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**Original** Article

## Pollination ecology of *Rhynchosia beddomei* Baker (Fabaceae), an endemic medicinal shrub in the southern Eastern Ghats, Andhra Pradesh, India

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## Abstract

*Rhynchosia beddomei* is a deciduous endemic shrub. The present study on the pollination ecology of this shrub provided information to understand its endemic status. The study showed that the flowers are hermaphroditic with explosive pollination mechanism, self-compatible, facultatively xenogamous and melittophilous. The flowers not visited by bees fall off while those visited and pollinated by them set fruit. The beetle, *Callosobruchus maculatus* causes pod infestation. Pods dehisce explosively to disperse seeds, which germinate during rainy season. But their growth is suppressed due to soil moisture deficiency amplified by nutrient deficient rocky terrain. Therefore, this shrub is unable to populate effectively. Leaf cut during leaf flushing time by locals for medicinal purposes is an additional factor affecting the vegetative growth, flowering and fruiting rate of this shrub. Its use for traditional medicine needs to be regulated in order to conserve and manage the existing population.

Keywords: Rhynchosia beddomei, hermaphroditism, facultative xenogamy, melittophily, pod infestation, seed dispersal

## 1. Introduction

*Rhynchosia* L. commonly known as snout bean, is a member of the legume family Fabaceae, Tribe Phaseoleae and Subtribe Cajaninae, a group closely related to beans (*Phaseolus*), pigeon peas (*Cajanus*) and grams (*Vigna*) (Jayasuriya, 2014; Lackey, 1981). The genus consists of approximately 200 species and occurs in warm temperate and tropical regions of both the western and eastern hemispheres. In the western hemisphere, it is distributed in the Americas from Greater Antilles and Mexico to northeastern Argentina, while in the eastern hemisphere it is distributed in Egypt, China, Japan, North Korea, Afghanistan, Iran, India, Pakistan, Vietnam, Saudi Arabia and Turkey (Grear, 1978). In India, the Seshachalam hills of southern Eastern Ghats in Andhra

Pradesh has twelve species of this genus. They include R. beddomei, R. rufescens, R. suaveolens, R. cana, R. albiflora, R. capitata, R. courtollensis, R. densiflora, R. heynei, R. minima, R. rothii, R. rufescens, R. suaveolens and R. viscosa. These species are either climbers or shrubs (Madhava Chetty, Sivaji, & Tulasi Rao, 2008). Of these, R. beddomei is a rare and endemic medicinal species and restricted to a few areas such as Talakona, Japalitheertham, Gogarbham in Sesha-chalam hills of Chittoor District, and Andhra Pradesh (Padmavathi, David, Rao, & Rama Gopal, 2012). Nair and Sastry (1998) also documented that this plant is distributed in Seshachalam hills of Eastern Ghats in Andhra Pradesh, India. Pullaiah (2006) reported that this plant is distributed in parts of Kadapa, Chittoor and Anantapur districts of Andhra Pradesh. In Chittoor district, it is common in Talakona forest and Japaliteertham, Gogarbham area, Sandralamitta and near deer park of Tirumala hills. Sudhakar Reddy, Reddy, Pat-tanaik and Raju (2006) mentioned that its present status is "vulnerable" in the IUCN Red Data Book of Indian plants, mainly because of its restricted distribution in Tirumala hills

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and small number of individuals left in the world. Prasad and Narayana Swamy (2014) reported a new species, *Rhynchosia ravii*, which is closely related to *Rhynchosia beddomei*. It has a small population in the spurs of the dry deciduous forest with a grassy under-storey of the southern Eastern Ghats in Ananthapuram district and Mangapatnam area in Kadapa district, Andhra Pradesh. In vegetative condition, the two species are morphologically similar, but *R. ravii* is distinguishable by the presence of glandular hairs. The display of the dense, short, fine indumentum of greyish white hairs on all the vegetative parts in both these species has led the taxonomists to treat both as *R. beddomei*.

Several authors have reported that the *Rhynchosia* species studied by them are self-fertile, but out-crossing is promoted by a valvular pollination mechanism. Further, *Rhynchosia* sp. is primarily pollinated by *Hypanthidium* sp. and *Centris* sp. in Brazil and by *R. sublobata* by *Xylocopa* bees in Zambia (Craufurd & Prins, 1979; Etcheverry *et al.*, 2011; Franco, 1995). There is no further information available on the reproductive ecology of all the other *Rhynchosia* species.

