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POLLINATION BIOLOGY OF TEN MEDICINALLY IMPORTANT ANGIOSPERMS OF WEST BENGAL (INDIA)

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ABSTRACT

The present paper gives flower morphology, anthesis, pollen production, foraging nature of flower visitors, pollination mechanisms, *in vitro* and *in vivo* pollen germination and stigma receptivity of ten medicinal plants viz., *Aegle marmelos* (L.) Correa (Rutaceae), *Anthocephalus chinensis* (Lamk.) A. Rich. (Rubiaceae), *Averrhoa carambola* Linn. (Oxalidaceae), *Cassia fistula* Linn. (Caesalpiniaceae), *Catharanthus roseus* (Linn.) G. Don. (Apocynaceae), *Dillenia indica* Linn. (Dilleniaceae), *Madhuca indica* Gmelin. (Sapotaceae), *Pterospermum acerifolium* Willd. (Sterculiaceae), *Terminalia arjuna* (Roxb. ex DC.) Wt. & Arn. (Combretaceae) and *Vitex negundo* Linn. (Verbenaceae). Species-wise variation on flower morphology, flower and pollen anthesis were observed. Pollen production flower^{-1} ranged from 4000 to 613150 and log value for pollen production flower^{-1} varied from 3.60 (in *Vitex negundo*) to 5.79 (in *Aegle marmelos*). Apertural types and exine ornamentation pattern of pollen grains were noticed to be specie-specific. During day time different members of Thysanoptera, Hymenoptera, Lepidoptera, Diptera and Coleoptera visited the flowers and enhanced pollination success rate in terms of fruit/seed set which was ascertained by netting and bagging process. *In vitro* pollen germination study indicated that the requirements of sucrose and minerals like boron, calcium, magnesium and potassium for optimum germination varied from species to species. Stigmas were more receptive during first day after anthesis in *A. marmelos, A. chinensis, M. indica, T. arjuna and V. negundo.* However, *A. carambola, C. roseus* and *D. indica* showed more receptive stigmas during 2nd day after anthesis whereas stigma were more receptive during 3rd day after anthesis in case of *C. fistula* and *P. acerifolium*.

Keywords: Anthesis, floral biology, insect behaviour, pollen viability, stigma receptivity

INTRODUCTION

Pollination, the transfer of pollen grains from anther to receptive stigma, has a significant contribution to gene transfer and sexual reproduction of flowering plants. Plant-pollinator interactions are crucial in determining the community structure and function. Successful seed and fruit formation is largely dependent on pollination success rate (PSR) which subsequently depends upon the availability of good pollinators, pollen amount, load, viability, stigma form, surface and receptivity. Scientists throughout the world are now worried about species extinction. Survival and propagation of plant species in altered environment needs some new adaptive features gained from plant-pollinator behavior. Agriculturalists now fear from minimum crop production which may be due to the non-availability of pollinators because the pollinators could be wiped out owing to industrialization, urbanization and intense pesticide

application in crop fields. Pollination is the mechanism, which leads to the first male-female interaction and subsequent recognition of events to determine compatible or incompatible pollen. In angiosperms this process is complex as it is associated with the behaviour of pollen vectors and floral characteristics to promote maximal pollination success. As angiosperm flowers vary greatly in structure and morphology, different types of pollinators have been observed. Recently, some information on pollination biology of some angiosperms have been made available by Atluri *et al*. (2000); Castro *et al*. (2008); Thien *et al*. (2009) and Singh and Dhakre (2010). In spite of having large medicinal importance little attention has been paid to the pollination biology of selected taxa which is essential to understand the evolution and survival of the species to develop effective conservation strategies and optimum utilization of the economic potential of the plant resources. Keeping this view a detailed study on the floral biology, pollination mechanism, pollen germination and stigma receptivity of ten selected medicinal plants like *Aegle marmelos, Anthocephalus chinensis, Averrhoa carambola, Cassia fistula, Catharanthus roseus, Dillenia indica, Madhuca indica, Pterospermum acerifolium, Terminalia arjuna* and *Vitex negundo,* growing at various places of West Bengal, has been undertaken.

