica* L. (100%)
S7	March	*__PDP__: *Dalbergia sissoo* Roxb. ex DC. (100%)
S8	April	*__PDP__: *Verbascum thapsus* L. (100%)
S9	April	*__PDP__: *Lannea coromandelica* (Houtt.) Merr. (100%)
S10	May	*__PDP__: *Murraya koenigii* (L.) Spreng. (100%)
S11	July	*__PDP__: *Amaranthus* spp. (100%)
Jakh (1400m asl)		
S12	February	*__PDP__: *Brassica* spp. (61%); **SP**: *Prunus-Pyrus* type (35%); **IMP***: Coriandrum sativum* L. (3%); **MP**: *Citrus* spp. (1%)
S13	March	*__PDP__: *Coriandrum sativum* L. (100%)
S14	March	*__PDP__: *Mangifera indica* L. (100%)
S15	March	*__PDP__: *Juglans regia* L. (65%); **SP**: *Citrus* spp. (23%); **IMP**: Rosaceous type (11%); **MP**: Asteraceous type (1%)
S16	March	*__PDP__: *Sonchus* spp. (95%); **IMP***: Grevillea robusta* A. Cunn. ex R.Br. (5%)
S17	April	*__PDP__: *Citrus* spp. (100%)
S18	April	*__PDP__: *Juglans regia* L. (60%); **SP**: *Citrus* spp. (35%); **IMP**: Asteraceous type (4%); **MP**: Acanthaceous type (1%)
S19	April	****SP**: *Tropaeolum majus* L. (37%), Asteraceous type (34%) and *Verbascum thapsus* L. (29%)
S20	May	*__PDP__: *Ageratum conyzoides* L. (100%)
S21	June	*__PDP__: Asteraceous type (100%)

S22	July	***PDP**: *Zea mays* L. (100%)

Note: *Unifloral sample, **Multifloral sample. **PDP**=predominant pollen (more than 45% of pollen grains counted), **SP**=secondary pollen (16–45%), **IMP**=important minor pollen (3–15%) and **MP**=minor pollen (1–3%).

