Original paper

MORPHOLOGY AND POLLEN CHEMISTRY OF SEVERAL BEE FORAGE TAXA OF FAMILY ROSACEAE FROM GARHWAL HIMALAYA, UTTARAKHAND, INDIA

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Received: 28 September 2017; accepted 1 July 2018
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Abstract

Pollen grains vary widely in pollen shape, size, aperture type and exine sculpture among the taxa and within the taxon, which make them taxonomically important. They also contain several proteins, lipids and vitamins which are essential for the growth and developments of developing bee larvae. Quantification of these chemical constituents is important for the dietary purposes of honey bees. The present study deals with the morphology and chemical constituents of hand-collected pollen from four bee forage plants viz. *Prunus cerasoides* D.Don, *Prunus persica* (L.) Batsch, *Pyrus pashia* Buch.-Ham. ex D.Don and *Rosa brunonii* Lindl. from Garhwal Himalaya, Uttarakhand. The family represents a homogenous group with a tricolporate pollen aperture type in all the studied taxa. Pollen shape varied from sub-prolate to prolate-spheroidal with a smaller pollen size observed in *Pyrus pashia* (26.53±0.30 µm polar view and 24.20±1.04 µm equatorial view) and a larger one in *Prunus persica* (38.39±3.06 µm polar view and 36.41±1.34 µm equatorial view). Exine sculpture was psilate to striato-reticulate. Maximum crude protein (68.33±0.14 mg/g) and starch content (32.98±0.67 mg/g) were recorded in pollen of *Prunus cerasoides* and maximum free amino acid (13.78±0.71 mg/g) in *Pyrus pashia*. All chemical constituents were found to be significant except the amino acids which were non-significant at the 0.05* level. Results showed that pollen grains of these Rosaceous members contained high amount of crude protein and phenolic content as a bee food source for brood, which makes this family economically important.

Keywords: biochemical analysis, bee forage, pollen morphology, Rosaceae

INTRODUCTION

Garhwal possesses luxuriant and varied vegetation because of its unique setting within the Indian Himalayan Region. It represents subtropical-to-temperate type of vegetation and almost every plant has economic values nutritional, aesthetic or medicinal. The family Rosaceae consisting of 85 genera and about 3000 species, including many apomictic lines (Mabberley, 2008), provides good forage to honeybees. India has 30 genera and 212 species belonging to Rosaceae, out of which 17 genera and 45 species have been reported from the Garhwal region (Gaur, 1999). The apicultural value of the Rosaceous members is extremely high (Inove, 1957; Tiwari, Tiwari, & Ballabha, 2009), and these are considered as important bee forage sources in the Garhwal Himalaya (Gaur, 1999; Tiwari et al., 2009, 2012). Their pinkish - white flowers are a rich source of nectar and pollen for bees (Sharma, 1970; Crane, Walker, & Rosemary, 1984; Gaur & Nawani, 1989; Pratap, 1997; Gaur & Tiwari, 2001).

Honeybees visit plants' flowers to collect nectar and pollen as their food, which are collectively termed as 'bee forage' or 'bee pasturage' (Singh, 1982). Nectar provides an energy source to honeybees, while all other nutritional needs for developmental stages are fulfilled from the pollen grains. Pollen grains contain carbohydrates, amino acids, proteins, lipids, vitamins, minerals, phenolic compounds, flavonoids and concentrations of phytosterols and are also

rich in phytochemicals (Balch & Balch, 1990; Broadhurts, 1999; Carpes, 2008). The chemical composition of pollen grains can vary due to their botanical and geographic origin (Almaraz-Abarca et al., 2004). Wandering from flower to flower, honeybees collect their food and simultaneously act as the primary pollinators for about three-quarters of crops that require animal pollination (Free, 1993; Pashte & Kulkarni, 2015).

These pollen grains play a fundamental role in the health and vigor of honeybees by supplying the dietary requirements (Roulston & Cane, 2000; Brodschneider & Crailsheim, 2010). It does likewise in larval development and adult reproduction in honeybees (Roulston & Cane, 2002). The concentration of chemical constituents in pollens varies from species to species, which also depends on climatic conditions and the nutrition conditions of plants (Mondal, Pauri, & Mandal, 1998).

