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Reproductive biology of *Kannanthali* [*Exacum bicolor* (Roxb.)]; An endangered, potential native ornamental of the lateritic hillocks of Kerala

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Abstract

Exacum bicolor (Roxb.) is an herbaceous perennial endemic to peninsular India and enlisted as an endangered plant species. Since a thorough insight into the reproductive biology of a species is necessary to aid conservation and crop improvement, the present study was formulated to assess the floral biology and reproductive behaviour of the plant. It was found that the bisexual flowers of this plant possess pollen grains that remain viable for nine days after anthesis exhibiting maximum viability on the first day of anthesis (84.61%) and 84.18 per cent viability on the previous day of anthesis. Stigma was found to be receptive upto four days after anthesis. Maximum seed weight per capsule (17.21 mg) was realised from flowers pollinated on the third day of flower opening. There was 100 per cent fruit set in flowers that were open pollinated as well as in the flowers which were either controlled out-crossed or self-pollinated under control. Fruit set was not observed on bagging the flowers to avoid natural out-crossing, and on open pollination. Hence, it became evident that *Exacum bicolor* (Roxb.) is primarily a cross-pollinated species, although no self-incompatibility mechanism operates in the species. Also, natural self-pollination is not favoured for procreation; reproduction *via* apomixis is absent in the species.

Keywords: *Exacum bicolor*, Floral biology, Pollen viability, Pollination, Stigma receptivity

Introduction

Native plant species can be an important component for a flourishing sustainable landscape (Martino et al., 2020). *Exacum bicolor* (Roxb.) is a beautiful native herbaceous perennial, endemic to peninsular India (Sreelatha et al., 2007). About five to eight decades earlier, *E. bicolor* was found in abundance in Kerala, Southern India. However, mainly owing to the indiscriminate habitat destruction, it is now enlisted as an endangered plant species (Brilliant et al., 2012; Sreelatha et al., 2007).The epithet *E. bicolor* has been chosen instead of *E. tetragonum*, since the present study focussed on the South Indian population unique to Kerala, characterised by large sized flowers with long curved anthers and calyx lobes that are broader at the base and tapering abruptly to a long point at the apex (Figure 1). The plant grows to a height of 25 - 120 cm with quadrangular stem which becomes hard when mature. The mature sturdy stem ensures that the plants do not lodge even during heavy rains. Inflorescence consists of dichasial cyme (John et al., 2001) with an average of 40 flowers per plant and 10– 20 buds in bloom at any given time, during the flowering period. Field life of individual flower

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Figure 1. *Exacum bicolor*; (**a**) the plant, (**b**) flower and (**c**) calyx

ranges from 8 to 10 days. Flowering commences from July and extends up to January. Anthesis starts in the morning and the open blooms close in the evening for two days from the first day of anthesis and remain open thereafter (Sreelatha et al., 2007).

Individual flowers are bisexual and tetramerous with four winged sepals and four petals. Petals are white in colour with purple tip. Androecium consists of four bright yellow oblong-shaped stamens, which dehisce at terminal pores and extend downwards, attracting pollinators. Gynoecium consists of a capitate stigma, long style which extends above anthers, and a two-celled ovary. Seeds of the species are tiny and takes minimum of two weeks for germination and seedlings possess long nursery period of six months for transplanting. Flowers are multi-hued. Altogether, a single flower is a blend of white, purple, and yellow colours which makes the plant more attractive and a beautiful option for gardening (John et al., 2001). Besides ornamental properties, the plant also possesses medicinal properties such as febrifuge, tonic, and stomachic properties (Rao, 1914). It is considered to possess antifungal (Khare, 2007), anthelmintic (Ashwini and Majumdar, 2014), antioxidant, thrombolytic and anti-inflammatory (Ashwini et al., 2015) attributes.

Dry grasslands of plains and high altitudes, and scrub savannas are the natural habitats of *E. bicolor* (Sreelatha et al., 2007). Occurrence of plants in isolated pockets in a huge expanse of grasslands is another distinct feature of the plant (Sreelatha and Baburaj, 2010). Habitat survey reveals that majority of the habitats in Kerala are under private possession and are likely to be exposed for exploitation mainly for laterite mining, soil excavation, quarrying etc. Study conducted during 2015 reported a habitat loss of 50 per cent in Malappuram, Thrissur, and Palakkad districts of Kerala over a period of 10 years (Sreelatha and Narayanankutty, 2017). This implies the eminence of *ex situ* conservation of the species. Production of good amount of quality seeds is thus an important factor for the *ex situ* conservation of the species.

