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SCANNING ELECTRON MICROSCPIC STUDIES OF *MURRAYA KOENIGII* L. (RUTACEAE)

VINEETA CHAUDHARY and ANITA RANA
Department of Botany, School of Life Sciences, Khandari Campus, Dr. B.R. Ambedkar University, Agra - 282002 (U.P.), India.
e-mail : vineetachaudhary@ymail.com

SEM studies showed that in *Murraya koenigii* L. the anther was dorsifixed, dithecous and bilobed. They dehisce longitudinally, pistil was short and syncarpous. Stigma was sticky, capitate, papillate. Pollen grains were spherical, mono to tetracalporate with reticulated exine pattern. The unicellular trichomes were present on the inner and outer surface of calyx, corolla, pedicel, anther, ovarian surface and lamina of leaves. Stomata were present on upper and lower surfaces of petals, upper and lower surfaces of leaves.

Keywords : Anther; *Murraya koenigii* L.; Pollen grains; SEM studies; Stomata; Trichomes.

Introduction

Murraya koenigii L. commonly known as Kurrypatta, Mithaneem and Barsanga belongs to family Rutaceae is a handsome, short and shrubby tree of about 2 - 5 meters height. It is a native of India. In India 16 genera and 75 species of family Rutaceae are recorded[1].

The genus *Murraya* comprises of 11 species of shrub or small trees distributed in the tropics and sub-tropical regions[2]. In India, only two species of *Murraya* i.e. *exotica* and *koenigii* are reported[3]. *Murraya koenigii* L. is common and found in Himachal Pradesh in the areas lying between 800-1450 meters above the sea level.

Indian species of *Murraya* have long been used in the indigenous system of medicine as tonic, carminative, abortive to treat vomiting, diarrhoea, dysentery, rheumatism, hysteria, body-aches and venereal diseases[4-10] and as an important ingredient of several anti-diabetes, herbal formulation and drugs[11,12]. Since *Murraya koenigii* is of considerbale medicinal importance, scanning electron microscopic studies were undertaken for its characterisation.

Material and Methods

Structure of anther and pistil was studied by SEM (Scanning - Electron Microscopic) studies. The structure of trichomes was also studied.

The following steps are taken :

a) Samples are fixed in 3% glutaraldehyde in 0.1 phosphate buffer at pH 6.8 for 8-20 hours at room temperature. Aspirator was used for quick penetration.
b) Samples are ished in the same buffer by three changes.
c) Post fixation is done in 1% osmic acid in same buffer for 4 hours at 4°C.
d) Ished in 75% ethyl alcohol and kept in the same for 12 hours at room temperature.
e) Passed through the graded series of ethyl alcohol for 1 hour at room temperature.
f) Placed in 100% ethyl alcohol (prepared by the action of heated copper sulphate in absolute alcohol) for 12 hours at 4°C.
g) Transferred to a mixture of 1:1 100% ethyl alcohol and isoamyl acetate (3-methyl butyl acetate) and kept at room temperature for 30 minutes.
h) Transferred to pure isoamyl acetate making 2 changes at an interval of 30 minutes at room temperature.
i) Samples are placed in small basket made up of iron-wire for drying.
j) Drying of samples is done with liquid carbon dioxide in a HCP-2 Hitachi critical point dryer at 1000 lb per inch.
k) Dry samples are placed in a dessicator.
l) Samples were mounted on brass stubs with the help of both side adhesive tapes.
m) Samples are coated with gold (20 mm coating) in a SCD 0.2 sputter coating unit (Polaron Equipment Ltd., Walford, England).
n) Observations and photographs were taken in LEO EM-SEM at All India Institute of Medical Sciences (AIIMS), New-Delhi.

Results and Discussion

Anther and Pollen Morphology : The SEM studies showed that in *Murraya koenigii* L. the anther was dorsifixed, dithecous and bilobed. The anther dehisce longitudinally

(Figs. 4-5). Pistil was short and syncarpous. Stigma was sticky, capitate, papillate with a terminal style and ovary was bilocular and bicarpellary.

