Scanning Electron Microscopic Study on Pollens of 8 Bee Floral Resources from Kangra Hills, Himachal Pradesh, India

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Abstract — *Scanning electron microscopic study on pollens of eight important bee floral resources collected from Kangra hills of Himachal Pradesh were carried. Pollens grains were investigated for size, shape, types of pores, exine sculptures and aggregation. Size of studied pollens varies from 14 µm X 12.8 µm in Eucalyptus sp. to 48.5 µm X 40 µm in Grevillea robusta. Shapes present were round, oval, triangular and types of aperture were pore in Taraxacum officinalis and Grevillea robusta whereas others were colpa and colporate. Six different types of exine sculpture were present i.e. fenestrate in Taraxacum officinalis; verrucate in Largerstroemia indica; Reticulate in Plectranthus rugosus and Grewia optiva; Faveolate in Melia azaderach; Scabrate in Eucalyptus sp. and Grevillea robusta; Striate in Litchi chinensis. Aggregation present were of single types in all pollen grains. This Scanning Electron Microscopic studies helpful in accurate identification of pollen types present in honey samples, construction of reference pollen data of particular place and therefore useful in palynology and melissopalynology.*

Keywords — *Scanning electron microscopy, Pollens grain, Palynology, melissopalynology.*

I. INTRODUCTION

Study of pollen and spores are known as palynology. It is an important branch of science having multiple applications in different fields i.e. in taxonomy to characterize different plant species in same or different hierarchical classification group; in aeropalynology which is dissemination of pollen in air and their impact on human health; in study of climate change; in forensic sciences.to find out the connection between people or objects to crime scene and in melissopaynology (i.e. study of pollen present in honey samples [1] [2] [3]) where identification of pollen present in the honey gives the information about floral sources visited by the honey bees and therefore helpful in qualitative as well as quantitative analysis of honey samples [4].

Quantitative analysis in melissopalynology is used for characterize the honey samples as unifloral or multifloral depending upon the predominance of a particular pollen type of single floral sources or combination of pollens from various floral sources. Hence different honey sample contains the specific pollen spectra which reflects the vegetation type of particular place of honey collection and gives the geographical origin of honey [5]. It is also used to test the purity of honey and to validate the claim for a unifloral honey source [6] [7]. The element content present in honey also gives the information about the abundance of minerals within specific bee foraging area [8] [9]. Thus melissopalynology provides the reliable and valuable information regarding floral resources of honey samples. This information is useful to beekeepers, entrepreneur of honey industries, scientist, researchers and general public. It is also helpful in conservation of ecosystem and maintaining biodiversity.

In melissopalynology light microscopy is used generally to identifying the pollen spectrum of honey samples and reference pollen slides [10] [11]. The use of SEM for identifying the pollen grain was not common earlier. However it is used for morphological comparison of pollen and in taxonomy, it is the resolution of the SEM that gives the clear-cut texture view of the pollen and differentiate it in different taxa. Therefore SEM studies useful in palynology along with melissopalynology. There is the less information available on the studies of pollen by SEM. Van Laere et. al., 1969 advocated the use of SEM in analysing the honey samples; Chen and Shen 1990 studied the Formosan honey with SEM, Paudyal and Gautam, 2012 used it to study pollen taxa in honey from Autochtone region [9] [12] [13]. But very little work has done on studies of pollen from bee floral resources. Hence present studies give us an insight of Scanning Electron Microscopic studies of pollen of eight bee floral resources from Kangra hills of Himachal Pradesh.

II. MATERIALS AND METHODS

For the scanning electron microscopic studies, the pollen grains/anthers of identified important bee floral resources (Table-1) were collected in glass vials and preserved at sub-zero temperature. Most of the specimens used during investigations were obtained from plants growing in natural conditions. In all cases, pollen samples were obtained only from the most mature specimens available. While doing the scanning, the anthers were placed on the slides and teased thoroughly in alcohol. The pollen grains were then air dried and mounted on metal stub with the help of double stick scotch tape. Uniform dusting of Gold metal was done with the help of fine coated ion Sputter J.F.C-1100. The pollen grains were scanned at accelerating voltage of 15 to 20 KV in a Scanning Electron Microscope, "JSM 6100" at Regional Sophisticated Instrumentation Centre, Panjab University, Chandigarh.