An in-depth knowledge on the reproductive biology of a plant is vital for understanding the reproductive strategies of the plant which in turn helps in the production of quality seeds and seedlings (Rodrigues et al., 2017). It also helps to elevate knowledge about the genus and to implement conservation strategies (Lemos et al., 2020). Plants which habitats in the isolated pockets or small patches may experience 'allee effect' (a positive correlation between population size and fitness) through reduced reproductive success and in breeding depression (Groom, 1998). In high altitude regions, many plants develop some reproductive strategies such as prolonged flower longevity, anthesis, and stigma receptivity for reproductive assurance (Torres-Díaz et al., 2011). Many of such

reproductive strategies such as herkogamy, delayed autonomous selfing, and dichogamy were also reported in Gentianaceae family (Duan et al., 2010; Goodwillie and Webber, 2018; Kissling and Barret, 2010; Lemos et al., 2020 and Lloyd and Webb, 1986).

Perusal of the literature reveals that studies on reproductive biology of *Exacum bicolor* is lacking. Thus, the present study was undertaken to amplify the knowledge on the floral biology of the species, to elucidate the floral fecundity on various days subsequent to the first day of anthesis, to assess the optimum stage of flower to attain higher reproductive success, and also understand the pollination behaviour of the plant which is crucial for its conservation and aid further crop improvement programmes.

Materials and Methods

The study was conducted in the Department of Floriculture and Landscaping, College of Agriculture, Kerala Agricultural University, Vellanikkara, Thrissur, Kerala, during October 2021 to December 2022. Seedlings were transplanted in eight-inch pots which were kept in open condition. Plants were given foliar application of 19:19:19 @ 5g/L at fortnightly intervals after transplanting. The plants started flower bud initiation two months after transplanting. Parameters that would help delineate the pollination behaviour of *Exacum bicolor* were recorded as described hereunder.

Anthesis time

Anthesis time was noted by monitoring the time of bud opening. Well-grown, plum buds were tagged on the previous day of anthesis. These buds were monitored between 3.30 a.m. to 6.30 a.m. at half an hour interval for 18 days during October–November 2021, to note the exact time of anthesis. The date and time of anthesis were recorded. The total number of flowers that had bloomed within each given time interval was also noted. A total of 134 flowers were observed. The time at which maximum number of buds opened was considered as the peak anthesis period.

Pollen viability

Pollen viability was assessed following acetocarmine staining technique (Slomka, 2010). Pollen was collected using a needle and was placed on a drop of 1% acetocarmine on a clean slide and stirred for a minute for dispersing the pollen. A cover slip was kept over the acetocarmine pollen mixture and was observed under a compound microscope (Magnus CH2Oi) after five minutes. Viable pollens were pink coloured and non-viable ones were transparent coloured. Viability of pollen collected from immature buds (12 days after bud initiation), fully developed flower buds (on the previous day of anthesis; D0), and flowers in bloom immediately upon anthesis (*i.e.,* on the 1st day of anthesis; D1) and on nine days subsequent to the first day of anthesis designated henceforth as D2, D3… D9 consecutively was examined. Pollen viability was examined at hourly intervals between 8.00 a.m. to 5.00 p.m. on all days designated above.

Stigma receptivity

As an initial step for testing stigma receptivity, flowers were emasculated on the previous day of anthesis, bagged, and pollinated on the next day. Twenty flowers each were pollinated from 7.00 a.m. to 11.00 a.m. at hourly intervals and recorded per cent capsule formation, capsule weight, and seed weight per capsule. The study was taken up during October 2021 to December 2021. The best time of pollination was selected for further study during the next season (October 2022 to December 2022) to standardise the age of flower after anthesis. This was assessed by pollinating the emasculated flowers at different stages *viz*. immature bud (12 days after bud initiation), previous day of opening (D0), the day of anthesis (D1) and for three days subsequent to the first day of anthesis designated henceforth as D2, D3 and D4. The time of pollination on each day was also spaced (at 8.00 a.m., 9.00 a.m. and 10.00 a.m.), in order to assess the optimum time of pollination that would result in maximum fruit and

seed set. Buds were emasculated, bagged with butter paper covers, and tagged on the previous day of opening. Fresh pollen grains collected from D1 flowers from different plants were dusted on to the stigmatic surface at the respective timings on each day. The flowers were bagged immediately after pollination to avoid out-crossing.The number of capsules formed, capsule weight, and seed weight per capsule were recorded two months after pollination. Ten flowers were pollinated for each treatment and each treatment was replicated thrice.