Pollen grains (Fig. 1-3) were spherical, mono to tetracolporate with reticulated exine pattern. Pollen grains in Rutaceae were 3-6 colporate, suboblate-perprolate; longest axis 16µ in *Phlebalium;* 17µ in *Lunasia amara;* 100µ in *Ravenia rullioides.*

According to Sharma[13] in *Aegle marmelos,* the SEM studies showed that the anther was basifixed, dithecous and bilobed. The anther dehisce longitudinally. The pollen grains were tetracolporate, spherical with 60 µm in diameter. In *Murraya exotica,* the SEM studies revealed that anther was dorsifixed, dithecous and dehisce longitudinally. The pollen grains were spheroidal, tricolporate with finely reticulate exine[14] and in *Citrus lemon,* the SEM studies showed that the anthers were basifixed[15]. Anther dehiscence was of longitudinal type. The pollen grains were numerous, tetracolparate and around 45 µm in size.

Mohl[16] is first to initiate the studies in pollen morphology of Bignoniaceae. Pollen morphology of Bignoniaceae has received considerable attention in the recent past and several palynologists have made significant contribution to the knowledge of pollen morphology of several members of Bignoniaceae[17-27].

Tricolpate pollen grains have also been reported by Mehra and Kulkarni[28] in *Millingtonia hortensis* and *Tabebuia rosea,* Singh[29] in *Withania somnifera,* tricolporate in *Haplophragma adenophyllum*[30], *Bombex ceiba,* 4-5 colporate in *Crataevea religiosa*[32]. Elliptical to sub circular pollen with spinous reticulate exine was reported by Tomar and Chauhan[33] in *Salvadora persica.* Similar observations are made by Vijayaraghavan and Sudesh[34] in *Psophocarpus tetragonolobus, Tabermontana divarticata*[35] and in *Cucumis* species[36].

Lim and Tinggie[37] have reported that pollen grains of *Severinia buxifolia* (Rutaceae) are tetracolporate and bicelled. Prakash[38] has reported that pollen grains of *Zieria prostrata* (Rutaceae) were tricolpate and bicelled.

Pistil

SEM studies showed that the pistil was short, syncarpous. The stigma was short, bright, sticky, capitate and papillate with a terminal style. Style was elongate, cylindrical, thick and articulate. Ovary was hypogynous, bilocular, bicarpellary and was seated on the disk, two celled with one or rarely two stigma (Figs. 6-10).

According to Sharma[13] in *Aegle marmelos,* the pistil was short, syncarpous. Stigma was sticky, capitate, papillate with a terminal style and ovary was multilocular and polycarpellary. In *Murraya exotica,* the stigma was bilobed or trilobed with small papillae on its surface. Style was solid and bilobed or trilobed. Ovary was bilobed or trilobed in *Citrus lemon*[15], stigma was wet, capitate, globose and yellowish. Style was long, yellowish and cylindrical. Ovary was superior, polycarpellary, syncarpous, multilocular and contains 10 locules and each locule contained 3 ovules. Similar observations have also been reported by Singh[39] in *Pyrostegia venusta* and Shakya[40] in *Campsis grandiflora.* The stigma in presently studied plant was bilobed or trilobed and was of wet type.

The structural and physiological feature of pollen capturing surface vary considerably between families[41]. However, Chauhan *et al.*[25] and Rana[42] reported that stigmatic surface of completely fruitless plants of *Cresentia cujete* and *Kigelia pinnata,* respectively growing at Agra has medium sized and compactly arranged papillae with swollen tips. According to them, this may be the cause of inhibition of normal *in vivo* pollen germination and ultimately fruitlessness in *C. cujete* and *Kigelia pinnata* growing at Agra. Similarly, differences in the morphology of stigmatic papillae in *Tecoma stans* plants showing seasonally transient sterility have been reported by Singh and Chauhan[43].

Trichomes

SEM studies showed the presence of unicellular trichomes on various floral parts and extra floral parts of *Murraya koenigii* L. i.e. calyx, corolla, pedicel, anther, ovarian surface and lamina of leaves (Figs. 11 - 18).