The attraction of pollinators towards the flowers depends on the pollens' protein concentration of (Roulston & Cane, 2000). Higher dietary pollen protein may ensure survival (Levin & Haydak, 1957), size (Regali & Rasmont, 1995), and longevity of bees (Schmidt, Thoenes, & Levin, 1987). Composition of amino acids defines more accurate nutritional value of pollen as compared to concentration of protein (Basuny, Arafat, & Soliman, 2013), as pollen contains all essential and nonessential amino acids which are building blocks of proteins. Pollen also has high contents of phenolic compounds (Campos et al., 2003), as it is an important site for phenolic synthesis and accumulation (Almaraz-Abarca et al., 2004). Studies have been conducted on bee-collected pollen because bees easily collect pollen (Villanueva et al., 2002; Almeida-Muradian et al., 2005), and a few have dealt with protein-content of hand-collected pollens (Roulston, Cane, & Buchmann, 2000; Saa-Otero, Diaz-Losada & Fernandez-Gomez, 2000; Manning, 2001; Cook et al., 2003; Human & Nicolson, 2006). This study was aimed to evaluate pollen morphology and chemistry for crude protein, free amino acids, soluble sugars, starch and phenolic contents of four bee forage taxa of Rosaceous pollen.

MATERIAL AND METHODS

The study was held in the Pauri district at 29° 45′ to 30° 15′ north latitude and 78° 24′ to 79° 23′ east longitude in Garhwal Himalaya, Uttarakhand in the year 2016 (Fig. 1). We selected four taxa (n=4) i.e., *Prunus cerasoides* D. Don, *Prunus persica* (L.) Batsch., *Pyrus pashia* Buch.-Ham. ex D. Don and *Rosa brunonii* Lindl. (Fig. 2-5). Anthers, the polliniferous material, were collected at the time of anthesis from the wild populations growing in the Pauri district. They were air dried, crushed and sieved (sieve size 100-150 µm). Pollen grains were collected in glass tubes and stored at -20°C for further analysis.

Pollen morphology

Pollen samples of the four bee forage species were prepared for light microscopy following Erdtman (1952). Light microphotography was performed by Magnus pro 3.7 and Scan Electron Microphotography was done with SEM (Model JSM-6610 LV-JEOL, Japan). All measurements polar axis, equatorial diameter, exine thickness, aperture length and breadth were taken in replicates (N=10 to 15).

Determination of Biochemical components

Soluble sugars and starch contents

Soluble sugars and starch in pollen samples were analyzed according to Yemm & Willis (1954). The pollen samples were weighed (50 mg) and 5 ml absolute ethanol was added. The mixture was centrifuged at 3000 rpm for 15 minutes. 100 µl supernatant was taken in a test tube and diluted with 900 µl of double distilled water for determining the soluble sugars in sample. 4 ml anthrone reagent (0.2%) was added in triplicates. The absorbance was recorded with spectrophotometer (Beckman Coulter 730 UV/VIS) at 620 nm. Starch content was determining by retaining the residue in centrifuge tube and store for 24 hours. After 24 hours, the residue were treated with 5 ml perchloric acid (52%) and centrifuged at 3500 rpm for 15 minutes. A similar process was applied for the detection of starch. Distilled water was taken as the control

