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Reproductive biology of *Convolvulus microphyllus* (Convolvulaceae) — A memory stimulating drug plant of Ayurveda

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Abstract

***Convolvulus microphyllus* Sieb.ex Spreng is a reputed memory improving drug of 'Ayurveda'. The present paper reports some aspects of reproductive biology of the species. Atmospheric temperature and relative humidity played an important role in anthesis pattern in the species. Flower longevity was about 4-6 hours in all the months studied and insects belonging to *Apis* spp. played an important role in pollen transfer. Maximum pollen germination was found in pollen collected after about 1 hour of anther dehiscence initiation. Stigma was not receptive in *C. microphyllus* in a freshly opened flower and peak receptivity was at about two to four hours after anthesis. However, stigma retained receptivity till flower closure (4 hours). Pollination experiments clearly revealed the predominance of cross pollination in the species. The information gathered by the present study could be effectively utilized in planning the breeding programmes in the species.**

Key words: Reproductive biology, *Convolvulus microphyllus*, Brewbaker and Kwack's medium, decolourised aniline blue, geitonomy

Introduction

Convolvulus microphyllus Sieb.ex Spreng, popularly known as *Shankupushpi* (Fig. 1a) is used as a reputed memory improving drug of 'Ayurveda'. The whole plant is used as raw drug for the preparation of herbal formulations as a nervine tonic for improvement of memory and intellect. It also has the property to reduce mental tension and used as psycho-stimulant and tranquilizer in 'Ayurveda'.

The species is not brought under commercial cultivation and the raw drug is collected from its natural habitat and hence the quality of the raw drug is not assured. The knowledge on flowering characters would help the plant breeder for developing improved *Shankupushpi* varieties with desirable chemical quality. However, no information on the above aspects is available in literature. So, reproductive biology of this species was studied and results are communicated here as under.

Materials and methods

The present study was conducted in the population of *C. microphyllus* naturally distributed in the campus of National Research Centre for Medicinal and Aromatic Plants (NRCMAP), Boriavi (22.5° N and 73.0° E) Anand, Gujarat, India which comes under semi arid agro-climatic zone of India. Measurements of various parts of flower such as calyx, corolla, androecium and gynoecium were carried out in 100 fully opened flowers (Fig. 1b), randomly selected from the population. Fully matured buds prior to anthesis were observed to study the different stages of anthesis during the middle of December to April end (2004-05) in ten randomly selected plants in the population. Atmospheric temperatures (maximum and minimum) were also noted during this period. Data were recorded on anther dehiscence, pollen dispersal, time duration from anthesis to pollen dispersal and time taken for flower closure.

Observations were also made on insects visiting the open flowers (during 7.00 am to 1.00 pm) to find out the role of different floral visitors in pollen transfer. Floral visitors were collected from the population and observed under a stereo microscope for the presence of pollen grains on their body parts. Pollen measurement was conducted in freshly collected pollen grains with ocular micrometer on a microscope [1]. Surface ornamentation was conducted by acetolysis method [2-4] using mature pollen grains. Pollen germination was studied in Brewbaker and Kwack's medium [5]. Effect of different sucrose concentration (0-35 per cent) was tested in liquid as well as in semi-solid Brewbaker and Kwack's medium. Sitting drop method [6] was used in the case of liquid medium. Pollen grains collected from 25 flowers selected randomly from the population were mixed thoroughly and used for the study. Readings were taken after 30 minutes of incubation at 28±2°C

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in all the pollen germination studies. Germination of pollen grains from anthers of different stages of anthesis, intact and fully matured anthers, dehiscence initiated anthers and also from anthers of pollen presentation stage was tested.

