Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 7, Issue, 05, pp.15505-15508, May, 2015

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

# MORPHOLOGY, ANATOMY, ULTRASTRUCTURE AND ANT-PATROLLING OF EXTRAFLORAL NECTARIES (EFNS) IN THREE SPECIES OF MIMOSACEAE

**Debasree Ghosh, *Amal Kumar Mondal and Sanjukta Mondal (Parui)**

Plant Taxonomy, Biosystematics and Molecular Taxonomy Laboratory, Department of Botany and Forestry, Vidyasagar University, Midnapore-721102, West Bengal, India

**ARTICLE INFO**

*Article History:*
Received 15th February, 2015
Received in revised form
20th March, 2015
Accepted 06th April, 2015
Published online 25th May, 2015

*Key words:*
Mimosaceae, Extrafloral nectaries, Morpho-anatomy, Ultrastructure, Ant-patrolling.

**ABSTRACT**

The Extrafloral nectaries are nectar-secreting structures that are especially common in the family Fabaceae. Light and electron microscopic structure reveals that the morphologically well-differentiated petiolar extrafloral nectaries of *Acacia auriculiformis*, *A. catechu*, *A. mangium* have an complex anatomical structures consisting of an epidermis, central secretory region and vascular region. The categories of EFNs following a structural-topographical classification, and characterize the vascularized and complex nectaries and secretory trichomes. Four species possess more than one morphotypes of gland structure. Observations and experimental field studies also support the anti-herbivore role of EFN-gathering ants in these plants.



## INTRODUCTION

Extrafloral nectaries (EFNs) occur in 93 flowering plants and five fern families and can be abundant among the flora of many habitats worldwide, especially in the tropics (Bentley, 1977; Koptur, 1992; Oliveira and Freitas, 2004). The nectar-secreting glands are structurally diverse and occur on virtually all above ground plant organs; they are especially common on the leaf blade, petiole, young stems, stipules and on reproductive structures such as buds, calyx, inflorescence axis, lower peduncles and fruits (Koptur, 1992; Elias, 1983). The extrafloral nectaries of common in the two sub families; Caesalpinoideae and Mimosoideae (Bhattacharyya and Maheshwari 1971). Anatomical data of EFNs for these plant species are not-mentioned earlier; although general morphological information on the glands of these genera is available (Diaz-Castelazo *et.al.* 2005; Pascal and Mckey, 2000; Lersten and Curtis, 1994). Numerous experimental field studies have shown that aggression towards hervivores by EFN - gathering insects, most especially ants, can positively affect plant fitness by decreasing herbivore damage (Wackers and Bonifay 2004). In the current paper focused on the morphology, anatomy of the EFNs presents in 4 species and discussed their ecological relevance. We categorize the gland that was originally proposed by Keeler (Keeler, 2008) and Zimmermann (Zimmermann, 1932).

***Corresponding author: Amal Kumar Mondal,***
*Plant Taxonomy, Biosystematics and Molecular Taxonomy Laboratory, Department of Botany and Forestry, Vidyasagar University, Midnapore-721102, West Bengal, India.*

## MATERIALS AND METHODS

The study was carried out in the Midnapore ($21^{0}52^{/}$N, $87^{0}22^{/}$E) district of West Bengal, India. The collected EFN-bearing material was collected from 3 species of *Acaia* genus. The protocol for locating EFN-bearing plants was based on: world list of plants with extrafloral nectaries (Keeler, 2008). EFN-bearing plant parts were also collected for morphological studies and were fixed and stored in 70% ethanol. The material was dehydrated with a graded ethanol series and tissue samples were infiltrated and embedded in polyethylene glycol. Using a Leica rotary microtome, 30-µm sections for each plant were obtained. For anatomical studies, dehydrated sections were diaphanized using 10% sodium hydroxide solution and 20% hypochlorite, stained with safranine (1% alcohol solution), and mounted in glycerinated gelatin. Observations and photomicrography of treated nectary tissues was accomplished using a Leica-DM-1000LED microscope and also Stereozoom microscope Leica-S8APO. For scanning electron microscopy (SEM), sections were fixed in a solution of 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH7.2) (Karnovsky. 1965) for 48 h, and post fixed for 18 h in 1% osmium tetroxide in the same buffer, buffer washed and then dehydrated and stored in 70% ethanol. After dehydration in an ethanol series, sections were dried to critical- point dried using $CO_2$ and coated with a thin layer of gold. Samples were then examined at the Microanalysis center of the A.J.C. Bose Institute, Kolkata-9 with a scanning electron microscope (Quanta 200) at an accelerating voltage of 15-20 kV.

