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Pollination Biology of *Turnera ulmifolia* Linn.

***Abstract*: The present paper deals with the study of flower morphology, anthesis, pollen production, pollen/ovule ratio, foraging behaviour of flower visitors and pollen germination (*in vitro* and *in vivo*) of *Turnera ulmifolia* Linn. (Turneraceae) which is a medicinally important perennial, extra floral nectaries bearing plant. The flowers open in between 6.00 to 8.00 hrs. As soon as the flowers open, different insects like *Trigona* sp., *Ceratina viridissima*, *Amegilla* sp., *Camponotus compressus* (ant), and butterflies (members of Nymphalidae, Papilionidae, Pieridae) and flies visit the flowers for collecting their food materials. A single flower produces an average of 6,26,340 pollen and pollen/ovule ratio was obtained 8947:1. During the visit the insects carry a considerable amount of pollen attached to their body parts and help in pollination. Although the flowers are visited by different flower visitors, about 5% pollen grains were trapped in the atmosphere.**

Key words: Pollen production, pollen/ovule ratio, flower visitors, pollen germination, *Turnera ulmifolia* Linn.

For utilization of plant resources in a judicial way a thorough knowledge is required of its reproductive needs including pollination. The evolutionary success and survival of angiosperms have been determined by the efficiency of their reproductive performance. So, a detailed knowledge about floral biology and pollination is a prerequisite for the systematic study of reproductive biology in relation to the development of various conservation protocols. *Turnera ulmifolia* Linn. (Turneraceae) is a medicinally important plant. Leaves are used in indigestion, biliousness, dysentery; fresh plants yield a mixture of cyano-hydringlucosides, deidaclin and tetraphyllin; seeds contain fatty acids including vernolic, malvalic and octanoic acids. In the present investigation an attempt has been made to find out the correlation between floral rewards, insect visits and fruit-set of *Turnera ulmifolia.*

Materials and methods: Morphology of acetolysed pollen[1] and receptive stigmas[2] were studied by Scanning Electron Microscope at USIC, Burdwan University. For the SEM of pollen and stigmas, these were collected during receptive period, washed in 0.015M phosphate buffer (pH 7.2), fixed with 2.5% glutaraldehyde for 2 hrs. dehydrated in ethanol series and finally critical point dried prior to gold coating. Microphotographs were taken from S-530 Hitachi Model. Flowering period, flower colour, odour and other floral characters were visually observed through extensive field exploration. Anthesis and other phenological characters were studied following the method as suggested[3&4]. Pollen productivity were studied following the procedure of Mandal and Chanda[5] . Pollen-ovule ratio were estimated as suggested by Cruden[6] .and Shivanna and Rangaswamy[7] . Ten plants at random of the same species and same age located at different places on the University campus and areas of Santiniketan, Birbhum (87°41′ and 87°42′east and 23°42′north latitude) were selected and observed for a period of three (3) years. *In vitro* pollen germination was conducted to the effect of different nutrients like sucrose and boric acid at various concentrations. The experimental set up was done as per the method of Shivanna and Rangaswamy[7]. Stigma receptivity and *in vivo* pollen germination were measured following the procedure[8] using 0.05% aniline blue dissolved in 0.05M NaH_2PO_4 as staining reagent for *in vivo* pollen tubes. The flower visitors observed to pollinate the flowers were collected, preserved in our laboratory and identified using the specimens that were already identified from Zoological Survey of India, Kolkata. Insect visits were observed visually at 2 hours intervals in each day. Contributions of flower visitors to fruit setting were quantified by comparing fruit set between bagged and unbagged flowers. Bagging and netting of flowers was done at the bud stage. For these observations ten different plants from the same locality and ten different inflorescence of a single plant were taken. Atmospheric pollen trapping was done by Parkins[9] designed 'Rotorod' sampler. The

Fig. 1: *T. ulmifolia* plant with flowers

Fig. 2: Longitudinal anther dehiscence

percentage of particular pollen was calculated by the following formula:

$$\text{Percentage of pollen grains} = \frac{\text{Number of particular pollen grains trapped}}{\text{Total number of pollen grains trapped}} \times 100$$