MATERIALS AND METHODS

Different flowering phenology parameters like anthesis, anther dehiscence and pollen release were studied following the method of Mathur and Mohan Ram (1986). The flower buds of different plants were tagged with thread and observed at every 2 h interval for 24 h. Ten different plants of each species growing at different places of lateritic belt (Birbhum, Burdwan, Murshidabad, Nadia, Bankura and Purulia districts) of West Bengal were selected for this observation. The flower visitors and their mode of visiting were observed for more than 3 subsequent years at different seasons, times of a day and photographs were taken by using Nikon camera fitted with telezoom lens. Ten flowers/inflorescences plant^{-1} were labeled for the study of flowering phenology and pollination mechanism in each case. To assess the biological potentialities i.e. productivity of pollen grains flower^{-1} of selected plants, the method of Mandal and Chanda (1981) was followed. For detailed morpho-features of pollen grains with the help of light microscope, temporary and permanent slides were prepared as per Erdtman (1960).

After flower opening, many flower visitors were found to visit flowers for fulfilling their demand of nourishment. Nature and behaviour of flower visitors were critically observed visually. Mode and period of visiting time was noticed minutely. The flower visitors, especially different types of insects, were collected with a hand-held insect catching net from 600 to 1800 h over 3 consecutive days. Observations were made on the timing of visitation, foraging behaviour and the number of insect visitors. Insects were killed in a glass container containing cotton saturated with CCl_4 and preserved in 70% ethanol. The hard bodied insects were dried and preserved intact in insect boxes containing naphthalene dust. The identity of insects was confirmed by an eminent entomologist Prof. Dr. P. Halder, Dept. of Zoology, Visva-Bharati University, West Bengal. Some insects were identified with the help of reference materials, preserved in the laboratory, previously identified by the Zoological Survey of India, Kolkata.

To obtain surface ornamentation of pollen grains through scanning electron microscopy (SEM), the acetolysed pollen samples (Erdtman, 1960) were mounted on the aluminum stubs and successively sputter coated with gold. After gold coating it was viewed and photographs taken using microscope (P SEM 500) at low voltage from RSIC Bose Institute, Kolkata. To study the receptive stigma surface pattern by SEM, the method suggested by Ciampolini *et al.* (1996) was followed with slight modification. To know the effect of different nutrients like sucrose, H_3BO_3, $Ca(NO_3)_2.4H_2O$, KNO_3 and $MgS0_4.7H_2O$ at various concentrations and to study the pollen viability, *in vitro* pollen germination of selected taxa were carried out. For this, different grades of sucrose (1-50%), H_3BO_3 (25-1200 µg ml^{-1}), Ca $(NO_3)_2.$ $4H_2O$ (25-800 µg ml^{-1}), KNO_3 (25-500 µg ml^{-1}) and $MgSO_4.7H_2O$ (25 -1000 µg ml^{-1}) were prepared and used either individually or in combination as applicable. The above mentioned medium

was utilized and pollen grains germinated in these modified medium (Brewbaker and Kwack, 1963). A drop (50 µL) of each solution was kept into each groove of groove slides individually or in combination. The fresh pollen samples immediately collected from nascent dehisced anthers of same or different flowers were put with platinum needle into each groove containing solution. The slides were then kept into petridishes lined with moist filter paper at laboratory condition. The observations were taken at different time intervals under Olympus microscope at low magnification (10x10X). Random counting of at least 500 pollen grains (germinated and un-germinated) at different microscopic fields was done and average percentages of germinated pollen grains calculated (Shivanna and Rangaswamy, 1993). To study the *in vivo* pollen behaviour and germination along with stigma receptivity the method of Bhattacharya and Mandal (2000) was followed.

RESULTS AND DISCUSSION

Specific floral features suggested that the pollen anthesis took place before, after or simultaneously with flower anthesis (Table 1). If the anthers open before anthesis, the pollen is only exposed once the flower opens. If flower opens first, pollen grains are limited by anther dehiscence (Pacini, 1992). The anthesis and pollen release are important criteria for subsequent dispersal of pollens which is controlled by many physical, physiological and biological factors (McCartney, 1994). Both anthesis and anther dehiscence are species dependent and are influenced by environmental conditions (Nepi and Pacini, 1993; Lisci *et al.,* 1994). Pollen production flower^{-1} ranged from 4,000 to 6,13,150 and log value for pollen production flower^{-1} varied from 3.60 (in *Vitex negundo*) to 5.79 (in *Aegle marmelos*). Apertural types and exine ornamentation pattern of pollen grains were of different types (Table 1, Fig. 1). The biological potentiality of an individual flower in an inflorescence could be known by counting the total pollen grains flower^{-1}. The pollen number may differ from individual anther of a single flower. It also differs