According to Rathore[14] in *Murraya exotica,* SEM studies showed the presence of unicellular trichomes on various floral parts and extra floral parts of *Murraya exotica* L. i.e. calyx, corolla, anther lobe, stigma, style, ovarian surface and upper and lower surfaces of leaves. In *Aegle marmelos*, glandular and non glandular trichomes are observed on various floral parts[13]. SEM observations in present study showed the presence of unicellular and peltate trichomes on the outer and inner surface of calyx, corolla and lower ovarian surface. It was also noted that inner surface of corolla showed the presence of sunken trichomes. Anther surface also showed the presence of unicellular and peltate trichomes.

Bahadur[44] have reported the presence of floral nectaries on various floral parts and which are used in taxonomic and phylogenetic consideration.

Bignoniaceae is characterized by the presence of both floral and extra floral trichomes[45,46]. Four types of glandular and two types of non- glandular trichomes have been reported in 5 species of Bignoniaceae[47]. Recently the presence of nuptial and extra nuptial nectaries in 15

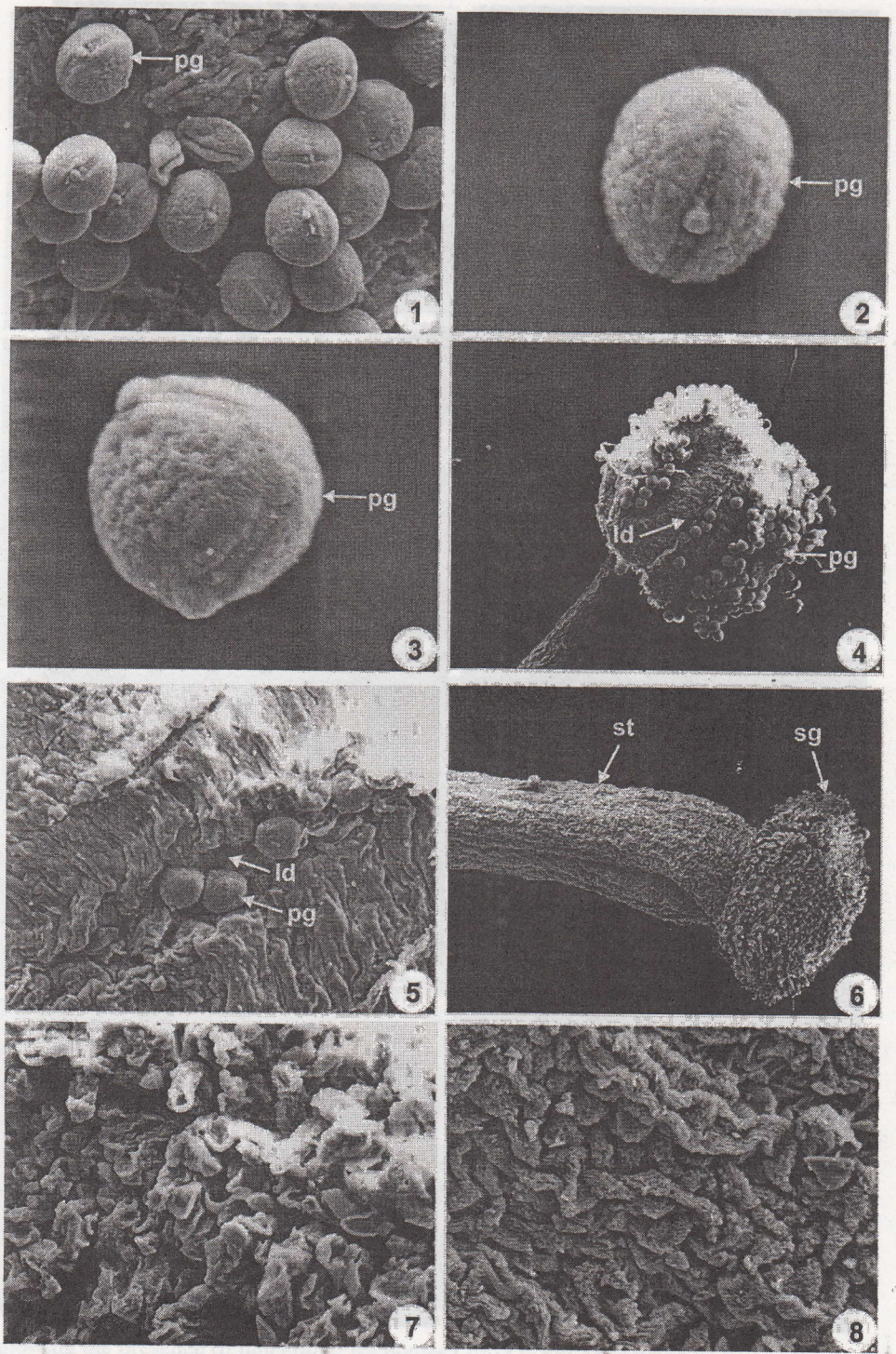

Fig.1. Showing spherical mono to textracolporate pollen grains (586 X); **Fig.2.** Showing magnified view of monocolporate pollen grain (200 X); **Fig.3.** Showing magnified view of bicolporate pollen grain (200X); **Fig.4.** Anther showing longitudinal dehiscence (400 X); **Fig.5.** Anther showing magnified view of longitudinal dehiscence (170X); **Fig.6.** Showing style and papillate stigma (112X); **Fig.7.** Magnified view of stigma (180X); **Fig.8.** Magnified view of style (180X).

