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MORPHOMETRIC VARIATION OF APIS CERANA FABRICIUS AND ALTITUDINAL EFFECTS ON HONEY COMPOSITION IN BAROT VALLEY, WESTERN HIMALAYA

Kritika Sood[1], Raj Kumar Thakur[2], Kritika Saklani[3]*

[1]M.Sc., Department of Entomology, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India, Email: kritika.22sood@gmail.com, Orcid ID : 0009-0005-1670-0911
[2]Former Head, Department of Entomology, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India, Email: rkt_apic@rediffmail.com, Orcid ID: 0009-0009-0874-3929
[3*]Ph.D. Scholar, Department of Entomology, Punjab Agricultural University, Ludhiana, Punjab, India , Email: kritikasaklani99@gmail.com, Orcid ID : 0009-0009-9221-4068

ABSTRACT:
The study investigated morphometric variation in Apis cerana F. and the influence of altitude on honey composition in Bharot Valley, Western Himalaya. Worker bees were sampled from three elevations (Jogindernagar, Multhan, Chelra Di Malahn). Morphological traits including head, thorax, abdomen, body length, proboscis, antenna, wing dimensions, and hamuli number increased with elevation, and body pigmentation darkened at higher altitudes. Physicochemical analysis of honey showed all samples met FSSAI and international standards. pH, moisture, electrical conductivity, and HMF indicated good quality and maturation. Low sucrose and appropriate fructose:glucose ratios confirmed fully ripened floral honey. Multhan honey exhibited comparatively higher vitamin C, phenolics, proline, and minerals, suggesting richer biochemical profiles linked to floral origin. Acidity remained within permissible limits. Proline exceeded Codex standards, confirming natural origin. Overall, A. cerana honey from Barot Valley is high quality with favorable nutrition. Altitudinal variation supports region-specific characterization of Himalayan honey and potential for niche commercialization.

KEYWORDS: Worker bee morphology; elevational gradient; physicochemical properties; mineral content; honey quality

1. INTRODUCTION

The Asiatic honey bee, Apis cerana Fabricius, is a key pollinator in many Asian ecosystems and plays a vital role in sustaining both natural vegetation and agricultural productivity (Ji et al., 2023). Honey bees contribute significantly to global food security, being responsible for the pollination of nearly three-fourths of cultivated crop species. The evolution and diversification of honey bees have been strongly shaped by ecological and geographical heterogeneity (Sheoran et al., 1998). Geographic barriers such as mountain ranges, along with variations in altitude, temperature, floral availability, and anthropogenic influences, contribute to phenotypic differentiation among populations (Zhu et al., 2011). As a result, A. cerana exhibits considerable morphological variability, particularly in traits such as body size, wing dimensions, and appendage structure, which facilitate adaptation to local environmental conditions (Abou-Shaara et al., 2012).

Morphological characteristics represent an important interface between genotype and environment and are widely used to assess population variability and adaptation (Ajao et al., 2014; Chen et al., 2018). Functional traits such as body size, proboscis length, and hind leg structure influence foraging efficiency and pollen-carrying capacity (Narayanan et al., 1960), while wing dimensions are directly associated with flight performance and honey production potential (Szentgyörgyi et al., 2016). Wing morphometry has also been extensively applied in the classification of honey bee races and populations (Ibrahim and Chandel, 2019). Regional differences in floral abundance and diversity further interact with these morphological traits to influence foraging behaviour and productivity (Alburaki and Alburaki, 2008). Previous morphometric studies on A. cerana have documented substantial regional variation, highlighting the role of environmental pressures in shaping population structure (Zhu et al., 2009; Zhu et al., 2011). Understanding such variation is essential for interpreting adaptive responses and for designing conservation strategies under changing environmental conditions (Balanya et al., 2009).

Honey, a natural product synthesized by bees from floral nectar, is valued for its nutritional, therapeutic, and commercial importance (Iglesias et al., 2004). During honey production, nectar undergoes enzymatic transformation, dehydration, and maturation within the hive, resulting in a complex mixture of bioactive compounds (Council, 2002). Carbohydrates, primarily fructose and glucose, constitute the major fraction of honey, accounting for approximately 82% of its composition (Chang et al., 1988). In addition, honey contains a wide range of minor constituents, including organic acids, enzymes, amino acids, vitamins, minerals, and phenolic compounds, which collectively determine its physicochemical properties and biological activity (Bogdanov et al., 2008). These components influence key attributes

such as flavour, colour, viscosity, crystallization behaviour, and shelf stability, while also contributing to antioxidant and antimicrobial properties (deAlmeida-Muradian et al., 2013).

The composition of honey is influenced by multiple interacting factors, including floral source, bee species, climatic conditions, and geographical origin, as well as post-harvest handling and storage practices (Tornuk et al., 2013; Escuredo et al., 2014). Since honey bees forage over large areas, honey integrates environmental inputs from soil, vegetation, and atmospheric conditions. Consequently, mineral composition and associated parameters such as electrical conductivity are considered reliable indicators of geographical origin and environmental characteristics (Przybylowski and Wilczynska, 2001; Atrouse et al., 2004). Physicochemical characterization of honey is therefore essential for assessing quality, authenticity, and market value, with parameters such as moisture, acidity, pH, sugar profile, and mineral content directly influencing its stability, sensory properties, and nutritional significance (Devi et al., 2021; Ratiu et al., 2019).

Increasing demand for natural honey, coupled with limited supply, has led to widespread adulteration through the addition of sugar syrups, thereby compromising quality and consumer trust (Se et al., 2018). This has necessitated the establishment of strict quality standards and regulatory frameworks at national and international levels (Thrasyvoulou et al., 2018). In India, honey holds considerable cultural and medicinal importance and is widely used for its therapeutic properties, including antimicrobial, anti-inflammatory, and antioxidant effects (Minhas et al., 2016; Nweze et al., 2020). Its applications extend to the food, pharmaceutical, and cosmetic industries (Crane, 1979), further driving demand for high-quality, authentic products (Pasias et al., 2017). The bioactivity of honey is largely attributed to compounds such as phenolics, proline, and ascorbic acid, whose concentrations are influenced by floral origin and environmental conditions (Oryan et al., 2016; Brunet et al., 2014; Anklam 1998; Abu-Tarboush et al., 1993; Dustmann, 1979).

Given the ecological and economic significance of A. cerana, morphometric analysis provides a valuable tool for assessing population diversity and adaptive variation across regions. The present study investigates the morphometric characteristics of A. cerana workers and the physicochemical and mineral composition of their honey across different altitudinal locations in the Barot Valley, Himachal Pradesh. By integrating morphological and compositional analyses, the study aims to evaluate regional variation, assess honey quality in relation to established standards, and provide baseline information relevant to conservation, sustainable management, and value addition of Himalayan honey.

MATERIAL AND METHODS

Study area and sample collection

Field sampling was conducted in Barot Valley, Himachal Pradesh, India, a remote Himalayan valley located along the Uhl River and flanked by the Dhauladhar mountain range. The valley spans an elevation gradient of approximately 1210 to 2150 m above mean sea level, between 32°8’1.096” N longitude and 75°55’28.37” E latitude. The region experiences a temperate climate with an average annual temperature of around 13 °C and winter temperatures that may drop below freezing, often accompanied by snowfall. Traditional beekeeping with the Asiatic honey bee, A. cerana, remains widely practiced in the region, making it a suitable landscape for studying altitudinal variation in bee morphology and honey characteristics.