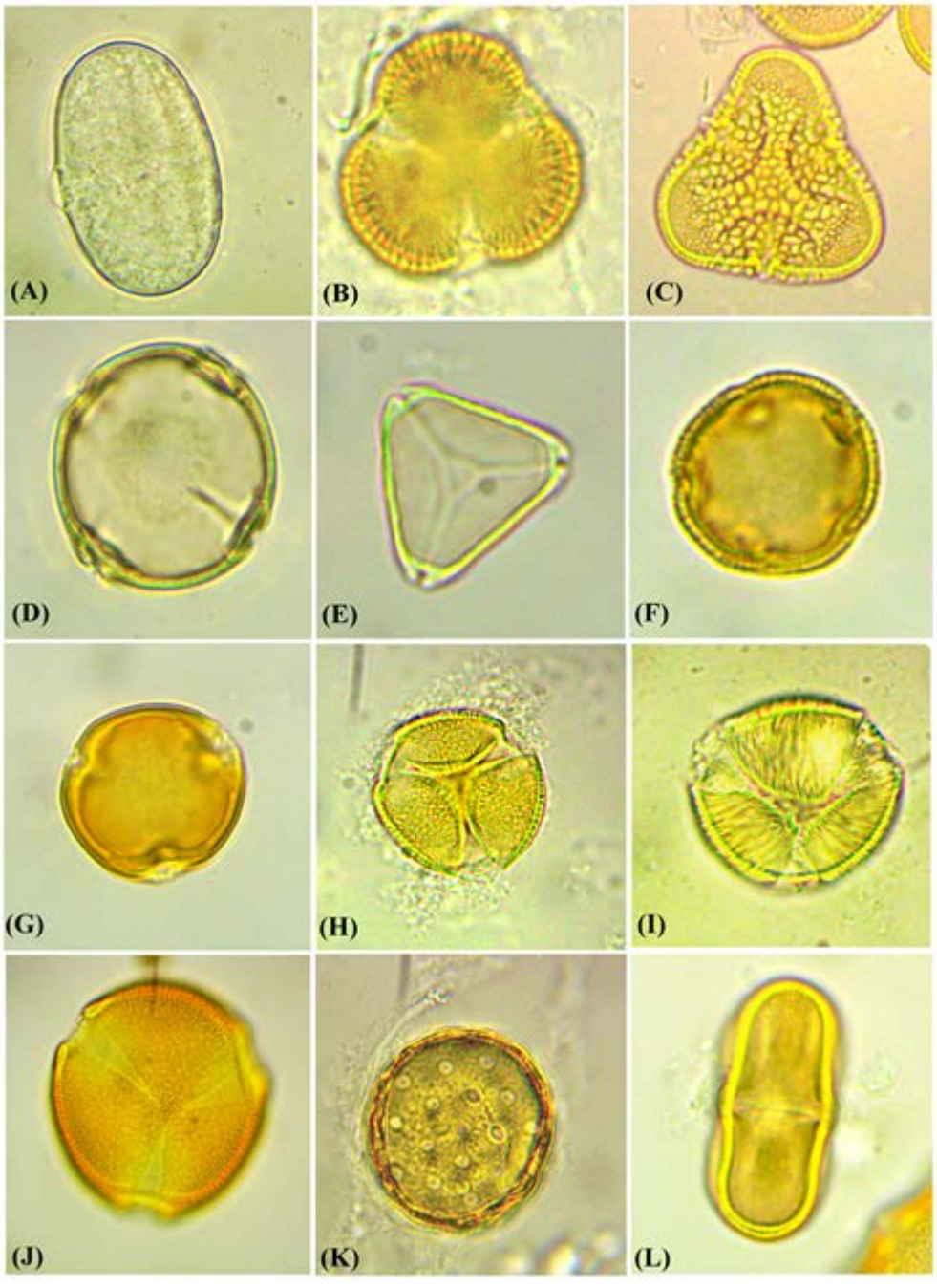

Figure 2. Microphotographs of the identified samples (400X & 1000X magnification): **A**. *Justicia adhatoda* (1000X), **B**. *Brassica* spp. (1000X), **C**. *Bombax ceiba* (400X), **D**. *Melia azedarach* (1000X), **E**. *Melaleuca viminalis* (1000X), **F**. *Phyllanthus emblica* (1000X), **G**. *Dalbergia sissoo* (1000X), **H**. *Verbascum thapsus* (1000X), **I**. *Lannea coromandelica* (1000X), **J**. *Murraya koenigii* (1000X), **K**. *Amaranthus* spp. (1000X), **L**. *Coriandrum sativum* (1000X).

