Scanning Electron Microscopic Studies on Surface Pattern of the Pollen Loads from *Apis cerana* in Jajarkot District

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Abstract

Over nineteen species of pollen flora belonging to thirteen families were recovered from four pollen load samples from honey bee *Apis cerana* collected in Jajarkot district, mid-western region, Nepal. The pollen morphology was investigated using light microscopy along with scanning electron microscopy for correct identification of pollen to its lower taxonomic level and to contribute to melissopalynological studies originating from the native apiflora. The palynological assemblage identified to the generic and some up to species level belong to *Alnus* sp., *Artemisia* sp., *Brassica* sp., *Cornus* sp., *Diploknema* sp., *Fraxinus* sp., *Ilex* sp., *Jasminum* sp., *Justicia* sp., *Ligustrum* sp., *Myrica esculenta*, *Salix* sp., *Strobilanthes* sp., and *Urtica* sp. Some of the pollen grains identified to only family level, belong to, Acanthaceae, Compositae, Lamiaceae and Rosaceae. The identified pollens clearly reflect the botanical and geographical origins of the pollen load samples. Palynomorphological investigation included the description of pollen symmetry, polarity, ornamentation, aperture, shape and size. The results for the pollen assemblages and nectariferous plant sources of Jajarkot district are discussed.

Key words: pollen flora, palynomorphology, botanical origin, nectar source, honey bee

Introduction

The study of pollen composition is an effective tool for studying the interaction between honey bees and vegetation, and has also been stressed its importance in establishing apiculture-based honey industries. Analysis of pollen of different species of the floral nectar sources gathered by honeybees to produce honey enable the botanical origin of honey and pollen load specimens to be identified (Dustman & von der Ohe 1993), its type and quality (Seethalakshmi 1980, Atri 2010) from different countries (Zander 1941). The honey bees frequently make use of the resources available. In the growth and development of honey bees, nectar is the source of carbohydrates, whereas proteins are provided by the pollen (Lin et al.1990). The honey bees frequently make use of the resources available close to the site of the hives and analyzing the proportional representation of different pollen types allows the characterization of honey from different regions, in terms of flora and vegetation. Therefore, assessment of the botanical taxa that are the source of honey is of high practical and scientific importance (Maurizio 1951, Louveaux et al. 1978, Molan 1998, Terrab et al. 2003) in the food control (Lieux 1975, Louveaux et al. 1978, Moar 1985). For this reason, it is necessary to protect consumers from the fraudulent mislabeling of inferior honeys (Dustmann & Bote 1985, 1987, Dustman & von der Ohe 1993).

Both light microscopic (LM) and scanning electron microscopic (SEM) observations of pollen loads from
a particular locality reflects the geographical origin of a particular type of honey, since its pollen spectrum reflects the floral situation of the place where that particular honey was produced (Louveaux et al. 1978). This helps the beekeepers in the proper management of bee colonies to enhance honey production.

The bee flora of Nepal have been previously surveyed (Kafle 1984, 1992, Maskey 1989, 1992, Partap & Verma 1996, Partap 1997). However, pollen analytical studies of Nepali honeys are mostly fragmentary, although the honey and pollen load samples from different parts of Nepal have been melissopalynologically studied by some workers (ICIMOD 1996, Partap 1997, Joshi 1999) using LM to identify and interpret the pollens present in a particular honey. SEM studies on surface pattern of pollen loads of honey bees are rarely studied in Nepal. Therefore, SEM studies led us to investigate the possibility of identifying pollen loads of A. cerana bees to a lower taxonomic level from Jajarkot district. Because the district has considerable potentiality for bee keeping ventures for the production of good quality honey, the present study is aimed to understand and identify the composition of vegetation especially nectariferous plants around the bee hives of the A. cerana bees of Jajarkot district through SEM observation of pollen loads.