Pollen viability duration was conducted to find out an easier method of storing pollen grains for using in pollinations. Pollen grains collected from anthers at pollen presentation stage were stored at room temperature (28±2°C) as well as in the refrigerated condition (4±2°C) in humidity chambers [7]. Germination was recorded at 2 hour intervals for the stored pollen grains using Brewbaker and Kwack's medium till the germinability was totally lost in both the treatments. For the pollination experiments, emasculation was done after anthesis in the morning hours since there was sufficient time gap between anthesis and anther dehiscence. The stamens are epipetalous and male reproductive part was removed along with petals with the help of forceps. Efficiency of the emasculation method was checked in the emasculated flowers by a hand lens. Bagging of the flowering twigs were done with butter paper bags after emasculation. Stigma receptivity was studied in emasculated flowers from 8.00 am to 4.00 pm at 2 hours intervals by decolourised aniline blue method [8-10]. Hand pollination was carried out with viable pollen grains using forceps and the inflorescence was bagged after pollination. In each treatment about 60 flowers from ten plants were used randomly from the population and pistils were collected and fixed in the evening.

Different pollination methods tested were: self-pollination by bagging (in order to allow spontaneous self pollination i.e. S_{sp}), self-pollination by hand within a flower (using the pollen grains of the same flower i.e. S_w), self-pollination between different flowers within the same plant or geitonogamy (flowers were emasculated and hand pollinated using the pollen grains of different flowers of the same plant i.e. S_g), artificial cross-pollination (flowers were emasculated and hand pollinated using the pollen grains of different plant i.e. C), and open pollination i.e. (O) All the hand pollinations were done during 10.00 am to 10.30 am and bagging was done after pollination. Pistils were collected in the evening and in vitro pollen tube growth was studied by decolourized aniline blue method in all the tests. Data were expressed in percentage of pistils with pollen tube growth in the style to the total number of pistils pollinated. The result was also compared with the ratio of the number of pollen tube growth in the style to the number of ovules since the ratio will give an indication of chances of ovule to get fertilized. An average of about 50 pistils was used in each treatment. Fruit set success in per cent (number of fruits divided by number of flowers pollinated expressed in per cent) was also calculated in another set of experiment using 50 flowers in each treatment i.e., S_w, S_g and C. Pollinated flowers were marked with fabric paint. The bags were removed after 24 hours of pollination for better fruit formation.

Results and discussion

Diameter of flower ranged from 10.75 mm to 17.5 mm; length of peduncle between almost absent to 25.59 mm, length of pedicel from 0.35 mm to 3.56 mm, length of sepals from 3.18 mm to 6.52 mm, length of corolla from 7.73 mm to 13.37 mm, stigma from 2.77 mm and 6.43 mm, style from 1.07 mm and 3.17 mm, length of anthers from 0.87 mm to 2.21 mm and filament from 1.08 mm to 6.56 mm in the population. Measurements of different floral parts revealed that mean values of the floral parts varied greatly among the plants in the population. These wide ranges of variability within the population could be due to the predominant cross pollinating nature of the species. This indicated that there is a scope for selection within the population. Lal *et al.* [11] made a study of genetic divergence in 52 germplasm of *Shankhupushpi* collected from different parts of India and grouped them into different clusters and reported a wide variability for days to 50% flowering, number of branches, branch length, spread area, leaf characters and herb yield.

The time of anthesis varied during the period under study in the population (Table 1). In cooler months, *viz.*, December, January and February, anthesis initiated comparatively later in the morning (between 8.30 am to 8.45 am). However, anthesis initiation was recorded at 7.00 am in March, which was further earlier i.e., at 6.30 am in the month of April because of early sunrise and increase in atmospheric temperature (Fig. 2). Pollen dispersal took about 1.00 to 1.30 hours after initiation of anthesis in cooler months while in warmer months (March and April), it took only 15-30 minutes. However, flowers closed (flower longevity) within 4-6 hours after anthesis during all the months. Parameters of floral biology are influenced by a number of environmental factors, i.e., temperature, light and humidity [12]. In the present study also, anthesis pattern varied during cooler months. This could be correlated to the weather data and inferred that atmospheric temperature and relative humidity played an important role in the pattern of anthesis in *Shankhupushpi*.