Table 1: Floral biology of selected plants

Name of the plants with family	Flower (corolla) type	Flower anthesis pattern and time (h)	Pollen anthesis time (h)	Pollens anther^{-1} (No.)	Pollens flower^{-1} with log value (No.)	Type of pollen aperture
Aegle marmelos (Rutaceae)	Open	Forenoon (6.00-9. 00)	6.00 - 9.00	12263	613150 (5.79)	3-colporate
Anthocephalus chinensis (Rubiaceae)	Tubular	Early morning (5. 00-6. 00)	3.00 - 5 00	1141	4566 (3.66)	3-colporate
Averrhoa carambola (Oxalidaceae)	Sub-tubular	Forenoon (6.00-9.00)	10.00-12.00	1400	7000 (3.85)	3-colporate
Cassia fistula (Caesalpiniaceae)	Open	Evening (17.00-20.00)	6.00 - 8.00	14834	148345 (5.17)	3-colpate
Catharanthus roseus (Apocynaceae)	Tubular	Evening (21.00-1.00)	4.00-10.00	1543	7715 (3.89)	3-colpate
Dillenia indica (Dilleniaceae)	Open	Forenoon (9.00-11.00)	10.00-12.00	664	106904 (5.02)	3-4-colpate
Madhuca indica (Sapotaceae)	Tubular	Mid day (5.00-12.00)	6.00-15.00	4225	101400 (5.00)	4-5-colporate
Pterospermum acerifolium (Sterculiaceae)	Open	Evening-mid night (17.00-24.00)	18.00 - 2.00	9036	135540 (5.13)	3-porate
Terminalia arjuna (Combretaceae)	Sub-tubular	Forenoon (6.30-9.30)	8.00-10.00	5766	57658 (4.76)	3-colporate
Vitex negundo (Verbenaceae)	Tubular	Forenoon (8.00-11.00)	8.00-11.00	1000	4000 (3.60)	3-colpate

from anther to anther, flower to flower and plant to plant with reference to dispersal (Mandal and Chanda, 1981), in anther and flower (Mandal and Ray, 1984), in anther, flower and whole plant (Mandal and Yanzone, 1987). It also differs in terms of anther number, anther length, filament length, pollen grains size and mode of anther dehiscence (Mondal and Mandal, 1998). The variation in pollen productivity may be due to the difference in the number of pollen grains anther^{-1}, anthers flower^{-1}, flowers inflorescence^{-1} and inflorescences tree^{-1}. After flower and pollen anthesis different flower visitors (Fig. 1) belonging to Thysanoptera, Hymenoptera, Lepidoptera, Diptera and Coleoptera visited the flowers for searching their forage materials. Social bees of the genera *Apis*, *Bombus*, *Trigona* and the solitary bees like *Xylocopa* and *Megachile* and other insects *Ceratina viridissima, Eumenes conica,*

