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SCANNING ELECTRON MICROSCOPIC STUDY ON POLLENS OF EIGHT BEE FLORAL RESOURCES FROM HAMIRPUR HILLS, HIMACHAL PRADESH, INDIA

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KEYWORDS

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Pollens grain

Palynology

Melissopalynology

ABSTRACT

Scanning electron microscopy study on pollens of eight important bee floral resources collected from Hamirpur 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 14um X 11um in *Woodfordia fruticosa* to 113umX 108um in *Cucurbita maxima*, shapes present were round, oval, elongated, triangular and types of apeture were pore in *Adhatoda vasica* and *Cucurbita maxima* whereas others were colporate. Five different types of exine sculpture *viz*. faveolate in *Woodfordia fruticosa*; scabrate in *Cassia fistula* and *Psidium gaujava*; reticulate in *Adhatoda vasica* and *Caesalpinia decalpetala*; echinate in *Ageratum conyzoides* and *Cucurbita maxima;* striate in *Prunus cerasoides* were reported during the study. Aggregation was reported as single type in all pollen grains. This Scanning Electron Microscopic studies helpful in construction of reference pollen data of particular place and can useful in palynology and melissopalynology.

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1 Introduction

Palynology is an interesting branch of science with vast application as it includes analysis of spores and pollens which contains lots of information along with them and are present in: fossils, around us in the air and in the honey samples etc. Bee floral resources provide pollen materials to honeybees, as they visit them to collect nectar/honeydew, and stored in honeycomb where with some specific substances added by honeybees, the nectar/honeydew transformed into honey along with pollens (White & Landis, 1980). While foraging on the plant for nectar and pollen, honeybees incidentally also reciprocate pollination services on this planet, which is significant part of evolutionary process on the earth (Deodikar, 1962; Martin, 1992). Palynological studies of some bee floral resources through scanning electron microscopy provide us an insight for taxonomic problems, morphological studies and in melissopalynology.

Among the honey producing countries India ranked seventh and having largest average beehives in the world (Michener, 2007; Van Engelsdorp et al., 2009; Balachandra et al., 2014) and there is a significant scope of growth in this field, so the SEM studies of honey pollen resources will also benefits melissopalynology. Light microscopy (LM) which is traditionally used in palynology/ melissopalynology to identify and interpret the pollen spectrum has its limitations however the SEM studies is useful in study of pollen morphology as well as in melissopalynology (Van Laere et al., 1969; Sivaguru et al., 2012; Pramanick et al., 2015; Nikolic & Milatovic, 2017)

There were numerous reports available on palynology by LM. However, SEM studies were carried out by Ferguson (1981), Dustmann & Ohe (1993), Perveen et al. (1994), Aftab & Perveen (2006) and Paudyal & Gautam (2012) from different parts of the world. But still very less work has carried on SEM studies of pollen of bee floral resources. Therefore, present study gives us an account of Scanning Electron Microscopy of Pollens of eight important bee floral resources from Hamirpur hills of Himachal Pradesh.

2 Materials and Methods

For the scanning electron microscopic studies, the pollen grains/anthers of identified important bee floral resources (Table 1) were collected (during 2013) 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 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

Table 1 Pollen morphological details of different Scanned electron micrograph of some important honey plant resources of Hamirpur hills.

