Abrahamia
Vol. 3
20-29
2017
ISSN: 2395-4108

Reproductive Ecology of *Impatiens phoenicea* Bedd.: an Endemic and Endangered balsam of Western Ghats

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Abstract

Impatiens phoenicea Bedd. is an endangered balsam, growing in isolated pockets of Western Ghats of Kerala and Tamil Nadu, India. In order to understand its narrow distribution and endangerment, the reproductive ecology covering floral phenology, pollination, breeding systems and seed germination. The scarlet coloured flowers bloom in the early morning between 0330–0740 h. and the species is adapted to entomophilous pollination. Pollen viability and stigma receptivity are temporally isolated; pollen viability is highest (72%) on the day of anthesis and stigma is receptive on the 3rd day. Field observations confirmed that, the flowers offer both, pollen and nectar to the visitors. The visitors include honeybees and butterflies, but honeybees served as better pollinators. Various hand pollination experiments confirmed that, the species is self-incompatible. The fruit set rate in natural pollination is low (35%) but manual xenogamous pollinations enhanced the fruit set up to 66%. Its dependence on specialized habitats, scarcity of pollinators, low insect visitation rate, delayed stigma receptivity and low percentage of seed germination could be the reasons for its limited distribution and endangerment in the Western Ghats.

Key words: Anthesis, *Impatiens phoenicea*, Phenology, Pollination, Western Ghats

Introduction

Impatiens, the sub-cosmopolitan genus belonging to the family Balsaminaceae, is one of the largest groups among flowering plants comprising more than 1000 species globally (Grey Wilson, 1980; Sreekala ,2016). The high range is one of the richest areas in the Western Ghats with respect to the species of balsams. The highly undulating mountainous configuration and the formation of a well marked upland shola forest with very high rainfall provide suitable habitat for *Impatiens* (Barnes, 1939). Many species of *Impatiens* are found restricted to specific altitudinal zones and most of the exclusive endemics are found restricted in isolated pockets in the high altitudes (2000m asl) in Western Ghats. They are mainly distributed in the tropics and subtropics of the old world, but a few species occur in the temperate Eurasia and North America. In India, the genus *Impatiens* is represented by 211 species mainly distributed in three major centers of diversity i.e., Western Himalayas, Hills of North Eastern States and Western Ghats. There are 94 balsam species so far been recorded from Peninsular India, of which more than 86 are endemic and confined to Western Ghats and 30 species of *Impatiens* have already in threatened category including 19 critically endangered ones (Vajravelu and Daniel, 1983). Though, the ideal climatic conditions prevailing in the Western Ghats region provide suitable habitat for balsams, their populations are

rapidly declining due to various biotic and abiotic factors. The reproductive inefficiency may also lead to the rarity of balsams in certain cases.

Any conservation approach has to be based on an in-depth study and understanding of plant and environment including reproductive ecology which determines the fitness of the species in a given community (Monika and Bhatnagar, 2004). Adequate knowledge on reproductive biology is essential for conservation, management and recovery of endemic and endangered species. By studying the reproductive biology of RET species, we can understand the causal factors inducing rarity and can overcome these factors through scientific intervention so as to protect the plants from endangerment. The selected *Impatiens* species is not only an endemic to Western Ghats but also endangered in their habitats and hence the investigation assumes great significance in understanding their reproductive problems or success in nature.

In this context, a detailed study on reproductive biology of *Impatiens phoenicea* Beddome was carried out. The results of the present study help to understand the ecological and biological requirements which bearing on conservation of rare and endemic balsams in India. The present investigation definitely serves as an important model system for achieving twin objective of conservation and sustainable utilization of wild balsams, which are at present at the verge of extinction.

Study area

Palni Hills, a part of Southern Western Ghats, extend as an eastward spur with tropical forest. The prevalence of tropical climate condition and high rainfall make it as home to many endemic species. It is located at the middle of the Western Ghats in Tamil Nadu and receives a maximum rainfall from north-east monsoon and also a least amount during south-west monsoon. Further, the region may also receive rainfall occasionally during summer. It receives an average rainfall of 1650 mm and the temperature prevails is ranging from 8^0C – 20^0C. The reproductive biology of *I. phoenicea* was undertaken in the forest areas of Bombay shola, Vattakkanal shola and Mathikettan shola of Kodaikanal hills of Dindigual district, Tamilnadu during 2012-2015.