Sampling was carried out during 2021-2022 at three representative locations along the altitudinal gradient of the valley: Jogindernagar (1220 m asl; low elevation), Multhan (1852 m asl; mid elevation), and Chelra Di Malahn (2117 m asl; high elevation). These sites were selected based on the presence of actively managed colonies maintained under traditional beekeeping practices. For morphometric analysis, a total of 70 foraging worker bees were randomly collected from the entrance of seven colonies at each location. The collected individuals were immediately transferred into vials containing 70% ethanol to preserve morphological structures prior to laboratory measurements.

Honey samples were obtained during the peak nectar flow period from mature capped combs of the same apiaries. At each location, seven independent honey samples (approximately 100 g each) were collected from different colonies, resulting in a total of 21 samples across the three altitudinal zones. Immediately after collection, the honey samples were homogenized and stored in sterile, airtight glass containers to prevent contamination or moisture absorption prior to physicochemical analysis.

Morphometric analysis

Worker specimens of A. cerana preserved in ethanol were carefully dissected and mounted on clean glass slides for morphometric examination. Measurements were obtained using a stereo microscope (Olympus SZ61) fitted with a calibrated ocular micrometer (0.67× magnification). Morphological traits representing major body regions were recorded for each individual. Head length was measured from the upper margin of the head to the lower edge of the clypeus. Thorax and abdomen lengths were determined along the dorsal midline. Wing measurements included the length of the forewing and hindwing from the articulatory base to the distal tip, while wing breadth was taken at the widest point of each wing. Additional characters measured included proboscis length (cardo to labellum) and antennal length. The number of hamuli (hooks) present along the margin of the hindwing was also counted under magnification. Body coloration of individual bees was assessed visually and categorized along a gradient from lighter to darker pigmentation. All morphometric measurements were expressed in millimeters (mm).

Physicochemical Parameters

Pollen density, Moisture, pH, Electrical Conductivity (EC), Colour

Pollen density was estimated following the method described by ISI, 1974. Briefly, 10 g of honey was diluted with 100 mL of distilled water and centrifuged to obtain the pollen sediment. The recovered sediment was stained and pollen

grains were enumerated using a hemocytometer under a microscope. Moisture content of the honey samples was determined by the oven-drying method. Approximately 5 g of honey was dried in a hot air oven at 103 °C until a constant weight was achieved, and moisture percentage was calculated as described by Ranganna, 2007. For pH determination, 10 g of honey was dissolved in 100 mL of distilled water and the pH of the solution was recorded using a calibrated digital pH meter (EcoTestr pH 2) according to the procedure outlined by AOAC, 2012. Electrical conductivity (EC) was measured using a calibrated conductivity meter (Thermo Scientific EUTECH CON 150). The electrode was immersed in a solution prepared by dissolving 10 g of honey in 20 mL of distilled water, and conductivity readings were recorded following the method described by Jackson, 2005. Honey colour was assessed spectrophotometrically by measuring the absorbance of a honey solution at 560 nm using a Spectronic-20 spectrophotometer (Bausch and Lomb). Distilled water was used as the blank, following the procedure of Townsend, 1969.

Sucrose, Fructose and Glucose, Fructose: Glucose ratio

Sucrose content of honey samples was determined after acid inversion of the honey solution. For this purpose, Fehling's solution A and B were used along with hydrochloric acid (specific gravity 1.18 at 20 °C) to estimate sucrose concentration. A standard invert sugar solution was prepared by dissolving 0.95 g sucrose in 500 mL distilled water, followed by the addition of 2 mL concentrated hydrochloric acid. The solution was subsequently neutralized with sodium carbonate prior to analysis. Total reducing sugars were then calculated according to the standard ISI, 1974 method.

The concentrations of fructose and glucose in the honey samples were also determined using the same standard analytical protocol (ISI, 1974). Based on the quantified values of these sugars, the fructose-to-glucose (F:G) ratio was calculated using the following relationship:

Fructose: Glucose ratio =Fructose (per cent by weight)Glucose (per cent by weight)

Acidity, HMF (Hydroxy methyl furfural) content, Total phenols, amino acid, Vitamin C

Free, lactonic and total acidity of honey samples were determined using the titrimetric procedure described by AOAC, 1984. Free acidity was measured by titrating the honey solution with standard alkali, while lactonic acidity was estimated after neutralization and subsequent hydrolysis of lactones. Total acidity was calculated as the sum of free and lactonic acidity, and the results were expressed as milliequivalents of acid per kilogram of honey.

Hydroxymethylfurfural (HMF) content was initially screened qualitatively using the Fiehe test, where the development of a cherry red colour after reaction with resorcinol-hydrochloric acid indicated the presence of HMF (ISI, 1974). Quantitative estimation of HMF was performed following the method of Schade et al., 1958. Ether extracts of honey samples were evaporated, reacted with resorcinol reagent, and the absorbance of the resulting coloured complex was observed at 490 nm using a spectrophotometer with distilled water as the blank. HMF concentration was determined from a standard calibration curve and expressed as mg/kg.

Total phenolic content of the honey samples was estimated using the Folin-Ciocalteu colorimetric method (Bray and Thorpe, 1954). The absorbance of the developed colour was measured at 650 nm with a spectrophotometer, and phenolic concentration was calculated from a catechol standard curve. The results were expressed as mg phenols per 100 g of honey on fresh weight basis.

Proline the major amino acid in honey, was determined according to the method described by Bogdanov, 2009. Diluted honey samples were reacted with formic acid and ninhydrin reagent and heated in a boiling water bath for 15 min. After cooling, the reaction mixture was diluted with aqueous isopropanol and the absorbance was read at 520 nm. Proline concentration was calculated from a standard curve and expressed as mg per 100 g honey.

Vitamin C content was determined by titration with 2,6-dichlorophenol-indophenol dye following the AOAC, 2004 method. Honey extracts prepared in 3 % metaphosphoric acid were titrated until a faint pink endpoint. The vitamin C concentration was calculated using the standard formula

Vitamin C (mg/100g) = titer value × dye factor × volume made × 100Aliquot of extract taken for estimation × Wt. of sample taken for estimation

Mineral content

For mineral determination, honey samples were first dried and subjected to wet digestion. Approximately 1 g of each sample was digested using a diacid mixture of nitric acid (HNO_3) and perchloric acid ($HClO_4$) in a 4:1 ratio until a clear digest was obtained. The resulting solution was diluted with distilled water and used for subsequent mineral analysis.

Major mineral elements, including potassium (K), calcium (Ca), and sodium (Na), were quantified using a flame photometer. Trace and secondary elements such as zinc (Zn), iron (Fe), manganese (Mn), and magnesium (Mg) were determined using atomic absorption spectrophotometry. Phosphorus (P) content was estimated colorimetrically using a Spectronic 20-D spectrophotometer following the procedures described by Jackson, 1973 and Sharma et al., 1987.

Data analysis

Morphometric measurements of worker bees were summarized as mean ± standard error for each sampling location. Differences among altitudinal sites were evaluated using one-way analysis of variance (ANOVA). Similarly, the physicochemical and mineral characteristics of honey samples were analyzed using one-way ANOVA to assess variation among the three locations, with seven independent replicates per site. Whenever the ANOVA indicated significant differences at $P \leq 0.05$, treatment means were compared using Duncan's Multiple Range Test (DMRT) to

determine pairwise differences among sites. All statistical analyses were performed using the statistical software OPSTAT (Sheoran et al., 1998).