Table 2: Flower visitors, forage materials and visited plants

Name of the visitors	Visiting period	Forage materials	Visited plants
Thysanoptera			
Heterothrips sp.	Day & night	Pollen & nectar	C*assia fistula*
Thrips hawaiiensis	Day & night	Pollen & nectar	*Anthocephalus chincnsis, Madhuca indica, Pterospermum acerifolium*
Haplothrips	Day & night	Pollen & nectar	*Pterospermum acerifolium*
Megalothrips sp.	Day & night	Pollen & nectar	*Anthocephalus chinensis, Pterospcrmum acerifolium*
Hymenoptera			
Apis sp.	Day	Pollen & nectar	*Aegle marmelos, Anthocephalus chinensis, Averrhoa carambola, Dillenia indica, Terminalia arjuna, Vitex negundo*
Xylocopa sp.	Day	Pollen & nectar	*Cassia fistula, Dillenia indica, Vitex negundo*
Mcgachile sp.	Day	Pollen & nectar	*Aegle marmelos, Anthocephalus chinensis, Averrhoa carambola, Dillenia indica, Terminalia arjuna, Vitex negungo*
Vespa sp.	Day	Nectar	*Aegle marmelos, Vitex negundo*
Ceratina viridissima	Day	Pollen & nectar	*Vitex negundo*
Eumenes conica	Day	Pollen & nectar	*Cassia fistula, Dillenia indica, Vitex negundo*
Bombus sp.	Day	Pollen & nectar	*Cassia fistula*
Trigona sp.	Day	Pollen & nectar	*Anthocephalus chinensis. Dillenia indica*
Euphosia sp.	Day	Pollen & nectar	*Dillenia indica, Terminalia arjuna*
Perdita sp.	Day	Pollen & nectar	*Terminalia arjuna, Vitex negundo*
Formicidae	Day & night	Nectar	*Aegle marmelos, Averrhoa carambola, Madhuca indica*
Ichneumonidae	Day	Pollen & nectar	*Dillenia indica*
Lepidoptera			
Papilionidae	Day	Nectar	*Aegle marmelos, Anthocephalus chinensis, Dillenia indica, Vitex negundo*
Nyinphalidae	Day	Nectar	*Aegle marmelos, Anthocephalus chinensis, Dillenia indica, Vitex negundo*
Pieridae	Day	Nectar	*Anthocephalus chinensis, Terminalia arjuna, Vitex negundo*
Sphingidae	Day	Nectar	*Catharanthus roseus*
Diptera			
Musca sp.	Day	Nectar	*Aegle marmelos. Vitex negundo*
Coleoptera	Day	Pollen & nectar	*Terminalia arjuna, Vitex negundo*

Euplusia, Perdita and some members of Formicidae, Ichneumonidae and the members of Papilionidae, Nymphalidae, Pieridae and Sphingidae were also observed to visit the flowers (Table 2). During forage time the flower visitor act as pollinators, the pollen being deposited either on their ventral side or dorsal sides. The contribution of bees as pollinators has long been recognized (Singh and Dhakre, 2010). As per the conservative estimates insects accomplish 80% of pollination (Ish-Am and Eisikowitch, 1993). The flowers are exploited by butterflies for nectar only. While foraging flowers for nectar, the pollen grains adhere to their body parts such as proboscis, head, thorax, legs and wings, though the exact pollen region depend on the floral architecture (Fig. 3). The pollen adhered is likely to be transferred on the stigmatic surface when the butterflies move to another non-specific flower, thus performing pollination (Balasubramanian, 1992). The pollinating activities of ants are irregular and unreliable. The Coleopterans (beetles) are the oldest group of insects which are accidental visitors. Their body is not so well accustomed for pollen transport. The mouth-parts of beetles are modified for pollen chewing. However, the roles of thrips, Coleopteran and Dipterans in pollination have long been established (Faegri and vander Pijl, 1980).

Medium composition for optimum *in vitro* pollen germination varied greatly depending upon the species. The pollens of *Anthocephalus chinensis* germinated in low sucrose concentration (5%) whereas sucrose requirement was high (30%) for *Pterospermum acerifolium* pollen germination. The sucrose concentration for *in vitro* pollen germination varied from 1 to 30% depending upon species (Table 3). It appears that sucrose maintains osmotic pressure of the medium, and also acts as a substrate for pollen metabolism (Shivanna and Johri, 1989). This is attributed to the fact that sucrose alone is necessary for proper pollen nutrition, osmotic control and possibly for other reasons but sucrose in combination with H_3BO_3 promoted pollen germination because boron makes a complex with sucrose which may be easily translocated than sucrose alone. The role of boron has been confirmed in germinating pollen and growing pollen tubes (Shivanna and Johri, 1989). This view gets support from the findings of Kaliamoorthy *et al.* (1996); Castro *et al*. (2008); Thien *et al*. (2009) and Singh and Dhakre (2010). Species-based variation on morphology and receptivity of stigmas was noticed (Table 4; Fig. 2). Stigma receptivity measured through *in vivo* pollen germination (Figs. 2 & 4) and pollen behaviour study which was found to be highest (70.5%) in *Catharanthus roseus* during second day after anthesis whereas it was found to be lowest (48.5%) in *Aegle marmelos* during first day after anthesis (Table 4). Receptivity of the stigma as well as stigma surface and *in vivo* pollen behaviour in the context of pollen-stigma interaction are critical factors for the successful completion of post-pollination events. Receptivity is generally maximal soon after anthesis. The period of receptivity varies from