### Study of Ant patrolling

The frequency of visited ants (an indirect measure of gland activity) in the 6 plant species was recorded. The number of ants patrolling of each plant was considered as an estimator of the level of biotic defense. The ant patrolling was monitored during three consecutive days, every 2 hours during a 12 hours period (07.00-19.00 h), counting for 1 min the number of ants in each plant. The total number of ants was divided by the total number of leaves per plant to determine the ratio of ant patrolling, independent of plant size (Boege and Marquez-Guzman 2013).

## RESULT AND DISCUSSION

### Description of extrafloral nectaries (EFNs)

**Morphological characters**: A prominent spot of EFN is present on the adaxial surface of each petiole and leaf base only in the two studied taxa of *Acacia catechu* (Fig.1). The other two species of *Acacia* possess very small formless nectaries on the base of the phyllode only. The detailed morphological features of these four taxa are compared in Table 1. Minute non-secretory trichomes are distributed around the nectary of *Acacia mangium* (Fig.2).

### Anatomical characters

In longitudinal section EFNs of the two taxa *A. mangium* and *A. auriculiformis* have three regions: the one layered epidermis, formed by cells of distinct shapes and same sizes, below the epidermal region parenchymatous cell layers are compactly arranged, a central region composed of a distinct pore by which secretion is shed outside (Fig.1), Although the cross sections of the EFNs of other species have both epidermis and parenchymatous tissues are acts as secreting regions. Elevated nectaries on the leaf rachis base are very common in Fabaceae. This EFN morphotype is morphologically and anatomically well known and has been reported for many leguminous plants (Pascal *et al*. 2000; Paiva *et al*. 2001).

### Ant patrolling

The EFNs of studied plants were found to be visited by many foraging ants as well as many 'non-ant' visitors like beetles, wasps, jumping spiders and flies. But here we only measure the rate of ant patrolling of different studied plants. In case of *A.mangium* ant patrolling ratio is high (0.109), but in other species of *Acacia* this ratio is low like *Acacia catechu* (0.132) and *A. auriculaeformis* (0.44). Macroevolutionary (between–species) patterns associated with the evolution of physical

**Table 1. Morphology and distribution (taxonomical and on plant tissues) of EFNs in 3 species of Mimosaceae**

| Sl. No. | Name of the plants | Morphotype of EFNs | Site (location) | Distribution | Size | Shape | Colour | Surface |
|---|---|---|---|---|---|---|---|---|
| 1 | *Acacia catechu* Willd. | Elevated nectaries | Petiole | Single | Small | Round | Green | Smooth |
| 2 | *Acacia auriculaeformis* Benth. | Formless nectaries | Base of the phyllode | Single | Very Small | Lanceolate | Red | Smooth |
| 3 | *Acacia mangium* Willd. | Formless nectaries | Base of the Phyllode | Single | Very Small | Lanceolate | Yellow | Hairy |

**Table 2. Anatomical Characterization of EFNs in 3 species of Mimosaceae**

| Sl.No. | Plant Name | P/A of pigment | Pigment colour | Composition of central zone | Hair type | Hair Shape | Trichome Type | Trichome Shape | pore present/Absent |
|---|---|---|---|---|---|---|---|---|---|
| 1 | *Acacia catechu* Willd. | A | NA | Parenchyma mixed with transfusion tissue | unicellular | Scale like | NA | NA | NA |
| 2 | *Acacia auriculaeformis* Benth. | P | Reddish orange and green | A distinct pore surrounded by small cells also surrounded by transfusion tissue | NA | NA | NA | NA | P |
| 3 | *Acacia mangium* Willd. | P | Reddish orange | A distinct pore surrounded by small cells also surrounded by transfusion tissue | Unicellular | Scale like | NA | NA | P |