RESULTS

Body length

The mean body length of worker bees of A. cerana collected from different altitudinal locations of Barot Valley ranged from 11.89-13.19 mm. The lowest mean body length was recorded in workers from Jogindernagar (11.89 mm), followed by Multhan (12.12 mm), with both populations being statistically similar. In contrast, workers from Chelra Di Malahn exhibited the greatest body length (13.19 mm), which was significantly higher than those from the other two locations.

The lengths of the head, thorax, and abdomen varied from 2.92-3.18 mm, 3.57-4.01 mm, and 5.72-6.03 mm, respectively. Head length did not differ significantly among the three locations, with measurement of 3.18 mm in Chelra Di Malahn, 3.01 mm in Multhan, and 2.92 mm in Jogindernagar. However, thorax length showed significant variation across sites, with the highest value recorded in Chelra Di Malahn (4.01 mm), followed by Multhan (3.89 mm), while the lowest value was observed in Jogindernagar (3.57 mm). A similar trend was observed for abdomen length, where workers from Chelra Di Malahn recorded the highest value (6.03 mm), followed by Multhan (5.99 mm), with both being statistically similar. The lowest abdomen length was observed in Jogindernagar (5.72 mm), which differed significantly from the other two locations (Table 1).

Table 1. Variation in total body length of A. cerana workers

Locations	Head length (mm)		Thorax length (mm)		Abdomen length (mm)		Body length (mm)	
	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	Range
Jogindernagar	2.92 ± 0.021b	2.84-2.93	3.57 ± 0.05b	3.37-3.77	5.72 ± 0.07b	5.41-5.99	11.89 ± 0.17b	11.48-12.63
Multhan	3.01 ± 0.01b	2.99-3.07	3.89 ± 0.02a	3.82-3.99	5.99 ± 0.06a	5.77-6.29	12.12 ± 0.27b	11.16-13.40
Chelra Di Malahn	3.18 ± 0.06a	3.03-3.36	4.01 ± 0.03a	3.92-4.10	6.03 ± 0.06a	5.77-6.31	13.19 ± 0.10a	12.84-13.47
C.D. (0.05)	0.12	-	0.10	-	0.13	-	0.61	-

Proboscis and antennal length

The mean proboscis length of worker bees varied from 5.11-5.34 mm across the studied locations. The longest proboscis was observed in workers from Chelra Di Malahn (5.34 mm), followed by Jogindernagar (5.22 mm), whereas the shortest length was recorded in bees from Multhan (5.11 mm) (Table 2).

Table 2. Variation in proboscis and antennal lengths of A. cerana workers

Locations	Proboscis length (mm)		Antennal length (mm)	
	Mean ± S.E.	Range	Mean ± S.E.	Range
Jogindernagar	5.22 ± 0.02ab	5.18-5.31	3.89 ± 0.01b	3.86- 3.91
Multhan	5.11 ± 0.05b	4.94-5.30	3.89 ± 0.01b	3.85- 3.94
Chelra Di Malahn	5.34 ± 0.06a	5.11-5.59	4.02 ± 0.01a	3.99- 4.07
C.D. (0.05)	0.14	-	0.02	-

Antennal length ranged from 3.89-4.02 mm. Workers collected from Chelra Di Malahn exhibited the greatest antennal length (4.02 mm), which differed significantly from the other locations. The shortest antennae were recorded in workers from Multhan and Jogindernagar (3.89 mm), which were statistically at par (Table 2).

Wing dimensions and hamuli

Forewing length and breadth ranged from 8.68-8.78 mm and 2.79-2.96 mm, respectively. The longest forewings were recorded in workers from Chelra Di Malahn (8.78 mm), whereas the shortest were observed in Jogindernagar (8.68 mm), which were statistically at par with Multhan (8.71 mm). Similarly, forewing breadth was highest in Chelra Di

Malahn (2.96 mm), followed by Jogindernagar (2.92 mm), while the lowest value was observed in Multhan (2.79 mm), with all differences being statistically significant (Table 3).
Hindwing length and breadth ranged from 5.91-6.19 mm and 1.59-1.73 mm, respectively. The longest hindwings were recorded from Chelra Di Malahn (6.19 mm), which was statistically similar to Multhan (6.19 mm), whereas the shortest were observed in Jogindernagar (5.91 mm). In terms of hindwing breadth, the highest value was observed in Chelra Di Malahn (1.73 mm), followed by Jogindernagar (1.61 mm), while Multhan recorded the lowest value (1.59 mm), which was statistically similar to Jogindernagar (Table 3).

Table 3. Variation in wing dimensions of A. cerana workers

Locations	Forewings				Hindwings			
	Length (mm)		Breadth (mm)		Length (mm)		Breadth (mm)	
	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	Range	Mean ± S.E.	Range
Jogindernagar	8.69 ± 0.17b	8.62-8.76	2.92 ± 0.02b	2.90- 2.98	5.91 ± 0.01b	5.88 - 5.93	1.61 ± 0.01b	1.59 - 1.66
Multhan	8.71 ± 0.02b	8.60-8.78	2.79 ± 0.01c	2.76- 2.85	6.19 ± 0.03a	6.05 - 6.30	1.59 ± 0.01b	1.54 - 1.61
Chelra Di Malahn	8.78 ± 0.04a	8.68-8.94	2.96 ± 0.01a	2.95- 2.98	6.19 ± 0.02a	6.10 - 6.23	1.73 ± 0.01a	1.69 - 1.76
C.D. (0.05)	0.07	-	0.03	-	0.07	-	0.03	-

The number of hamuli on the hindwing margin ranged from 18.27 to 19.33. The highest number was recorded in workers from Chelra Di Malahn (19.33), which was statistically at par with Jogindernagar (18.91). The lowest value was observed in Multhan (18.27), which differed significantly from the other locations (Table 4).

Table 4. Variation in number of hamuli

Locations	Hamuli number	
	Mean ± S.E.	Range
Jogindernagar	18.91 ± 0.15a	18.33- 19.33
Multhan	18.27 ± 0.01b	18.00- 18.50
Chelra Di Malahn	19.33 ± 0.23a	18.17- 19.67
C.D. (0.05)	0.52	-

Body colour
Body colour of worker bees varied from yellowish-brown to brownish-black. Bees collected from the lower elevation site at Jogindernagar (1220 m amsl) exhibited comparatively lighter coloration, whereas individuals from higher elevations, particularly Multhan (1852 m amsl) and Chelra Di Malahn (2117 m amsl), showed darker pigmentation.