Table 3: Medium composition for optimum *in vitro* pollen germination of selected plants

Name of the plants	Optimum pollen germination (%)	Pollen tube length (µm)	Medium composition
Aegle marmelos	69	275	15% sucrose + 400 µg ml^{-1} boric acid + 300 µg ml^{-1} calcium nitrate
Anthocephalus chinensis	91	642	5% sucrose + 100 µg ml^{-1} boric acid
Averrhoa carambola	76	317	25% sucrose + 300 µg ml^{-1} boric acid
Cassia fistula	78	1945	10% sucrose + 300 µg ml^{-1} boric acid
Catharanthus roseus	87	1865	20% sucrose + 200 µg ml^{-1} boric acid
Dillenia indica	74	526	20% sucrose + 300 µg ml^{-1} boric acid
Madhuca indica	85	375	5% sucrose + 500 µg ml^{-1} boric acid
Pterospermum acerifolium	64	347	30% sucrose + 700 µg ml^{-1} boric acid
Terminalia arjuna	58	169	25% sucrose + 100 µg ml^{-1} boric acid
Vitex negundo	76	359	10% sucrose + 400 µg ml^{-1} boric acid

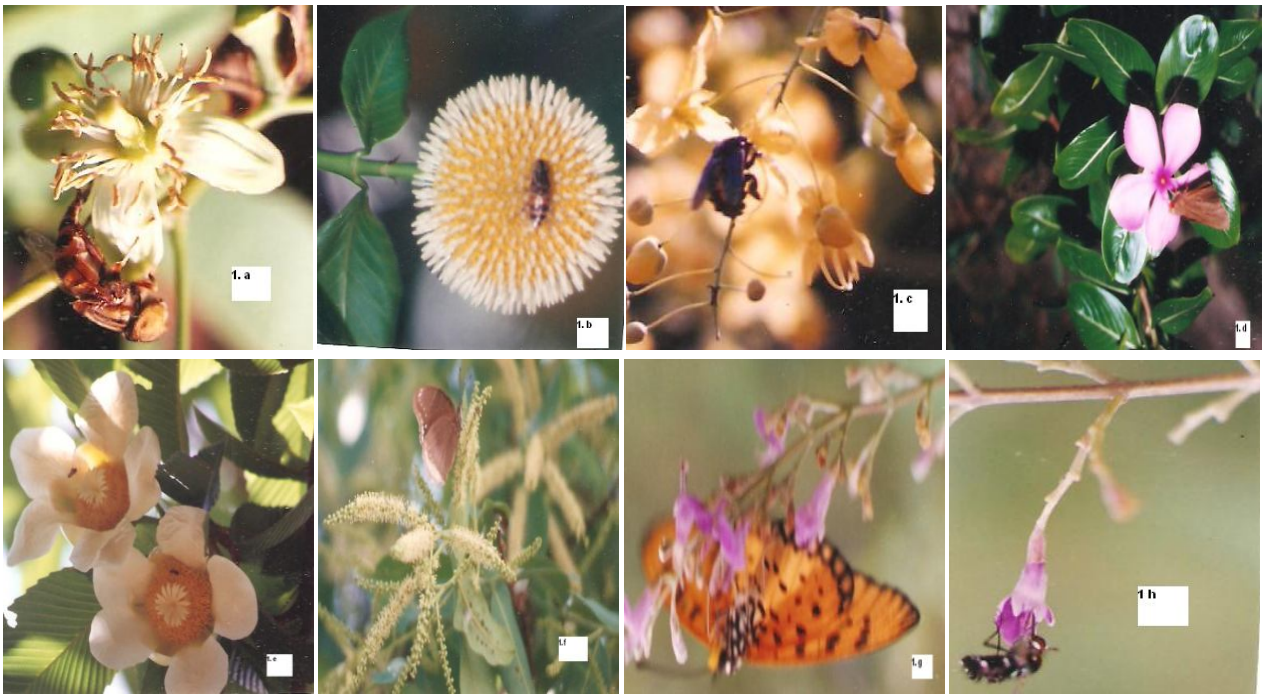