A number of insects were found visiting the flowers in this species (Fig. 1c). The major insects visiting the species were Indian rock bee (*Apis dorsata*; Family: Apidae; Order: Hymenoptera), Italian bee (*A. melifera*; Family: Apidae; Order: Hymenoptera), House fly (*Musca domestica* Family: Muscidea; Order: Diptera),

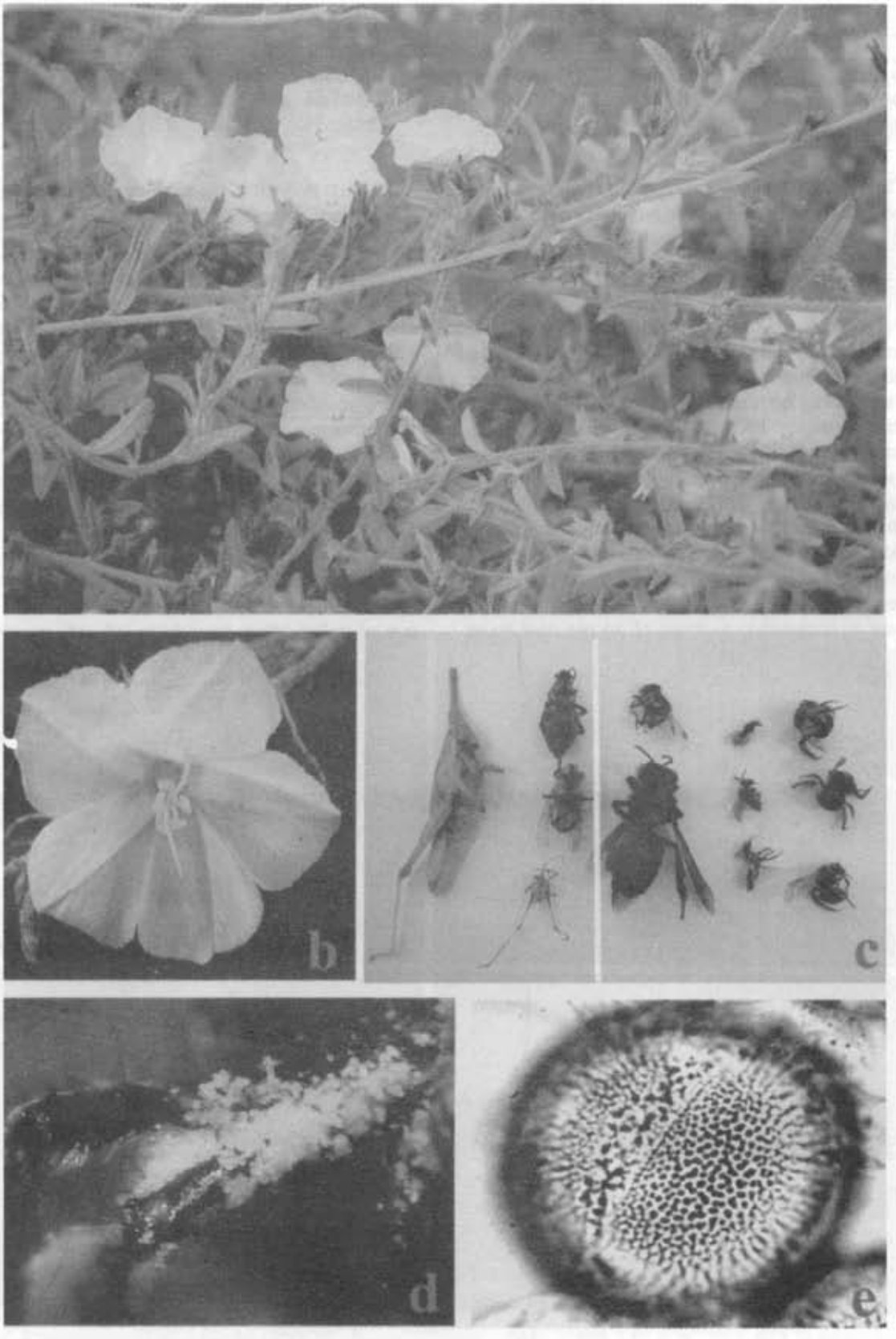

Fig. 1. a: *Convolvulus microphyllus* Sieb. ex Spreng plant habit; b: Flower of *C. microphyllus* at anthesis; c: Floral visitors (pollinators on the right side of the line) of *C. microphyllus*; d: Pollen attached on leg of *Apis* sp.; e: Reticulate pollen ornamentation in *C. microphyllus*

