POLLINATION ECOLOGY OF SOME ASCLEPIADS (ASCLEPIADACEAE) FROM PAKISTAN

by

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By

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ABSTRACT

Pollination ecology of 10 Asclepiads viz. *Calotropis procera* (Ait.) Ait.f. ssp. *hamiltonii*, *Glossonema varians* (Stocks) Hook. f., *Oxystelma esculentum* (Linn. f.) R. Brown, *Pentatropis nivalis* (Gmel.) Field and Wood, *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L. of tribe Asclepiadeae and *Caralluma edulis* (Edgew.) Benth., *Caralluma tuberculata* N.E.Brown, *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Decne. of tribe Ceropegieae has been carried out from Pakistan. In this connection floral phenology and morphology, location of germination locus, in-vitro pollinium germination, floral visitors and their pollinium load, level of pollinator activity in natural populations, mechanism of pollinarium removal and pollinium insertion, pollen ovule ratio and ratio of pollen grains per pollinium to ovules per carpel have been studied.

Andromonoecy is being reported for the first time in *Glossonema varians* (Stocks) Hook. f. and in the family. Flower life of the studied taxa is usually short ranges only from 1-3 days.

A variety of structural adaptations have been revealed in response to the mode of pollination, particularly in pollinium morphology and orientation, stigmatic chamber, coronal elements, nectaries and nectar location and presentation.

It has been demonstrated that pollinarium removal and pollinum insertion are specialized processes rather than a chance and exhibit definite relationship with the specialized floral modifications. Apart from presence of residual corpusculum and narrow-

ness of stigmatic chambers, other three factors like closing of stigmatic chamber, presence of glandular papillae in the stigmatic chamber and anther wings are reported to be responsible for the proper and successful insertion of the pollinium into the stigmatic chamber. A correlation between pollinarium removal and pollinium insertion exist and thus revealed that pollinium insertion rates are the positive function of removal rates.

Pollinators (insects) are species as well as tribe specific. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali is regarded as *Xylocopa* blossom (Melittophilous), *Glossonema varians* (Stocks) Hook. f. and *Oxystelma esculentum* (Linn. f.) R. Brown as bee and wasp blossom (Melittophilous), *Pentatropis nivalis* (Gmel.) Field and Wood, *Pergularia daemia* (Forssk.) Chiov., and *Pergularia tomentosa* L. as bee and moth blossom (Melittophilous and Phalaenophilous), *Ceropegia bulbosa* Roxb. as fly blossom (Myophilous) and *Leptadenia pyrotechnica* (Forssk.) Decne. as fly and beetle blossom (Myophilous and Cantharophilous).

The germination locus of the pollinium seems to be correlated with the flower structure and pollination mechanism of the species. Pollinium of each species required a specific sugar conentration for optimal germination (in vitro - pollinium germination) which is more or less similar to its floral nectar sugar concentration.

A relationship has also been observed between pollinium vigor (in - vitro), pollinarium removal and pollinium insertion with respect to flower age. However, pollinium vigor and pistil receptivity did not change significantly with the flower age.

Low pollen ovule ratios seems to be the characterstic feature of Asclepiads. Pollen ovule ratios have been correlated with a number of floral characters, pollinator type and pollinator activity.

Number of floral and particularly reproductive characters such as pollinium orientation and morphology, germination locus, pollinator type, pollinator activity and pollen ovule ratios provide additional evidence in support of the tribal classification.

It has been concluded that Asclepiads possess a high degree of synorganization of variety of structural elaborations and functional process of pollination.

خلاصہ

پاکستان سے مدار کے دو قبائل کے پودوں جیسا کہ *Calotropis procera*
Glossonema varians (Stocks) Hook.f., (Ait.) Ait.f.ssp.*hamiltonii*,
Pentatropis nivalis *Oxystelma esculentum* (Linn. f.) R. Brown,
Pergularia , *Pergularia daemia* (Forssk.) Chiov., (Gmel.)
tomentosa L. قبیلہ Asclepiadeae کے اور *Caralluma edulis* (Edgew.) Benth
Ceropegia bulbosa Roxb. *Caralluma tuberculata* N.E.Brown,
Leptadenia pyrotechnica (Forssk.) Decne.
Ceropegieae قبیلہ کے) کی محوالیات زیرگی کا تجزیہ کیا گیا ہے ۔

اس سلسلے میں زہری ماحول حساسیات اور شکلیات ، روئیدگی کی ناف کا تعین ۔ زیرہ تودے کی روئیدگی *in-vitro* میں ۔ زہری ملاقاتیوں اور ان کے زیرے تودے کے وزن ۔ فطری آبادی میں زیرگی حشریات کی سرگرمیاں اور زیرہ تودے کی علیحدگی اور ادغام کا عمل ، زردانہ تخمک کی نسبت ، زردانے فی زیرہ تودہ کی نسبت اور تخمک فی ثمرہ برگ معلوم کیے گئے ہیں ۔

Andromonoecy) پہلی بار *Glossonema varians* (Stocks) Hook. f.
میں اور اس خاندان میں رپورٹ کی جا رہی ہے ۔

تحقیقاتی پودوں کی پھواست بہت ہی تھوڑے عرصہ کی ہے ۔ صرف ایک سے تین ۳ ۔

موجودہ تحقیق میں مختلف اقسام کی ساخت کی مطابقت بھی ظاہر ہوتی ہے زیرگی کی بہت کے ردعمل میں ۔ خاص طور پر زیرہ تودے کی شکلیات اور سمعت میں ۔ سربقچوی خانہ ، اکنابلی عناصر ۔ شہدانوں اور شہد کے مقام اور نمائش سے بھی ثابت ہوا ہے کہ زیرہ تودے کی علیحدگی اور ادغام اختصاصی عمل میں قدرے امکانی سے ۔ اور اختصاصی زیری ردوبدل سے خاص تعلق ظاہر کرتے ہیں ۔

باقی مانندہ زرہ کی موجودگی اور سربقچوی خانہ کی باریکی کے علاوہ تین دوسرے عناصر مثل سربقچوی خانہ کا بند ہونا ، سربقچوی خانہ میں محدودی شالیل

حاند کی موجودگی اور پر والے زردان ہی اصل میں سربقچوی خانہ میں زیرہ تودے کے کامیاب ادغام کے ذمہ دار ہیں ۔ (نحل)

Calotropis کو " شگوفہ بھنورا " کہا جاسکتا ہے ، اسی طرح *Glossonema* اور *Oxystelma* کو نحل اور بھڑ کا شگوفہ *Pentatropis* اور *Pergularia* کو نحل اور پروانہ کا شگوفہ ، *Ceropegia* کو مکھی کا شگوفہ اور *Leptadenia* کو مکھی اور بھنورا شگوفہ کہا جاسکتا ہے ۔

زردانہ تعمک نسبت کی کمی مدار کے پودوں کی امتیازی صفت معلوم ہوتی ہے زردانہ تعمک نسبت کا بہت سے زہری صفات اور زیرگی حشریات کی اقسام اور ان کی سرگرمیوں سے گہرا تعلق ہے ۔

بہت سے زہری صفات خاص طور پر تولیدی صفات جیسے زیرہ تودے کی سمت اور شکل ، روئیدگی کا مقام ، زیرگی حشریات کی اقسام ، رودن کی سرگرمیاں اور زردانے تعمک نسبت قبائلی درجہ بندی کی تائید میں زائد ثبوت فراہم کرتی ہیں ۔
میں زیرہ تودے کی اعلی روئیدگی کے لیے مخصوص تمرکز کی شکر ضروری ہوتی ہیں جو کہ زہری شہد کی تمرکز کے متشابہ ہے ۔

CHAPTER 1

GENERAL INTRODUCTION

1.1 **Introduction:**

The family Asclepiadaceae is commonly known as "Milkweed family". The opposite exstipulate leaves, gamopetalous corolla, staminal column, corona, gynostegium, pollinarium/tetrads, follicular fruits, flattened , silky comose seeds are the characterstics of the family.

1.2 **Taxonomic History and Classification:**

Linnaeus was the first to list a number of Asclepiads such as *Asclepias*, *Ceropegia*, *Cynanchum*, *Pergularia* etc in his "Species Plantarum" (1753) and "Genera Plantarum" (1754). Jussieu (1789) placed all known Asclepiads with Apocynads under the natural order Apocyneae (family Apocyraceae). Brown (1810) separated his natural order Asclepiadeae from the Apocyneae of Jussieu on the basis of the highly specialized pollinia of the former. He divided the family into the following three groups as "subordo".

1. Asclepiadeae verae
2. Secamone group
3. Periploceae

Endlicher (1838) followed Brown (1810) and proposed a system that now appears to reflect suprageneric relationships within the Asclepiadaceae more accurately than does any other. However, he placed the "Periploceae' first, the "Secamoneae" second and the "Asclepiadeae verae" third [Rosatti, (1989) pointed out that acc-

"Asclepiadeae verae" third [Rosatti, (1989) pointed out that according to Article 18.3 (ICBN, 1988) these names should be treated as names of subfamilies] The "Asclepiadeae verae" was divided into three tribes differing in orientation of the pollinia, i.e. "Cynancheae" (pendulous), "Gonolobeae" (horizontal) and "Pergularieae" (erect). The "Pergularieae" was separated into two subtribes, "Hoyeae" and "Stapelieae", with and without terminal membranous anther appendages, respectively. On the basis of the corolla and corona characters, "Cynancheae" into (6 groups) and the two subtribes of "Pergularieae" were divided into (2 groups each) groups of unspecified rank.

Decaisne (1844) recognized Brown's 3 groups (subordo) as tribes with the addition of three new ones, i.e. Periploceae, Secamoneae, Asclepiadeae verae, Gonolobae, Stapeliae and Ceropegieae.

Bentham (1869) divided the family into two groups as subordo "Periploceae" and subordo "Euasclepiadeae". The Euasclepiadeae was further divided into 6 tribes i.e. Secamoneae, Cynancheae, Gonolobeae, Marsdenieae, Ceropegieae and Stapelieae.

Schumman (1895) recognised 2 groups in the family as subfamilies the "Periplocoideae" and the "Cynanchoideae" which were further divided into 5 tribes. The tribe "Tylophoreae" was further distinguished into 2 subtribes as " Ceropeginae" and "Marsdeninae".

Schlechter (1905) for the first time separated the subfamily "Periploceae" from the "Asclepiadaceae" as an independant family the "Periplocaceae".

Woodson (1964) followed Schumann's (1895) treatment however he raised the rank of Schumann's two subtribes i.e. "Marsdenieae" and "Ceropegieae" into tribes.

Woodson (1954) considered the structure of the pollinia much more important than their orientation and on this basis recognized 3 tribes namely " Aclepiadeae", "Tylophoreae" and "Gonolobeae" in the subfamily " Asclepiadoideae" in N. America.

Bullock (1958) accepted the Schlechter's (1905) separation of the "Periplocaceae" as an independent family. He divided the family " Asclepiadaceae " into 2 subfamilies the " Secamonoideae" and the "Asclepiadoideae", in later. 5 tribes were recognized and Stapelieae was including in the Ceropegieae.

A number of worker like Hutchinson (1959), Santapau and Irani (1962), Riley (1963), Airy Shaw (1973), Huber (1973, 1983) and Field (1980) etc. accepted Schlehter's (1905) and Bullock's (1958) treatment. However, Takhtajan (1967), Cronquist (1968), Markgraf (1972), Rechinger f. (1973), Spellman (1975), Stevens (1976) and Ali (1983) have not recognized these two groups as separate families and consider them as 2 subfamilies of the family Asclepiadaceae. Whereas Rosatti (1989) followed Endlicher's system (1838) of dividing the family into 3 subfamilies. Recently, Bruyn's and Forster (1991) also recognized 3 subfamilies following Endlicher (1838) . However they proposed 4 tribes in subfamily Asclepiadoideae i.e. Asclepiadeae, Gonolobeae, Marsdenieae and Staplieae (including Ceropgieae).

1.3 Evolution and relationship with other families:

One of the most significant families of flowering plants from the evolutionary point of view is the Asclepidaceae. Bentham and Hooker (1876) in their system of classification placed the Asclepiadaceae together with the Oleaceae, Selvadoraceae, Apocynaceae, Loganiacee and Gentianceae in the order Gentianales. Engler (1887) placed this family with Apocynaceae in Contorteae. Hallier (1905) included Asclepiadaceae with Apocynaceae, driving them from Linaceae. Hutchinson (1969) split up the order Gentianales into 3 separate orders, Gentianales, Loganiales and Apocynales. In Apocynales, he recognized 4 families, Apocynaceae, Asclepiadaceae, Plocospermataceae and Periplocaceae, mainly on their pollen characters. According to him, Apocynales are more advanced than Longaniales and treated Asclepiadaceae as a "Climax" family, in which the pollen has become waxy and collected into masses (Pollinia) as in Orchidaceae of the Monocotyledons. Rosatti (1989) proposed a suborder Apocyninceae with in Gentianales which includes Apocyniaceae and Asclepiadaceae only.

The Asclepiadaceae is most closely related to and is more likely to be derived from Apocynaceae. As pointed out by Cronquist (1968), in various aspects of floral morphology there are evolutionary progressions from the Plumerioideae to the Apocynoideae of the Apocynaceae, and from there into the Asclepiadaceae, in which the sequence proceeds from the Periplocoideae to the Secamonoideae and the Asclepiadoideae.

The Apocynaceae and Asclepiadaceae share a number of features including those having to do with morphology, embryology, anatomy and chemisty; many of the frequently cited differences may not hold after the plants are more thoroughly known. The apparent lack in the Apocynaceae and universal presence in the Asclepiadaceae of special structures for the pollen transport has been widely believed to be the single most reliable difference between the families.

1.4 Pollination History:

Pollination in Asclepiadaceae, specially in subfamily Asclepiadoideae comprises some of the most complex floral mechanisms is angiosperms. The flower structure and mode of pollination in Asclepiadaceae (*Asclepias*) have been studied by Koelreuter and Sprengel in the eighteenth century and Brown, Delphino, Hildebrand, Corry and Mullar in the nineteenth century (Galil and Zeroni, 1965). Reexamination of floral morphology of *Secamone* sp. and *Asclepias* sp. by Safwat (1962) and Galil and Zeroni (1965, 1969), respectively, have dealt with the functional and evolutionary significance of the extremely complex floral modifications in the Asclepiadaceae.

Quantitative studies of the reproductive biology of *Asclepias* were atttempted in 1940's, when potential commercial uses of milkweeds were investigated (Fischer, 1941; Whiting, 1943; Stevens, 1945; Moore, 1946a and b, 1947; Sparrow and Pearson, 1948).

Frost (1965) and Macior (1965) have shown that species of *Asclepias* may differentially attach pollinaria on various parts of a pollinator's body and have suggested that this type of mechanical isolating mechanism may play a role in speciation in the genus.

Analysis of nectar production and pollinator behaviour was done for several species of *Asclepias* (Galil and Zeroni, 1965;

Willson and Bertin, 1979; Willson, Bertin and Price, 1979; Bertin and Willson, 1980; Wyatt, 1980b; Morse , 1981, 1982; Fritz and Morse, 1981; Southwick, 1983; Sothwick and Soutwick , 1983) and Kephart, 1983).

Nectaries of *Asclepias* and *Vincetoxicum* was studied by (Galil and Zeroni , 1969; Schnephf and Chirst, 1980 and Christ and Schnepf, 1985) while extrafloral nectaries of *Calotropis* and *Wattakaka* by Arekal and Ramakrishna (1980). Formation (as a secretion) of translator of *Asclepias* and *Gomphocarpus* was studied by Schnepf, Witzig and Schill (1979).

Detailed studies of spatial and temporal aspects of the floral display and relative importance of pollen vs resource limitation in restricting fruit level was also completed for several *Asclepias* species (Willson and Ratheke, 1974; Wyatt, 1976, 1980a, 1981 and 1982; Lynch, 1977; Willson and Price, 1977, 1980; Chaplin and Walker 1982; Bookman, 1983; Shannon and Wyatt, 1986).

The mechanics of pollination and the importance of mechanical vs. physiological reproductive isolation were studied by Wyatt (1976, 1978), Kephart and Heiser (1980), Beare and Perkins (1982), Morse and Fritz (1985).

Information regarding demography (Wilbur, 1976; Wyatt and Antonovics, 1981) and breeding systems (Wyatt, 1981; Kephart, 1981) was also gathered.

Morphogenesis and synorganization of stamens, morphology and evolution of corona in Asclepiadaceae was studied by Kunze (1981, 1982). The morphology, ontogeny and histochemistry of the twin pollinia and the translator apparatus of Asclepiads was studied by El-Gazzar, Hamza and Badawi (1974), Vijayaraghavan and Shukla (1976a, b; 1977 a,b); Vijayaraghavan and Cheema (1977), Shill and Jäckel (1978), Rao (1984), Rehman and Wilcock (1989) etc.

Volk (1950), Galil and Zeroni (1969), Linskens and Suren (1969), Jaeger (1971), Saoji and Chitaley (1975), Rao and Kumari (1979), Sreeedevi and Namboodiri (1982), Khatoon and Ali (1983), Schill and Dannenbaum (1984) and Eisikowitch (1986) studied the pollinium germination of some Asclepiads.

Although the pollination of the Asclepiads was investigated since the 1st. thorough study by Brown (1810), but the study was restricted only to few genera and by far the most attention was given to the genus *Asclepias*.

Pollination ecology of *Calotropis procera* (Ait.) Ait. f. was carried out by Jaeger (1971) in Senegal, Schremmer (1972) in Egypt, Eisikowitch (1986) in Israel, Bhatnagar (1986) in India and Ali and Ali (1989) in Pakistan, whereas *C. gigantea* by Van der Pijl (1954) in Java, Wanntorp (1974) in Ceylon and Ramakrishna et al. (1979) in India.

From Indo-Pak subcontinent morphology, ontogeny, histochemistry of twin pollinia, pollinium germination and floral morphology for several genera was carried out by Bhatnagar (1975), Vijayaraghavan and Shukla (1976 a , b), Vijayaraghavan and Cheema (1977), Deshpande and Joneja, (1982), Saoji and Chiltaley (1975), Rao and Kumari (1979), Sreedevi and Namboodiri (1982) and Khatoon and Ali (1983).

Pollination ecology pertaining to the pollinators and their behaviour of the genera like *Calotropis*, *Oxystelma*, *Holostemma*, *Asclepias, Marsdenia, Cosmostigma, Stapelia , Telosma, Pergularia, Cynanchum, Tylophora, Leptadenia and Gymnema* was carried out by Chaturvedi and Pant (1986). Chaturvedi (1987, 1989) and Bhatnagar (1986) from India. Pollination of *Sarcostemma* was studied by Liede and Whitehead (1991) from S. Africa and Kunze and Liede (1991) from Mexico.

Floral morphology with respect to pollination of seven genera (*Secamone, Tylophora, Leptadenia, Sisyranthus, Astephanus, Microloma and Vincetoxicum)* was studied by Kunze (1991).

The morphological studies and artificial pollination (in greeen house) of stapeliads was carried out by Bayer (1978a,b), Barad (1978), Manders (1979, 1980) and Jahnke (1989). While the pollinators of *Ceropegia* species were analysed by Vogel (1961) and Bayer (1978c).

This account shows that only few of more than 250 genera have been analysed thoroughly with respect ot their pollination ecology. So the present study is carried out from Pakistan to analyze the different aspect of the pollinaiton ecology of the ten species, belong to 8 genera, representing 2 tribes viz. *Calotropis Procera* (Ait.) Ait. f. ssp.*hamiltonii* (Wight) Ali, *Ceropegia bulbosa* Roxb., *Glossonema varians* (Stocks) Hook. f., *Caralluma edulis* (Edgew.) Hook. f., *Caralluma tuberculata* N. E. Br., *Pentatropis nivalis* (Gmel.) Field and Wight, *Pergularia daemia* (Forssk.) Chiov., *Pergylaria tomentosa* L., *Oxystelma esculentum* (L. f.) R. Br. and *Leptadenia pyrotechnica* (forssk.) Dcne.

1.5 Geographical Distribution:

The Asclepiadaceae is fairly large family with more than 250 genera and 3000 species (Rosatti, 1989), principally distributed in the tropical and subtropical regions of the world, with considerable representative in more temparate regions notably in South Africa. Represented in Pakistan by 23 genera and 41 species, however c.10 species are either under collected or need confirmation.

Distribution pattern of the studied taxa is as follows:

1. ***Calatropis procera*** **(Ait.) Ait. f. ssp.** ***hamiltonii*** **(Wight) Ali:**

It is distributed in Pakistan , India , Afghanistan ,Iran, Iraq and Oman. It is present in tropical to dry sandy part of Africa, extended the Mediterranian belt , Jordan, Arabia, Palestine , Abu Dhabi and introduced into the West Indies and tropical South and Central America (Ali, 1980, 1983; Spellman, 1975b).

It is distributed in all over the Pakistan with the exception of high altitudes and cold weather. It is associated with high temperature and found in a variety of habitats such as desert, semi-desert, near water channels, irrigated fields, settlements, disturbed areas, along roadside, ruined lands, sandunes etc. According to Puri et al. (1963), *C. procera* (Ait.) Ait f. is an indicator of lime or gypsum in the soil and in Rajasthan its occurance is related to calcium content of the soil. Gillet (1968) called it as anthropophilous, nitratophilous, psammophyte and pointed a close relationship between its density and organic matter in the soil.

2. *Glossonema varians* (Stocks) Hook. f. :

G. varians (Stocks) Hook.f. is distributed in Pakistan, Persia and Arabia. In Pakistan it is restricted to Sindh and Balochistan. In Sindh it is found in Karachi, Hyderabad and in Balochistan in Bela, Panjgur, Kalat and Quetta. Usually found in sand, in cracks, in limestone and often on rocky elevated ground.

3. *Oxystelma esculentum* (Linn. f.) R. Br.

O. esculentum (L. f.) R.Br. is distributed in Pakistan, Indonesia, Ceylon, Iraq and Egypt. In Pakistan it is profusely dense in the waterlogged area along the canals and rivers of Sindh and Punjab, however, it has also been reported from NWFP, along the bank of Kabul and Swat rivers in Peshawar and Swat respectively and in dry bed of Bella river in Balochistan.

It is a waterlogged and saline loving plant usually climbed on *Tamarix indica, Prosopis glandulosa, Salvadora pursica* and on a number of halophytic herbs.

4. *Pentatropis nivalis* (Gmel.) Field and Wood :

It is distributed in Pakistan, India, Afghanistan, Iran, Arabia and tropical Africa. In Pakistan, it occur in the plains of Punjab, Sindh and Balochistan. It usually found twining and corkscrewing the *Acacia, Prosopis, Salvadora*, cultivated hedges of *Clerodendrum philmoides*, cultivated *Ficus* trees along road sides, and even seeen climbing up the wall of houses. It exhibit variety of habitat viz. in near dry stream beds, saline soil with halophytic plants, clayey-gravely soil, cultivated lands in gardens, and in distrub areas.

5. ***Pergularia daemia*** **(Forssk.) Chiov.:**

P. daemia (Forssk.) Chiov. is distributed in Pakistan, India, Ceylon, Burma, Malaya, Afghanistan through Arabia to South and tropical Africa. In Pakistan widely distributed from plains upto 3000 ft. It is very common in Sindh and Punjab and particularly in Karachi, Thatta, Lahore, Rawalpindi, Jhelum also reported from Kashmir and Hazara (Ali, 1983). It is a common inhabitant of clayey-calcarious,saline soil, found twining on hedges of cultivated fields on *Prosopis* sp., *Tamarix* sp. in saline areas.

6. ***Pergularia tomentosa:*** **L.:**

It is distributed in Pakistan ,Afghanistan, Iran, Arabia, Egypt and Libya. In Pakistan, it occurance is more or less restricted to the southern Balochistan. Particularly in Makran Division, Bella and Khuzdar area. However, also reported from Thana Bullah Khan's hilly area (Dadu Dist.; Sindh) which is adjacent to Bella Dist. and only once reportd from Thatta Dist. (Ali, 1983). It occur in dry areas with gravel sandy and silty soil. In Balochistan between patches of *Nannorhops* sp. and occasionly on the hills slopes near the ground level.

7. ***Caralluma edulis*** **(Edgew.) Hook. f.:**

It is distributed in Pakistan and India (Punjab). In Pakistan reported from Sindh (karachi), Punjab (Rawalpindi) and Multan) and Balochistan. However, I have collected it from Ghickh

(Makran Division) and Kharan Dist. (Balochistan). It usually occur on hard rocky-gravel land, and very difficult to find out as it grows under the bushs of *Aerva sp.*, *Peltophorum* sp., *Rumex* sp. etc.

8. ***Caralluma tuberculata*** N. E. Br. :

It is distributed in Pakistan, Afghanistan, Persia and Arabia (Hook, 1883). In Pakistan, reported from 2500-4000 ft, in Swat, Wazirstan, Kurram, Peshawar and Salt range (Ali, 1983). It is usually found amongst large stones, in the clefts of rocks and among the roots of large bushes. However, I have collected it from Balochistan (Kharan Dist.) found with *C. edulis* with in the same locality.

9. ***Ceropegia bulbosa*** Roxb. :

It is distributed in Pakistan and India. In Pakistan, it is reported from Punjab (Jhelum, Multan and Lahore) only. However, I have found it in Sindh also, particularly in Karachi, viz. Karachi University Campus, and Super Highway area (Angaro Goth) c. 10-15 Km from Karachi University Campus. It usually occur in the bushes of *Ziziphus numularia*, *Lycium edgewortii* on sandy gravel soil.

10. ***Leptadenia pyrotechnica*** (Forrsk.) Dcne. :

It is distributed in Pakistan, India, Iran, Arabia, Egypt, Sudan, Somalia, Chad, Libya and Algeria. It occur in the desert and semidesert area of Pakistan and has been reported from Quetta. Sibi, Makran, Bella (Balochistan). D.I.Khan, Multan (Punjab), Karachi. Thatta. Gharo. Hyderabad. Tharparkar (Sindh). It requires a gravely subsoil and found on low sandunes or on loose sandy soil or sandy flasks of limestone. According to Puri et al. (1983) it is an indicator of very hot, arid zone.

1.6 Economic Importance:

The Asclepiads are usually considered to be of relatively little economic importance. Most species of Asclepiads are more or less poisonous and some have proved useful as medicinal plants. The roots of *Asclpias tuberosa* L. has proved efficacious in pleurisy and other chest complaints (Wren, 1956). The roots of *Asclepias curassavica* L. is used as a purgative in West Indies while as remedy in piles and gonorrhoea in India (Chopra et al., 1956). Its seeds contain a semi-drying oil, used in the manufacture of liquid soap and indispensable in the textile industry, while the residual cake from the oil extraction has considerable food value (Pobedimova, 1952).

Calotropis species have long been used as a source of very useful commercial fibre (Mador). The floss from the seeds are used for stuffing the pillows and mattresses in India (Ali, 1983). The latex, stem, leaves and root extracts are used medicinally for many purposes e.g. as purgative, antispasmodic, cholagogue, cure of cough, cold asthma, cholera etc, (Sastri 1950; Baquar, 1989).

Some species of *Marsdenia* are commercially valuable. *M. cundurango* yields the condurango bark of S. America (used as a stomachic) and condurango wine. The leaves of *M. tinctoria* yield a quality dye known as Indigo. *M.tenacissima* yield a valuable fibre "Rajmahal hemp", which is used to make bowstrings, cordage and netting in India. *Caralluma edulis* is used as vegetable and cure in blood diseases (Ali, 1983; Sastri, 1950). *Caralluma tuberculata* also used as vegetable and juice of the stem as febrifuge, stomachic and carminative (Baquar, 1989). The tuberous roots and leaves of *Ceropegia bulbosa* are also eaten as food (Ali, 1983). The follicles of *G. varians* are eaten and have cooling properties.

Young twigs and fruits of *L. pyrotechnica* are used as food and roots as vegetable (Uphoff, 1968; Sastri, 1950). The plant yields a fibre used for rope making. It also provide fodder for cattles, horse and camels (Ali, 1983). Different parts of the *O. esculentum* are used in throat and mouth infections, jaundice and as wash for ulcers (Chopra et al., 1956; Baquar, 1989). *P. nivalis* possess emetic properties and also used in gonorrhoea (Baquar, 1989, Deshaprabhu, 1966).

The plant extract of *P. daemia* is useful in inflamatory conditions, uterine and menstrual disorders, leucoderma, urinary discharge, eye troubles, etc (Baquar, 1989, Deshaprabhu, 1966). *Gymnema sylvestre* has been used in the treatment of diabetes. *Tylophora indica* is well known for the treatment of asthma. The large fleshy roots of *Brachystelma buchanani* are used in healing wounds in Central Africa.

Several plants are used as ornamentals and among them some remarkable genera are *Asclepias*, *Caralluma*, *Ceropegia*, *Dischidia*, *Hoya*, *Stapelia*, *Stemphanotis* etc.

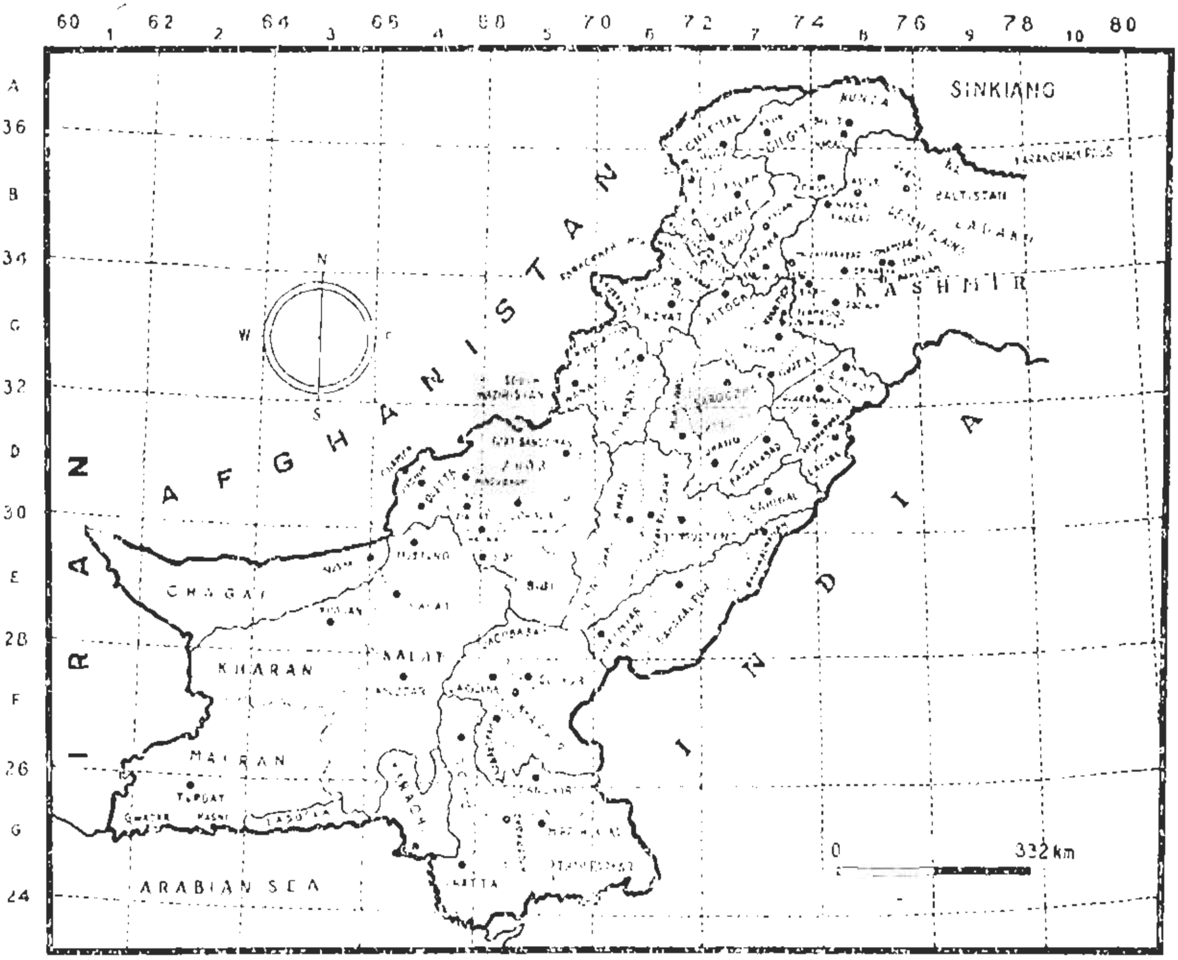


Fig.1· Map of Pakistan and Grid system (after E. Nasir & S.I. Ali (eds.), *Flora of Pakistan*).

[illegible] Study area:

Pakistan is situated in South Asia. It lies between 23°.30' and 37°.45' north and 61° and 75°.30' east longitude. Its maximum length from north to south is over 1000 miles and from east to west about 550 miles. Total area of the country is 310403 square miles.

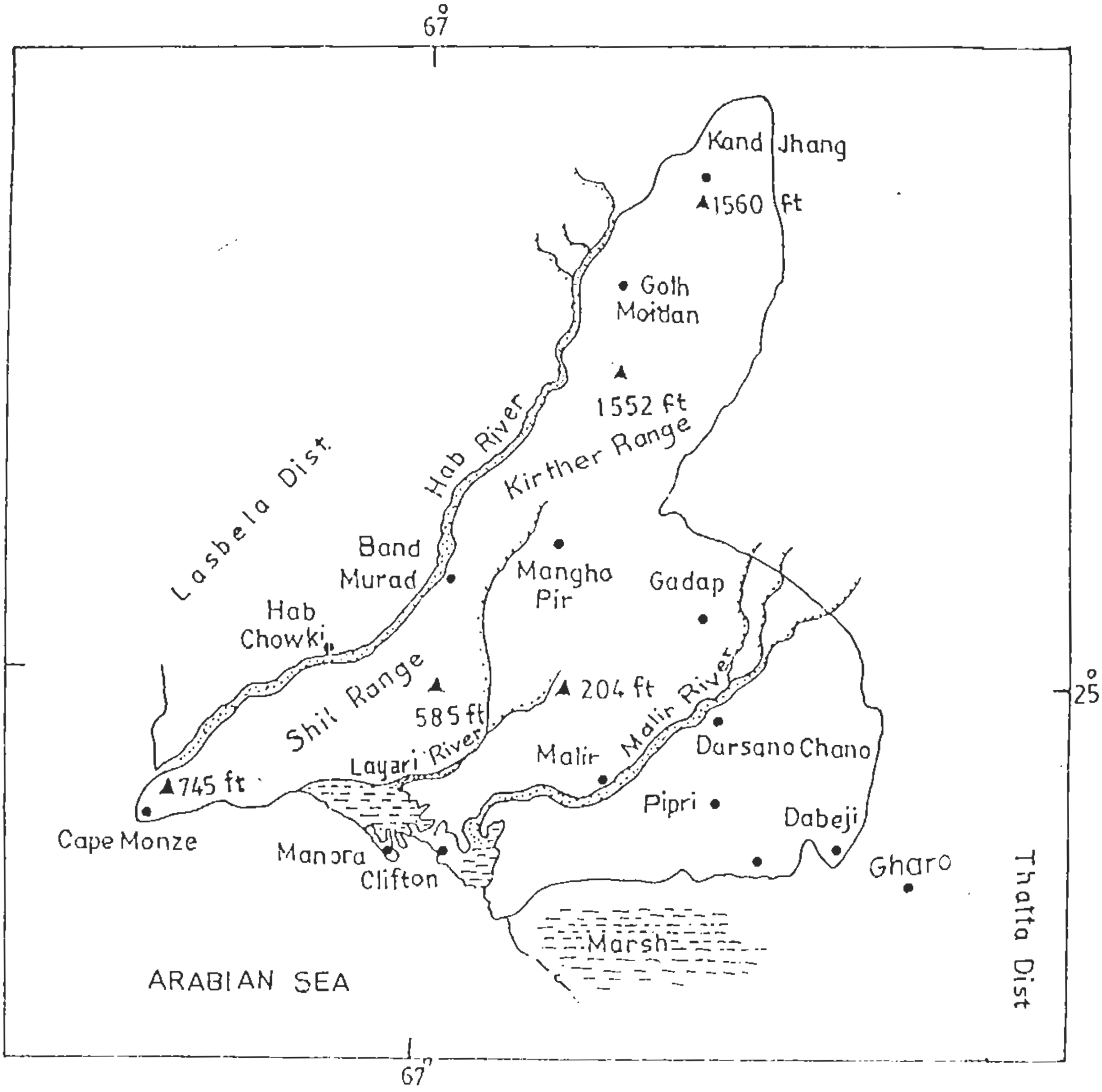
Pakistan boardered in the west by Iran , in the north west by Afghanistanm, in the north by China and in the east by India. Southern boundary is represented by the coast on the Arabian sea (Fig. 1).

Geographically, it is subtropical however, its greater part has tropical conditions and the vegetation resembles remarkbly to tropical region rather than subtropical one.

Present study is caried out from the southern part of Pakistan mainly from Karachi (Karahci District), Gharo (Thatta District, Sindh province) and Khuzdar (Khuzdar District, Balochistan province (Fig.2. 3).

Karachi:

Karachi lies in the southern part of Pakistan between the latitude 24.50' - 25.15' north and between the longitude 65.51° - 67.40' east. It is boardered in the west by Lasbelle district and in the north-east by Thatta Dist. Arabian sea is towards south while swampy area of Indus delta lies in the south - east corner. (Fig. 2). The area of Karachi is 1357 square miles and comparises of the city of Karachi (566 sq miles) 91 villages and 5 islands (Hussain, 1984).



MAP-1. PHYSIOGRAPHIC MAP OF KARACHI AND GHARO

According to Naqvi (1956) and Chaudhri (1981), climate of Karachi is subtropical maritime desert. Qadri (1967) determined the bioclimamte of Karachi under the category of desert bush formation.

The climamte remains moderate and pleasant throughout the year due to the presence of sea. Broadly speaking, climate of Karachi can be divided into two distinct seasons. i.e. winter and summer. Hot and humid period is extremely variable and unpredictable and occurs during the summer from June to September.

The coastal regions and inland regions show a considerable variation in temperature. The area which are close to sea have maritime trend but inland regions show continental trend. The hottest months are May and June, when the mean maximum temperature is 95.5°F and the mean minimum is 78.3 - 82.4°F. Winter is not very severe. The coldest month is January, with a normal maximum temperature of 75.5°F and normal minimum daily temperature of 57.4°F.

The average annual rainfall is 150-200 mm, most of which received during the monsoon period from June to September. Rainfall at Karachi is not only scanty but also very variable. There are years where no rainfall occurs at all and few years have above average rainfall. The great fluctuations in the rainfall make plant life very difficult and highly specialized.

For great part of the year the relative humidity around Karachi is high. It ranges from 53-86% in the morning and 53-81 % in the afternoon .

Strong coastal wminds are the characterstic feature of the region. Mean wind speed varies from 5.3 m.p.h. in November to 12.8 m.p.h. in July. Remarkble strong and steady wind blows mostly from south - west or westward directions from March till October. From May - September, it blows with an average rate of 12-15 m.p.h. In the coastal regions, due to high wind velocities (i.e 25 m.p.h.), the crowns of trees are usually orientated towards the prevailing wind direction. The strong wind constantly carries sand particles and sodium chloride crystals from the beach to the interior.

Karachi represents quite a variety of habit such as islands, sea coast, salt swamps, sandunes, rocky places, semidesert, saline soils, disturbed areas, cultivated fields, dry streams beds, sandy plains, hillocks etc.

According to the physiographic features Karachi can be divided into the following three broad categories.

(1) Hilly region

Low detached calcareous hills are quite common in this area. Western boarder of the upper Sindh is skirted by Kirther range and Pab range. The shil range extends from Cape Monze to Mangopir which is a series of hills and ridges present in a parallel manner. The hills are dry and the available water to the plant is only due to little amounts of rainfall which collects in the underlying bed rocks. The characteristic vegetation on the hills are xerophytic, growing on the slopes as well as on the hills. The most commmon species which are found on the top of the hills are *Iphiona grantioides* (Boiss.) A. Anderberg, *Blepharis scindica* (Stocks) T. And.;*Aristida mutabilis* Trin. & Rupr.; and *A. adscensionis* L. While, *Commiphora wightii* (Arn). Bhandar, *Euphorbia* *caducifolia* Haines, *Grewia tenax* (Frossk.) Fiori, *G. villosa* Willd. are the major constituents of the vegetation on the slopes.

(2) Alluvial plains

Soil between the hills is alluvial in nature and has been deposited by the Hab, Lyari and Malir rivers and by the seasonal water flow, creating flat and sloping plain and low lying areas. Except for the Hab, there are no perennial rivers in the neighbourhood and even the Hab form a series of shallow pools for most of the year. Lyari river with its tributaries flow through the thickly populated parts of the city and ultimately falls into the sea near Kemari. Malir river draining the Mol plateau in Kohistan has the Mol and Khadeji as its chief tributaries. After thin confluence the large stream of Malir river is formed which ultimately falls into Ghizri creek towards the east of Kemari. Rainfall being very scanty, these rivers are dry for the majfor part of the year but they are a powerfufl underground source of fresh water supply. On the alluvial plains pioneer plants are *Corchorus depressus* (L.) Stocks, *Launaea nudicaulis* (L.) Hk. f.; *Salvia santolinifolia* Boiss.; *Sida ovata* Forssk.; alongwith the grasses like *Aristida mutabilis* Trin. & Rupr.; and *Ochthochloa compressa* (Forssk.) Hilu followed by herbs of *Cassia holoseriacea* Fres.; *Cassia senna* L.; *Aerva javanica* (Burm.F.) Juss. and *Indigofera oblongifolia* Forssk. These plants are succeeded by shrubs of *Cordia gharaf* (Forssk.) Ehrenb.: *Grewia tenax* (Forssk.) Fiori and *Commiphora wightii* (Arn.) Bhandari.

On the soil with good depth, better texture and moisture condition large shurbs like *Ziziphus numularia* (Burm.f.) Wight & Arn.; and *Euphorbia caducifolia* Haines are common. On the fine textured soil *Euphorbia caducifolia* Haines. with *Capparis decidua* (Forssk.) Edgew.: which is later dominated by *Prosopis cineraria* (L.) Druce and *Salvadora oleoides* Decne. as climax species. On the soil with good moisture content *Acacia nilotica* (L.) Delile is fairly common.

Along the gradually sloping areas on shores as well as on the inlands are found sand dunes formed by the sand blown from the coast. Sand dunes are particularly met with in the region of Korangi Creek , Clifton, Sandspit, Paradise point, Hawksbay etc. The height of the dunes reach upto fourty feet. Inland sand dunes are mostly found on the plains of Pipri, Landhi, Mangopir areas.

The common plants which are found on sand dunes are *Ipomoea pes-caprae* (L.) Swt., *Cyperus arenarius* Retz., *Citrullus colocynthis* (L.) Schard., *Heliotropium curassavicum* L., *Atriplex stocksii* (Wt.) Boiss., *Salsola baryosma* (R. & S.) Dandy and *Aerva pseudo-tomentosa* Blatt. & Hall. Phytogeographically the study area lies in Saharo-sindian region (Ali & Qaiser, 1986).

3. Coasatal Areas

Karachi has a long coastline and has some fine beaches like Clifton, Sandspit, Hawksbay, Paradise Point, Korangi Creek and China creek. These beaches provide excellently calm water for mangrove vegetation. Aress near the sea coast suffer from physiological dryness due to the salinity of the soil. Main islands of Karachi include Manora, Bhit, Baba etc. The most important vegetation of this area is mangrove. Most common plants among these mangrove vegetation are *Avicennia marina* (Forssk.) Vierh., and rare individuals of *Aegiceras corniculata* (L.) Blanco.

Towards the sea coast on the swampy, muddy and waterlogged areas *Athrocnemum indicum* (Wild.) Moq., *Urochondra setulosa* (Trin.) C. E. Hubbard, and *Limonium stocksii* (Bioss.) O. Ktze., grow pure. Away from the sea other plants like *Tamarix indica* Willd., *Suaeda fruticosa* (L.) Forssk., and *Prosopis juliflora* (Swartz.) DC., are found.

Gharo :

Gharo lies in the Southern part of Pakistan between the latitude 24°.44′ north and logitude 67°.37′east. It is situated c. 50 km from Karachi on Indus highway in the Thatta District. It is boardered in the North west by Karachi, in the West and South by swampy area of indus delta and in the East by Thatta city (Fig. 2).

The climate is very hot and arid. The mean summer temperature (May - August) ranges from 30 - 35°C while in winter (December - January) it ranges from 17.5 - 20°C. The average rainfall is below 125 mm.

Gharo represent a variety of habit such as inlands, salt-swamps, saline soils sand dunes and cultivated fields.

Due to seepage of water through canal banks, large area of the land is suffering from waterlogging and salinty. In waterlogged ditches, ponds and lakes *Zannic palustris*, *Potamogeton crispus*, *Vallisneria spiralis* are found at submerge stages, while among floating plants *Marsielia minuta*, *Lemmna polyrhiza*, *Nelumbium speciosum*, *Ipomoea aquatica*, *Azolla pinnata* and *Eichornia* *crassipus* are found, among amphibious plants *Paspalum paspaloided*, *Scirpus maritimus*, *Juncus laevigatus*, *Fibmbrystylis dichotoma*, *Polypogen* sp. etc. are found *Cyperus kyllinga*, *Typha angustifolia*, *Phragmites Karka* etc. are ultimate plants of ditches. Ponds or lakes before they get filled up and on drying *Saccharum spontaneum* and *Tamarix indica* take their place.

Due to high rate of evapotranspiration on waterlogged soil, concentration of salts on the soil surface become very large. In the saline area *Salsola baryosma*, *Suaeda monoica*, *S.fruticosa*, *Halyoxylon recurvum*, *Heliotropium rariflorum*, *Atriplex griffithii*, *Tamarix indica* and *Salvadora persica* are found.

On the coastal saline swamps common plants are *Cressa cretica*, *Salsola baryosma*, *Suaeda monoica*, *Tamarix indica*, *Salvadora persica* etc.

Due to sand borne wind, sand dunes are also formed in this area. Sanddunes are colonized by *Panicumm antidotale*, *Aerva javanica*, *Leptadenia pyrotechnica*, *Siziphus nummularia*, *Prosopis glandulosa* and *Saccharum benghalense*.

Khuzdar :

Khuzdar city is the head quarter of Khuzdar district (Kalat Division). It lies in the western part of Balochistan between latitude 27°.58' north and longitude 66°-38' east (at an height of upto 1000 meter above sea level). It is boardered in the east by Sindh Province (Larkana and Dadu Districts), in the east by Kachhi District, in the north by Kalat District, in the west by Kharan and Panjgur District and in the south by Lasbela district (Fig. 3). Khuzdar is irregular in shape and charaterized by low

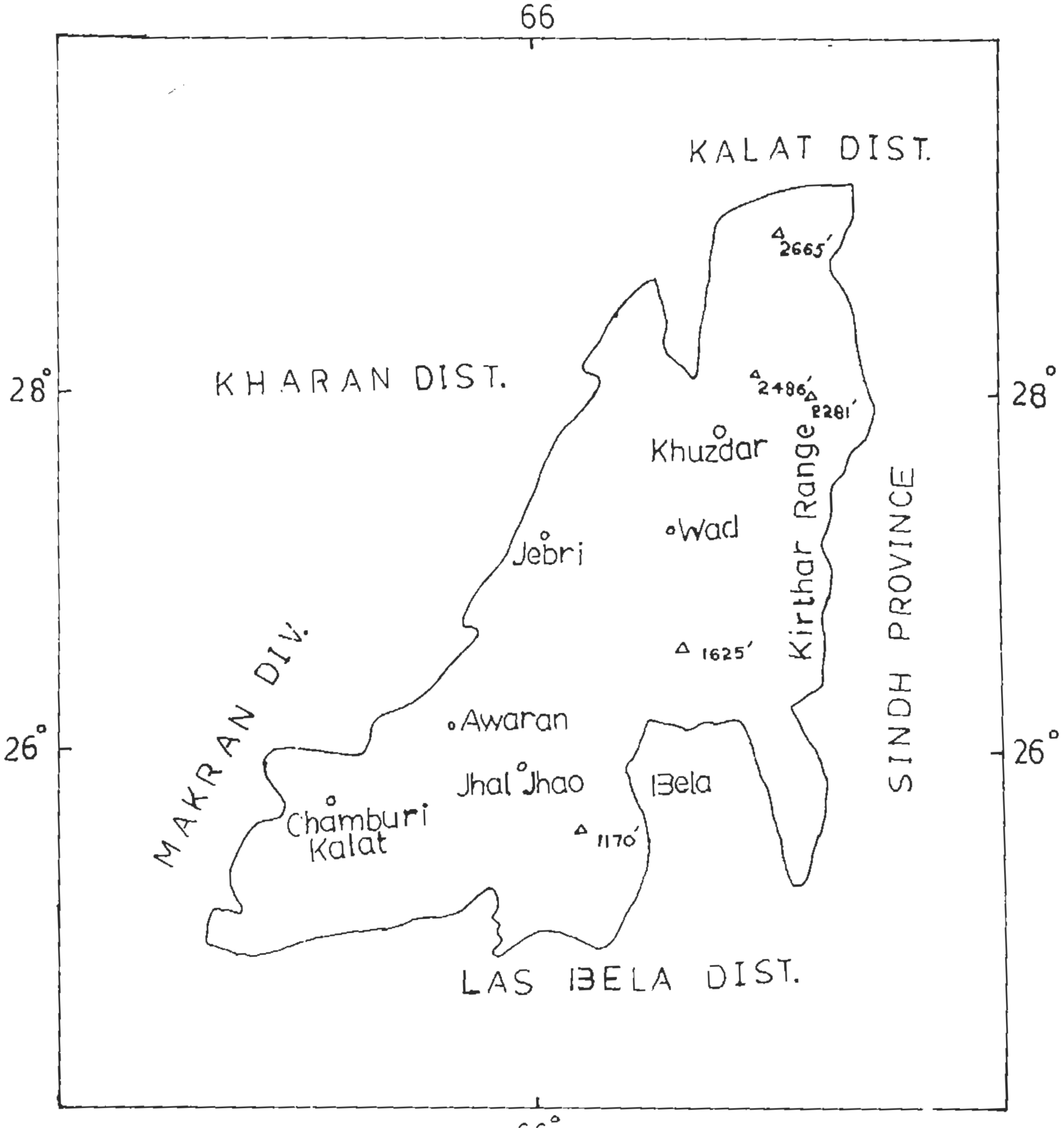


FIG. 3. PHYSIOGRAPHIC MAP OF KHUZDAR

to high mountain ranges and dry stream beds. and drained by Kolachi river.

The climate of the area is classified as arid subtropical continental high land characterized by very hot summer. The mean summer temperature (June - August) is ranges from 30 - 35°C and the mean winter temperature (December - February) from 75 - 10°C. The average annual rainfall is below 125 mm. The area experiences cold gusty winds during winter, which below from north and north-west. The soil is mixed calcareous alluvium gravelly derived mainly from the sedimentary rocks of the mountain ranges and deposited by mountain streams and washed from the steep mountain slopes by run - off. The main rocks are limestone, shale and part sandstone.

Vegetation is classified as dry scrub by Hussain (1984). The desertic and semi-desertic conditions give rise to xerophytic type of scrub vegetation, which are scanty in most of the localities. The tree species are badly affected by felling and grazing. Vegetation is mainly comprises of *Haloxylon griffithii*, *Salsola Kali*. *Alhaji maurorum*, *Tamarix sp.*, *Euphorbia caducifolia*, *Periploca apylla*, *Namorphs ritichiana*, *Ziziphus numularia* etc.

Terminology:

A profusion of terminology has developed in the Asclepiad literature over time relating to their complicated floral structure. Several authors have proposed synonym terms describing the floral structure, particularly in relation to the pollinia and pollination mechanism. Bookman (1981) and Newton (1984) atttempted to standarizes the terms and Bookman's (1981) proposals are generally adopted here (Table. 1).

Table 1. Terms used in Asclepiads floral morphology with sources.

Cucullus	Brown, 1810; Galil and Zeroni, 1965; Eisikowitch, 1986.
Bifid fleshy pad	Galil and Zeroni, 1965.
Alternating lobule	Woodson, 1954.
Gynostegium	Corry, 1883b; Woodson, 1954, Lynch, 1977; Eisikowitch, 1986; Ali, 1983; Wyatt, 1978; Beare and Perkins, 1982.
Stigma head	Woodson, 1954; Kunze, 1990; Kunze and Liede , 1991; Rosatti, 1989; Wyatt, 1976; Lynch 1977.
Stigma	Ali, 1983.
Style table	Corry, 1883b: Robertson, 1887a, 1887b.
Style-stigma head	Rosatti, 1989.
Pollinium	Leggett, 1872; Corry, 1883a, 1883b. Robertson, 1886; 1887a, 1887b; Frye. 1901, 1902; Moore, 1946a, 1946b, 1947: Sparrow and Pearson, 1948: Maheswari Devi, 1964; Willson and Rathcke, 1974; Wyatt, 1976; Rosattti, 1989; Newton, 1984; Kunze 1990; Lynch 1977; Eisikowitch, 1986; Morse and Fritz, 1985.
Pollinium sac	Woodson, 1954.
Pollen sac	Macior, 1965.
Pollen mass	Stevens, 1945.

Translator arm	Sparrow and Pearson, 1948; Holm, 1950; Woodson, 1954; Safwat, 1962; Marior, 1965; Beare and Perkins, 1982; Galil and Zeroni, 1969; Wyatt, 1976, 1978; Lynch, 1977; Agnew, 1976; Ali, 1983; Rosatti, 1989.
Stalk	Brown, 1937.
Retinaculum	Robertson, 1886, 1887a, 1887b; Moore, 1946a, 1946b, 1947; Biswas, 1957; Maheswari Devi, 1964; Vijayaraghavan and Cheema, 1977; Agnew, 1976; Proctor & Yeo, 1973; Ali, 1983; Willis, 1925.
Connective	Dyer, 1971 & 76.
Caudiclo (caudicule)	Corry, 1883b; Robertson, 1887b; Frye, 1901,1902, El-Gazzar and Hamza, 1973. Leach, 1976; Lavranos, 1977; Jackson, 1953; dyer, 1971 & 1976; Bullock, 1966; Bayer, 1978; Newton, 1984.
Corpusculum	Beare and Perkins, 1982; Corry, 1883b; Robertson, 1886; 1887a, 1887b; Frye, 1901, 1902; Stevens, 1945; Safwat, 1962; Maheswari Devi, 1964; Wyatt, 1976, 1978; Agnew, 1976; Kernere and Oliver, 1904; Proctor & Yeo, 1973; Willis, 1925; Kunze, 1990; Kunze and Liede, 1991; Rosatti 1983; Lynch, 1977; Vijayaraghavan and Cheema, 1977; Ali, 1983.
Pollen carrier	Brown, 1937; Bullock, 1966.
Carrier	Dyer, 1971, 1976; Lavranos, 1977.
Clip	Kerner and Oliver, 1904.

Translator	Leech, 1976; [illegible], 1977; Haver, 1978; Willis, 1925.
Retinaculum	Jackson, 1953.
Gland	Rothrock, 1868; Kirkpatrick, 1870; Leggett, 1870; Woodson, 1954; Macior, 1965; Willson and Rathcke, 1974.
Corpuscle	El-Gazzar and Hamza, 1973; Bullock, 1966; Newton, 1984.
Translator apparatus	Holm, 1950; Vijayaraghavan and Cheema, 1977.
Translator	Maheswari Devi, 1964; Procter & Yeo, 1973; Reese, 1973; Kunze, 1990.
Pollen carrier	Reese, 1973; Bailly, 1962.
Pollinarium	Lynch, 1977; Wyatt, 1978; Kephart and Heiser, 1980; Rosatti, 1989; Kunze, 1990; Moore and Fritz, 1985; Beare and Perkins, 1982.
Pollinia	Moore, 1946a, 1946b, 1947; Woodson, 1954; Willson and Ratcke, 1974; Wyatt, 1976; Willson and Price, 1977; Beare & Perkins, 1982; plus others.
Bi-pollinium	Newton, 1984.
Twin pollinia	El-Gazzar and Hamza, 1973.
Paired pollinia	Eisikowitch, 1986.
Anther wings	Corry, 1883b; Woodson, 1954; Rosatti, 1989; Kunze, 1990, Wyatt, 1976; Bear & Perkins, 1982; Lynch, 1977.
Anther flaps	Galil and Zeron, 1965.
Alar fissure	Corry, 1883b; Robertson, 1886; Beare and Perkins, 1982; Morse & Fritz, 1965.

Stigmatic chamber	Woodson, 1954; Kunze, 1990; Rosatti, 1989; Wyatt, 1976, 1979; Boare & Perkins, 1982; Eisikowitch, 1986.
Alar chamber	Corry, 1883b; Frye, 1901.
Knee band	Leggett, 1870.
Bend	Wyatt, 1976, 1978.

Adopted from (Bookman, 1981), however some recent references are incorporated. Synonyms are indented beneath preferred terms.

CHAPTER 2

FLORAL PHENOLOGY AND MORPHOLOGY

INTRODUCTION

Asclepiadaceae is one of the most significant flowering plant family from the evolutionary point of view. The interest in this group is derived from the floral structure of its members which exhibit extreme adaptation to insect pollination. That's why its floral structure and mode of pollination is the subject of much interest earlier from the eighteenth century Sprengel (1793), Brown (1833), Delphino (1865), Corry (1883) etc. Safawat (1962) and Galil and Zeroni (1965, 1969) reexamined the floral morphology of *Secamone* and *Asclepias* species respectively and highlighted the functional and evolutionary significance of their extreme complex floral modifications.

Apart from major adaptations even the minor differences in the arrangement and form of floral parts particularly of those involved in the pollinistion may play an important role in the specification of the pollinators and pollination mechanisms as described by Wanntorp (1974), Lynch (1977) and Kunze (1991).

In this family the structure and pecularity of the androecium particularly the presentation of pollen grains as tetrads or pollinium: number, orientation, location and morphology of pollinium, anther structure etc. provide key characters at subfamilial and tribal classification (Brown, 1810; Hooker, 1885; Bentham, 1869; Schumann, 1895; Good, 1974; Bruyns and Forster, 1991.

On germination of pollinium , the pollen tubes emerge from a specific region. This germiantion zone of the pollinium is designated as "Germination locus" (Rao and Kumari, 1979; Khatoon and Ali, 1983) or "Germination furrow" (Sreedevi and Namboodiri, 1982) to distinguish it from the germ pore of pollen grains. On the sectioning of the pollinium, tiny notches, pores and slits in *Asclepias curassavica*, *Pergularia daemia* (Forssk.) Chiov. and *Calotropis procera* (Ait.) Ait. f. have been reported by Galil and Zeroni (1969), Vijayaraghavan and Shukla (1976a) and Khatoon and Ali (1983) respectively. Sreedevi and Namboodiri (1982) discussed the non-visibility of germination locus in ungerminated pollinia of *Asclepias* and *Calotropis* in light microscope, appearance of various sizes apertures in scanning electron microscope and a continuous slit on acetolysis, at the germiantion locus.

Although, Chaturvedi (1987, 1989) and Bhatnagar (1986) studied the pollination ecology of at least 10 genera of this family from India but they mostly emphasized on the pollinators while information about floral morphology pertaining to pollination is lacking.

Thus in the present study, morphology and phenology of the members of subfamily Asclepiadoideae belonging to tribe *Asclepiadeae* viz. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) Hook. f., *Oxystelma esculentum* (L. f.) R. Br., *Pentatropis nivalis* (Gmel.) Field and Wood, *Pergularia daemia* (Forssk.) Chiov. and *Pergularia tomentosa* L. and tribe Ceropegieae viz. *Caralluma edulis* (Edgew.) Hook. f., *Caralluma tuberculata* N. E. Br., *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Dcne. is carried out, to under-stand the floral adaptations in response to their pollination mechanism and pollinators behaviour

MATERIALS AND METHODS

Morpgology:

Fresh flowers of all the studied taxa (except *Caralluma edulis* (Edgew.) Hook. f.; *Carallima tuberculata* N. E. Br. and *Pergularia tometosa* L.) were collected from Karachi University Campus (KUH), Malir (MAL), Darsanochino (DAR) and Mangopir (MAN) while of *Caralluma edulis* (Edgew.) Hook. f. and *C. tuberculata* N. E. BR. from cultivated plants [Stems purchased from local market and planted at Department of Botany, Universituy of Karachi] and preserved (in 50% alcohol) flowers of *Pergularia tomentosa* L. (collected from Khuzdar) were used for the morphological studies .