Fig. 1: Insects visiting various flowers (upper row from left to right) i. Bee on *Aegle marmelos;* ii. Honey bee on *Anthocephalus chinensis;* iii. *Xylocopa* on *Cassia fistula*; iv. Butterfly on *Catharanthus roseus*; (lower row from left to right) i. Honey bee on *Dillenia indica;* ii. Butterfly on *Terminalia arjuna*; iii. Butterfly on *Vitex negundo* and iv. Honeybee on *Vitex negundo*

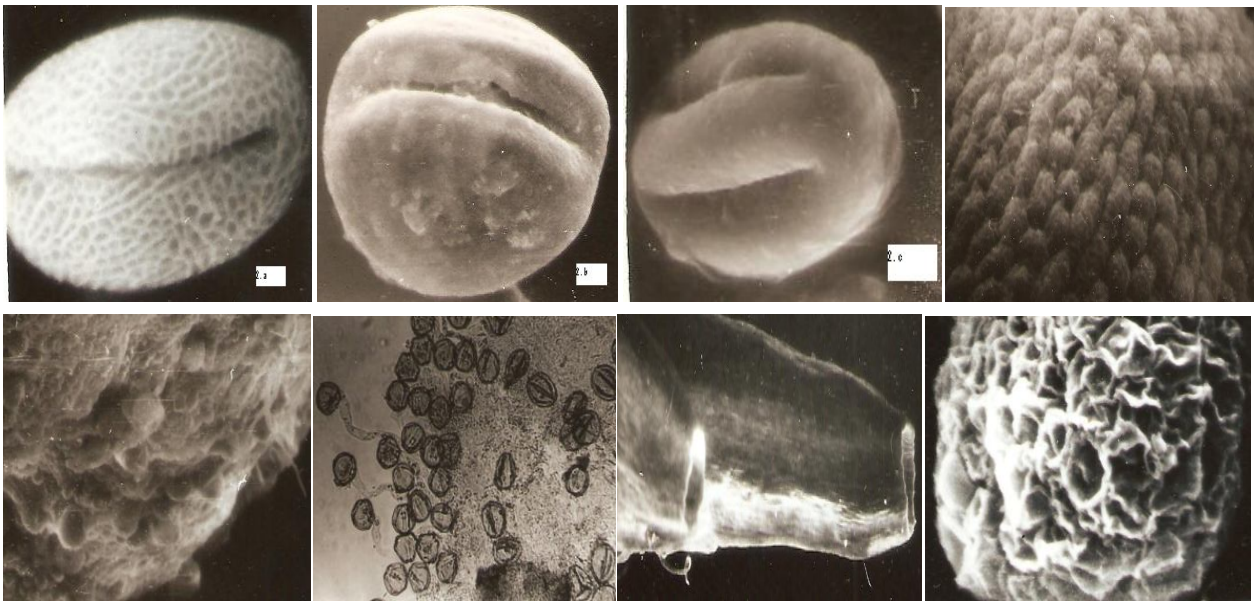

Fig. 2: SEM (Upper row from left to right) i. *Aegle marmelos* pollen grain (3-colporate with reticulate exine) X200; ii. *Cassia fistula* pollen grain (3-colporate with psilate exine) X200; iii. *Vitex negundo* pollen grain (3-colpate with psilate exine) X250; iv. Stigma surface of *Aegle marmelos* showing clavate shaped papillae cells X300; (Lower row from left to right) i. Stigma surface of *Pterospermum acerifolium* showing *in vivo* pollen and lobed papillae cells X300; ii. *In vivo* germinating pollens on *Catharanthus roseus* stigma X400; iii. Hollow stigma surface of *Terminalia arjuna* showing long unicellular papillae cells X250; iv. Stigma surface of *Dillenia indica* showing rhomboidal papillae cells X300.