Pollination behaviour

Pollination behaviour was studied by examining different methods of pollination such as open pollination, controlled out-crossing, forced open pollination, self-pollination, and controlled selfing. Healthy unopened buds were tagged and kept as such to assess the extent of open pollination. For controlled out-crossing, healthy plump buds were emasculated, tagged, and bagged on the previous day of anthesis. They were hand pollinated on the next day using pollen from D1 flowers collected from other plants. Seed set through forced open pollination was studied by emasculating and tagging the flower buds on the previous day of anthesis. These emasculated buds were left unbagged to permit natural out-crossing. Natural autogamy (self-pollination) was examined by bagging the buds on the previous day of flower opening and allowing for autonomous selfing and fertilization to occur. In the case of controlled selfing, buds were tagged on the previous day of anthesis and bagged with butter paper cover. They were selfed the next day. Observations on pollination methods were analysed by examining 10 flowers for each method of pollination which was replicated thrice.

In the relevant treatments, the bags used to prevent contamination from foreign pollen were removed after eight to nine days of pollination. Observations on capsule formation, capsule weight and seed weight per capsule were recorded. Per cent capsule formation was worked out fourteen days after tagging each flower, when the fruit formation by the enlargement of ovary was clearly visible. Mature capsules were harvested once the colour of the capsule turned brownish and dry. Capsule weight was recorded immediately after harvest by removing the remnant calyces and other floral parts. Individually labelled capsules were stored in transparent polythene bags and seed yield (mg) was observed once the capsule dehisced and released the seeds.

In order to authenticate the pollinating agents and analyse whether the plant is anemophilous or entomophilous, a few potted plants were kept protected in 40 mesh-size insect-proof cages (Figure 2) in an open area and the fruit set in the caged plants was observed. The major floral visitors in the field were also beheld, during the study period. As herkogamy is reported in many Gentianaceae plants, the spatial arrangement of stigma and anthers was studied to detect the occurrence of the herkogamy that exists in *Exacum bicolor*.

Observed data were statistically analysed by completely randomized design for pollination behaviour, and factorial completely randomized

Figure 2. Plants kept in insect proof cages for autonomous selfing

design for pollen viability, and stigma receptivity by WASP 2.0 software.

Results and Discussion

Anthesis time

As per the observations given in Figure 3, the anthesis in *Exacum bicolor* was observed to occur between 4.00 a.m. and 5.00 a.m. with the peak anthesis occurring at 5.00 a.m. (56.25 %). Anthesis was found to be absent on or before 3.30 a.m. as well as after 6.00 a.m. The anthesis was observed during October and November 2021. A similar trend of anthesis in the early morning was also reported in other species of Gentianaceae (Hentrich et al., 2010).

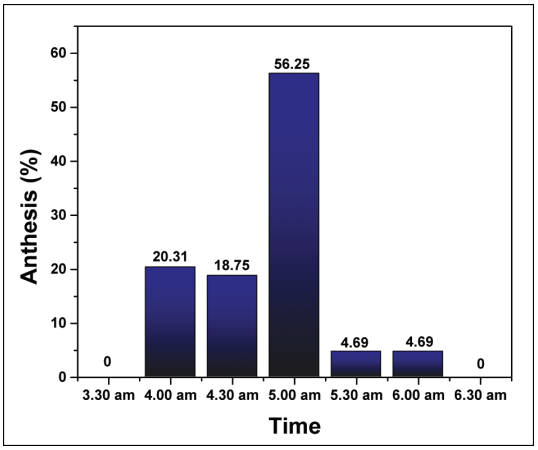

Figure 3. Anthesis in *Exacum bicolor*

Pollen viability

Pollination success depends strongly on pollen viability and stigma receptivity. The results of pollen viability tests (Table 1) indicated that irrespective of time, pollen viability was significantly higher in the bud D0 (84.18 %) and the flower D1 (84.61%), followed by D2 (80.46%). There was a gradual reduction in pollen viability from D5 (74.26%) to D9 (45.73%). It was surprising that 67.47 per cent pollen viability was recorded for immature buds just after 12 days of bud emergence. Irrespective of flower age, significantly higher pollen viability was observed between 8.00 a.m. and 11.00 a.m. and the lowest pollen viability was witnessed at 5.00 p.m. (66.91%).