Phenology:

Mature floral buds, just prior to anthesis, were tagged at the above mentioned localities and observed for phenological changes.

Germination locus:

Pollinia from fresh or preserved (in 50% alcohol) flowers of the studied taxa were acetolysized according to the method of Erdtman (1952). The acetolysized pollinia were mounted on stubs with double adhesive tape, gold coated and viewed for germination loci under a JEOL JSM-35. Scanning Electron Microscope.

Translator	Leech, 1976; [illegible], 1977; Bayer, 1978; Willis, 1925.
Retinaculum	Jackson, 1933.
Gland	Rothrock, 1860; Kirkpatrick, 1870; Leggett, 1870; Woodson, 1954; Macior, 1965; Willson and Rathcke, 1974.
Corpuscle	El-Gazzar and Hamza, 1973; Bullock, 1966; Newton, 1984.
Translator apparatus	Holm, 1950; Vijayaraghavan and Cheema, 1977.
Translator	Maheswari Devi, 1964; Procter & Yeo, 1973; Reese, 1973: Kunze, 1990.
Pollen carrier	Reese, 1973; Bailly, 1962.
Pollinarium	Lynch, 1977; Wyatt, 1978; Kephart and Heiser, 1980; Rosatti, 1989; Kunze, 1990; Moore and Fritz, 1985; Beare and Perkins, 1982.
Pollinia	Moore, 1946a, 1946b, 1947; Woodson, 1954; Willson and Ratcke, 1974; Wyatt, 1976; Willson and Price, 1977; Beare & Perkins, 1982; plus others.
Bi-pollinium	Newton, 1984.
Twin pollinia	El-Gazzar and Hamza, 1973.
Paired pollinia	Eisikowitch, 1986.
Anther wings	Corry, 1883b; Woodson, 1954; Rosatti, 1989; Kunze, 1990, Wyatt, 1976; Bear & Perkins, 1982; Lynch, 1977.
Anther flaps	Galil and Zeroni, 1965.
Alar fissure	Corry, 1883b; Robertson, 1886; Beare and Perkins, 1982; Morse & Fritz, 1985.

Stigmatic chamber	Woodson, 1954; Kunze, 1990; Rosatti, 1989; Wyatt, 1976, 1978; Peare & Perkins, 1982; Eisikowitch, 1986.
Alar chamber	Corry, 1883b; Frye, 1901.
Knee band	Leggett, 1870.
Bend	Wyatt, 1976, 1978.

Adopted from (Bookman, 1981), however some recent references are incorporated. Synonyms are indented beneath preferred terms.

CHAPTER 2

FLORAL PHENOLOGY AND MORPHOLOGY

INTRODUCTION

Asclepiadaceae is one of the most significant flowering plant family from the evolutionary point of view. The interest in this group is derived from the floral structure of its members which exhibit extreme adaptation to insect pollination. That's why its floral structure and mode of pollination is the subject of much interest earlier from the eighteenth century Sprengel (1793), Brown (1833), Delphino (1865), Corry (1883) etc. Safawat (1962) and Galil and Zeroni (1965, 1969) reexamined the floral morphology of *Secamone* and *Asclepias* species respectively and highlighted the functional and evolutionary significance of their extreme complex floral modifications.

Apart from major adaptations even the minor differences in the arrangement and form of floral parts particularly of those involved in the polliniation may play an important role in the specification of the pollinators and pollination mechanisms as described by Wanntorp (1974), Lynch (1977) and Kunze (1991).

In this family the structure and pecularity of the androecium particularly the presentation of pollen grains as tetrads or pollinium: number, orientation, location and morphology of pollinium, anther structure etc. provide key characters at subfamilial and tribal classification (Brown, 1810; Hooker, 1885; Bentham, 1869; Schumann, 1895; Good, 1974; Bruyns and Forster, 1991.

On germination of pollinium , the pollen tubes emerge from a specific region. This germiantion zone of the pollinium is designated as "Germination locus" (Rao and Kumari, 1979; Khatoon and Ali, 1983) or "Germination furrow" (Sreedevi and Namboodiri, 1982) to distinguish it from the germ pore of pollen grains. On the sectioning of the pollinium, tiny notches, pores and slits in *Asclepias curassavica*, *Pergularia daemia* (Forssk.) Chiov. and *Calotropis procera* (Ait.) Ait. f. have been reported by Galil and Zeroni (1969), Vijayaraghavan and Shukla (1976a) and Khatoon and Ali (1983) respectively. Sreedevi and Namboodiri (1982) discussed the non-visibility of germination locus in ungerminated pollinia of *Asclepias* and *Calotropis* in light microscope, appearance of various sizes apertures in scanning electron microscope and a continuous slit on acetolysis, at the germiantion locus.

Although, Chaturvedi (1987, 1989) and Bhatnagar (1986) studied the pollination ecology of at least 10 genera of this family from India but they mostly emphasized on the pollinators while information about floral morphology pertaining to pollination is lacking.

Thus in the present study, morphology and phenology of the members of subfamily Asclepiadoidae belonging to tribe *Asclepiadeae* viz. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) Hook. f., *Oxystelma esculentum* (L. f.) R. Br., *Pentatropis nivalis* (Gmel.) Field and wood, *Pergularia daemia* (Forssk.) Chiov. and *Pergularia tomentosa* L. and tribe Ceropegieae viz. *Caralluma edulis* (Edgew.) Hook. f., *Caralluma tuberculata* N. E. Br., *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Dcne. is carried out, to under-stand the floral adaptations in response to their pollination mechanism and pollinators behaviour

MATERIALS AND METHODS

Morpgology:

Fresh flowers of all the studied taxa (except *Caralluma edulis* (Edgew.) Hook. f.; *Carallima tuberculata* N. E. Br. and *Pergularia tometosa* L.) were collected from Karachi University Campus (KUH), Malir (MAL), Darsanochino (DAR) and Mangopir (MAN) while of *Caralluma edulis* (Edgew.) Hook. f. and *C. tuberculata* N. E. BR. from cultivated plants [Stems purchased from local market and planted at Department of Botany, Universituy of Karachi] and preserved (in 50% alcohol) flowers of *Pergularia tomentosa* L. (collected from Khuzdar) were used for the morphological studies .

Phenology:

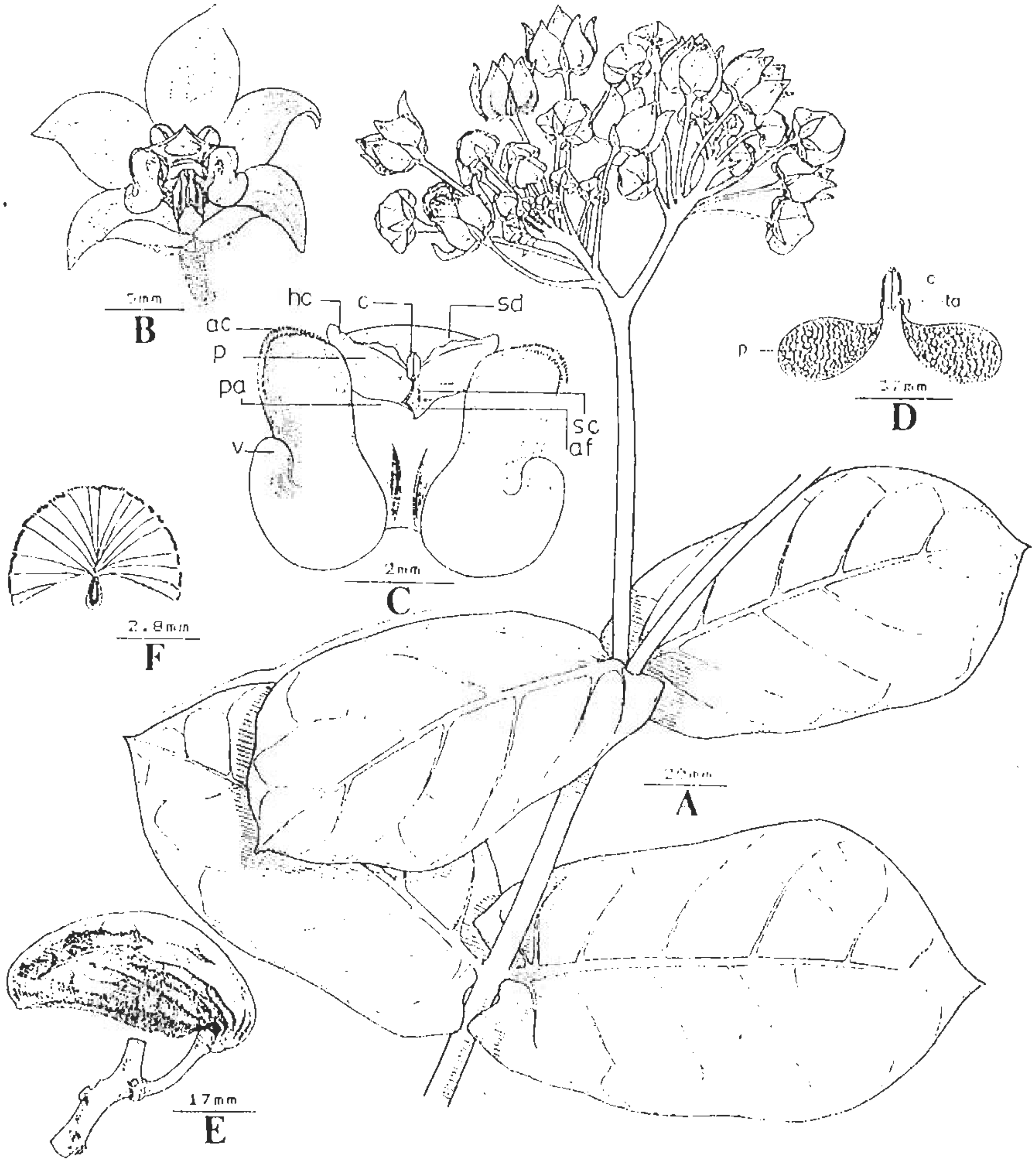
Mature floral buds, just prior to anthesis, were tagged at the above mentioned localities and observed for phenological changes.

Germination locus:

Pollinia from fresh or preserved (in 50% alcohol) flowers of the studied taxa were acetolysized according to the method of Erdtman (1952). The acetolysized pollinia were mounted on stubs with double adhesive tape, gold coated and viewed for germination loci under a JEOL JSM-35. Scanning Electron Microscope.

Fig. 4. Calotropis procera ssp. hamiltonii: A, Habit ; B, Flower; C, Side view of gynostegium and two corona lobes; D, Pollinarium; E, Fruit; F, Seed.

(ac, apex of corona lobe; af, anther wing; c, corpusculum; cl, corona lobe; hc, hayline outgrowth of connective; pa, pad; po, pollinium; sc, stigmatic chamber; sd, stigma head; ta, translator arm; v, vesicle)


5mm
B
hc
c
sd
ac
p
pa
v
sc
af
2mm
C
c
ta
p
3.7mm
D
2.8mm
F
20mm
A
17mm
E

OBSERVATIONS AND RESULTS

Tribe : Asclepiadeae

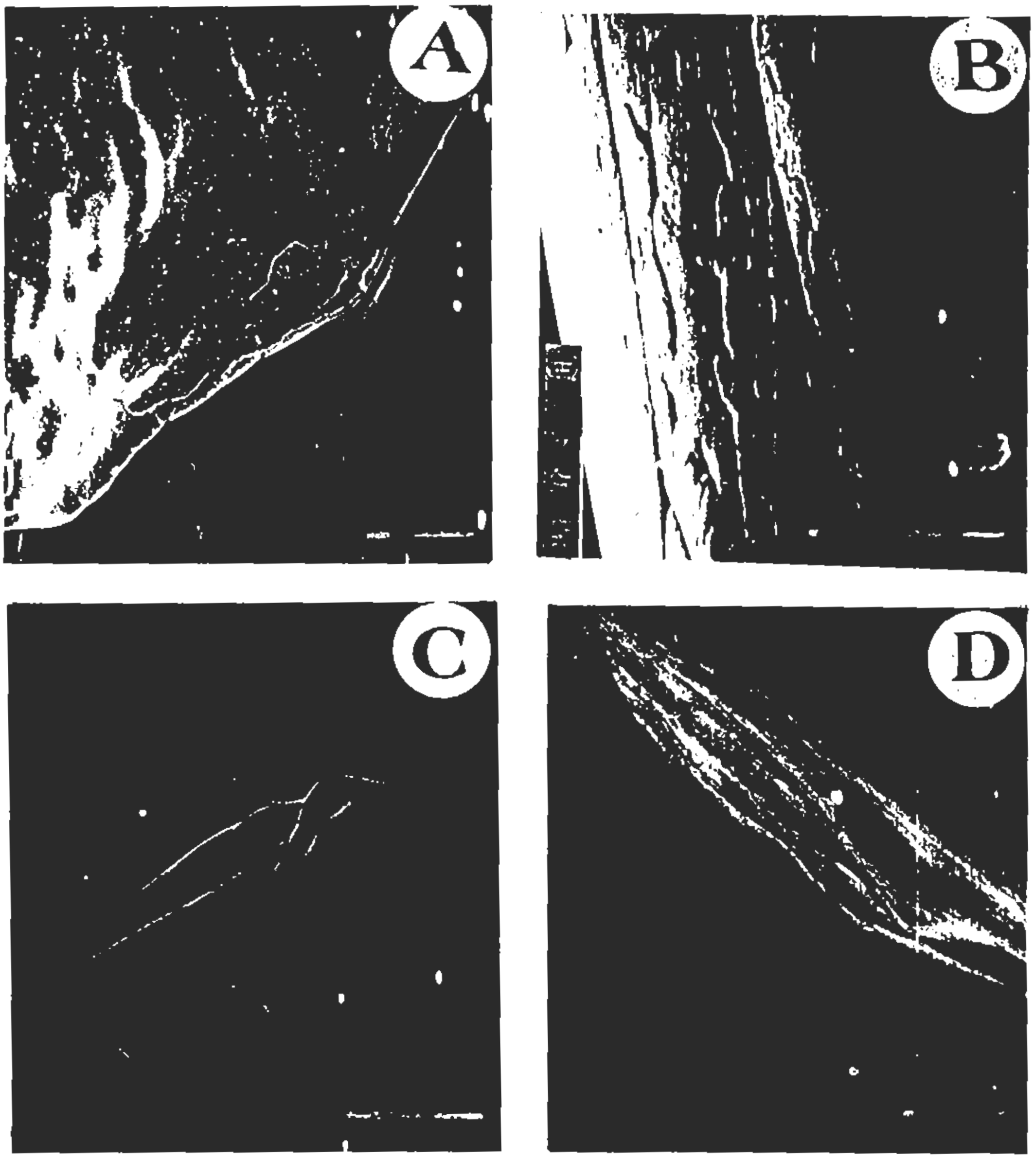
1. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali:

It is an erect shrub or small tree upto 3m tall, much branched from the base with with soft, corky bark and milky latex. Young stems and leaves are covered with white cottony tomentum. Leaves are opposite , decussate, broadly ovate, ovate-oblong, elliptic or obvate, entire, with cordate base and acute apex, subsessile, somewhat fleshy. Flowers are arranged in terminal and axillary umbelloid cymes (Fig. 4).

Flowers are perfect, actinomorphic and pedicelate. Calyx 5 lobed, lobes green ± rhomboidal. Corolla also 5 lobed, lobes white with purple tips, campanulate, broadly ovate. The androecium consists of five stamens, that adnate to the stigma head to form the gynostegium. The filaments are fused to form the staminal column that surround but not joined with ovaries and styles. The corona is made up of five fleshy, purple, laterally compressed lobes, originating from the staminal column. Each lobe is consist of a recurved vesicle at the base and a bifid apex with a cleft outside. A bifid (V - shaped) fleshy pad is situated below each stigmatic chamber, between two corona lobes (Fig. 4B). The five bilocular anthers united and expand laterally to form anther head. mostly concealing the lateral surfaces of stigma head. An apcial hyaline flap from each anther, cover the stigma head at perophery. Laterally, anther margins are boardered by hard, leathery anther wings that arranged parallel with the wings of

Fig. 5. Germination loci showing slits, apertures and notches. A & B, Calotropis procera ssp. hamiltonii: Exocentrolateral; C, Glossonema varians: Exoproximolateral; D, Oxystelma esculentum: Exosubdistolateral.

Scale bar: A & C = 100 μm; B = 10 μm; D = 20 μm.


A
B
C
D

adjacent anthers to form five slits or alar fissures over the five lateral vertical furrows of the stigma head, thus resulting in the formation of stigmatic chambers (Fig. 4C). At the apex of each slit or alar fissure a dark brown 450 x 150µm corpusculum is present, that attached laterally to two translator arms. Each translator arm is c. 380 µm long and attached to a pollinium. The corpusculum, translator arms and the two attached pollinia form a pollinarium (Fig. 4C, D).

Each pollinium is a flat wing like body, 1407 x 600 µm, narrow at its base close to the translator arm and wide at the apex, without any extra pollinial appendages. The pollinium is pendulous in orientation and situated in the basal part of the anther (Fig. 4C, D). The germination locus of the pollinium is exocentrolateral and appeared as a slit after acetolysis (Fig.5A, B).

Gynoecium consists of two free, superior ovaries and two separate styles, united apically into stigma head. Styles c.2500 µm long, terete. Stigma head is broad, c.5107 µm diameter, pentagonal. Five stigmatic chambers are situated below the lobes of stigma head alternating the anthers. Stigmatic chambers are beak like, due to c. 1500 µm long, hard, raised, anther wings. Each stigmatic chamber encloses the true receptive stigmatic surfaces of the gynoecium and the secretory tissues i.e. nectaries (for detail see chapter 7).

Stigmatic chambers are connected to the corona lobes by delicate spongy capillary passages. Through this capillary system the nectar secreted by the nectaries in the stigmatic chambers flows into the corona lobes. Corona act as the nectar holder and allows the incoming insects to such the necter through the pore at its apex.

Follicles are produced singly or paired per flower. They are inflated with an air sac round the seed producing region, recurved and 6-10 x 3-5 cm in size (Fig. 4E). Seeds flat, broadly ovate, 8.1 x 5.15 mm, minutely tomentose, comose (Fig. 4F)and each weigh about 1.15 mg.

The plants flowers all the year round, however peak flowering occurs from May - August while maximum fruit formed from August - October. The flower anthesis usually occur in the early morning or late evening. The flower remain in bloom for about 3 days, rarely upto 4th. day. Unpollinated flowers soon droped along with the pedicle, leaving scarce of pedicle on the peduncle. while in the pollinated (fertilized) flowers, swelling of ovaries is evident within 3 - 4 days of flower closing.

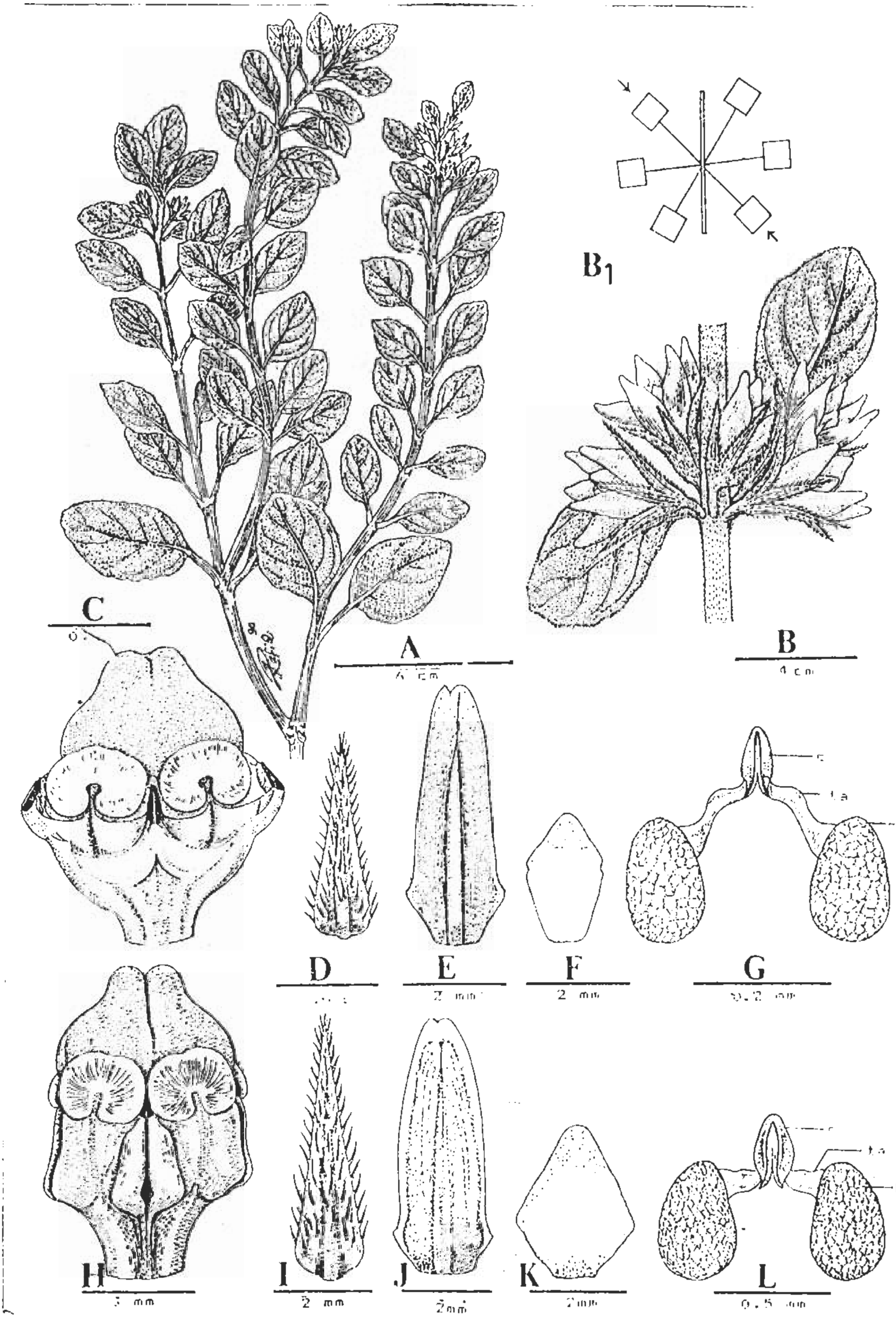
Generally the structure and occurence of androecium and gynoecium and its component parts as described for *Calotropis* are more or less similar throughout the studied taxa so they are not mentioned in following text to avoid repetetion.

Glossonema varians (stocks) Hook. f.:

Glossonema varians (stocks) Hook. f. is a perennial herb, branched from the base with milky latex. Stem is prostrate or ascending, thinly-densely pubescent with white hairs. Leaves are opposite, petiolate, somewhat fleshy, grey-green, broadly ovate-circular, pubescent with acute, obtuse or mucronate, retuse tip, undulate-weavy margin and obtuse to subcordate base. Flowers are

Fig. 6. Glossonema esculentum: A, Habit ; B & B_1, Inflorescence (arrow indicating ♀ flowers); A-G parts of ♂ flower: C, Gynostegium; D, Sepal; E, Petal; F, Corona; G, Pollinarium; H-L parts of ♀ flower: H, Gynostegium; I, Sepal; J, Petal; K, Corona; L, Pollinarium.

(c, corpusculum; p, pollinium; ta, translator arm)

B1
A
6 cm
B
4 cm
C
0.
D
E
2 mm
F
2 mm
G
0.2 mm
c
f.a
p
H
3 mm
I
2 mm
J
2mm
K
2mm
L
0.5 mm
c
f.a
p

arranged in subsessile umbellate, 4-8 flowered axillary cymes (Fig. 6A, B).

The plant is andromonoecious with two perfect and 2-6 staminate flowers per inflorescence (Fig. 6B$_1$).

Staminate flowers:

Flowers are actinomorphic, c.8.33 mm long with 3.2 x 1 mm pedicel. Calyx and corolla are more or less similar to that of perfect flowers but relatively smaller in size i.e. 4.04 x 0.96 mm and 5.04 x 2.13 mm respectively (Fig. 6D, E). The corona lobes are obtrullate, 2.57 x 1.79 mm, and bifid (Fig. 6F). Staminal column 1.12 mm long. The reniform anther flap 639 x 853 µm, covers the periphery of stigma head but not the corpusculum. Corpusculum dark brown, 250 x 117 µm , translator arms relatively much elongated, 318 x 193 µm in size, Each polinium is 460 x 291 µm, similar to the perfect flowers (Fig. 6G).

Stigma head is relatively large 1.46 x 1.75mm, style absent and ovaries rudimentry. Nectaries are situated in the interstaminal portion where stigmatic chambers are found in perfect flowers. Detail of nectar secretion and availability is similar to that of perfect flower.

Usually solitary follicles are produced per flower. They are usually inflated , 3-6 x 1-2 cm, ellipsoid, ± attenuate at apex, echinate. Seeds 5.5 x 4.5 mm, brown, broadly ovate, flattened, comose and each weigh about 0.26 mg.

Perfect flowrs:

Flowers are actinomorphic, c.9.39 mm long (excluding pedicle) with 3.6 x 1.67 mm pedicle. Calyx 5-lobed, lobes 5.24 x 1.19

mm. green, triangular-subulate, hairy and with acute tips (Fig. 6I). Corolla also 5-lobed, yellow with purplish ting, campanulate, tube short. lobes 5.61 x 2.11 mm. oblong-ovate, obtuse-retuse, obtusely keeled at base (Fig. 6J). The corona is made up of five cream, thin lobes, attached to the corolla and surrounds the gynostegium along with the staminal column. Each lobe is 3.14 x 2.07 mm, trullate, thin, entire, glabrous, smooth alternating with the stigmatic chambers and corolla lobes (Fig. 6K). Staminal column is short about 1.32 mm long. An hyaline, reniform, 723 x 1033 µm anther flap from each anther, cover the corpusculum and periphery of the stigma head (Fig. 6H). Corpusculum is black brown, 269 x 147 µm and translator arms 148 x 59 µm in size.

Each pollinium is yellow, ovate, 502 x 296 µm, without any extra pollinial appendages (Fig. 6L). The pollinium is pendulous in orientation and situated in the basal part of the anther. The germination locus of the pollinium is exproximolateral which appeared as a slit after acetolysis (Fig. 5C).

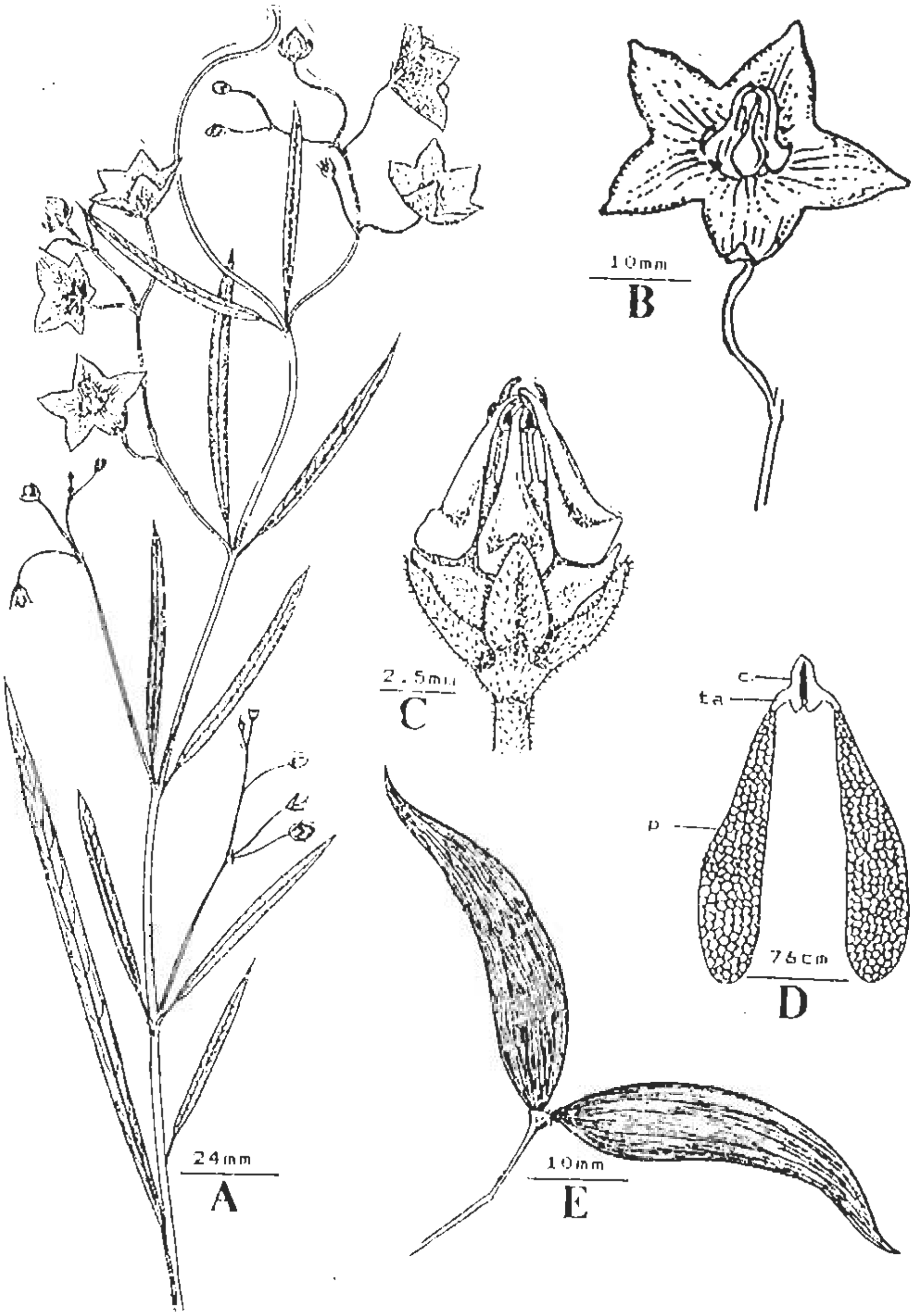
Styles short, terrate, c. 1325 µm long. Stigma head green, fleshy, conical, 1.36 x 1.57 mm, clavate or peltate. Anther wings forming the alar fissure are 525 µm long.

Nectaries situated with in the stigmatic chambers secrete nectar that accumulate below stigmatic chambers betweeen staminal column and corona lobes(see chapter 7 for detail). A notch or small gap is present between the corona lobes in front of the stigmatic chamber, so that insects could reach the nectar.

G. varians (Stocks) Hook. f. regenerates from the perennating rootstock after rain. Plants usually flowers once a year.

Fig. 7. Oxystelma esculentum: A, Habit ; B, Flower; C, Gynostegium and corona lobes (Petals removed); D, Pollinarium; E, Fruit.

(c, corpusculum; p, pollinium; ta, translator arm)


24mm
A
10mm
B
2.5mm
C
c.
ta
p
76cm
D
10mm
E

Flower anthesis starts from the basal infloresence of a stem and continue towards apex. In an inflorescence first the perfect flowrs open at c. 6.0 - 6.30 a.m., on next morning (after 24 hour) the 2nd perfect flower opens. Then the staminate flowers opens regularly with an interval of 12 - 24 hours, mostly one and rarely two at a time.

The flower remain in bloom for an average three days (3-4 days) and on the 3rd. day of anthesis, the colour of corolla and corona fades and stigma head become dark brown. The plant remain in bloom for about 1½ - 2 months. Usually not more than 3 - 4 fruits are produced per plant. After their dehiscence the leaves and stems dry and wither and only the perennating rootstock remains.

Oxystelma esculentum **(L. f.) R. Br.:**

O. esculentum (L. f.) R. Br. is a twining perrenial herb. Stem much branched, slender, glabrous with milky latex. Leaves are peliolate, deciduous, pale green, glabrous, linear or linear lanceolate with acute tip. Flowers are arranged in pedunculate, lateral sub-umbellate or racemoid cymes (Fig. 7A).

Flowers are perfect, actinomorphic and pedicelate (Fig. 7B). Calyx consists of five, green, glabrous, lanceolate lobes with acute tip. Corolla shallowly campanulate to almost rotate, differentiated into a small, green tube with fark purple lines opposing the corona lobes and into five broad lobes. Lobes are glabrous outside, densely pubescent at margins, free at half way of their length , with white margins and tips and purple line pattern on white background. Corona are white and in two series.

The outer one forms a densely pubescent ring or annulus at the base of the staminal column. The inner corona is made up of five ovate - lanceolate segments, attached to the staminal column and their tips over arch the stigma head (Fig. 7C). An hyaline flap from each anther apex cover the periphry of stigma head. Corpusculum brown, 280 x 100µm, and translator arms c.143 µm in length.

Each pollinium is yellow, 1430 x 250 µm, without any extra pollinial appendages (Fig. 7D). The pollinium is located in the basal part of the anther and pendulous in orientation. The germination locus is exocentrolateral along with notches and small slits (Fig. 5D).

Stigma head is c.1625 µm in diameter, ± circular, styles are comparatively long, c. 3000 µm in length. Stigmatic chambers are boardered by c. 2062 µm long anther wings and are continuous to the base of the staminal column through the depression or furrow below them.

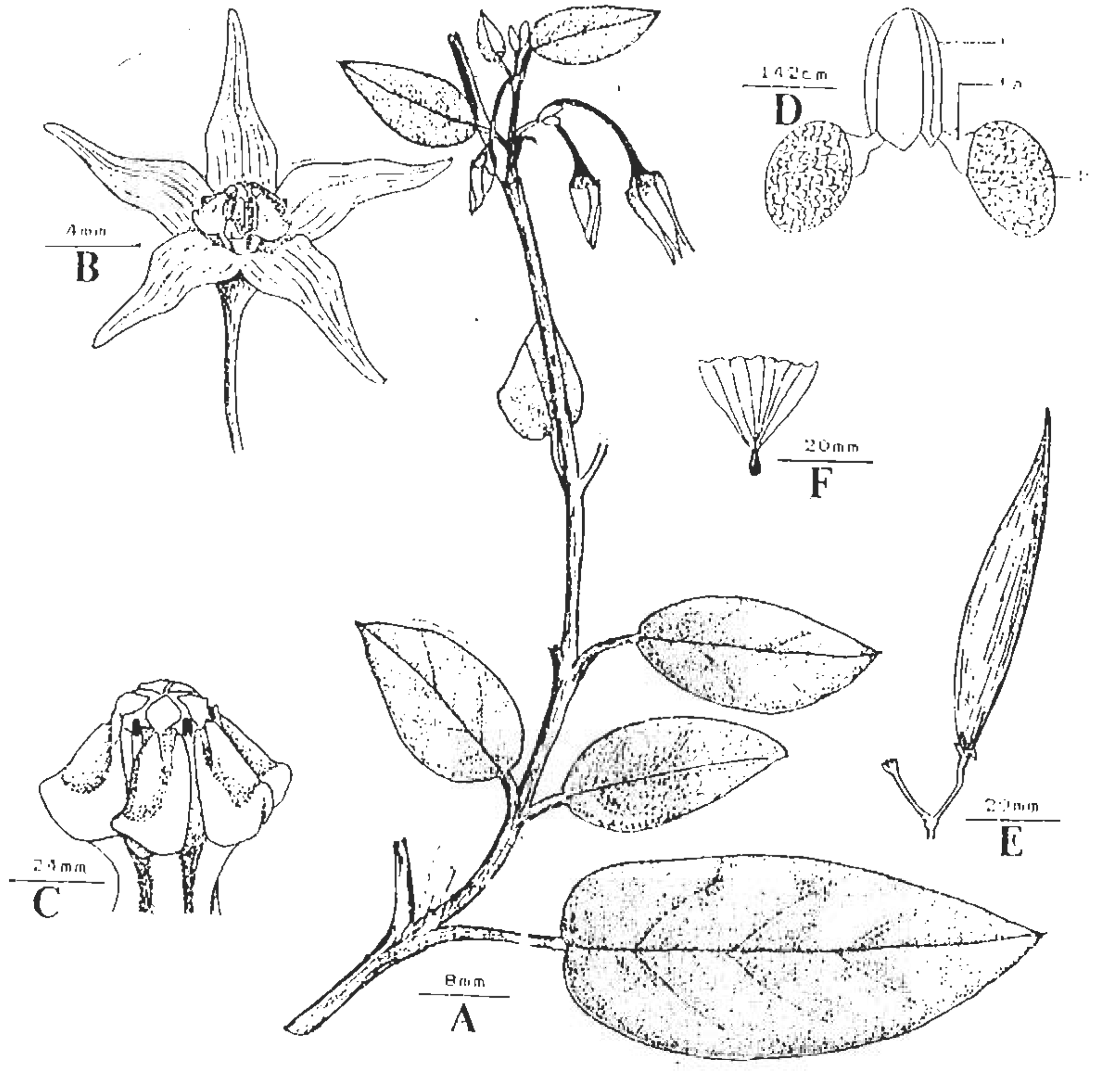
Nectar secreted in the stigmatic chambers, accumulates in the staminal furrows between staminal column and outer corona.

Solitary - paired follicles are produced per flower. They are 3.8 - 7 cm long, glabrous, oblong and obtuse or lanceolate, acute (Fig. 7E). Seeds c. 3.5 mm long, broadly ovate, comose.

Plants usually flowers once a year during July - September, when water stands in the area (low lying and saline) after the rain or through the seepage of canals. Flower open early in the morning and remain in anthesis for 3 - 4 days. After the dehiscence of fruits in September - October, aerial parts of plant dry and wither, only perennating rootstocks survive the winter sea-

Fig. 8. Pentatropis nivalis: A, Habit ; B, Flower; C, Gynostegium and corona lobes (Petals removed); D, Pollinarium; E, Fruit; F, Seed.

(c, corpusculum; p, pollinium; ta, translator arm)


4mm
B
1.42cm
D
20mm
F
2.4mm
C
20mm
E
8mm
A

son.

Pentatropis nivalis **(Gmel.) Field and Wood:**

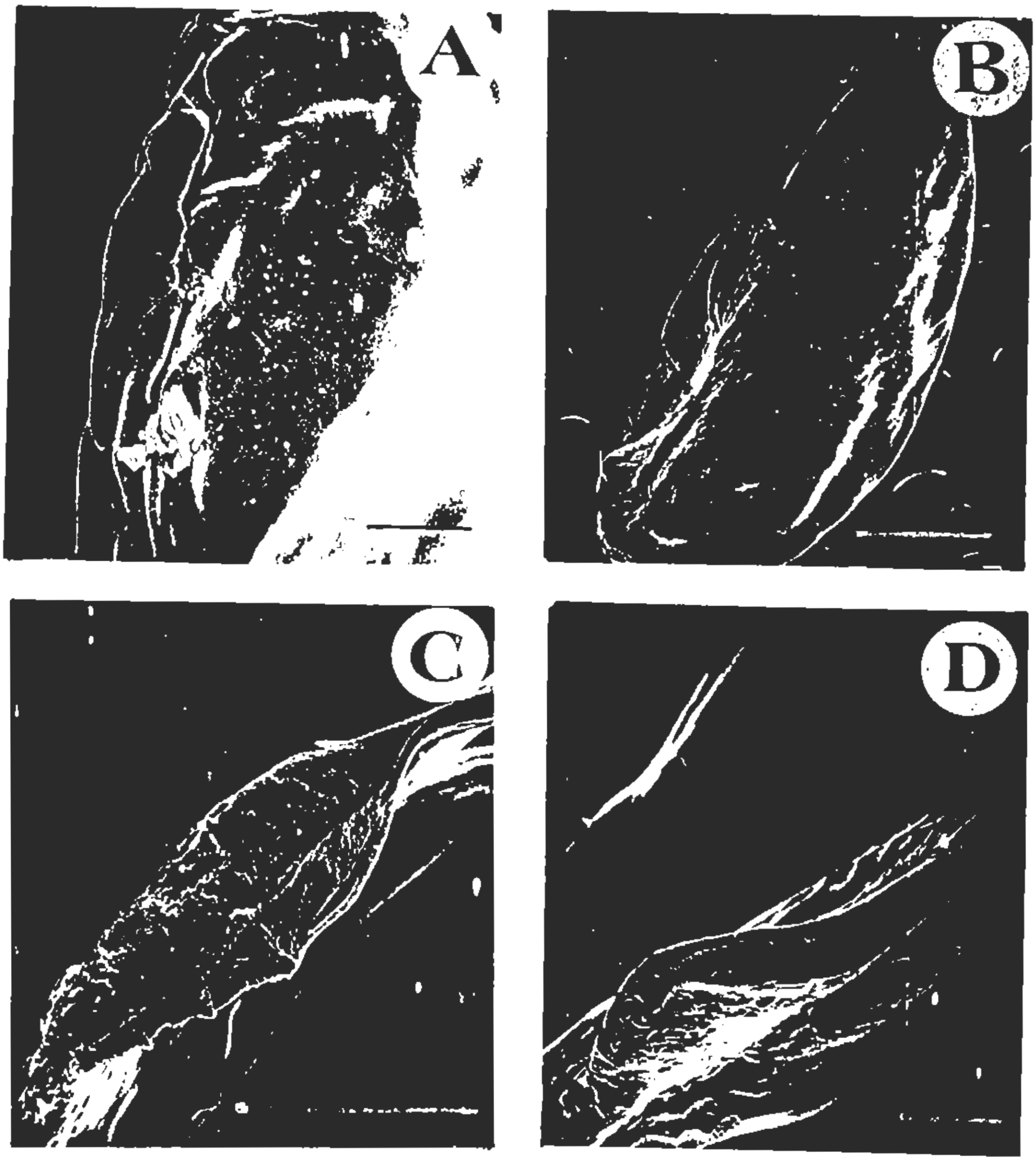
P. nivalis (Gmel.) Field and Wood, is a twining perennial herb, yellowish green, glabrous or glabrescent sap water like, older stems with rough corky bark. Leaves are opposite, petiolate, fleshy, linear, oblong, ovate or elliptic, glabrous with acute or obtuse , mucronate tip, rounded or subcordate base and entire margin. Flowers are arranged in few-flowered axillary clusters (Fig. 8A).

Flowers are perfect, actinomorphic and pedicelate. Calyx 5-lobed, lobes green, ovate-lanceolate. Corolla also 5-lobed, lobes pale yellow or pale greenish yellow with purple tinged at base, spreading and spiralling, linear-oblong to linear-ovate, glabrous outside, minutely puberulous within (Fig. 8B). Corona in one series, consisting of five laterally compressed segments, alternating with the stigmatic chambers and corolla lobes, attached towards the top of the staminal column. Segments are with spreading spurred base and acute incurved tip. An hyaline flap from each anther cover the periphery of stigma head (Fig. 8C). Anther wings are hard, straight, elongated, about 1375 µm in length, boardered the stigmatic chambers.

Corpusculum brown, 637 x 220 µm and translator arms are 105 µm long. Each pollinium is yellow, 350 x 243 µm, without any extra pollinial appendages (Fig. 8D). The pollinium is pendulous in oriention and situated in the basal part of the anther. A slit like germination locus is situated in exoproximolateral region of the pollinium (Fig. 9A).

Fig. 9. Germination loci showing slits, apertures and notches. A, Pentatropis nivalis: Exoproximotrolateral; B & C, Pergularia daemia: Exosubdistolateral; D, Pergularia tomentosa: Exosubdistolateral.

Scale bar: A & D = 20 μm; B & C = 10 μm.


A
B
C
D

Styles terete, c. 1875 µm long. Stigma head greenish, ± rounded, c.1310 µm in diameter. Stigmatic chambers are much elongated widest at the base, gradually narrowing towards apex.

Nectar secreted in the stigmatic chambers, accumulates in the long narrow depression or furrow which is continuous from the apex of the stigmatic chambers to the base of the staminal column, outwardly boarded by corolla.

Follicles are produced solitary or paired per flower. They are lanceolate, glabrous, 5-7.5 x 1 cm , narrowed into a long acuminate tip or beak (Fig. 8E). Seeds 5.5 x 2.9 mm, ovate, flattened, minutley irregularly crenate at the lower end, comose (Fig. 8F) and each weigh about 0.25 mg.

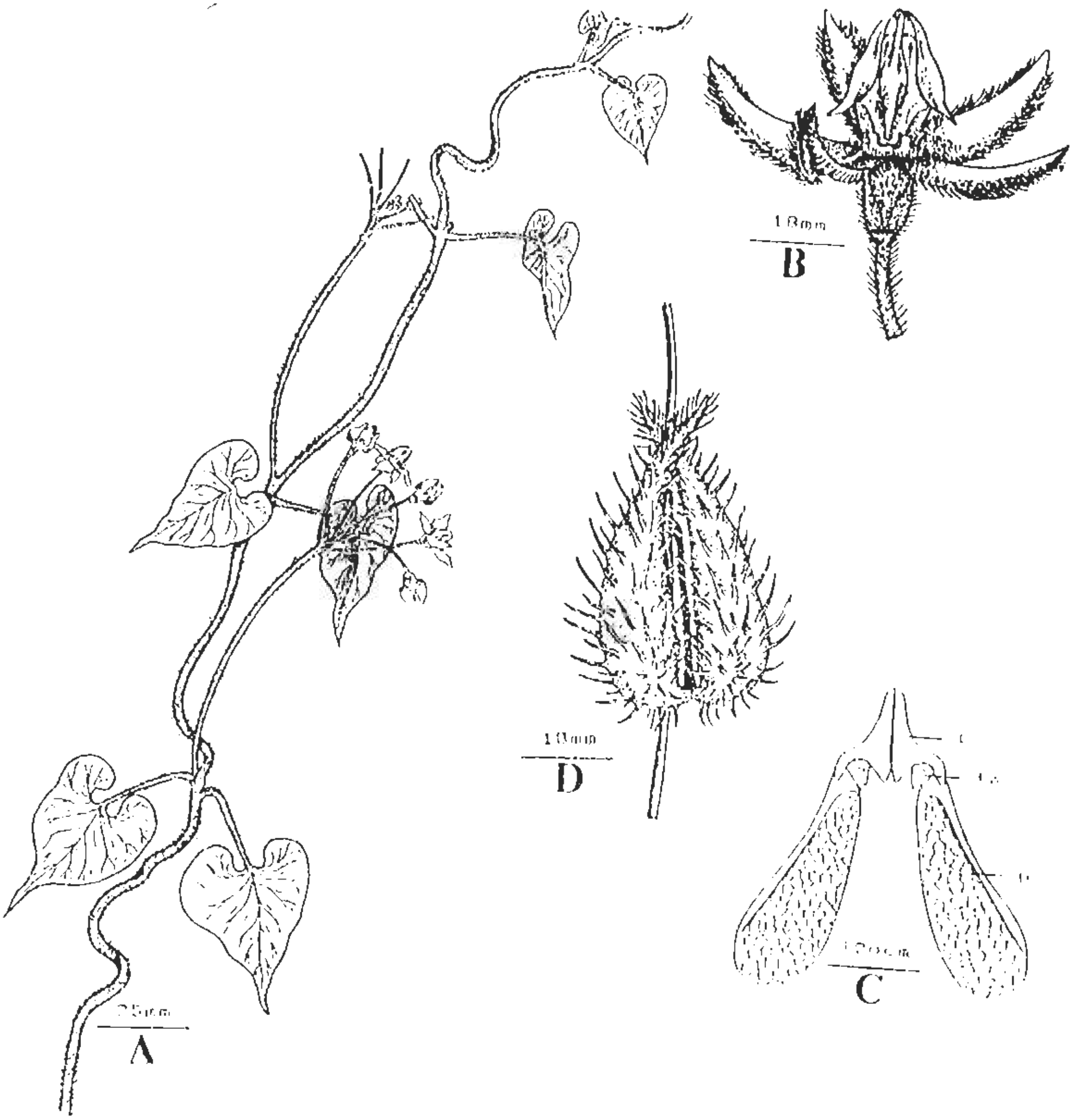
The plants usually flowers once a year after rain. The individual flower opens at 6.00 - 6.30 p.m in the evening, remain open through out the night and closed in the morning at 5.30 - 6.0. a.m. The flower usually open on three continuous nights and drop on the next day if unpollinated.

Pergularia daemia: **(Forssk.) Chiov.**

P. daemia (Forssk.) Chiov., is a perennial twining herb with spreading hairs and milky latex. Leaves are opposite, petiolate, broadly ovate or suborbicular with acute-acuminate tip and cordate base, glabrous or shortly pubessent above, usually velvety pubescent below. Flowers are arranged in lateral cymes (Fig. 10A).

Flowers are perfect, actinomorphic and pedicelate. Calyx consists of five, green ovate-lanceolate, hairy lobes with acute

Fig. 10. Pergularia daemia: A, Habit ; B, Flower; C, Pollinarium; D, Fruit.
(c, corpusculum; p, pollinium; ta, translator arm)


2.5cm
A
1.0mm
B
1.0mm
D
C

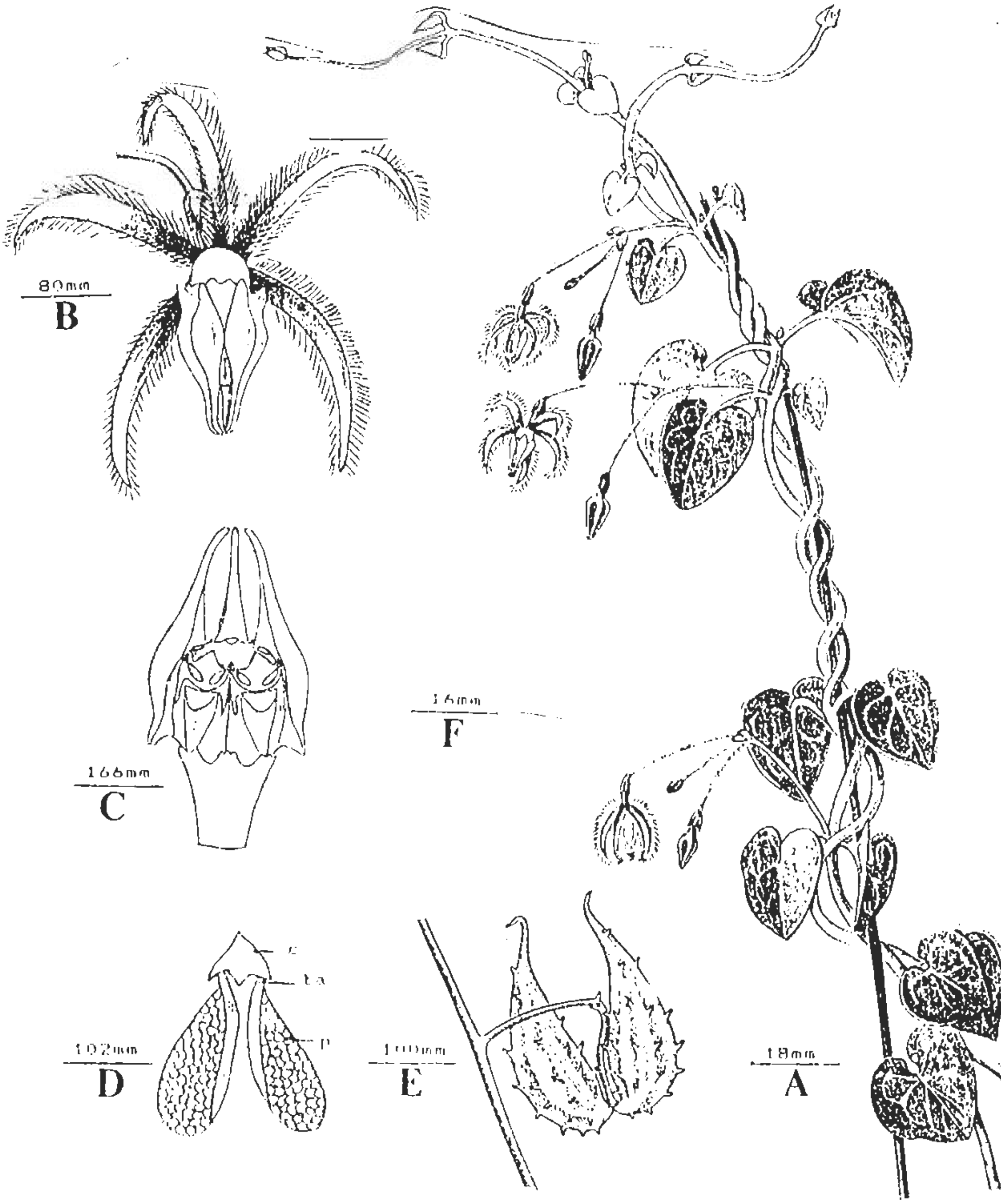
tips. Corolla is differentiated into c. 3.5 mm long basal tube that surrounds the base of corona and staminal column and into 5-lobes. Each lobe is dull white or greenish white, spreading, ovate - oblong, pubescent and hairy on the margins of the outer surface, The corona is white, smooth, shining and in two series. The outer one is membraneous, forming a continuous ring or annulus at the base of the staminal column, become 3-lobed just beneath each stigmatic chamber. The inner corona is consist of five fleshy lobes, attached to the apex of the staminal column. Each lobe has two spurs, one directed outward and downward, the other upward with the tip over the stigma head. An hyaline flap from each anther cover the periphery of stigma head.

Corpusculum brown, 240 x 155 µm and translator arms are quite short only c.50 µm long (Fig. 10C). Each pollinium is ± obovate, narrow at the apex close to the translator arm and wide at the base, 755 x 255 µm, with a false wing like extra - pollinial appendage on exolateral portion (Fig. 10C). The germination locus is exosubdistolateral with notches and an elongated slit (Fig. 9B, C).

Styles terete, c.4475 mm long, the two styles are united ± halfway of their length and continue as a single style (i.e. forming a compitum) toward the apex i.e. stigma head. Five vertical furrows or depressions runs along the length of th staminal column. Each furrow at its apical portion constitue true receptive stigmatic surface, boarded by the anther wings of two adjacent anthers forming a stigmatic chamber. While the middle-basal portion is overachis by the staminal outgrowth, leaving a slot in

Fig. 11. Pergularia tomentosa: A, Habit ; B, Flower; C, Gynostegium and corona lobes (Petals removed); D, Pollinarium; E, Fruit; F, seed.

(c, corpusculum; p, pollinium; ta, translator arm)


80mm
B
166mm
C
16mm
F
c
t.a
p
102mm
D
100mm
E
18mm
A

[illegible] [illegible], which seems to be continuous till the [illegible] fissure of the stigmatic chamber.

Follicles are mostly produced in pairs per flower. They are lanceolate, 5-8 x 1.2-1.3 cm, beaked, echinate and puberulous (Fig. 10D). Seeds 7.2 x 5.2 mm, ovate, velvety pubescent, with truncate tip and crenate margin, comose and each with an average weight of 1.35 mg.

In dry areas, plants usually flower once a year after rain. Where as in the well irrigated areas i.e. along the hedges of cultivated fields and gardens, they may flower twice a year, firstly from March - June and secondly from August - mid September.

Individual flower opens at 6 - 6.30 p.m. in the evening and closed in the morning. The flower usually open on three continuous nights. However the pollinated flower does not complete its anthesis period and remain close on the next night.

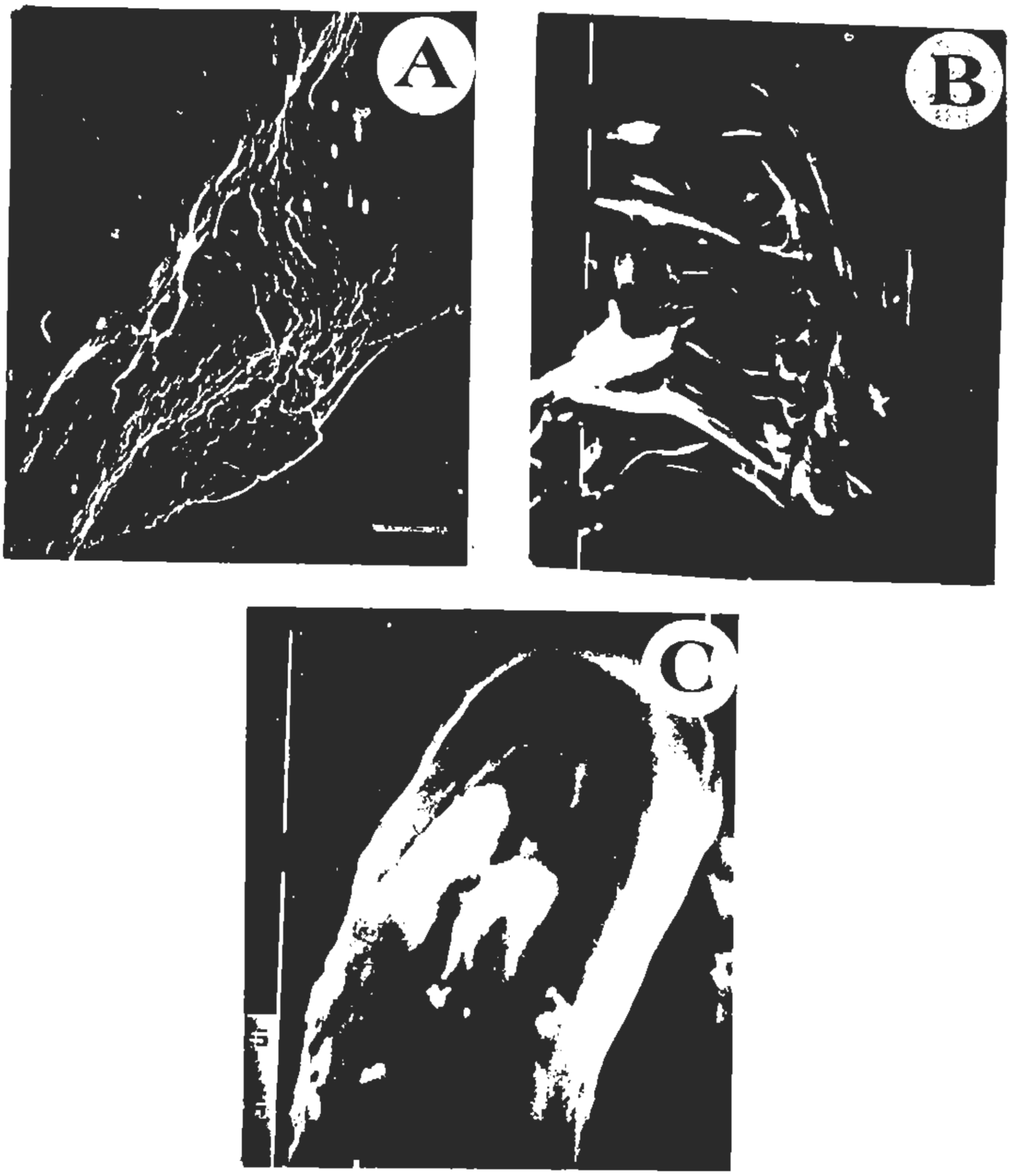
After fruit dehiscence, aerial parts of the plant wither and only perennating rootstocks remains through which plant regenerate itself on incoming favourable season.

***Pergularia tomentosa* L.:**

*P.tomentosa*L., is a twinning perennial herb, branches covered with dense ash-coloured pubescence and appressed hairs, with milky latex. Leaves are opposite, petiolate, ovate to almost circular with acute-accuminate tip, cordate base and entire margin, pubescent or velvety below. Flowwers are arranged in pedunculate, axillary umbellate cymes (Fig. 11A).

Fig. 12. Germination loci showing slits, apertures and notches. A, Pergularia tomentosa: Exosubdistolateral; B & C, Leptadenia pyrotechnica: Distal.

Scale bar: A = 100 μm; B & C = 10 μm.


A
B
C

Flowers are perfect, actinomorphic and long pedicelate. Calyx consists of five green, elliptic, acute, pubescent lobes. Corolla is greenish or reddish brown or white with much elongated tube. Descriptions of corolla, corona, androecium and gynoecium are more or less similar to that of *Pergularia daemia* (Forssk.) Chiov. with the exception of sizes (Fig. 11B, C). Thus the pollinium is 683 x 257 µm, corpusculum 223 x 163 µm in size while translator arms c.30 µm long. The germination locus is exosubdistolateral with slit and notches (Fig. 9D, 12A). The diameter of stigma head is 1500 µm and anther wings and styles are 1250 µm and 4000 µm in length, respectively.

Follicles are usually produced in pairs, 5 - 7.5 x 1.2 - 1.30 cm erect, ovoid, grey - tomtose, softly spine - echinulate, tapering towards the apex (Fig. 11E). Seeds 8.9 x 6.9 mm, ovate, flattened, with minutely toothed margin, velvety pubescent, comose (Fig. 11F) and each with a weight of 1.4 mg.