Madhav Chetty et al. (2008) reported that R. beddomei is useful for certain medicinal purposes, as abortifacient, anti-bacterial, anti-fungal, anti-diabetes and hepatoprotective. Rama Rao and Henry (1996) noted that the leaves of R. beddomei are used for wounds, cuts, boils and rheumatic pains by the Adivasi tribes (Sugali, Yanadi, Erukala) inhabiting the forests of Eastern Ghats of Andhra Pradesh, India. Gunasekar (1980) noted that this plant contains flavor-noid compounts, such as flavones, flavonols and flavanones. Bakshu and Venkata Raju (2001) mentioned that the leaves of this plant possess significant antimicrobial activity. These various medicinal uses by locals might have led to the endemic status of this shrub. Since the plant is now endemic in the southern Eastern Ghats, it is important to study its pollination ecology to understand its reproductive aspects and use that knowledge to take measures for the conservation and management of this shrub in its natural area. The aim of this study was to clarify the flowering phenology, floral biology, sexual system, breeding system, pollination mechanism, pollinators. and fruiting ecology. Further, it also aimed at understanding the role of floral rewards in attracting and promoting insect visitation rate, and pollen carryover capacity of insects to evaluate their relative importance as pollinators. Lastly, the impacts of flower predation and fruit infestation rates on the success of sexual reproduction are addressed.

## 2. Materials and Methods

## 2.1 Study site

The population of *Rhynchosia beddomei* located in the Seshachalam Hill Range in the southern Eastern Ghats of Peninsular India (13°40'N latitude and 79°19'E longitude, and 2,443 ft altitude) was studied during June 2014-June 2016.

# 2.2 Phenology, floral morphology, floral biology and pollinators

Field trips were conducted to record phenological aspects. Ten virgin inflorescences were tagged and followed daily to record the life span, anthesis schedule and anther

dehiscence. Twenty-five fresh flowers were used to record the floral morphological details. The protocols mentioned in Dafni, Kevan and Husband (2005) were used to record nectar volume, sugar concentration, sugar types and the sugar content per flower, pollen output, pollen-ovule ratio and stig-ma receptivity. Mean  $\pm$  s.d of ten flowers each for nectar volume and sugar concentration and pollen output are re-ported. Insects foraging at the flowers were observed through-out the day on four days for their foraging behavior and pollination potential. Specifically, foraging visits of insects were recorded for fifteen minutes at each hour throughout the day on each of the four days, and the percentage of foraging visits made by each insect species and the percentage of foraging visits of each insect category were calculated. Ten specimens of each insect species were captured during peak foraging period, brought to the laboratory, washed in ethyl alcohol, stained with aniline-blue on a glass slide and ob-served under microscope to count the number of pollen grains present. From this, the average number of pollen grains carried by each insect species was calculated to estimate the pollen carryover efficiency. A sample of 500 flowers collected randomly from twenty plants was used to record the flower predation rate by the Chrysomelid beetle, Callosobruchus maculatus.

#### 2.3 Breeding experiments

Five flowers each on ten plants were used for autogamy, geitonogamy and xenogamy. The flowers were finemesh bagged without hand pollination for autonomous autogamy. The flowers were pollinated with the pollen of the same flower manually and bagged for autogamy. The emasculated flowers were hand-pollinated with the pollen of a different flower on the same plant and bagged for geitonogamy. The emasculated flowers were pollinated with the pollen of a different plant and bagged for xenogamy. Seventy virgin inflorescences consisting of four hundred eighty seven flowers on twenty plants were tagged and exposed to insect activity for open-pollinations. All these categories of polli-nations were followed to record fruit set rate in each mode. Four hundred and fifty fruit were randomly collected from twenty-five plants to record fruit infestation rate. Fruit maturation period, dehiscence and seed dispersal aspects were observed from the initiation of fruit formation to seed dispersal time at 2-day intervals in the field. Further, field observations were continued at one-week intervals to record seed germination and seedling establishment aspects.

## 3. Results

## 3.1 Phenology

It is an erect, perennial shrub, 1.5 m tall with tomentose branchlets that grows in rocky areas with red soil. The plant re-grows from the perennial rootstock and the seed during wet season from July to November. The flowering occurs during December-March with a peak in January. The plants wither away in April. At plant level the flower production rate gradually increased from the 1st week of December to 2nd week of January and decreased gradually towards the 2nd week of March (Figure 1). The flower output per plant averaged 8,415, out of which 33% was recorded in December, 56% in January, 10% in February and 1% in March (Table 1). The flowers are borne in pedunculate axil-lary and terminal racemes, which anthese for 3-5 days pro-ducing 5-8 flowers ( $5.24 \pm 2.1$ ); they remain open for 3 hours only.