Name of the Plant	Common Name	Family	Honey Potentiality	Distribution	Pollen morphology
Adhatoda vasica Nees	Basuti	Acanthaceae	N^2P^2	Throughout	41.2µm x 26.0µm; elongated; porate, two pores; fine reticulate; single.
Ageratum conyzoides L.	Goat weed	Asteraceae	N^3P^3	Throughout	15.2µm x 14.8µm; triangular/ round; colporate, tricolporate; echinate; single.
Cucurbita maxima L.	Great pumpkin	Cucurbitaceae	N^1P^1	Throughout	113µm x 108µm; round; porate; echinate; single.
Cassia fistula L.	Indian laburnum	Fabaceae	N^3P^3	Valley & low hills	28.5µm x 20.3µm; elongated; colporate, tricoporate; scabrate; single.
Caesalpinia decalpetala (Roth.) Alston.	Kingari	Fabaceae	N^2	Valley & low hills	40µm x 30.3µm; oval; colporate, tricolporate; reticulate; single.
Woodfordia fruticosa (L) kurz.	Dhawi	Lytheraceae	N^1P^1	Throughout	14µm x 11µm; round/oval; colporate; faveolate; single.
Psidium guajava L.	Gauva	Myrtaceae	N^1P^1	Valley and low hills	14µm x 12.4µm; triangular; tricolporate; scabrate; single.
Prunus cerasoides D. Don.	Wild Cherry	Rosaceae	N^1P^1	Mid & High hills	30.5µm x 28.7µm; triangular; colporate, tricolporate; striate; single.

dusting of Gold metal was done with the help of fine coated ion Sputter J.F.C-1100 (Donmez, 1999). 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.

Physical appearance of Pollen grains analyzed through SEM includes: type of apertures present along with its number, shape and size of pollens, type of exine and structural ornamentation of sexine. Thus size of pollen, shape, symmetry, polarity, type and number of aperture and ornamentation of exine were investigated from this palynomorphological study. The descriptive terminology is followed as by Sawyer (1981) and Vorwohl (1990).

3 Results

In present scanning electron microscopic investigation pollens of eight different bee floral resources belonging to Division Angiosperm were studied. Size of the pollens investigated varied from 14µm x 11µm in *Woodfordia fruticosa* (Figure 6) to 113µm x 108µm in *Cucurbita maxima* (Figure 3). Shapes of pollens varied from round in *C. maxima*, elongated in *Adhatoda vasica* (Figure 1), *Cassia fistula* (Figure 4)*;* triangular in *Ageratum conyzoides* (Figure 2), *Psidium guajava* (Figure 7), *Prunus cerasoides* (Figure 8)*;* oval in *W. fruticosa*, *Ceasalpinia decalpetala* (Figure 5). There were two type of apertures

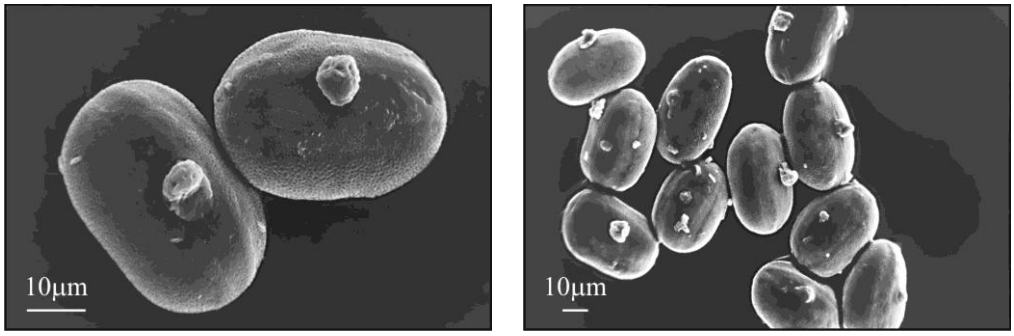

Figure 1 *Adhatoda vasica* Nees EV (X^{1100}) EV (X^{550})