Materials and Methods

Mature individuals of *I. phoenicea* was periodically monitored for different phenological events covering leaf flushing, flowering, anthesis, fruit initiation and development and seedling establishment in the natural habitat. Seed germination was also observed in the natural habitat during the South West Monsoon season. Flowering phenology was observed on a day to day basis in the natural habitat according to the method suggested by (Dafni *et al.,* 2005). The initiation, development and maturation of inflorescence and flowers were observed on daily basis during the flowering season and number of flowers produced per inflorescence, lifespan of flowers, time of anthesis, time of anther dehiscence, nectar production and stigma receptivity were recorded by continuous observation. Further, the morphological characters of flowers were analyzed by collecting the flower at different stages and observed under the dissection microscope. The observations were based upon the spatial and temporal arrangement of mature male and female sexual parts within the flowers. Morphology of the pollen grains was studied through acetolysis method. Pollen-ovule ratio was worked out according to the method suggested by (Cruden, 1977).

Pollen viability was assessed by 2,3,5-triphenyl tetrazolium chloride (TTC), Di Amino Benzidine and FCR test (Shivanna and Rangaswamy,1992 ; and Dafni 2005). Pollen germination was studied by *in vitro* with different concentration of sucrose in modified Brewbaker's medium in order to analyze the viability at different time period. The male maturity was assessed at the period of pollen maturation and anther dehiscence and female maturity as stigma receptivity during which stigma evolves bubbles of oxygen when submerged in hydrogen peroxide.During the flowering period in general and at the time of anthesis in particular, the plant was continuously monitored in day and night to record the different pollinators visiting the flowers. The foraging behavior of floral visitors were analyzed by photography and visual observation through high resolution binoculars. Pollination efficiency of different pollinators was checked by observing the viable pollen load on different body parts of insects under a microscope. After each visit of the pollinators, the marked stigmas were observed carefully by hand lens (40x) and confirmed the viable pollen transfer to the stigma by various tests. The foraging period and the type of food collected by different pollinators/visitors were also recorded by close observation. Several aspects of pollinators behavior *viz*., contact with the male and female parts of the flower, length of visit, foraging and the number of flowers visited by the individual pollinators were recorded.

Different breeding experiments including open pollination, autogamy, geitonogamy and xenogamy were carried out during the three subsequent years with three flowering seasons. Open pollination (Control): flower buds were tagged and observed the fruit set. Autogamy: mature flower buds were tagged and bagged with a cloth mesh bag and fruit set at maturity was recorded. Geitonogamy (manual self pollination) : mature flower buds were tagged and bagged, the buds upon opening were hand self pollinated with the pollen collected from the flowers of the same plant, re-bagged and observed the fruit set. Xenogamy (manual cross pollination): mature flower buds were tagged and bagged, the buds upon opening were hand cross pollinated with pollen collected from flowers of 2 or 3 different plants and then re-bagged and fruit set observed.

Fruit initiation, development, maturation and dispersal of seeds from the mother plant were carefully observed. The seeds were collected and preserved for further studies. The viability of the seeds was analysed as per the method suggested by Enescu (1991). The seeds were allowed to germinate in the natural habitat (1×1 m plots). They were also collected and stored under laboratory conditions and allowed to germinate in plastic trays filled with garden soil.

Results

Impatiens phoenicea was discovered and described by Beddome in 1958 and also subsequently included in his Icon (1868-1874). It grows as undergrowth in high altitude sholas of Anaimudi and Palni hills above 1500m. The extent of occurrence was estimated both in Anaimudi and Kodaikkanal hills as 50 km^2 and 20 km^2 respectively by taking into account of maximum distance between the populations. The area of occupancy was to less than 2 km^2 and the mature individuals were ± 350 in both the distributional areas. By considering the above causal factors, the species was already be treated under endangered category (EN) by the Botanical Survey of India but the present study suggested that it should be included under the category of Critically Endangered (CR). The description of the species is given below. The present investigation was carried out in three selected populations in Kodaikanal during 2012-2015.