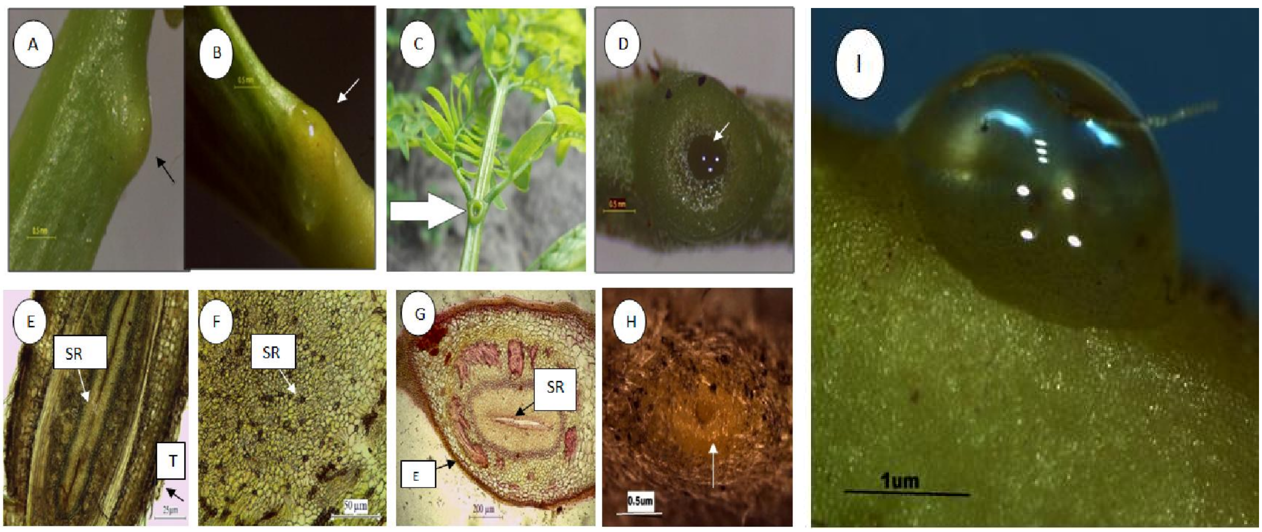


**Figure.1. (A) Stereomicroscopic picture of Acacia auriculaeformis. (B) Stereomicroscopic picture of Acacia auriculaeformis. (C) EFN position on plant parts of Acacia catechu. (D) Stereomicroscopic picture of Acacia catechu. (E) T.S. of EFNs of Acacia mangium. (F) T.S. of EFNs of Acacia catechu. (G) T.S. of EFNs of Acacia auriculiformis. (H) Stereomicroscopic picture of Acacia mangium. (I) Stereomicroscopic picture of Acacia auriculaeformis showing a drop of secretion from EFN gland. [T- Trichome, SR- Secretory Region, E- Epidermis].**