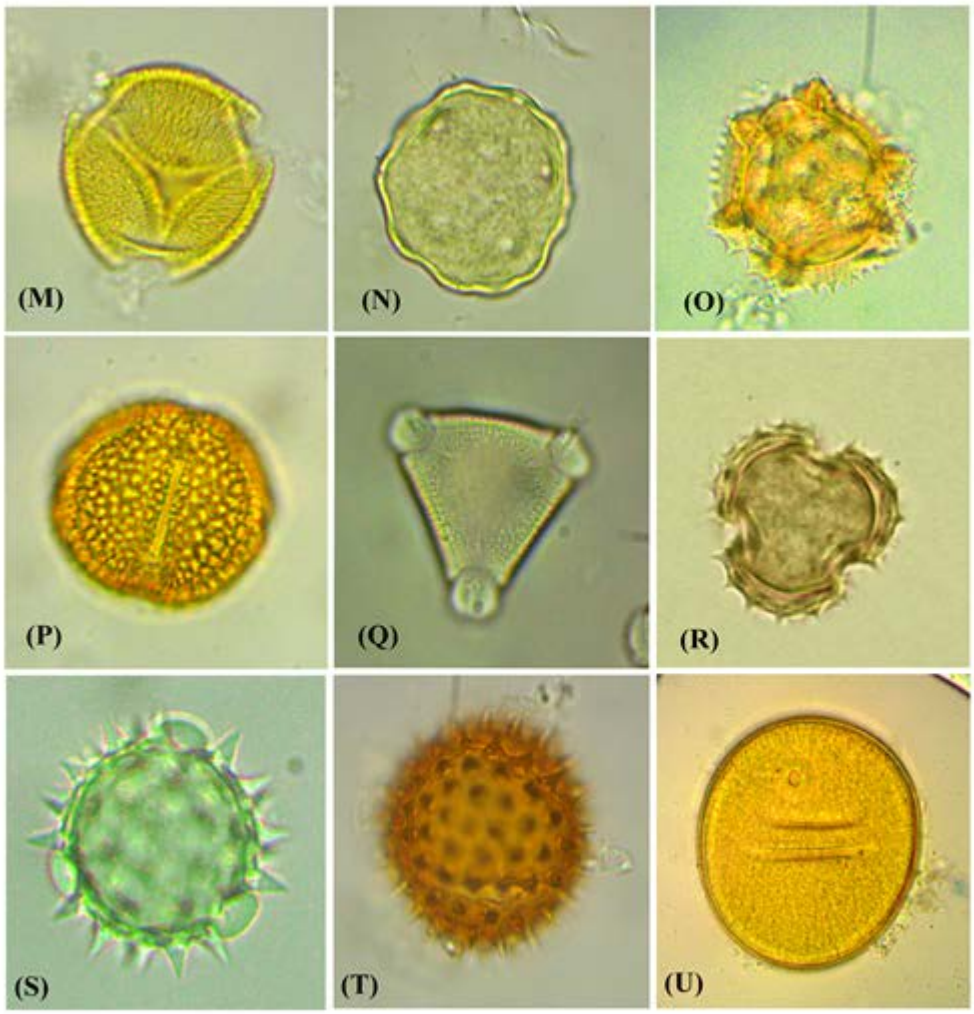

Figure 3. Microphotographs of the identified samples (400X & 1000X magnification): **M**. *Mangifera indica* (1000X), **N**. *Juglans regia* (1000X), **O**. *Sonchus* spp. (1000X), **P**. *Citrus* spp. (1000X), **Q**. *Tropaeolum majus* (1000X), **R**. *Ageratum conyzoides* (1000X), **S.** *Coreopsis* spp. (1000X), **T**. *Tagetes erecta* (1000X), **U**. *Zea mays* (400X).

3.2. Chemical composition

Chemical composition (soluble sugars, starch, crude protein, free amino acid, and phenolic contents) of bee pollen samples is represented in Table 2. Soluble sugars ranged from 0.2 to 26.09 mg/g with a mean of 16.56±6.35 mg/g while starch content varied from 0.22 to 11.04 mg/g with a mean of 4.34±2.73 mg/g. Highest soluble sugars were found in sample S14- *Mangifera indica* (26.09±0.48 mg/g) and S18- *Juglans regia* (25.25±0.06 mg/g). Maximum starch content was found in S22- *Zea mays* (11.04±0.43 mg/g). Our findings showed high soluble sugars and low starch content in bee pollen samples and are in line with the findings of

Todd & Bretherick (1942) who reported that bee-collected pollens have high carbohydrate (21% to 48%) and low starch content (2.55%) this occurs due to the addition of honey or nectar by the honeybees to bind the pollen pellets together (Stanley & Linskens, 1974; Herbert & Shimanuki, 1978).

Crude protein of bee pollen samples ranged from 13.40 to 191.41 mg/g with a mean of 63.77±38.11 mg/g. Highest protein content was found in sample S3- *Bombax ceiba* (191.41±2.63 mg/g) followed by S19- *Tropaeolum majus* (106.86±0.61 mg/g) and S9- *Lannea coromandelica* (104.26±0.51 mg/g). The range obtained for crude protein in analyzed samples was found in accordance to the findings of Celemli et al. (2017) who recorded the values between 32–176 mg/g from Turkey, while it varied with the findings of Otero et al. (2009) from Spain, who observed a quite low range 0.18–2.37 mg/g. Free amino acid content was found in range from 2.01 to 6.48 mg/g with a mean of 3.90±1.25 mg/g. Maximum content was found in S9- *Lannea coromandelica* (6.48±0.32 mg/g). Our findings revealed low amino acid content in bee pollen samples in comparison to the findings of Mondal et al. (1998) and Tidke and Nagarkar (2015) who recorded the range between 5 to 16.5 mg/g from West Bengal and 6.3 to 30 mg/g from Maharashtra. Stanley and Linskens (1974) reported that pollen amino acid content varies with environmental and nutritional conditions as well as with storage conditions.