Plant flowers once a year from March - July and in contrast to *P.daemia* (Forssk.) Chiov. its aerial parts do not die off after fruit dehiscence. Other phenological aspects are similar to *P. daemia* (Forssk.) Chiov.

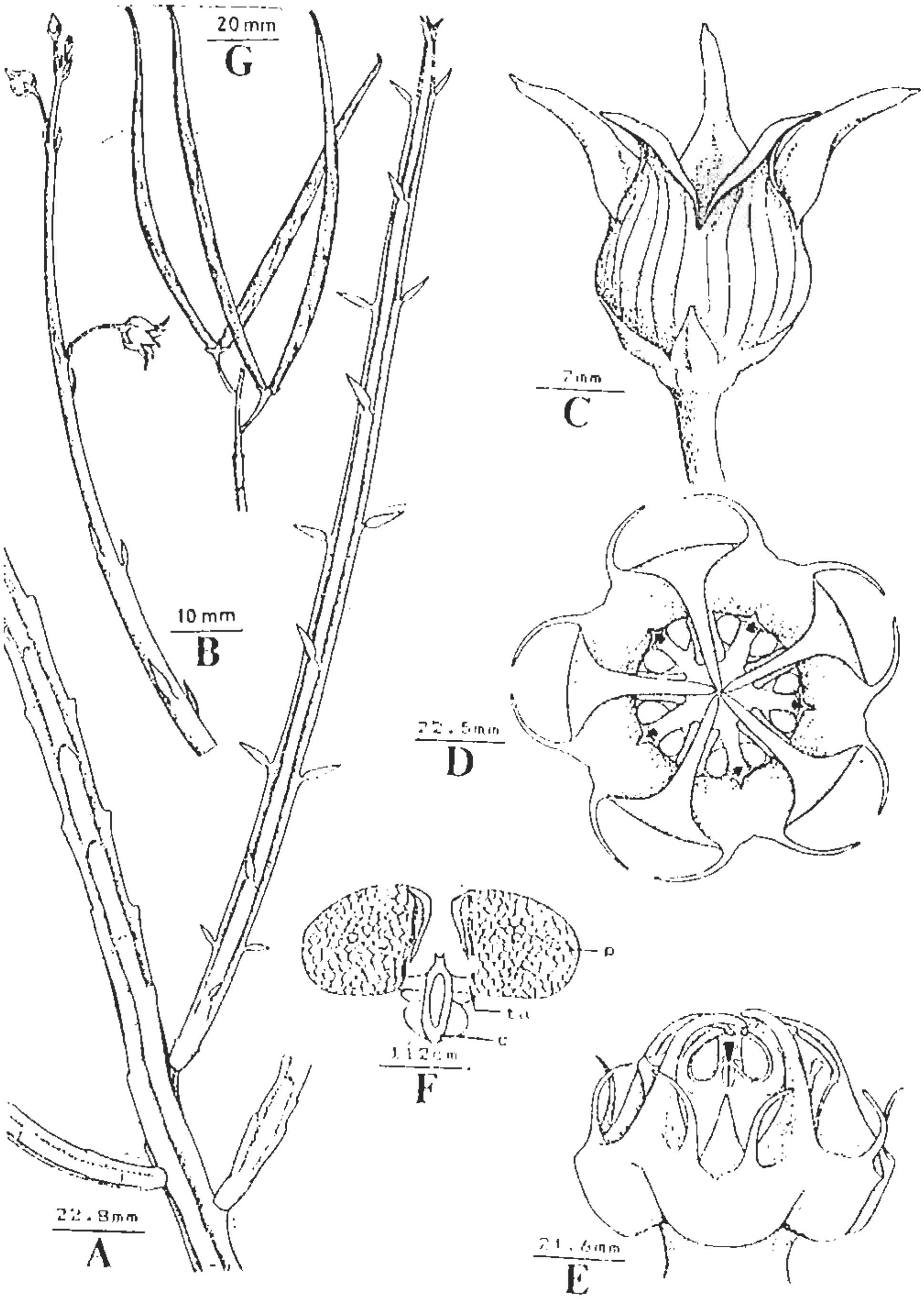
Tribe Ceropegieae:

Caralluma edulis **(Edgew.) Hook. f.:**

C. edulis (Edgew.) Hook. f. is a small succulent, perennial, branched herb. Stem is erect, branches subterate, 4-grooved and with clear water like sap. Leaves (when present) are small,

Fig. 13. Caralluma edulis: A, Vegitative branch; B, Flowering branch; C, flower; D & E, Gynostegium and corona lobes (side and top view); F, Pollinarium.

(c, corpusculum; p, pollinium; ta, translator arm)


20 mm
G
7mm
C
10 mm
B
22.5mm
D
p
t.a
c
112cm
F
22.8mm
A
21.6mm
E

linear with acute apex. Flowers are arranged in axile of scale like bracts in pairs or solitary (Fig. 13A, B).

Flowers are small, pedicelate, perfect and actinomorphic. Calyx 5-lobed, lobes are greenish yellow, ovate-linear, glabrous with acute tip. Corolla campanulate, yellowish, could be distinguish into a broad tube with vertical purplish lines on yellowish background and into 5-lobes. Lobes are ovate-lanceolate with ± recurved acute tips (Fig. 13C). Corona in two series, attached to the staminal column. Outer corona is compose of five deeply bifid segments forming the nectar holder or pouch below the stigmatic chambers. Inner coron consists of five linear segments, incumbment on the anthers and the stigma head (Fig. 13D, E). Staminal column is short and attached to the corolla. Anthers are short, subquadrate, lying horizontally in deep grooves on the stigma head, without apical hyaline membranous flaps over stigma head. Corpusculum 205 x 65 µm and translator arms are c.60 µm in size. The pollinarium i.e. corpussculum, translator arms and pollinia is not concealed by anther (Fig. 13D, E).

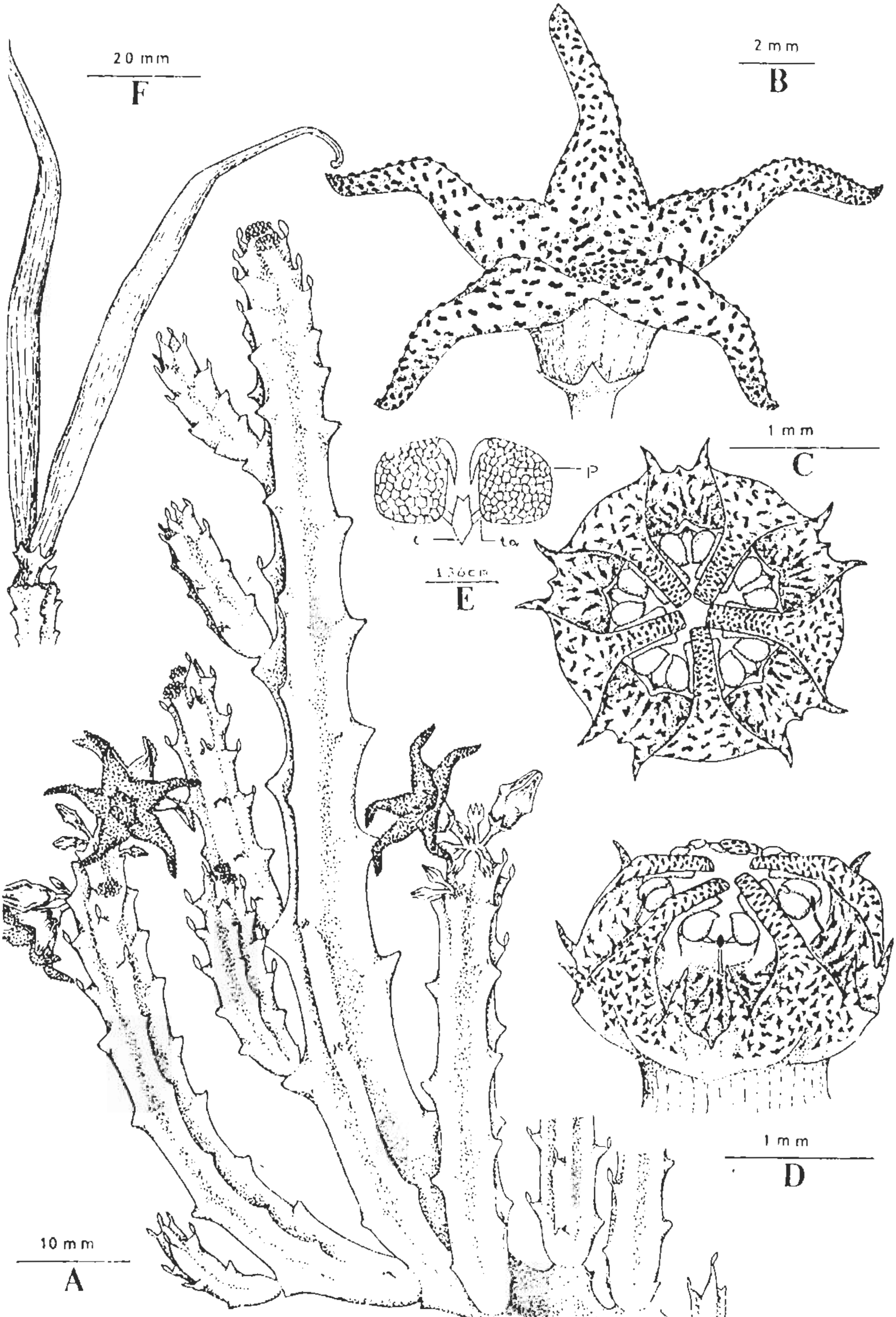
Each pollinium is yellow, 447 x 342 µm, with extra pollinial appendage on the distal end (Fig. 13F). The pollinium is erect in orientation and located in the apical part of the anther. The germination locus of pollinium is distal and the extra pollinal appendage act as the site of germination.

Stigma head is green, c.1625 µm in diameter, anther wings c.250 µm in length while style is ± absent or very short.

Nectar is secreted in the stigmatic chambers and accumlates below in the nectar pouches formed by the outer corona.

Fig. 14. Caralluma tuberculata: A, Habit; B, flower; C & D, Gynostegium and corona lobes (side and top view); E, Pollinarium.

(c, corpusculum; p, pollinium; ta, translator arm)


20 mm
F
2 mm
B
1 mm
C
p
c
ta
136cm
E
1 mm
D
10 mm
A

Mostly paired follicles are produced per flower. They are narrowly fusiform, 8.5 -11 cm. smooth with acute tips. Seeds are 7.6 x 4.2 cm in size and each with an average weight of 0.42 mg.

Plants usually flowers from July - September. Flower open in the morning and remain in anthesis for 24 - 48 hours.

Caralluma tuberculata N. E. Br.:

The morphology of *C. tuberculata* N. E. Br., is similar to *C. edulis* (Edgew.) Hook. f. However it differ from *C. edulis* Hook. f. on the basis of angular stem, terminal cymose inflorescence and flower colour (Fig. 14A, B). Corona, corolla lobe and interior of the tube are dark maroon with small glandular surface while the limb of the tube is yellow with maroon glands (Fig. 14B, C).

Pollinium is yellow 337 x 280 µm (Fig. 14E), translator arms c. 50 µm and corpusculum 170 x 60 µm in size.

The stigma head is whitish, c. 1250 um in diameter and the anther wings boardering the stigmatic chamber are c. 275 µm in length.

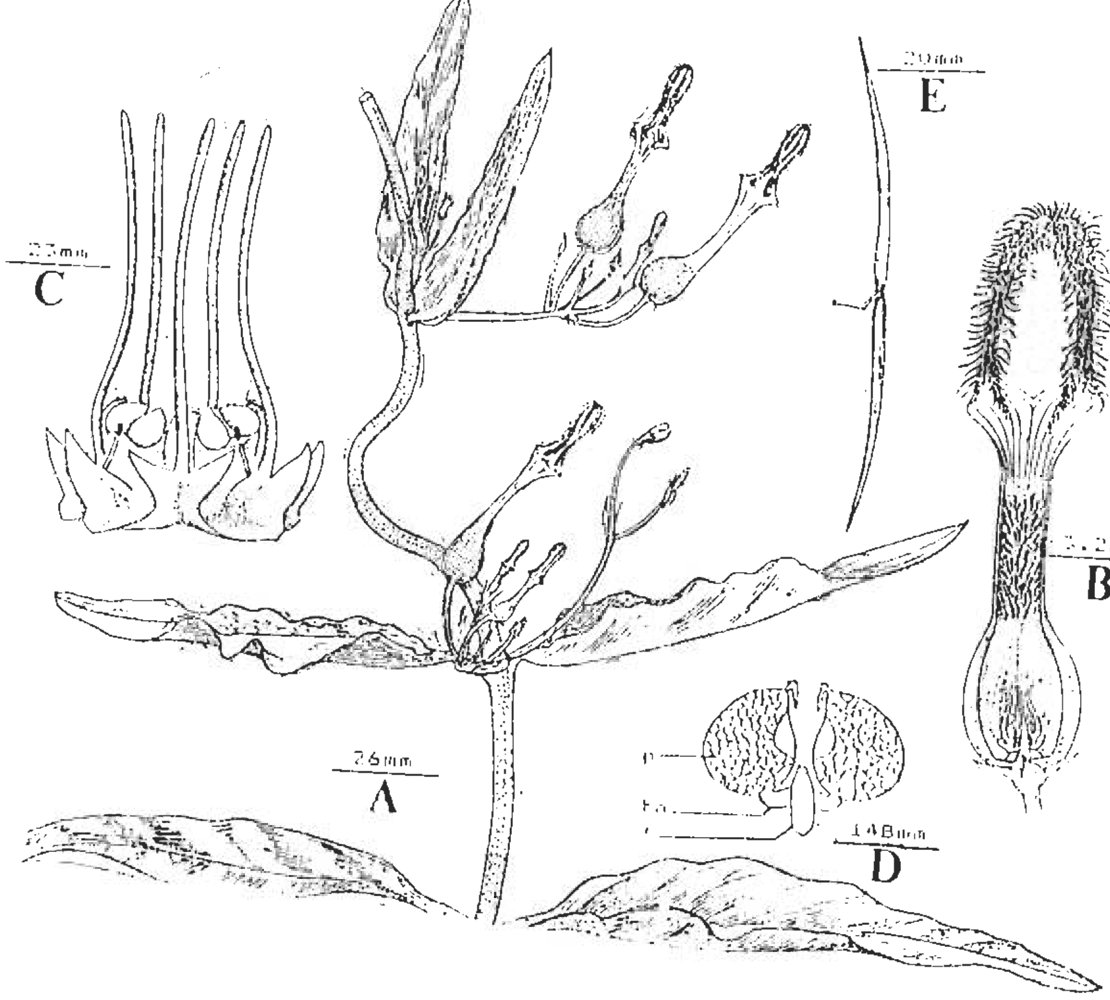
Follicles are usually in pairs, 8 - 10.5 cm, fusiform smooth and gradually tapering toward the tip (Fig. 14F). Seeds 7.9 x 3.25 µm, ovate. flattened with winged margins, comose and each with an average weight of 0.67 mg.

Plants usually flowers from February - May. Flower open in the morning and closes after 24 hours only.

Ceropegia bulbosa Roxb.:

C. bulbosa Roxb. is a perennial, tuberous herb. Tuber is de-

Fig. 15. Ceropegia bulbosa: A, Habit; B, flower (cut open to show the inner portion); C, Gynostegium and corona lobes (side view); D, Pollinarium; E, Fruit.
(c, corpusculum; p, pollinium; ta, translator arm)

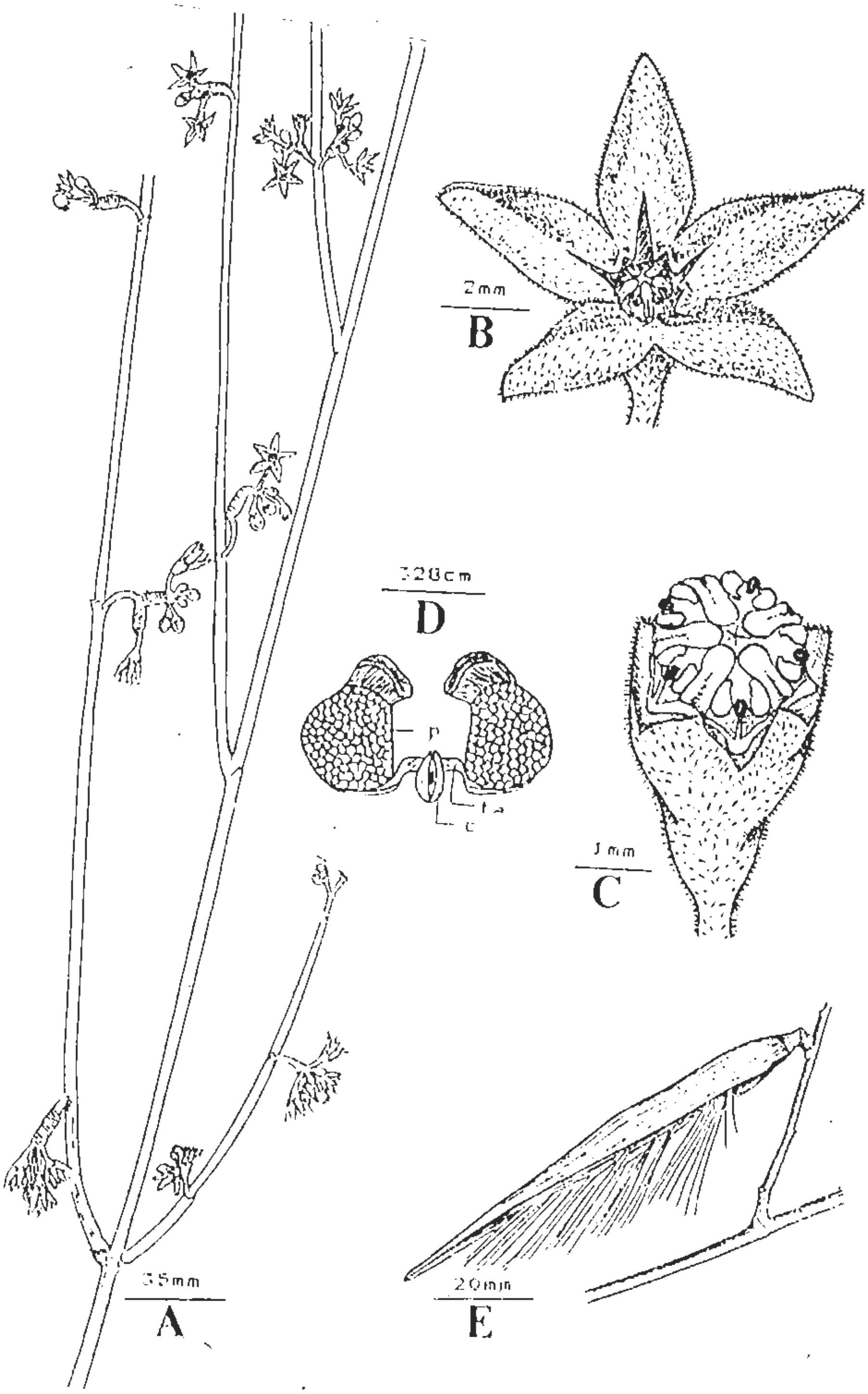

2.3mm
C
2.0mm
E
3.2mm
B
2.6mm
A
p
t.a.
r
1.48mm
D

pressed - globose. Stem is twining, slender, glabrous or with few scattered hairs. Leaves are opposite, narrowly ovate to ovate - oblong to broadly ovate, lanceolate - linear, petiolate, green, sometime reddish tinged, fleshy glabrous, with entire margin, mucronate apex and obtuse - subcordate base. Flowers are arranged betweeen the petioles in 2 - 7 flowered pedunculate cymes (Fig. 15A).

Flowers are perfect, pedicelate, erect and actinomorphic. Calyx 5-lobed, lobes are green, linear-lanceolate with acute tip. Corolla is distinguished into a long tube and five lobes . Corolla tube consists of a pale green globose base and a long narrow hairy (inside) tube which broaden into a funnel shaped top. Corolla lobes are purple with green tip, linear, fused together at the tips to form a cage, with long purple hairs on reflexed margins (Fig. 15B). Corona is in two series attached to the staminal column. Outer corona consistss of five ± long, erect, filiform segments alternating the stigmatic chambers. Staminal column is short, located centrally in the inflated globose basal section of the corolla. Anthers are short, lying horizontally in deep grooves on the stigma head, without apical hyaline flaps. Pollinarium is exposed and not concealed by the anther (Fig. 15C). Corpusculum is 110 x 40 µm and translator arms are c.50 µm in length.

Each pollinium is yellow, 257 x 237 µm , with extra pollinial appendage at distal end (Fig. 15D). The extra pollinial appendage acts as the germination site, so the germination locus is distal. The pollinium is erect in orientation and located in the

Fig. 16. Leptadenia pyrotechnica: A, Habit; B, flower; C, Gynostegium and corona lobes ; D, Pollinarium; E, Fruit. (c, corpusculum; p, pollinium; ta, translator arm)


2mm
B
320cm
D
p
t a
c
1mm
C
35mm
A
20mm
E

apical part of anther (Fig. 15C).

Stigma head is small, green, c. 1437 µm in diameter, anther wings c.144 µm in length and each style is c.250 µm long.

Nectar is secreted in the stigmatic chambers and accumulates in the outer corona (nectar pouches).

Mostly paired follicles are produced per flower. They are cylindrical, 10 x 0.4 cm, smooth with acute tips. Seeds are ovate, brown, 607 x 3.0 mm, glabrous with paler margins, comose and each c.0.34 mg in weight.

Tubers initiate aerial parts of plant soon after rain. Plants usually flowers within 15-20 days after rain. Flower anthesis occur in late afternoon at c. 5.0 p.m. and it survive only for 24 hours. Plant remain in bloom for only 1-1½ month and after dehiscence of fruits, aerial portion dry off and only underground tuber remains.

***Leptadenia pyrotechnica*: (Forssk.) Dcne.**

L. pyrotechnica (Forssk.) Dcne. is an erect, much branched, generally leafless shrub with clear yellowish sap. Branches are wand like, erect, green, slender and spinescent. Leaves (when present) are subsessile, linear to linear - lanceolate, glabarous with acute tips and soon deciduous. Flowers are arranged in short pedunculate, unbellate cymes (Fig. 16A).

Flowers are perfect, small, short pedicelate and actinomorphic. Calyx deeply five lobed, lobes are ovate, fine tomentose with subacute tip. Corolla greenish - yellow, rotate or funnel shaped, with elliptic - triangular acute lobes, convex above, concave and weakly revolute - margined at back, fine tomentose, each lobe with a triangular like depression at the base (Fig. 13B). Corona is in two series. Outer corona consists of five fleshy segments, attached to the base of the corolla alternating with its lobes, incumbent on the stigma head. Inner corona is reduced to an undulate ring or annulus on the base of staminal column, below the stigmatic chambers (Fig. 16C). Anthers are short, incumbent on the stigma head and without apical hyaline flaps. Pollinarium is exposed and not concealed by the anther (Fig. 16C). Corpusculum is 80 x 50 µm, and translator arms are c. 110 µm in length.

Each pollinium is yellow, 250 x 170 µm, with extra pollinial appendage at distal end (Fig. 16D). The extra pollinial appendage acts as the germination site, so the gomination locus is distal. On acetolysis, notches and slits are observed at the germination locus (Fig. 12B, C). The pollinium is located in the apical part of the anther and its orientation is erect.

Stigma head is small, green, c.750 µm in diameter, anther wings c. 175 um and each style is c. 175 µm long. The annulus formed by the inner corona and portion between staminal column and corolla act as the nectar holder which is secreted by the nectaries within the stigmatic chambers.

Single-paired follicleas are produced per flower. They are ovate-oblong, oblong, 7.5 - 11.5 cm x 8 7 mm, smooth and tapering towards apex. Seeds are ovate, 0 x 3.25 mm, brown, comose and each c. 0.37 mg in weight.

Plants flowers all the year round with maximum flowering after rain. Flower usually open in the evening at 5.30 p.m. or early morning. The life of a flower is of ± 3 days.

DISCUSSION

All the studied taxa are perennial, mostly herbs few shrubs, about half of them survive unfavourable season as the perennating rootstock or tuber. All of them bloom for a short period after rain with the exception of *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali and *Leptadenia pyrotechnica* (Forssk.) Dcne.

The life of flower is only 24 hours in *C. edulis* (Edgew.) Hook. f., *C. tuberculato* L. and *Ceropegia bulbosa* Roxb. while Bayer (1978) reported 2-3 days in *Ceropegia ampliata* E. Meyer. In all the other studied taxa flower life is 3 days long. Similarly, life of *Calotropis procera* (Ait.) Ait. f. flower is reported as 2- 3 days by Eisikowitch (1986) from Israel and Saoji and Chitaley (1975) from India. According to Liede and Whitehead (1991), the flower life of *Sarcostemma viminale* R.Br. is 4-5 days and of *Asclepias* species 5-7 days (Lynch, 1977; Bookman, 1983; Morse, 1987; Shannon and Wyatt, 1988c). Thus the flower life of the studied taxa is quite short as compare to the *Sarcostemma* and *Asclepais* species.

Glossonema varians (Stocks) Hook. f., is andromonoecious with perfect and staminate flowers on a single inflorescence, whereas all the other taxa are hermaphrodite with perfect flowers. Andromonoecious condition is being reported for the first time in the family. Although, Beare and Perkins (1982) in *Asclepias tuberosa* L. reported male and female plants (i.e. dioecy) on the basis of their flowers, functioning as pollinia donors (staminate) and recepients (pistillate) due to differences in the size of alar fissures and pollinia, however, flowers of both type of plants are bisexual (i.e. perfect).

Sreedevi and Namboodiri (1982) have stated that the position of the germination locus is genus specific. However, the present study indicates that the two or more genera and species have similar germination locus. For instance, *Pentatropis nivalis* (Gmel.) Field and Wood. and *Glossonema varians* (Stocks) Hook. f. both have exoproximal germination locus; *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L. and *Oxystelma esculentum* (L.f.) R. Br., all with exosubdistolateral while *Caralluma edulis* (Edgew.) Hook. f., *Caralluma tuberculata* N. E. Br., *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Dcne. are with distal germination locus. Furthermore, in some genera, like *Calotropis*, where information about more than one species is available, intrageneric variation has also been observed. In *Calotropis gigantea*, the germination locus is exodistolateral (Saoji & Chitaley, 1975; Rao & Kumari, 1979), whereas in *Calotropis procera* (Ait.) Ait. f., it is exocentrolateral. It has also been observed that the extra pollinial appendages present on the pollinium of *C. edualis* (Edgew.) Hook. f., *C. tuberculata* N. E. Br., *Ceropegia bulbosa* Roxb. and *L. pyrotechnica* (Forssk.) Dcne.

[illegible] as the germination [illegible].

Since Sprengel (1793) the coronal segments (cuculli) were considered as the nectaries of the flower, although Stadler (1886) pointed out that along with coronal segments, stigmatic chambers also secrete nectar. It is Galil and Zeroni (1965), who cleared the concept and proved that nectaries are situated with in the stigmatic chamber and coronal appendages only act as nectar holders.

According to Schick and Remus (1984) and Kunze (1990, 1991), the nectaries are located on the receptacle between the filaments i.e. interstaminal and may referred as "Focal nectaries" according to Kunze (1991).

In the present study nectaries are located with in the stigmatic chambers. In *Calotropis procera* (Ait.) Ait. f., nectar is secreted in the stigmatic chamber and flow to the coronal lobes via fleshy pads fitted between them. Thus in *Calotropis procera* (Ait.) Ait. f., the capillary system for nectar flow is almost similar to *Asclepias* species as described by Galil and Zeroni (1965). The storage of nectar in coronal segments of *Calotropis* has also been reported by Wanntorp (1974) and Fisikowitch (1956).

In perfect flowers of *Glossonema varians* (Stocks) Hook. f., nectaries are situated within the stigmatic chambers whereas in staminate flowers at the same position of staminal column, where stigmatic chambers are situated in perfect flowers. In both type of flowers, nectar from stigmatic chambers flow and accumulates at the base and between the staminal column and coronal appendages.

In *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L., *Pentatropis nivalis* (Gmel.) Field and Wood and *Oxystelma esculentum* (L. f.) R. Br. the staminal or interfilament furrows are continuous with the stigmatic chambers and act as nectar reservoirs.

In *Caralluma edulis* (Edgew.) Hook. f., *Caralluma tuberculata* N. E. Br., *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Dcne., nectaries are located in the stigmatic chambers while the outer corona in the form of pouch or bowl (in above first three species) and annulus(in the last one) serve as nectar reservoir.

As mentioned earlier, the tribes of subfamily Asclepiadoideae are separated on the characters of pollinia and anthers (Brown, 1902, 1907; Schill and Jäkel, 1978; Bruyns and Forster, 1991 etc). In the present investigation the two tribes studied are also separated on these characters, however a new character i.e. style lenth is seem to be tribe specific. Thus tribe Asclepiadeae is characterized by pendulous pollinium located in the basal portion of the anther, with exolateral germination locus, mostly without extra pollinial appendages (if present, then only on the exolateral side e.g. in *Pergularia*), translator arms and pollinium encloses by anther, anther not incumbent on stigma head but only an apical hyaline flap cover the periphery of stigma head and relatively with long styles ranges from 1325-1475 um.

The tribe Ceropegieae is characterized by erect pollinium located in the apical portion of the anther, with distal germination locus, with distal extra pollinial appendages, translator arms and pollinium not covered by anther, anther inconmbment on stigma head without apical hyaline flap and wihtout to vary short syle, ranges from 0- 250 µm.

CHAPTER 3

INSECTS (POLLINTORS AND VISITORS).

INTRODUCTION

The entomophilous pollination of Asclepiads provides one of the most facinating chapters in biology (Woodson, 1954). Apparently, Sprengel (1973) was the first to observe the extraction of pollinaria by insects. Pollination in Asclepiads is carried out by butterflies and bees and hence the type genus of the family *"Asclepias"* is known as butterfly weed. Following Sprengel, Brown (1883), Treviranus (1846), Hildebrand (1866), Delphino (1867), Muller (1873, 1882), Robertson (1886, 1887) etc. studied the pollination behaviour a recorded the number of insects on *Asclepias* species. Pollination mechanism and the foraging behaviour of the pollinators (insects) of the American species of *Asclepias* was carried out by many workers like Woodson (1954), Frost (1965), Macior (1965), Wyatt (1976), Lynch (1977), Bookman (1981), Beare and Perkins (1982) etc.

From Indo-Pak subcontinent pollination ecology pertaining to insect visitors (pollinators) of *Calotropis* species was carried out by Ramakrishna et al. (1979), Pant et al. (1982), Bhatnagar (1986) and Ali and Ali (1989). *Pergularia daemia* (Forssk.) Chiov. was pollinated by bee *Apis dorsata* (Vijayaraghavan and Shukla, 1980) whereas *Cynanchum canescens* (Willd.) Schum. also by a bee *Campsomeris* species (Chaturvedi, 1987). Pant et al. (1982) reported seventeen species of pollinators including bees, a few ants, beetles and nocturnal moths on the flowers of *Calotropis procera*, *Leptadenia reticulata*, *Sarcostemma secamone* and *Wattakaka volubilis*. According to Bhatnagar (1986), the members of the tribe Asclepiadeae i.e. *Calotropis procera*, *Calotropis gigantea*, *Oxystelma secamone* and *Holostemma adakodien* (= *H. annulare*) are pollinated by *Xylocopa* species (Carpenter bee, Hymenoptera) , of tribe Marsdenieae i.e. *Marsdenia tenacissima* and *Cosmostigma racemosa* and of tribe Stapelieae i.e. *Stapelia* species by Carrion and dung flies (Diptera) with the exception of *Pergularia daemia*, *Telosma pallida* (tribe Asclepiadeae) pollinated by nocturnal moths and *Asclepias curassavica* (tribe Asclepiadeae) pollinated by wasp and butterfly.

As it is evident from the cited literature that very few reports exists on the foraging behaviour of the pollinators of the Asclepiads from this region particularly from Pakistan. So present attempt has been made to study the insect visitors and pollinators of the following taxa viz. *Calotropis procera* (Ait.0 Ait. f. ssp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) hook. f., *Oxystelma esculentum* (L. f.) R. Br., *Pentatropis nivalis* (Gmel.) Field and Wood, *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L. (tribe Asclepiadeae), *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Dcne. (tribe Ceropegieae) in their natural habitat.

MATERIAL AND METHODS

The insects that visited the flowers were collected and their foraging behaviour was noted. The study was made from

Table 2. Pollinaria load on Insects (Pollinators)

Taxon	Insects	Sample size	Mouth part	Claws	Tibiae	Tarsi	
			B	B	B	B	D
Tribe Asclepiadeae							
Calotropis procera	Hymenoptera: Anthophoridae						
subsp. *hamiltonii*	*Xylocopa pubescens* Spin.	5	-	-	-	30	-
	Xylocopa pubescens Spin.	14	-	-	-	21	1
	Xylocopa fenestrata (F.)	28	-	3	-	64	4
	Apidae						
	Apis florea (F.)	10	3	-	5	32	2
Total		57	3	3	5	147	7
Glossonema varians	Hymenoptera : Apidae						
	Apis florea (F.)	6	20	-	-	-	-
	Sphecidae						
	Bembix sp.	8	4	-	-	-	-
	Scoliidae						
	Scolia quadripustulata Fabr.	12	6	-	-	-	-
	Vespidae						
	Polistes wattii Cam.?	5	2	-	-	-	-
	Coleoptera: Dermestidae						
	Attagenus fasciatus (Thaunb)	8	-	2	-	-	-
Total		39	32	2	-	-	-
Oxystelma esulentum	Hymenoptera: Apidae						
	Apis florea (F.)?	20	-	14	-	-	-
	Anthophora sp. ?	21	-	20	-	-	-
	Vespidae						
	Polistes wattii cam. ?	30	-	35	-	-	-
Total		71	-	69	-	-	-
Pentatropis nivalis	Hymenoptera : Apidae						
	Apis florea (F.)	26	4	2	-	-	-
	Lepidoptera: Noctuidae						
	Mythimna consanguis Guen.	15	7	-	-	-	-
Total		54	12	-	-	-	-

Table 2. contd....

Taxon	Insects	Sample size	Mouth part	Claws	Tibiae	Tarsi	
			B	B	B	B	D
Pergularia daemia	Hymenoptera : Apidae						
	Apis florea (F.)	6	-	8	-	-	-
	Braunsapis cf. mixta (Smith)	24	-	5	-	-	-
	Vespidae						
	Ropalidia spatulata V.V.	14	-	10	-	-	-
	Lepidoptera : Noctuidae						
	Thysanoplusia orichalcea Fab.	20	-	24	-	-	-
	Agrotis biconica Kollar	23	-	16	-	-	-
Total		87	-	49	-	-	-
Pergularia tomentosa	Lepidoptera: Noctuidae						
	Helicoverpa armigera Hub.	20	-	10	-	-	-
	Noctuidae sp.	20	-	6	-	-	-
Total		40	-	16	-	-	-
Tribe Ceropegieae							
Ceropegia bulbosa	Diptera : Ceratopogenoideae						
	Forcepomya sp.	108	28	-	-	-	-
Leptadenia pyrotechnica	Diptera : Otitidae						
	Physiphora sp	40	35	-	-	-	-
	Calliphoridae						
	Cosmina prasina (Brarer + Berg)	30	21	-	-	-	-
	Chrysomya albiceps (Wied)	30	18	-	-	-	-
	Mosquito like fly	8	1	8	-	-	-
	Coleoptera : Dermestidae						
	Attagenus fasciatus (Thumb.)	21	7	-	-	-	-
	Attagenus i. posticalis Fairm.	8	3	-	-	-	-
	Coccinellidae						
	Brumoides suturalis (F).	26	8	-	-	-	-
	Hymenoptera:						
	Crematogaster sp.(Black ant)	100	-	8	-	-	-
	Winged Black Ant (Small)	10	3	-	-	-	-
Total		273	96	16	-	-	-

Frequency and location of Basal (B) and Distal (D) corpuscula on insects.

Table 3. Pollinating insects

Taxa	Tribe Asclepiadeae						Tribe Ceropegieae	
Insects	*Calotropis procera*	*Glossonema varians*	*Oxystelma esculentum*	*Pentatropis nivalis*	*Pergularia daemia*	*Pergularia tomentosa*	*Ceropegia bulbosa*	*Leptadenia pyrotechnica*
Agrotis biconica	-	-	-	-	+	-	-	-
Anthophora sp.	-	-	+	-	-	-	-	-
Apis florea	+	+	+	+	+	-		-
Attagenus fasciatus	-	-	-	-	-	-	-	+
Attagenus cf.*posticalis*	-	-	-	-	-	-	-	+
cf.*Crematogaster* sp.	-	-	-	-	-	-	-	+
Black ant (winged)	-	-	-	-	-	-	-	+
Bembix sp.	-	-	-	+	-	-	-	-
Braunsapis sp.	-	-	-	-	+	-	-	-
Brumoides suturalis	-	-	-	-	-	-	-	+
Chrysomya albiceps	-	-	-	-	-	-	-	+
Cosmina prasina	-	-	-	-	-	-	-	+
Forcipomyia sp.	-	-	-	-	-	-	+	-
Helicoverpa armigera	-	-	-	-	-	+	-	-
Mythimma consanguis	-	-	-	+	-	-	-	-
Noctuidae sp.	-	-	-	-	-	+	-	-
Physiophora sp.	-	-	-	-	-	-	-	+
Polistes wattii	-	+	+	-	-	-	-	-
Ropalidia spatulata	-	-	-	-	+	-	-	-
Scoliaquadripustulata	-	+	-	-	-	-	-	-
Thysanoplusia orichalcea	-	-	-	-	+	-	-	-
Xylocopa fenestrata	+	-	-	-	-	-	-	-
Xylocopa pubescens	+	-	-	-	-	-	-	-

Information about the pollinator of *Caralluma edulis* and *Caralluma tuberculata* is not available.

Darsanochino, Karachi University Campus, Khuzdar, Malir and Mangopir populations. The study was limited to the periods when weather conditions were favourable for the foraging activities of the visitors. All the insects were taken between 8.0 a.m. - 7.0 p.m. for diurnal and from 7.0 p m. - midnight for nocturnal taxa. The number of pollinaria and position of corpuscula either directly (basal) or indirectly (distal) attached to different parts of the insect's body was also determined. The insects carrying pollinaria were evaluated as pollinators.

The insects were get identified from Entomological Section of British Museum, Natrual History, London.

OBSERVATIONS AND RESULTS

Floral visitors (insects) in the studied taxa belong to order Hymenoptera, Coleoptera, Diptera, Lepidoptera and Hemiptera. The Hymenoptera are represented by 6 families, 18 species and 400 specimens, Coleoptera by 6 families, 7 species and 100 specimens, Diptera by 4 families, 7 species and 236 specimens, Lepidoptera by 7 families, 14 species and 130 specimens while Hemiptera by 1 families, 1 species and 1 specimens (Table 2 - 4).

Tribe Asclepiadeae

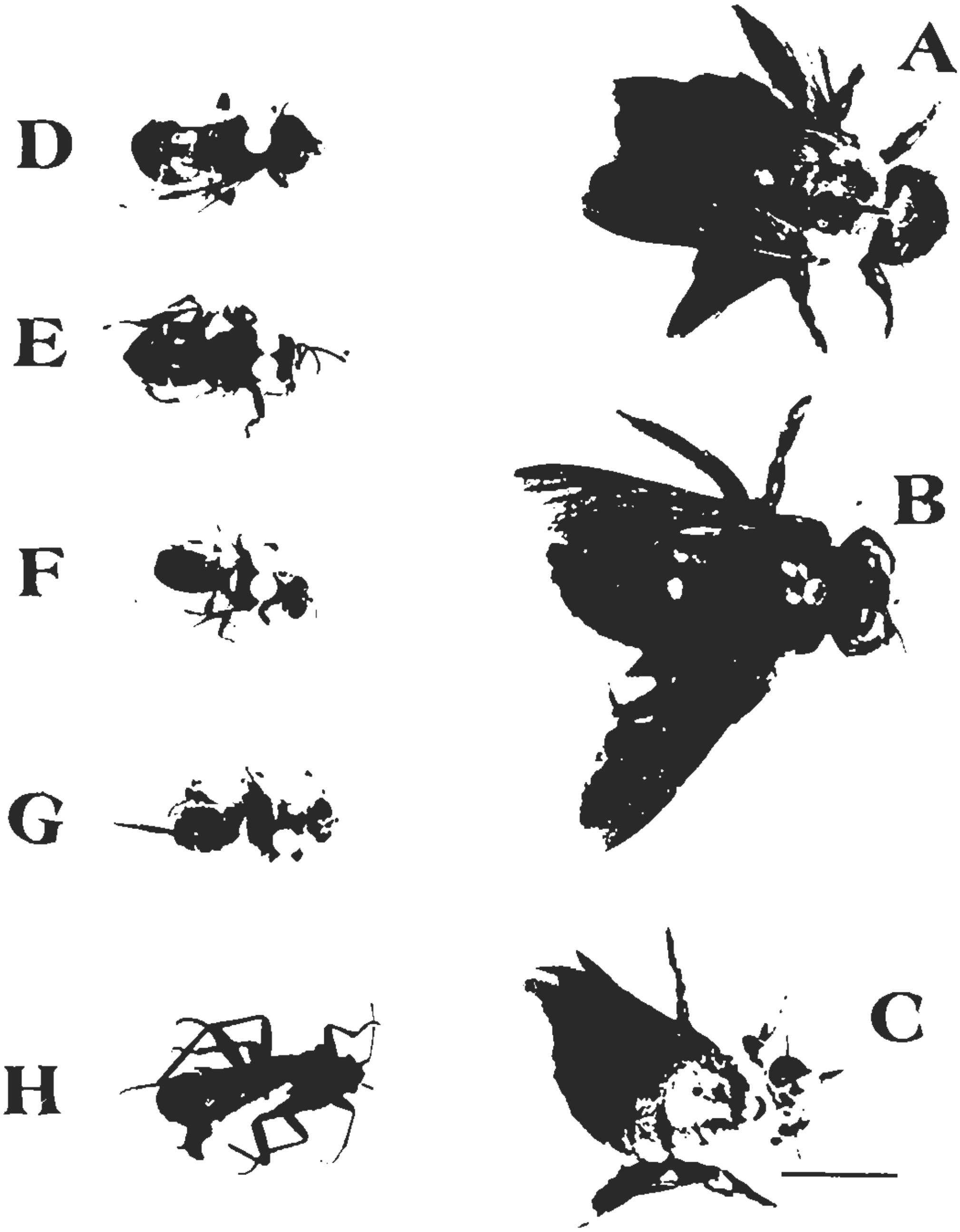
1. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali:

Pollinators:

Xylocopa pubescens, *Xylocopa fenestrata*, and *Apis florea* are

Fig. 17. Calotropis procera ssp. hamiltonii: Insects visitors. A & B, Xylocopa pubescens (♂ & ♀); C, Xylocopa fenestrata (♀); D & E, Apis florea (♀); F & G, Vespidae species; H, Spilostethus pandurus.

Scale bar = 10 mm


A
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the main pollinators .

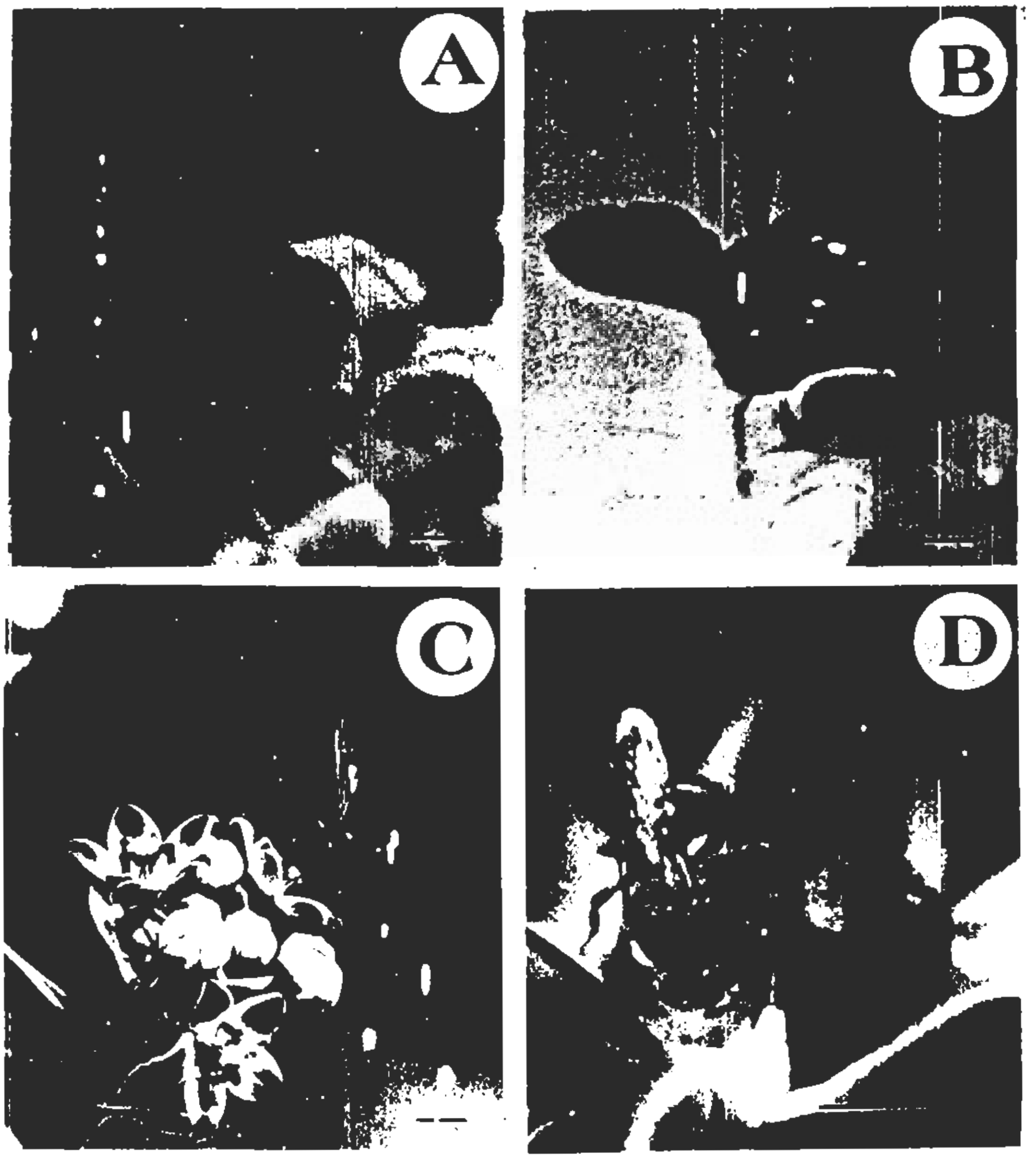
A. *Xylocopa* species (Fig. 17A - C):

The two *Xylocopa* species *Xylocopa pubescens* Spin. (♀ & ♂) and *Xylocopa fenestrata* (F.) (♂), altogether constitute 82.75% of the total number of all the pollinaria bearing insects (Table 2). Both the species of *Xylocopa* exhibit a diurnal foraging pattern. The first visit to the plant start at about 7.30 a.m. and continue to about 7.0 p.m. *Xylocopa* species gradually increase in number from about 9.0 a.m., reaching peak frequency between 10.0 - 11.0 a.m., fluctuating at this level from 12.0 - 2.0 p.m. and again reaching the peak frequency between 3.0 - 5.0 p.m. and declining slowly thereafter.

The *Xylocopa* species land on the flower particularly on the broad stigma head. Front legs usually grasp the corona lobes from the base while middle and hind legs often rest over the anther wings and stigmatic chambers. Particularly tarsal segments of legs frequently come in contact with the corpuscula during foraging (Fig. 18A - C). As the result, the corpusculum gets attached to a leg hair and removal of pollinarium occurs. When the insect (carrying pollinaria) visit another flower the pollinium can be lodged in the stigmatic chamber.

The emphasis seems to be on nectar gathering. The insect inserts its proboscis in the opening at the apex of the corona lobe to suck the nectar (Fig. 18C). *Xylocopa* usually exploits one corona lobe of a flower, but sometimes also two or three. It prefer to fly from one plant to another making angling flights and also to another inflorescence of the same plant, very rarely it changes to other flowers of the same inflorescence.

Fig. 18. Calotropis procera ssp. hamiltonii: Showing the foraging behaviour of insects. A - C, Xylocopa pubescens (arrow indicating the inserted proboscis of insect in the opening at the apex of corona lobe); D, Apis florea.
Scale bar: = 5 mm;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X. pubescens (♀ & ♂) carried 17.5% and 14.3% while *X. fenestrata* (♂) 42.5% of the pollinaria (out of the total pollinaria carried by all insects). *X. pubescens* (♀) carried not a single indirectly attached corpusculum. 91.6% directly and 8.3% indirectly attached corpuscula were carried by *X. pubescens* (♀), 94.3% and 5.6% respectively by *X. fenestrata* (♂) (Table 2). The maximum pollinaria are located on tarsal segments and fewer on tibiae and claws while non on the mouth parts (Table 2; Fig. 19, 20).

B. *Apis florea* F. (Fig.17D, E):

Apis florea workers visit the flowers whole day and land on the flower particularly on the stigma head. The insect inserts its proboscis in the opening at the apex of the corona lobe to suck the nectar, sometimes the insect tries to suck the nectar from the recurved vesicle and boarders of fleshy pads also (Fig. 18D). but without any success. During foraging, the body of the insect rests on the stigma head while its legs particularly the middle and hind legs rest over the stigmatic chambers. Tibial and particularly tarsal segments of legs of insects frequently come in contact with the stigmatic chambers and corpuscula.

Apis florea carried 25% of the pollinaria counted for all insects. 95.2% pollinaria were attached directly and 4.7% indirectly with most of them on the tarsal segment (Table, 2; Fig. 19D).

Fig. 19. Calotropis procera ssp. hamiltonii: Xylocopa pubescens. A, Pollinaria attached on front and middle legs; B - C, Pollinaria attached on the tarsal segment of leg. Scale bar: A = mm;

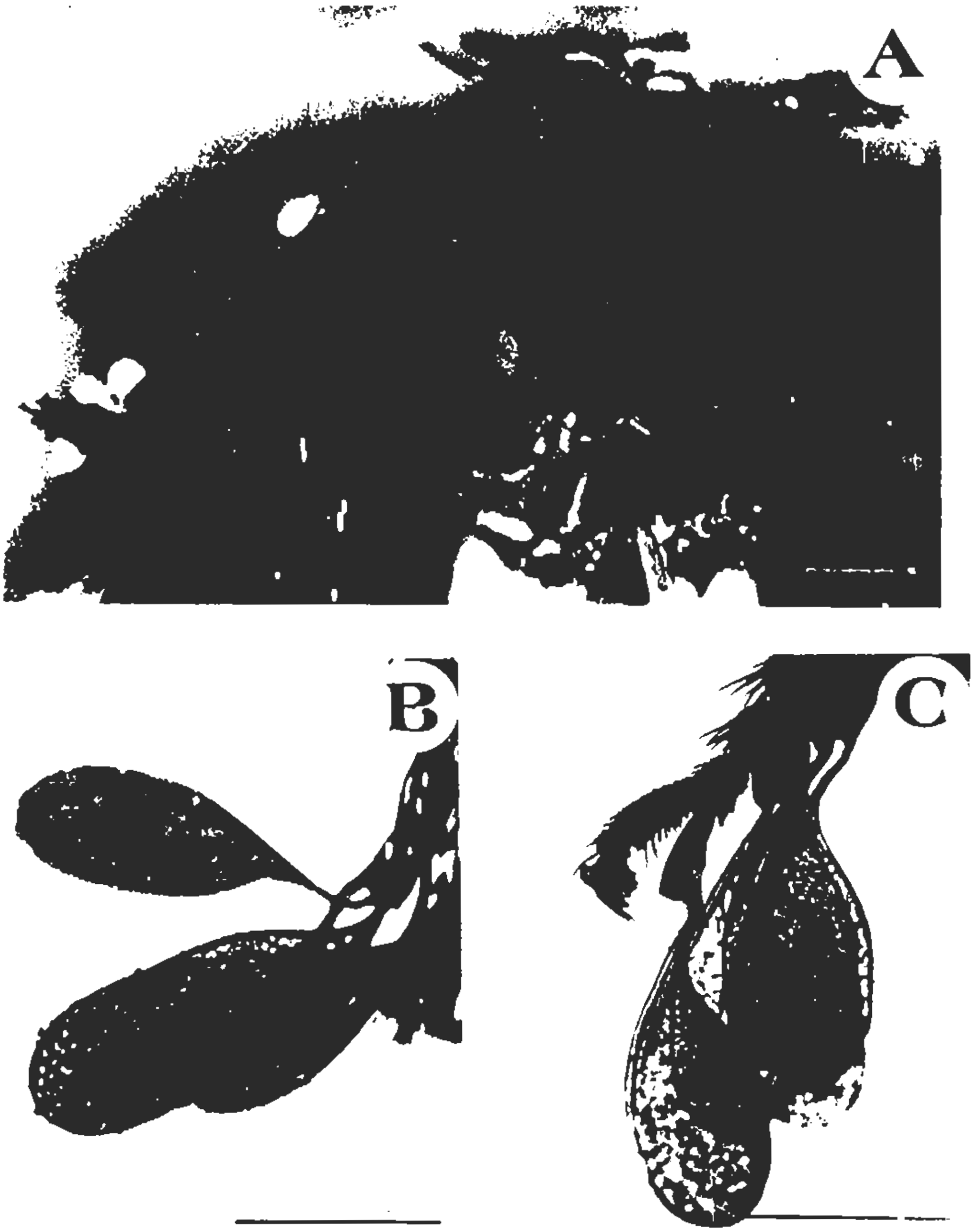
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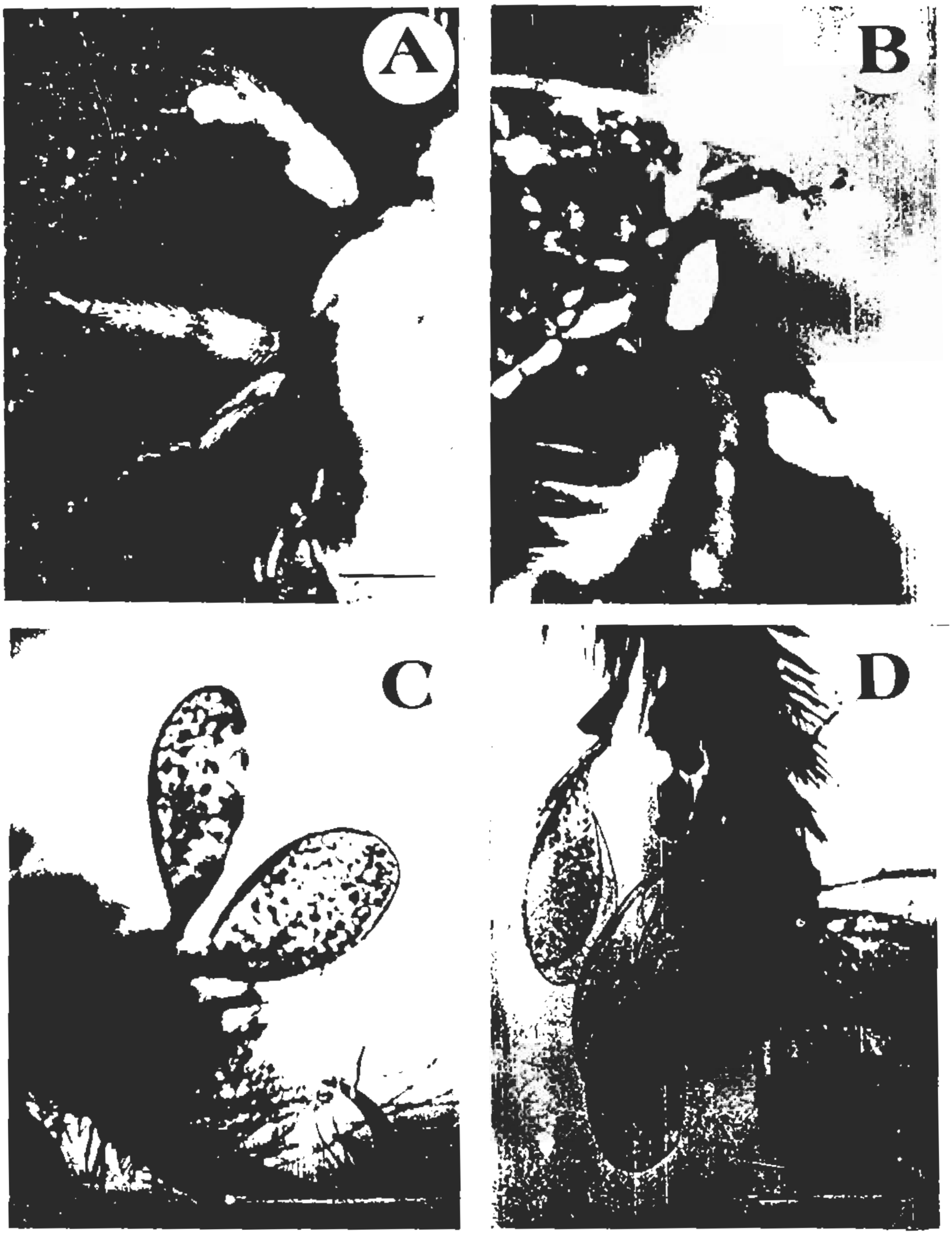
Fig. 20. Calotropis procera ssp. hamiltonii: A - C, Xylocopa fenestrata. A, Pollinaria attached on front legs; C, Pollinarium attached on the tarsal segment of leg; D, Apis florea, Pollinaria attached on tarsal and tiabial segment of leg.

Scale bar: = 5 mm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Non - pollinating insects:

A. *Amegilla comberi* (Ckll.):

A. comberi (Ckll.) a rare visitor, has two types of foraging behaviour. Firstly it does not land on the flower but remain in the air by vigorous wings movement and suck the nectar from the opening at the apex of corona lobe through its long proboscis. Secondly, it lands on the flower between the two petals, front and middle legs grip the petals from above and hind legs from below and suck the nectar from the apical opening of the corona lobe.

B. *Vespidae* species (♀ & ♂) (Fig. 17F, G):

Vespidae species have been observed on plant all the year round. They are also found on the plant during the (dry) winter season. It land on a petal of the flower and try to suck nectar from the base of the pads. It usually found on both the surfaces of the leaves. The plant act as the site for their copulation also.

C. *Spilostethus pandurus* (Scopoli) (♀ & ♂) (Fig. 17H):

S. pandurus (Scopoli) is quite common on the plant. Like *Vespidae* species, it also copulate and found on the plant all the year round, particularly abundant in fruiting period.

Rare visitors were Diptera (small flies) and Lepidoptera (small butterflies). All these insects may be regarded as oppurtunists that merely exhaust the floral rewards without providing pollination.

Fig. 21. Glossonema varians: Insects visitors. A_{1-2}, Apis florea (♀); A_{3-4}, Scolia quadripustulata (♂ & ♀); B & C, Scolia quadripustulata. Pollinaria attached on mouth parts. Scale bar: = 10 mm.