Description of the plant

Herbs, suffruticose, erect, some time prostrate slightly, 1-1.5m high, branched, glabrous with runner roots growing in shady places (Fig.1 A). Leaves alternate, lanceolate or ovate, 5-6 × 3-3.5cm, attenuate at both ends, bristly crenate-serrate, incurved, dark green, shining; petioles 1.5-2.5cm long. Inflorescence recemes, axillary; peduncles solitary, longer than leaves, slender, erect; bracts at base of pedicels, ovate, cordate, persistent. Flowers 2.5cm long, bright scarlet red with yellow in center (Fig. 1B). Lateral sepals ovate, acuminate. Lip with spur blood crimson, trumpet shaped, incurved with swollen tip, 2-4cm long. Standard broad, ovate, scarlet. Lateral petals red with yellow at base; basal lobes cordate, overlapping the distal lobes, oblong. Anthers 5, coherent at apex. Ovary superior, 5 celled; ovules 5-7; placenta axile. Capsules erect, seeds compressed, furrowed, 3mm long, light brown (Table 1).

Floral phenology

In *Impatiens phoenicea,* vegetative buds begin to appear during May with the onset of South West monsoon. Each new branch produces 12-15 leaves. The new inflorescence primordia appears in the first week of July. It starts flowering in the third week of July and extend up to December with maximum bloom in October. The average life span of individual flower is 3- 4 days. The plants were in flower for a total of 180 days in a year. The lower most bud of the inflorescence is resupinating with 5-6 days from initiation. The flower bud attains its original position by the turning of 180^0 and bloom normally. The actual mechanism of resupination is still unknown and it may due to physiological process.

The inflorescence is an axillary raceme and distributed all most all the leaf axis during peak flowering season. Each plant produces on an average of 13±3.2 inflorescences and each inflorescence produces about 7-9 flowers. The flower buds took 9-12 days from initiation to full bloom. At the peak flowering time, 10.84± 1.68 flowers open in each day per plant in the early morning between 0330-0740h. Anther dehiscence was observed one day before flower opening which in turn confirmed the protandrous condition of the flowers. The fruiting period starts from August and extended up to January. The development of fruit is completed within 15-18 days after pollination (Fig.1C).

Pollen viability

Pollen grains are ovate or rectangular. An average length and breadth of the pollen grains was 38.36 ± 3.37µm and 24.7 ±4.32µm. Each flower produces 5 stamens and an ovary with five cells. The mean number of pollen grains and ovules per flower were 28860 ±21.14 and 14 ±1.09.

Hence, the pollen–ovule ratio had been worked out as 1990:1 (Table.1). The floral characters and protandrous nature favors cross-pollination. The acetocarmine-glycerin staining technique revealed that 68% pollen grains were fertile. Pollen viability test by Diamino benzidine (DAB) confirmed that pollen from freshly dehisced anthers showed 72% viability. Pollen viability was also assessed by Fluorochromatic reaction (FCR) test and 2, 3,-5 triphenyl tetrazolium chloride (TTC) test and both tests confirmed that 66% and 68% pollen grains were viable respectively on the day of anthesis and gradually loses its viability on successive days (Fig.1D). Only 2-9% of pollen grains were viable on the third day of anthesis when stigma become receptive. *In vitro* pollen germination studies using sucrose medium at different concentrations revealed that 63% of pollen grains were germinated and attained the tube length of 579± 0.240µm in 10% sucrose medium. Germination percentages were significantly low in higher concentration of sucrose medium. When the medium contains sucrose (10%) and boric acid (200µg/l) about 36% of the pollen grains were germinated with the tube length of 327±0.713µm. The pollen germination in different concentrations (25-200 µg/l) of $CaNO_3$ revealed that 42% of the pollen grains were germinated with an average tube length of 464±0.276µm in 100mg/l of Ca NO_3. The highest percentage of pollen germination of 69% along with 928±0.467µm tube length was noticed in Brewbakers medium after 4hrs of incubation.