Phenolic contents of analyzed samples varied from 5.10 to 35.50 mg GAE/g with a mean of 17.39±8.33 mg GAE/g. Highest phenolic contents were found in S5- *Melaleuca viminalis* (35.50±0.29 mg GAE/g) followed by S7- *Dalbergia sissoo* (31.75±0.09 mg GAE/g). The mean obtained for phenolic contents in analyzed samples was found similar to the values obtained by Feas et al. (2012) and Domenici et al. (2015) who reported 16.4±2.0 mg GAE/g from Portugal and 19.82±0.47 mg GAE/g from Italy. Higher phenolic content values were recorded by Atsalakis et al. (2017) from Greece (34.7 ±1.03 mg GAE/g) while lowest values were recorded by Morais et al. (2011) from Portugal (12.88 ±0.01 mg GAE/g) and Margaoan et al. (2013) from Transylvania (6.55± 0.075 mg GAE/g) in comparison to our values. Thus bee pollen's composition is greatly influenced by its botanical and geographical origin, edaphic and climatic factors, storage and preservation techniques, nutritional status of plants, beekeeper's activities, etc. (Szczesna et al., 2002; Feas et al., 2012).

Table 2. Chemical composition of bee (*Apis cerana*) pollen samples from Garhwal Himalaya, Uttarakhand.

S. No.	PDP	TSS (mg/g) /SD	TS (mg/g) /SD	CP (mg/g) /SD	TAA (mg/g) /SD	TPC (mg GAE/g) /SD
S1	*Justicia adhatoda*	14.19^{ef} ±0.39	2.28^{bc} ±0.87	76.91^{j} ±0.21	2.71^{bc} ±0.04	12.31^{f} ±0.48
S2	*Brassica* spp.	15.6^{g} ±0.31	0.27^{a} ±0.19	68.54^{i} ±2.62	2.85^{cd} ±0.56	26.11^{l} ±0.38
S3	*Bombax ceiba*	17.37^{i} ±0.13	0.67^{a} ±0.12	191.41^{o} ±2.63	4.62^{gh} ±0.30	7.06^{b} ±0.02
S4	*Melia azedarach*	19.16^{j} ±0.05	7.12^{j} ±0.06	13.40^{a} ±0.31	4.39^{gh} ±0.34	11.16^{e} ±0.77
S5	*Melaleuca viminalis*	13.79^{e} ±0.51	6.45^{i} ±0.47	55.77^{gh} ±0.21	4.29^{fg} ±0.20	35.5^{o} ±0.29
S6	*Phyllanthus emblica*	22.98^{m} ±0.15	6.46^{i} ±0.38	58.34^{h} ±1.15	3.66^{e} ±0.19	14.26^{g} ±0.09
S7	*Dalbergia sissoo*	17.30^{i} ±0.08	2.34^{bc} ±0.02	68.81^{i} ±0.52	2.01^{a} ±0.55	31.75^{n} ±0.09
S8	*Verbascum thapsus*	15.40^{g} ±0.28	3.64^{ef} ±0.15	68.46^{i} ±2.10	3.81^{ef} ±0.51	5.10^{a} ±0.19
S9	*Lannea coromandelica*	22.02^{l} ±0.11	6.05^{i} ±0.04	104.26^{m} ±0.51	6.48^{j} ±0.32	17.53^{h} ±0.38
S10	*Murraya koenigii*	14.67^{f} ±0.42	6.01^{i} ±0.33	56.39^{h} ±1.58	5.86^{i} ±0.36	28.61^{m} ±0.62
S11	*Amaranthus* spp.	0.20^{a} ±0.19	5.88^{i} ±0.02	35.10^{d} ±1.67	5.62^{i} ±0.46	10.45^{de} ±0.24
S12	*Brassica* spp.	15.92^{gh} ±0.14	3.09^{de} ±0.59	53.46^{g} ±0.95	4.38^{gh} ±0.01	18.65^{i} ±0.05
S13	*Coriandrum sativum*	16.42^{h} ±0.22	2.19^{b} ±0.60	31.55^{c} ±2.23	3.81^{ef} ±0.17	22.37^{j} ±0.81
S14	*Mangifera indica*	26.09^{o} ±0.48	4.48^{gh} ±0.26	96.62^{l} ±2.35	2.17^{ab} ±0.16	16.7^{h} ±0.14
S15	*Juglans regia*	21.75^{kl} ±0.20	0.22^{a} ±0.23	50.11^{f} ±1.58	2.59^{bc} ±0.44	14.42^{g} ±0.29
S16	*Sonchus* spp.	23.35^{m} ±0.09	3.96^{fg} ±0.20	90.1^{k} ±0.42	4.80^{gh} ±0.25	8.37^{c} ±0.24
S17	*Citrus* spp.	11.34^{d} ±0.84	5.06^{h} ±0.18	37.06^{d} ±1.69	4.94^{h} ±0.21	28.38^{m} ±1.26
S18	*Juglans regia*	25.25^{n} ±0.06	4.71^{h} ±0.06	46.34^{e} ±1.46	3.71^{e} ±0.20	14.17^{g} ±1.15
S19	*Multifloral*	21.28^{k} ±0.15	2.86^{cd} ±0.34	106.86^{n} ±0.61	3.25^{de} ±0.15	14.01^{g} ±0.14
S20	*Ageratum conyzoides*	6.75^{c} ±0.62	1.94^{b} ±0.06	15.84^{a} ±0.84	4.88^{h} ±0.23	23.74^{k} ±0.62
S21	Asteraceous type	17.83^{i} ±0.09	8.84^{k} ±0.09	49.90^{f} ±0.62	2.60^{bc} ±0.04	12.12^{f} ±0.40
S22	*Zea mays*	5.61^{b} ±0.44	11.04^{l} ±0.43	27.71^{b} ±1.78	2.34^{abc} ±0.12	9.94^{d} ±0.25
Total		**16.56 ±6.35**	**4.34 ±2.73**	**63.77 ±38.11**	**3.90 ±1.25**	**17.39 ±8.33**

