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## POLLINATION ECOLOGY OF AN ENDEMIC MEDICINAL SHRUB, *RHYNCHOSIA BEDDOMEI* BAKER (FABACEAE)

A J Solomon Raju<sup>1</sup>, Ch. Prasada Rao<sup>2</sup> and M. Mallikarjuna Rao<sup>3</sup>

<sup>1,2</sup>Department of Environmental Sciences, Andhra University, Visakhapatnam 530 003, India

<sup>2</sup>Department of Botany, Andhra University, Visakhapatnam 530 003, India

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### Abstract

*Rhynchosia beddomei* is an endemic deciduous shrub. It flowers during December-March with peak flowering during January. The flowers are hermaphroditic, nectariferous, self-compatible and have explosive pollination mechanism adapted for pollination by bees, especially carpenter bees. They do not fruit through autonomous selfing but fruit through manipulated selfing, geitonogamy and xenogamy mediated by pollen vectoring bees. The flowers not visited by bees fall off while those visited and pollinated by them set fruit. An unknown insect, possibly bruchid beetle uses the floral buds for its breeding and the adults emerge from the exit hole made by them on the pod. Seed dormancy is not tested but field observations indicated that seeds germinate in the vicinity of the parental plants during rainy season. But their growth soon suppressed due to intermittent rains and long dry spells within rainy season coupled with rocky terrain with less soil and poor in nutrient and moisture content. Therefore, *R. beddomei* with specialized explosive pollination mechanism, self-compatibility and melittophily is unable to populate itself. Leaf cut during leaf flushing time by locals for medicinal purposes might be an additional factor affecting the vegetative growth, flowering and fruiting rate of this shrub. Its use for traditional medicine is to be regulated in order to conserve and manage the existing population.

**Key words:** *Rhynchosia beddomei*, hermaphroditism, explosive pollination mechanism, melittophily, pod infestation, medicinal value

### 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*) (Lackey 1981; Jayasuriya 2014). The genus consists of approximately 200 species and occurs in both the eastern and western hemisphere in warm temperate and tropical regions (Grear 1978). In the Eastern Ghats, twelve species of this genus have been reported to be occurring almost in one region, Seshachalam hills of southern Eastern Ghats of Andhra Pradesh. 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 et al. 2008). Of these, *R. beddomei* is a rare and endemic medicinal species and restricted to a few areas such as Talakona, Japalitheertham, Gogarbham in Seshachalam hills of Chittoor District, Andhra Pradesh (Padmavathi et al. 2012). Nair & Sastry (1998) also documented that this plant is distributed in Seshachalam hills of Eastern Ghats of Andhra Pradesh, India. Pullaiah (2006) recorded 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 Japalitheertham, Gogarbham area, Sandralamitta and near deer park of Tirumala hills. Sudhakar Reddy et al. (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 and small number of individuals left in the world. Prasad & Narayana Swamy (2014) reported a new species, *Rhynchosia ravii* which is closely related to *Rhynchosia beddomei*. It is a small population that occurs 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 the species has led the taxonomists to treat both as *R. beddomei*.

Franco (1995) provided floral details of *Rhynchosia* in Brazil. He has not mentioned the species in his work. He reported that it is autogamous which is limited by spatial segregation between stigma and anthers. Levels of out-crossing are maintained by retention of a pollination mechanism. *Hypanthidium* sp. and *Centris* sp. are the primary pollinators and the pollen is deposited on the ventral part of their abdomen when the flower is probed. Craufurd & Prins (1979) reported that *Rhynchosia sublobata* is self-compatible and pollinated by *Xylocopa* bees. Etcheverry et al. (2011) reported that *Rhynchosia edulis* and *R. senna* var. *texana* display valvular pollination mechanism; the former is facultative xenogamous while the latter is obligately xenogamous. There is no other information on flowering phenology, breeding systems, pollen presentation mechanisms, pollination

mechanisms, pollinators and fruiting ecology of any species of *Rhynchosia*.

Madhav Chetty et al. (2008) reported that *R. beddomei* is useful for certain purposes such as abortifacient, anti-bacterial, anti-fungal, diabetes and hepato-protectivity. Rama Rao & Henry (1996) noted that the leaves of *R. beddomei* are used for wounds, cuts, boils and rheumatic pains by Adivasi tribes (Sugali, Yanadi, Erukala) inhabiting the forests of Eastern Ghats of Andhra Pradesh, India. Gunasekar (1980) noted that this plant contains flavonoid compounds, such as flavones, flavonols and flavanones. Bakshu & 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 an endemic in the southern Eastern Ghats, its pollination ecology has been studied in detail to understand its flowering phenology, pollen presentation and pollination mechanisms, pollinators, and fruiting ecology. This information is useful to take measures for the conservation and management of this shrub in its natural site.