Table 1.
Floral characters of I. phoenicea

Sl. No	Floral characters	Observation
1	Flowering period	July- December
2	Flower type	Zygomorphic
3	Flower colour	Scarlet red
4	Odour	Mild fragrance
5	Nectar	4.1 ±1.43µl
6	Anthesis time	0330–0740 h.
7	Anther dehiscence time	One day before anthesis (protandrous)
8	Mean no. of anthers/ flower	5
9	Mean no. of pollen grains/ flower	28860± 21.14
10	Mean no. of ovules / flower	14± 1.09
11	Pollen – Ovule ratio	1990:1
12	Pollen type	4- colpate
13	Pollen size	38.36±3.37 × 24.7 ±4.32 µm
14	Stigma type	Wet and non-papillate
15	Fruit type	Capsules

Stigma receptivity and in vivo pollen germination

The stigma receptivity was assessed by visual observation and by carrying out hand pollination experiments. Stigmas at different stages of maturity starting from 24hrs before anthesis to 48hrs after anthesis were processed for studying pollen germination. The star shaped stigmatic lobes spread and expose their receptive surface after shedding of androecium .Maximum percentage of pollen germination was observed in stigmas of 48hrs after anthesis and this stage also showed maximum number of pollen tubes entering into the stigma (Fig.1E). The stigma continued to remain receptive for another 24hrs beyond which the receptivity declined. On the basis of these studies, flowers that showed good stigma receptivity (48hrs after anthesis) were used for all manual pollination experiments. In *I. phoenicea,* the flowers open in the early morning between 0330-0740h. The stigma is wet and non-papillate type. Non specific surface stigma esterases were observed on the stigma mainly on the stigmatic lobes and slightly on the stigmatic head. Significant increase of esterases was observed after 48hrs of anthesis. The presence of adequate esterases over stigma surface is coincides with its receptivity.

Manual *in vivo* xenogamous pollination on receptive stigmas was carried out and the pistils were processed for studying details of pollen germination and tube growth. The pistils of 48 hrs after pollination were processed for studying pollen adhesion and germination through Scanning Electron Microscopy (SEM) (Fig.1F). There was no pollen adhesion and germination on the stigmas in flower buds 24hrs before anthesis. Stigmas of 48hrs after anthesis supported pollen adhesion as well as germination. Maximum percentage (45%) of *in vivo* pollen germination and tube elongation (631µm) was noticed after 48hrs of anthesis. The percentage of stigma receptivity and *in vivo* pollen germination gradually decreased after 60hrs of anthesis. The pollen tubes were confined to stigma for 3-4hrs

after pollination and then entered the pistillate tissues. Pollen tubes reached the base of the pistil 6hrs after pollination and entered into the ovules around 8hrs and subsequently fertilization takes place. The fertilized ovules developed into seeds.

Pollination biology

In *Impatiens phoenicea*, the flowers are very attractive due to scarlet red in colour with small amount of nectar (4.1±1.43µl). Natural pollination is effected by honeybees and butterflies. Most of the flowers bloom in the early morning between 0330-0740h and remain fresh till in the evening. Honeybees are the most important pollinators and they visit more flowers than any other pollinators. They forage for both pollen and nectar and carried the pollen loads on the head, thorax and hind legs and thereby transferred the pollen grains from one flower to another during the day time (0630-1300 h). Honey bees are entering into the lip region and collecting the nectar. During foraging, they transfer the pollen grains from one flower to another of the same plant or flowers of different plants within and between the populations (Table 2).

Butterflies were active during day time and spent 2-4 seconds in each flower. The butterflies play an important role in the pollination of *I. phoenicea*. During day time and in fine weather four species of butterflies were actively foraging. The butterflies effectively transferred the pollen grains from one flower to another by touching the stigma with its head while collecting nectar from the lip region. Butterflies land on the two wing petals, slightly bend the body and insert the proboscis in the long and deep saccate lip regions for nectar. During the nectar harvesting, pollen grains stick on the head of the butterflies and transferred the pollen grains to the stigma.

All insects are pollen carriers and their frequent inter plant movement facilitates cross-pollination. However, their overall visits are not enough to pollinate all the flowers in the populations. It is corroborated by the fact that only 42 insects are visiting nearly 1,000 flowers per hour.