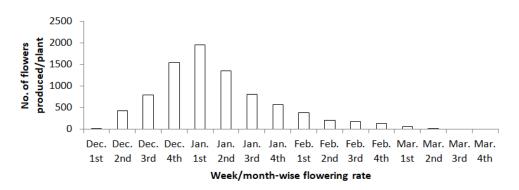
#### **3.2 Flower morphology**

The flowers are small (9.8  $\pm$  0.4 mm long and 8.3  $\pm$ 0.4 mm wide), yellow, odorless, papilionaceous, zygomorphic and bisexual. The calyx is green with purplish tinge and consists of 5 free oblong, obtuse sepals; the upper two sepals are longer than the lower 3 sepals. The corolla is bright yellow, pubescent and papilionaceous. The standard petal is large, obovate with reddish-brown lines at the bottom of the midregion, which serves as nectar guide; the petal base is clawed and consists of two inflexed fingernail auricles. This petal envelops the rest of the petals in bud but reflexes when the flower blooms. Two adjacent petals called wing petals surround the two bottom petals, called keel petals. The keel petals form a proximal cylindrical part and a distal part consisting of a pressed angular pouch, with an acute porate tip in which the stamens and stigma are housed. The keel and the wing petals are attached by means of two notched folds. The stamens are ten and diadelphous (Figure 4e). The distal parts of the filaments are free and tipped with uniform dithecous anthers. The ovary is sessile, green, villous and lies in the sheath of the filaments along the cylindrical part of the keel. It is monocarpellary and monolocular with a single ovule arranged on marginal placentation (Figure 4g). It has a long

glabrous style with a capitate wet shiny stigma, which is situated slightly above the anthers. The distal portion of free filaments and style and stigma are incurved and clamped into the keel petals.

#### 3.3 Floral biology

Mature buds open during 1200-1500 h (Figure 4a,b). Unfolding of the standard petal and wing petals indicates flowering opening. The keel petals do not unfold and remain in their original position as in mature bud stage (Figure 4c and d). All anthers in a flower dehisce synchronously by longitudinal slits in mature bud stage. The pollen output is  $728 \pm 38.44$  ( $\overline{x}$  $\pm$  s.d.) per anther and 7,280 per flower. The pollen-ovule ratio is 7,280:1. The pollen grains are monads, spheroidal,  $37.35 \pm$ 4.37  $\mu$ m ( $\overline{x} \pm s.d.$ ) in size, powdery and tricolporate, angulaperturate with reticulate exine (Figure 4f). A nectariferous disc is present at the base of the ovary. The nectar secretion begins during mature bud stage and ceases an hour after anthesis. A flower produces  $1.6 \pm 0.26 \,\mu l \,(\bar{x} \pm s.d.)$  of nectar with 0.54 mg of sugar; the sugar concentration is 30% (Range 29-34%) consisting of sucrose, glucose and fructose with the first as dominant. Nectar is deeply concealed and it is open through the windows between the free filaments at the flower base. The stigma is receptive during anthesis and remains so for about three hours. After three hours of anthesis, the standard, wing and keel petals gradually move close to each other enclosing the reproductive organs. The closed flowers remain so even during most part of the fruit development. The calyx initially encloses the ovary and subsequently turns light brown and discloses the ovary since the latter gradually bulges and develops into a seeded pod.



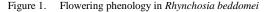


Table 1. Flower production rate at plant level in Rhynchosia beddomei

Week of observation	December	January	February	March
1st week	$15.7\pm2.12$	$1945.4 \pm 32.51$	$379.8 \pm 5.28$	$59.8 \pm 1.72$
2nd week	$421.3 \pm 12.81$	$1356.1 \pm 8.16$	$210.2 \pm 4.79$	$19.2\pm0.91$
3rd week	$789.7 \pm 3.61$	$801.4 \pm 4.14$	$169.6 \pm 7.68$	
4th week	$1536.2 \pm 21.73$	$578.3 \pm 3.21$	$132.1 \pm 23.71$	
Total flowers produced	2762.9	4681.2	891.7	79.0

Values are given as  $\overline{x} \pm s.d.$ 



Figure 4. Rhynchosia beddomei: a. & b. Mature buds, c. Stamens and Stigma housed in keel petals, d. Floral parts, e. Diadel-phous stamens and capitate stigma placed above the height of anthers, f. Ovule, g. Thrips, h. Apis dorsata, i. Apis cerana, j. Apis florae, k. Nomia sp., I. Xylocopa pubescens, m. Popillia impressipyga, n. Fruit with exit hole drilled by Callosobruchus maculates, o. & p. Explosive Fruit dehiscence, q. Seeds.