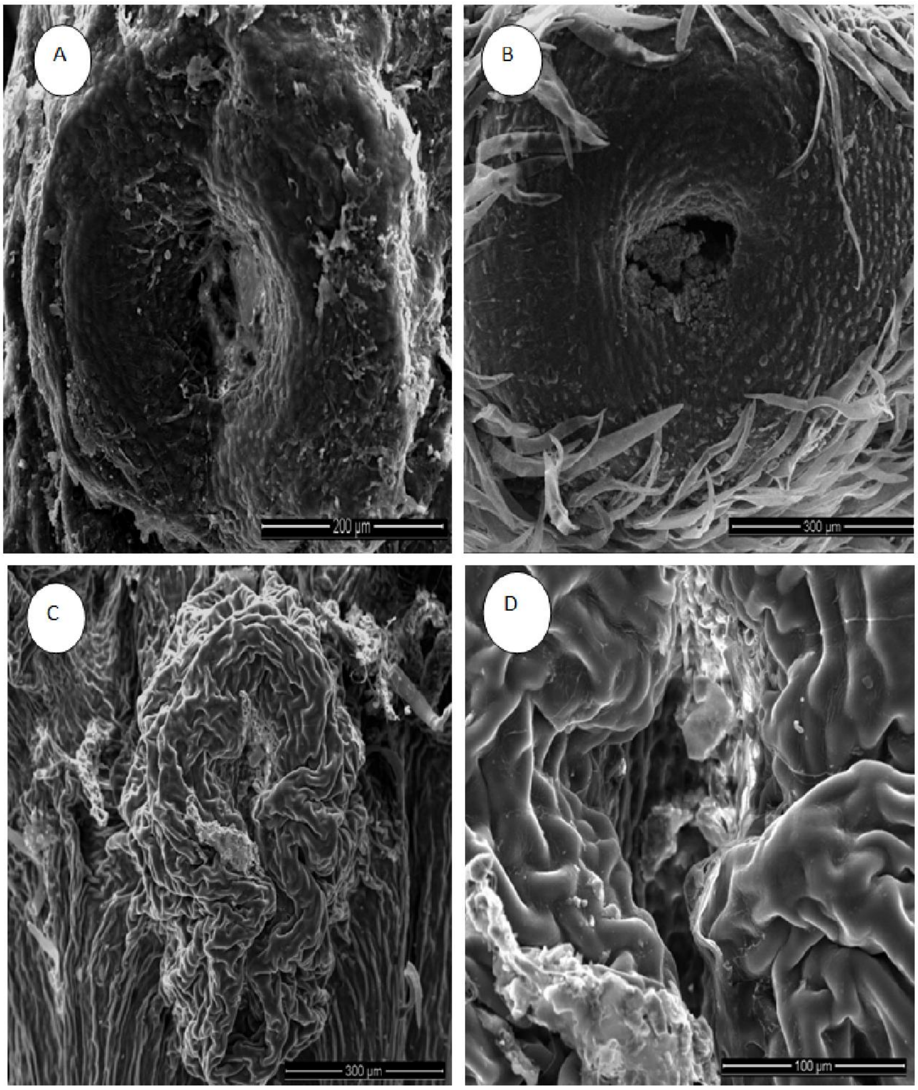


**Figure. 2. (A) Single circular elevated nectary of Acacia catechu, scale bar= 200µm. (B) Single circular elevated nectary of Acacia mangium surrounded by many unicellular non-secretory trichomes, note the concave nectary surface, scale bar=300µm. (C) Single circular sub-sessile nectary of Acacia auriculaeformis, scale bar=300µm. (D) Acacia auriculaeformis, notice secretory region of EFN gland, scale bar=100µm.**

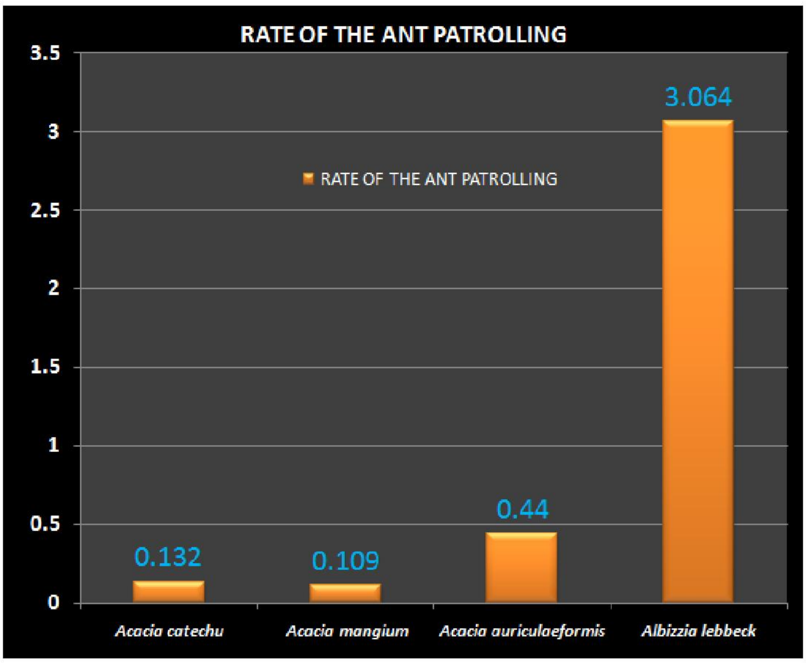


**Figure 3. Graphical representation of ant-patrolling data of three *Acacia* taxa**

plant-traits, and the co-evolution of their hervivores, also provide some of the strongest support for the hypothesis of plant defenses (Agrawal, 2007).

**Conclusion**

Two distinct kind of EFN morphologies exist in *Acacia*, which we here interpreted in relation to their morphological differentiation and specialization of EFNs. Morphological characterization of this kind of nectaries is, however, not so simple. The anatomical characterization of the secretory structures which exist on the plant organ is highly significant. The similarities of morphological and anatomical characters present in high ratio between three members of the genus *Acacia*. These EFNs were globular and elevated, sessile glands, with a central secretory pore present. In *A. mangium* and *A. auriculaeformis* shows the presence of EFNs gland only on the base of the phyllode only. Beside this unicellular hairs were present over the surface of the EFNs of *A.mangium*. The ant-patrolling ratio is also varies. It is recommended that the morphological as well as structural similarity present between studied plant species. We will also hopefully cast further light on ecological relevance of these glands in genus *Acacia*, especially with respect to their attractiveness to multiple visitors. So, the present study is one of a very few (Heil *et al*. 2004; Weber *et al*.2012) to investigate how EFN attributes such as gland morphology influences ant preference and understand the evolutionary role in a phylogenetic framework.