Physical properties
Pollen density, Moisture, pH, Electrical Conductivity (EC), Optical density (OD)
The pollen density of honey samples ranged from 74,435-77,726 pollen grains per 10 g of honey. The highest pollen density was recorded in samples from Chelra Di Malahn (77,726 pollen grains/10 g), followed by Jogindernagar (75,319 pollen grains/10 g), while the lowest value was observed in samples from Multhan (74,435 pollen grains/10 g) (Table 5).
Moisture content of honey samples varied between 17.05-18.13 per cent. The highest moisture level was recorded in honey from Multhan (18.13%), followed by Chelra Di Malahn (17.89%), whereas the lowest value was observed in samples from Jogindernagar (17.05%). Moisture content differed significantly among the three locations and remained within the permissible limit (<20%) prescribed by the Food Safety and Standards Authority of India (FSSAI, 2018) (Table 5).
The pH of honey samples ranged from 3.98-4.49. The highest pH value was recorded in samples from Multhan (4.49), followed by Chelra Di Malahn (4.28), while the lowest value was observed in honey from Jogindernagar (3.98). The pH values differed significantly among the sampling sites and fell within the acceptable range for honey (3.90-6.10) specified by FSSAI (2018) (Table 5).
Electrical conductivity (EC), which reflects the mineral and organic acid content of honey, ranged from 0.46-0.52 mS/cm. The highest EC value was recorded in honey from Multhan (0.52 mS/cm), whereas the lowest value was

observed in samples from Jogindernagar (0.46 mS/cm). Honey from Chelra Di Malahn recorded an intermediate EC value of 0.49 mS/cm, which was statistically comparable with the other two locations (Table 5).
The optical density (OD) of honey samples measured at 560 nm ranged from 0.78-1.02. The highest absorbance was observed in honey from Chelra Di Malahn (1.02), which was statistically at par with samples from Multhan (0.93), while the lowest value was recorded in honey from Jogindernagar (0.78). According to the colour classification described by White (1975), all analysed samples fell within the absorbance range of <3.008 at 560 nm, corresponding to honey colours ranging from white to amber-white (Table 5).

Table 5. Physical properties of A. cerana honey from Barot valley

Locations	**Physical Parameters**				
	Pollen Density (pollen grain per 10g)	**Moisture (%)**	**pH**	**EC (mS/cm)**	**Optical density at 560 nm**
Jogindernagar	75319b	17.05c	3.98c	0.46b	0.78b
Multhan	74435c	18.13a	4.49a	0.52a	0.93a
Chelra Di Malahn	77726a	17.89b	4.28b	0.49ab	1.02a

Chemical properties

Sucrose, Fructose and Glucose, Fructose: Glucose ratio

Sucrose content of honey samples ranged from 3.86-4.05 per cent across the three locations. The highest sucrose concentration was recorded in honey from Chelra Di Malahn (4.05%), followed by Multhan (3.97%), while the lowest value was observed in samples from Jogindernagar (3.86%). The differences among locations were statistically significant, and all values remained within the permissible limit (<5%) prescribed by the Food Safety and Standards Authority of India (FSSAI, 2018) (Table 6).
Glucose content varied from 23.73-24.61 per cent. The highest glucose concentration was observed in honey from Chelra Di Malahn (24.61%), which was statistically similar to samples from Multhan (24.51%). The lowest glucose level was recorded in honey from Jogindernagar (23.73%) (Table 6).
Fructose content ranged from 26.70-27.75 per cent. The highest fructose concentration was recorded in honey from Jogindernagar (27.75%), followed by Chelra Di Malahn (26.86%), while the lowest value was observed in samples from Multhan (26.70%). Significant differences were observed among the locations (Table 6).
The fructose-to-glucose (F:G) ranged from 1.06-1.16. The highest ratio was recorded in honey from Jogindernagar (1.16), followed by Chelra Di Malahn (1.09) and Multhan (1.06). The F:G ratios observed in the present study were within the acceptable range (0.95-1.5) specified by FSSAI (2018) (Table 6).

Table 6. Chemical properties of A. cerana honey from Barot valley

Locations	**Chemical Parameters**								
	Sucrose (%)	**Glucose (%)**	**Fructose (%)**	**F: G**	**Acidity (mili-equi/kg)**	**HMF (mg/100g)**	**Total phenols (mg/100g)**	**Amino acid (mg/100g)**	**Vit C (mg/100g)**
Jogindernagar	3.86c	23.73b	27.75a	1.16a	31.57c	3.43c	78.07b	71.61b	20.60c
Multhan	3.97b	24.51a	26.70c	1.07c	33.55a	3.56b	78.50a	71.88a	21.28a
Chelra Di Malahn	4.05a	24.61a	26.86b	1.09b	32.79b	3.98a	78.43a	71.89a	20.99b

Acidity, HMF (Hydroxy methyl furfural) content, Total phenols, amino acid, Vitamin C

The acidity of honey samples ranged from 31.57-33.55 milliequivalents/kg. The highest acidity was observed in honey collected from Multhan (33.55 meq/kg), followed by Chelra Di Malahn (32.79 meq/kg), while the lowest value was

recorded in samples from Jogindernagar (31.57 meq/kg). All analysed samples were within the acceptable limit (<50 meq/kg) specified FSSAI (2018), indicating good freshness and absence of fermentation (Table 6).
Hydroxymethylfurfural (HMF) content varied from 3.43-3.98 mg per 100 g of honey. The highest HMF concentration was recorded from Chelra Di Malahn (3.98 mg/100 g), followed by Multhan (3.56 mg/100 g), whereas the lowest value was observed in samples from Jogindernagar (3.43 mg/100 g). The differences among locations were statistically significant (Table 6).
Total phenolic content ranged from 78.07-78.50 mg per 100 g. The highest phenolic concentration was recorded in samples from Multhan (78.50 mg/100 g), which was statistically similar to Chelra Di Malahn (78.43 mg/100 g). The lowest value was recorded in honey from Jogindernagar (78.07 mg/100 g) (Table 6).
Total amino acid varied from 71.61-71.89 mg per 100 g. The highest concentration was observed in honey samples from Chelra Di Malahn (71.89 mg/100 g), which was statistically at par with Multhan (71.88 mg/100 g). The lowest value was recorded in honey from Jogindernagar (71.61 mg/100 g) (Table 6).
Ascorbic acid (vitamin C) content ranged from 20.60-21.28 mg per 100 g. The highest concentration was recorded in samples from Multhan (21.28 mg/100 g), followed by Chelra Di Malahn (20.99 mg/100 g), while the lowest value was observed in honey from Jogindernagar (20.60 mg/100 g). The differences among locations were statistically significant.

Mineral content
The mineral composition of honey samples varied among the three sampling locations. Honey collected from Multhan showed comparatively higher concentrations of several mineral elements, including magnesium (0.05%), calcium (0.29%), potassium (10.2%), zinc (1.13%), phosphorus (94 ppm), sodium (182 ppm), and manganese (3.9 ppm). In contrast, the highest iron concentration was recorded in honey samples from Jogindernagar (76.5 ppm) (Table 7).