Table 2
ollinators and their foraging behaviors in Impatiens phoenicea

Sl. No.	Visitors	Visiting time	Foraging nature	Foraging hours	Percentage of visit	M.N.I.V	Stigma touch
1	*Apis cerana indica*	Day	Nectar & pollen	0630-1300	29.10	12.32 ± 1.08	++
2	*Apis dorsata*	Day	Nectar & pollen	0900-1200	23.25	9.84±1.20	+++
3	*Mycalesis mineus*	Day	Nectar	0730-1100	15.45	6.54±0.97	++
4	*Rpthima baldus*	Day	Nectar	0600-1400	12.80	5.42±0.54	++
5	*Hasora chromus*	Day	Nectar	0800-1200	9.80	4.15±0.99	++
6	*Badamia exclamationis*	Day	Nectar	0900-1600	5.63	2.38±0.28	++
7	*Delias eucharis*	Day	Nectar	0700-1200	3.97	1.68±0.54	++

M.N.I.V- Mean no. of individuals visited per hour/ Population- : + - Poor, ++ - Good, +++ - V. good

Breeding systems

Details of breeding systems were studied under controlled field pollination experiments and subsequent observation on fruit and seed set. Three types of manual field pollinations were carried out such as autogamous self, geitonogamous and xenogamous. Fruit set was not observed in autogamous self- pollination. The percentage of fruit set under open pollination was (35%) markedly lower than that of manual pollination. The geitonogamous and xenogamous pollination (within the population) have resulted in 33% and 38% of fruit set out of 100 flowers. The artificial cross-pollination experiments were conducted with the following distinct populations

Test 1 (T_1) - Bombay Shola × Vattakkanal Shola

Test 2 (T_2) - Vattakkanal Shola × Mathikettan Shola

Test 3 ($T_{3)}$ - Mathikettan Shola × Bombay Shola

Among the three xenogamous experiments, the cross between the populations of Bombay Shola× Vattakkanal Shola produced 33 fruits (66%) out of 50 flowers pollinated. The cross between Vattakkanal Shola × Mathikettan Shola and Mathikettan Shola × Bombay Shola produced 28 (56%) and 31 fruits (62%) respectively(Table 3). The mean number of seeds per capsule in xenogamous pollinations (at different cross) was 8-9. The seed setting percentages were ranging from 51.1 -66.1%. The average weight of individual seeds produced by natural pollination and xenogamous pollination was ± 0.4 and ± 0.6mg respectively.

Table 3
Fruit set in different modes of pollination in Impatiens phoenicea

Sl. No.	Breeding experiments	No. of Flowers pollinated/ observed	No. Flowers sets fruit	% of fruit set	Mean No. of seeds/ capsule	% of Seed set
1	Open pollination	100	35	35	7.3 ± 1.26	52.10
2	Autogamous self pollination	100	0	0	0	0
3	Geitonogamous pollination	100	33	33	7.7 ± 1.49	56.4
4	Xenogamous pollination within the population	100	38	38	8.04 ±1.48	57.4
5	Bombay Shola× Vattakkanal Shola	50	33	66	9.25 ±0.98	66.1
6	Vattakkanal Shola × Mathikettan Shola	50	28	56	8.65 ±1.12	61.8
7	Mathikettan Shola × Bombay Shola	50	31	62	9.01 ±1.87	64.4

Fruit and seed biology

The fruit development took 15-18 days after pollination. The average length of the ovary at the time of pollination is ± 0.8cm. The ovary showed slight enlargement after 5-7days of pollination and attains its maximum size 1.7cm after 15-18 days of pollination. When the capsule matured, the fruit wall ruptured and the seeds ejected from the mother plant to a distance of 1.3-1.5m. Average number of ovules developed from each ovary was 14 and the mean number of seeds developed from each ovary was only 7. The ovule-seed ratio was 2:1, which clearly indicates that only half of the ovules get fertilized and developed into seeds. Poor fruit set may be due to various reasons such as scarcity of pollinators, insufficient pollen transfer to the stigma during the receptive period, ovule abortion etc.