Note: S. No. = Sample Number, PDP = Predominant pollen type, TSS = Total soluble sugars, TS= Total starch, CP = Crude protein, TAA = Total amino acids, TPC= Total phenolic contents, SD = Standard deviation. Mean value in the same column with different lowercase letters indicate significant difference ($p<0.05$).

3.3. Statistical Results

Statistically significant differences ($p<0.05$) were observed among the analyzed samples with the help of ANOVA; mean values with different lowercase letters within the same column represent significant differences (Table 2). A negative or very weak correlation was observed among the chemical parameters however, a moderate correlation was observed between soluble sugars and crude protein ($r=0.400$) (Table 3).

Table 3. Pearson's correlation analysis between the chemical parameters of bee pollen samples.

	Total soluble sugars	**Total starch**	**Crude protein**	**Total amino acid**	**Total phenolic contents**
Total soluble sugars	1				
Total starch	-0.192	1			
Crude protein	**0.400***	-0.369*	1		
Total amino acid	-0.223	0.100	0.032	1	
Total phenolic contents	-0.128	-0.119	-0.256	0.065	1

A dendrogram obtained by HCA was used to calculate the similarities between the samples with the help of Euclidean distance (Fig. 4). Four clusters were suggested however, the first cluster contained only a single sample i.e., S3 due to high differences in its chemical composition in comparison to other samples. Cluster 2 involves 4 samples (S9, S14, S16 and S19). Cluster 3 contained maximum number of samples i.e., n = 11 (S1, S2, S5, S6, S7, S8, S10, S12, S15, S18 and S21) due to their similarity in chemical composition while cluster 4 (n = 6) included S4, S11, S13, S17, S20 and S22.