## **Materials and Methods**

### **Study site**

The study region is the southernmost region of Andhra Pradesh and is an integral part of Southern Eastern Ghats of Peninsular India. The area is located at 13°40'N latitude and 79°19'E longitude, and at an elevation of 2,443 ft. The exact study area is the forest cover of Tirumala Hills a constituent of Seshachalam Hill Range in Chittoor District, Andhra Pradesh. The entire region represents the deciduous forest ecosystem. The site is characterized by a combination of rocky, undulating and steep terrain with some litter content formed from grass and other herbaceous plants. This forest is known as hot-spot for some rare, endangered, vulnerable, threatened and endemic plants (Madhava Chetty et al. 2008). In this area, *Rhynchosia beddomei* was selected for study during 2014-2015.

### **Flowering and floral biology**

Flowering season was defined based on regular field trips made. Ten plants were tagged and followed for quantifying the flower production rate daily. The flower count was computed and provided week-wise during flowering season. Twenty inflorescences were tagged and followed to record the length of flowering and the number of flowers produced. Observations regarding the organization of inflorescences, the spatial positioning of flowers, and their position (terminal, axillary, etc.) on the plants were made since these features are regarded as important for foraging and effecting pollination by flower-visitors. The flower life was recorded by marking twenty five just anthesed flowers and following them until fall off. Anthesis was initially recorded by observing twenty five marked

mature buds in the field. Later, the observations were repeated five times on different days in order to provide accurate anthesis schedule. Similarly, the mature buds were followed for recording the time of anther dehiscence. The presentation pattern of pollen was also investigated by recording how anthers dehisced and confirmed by observing the anthers under a 10x hand lens. The details of flower morphology such as flower sex, shape, size, colour, odour, sepals, petals, stamens and ovary were described based on twenty five flowers randomly collected from five plants. Observations regarding the position and spatial relationships of stamens and stigma in mature bud, at anthesis and after during the flower-life with reference to self and/or cross-pollination were made very carefully.

### **Pollen output**

Thirty mature but un-dehisced anthers from five different plants were collected and placed in a Petri dish. Later, each time a single anther was taken out and placed on a clean microscope slide (75 x 25 mm) and dabbed with a needle in a drop of lactophenol-aniline-blue. The anther tissue was then observed under the microscope for pollen, if any, and if pollen grains were not there, the tissue was removed from the slide. The pollen mass was drawn into a band, and the total number of pollen grains was counted under a compound microscope (40x objective, 10x eye piece). This procedure was followed for counting the number of pollen grains in each anther collected. Based on these counts, the mean number of pollen produced per anther was determined. The mean pollen output per anther was multiplied by the number of anthers in the flower for obtaining the mean number of pollen grains per flower. The characteristics of pollen grains were also recorded.

### **Pollen-ovule ratio**

The pollen-ovule ratio was determined by dividing the average number of pollen grains per flower by the number of ovules per flower. The value thus obtained was taken as pollen-ovule ratio (Cruden 1977).

### **Nectar characters**

The presence of nectar was determined by observing the mature buds and open flowers. The volume of nectar from 10 flowers was used to determine the average volume of nectar per flower and was expressed in  $\mu\text{l}$ . The flowers used for this purpose were bagged at mature bud stage, opened after cessation of nectar secretion and squeezed nectar into micropipette for measuring the volume of nectar. Nectar sugar concentration was determined using a Hand Sugar Refractometer (Erma, Japan). Ten samples were used for examining the range of sugar concentration in the nectar. For the analysis of sugar types, paper chromatography method described by Harborne (1973) was followed. Nectar was placed on Whatman No.