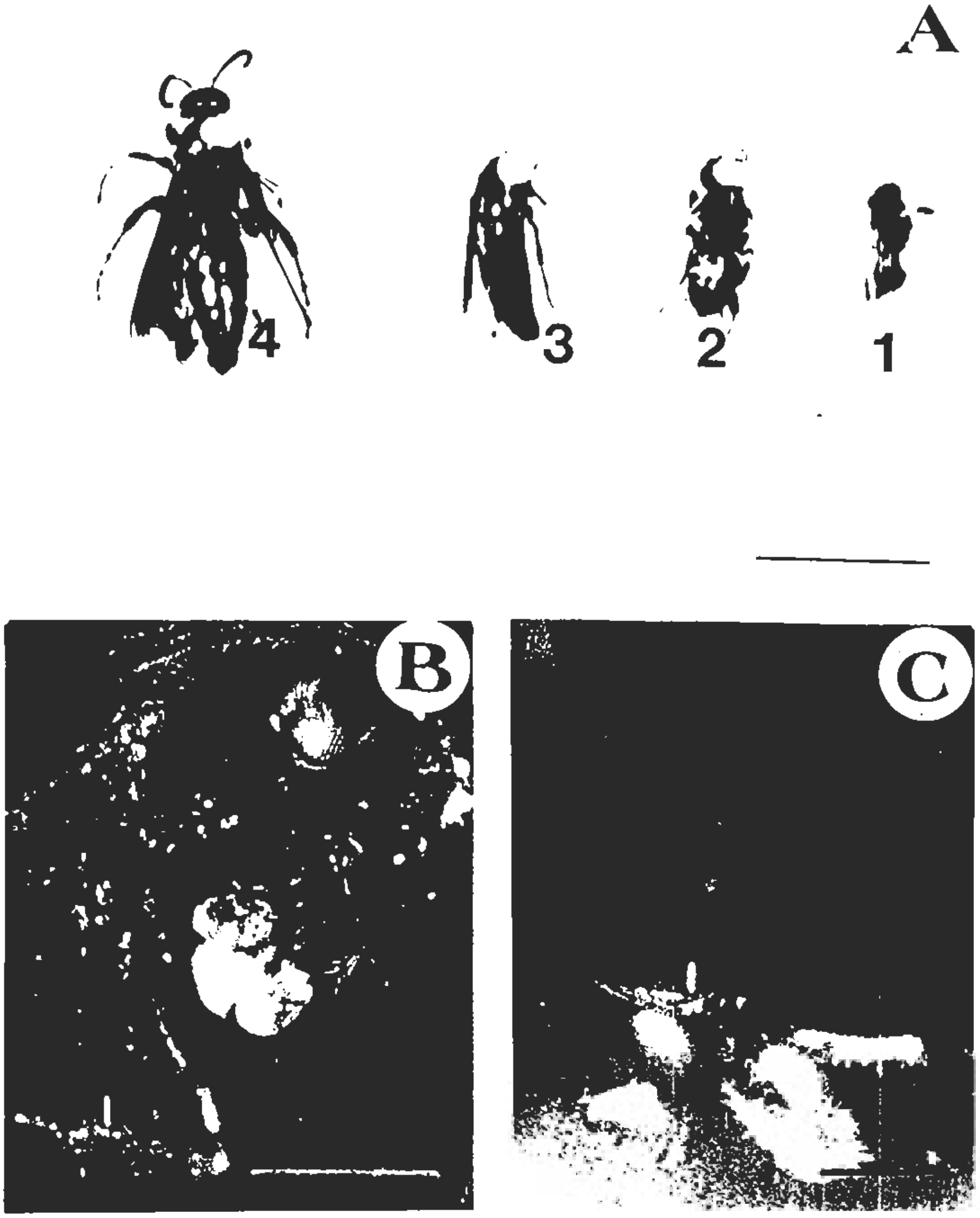
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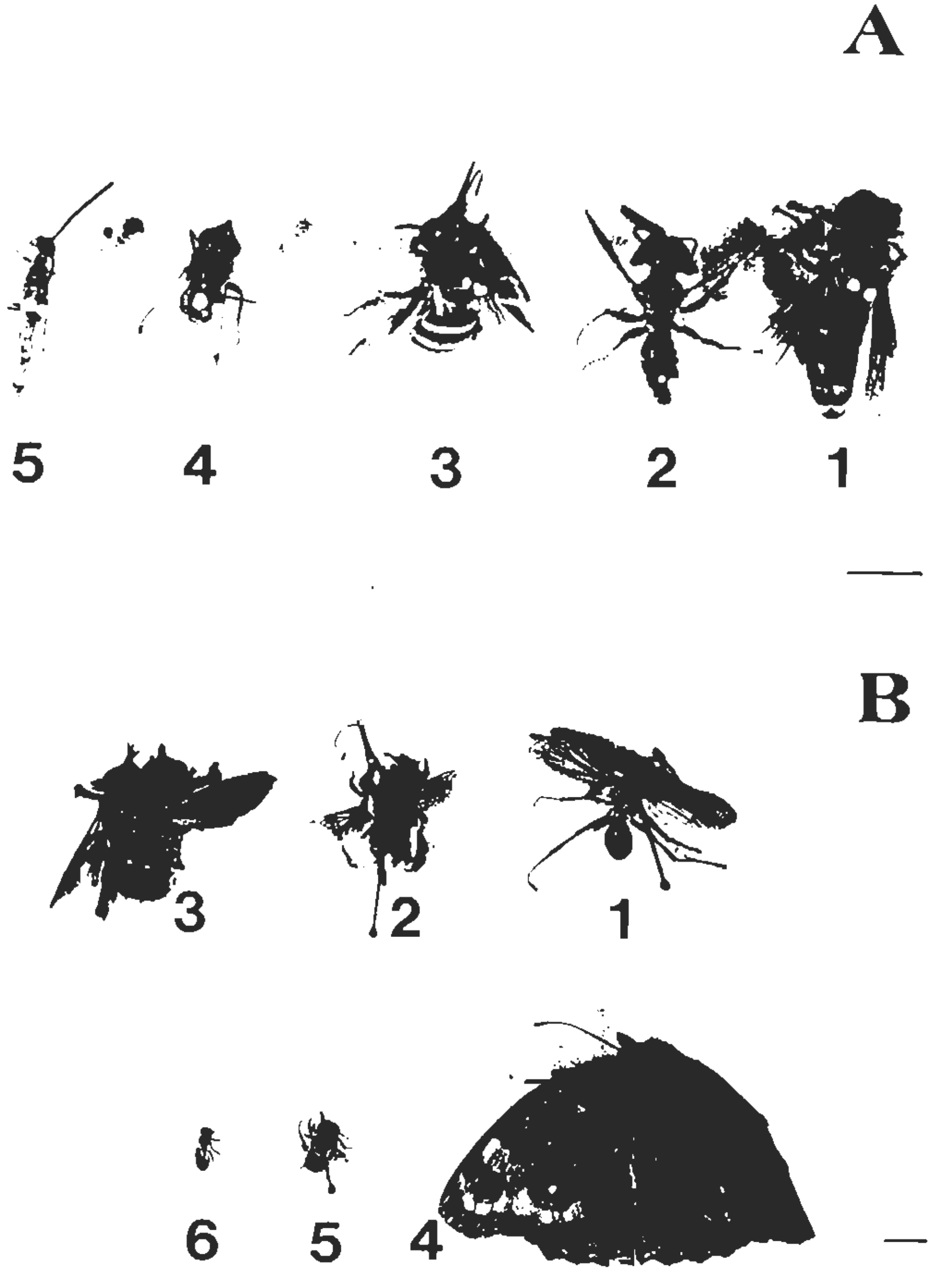
Fig. 22. Glossonema varians: Insects visitors. A_1 , Bembix sp.(♂); A_2, Tachysphex sp. (♂); A_3, Anthophora sp. (♂); A_4, Rhyncomya nigripes; A_5, Meria sp. (♂). **Oxystelma esculentum:** Insects visitors. B_1, Polistes wattii (♀); B_2, Anthophora sp. (♂), B_3, Xylocopa sp. (♂), B_4, Junonia almana, B_5, Nomia sp. (♂), B_6, Tachysphex sp. (♂).

Scale bar: = 5 mm.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2. *Glossonema varians* (Stocks) Hook. f.:

A variety of insects visit *Glossonema varians* (Stocks) Hook. f. flowers (Fig. 21A, 22A).

Pollinators:

A. *Apis florea* (F.) (♀) (Fig. 21A_1 - A_2):

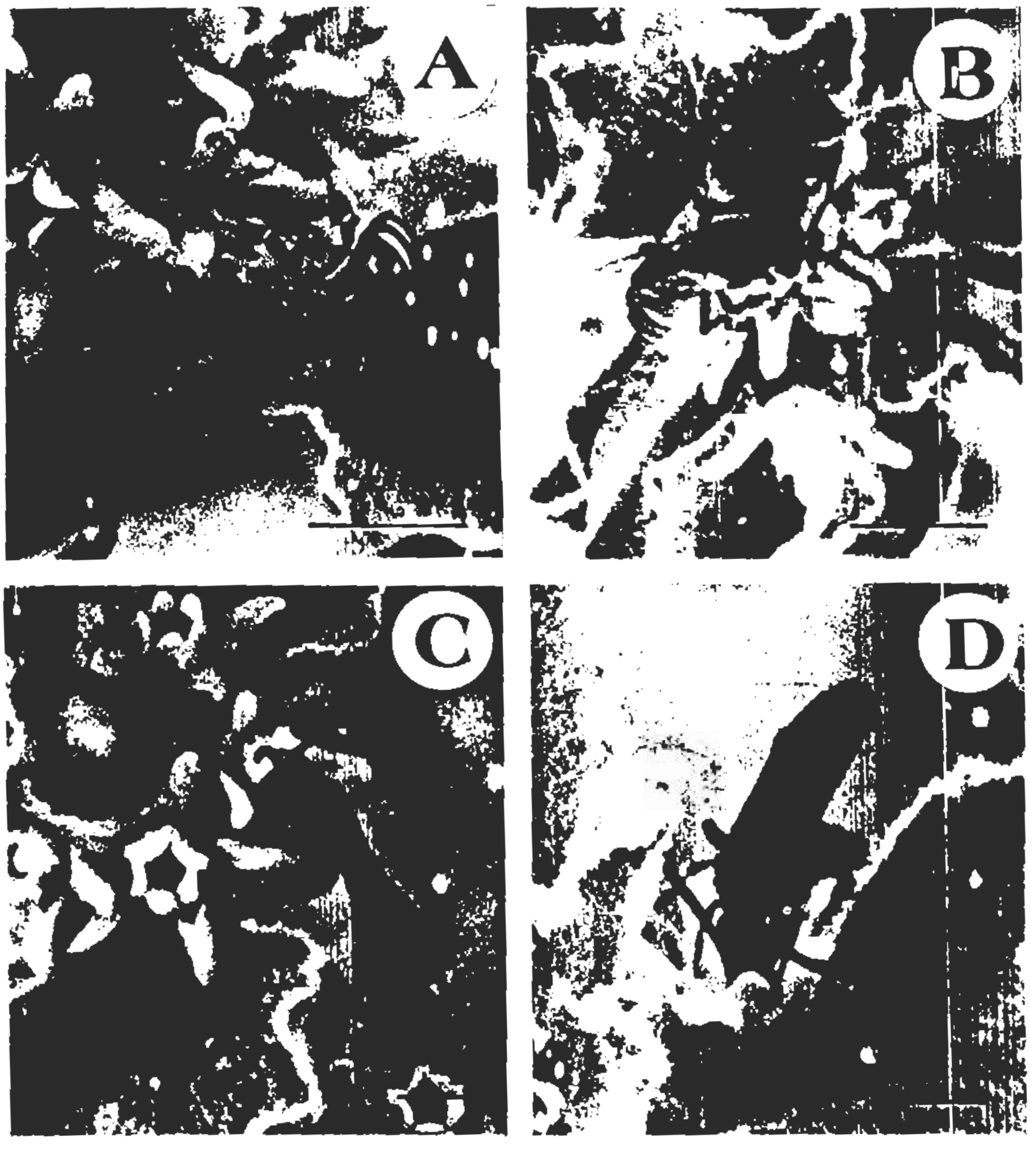
A. florea (F.) constitute about 16.7% of the total number of all the pollinaria bearing insects. The visit to the plant started at about 9.0 a.m. and continue to about 5.0 p.m., however the maximum number was observed between 11.0 a.m. - 1.0 p.m.

It lands on the flower with front and middle legs rest on the corona, hind legs on petals while thorax and abdomen on the stigma head (Fig. 23A - B). The emphasis seems to be on nectar gathering from the base of the staminal column and during nectar sucking corpusculum get attached to the mouth parts and removal of pollinarium occurs. When this insect visit another flower the pollinium can be loged in the stigmatic chamber.

A. florea (F.) usually exploits all the nectaries of a flower by moving in a circle on the flower. It also visit all the flowers of an inflorescence and walked towards other inflorescence of the same plant. Its inter-plant movement is rather slow and spend much of the time on a flower/infloresence/plant than the other pollinators.

About 50% of the captured individuals carried pollinaria with an average of 3.33 per insect. Insects carried 47.62% of the pollinaria counted for all insects and all of them were directly attached to the mouth parts only (Table 2).

Fig. 23. Glossonema varians: Foraging behaviour of insects.
A - B, Apis florea, C - D, Scolia quadripustulata.
Scale bar: = 5 mm.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B. *Scolia quadripustulata* Fabr. (♀ & ♂) (Fig. 21A_3 - A_4):

S. quadripustulata Fabr. constitute about 50% of the total number of all the pollinaria bearing insects. Their behaviour is more or less similar to that of *Apis florea* F. (Fig. 23C, D). However, their intra and inter plant movements are quite fast and they visit more flowers as compare to other insects. They outnumber the other insects in frequency and constancy on flowers. About 75% of them (of captured individuals) carried pollinaria with an average of 0.75 per insect. They carried 38.1% of the pollinaria counted for all insects and all of them were directly attached to the mouth parts (Table 2; Fig. 21B, C).

C. *Bembix* species (♀) (Fig. 22A_1):

Bembix species constitute about 22.2% of the total number of all the pollinaria bearing insects. Their behaviour (Fig. 24A, B) is also similar to *Apis florea* F. However, after visiting few flowers, they usually rest on leaves, stems or even on ground and groom their mouth with legs in order to get rid of the attached pollinaria. About 50% of them (of captured individuals) carried pollinaria with an average of 0.5 per insect. They carried 9.52% of the pollinaria counted for all insects and all of them directly attached to the mouth parts (Table 2).

D. *Polistes wattii* Cam. (♀):

P. wattii Cam. are less frequent visitors and constitute only 11.1% of the total number of all the pollinaria bearing insects. Their inter-plant movements are more frequent as compare

Fig. 24. Glossonema varians: Foraging behaviour of insects.

A - B, Bembix sp., C - D, Polistes watti.

Scale bar: 5 mm.


A
B
C
D

to intra-plant ones. Their foraging behaviour is also similar to *Apis florea* F. (Fig. 24C, D). About 40% of the captured individuals carried pollinaria with an average of 0.4 per insect. They carried only 4.75% of the pollinaria counted for all insects and all directly attached to the mouth parts (Table, 2).

Non - pollinating insects :

A variety of insects visit flowers of *Glossonema varians* (Stocks) Hook. f. that do not carry pollinaria (Table 4).

A.*Attagenus fasciatus* (Thaumb.):

A small beetle, suck the nectar while sitting in between petals and corona segments. Due to their small size and behaviour, they are not able to act as pollinators, however pollinaria are located on the claws (legs) of two individuals out of eight examined.

Other insects like, *Taachysphex* species (Fig. $22A_2$), *Anthophora* species (Fig. $22A_3$), *Rhyncomya nigripes* Segny. (Fig. $22A_4$), *Meria* species (Fig. $22A_5$), *Bombylisoma* species, *Lasioglossum* species, are common visitors. These insects land on the petals and suck the nectar without touching the corpuscula.

3. ***Oxystelma esculentum* (L.f.) R. Br.:**

A variety of insects visit *Oxystelma esculentum* (L.f.) R. Br. flowers (Table 2 - 4).

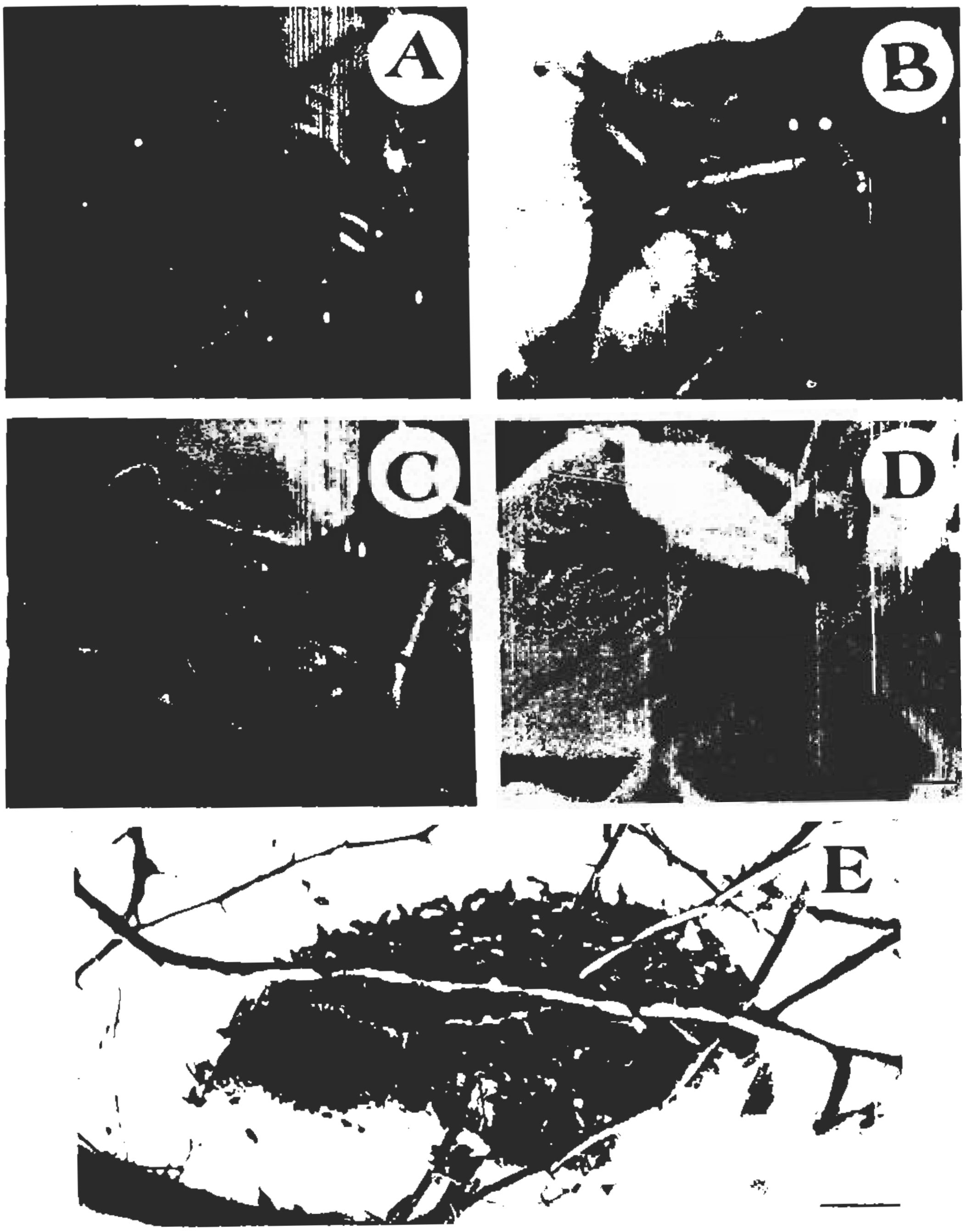
Pollinators:

A.*Polistes wattii* Cam. (♀) (Fig. $22B_1$):

P. wattii Cam. constitute about 52.17% of the total number

Fig. 25. Oxystelma esculentum: Foraging behaviour of insects. A - C, Polistes watti; D, Nomia sp.; E, Hive of Polistes watti.

Scale bar: = 5 mm.


A
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E

of all the pollinaria bearing insects. The visit to the plant started at about 9.0 a.m. and continue to about 5.0 - 6.0 p.m.

Insect usually fly over many flowers and land only on the selected ones. As insect alight, the flower tilt downward due to its weight so insect first hold the petals with its legs (Fig. 25A) and then grasp the coronal segments by its fore and middle legs while hind legs still holding the petals, and suck the nectar from the base of staminal column (Fig. 25B, C). During nectar sucking, its legs particulrly claws come in contact with the corpuscula and removal of pollinaria occurs. Later when this insect visit another flower the attached pollinium may remain in the stigmatic chamber and insertion occurs. It has been observed that insects legs seem to be stuck in the stigmatic chambers and force is applied by them to get free.

Insects usually rest on leaves or branches after visiting the flower, may be scared of previous traping, after visiting 1 - 3 flowers, they fly towards their hive, built in the same locality near the *Oxystelma* population (Fig. 25E).

About 80% of the captured individuals carried pollinaria with an average of 1.2 per insect. They carried 50.72% of the pollinaria counted for all insects. All the pollinaria are directly attached on the claws with 71.42% on middle and 28.57% on fore legs (Table 2).

B.*Apis florea* (F.) (♀):

A. florea (F.) constitute about 21.74% of the total number of all the pollinaria bearing insects. Their behaviour is more or less similar to *Polistes wattii* Cam. However, they directly land on the flower and settle on the stigma head, holding the corona segments with all the three pairs of legs. They usually move in a circle within the flower in search of nectar. Their inter-plant movement is slow as they visit several flowers of a plant either by flying or walking on stems.

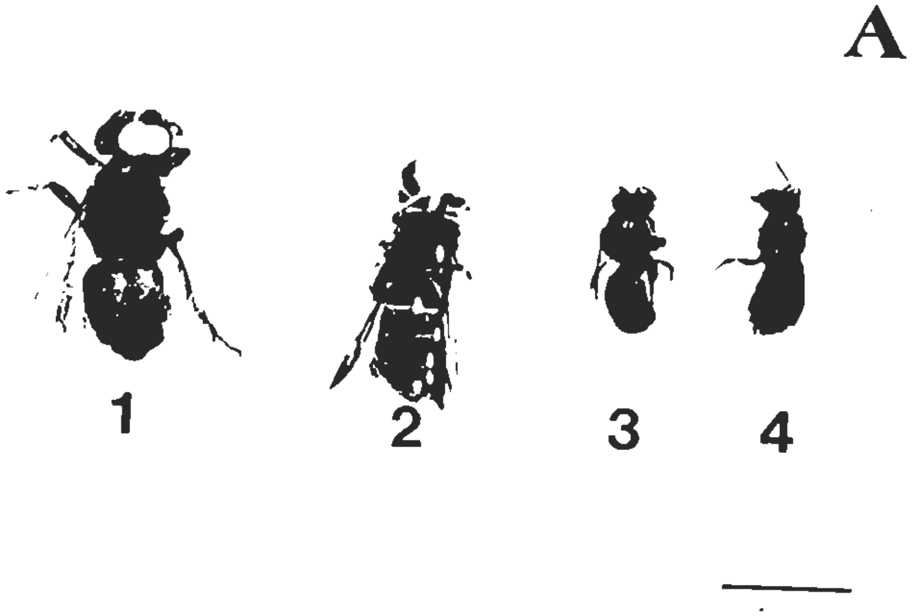
About half (50%) of them (captured) carried pollinaria with an average of 1.2 per insect. They carried about 34.78% of the pollinaria counted for all insects. All the pollinaria are directly attached on the claws of all three pairs of legs (Table 2).

C. *Anthophora* species (Fig. $22A_2$):

Anthophora species constitute about 26.09% of the total number of all the pollinaria bearing insects. Its behaviour is different from two above mentioned pollinators (insects). It usually hover around the flower and from time to time become still in air (by vigorous wings movement and making sound) and then land on the flower. During nectar sucking, its middle legs come in contact with corpuscula while front and hind legs rest on the corona segments. It usually visit 1 - 2 flowers of a plant and then move towards the other plant.

About 57.41% of the captured individuals carried pollinaria with an average of 0.95 per insect. It carried about 28.98% of the pollinaria counted for all insects. All the pollinaria are directly attached on the claws of the middle legs (Table 2).

Fig 26. Pentatropis nivalis: Insects visitors. A_1, Apis florea; A_2, Rhopalomeliossa sp.; A_{3-4}, Lasioglossum sp.; B, Apis florea. Pollinarium attached on the claw of leg. scale bar = 5 mm.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B

Non - pollinating insects:

A number of insects visit *Oxystelma esculentum* (L. f.) R. Br. flower without performing pollination.

A. *Xylocopa* species (♀) (Fig. $22B_4$):

It is quite rare visitor and similar in behaviour to *Polistes wattii* Cam.

B. *Junonia almona* (Fig. $22B_4$):

It is very commom, land on the petals and suck the nectar by its long proboscis without touching the corpuscula.

C. *Nomia* species (♀) (Fig. $22B_5$; 25D):

D. *Tachysphex* species (Fig. $22B_6$)

E. *Rhyncomya* species:

These insects are very common on the flowers and suck the nectar without touching the corpuscula. Thus, due to their small size and behaviour not able to act as pollinators.

4. *Pentatropis nivalis* (Gmel.) Field and Wood:

A variety of both diurnal and nocturnal inects visit *Pentatropis nivalis* (Gmel.) Field and Wood flowers (Table 2 - 4).

Pollinating insects:

Diurnal:

Apis florea(F.) (♀) (Fig. $26A_1$):

A florea (F.) constitute about 63.16% of the total number of all the pollinaria bearing insects. Its visit to the plant started at about 5.30 p.m. and conitnue till 7.30 p.m. It land directly on the flower and grasp the coronal segments with the legs and suck the nectar from the base of the staminal column. During necatr sucking, legs contacted the corpuscula and pollinaria removal occur as insect leave the flower, when same insect visit another flower, the pollinium attached to its leg get inserted into the stigmatic chamber.

A. florea (F.) usually visit many flowers of a plant so inter - plant movement is less. About 30.77% of the captured insects carried pollinaria with an average of 1.25 per insect. It carried about 68.18% of the pollinaria counted for all insects. All the pollinaria are directly attached to the claws of legs (Table 2; Fig. 25B).

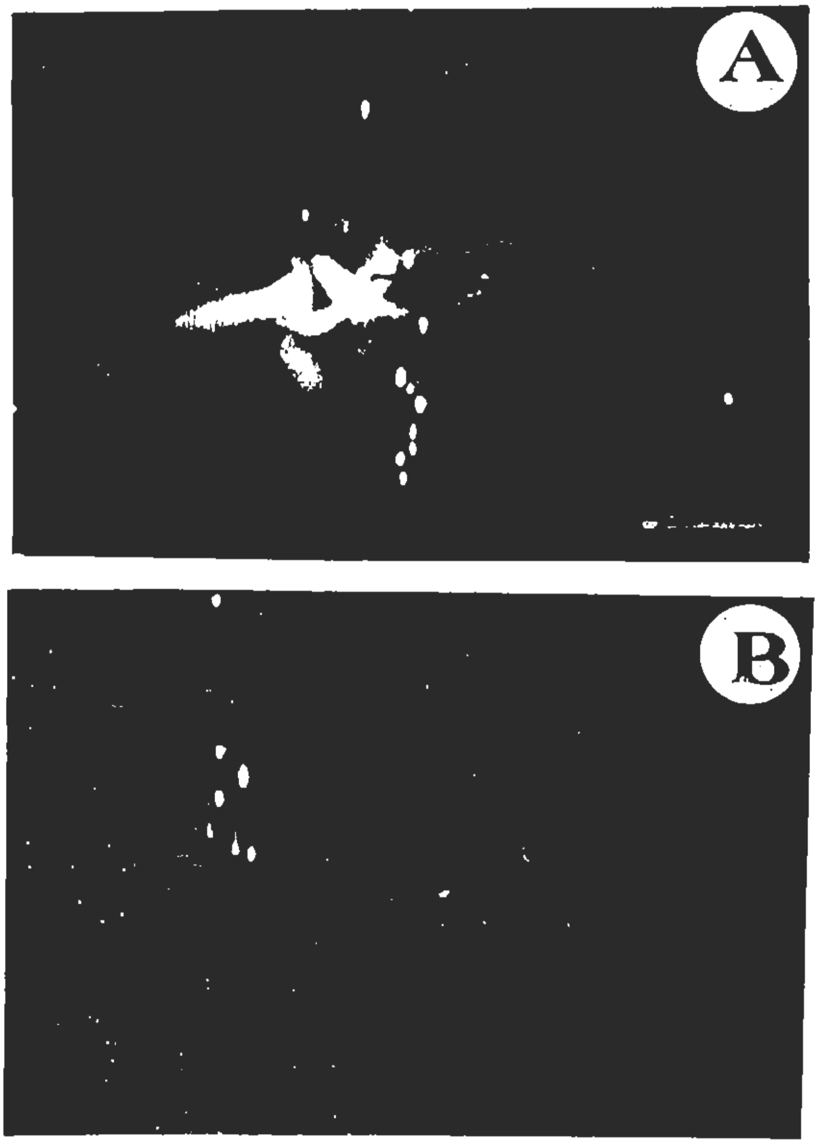
Nocturnal:

A. *Mythimna consanguis* Fab.:

M. consanguis constitute about 36.84% of the total number of all the pollinaria bearing insects. It starts visiting the plant at about 8.0 - 8.30 p.m. and observed till 12.0 a.m. [observations confined upto midnight only].

It alight on the hanging (inverted) flower and grasp the corona segments with thorax resting on the stigma head and proboscis inserted at base of the staminal column to suck the nectar. During nectar sucking, corpuscla get attached to the legs and pollinaria removed when insect leave the flower. Insect with attached pollinarium, when visit another flower, the pollinium get inserted into the stigmatic chamber and in this way insewrtion of pollinium occurs.

Fig 27. Pentatropis nivalis: Insects caught by the corpuscula of flower. A, Common house fly; B, a small ant.
Scale bar = 5 mm.


A
B

It frequently move between plants and visit only 2 - 3 flowers of a plant. About 46.67% (captured individuals) carried pollinaria with an average of 1.0 per insect. It carried about 31.82% of the pollinaria counted for all insects. All the pollinaria are directly attached to the claws of legs, particularly of middle leg (Table 2).

Non - pollinating insects:

A variety of insects visit its flowers without performing pollination. Among them *Rhopalomeliossa* sp. (Fig. 26A$_2$), *Lasioglossum* sp. (Fig. 26A$_3$ - $_4$). Most of the small insects ar caught on the flower by corpuscula (Fig. 27A, B) and become th food of spiders.

5. ***Pergularia daemia*** **(Forssk.) Chiov.:**

P. daemia (Forssk.) Chiov. is visited by a number of bot diurnal and nocturnal insects (Table 2 - 4; Fig. 28, 30).

Pollinating insects:

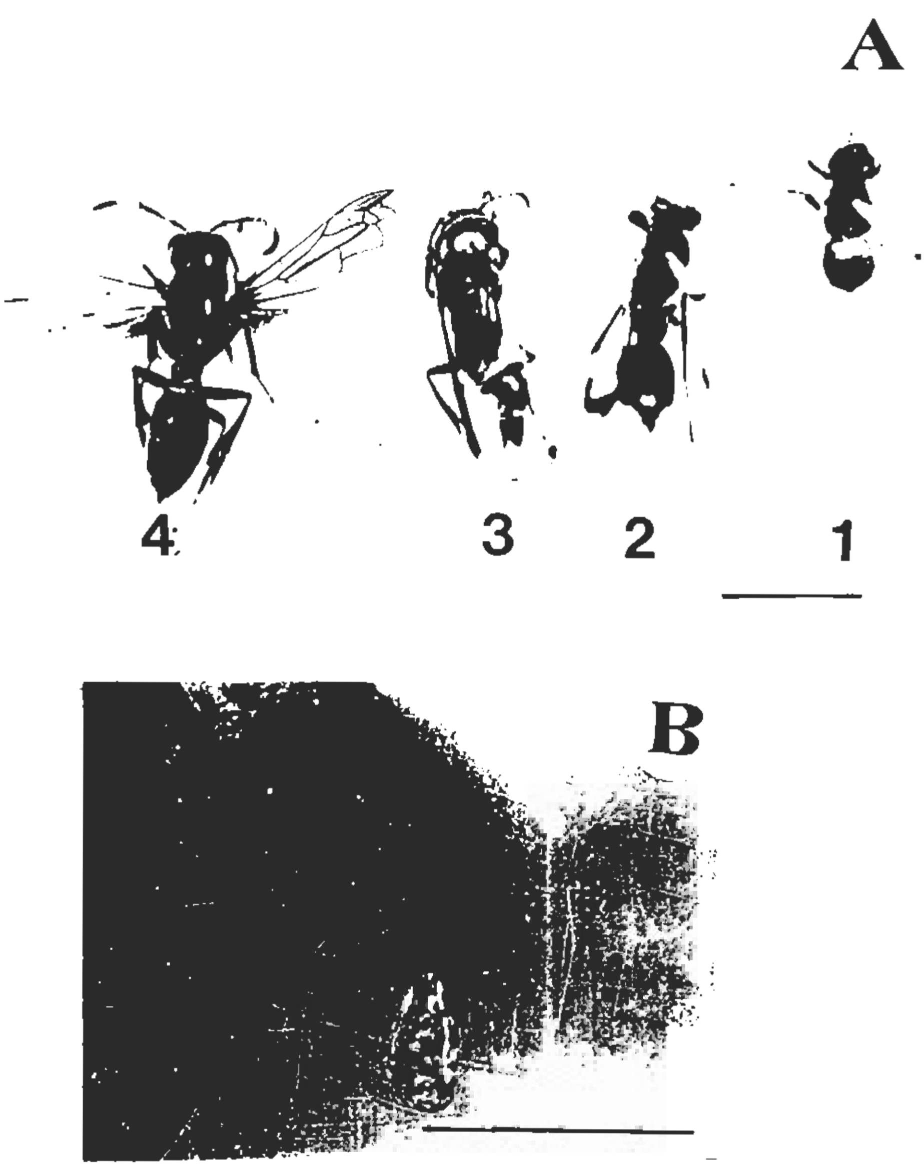
Diurnal:

A. *Apis florea* (F.) (♀) (Fig. 28A$_1$):

A. florea (F.) constitute about 13.64% of the total numbe of all the pollinaria bearing insects. It started visiting th plant at about 5.30 p.m. and continue to about 7.30 p.m. I directly land on invertly hanging flower, grasp the corona segments with all three pairs of legs with thorax resting on th stigmma head and proboscis is inserted at the base of the stami

Fig 28. Pergularia daemia: Insects visitors (diurnal). A_1, Apis florea, A_{2-3}, Ropalidia spatulata, A_4, Polistes watti; B, Ropalidia spatulata. Pollinriumm attached to the claw of leg.

Scale bar = 5 mm.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nal column to suck the nectar (Fig. 29A, B). During nectar sucking, corpuscula get attached to the legs and pollinaria removed from the flowers as insect left the flower. When the same insect (with attached pollinaria) visit another flower the whole process is repeated and pollinium may be inserted in the stigmatic chamber of the flower.

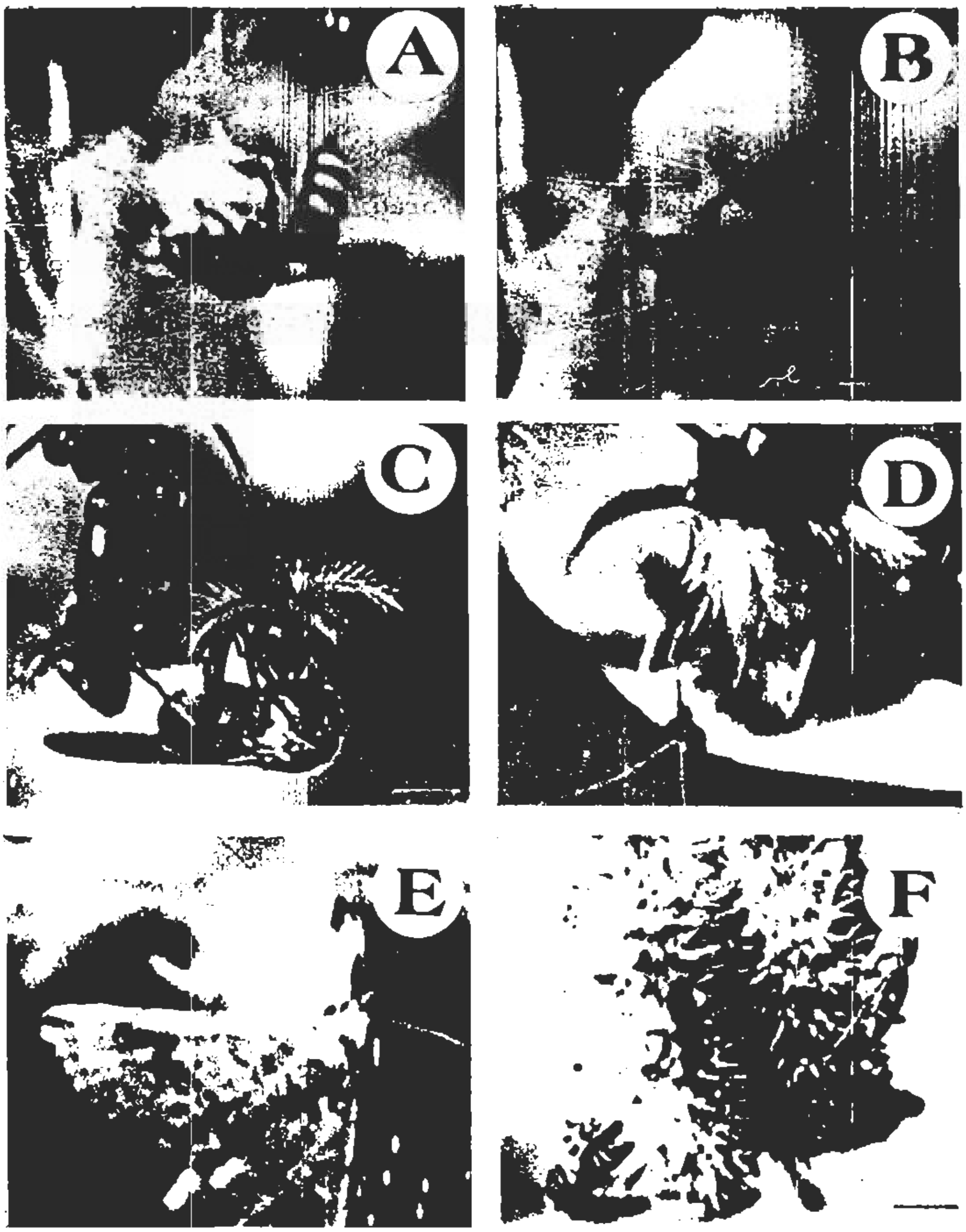
It is less frequent between plants as it spend much time on the flowers of a plant. All the captured individuals carried pollinaria with an average of 1.33 per insect. It carried about 13.56% of the pollinaria counteo for all insects. all the polli aria are directly attached on the claws of middle and hind legs (Table 2).

B. *Ropalidia spatulata* V. V. (♀) (Fig. 28A$_{2-3}$) :

Ropalidia spatulata V. V. is active in only Malir population and constitute abcut 22.73% of the total number of all the pollinaria bering insects. They usually touch the flowers of many inflorescence and land on the selected ones. They explore only single nectary of a flower. aaAlthough their movement is slow but they usually check or touch most of the flowers of a plant as well as population. Teir foraging behaviour is similar to that of *Apis florea* (F.).

About 71.43% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried about 16.95% of the pollinaria counted for al the insects. All of the pollinaria are directly attached to the claws of the legs, particularly middle leg (Table, 2; Fig. 28B).

Fig 29. Pergularia daemia: Foraging behaviour of insects. A - B, Apis florea; C, Amerila rhodopa; D, Raparna conciephala; E, Triphassa macrarthralis; F, Spilostethus pandurus. Scale bar = 5 mm.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C. *Braunsapis mixta (Smith)* (♀):

Braunsapis mixta (Smith) is the most common insect on *Pergularia daemia* (Forssk.) Chiov. flowers but constitute only 11.36% of the total number of all the pollinaria bearing insects. They are of small size as compared to other pollinators. They generally, enter into the flower, such the nectar from the base of the stigmatic chamber while grasping the coronal lobes. The hind leg get into the stigmatic chamber and as insect pull that leg, corpusculum get attached to its claw and pollinarium removed from the flower. Similarly, the pollinium get inserted when this process is repeated by pollinarium baering insect on other flower.

Only 21.0% OF the captured individuals carried pollinaria with an average of 1.0 per insect. They carried only 8.47% of the pollnaria counted for all the insects. All the pollinaria are directly attached on the claws of hind legs.

Nocturnal:

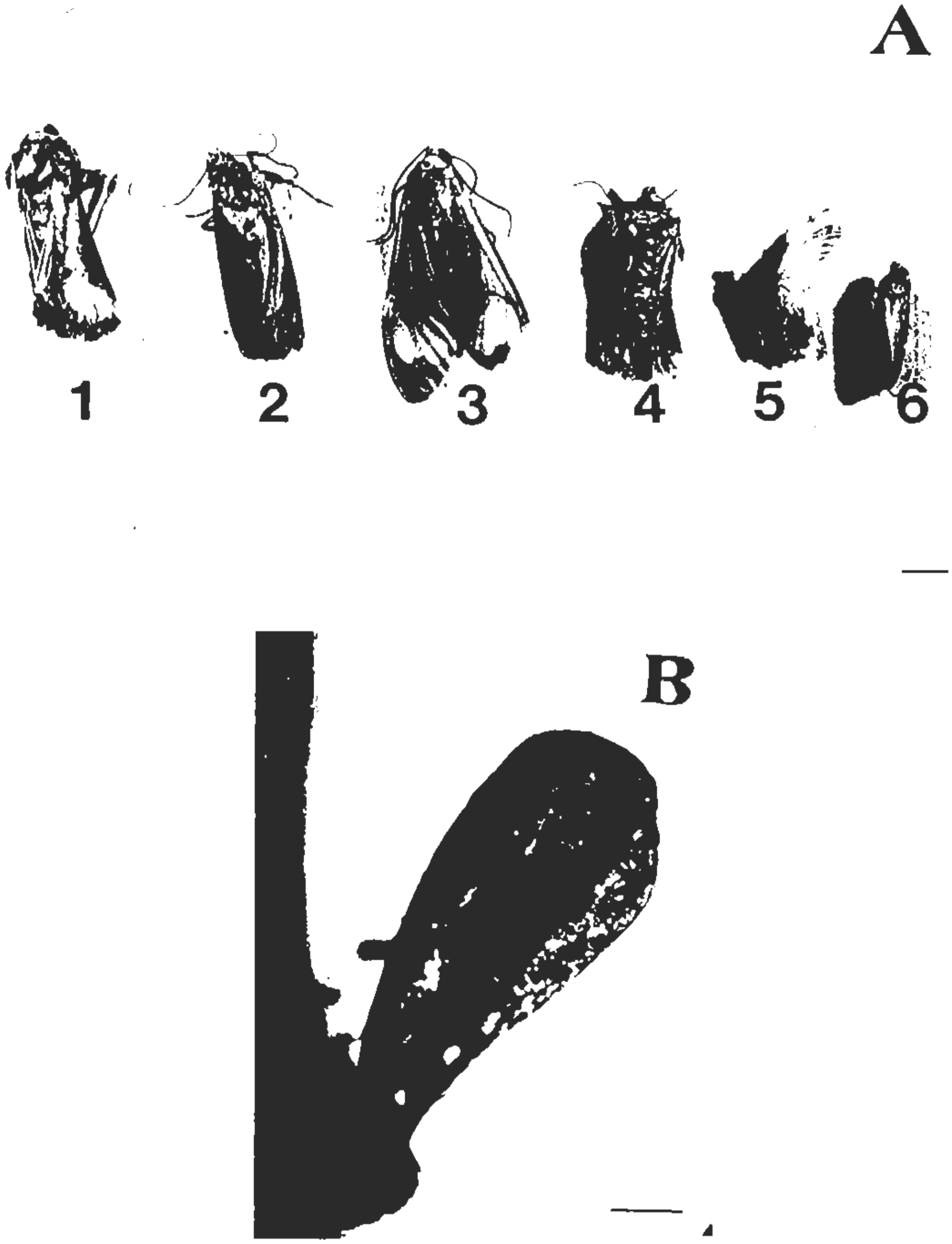
A. *Thysanoplusia orichalcea* Fabr. (Fig. $30A_1$):

T. orichalcea Fabr. constitute about 34.09% of the total number of all the pollinaria bearing insects. Its visit to the plant started at 8.0 - 8.30 p.m. and continue to about 12.0 a.m. [observations confined to this time only].

It lands on inverted hanging flower in such a way that front and middle legs grasp the corona segments, hind legs rest on the petals, stigma head comes underneath of thorax and proboscis

Fig 30. Pergularia daemia: Insects visitors (nocturnal). A_1, Thysanoplusia orichalcea; A_2, Agrotis biconoca; A_3, Amerila rhodopa; A_4, Mythimna consanguis; A_5, Raparna concicephala; A_6, Triphassa macrarthralis; B, Thysanoplusia orichalcea. Pollinarium attached on the claw of leg.

Scale bar = 5 mm.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inserted at the base of the staminal column to suck the nectar. During this process. the corpuscula get attached to the legs and pollinaria removed from the flower along with the legs of the insects. when this insect (with attached pollinaria) visit another flower, the whole process is repeated and pollinium may be inscreted in the stigmatic chamber of the flower.

T. orichalcea Fabr. is very frequent in inter - plant movements. It is very eagile and quick, usually visit 1 - 2 flowers of a plant, and come back to the first plant after visiting several plants of the population. About 75% of them (captured) carried pollinaria with an average of 1.6 per insect. It carried about 40.68% of the pollinaria counted for all insects. All the pollinaria are directly attached to the claws of front and middle legs (Table 2; Fig. 30B).

B. *Agrotis biconica* Kollar (Fig. 30A_2):

Agrotis biconica Kollar constitute about 18.18% of the total number of all the pollinaria bearing insects. Their visiting time and behaviour is similar to that of *T. orichalcea* Fabr.

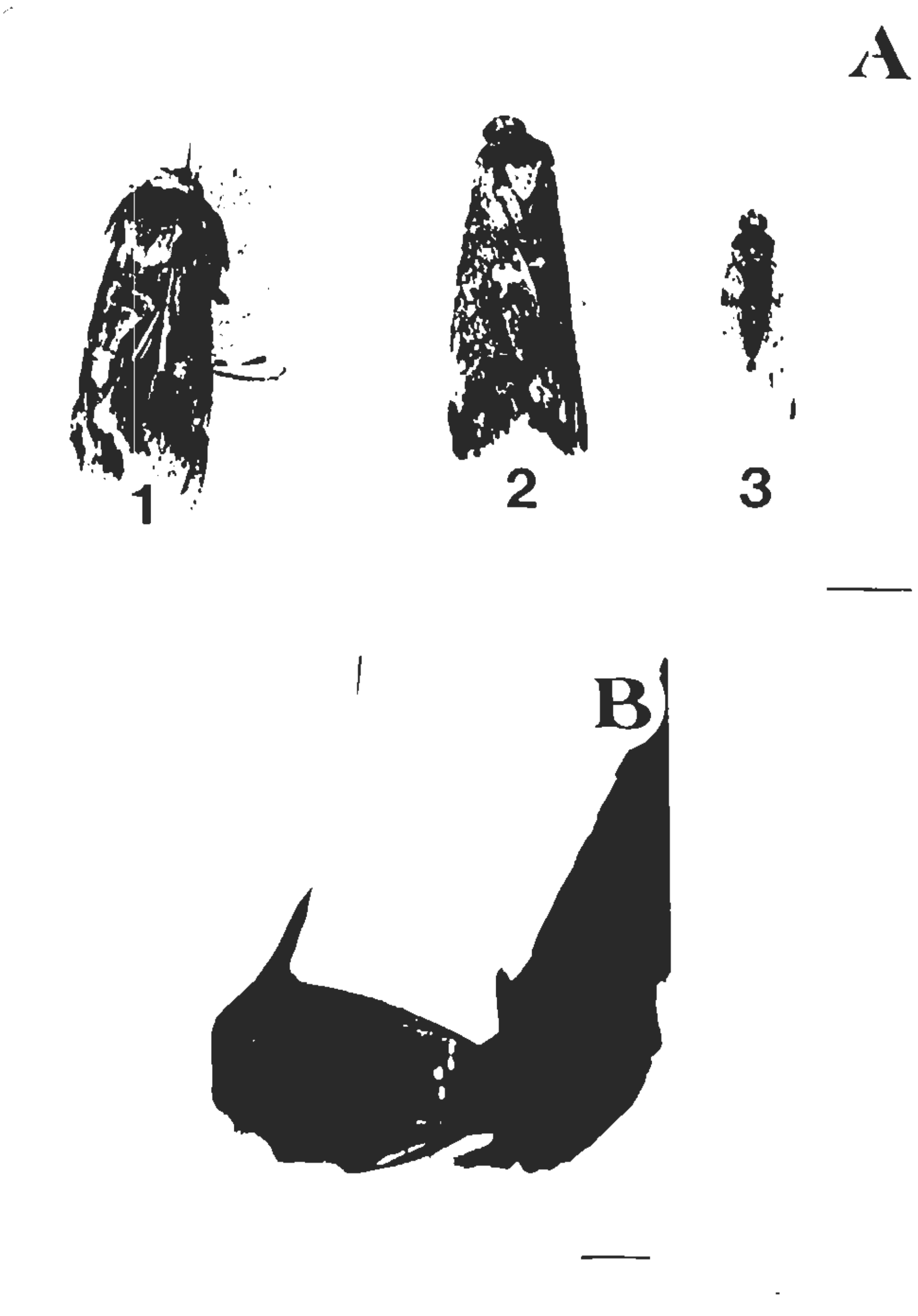
Non - Pollinating insects:

Diurnal:

Polistes watti Cam. (Fig. 28A_4) and Muscidae sp. (Diptera) visit the plant between 5.0 - 7.30 p.m. They usually suck the nectar without providing any benefit i.e. pollination to the plant. *Spilostethus pandurus* Segny. is also present on the plant particularly in fruiting season on fruits.

Fig 31. Pergularia tomentosa: Insects visitors (nocturnal). A_1, Helicoverpa armigera; A_2, Noctuidae sp.; A_3, Duzulla subhyalinalis; B, Helicoverpa armigera. Pollinarium attached on the claw of leg.

Scale bar = 5 mm.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Nocturnal:

A. *Amerila rhodopa* Walker (Fig. $30A_3$) :

Amerila rhodopa Walker is quite common on the flowers. They land on the inflorescence rather on the flower. The prasp the other flowers of an inflorescence with middle and hind legs, while the visiting flower with the fore legs. Insert its proboscis at the base of stigmatic chamber and suck the nectar (Fig. 29C). Non of its leg come in contact with **thecorpusculum** so removal of pollinaria not occur.

B. *Mythimna (Aletis) consanguis* Fabr. (Fig. $30A_4$)

C. *Rapaena conicephala* Staud. (Fig. 29D; $30A_5$)

D. *Triphassa macrarthralis* Hamp. (Fig. 29E; $30A_6$)

E. *Agdistis* sp.

F. Noctuidae

All these insects visit flowers regularly but none of them carry pollinaria.

6. ***Pergularia tomentosa*** .

It has also been visited by a number of insects particularly by moths Table 2 -4; Fig. 31, 32).

Pollinting insects:

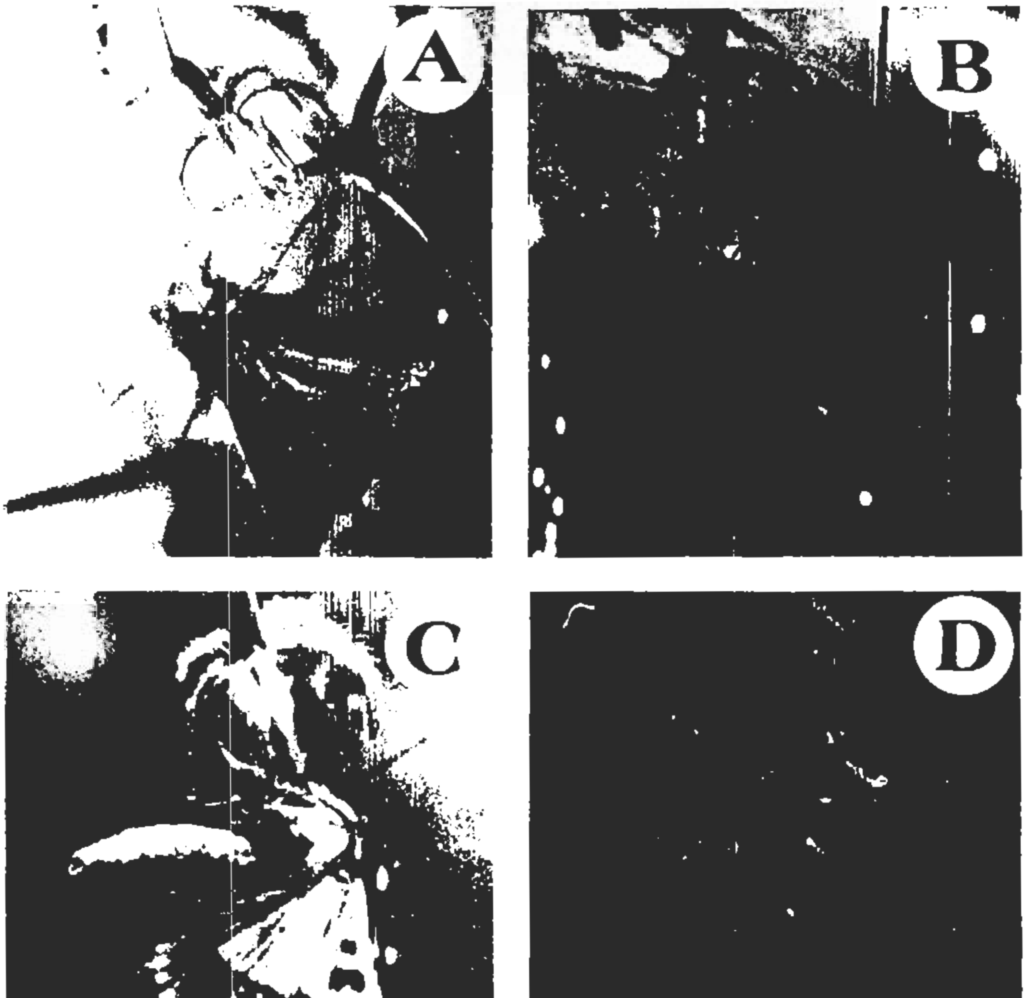
Nocturnal:

A. *Helicoverpa armigera* Hübner (Fig. $31A_1$):

Helicoverpa armigera Hübner constitute about 62.5% of the total number of all the pollinaria bearing insects. Generally, the insect land on the stem or leaves near the visiting flower

Fig 32. Pergularia tomentosa: Foraging behaviour of insects. A - B, Helicoverpa armigera; C, Noctuidae sp.; D, Duzulla subhyalinalis.

Scale bar = 5 mm.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and then pull the flower towards itself by grasping the coronal lobes with the front leg and then insert its proboscis at the base of stigmatic chamber (Fig. 32A, B). During grasping, the legs slipped into the stigmatic chambers and contacted the corpusculum, After nectar sucking, when insect pull its leg from the stigmatic chamber, the corpusculum get attached to it and come out along the leg. When this process is repeated by the pollinaria bearing insect, pollinum get inserted into the stigmatic chamber.

They usually visit 1-2 flower on a plant and their interplant movements are frequent than the itra-plant ones. About 50% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried about 62.55% of the pollinaria counted from all the pollinators. All the pollinaria are directly attached to the claws of front legs (Fig. 31B).

B. Noctuidae sp. (Fig. 31A_2):

It constitute about 37.5% of the total number of all the pollinaria bearing insects. Generally, insect directly land on the flower, grasp the corona lobes with front and middle legs and petals with hind legs, insert the proboscis at the base of the stigmatic chamber and suck the nectar (Fig. 32C). The rest of the process of pollinarium removal and pollinium insertion is similar to that of *H. armigera* Hübner.

They are also common and about 30% of the captured idividuals carried pollinaria with an average of 1.0 per insect. They carried about 37.5% of the pollinaria counted on all the pollina-

Fig. 33. Ceropegia bulbosa: Forcipomya sp. carrying pollinarium (arrow) on the mouth part.

Scale bar = 0.5 mm.

tors. All the pollinaria are directly attached on the claws with 60% on front and 40% on middle legs.

Non - Pollinating insects:

Duzulla subhyalinalis Hampson (Fig. $31A_3$):

Duzulla subhyalinalis Hampson usually land on the petals. Legs grasp the petals while rest of the body rest on the petal tips. Inser its long proboscis at the base of stigmatic chamber and suck the nectar (Fug. 32D). Legs never touch the corpusculum so no removal of pollinarium occur.

Tribe Ceropegieae

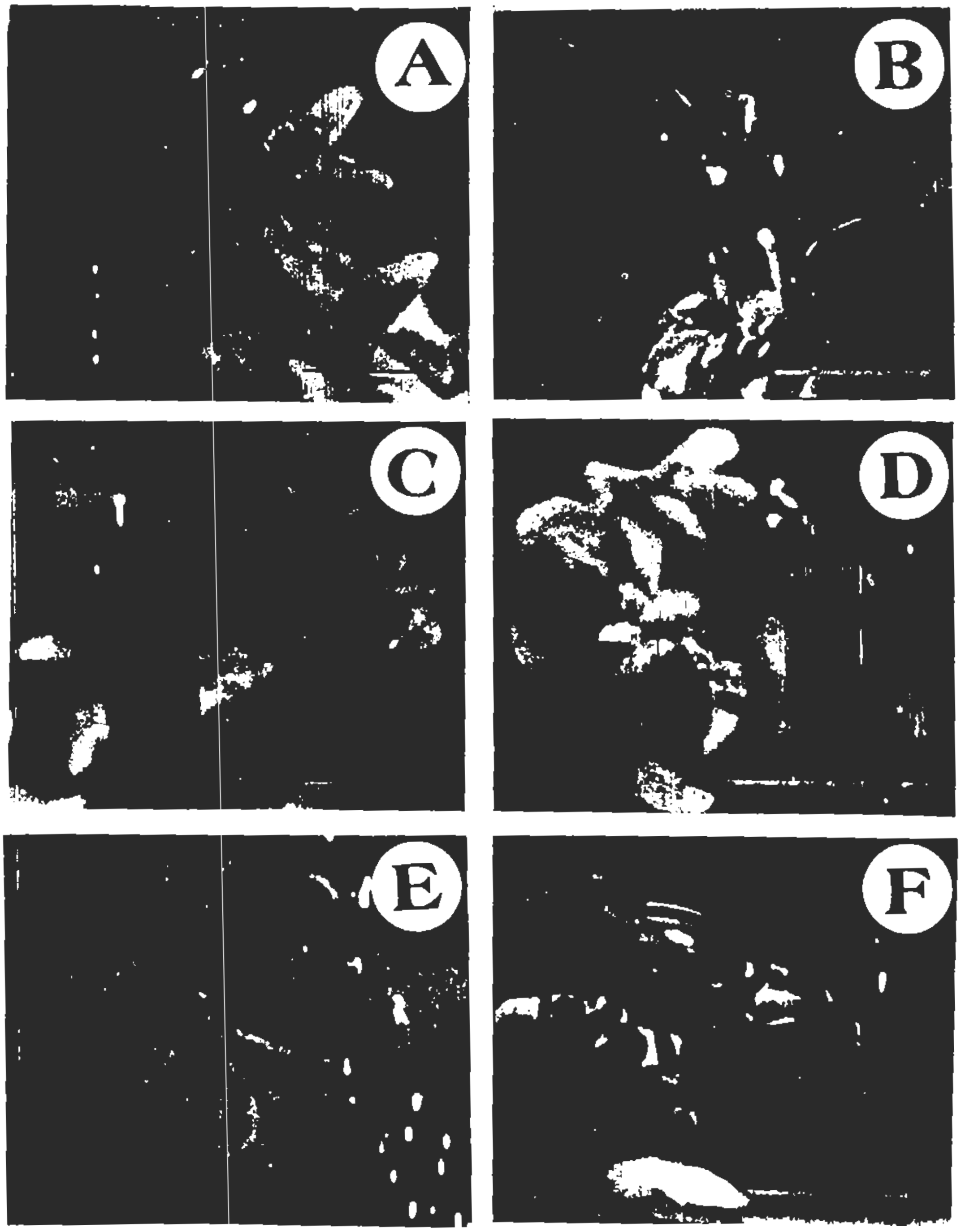
7. *Ceropegia bulbosa* Roxb. (Fig. 33):

Ceropegia bulbosa Roxb. is visited and pollinated by single insect " *Forcepomya* sp. Insect hovered around the flower, alight on the perianth lobe and slipped into the trap where it prisoned for about 18-24 hours. In prison insects get the nectar from the stigmatic chambers and the coronal cups below it, and in this process the corpusculum get attached on lower side of the mouth of the insect. When this process repeated on the other or same flower, the removed pollinium may be inserted into the stigmatic chamber.

About 25.93% of the captured insects carried pollinaria directly on the mouth parts with an average of 1.0 per insect.