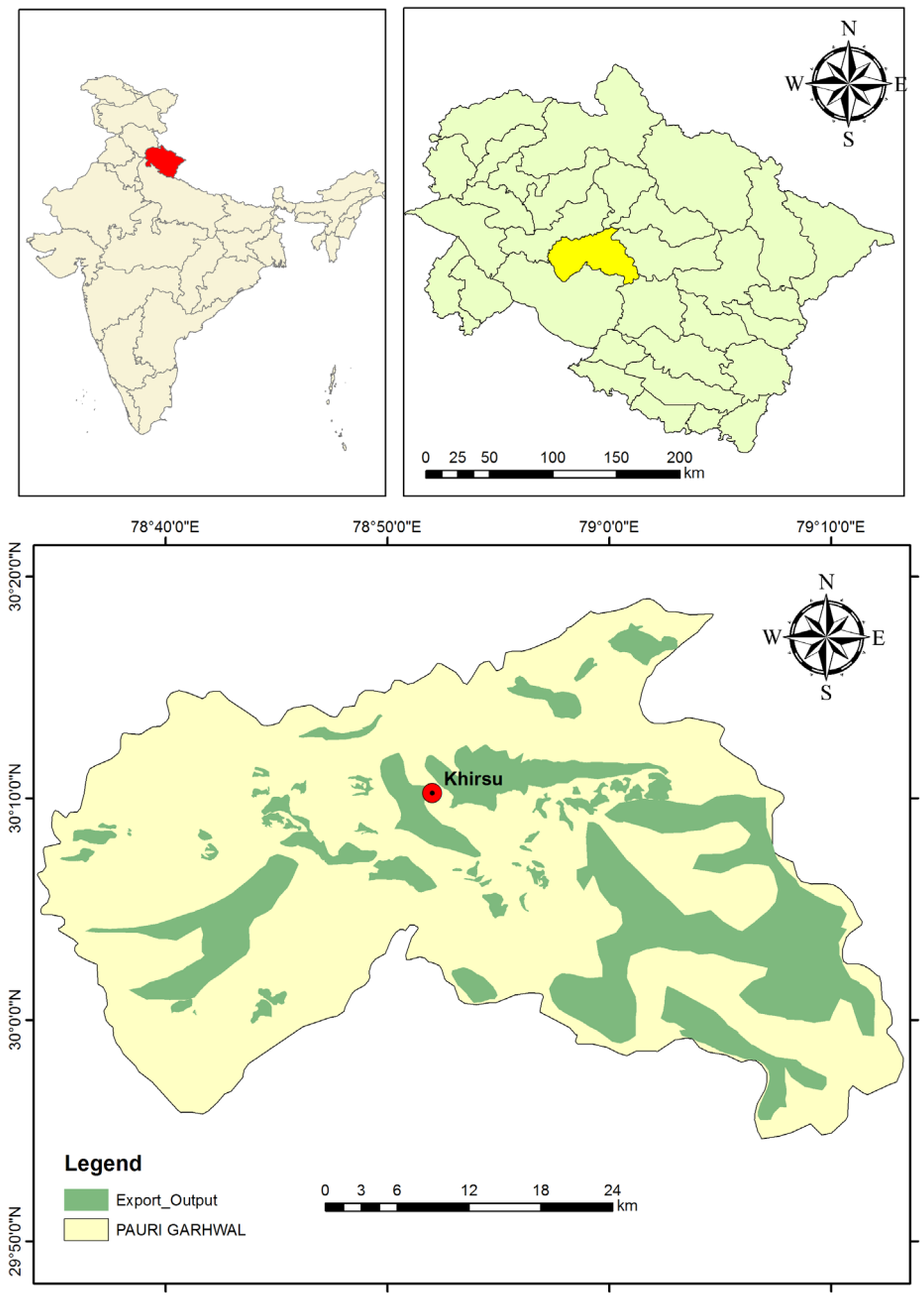

Fig. 1. Map of study area, marked areas locate the location from where polliniferous material was collected.

solution while dextrose used as a standard solution.

Crude protein

The crude protein of pollen samples was analyzed by following Bradford (1976). The samples were weighed (50 mg) and 5 ml HCl buffer was added to it. The mixture was centrifuged at the 10,000 rpm for 10 minutes. Supernatant 100 µl was taken in a test tube and 4.9 ml Bradford dye added to it, in triplicates. The absorbance was recorded with spectrophotometer (Beckman Coulter 730 UV/VIS) for pollen samples at 595 nm. Distilled water was taken as the control (empty) solution while Bovine serum albumin (BSA) used as a standard solution.

Free amino acids

Free amino acids of pollen samples were analyzed according to Dukhanina et al. (2006). 100 mg of pollen sample was weighed and 5 ml ethanol (80%) added to it, and next further cen-

Fig. 2-5. Rosaceous taxa *Prunus cerasoides* (2), *Prunus persica* (3), *Pyrus pashia* (4), *Rosa brunonii* (5).

trifuged at 3000 rpm for 10 minutes. Supernatant 100 µl was taken in a test tube and diluted with 900µl double distilled water and 2 ml ninhydrin reagent added to it. The mixture was then boiled in a test tube at a temperature of 70-80°C for 20-30 minutes, further cooled and absorbance was recorded with spectrophotometer (Beckman Coulter 730 UV/VIS) for pollen samples at 570nm. Distilled water was taken as the control solution while Glycine used as a standard solution.