**Methodology**

The study was comprised of analysis of four pollen load samples from A. cerana honeybee. The pollen loads were obtained from four randomly selected locations from Jajarkot district in 2001 and taken to the palynology section of the Institute of Palaeontology, Vienna University, Austria in sealed vials during the same year for scanning electron microscope (SEM) observation. In order to collect the pollen, the samples were diluted with distilled water and centrifuged at 3000 rpm (rotation per minute) for 3 minutes. The samples were washed with glacial acetic acid two times and proceeded for acetolysis to remove the cellulose and cell content in the pollen grains. This included the treatment with a solution containing acetic anhydride (CH₃CO)₂O and concentrated sulphuric acid (H₂SO₄) in a ratio of 9:1 (Erdtman 1954). The samples were then kept in water bath for 5 minutes and washed with glacial acetic acid and water respectively. At the end, the samples were mounted in glycerin jelly and proceeded for light microscopic (LM) study.

After examining the pollen, under light microscope (LM) and taking LM photographs, the same pollen grains were brought to the edge of the glycerin using specially adapted needles with a human hair glued at the tip (Zetter 1989). With the help of needles the pollens were transferred to a SEM stub to which a drop of absolute ethanol (C₂H₅OH) had been applied with a pipette simultaneously. The SEM stub was kept under a binocular microscope at the required magnification. The pollen grains were then coated with gold in a BIORAD Sputter Coater for four minutes. The samples then followed by examination of the pollen with Jeol JSM 6400 SEM at 10 kV at different magnifications and orientation. SEM photographs were taken with the camera attached to the microscope using AGFA APX 100 (100 ASA) black and white film. This was followed by development of the film and photographs in the dark room.

Pollen grains were generally identified according to their physical appearance. The criteria of identification used according to the position and number of apertures, the shape and size of the pollen grains as a whole, fine structures (ornamentation) on the sexine, and the sculptured exine. There are mainly three types of apertures; (i) porate, having isodiametric pores and (ii) colpate, having pores that are long, boat shaped
with pointed ends. Sometimes the pore and colpus in a pollen grains combined together to form (iii) colporate apertures. If arranged equidistantly round the equator of the pollen grain; they are assigned the prefix; zono-. If scattered all over, the prefix panto-. The number of apertures were also indicated by prefixes; mono- for one aperture, di- for two apertures, tri- for three apertures, tetra for four and penta or poly- for numerous apertures. Morphological classes of pollen grains on the basis of aperture and symmetry could be viewed from equatorial as well as polar axis. Each pollen grain varied in their ultra-structures at the species level which helps for accurate taxonomical groupings (Erdtman 1952). The SEM study is very helpful to identify the pollen to lower taxonomical level as the details in the tectum can be measured in microns in high magnification (Ferguson et al. 2007).

Results

Palynological assemblages


Since, SEM investigation of pollen is poorly done in Nepal, in present study the pollen morphology was investigated using light microscope along with scanning electron microscopy for correct identification of pollen to its lower taxonomic level and to contribute melissopalinological studies originating from the native apiﬂora. Thus, a binomial was only given to the pollen when it could be conﬁrmed by examining well identiﬁed comparative material from herbarium specimens. In present study, a diverse spectrum of 19 pollen types belonging to 13 families was recovered from four pollen load samples, collected from the Jajarkot district, mid-western region, Nepal (Table 1). The pollen assemblages were belonging to angiosperms only. Palynomorphological identiﬁcation included the description of pollen symmetry, polarity, ornamentation, aperture, shape and size of the pollens to the generic and some up to species level belong to Alnus sp., Artemisia sp., Brassica sp., Cornus sp., Diploknema sp., Fraxinus sp., Ilex sp., Jasminum sp., Justicia sp., Ligustrum sp., Myrica esculenta, Salix sp., Strobilanthes sp., Urtica sp. etc. In the present study, only the generic name was used. Some of the pollen could not be identiﬁed beyond the family level. The LM and SEM photographs pollen taxa are shown in Figs. 2-5.