Fig. 34. Leptadenia pyrotechnica: Foraging behaviour of insects. A, Physiophora sp.; B, Chrysomya albiceps; C & D, Attagenus cf. posticalis; D, Black ant; E, Fly (Unidentified).

Scale bar = 5 mm.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8. ***Leptadenia pyrotechnica* (Forssk.) Dcne.**

Leptadenia pyrotechnica (Forssk.) Dcne. is visited by a number of insects. Most of them are Diptera and Coleoptera, which are smaller in size as compare to the visitors of above mentioned taxa. (Fig. 34, 35).

Pollinating insects

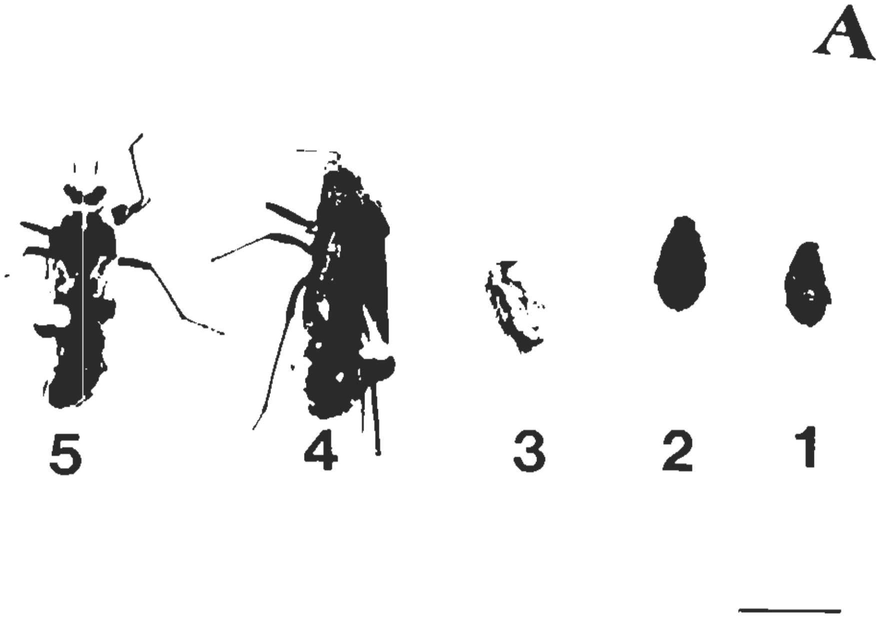
A. *Physiophora* sp. (Fig. 34A):

Physiophora sp. constitute about 33.33% of the total number of all the pollinaria bearing insects. They vist the plant continously whole the day long from 8.0 a.m. - 7.o p.m. The insects does not land directly on the flower instead it land on the stem near the flower than walk towards the the visiting flower. Insect alight on the flower with its legs resting on petals, thorax and abdomen on the stigma head while proboscis inserted in the nector pouch located below stigmatic chamber (Fig. 34A). During nectar sucking, the fine hairs on the mouth parts brush against the corpusculum and as a result corpusculum get attached to these hairs. When insect leave the flower, the removal of pollinarium occur as corpusculum also reoved from the flower along with the insect's mouth. Similarly when this process is repeated the insertion of pollinium occur.

Insect usually rest on the stem after visiting a flower. It visit nearly all the flowers of an inflorescence and then walk on stem towards the other inflorescence. Insects are more active between inflorescence of a plant rather than between plants. Plant act as a site for their copulation also. Insects are often

Fig. 35. Leptadenia pyrotechnica: Insects visitors. A_{1-3}, Attagenus fasciatus; A_{4-5}, Spilostethus pandurus; B, . Attagenus fasciatus. Pollinaria (arrow) attached on the mouth part.

Scale bar = 5 mm.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observed to brush their mouth parts with legs and may result in brushing of attached pollinaria also. About 37.5% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 33.25% of the pollinaria counted for all pollinating insects. All the pollinaria are attached directly to mouth parts.

B. *Chrysomya albiceps* (Widemann) (Fig. 34B):

Its foraging behaviour is similar to *Physiphora* sp. It constitute about 17.14% of the total number of all the pollinaria bearing insects. About 80% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 16.07% of the pollinaria counted on all insects. All the pollinaria are attached directly on the mouth parts.

C. *Cosmina prasina* (Br. + Berg.) (Fig. 34B):

It constitute about 20% of the total number of all the pollinaria bearing insects. About 70% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 18.75% of the pollinaria counted on all insects. All the pollinaria are attached directly on the mouth parts.

D. *Attagenus fasciatus* (Thunb.) (Fig. 35A_{1-3}):

It is quite small beetle and costitute about 6.67% of the total number of all the pollinaria bearing insects. About 33.33% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 6.25% of the pollinaria counted on all insects. All the pollinaria are attached directly on the mouth parts (Fig. 35B). It usually cofined on a single plant only as their iter-plant movement is negligible.

E. *Attagenus* cf. *posticalis* Fairm. (Fig. 34C, D):

Its behaviour is similar to *A. fasciatus* (Thunb.). It constitute about 2.86% of the total number of all the pollinaria bearing insects. About 37.5% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 2.68% of the pollinaria counted on all insects. All the pollinaria are attached directly on the mouth parts.

F. *Brumoides suturalis* (F.):

It constitute about 7.62% of the total number of all the pollinaria bearing insects. About 30.77% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 7.14 % of the pollinaria counted on all insects. All the pollinaria are attached directly on the mouth parts.

G. cf. *Crematogaster* sp. (Black ant) (Fig. 34E):

It is very common and foraging behaviour is similar to other pollinators. It constitute about 7.62% of the total number of all the pollinaria bearing insects. About 8% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 7.14% of the pollinaria counted on all insects. All the pollinaria are attached directly on the mouth parts.

H. Winged Blach Ant (small);

It constitute about 2.86% of the total number of all the pollinaria bearing insects. About 30% of the captured insects carried pollinaria with an average of 1.0 per insect. They carried 2.86% of the pollinaria counted on all insects. All the pollinaria are attached directly on the mouth parts. Apart from walking, it also fly from inflorescence to inflorescence on same as well as on plants.

Non - Pollinating Insects:

A number of small insects visit flowers including, Lepidoptera (Noctuidae sp., Gelechoidea sp., *Eretmocera* sp., *Utetheisa* sp.), Diptera (unidentified, Fig. 34F), Coleoptera (Mordellidae sp.,Bruchidae sp., Curculionidae sp., and *Spilostethus pandurus* Segny. Fig. $35A_{4-5}$).

DISCUSSION

Calotropis procera (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali:

According to Van der Pijl (1954) flowers adapted for polination by *Xylocopa* (*Xylocopa* blossom) should be spacious, solid with hidden nectar which is mostly accessible only by force, strong walled nectar room separated from anteroom, nototribic pollen applicaiton and nectar not abundant. He wonder why *Calotropis* flowers so different from usual *Xylocopa* blossoms are exlusively visited by *Xylocpa* . However, if one regards (according to Schick and Remus, 1984) the five sectors of the flowers as meranthia, so with the exception of nototribic condition, the deviation is not so spectacular and fulfill all the esssential characterstics stated by Van der Pijl.

The genus *Calotropis* is reported to be mainly pollinated by *Xylocopa* species. *Calotropis procera* is pollinated by *Xylocpa* sp. in Senegal (Jaeger, 1971), by *X.leucothorax* in Egypt (Schremmer, 1972), by *X.pubescence* and *X.sulcatipes* in Israel (Eisikowitch, 1986) and *Calotropis gigaentea* by *X.latipes* in Java (Van der Pijl, 1954) by *X.tenuiscapa* in Ceylon (Wanntorp, 1974), by

X.collaris and *X.dissimilis* in India (Ramakrishna and Aerekal, 1978). However, in contrast to above reports, Pant *et al* (1982) has reported *X.fenestrata* as the minor pollinator of *C.procera* in India.

In the present study, *X.puybescence* (♀ & ♂) and *X.fenestrata* (♀) are the main pollinators and their behaviour of nectar collection by inserting the proboscis in the slit or pore at the apex of coronal segemts is in accordance with Schremmer (1972).

Present study confirmed Schremmer's (1972) opinion that *C.procera* can be regarded as Xylocopa flower. It is also evident from the present study and available literature that a certain association exists between *Xylocopa* and *Calatropis*. It seems that *Calotropis* depend for its pollinatioan on *Xylocopa*, however, the exclusive dependence of *Xylocopa* on *Calotropis* can't be acertain as it also visit a number of other plant species (Van der Pijl, 1954).

Glossonema varians (Stocks) Hook. f.:

G.varians (Stocks) Hook. f. is visited by a variety of insects may be due its somewhat open nectar presentation i.e. easily accessible. *Apis florea* and *Scolia guadripustulata* the major while *Polistes wattii* and *Bembix* sp. are the minor pollinators. Pollinaria are located only on mouth parts of all the pollinators. Although, *Apis florea* carries an average of 3.3 pollinaria per individual but it is more or less restricted to a single plant and thus may perform more geitonogamy than Xenogamy. Where as *Scolia quadripustulata* carries only 1.3 pollinaria per individual but it is quite active between plants, so apparently perform more xenogamy.

Thus *S.quadripustulata* may be regarded as the main pollinator of *G. varians* as it is confined to this species only.

Oxystelma esculentum (L.f.) R. Br.:

O.esculentum (L.f.) R. Br. is also visited by a variety of insects, however, only Hymenoptera carry the pollinaria . *Polistes wattii* is the major pollinator alongwith *Apis florea* and *Anthophora* sp. *Polistes wattii* (Wasp) carried pollinaria on claws of middle and for legs whereas *Apis florea* carried polliniaria pollinaira on claws of middle legs while *Anthophora* sp. on claws of middle legs only. *P. wattii* make its hive near *O. exculentum* population and are the regular and abundent visitors than other insects. On all the three pollinators pollinaria are located on the claws only.

***Pergularia daemia* (Forssk.) Chiov.:**

Vijayaraghavan and Shukla (1980) reported *P.daemia* as diurnal and *Apis dorsata* Fabr. as the major pollinator. However, it is nocturnal i.e. its flower open in the evening and close in the morning. Flowers seems to be partly adapted for the day (evening) pollination by Hymenopter. (Bees) and partly for the night pollination by the Lepidoptes (Moths). In the evening it has been pollinated by *Apis florea*, *Ropalidia spatulata* and *Braunsapis mixta*. *R.spatulat* is active only in Malir population where as other two are found in all the studied populations. Pollinaria are located on the claws of middle and hind legs of these insects as forelegs are usually used to grasp the corona segments of the inverted flowers. The characters of flowers i.e. opening in evening and closing in day light, white-green colour, faint fruity smell are predoimnantly moth pollinated. A number of moths visit the flowers at night. *Thysanoplusia orichalcea* Fab. seems to be the main pollinator alongwith *Agrotis biconica* Kellar. All the pollinaria are located on the claws of middle and front legs.

It has been noted that both *Hymenoptera* and *Lepidoptera* of fairly large size act as pollinators while small insects do not carry pollinaria and regared as nectar theivers.

Our study also confirm the finding of Bhatnagar (1986) that *P.daemia* is pollinated by moths.

Pergularia tomentosa **L. :**

P.tomentosa L. is also a nocturnal and its flowers also open in the evening and closed in the morning It is also visited by a number of insects but only *Helicoverpa armigera* (moth) and Noctuidar sp. (moth) act as the pollinators. All the pollinaria are located on the claws of front and middle legs.

Pentatropis nivalis **(Gmel.) Field and Wood:**

It is also noctruanl and visited by both diurnal (in the evening) and nocturnal (in night) insects. In the evening only *Apis florea* carry pollinaria and act as pollinator while at night *Mythimma cansanguis* is the major pollinator. both insects carried pollinaria on the claws of legs, particulary of middle legs

Ceropegia bulbosa **Roxb. :**

Forcepomya sp. (Diptera, Ceratopogonoidae) is the only visitor and pollinator of *C.bulbosa*. Members of Ceratopogonoidae (biting midges) has also been reporte by Faegri and Van der Pijl (1971) , Percival (1966), Proctor and Yeo (1973) for *Ceropegia* species. Bayer (1978) reportd four types of small flies (Diptera) visitors of *Ceropegia amplicatea* E.Meyer.

Forcepomya sp. trapped (or prisoned) in the flower for about 18 - 24 hours and carried pollinaria on mouth parts.

Leptadenia pyrotechnica (Forssk.) Dcne.:

Pant et al. (1982) pointed out *Crematogaster* sp. (Black ant), *Evylaes* sp. and *Micrapis florea* as the pollinaria carrying insects of *L. reticulata*. They have also suggested that small size of its flower favour pollination by black ant. However, in the present study, Diptera like, *Physiophora* sp., *Chrysomya albiceps*, *Cosmina presina* and unidentified mosquito like fly, Coleoptera like, *Attagenus fasciatus* , *Brumoides suturalis* and Hymenoptera like *Crematogaster* sp. and winged small black ant are found to carry pollinaria of *L.pyrotechnica*. Pollinaria are located on the mouth parts of all these insects. The Diptera, *Physiophora* sp. *Cosmina presina* and *Chrysomya albiceps* are the main pollinators.

The major pollinators (insects) seems to be species specific (Table 3). *Apis florea* (F.) is the common pollinator of *Calotropis procera* (Ait.) Ait. f. spp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) Hook. f., *Oxystelma esculentum* (L.f.) R.Br., *Pentatropis nivalis* (Gmel.) Field and Wood, and *Pergularia daemia* (Forssk.) Chiov.. It is note worthy that *Apis florea* (F.)

visit and pollinate members of tribe Asclepiadeae. *Polistes wattiii* Cam. is the common pollinator of *Glossonema varians* (Stocks) Hook. f. and *Oxystelma esculentum* (L.f.) R.Br., whereas *Attagonus fasciatus* act as the minor pollinator of *Glossonema varians* (Stocks) Hook. f. and *Leptadenia pyrotechnica* (Forssk.) Dcne. Most of the corpuscula are directly attached to the insects, without forming chains or clusters as repoted from *Asclepias* species.

Long anther wings and staminal column are related to leg-pollination i.e. corpuscula are attached on the legs of insects, particularly on tarsi and claws, whereas short anther wings and staminal column to mouth-pollination i.e. corpuscula attached to the mouth parts of insects.

Pollinators also seems to be tribe specific. Hymenoptera and Lepidoptera are the sole pollinators of the members of tribe Asclepiadaceae while Diptera and Coleoptera are the pollinators of tribe Ceropegieae.

CHAPTER 4

POLLINATOR ACTIVITY AND POLLINATION SUCCESS

INTRODUCTION

As stated earlier the Asclepiads possess a mechanism for pollen transfer which requires insects to remove the pollinaria and later to insert these pollinia into the receptive stigmatic chambers on other flowers. Pollinarium removal (extraction) rate allows an estimate of insects activity in Asclepiads (Willson & Rathke, 1974). However, for the estimation of pollination success the rate of pollinia insertion has to be determined . Level of pollinator activity and pollination success (determined on the basis of rate of pollinaria removal and pollinia insertion) have been carried out by many workers such as Wyatt, 1976 and 1978; Beare and Perkins, 1982 on *Asclepias tuberosa* L.; Wyatt and Shannon, 1986 on *Asclepias exaltata* L.; Lynch, 1977 on *Asclepias solanoana* Woods; Willson & Rathke, 1974; Fritz and Morse, 1981; Morse and Fritz, 1983 on *Asclepias syriaca* L.; Chaturvedi, 1987 on *Cynanchum canescens* (Willd.) Schum.; Kunze and Liede, 1991 on *Sarcostemma clausum* (Jacq.) Schultes and *Sarcostemma pannosum* Decne. and Liede and Whitehead, 1991 on *Sarcostemma viminale* R.Br.

In the present study, level of pollinator activity and pollination success as well as relationship of pollinia insertions into stigmatic chambers with or without removal of residual pollinaria (pollinaria present above stigmatic chambers) in different populations of *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, *Ceropegia bulbosa* Roxb., *Glossonema varians* (Stocks) Hook.f., *Leptadenia pyrotechnica* (Forssk.) Dcne., *Oxystelma esculentum* (L.f.) R.Br., *Pentatropis nivalis* (Gmel) Field & Wood, *Pergularia daemia* (Forssk.) Chiov., and *Pergularia tomentosa* L. has been carried out.

MATERIAL AND METHODS

Flowers (N=100) of above the mentioned eight species were randomly collected from different populations (Table 5) and preserved in 50% ethyl alcohal. Later the preserved flowers were dissected and the number of pollinaria removed (extracted) and the number of pollinia inserted into the stigmatic chambers were scored under the dissecting microscope. Presence or absence of residual pollinaria above the inserted pollinia were also recorded. Data was statisticaly analysed on Personal Computer (PC) by Statistical package "COSTAT" version 3.03 (CoHort Software, Berkeley, California, USA) and according to Sokal & Rolhf (1981).

Table 5. Pollinaria removal rate

Taxa	Population studied	No. of flowers examined	Flowers with removed pollinaria %	Pollinaria removed	
				%	per flower
Tribe: Asclepiadeae					
Calotropis procera ssp. *hamiltonii*	DAR	50	86.0	44.4	2.22
	KUC	50	14.0	3.6	0.18
	MAN	45	24.44	7.1	0.35
	NED	125	6.4	1.8	0.09
	NIPA	50	18.0	6.0	0.30
	SUP	50	14.0	10.8	0.04
Total		370	211.62	8.86	0.04
Glossonema varians	KUC	(?) 109	85.32	62.20	3.11
		(?) 58	86.21	57.24	2.86
Total		167	85.63	60.48	3.02
Oxystelma esculentum	GHA	80	47.5	4.0	0.70
	KUC	23	95.65	57.39	2.87
	SAJ	36	5.55	1.11	0.05
Total		139	44.60	18.82	0.89
Pentatropis nivalis	KUC	304	45.06	14.29	0.71
	MAL	110	28.18	8.0	0.46
Total		414	40.48	12.61	0.63
Pergularia daemia	KUC	231	36.79	13.27	0.64
	MAL	166	13.85	3.61	0.18
Total		397	27.20	9.15	0.45

Table 5. contd....

Taxa	Population studied	No.of flowers examined	Flowers with removed pollinaria %	Pollinaria removed	
				%	per flower
Pergularia tomentosa	KHZ	184	67.39	35.65	1.78
Tribe: Ceropegieae					
Ceropegia bulbosa	KUC	56	57.14	165.71	3.3
	SUP	44	75.0	34.09	1.7
Total/Average		100	65.0	51.80	2.59
Leptadenia pyrotechnica	DAR	100	76.0	42.40	2.12
	GHA	288	92.01	57.29	2.86
	KUC	224	75.4	30.17	1.51
	MAN	100	80.0	44.80	2.24
Total/Average		712	82.86	44.91	2.25

Abbreviations:

% = Percentage
DAR = Darsano Chino
GHA = Gharo
NED = NED University
SUP = Super Highway
KUC = Karachi University Campus
NIPA = Near NIPA round about
MAN = Mangopir
KHZ = Khuzdar

Table 6. Pollinia insertion rate

Taxa	Population studied	No.of flowers examined	Flowers with inserted pollinia %	Pollinia inserted	
				%	per flower
Tribe: Asclepiadeae					
Calotropis procera ssp. *hamiltonii*	DAR	50	48.0	16.4	0.82
	KUC	50	2.0	0.4	0.02
	MAN	45	4.4	0.88	0.35
	NED	125	2.4	0.48	0.09
	NIPA	50	8.0	2.0	0.30
	SUP	50	2.0	0.4	0.04
Total		370	9.46	2.86	0.14
Glossonema varians	KUC	109	5.5	1.65	0.08
Oxystelma esculentum	GHA	80	23.75	6.25	0.31
	KUC	23	78.26	26.96	1.35
	SAJ	36	11.11	2.22	0.11
Total		139	30.93	8.63	0.57
Pentatropis nivalis	KUC	304	6.91	1.64	0.10
	MAL	110	6.36	2.18	0.11
Total		414	6.75	1.79	0.10
Pergularia daemia	KUC	231	11.69	3.03	0.15
	MAL	166	5.42	1.08	0.05
Total		397	9.07	2.22	0.11

Table 6 contd....

Taxa	Population studied	No.of flowers examined	Flowers with inserted pollinia %	Pollinia inserted	
				%	per flower
Pergularia tomentosa	KHZ	184	39.13	11.85	0.59
Tribe: Ceropegieae					
Ceropegia bulbosa	KUC	56	42.86	13.21	0.66
	SUP	44	9.09	2.27	0.11
Total		100	28.0	8.4	0.42
Leptadenia pyrotechnica	DAR	100	22.0	5.0	0.25
	GHA	288	8.68	2.15	0.11
	KUC	224	12.5	3.21	0.16
	MAN	100	23.0	5.6	0.28
Total		712	13.76	3.37	0.17

For abbreviations see Table 5

OBSERVATIONS AND RESULTS

Tribe Asclepiadeae

1. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali:

The six populations were studied which exhibited considerable variation in pollinaria removal and pollinia insertion rate (Table 5, 6). In KUC population, 3.6% pollinaria (of the total pollinaria available) were removed in 14% flowers with an average of 0.18 pollinaria per flower whereas 0.4% pollinia (of the total pollinia available) were found to be inserted in 2% flowers with an average of 0.02 pollinia per flower. In NED population, 1.8% pollinaria were removed in 6.4% flowers with an average of 0.09 pollinaria per flower and 0.48% pollinia were inserted in 2.4% flowers with an average of 0.024 pollinia per flower. In NIPA population, 6% pollinaria were removed in 18% flowers with an average of 0.30 pollinaria per flower and 2% pollinia were inserted in 4.44% flowers with an average of 0.1 pollinia per flower. In MAN population, 7.1% pollinaria were removed in 24.44% flowers with an average of 0.35 pollinaria per flower and 0.88% pollinia were inserted in 4% flowers with an average of 0.04 pollinia per flower. In DAR population, 44.4% pollinaria were removed in 86% flowers with an average of 2.22 pollinaria per flower and 16.4% pollinia were inserted in 48% flowers with an average of 0.82 pollinia per flower. In SUP population, 0.8% pollinaria were removed in 4% flowers with an average of 0.04 pollinaria per flower and 0.4% pollinia were inserted in 2%

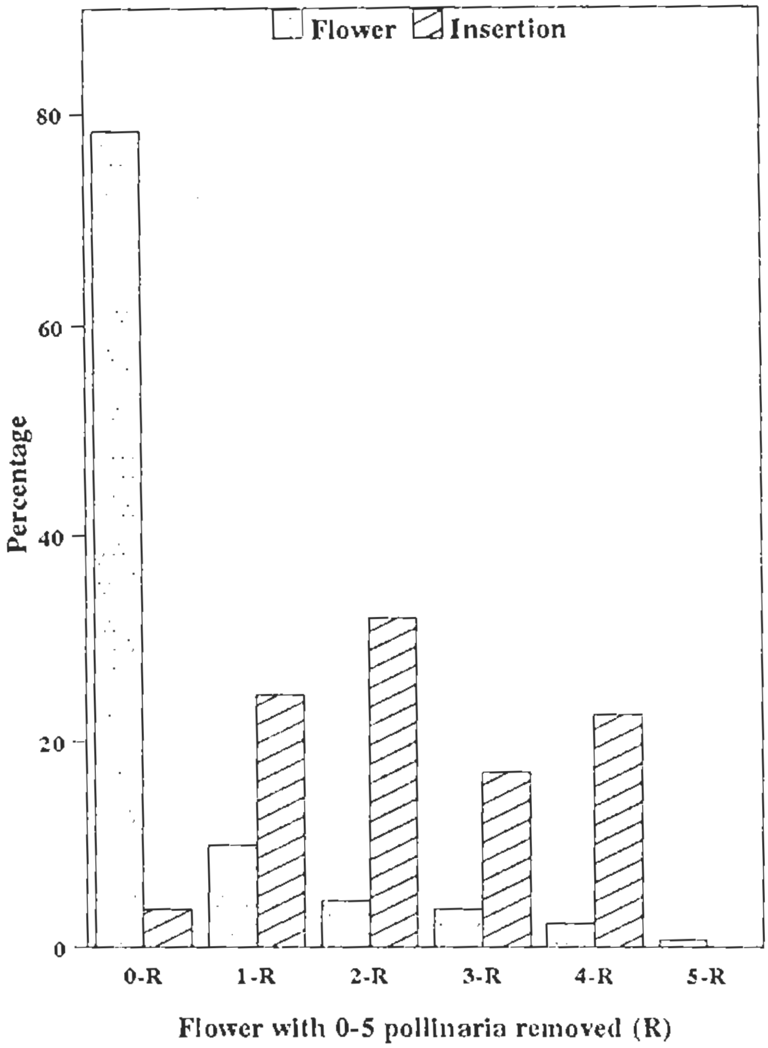


Fig. 36. ***Calotropis procera*** **ssp. *hamiltonii*:** Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

flowers with an average of 0.02 pollinia per flower (Table 5, 6).

Overall in all the six populations 8.88% pollinaria (of the total pollinaria available) were removed in 21.62% flowers with an average of 0.44 pollinaria per flower. Most of the flowers (67.50% of the flowers with removed pollinaria) were with one and two pollinaria removals (Fig. 36). Whereas 2.86% pollinia (of the total pollinia available) were inserted in 9.46% flowers with an average of 0.14 pollinia per flower. Most of the pollinia i.e. 57% (of the inserted pollinia) were inserted in flowers with one and two pollinaria removed (Fig. 36). The probability of removal was more than twice that of an insertion.

Significantly higher number of insertions (66 %, χ^2 = 37.52, P<0.001, Table 7) were found in the stigmatic chambers with residual pollinaria present as compared to stigmatic chambers (34%) with residual pollinaria (removed) absent.

Multiple insertions (insertions into more than one stigmatic chambers of a flower) were only 22.64% of all insertions. Of which 58.33% were double (insertion in two out of five stigmatic chambers of a flower), 33.33% triple (insertion in three stigmatic chambers of a flower) and 8.33% with insertions in four stigmatic chambers of a flower (Table 8).

2. *Glossonema varians* (Stocks) Hook. f.:

Removal and insertion rates for *G. varians* (Stocks) Hook. f. were studied from KUC population only (Table 5). In male flowers, 57.24% pollinaria in 86.21% flowers with an average of 2.86 per flower were removed (Table 5). Of the flowers with removed polli-

Table 7: Pollinia insertion into stigmatic chambers with and without residual pollinaria

Taxa	Pollinia insertion with residual pollinaria*		x^2	Probability
	Present No/%	Absent No./%		
Tribe: Asclepiadeae				
Calotropis procera ssp. *hamiltonii*	35 / 66.04	18 / 33.96	37.52	$P < 0.001$
Glossonema varians	6 / 66.66	3 / 33.33	3.12	ns
Oxystelma esculentum	38 / 63.33	22 / 36.66	12.55	$P < 0.001$
Pentatropis nivalis	22 / 59.46	15 / 40.54	24.91	$P < 0.001$
Pergularia daemia	33 / 75.0	11 / 25.0	12.57	$P < 0.001$
Pergularia tomentosa	62 / 56.88	47 / 43.12	3.46	ns
Tribe: Ceropegieae				
Ceropegia bulbosa	1 / 26.19	31 / 73.81	5.06	$P < 0.05$
Leptadenia pyrotechnica	87 / 72.50	33 / 27.50	14.27	$P < 0.001$
Total of all taxa	315/61.76	195/38.23	31.99	$P < 0.001$

* Pooled data from all population of each taxon

ns = non - significant

Table 8: Multiple Insertion

Taxa	Total No.of Pollinia inserted	Total insertion %	Multiple insertion			
			2-insertion %	3-insertion %	4-insertion %	5-insertion %
Tribe: Asclepiadeae						
Calotropis procera ssp. hamiltonii	53	22.64	58.33	33.33	8.33	-
Glossonema varians	9	11.11	100	-	-	-
Oxystelma esculentum	60	26.67	93.75	6.25	-	-
Pentatropis nivalis	37	24.32	100	-	-	-
Pergularia daemia	44	15.91	85.71	14.28	-	-
Pergularia tomentosa	109	28.44	87.09	9.68	-	3.22
Tribe: Ceropegieae						
Ceropegia bulbosa	42	33.33	100	-	-	-
Leptadenia pyrotechnica	120	16.66	90	10	-	-

Key: - = absent (or not inserted)

naria. 32% flowers were with three and 30% with four removals (Fig. 37). In hermaphrodite flowers, 62.20% pollinaria in 85.32% flowers with an average of 3.11 per flower were removed (Table 5). Of the flowers with removed pollinaria, 35.48% flowers were with five and 22.59% with three removals (Fig. 37). Altogether in both type of flowers, 60.48% pollinaria in 85.63% flowers with an average of 3.02 per flower were removed (Table 5). In 72.03% flowers (of total flowers with removals) three - five pollinaria were removed (Fig. 37).

About 33.33% pollinia (of total inserted pollinia) were inserested in flowers with three, 22.22% in flowers with two and none, 11.11% in flowers with four and five and not a single pollinium in flowers one pollinarium removed (Fig. 37). In hermaphrodite flowers only 1.65% pollinia in 5.5% flowers with an average of 0.1 per flower were inserted (Table 6). The probability of removal was four times than that of an insertion.

Although higher number of insertions (66.66%) were in stigmatic chambers with residual pollinaria present than absent but statisticallv the difference was insignificant (x^2 = 3.123, ns, Table 7). Only a single double insertion (11.11%) was observed (Table 8).

3. ***Oxystelma esculentum***(L.f.) R.Br.:

Removal and insertion rates varied greatly in all the three studied populations (Table 5, 6). In GHA population, 14% pollinaria were removed in 47.5% flowers with an average of 0.7 per flower while 6.25% pollinia were inserted in 23.75% flowers with

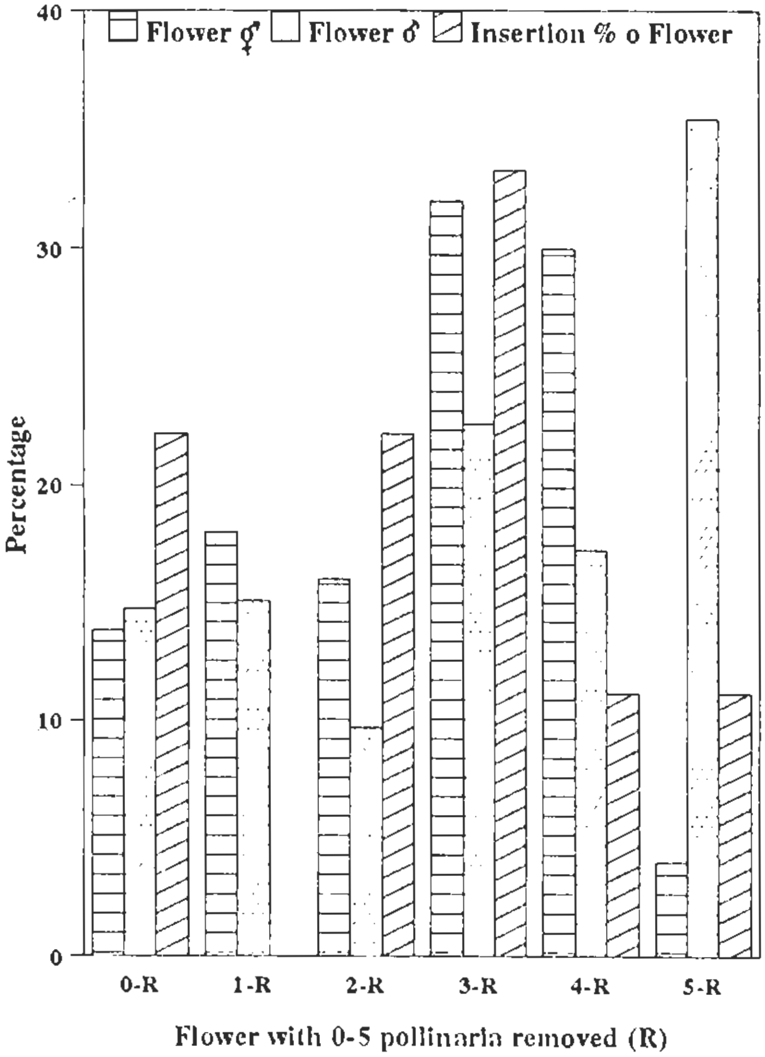


Fig. 37. Glossonema varians: Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

an average of 0.31 per flower. In KUC population, 57.39% pollinaria were removed in 95.65% flowers with an average of 2.87 per flower while 26.96% pollinia were inserted in 78.26% flowers with an average of 1.35 per flower. In SAJ population, 1.11% pollinaria were removed in 5.55% flowers with an average of 0.05 per flower while 2.22% pollinia were inserted in 11.11% flowers with an average of 0.11 per flower (Table 5, 6).

Overall in all the three populations, in 44.60% flowers only 18.82% pollinaria with an average of 0.89 per flower were removed (Table 5). In 46.77% flowers (of flowers with removed pollinaria) one, in 24.19% flowers two and in rest of the flowers three pollinaria were removed (Fig. 38). Whereas in 30.93% flowers about 8.63% pollinia with an average of 0.57 per flower were inserted (Table 6). About 50% pollinia (of total pollinia inserted) were inserted in flowers with one and three while 30% in flowers with none and two pollinaria removal (Fig. 38). The probability of removal was 1.5 times than that of an insertion.

Significantly higher number of insertions (63.33%, x^2 = 12.55, P<0.001, Table 7) were in stigmatic chambers with residual pollinaria present than in stigmatic chambers with removed (absent) residual pollinaria. Multiple insertions were 26.67% of the total insertions, of which 93.75% were double and 6.25% triple (Table 8).

4. *Pentatropis nivalis* (Gmel.) Field and Wood :

Two populations of *P. nivalis* (Gmel.) Field and Wood were studied (Table 5, 6). In KUC population, 14.28% pollinaria were removed in 45.06% flowers with an average of 0.71 pollinaria per

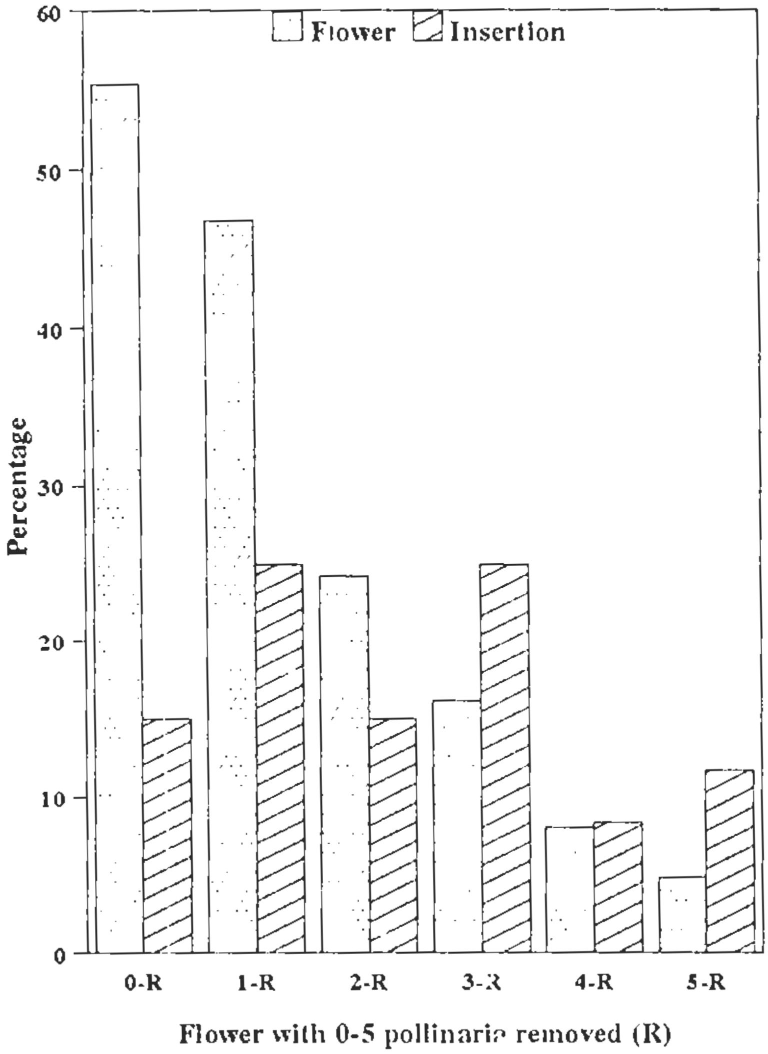


Fig. 38. Oxystelma esculentum : Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

flower while 1.64% pollinia were inserted in 6.91% flowers with an average of 0.08 per flower. In MAL population, 8% pollinaria in 26.16% flowers with an average of 0.4 per flower were removed while 2.18% pollinia in 6.36% flowers with an average of 0.11 per flower were inserted (Table 5, 6).

Altogether in both the populations 12.61% pollinaria in 40.48% flowers with an average of 0.63 per flower were removed (Table 5). One pollinarium was removed in 62.5% flowers (of the flowers with removed pollinaria), two in 26.77% while three - five in rest of the remaining flowers (Fig. 39). Whereas 1.79% pollinia in 6.75% flowers with an average of 0.10 per flower were inserted (Table 6). Nearly half (43.24%) of the inserted pollinia were found in flowers with one pollinarium removed and not a single pollinium was inserted into flowers with five pollinaria removed (Fig. 39). The probability of removal was more than six times that of an insertion.

Significantly higher number of insertions (59.5%, x^2 = 24.914, P<0.001, Table 7) were in the stigmatic chambers with residual pollinaria present than in stigmatic chambers with removed (absent) residual pollinaria. Multiple insertions were 24.32% of the total insertions (Table 8) and all of them were double.

5. ***Pergularia daemia*** (Forssk.) Chiov.:

In two populations of *P. daemia* (Forssk.) Chiov. the rate of pollinaria removal and pollinia insertion was determined (Table 5, 6). In KUC population, [illegible].27% pollinaria (of the available pollinaria) were removed in 36.79% flowers with an average of

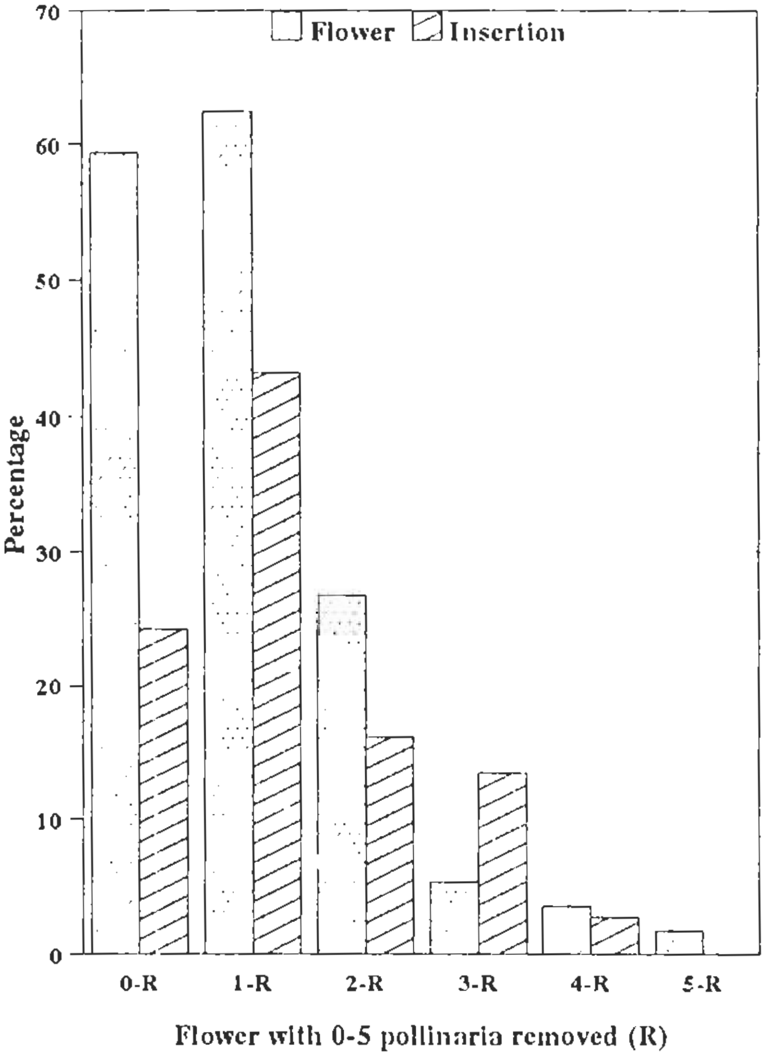


Fig. 39. ***Pentatropis nivalis*** **:** Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

0.64 pollinaria per flower while 3.03% pollinia (of the available pollinia) were found inserted in 11.69% flowers with an average of 0.15 pollinia per flower. Whereas in MAL population, only 3.61% pollinaria were removed in 13.85% flowers with an average of 0.18 pollinaria per flower and in 5.42% flowers, 1.08% pollinia were found inserted with an average of 0.05 pollinia per flower (Table 5, 6).

Altogether in both the populations, 9.15% pollinaria were removed in 27.20% flowers with an average of 0.45 pollinaria per flower (Table 5). Of the flowers with removed pollinaria, 58.09% flowers were with one, 25.71% with two, while 16.91% were with 3-5 pollinaria removals (Fig. 40). Whereas 2.22% pollinia were inserted in 9.07% flowers with an average of 0.11 per flower (Table 6). Of the inserted pollinia, 22.73% were inserted in flower with no pollinaria removal, 20.45% in flower with 1 & 3 pollinaria removal, 15.91% in flower with 2 & 4 pollinaria removal and 4.54% in flower with all 5 pollinaria removal (Fig. 40). The probability of removal was more than three times that of an insertion.

Significantly higher number of insertions (75 %, $x^2 = 12.57$, $P<0.001$, Table 7) were in the stigmatic chambers with residual pollinaria present than in stigmatic chambers with residual pollinaria (removed) absent. Multiple insertions were only 15.91% of the total insertions, of which 85.71% were double and 14.28% triple (Table 8).

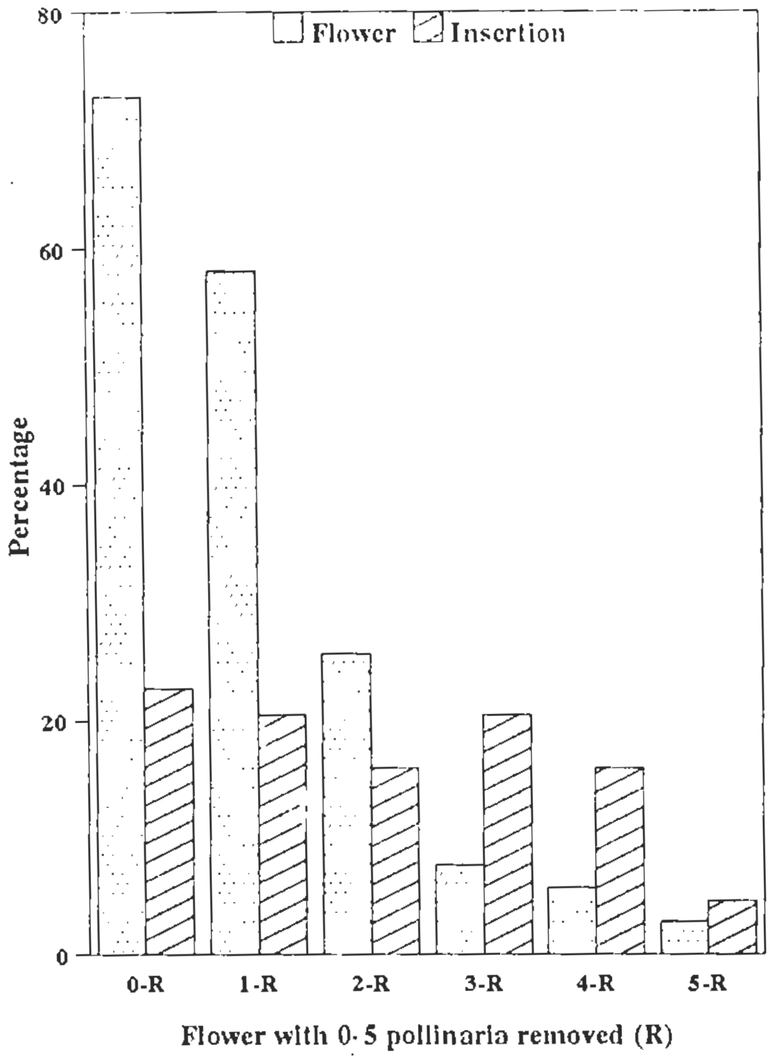


Fig. 40. Pergularia daemia: Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

6. ***Pergularia tomentosa*** **L.:**

Removal and insertion rate for *P. tomentosa* were studied from KHZ population only. In 67.39% flowers 35.65% pollinaria with an average of 1.78 per flower were removed able 5). In 27% flowers (of the total flowers) one, in 20% flowers two, in 24% flowers three, in 19% flowers four and in 11% flowers all five pollinaria were removed (Fig. 41). While 11.85% pollinia were inserted in 39.13% flowers with an average of 0.59 per flower (Table 6). About 51% of the inserted pollinia were in flowers with two - three and 30% with four - five pollinaria removal (Fih. 41).

Number of insertions in the stigmatic chambers with and without residual pollinaria were insignificant (Table 7). Multiple insertions were 28.44% of the total insertions, of which 87% double, 9.68% triple and 3.22% were with five insertions (Table 8).

Tribe Ceropegieae

7. ***Ceropegia bulbosa*** **Roxb.:**

From two populations of *C. bulbosa* Roxb. rate of pollinaria removal and pollinia insertion were studied (Table 5, 6). In KUC population 65.71% pollinaria in 57.14% flowers were removed with an average of 3.3 per flower while 13.21% pollinia in 42.86% flowers were inserted with an average of 0.66 per flower. In SUP population, 34.09% pollinaria in 75% flowers were removed with an average of 1.7 per flower, while 2.27% pollinia in 9.09% flowers were inserted with an average of 0.11 per flower (Table 5, 6).

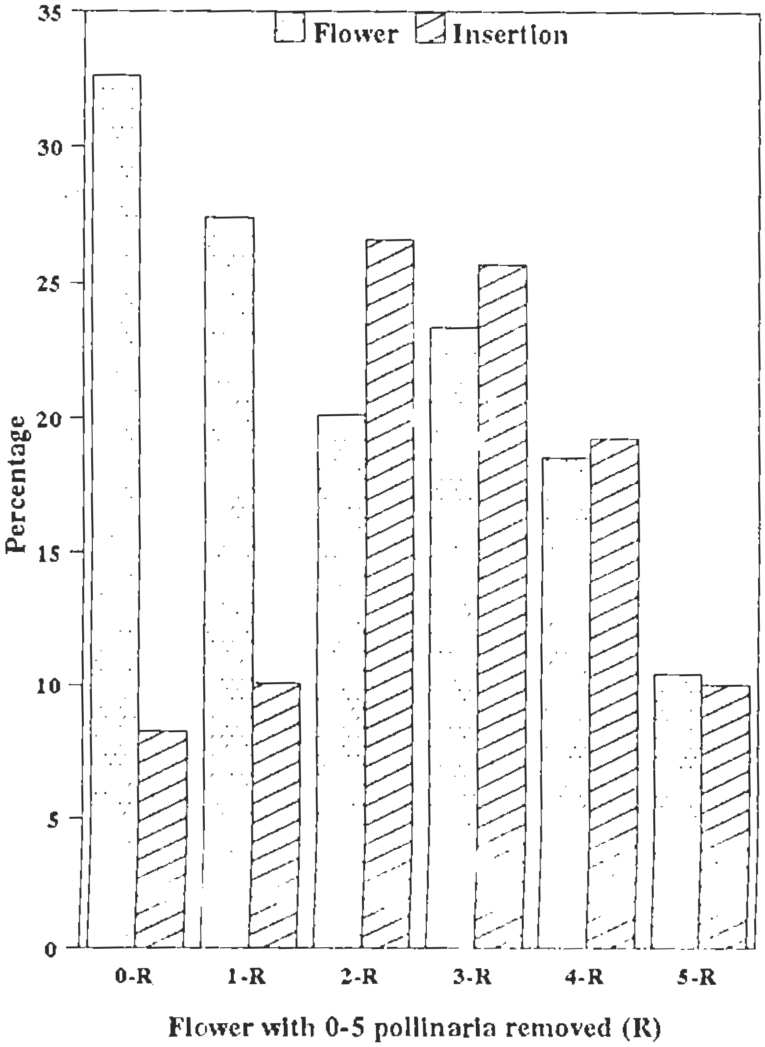


Fig. 41. *Pergularia tomentosa*: Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

Altogether in both the populations, 51.8% pollinaria in 65% flowers were removed with an average of 3.0 per flower. In 33.86% flowers (of total flowers with removal) five and in 21.54% flowers two pollinaria were removed (Fig. 42). Whereas 8.4% pollinia in 28% flowers were inserted with an average of 0.42 per flower. Slightly more than half (53.84%) of inserted pollinia were in flowers with four - five pollinaria removed (Fig. 42).

It is the only case where number of insertions significantly exceeds (73.81%, $x^2 = 5.06$, $P<0.05$, Table 7) in stigmatic chambers with residual pollinaria (removed) absent than present. Multiple insertions were 33.33% of the total insertions and all of them were double (Table 8).

8. ***Leptadenia pyrotechnica*** **(Forssk.) Dcne.:**

Four populations of *Leptadenia pyrotechnica* (Forssk.) Dcne. were studied for pollinia insertion and pollinaria removal rates (Table 5, 6). In DAR population, 42.2% pollinaria in 76% flowers were removed with an average of 2 12 per flower, while 5% pollinia in 22% flowers were inserted with an average of 0.25 per flower. In GHA population, 57.29% pollinaria in 92.01% flowers were removed with an average of 3.0 per flower, while 2.15% pollinia in 8.68% flowers were inserted with an average of 0.11 per flower. In KUC population, 75.45% pollinaria in 30.17% flowers were removed with an average of 1.51 per flower, while 3.21% pollinia in 12.55% flowers were inserted with an average of 0.16 per flower. In MAN population, 44.8% pollinaria in 80% flowers were removed with an average of 2.24 per flower, while 5.6% pollinia in 23% flowers were inserted with an average of 0.28 per

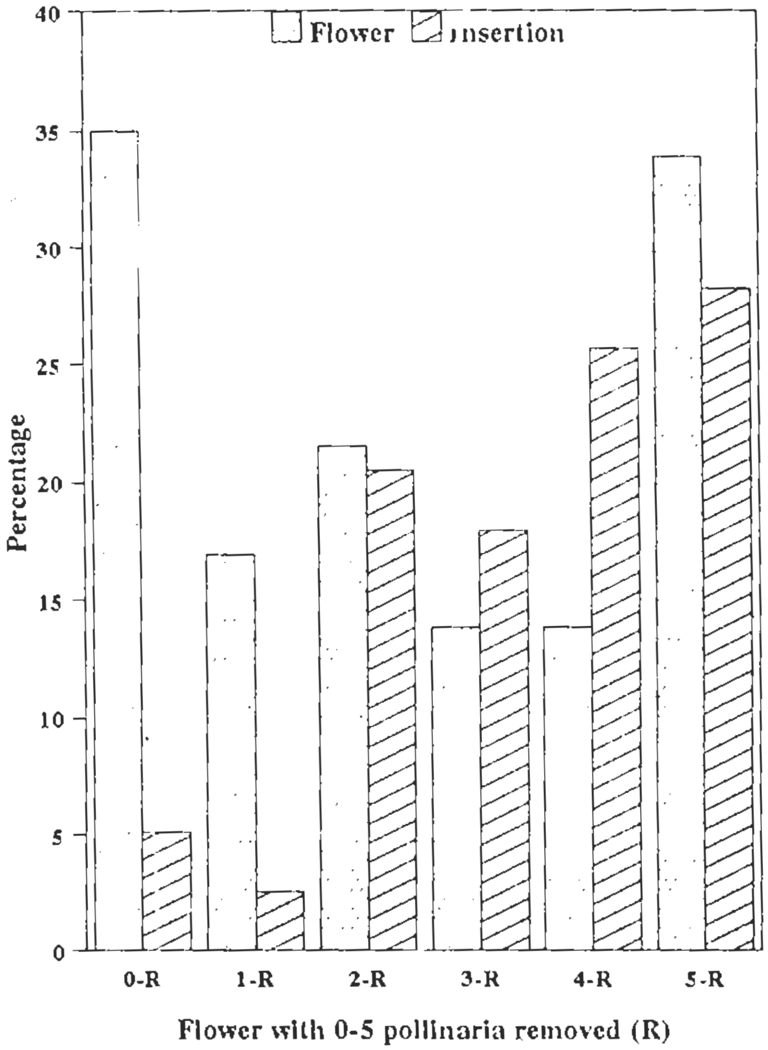


Fig. 42. Ceropegia bulbosa: Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

flower (Table 5, 6).

Overall in all four populations, 44.91% pollinaria in 82.86% flowers were removed with an average of 2.25 per flower. In nearly half of the flowers(49.49% of total flowers with removal) one - two pollinaria were removed (Fig. 43). Whereas 3.37% pollinia in 13.76% flowers were inserted with an average of 0.17 per flower. About 46.6% of the inserted pollinia were in flowers with one - two pollinaria removal (Fig. 43). The probability of removal 6 - 13 times than that of an insertion.

Significantly higher number of insertions (72.5%, x^2 = 14.27, P<0.001, Table 7) were in stigmatic chambers with residual pollinaria present than (removed) absent. Multiple insertions were 16.75 of the total insertions, of which 90% were double and 10% triple (Table 8).

DISUSSION

A considerable variation has been observed among populations of the studied taxa in the level of pollinator activity and pollination success. The highest level of pollinator activity and pollination success has been detected in DAR population of *Calotropis procera*, in KUC population of *Pergularia daemia,Oxystelma esculentum* and *Ceropegia bulbosa* as compare to other populations. Pollinator activity was high in KUC population while pollination success is nearly equal in both the studied populations of *Pentatropis nivalis*. Similarly for *Leptadenia pyrotechnica* pollinator activity was highest in GHA population and pollination success in MAN population. Such wast differences have also been reported

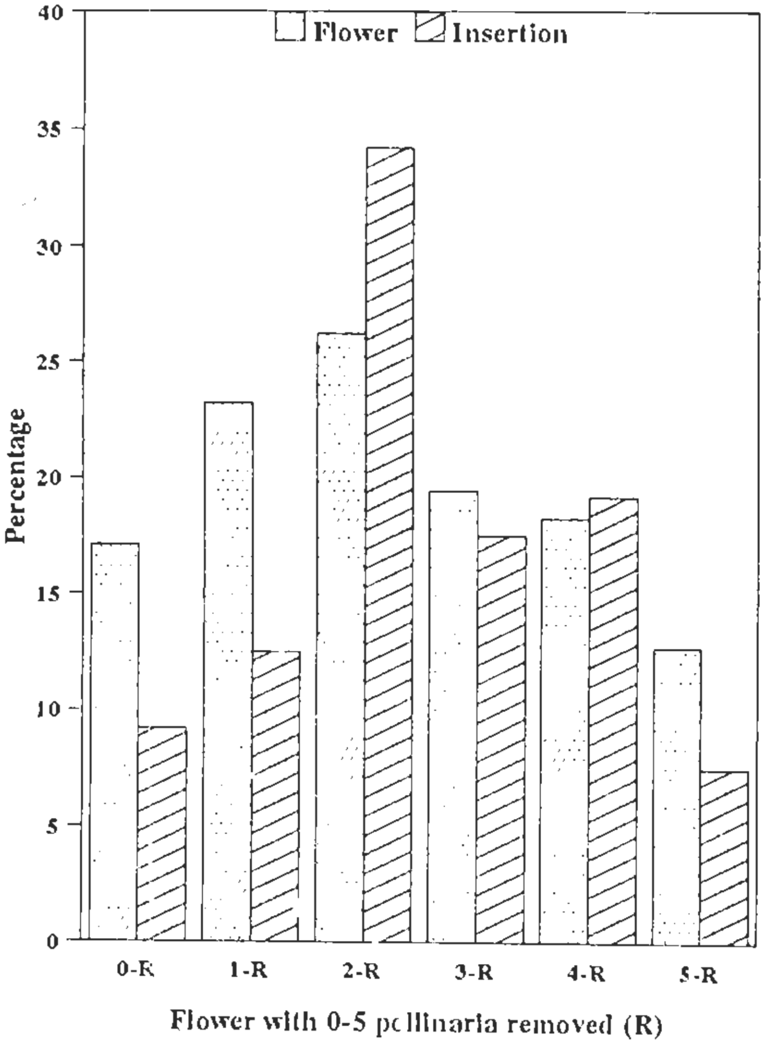


Fig. 43. Leptadenia pyrotechnica: Flower (%) with 0-5 pollinaria removed and pollinia insertion in relation to pollinaria removal.

among populations of *Asclepias* species by Willson and Rathke (1974), Wyatt (1976 & 1978), Lynch (1977), Wyatt and Shannon (1986) and for *Sarcostemma* species by Kunze and Liede (1991) and Liede and Whitehead (1991). According to Wyatt (1976), differences among different populations in pollinator activity and pollination success may be due to the availability of the pollinators, colony size, nearest colony and nearest neighbour distances, inflorescence size, form and due to some other parameters.

Present study also indicates that different taxa exhibit different rates of pollinaria removal and pollinia insertion. Whereas on the tribal basis these differences were insignificant (Table 9). Two groups could be recognized of the studied taxa on the basis of attachment of pollinaria on insects (pollinators) body. The first group comprised of *C.procera*, *P.daemia*, *P.tomentosa*, *P.nivalis* and *O.esculentum* where pollinaria were attached on legs of insects, whereas second group comprised of *C.bulbosa*, *G.varians*, and *L.pyrotechnica* where pollinaria were attached on mouth parts of insects. First group has significantly low pollinaria removal rate than second group while rate of pollinia insertion was non-significant in both groups (Table 10).

This factor (i.e. attachment of pollinaria on different body parts of insects) may even be responsible for interspecific differences in removal and insertion rates. For instance, Kunze & Liede (1991) reported high removal and insertion rates for *Sarcostemma clausum* (Jacq.) Schultes and *S.pannosum* Decne. (in both species pollinaria attached on mouth parts of insects) from Mexico, whereas low rates of pollinaria removal and pollinia

Table 9. Student's t - test between Tribe Asclepiadeae (1) and Tribe Ceropegieae (2)

Parameters	Tribe	Mean	No.of samples	t - value	Probability
% of flowers with removed pollinaria	1	47.73 ± 24.36	6	1.41	ns
	2	73.93 ± 12.65	2		
% of flowers with inserted pollinaria	1	18.19 ± 17.13	6	0.20	ns
	2	20.88 ± 10.07	2		
% of pollinaria removed	1	24.17 ± 20.26	6	1.59	ns
	2	48.35 ± 4.87	2		
% of pollinia inserted	1	4.94 ± 4.54	6	0.26	ns
	2	5.88 ± 3.56	2		
Pollinaria removed per flower	1	1.19 ± 1.01	6	1.61	ns
	2	2.42 ± 0.24	2		
Pollinia inserted per flower	1	0.27 ± 0.25	6	0.126	ns
	2	0.29 ± 0.18	2		

± = Standard deviation
ns = non - significant at 5% level

Table 10. Pollinaria removal and pollinia insertion rate between Group 1 (pollinaria attached on legs of insects) and Group 2 (pollinaria attached on mouth parts of insects).

Taxa	% of flowers with		% of total		Per flower	
	pollinaria removed	pollinia inserted	pollinaria removed	Pollinia inserted	Pollinaria removed	pollinia inserted
Group 1						
Calotropis procera ssp hamiltonii	21.62	9.46	8.86	2.86	0.44	0.14
Oxystelma esculentum	44.60	30.93	18.82	8.63	0.89	0.57
Pentatropis nivalis	40.48	6.75	12.61	1.79	0.63	0.10
Pergularia daemia	27.20	9.07	9.15	2.22	0.45	0.11
Pergularia tomentosa	67.39	39.13	35.65	11.85	1.78	0.59
Mean	40.26 ± 17.63	19.06 ± 17.85	17.01 ± 10.79	5.47 ± 4.75	0.84 ± 0.54	0.30 ± 0.26
Group 2						
Ceropegia bulbosa	65.0	28.0	51.80	8.4	2.59	0.42
Glossonema varians	85.63	5.5	60.48	1.65	3.02	0.08
Leptadenia pyrotechnica	82.86	13.76	44.91	3.37	2.25	0.17
Mean	77.83 ± 11.19	15.75 ± 11.38	52.40 ± 7.80	4.47 ± 3.51	2.62 ± 0.38	0.22 ± 0.18
t - value	3.27	0.43	4.92	0.35	4.96	0.49
Probability	0.05	ns	0.01	ns	0.01	ns

± = Standard deviation
ns = non - significant

insertion were reported in *S.viminale* (L.) R. Br. (pollinaria attached on legs of insects) from S.Africa by Liede & Whitehead (1991). One may argued as Liede & Whitehead (1991) did that as both studies were carried out in different regions so low removal and insertion rates from S.Africa might be due to low abundance of anthophil insects in arid areas. However, this may not be the case in the present study as all the taxa were studied from the same region.