From the interaction of flower age and time, significantly higher pollen viability was observed for D0 and D1 from 8. 00 a.m. to 1.00 p.m. On D2, significantly higher pollen viability was observed up to 11.00 a.m., and on D3 and D4 significant pollen viability was noted up to 9.00 a.m. and gradually decreasing thereafter.

The results indicated that pollen viability decreased gradually with aging of flower. The results are in

Table 1. Pollen viability with flower age and time in *Exacum bicolor*

Flower age (A)	Time (B)										
	8.00 am	9.00 am	10.00 am	11.00 am	12.00 Noon	1.00 pm	2.00 pm	3.00 pm	4.00 pm	5.00 pm	Mean A
Immature bud	74.49	69.70	74.36	77.25	65.84	64.19	63.96	64.69	61.47	58.81	67.47
D0	90.14	90.13	89.00	84.53	85.72	85.69	81.02	74.98	80.59	80.05	84.18
D1	83.97	86.51	88.77	86.57	87.63	83.92	77.47	86.80	84.72	79.72	84.61
D2	82.88	85.65	85.22	85.77	76.77	77.42	78.06	74.73	76.41	81.67	80.46
D3	82.20	82.11	79.34	79.77	80.04	79.12	79.92	79.00	79.67	75.58	79.67
D4	82.21	89.02	77.05	80.40	83.09	74.84	74.45	82.07	78.87	81.88	80.39
D5	76.14	83.02	71.99	71.40	72.67	73.39	73.79	74.86	78.64	66.74	74.26
D6	73.39	72.31	66.73	66.50	66.85	66.46	71.54	74.92	77.28	68.76	70.47
D7	57.13	56.89	51.88	49.96	48.22	56.81	62.93	63.34	62.24	53.64	56.30
D8	52.31	49.09	61.03	60.69	59.98	54.67	56.77	55.39	50.03	49.26	54.92
D9	49.97	50.90	46.65	45.07	46.14	45.59	45.87	44.53	42.64	39.93	45.73
Mean B	73.16	74.12	72.00	71.63	70.27	69.28	69.61	70.48	70.23	66.91	
Factors	C.D.	SE(d)	SE(m)								
Factor(A)	2.90	1.46	1.03								
Factor(B)	2.77	1.39	0.99								
Factor(A X B)	9.17	4.62	3.27								

close conformity with the research findings on *Gentianopsis paludosa* in which pollen viability was found to be highest on the first day of anthesis and decreased gradually on later days (Duan et al., 2010) and in *Gentiana penumonanthe* in which viable pollen grains were observed up to eight days with a gradual decrease in viability over flower age (Petanidou et al., 2001). However, a considerable pollen viability of more than 40 per cent in *E. bioclor* even on the ninth day of flower opening (D9) could be observed. It may be a reproductive strategy of plants that occur in isolated patches in a vast stretch of grasslands where limited pollination due to reduced pollinator visits (Groom, 1998) is common. Under such conditions, flowers also remain open for a longer period, as a flower longevity compensation strategy for pollination limitation (Arroyo et al., 2017). This might also be applicable to *E. bicolor* in which flowers are reported to have a longer longevity (Sreelatha et al., 2007).

Stigma receptivity

The results of the preliminary study conducted during October to December 2021 to screen out the best time for pollination indicated (Table 2) 100 per cent fruit set for all the time spaces of pollination *i.e.*, from 7.00 a.m. to 11.00 a.m. However, maximum capsule weight and seed weight per capsule were observed at 9.00 a.m. (100.33 mg and 16.50 mg respectively), 10.00 a.m. (98.70 mg and 14.73 mg respectively) and 8.00 a.m. (97.20 mg and 13.82 mg respectively). Therefore, time intervals of 8.00 a.m., 9.00 a.m. and 10.00 a.m. were selected for further comparison of stigma receptivity with flower age after anthesis.