Pollen grains were generally recognized according to their physical appearance i.e. as per number and position of aperatures, the shape and size of the pollen grain, structure ornamentation on the sexine and the sculptured exine. Thus, palynomorphological investigation includes pollen size, shape, symmetry, polarity, aperture number, aperture type and exine ornamentation. The descriptive terminology is followed as by [14] [15].

RESULTS AND DISCUSSION

Table I contained the macromorphological results obtained for the different morphological parameter of each bee floral resources.

In present investigation pollens of 8 different bee floral resources belonging to Division Angiosperm were studied. Size of the pollens investigated varied from 14µm X 12.8 µm in *Eucalyptus* sp. (Figure 5) to 48.5 µm X 40 µm in *Grevillea robusta* (Figure 6). Shapes of pollens varied from round in *Taraxacum officinalis* (Figure 1), *Largerstroemia indica* (Figure 3), *Plectranthus rugosus* (Figure 2), *Melia azaderach* (Figure 4); triangular in *Eucalyptus* sp., *Grevillea robusta*, *Litch chinensis* (Figure 7); oval in *Grewia optiva* (Figure 8). The spherical and triangular shape present in pollen types are similar to those found in the pollens of family Rhamnaceae, Moringacea, and Caesalpiniaceae [16]. Oval shape of the pollen in *Grewia optiva* corresponds to the pollen of the genus *Sonchus asper*, an important bee floral resource [17]. There were three type of apertures present in various sporomorphs: pore in *Taraxacum officinalis* (Figure 1) and *Grevillea robusta*; Colpate (when only furrow present) in *Plectranthus rugosus*; colporate (when both colpa and furrow present) in *Largerstroemia indica, Melia azaderach, Eucalyptus* sp., *Litch chinensis* and *Grewia optiva*. The coplpate pollen found in the present studies is also the characterstics feature of the pollens belongs to the family Mangoliaceae, Nymphaeceae [18]. Similarily colporate pollens types are also found in the Anacardiaceae, Apocynaceae, Bignoniaceae [16].

Exine sculpturing of six different types were found: fenestrate (when window like hole present on exine) in *Taraxacum officinalis*; verrucate (when warts present were of more than 1 µm) in *Largerstroemia indica*; Reticulate (when net like exine present) in *Plectranthus rugosus, Grewia optiva* ; faveolate (when little pits present) in *Melia azaderach*; scabrate (when little warts present) in *Eucalyptus* sp, *Grevillea robusta*; striate (when striation present) in were found in *Litchi chinensis*. Reticulae exine sculptural ornamentation present in *Plectranthus rugosus* is also reported in pollen of *Caesalpinia pulcherrima* and Bignoniaceae and Moringaceae by [16][19] and Scabrate in pollens of different species belong to genus *Piper* from Thailand [20] Besides this, aggregation present was found single as in *Taraxacum officinalis, Largerstroemia indica, Plectranthus rugosus, Grewia optiva* etc. Thus, this studies provide us a better way to understand pollen morphology and such palynomorphological investigations helps in correct identification of pollens up to its lower taxonomic level and also contributes to melissopalynology originating from native apiflora [21] [22].

Table I

POLLEN MORPHOLOGICAL DETAILS OF DIFFERENT SCANNED ELECTRON MICROGRAPH OF SOME IMPORTANT BEE FLORAL RESOURCES OF KANGRA HILLS.

NAME OF THE PLANT	COMMON NAME	FAMILY	HONEY POTENTIALITY	DISTRIBUTION	POLLEN MORPHOLOGY
Taraxacum officinalis Weber	Dandelion	Asteraceae	N^1P^1	Throughout	30µm x 29.5µm; Round; porate, fenestrate; single.
Largerstroemia indica Linn.	Pride of India	Lytheraceae	N^2P^2	Throughout	24.5µm x 22.3µm; Round; colporate, tricolporate; Verrucate; single.
Plectranthus rugosus	Shain	Lamiaceae	N^1P^2	Midhills and High	23.6µm x 21.2µm; round(PV);