Results and Discussion: **Floral Biology and Pollination Mechanism:** Flowers (38x22 mm) axillary, solitary, large and yellow coloured open in the morning (6.00 - 8.00 hrs.) having longitudinal anther dehiscing pattern (Fig. 2). A single flower produces an average of 6,26,340 pollen grains with the occurrence of 8,947 pollen per ovule (Table 1). Pollen 3-colporate, sub-prolate, P/E ±78 × 71.50 µm, polar out line triangular, equatorial outline elliptic, colpi ±61.75 µm long, ±9.75 µm wide, exine ±7.5 µm thick, sculpturing reticulate (Figs. 4, 5). As soon as the flowers open, different insects like *Trigona* sp., *Ceratina viridissima, Amegilla* sp., *Camponotus compressus* (Ant) and butterflies (members of Nymphalidae, Papilionidae, Pieridae) and flies visit flower for collection of pollen and nectar as their food materials (Table 2, Figs. 7,8,9). So, the pollen grains transported effectively by these bees which can easily be landed over large petals. Bees like *Apis* sp., *Ceratina viridissima, Amegilla* sp. continuously visit the flowers in purpose of searching their food materials and during this time thepollen grains are adhered to the body parts of them and transferred to another receptive stigmas. The fruit yielding potentiality

Table 1: Flowering Phenology of *Turnera ulmifolia.*

Floral parameters	Observations
Flowering period	March-October
Flower shape	Regular
Flower size	38×22mm.
Flower colour	Yellow
Odour	Present
Nectar	Present
Flower opening time	6.00-8.00 hrs.
Anther dehiscence time	Just after flower opening
Mean no. of anther per flower	5
Mode of anther dehiscence	Longitudinal
Mean no. of pollen per anther	125268
Mean no. of pollen per flower	626340
Mean pollen per ovule	8947
Pollen morphology	3-colporate, Pollen diam. 78 × 71.50µm. Exine 7.5µm, Colpi length 61.75 µm., Colpi thickness 9.75 µm. trianguular in polar view, elliptical in equatorial view.
Stigma type	Wet type
Fruit-setting:	
Natural open flowers	54.50%
Netting flowers	4.50 %
Bagging flowers	Nil

Table 2: List of flower visitors of *Turnera ulmifolia.*

Name of flower visitor	Visiting time	Forage matter
Hymenoptera		
Apidae		
Trigona sp.	Day	Pollen and nectar
Ceratina viridissima	Day	Pollen and nectar
Anthophoridae		
Amegilla sp.	Day	Pollen and nectar
Formicidae		
Camponotus compressus (Ant)	Day and night	Pollen and nectar
Lepidoptera		
Nymphalidæ	Day	Nectar
Papilionoidæ	Day	Nectar
Pieridae	Day	Nectar
Diptera	Day	Nectar

of open flowers are higher (54.50%) than netted flowers (4.50%) and no fruit formation in bagged condition (Table 2). Although the flowers are visited by different flower visitors, about 5% pollen grains were trapped in the atmosphere.

Fig. 3: Style free, stigma flabellately Trifid

***B. In vitro* Pollen Germination:** 5% sucrose solution showed 51% germination with 428µm pollen tube development while 64% pollen germination with 750µm tube development was obtained in 200ppm boric acid solution. It is also observed that the addition of boric and sucrose solution results in the increase of pollen germination percentage as well as pollen tube development.The best result i.e. 75% germination with 1505µm tube development was obtained in 5% sucrose solution supplemented with 200ppm boric acid. 34% germination with 245µm tube development was found in 300ppm of $MgSO_4$ solution (Tables 3,4,5,6, Fig. 10).