## 3.4 Pollination mechanism

The reproductive column is held under pressure within the keel part in open flowers and it is exposed when the pollinator presses against the wing and the keel petals. When insects land on the wing petals, the latter cause the keel petals to release the reproductive column explosively. Consequently, the reproductive column snaps forward against the standard petal causing most of the pollen to be instantly released and the pollen thus released comes into contact with the ventral side of the insect body. Since the incurved stigma is situated above the height of the anthers, it strikes the insect body first due to which cross-pollination occurs if the insect visited the other flowers previously and carried pollen on its ventral side, and also then the pollen ejected from the anthers powders the ventral side of the insect instantly. With the departure of the insect, the keel moves forward partly covering the stamens and stigma. The downward movement of keel petals occurs with each subsequent foraging visit by appropriate insects. Tripping of keel boat can also occur due to heavy rain or high temperatures that weaken turgidity of the restraining keel tissues. In flowers with un-tripped keel petals, the reproductive column remains inside and falls off without fruit set.

## 3.5 Breeding systems

Fruit set is absent in autonomous autogamy. It is 18% in hand-pollinated autogamy, 34% in geitonogamy, 82% in xenogamy and 34% in open-pollination (Table 2). Individual inflorescences produce  $3.42 \pm 1.2$  fruits, which account for 46% of the average number of flowers produced.

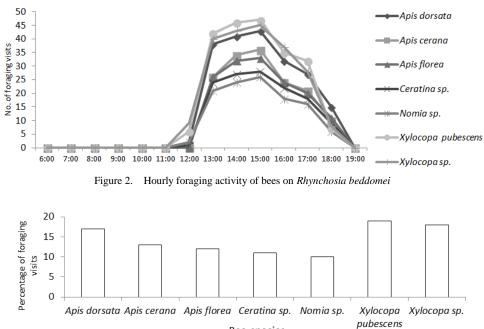
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Pollination mode	No. of flowers pollinated	No. of fruits formed	Fruit set (%)
Autogamy (un- manipulated and	50	0	0
bagged) Autogamy (hand- pollinated and bagged)	50	9	18
Geitonogamy	50	17	34
Xenogamy Open-pollination	50 487	41 167	82 34

#### 3.6 Bee pollinators and pollination

Thrips were found to use buds for their breeding. They had access to nectar only during bud phase due to the concealment by keel petals. They remained inside the flowers even after anthesis because the standard petal locked the nectar windows. Thrips departed from the flowers with tripped keel petals through nectar windows; they came out from flowers with un-tripped keel petals only when the latter withered. They fed on both nectar and pollen. Thrips depleted the nectar volume compelling the bees to visit and pollinate many flowers promoting cross-pollination rate.

With the initiation of anthesis from 1200 h onwards, bees began to visit the flowers and continued their foraging activity until 1800 h with peak foraging activity during 1400-1500 h (Figure 2). The foraging activity pattern indicated a gradual increase in foraging visits concomitant with the gradual increase in the anthesis rate, and later there is a gradual decrease in foraging visits concomitant with the gradual increase in the number of closed flowers. The bees were Apis dorsata (Figure 4h), A. cerana (Figure 4i), A. florea (Figure 4j), Ceratina sp., Nomia sp. (Figure 4k), Xylocopa pubescens (Figure 4l) and Xylocopa sp. (Table 3). Apis collectively made 42%, Xylocopa 37% and the others 21% of the total foraging visits (Figure 3). The body washings of foraging bees showed that they carried on average maximally 281.1 pollen in the case of X. pubescens, and minimally 102.1 pollen in the case of Nomia sp. (Table 4). The pollen carrying capacity was found to be related to the size of the bee body. The bees landed on the wing petals and the keel, with their head near the standard. They then exerted pressure with legs on the wing petals until these and the keel petals bent downwards, and then proceeded to collect nectar during which the bee's abdomen became pollen smothered. The pollen collecting bees took a U-turn after nectar collection and proceeded towards the stamens to collect pollen. A beetle, Popillia impressipyga, was found to feed on all floral parts and its flower predation rate was 19.6% (Figure 4m).