**Acknowledgement**

We are thankful to Botanical Survey of India (BSI), Shibpur, Howrah (for proper plant identification with voucher information), Bose Institute, Kolkata, Pradip Karmakar (Assistance with SEM), USIC, Vidyasagar University (Assistance with paraffin microtome sections), UGC-DRS-SAP Laboratory [use of light microscope with digital camera(Leica DM-1000 LED)] UGC-DRS-SAP Department, Department of Botany and Forestry, Vidyasagar University (use of Stereozoom microscope with digital camera), Dr. Sanjukta Mondal (Parui) (Stimulating conclusion), Dr. Amal Kumar Mondal (valuable comments on manuscript). Our work was funded by the UGC-New Delhi in the form of Major Research Project (Ref.No.F.N.42-917/2013 (SR) dated 14.3.13). This work was supported by the Department of Botany and Forestry, Vidyasagar University, Midnapore-721102 and Lady Brabourne College, Kolkata-17.

## REFERENCES

Agrawal A.A. 2007, Macroevolution of plant defense strategies. *Trends in Ecology and Evolution.*, 22:103-109.

Bentley BL.1977.The protective function of ants visiting the extrafloral nectaries of *Bixa orellana* (Bixaceae). *Journal of Ecology.,* 65: 27-38.

Bhattacharyya B., Maheshwari J.K. 1971. Studies on extrafloral nectaries of the Leguminales, Papilionaceae, with a discussion on the systematics of the Leguminales, *Indian National Science Academy*.,37: 11-30.

Boege K., Villamil N., Marquez-Guzman J. 2013.Understanding ontogenetic trajectories of indirect defense: ecological and anatomical constraints in the production of extrafloral nectaries, *Annals of Botany.*, 112: 701-709.

Diaz-Casteiazo C., Rico-Gray V.,Ortega F., Angeles G. 2005. Morphological and secretory characterization of extrafloral nectaries in plants of coastal Veracruz, Mexico, *Annals of Botany.*, 96: 1175-1189.

Elias TS. 1983. Extrafloral nectaries: their structure and distribution. In Bentley B, Elias T, eds. The biology of nectaries, New York, NY: Columbia University Press., 174-203.

Heil M., Greiner S., Meimberg H. 2004. Evolutionary change from induced to constitutive expression of an indirect plant resistance, *Nature.,* 430:205-208.

Karnovsky M.J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy, *Journal of Cell Biology.*, 27: 137-138.

Keeler K.H. 2008, World list of plants with extrafloral nectaries. http://biosci-labs.unl.edu/Emeriti/Keeler/extrafloral/cover.htm.

Koptur S. 1992.Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E, ed. Insect-plant interactions, Boca Raton., FL: CRC Press, 81-129.

Lersten N.R., Curtis J.D. 1994. Leaf anatomy in Caesalpinia and Hoffmannseggia (Leguminosae, Caesalpinioideae) with emphasis on secretory structures, *Plant systematics and Evolution.*, 192: 231-255.

Oliveira P.S., Freitas A.V.L. 2004. Ant-Plant-herbivore interactions in the neotropical cerrado savanna, *Naturwissenschaften.,* 91:557-570.

Paiva E.A.S., Moraes H., Isaias R.M.S., Rocha D.M.S., Oliveira P.E. 2001.Occurrence and structure of extrafloral nectaries in *Pterodon pubescens* Benth. And *Pterodon polygalaeflorus* Benth. (Fabaceae-Papilionoideae), *Pesquisa Agropecuaria Brasileira.*,36:219-224.

Pascal LM., Motte-Florac EF., Mckey DB.2000. Secretory structures on the leaf rachis of Caesalpinieae and Mimosoideae (Leguminosae): implications for the evolution of nectary glands, *American Journal of Botany*., 87:327-338.

Wackers F.L., Bonifay C. 2004.How to be sweet? Extrafloral nectar allocation by *Gossypium hirsutum* fits optimal defense theory predictions, *Ecology.*, 85: 1512-1518.

Weber MG., Clement WL., Donoghue M.J, Agrawal AA. 2012.Phylogenetic and experimental tests of interactions among mutualistic plant defense traits in *Viburnum* (Adoxaceae),*The American Naturalist.,* 180:450-463.

Zimmermann JG. 1932. Uber die extrafloralen Nektarien der Angiospermen, *Beihefte zum Botanischen Zentralblatt*., 49:99-196.

*******