1 of filter paper along with standard samples of glucose, fructose and sucrose. The paper was run ascendingly for 24 hours with a solvent system of n-butanol-acetone-water (4:5:1), sprayed with aniline oxalate spray reagent and dried at 120°C in an electric oven for 20 minutes for the development of spots from the nectar and the standard sugars. Then, the sugar types present and also the most dominant sugar type were recorded based on the area and colour intensity of the spot. The sugar content/flower is expressed as the product of nectar volume and sugar concentration per unit volume, mg/µl. This is done by first noting the conversion value for the recorded sugar concentration on the refractometer scale and then by multiplying it with the volume of nectar/flower. Table 5.6 given in Dafni et al. (2005) was followed for recording the conversion value to mg of sugars present in one µl of nectar. During the period of open state of flowers, the prevailing ambient temperature was recorded using field thermometer and relative humidity using hygrometer.

#### **Stigma receptivity**

In visual method, the stigma physical state (wet or dry) was considered to record the commencement of receptivity. H<sub>2</sub>O<sub>2</sub> test as given in Dafni et al. (2005) was followed for the confirmation of stigma receptivity period.

#### **Breeding Systems**

Mature flower buds of some inflorescences on different individuals were tagged and enclosed in paper bags. They were tested in the following way and the number of flower buds used for each mode of pollination was given in Table 1.

1. The flowers were fine-mesh bagged without hand pollination for autonomous autogamy.
2. The stigmas of flowers were pollinated with the pollen of the same flower manually by using a brush; they were bagged and followed to observe fruit set in manipulated autogamy.
3. The emasculated flowers were hand-pollinated with the pollen of a different flower on the same plant; they were bagged and followed for fruit set in geitonogamy.
4. The emasculated flowers were pollinated with the pollen of a different individual plant; they were bagged and followed for fruit set in xenogamy.

All these categories of flower pollinations were followed for fruit set. If fruit set is there, the percentage of fruit set was calculated for each mode.

### **Flower-visitors**

After making preliminary observations on flower visitors, the categories of insects were identified. The flower foragers included only bees and one beetle; the bees were forage collectors while the beetle was predator on flowers. The hourly foraging visits of each bee species were recorded on 3 or 4 occasions depending on the possibility and the data was tabulated to use the same for further analysis. Fully blooming plants were selected to record the foraging visits of bees. The data obtained was used to calculate the percentage of foraging visits made by each bee species per day and also to calculate the percentage of foraging visits of each bee species per day in order to understand the relative importance of each bee species. Their foraging behaviour was observed on a number of occasions for the mode of approach, landing, probing behaviour, the type of forage collected, contact with essential organs to result in pollination, inter-plant foraging activity in terms of cross-pollination. A sample of 500 flowers collected randomly from twenty plants was used to record the flower predation rate by the beetle.

### **Determination of pollen carryover efficiency of bees**

Ten specimens of each bee species were captured from flowers and brought them to the laboratory. The pollen loads if present in the corbiculae of these bees, they were removed prior to pollen analysis. Each specimen was washed first in ethyl alcohol and the contents stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present. From this, the average number of pollen grains carried by each bee species was calculated to know the pollen carryover efficiency of different bee species.

### **Natural fruit set, seed dispersal and seedling ecology**

A sample of flowers on different plants were tagged prior to anthesis and followed for fruit set rate in open-pollinations. Fruit maturation period, fruit dehiscence and seed dispersal aspects were observed to the extent possible. Ten inflorescences on five plants were tagged and followed to record fruit set rate in open pollination against the total number of flowers produced. Field observations indicated the infestation of fruits; four hundred fifty fruits were randomly collected from fifteen plants to record fruit infestation rate. In infested fruits, seed was the target and it appeared that the insects used it as food source for its breeding. Field observations were also made on fruit and seed dispersal modes, seed germination and seedling establishment to the extent possible.

### **Photography**

Plant habitat, flowering inflorescences, flower and fruit details, and foragers were photographed with Nikon D40X Digital SLR (10.1 pixel) and TZ240

Stereo Zoom Microscope with SP-350 Olympus Digital Camera (8.1 pixel). Olympus Binoculars (PX35 DPSR Model) was also used to make field observations. Magnus Compound Microscope - 5x, 10x, 40x and 100x magnification was used for studying the pollen characteristics.

## Results

### Phenology

It is an erect, perennial shrub, 1.5 m tall with tomentose branchlets that grows in rocky areas with red soils (Figure 4a). The plant re-grows from below ground perennial root stock and from the seed during wet season from July to November during which growth and leaf flushing occurs. The leaves are trifoliate with reticulate venation. The leaflets are ovate-lanceolate, slightly silvery, silky and coriaceous. The flowering occurs during December-March with peak flowering in January (Figure 4b). The plants wither and disappear in April. The flowering phenology at individual plant level indicated that the flower production rate gradually increased from the 1st week of December to 2nd week of January and then onwards gradually decreased until 2nd week of March (Figure 1). The flowering ceased completely by the end of 2nd week of March. The flower output per plant averaged to 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 axillary and terminal racemes; individual racemes are 5-8 flowered ( $5.24 \pm 2.1$ ) which open over a period of 3-5 days (Figure 4c,d).