The taxa with pollinaria attached on legs of pollinators tend to have more effective pollinator service i.e. low removals and high insertions of the removed pollinaria as compared to taxa with pollinaria attached on mouth parts of pollinators. Wyatt (1976) also pointed out that in *Asclepias tuberosa* L. attachment of pollinaria on mouth parts of pollinators may lead to more wastage of pollinia as compare to pollinaria attached on their legs. Thus, the floral structure in response to the attachment of pollinaria on different parts of insects body and the difference and specificity of pollinators seems to be the palusible cause of difference in pollinaria removal and pollinia insertion rates among the two groups.

Significant correlation has occurred between number of pollinaria removed and number of pollinia inserted at all the three levels (i) per flower (r = 0.591, P<0.01. Fig. 44), (ii) % of pollinaria/pollinia (r = 0.[illegible] P<0.01, Fig 45) and (iii) % of flower (r = 0.575, P<0.01, Fig. 46). Thus present study supports Wyatt (1976 & 1978), Lynch (1977) and Beare & Perkins (1982) that insertion rates are the positive function of removal rates.

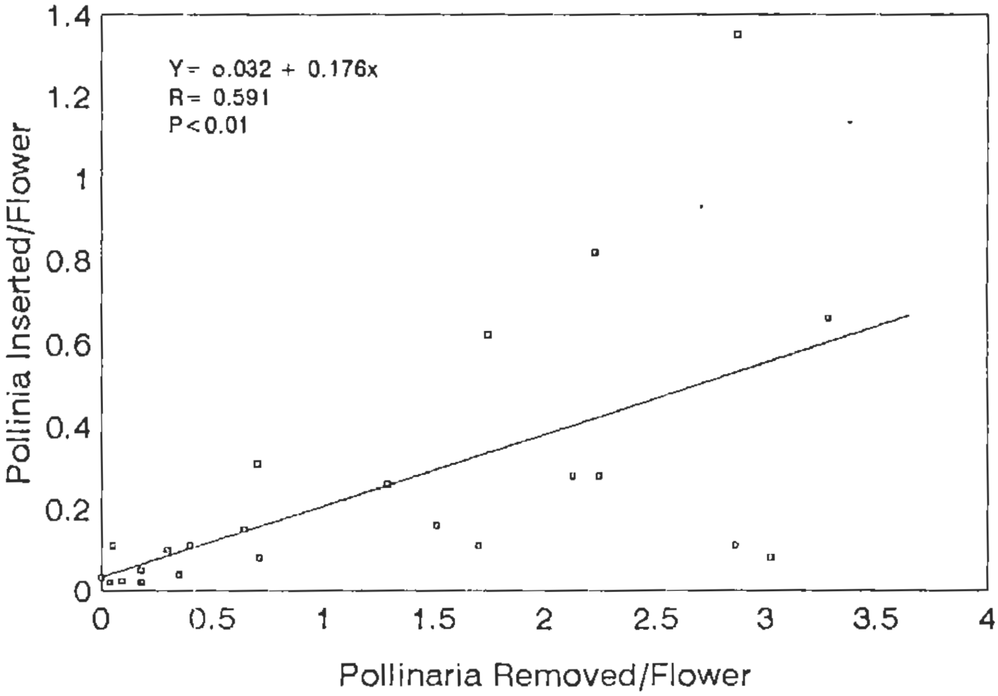


Fig. 44. Relationship between pollinia inserted per flower and pollinaria removed per flower. Points represents 21 populations belonging to 8 taxa.

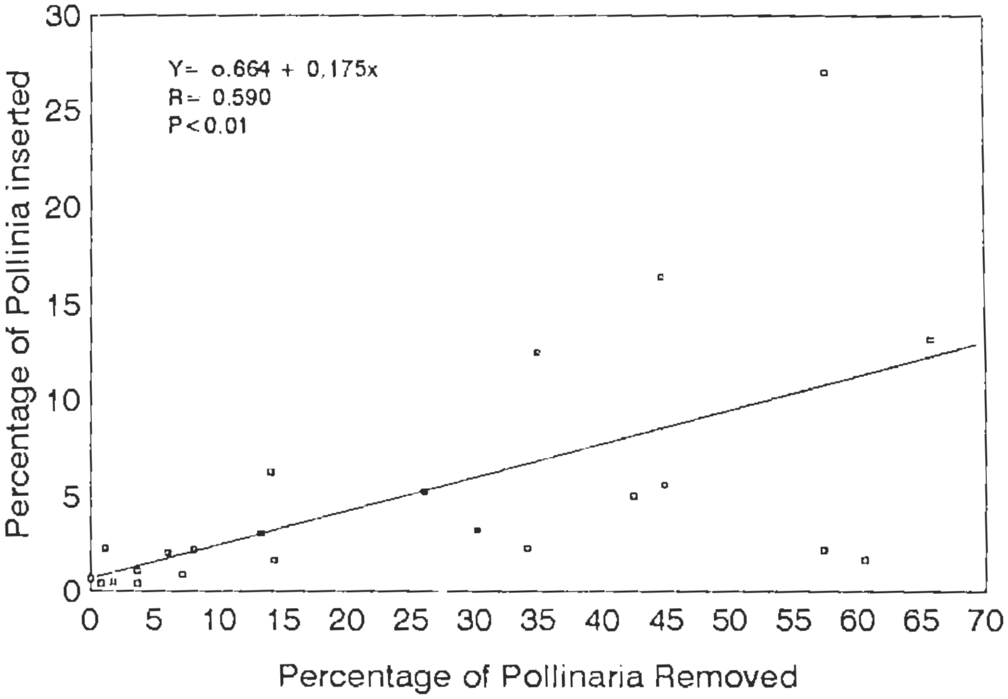


Fig. 45. Relationship between percentage of pollinia insertion an percentage of pollinaria removal. Points represent 21 populations of 8 taxa.

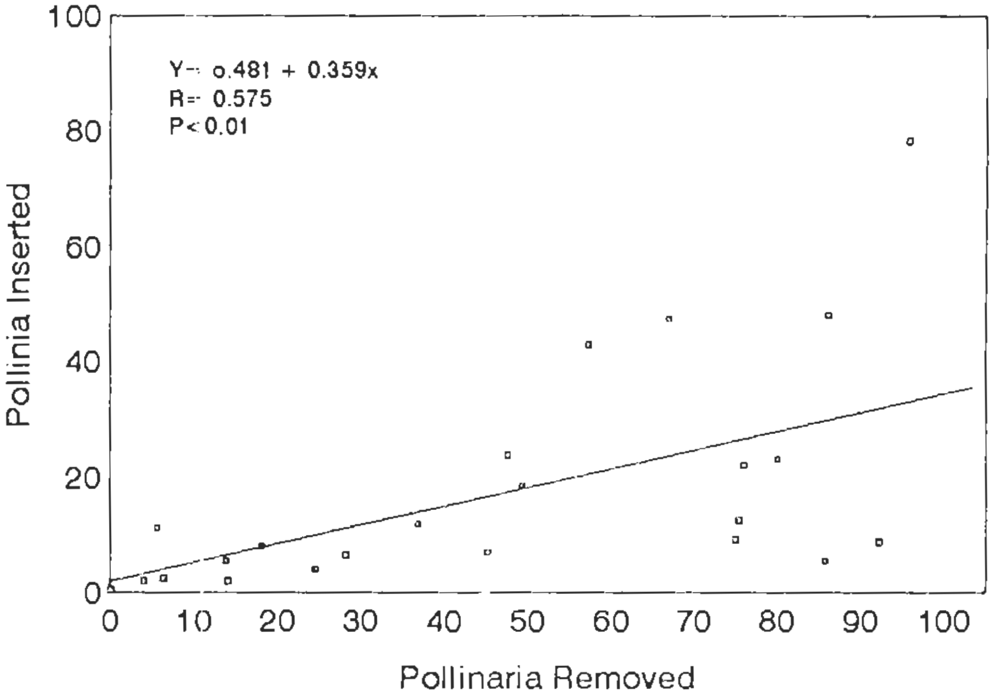


Fig. 46. Relationship between percentage of flowers with pollinia inserted and pollinaria removed. Points represent 21 populations belonging to 8 taxa.

Wyatt (1976) proposed a hypothesis that successful pollinia insertions are primarily into stigmatic chambers with residual pollinaria still present (intact). His hypothesis was supported by number of workers viz. Lynch (1977), Wyatt (1978), Beare and Perkins (1982), Ali and Ali (1989) and Liede and Whitehead (1991). In the present study, out of 8 in 7 taxa significantly (x^2 = 31.99 , P<0.001, Table 7) more insertions were into stigmatic chambers with residual pollinaria still present. Thus our data also supports Wyatt's (1976) hypothesis. In his opinion, this process may effect some degree of temporal separation of the male and female function of the flower. As the probability of insertion is greater for flowers with residual pollinaria still present, outcrossing is encouraged since insertions are likely to be from flowers from which pollinaria have been removed to other flowers with residual pollinaria present.

The only exception is *Ceropegis bulbosa*, where insertions of pollinia significantly exceeds (x^2 = 5.06, P<0.05, Table 7) in stigmatic chambers with residual pollinaria (removed) absent. This can be explained in view of Wyatt's (1976) suggestion that pollinarium removal may occur after a successful insertion. Thus as in *C.bulbosa* pollinators prisoned in flower for 15-20 hours, so there were more chances of pollinaria removals after successful insertions.

Multiple insertions were rare and exhibited only 23.14% of the total insertions. Our findings also coincide with most of the earlier observations viz. 10% in *A.solonana* by Lynch (1977), and 21% in *S.viminale* by Liede & Whitehead (1991), except *Cynanchum* *canescens* where 93% multiple insertions has been reported by Chaturvedi (1987). Double insertion (i.e. two separate pollinia inserted in two stigmatic chamber out of five of a flower) seems to be the dominant i.e. 89% of all the multiple insertions. Liede & whitehead (1991) also reported double insertion (61%) as the major multiple insertion in *S.viminale* while according to Chaturvedi (1987) triple insertion (44%) was highest in *C.canescens*.

About 9.3% triple and 0.85% each 4 & 5 insertions have also been observed. However, Wyatt and Shannon (1986) and Liede and Whitehead (1991) have not reported 4-5 insertions while Chaturvedi (1987) found them in 11% and 6% flowers, respectively.

Chaturvedi (1987 in *C.canescens* also reported more than one pollinium insertion in single stigmatic chamber in 6% flowers. In the present study only in two flowers of *O.esculentum* 3-4 pollinia were inserted in single stigmatic chamber. In *P.nivalis* a unique situation exists, a part from single pollinium insertion, sometimes (65% of total insertions) entire pollinarium (i.e. two pollinia with arms and gland) was inserted in single stigmatic chamber.

The probability of removal was more than 9 times (pooled value of all taxa) that of an insertion. Thus high number of removals and relatively low number of insertions seems to be the characteristic feature of the Asclepiads as nearly all the studies on the members of this family reported similar results. Wyatt (1976) suggested that it might be due to that certain floral visitors functioned primarily as non-inserters. While according to Beare and Perkins (1982) due to such a narrow mechanical margin of error, insertions are simply rare events compared to pollinaria removals or perhaps large number of removals are necessary in order to increase the probability of successful insertions.

CHAPTER 5

POLLINARIUM REMOVAL AND POLLINIUM INSERTION MECHANISM

INTRODUCTION

It was generally believed that no specific mechanism is employed by Asclepiads for pollinaria removal and pollinia insertion. The insect merely places its legs in anthEr slit and by a chance process the pollinium is left there and no spcecial pollinia insertion mechanism operates other than the compatible dimensions of pollinia and stigmatic chambers.

Wyatt (1976) was the first to explain the mechanism of pollinia insertion. Acccording to him, the corpusculum of an intact pollinarium play an important role in pollinium insertion. The groove of an intact pollinarium located at the apex of the anther slit (stigmatic chamber) act as the point of resistance for the translator arm of the inserting pollinium and this result in the breakage of translator arm at knee bend leaving the pollinium inserted in the stigmatic chamber. Wyatt's (1976) hypothesis was tested and verified by himself (Wyatt, 1978) and others (Bookman, 1981; Beare and Perkins, 1982 etc.).

Kunze (1991) pointed out the adaptation of several floral structures in relation to pollination. According to him pollinaria removal and pollinia insertion by the insects (pollinators) is aided by various structural devices such as hair, corona elements, and position of nectary all guiding the proboscis (or leg) towards the entrance of the anther slit. Even anther slit often displays structural differentiation in adaptation to pollinium insertion and guiding of the insect's probocis or leg.

MATERIALS AND METHODS

Insect behaviour in relation to pollinaria removal and pollinia insertion of *Calotropis procera* (Ait.) Ait. f. ssp. hamiltonii (Wight) Ali, *Glossonema varians* (Stocks) Hook. f., *Oxystelma esculentum* (L.f.) R.Br., *Pentatropis nivalis* (Gmel.) Field and Wood, *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L., *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forsk.) Dcne. was studied as described in chapter 3.

Pollinated flowers of the above mentioned species were selected from the flowers used for the study of pollinator activity (see Chapter 4). Floral samples were examined in Scanning Electron Microscope as well as in Light Microscope.

OBSERVATIONS AND RESULTS

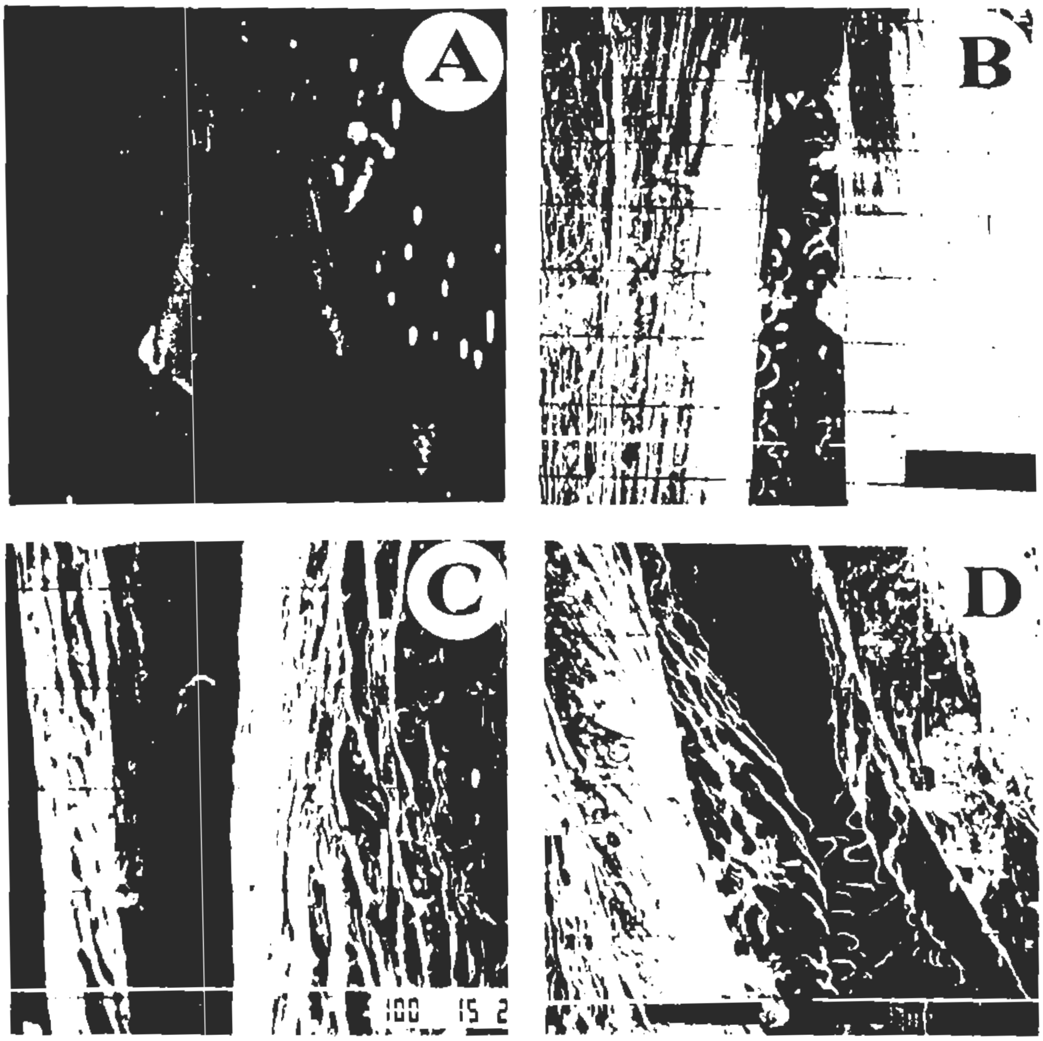
Tribe Asclepiadeae

1. *Calotropis procera* (Ait.) Ait. f. ssp. hamiltonii (Wight) Ali

Pollinarium Removal Mechanism:

In *C.procera* (Ait.) Ait. f. ssp. hamiltonii (Wight) Ali the nectar is of concealed type and as already mentioned presented in

Fig. 47. Calotropis procera ssp. hamiltonii: A, Open stigmatic chamber with intact corpusculum and an inserted pollinium, note the position of the broken translator arm (arrow) in nature it fit in the groove of corpusculum, here the original position is altered; B, Anther wings with striae and nectaries (glands) within the stigmatic chamber. **Glossonema varians:** C, Anther wings with striae; D, nectaries (glands) with in the stigmatic chamber.
Scale bar: A = 10 µm; B - D = 100µm.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the staminal segments. The insect (Pollinator) land on the stigma head and insert its proboscis in the opening at the apex of coronal segment to suck the nectar. During nectar sucking, the insect press the coronal segment and the fleshy pads in order to get more nectar, it also rotate on the stigma head to exploit all the coronal segments of a flower. The pressing and repressing of the pads and rotation on the stigma head involves the movement of legs over stigmatic chambers. In these processess tarsal and tibial segments of the insect's leg frequently come in contact with the corpuscula. Thus a corpusculum gets attached to a leg hair and alongwith the leg the corpusculum with the attached pollinarium is pulled upward freeing the pollinia from their anther sacs.

Pollinium Insertion Mechanism:

The stigmatic chamber is beak like and tightly closed by thick and hard anther wings, so that the opening of the stigmatic chamber must be the first step in the process of pollinium insertion. Observations revealed that during the pressing of the pads by the insect, the portion above the pads and below the anther wings of the stigmatic chamber (arrow in Fig. 4C) is also pressed, as a result anther wings get apart (are separated) and stigmatic chamber open (Fig. 47A). The previously removed pollinarium become positioned on the hair of insects's legs through the curvature of the translator arm so that the exolateral surface of the pollinium with the germination locus extends away from the insect's leg. The striae running diagonally along the edges of anther slit are observed (Fig. 47B), that act as guiding structure to keep the clenched insect part i.e. hair of tibiae or tarsi inside the stigmatic chamber. The pollinium (not the translator arm) enters into the stigmatic chamber and the translotor arm breaks at the bend below the corpusculum as the insect lifts its leg, leaving the pollinium within the stigmatic chamber.

Two factors seems to be responsible for the breakage of the translator arm at the Knee bend.

(i) Presence of an intact corpusculum:

When the translator arm of an inserting polliniumm comes in contact with the intact corpusculum, the groove of the intact corpusculum acts as a point of resistance (Fig.47A) and the break at the knee bend occurs, as the insect pull its leg (Wyatt, 1976; Beare and Perkins, 1982).

(ii) Closing of the stigmatic chamber:

In the absence of intact pollinarium, movement of the anther wings may also provide succcessful pollinium insertion. Thus, as an insect stops pressing the pads, anther wings come back to their original position and the stigmatic chamber is closed, grasping the inserted pollinium. When insect pull its leg, the break occur at the knee bend.

(iii) Presence of glandular papillae:

The inner surface of the anther wings are provided with glandular papillae (Fig. 47B). These papillae apart from secretion of nectar may also grasp or hold the inserting pollinium as the stigmatic chamber closes, and results in the breakage of translator arm.

2. *Glossonema varians* (Stocks) Hook. f.:

Pollinarium Removal Mechanism:

In *G.varians* (Stocks) Hook. f. the nectar is of semi-open type i.e. nectar is secreted with in the stigmatic chamber in hermaphrodite flowers and between anthers below corpusculum (where stigmatic chamber is present in hermaphrodite flower) in male flowers. Nectar is then accumulates at the base of the staminal column in a ring or annulus formed by the inner corona segments through a wide gap or opening of anther slit at the base of stigmatic chamber. The annulus is surrounded by the outer coronal segments, leaving only a small gap between them in front of each stigmatic chamber for the insects to reach the nectar.

Insect (pollinator) usually land on the stigma head and insert its proboscis into the gap between the corona segments. Its front and sometimes middle legs press the corona segments to widen the gap and reach the base of the annulus. During this process, the proboscis (lower or vertical side) come in contact with the corpusculum which get attached to the proboscis. After nectar sucking, insect withdraw its proboscis from the coronal gap and thus alongwith proboscis the corpusculum with the attached pollinarium is pulled upward and so removed from the flower. Sometimes, insect (particularly, *Scolia quadripustulata*, land on the petals instead of stigma head and insert its proboscis into the gap while widening the gap with the help of front legs. this time, the corpusculum get attached to the dorsal side of proboscis instead of ventral side.

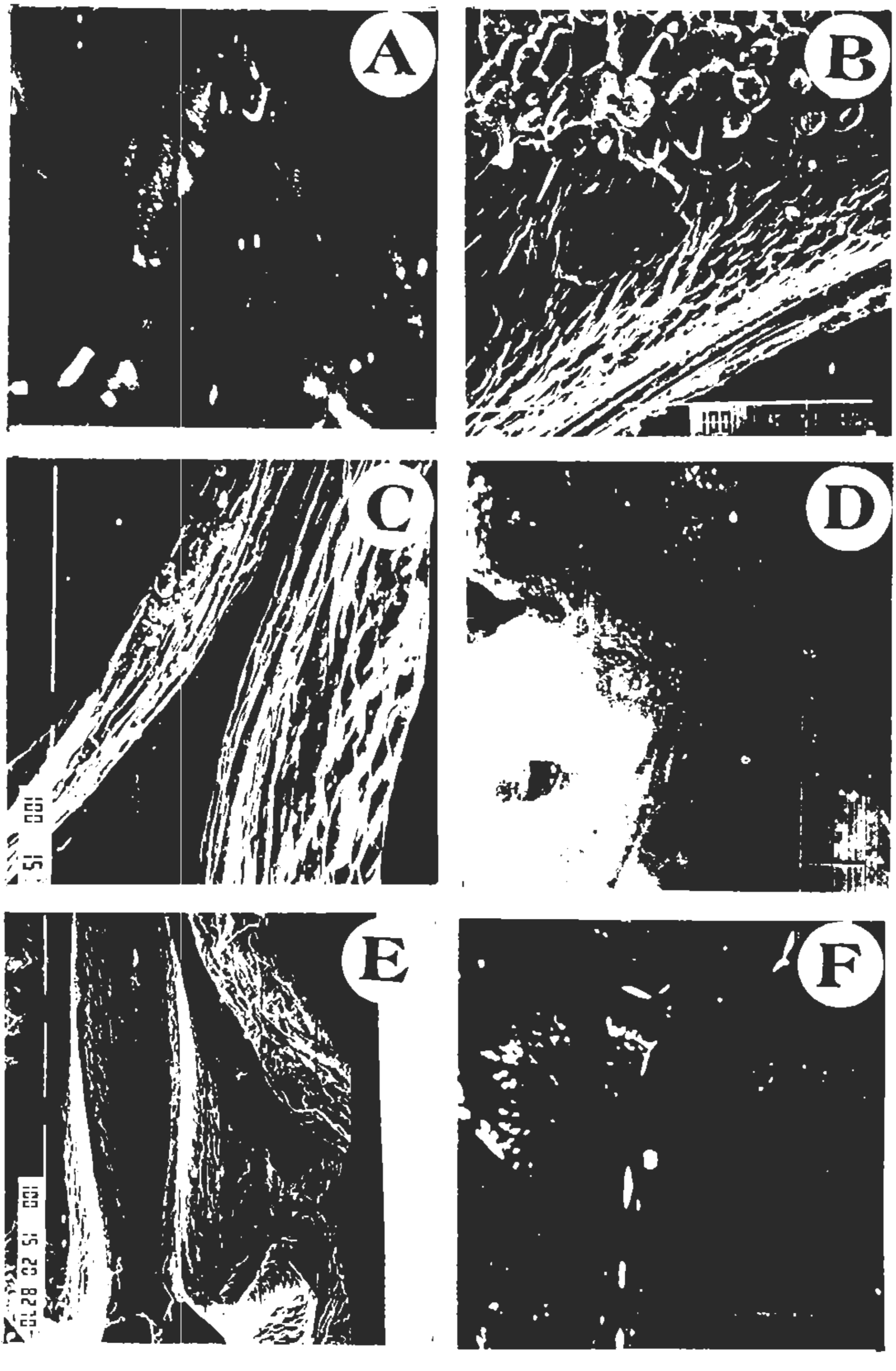
Pollinium Insertion Mechanism:-

The attached pollinarium on insect's proboscis, positioned itself through the curvature of translator arm in such a way that the exolateral surface of the pollinium with germination locus extends away from the insect's proboscis. During nectar sucking, the pollinium of a attached pollinarium on insect's proboscis may get inserted into the basal opening of anther slit. As insect pull its proboscis upward in order to leave the flower or coronal gap, the pollinium alongwith the proboscis also pushed upward into the stigmatic chamber guided by the striae present on the edges of anther wings (Fig. 47C) and thus a break occur at Knee bend of translator arm and pollinium is successfuly inserted into the upper portion of stigmatic chamber. The groove of corpusculum of an intact polinarium at the apex of the stigmatic chamber may act as the point of resistance as well as the narrowing of the stigmatic chambers and glandular papillae (Fig. 47D) may be responsible for the breakage of the translator arm.

The long translator armed pollinaria of male flower seems to have advantage over short armed pollinaria of hermaphrodite flower as long armed pollinaria attached to insect's proboscis positioned farthest away from proboscis due to curvature of arms than the short armed pollinaria and thus have greater chances of being inserted first into the stigmatic chambers. Thus lenght of the translator arm is an adaptation to promote insertion of male flower's pollinia as compare to hermaphrodite ones.

Whole of the pollinium is inserted in the stigmatic chamber and not a single case of more than one pollinium insertion in a

Fig. 48. Oxystelma esculentum: A, Stigmatic chamber with inserted pollinium, note the position of pollinium quite below and not the intact corpusculum; B, Anther wing with striae and inner portion of stigmatic chamber with glandular nectaries. **Pentatropis nivalis:** C, Anther wings with striae. **Pergularia daemia:** D, Stigmatic chamber with inserted pollinium, broken translator arm of pollinarium fitted in the groove of intact corpusculum; E, Inner portion of the stigmatic chamber and striae at anther wings. **Pergularia tomentosa:** F, Stigmatic chamber with inserted pollinium, note the attachment of inserted pollinium to the intact corpusculum. Scale bar: A, D & F = 1000 μm; B, C & E = 100 μm.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stigmatic chamber is observed.

3. *Oxystelma esculentum* (L.f.) R.Br.:

In *O.esculentum* (L.f.) R.Br. the nectar is of more or less concealed type. Nectaries are located within the stigmatic chambers and may be extended below into the furrow of two corona segmetns. The inter coronal furrow form continuous depression along the length of staminal column, narrowing towards apex into stigmatic chamber, which is bounded by anther wings and broadening towards the base, bounded by the margins of coronal segments. Thus a continuous slit alongwith anther slit is formed on the inter coronal furrow. The base of the staminal column is bounded by the outer corona in the form of a ring or annulus. The nectar is stored or presented in the inter coronal furrow which is covered by the anther wings and margins of coronal segments and by annulus at the base, leaving only a long narrow slit for insects to get the nectar.

Pollinarium Removal Mechanism:

The flowers are not in upright position on inflorescence but rather at an angle of 180° to that of stalk. As insect land on the flower, it tilt downward due to insect's weight. So insect first try to hold its grip on the flower by holding the petals with legs. After holding the flower, it move into the flower and try to hold or grasp the coronal segments, with its fore and middle legs and insert the proboscis in the slit below the stigmatic chamber to suck nectar; however , due to smooth, shiny and slipery surface of coronal segments, legs slipped into the slits located on inter coronal furrows. The legs are then held into the anther slits due to narrowing of the furrow, thus insect get hold on the flower. After nectar sucking, when insect pull its legs out of anther slit (stigmatic chamber), the corpusculum at the apex of anther slit attached to the claw, and so along with leg, the pollinarium is removed from the flower.

Pollinium Insertion Mechanism:

No movement of translator arm of the removed pollinarium has been observed. After removal of the pollinarium the two pollinia tilt downward and simply hangdown on their arms from the corpusculum and in this way the exolateral (germination locus) surface extends away from insect's leg.

When an insect, with pollinarium attached to its leg, visit another or same flower, one of the pollinium of the attached pollinarium, get inserted into the stigmatic chamber alongwith the leg. Diagonally running striae are present at the edges of anther wings (Fig. 48B) which directed the insect leg into the anther slit. It has been observed that upward movement of inserted pollinium is stoped below the apex of stigmatic chamber, and seems that inserted pollinium fits at this narrow portion of stigmatic chamber (Fig. 48A). As insect pull its leg, the translator arm break, leave the pollini.. in the stigmatic chamber.

Thus, intact corpusculum do not play any role, rather the narrowness of stigmatic chamber is responsible for the breakage of translator arm of inserted pollinium (Fig. 48A).

Whole pollinium is inserted in the stigmatic chamber and in contrast to above three species, in only 2 flower 2-3 pollinia are found to be inserted in a stigmatic chamber.

4. *Pentatropis nivalis* (Gmel.) Field and Wood

5. *Pergularia daemia* (Forssk.) Chiov.

6. *Pergularia tomentosa* L.

The flower of all these three species are in inverted positon i.e. hanging upside-down on infloresecensce. The mechanism of pollinarium removal and pollinium insertion in relation to flower structure is more or less similar to that of *Oxystelm esculentumm* (L.f.) R.Br. (Fig. 48C-F). However, in *Pentatropis nivalis* (Gmel.) Field and Wood and in both *Pergularia* species two factors may be responsible for the breakage of translator arm of inserting pollinium.

(i) **Presence of intact corpusculum:**

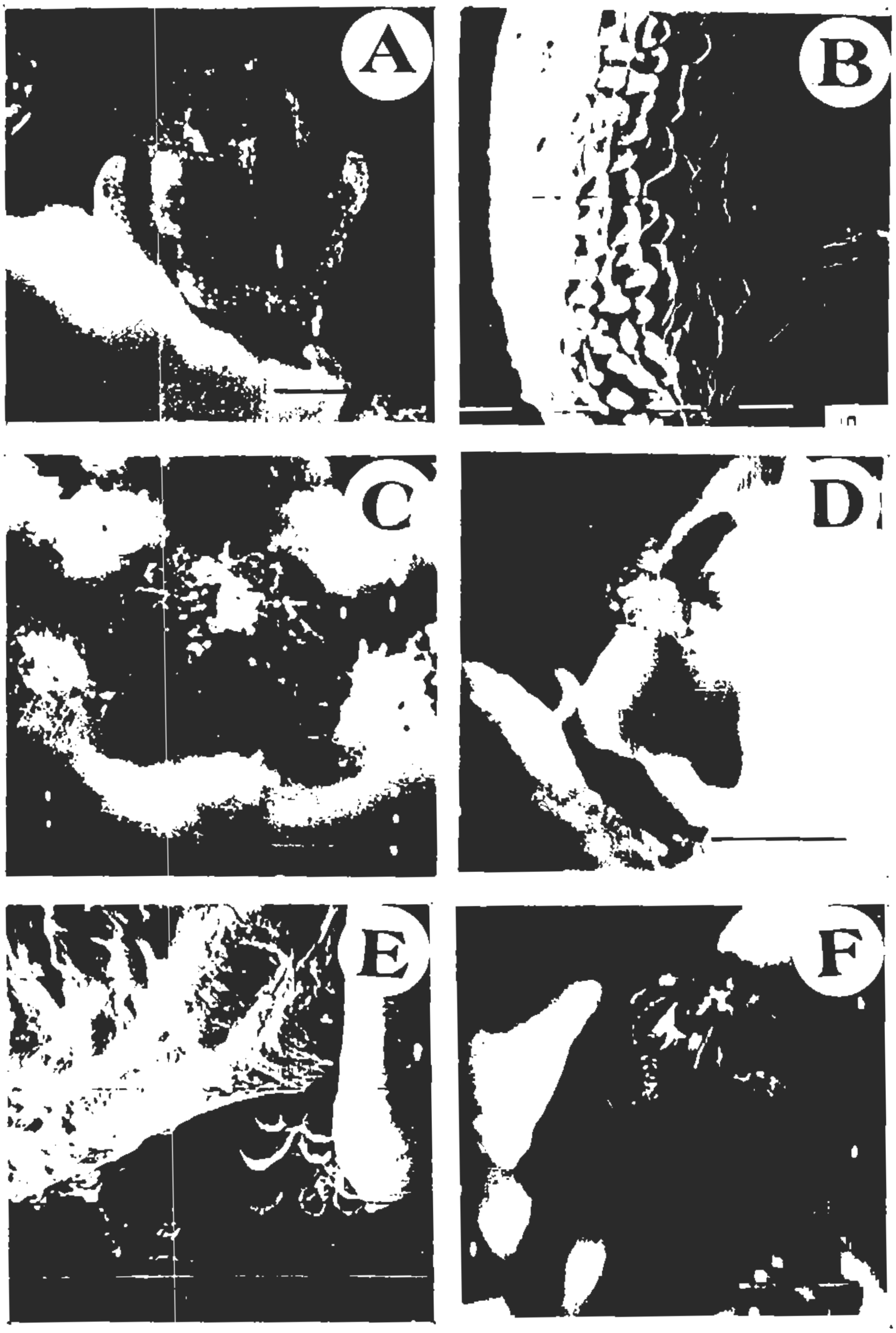
Intact corpusculum work in similar fashion (Fig. 48D, F) as described for *C.procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali.

(ii) **Narrowness of stigmatic chamber:**

Narrowness of the stigmatic chamber toward the apex also resist the inserting pollinium to remain in the stigmatic chamber, similar to *Oxystelma esculentum* (L.f.) R.Br. (Fig.48D, F). However, in *Pentatropis nivalis* (Gmel.) Field and Wood, apart from single pollinium, sometimes whole of the pollinarium (i.e. Corpusculum with translator arms and both pollinia) is also inserted in a stigmatic chamber may be due to the loose attachment of corpusculum on insect leg (claw).

Fig. 49. Ceropegia bulbosa: A, Inserted pollinium; B, Inner portion of stigmatic chamber with glandular nectaries. **Leptadenia pyrotechnica:** C, Inserted pollinium (double). **Caralluma edulis:** D, Inserted pollinium, E, Glandular nectaries at the base of stigmatic chamber. **Caralluma tuberculata:** F, Inserted pollinium (double).

Scale bar: A & F = 1000 μm; C & D = 500 μm; B & E = 10 μm.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Tribe Ceropegieae

7. *Ceropegia bulbosa* Roxb.:

In *C.bulbosa* Roxb. the nectar is of open-type however, flower itself is closed type with a tube and traping mechanism. The nectar is secreted in the stigmatic chamber and accumulates in the cups formed by outer corona just below each stigmatic chamber (Fig. 68A). Like *Glossnema varians* (Stocks) Hook.f., a wide opening is present at the base of stigmatic chamber from which nectar flow towards the coronal cups.

Pollinarium removal mechanism:

The insects are trapped in the flower for 18-24 hours. Insect usually sit on the stigma head and from there try to suck the nectar from the open base of the stigmatic chamber and also from the coronal cups below stigmatic chambers. During this process, the lower portion of the mouth parts come in contact with the corpusculum present at the apex of anther slit (stigmatic chamber). Thus the corpusculum get attached to the mouth parts and as insect lift its head from the stigmatic chamber, pollinarium due to attached corpusculum also removed from the flower.

Pollinium insertion mechanism:

The two pollinia of a pollinarium attached to the insect's mouth part tilt downward and come in hanging position so that the external appendage of pollinium (i.e. germination locus) can catch the anther slit like a hook. When insect with attached pollinarium visit a flower. The above mentioned process is repeated. But now this time, one of the pollinium of the attached pollinarium get caught in the basal open portion of the anther slit (stigmatic chamber) and as insect pull its head, break occur at the translator arm and the pollinium is left in the stigmatic chamber (Fig. 49A). The size of the external pollinial appendage is such that it fit into the basal open portion of anther slit; Further, upward movement of inserting pollinium is stoped due to narrowness of stigmatic chamber, due to contact of broad portion of pollinium with the anther wsings and may be also due to the presence of glandular papillae on the inner surface of anther wings (Fig. 49B). It my also be possilbe that glandular papillae fit in the ridges and furrows of the inserting extra pollinial apendage, thus resist upward movement and result in the breakage of translator arm.

Thus only the external pollinial appendage is inserted in the stigmatic chamber (Fig. 49A).

8. *Leptadenia pyrotechnica* (Forssk.) Dcne.:
9. *Caralluma edulis* (Edgew.) Hook.f.
10. *Caralluma tuberculata* N.E.Br.

In *L.pyrotechnica* (Forssk.) Dcne. the nectar is also of open type and from the stigmatic chamber flow and accumulates in the coronal cups located below each stigmatic chamber. The mechanism of pollinarium removal and pollinium insertion is more or less similar as described for *Ceropegia bulbosa* Roxb. (Fig. 49C).

Although, the behaviour of insects (pollinators) could not be studied in *C.edulis* (Edgew.) Hook.f. and *C.tuberculata* N.E.Br. but on the basis of more or less similar pollinarium morphology, nectar system and gynostegium structure, it can be predicted that *Caralluma* species may also have similar pollinarium removal and pollinium insertion mechanism (Fig. 68C-F) as described for *Ceropegia bulbosa* Roxb. and *L.pyrotechnica* and may be evident from the presence of similar glandular papilae on the inner surface of anther wings of *C.edulis* (Edgew.) Hook.f. (FIG. 49E) as observed in *Ceropegia bulbosa* Roxb. and *L.pyrotechnica* (Forssk.) Dcne.

However, in contrast to *Ceropegia bulbosa* Roxb. the external pollinial appendage of above 3 species enter properly in upper portion of the stigmatic chamber (Fig. 49C, D & F).

DISCUSSION

Present study has revealed a variety of structural adaptations to highly specialized functions pertaining to pollination, such as:

Stigmatic chambers:

Stigmatic chambers show different structural modifications in response to the mode of pollination. For instance, in the taxa like *Calotropis procera* (Ait.) Ait.f. ssp.*hamiltonii* (Wight) Ali, *Oxystelma esculentum* (L.f.) R. Br., *Pentatropis nivalis* (Gmel.) Field and Wood, *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L., where entire pollinium or pollinarium is to be inserted, the stigmatic chambers become quite deep, elongated, gradually nawrrowing towards apex and posses diagonally running striae at the edges of anther wings as an guiding structure to keep the insect leg or proboscis inside the anther slit. Simalarly striae on the anther wing edges are also reported in *Astephanus triflorus* Schltr., *Microloma calycinum* E.Mey. and several species of *Sarcostemma* by Kunze and Liede (1991) and Kunze (1991).

Whereas in the taxa like, *Caralluma edulis* (Edgew.) Hook.f., *Caralluma tuberculata* N.E.Br., *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Dcne., where only the germination locus (Extra pollinial appendage) is to inserted, the stigmatic chamber become shallow, short i.e. matches with the size of germination locus, without striae on edges of anther wings and with glandular papillae on the inner surfaces of anther wings which not only secerete nectar but also hold the germination locus of the inserting pollinium.

Coronal segments:

Coronal segments also show great deal of modifications. Apparently they seem to provide foot hold to insects, however, they actually guide the insect's leg or proboscis towards the entrance of anther slits by their smooth, shiny, slippery surface and ridges or furrows pointing towards anther slits. Coronal segments mainly act as the nectar reserviours or storage and provide access to it. Delphino (1865) has already related the attachment of pollinaria on insect's body parts (i.e. leg or proboscis) with the location and access to nectar. According to him, taxa with nectar stored in coronal segments located at the back of anther have leg-pollination while taxa with location and access to nectar directly below the entrance of anther slit have proboscis-pollination.

Present study also show and support this type of relationship in several taxa but few exceptions are also observed. For instance in *Oxystelma esculentum* (L.f.) R.Br., *Pentatropis nivalis*, (Gmel.) Field and Wood, *Pergularia daemia* (Forss k.) Chiov. and *Pergularia tomentosa* L. although the nectar and its access is located below the anther slit but still they are leg-pollinated. It seems that these taxa have adapted to this way (on leg of insect) of pollinaria removal in several structural aspects. Not only is the entrance to anther slit become farther away but also the access to the nectar has shifted laterally towards the side of the stamens and at the base of the staminal column. The inter-staminal furrow (nectar reserviour) itself is covered by the inflexed rim or margins of coronal segments. Thus the pollinator, clinging with its legs to the gynostegium, is forced into an oblique position towards the anther slit which favours the legs getting caught rather than the proboscis.

The following factors are found to be responsible for the breakage of the translator arm of an inserting pollinium:

i) Presence of an intact corpusculum

ii) Narrowness of the stigmatic chamber towards apex

iii) Closing of the stigmatic chamber

iv) presence of glandular papillae within the stigmatic chamber

v) Anther wings may act as the point of resistance for the broaden portion of inserting pollinium

First factor has been reported by Wyatt (1976, 1978) and Beare and Perkins (1982) in *Asclepias* species, the second by Kunze (1991) in *Sisyranthus* species, however rest of the factors are reported for the first time. These factors operate singly or in combination of two or three, may depend and vary from species to species as described in results.

Thus it could be concluded that Asclepiads possess specialized mechanism of pollinarium removal and pollinium insertion due to a high degree of synorganization of variety of structural elaborations and functional process of pollination.

CHAPTER 6

THE EFFECT OF SUGAR CONCENTRATION ON POLLINIUM GERMINATION

INTRODUCTION

As stated earlier the pollen grains in the members of sub-family Asclepiadoideae (family Asclepiadaceae) are produced in paired packages known as "Pollinia". These pollinia are removed from the flowers by the pollinating insects. On a subsequent visit to another flower, a pollinating insect inserts the pollinium into a stigmatic chamber where it may remain and so effect pollination (Wyatt, 1976; Ali and Ali, 1989 etc.).

The pollinium normally germinate in a solution of nectar, which is produced by the nectaries located in the stigmatic chamber (Corry, 1884; Galil and Zeroni, 1965; Eisikowitch, 1986; Shannon and Wyatt, 1986; Kevan et al., 1989 and Eisikowitch et al., 1990). In Asclepiads, a wide range of floral nectar sugar concentration has been reported by Willson and Bertin (1979), Morse and Fritz (1983), Southwick (1983), Southwick and Southwick (1983), Punchihewa (1984), Eisikowitch (1986), and Wyatt and Shannon (1986).

As it has been known that each taxon required a specific sugar concentration for its pollen germination and in Asclepiads nectar acts as the natural germinating medium so the variation in its sugar concentration may effect the pollinium germination.

Thus in-vitro pollinium germination of the following species viz. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii*, (Wight) Ali, *Caralluma edulis* (Edgew.) Benth., *Caralluma tuberculata* L., *Ceropegia bulbosa* Roxb., *Glossonema varians* (Stocks) Hook. f., *Leptadenia pyrotechnica* (Forssk.) Decne., *Pentatropis nivalis* (Gmel.) Field and Wood, and *Pergularia daemia* (Forssk.) Chiov., has been carried out to study the effect of sugar concentration on pollinium germination.

MATERIAL AND METHODS

Nectar Sugar Concentration:

25 mature floral buds (just prior to opening) were bagged (nylon-nested/mashed) and tagged in early morning (6-7 a.m.) and afternoon (5-6 p.m.) for diurnal and nocturnal species respectively. Bagged were removed at the time of maximum insect activity and nectar was withdrawn with microcapillaries and its sugar concentration immediately assessed with a hand-held refractometer (Bellingham & Stainly, U.K.) from the concentration of equivalent sucrose solution.

Pollinium Germination:

Pollinium germination was carried out under laboratory conditions by the method of Khatoon & Ali (1983), but instead of using agar and glucose, sucrose alone was used. As nectar is the natural germinating medium for pollinia and it is dominated by sucrose (Percival, 1961; Willson and Bertin, 1979; Southwick, 1983; Wyatt and Shannon, 1986c), so liquid medium of the sucrose was used in order to remain close to the natural conditions for germination. A series of sucrose solution ranging from 5-40% w/v, along with 0.003% $Ca(No_3)_2$, 0.002% $MgSo_4$, 0.001% KNo_3 and 0.001% boric acid were prepared.

Twenty pollinia (two / flower) from fresh flowers of each species were transferred to each sucrose solution at room temperature (30-35^{o}c) in a moist chamber for 12 hours. Pollinia of nocturnal species i.e. *Pentatropis nivalis* (Gmel.) Field and Wood and *Pergularia daemia* (Forssk.) Chiov., were inoculated at 8-9 p.m. whereas of diurnal species at 8-9 a.m. Percentage of germinated pollinia was calculated, and from each germinated pollinium the number of germinated pollen grains (assessed by counting the number of pollen tubes grown from the pollinium) and the length of the longest pollen tube was measured microscopically. Changes in the sucrose concentration of the media were assessed by a hand-held refractometer.

OBSERVATIONS AND RESULTS

Nectar Sugar Concentration:

In *Calotropis procera*(Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali floral nectar sugar concentration ranged from 30-52% with an average of 37%. In *Pentatropis nivalis* (Gmel.) Field and Wood and *Pergularia daemia* (Forssk.) Chiov. average nectar sugar concentration was 24% and 17% which ranged from 20-32% and 15.8-18.3% respectively. Sugar concentration in rest of the studied species was not dectected, due to very small amount of nectar production.

Fig. 50.. In - vitro pollinium germination and germination loci. A & B, Calotropis procera ssp. hamiltonii: Exocentrolateral; C, Glossonema varians: Exoproximolateral; D, Oxystelma esculentum: Exosubdistolateral; Pergularia daemia : Exosubdistolateral.

Scale bar: A = 100 μm; B - D = 200 μm.

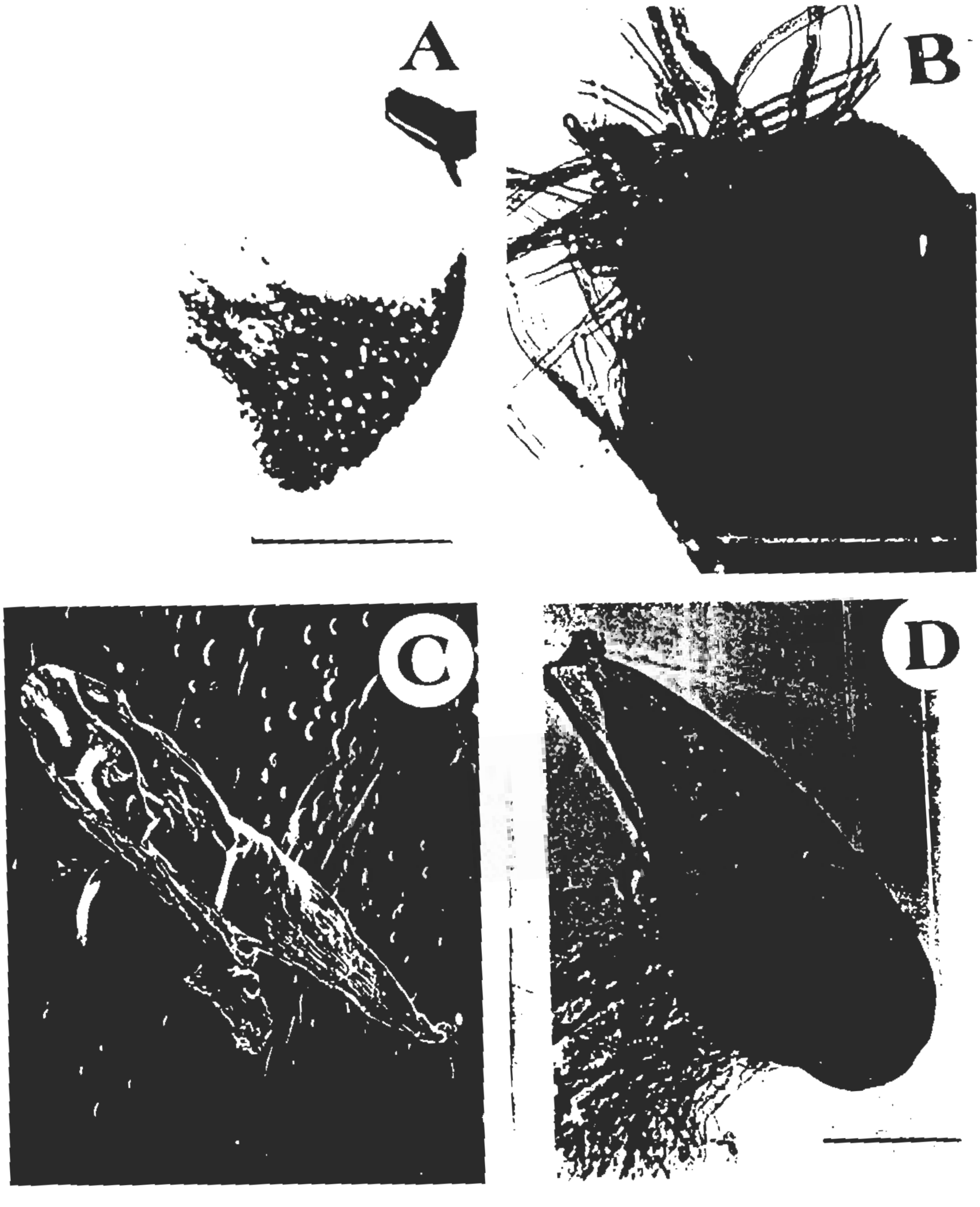

A
B
C
D

Fig. 51.. In - vitro pollinium germination and germination loci. A, Caralluma edulis: Distal; B, Ceropegia bulbosa: Distal; C, Leptadenia pyrotechnica; Distal.
Scale bar: A & C = 200 μm; B = 100 μm.


A
B
C

Germination Locus:

On germination of pollinium, the pollen tubes emerge from a specific region known as "Germination locus". In *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali the germination locus is exocentrolateral, in *Glossonema varians* (Stocks) Hook.f. and *Pentatropis nivalis* (Gmel.) Field and Wood exoproximolateral, in *Pergularia daemia* (Forssk.) Chiov. exodistolateral, in *Caralluma edulis* (Edgew.) Benth., *Caralluma tuberculata* L., *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Decne. distal (Fig. 50, 51).

Pollinium Germination:

Tribe Asclepiadeae

In *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, 30% sugar (Sucrose) concentration was found to be optimal for pollinium germination i.e. pollen germination (maximum number of germinated pollen grains/pollinium) and pollen tube growth (healthy and longest pollen tube, Fig. 52). From 5-20% sugar concentration, pollinium germination started with in 1st. hour of inoculation, whereas at high sugar concentration i.e. 25-40%, late germination was observed. At 5% and 30% sugar concentration, 100% pollinia were germinated. At low sugar concentrations i.e. 5-15%, pollen tubes little bit coiled and become beaded whereas at high concentrations i.e. 20-40%, pollen tubes elongate normally but very best and straight at 25% and 30% concentration.

In *Glossonema varians* (Stocks) Hook. f., in hermaphrodite flowers best pollinium germination in 15% sugar concentration and

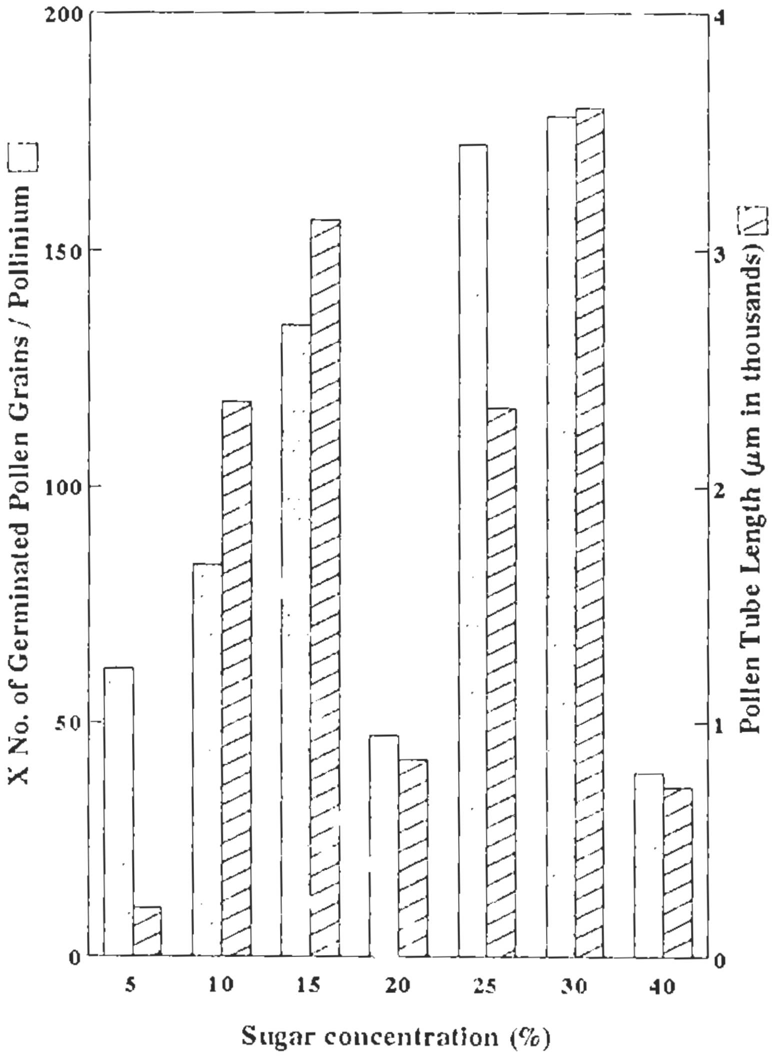


Fig. 52. Calotropis procera ssp. hamiltonii: In - vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

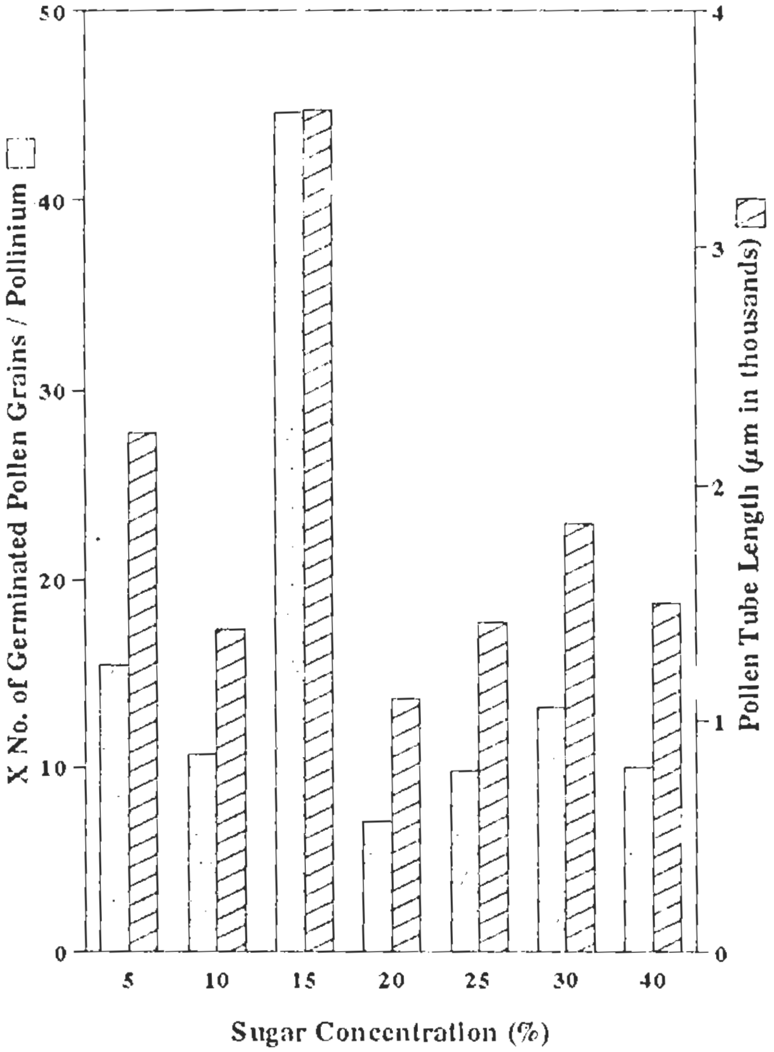


Fig. 53. Glossonema varians (⚥): In - vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

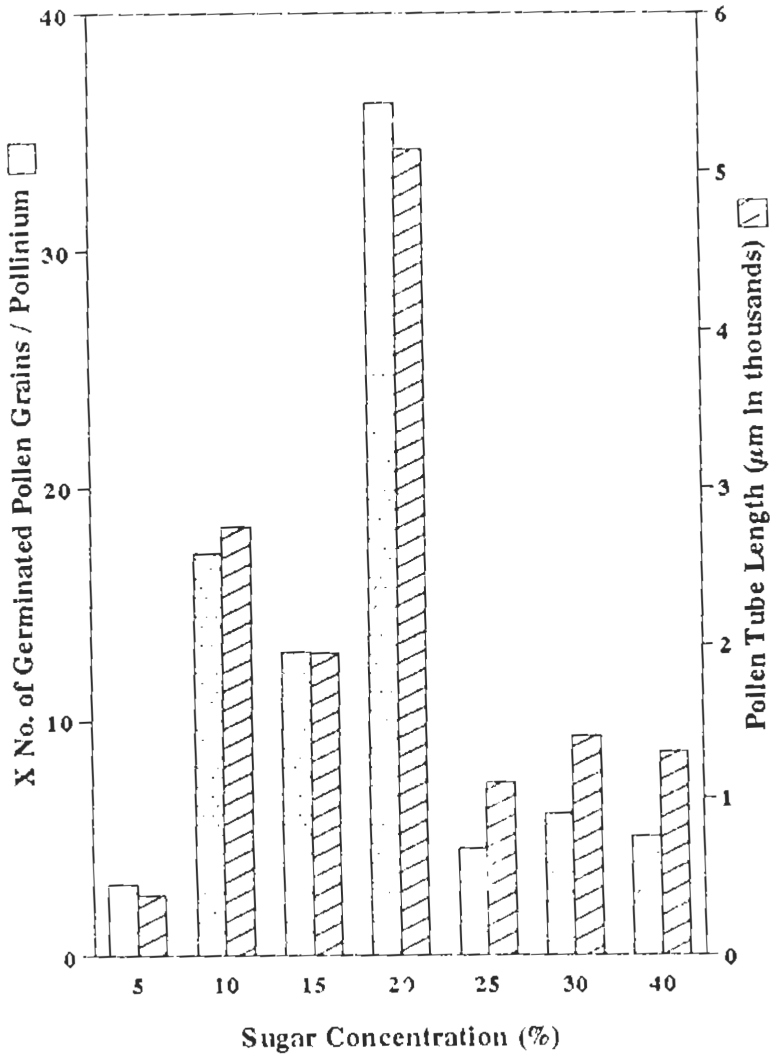


Fig. 54. Glossonema varians (♂): In - vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

in male flowers in 20% concentration was observed (Fig. 53, 54). Similar to the above species, pollinium germination started earlier in lower sugar concentrations with malformation of the pollen tubes.