There was no capsule formation for immature buds which were pollinated on 12 days after bud initiation. There was 100 per cent capsule formation for the treatments *viz*. D0, D1, D2, D3 and D4.The results of stigma receptivity test, conducted during October to December 2022, with respect to variation in capsule weight and seed weight per capsule related with flower age and time is depicted in Table 3. Irrespective of time of pollination, significantly greater capsule weight was recorded on D3 (117.12 mg) and D2 (113.70 mg). However, significantly greater seed weight per capsule (17.21 mg) was

Table 2. Recovery of capsules and seeds with respect to time of pollination (October -December 2021) in *E. bicolor*

Treatments	Capsule formation (%)	Weight of capsule (mg)	Weight of seeds per capsule (mg)
7.00 am	100	84.73	10.29
8.00 am	100	97.20	13.82
9.00 am	100	100.33	16.50
10.00 am	100	98.70	14.73
11.00 am	100	81.35	10.24
C. D	-	6.03	1.51
C. V	-	10.39	18.38

Table 3. Capsule and seed recovery in relation to age of flower and time of pollination (October -December 2022) in *E. bicolor*

Flower age (A)	Weight of capsule (mg) Time of pollination (B)			Mean (A)	Weight of seeds per capsule (mg) Time of pollination (B)			Mean(A)
	8.00 am	9.00 am	10.00 am		8.00 am	9.00 am	10.00 am	
D0	82.40	101.28	99.85	94.51	9.30	12.80	10.67	10.92
D1	102.56	104.71	103.77	103.68	14.69	15.40	13.44	14.51
D2	110.81	116.63	113.67	113.70	14.35	15.57	14.89	14.94
D3	116.99	119.49	115.89	117.12	16.45	18.56	16.62	17.21
D4	104.57	104.59	104.77	104.64	12.61	12.77	11.87	12.41
Mean	103.47	109.14	107.59		13.48	15.02	13.50	
Factors C.D.	C. D.							
Factor (A)	5.58	1.57						
Factor (B)	4.32	1.22						
Factor (A X B)	9.67	2.72						

noticed for D3. The lowest capsule weight (94.51 mg) and seed weight per capsule (10.92 mg) were recorded for D0. In the case of time of pollination, irrespective of flower age, significantly greater capsule weight was recorded for pollination at 9.00 a.m. (109.14 mg) and 10.00 a.m. (107.59 mg) and seed weight per capsule at 9.00 a.m. (15.02 mg).

From the interaction of flower age and time, significantly greater capsule weight was observed on day three (D3) from 8.00 to 10.00 a.m. (116.99 mg, 119.49 mg and 115.89 mg respectively) and day two at 9.00 a.m. and 10.00 a.m. (116.63 mg and 113.67 mg respectively) while seed weight per capsule was the highest for D3 at 8.00 a.m., 9.00 a.m. and 10.00 a.m. (16.45 mg, 18.56 mg and 16.62 mg). The results indicated maximum stigma receptivity on third day (D3) of flower opening and the lowest receptivity for buds on the previous day of opening. The results also implies that stigma is receptive even on the fourth day of flower opening. However, considering the most relevant parameter *i.e.*, seed weight per capsule, it could be inferred that pollination of flowers between 8.00 a.m. to 10.00 a.m. on third day of anthesis (D3) assures good seed set. Capsule formation in stigma receptivity test is given in Figure 4.

Figure 4. Capsule formation in stigma receptivity test

As the plant is an inhabitant of isolated pockets and discontinuous occurrence is a characteristic feature of the genus *Exacum*, prolonged stigma receptivity and flower longevity can be a strategy of the plant, for reproductive assurance. Under conditions of isolated and fragmented patches, pollinators may fail to get attracted towards the population and thus there might be pollination limitation due to reduced pollen transfer (Groom, 1998). Many of such reproductive strategies were also reported in other species of Gentianaceae family (Goodwille and Webber, 2018).