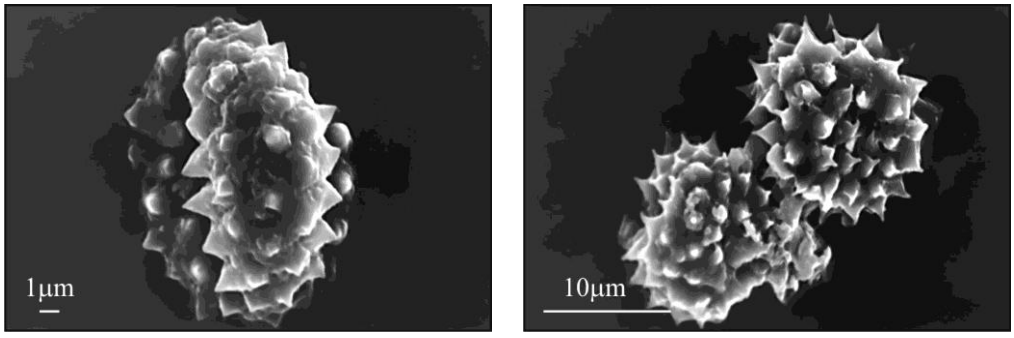

Figure 2 *Ageratum conyzoides* L. EV (X^{4000}) EV (X^{2500})

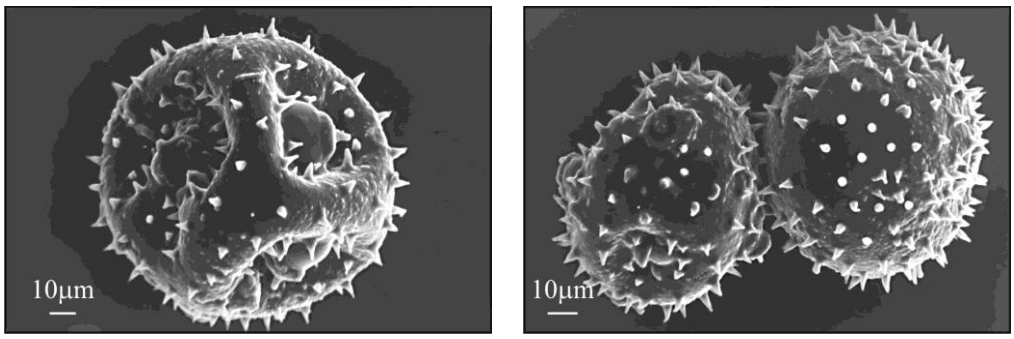

Figure 3 *Cucurbita maxima* L. PV (X^{550}) PV (X^{450})

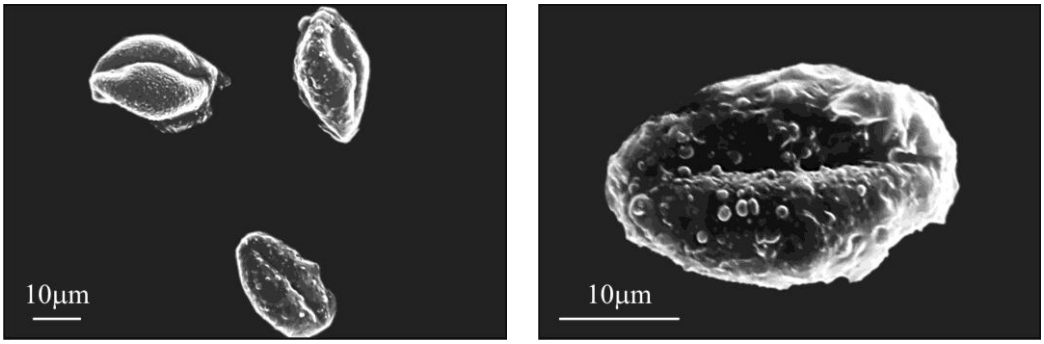

Figure 4 *Cassia fistula* L. EV (X^{900}) EV (X^{2300})