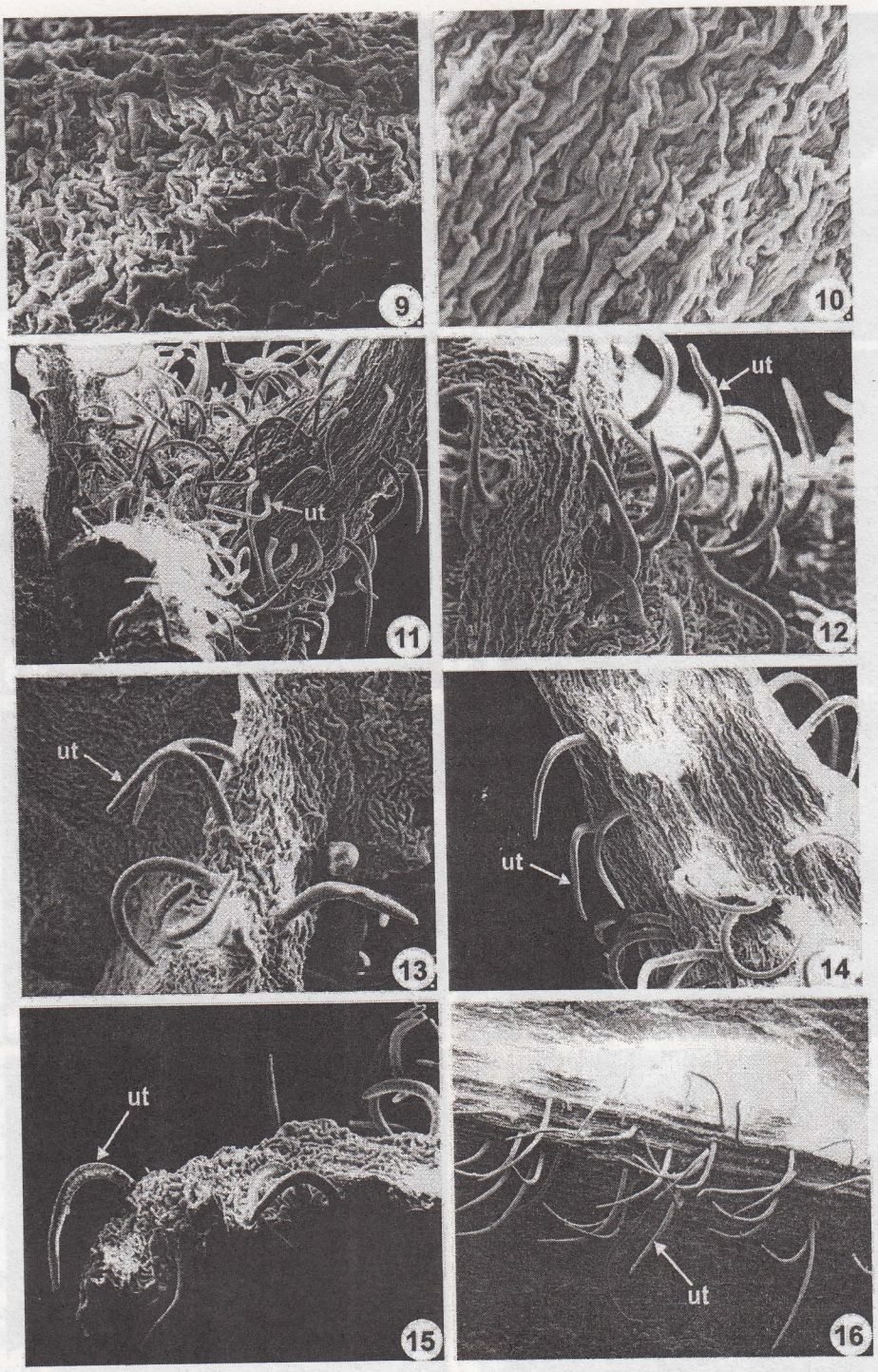

Fig.9. Magnified view of ovary (200X); **Fig.10.** Magnified view of filament (170X); **Fig.11.** Pedicel and sepal showing the presence of unicellular trichomes (115X); **Fig.12.** Magnified view of pedicel and sepal showing the presence of unicellular trichomes (170X); **Fig.13.** Magnified view of sepal showing the presence of unicellular trichomes (180X); **Fig.14.** Magnified view of pedicel showing the presence of unicellular trichomes (170X); **Fig.15.** Petal showing the presence of unicellular trichomes (210X); **Fig.16.** Lamina of leaf showing the presence of unicellular trichomes (85X).