Table 7. Mineral content of A. cerana honey from Barot valley

Locations	Minerals							
	Mg (%)	Ca (%)	K (%)	Zn (%)	P (ppm)	Na (ppm)	Fe (ppm)	Mn (ppm)
Jogindernagar	0.04b	0.21b	8.00b	0.10c	53c	173b	76.5a	3.2b
Multhan	0.05a	0.29a	10.2a	1.13a	94a	182a	56.1c	3.9a
Chelra Di Malahn	0.036c	0.16c	8.40b	0.12b	75b	165c	66.6b	2.6c

DISCUSSION
A. cerana, an important pollinator in Asian ecosystems, exhibits remarkable ecological adaptability and occurs across diverse regions and climatic zones. This wide distribution contributes to considerable morphological variation among its populations. Several environmental factors, including subspecies differentiation, climatic conditions, altitude, latitude, seasonal variation and temperature have been reported to influence body size and morphological characteristics (Baskaran, 2016; Nunes et al., 2012; Techer et al., 2017). Intraspecific variation in body size is considered an indicator of environmental conditions and resource availability (Banaszak-Cibicka et al., 2018), while worker body weight and size can also respond to nectar and pollen availability and seasonal fluctuations (Hepburn et al., 2001; Liu et al., 2018; Tan et al., 2006). Previous studies have reported a positive relationship between body size and altitude (Dar and Ahmad, 2017), supporting the present findings that morphological traits of A. cerana populations from Barot Valley vary along the altitudinal gradient.
Proboscis length, a key trait associated with nectar foraging efficiency, also showed regional variation in earlier studies. Proboscis lengths ranging from 5.26-5.72 mm have been reported across different agro-climatic zones, while 5.35-5.46 mm were documented in Kashmir and 5.11-5.45 mm in Northern China (Dar and Ahmad, 2017; Radloff et al., 2005). Variation in proboscis length is generally associated with adaptation to local floral resources and nectar accessibility. The antennae of honey bees are key sensory organs involved in environmental perception and foraging behavior (Kumar et al., 2016). The antennal length recorded in the present study aligns with earlier reports from Shimla (4.01-4.17 mm) (Mattu and Verma, 1984), but is greater than values reported for A. cerana populations from Tamil Nadu (3.04 mm) (Baskaran, 2016), indicating clear geographic variation. While such differences have been attributed to altitude-related environmental pressures (Dar and Ahmad, 2017), the present data do not directly establish a causal relationship. Morphological variation may also arise from genetic differentiation or developmental factors.
The recorded values of forewing length and breadth were comparable with those reported from different parts of India, including Himachal Pradesh, Kashmir and Allahabad (Indhu et al., 2023; Dar and Ahmad, 2017; Ken et al., 2003). Wing

dimensions are influenced by environmental variables such as altitude, latitude, climate and seasonal conditions (Montero-Mendieta et al., 2019, Dar and Ahmad, 2017). Larger wing size can enhance flight efficiency in cooler mountainous environments where air density and temperature differ from lowland regions. Hindwing measurements recorded in the present investigation were also consistent with some reported ranges (Indhu et al., 2023). The number of hamuli recorded in the present study falls within the range reported by earlier studies, 18.43-18.96 (Mattu and Verma, 1984), 18.58-20.73 (Dar and Ahmad, 2017), 18.64-19.90 (Ibrahim et al., 2019), and 17.55-19.60 across different agro-climatic zones of Himachal Pradesh (Singh, 2020). Notably, previous investigations (Akahira and Sakagami, 1959; Singh, 1989) have reported no significant association between altitude and hamuli number, suggesting that this trait remains relatively conserved and less influenced by environmental gradients. The present findings are in agreement with these observations, reinforcing the view that hamuli count is a stable morphological characteristic with limited ecological plasticity.

Body colour exhibited noticeable variation among sampling locations. Darker body pigmentation has frequently been associated with higher altitudes, where darker coloration may improve thermoregulation by facilitating heat absorption under cooler environmental conditions (Indhu et al., 2023; Wang et al., 2021). Such pigmentation differences likely represent adaptive responses that enhance survival in cooler mountainous habitats.

Physicochemical characteristics of honey are widely used indicators of honey quality, authenticity, and market value (Grassi et al., 2025). In the present study, honey samples collected from Barot Valley locations (Chelra Di Malahn, Multhan, and Jogindernagar) exhibited physicochemical properties within the limits prescribed by the Food Safety and Standards Authority of India (FSSAI, 2018). Variations observed among locations may be attributed to differences in floral sources, climatic conditions, and micro-environmental factors that influence nectar composition and bee foraging behaviour.

Pollen content is widely recognized as a reliable indicator of honey authenticity and processing history. The pollen density recorded in the present study falls within previously reported ranges, including a mean of 71,250 pollen grains per 10 g (Gupta, 2019) and a broader range of 20,000-126,000 pollen grains per 10 g from the lower hills of Himachal Pradesh (Yadav, 1995), indicating that the observed values are regionally consistent. Variation in pollen density is primarily influenced by floral diversity, climatic conditions, and the geographical location of apiaries (Cherian et al., 2011). Based on absolute pollen counts, all samples fall within Group II (20,000-100,000 pollen grains) as defined by Louveaux et al., 1978, classifying them as moderately pollen-rich honeys.

The pH values of the analysed honey samples fell within the typical acidic range of 3.90-6.10 as reported for natural honey by FSSAI, 2018. Honey acidity is primarily due to the presence of organic acids and contributes to its antimicrobial stability and resistance to microbial spoilage (Hajian-Tilaki et al., 2024; Da Silva et al., 2016). Similarly, EC values recorded were also within the acceptable limit (≤0.8 mS/cm) (FAOSTAT, 2020) for floral honey, indicating normal levels of mineral salts, organic acids, and proteins. Electrical conductivity is often correlated with mineral composition and botanical origin, reflecting the environmental characteristics of the region where nectar is collected (Addi and Bareke, 2021; Can et al., 2015). Moisture content is an important quality parameter influencing honey stability, fermentation susceptibility, and storage characteristics (Dung et al., 2005). All honey samples analysed in the present study contained moisture levels within the permissible limit of 20 % recommended by Codex Alimentarius Commission, 2001, indicating proper maturation of honey within the hive. Variations in moisture content among the locations may be associated with local climatic conditions, humidity levels, and nectar composition.

Sugars constitute the major component of honey, primarily in the form of fructose and glucose along with minor amounts of disaccharides and oligosaccharides (Bogdanov et al., 2004). In the present study, fructose content was higher than glucose in all honey samples collected, resulting in F:G ratios >1.0. Such ratios indicate a lower tendency for crystallization and contribute to better storage stability of honey (Abselami et al., 2018). The sucrose content of the analyzed samples remained below the maximum limit of 5 % recommended by FSSAI, suggesting adequate enzymatic conversion of nectar sucrose into fructose and glucose during honey maturation. This enzymatic hydrolysis is primarily facilitated by invertase enzymes secreted by honeybees during nectar processing within the hive (Al-Farsi et al., 2018). Low sucrose levels therefore indicate well-ripened honey and support the authenticity of the analysed samples (Anklam, 1998).

Acidity values recorded for honey samples from Chelra Di Malahn, Multhan, and Jogindernagar were within the maximum limit of 50 meq/kg specified by international standards (FAOSTAT, 2020), indicating good quality and absence of significant deterioration. Acidity contributes to the characteristic flavour of honey and enhances its resistance to microbial growth (Rani and Verma, 2025). The relatively higher acidity observed in Multhan samples may reflect increased organic acid content, while lower values in Jogindernagar suggest comparatively fresher honey (Gomes et al., 2010; Habib et al., 2014; Shobham and Nayar, 2017). However, as all values remain within acceptable limits, these differences are more plausibly attributed to variation in floral origin and nectar composition rather than pronounced fermentation effects.

HMF levels were very low (<4 mg/kg), indicating minimal heating and good freshness of the honey samples. HMF formation generally increases during prolonged storage or thermal processing of honey; therefore, low HMF content confirms that the samples were fresh and not subjected to excessive heating or adulteration (Choudhary et al., 2021; Godoy et al., 2022).

Bioactive compounds such as phenolic substances, amino acids, and vitamin C contribute to the nutritional and antioxidant properties of honey.