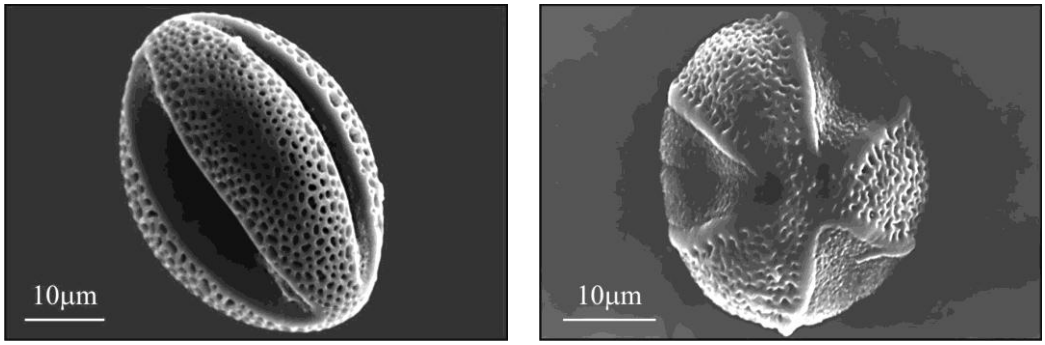

Figure 5 *Caesalpinia decalpetala* (Roth.) Alston. EV (X^{1500}) PV (X^{2000})

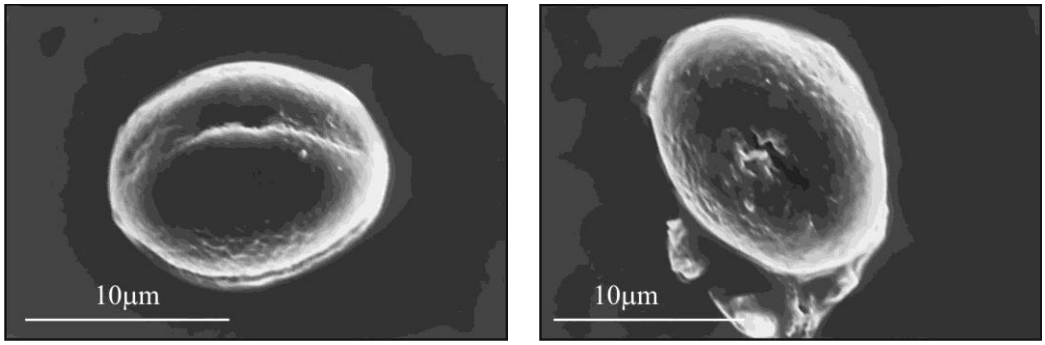

Figure 6 *Woodfordia fruticosa* (L) kurz. EV (X^{1400}) EV (X^{3700})

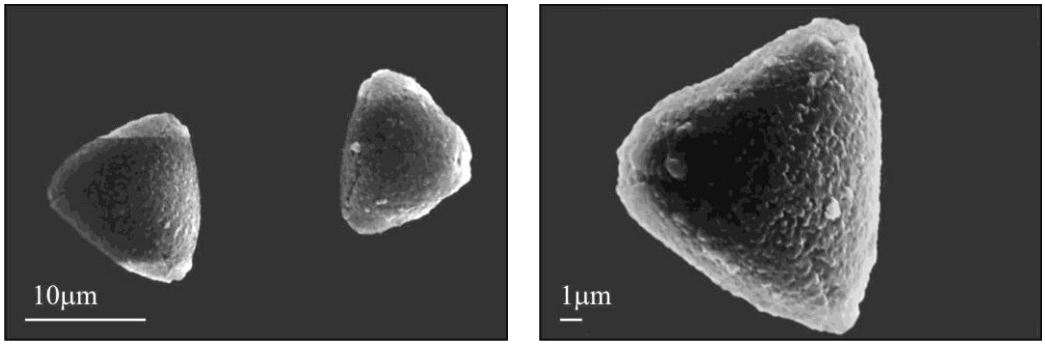

Figure 7 *Psidium guajava* L. PV (X^{2000}) PV (X^{4000})

