



SCANNING ELECTRON MICROSCOPY STUDIES ON PLECTRANTHUS- AN IMPORTANT BEE FORAGE PLANT OF HIMACHAL PRADESH, INDIA

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(Date of Receiving-09-01-2021; Date of Acceptance-30-03-2021)

ABSTRACT

The present investigations were conducted on two important nectar and pollen yielding plants viz. *Plecranthus coesta* and *Plecranthus rugosus* from different agro-climatic zones of Himachal Pradesh. The bee forage plants were collected, identified, classified and differentiating characters of two species were noted. Apart from this, pollen grains of *P. coesta* and *P. rugosus* were also studied using light and scanning electron microscope. The pollen grains were observed in terms of aggregation, shape, shape class, size, aperture, polarity, symmetry, surface pattern/exine complexity. Both *P. coesta* and *P. rugosus* had solitary medium sized, hexocolpate, isopolar and radially symmetric grains. However, the pollen grain of two species varies in shape and exine complexity. *P. coesta* pollens were round/ circular and were prolate, whereas in *P. rugosus* pollens were round/oval and subprolate-prolate. Variation was also found in the exine complexity of the two species. Exine was tectate, tectum was nearly reticulate / scabrate in *P. coesta*. But in *P. rugosus* and exine was either tectate or semitectate, tectum was reticulate, microreticulate.

Keywords: Bee forage, Pollen morphology, SEM.

INTRODUCTION

Himachal Pradesh is a state in northern part of India. It is nestled in the heart of Western Himalayas between $30^{\circ} 22' 40''$ to $30^{\circ} 12' 40''$ North latitude and between $75^{\circ} 45' 55''$ and $79^{\circ} 04' 20''$ East longitude. Himachal Pradesh shares borders with Jammu and Kashmir and Ladakh to the north, Punjab to the west, Haryana to the southwest and Uttarakhand and Uttar Pradesh to the south. The state also shares an international border to the east with the Tibet. The total area of state is 55,673 sq. km (Balokhra, 2020).

Himachal Pradesh is a mostly a mountainous state and is biodiversity hotspot, possesses a rich biodiversity of immense potential. There are four major agro-climatic zones in Himachal Pradesh which can broadly be classified as sub-tropical (low lying hills), sub-temperate (mid hills), temperate (high hills and interior valleys) and cold and dry zones.

The genus *Plectranthus* L Her. belongs to family Lamiaceae, subfamily Nepetoideae, tribe Ocimeae, subtribe Plectranthinae, comprising of about 300 species distributed throughout the tropical and warm regions of the Old World including Africa, India and Australia (Retief, 2000; Rice *et al.*, 2011). The genus *Plectranthus* serves as important bee forage plant. Studies were conducted on different species of *Plectranthus*. Singh and Singh (1971) reported *P. rugosus* as the major honey plant resource from Kashmir valley. Gupta *et al.*, 1984 recognised *Plectranthus* as forage for *Apis cerana indica* and *Apis mellifera*. Sharma (1989) conducted melissopalynological studies on honey samples and pollen loads collected from 30 localities of Himachal Pradesh and identified *Plectranthus* as major

nectar and pollen source. Sharma (1996) also identified it as important spromorph in Jammu and Kashmir contributing to unifloral honeys. Rana *et al.*, (1997) listed bee flora and surveyed potential areas for beekeeping in all the 12 districts of Himachal Pradesh. Surplus honey was collected during the blooming of *Plectranthus* sp. in the months of August–October in Shimla, Kinnaur and Chamba districts. Gatoria *et al.*, (2002) studied the potential of beekeeping in India and listed *Plectranthus* sp as important floral resources in different regions of India. *P. rugosus* was also found as important bee pasturage plant (Abrol *et al.*, 2015). Sharma and Ahmad (2019) performed physicochemical analysis of honey from different areas of Jammu. They documented *P. rugosus* as potential source of honey harvested during mid-August to First week of November. This mainly due to high nectar content (11.00 $\mu\text{l}/\text{flower}$ and nectar sugar content (32.14 gm/l) in *P. rugosus* as reported by Ara *et al.*, (2019). Similarly, *P. coesta* produces large amounts of nectar and is a major honey plant in northwestern India and NWPF of Pakistan (Singh, 1962; Shahid and Qayyum, 1997, Verma, 1990).