Pollination behaviour

Fruit set in pollination behaviour experiment is depicted in Figure 5 and Table 4. Except in self-pollination and forced open pollination, 100 per cent capsule formation was observed in the other modes of pollination. Seed set was absent in self-pollinated and forced open pollinated flowers. Capsule weight was found significantly higher in open pollination (124.80 mg), and controlled selfing (116.40 mg), followed by controlled out-crossing (108.30 mg). Seed weight per capsule was recorded significantly higher in controlled out-crossing (23.20 mg) and open pollination (20.67 mg) followed by controlled selfing (18.00 mg).100 per cent fruit set in controlled selfing and controlled out-crossing indicated that the plant is both self and cross-compatible.

The lack of fruit set in bagged flowers to promote self-pollination/apomixis revealed that the phenomenon of apomixis was absent in *E. bicolor*. The results also strongly pointed out that self-

Table 4. Recovery of capsules and seeds with respect to pollination methods in *E. bicolor*

Treatment	Capsule formation (%)	Weight of capsule (mg)	Weight of seeds per capsule (mg)
Open pollination	100	124.80	20.67
Controlled selfing	100	116.40	18.00
Controlled out-crossing	100	108.30	23.20
Forced open pollination	No fruit set	-	-
Self-pollination	No fruit set	-	-
C.D.	-	11.05	2.75
C.V	-	8.58	12.07

Figure 5. Fruit set in various pollination methods; (a) open pollination, (b) controlled selfing, (c) controlled out-crossing, (d) forced open pollination, and (e) self pollination

pollination in the species might be prevented owing to herkogamy. It was observed that the stigma was positioned well above the stamen at all stages of flower development (Table 5 and Figure 10). It was also evident that even in the immature bud (12 days after bud emergence) the stigmatic surface was positioned above the stamens (1.27 mm). At this stage the pollens were viable (Table 1). However, capsule formation was found to be nil, indicating that, the stigma was not receptive in the immature bud just after 12 days of bud emergence. From the day the stigma became receptive (D0), the distance between the stigma and stamens was found to have increased to 1.40 mm. Subsequently, the distance progressively increased to 6.02 mm on D3 when the stigma receptivity was found to be the highest. Herkogamy was also reported in other species of Gentianaceae (Duan et al., 2010). It was also observed that stamens were curved at an angle and faced away from the stigma, lowering the chance of pollen dispersal on the stigmatic surface (Figure 6 a and b). This spatial isolation between the stigmatic surface and stamen can be considered as an additional factor that prevents the spontaneous selfing in the species. It is therefore evident that *Exacum bicolor* is inherently a cross pollinated species.

So, unless pollinated by a pollinator there would not be any fruit set. Hence, in order to ascertain the pollinator, some potted plants were kept in insect proof cages in an open area where wind was not disrupted. From this it was observed that, all the plants kept in the cages did not set fruits while the nearby plants kept in the open had 100 percent fruit

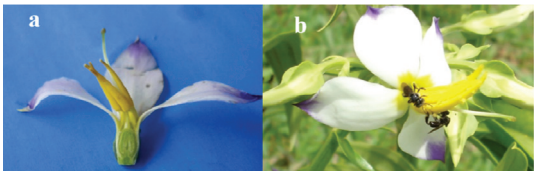

Figure 6.(a), (b) Curved stamens and straight stigma in fully opened flower of *Exacum bicolor*

Figure 7. Comparison of fruit set; (a) plants in insect proof cage, and (b) plants in open field

Table 5. Spatial isolation of anther and stigma in *Exacum bicolor*

Age of flower	Length of stigma(mm)	Length of stamen(mm)	Distance between the stigma and stamen (mm)
Immature bud	6.28	7.55	1.27
D0	13.25	14.65	1.40
D1	13.89	16.17	2.28
D2	14.22	19.27	5.05
D3	13.80	19.82	6.02
D4	13.71	18.00	4.29
D5	14.97	18.76	3.79
D6	14.50	17.81	3.31
D7	13.88	16.95	3.07