(Wall.ex Benth)				hills	Colpa, Hexacolpate; Reticulate; single.
Melia azaderach L..	Chinaberry	Meliaceae	P^2	Valley & low hills	26.2µm x 23.8µm; Round(EV); colporate, Tetracolporate; Faveolate; single.
Eucalyptus sp..	Eucalyptus	Myrtaceae	N^1P^1	Valley & low hills	14µm x 12.8µm; Triangular(PV); colporate, trisyncolporate; Scabrate; single.
Grevillea robusta A. Cunn. ex R. Br.	Silky oak	Proteaceae	N^1P^1	Valley, low and mid hills.	48.5µm x 40µm; Triangular(PV); Triporate; Scabrate; single.
Litchi chinensis Sonner.	Litchi	Sapindaceae	N^1P^1	Valley, low and mid hills	16.7µm x 14.6µm; triangular; Colporate, tricolporate; Striate; single.
Grewia Optiva Dumm ex. Burret.	Beolor Bhemal	Tiliaceae	N^1P^1	Throughout	39.1µm x 32µm; Oval; Colporate, Reticulate; single.

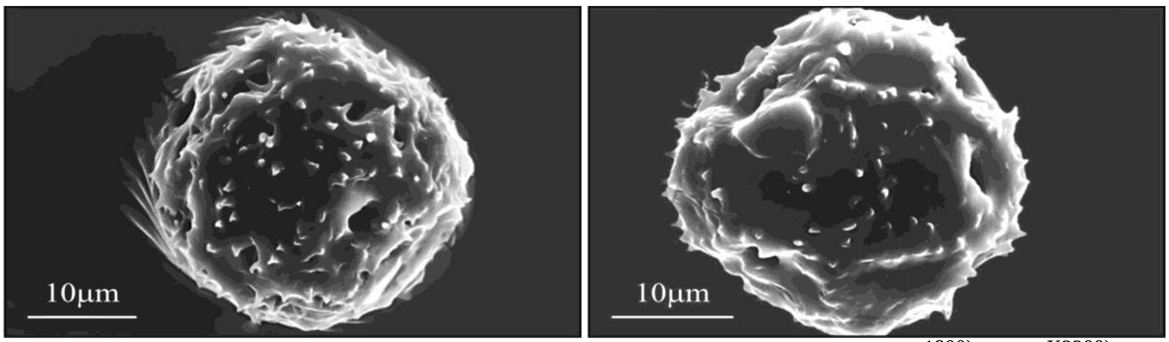

Figure 1 *Taraxacum officinalis* Weber Nees PV ($X^{1800)}$ PV ($^{X2200)}$

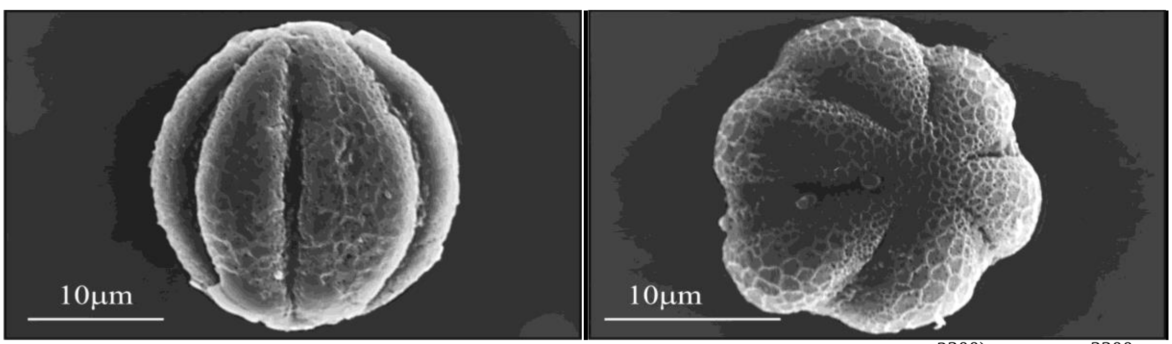

Figure 2 *Plectranthus rugosus* (Wall.ex Benth). EV ($X^{2300)}$ PV (X^{3300})