<table>
<thead>
<tr>
<th>Families</th>
<th>Name of the identified generas</th>
<th>Identified as gen. et spec. indet.</th>
<th>Total genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acanthaceae</td>
<td>Strobilanthes sp. (1), Strobilanthes sp. (2)</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Aquifoliaceae</td>
<td>Ilex sp.</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Betulaceae</td>
<td>Alnus sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Brassicaceae</td>
<td>Brassica sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Compositae</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cornaceae</td>
<td>Cornus sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lamiaceae</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Myricaceae</td>
<td>Myrica sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Oleaceae</td>
<td>Fraxinus sp., Jasminum sp.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Rosaceae</td>
<td></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Salicaceae</td>
<td>Salix sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Sapotaceae</td>
<td>Diploknema sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urticaceae</td>
<td>Urtica sp.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
<td>19</td>
</tr>
</tbody>
</table>

Table 1. Floral diversity of the pollen taxa in four pollen loads from Jajarkot district
Morphological description of individual palynomorphs

Angiosperms

Family Acanthaceae

*Strobilanthes* sp. (1)

Fig. 2 (1-3)

**Shape:** Prolate.

**Size:** Polar axis 80 µm, equatorial axis 36 µm.

**Aperture:** Tricolporate, colpi shallow and distinct.

**Exine:** 4 µm, sexine thicker than nexine, pseudocolpi uniformly present throughout the grain dividing the sexine into longitudinal strips, sexine bireticulate, each longitudinal sexine strip having an uneven coarse reticulum with a depressed reticulated tectum.

*Strobilanthes* sp. (2)

Fig. 2 (4-6)

**Shape:** Prolate.

**Size:** Polar axis 85 µm, equatorial axis 29 µm.

**Aperture:** Tricolporate.

**Exine:** 5 µm, sexine thicker than nexine, sexine divided into longitudinal stripes, sexine bireticulate, each longitudinal sexine strip having an uneven coarse reticulum with a depressed reticulated tectum.

Acanthaceae gen. et spec. indet. (1)

Fig. 2 (7-9)

**Shape:** Prolate.

**Size:** Polar axis 76 µm, equatorial axis 29 µm.

**Aperture:** Tricolporate.

**Exine:** 5 µm, sexine thicker than nexine, sexine divided into longitudinal stripes, sexine reticulated. The sizes of reticulation are different in stripes. Some stripes are without reticulum having randomly distributed granules on the surface.

Acanthaceae gen. et spec. indet. (2)

Fig. 2 (10-12)

**Shape:** Prolate.

**Size:** Polar axis 60 µm, equatorial axis 27 µm.

**Aperture:** Tricolporate.

**Exine:** 3 µm, sexine thicker than nexine, sexine divided into longitudinal but unequal stripes, sexine reticulated.

Family Aquifoliaceae

*Ilex* sp.

Fig. 3 (13-15)

**Shape:** Prolate, sub-circular in polar view.

**Size:** Polar axis 36 µm, equatorial axis 24 µm.

**Aperture:** Tricolporate.

**Exine:** 3 µm, atectate, clavate and pilate, pila with fine striate ornamentation.

Family Betulaceae

*Alnus* sp.

Fig. 3 (16-18)

**Shape:** Oblate, penta-angular in polar view.

**Size:** 17-20 µm

**Aperture:** Pentaporate, pori vestibulum type, neighbouring pori connected by archs or bands of nexinous thickening.

**Exine:** 1.5 µm, tectum consists of irregular rugulae with very small spinules (microechinate). Sexine slightly thicker than nexine.

Family Brassicaceae

*Brassica* sp.

Fig. 3 (19-21)

**Shape:** Prolate, circular in polar view.

**Size:** Polar axis 30 µm, equatorial axis 20 µm.

**Aperture:** Tricolpate.

**Exine:** 1.3 µm, tectum uniformaly reticulate, the colpi area is granulate.

Family Compositae

*Artemisia* sp.