Bee species

Figure 3. Percentage of foraging visits of individual bee species on Rhynchosia beddomei

Table 3. List of insect foragers on Rhynchosia beddomei

Order	Family	Sub-family	Genus	Species	Common Name	Foraging schedule	Forage collected
Hymenoptera	Apidae	Apinae	Apis Apis Apis	dorsata F. cerana F. florea F.	Rock honey bee Asiatic honey bee Dwarf honey bee	1200-1800 1300-1800 1300-1800	Nectar + Pollen Nectar + Pollen Nectar + Pollen
		Nomiinae	Ceratina Nomia	sp. sp.	Small carpenter bee Alkali bee	1200-1800 1200-1800	Nectar + Pollen Nectar + Pollen
		Xylocopinae	Xylocopa Xylocopa	<i>pubescens</i> Spinola sp.	Large carpenter bee Larger carpenter bee	1200-1800 1200-1800	Nectar Nectar
Coleoptera	Scarabaeidae	Rutelinae	Popillia	<i>impressipyga</i> Ohaus	Flower-feeding beetle	1200-1800	All floral parts

 
 Table 4.
 Pollen recorded in the body washings of bee foragers on Rhynchosia beddomei

	Sample	Number of pollen grains			
Bee species	size – (N)	Range	Mean	S.D	
Apis dorsata	10	260-340	281.1	31.48	
Apis cerana	10	120-321	214.3	97.23	
Apis florea	10	81-286	184.6	82.06	
<i>Ceratina</i> sp.	10	64-197	117.2	49.06	
Nomia sp.	10	47-158	102.1	37.53	
Xylocopa pubescens	10	476-870	643.2	95.2	
<i>Xylocopa</i> sp.	10	321-569	432.1	68.1	

#### 3.7 Fruiting behavior

Fruits mature within three weeks. They are initially green, later brown to dark brown when ripe and dry. They are non-fleshy, hairy, glandular, circular to narrowly oblong and compressed one-seeded pod. Fruit infestation rate is 21.55%.

The infested pods showed different stages of the bruchid beetle, *Callosobruchus maculatus*. Infested pods contained characteristically one larva or pupa anchored to the seed part. This beetle was found to use the floral buds for breeding because the mature pods ready for dehiscence contained exit holes through which adult insects came out (Figure 4n). However, the adult insect pest left the pod before pod dehiscence.

#### 3.8 Seed ecology

Mature and dry fruit with bi-valvate configuration dehisce elastically and explosively to disperse seeds (Figure 40, p). Then, the seeds of completely dry fruit are released into the air but those of incompletely dry fruit remain attached to the fruit wall. The seeds are reddish brown to black, compressed, sub-reniform, glabrescent, and shiny with a prominent strophiole (Figure 4q). The seeds germinate during the rainy season, but their continued growth is regulated by the extent of soil moisture.

## 4. Discussion

*Rhynchosia beddomei* is a deciduous shrub that proliferates by asexual and sexual modes. The flowers display synchronous hermaphroditism or homogamy. Autonomous autogamy does not occur despite self-compatibility, but autogamy is functional through pollinators. It appears that the stigma, although receptive, blocks the germination of the self-pollen while it is in keel petals and hence, it essentially requires the rupture of its surface by a pollinator to allow the self- or cross-pollen to germinate. Such a stigmatic regulatory function appears to have evolved to discourage selfing and promote outcrossing (Lloyd & Schoen, 1992). The fruit set rates in geitonogamy and xenogamy tests indicate that the plant is facultative xenogamous, a breeding system that is flexible and keeps the options open for both selfing and out-crossing mediated by pollen vectors.

*R. beddomei* has typical papilionaceous corolla; the flag petal serves as a visual attractant, wing petals provide landing platform and keel petals protect the entire length of reproductive column. The flowers are typical of pollination by bees since they are zygomorphic, bright yellow with nectar guide, hidden nectar at the corolla base and hidden pollen in keel petals (Faegri & van der Pijl, 1979).