Table 1. Time of anthesis in *C. microphyllus* during different months

Activity	December	January	February	March	April
Anthesis initiation	8.10 am	8.35 am	8.45 am	7.00 am	6.30 am
Pollen dispersal	9.25 am	9.45 am	10.00 am	7.30 am	6.45 am
Flower closure	2.15 pm	3.00 pm	12.30 pm	11.00 am	10.30 am

Shield bug and Grasshopper. The body parts of the collected insects observed under the stereo microscope showed pollen grain only from the *Apis* spp. (Fig. 1d) and hence revealed their role in pollen transfer during their visit in the species.

Pollen grain size ranged from 29.04 to 33.20 µm in the species. Ornamentation of pollen surface was of reticulate type and shape was spherical (Fig. 1e). Pollen size could be classified as medium type in *C. microphyllus* as per Erdtman [13]. Appropriate media that give consistent results of pollen germination to test pollen viability have not been established for several plant families [14]. Brewbaker and Kwack's liquid medium with 30 per cent sucrose was the best for pollen germination in *C. microphyllus* (Table 2). In semisolid medium, 25 per cent sucrose showed maximum pollen germination (36.63%), however, germination percentage was lower in comparison to those of 30 per cent liquid medium (56.25%). The present study revealed that in *C. microphyllus*, pollen viability can easily be tested by Brewbaker and Kwack's liquid medium with 30 per cent sucrose.

Pollen germination was low in the pollen grains collected during the different stages of anthesis (only up to 12.90±1.79%). There was no or very limited germination in bud, half opened flower and 3/4th opened flower (0.0 to 3.13%). Germination started only in flowers which were completely opened (12.90±1.79%).

Germination in the pollens collected during anther dehiscence stage was 15.51±6.32% and maximum germination was noted in the pollens collected after about one hour of anther dehiscence (54.01±6.69). There was no pollen germination in pollens collected from intact anthers. The efficiency of *in vitro* pollen germination may be affected by the time of collection and storage conditions. Leduc *et al.* [15] also reported that pollen collected at different floral development had different pollen viability. In *C. microphyllus*, pollen grains were not viable in the intact anthers and pollen germination was very low in the dehiscence initiated anthers and maximum pollen germination was found only after about 1 hour of anther dehiscence initiation (i.e. pollen presentation stage). The time and storage age of pollen affect its viability [16, 17]. In this study, it was found that pollen germination remained more than eight hours of storage in pollen stored at room temperature, while at refrigerated condition it remained up to about 28 hours of storage (Fig. 3).

For successful hybridization, it is also important that the pollen transfer should be during the period of stigma receptivity. If the stigma is not receptive, pollen may not adhere to or may not germinate [18]. Stigma