Several studies were conducted in different parts of the world on bee flora. But, not much work has been done in Himachal Pradesh. Therefore, this study attempts to identify two different species of *Plecranthus* in different zones of Himachal Pradesh. Apart from this pollen morphology of two species was also investigated through light and scanning electron microscopy (SEM). As SEM provides better depth of focus and gives the clear-cut view of exine ornamentation of the pollen for its morphological comparison and also in taxonomy. Under high magnification it is also possible to measure the tectum

of pollens upto microns with the help of SEM. Thus, it is helpful in recognizing the pollens to their species level (Ferguson *et al.*, 2007).

MATERIALS AND METHODS

During these investigations (field and laboratory studies were done as a part of Ph.D Work during 2009 to 2012). However, extended field work was also done between 2012-2016 in order to study the bee flora from different agro-climatic zones Himachal Pradesh.

For the preparation of pollen slides standard acetolysis method was followed as suggested by Erdtman (1952). The pollen slides were then observed under light microscope. In scanning electron microscopic studies, the pollen grains/anthers of identified pollen taxa were collected in glass vials and preserved at sub-zero temperature. Pollen samples were obtained from the mature specimens. For scanning, an adhesive (a plastic dissolved in a volatile solvent) is applied to the smooth metal surface of the microscope stage, and a small quantity of pollen residue obtained above or of pollen collected directly from the plant is placed on the adhesive and teased thoroughly in alcohol. The pollen grains were then air dried in vacuum evaporator and coated first with carbon and then with gold 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. Pollen grains are then ready for observation (Laere *et al.*, 1969). Thus, shape and size of pollen, type and number of aperture, surface pattern and ornamentation of exine were investigated from this palynomorphological study. The descriptive terminology is followed as by Sawyer (1981) and Vorwohl (1990).

RESULTS AND DISCUSSION

Plectranthus coesta (Buch.-Ham. ex D.Don) Kudo

Common name: Java Isodon

Local Name: Chhichri

Synonyms: *Elsholtzia javanica* Blume

Isodon coetsa (Buch.-Ham. ex D.Don) Kudo

Ocimum coetsa (Buch.-Ham. ex D.Don) Spreng.

Rabdossia coetsa (Buch.-Ham. ex D.Don) H.Hara

Description: Wild, strongly aromatic, erect, branched, perennial herb/shrub up to 1.5-2 metres tall (Fig. 1a). Common in slopes, forest edges and along cultivated fields. Leaves thinner, opposite ovate or ovate-lanceolate, acute at apex and about 5-10 cm long. Flowers small, lavender-blue in cymes arranged in spreading panicles. It is visited by both *Apis cerana* and *Apis mellifera*. Flowering occurs in September-October. Found at the elevation from 1,800-3,300 m.

It is major source of nectar and medium source of pollen (N^1P^2).

Pollen grain: Monad, round/ circular and prolate, medium

sized, size ranges from $27.8\mu\text{m} \times 25.4\mu\text{m}$, hexocolpate (zonocolpate), isopolar, radially symmetric. Exine was tectate. Tectum was nearly reticulate/ scabrate, sexine thicker than nexine (Fig. 1b,1c,1d)

***Plectranthus rugosus* Wall. ex Benth.**

Common name: Wrinkled leaf Isodon

Local Name: Chhichri, Shain

Synonyms: *Isodon rugosus* (Wall. ex Benth.) Codd

Isodon plecranthoides Schrad. ex Benth.