Undehisced mature capsules were covered with paper bags and the seeds were collected. Percentage of moisture content at the time of seed dispersal was 67% and it was gradually reduced after 1-4months and stabilized up to 15 months. The viability test confirmed that 68% of the seeds were viable at the time of capsule dehiscence and thereafter the viability reduced on successive months. The seeds were maintaining its viability up to 15 months. The seed germination studies confirmed that the seeds of *I. phoenicea* exhibits dormancy period to overcome the dry months of January to May. Freshly harvested seeds show no germination. The germination of the seeds was only 28% in both natural habitat (1 x 1m plot) and in laboratory conditions of 50 x 50cm plastic trays filled with sand and garden soil after 10-12 months of harvest. However, the seeds were losing their viability on successive months. The seedling emerged in the month of June and continued up to July. Due to heavy monsoon coupled with humid climate the seedlings were affected by damping off disease which reduces 80% of the total seedlings emerged during south west monsoon. Further, the seedlings were heavily browsed by herbivores which reduce the establishment further. The above reasons were responsible for poor seedling recruitment in natural condition.

Discussion

The studies on reproductive biology of angiosperms have received much attention in recent years (Bawa *et al*., 1985; Shukla and Pandey, 1991) but little attention has been paid on endangered species (Aspinwall and Christian, 1992; Sreekala *et al*., 2008; Ramasubbu et al., 2011). Detailed information on the reproductive biology of RET plants is essential for developing effective strategies for their conservation and sustainable utilization. Some of the results emerging from the present work are discussed in the following pages in the light of available literature. Phenology is the study of phenol-events which are critical for the survival and reproduction of plant species (Rathcke and Lacey, 1985). Therefore, the detailed information regarding the phenology is a prerequisite for the studies on the breeding systems and silviculture practices. Community based phenological patterns have been already explored by several workers whereas population based phenological studies are rather limited despite the fact that such evens have direct bearing on the survival and establishment (Primack, 1980; Bawa *et al*., 1990).

The genus *Impatiens* L. is represent the true balsam and their members are most fascinated with peculiar flower structures and spectacular colours. Knowledge on phenology and floral morphology is essential for conducting studies on pollination biology and breeding systems. About 62% of *Impatiens* species growing in the Western Ghats flower in July-December, 16% during April-June and 15% between January- March. Interestingly 18% of the *Impatiens* flowers throughout the year if conditions are favourable (Pandurangan and Sreekala, 2008; Rajalal *et al*., 1996). In the present study, the candidate species starts flowering in the month of July, extended up to December and reaches a peak during October. Anthesis commenced in the early morning between 0330-0740h. Bhaskar and Razi (1974) had already reported that, majority of the wild *Impatiens* growing in the high altitude areas are night blooming and have wide range of timing with regard to pollen germination. The anther dehiscence takes place one day before flower opening which in turn confirmed the protandrous condition of the flowers. This observation was similar to that of *I. reptans* in China (Tian *et al.,* 2004) and *I. henslowiana* (Sreekala *et al*., 2007).

Knowledge on floral morphology is essential for the studies on breeding systems particularly the pollination biology. In *Impatiens,* the morphology, size and shape of the flowers vary between the species, but the breeding system of the species is almost same (Grey Wilson, 1980). The number of pollen grains varies between the species. The pollen grain number is to be positively related to ovule number which reflects the pollination behaviour of the species and this relationship was observed across families (Cruden and Miller-Ward, 1981) and within families (Kirk and Ghats, 1993). The variation in pollen grains and ovule number may occur within the individuals or among populations. There is a strong correlation between pollen-ovule ratio and breeding systems. Pollen viability is a critical for any studies on pollen biology. Treating the pollen grains with non-vital stains such as acetocarmine, aniline blue in lactophenol essentially imparts colours to the contents of the pollen as well as fixed/dead pollen. It may be useful to determine the degree of pollen sterility in plants of hybrid origin or those grown under unfavourable conditions (Alexander, 1980). From time to time, pollen viability tests have been fine tuned and constantly upgraded. In the present investigation, 2, 3, 5- triphenyl tetrazolium chloride (TTC) test, diamino benzidine (DAB) test and Fluorochromatic reaction (FCR) test were used for assessing pollen viability. *In vitro* pollen germination test was also incorporated in the present study. In many plants, this test shows correlation with fruit set and seed set (Akihama *et al*., 1978). In the present investigation, maximum pollen viability was noticed on the day of anthesis but it gradually decreased on successive days. This observation was similar to that of *I. reptans* in China (Tian *et al.,* 2004).