In *Pentatropis nivalis* (Gmel.) Field and Wood, at 10% sugar concentration best pollen germination and pollen tube growth was observed (Fig. 55). Similar to *C.procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali germination started earlier at lower concentrations as compared to higher ones. At 5%, 10% and 25% sugar concentrations 100%, at 15% and 40% concentration 60% pollinia and at 20% and 30% concentration 80% pollinia were germinated . From 5%-20% concentration normal germination whereas from 25%-40% concentration coiling and beaded formation was observed.

In *Pergularia daemia* (Forssk.) Chiov., best germination and pollen tube growth was observed at 15% sugar concentration (Fig. 56). At all the sugar concentrations 100% pollinia were germinated. Pollen tubes elongates normally from 5-15% sugar concentrations while from 20-40% concentrations either the pollen tubes failed to emerged from the pollinium or if emerged than did not elongate much, coiled and ultimately bursted.

Tribe Ceropegieae:

In *Caralluma edulis* (Edgew.) Benth. *Caralluma tuberculata* L. and *Ceropegia bulbosa* Roxb. best pollen germination was observed at 20 and 25% sugar concentration, respectively (Fig. 57, 58 & 59). In all the three species 100% pollinia were germinated in all the sugar concentrations with slight malformation of pollen

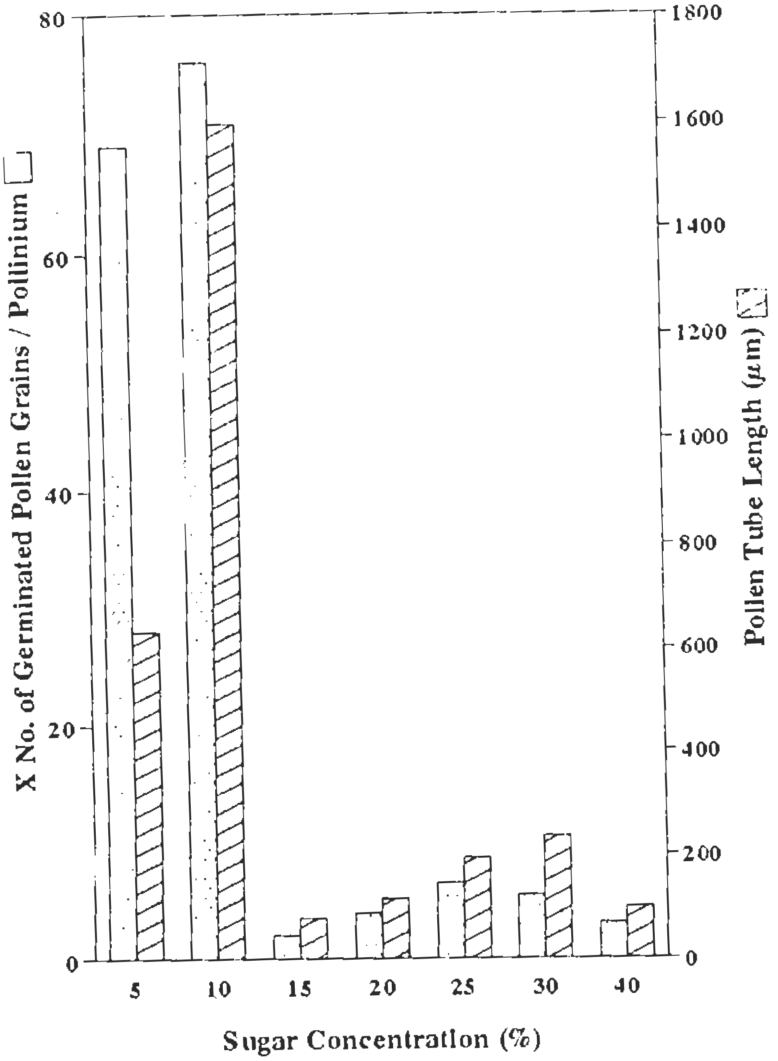


Fig. 55. Pentatropis nivalis: In-vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

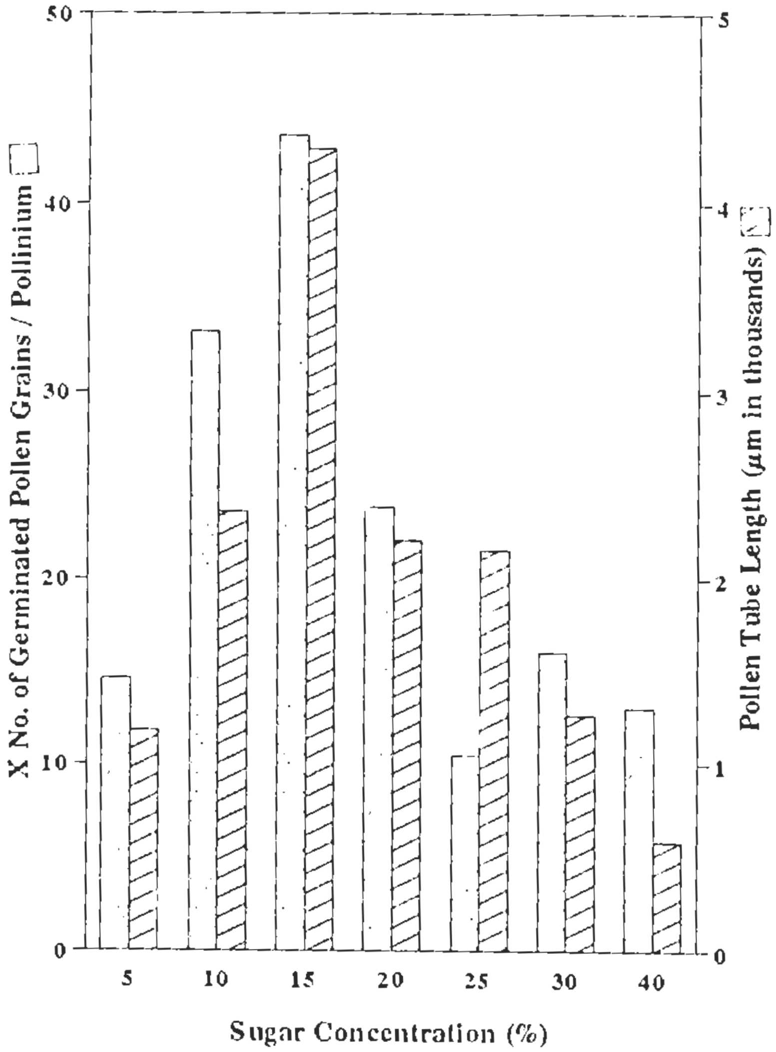


Fig. 56. Pergularia daemia: In - vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

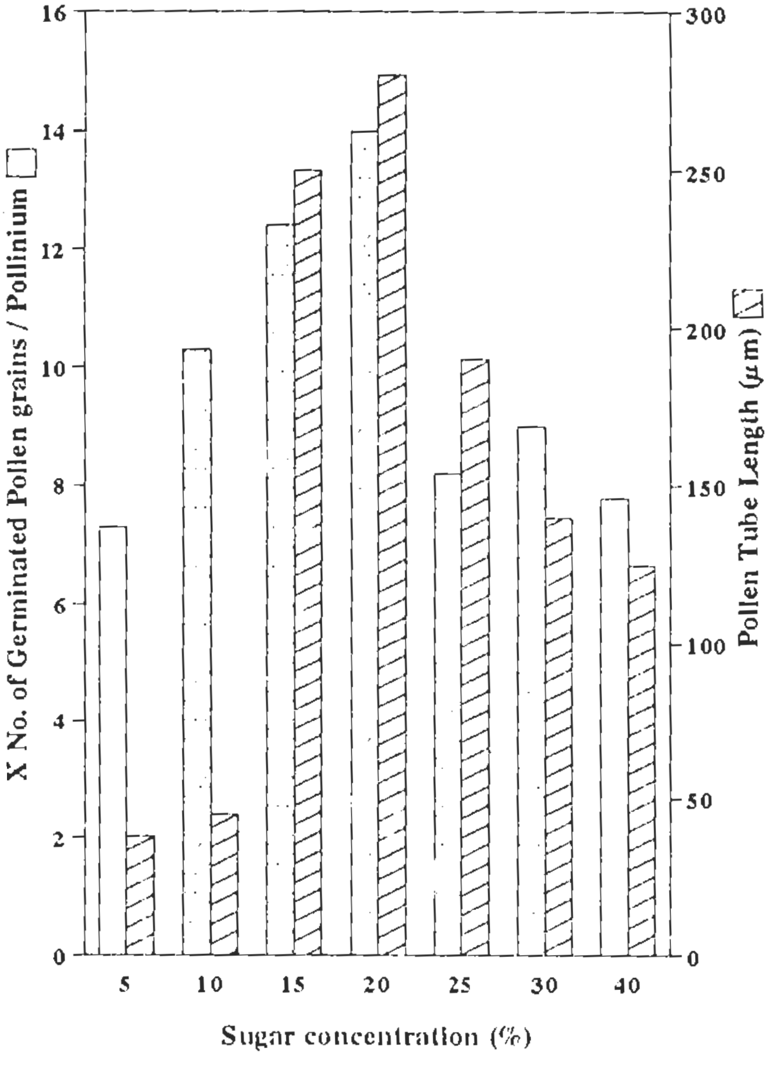


Fig. 57. Caralluma edulis: In - vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

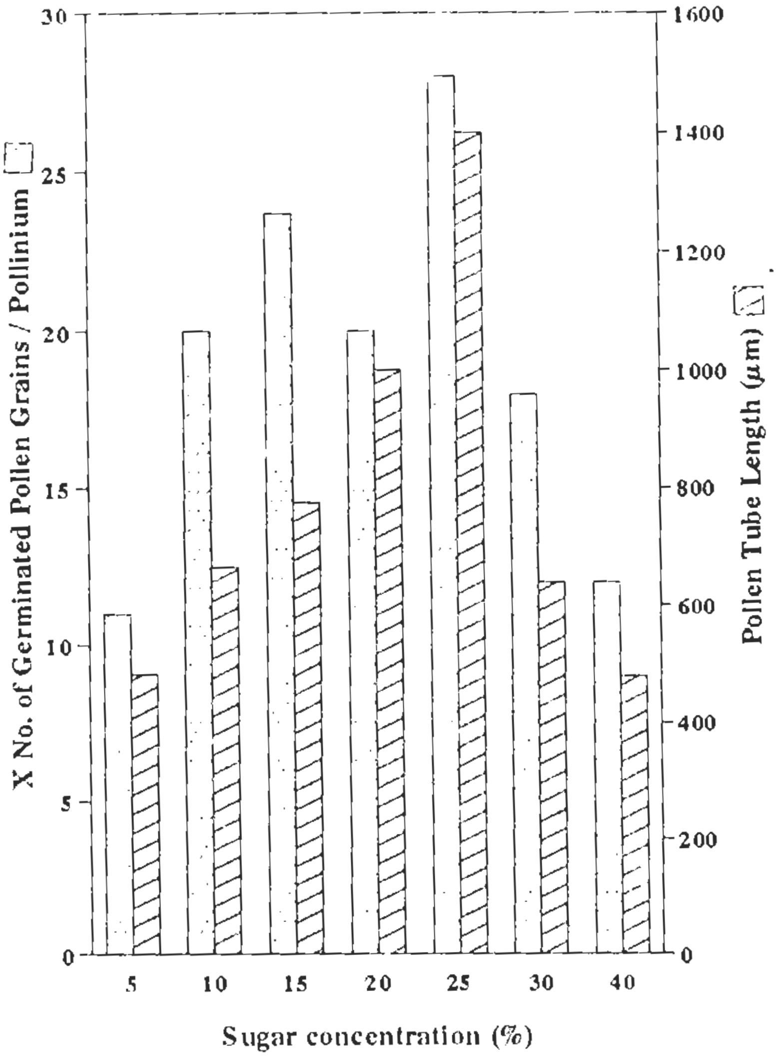


Fig. 58. Caralluma tuberculata: In-vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

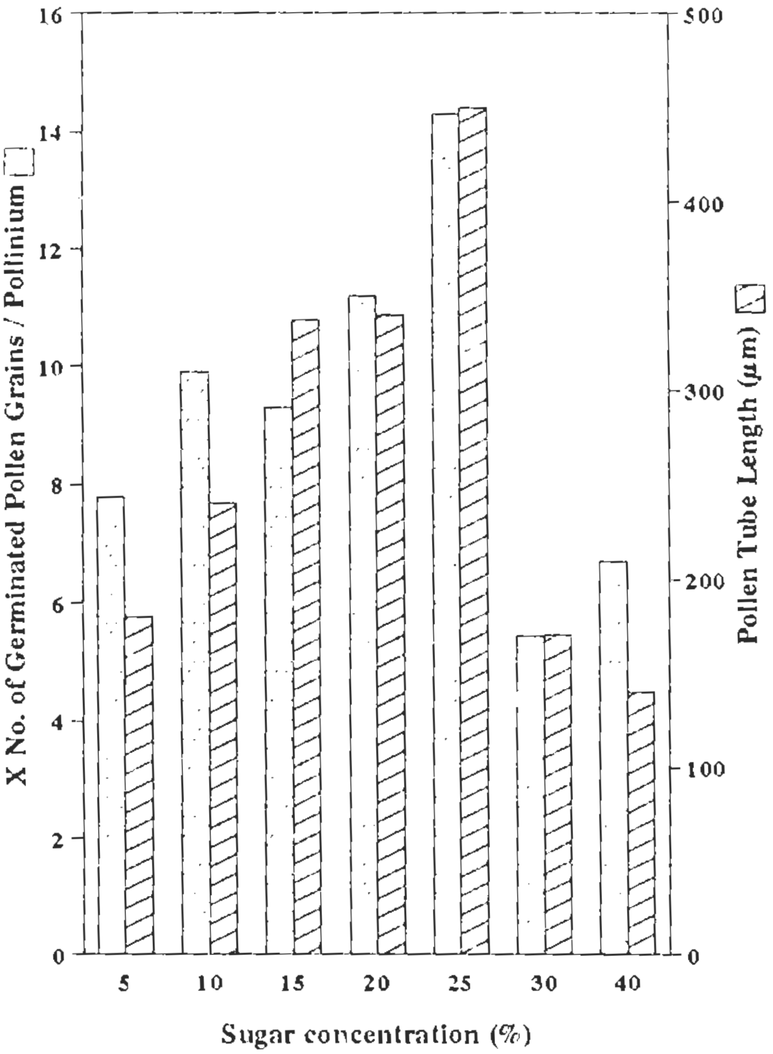


Fig. 59. Ceropegia bulbosa: In - vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

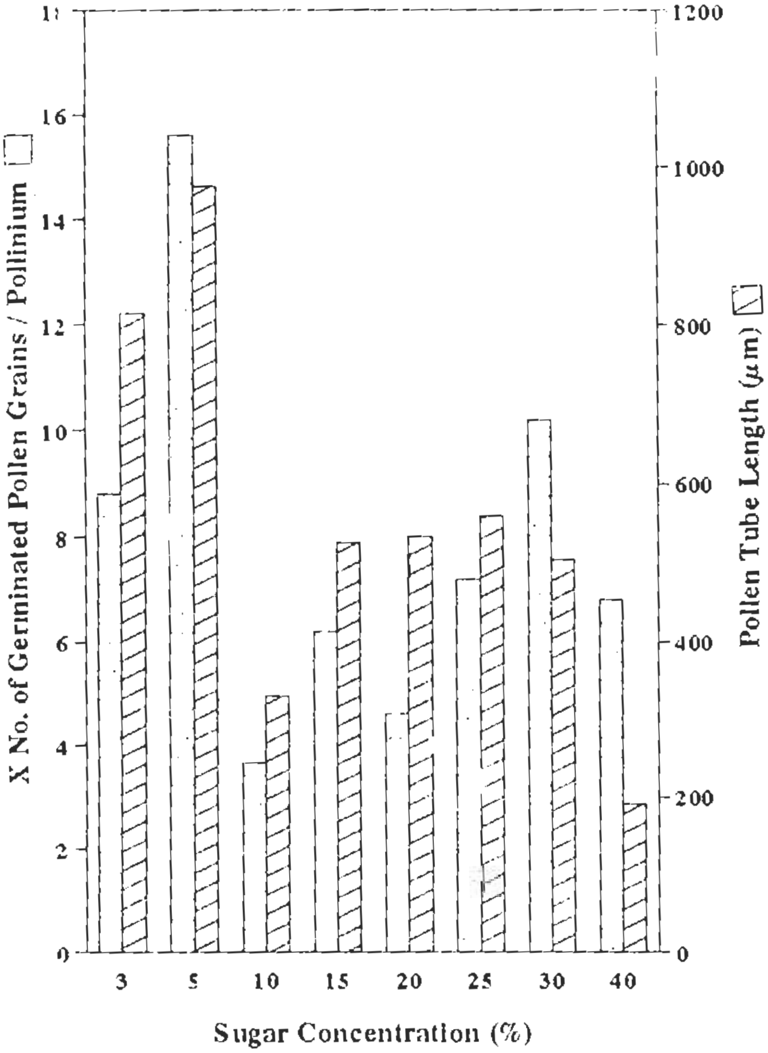


Fig. 60. Leptadenia pyrotechnica: In - vitro pollinium germination (average number of germinated pollen grains / pollinium and average pollen tube length) at different sugar (sucrose) concentration.

tubes in lower concentrations.

In *Leptadenia pyrotechnica* (Forsk.) Decne. at 25% sugar concentration, best pollen germination and pollen tube growth was observed (Fig. 60). At all the sugar concentrations, 100% pollinia were germinated. Pollinium germination in 5-15% concentration started within 1st. hour of inoculation but almost all of the pollen tubes coiled and bursted while in 20-40% concentrations late germination with normal pollen tube growth was observed.

DISUSSION

The position of germination loci on the pollinia of the studied taxa are similar to our findings assessed by acetolysis method (Chapter 2).

The effect of sugar (sucrose) concentrations on pollinium germination was marked. Although, pollinia of all the eight species studied can germinate in a wide range of sugar concentration but each species required a specific sugar concentration for optimal pollinia germination. Thus, maximum pollinium germination (highest % of germinated pollinia), pollen grain germination (maximum number of germinated pollen grains/pollinium) and pollen tube growth (longest pollen tube) in *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali was at 30%, in *Glossonema varians* (Stocks) Hook. f. at 15% (in hermaphrodite flowers) and at 20% (in male flowers), in *Pentatropis nivalis* (Gmel.) Field Wood at 10% and in *Pergularia daemia* (Forssk.) Chiov. at 15% sugar concentration, in *Caralluma edulis* (Edgew.) Benth. at 20%, in *Caralluma tuberculata* L., *Ceropegia bulbosa* Roxb. and *Leptadenia* *pyrotechnica* (Forssk.) Decne. at 25%.

Our results coincides with Kevan et al., (1989) and Eisikowitch et al., (1990) who reported that germination of pollinium was started earlier at lower sugar concentrations as compared to the higher ones. Like *Asclepias syriaca* L. (Eisikowitch et al., 1990) in *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, *Caralluma edulis* (Edgew.) Benth., *Caralluma tuberculata* L., *Ceropegia bulbosa* Roxb., *Glossonema varians* (Stocks) Hook. f. and *Leptadenia pyrotechnica* (Forssk.) Decne. pollen tubes bursts at 5% sugar concentration while in *Pentatropis nivalis* (Gmel.) Field and Wood and *Pergularia daemia* (Forssk.) Chiov. abnormality and bursting of pollen tubes was observed above 20% sugar concentration. In *Asclepias syriaca* L. inhibition of pollinium germination was reported above 30% sugar concentration by Eisikowitch et al., (1987, 1990), however in the present study similar to Eisikowitch et al., (1986) pollinium germinated even at 40% sugar concentration in all the studied species, although percentage of pollinium germination was reduced and in *Pentatropis nivalis* (Gmel.) Field and Wood and *Pergularia daemia* (Forssk.) Chiov. even pollen tubes bursted at this concentration.

The optimal sugar concentration required for in-vitro pollinium germination was more or less similar to the sugar concentration observed in the floral nectar. Thus in the floral nectar of *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali average sugar concentration was 37%, whereas in-vitro its pollinia germinated best in 30% sugar concentration. Similarly, in *Pergularia daemia* (Forssk.) Chiov. floral nectar sugar concentration was 17% and its pollinia best germinated at 15% sugar concentration. This similarity in sugar concentration of artificial media (that gave best germination) and floral nectar have also been reported by Shannon and Wyatt (1986) for *Asclepias exaltata* L. and Eisikowitch et al., (1990) for *Asclepias syriaca* L. However, the case of *Pentatropis nivalis* (Gmel.) Field and Wood is exceptional, in which its pollinia best germinated at 10% sugar concentration while on average 24% sugar concentration was found in floral nectar.

CHAPTER 7

EFFECT OF FLOWER AGE ON POLLINIUM VIGOR AND PISTIL RECEPTIVITY

INTRODUCTION

In many plant species including Asclepiads, a large number of ovaries and initiated fruits fail to form fruit as a result of several ecological and proximate factors (Kephart and Heiser, 1980; Willson and Price, 1980; Stephenson, 1981; Wyatt, 1982). Among many, pistil receptivity and pollen vigor also seems to the important factors at the time of pollination and as obvious these two factors may likely be changed with the age of the flower (Morse, 1987)

Although, the effect of flower age on pollen viability and pistil receptivity have been studied on a number of plant species (Karaai, 1962; Linskens,1969; Frankel and Galun, 1977; Stephenson and Bertin, 1983; Primack, 1985) but unfortunately, very few reports are available on Asclepiads e.g. on *Asclepias speciosa* Torr. (Bookman, 1983), on *Asclepias exaltata* L. (Shannon and Wyatt, 1986b), on *Asclepias syriaca* L. (Eisikowitch et al., 1987; Morse, 1987).

However, the unique morphology of Asclepiads flowers particularly the transfer of pollen between plants in the form of pollinium, makes it possible to quantify the aspects of pollination that are difficult to study in other plants without pollinium. For instance, pollination rates can be scored easily in the laboratory and field by examining the flowers and recording the number of pollinaria removed and pollinia inserted. Thus, functional sexuality of the plants can also be determined as removal rates give some indication of relative male success and insertion rates of relative female success (Wyatt and Shannon, 1986c).

Bookman (1983) and Morse (1987) used rate of pollinaria removal and pollinia insertion not only for sex determination of flower but also for the study of changes in pistil receptivity and pollinium vigor with respect to the flower age.

In the present study, an attempt has been made to determine the pollinium vigor with respect to flower age by in vitro pollinium germination and both pollinium vigor and pistil receptivity by calculating the frequency of pollinaria removed and pollinia inserted in different aged flowers of *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) Hook. f., *Leptadenia pyrotechnica* (Forssk.) Decne. and *Pergularia daemia* (Forssk.) Chiov.

MATERIAL AND METHODS

Flower age:

Described in detail in Chapter 2.

Pollinium germination:

In-vitro pollinium germination was carried out in sucrose solution of particular concentration, that give best results for each species (as observed in chapter 5). Germination of pollinium (N=20) from each 1-3 days aged flowers of each studied species

Table 11. One - way ANOVA of mean number of germinated pollen grains/pollinium of 1 - 3 days old flowers

Taxa	Days	One - way ANOVA					
		S.Var	SS	df	MS	F	Probability
Calotropis procera ssp. *hamiltonii*	All 3- days	Days	1075.7	2	537.85	0.0097	ns
		Error	664508	12	55375.7		
	D_1 vs D_2	Days	672.2	1	672.2	0.355	ns
		Error	481685.2	8	60210.16		
	D_1 vs D_3	Days	19.6	1	19.6	0.005	ns
		Error	338187	8	42273.4		
	D_2 vs D_3	Days					
		Error					
Glossonema varians (?)	All 3- days	Days	807.6	2	403.8	6.11	P<0.025
		Error	792.8	12	66.067		
	D_1 vs D_2	Days	756.9	1	756.9	9.26	P<0.025
		Error	653.6	8	81.7		
	D_1 vs D_3	Days	396.9	1	396.9	4.84	ns
		Error	656	8	81.7		
	D_2 vs D_3	Days	57.6	1	57.6	1.67	ns
		Error	276	8	34.5		
Glossonema varians (?)	All 3- days	Days	7840	2	3920	83.40	P<0.001
		Error	564	12	47		
	D_1 vs D_2	Days	6760	1	6760	118.59	P<0.001
		Error	456	8	57		
	D_1 vs D_3	Days	4840	1	4840	254.74	P<0.001
		Error	152	8	19		
	D_2 vs D_3	Days	160	1	160	2.46	ns.
		Error	520	8	65		

Table 11. contd....

Taxa	Days	One - way ANOVA					
		S.Var	SS	df	MS	F	Probability
Leptadenia pyrotechnica	All 3- Days	Days	920.9	2	460.45	13.11	$P<0.005$
		Error	421.4	12	35.112		
	D_1 vs D_2	Days	0.1	1	0.1	0.003	ns
		Error	288.4	8	36.05		
	D_1 vs D_3	Days	685.2	1	685.2	23.0	$P<0.005$
		Error	238.2	8	29.77		
	D_2 vs D_3	Days	697.5	1	697.5	19.56	$P<0.005$
		Error	285.2	8	35.65		
Pergularia daemia	All 3- days	Days	6836.4	2	3418.2	31.26	$P<0.001$
		Error	1312	12	109.33		
	D_1 vs D_2	Days	1368.9	1	1368.9	10.438	$P<0.025$
		Error	1049.2	8	131.15		
	D_1 vs D_3	Days	2073.6	1	2073.6	58.82	$P<0.001$
		Error	282	8	35.25		
	D_2 vs D_3	Days	6812.1	1	6812.1	42.15	$P<0.001$
		Error	1292.8	8	161.6		

Table 12. One - way ANOVA of mean pollen tube length (um) of the germinated pollen grains/pollinium of 1 - 3 days old flowers.

Taxa	Days	One - way ANOVA					
		S.Var	SS	df	MS	F	Probability
Calotropis procera ssp. *hamiltonii*	All 3- days	Days	12420253.32	2	6210126.65	3.30	ns
		Error	22564920	12	1880410		
	D_1 vs D_2	Days	1953640	1	1953640	3.86	ns
		Error	4244920	8	530615		
	D_1 vs D_3	Days	4422250	1	4422250	1.59	ns
		Error	338187	8	42273.4		
	D_2 vs D_3	Days	12254490	1	12254490	5.26	ns
		Error	18651920	8	2331490		
Glossonema varians (?)	All 3- days	Days	481363.33	2	240681.66	16.4	$P<0.001$
		Error	175842	12	14653.5		
	D_1 vs D_2	Days	2560	1	2560	0.13	ns
		Error	159160	8	19895		
	D_1 vs D_3	Days	390062.5	1	390062.5	19.64	$P<0.005$
		Error	15888.2	8	19860.25		
	D_2 vs D_3	Days	3296422.5	1	3296422.5	783.88	$P<0.001$
		Error	33642	8	4205.25		
Glossonema varians (?)	All 3- days	Days	1353813.33	2	677406.67	59.7	$P<0.001$
		Error	136160	12	11346.67		
	D_1 vs D_2	Days	967210	1	967210	66.89	$P<0.001$
		Error	115680	8	14460		
	D_1 vs D_3	Days	1062760	1	1062760	82.16	$P<0.001$
		Error	103480	8	12935		
	D_2 vs D_3	Days	2250	1	2250	0.33	ns
		Error	53160	8	6640		

Table 12. contd....

Taxa	Days	One - way ANOVA					
		S.Var	SS	df	MS	F	Probability
Leptadenia pyrotechnica	All 3- Days	Days	2004520	2	1002260	14.98	$P < 0.001$
		Error	802520	12	66876.7		
	D_1 vs D_2	Days	38440	1	38440	1.21	ns
		Error	254520	8	31815		
	D_1 vs D_3	Days	1246090	1	1246090	15.16	$P < 0.005$
		Error	657520	8	82190		
	D_2 vs D_3	Days	1722250	1	1722250	19.88	$P < 0.005$
		Error	693000	8	86625		
Pergularia daemia	All 3- days	Days	22543453.3	2	112712726.6	248.6	$P < 0.001$
		Error	5440320	12	453360		
	D_1 vs D_2	Days	492840	1	492840	102.9	$P < 0.001$
		Error	38300	8	4787.5		
	D_1 vs D_3	Days	13806250	1	13806250	32.4	$P < 0.001$
		Error	3408440	8	426055		
	D_2 vs D_3	Days	19516082	1	19516082	58.8	$P < 0.001$
		Error	2653600	8	331700		

Abbreviations:

D_1 = Day one

D_2 = Day two

D_3 = Day three

S. Var = Source of variation

SS = Sum of squares

df = Degree of freedom

MS = Mean of sum of squares

F = Value of F ratio

ns = non significant

was carried out as described in chapter 6.

Pollinarium removal and pollinium insertion rate:

At least 25 mature buds (just prior to opening) were tagged on each of the 5 individuals of each species in Karachi University Population (KUH). After every 24 hours (in morning for diurnal and in evening for nocturnal species) the number of pollinaria removed and pollinia inserted on each (tagged) flower was counted with the help of 10x hand lens in the field through the life of the flower.

OBSERVATIONS AND RESULTS

In *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, in vitro pollinium viability do not change significantly over the 3- day life of the flower (Table 11, 12). However, the maximum vigor (i.e. maximum number of germinated pollen grains/pollinium and longest pollen tube length) was observed on day 2 of the flower anthesis (Fig. 61).

Similarly, maximum pollinaria removal (i.e. 0.62 per flower and 70.45% of the total removed pollinaria on all three days) and pollinia insertion (i.e. 0.09 per flower and 90% of the total inserted pollinia on all three days) was observed on 2nd. day of flower anthesis (Fig. 62). Thus, the maximum number of pollinaria removed and pollinia were inserted on day 2 of flower anthesis.

In *Glossonema varians* (Stocks) Hook. f., pollinium vigor of both hermaphrodite and male flower changed significantly with the

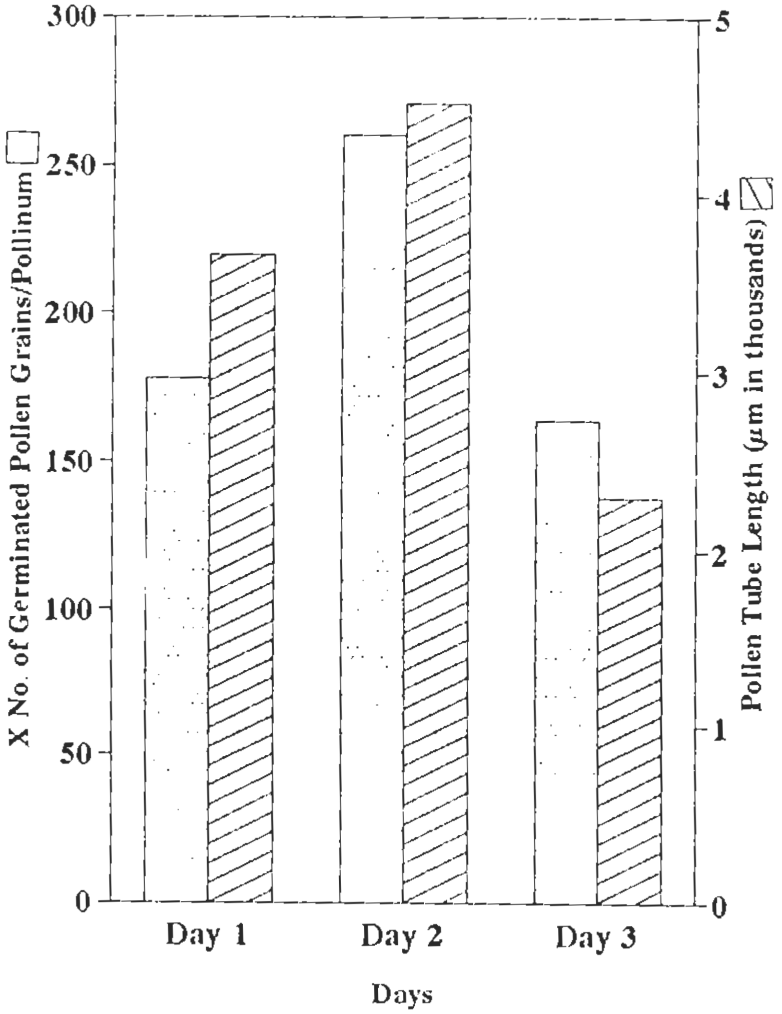


Fig. 61. Calotropis procera **ssp. hamiltoni:** In - vitro pollinium germinaticn (average maximum number of germinated pollen grains / pollinium and average maximum pollen tube length) over the life time (days) of flowers.

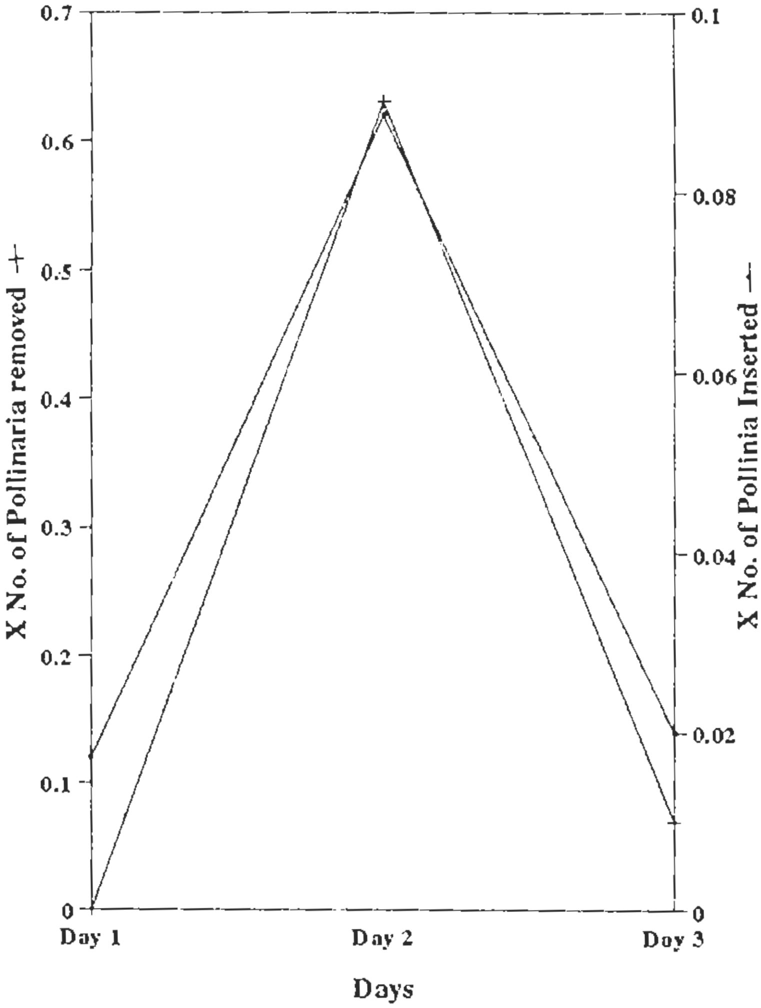


Fig. 62. Calotropis procera ssp. hamiltoni: Mean number of pollinaria removed and pollinia inserted per flower for each day after flower opening.

age of the flower (Table 11, 12). For hermaphrodite flower, average number of germinated pollen grains/pollinium significantly decreased between ages 1- & 2- days and non-significantly increased between 2- & 3- days, while pollen tube length increased significantly only between 2- & 3- days (Table 11, 12). For male flower, both average number of germinated pollen grains/pollinium and pollen tube length decreased significantly only between ages 1- & 2- days of flower anthesis (Table 11, 12).

Thus for both hermaphrodite and male flowers maximum vigor was observed on 1- day of anthesis (Fig. 63).

In hermaphrodite flowers, maximum pollinaria removal (i.e. 3.96 per flower and 45.73% of the total removed pollinaria on all three days) was observed on 3rd. day while maximum pollinia insertion (i.e. 0.12 per flower and 48% of the total inserted pollinia on all three days) on 1st. day of flower anthesis (Fig. 64).

In male flowers, maximum pollinaria removal (i.e. 4.25 per flower and 53.46% of the total removed pollinaria on all three days) took place on 3rd. day of flower opening (Fig. 64).

In Leptadenia pyrotechnica (Forsk.) Decne., pollinium vigor also did not changed significantly with the flower age (Table 11, 12). Only the pollen tube length decreased significantly between 2- & 3- day of flower opening (Table 12). Hence, the pollinium vigor was more or less remain unchanged over the 3- day life of flower (Fig. 65).

However, the maximum pollinaria removal (i.e. 2.1 per flower and 42.51% of the total removed pollinaria on all three days) and

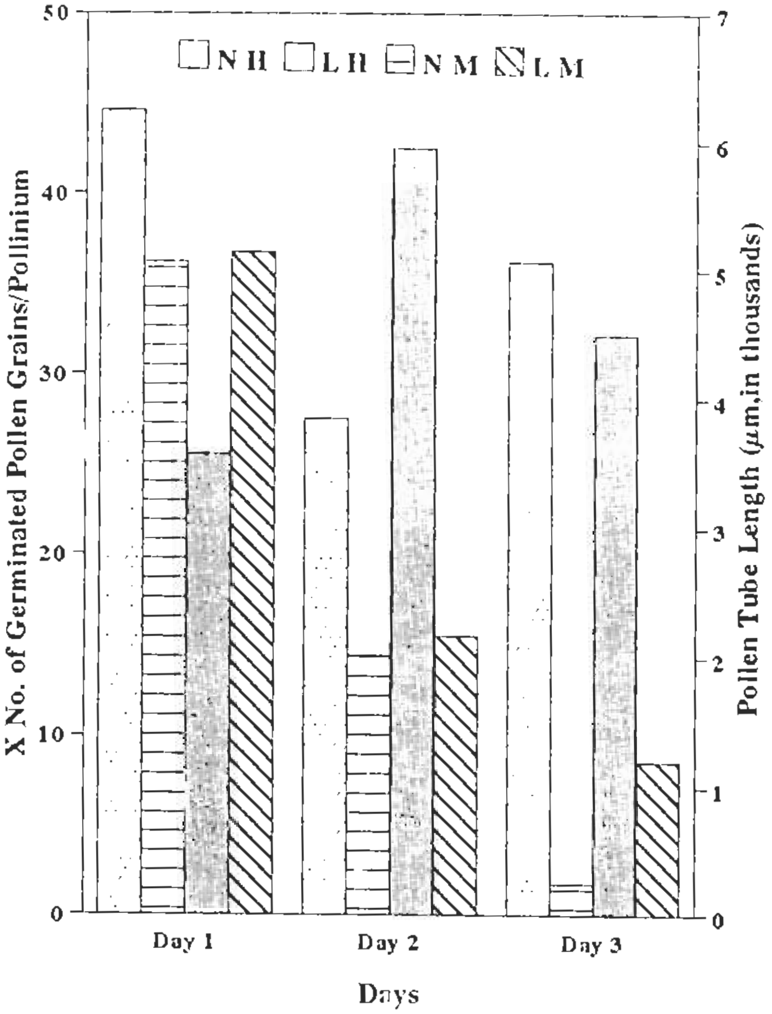


Fig. 63. Glossonema varians: In - vitro pollinium germination (average maximum number of germinated pollen grains / pollinium and average maximum pollen tube length) over the life time (days) of flowers (N = Number, L = Length, M = Male flower, H = Hermaphrodite flower).

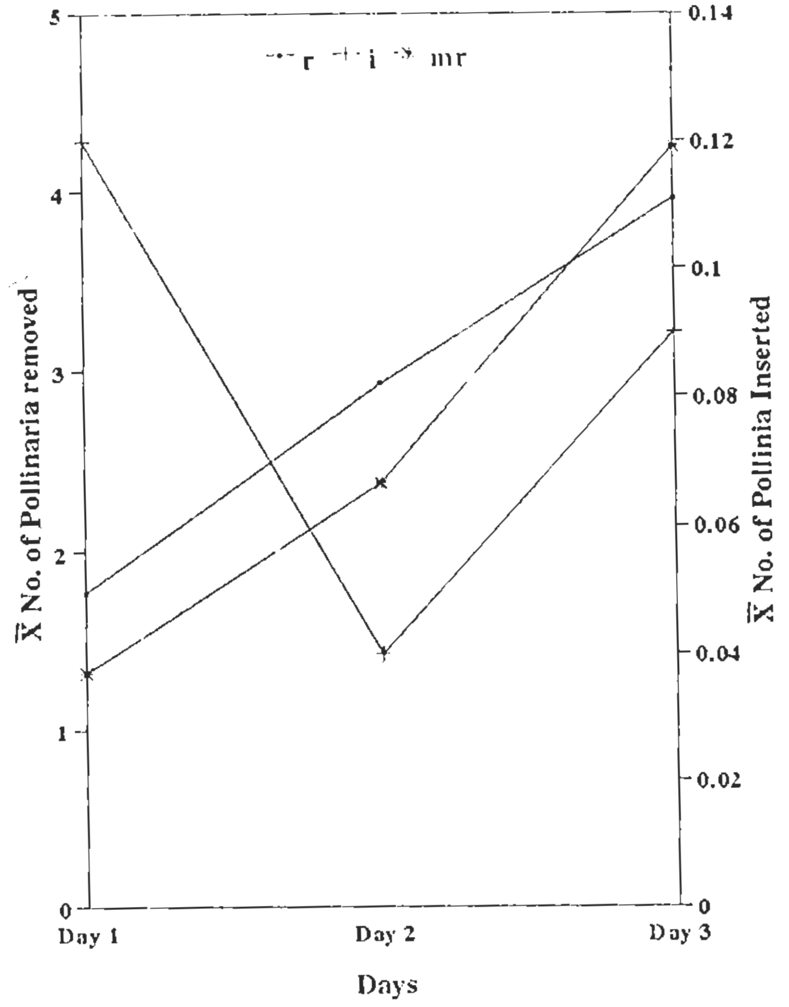


Fig. 64. Glossonema varians: Mean number of pollinaria removed and pollinia inserted per flower for each day after flower opening.
(r = Removal, i = Insertion, mr = Removal in male flowers)

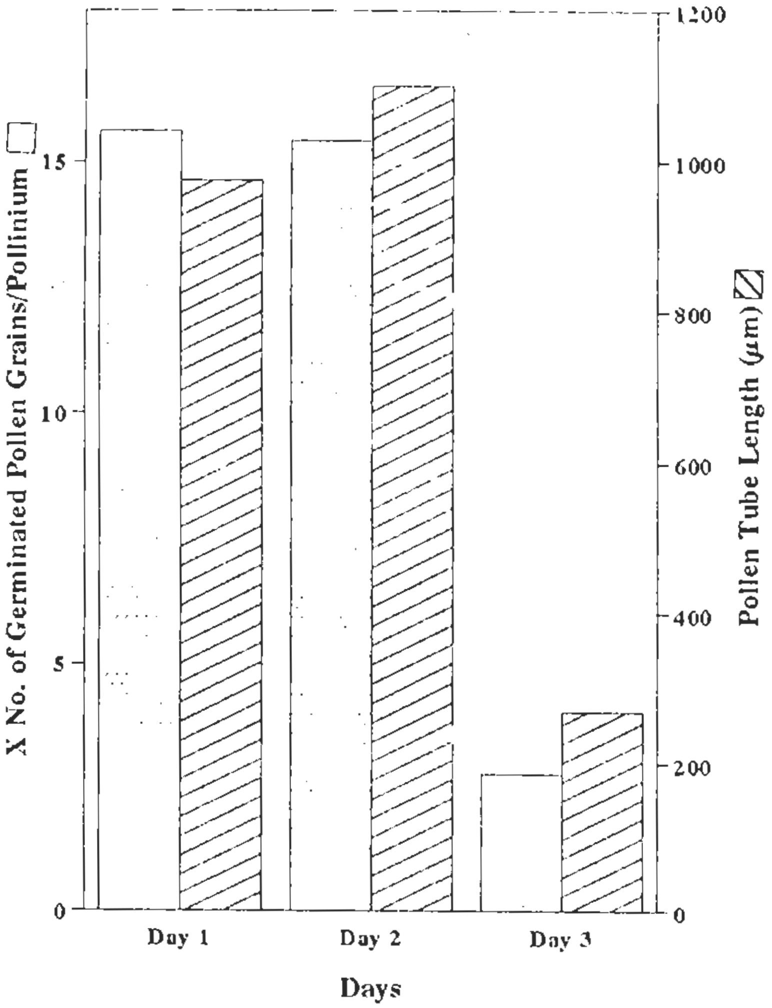


Fig. 65. Leptadenia pyrotechnica: In - vitro pollinium germination (average maximum number of germinated pollen grains / pollinium and average maximum pollen tube length) over the life time (days) of flowers.

pollinia insertion (i.e. 0.08 pollinia per flower and 60.32% of the total inserted pollinia on all the three days) was observed on 3rd. day of flower anthesis (Fig. 66).

In *Pergularia daemia* (Forssk.) Chiov. like *Glossonema varians* (Stocks) Hook. f., pollinium vigor changed significantly with respect to the flower age (Table 11, 12). The mean number of germinated pollen grains per pollinium and pollen tube length increased significantly between the ages 1- & 2- days while decline significantly betwoen 2- & 3- days of flower anthesis. Hence, the maximum pollinium vigor was observed on 2nd. day of flower opening (Fig. 67).

The maximum pollinaria removal (i.e. 0.70 per flower and 38.25% of the total removed pollinaria on all three days) and pollinia insertion (i.e. 0.18 per flower and 45% of the total inserted pollinia on all three days) was observed on 2nd. day of flower opening (Fig. 68).

DISCUSSION

The pollinium vigor has not been effected with respect to flower age in *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali and *Leptadenia pyrotechnica* (Forssk.) Decne. Similar results have also been reported for *Asclepias exaltata* L. (Shannon and Wyatt, 1986b) and *Asclepias syriaca* L. (Eisikowitch et al., 1987, Morse, 1987). However, in accordance with Bookman

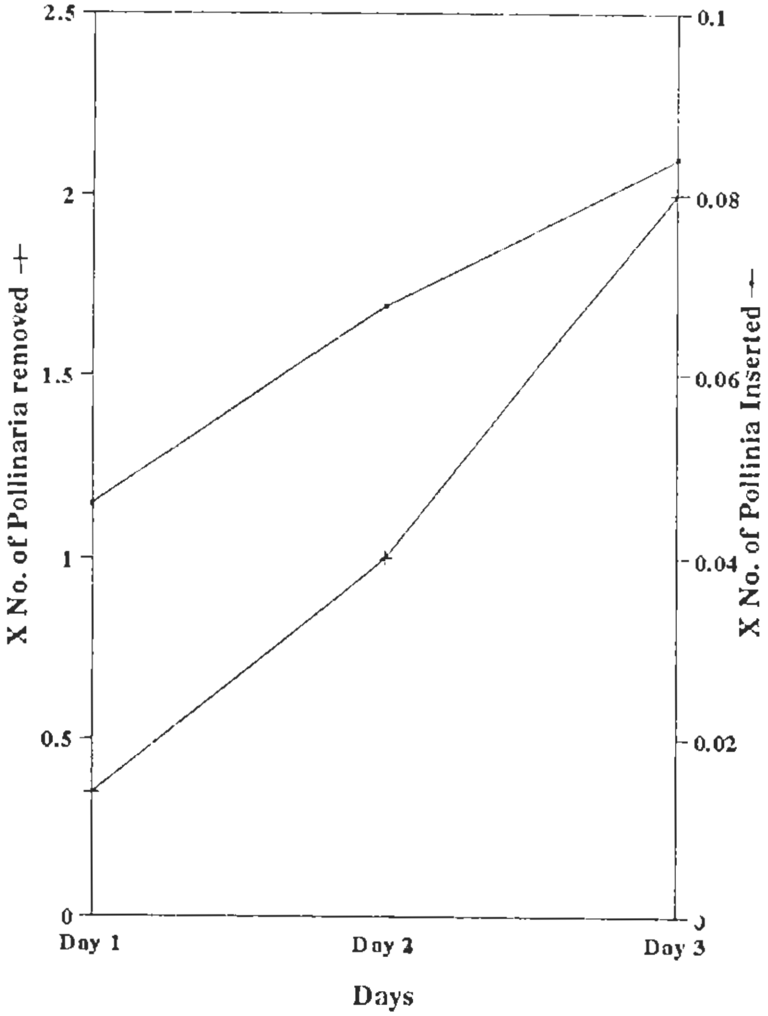


Fig. 66. Leptadenia pyrotechnica: Mean number of pollinaria removed and pollinia inserted per flower for each day after flower opening.

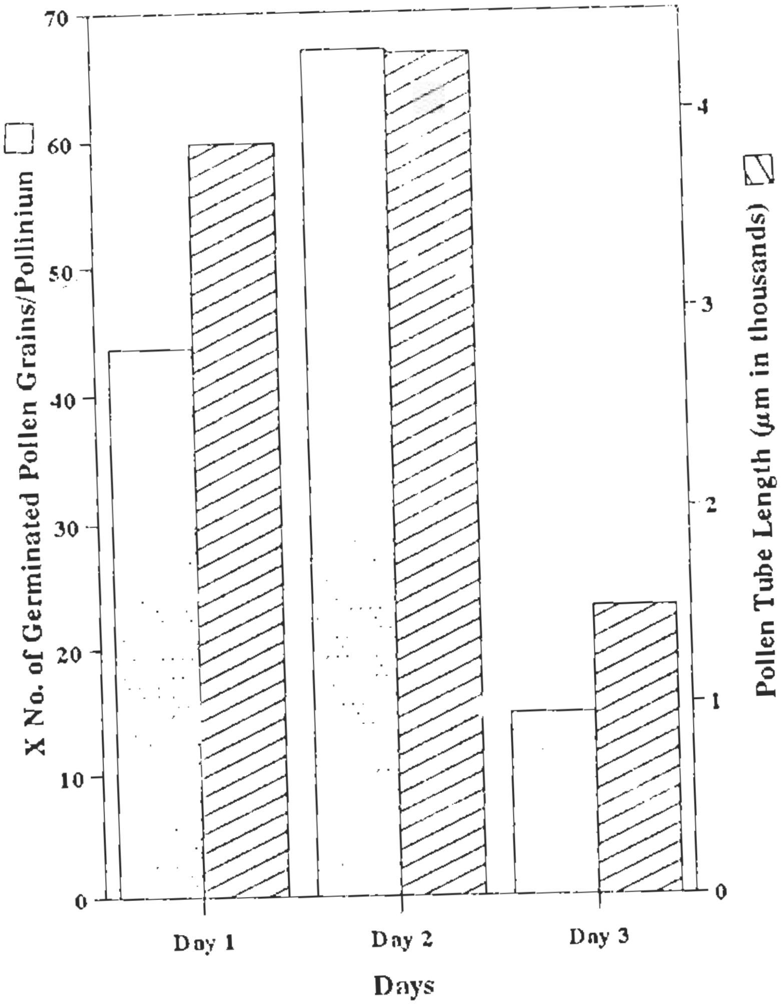


Fig. 67. Pergularia daemia: In - vitro pollinium germination (average maximum number of germinated pollen grains / pollinium and average maximum pollen tube length) over the life time (days) of flowers.

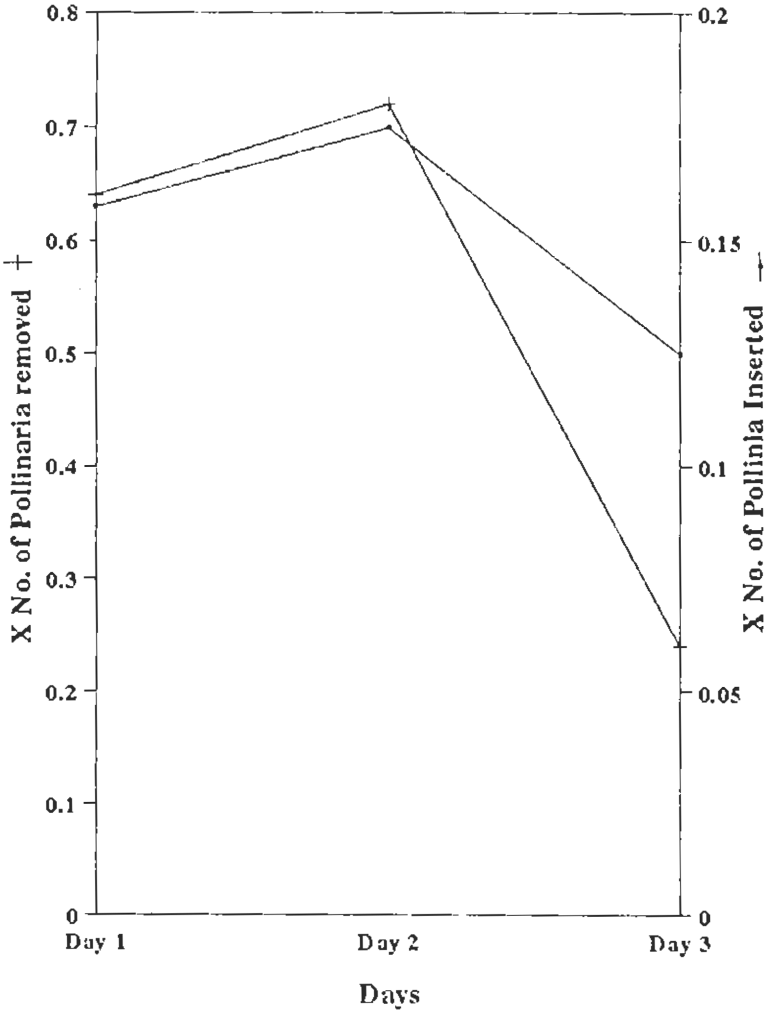


Fig. 68. Pergularia daemia: Mean number of pollinaria removed and pollinia inserted per flower for each day after flower opening.

(1987) for *Asclepias speciosa* Torr., the pollinium vigor has significantly altered with respect to the flower age in *Glossonema varians* (Stocks) Hook. f. and *Pergularia daemia* (Forssk.) Chiov.

In both *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali and *Pergularia daemia* (Forssk.) Chiov. maximum pollinium vigor was observed on 2nd. day, in *Glossonema varians* (StocKs) Hook. f. on 1st. day while in *Leptadenia pyrotechnica* (Forssk.) Decne. equally on both 2nd. and 3rd. day of flower anthesis.

Pollinaria removal and pollinia insertion by insects were highest on 2nd. day in *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali and *Pergularia daemia* (Forssk.) Chiov., on 3rd. day in *Leptadenia pyrotechnica* (Forssk.) Decne., thus similar to the period of greater vigor in vitro. However, in *Glossonema varians* (Stocks) Hook. f. a different pattern was observed, i.e. maximum pollinaria removed on 3rd. day and maximum pollinia inserted on 1st. day of flower opening. The maximum pollinaria removal and pollinia insertion in the middle period of flower age was also reported for *Asclepias speciosa* Torr. (Bookman, 1983) and for *Asclepias syriaca* L. Morse, 1987). However, the maximum pollinaria removal and pollinia insertion on the last day of flower life in *Leptadenia pyrotechnica* (Forssk.) Decne. and *Glossonema varians* (Stocks) Hook. f. is being reported for the first time.

Thus similar to *Asclepias syriaca* L. (Bookman, 1983) a relationship between pollinium vigor, pollinaria removal and pollinia insertion has been existed in all the three species with the exception of *Glossonema varians* (Stocks) Hook. f., where relationship has been noted between pollinium vigor and pollinia insertion only. In *Glossonema varians* (Stocks) Hook. f., maximum pollinaria removal on 3rd. day and maximum pollinia insertion on 1st. and 3rd. days, corresponding with in-vitro drop in pollinium vigor suggests that the decline in pollinium vigor with flower age is probably of less importance in the field than indicated experimentally.

Our results also corroborate with Kephart (1981) for *Asclepias incarnata* and Bookman (1983) for *Asclepias speciosa* Torr. as present study also provide evidence of homogamy since synchrony of sex functions was indicated by the approximate synchrony of pollinaria removals and pollinia insertions with respect to flower age. However. in *Glossonema varians* (Stocks) Hook. f. female function precede in hermaphrodite flowers as in these flowers pollinia insertion was greatest on 1st. day of anthesis while pollinaria removal on 3rd. day in both hermaphrodite and male flowers.

The non-synchrony in sex functions of *Glossonema varians* (Stocks) Hook. f. may be on one hand, due to the andromonoecious condition of plant as compared to the hermaphrodite of other studied species and on other hand due to flower phenology as within an inflorescence hermaphrodite flowers open earlier than the male flowers. However the reason of this phenomenon is remain to be tested on experimental grounds.

It has been concluded from the present study that generally pollinium vigor and pistil receptivity have not been effected with the flower age. However in-vivo pistil receptivity as well as pollinium vigor with respect to flower age must be studied before deriving any conclusion.

CHAPTER 8

THE POLLEN TUBES PATH AND THE RELATIONSHIP BETWEEN FIVE STIGMATIC CHAMBERS AND TWO OVARIES OF A FLOWER.

INTRODUCTION

The Asclepiadaceae is an interesting family from the view point of floral structure.The structure and mode of fertilization of the flower in the genus *Asclepias* have received attention of many botanists including Sprengel (1793), Brown (1833), Delpino (1865),Darwin (1877), Corry (1883a,b), Frye (1901,1902), Moore (1946a,b; 1947), Sparrow and Pearson (1948), Woodson (1954), Macior (1965), Bookman (1981) and Kunze (1991).

An important feature of this family is the presence of a gynostegium. Generally, two free superior ovaries are jointed by their styles to form a gynostegium with five lateral stigmatic surfaces.These surfaces are enclosed by wings of adjacent anthers to produce five stigmatic chambers. The relationship between these five stigmatic chambers and two ovaries is the most important factor in fertilization and fruit set determination. According to Corry (1883a) all the pollen tubes from a single pollinium enter the same (single) ovary. Later, this observation was confirmed by Frye (1902), Moore (1947), Woodson (1954), Wyatt (1976) and Bookman (1981, 1984). Sparrow and Pearson (1948) stated that for the fertilization of both the ovaries of a flower the two pollinia must be located in the stigmatic chambers in such a way that the pollen tubes from one pollinium will be directed towards one carpel and those from the other pollinium towards the second carpel given that the two adjacent chambers belong to one carpel and the other three to the second carpel. Sage et al., (1990) are the first to confirmed Sparrow & Pearson's observation anatomiaclly in *Asclepias amplexicaulis* Sm.

Unfortunately, most of these studies are confined to the genus *Asclepias,* whereas path of the pollen tubes has been investigated in very few members of this family (Kunze, 1991). *Calotropis procera*(Ait.) Ait.f.ssp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) Hooh.f. *Oxystelma esculentum* (L.f.) R.Br., *Pentatropis nivalis* (Gmel.) Field & Wood *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L., *Caralluma edulis* (Edgew.) Hook.f., *Ceropegia bulbosa* Roxb., and *Leptadenia pyrotechnica* (Forssk.) Dcne. are selected for the present study as no published information is available on these aspects of these taxa.

MATERIAL AND METHODS

Pollinated flowers of all the taxa (except *Caralluma* species) were selected from the flowers used to study the pollinator activity (see chapter 4), While pollinated flowers of both *Caralluma* species were collected from cultivated (Depatrment of Botany, University of Karachi) plants.

Three types of pollinated flowers were selected:

1. Flowers with single insertions i.e. insertion of a single pollinium in any one of the five stigmatic chambers of a flower.

2. Flowers with double adjacent insertions i.e. insertion of two pollinia into two neighbouring stigmatic chambers of a flower.