The total phenolic content observed, reflects the presence of bioactive phytochemical compounds derived from floral nectar. Variation among locations is primarily attributed to differences in botanical origin and environmental conditions,

which strongly influence chemical profile of the nectar (Anklam, 1998; Aljadi and Kamaruddin, 2004). The comparatively higher phenolic content in Multhan and Chelra Di Malahn samples indicates greater antioxidant potential, consistent with the established role of phenolic compounds in determining the functional properties of honey (Gheldof et al., 2002).

Proline, the dominant free amino acid in honey, is introduced largely through bee-derived secretions during nectar conversion and is widely used as an indicator of honey maturity and authenticity (Da Silva, 2016). In the study conducted, proline levels ranged from 716.1 to 718.9 mg/kg, substantially exceeding the minimum standard of 180 mg/kg set by the Codex Alimentarius Commission, 2001, confirming proper ripening and absence of adulteration. The observed variation among locations is more likely associated with differences in floral sources and foraging conditions than with processing or storage factors, given that all samples showed sufficiently high values. Considering that proline constitutes a major fraction of total amino acids in honey, its elevated levels further support the overall quality and biochemical integrity of the samples (Iglesias et al., 2006; Truzzi et al., 2014).

Vitamin C content varied across locations, with Multhan honey exhibiting comparatively higher levels, likely reflecting differences in floral origin. Although more labile than phenolic compounds, vitamin C contributes to the overall antioxidant capacity of honey and may act synergistically with other bioactive constituents (Da Silva et al., 2016). The observed variation is therefore primarily attributed to differences in botanical sources rather than post-harvest factors.

Mineral composition of honey is strongly influenced by soil properties, plant species, and environmental conditions of the foraging area (Escuredo et al., 2013; Madejczyk and Baralkiewicz, 2008). It was recorded that electrical conductivity increased in parallel with mineral content, indicating that EC is primarily governed by the concentration of dissolved ionic constituents. The relatively higher mineral levels observed in Multhan honey further support this relationship. Overall, these variations reflect the combined influence of geographical and botanical factors in the Barot Valley.

The results indicate that variation in environmental conditions, altitude, and floral diversity across Barot Valley is associated with differences in both worker morphology of A. cerana and the physicochemical characteristics of the honey. These patterns underscore the ecological responsiveness of A. cerana and the strong influence of regional factors on honey composition. The findings provide a basis for region-specific characterization of honey quality, which may support value addition and targeted marketing of locally produced honey.

CONCLUSION

The present study demonstrates that variation across elevations in the Barot Valley is associated with differences in both the morphology of A. cerana workers and the physicochemical characteristics of their honey. Bees from higher elevations exhibited comparatively larger body size and darker coloration, suggesting possible adaptation to local climatic conditions, although genetic influences cannot be excluded. Physiochemical analysis confirmed that all honey samples met established quality standards, reflecting proper ripening and overall compositional integrity. Overall, these findings provide baseline data for region-specific characterization of A. cerana populations and their honey, with implications for conservation, sustainable management, and potential value addition of Himalayan honey.

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Author Contributions

KSo: Conducted field sampling, laboratory analyses, performed the morphometric measurement, statistical analysis; RKT: Designed the study and supervised the research; KSa: prepared manuscript, supported project management and reviewed manuscript.

Conflict Of Interest

The authors declare no conflict of interest.

REFERENCES

1. Abou-Shaara, H.F., Al-Aw, M.A., & Eid, K.S. (2012). Stability of honey bee morphological characters within open populations. Uludag Aricilik Dergisi, 12(1), 31–37.
2. Abselami, A., Tahani, A., Syndic, M., Fauconnier, M.L., Bruneau, E., & El Bachiri, A. (2018). Physicochemical properties of honeys from different floral sources in eastern Morocco. Journal of Materials and Environmental Science, 9, 879–886. https://doi.org/10.26872/jmes.2018.9.3.97
3. Abu-Tarboush, H.M., Al-Kahtani, H.A., & El-Sarrage, M.S. (1993). Floral-type identification and quality evaluation of selected honey types. Food Chemistry, 46(1), 13–17.
4. Addi, A., & Bareke, T. (2021). Botanical origin and characterization of monofloral honeys in southwestern Ethiopian forests. Food Science and Nutrition, 9(9), 4998–5005.