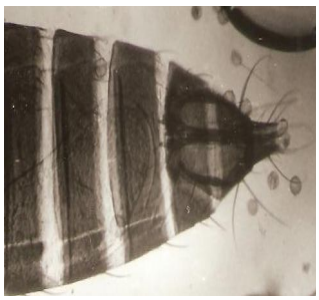

Fig. 3: Pollen grains adhered to the body surface of thrips collected from A) *Pterospermum acerifolium* flower X100; B) *Madhuca indica* flower X100

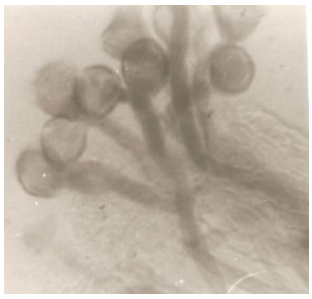
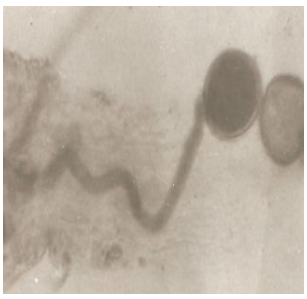

Fig. 4: *In vivo* germinating pollens on A) *Averrhoa carambola* stigma X400; B) *Vitex negundo* stigma X400

Table 4: Stigma receptivity and *in vivo* pollen germination of selected plants

Name of the plants	Maximum stigma receptive period	*In vivo* germinating pollen (%)	Pollen tube length (μm)
Aegle marmelos	First day after anthesis	48.46	216.6
Anthocephalus chinensis	First day after anthesis	52.69	116.5
Averrhoa carambola	Second day after anthesis	60.83	204.6
Cassia fistula	Third day after anthesis	49.46	106.5
Catharanthus roseus	Second day after anthesis	70.52	285.8
Dillenia indica	Second day after anthesis	56.28	96.2
Madhuca indica	First day after anthesis	66.66	392
Pterospermum acerifolium	Third day after anthesis	53.28	247
Terminalia arjuna	First day after anthesis	63.41	123
Vitex negundo	First day after anthesis	60	175

Table 5: Pollination success rate in terms of fruit seed^{-1} set (%) of selected plants under variable conditions

Name of the plants	Netting condition	Bagging condition	Control condition
Aegle marmelos	-	-	12.5
Anthocephalus chinensis	46.6	5.0	90.0
Averrhoa carambola	-	-	67.6
Cassia fistula	0.5	-	12.8
Catharanthus roseus	27.3	26.9	87.5
Dillenia indica	-	-	96.0
Madhuca indica	26.6	21.5	39.0
Pterospermum acerifolium	3.5	-	14.6
Terminalia arjuna	-	-	4.0
Vitex negundo	-	-	61.3

- = No pollination success

species to species and may be influenced by temperature and humidity (Kaliamoorthy *et al.*, 1996; Bansal *et al.*, 1997; Castro *et al*., 2008; Thien *et al*., 2009 and Singh and Dhakre, 2010). No fruits/seeds set up were noticed in *Aegle marmelos, Averrhoa carambola, Dillenia indica, Terminalia arjuna* and *Vitex negundo* under netting and bagging conditions indicating the role of pollinators in successful pollination of these plants. Minor role of flower visitors were observed in successful pollination and fruit set of *Anthocephalus chinensis, Cassia fistula, Catharanthus roseus, Madhuca indica* and *Pterospermum acerifolium* as the extent of fruit/seed set was negligible in these taxa. Moreover, all plants showed higher degrees of fruit/seed set under controlled/open flowers conditions compared to the flowers

under netting and bagging conditions (Table 5) thereby reflecting enhanced pollination success rate in terms of fruit/seed set up. This finding is concomitant with the earlier works (Mathur and Mohan Ram, 1986; Atluri *et al.,* 2000; Bhattacharya and Mandal, 2000; Castro *et al*., 2008 and Thien *et al*., 2009).

Conclusion: From present study it may be concluded that species base variation on flower morphology, floral and pollen anthesis, pollen morphology, pollen productivity in relation to biological efficiencies of plants, standardization of medium composition for obtaining maximum *in vitro* germinating pollens, morphology and receptivity of stigmas, *in vivo* pollen behaviour were noticed reflecting the adaptability of plants for their sustainable occurrence under critical environment. Pollination success rate could be enhanced by exposing the open flowers to a large number of flower visitors and pollinators which of course are essential for conservation of floristic gene pool.

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