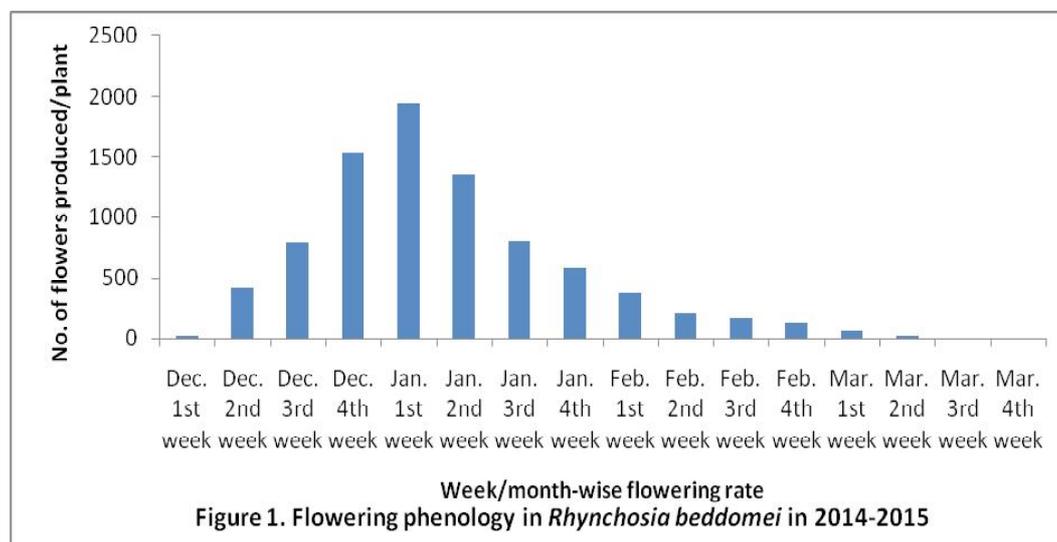


Table 1. Flower production rate at plant level in *Rhynchosia beddomei*

Week of observation	December	January	February	March
1st week	15.7 ± 2.12	1945.4 ± 32.51	379.8 ± 5.28	59.8 ± 1.72
2nd week	421.3 ± 12.81	1356.1 ± 8.16	210.2 ± 4.79	19.2 ± 0.91
3rd week	789.7 ± 3.61	801.4 ± 4.14	169.6 ± 7.68	—
4th week	1536.2 ± 21.73	578.3 ± 3.21	132.1 ± 23.71	—
Total flowers produced	2762.9	4681.2	891.7	79.0

### 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 ( $6.1 \pm 0.7$ ) than the lower 3 sepals ( $5.8 \pm 0.3$ ). The corolla is bright yellow, pubescent, specialized and consists of upper standard petal, two wing petals and two keel petals. The standard petal is large, obovate with reddish-brown lines at the bottom of the mid-region which serves as nectar guide (Figure 4e); the petal base is clawed and consists of two inflexed fingernail auricles. The standard petal envelops the rest of the petals in bud but reflexes when the flower blooms. The two adjacent petals ( $7.6 \pm 0.5$  mm long and  $3.8 \pm 0.4$  mm wide), called wing petals surround the two bottom petals, called keel petals ( $7.4 \pm 0.5$  mm long and  $3.6 \pm 0.4$  mm wide). 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 (Figure 4h). The keel and the wing petals are attached by means of two notched folds. The wing petals serve as alighting platform for insects visiting the flowers. The stamens are ten,  $6.8 \pm 0.4$  mm long, diadelphous; nine filaments are fused by the basal part into a sheath open along the upper side while the tenth filament is free and lies on the others (Figure 4j). The distal parts of the filaments are free and contain 1 mm long uniform dithecous anthers. The ovary is sessile, green, villous,  $2.6 \pm 0.4$  mm long (Figure 5d) and lies in the sheath of the filaments ( $5.7 \pm 0.4$  mm long) along the cylindrical part of the keel. It is monocarpellary and monolocular with a single ovule arranged on marginal placentation (Figure 5f). It has a long glabrous style with a capitate wet shiny stigma (Figure 5e), both together account for a length of  $4.8 \pm 0.3$  mm (Figure 4i; 5c). The stigma is situated slightly above the anthers. The distal portion of free filaments and style and stigma are incurved and clamped into the keel petals.