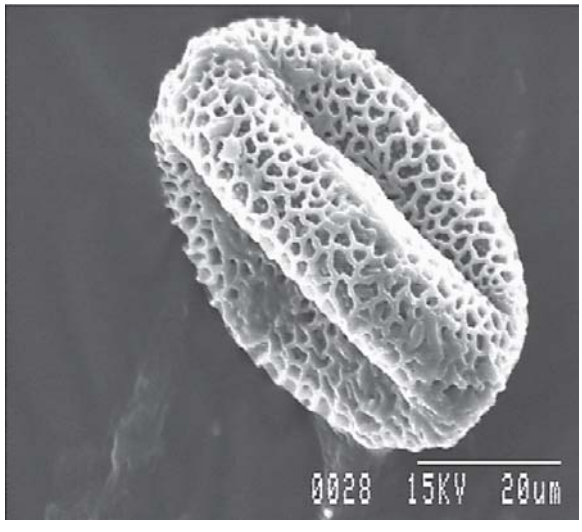

Fig. 4: Pollen - equatorial view with one of three colpi (SEM-1500x)

C. Stigma Receptivity and *In vivo* Pollen Germination: Stigma flabellately trifid, style three and free. Long, hair like structures are found all over the style. Cells of the stigma head forming long, thin, filamentous structures, which looks like a brush (Figs.3, 6). The pollen grains are adhered to this long, membranous, filamentous structures. Vascular strands reaches up to the lower portion of stigma head. Stigma is of wet type. A critical observation on the stigma characters taking successive days after anthesis revealed that stigmas remained receptive more (80%) during second day after anthesis and showed 78% *in vivo* germinating pollen along with 1554µm pollen tube over stigma head (Table 7, Fig. 11).

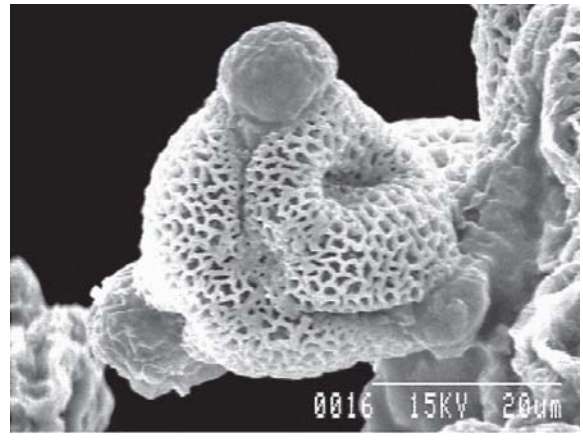

Fig. 5: Tricolporate, reticulate pollen from polar view (2000x)

The actinomorphic, wheel-shaped flowers of *T. ulmifolia* open early in the morning after that pollen presentation time starts. Flower and pollen anthesis may depend upon physical, physiological and biochemical factors of a plant as well as on climate conditions. As soon as the flowers open different flower visitors visit the flowers for collecting their forage materials i.e. pollen and nectar. Extra floral nectaries (EFN) are located at both sides of the petiole. The nectar produced is a balanced solution of sucrose, glucose and fructose. Ants, wasps, honey bees,

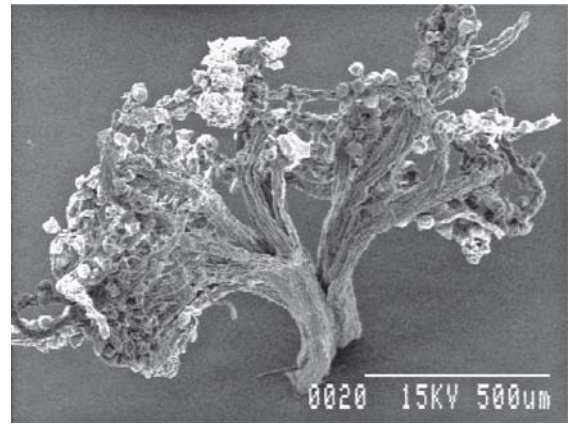

Fig. 6: Stigma (80x)

Table 3: Effect of sucrose on *in vitro* pollen germination of *T. ulmifolia*

Concentration (%)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen Tube Length(µm)	Germination (%)	Pollen Tube Length(µm)	Germination Germination(%)	Pollen Tube Length(µm)
5	35	272	40	340	51	428
10	28	229	35	280	44	355
15	20	198	24	210	30	265
20	15	175	19	186	25	214
25	5	124	8	147	14	178
30	2	65	5	86	10	105