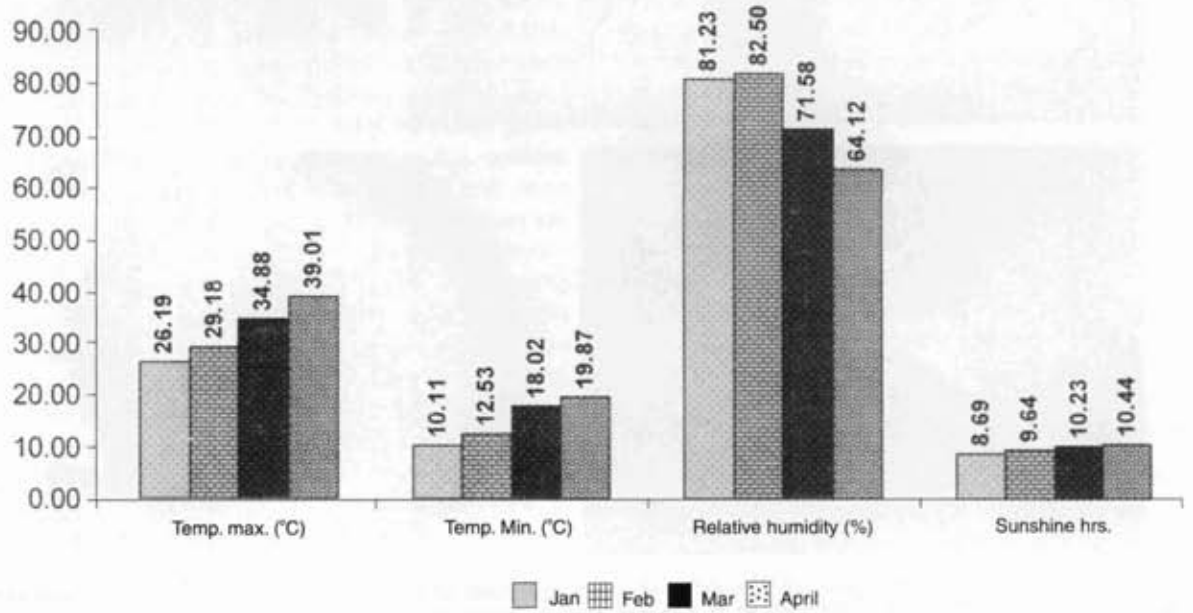

Fig. 2. Weather data during the period of study

Table 2. Standardization of pollen germination medium in *C. microphyllus*

Media	Sucrose concentration in Brewbaker and Kwack's media				
	15%	20%	25%	30%	35%
Liquid media	0.0	10.29±2.51	37.63±3.03	56.25±3.69	29.12±2.63
Semi solid media	9.28±0.74	18.65±1.67	36.63±2.59	26.93±2.21	13.19±2.30

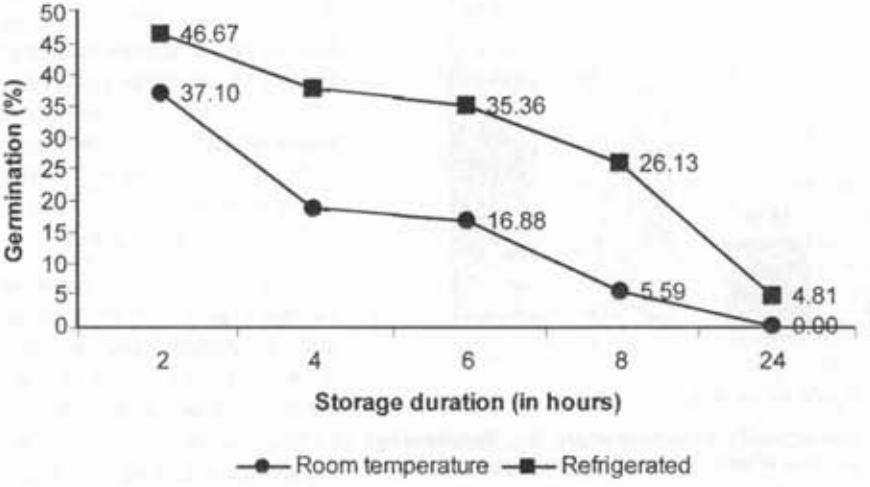

Fig. 3. *In vitro* pollen germination under storage conditions

receptivity study by decolourised aniline blue method in the pistils showed maximum receptivity during 10.00 am to 12.00 noon (Fig. 4a,b,c). There was no pollen tube growth in pistils pollinated at 8.00 am. The present study thus revealed that the stigma was not receptive in a freshly opened flower. In *C. microphyllus*, receptivity initiated in the flower after about one hour of anthesis and peak receptivity was after about two to four hours of anthesis. However, stigma retained receptivity till flower closure (Fig. 4d). Hence for