Ocimum densiflorum Roth

Rabdossia rugosa (Wall. ex Benth.) H.Hara

Description: Wild, aromatic, erect, bushy and branched shrub/ undershrub about 1-2 metres tall (Fig. 2a). Common and gregarious on steep stony, open and dry slopes. It is also cultivated as an ornamental plant for landscaping and as ground cover to prevent soil erosion in hilly slopes. Branchlets are densely covered with hairs. Leaves thicker, opposite, white-grey tomentose beneath, broad, ovate or oblong, wrinkled and about 1-4 cm long. Leaf margins are toothed. Flowers small, white, spotted with purple and borne in cymes on lateral slender branches. Flowering occurs in August-November. Its flowers are visited by both *Apis cerana* and *Apis mellifera*. Found at the elevation from 900-2,500 m.

It is a major source of nectar and pollen (N^1P^1).

Pollen grain: Monad, round/oval and subprolate-prolate, medium sized grains, size ranges from $30.5\mu\text{m} \times 28.6\mu\text{m}$, hexocolpate, isopolar, radially symmetric. Exine was tectate, semitectate. Tectum was reticulate, microreticulate, sexine thicker than nexine (Fig. 2b,2c,2d).

Discussion

Pollen grains of *P. coesta* and *P. rugosus* of family Lamiaceae were observed through light and scanning electron microscopy (Fig. 1b-1d & 2b-2d). Different pollen characters were studied included pollen aggregation, pollen shape, shape class (100 P/E) size, aperture, polarity, symmetry, surface pattern and exine ornamentation. Both *P. coesta* and *P. rugosus* had solitary grains (Monads). The shape of pollen grains varies from round/ circular in *P. coesta*, whereas it is round/oval in *P. rugosus*. The shape class of pollens was arrived at using ratio of the polar and equatorial axis diameter measurements. The pollens observed were prolate (133-200) in *P. coesta* and subprolate-prolate (114-133 to 133-200) *P. rugosus*. In both species pollen grains were medium sized but in *P. coesta* size (length x breadth) ranges from $27.8\mu\text{m} \times 25.4\mu\text{m}$, whereas, in *P. rugosus*, it was $30.5\mu\text{m} \times 28.6\mu\text{m}$. However, the pollen of both species were hexocolpate (with six colpa), isopolar and radially symmetric. Exine was tectate (continuous tectum) in *P. coesta* and it can be either tectate or semitectate (discontinuous tectum) in *P. rugosus*. Tectum was nearly reticulate (sculpturing elements forming reticular pattern)/ scabrate (radial projections of sculpturing elements having no dimensions $\geq 1\mu\text{m}$), in *P. coesta* but in *P. rugosus*, tectum was reticulate,



Figure. 1a: *Plectranthus coesta*

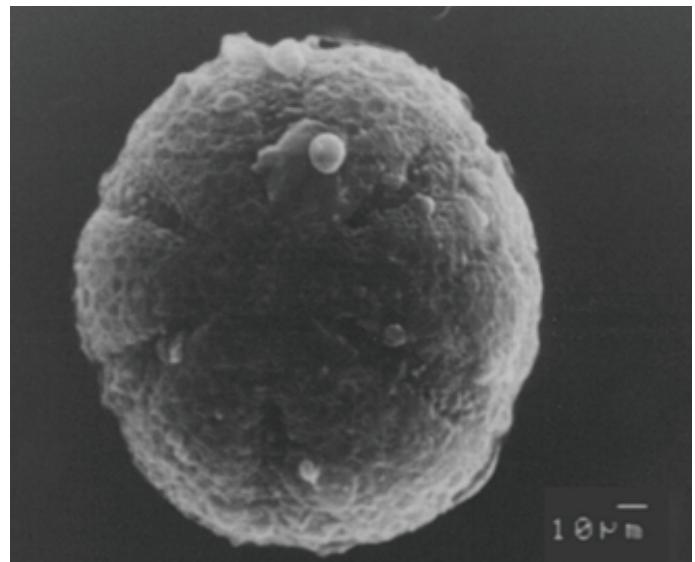


Figure. 1d: *Plectranthus coesta*, PV (X2,700)