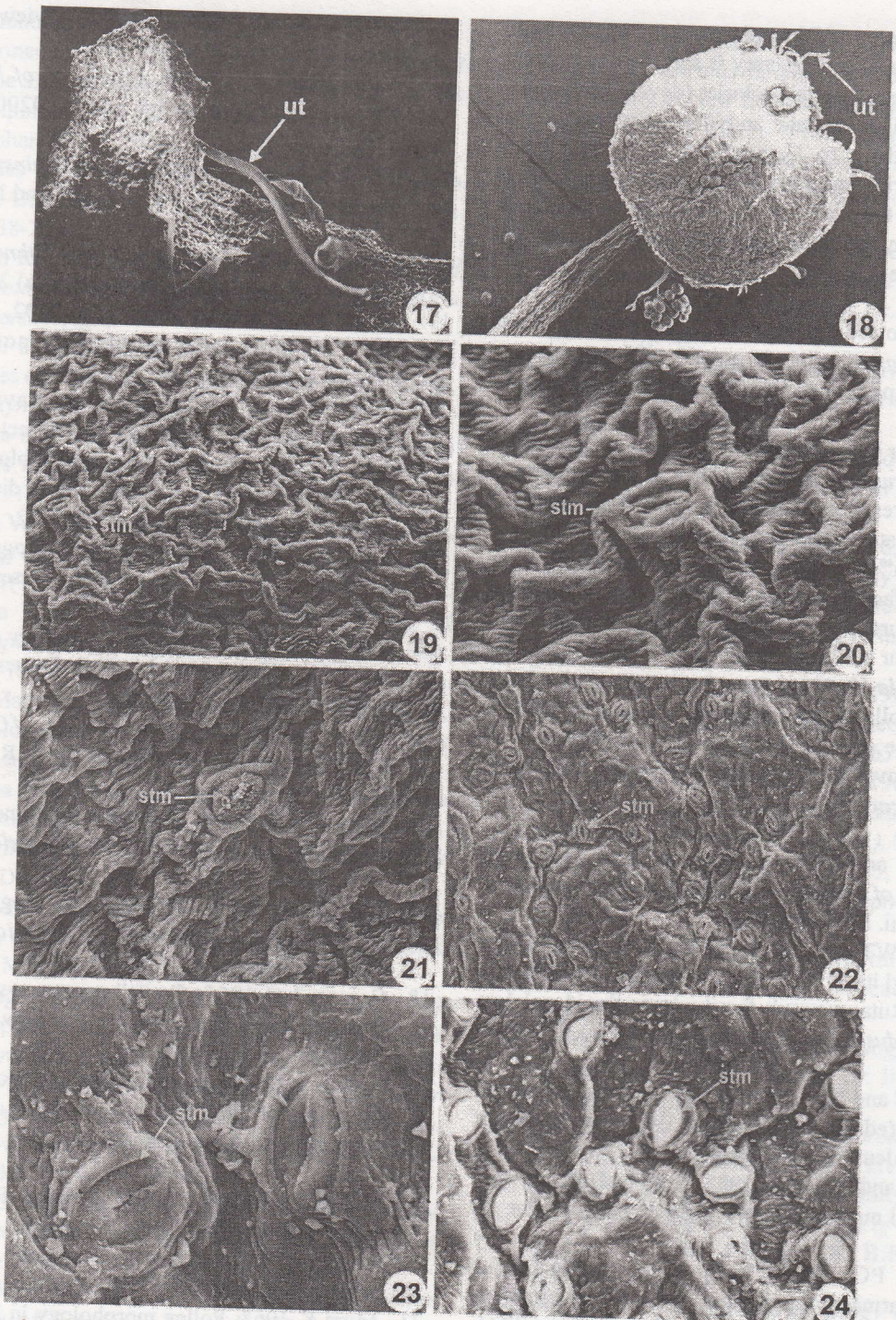

Fig.17. Ovary showing the presence of unicellular trichomes (200X); **Fig.18.** Anther surface showing the presence of unicellular trichomes (210X); **Fig.19.** Upper surface of petal showing the presence of stomata (400X); **Fig.20.** Magnified view of stomata showing the upper surface of petal (170X); **Fig.21.** Lower surface of petal showing the presence of stomata (380X); **Fig.22.** Upper surface of leaf showing the presence of stomata (250X); **Fig.23.** Magnified view of stomata showing the upper surface of leaf (169X); **Fig.24.** Lower surface of leaf showing the presence of stomata (598X).

species of 12 genera of Bignoniaceae has been reported by Galetto[48]. The nuptial nectary is supplied by phloem branches. The extra-nuptial nectaries (devoid of vascular tissue) are found on the outer and inner calyx surface.

According to Gupta[30] in *Haplophragma adenophyllum,* trichomes were present on the inner surface of corolla and on the ovarian surface. Unicellular trichomes were present on the outer surface of corolla, ovarian surface and style.

Stomata

SEM studies showed the presence of stomata on various parts of *Murraya koenigii* L. i.e. upper and lower surfaces of petals, upper and lower surfaces of leaves (Figs. 19-24).

Similar observations have also been reported by Kumar[15] in *Citrus lemon* L. According to him, numerous stomata were present on the style. Rathore[14] also reported numerous stomata on leaves of *Murraya exotica* L.

Mehta[49] found the stomata distributed all over the corolla surface in *Adhatoda vasica* Nees. According to her the mature stomata were typically caryophyllous with two to four subsidiary cells; Yadav[50] in *Colliondra haematocephala* Hassk observed stomata on the outer surface of corolla; Singh[51] observed the stomata on the ovarian surface of *Clerodendrum* species. Presence of stomata on the ovarian surface was also reported by Singh[52] in *Tecomaria capensis.*