Fig. 3 (22-24)

**Shape:** Prolate, circular in polar view, lobate.

**Size:** Equatorial axis 20 µm.

**Aperture:** Tricolporate, colpi nearly as long as polar axis.

**Exine:** 3 µm, sexine much thicker than nexine, distinctly stratified, sexine in mesocolpium thicker than in the colpi area forming a margo, sexine
microechinate and granulate, granules uniformly
distributed between the spinules.

**Compositae gen. et spec. indet. (1)**
Fig. 4 (25-27)
*Shape:* Sub-prolate to prolate
*Size:* 28 µm
*Aperture:* Tricolporate.
*Exine:* 6-7 µm (with spines), sexine thick, perforate
and echinate, the basal part of spine broadly
perforate with perforations of various shape and
sizes, surface granulate, spines 2-3 µm in length.

**Compositae gen. et spec. indet. (2)**
Fig. 4 (28-30)
*Shape:* Prolate to spheroidal, lobate.
*Size:* 25 µm.
*Aperture:* Tricolporate.
*Exine:* 1 µm, sexine as thick as nexine, spiny and
granulate, tectum and spines with only very few
perforations, surface granulate.

**Family Cornaceae**
*Cornus* sp.
Fig. 4 (31-33)
*Shape:* Prolate.
*Size:* Polar axis 20 µm.
*Aperture:* Tricolporate.
*Exine:* 1.5-2 µm, tectate, tectum micro-echinate, colpi
nearly as long as polar axis.

**Family Labiatae**
*Labiatae* gen. indet.
Fig. 4 (34-36)
*Shape:* Oblate, semicircular in polar view.
*Size:* Equatorial axis 34-40 µm.
*Aperture:* Hexacolporate, colpi long and 6-7 µm wide.
*Exine:* 1.5 µm, sexine slightly thicker than nexine,
sexine supratectum, muri less than 1 µm, lumina
large up to 2 µm.

**Family Myricaceae**
*Myrica* sp.
Fig. 5 (37-39)
*Shape:* Oblate, triangular in polar view.
*Size:* 20 µm.
*Aperture:* Triporate.

**Family Oleaceae**
*Fraxinus* sp.
Fig. 5 (40-42)
*Shape:* Prolate, circular in polar view.
*Size:* Polar axis 18 µm, equatorial axis 15 µm.
*Aperture:* Tricolporate, colpi are long with small
circular endoapertures.
*Exine:* 1 µm, sexine thicker than nexine, tectum
reticulate, lumina are heterobrochate, triangular to
polygonal in shape.

**Family Rosaceae**
*Jasminum* sp.
Fig. 5 (43-45)
*Shape:* Prolate, circular in polar view.
*Size:* Equatorial axis 39 µm.
*Aperture:* Tricolporate, colpi long, small circular
endoapertures.
*Exine:* 1 µm, sexine thicker than nexine, tectum
reticulate, lumina are heterobrochate, triangular to
polygonal in shape

**Family Rosaceae**
*Rosaceae* gen. indet. (1)
Fig. 5 (46-48)
*Shape:* Prolate, circular in polar view.
*Size:* Polar axis 37 µm, equatorial axis 27 µm.
*Aperture:* Tricolporate, colpi long.
*Exine:* 1-1.5 µm, sexine is thicker than nexine, striae,
striations sometimes joined with each other and
variable in size, striations granulated.

*Rosaceae* gen. indet. (2)
Fig. 6 (49-51)
*Shape:* Prolate, circular in polar view.
*Size:* Polar axis 36 µm, equatorial axis 30 µm.
*Aperture:* Tricolporate, colpi long.
*Exine:* 1-1.5 µm, sexine is thicker than nexine, striae,
striations sometimes joined with each other and
variable in size.