In *I. phoenicea*, the stigma and style are not much differentiated and the small portion above the ovule is normally considered as style. The stigma is wet and non-papillate and having five stigmatic lobes. The stigma becomes receptive only after the shedding of androecium. At that time, the coherent stigmas spread and the star shaped receptive surface is exposed. The stigma receptivity starts after 2 days of anthesis and continued its receptivity up to 24hrs. The quantity of stigmatic exudates is very less on the day of anthesis and gradually increases in two days after anthesis. The presences of esterases become more in stigmas after anthesis which often coincides with more receptivity (Stone *et al*., 1995; Bhattacharya and Mandal, 2004). Thus, there may have been correlation between esterase location and stigma receptivity of selected species. The present investigation is in accordance with findings of Baker *et al*., (1974), Ghosh and Shivanna (1984) and Shivanna and Owens (1989) who showed that

there is a positive correlation between presence of esterase and stigma receptivity.

The balsams are highly evolved members among the order Geraniales as evident from the marked zygomorphic flowers and nectiferous spur. The arrangement of stamens and its protandry nature, pistil and spur are markedly adopted for cross pollination (Bhaskar and Razi, 1974) and hence most of the species of *Impatiens* reproduce by means of cross pollination including *I. phoenicea*. It is well known that the flowers of *Impatiens* have enormous diversity in structure, shape, colour etc., which attract different pollinators. In the present study, the selected species of *Impatiens* are pollinated by honey bees and butterflies etc. In different climatic regions, species of pollinators are also varying. In subtropical regions of Africa, the *Impatiens* species are pollinated by humming birds as well as insects. In temperate zones, pollinators are bumble bees and humming birds (Rust, 1977, 1979; Heinrich, 1979). In Sumatra and Japan, hawk moths and honey bees are major pollinators of *Impatiens* (Kato, 1988; Kato *et al*., 1991).

Breeding system includes all aspects of sex expression that effect the relative genetic contribution to the next generation of individuals within the species (Wyatt, 1983). Flowering plants display a wide variety of breeding systems from strict outcrossing to strict autogamy (Bawa and Beach, 1981). The breeding systems influence the genetic structure of the populations (Richards, 1986). The evolution of a particular breeding system is shaped largely by the dynamics of pollination mechanisms.

In *I. phoenicea* both geitonogamy and xenogamy were successful. The percentage of fruit set in artificially cross pollinated flowers are higher than that resulting from natural pollination, which indicates that some external agents are required for successful pollination. This observation was similar to that of *I. henslowiana* (Sreekala *et al*., 2007). Out crossing is very significant in the pollination biology of *Impatiens* which ensures better seed output.

The fruit development took 12-18 days for attaining maturity after successful pollination. In *I. reptans* in China, the distribution of seeds from the mother plant was between 0.50-1m only. Most of the seeds were dispersed around the mother plant and some of them were deposited into the stream (Tian *et al*., 2004). Therefore, the percentage of germination of these seeds was low in natural condition. It has small seeds which are exarillate and exalbuminous. Seeds of *I. coelotropis* and *I. platyadena* remain viable for 9-11 months. Seed viability could not further improved by storing them under low temperature (Ramasubbu 2010). The seeds of *I. phoenicea* remain viable 5-7 months under laboratory conditions. They do not germinate during the ensuing monsoon and loses its viability completely by the next season. In the present study, the percentage of seed germination was only 10-30%.

Conclusion

In *I. phoenicea,* 35% fruit set was observed in open/natural pollination. The percentage of capsules developed through xenogamy and geitonogamy was 38% and 33% respectively. Manual xenogamous pollination between populations enhanced the fruit set rate up to 66%. The flower-fruit ratio was also very low. The extent of occurrence is restricted to less than 20km^2 in Kodaikanal and hence, the availability of distinct population is a limiting factor for gene flow which greatly affects the sexual reproduction. Scarcity of pollinators, low insect visitation rate and fragmentation of populations are adversely affecting the fruit set in the natural habitat of *Impatiens phoenicea*.

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Figure-1:Reproductive dynamics of *Impatiens phoenicea*