### Floral biology

Mature buds open during 1200-1500 h (Figure 4f,g). 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. All the ten anthers in a flower dehisce at the same time by longitudinal slits in mature bud stage. The number of pollen grains per anther is  $728 \pm 38.44$  and per flower is 7,280. The pollen-ovule ratio is 7,280:1. The pollen grains are monads, spheroidal,  $37.35 \pm 4.37 \mu\text{m}$  in size, powdery and tricolporate, angulaperturate with reticulate exine (Figure 5a,b). A nectariferous disc is present at the base of the ovary. The initiation of nectar secretion occurs during mature bud stage and its cessation occurs an hour after anthesis. Individual flowers produce  $1.6 \pm 0.26 \mu\text{l}$  of nectar with 0.54 mg of sugar. The nectar sugar concentration is 30% (Range 29-34%) consisting of sucrose, glucose and fructose with the first as dominant. The ambient temperature during this period varied from 24 to 31°C while the relative humidity varied from 90 to 70%. Nectar is deeply concealed and it is open through two windows between the joined and the free filaments at the flower base. These windows allow access to the nectar. The stigma attains receptivity during anthesis and remains receptive 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 mature buds that opened at 1200 h close back at 1500 h, those opened at 1300 h close back at 1600 h and those opened at 1400 h close back at 1700 h and those opened at 1500 h close back at 1800 h. 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.

### **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 causes 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. If it is the first visit for the insect to the flower, then it effects self-pollination upon explosive release of reproductive column from the keel boat. With the departure of the insect from the flower, the reproductive column does not return back to its former position but the keel moves forward partly covering the stamens and stigma. The downward movement of keel petals occurs

in each subsequent foraging visits by appropriate insects. Tripping of keel boat can also occur due to heavy rain or high temperature that weaken turgidity of the restraining keel tissues. But, the tripping due to these two factors is ruled out since the plant flowers during winter season when heavy rains do not normally occur and the temperature usually stands low. If the flower is untouched or tripping to keel did not occur, the reproductive column is never exposed and remain enclosed in the keel boat. Such flowers fall off subsequently upon withering without fruit set.

### Breeding systems

In mature buds, anthers dehisce but autonomous autogamy does not occur. Fruit set is absent in un-manipulated autogamy, 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.

Table 2. Results of breeding systems in *Rhynchosia beddomei*

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

### Bee pollinators and pollination

Thrips were found in the buds and flowers (Figure 5g). Their presence in the buds indicated that they use them for breeding. During bud phase, the thrips did not have access to anthers which are concealed in the keel petals but they did have access to the nectar secreted around the ovary. They remained inside the flowers even after anthesis because the standard petal locked the nectar windows. When the keel petals were tripped by insects, the nectar was exposed through the two windows situated between the joined and the free filaments at the flower base and the keel petals released the reproductive column. Consequently, the thrips were able to take exit either from the nectar windows or via keel petals. The thrips take the exit from the un-tripped flowers only when the latter wither away. During mature bud stage, the thrips had access to nectar but not to pollen which was accessible only when keel petals were tripped in open flowers. They fed on both nectar and pollen. The nectar feeding activity by thrips deplete the

available nectar at flower or plant level and compels the actual flower foragers to visit many flowers and in consequence, the thrips feeding activity appeared to be promoting flower visitation rate and cross-pollination rate by flower foragers.