*R. beddomei* flowers have explosive pollination mechanism and deliver pollen directly from the anthers to the bee's body when keel petals are tripped by the foraging bee; this type of pollen delivery is referred to as primary pollen presentation (Howell, Slater, & Knox, 1993). This pollination mechanism is efficient due to commencement of anthesis from noon onwards when the ambient air is dry. Accordingly, the bees also commenced their foraging activity from noon onwards and until the flowers close. The concealment of the stamens within the keel petals until it is tripped is an advantage for the plant to secure the pollen against unusual rains and ambient moisture conditions during winter season (Peter, Dold, Barker, & Ripley, 2004).

Percival (1961) stated that plants with deep-tubed flowers tend to produce sucrose-rich nectar. However, in R. beddomei the flowers are short-tubed presenting sucrose-rich nectar because the nectar is not exposed for the breakdown of sucrose into hexoses. Honeybees prefer those flowers with sucrose as chief constituent of nectar (Kevan, 1995). The flowers pollinated by long-tongued bees produce sucrose-rich nectar (Baker & Baker, 1990). Similarly, R. beddomei with melittophilous pollination syndrome also produces sucrose-rich nectar, which is utilized exclusively by long-tongued bees such as Apis, Ceratina, Nomia and Xylocopa. Bee-flowers tend to produce a small volume of nectar with higher sugar concentration than the nectar of flowers pollinated by other animals (Cruden, Hermann, & Peterson, 1983). Honeybees prefer sugar concentrations of 20 to 40% in the nectar (Waller, 1972), or according to Baker and Baker (1983) from 30 to 50%. R. beddomei produces a small volume of nectar with 30% sugar concentration. Therefore, R. beddomei flowers with explosive pollination mechanism, primary pollen presentation, and hidden nectar and pollen, have evolved to discourage other foragers from visiting the flowers and to ensure that the bees get the floral rewards.

In *R. beddomei*, the keel tripping process is not selfactivated to effect pollination. The flowers depend on bees for tripping the keel petals to trigger explosive pollination. The flowers that were not tripped by external agents subsequently fall off. Of the bees recorded, carpenter bees and rock honey bees, being large in size, were more efficient in tripping the flowers than other bees and hence were the principal pollinators. Other bees, although tripping the flowers and effecting pollination, ranked lower as pollinators.

Cruden (1977) used the pollen-ovule (P/O) ratio as an indicator of the breeding system of plants. He provided P/O ratios for different breeding systems - 168.5 + 22.1 for facultative autogamy, 798.6 + 87.7 for facultative xenogamy, and 5859.2 + 936.5 for xenogamy. *R. beddomei* shows higher P/O ratio than even the xenogamy range assigned by Cruden (1977). This very high P/O ratio appears to be a result of pollen collection by certain bees and the beetle, *Popillia impressipyga*. Therefore, it is inevitable that *R. beddomei* needs to produce high P/O to compensate for the pollen loss to pollen collectors, and to ensure the function of its vector-dependent facultative xenogamous breeding system.

Bruchid beetles primarily utilize beans from the family Fabaceae as their hosts (Johnson, 1981). *Callosobruchus* beetles utilize seeds of legumes of the tribe Phaseoleae (Fabaceae), such as *Vigna, Cajanus, Rhynchosia acuminatifolia* and *Phaseolus*. Phaseoleae species lack the toxic secondary compound L-canavanine, observed in other Faboideae (Bisby, Buckingham, & Harborne, 1994), and hence the host range of *Callosobruchus* is limited to Pha-seoleae (Oliveira, Sales, Machado, Fernandes, & Xavier, 1999). In *R. sublobata*, the bruchid beetle breeds in the seeds that are infested (Craufurd & Prins, 1979). The bruchid beetle, *Callosobruchus maculatus* uses the flower buds of *R. bed-domei* for its breeding and adults emerge from exit holes on pods that are still attached to the plant.

Ali, Tanveer and Nadeem (2012) also reported that in Rhynchosia capitata, the seed has physical dormancy due to impermeable seed coat, which enables it to persist for long periods in soil. In R. beddomei, seed dormancy is not tested but field observations indicate that seeds germinate in the vicinity of the parental plants during the rainy season. However, their growth is soon suppressed by the rocky habitat, intermittent rains and long dry spells within the rainy season. The perennial rootstock seasonally resurrects and produces new growth. The seeds do not disperse far away from the parental site despite the explosive break-up of pods. R. beddomei, despite having specialized pollination mecha-nism with primary pollen presentation adapted to bee pollinators, is unable to populate effectively due to several limitations during its growth season. The findings reported here could form the basis of extended studies supporting measures for the conservation and management of this endemic species in its natural locations.

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