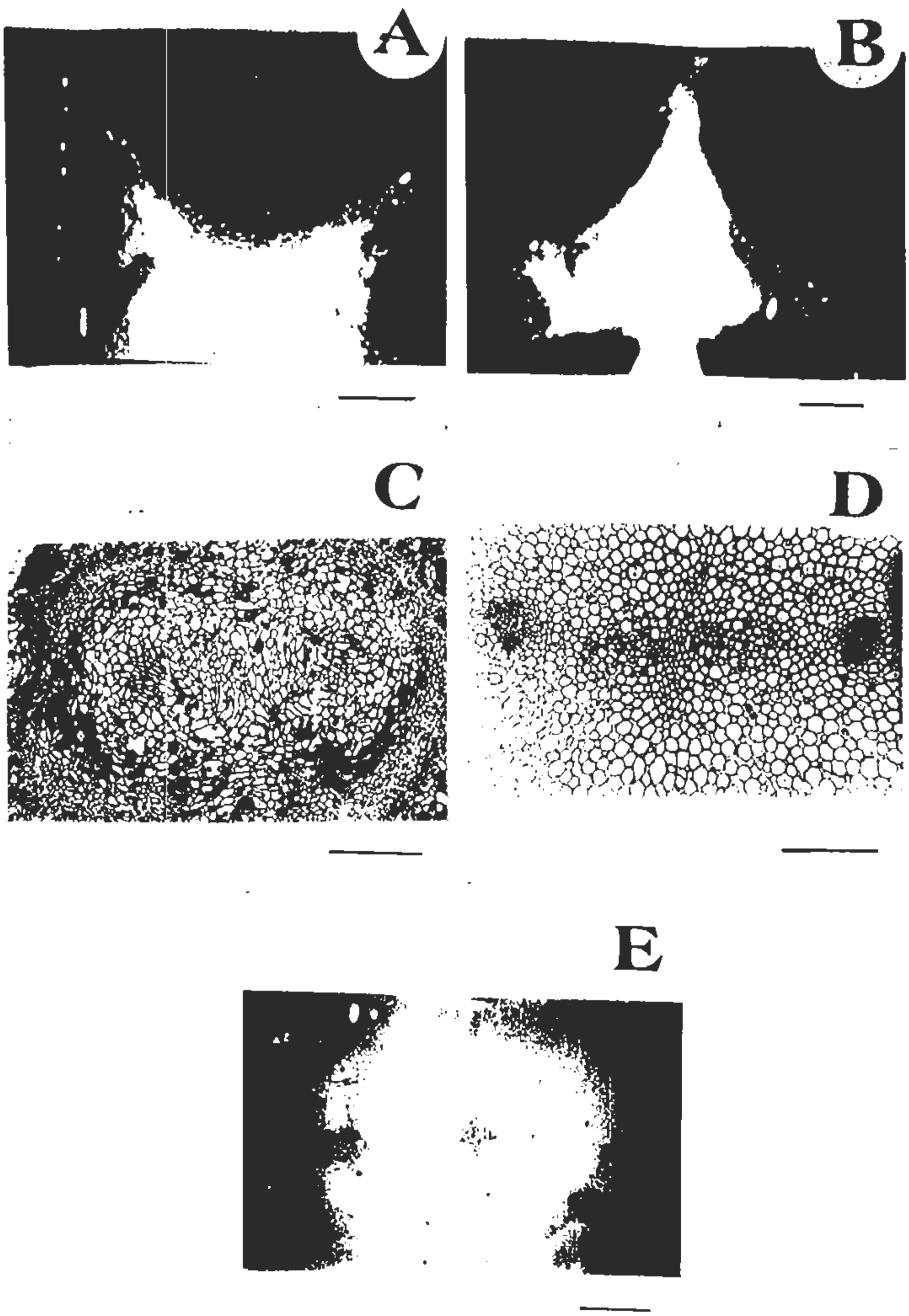
3. flowers with double opposite insertion i.e. insertion of two pollinia into two opposite stigmatic chambers of a flower.

The gynostegia of the flowers were stained in Aniline blue stain by the modified method of Lewis(1979). These gynostegia were fixed in 1:1:8 mixture of Formalin : 80% Acetic acid : Alcohal for 24 hours. They were then rinsed in water and cleared in 8M NaOH solution for 24 hours. Then they were rinsed with water and stained with 0.01% of water soluble aniline blue in 0.1M K_3PO_4 for 10 minutes. Gynostegia were then slightly squashed and mounted on the glass slides in few drops of Aniline blue stain, observed and photographed with a Zeiss U.V. photomicroscope using blue filter.

The gynostegia of *Calotropis procera*(Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali, *Pentatropis nivalis* (Gmel.) Field & Wood *Pergularia daemia* (Forssk.) Chiov., were fixed in Karnovosky's fixative (4% Paraformaldehyde and 3% gluteraldehyde in 0.05M phosphate buffer, pH$_7$) for 4 hours, dehydrated in acetone and embeded in Epoxy resin (Spurr,1969). Sections of 2μm thickness were cut on a Reichert-Jung Ultratome using glass knives and stained with 0.2% toludine blue buffered with benzoate buffer at pH$_{4.4}$ (O'Brien & McCully,1981). Sections were attached to the glass slides by drying them at 50^{o}c for at least 3 hours and slides were then made permanent. Slides were observed and photographed with Zeiss photomicroscope.

Fig. 69. Calotropis procera ssp. hamiltonii: Stigma head dissected from the middle into two havles. A, first half with two lobed inner portion of stigma head, two adjacent germinated pollinia and pollen tubes entering into a style; B, Second half with three lobed inner portion of stigma head with a germinated pollinum and pollen tubes entering into a style; C, T.S. of the middle portion of the stigma head showing two arc shaped vascular bundles surrounding the two stylar canals, stylar canals are filled with parenchymatous cells; D, T.S. of stigma head showing two separate rigs of vascular bundles and both stylar canals filled with pollen tubes (black dots); E, T.S. at the junction of styles and stigma head showing enterance of pollen tubes from two opposite stigmatic chambers.

Scale bar: A & B = 1000 μm; C - E = 200 μm.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OBSERVATIONS AND RESULTS

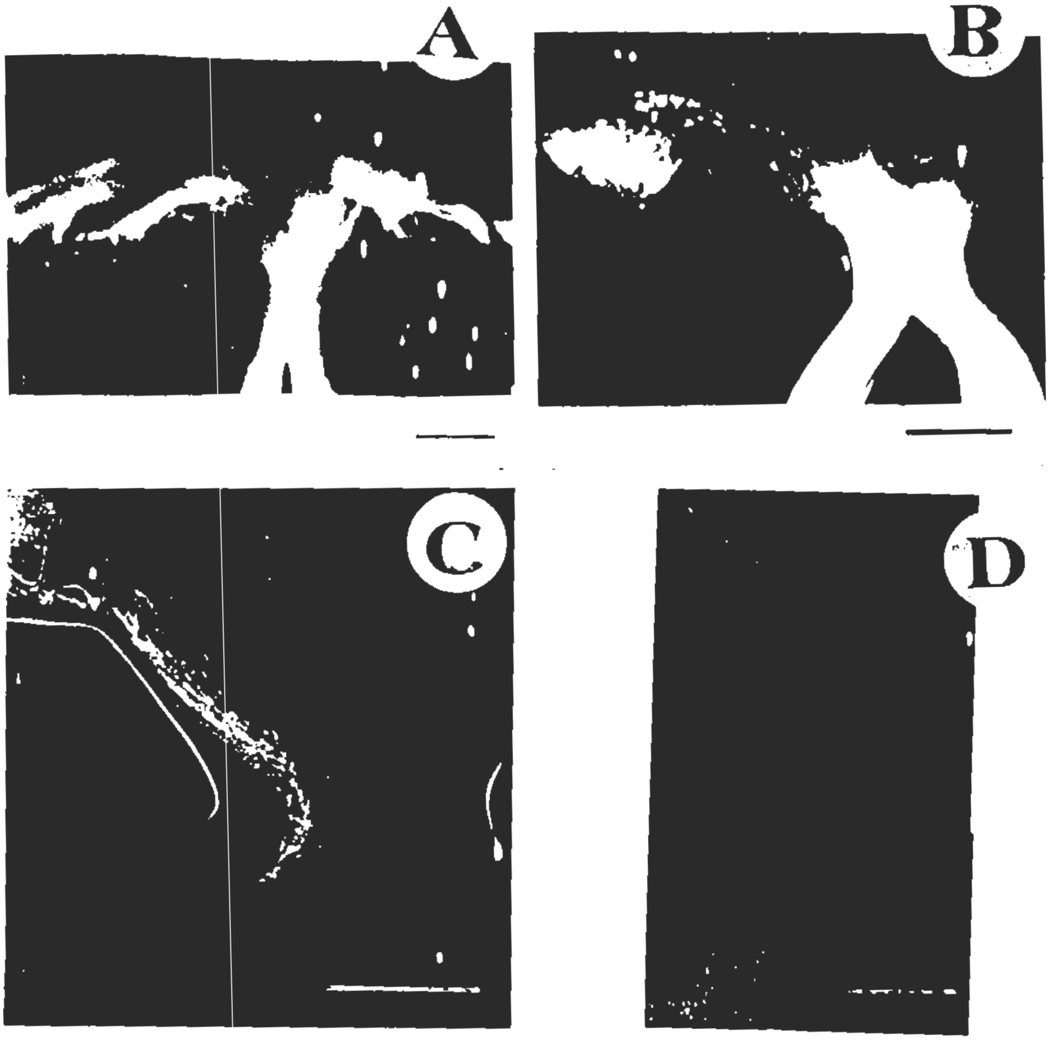
1. *Calotropis procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali:

In *Calotropis procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali. a furrow is present on the dorsal surface of the gynostegium. This furrow divides the gynostegium into two halves. Three stigmatic chambers (out of five) are associated with one half and remaining two stigmatic chambers to the other half of the gynostegium. Transverse sections through the gynostegia and longitudinal sections through the furrow revealed that all the three stigmatic chambers belonging to the one half of the gynostegium transmits pollen tubes to one carpel while the remaining two stigmatic chambers belongs to the other half transmits pollen tubes to the second carpel of the flower (Fig. 69A, B).

Serial transverse sections of the gynostegia revealed that near the dorsal surface, the vascular bundles are arranged in the form of a ring. At a lower level (towards ovary), the vascular bundles become two semi-circular or arc shaped with encurved margins towards the centre. Each of those semi-circular vascular bundles encircled the stylar canal of one of the two carpels (Fig. 69C). Each style is of open type and surrounded by stylar canal cells which are different from the surrounding cells. Further lower (towards ovary), the vascular bundles become rounded in shape, thus no longer surrounding the stylar canals which are now lying on their inner side close to the centre of gynostegium (Fig. 69D).

Fig. 70. Calotropis procera ssp. hamiltonii: A, Pollen tubes from the two opposite inserted (germinated) pollinia entering into their respective styles from the lower surface of stigma head; B & C, Pollen tubes from a single pollinium entering into a single style only; D, Pollen tubes from two pollinia inserted into adjacent stigmatic chambers entering into single style only.

Scale bar: A & B = 1000 μ; C = 100 μm; D = 300 μm.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Pollinium germination typically occurs within 12-24 hours after pollination. After germination, pollen tubes enter the gynostegium at the junction of stigmatic head and styles (Fig. 70A). Pollen tubes grow laterally, penetrating the stigmatic cells. Upon reaching the stylar canal, pollen tubes change their direction of growth and grow basipetally towards the ovary (Fig. 70B, C). As pollen tubes grow downward into the stylar canal, they displace the stylar canal cells.

Serial sections and Aniline blue stained material of Single insertion of pollinium indicate that pollen tubes arising from this pollinium always enter the style of a carpel belonging to it (Fig. 70B, C). Similarly, pollen tubes from both of the Double adjacent inserted pollinia always enter into the style of its corresponding (single) carpel (Fig. 70C). In both these cases pollen tubes were never observed to cross over into the other adjacent style as they grow basipitally towards the ovary. Where as in Double opposite insertions, the pollen tubes from one pollinium enter into the style of one carpel and pollen tubes from the other pollinium enter into the style of the other (second) carpel (Fig. 70D, D) Thus both the ovaries of a flower are fertilized. It is also evident from Fig.70D that the stylar region of the two carpels starts separating from each other at the centre and from the periphery of the gynostegium.

2. *Glossonema varians* (Stocks) Hook. f.
3. *Oxystelma esculentum* (L.f.) R.Br.

In both these species pollen tube path is similar to that of *Calotropis procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali

Fig. 71.Glossonema varians: A, Pollen tubes from a pollinium entering into a single style; B; Two ovaries of a flower one receving pollen tubes from a pollinium. Pentatropis nivalis: C, T.S. showing a slit in the middle of the upper portion of stigma head; D, T.S. of the stigma head showing anthers with pollinia, stihmatic chambers and vascular bundles in the middle; E, T.S. of stigma head showing vascular bundles surroynding the styler canals.

Scale bar: A = 1000 μm; B = 200 μm; C - E = 100 μm.

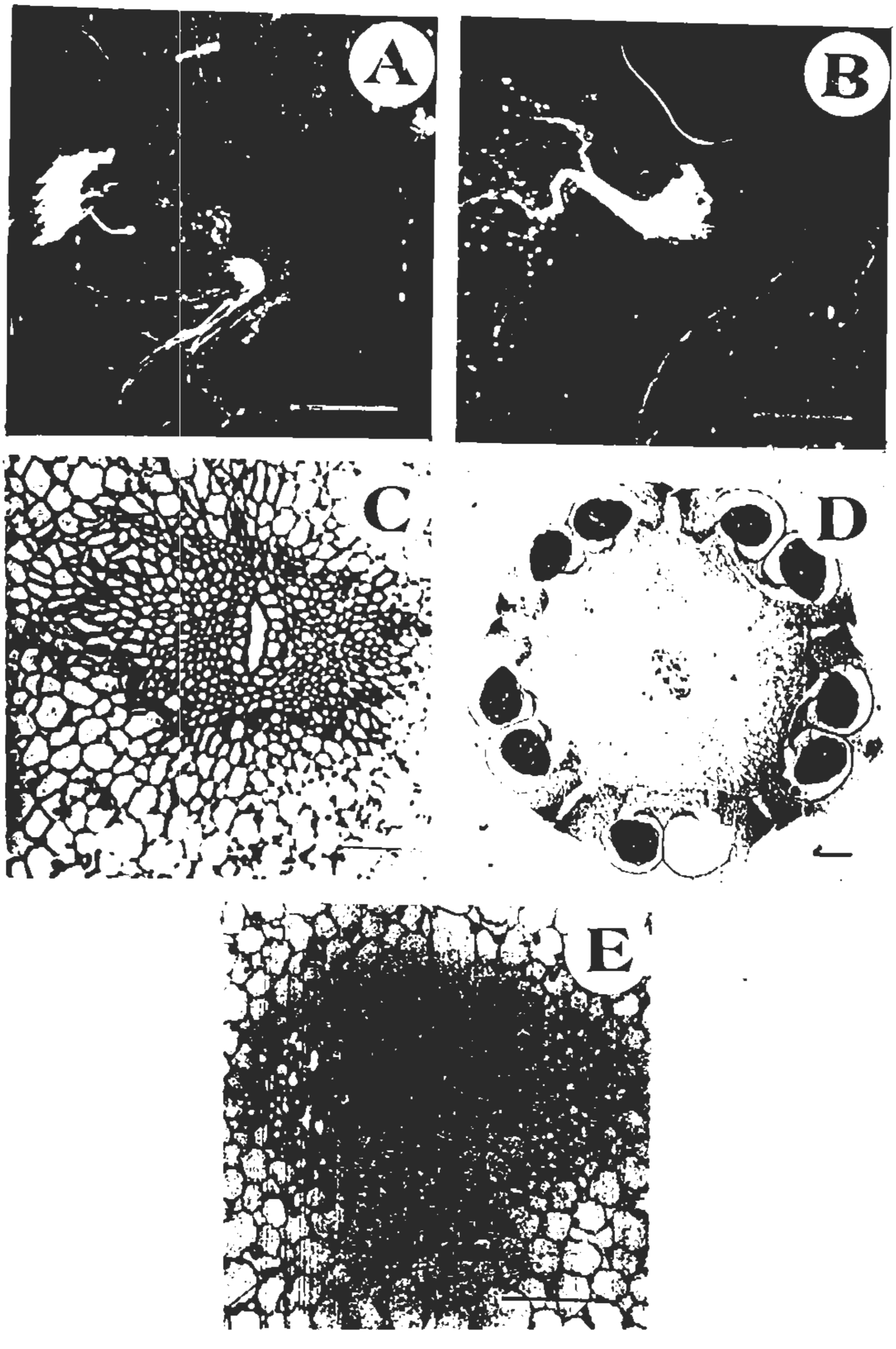
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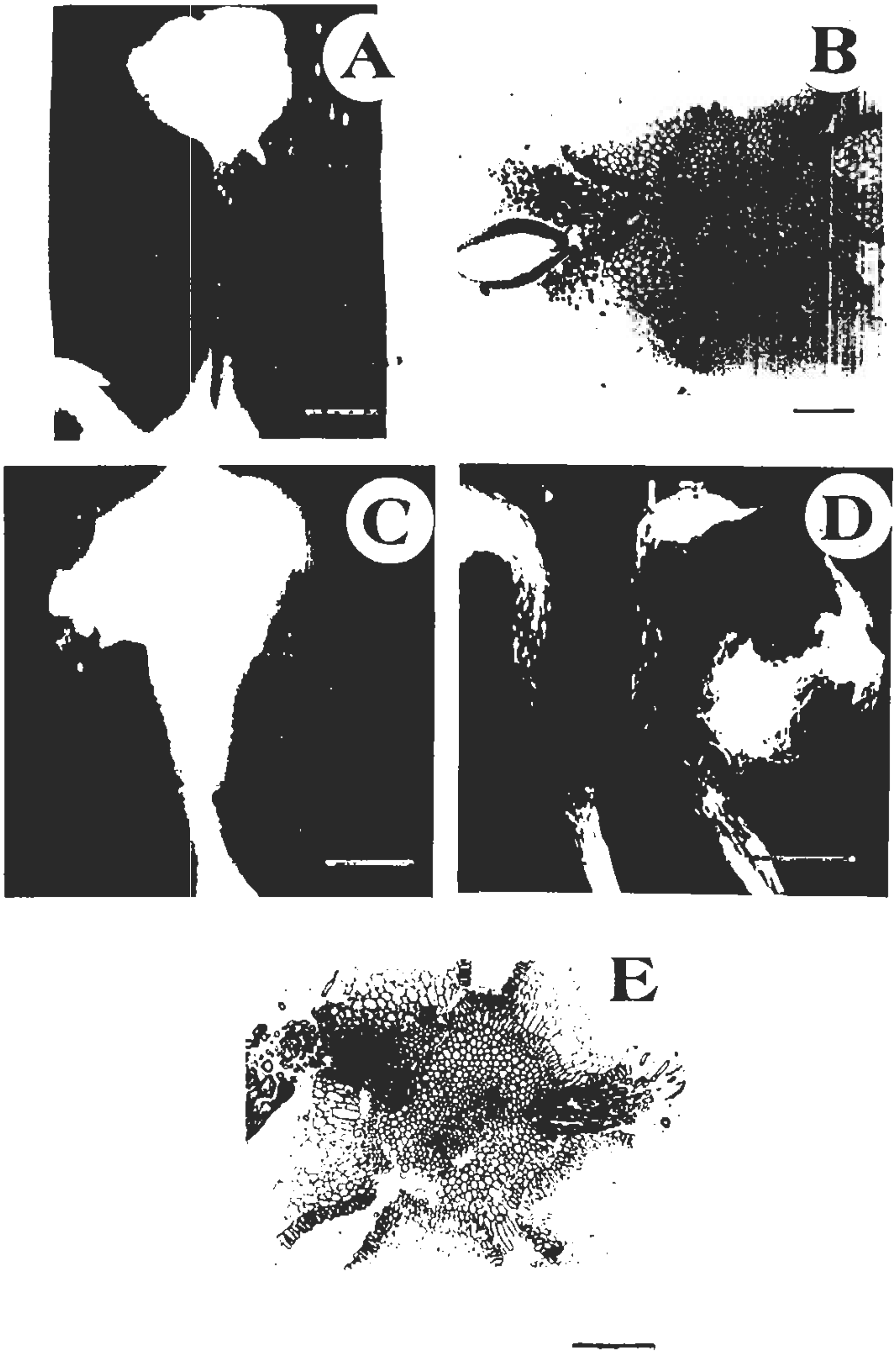
Fig. 72.Pentatropis nivalis: A, Single insertion of a pollinarium germinated at the base of stigma head lobe; B, T.S. of stigma head showing pollen tubes from a germinated pollinium entering into a style only; C, Double insertion of pollinaria; D & E, Pollen tubes from two inserted pollinaria into opposite stigmatic chambers entering ito both the styles of a flower.

Scale bar: A & C = 1000 µm; B = 100 µm; C & D = 200 µm.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(Fig. 71A, B; 72). However, in *Oxystelma esculentum* (L.f.) R.Br. pollen tubes do not enter into the stigma head, rather directly enter into the styles (Fig. 72A, B).

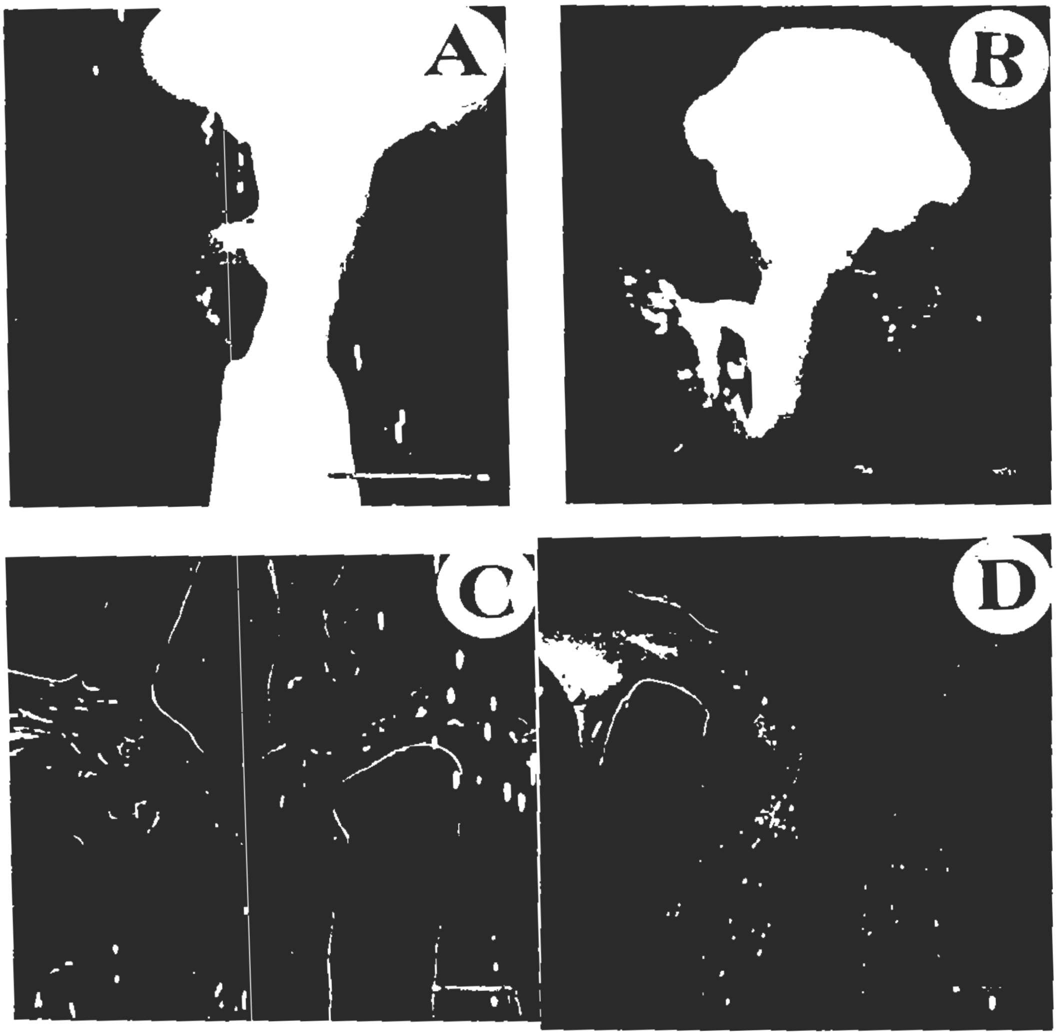
4. ***Pentatropis nivalis*** **(Gmel.) Field and Wood:**

Like *Calotropis procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali in *Pentatropis nivalis* (Gmel.) Field and Wood also the gynostegium is divided into two halves by the longitudinal furrow on the dorsal surface (Fig. 71C). One half of the gynostegium has three stigmatic chambers which are associated with one carpel and other half has the remaining two stigmatic chambers which are associated with the second carpel of the flower (Fig. 71D). Transverse sections through the gynostegia and longitudinal sections through the furrow revealed that all the three stigmatic chambers belongs to one half of the gynostegium transmits pollen tubes to one carpel while the remaining two stigmatic chambers belongs to the other half transmits pollen tubes to the second carpel of the flower. Serial transverse sections of the gynostegia shows that the vascular bundles are arranged in a ring (Fig. 71E). As we go lower towards ovary, they divides into two and become rounded in shape. Each of these vascular bundles occupy place in one of the gynostegium half. On inner side(towards the centre) of each vascular bundle lies the stylar canal. Stylar canal is of close type and filled with transmitting tissue (Fig. 71E).

Germination of pollinium and path of the pollen tubes of Single, Double adjacent and Double opposite insertions of pollinia are similar to that of *C.procera* (Ait.) Ait.f. ssp. *hamiltonii*

Fig. 73. Oxystelma esculentum: A, Single pollinium insertion; B, Double pollinium insertion, both photographs showing enterance of pollen tubes directly into the styles rather than from the base of stimga head; C, Pollen tubes from two pollinium inserted into adjacent stigmatic chambers of a flower entering into a style only: D, Pollen tubes from two pollinium inserted into opposite stigmatic chambers of a flower entering into both styles.

Scale bar: A & B = 1000 μm; C = 300 μm; D = 200 μm.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(Wight) Ali (Fig. 71E). The only difference is that in natural populations in *C.procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali single pollinium is inserted into a stigmatic chamber while in *Pentatropis nivalis* (Gmel.) Field and Wood apart from insertion of a single pollinium, in about 65% cases whole of the pollinarium i.e. corpusculam with two arms and two pollinia, is inserted by pollinators into a single stigmatic chamber and both of these pollinia germinate (Fig. 73A, B). Pollen tubes from a single stigmatic chamber of both of these germinated pollinia follow the similar path and enter into a single style only (Fig. 73B). It has never been observed that pollen tubes of a pair of pollinia from a single stigmatic chamber enter into both the styles of a flower (Fig. 73C-E).

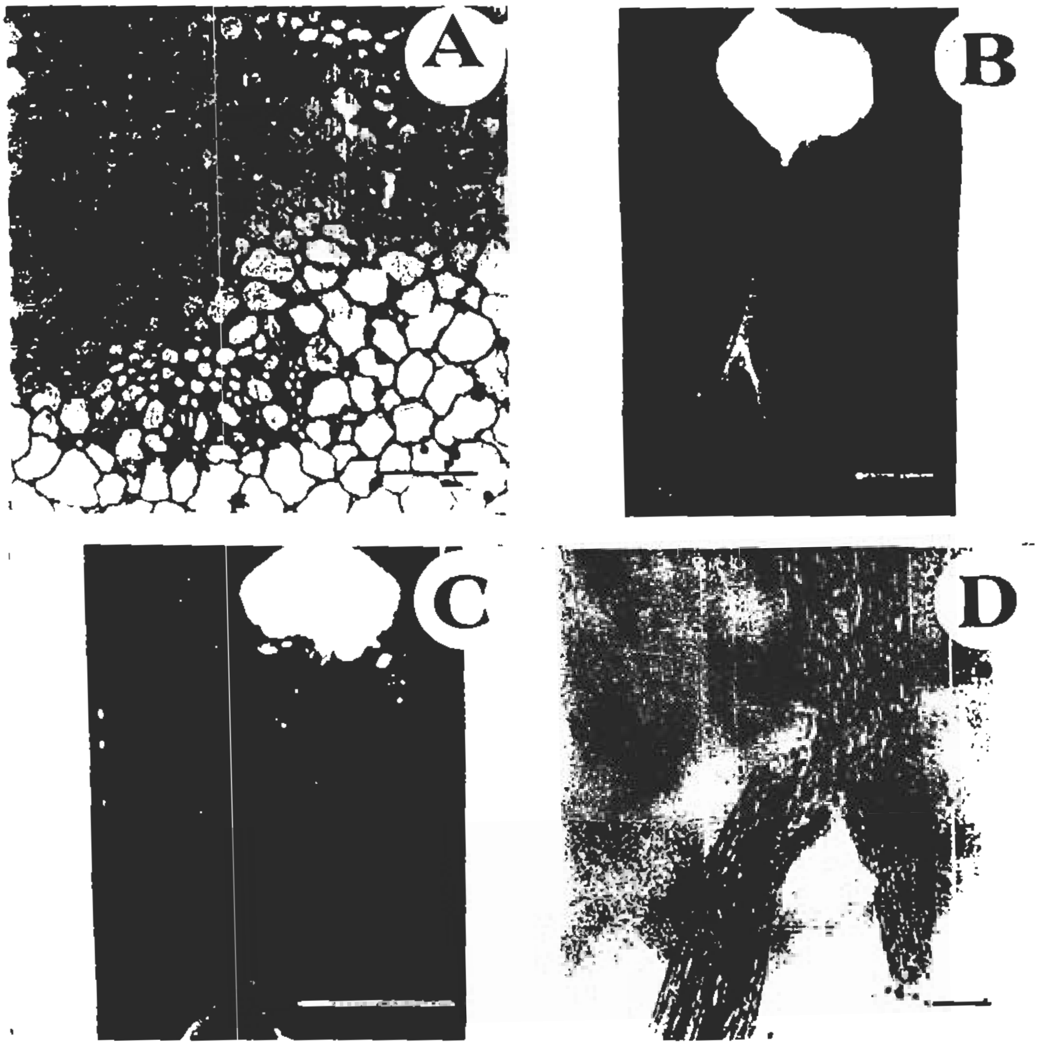
5.*Pergularia daemia* (Forssk.) Chiov.:

6. *Pergularia tomentosa* L.:

In *P.daemia* (Forssk.) Chiov. and the gynostegium is devoid of elongated furrow, instead a conical depression is present in the middle on the dorsal surface. thus gynostegium is not divided into two halves. Serial transverse sections of the gynostegium of *P. daemia* (Forssk.) Chiov. shows that vascular strands are arranged in a ring form (Fig. 74A) and further down towards ovary, they divides into two and become rounded in shape. In *P.daemia*, unlike other two taxa only a single stylar canal is present in the middle of the gynostegium just below the conical depression (Fig. 74A). The stylar canal is of close type and filled with transmitting cells. Nearly halfway below the stigmatic head towards ovary, the style alongwith the stylar canal

Fig. 74. Pergularia daemia: A, T.S. of stigma head showing vascular bundles surrounding the stylar canal region; B; Germinated pollinium at the base of stigma head and style bifercating into two; **Pergularia tomentosa:** C;Two germinated pollinia and style bifercating into two: Pergularia daemia: D - E, Pollen tubes from style distributing into bifercating portions.

Scale bar: A, D - F = 100 µm; B & C = 1000 µm.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divides into two (Fig. 74B-D). Now each style with its own vascular strands and stylar canal, joint with its respective ovary.

Pollinium germination starts within 12-24 hours after pollination. Pollen tubes from the germinated pollinium enter into the style at the junction of stigmatic head and style (Fig. 74B, C). Pollen tubes enter into the stylar canal and grow towards ovary as a single cord. At the point where style bifurcate, pollen tubes distributes and grow into both the styles (Fig. 74D, E) and fertilized both the ovaries.Thus pollen tubes from a single inserted pollinium in any one of the five stigmatic chambers always enter into both the styles and fertilized both the ovaries of a flower. So the result of single, double opposite and double adjacent insertions of pollinia is the same i.e. fertilization of both the ovaries of a flower.

Similarly *Pergularia tomentosa* L. has same pollen tube path as described for *Pergularia daemia* (Forssk.) Chiov.

7. ***Caralluma edulis*** **(Edgew.) Hook.f.:**

8. ***Caralluma tuberculata*** **N.E.Br.:**

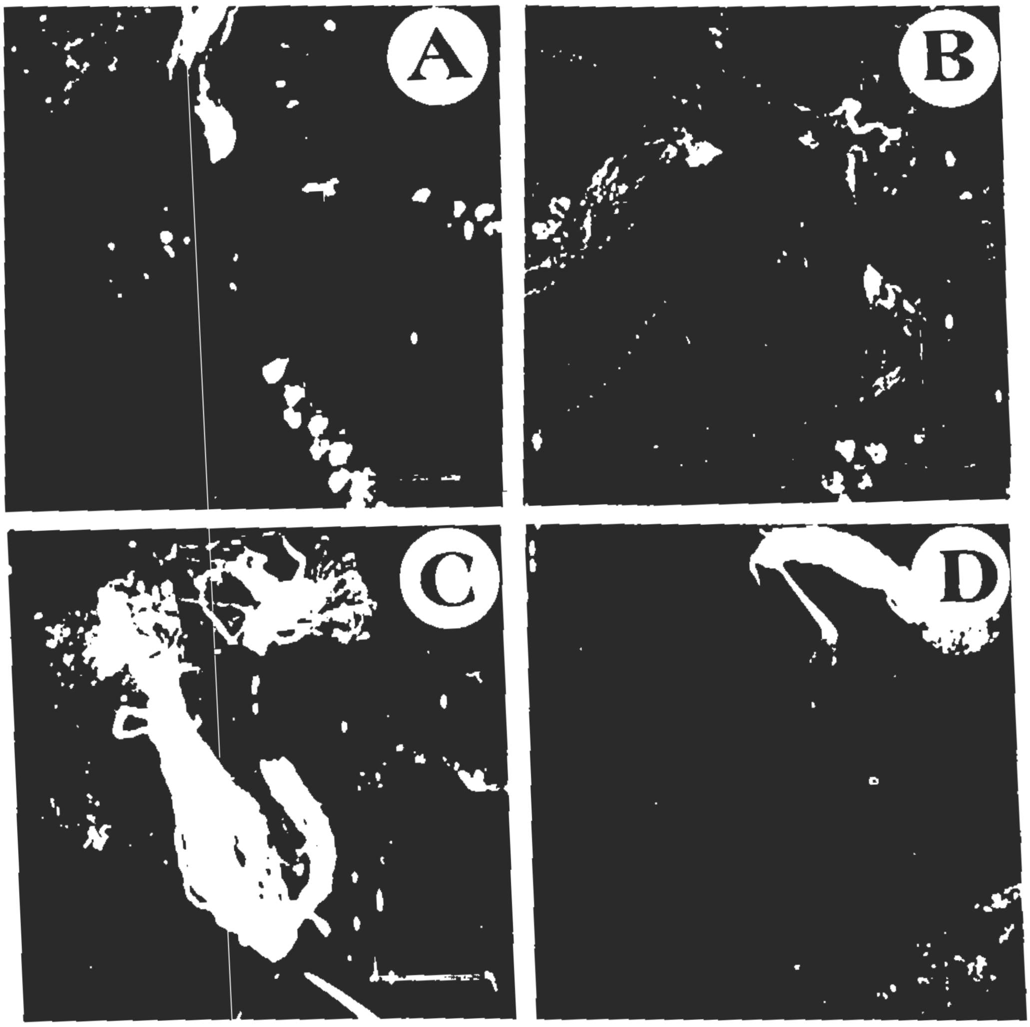
9. ***Ceropegia bulbosa*** Roxb :

In all these three species like *Pergularia daemia* (Forssk.) Chiov. pollen tubes from single inserted pollinium enter into both the ovaries of a flower and thus fertilized both the carpel (Fig. 75A-C). However, in contrast to *Pergularia daemia* (Forssk.) Chiov. in these species style is either very short or ± absent.

10. ***Leptadenia pyrotechnica*** **(Forssk.) Dcne.:**

In *Leptadenia pyrotechnica* (Forssk.) Dcne. similar to

Fig. 75. Caralluma edulis: A, Pollen tubes from a single pollinium entering into both the ovaries. **Caralluma tuberculata:** B, Pollen tubes from a two pollinium entering into both the ovaries. **Ceropegia bulbosa:** C, Pollen tubes from a two pollinium entering into both the ovaries. **Leptadenia pyrotechnica:** D, Pollen tubes from a single pollinium entering into single ovary only.
Scale bar: A - C = 500 μm; D = 1000 μm.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C.procera (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali pollen tubes from single pollinium insertion enter (fertilized) single ovary of a flower (Fig. 75D) while from double opposite insertion enter into both the ovaries of a flower.

DISCUSSION

This study supports the spatial relationship between the five stigmatic chambers and two ovaries of *Calotropis procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) Hook. f., *Oxystelma esculentum* (L.f.) R.Br., *Pentatropis nivalis* (Gmel.) Field and Wood and *Leptadenia pyrotechnica* (Forssk.) Dcne. flower as described by Sage et al., (1990) and Woodson (1954) for *Asclepias* flower i.e. three stigmatic chambers transmits pollen tubes to one ovary and the remaining two chambers transmits them to the second ovary.

On the dorsal surface of the gynostegium of *Calotropis procera* (Ait.) Ait.f. ssp. *hamiltonii* (Wight) Ali, and *Pentatropis nivalis* (Gmel.) Field abd Wood, a furrow is present. This furrow divides the gynostegium into two halves. Three stigmatic chambers associated with one style/ovary are on one side of this furrow and the two stigmatic chambers associated with the second style/ovary are on the opposite side. Thus presence of this furrow enables workers to predict which stigmatic chambers transmits pollen tubes to the particular styles and ovaries. Similar type of furrow on the upper surface of the gynostegium is reported by Sage et al.(1990) in *A. amplexicaulis* Sm., *A. exaltata* L., *A. Purpurascens* L., *A. speciosa* Torr., *A. syriaca* L., *A. viridiflora* Raf., and *A. asperula* (Dcne.)Woods. In *P. daemia* instead of a furrow, a conical depression is present in the middle of the gynostegium. Thus gynostegium is not divided and all the five stigmatic chambers transmits pollen tubes to both the ovaries. The conical depression has also been reported by Sage et al.(1990) in *A. tuberosa*L.but it is not certain that the conical depression divides the gynostegium of the flower of this taxon.

In *C. procera* ssp. *hamiltonii* and *P. nivalis* two separate styles are present. The styles of *C. procera* ssp. *hamiltonii* are of open type with open stylar canals, whereas the styles in *P. nivalis* are of close type with close stylar canals filled with transmitting tissue. *P.daemia* has single style which bifurcate more or less half way down towards ovaries. The style is of close type with close stylar canal filled with transmitting tissue.

Single insertion and double adjacent insertion of pollinia in flowers of *C. procera* ssp. *hamiltonii* and *P. nivalis* results in the fertilizatin of a single ovary. In both the cases pollen tubes were never observed to cross over during the growth down the stylar canal. Kunze(1991) suggested that as the two stylar canals are clearly separated from each other, pollen tubes from one pollinium could not cross over and fertilized only one ovary. According to him the strategy of these Asclepiads may be described as structural defined pollen tube allocation, since all the pollen tubes of one pollinium are guided to fertilized only one carpel. However, Sparrow and Pearson(1948) in *A. syriaca* and Broyles(1988) in *A. exaltata*, both workers observed one case each in which pollen tubes were transmitted from a single pollinium to both ovaries.

Double opposite insertion in *C. procera* ssp. *hamiltonii* and *P. nivalis* results in the fertilization of both the ovaries of a flower.In natural populations Asclepiads rarely mature follicles from both the ovaries of a flower. Sparrow and Pearson(1948) estimated only 5.4% and Moore(1947) reported 9.5% to 24.4% twin fruits in *A. syriaca*.Sage et al.(1990) has reported less than 5% twin fruits in *A. exaltata* and *A. tuberosa*.In natural populations. opposite double insertions occur more frequently than adjacent ones and twin fruits are initiated twice as frequent from opposite double insertions (Sparrow and Pearson, 1948; Wyatt,1976). Our study of relationship between stigmatic chambers and ovaries of a flower supports Wyatt's(1976) prediction that the probability of twin fruits is twice as great for opposite double insertions as for adjacent ones.

In *P. daemia* the pollen tubes from all the three types of pollinium insertions i.e. single, double opposite and double adjacent insertions, always fertilized both the ovaries of a flower. Thus *P. daemia* unlike other two studied taxa, possesses a compitum due to which pollen tubes are distributed to both the ovaries of a flower. Compitum has also been reported by Kunze(1991) in *Secamone alpinii* Schultes and *Tylophora* sp.

CHAPTER 9

POLLEN - OVULE RATIOS

INTRODUCTION

The pollen - ovule ratio (p/o) is the ratio of pollen grains produced per ovule. Cruden (1977) reported that the members of family Asclepiadaceae have low pollen - ovule ratios (p/o's) as compared to the other xenogamous species. As the pollen grains are incorporated in pollinia so the important comparison is not the p/o but the ratio of pollen grains in a pollinium to the number of ovules per carpel.

Reproduction of Asclepiads has been named as "Sweepstakes" by Cruden (1977) as they invest minimal energy in pollen production (i.e. pollinium), exhibit low fecundity but relatively high reproductive return when pollination is successful.

Cruden (1977) for the first time proved p/o's as the reliable indicator of breeding system. Later his work was tested and verified by a number of workers viz. Spira (1980) and Lord (1980) in Labiatae, Short (1981) in Compositae and Preston (1986) in Cruciferae. Further, pollen - ovule ratio is related to the pollinator type and pollinator activity (Cruden,1977), to the pollen - collecting area of the body of pollinator, to the area of the pollen grain surface and the stigma surface (Cruden & Jensen,1979; Cruden & Miller - Ward,1981 and Wyatt,1984). Small (1988) study pollen - ovule ratios with respect to mechanical pollination system (Explosive tripping arrangement) in comparison with other pollination systems in Leguminosae.

As very little (or scanty) data on pollen - ovule ratios of Asclepiads (Cruden,1977) has been available, so present attempt been made to study the pollen - ovule ratios in relation to tribal classification and other aspects of pollination systems of the following taxa viz. Tribe Asclepideae: *Calotropis procera* (Ait.) Ait.f. subsp. *hamiltonii* (Wight) Ali, *Glossonema varians* (Stocks) Hook.f., *Oxystelma esculentum* (L.) R.Br., *Pentatropis nivalis* (Gmel.) Field & Wood, *Pergularia daemia* (Forssk.) Chiov., *Pergularia tomentosa* L.; Tribe Ceropegieae: *Caralluma edulis* (Edgew) Benth., *Caralluma tuberculata* Brown, *Ceropegia bulbosa* Roxb. and *Leptadenia pyrotechnica* (Forssk.) Dcne.

MATERIAL AND METHODS

The number of pollen grains in a pollinium was determined mostly from preserved (in 80% Alcohol) flowers (N=10) of the above mentioned species. The pollen grains were first separated by teasing the pollinium with dissecting needles on a glass slide in few drops of Safaranine or Acetocarmine and then counted under compound microscope. Likewise, the number of ovules was determined by dissecting the ovaries (under dissecting microscope) of the same flowers used for pollen grains count.

Pollen - ovule ratios for the hermaphrodite species were determined by multiplying the number of pollen grains in a pollinium with the total number of pollinia (i.e.10) in a flower and dividing it by the number of ovules in an ovary multiplied by the total number of ovaries (i.e.2) in a flower.

To determine the pollen - ovule ratio of andromonoecious species (*G.varians*), it is essential to determine the ratio of male to hermaphrodite flowers (Cruden,1976 & Short,1981). To accomplish this, the number of male and hermaphrodite flowers were counted in 100 inflorescences of 10 individuals from Karachi University Campus (KUC) population. Thus the average percentage of male and hermaphrodite flowers for the population was calculated. The pollen - ovule ratio was determined according to the following formula of Cruden (1976).

$P/O = A/B \times C$

Where

A = Total number of pollen grains / flower (Average of both ⚥ & ♂ flowers).

B = Total number of ovules / flower × % of ⚥ flowers in the population.

C = % of ⚥ flowers in the population.

Methods for determination of pollination efficiency, pollinator type and measurements of floral parts have been described in previous chapters. Statistical analysis like Product - Moment Correlation Coefficient and Student's t - test etc. was performed on personal computer (PC) with statistical package "COSTAT".

RESULTS AND DISUSSION:

The pollen - ovule ratios in the studied taxa found are to be low and ranges from 5.89 - 26.5 while the ratio of pollen grains per pollinium to the number of ovules per carpel ranges from 1.2 - 5.3 (Table 13). Thus our data is in accordance with Cruden's (1977) observation that family Asclepiadaceae has low pollen - ovule ratios.

In andromonoecious species i.e. *Glossonema varians*(Stocks) Hook.f. the pollen - ovule ratio is 5.89 and the ratio of pollen grains per pollinium to ovules per carpel is 1.18 (for calculation see page 241). The total number of flowers per inflorescence varies from 4 - 8 with 2 hermaphrodite and 2 - 6 male flowers. However, average flowers per inflorescence were 5 with 2 hermaphrodite and 3 male flowers, whereas on average 40% hermaphrodite flowers were produced in the population.

Cruden and Miller - Ward (1981) and Small (1988) pointed out that pollen - ovule ratios have been inversely correlated with pollen grain size and stigmatic surface area. Similar correlations have also been observed in the present study, but instead of the above parameters pollen - ovule ratios found to be inversely correlated with pollinium length ($r = -0.6327$; $P < 0.05$), Calculation of Pollen - ovule ratio of andromonoecious species (*Glossonema varians*).

Mean No. of Pollen grains / Pollinium in ? flower = 165.0

Mean No. of Pollen grains / Pollinium in ? flower = 163.0

Mean No. of Pollen grains / Pollinium [in both flowers] = 164.0

Mean No. of Ovules / Ovary in ? flower = 139.0

So.

Total no. of Pollen grains / flower = 164 x 10 = 1640

Total no. of Ovules / flower = 139 x 2 = 278

% of ? flowers in the population = 40

Now according to formula

$$P/O = \frac{\text{Total No. of Pollen grains/flower}}{\text{Total no. of Ovules / flower} \times \text{\% of ? flowers in population}} \times \text{\% of ? flowers in population}$$

$$P/O = \frac{1640}{278 \times 40} \times 40$$

P/O = 5.89

Whereas, ratio of pollen grains per pollinium / ovules per carpel =

164/139 = 1.179

Table 14. Product - moment linear correlation between floral parameters (X - variable) and P/O (Y - variable).

Parameters	Correlation (r) S.E. of r	Slope (b) S.E. of b	Y Intercept (a)	df (n-2)	Probability level
No.of pollen grains/pollinium	-0.490627 0.308075	-0.014502 0.009106	15.261292	8	ns
No. of ovules/ ovary	-0.563714 0.292024	-0.018585 0.009628	14.926944	8	ns
Anther wing length (um)	-0.808840 0.207899	-0.007222 0.001856	18.97722	8	P > 0.01
Style length (um)	-0.717896 0.246126	-0.002611 0.000089	117.183817	8	P > 0.05
Pollinium length (um)	-0.632730 0.273782	-45.7118835 19.7825211	12112.562987	8	P > 0.05
Pollinium breadth (um)	-0.2.3226 0.3461175	-0.0110715 0.018252	15.727694	8	ns
Stigma head diameter (um)	-0.2.3226 0.346175	-0.010715 0.018252	15.72769	8	ns
Ratio of pollinium length/breadth	-0.585136 0.286708	-2.556627 1.252711	18.138716	8	ns
Seed length (mm)	-0.146820 0.373868	-0.686418 1.747923	8.254642	7	ns
Seed breadth (mm)	-0.345085 0.354746	-1.571803 1.615805	20.025248	7	ns
Seed weight (mg)	-0.396188 0.324621	-0.002602 0.001640	16.191958	8	ns
Efficiency	-0.165238 0.348693	-2.039179 4.303170	13.955289	8	ns

Key : ns = non-significant.

Anther wing length ($r = -0.8088$; $P<0.01$) and style length ($r = -0.7178$; $P< 0.05$; Table 14).

Similarly, negative correlations have also been found between pollen - ovule ratios and stigmatic disc diameter ($r = -0.2032$), Pollinium breadth ($r = -0.2032$), length and breath ratio of pollinium ($r = -0.5852$), seed breadth ($r = -0.3450$), seed weight ($r = -0.3961$) and positive correlation with seed length ($r = 0.146$), but these relationships are insignificant at 5% level (Table 14).

According to Cruden (1977) and Cruden & Miller - Ward (1981) pollen ovule ratios exhibit negative correlation with the pollinator type and pollination efficiency. Our study also support their suggestions ($r = -0.677$, although insignificant at 5% level) as in the taxa with low pollen - ovule ratios (6.0 - 11.79), pollinators are usually Hymenoptera (Bees) and Lepidoptera (Moths) which seems to be comparatively of large sizes, more reliable and efficient i.e. about 22.65% of pollinia have been removed by them of which 33.31% pollinia were inserted,thus more economic i.e. less wastage of pollinia as compare to the taxa with high pollen - ovule ratios (15 - 26.5) where pollinators are usually Diptera (Flies) of comparatively smaller sizes, less reliable and efficient i.e. about 48.35% pollinaria are removed and out of which only 10.35% pollinia have been inserted, thus less economic i.e. more wastage of pollinia (for detail see chapter 4).

A positive correlation have also been established between pollen - ovule ratios and mode of pollinium insertion. Taxa in which entire pollinium was inserted in the stigmatic chamber tend

Table 15. Pollen - ovule ratios and tribal classification

Parameters	Tribe	Mean	Variance	No.of samples	Student's t-Test	
					t-value	Probability level
No.of Pollen grains/pollinium	1	247.333	65290.667	6	1.492	ns
	2	90.0	970.667	4		
No.of ovules per ovary	1	193.167	48879.767	6	1.861	ns
	2	25.0	76.0	4		
P/O	1	8.712	5.121	6	3.372	P > 0.05
	2	18.40	29.607	4		
Pollen grains/pollinium per ovules/ovary	1	1.543	0.146	6	3.759	P > 0.05
	2	3.675	1.189	4		
Style length (μm)	1	2862.5	1476687.5	6	5.511	P > 0.01
	2	106.25	15989.583	4		
Stigmatic chamber length (μm)	1	1334	244504.4	6	5.499	P > 0.01
	2	211	3806.7	4		
Pollinium length (μm)	1	846.667	217944.3	6	2.673	P > 0.05
	2	322.75	8418.917	4		
Pollinium breadth (μm)	1	316.667	19628.267	6	12.673	P > 0.05
	2	257.25	5240.917	4		
Ratio of Pollinium length/breadth	1	2.776	2.447	6	0.878	ns
	2	1.256	0.027	4		
Stigmatic disc diameter (μm)	1	2157.333	2193761.067	6	1.408	ns
	2	1265.5	141544.333	4		
Seed length (mm)	1	5.867	10.131	6	1.398	ns
	2	7.8	0.9	4		
Seed breadth (mm)	1	4.117	5.689	6	0.685	ns
	2	3.425	0.281	4		
Seed weight (mg)	1	0.735	0.399	6	0.562	ns
	2	0.575	0.058	4		

Key: 1 = Tribe Asclepiadeae
2 = Tribe Ceropegieae
ns = non - significant

to have low pollen - ovule ratios whereas taxa in which only germination locus (external appendages) was inserted in the stigmatic chamber tend to have high pollen - ovule ratios.

Pollen - ovule ratios and the ratio of pollen grains per pollinium to the ovules per carpel seems to be specific at tribal level ($P < 0.05$; Table 15). Although the number of species studied in each tribe is relatively small but the trend is clear. The average pollen - ovule ratio is 8.71 ± (5.89 - 10.0) and 18.4 ± (15 - 26.5) and the ratio of pollen grains per pollinium to the ovules per carpel is 1.54 ± (1.2 - 2.0) and 3.67 ± (3.0 - 5.3) in the tribe Asclepiadeae and tribe Ceropegieae respectively (Table 15). It is also noteworthy that floral parameters like length of pollinium, style and stigmatic chamber that shows negative correlation with pollen - ovule ratio also exhibit significant tribal specificity (Table 15).

Intra and inter generic differences in the pollen ovule ratios may reflect differences in the kind and levels of activity (or efficiency) of their pollinators as suggested by Cruden (1976).

Occurrence of low pollen - ovule ratios in the family Asclepiadaceae may be explained in the light of Queller's (1984) hypothesis. According to him local mate competition may be the reason for low pollen - ovule ratios in the pollinaria bearing taxa. In these taxa, pollen grains are transferred as a unit called pollinium to a stigmatic surface (i.e. chamber) where they compete with each other for the available ovules. Thus, this local mate competition should select for fewer grains per anther sac than is usual in non - pollinial taxa.

CHAPTER 10

GENERAL DISCUSSION

Generally the studied taxa are found in stress habitats i.e. in deserts, sandunes, saline and dry rocky areas etc. All of them are perennials, about half of them survive unfavourable season as the perenating rootstocks or tuber. Most of them bloom for a short period after rain with the exception of *Calotropis procer* (Ait.) Ait. f. ssp. *hamiltonii* and *Leptadenia pyrotechnica* (Forssk.) Dcne . which flower all the year round.

The flower life of the studied taxa ranges from 1-3 days, and seems to be quite short as compare to 5-7 days of *Sarcostemma* and *Asclepias* flowers. *Pentatropis nivalis* (Gmel.) Field and Wood and both the *Pergularia* species are nocturnal, there flowers open in the night and closee in the day light, whereas other taxa are diurnal with flowers remain open throughout their life.

Andromonoecious condition is being reported for the first time in *Glossonema varians* (Stocks) Hook. f. and in the family. In *G. varians* (Stocks) Hook. f. two hermaphrodite and 2-5 male flowers are borne on the same inflorescence. Floral parts of the hermaphrodite flower are usually of large size as compare to floral parts of male flower, however the translator arm of male flower's pollinarium is long than the hermaphrodite one.

A variety of insects visit Asclepiads flowers, however the Hymenoptera, Lepidoptera and Diptera are regarded as the major while Coleoptera as minor pollinators. The major pollinators seems to be species specific. *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali is pollinated by *Xylocopa* species. It may be regarded as "Xylocopa blossom. *Pentatropis nivalis* (Gmel.) Field and Wood, and *Pergularia daemia* (Forssk.) Chiov. are mainly moth pollinated flowers, however, they are adapted to be pollinated by Hymenoptera (bees and wasps). *Glossonema varians* (Stocks) Hook. f. and *Oxysteelma esculentum* (L.f.) R. Br. are also pollinated by bees and wasps. *Ceropegia bulbosa* Roxb. is pollinated by flies while *Leptadenia pyrotechnica* (Forssk.) Dcne. by both flies and beetles.

In *Caloptropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali, pollinaria are attached on the tarsal and tibial segments of insects legs, in *Oxystelma esculentum* (L.f.) R. Br., *Pentatropis nivalis* (Gmel.) Field and Wood and both *Pergularia* species on the claws while in rest of the taxa on the mouth parts of the insects. Most of the corpuscula are directly attached to the insects, without forming chains.

In the light of the generalization made by Wyatt (1976), it is concluded that populations of all the taxa have low levels of pollinator activity. A considerable inter - population and inter - species variation has been observed in the level of pollinator activity and pollination success. Present study also support Wyatt (1976, 1978), Lynch (1977) and Beare and Perkins (1982) that insertions rates are the positive function of removal rates as significant correlation has been observed between pollinaria removal and pollinia insertion rates. Our study also provide evidence in support of Wyatt's (1976) hypothesis that successful pollinia insertions are primarily into stigmatic chambers with residual (intact) pollinaria still present.

In *Calotropis*, *Glossonema*, *Oxystelma*, *Pentatropis* and *Pergularia* entire pollinium (Pollinarium in *Pentatropis*) is inserted into the stigmatic chamber while in *Caralluma*, *Ceropegia* and *Leptadenia* only extra - pollinial appendage i.e. germination locus, is inserted and rest of the pollinium remain outside the stigmatic chamber.

In - vitro pollinium germination studies revealed a marked effect of various sugar (sucrose) concentration, as in low and high concentrations, germination was abnormal i.e. coiling, beading, malformation and even bursting of pollen tubes occured. Thus, pollinium of all the taxa can germinate in a wide range of sugar concentrations but each taxon required a specific sugar concentration for optimal pollinium germination. In accordance with Shannon and Wyatt (1986c), it has been observed that optimal sugar concentration required for in - vitro germination is more or less similar to the average floral nectar sugar concentaration of that particular taxon with the exception of *Pentatropis nivalis* (Gmel.) Field and Wood.

Germination locus of the pollinium of each taxon has been characterized by notches, slits and apertures of various sizes as reported by Galil and Zeroni (1969) and Sreedevi and Namboodri (1982). In - vitro pollinium germination studies also confirmed the location of germination locus on the pollinium of each species as obseved after acetolysis. It also observed that two or more species or genera have similar germination locus on their respective pollinium. Thus both *Glossonema varians* (Stocks) Hook.

f. and *Pentatropis nivalis* (Gmel.) Field and Wood have exoproximal. *Oxystelma esculentum* (L. f.) R. Br., *Pergularia daemia* (Forssk.) Chiov., and *Pergularia tomentosa* L. exosubdistolateral and *Caralluma edulis* (Edgew.) Hook. f., *Caralluma tuberculata* N. E. Br. and *Leptadenia pyrotechnica* (Forssk.) Dcne. have distal germination locus.

The pollinium vigor has been not effected with flower age in *Calotropis procera* (Ait.) Ait. f. ssp. *hamiltonii* (Wight) Ali and *Leptadenia pyrotechnica* (Forssk.) Dcne. while significantly altered in *Glossonema varians* (Stocks) Hook. f. and *Pergularia daemia* (Forssk.) Chiov. In response to flower age a relationship has been existed between pollinium vigor, pollinaria removal and pollinia insertion. Our results also provide evidence of homogamy since synchrony of sex functions was indicated by the approximate synchrony of pollinaria removals and pollinia insertions with respect to flower age.

Pollen - ovule ratio data support Cruden (1977) that Asclepiads have low p/o's. P/o's show significant inverse correlation with pollinium length, anther wing length and style length. It has also correlated with pollinator type and mode of pollinium insertion. For instance, taxa pollinated by Hymenoptera and Lepidoptera exhibit low p/o's while taxa pollinated by Diptera and Coleoptera have high p/o's. Similarly, taxa with entire pollimium inserted into stigmatic chamber possess low p/o's and taxa with only germination locus insertion have high p/o's.

P/o's and the ratio of pollen grains per pollinium to the number of ovules per carpel also seems to be tribe specific. In tribe Asclepiadeae p/o is upto 10.0 and in tribe Ceropegieae it is more than 15.0 while ratio of pollen grains per pollinium to the number of ovules per carpel in tribe Asclepiadeae is upto 2.0 and in tribe Ceropegieae it is more than 3.0.

Present study has revealed a variety of structural adaptations to highly specialized functions pertaining to pollination. Such as:

Stigmatic chambers:

They show different structural modifications in response to mode of pollination. Thus in the taxa where entire pollinium is inserted, the stigmatic chambers become deep, elongated, gradually narrowed towards apex and possess diagonally runnung striae at the edges of anther wings to guide the insect's leg or proboscis into the stigmatic chamber. Whereas in taxa where only germination locus is inserted, the stigmatic chambers become shallow, short, matches with the size of germination locus and with basal opening at anther slit for guiding purposes.

Nectaries:

Nectaries are located within the stigmatic chambers in the form of glandular papillae. Nectaries perform two functions, firstly it secrete nectar and secondly glandular papillae help in the proepr adjustment of inserting pollinium and also grasp it in order to be remain inserted within the stigmatic chamber.

Coronal segments:

Coronal segments also show great deal of modifications. They perform two functions. Firstly, they guide the insect's leg or proboscis towards the stigmatic chambers by their smooth, slippery surfaces and ridges and forrows pointing towards the stigmatic chambers. Secondly, they act as nectar reserviour and provide insects assess to it i.e. guide the insects towards nectar and nectaries.

Thus factors like intact corpusculum, narrowness of stigmatic chambers, movement of anther wings, glandular nectaries and even anther wings are responsible for the proper insertion of pollinium into stigmatic chambers.

Reproductive characters provide evidence in support of the tribal classifiction. Tribe Asclepiadeae is characterised by pendulous pollinium, with exolateral germination locus which is not surrounded by external appendages. The entire pollinium is inserted inside the stigmatic chamber with the exception of *Pentatropis nivalis* (Gmel.) Field and wood, where entire pollinarium get inserted. The ratio of pollen grains per pollinium to the ovules per ovary ranges from 1.0-2.0 and p/o is upto 10.0. The floral morphology particularly, pollinium orientation seems to be adapted for pollination by strong and large insects (mostly Hymenoptera and Lepidoptera), as the removal of pendulous pollinium is not easy and force is required for its removal. Whereas the tribe Ceropegieae is characterised by erect pollinium, with distal germination locus which is surrounded by external appendages. Only the germination locus is inserted in the stigmatic chamber. The ratio of pollen grains to ovules per carpel is more than 3.0 and p/o is more than 15.0. As pollinia are located in the apical portion of the anther, therefore they can be removed easily by small insects (mostly Diptera and Coleoptera).

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