Insect activity was not found at the inflorescences during forenoon period. Their activity was recorded only from noon time onwards due to the availability of fresh flowers. 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 that there is a gradual increase in foraging visits concomitant with the gradual increase in the anthesis rate as a function of time and later there is a gradual decrease in foraging visits concomitant with the gradual increase in the number of closed flowers. Insects that visited the flowers belonged to only one order, Hymenoptera, one family, Apidae and three sub-families, Apinae, Nomiinae and Xylocopinae, all belonging to bee category. Four bee species belonged to Apinae, one species to Nomiinae and two species to Xylocopinae. The bees included *Apis dorsata* (Figure 5h), *A. cerana* (Figure 5i), *A. florea* (Figure 5j), *Ceratina* sp., *Nomia* sp. (Figure 5k), *Xylocopa pubescens* (Figure 5l) and *Xylocopa* sp. (Figure 5m) (Table 3). *Apis* bees collectively made 42%, *Xylocopa* bees 37% and the other bees 21% of total foraging visits. Individually, *A. dorsata* made 17%, *A. cerana* 13%, *A. florea* 12%, *Ceratina* sp. 11%, *Nomia* sp. 10%, *Xylocopa* sp. 18% and *X. pubescens* 19% of total foraging visits (Figure 3). The body washings of foraging bees showed variation in the pollen carrying capacity; the average pollen recorded on *A. dorsata* was 281.1, *A. cerana* 214.3, *A. florea* 184.6, *Ceratina* sp. 117.2, *Nomia* sp. 102.1, *Xylocopa pubescens* 643.2 and *Xylocopa* sp. 432.1 (Table 4). The flowers were visited several times by bees but new visits lasted shorter than the first one. On certain occasions the bees abandoned their intention of browsing on previously visited flowers upon landing. With respect to their behavior, the bees landed on the wing petals and the keel, with their head near the standard. They then exerted a certain pressure with legs on the wing petals until these and the keel bent downwards, and then proceeded to collect nectar during which the bee's abdomen appeared pollen smothered (sternotribic pollen deposition). The pollen collecting bees took "U" turn after nectar collection and proceeded to the stamens to collect pollen. A coleopteran scarabaeid beetle, *Popillia impressipyga* (Figure 5n,o) was also found to feed on all floral parts, especially the stamens and stigma. The flower predation rate by this beetle is 19.6%.

Table 3. List of insect foragers on *Rhynchosia beddomei*

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

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

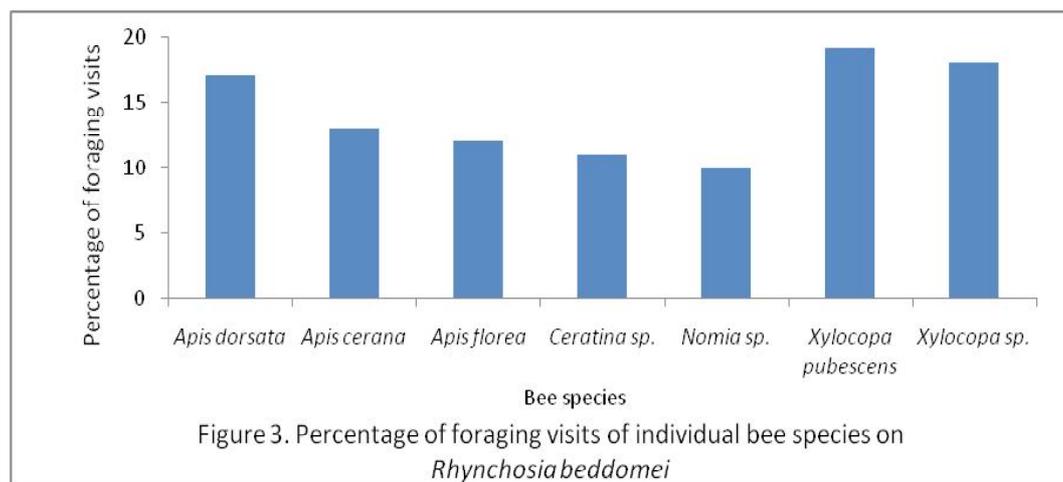
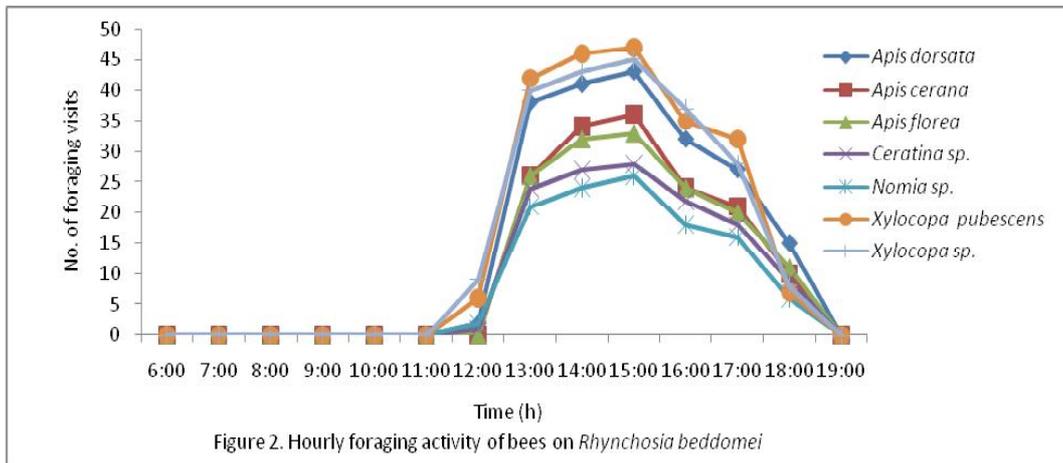