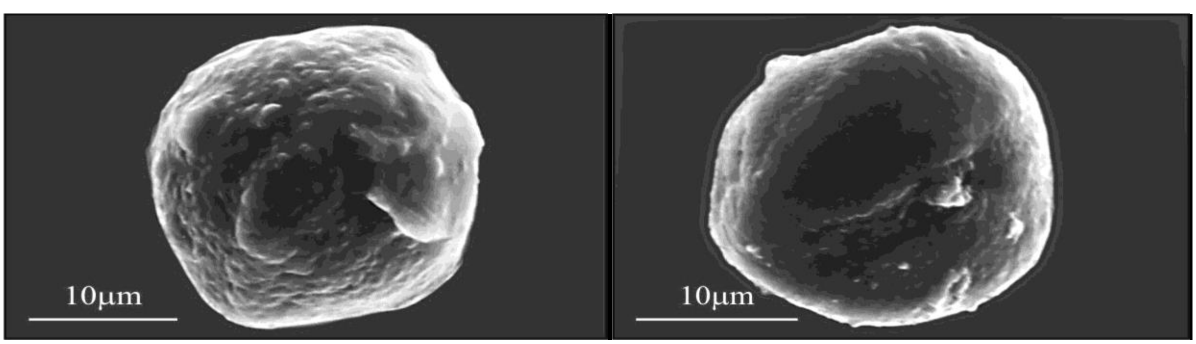

Figure 3 *Largerstroemia indica* Linn. EV ($X^{2000)}$ EV (X^{2700})

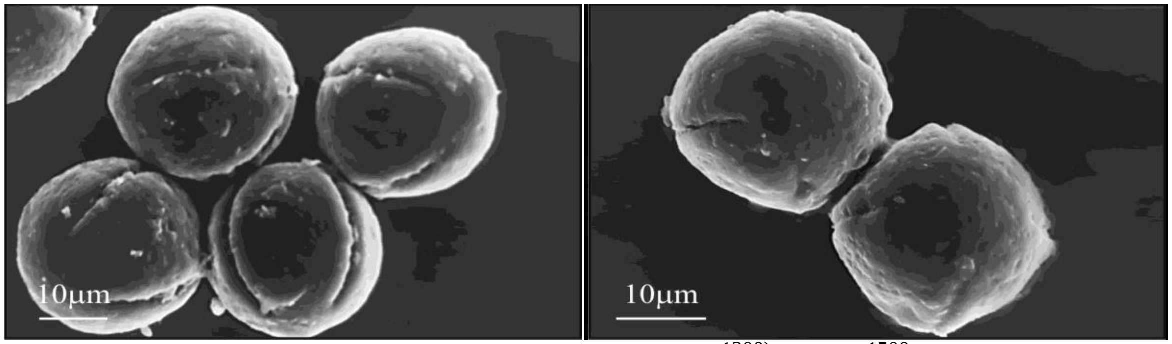

Figure 4 *Melia azaderach* L. EV (X^1300) PV (X^1500)

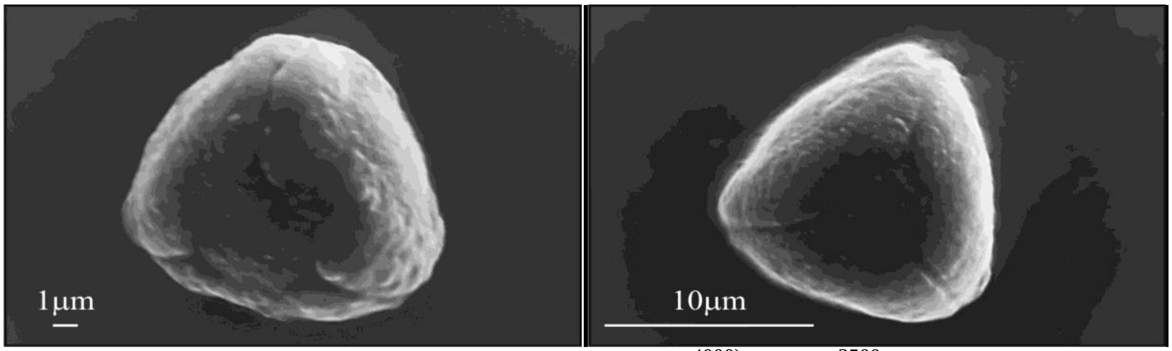

Figure 5 *Eucalyptus* sp. PV (X^4000) PV (X^3500)