Table 4: Effect of boric acid on *in vitro* pollen germination of *T. ulmifolia*

Concentration (ppm)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen Tube Length(µm)	Germination (%)	Pollen Tube Length(µm)	Germination Germination(%)	Pollen Tube Length(µm)
50	15	203	28	275	34	312
100	38	338	46	388	50	432
200	47	390	58	632	64	750
300	25	260	39	329	45	389
500	14	173	22	212	31	268

Table 5: Effect of sucrose and boric acid on *in vitro* pollen germination of *T. ulmifolia*

Concentration (% + ppm)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Pollen Tube Length(µm)	Germination (%)	Pollen Tube Length(µm)	Germination Germination(%)	Pollen Tube Length(µm)
5 + 50	24	261	30	328	35	375
5 + 100	38	342	47	452	52	658
5 + 200	51	645	62	688	75	1505
5 + 300	33	315	39	340	48	457
5 + 500	9	98	14	200	25	242

Table 6: Effect of different salts on *in vitro* pollen germination of *T. ulmifolia*

Concentration (ppm)	$Ca(NO_3)_2$		KNO_3		$MgSO_4$	
	Germination (%)	Pollen Tube Length(µm)	Germination (%)	Pollen Tube Length(µm)	Germination Germination(%)	Pollen Tube Length(µm)
100	5	75	–	–	11	115
200	12	134	8	94	22	182
300	30	224	19	178	34	245
500	17	158	10	115	15	150
600	3	60	–	–	6	88

butterflies, flies collect these food materials[10].. Among these *Amegilla* sp. may be considered as effective pollinators to this plant. They need greater energy for their existence because they are large sized. *Amegilla* sp. suddenly comes and land over large petals and try to collect nectar and yellow, sticky pollen mass. During forage time the viable pollen mass are attached to their forelegs and back-side of body. Stigmas are above the anther level, trifid and

Table 7: Stigma receptivity and *in vivo* pollen germination of *T. ulmifolia*

Period after flower opening	24 hrs.	48 hrs.	72 hrs.	Drooping stage
No. of stigmas observed	10	10	10	10
No. of stigmas showing germination	4	6	8	1
Stigma receptivity (%)	40	60	80	10
Mean No. of pollen retained on tigmas	430	445	480	275
Mean No. of germinated pollen	150	222	374	42
In vivo pollen germination (%)	35	50	78	15.27
Mean pollen tube length (μm)	337	670	1554	216

branched. So, the pollen grains transported by this bees can easily be landed over stigma of other flowers.Nectar foraging ants, butterflies also help in pollination. In bagged condition no fruit formation occurred and in nature fruit formation is better than netted condition. So, from the result of netting and bagging experiment it can be predicted that plants require the external agents for better production.

Fig. 7: Bee visiting the flower

Turnera ulmifolia (5% in 12-00 hrs.) showed high atmospheric pollen frequency. The present study gets support from the observations of other scientists[11-14], who have critically reviewed that entomophilous pollen grains may be airborne. The biological potentiality of individual flowers in an inflorescence could be calculated by counting the total pollen grains per flower, but it is very difficult to estimate absolute pollen production[5, 15]. However, pollen per flower and perovule is related to fertilization rate. In *T. ulmifolia* higher number of pollen per flower, as well as per ovule (Table 1), indicates its xenogamous nature which is genetically superior. This observation gets support from Cruden[6] .

Fig. 8: Butterfly visiting the flower

The maximum germination (75%) along with pollen tube growth (1505μm) occurs in 5% sucrose supplemented with 200 ppm boric acid solution (Table 5, Fig. 7). This is attributed to the fact that sucrose is necessary forproper pollen nutrition, osmotic control and in combination with boricacid promoted pollen germination because boron