References

1. Shukla P and Misra S 1997, *An introduction to taxonomy of Angiosperms.* Vikas Pub. House Pvt. Ltd. New Delhi. Pp. 466-470.
2. Swingle WT and PC Reece 1967, The botany of *Citrus* and its wild relatives of the orange subfamily (family Rutaceae, sub family Aurantinoideae) In : *Citrus Industry* (eds Reuther W. *et al.)* Vol. **1** Pp 190-430.
3. Nair K N and Nayar M P 1997, Rutaceae. In : *Flora of India* (eds Hajra PK *et al.)* Botanical Survey of India, Calcutta Vol. **4** 259-408.
4. Joseph S and Peter K V 1985, Curry leaf (*M. koenigii)* perennial nutritious, leafy vegetable. *Econ. Bot.* **39** 68-73.
5. XIOG PG and Wang NG 1991, Can ethnopharmacology contribute to the development of anti- fertility drugs? *J. Ethnopharmacology* **32** (1-3) 167-177.
6. Kinoshita T and Firman K 1996, Highly oxygenated flavonoids from *Murraya paniculata. Phytochemistry* **42(4)** 1207-1210.
7. Kumar V S, Sharma A, Tiwari R and Kumar S 1999, *Murraya koenigii* (Curry leaf): a review. *J. Med. Aromat. Plant Sci.* **21** 1139-1144.
8. Bhattacharjee S K 2000, *Handbook of Medicinal Plants,* Pointer Publishers, Jaipur 302003 (India) 231p.
9. Parrota JA 2001, *Healing plants of Peninsular India,* CABI publishing, Walling ford, UK and New York, 917 p.
10. Trivedi PC 2007, *Medicinal plants: Ethnobotanical Approach,* Agrobios Publication (India) 287, 158p.
11. Grover J K, Yadav S and Vati V 2002, Medicinal plants of India with antidiabetic potential. *J. Ethnopharmacol.* 81-100.
12. Vinuthan MK, Girish Kumar V, Ravindran JP, Prakash Jaya and Narayan K 2004, Effect of extracts of *M. Koenigii* leaves on the levels of blood glucose and plasma insulin in alloxan-induced diabetes rats. *Indian J. Physiol. Pharmacol.* 348-352.
13. Sharma L 2008, *Reproductive biology of Aegle marmelos L. (Rutaceae).* M. Phil. Disser., Dr. B.R. Ambedkar University, Agra.
14. Rathore M 2008, *Reproductive biology of Murraya exotica L. (Rutaceae).* M. Phil. Disser., Dr. B.R. Ambedkar University, Agra.
15. Kumar U 2008, *Reproductive biology of Citrus lemon L. (Rutaceae).* M. Phil. Disser., Dr. B.R. Ambedkar University, Agra.
16. Mohl H 1835, Sur la Structure et lesformes des grains de pollen. *Ann. Des. Sciences Natureles.* Seconde Serie. T. III Vol. **2** 304-306.
17. Erdtman G 1952, "Pollen morphology and plant taxonomy". *Angiosperms. J. Osmania University* (Science Faculty). **10** 166-174.
18. Gomex J C 1955, Contribucae a systematic das Bignoniaceae Brasileiras. *Arg. Do Serv. Forestral.* **9** 261-296.
19. Cranwell L 1962, Endemism and Isolation in the three kings Iseland New Zealand, with notes on pollen and spore type of the endemics. *Rec. Inst. Mus.* **5** 215-232.
20. Guinet P H 1962, Pollen, d Asiac Tropical Inst. Francis Pondicheri. Trave Sec. Scient. Tech. Tome. V. original not seen cited by: Buurman J. 1977. *Pollen et spore* **19** 447-519.
21. Mitra K 1968, Pollen morphology in Bignoniaceae in relation to taxonomy. *Bull. Bot. Surv. India* **13** 3-4.
22. Fergusan I K and Santisuk K 1973, Notes on pollen morphology of some Asiatic Bignoniaceae. *Kew Bull.* **29**(2) 187-194.
23. Suryakant 1973, Pollen morphological studies in

Bignoniaceae. *Palynol.* **IX** (1) 45-82.