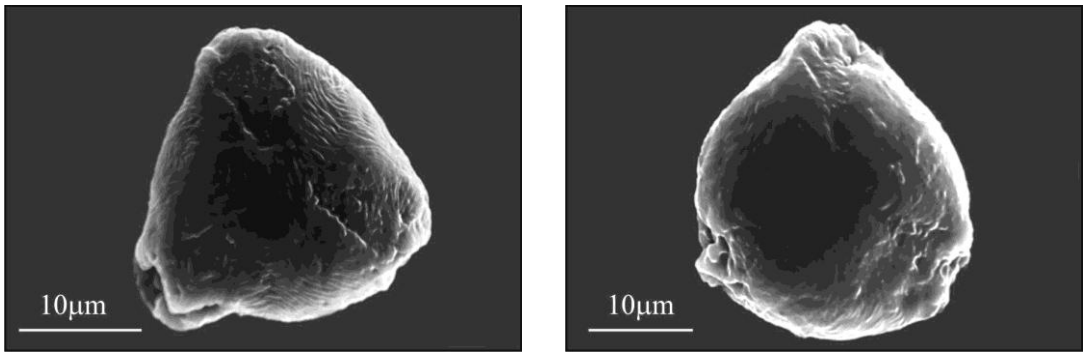

Figure 8 *Prunus cerasoides* D. Don. PV (X^{2000}) PV (X^{2000})

present in various sporomorphs: pore in *A. vasica*, *C. maxima*; colporate (when both colpa and furrow present) in *A. conyzoides*, *C. fistula*, *C. decalpetala*, *W. fruticosa*, *P. cerasoides*, *P. guajava*.

Exine sculpturing of five different types were found i.e faveolate (when little pits present) in *W. fruticosa*; scabrate (when little warts present) in *C. fistula*, *P. guajava*; striate (when striation present) in *P. cerasoides* Reticulate (when net like exine present) in *A. vasica*, *C. decalpetala*. Echinate (when spines present) were found in *A. conyzoides*, *C. maxima*. Besides this, aggregation present was found single as in *C. maxima*, *W. fruticosa*, *A. vasica* etc.. Thus, the studies of scanned electron micrographs of different pollen provides us a better way to understand pollen morphology and such palynomorphological investigations help in correct identification of pollens up to its lower taxonomic level. These studies also contribute to melissopalynology as pollens are associated with honey and they are essential food element for honeybees as it is their main protein source (Michner, 2007). Moreover pollen grains structural feature also help us to indicate its origin. (Paudayal & Gautam, 2011a; Paudayal & Gautam, 2011 b).

4 Discussion

Generally, Light microscopy (LM) is useful for identifying and interpreting the reference pollen slides prepared from identified bee floral resources and pollen spectrum of a particular honey sample so by using LM, shape and exine can be determined but ectexine structural pattern were not satisfactory due to its limited depth of focus. However, scanning electron microscopic (SEM) can facilitate to measure the tectum of pollens upto microns under high magnification. Thus it is helpful in recognizing the pollens to their species level (Ferguson et al., 2007). SEM studies also help us to identify the ultra structures of pollen surface, which makes it possible to find specific reorganization marks in different pollens. So, several different genera and species can be identified which could only be possible up to family level by light microscopy. Hence, this technique was also used by various researchers for investigating pollen grains isolated from honey samples (Van Laere et al., 1969; Chen & Shen, 1990; Mattu & Mattu, 2000; Paudayal & Gautam, 2012) and thus it is useful in melissopalynology.

The study of pollen structural pattern through Erdtmann's (1960) acetolysis method by using honey sample leads to damage and loss of pollen grains specially those which were fewer in number, as pollen of Euphorbiceae were under represented in its honey samples (Dustmann & Ohe, 1993). So present method of SEM studies of different pollen belongs to different plants is quite useful.

Recognition of plants pollen up to lowest level of classification through SEM can be helpful for development of well defined pollen bank/ pollen data bank for specified areas, which will serve as reference/verification source (Paudayal & Gautam, 2012) and present studies added few more steps to understand melissopalynology/ palynology. Taxonomical problems can be solved up to certain extent by such studies. These palynomorphological investigations were also applicable in genetic studies and helpful in tracing evolutionary history of certain vegetation, which provide an insight about ancient change in climate (Aftab & Perveen 2006). Besides this, it is also helpful in horticulture, Agriculture, forestry etc.

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