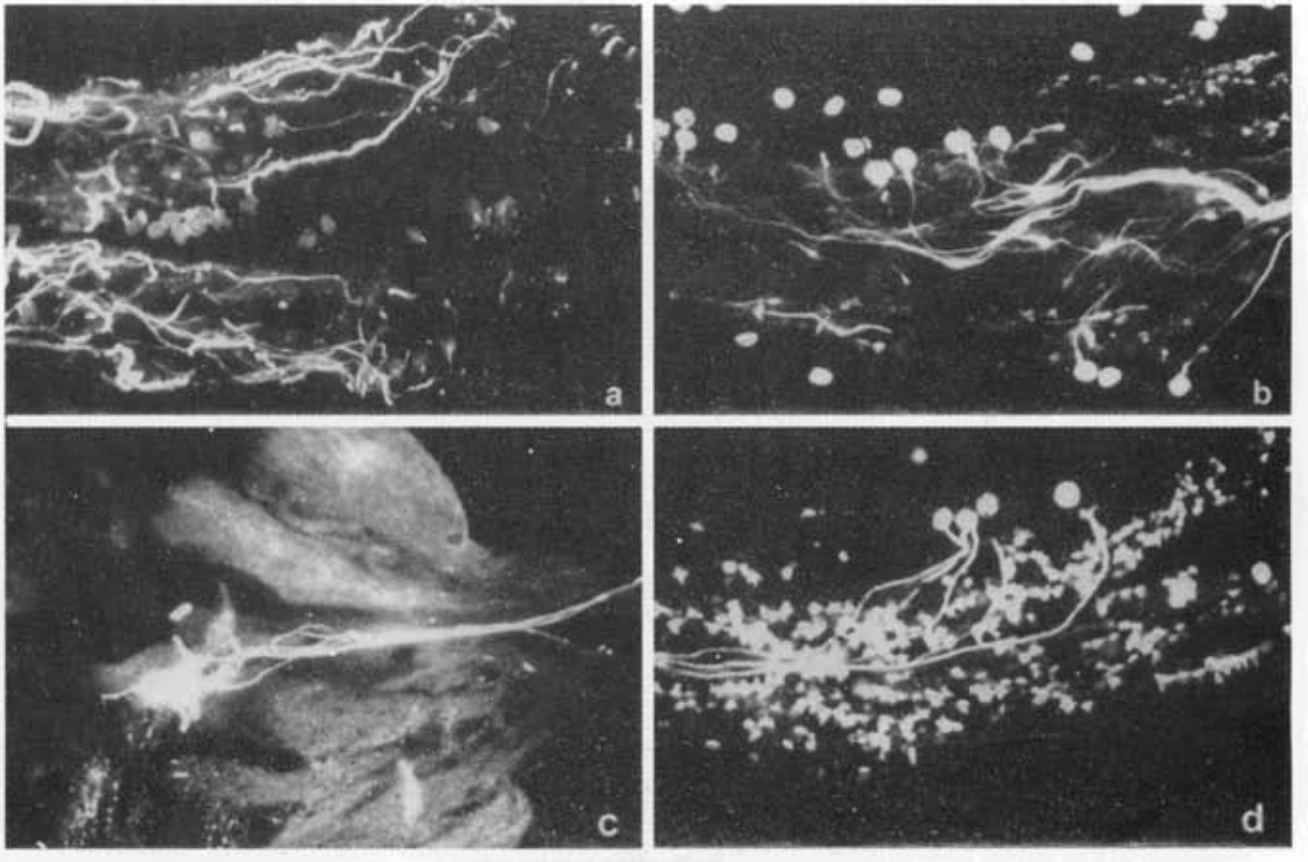

Fig. 4. Study of stigma receptivity in *C. microphyllus* by decolourised Aniline blue: a&b: Stigma pollinated at 10.00 am showing high pollen tube growth in the pistil (view at 5X & 10X); c: Pollen tube reaching ovule (view at 10X) and d: Stigma pollinated at 4.00am still retaining receptivity (view at 5X)