5. Ajao, A.M., Oladimeji, Y.U., Idowu, A.B., Babatunde, S.K., & Obembe, A. (2014). Morphological characteristics of Apis mellifera L. (Hymenoptera: Apidae) in Kwara State, Nigeria. International Journal of Agricultural Science, 4(4), 171–175.
6. Akahira, Y., & Sakagami, S.F. (1959). A biometrical study on the Japanese honey bee: Observations on populations of Kyushu (Apis cerana cerana F.). Journal of the Faculty of Science, Hokkaido University, 10, 353–362.
7. Alburaki, M., & Alburaki, A. (2008). Morphometrical study of Syrian honey bee (Apis mellifera syriaca). Emirates Journal of Food and Agriculture, 20(2), 89–93.
8. Al-Farsi, M., Al-Belushi, S., Al-Amri, A., Al-Hadhrami, A., Al-Rusheidi, M., & Al-Alawi, A. (2018). Quality evaluation of Omani honey. Food Chemistry, 262, 162–167.
9. Aljadi, A.M., & Kamaruddin, M.Y. (2004). Evaluation of phenolic content and antioxidant capacity of Malaysian floral honeys. Food Chemistry, 85(4), 513–518.
10. Anklam, E. (1998). A review of analytical methods for determining the geographical and botanical origin of honey. Food Chemistry, 63(4), 549–562.
11. AOAC. (1984). Official methods of analysis (12th ed.). Association of Official Analytical Chemists.
12. AOAC. (2004). Official methods of analysis (20th ed.). Association of Official Analytical Chemists.
13. AOAC. (2012). Official methods of analysis. Latimer, G.W. (Ed.). Association of Official Analytical Chemists International.
14. Atrouse, O.M., Oran, S.A., & Al-Abbadi, S.Y. (2004). Chemical analysis and identification of pollen grains from different Jordanian honey samples. International Journal of Food Science and Technology, 39, 413–417.
15. Balanyà, J., Huey, R.B., Gilchrist, G.W., & Serra, L. (2009). The chromosomal polymorphism of Drosophila subobscura: A microevolutionary tool to monitor global change. Heredity, 103(5), 364–377. https://doi.org/10.1038/hdy.2009.86
16. Banaszak-Cibicka, W., Fliszkiewicz, M., Langowska, A., & Żmihorski, M. (2018). Body size and wing asymmetry in bees along an urbanization gradient. Apidologie, 49, 297–306. https://doi.org/10.1007/s13592-017-0554-y
17. Baskaran, M. (2016). Variation in morphological characters of the Indian honey bee Apis cerana indica (Fabr.) from northern to southern India. Journal of Apicultural Research, 55(3), 221–227. https://doi.org/10.1080/00218839.2016.1225446
18. Bogdanov, S. (2009). Harmonised methods of the International Honey Commission. Bee Product Science, 5, 1–62.
19. Bogdanov, S., Jurendic, T., Sieber, R., & Gallmann, P. (2008). Honey for nutrition and health: A review. Journal of the American College of Nutrition, 27, 677–689.
20. Bogdanov, S., Ruoff, K., & Oddo, L.P. (2004). Physicochemical methods for the characterization of unifloral honeys. Apidologie, 35, 4–17. https://doi.org/10.1051/apido:2004047
21. Bray, H.G., & Thorpe, W.V. (1954). Standard methods of biochemical analysis. Kalyani Publishers.
22. Brunet, J., Čanadanović-Brunet, J., Ćetković, G., Šaponjac, V., Tumbas, V., & Stajčić, S. (2014). Evaluation of antioxidant properties of honey. Industrial Crops and Products, 6, 2–7.
23. Can, Z., Yildiz, O., Sahin, H., Turumtay, E.A., Silici, S., & Kolayli, S. (2015). Physicochemical properties, antioxidant capacities, and phenolic profiles of Turkish honeys. Food Chemistry, 180, 133–141.
24. Chang, H.G., Han, M.K., & Kim, J.G. (1988). Chemical composition of Korean honey. Korean Journal of Food Science and Technology, 20(5), 631–636.
25. Chen, C., Wang, H., Liu, Z., Chen, X., Tang, J., & Meng, F. (2018). Population genomics provides insights into the evolution and adaptation of the eastern honey bee (Apis cerana). Molecular Biology and Evolution, 35(9), 2260–2271. https://doi.org/10.1093/molbev/msy130
26. Cherian, K.J., Bhowal, M., & Godghate, S.D. (2011). Pollen and physicochemical analysis of honey produced by Apis cerana indica in Nagpur, India. Journal of Environmental Research and Development, 5, 542–550.
27. Choudhary, A., Kumar, V., Kumar, S., Majid, I., Aggarwal, P., & Suri, S. (2021). 5-Hydroxymethylfurfural formation, occurrence, and potential health concerns. Toxin Reviews, 40, 545–561. https://doi.org/10.1080/15569543.2020.1756857
28. Codex Alimentarius Commission. (2001). Revised Codex standard for honey (Codex Stan 12-1981, Rev. 2).
29. Council of the European Union. (2002). Council Directive 2001/110/EC relating to honey. Official Journal of the European Communities, L 10, 47–52.
30. Crane, E. (1979). Honey: A comprehensive survey. Heinemann.
31. Da Silva, P.M., Gauche, C., Gonzaga, L.V., Costa, A.C.O., & Fett, R. (2016). Honey: Chemical composition, stability, and authenticity. Food Chemistry, 196, 309–323.
32. Dar, S.A., & Ahmad, S.B. (2017). Morphometric variations and body colour patterns of Apis cerana in Kashmir. Journal of Entomology and Zoology Studies, 5(4), 364–371.
33. de Almeida-Muradian, L.B., Stramm, K.M., Horita, A., Barth, O.M., da Silva de Freitas, A., & Estevinho, L.M. (2013). Comparative physicochemical and palynological analysis of honey from Melipona subnitida and Apis mellifera. International Journal of Food Science and Technology, 48(8), 1698–1706.
34. Devi, S., Parihar, A., Thakur, M., Thakur, B., & Sharma, H.K. (2021). Antibacterial potential of honey from Himachal Pradesh. Archives of Microbiology, 203, 5029–5041.
35. Dung, N.T.P., Rombouts, F.M., & Nout, M.J.R. (2005). Development of defined mixed-culture fungal fermentation starter granulate. Innovative Food Science and Emerging Technologies, 6, 429–441.
36. Dustmann, J.H. (1979). Antibacterial effect of honey. Apiacta, 14(1), 7–11.

37. Escuredo, O., Dobre, I., Fernández-González, M., & Seijo, M.C. (2014). Influence of botanical origin on honey crystallization. Food Chemistry, 149, 84–90.
38. Escuredo, O., Míguez, M., Fernández-González, M., & Seijo, M.C. (2013). Nutritional value and antioxidant activity of honey from a European Atlantic area. Food Chemistry, 138, 851–856.
39. FAOSTAT. (2020). FAO statistics. Food and Agriculture Organization of the United Nations.
40. FSSAI. (2018). Food safety standards for honey. Food Safety and Standards Authority of India.
41. Gheldof, N., Wang, X.H., & Engeseth, N.J. (2002). Identification and quantification of antioxidant components in honey. Journal of Agricultural and Food Chemistry, 50, 5870–5877.
42. Godoy, C.A., Valderrama, P., & Boroski, M. (2022). HMF monitoring and honey quality. Food Analytical Methods, 15, 3162–3176.
43. Gomes, S., Dias, L.G., Moreira, L.L., Rodrigues, P., & Estevinho, L. (2010). Physicochemical and antimicrobial properties of Portuguese honey. Food and Chemical Toxicology, 48, 544–548.
44. Grassi, G., Capasso, G., Cillo, A., Miedico, O., Pompa, C., Nardelli, V., & Perna, A.M. (2025). Effect of altitude on polyphenols and antioxidant activity of wildflower honey. Applied Sciences, 15, 3255.
45. Gupta, N. (2019). Physicochemical variations in honey from different agroclimatic zones of Himachal Pradesh (Master's thesis). Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, India.
46. Habib, H.M., Al-Meqbali, F.T., Kamal, H., Souka, U.D., & Ibrahim, W.H. (2014). Physicochemical and biochemical properties of honey from arid regions. Food Chemistry, 153, 35–43.
47. Hajian-Tilaki, A., Kenari, R.E., Farahmandfar, R., & Razavi, R. (2024). Comparative study of physicochemical properties of Iranian multifloral honeys. Heliyon, 10, e23317.
48. Hepburn, H.R., Radloff, S.E., Verma, S., & Verma, L.R. (2001). Morphometric analysis of Apis cerana populations in the southern Himalayan region. Apidologie, 32(5), 435–447. https://doi.org/10.1051/apido:2001142
49. Ibrahim, M.M., & Chandel, Y.S. (2019). Morphometrics of Apis cerana across agroclimatic zones of Himachal Pradesh. Indian Journal of Entomology, 81(3), 406–410. https://doi.org/10.5958/0974-8172.2019.00116.0
50. Iglesias, M.T., De Lorenzo, C., Polo, M.C., Martín-Álvarez, P.J., & Pueyo, E. (2004). Amino acid composition for differentiating honeydew and floral honeys. Journal of Agricultural and Food Chemistry, 52, 84–89.
51. Iglesias, M.T., Martín-Álvarez, P.J., Polo, M.C., De Lorenzo, C., González, M., & Pueyo, E. (2006). Changes in free amino acids during honey storage. Journal of Agricultural and Food Chemistry, 54, 9099–9104.
52. Indhu, A., Lazar, J., Prasad, S., & Anupama, K. (2023). Pollinators in tropical ecosystems of southern India with emphasis on native pollinators Apis cerana indica and Tetragonula iridipennis. Indian Journal of Entomology, 85(5), 1187–1199. https://doi.org/10.55446/IJE.2021.369
53. Indian Standards Institution. (1974). Indian standard specification for honey (ISI 4941).
54. Jackson, M.L. (1973). Soil chemical analysis. Prentice Hall.
55. Jackson, M.L. (2005). Soil chemical analysis. Prentice Hall of India.
56. Ji, C., Shi, W., Tang, J., Ji, T., Gao, J., & Liu, F. (2023). Morphometrical analyses reveal diversity of the eastern honey bee (Apis cerana) in mountainous and island regions of China. Journal of Apicultural Research, 62(4), 647–655.
57. Ken, T., Fuchs, S., Koeniger, N., & Ruiguang, Z. (2003). Morphological characterization of Apis cerana in Yunnan Province, China. Apidologie, 34, 553–561. https://doi.org/10.1051/apido:2003049
58. Kumar, N.R., Sharma, R., & Anudeep. (2016). Scanning electron microscopic analysis of antennae in plain and hill populations of Apis cerana. Journal of Entomology and Zoology Studies, 4, 5–9.
59. Liu, Z., Tan, X., Orozco-terWengel, P., Zhou, X., Zhang, L., & Tian, S. (2018). Population genomics of wild Chinese rhesus macaques reveals demographic history and local adaptation. GigaScience, 7(9), giy106. https://doi.org/10.1093/gigascience/giy106
60. Louveaux, J., Maurizio, A., & Vorwohl, G. (1978). Methods of melissopalynology. Bee World, 59, 139–157.
61. Madejczyk, M., & Baralkiewicz, D. (2008). Mineral composition of Polish honeys. Analytica Chimica Acta, 617, 11–17.
62. Mattu, V.K., & Verma, L.R. (1984). Morphometric studies on Apis cerana indica: Effect of seasonal variation. Apidologie, 15, 63–74.
63. Minhas, S., Khanna, P., & Kaushik, R. (2016). Comparative study of ripened and unripened honey during storage. International Journal of Food Science and Nutrition, 1(3), 52–57.
64. Montero-Mendieta, S., Tan, K., Christmas, M.J., Olsson, A., Vilà, C., & Wallberg, A. (2019). Genomic basis of adaptation to high-altitude habitats in Apis cerana. Molecular Ecology, 28(4), 746–760. https://doi.org/10.1111/mec.14986
65. Narayanan, E.S., Sharma, P.L., & Phadke, K.G. (1960). Tongue length and hind wing hooks in Apis indica. Indian Bee Journal, 22, 81–88.
66. Nunes, L.A., Araújo, E.D., Marchini, L.C., & Moreti, A.C. (2012). Morphogeometric variation of Africanized honey bees (Apis mellifera) in Brazil. Iheringia Série Zoologia, 102, 321–326. https://doi.org/10.1590/S0073-47212012005000002
67. Nweze, A.J., Olovo, C.V., Nweze, E.I., John, O.O., & Paul, C. (2020). Therapeutic properties of honey. In Honey analysis: New advances and challenges (pp. 1–21).
68. Oryan, A., Alemzadeh, E., & Moshiri, A. (2016). Biological properties of honey in wound healing. Journal of Tissue Viability, 25(2), 98–118.
69. Pasias, I.N., Kiriakou, I.K., & Proestos, C. (2017). HMF and diastase activity in honey. Food Chemistry, 229, 425–431.