Total phenolic contents

Total phenolic contents were determined with the Folins Ciocalteau method (Singleton, Orthofer, & Lamuela–Raventós, 1999). 100 mg pollen sample was weighed to which 5 ml methanol (80%) was added, and further centrifuged at 5000 rpm for 10 minutes. Supernatant 100µl was taken in a test tube and diluted with 7.9 ml double distilled water. 500 µl Folin Ciocalteau reagent (2N) was added, in triplicates, and further kept at room temperature for 10 minutes after which 1.5 ml sodium carbonate 20% was added. These tubes were heated at 40°C for 20 minutes and immediately cooled, and absorbance was recorded with spectrophotometer (Beckman Coulter 730 UV/VIS) at 720 nm. Gallic acid was used as a standard, and the total phenolic contents were expressed as mg/g dry weight of pollen. Distilled water was taken as the control solution while gallic acid (GAE) used as a standard solution.

Statistical analysis

The analysis was carried out in triplicate, and the results were expressed as mean and standard deviation (SD) for both parameters (pollen morphology and pollen chemistry). ANOVA using SPSS software was done for pollen chemistry to prove the statistical significance of $p<0.05$.

RESULTS

The morphological characters of pollen samples showed that pollen grains were monad, bilaterally symmetrical, medium-sized and tricolporate (Fig. 6-9, 11-14, 16-19 and 21-24). Pollen size was found to be smaller in *Pyrus pashia*, whereas it was in *Prunus persica* varying from 26.53±0.30 µm to 38.39±3.06 µm in polar view and 24.20±1.04 µm to 36.41±1.34 µm in equatorial view (Tab. 1). Pollen shape class ranged from prolate-spheroidal to oblate-spheroidal. Exine thickness was 1.05±0.01 µm, 1.85±0.51 µm, 2.20±0.75 µm and 3.13±1.16 µm in

Table 1.

Details of pollen morphology of studied taxa

Name of species	PA (µm)	ED (µm)	P/E	Shape	AT	AS (L×B)	ET	ES
Prunus cerasoides	28.33±2.63	26.25±6.74	1.05	Prolate spheroidal	tricolpo-rate	3.18×7.59	3.13±1.16	Striato-reticulate
Prunus persica	38.39±3.06	36.41±1.34	1.05	Prolate spheroidal	tricolpo-rate	10.51×11.77	1.85±0.51	Psilate
Pyrus pashia	26.53±0.30	24.20±1.04	1.09	Prolate spheroidal	tricolpo-rate	5.37×9.18	1.05±0.01	Striate thin elongated-branched
Rosa brunonii	31.86±2.62	35.33±5.59	0.90	Oblate-spheroidal	tricolpo-rate	7.88×8.55	2.20±0.75	Striate parallel reticulate

PA= Polar axis, ED= Equatorial diameter, AT= Aperture type, AS= Aperture size, ET= Exine thickness, ES= Exine sculpture.

Table 2.

Details of the pollen chemistry ±SD (mg/g) in studied taxa

Plant species	Crude Protein	Free amino acids	Soluble Sugars	Starch	Total Phenolic Contents
Prunus cerasoides	68.33^{a} ±0.14	11.26^{a}±0.41	7.79^{b} ±0.17	32.98^{a} ±0.67	14.10^{b} ±0.17
Prunus persica	46.26^{b}±0.63	11.08^{a}±0.05	6.33^{c} ±0.05	10.32^{b} ±1.09	1.81^{d} ±0.07
Pyrus pashia	37.63^{c} ±3.35	8.10^{a}±1.53	12.31^{a} ±0.41	8.55^{c} ±0.18	3.60^{c} ±0.10
Rosa brunonii	39.25^{c}±5.50	13.78^{a}±0.71	1.78^{d} ±0.20	4.54^{d} ±0.11	31.98^{a} ±0.70
Mean±	**47.86±2.40**	**11.05±0.67**	**7.04±0.21**	**14.09±0.49**	**12.87±0.28**

Mean value in the same column with different letters indicates significant difference ($p<0.05$).

Pyrus pashia, *Prunus persica*, *Rosa brunonii* and *Prunus cerasoides*, respectively. Exine sculpture in all the four genera was striato-reticulate to psilate in *Prunus cerasoides* and *Prunus persica*, respectively (Fig. 10, 15, 20 and 25).

A chemical analysis of pollen samples revealed that maximum soluble sugars was recorded in *Pyrus pashia* while minimum in *Rosa brunonii*, with a mean of 7.04±0.21 mg/g. Starch contents varied from 4.54±0.11 mg/g (*Rosa brunonii*) to 32.98±0.67 mg/g (*Prunus cerasoides*), with a mean of 14.09±0.49 mg/g (Tab. 2).