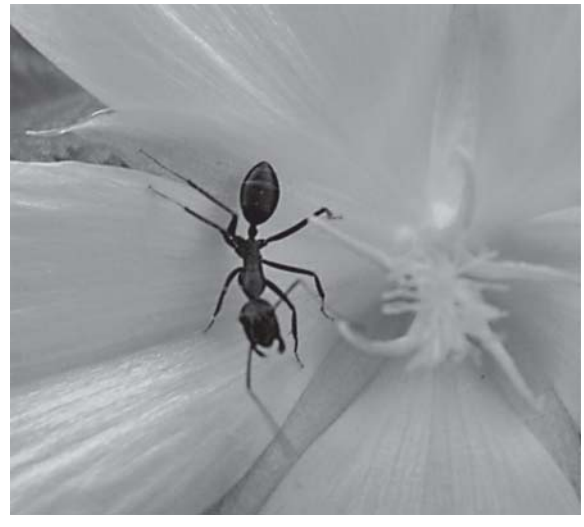

Fig. 9: Ant (*Camponotus*sp.) visiting the flower

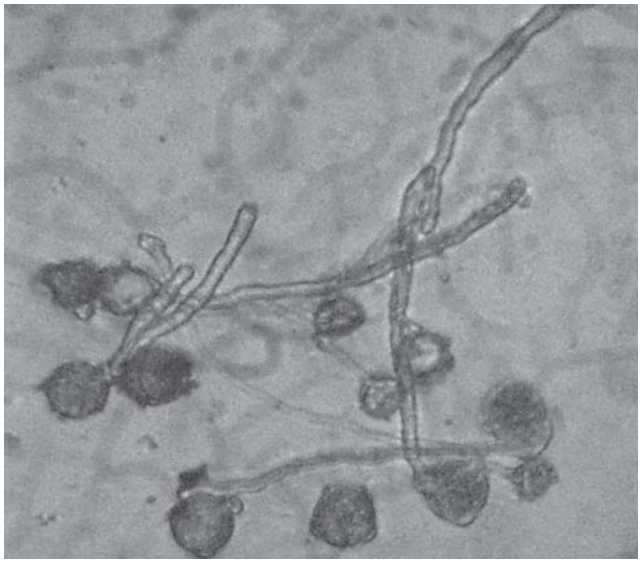

Fig. 10: *In vitro* pollen germination

makes a complexwith sucrose which may be easily translocable rather than sucrose alone. The role of boron has been confirmed in germinating pollen and growing pollen tubes which may enhance the sucrose uptake and stimulate germinating ability. This observation gets support from the findings of other scientists[16-25] .Stigma remains more receptive during the first day after anthesis (Table 7) (Fig. 8, 9). Generally receptivity reaches a maximum soon after anthesis[26] but the period of receptivity may vary from species to species[8]. The present findings are in conformity with the other scientists working at the global level[2, 23, 27-34].

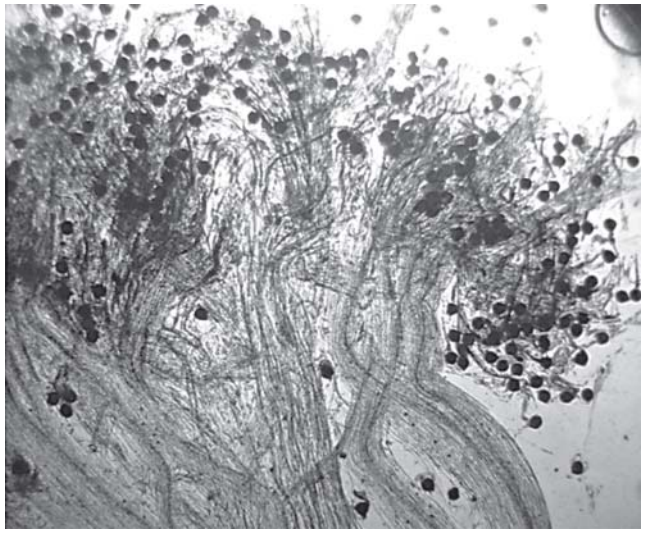

Fig. 11: Pollen germination through pistil tissue (LM- 5x)