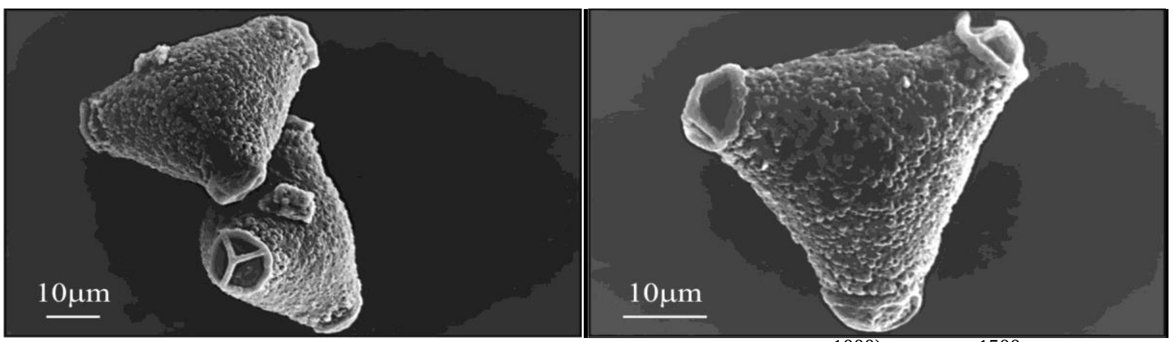

Figure 6 *Grevillea robusta* A. Cunn. ex R. Br. PV (X^1000) PV (X^1500)

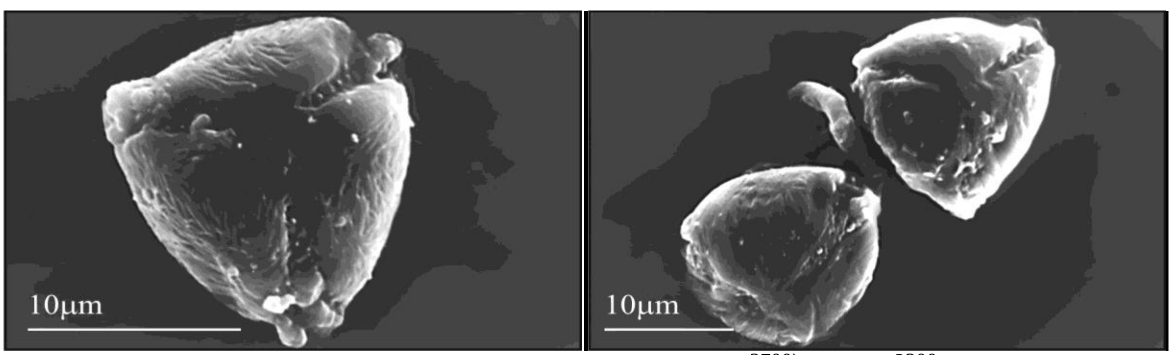

Figure 7 *Litchi chinensis* Sonner. PV (X^3700) PV (X^2300)

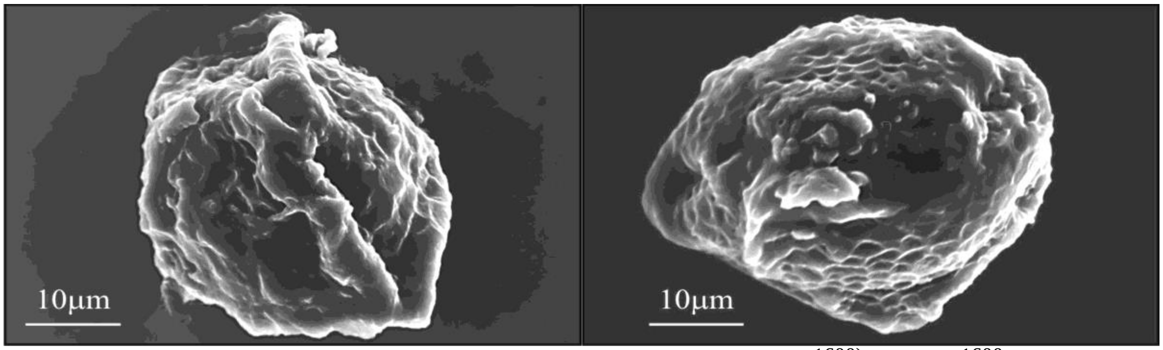

Figure 8 *Grewia Optiva* Dumm ex. Burret. EV (X^{1600}) EV (X^{1600})

III. CONCLUSION

This palynomorphological investigation helps in correct identification of pollens which cannot be very accurate with Light microscopy and thus helpful in melissopalynology [23]. Thus SEM studies provides well defined and accurate identified pollen records of reference pollen which serve the purpose of identification in melissopalynology as well as it is beneficial to beekeepers for scientific management of honeybees and also in conservation of bee floral resources.

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