24. Buurman J 1977, Contribution to pollen morphology of the Bignoniaceae with special reference to the tricolporate type. *Pollen et. spore* **19** 447-519.
25. Chauhan S V S, Singh K P and Kinoshita T 1987, Studies on floral abscission and fruit formation in some Bignoniaceae. *J. Fac. Agric. Hokkaido Univ.* **63** 238-245.
26. Bewli KS 1994, *Studies of pollen morphology and physiology in some Bignoniaceae.* Ph.D. Thesis. Agra University. Agra.
27. Bewli K S, Chauhan SVS and Singh Jolly 1995, Studies on pollen morphology in some Bignoniaceae. *J. Palynol.* **31** 137-149.
28. Mehra K P and Kulkarni AR 1985, Embryological studies in Bignoniaceae. *Phytomorphology* **35** (3-4) 239-351.
29. Singh Vandana 2009, Phenolongy and Reproductive biology of *Withania somnifera L. The J. Plant Reprod. Biol.* **1**(1) 81 - 86.
30. Gupta Renu 2005, *Reproductive biology of Haplophragma adenophyllum.* M. Phil. Dissertation. Dr. B. R. Ambedkar University. Agra.
31. Bhat Mansoor, Chauhan Seema and Rana Anita 2009, Phenology and Reproductive Biology of *Bombax ceiba* Linn. *The Int. J. Plant Repro. Biol.* **1**(2) 173-178.
32. Sharma 2005, Reproductive biology of *Crataevea religiosa* Forst. with special reference to floral galls. (Abs.) *Ind. Bot. Soc. XXIII* Conference of Bot. Survey India, Dehradun 197.
33. Tomar R K and Chauhan SVS 2003, Reproductive biology of *Salvadora persica* L. An endangered species of Brij Mandal. *XXVI* Conf. of I.B.S. Pp. 74-78.
34. Vijayraghvan MR and Sudesh 1994, Structure and Development of Anther and Pollen in *Psophocarpus tetragonolobus. Phytomorphology* **44** (1 and 2) 71-81.
35. Bansal Deepti 2005, *Phenology and Reproductive biology of Tabermontana divarticata* L. *(Apocynaceae).* M.Phil. Dissertation. Dr. B.R. Ambedkar University. Agra.
36. Beevy S S and Chan P K 2005, Pollen Morphology and Taxonomy of the Genus *Cucumis* L. *J.Indian bot.Soc.* **1** 121-126.
37. Lim AH Lan and Tinggie DA 1991, The reproductive biology of *Severinia buxifolia* (Poir.) Tenore. *Malaysian J. Sci.* **13** 1-12.
38. Prakash N 1994, *Aspects of the Reproductive Biology of Zieria prostrata (Rutaceae).* Department of Botany. University of New England. Armidale. NSW.
39. Singh Sweety 2005, *"Floral polymorphism and establishment of self incompatibility of Pyrostegia venusta".* M.Phil. Dissertation. Dr. B.R. Ambedkar University. Agra.
40. Shakya Archana 2005, *Reproductive biology of Campsis grandiflora L. with special reference to fruit formation.* M.Phil. Dissertation, Dr. B. R. Ambedkar University, Agra.
41. Heslop-Heslop-Harrison Y and Shivanna K R 1977, Receptive surface of angiosperm stigma. *Ann. of Botany* **41** 1233-1288.
42. Rana Anita 2004, Distribution and morphology of floral and extra-floral trichomes in *Kigelia pinnata* DC. *XXVIII* Conf. of I.B.S. Pp.56.
43. Singh Jolly and Chauhan SVS 1996, Morphological changes in the stigma of seasonally transient sterile *Tecoma stans. Phytomorphology* **46** 1-7.
44. Bahadur B 1998, *Nectary Biology.* Nagpur, India. Datt Sons.
45. Subramanian R B and Inamdar JA 1985, Occurrence, structure, ontogeny and biology of nectaries in *Kigelia pinnata* DC. *Bot. Mag. Tokyo.* **98** 67-74.
46. Subramanian R B and Inamdar JA 1986, Nectaries in *Bignonia illicium* L. Ontogeny, structure and function. *Proc. Indian Acad. Sci. (Plant Sci.).* **96** 135-140.
47. Mehra K P and Kulkarni A R 1985, Embryological Studies in Bignoniaceae. *Phytomorphology* **35** (3 and 4) 239-351.
48. Galetto L 1995, Nectary Structure and Nectar Characteristics in some Bignoniaceae. *Pl. Syst. and Evol.* **196** 99-121.
49. Mehta M 2007, *Reproductive biology of Adhatoda vasica* Nees. M. Phil. Disser., Dr. B.R. Ambedkar University, Agra.
50. Yadav M 2007, *Phenology and Reproductive Biology of Colliondra haematocephala* Hassk. M. Phil. Disser., Dr. B.R. Ambedkar University, Agra.
51. Singh R 2007, *Reproductive biology of Clerodendrum species.* M. Phil. Disser., Dr. B.R. Ambedkar University, Agra.
52. Singh 2007, *Reproductive biology of Tecomaria capensis.* M. Phil. Disser., Dr. B.R. Ambedkar University, Agra.