The maximum crude protein content was recorded in *Prunus cerasoides*, while minimum in *Pyrus pashia*, with a mean of 47.86±2.40 mg/g as shown in Tab. 2. It also depicts maximum free amino acids in *Rosa brunonii*, while minimum in *Pyrus pashia*, with an average of 11.05±0.67

mg/g. The maximum total phenolic contents were recorded in *Rosa brunonii*, while minimum in *Prunus persica*, with an average of 12.87±0.28 mg/g.

Pollen chemistry of all the taxa showed significant results ($p<0.05$) except for crude protein for *Pyrus pashia* and *Rosa brunonii.* In contrary, the amino acid content of all the pollen samples did not significantly differ ($p<0.05$) from one another.

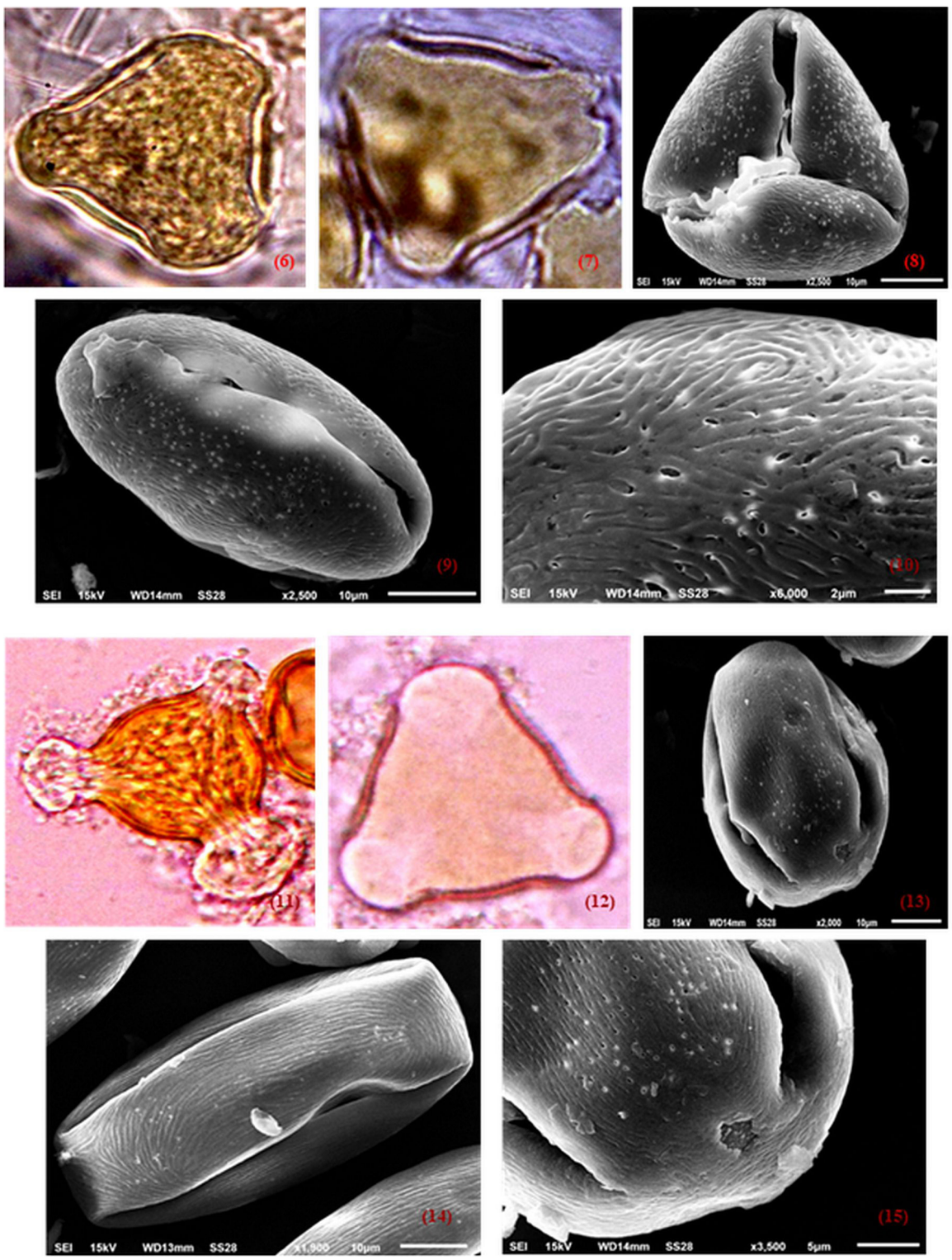

Fig. 6-15. Light and Scan Electron Microphotography of pollen grains *Prunus cerasoides* (6, 7 LM; 8-10 SEM). *Prunus persica* (11, 12 LM; 13-15 SEM).