**Family Salicaceae**
*Salix* sp.
Fig. 6 (52-54)
*Shape:* Prolate, circular in polar view.
*Size:* Equatorial axis 11 µm, polar axis 23 µm
*Aperture:* Tricolporate, colpi long broad reaching
nearly to poles.

**Exine:** 1 µm, sexine is thicker than nexine near and
around pori, sexine regularly microechinate.
Exine: 2 µm, reticulated, lumina broad in mesocolpium, smaller in apertural area.

**Family Sapotaceae**

*Diploknema* sp.

Fig. 6 (55-57)

**Shape:** Prolate.

**Size:** Polar axis 49 µm, equatorial axis 33 µm.

**Aperture:** Tetracolporate, colpi long and gradually broaden towards the poles, end of colpi rounded.

**Exine:** 1.5 µm, sexine as thick as nexine, sexine micro-rugulate, perforated and covered by irregularly distributed nano-granules.

**Family Urticaceae**

*Urtica* sp.

Fig. 6 (58-60)

**Shape:** Oblate.

**Size:** Equatorial axis 15-20 µm.

**Aperture:** Tricolporate, colpi short and barrow.

**Exine:** 1.5 µm, sexine as thick as nexine, sexine micro-echinate, perforated and covered by irregularly distributed nano-granules. Microechinae fused to form clusters

**Discussion**

The honey from different regions has a specific pollen spectrum depending on the floristic composition of the region. As a result of the pollen analysis of four pollen load samples from the Jajarkot district, the midwestern region of Nepal, the important apiflora have been identified and the plant species used by *A. cerana* as nectar source assessed. They are: *Strobilanthes* sp. (Acanthaceae), *Ilex* sp. (Aquifoliaceae), *Alnus* sp. (Betulaceae), *Brassica* sp. (Brassicaceae), *Artemisia* sp. (Compositae), *Cornus* sp. (Cornaceae), *Myrica* sp. (Myricaceae), *Fraxinus* sp. and *Jasminum* sp. (Oleaceae), *Salix* sp. (Salicaceae), *Diploknema* sp. (Sapotaceae), *Urtica* sp. (Urticaceae) including Labiatae and Rosaceae.

Joshi (1999) identified total 16 pollen types in honey samples from *A. cerana* in the Jajarkot district using LM. Whereas, the present study of pollen loads of the same honeybee in Jajarkot district using LM as well as SEM revealed 19 species of pollen flora belonging to 13 families. The pollen composition was mostly from the plants growing in subtropical climate zones. Most of the pollen in the palynological assemblages are derived from entomophilous plants, however, the honey samples also contained a significant number of airborne (anemophilous) pollens from some plant such as *Alnus, Salix* and *Myrica*.

Beekeeping is based on vegetation and generates multi-pronged employment through small and rural scale enterprises. It has the potential of preventing the outflow of rural folk from villages towards cities. Jajarkot is one of the districts in Nepal that has a rich potential of indigenous beekeeping. Therefore, this study not only reflects the palynological composition, botanical origins of the pollen loads of the particular area, but also provides knowledge of floral assemblage of the area where the *A. cerana* honey bees are foddering, and which may be useful for the local beekeepers in bee management and in promoting beekeeping development.

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Family Acanthaceae (1-12)
1. _Strobilanthes_ sp. (1), equatorial view, LM x 850
2. _Strobilanthes_ sp. (1), equatorial view, SEM x 1,000
3. _Strobolanthes_ sp. (1), details of the tectum x 10,000
4. _Strobilanthes_ sp. (2), equatorial view, LM x 850
5. _Strobilanthes_ sp. (2), equatorial view, SEM x 900
6. _Strobolanthes_ sp. (2), details of the tectum x 8,500
7. Acanthaceae gen. et spec. indet. (1), equatorial view, LM x 850
8. Acanthaceae gen. et spec. indet. (1), equatorial view, SEM x 1,000
9. Acanthaceae gen. et spec. indet. (1), details of the tectum x 8,500
10. Acanthaceae gen. et spec. indet. (2), equatorial view, LM x 850
11. Acanthaceae gen. et spec. indet. (2), equatorial view, SEM x 1,600
12. Acanthaceae gen. et spec. indet. (2), details of the tectum x 15,000