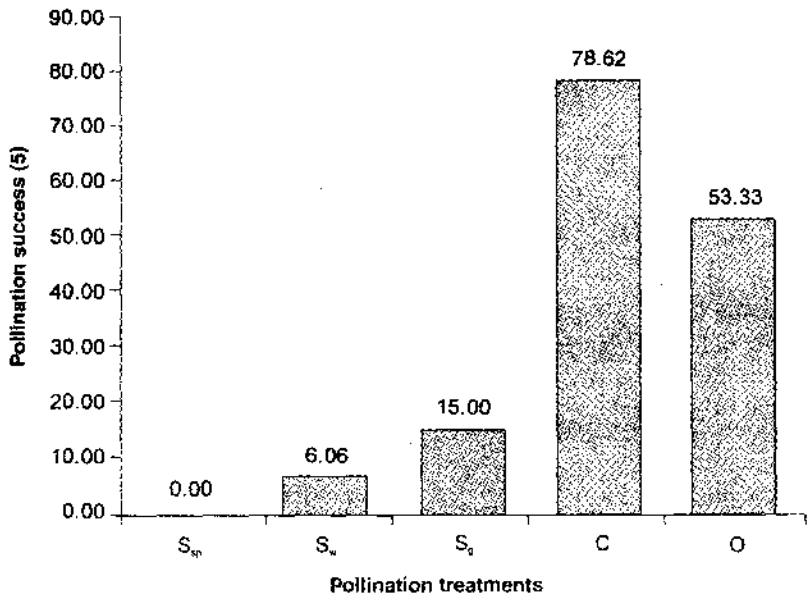

Fig. 5. Pollination success assessed by decolourised aniline blue study: S_{sp}: Spontaneous self by bagging; S_w: Self pollination within a flower; S_g: Self by geitenogamy, C: Cross pollination, O: Open pollination

successful seed production, pollination can be done in the species during 10.00 to 11.00 am.

Study of different methods of pollination showed that there was no or very few pollen tube growth in pistils collected from the self-pollination treatments i.e., in S_{sp}, S_w and S_g (Fig. 5). Pollen tube growths were observed in 0, 6.06 and 15.0 % of the total pollinated pistils respectively, in S_{sp}, S_w and S_g. In cross-pollination (C), percentage of pistils having pollen tube growth was maximum (85.29%). In open pollinated pistils (O), percentage of pistils having pollen tube growth was 53.33 per cent. Pollen tube ovule ratio was the highest in cross pollinations (7.27±1.40) and lower in self pollinations i.e., S_{sp}, (0), 0.59±0.15, S_w and S_g, 0.65±0.15. In open pollination, it was 2.11±0.42 (Fig. 6). Fruit set study revealed that S_w had 2.43 % fruit set and S_g had 6.38% as compared to 40.91% in cross pollination. Thus the pollination experiments conducted indicated clearly the predominance of cross pollination in the species.

The information gathered by the present study such as anthesis pattern, time of pollen dispersal, mode of pollen transfer, pollen viability duration, stigma receptivity schedule, emasculation technique and the predominant cross pollinating nature of the species could be effectively utilized in planning the breeding programmes in the species.

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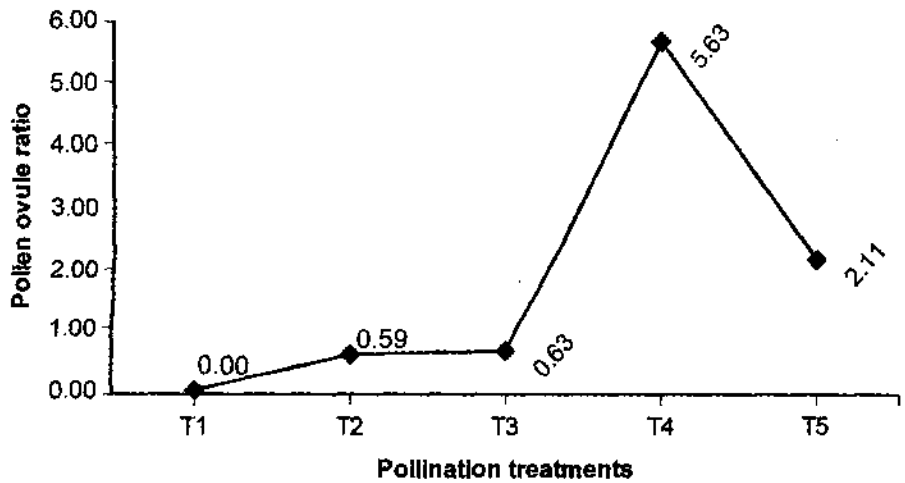

Fig. 6. Pollen ovule ratio in pollination experiment: T1: Self by bagging; T2: Self pollination between two flowers of the same plant (geitenogamy), T3: Self pollination between the flowers; T4: Cross pollination and T5: Open pollination

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