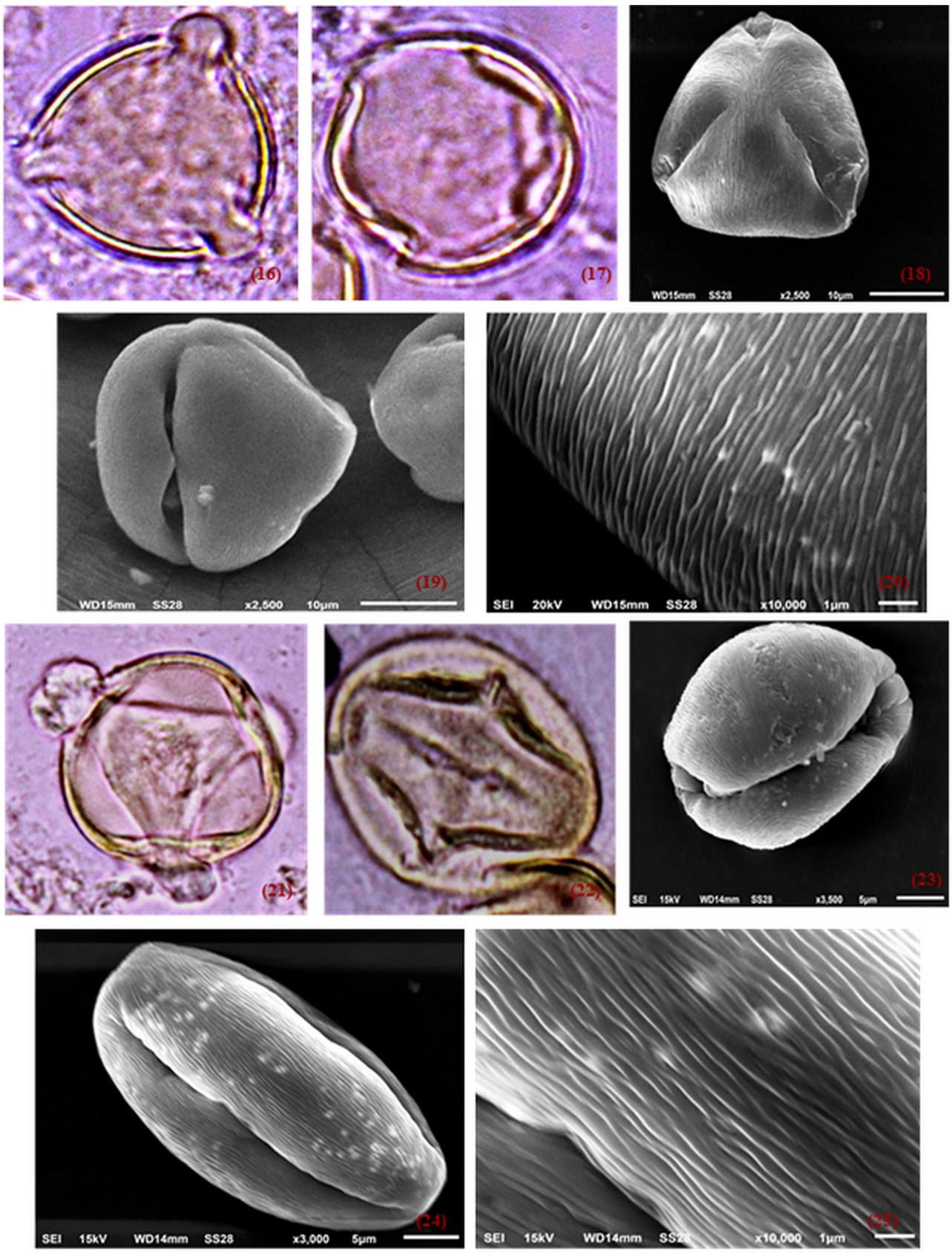

Fig. 16-25. Light and Scan Electron Microphotography of pollen grains *Pyrus pashia* (16, 17 LM; 18-20 SEM). *Rosa brunonii* (21, 22 LM; 23-25 SEM).