Family Aquifoliaceae (13-15), Betulaceae (16-18), Brassicaceae (19-21), Compositae (22-24)
13. _Ilex_ sp., equatorial view, LM x 850
14. _Ilex_ sp., equatorial view, SEM x 2,000
15. _Ilex_ sp., details of the tectum x 8,500
16. _Alnus_ sp., polar view, LM x 850
17. _Alnus_ sp., polar view, SEM x 2,500
18. _Alnus_ sp., details of the tectum x 8,500
19. _Brassica_ sp., polar view, LM x 850
20. _Brassica_ sp., Equatorial view, SEM x 2,500
21. _Brassica_ sp., details of the tectum, SEM x 13,500
22. _Artemisia_ sp., polar view, LM x 850
23. _Artemisia_ sp., polar view, SEM x 2,700
24. _Artemisia_ sp., details of the tectum SEM x 9,000
Family Compositae (25-30), Cornaceae (31-33), Lamiaceae (34-36)
25. Compositae gen. et spec. indet., polar view, LM x 850
26. Compositae gen. et spec. indet., polar view, SEM x 2,300
27. Compositae gen. et spec. indet., details of the tectum, SEM x 10,000
28. Compositae gen. et spec. indet., polar view, LM x 850
29. Compositae gen. et spec. indet., polar view, SEM x 2,200
30. Compositae gen. et spec. indet., details of the tectum, SEM x 6,000
31. Cornus sp., polar view, LM x 850
32. Cornus sp., oblique view, SEM x 3,000
33. Cornus sp., details of the tectum, SEM x 10,000
34. Lamiaceae gen. et spec. indet., polar view, LM x 850
35. Lamiaceae gen. et spec. indet., polar view, SEM x 1,500
36. Lamiaceae gen. et spec. indet., details of the tectum, SEM x 13,000

Family Myricaceae (37-39), Oleaceae (40-45), Rosaceae (46-48)
37. Myrica sp., polar view, LM x 850
38. Myrica sp., polar view, SEM x 2,700
39. Myrica sp., details of the tectum, SEM x 11,000
40. Fraxinus sp., polar view, LM x 850
41. Fraxinus sp., equatorial view, SEM x 4,000
42. Fraxinus sp., details of the tectum, SEM x 15,000
43. Jasminum sp., polar view, LM x 850
44. Jasminum sp., Polar view, SEM x 1,300
45. Jasminum sp., details of the tectum, SEM x 10,000
46. Rosaceae gen. et spec. indet., polar view, LM x 850
47. Rosaceae gen. et spec. indet., equatorial view, SEM x 1,000
48. Rosaceae gen. et spec. indet., SEM x 10,000
Family Rosaceae (49-51), Salicaceae (52-54), Sapotaceae (55-57), Urticaceae (58-60)

49. Rosaceae gen. et spec. indet., polar view, LM x 850
50. Rosaceae gen. et spec. indet., equatorial view, SEM x 1,500
51. Rosaceae gen. et spec. indet., SEM x 10,000
52. Salix sp., equatorial view, LM x 850
53. Salix sp., equatorial view, SEM x 3,500
54. Salix sp., details of the tectum, SEM x 11,000
55. Diploknema sp., equatorial view, LM x 850
56. Diploknema sp., equatorial view, SEM x 1,300
57. Diploknema sp., details of the tectum, SEM x 8,000
58. Urtica sp., polar view, LM x 850
59. Urtica sp., polar view, SEM x 3,500
60. Urtica sp., details of the tectum, SEM x 14,000

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