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1. G. Erdtman, *Svenesk. Bot. Tidskr*., **54**, 261 – 264 (1960).
2. F.Ciampolini, C. Faleri. D. Dipietro and M. Cresti, *Ann. Bot*. **78,** 759–764 (1996).
3. C. S. Reddi and A. Janki Bai, Jour. Bombay Nat. History Society, 77, 471-475 (1981).
4. G. Mathur and H. Y. Mohon Ram, *Phytomorphology,* **36**, 79-100 (1986).
5. S. Mandal, and S. Chanda, *Biol. Mem.*, **6**, 1-61 (1981).
6. R. W. Cruden, *Evolution,* **31**, 31-46 (1977).
7. K. R. Shivanna and N. S. Rangaswamy, Pollen Biology (Narosa Pub. House. New Delhi, 1993).
8. J. A. Joshi Rao and A. A. Saoji, *Jour. Palynol.* **25,** 45-50 (1989).
9. W. A. Perkins, The Rotorod Sampler (Standford Univ. USA, 1957) p. 186.
10. L. Torres-Hernandez, V. Rico-Gray, C. Castillo-Guevara and A. J. Vergara, *.Acta Zool. Mex. (n.s.)* **81**, 13-21 (2000).
11. S. T. Tilak, *Jour. Plant and Nat.*, **1**, 45-50 (1984).
12. S.N. Agashe, In Recent Researches in Ecology: Environment and Pollution (Ed. S.T. Tilak) : pp. 153-157 (1989).
13. E. K. Ong, M. B. Singh and R. B. Knox, *Aerobiologia*, **11**, 51–55 (1995).
14. A. Bhattacharya, S. Mondal. and S. Mandal, *Aerobiologia*, **15,** 1-5 (1999).
15 S. Mondal and S. K. Ray, *Geophytology*, **14**, 74–81 (1984).
16. J. K. Pal, B. K. Datta, S. Mandal and G. N. Bhattacharya, *Mendel*. **6**, 311–315 (1989).
17. S. Gupta, K.N. Bhattacharya and S. Chanda, *Jour. Palynol.*, **25**, 65–72 (1989).
18. S. Mandal, N. C. Barui and P. N. Ganguly, *Sci and Cult*., **48**, 115–116 (1982).
19. S. Mondal, K. N. Bhattacharya and S. Mandal, *Sericologia*, **37**, 349–352 (1997).
20. A. Bhattacharya and S. Mandal, *Sci. and Cult*., **65**, 327–328 (1999).
21. A. Bhattacharya and S. Mandal, S. *Curr.Sci*, **79**, 1706–1712 (2000).
22. A. Bhattacharya and S. Mandal, *Grana*, **43**, 48–56 (2004).
23. G. Mohi-ud-din, I. A. Nawchoo and B. Wafai, *Phytomorphology*, **57**, 109-116 (2007).

24. K. Biswas, S. Mondal and S. Mandal, *Sci. and Cult.*, **74**, 149-152 (2008).

25. K.Biswas, S. Mondal and S. Mandal, In Advances in Plant Biology (Eds. S. Mandal and S. Bhattacharya), Kolkata, pp. 374-384 (2009).

26. K. R. Shivanna and B. M. Johri, The angiosperm pollen structure and function (Wiley Eastern Ltd., New Delhi, 1985)

27. K. R. Shivanna and S. J. Owens, *Advances in Legume Biology,* **29**, 157–182 (1989).

28. M.Nepi and E. Pacini, *Annals of Botany*, **72**, 527 – 536 (1993).

29. R. Tandon, T. Manohara, N. Nijalingappa and K. R. Shivanna, Annals of Botany, **87**, 831-838 (2001).

30. S. Choudhury, S. Mondal, and S. Mandal, *Sci. and Cult.*, **74,** 463-465 (2008).

31. R. H. Luo, V. A. Dalvi, Y. R. Li and K. B. Saxena, *Jour. Plant Breed. and Crop Sci*., **6**, 254-257 (2009).

32. A. K. Sreekala, A. G. Pandurangan, R. Ramasubbu and S. K. Kulloli, Jour. Threatened Taxa, **3**, 1818-1825 (2011).

33. K. Aswani and M. Sabu, M. *Int. Jour. Plan t Repro. Biol.*, **4**, 5-8 (2012).

34. S. Chauhan, *Int. Jour. Plant Repro. Biol.*, **4**, 31-35 (2012).