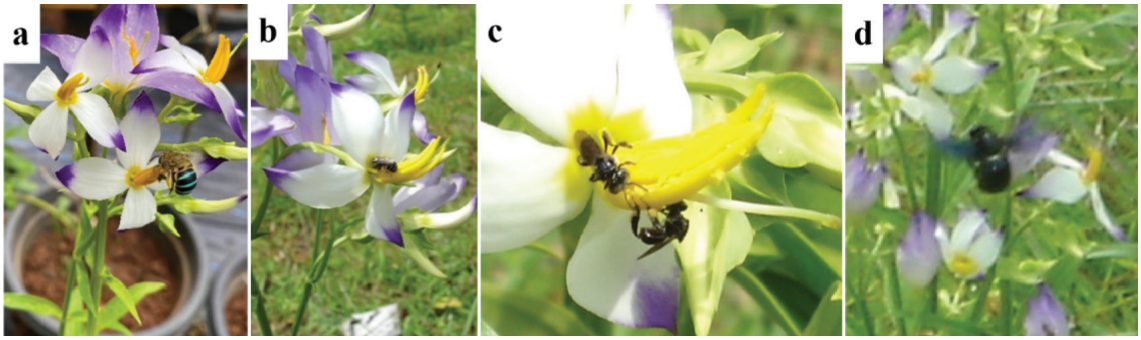

Figure 8. Floral visitors observed in the field; (a) Blue banded bee (*Amegilla* sp.), (b) Halictid bee (*Halictus* sp.), (c) Stingless bees (*Tetragonula* sp.), and (d) Large carpenter bee (*Xylocopa* sp.)

set (Figure7). Thus, it was concluded that *Exacum bicolor* is an entomophilous. Major pollinators observed during the study period were blue banded bees (*Amegilla* sp.), halictid bees (*Halictus* sp.), stingless bees (*Tetragonula* sp.) and large carpenter bees (*Xylocopa* sp.) (Figure8).

Surprisingly any fruit set was not observed in forced open pollination too, even though the flower buds were treated randomly in different plants. So, it could be ascertained that, flower visitors are purely attracted towards the yellow-coloured stamen with great reward of pollen grains present in *E. bicolor* and the pollinators could easily spot the flowers with or without the pollen source. A comparison of fruit set in open pollination and forced open pollination is depicted in Figure 9.

Conclusion

Exacum bicolor is an endemic endangered species. The individual flowers of *Exacum bicolor* possess a higher flower longevity and field life. More than 40 per cent of pollen grains were viable even on the ninth day after flower opening. The maximum viability was observed for the previous day of opening (D0) and on the anthesis day (D1). Stigma was found to be receptive upto fourth day (D4) of flower opening, which was the longest period observed during the study. However, the greatest seed weight per capsule was observed on day three (D3) of flower opening. Pollination of flowers on the third day of flower opening using pollen from flowers of the first day of opening or the previous day of opening ensures good seed set. The study proved that the plant is entomophilous and pollinated by blue banded bees, halictid bees, stingless bees, and large carpenter bees, and this supported 100 per cent fruit set in open pollination. However, the pollinators are too clever to identify the flowers without stamen and thus no fruit set in forced open pollination was observed. The 100 per

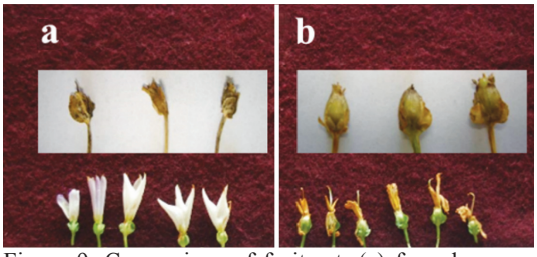

Figure 9. Comparison of fruit set; (a) forced open pollination, and (b) open pollination in *E. bicolor*

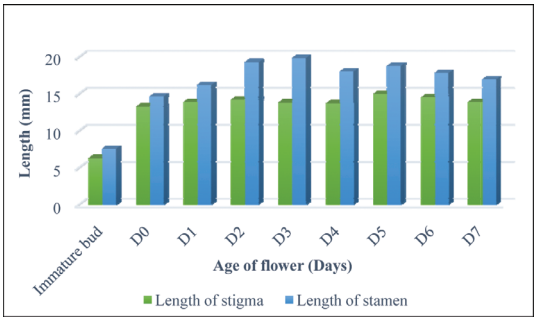

Figure 10. Length of stamen and stigma with age of flower in *E. bicolor*

cent fruit set and significantly greater seed weight per capsule for controlled out-crossing indicated the presence of heterosis which will be exploitable for future improvement programmes of the plant. Fruit set in controlled selfing ruled out the self-incompatibility; absence of fruit set in bagged flowers proved the absence of apomixis and spontaneous selfing.

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