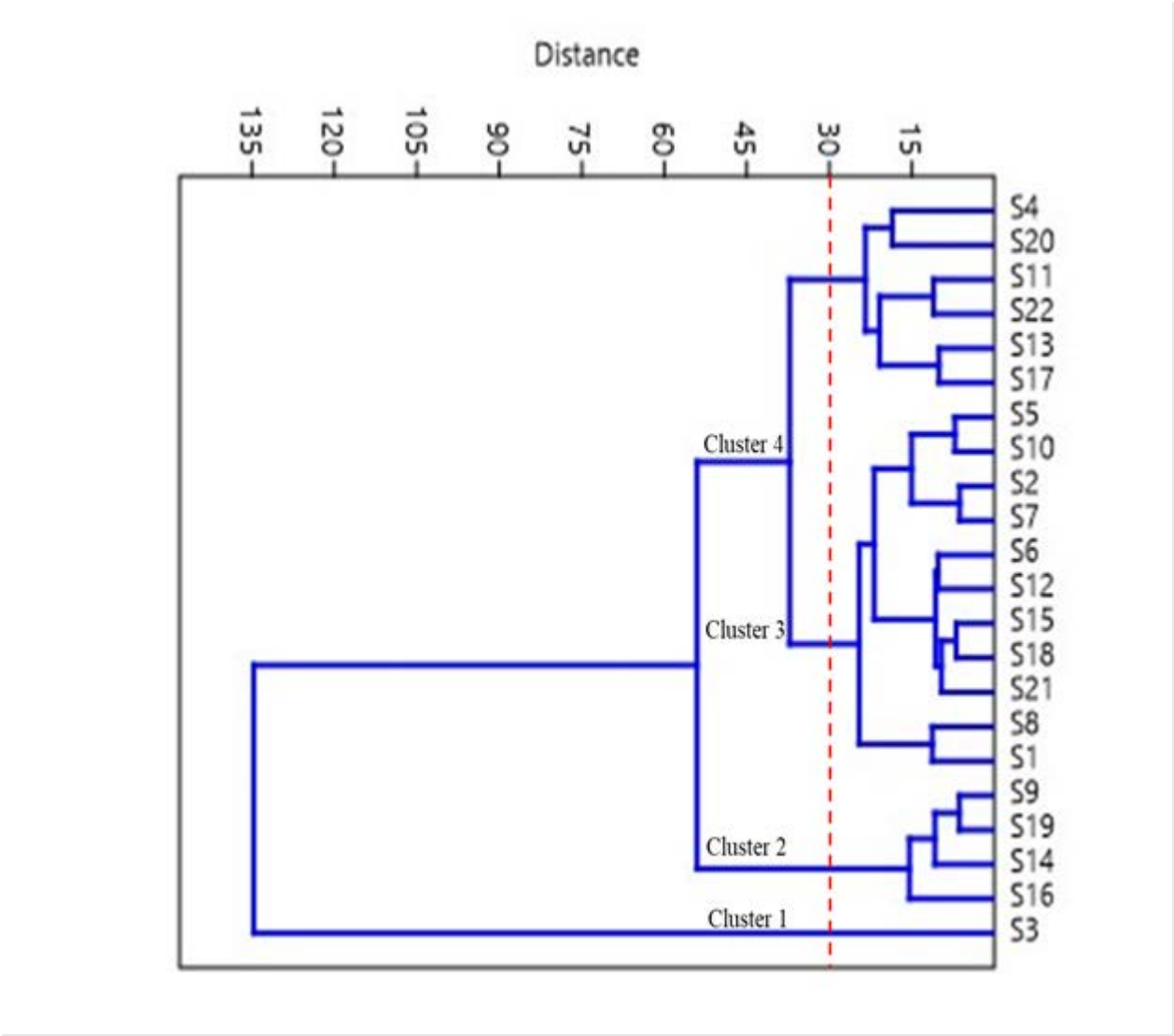

Figure 4. Dendrogram for bee pollen samples obtained by Hierarchical Cluster Analysis.

4. Conclusion

Present study revealed that the mountain village ecosystems of Garhwal region embody diverse vegetational range of plants which support rich bee forage. Several potential sites exist where beekeeping pursuit can be tapped excessively. Naturally growing plants like *Dalbergia sissoo*, *Juglans regia*, *Bombax ceiba*, *Murraya koenigii*, *Phyllanthus emblica*, etc. and some planted species like *Coriandrum sativum*, *Brassica* spp., *Amaranthus* spp., *Zea mays* are utilized and favoured by bees extensively. Plants like *Ageratum conyzoides* and *Tagetes erecta* bloomed throughout the year even in dearth period thus supports the beekeeping pursuit. It can be concluded from the present results that the studied bee pollen samples have high soluble sugars, protein, amino acids and phenolic contents, making it a good source of nutrition, medicine and dietary supplement for both humans and bees. Presence of phenolic contents may be useful in

the prevention of those diseases which are associated with free radicals. The study may be helpful to aware the local inhabitants and beekeepers to harvest bee pollen which must be properly analyzed for their quality before being used in food processing industries. Therefore, governments and private organizations need to work together to educate beekeepers about the benefits of bee pollen as well as to establish quality standards for it.

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