Figure. 2a: *Plectranthus rugosus*



Figure. 1b: Pollen grain, OS X 400,

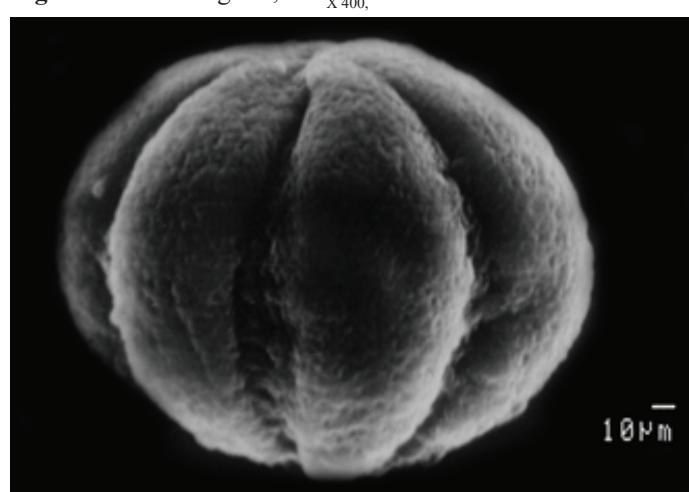


Figure. 1c: *Plectranthus coesta*, EV (X3,300)

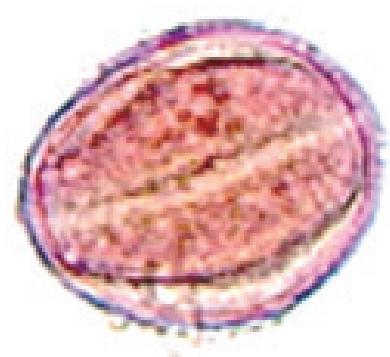


Figure. 2b: Pollen grain, OS X 400,



Figure. 2c: *Plectranthus rugosus*, EV_(X2,500)

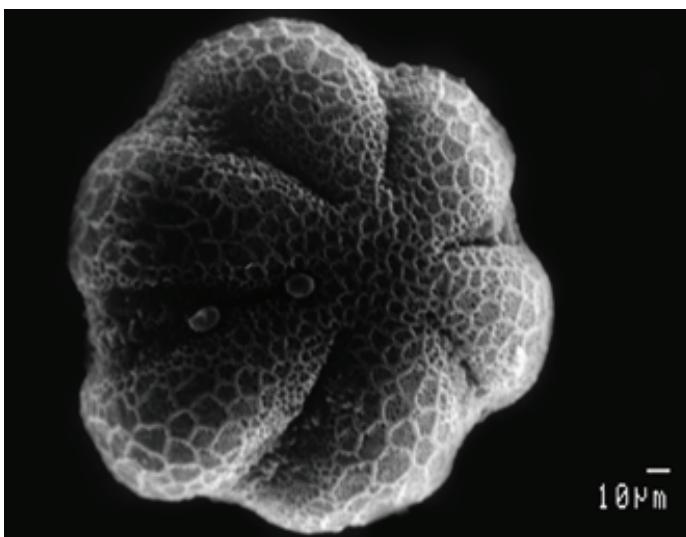


Figure. 2d: *Plectranthus rugosus*, PV_(X3,300)

microreticulate (appears reticulate but lumina less than 1 μm in diameter), Sexine was thicker than nexine in both species.

CONCLUSION

These studies can be helpful in resolving taxonomic disputes, aids to identify of plant on the basis of pollen grains and also helps to determine botanical and geographical origin of honeys. These palynomorphological investigations can be fruitful in the preparation of floral calendar particularly in autumn season and during dearth period. Moreover, scanning electron microscopic studies can be employed in correct identification of pollens which cannot be that accurate with Light microscopy (Ferguson et al., 2007).

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