DISCUSSION

The morphological characters of pollen from genera *Prunus* and *Pyrus* showed a prolate-spheroidal shape class. A similar pollen shape class was observed by Fogle (1977) and Geraci et al. (2012). A prolate shape was observed by Chwil (2015) in *Prunus persica*. The pollen size in genus *Prunus* was observed to be 28.33±2.63 µm for the polar view and 26.25±6.74 µm for the equatorial view, which was almost half of what had been previously recorded in the polar view and almost equal in the equatorial view. Chwil (2015) reported 50.93 µm for the polar view and 29.84 µm for the equatorial view in size. The size in *Pyrus pashia* was 26.53±0.30 µm for the polar view and 24.20±1.04 µm for the equatorial view, which was in line with the 31.23 µm polar and 28.35 µm equatorial views reported by Zamani, Attar, & Maroofi (2010) from Iran. Pollen size (31.86±2.62 and 35.33±5.59) in polar and equatorial view, aperture type (tricolporate) and exine sculpture (striate parallel reticulate) of *Rosa brunonii* in the present study was found to be nearer to previous reports by Zuraw et al. (2015), who had found that *Rosa* pollen size ranged from 29.2-34.2 µm in polar and 29.4-33.5 in equatorial view, tricolporate aperture and striate exine. The present study's P/E ratio of 1.09 is similar to the P/E ratio of 1.10 in *Pyrus pashia* reported by Zamani, Attar, & Maroofi (2010). Rosaceous pollen morphology has been found to be highly variable, even among the populations within the same species due to frequently occurring hybridization (Moore, Webb, & Collinson, 1991) which also varies with environmental conditions.

The mean crude protein of studied species was 47.86±2.40 mg/g (Tab. 2). The recorded value of 4.7% for crude protein agreed with the value reported by Buchmann (1986) and Roulston, Cane, & Buchmann (2000), who had announced that crude protein in hand- collected pollen varied from 2.5 % to 61 % of dry weight. The crude protein of hand-collected pollen in the present study was found to be considerably high which makes them of high quality. This high quality protein helps to maintain bee health and reduces their susceptibility to diseases (Basualdo et al., 2013). Rosaceae pollens also have a sufficient protein content to rear worker larve to the pupal stage. The crude protein content in the present study is more than the value reported by Brodschneider, Reissberger-Galle, & Crailsheim (2009), who found that 25 to 37.5 mg/g of protein was needed to rear one worker larva to the pupal stage. The recorded value for free amino acid content was similar with earlier reports by Bhunia & Mondal (2012), 5.35-7.85 mg/g for Nymphaeaceae. The amino acids contents for Rosaceae were 11.05±0.67 mg/g.

The soluble sugars were found to be 7.04±0.21 mg/g. The findings are in accordance with Todd & Bretherick (1942), who reported a carbohydrate content 1% to 37% of total dry mass in hand-collected pollen. The recorded value for starch content of 14.09±0.49 mg/g was similar with earlier reported (0% to 22%) values by Roulston & Buchmann (2000).

Total phenolic contents in Rosaceae were estimated as 12.87±0.28 mg/g, which are above eight times higher than the earlier reports of Nozkova et al. (2009), who reported 0.79 to 1.55 GAE mg/g phenolic contents in *Brassica napus* subsp. *napus* L. High phenolic contents in bee pollen becomes a natural source of antioxidant properties to honeybees which help them to avoid the disease. These phenolic contents in bee pollen also protect human beings from many such diseases as cancer, diabetes, cardiovascular diseases and atherosclerosis (Rzepecka-Stojko, 2015).

The pollens of Rosaceae are proteinaceous in nature and serve as a food source for bees. We also confirm high phenolic content in pollens which suggests that these are helpful in preventing oxidative damages to honeybees. The present study is useful in the development of the analytical standard for further studies on pollen chemistry. Furthermore, we suggest that the nutritional aspects of pollens such as amino acid profile and the correlation between the pollen phenolics and antioxidants can be taken into account in assessing the total dietary value

of these for bees and subsequently for human beings.

ACKNOWLEDGEMENTS

The research was supported by the University Grants Commission, Government of India, New Delhi for providing research fellowship to one of us (EC). We extend our gratitude to the head of the Department of Botany, HNB Garhwal University, for supporting this work. Thanks are also due to the head of the Department of Botany, University of Delhi, for providing necessary facilities.

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