70. Przybylowski, P., & Wilczynska, A. (2001). Honey as an environmental marker. Food Chemistry, 74, 289–291.
71. Radloff, S.E., Hepburn, R., & Fuchs, S. (2005). Morphometric affinities of Apis cerana in the Hindu Kush and Himalayan regions. Apidologie, 36(1), 25–30. https://doi.org/10.1051/apido:2004066
72. Ranganna, S. (2007). Handbook of analysis and quality control for fruit and vegetable products (2nd ed.). Tata McGraw Hill.
73. Rani, A., & Verma, S. (2025). Phytochemical composition and antioxidant potential of honey. International Journal of Plant and Environment, 11, 10–19.
74. Ratiu, I.A., Al-Suod, H., Bukowska, M., Ligor, M., & Buszewski, B. (2019). Correlation of physicochemical properties of honey. Molecules, 25, 1–15.
75. Schade, J.E., Marsh, G.L., & Eckert, J.E. (1958). Diastase activity and hydroxymethylfurfural in honey. Journal of Food Science, 23, 446–463.
76. Se, K.W., Ghoshal, S.K., Wahab, R.A., Ibrahim, R.K.R., & Lani, M.N. (2018). Detection of adulterants in stingless bee honey. Food Research International, 105, 453–460.
77. Sharma, V.A.K., Krishna, P., & Budihal, S.L. (1987). Soil resource mapping of different states in India. NBSS & LUP.
78. Sheoran, O.P., Tonk, D.S., Kaushik, L.S., Hasija, R.C., & Pannu, R.S. (1998). Statistical software for agricultural research. In D.S. Hooda & R.C. Hasija (Eds.), Recent advances in information theory, statistics and computer applications (pp. 139–143).
79. Shobham, K.K.C., & Nayar, J. (2017). Physicochemical analysis of commercial honey samples. Indian Journal of Nutrition, 4, 153.
80. Singh, M.P. (1989). Melissopalynology and identification of Himalayan honey bee races (Doctoral dissertation). Himachal Pradesh University, Shimla, India.
81. Singh, S.J. (2020). Molecular and morphometric variability in Apis cerana (Master's thesis). Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, India.
82. Szentgyörgyi, H., Czekońska, K., & Tofilski, A. (2016). Influence of pollen deprivation on wing asymmetry of honey bees. Apidologie, 47, 653–662. https://doi.org/10.1007/s13592-015-0415-5
83. Tan, K., Meixner, M.D., Fuchs, S., Zhang, X., He, S., & Kandemir, I. (2006). Geographic distribution of Apis cerana across ecological zones in China. Systematics and Biodiversity, 4(4), 473–482. https://doi.org/10.1017/S1477200006002015
84. Techer, M.A., Clémencet, J., Simiand, C., Turpin, P., Garnery, L., & Reynaud, B. (2017). Genetic diversity of insular honey bee populations. PLoS ONE, 12(12), e0189234. https://doi.org/10.1371/journal.pone.0189234
85. Thrasyvoulou, A., Tananaki, C., Goras, G., Karazafiris, E., Dimou, M., Liolios, V., Kanelis, D., & Gounari, S. (2018). Honey standards and legislation. Journal of Apicultural Research, 57(1), 88–96.
86. Tornuk, F., Karaman, S., Ozturk, I., Toker, O.S., Tastemur, B., Sagdic, O., Dogan, M., & Kayacier, A. (2013). Quality characterization of Turkish blossom honeys. Industrial Crops and Products, 46, 124–131.
87. Townsend, G.F. (1969). Optical density for honey colour classification. Journal of Apicultural Research, 8, 29–36.
88. Truzzi, C., Annibaldi, A., Illuminati, S., Finale, C., & Scarponi, G. (2014). Determination of proline in honey. Food Chemistry, 150, 477–481.
89. Wang, S., Gao, J., Liu, J., & Zhao, D. (2021). Morphometric characterization of Apis cerana hainana in China. Journal of Apicultural Research, 60(2), 337–340. https://doi.org/10.1080/00218839.2020.1753326
90. Yadav, S.P. (1995). Physicochemical characteristics of Indian honey with reference to pesticide residues (Doctoral dissertation). Dr. Y.S. Parmar University of Horticulture and Forestry, Solan, India.
91. Zhu, X., Zhou, B., Wu, X., Xu, X., & Wang, Q. (2009). Morphometric genetic analysis of Apis cerana cerana. Journal of Fujian Agriculture and Forestry University (Natural Science Edition), 38(1), 75–78.
92. Zhu, X., Zhou, B., Wu, X., Xu, X., & Xia, X. (2011). Genetic diversity of Apis cerana cerana based on microsatellite markers. Journal of Fujian Agriculture and Forestry