Figure 4. *Rhynchosia beddomei*: a. Habit, b. Flowering phase, c. & d. Flowering branch, e. A close up view of a flower with standard petal labeled with nectar guide, f. & g. Mature buds, h. Stamens and Stigma housed in keel petals, i. Floral parts, j. Diadelphous stamens and capitate stigma placed above the height of anthers.



Figure 5. *Rhynchosia beddomei*: a. & b. Pollen grains, c. Ovary with style and stigma, d. Ovary, e. Capitulate stigma, f. Ovule, g. Thrips, h. *Apis dorsata*, i. *Apis cerana*, j. *Apis florea*, k. *Nomia* sp., l. *Xylocopa pubescens*, m. *Xylocopa* sp., n. & o. *Popillia impressipyga*.

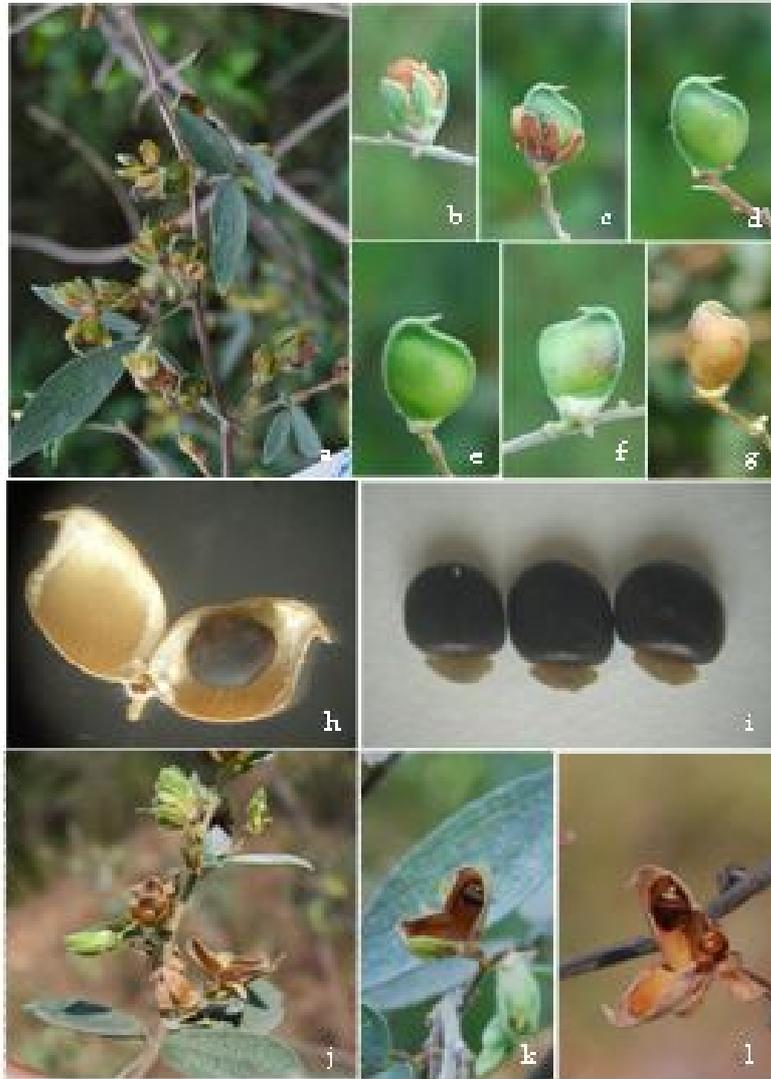


Figure 6. *Rhynchosia beddomei*: a. Fruiting branch, b-g. Different stage of fruit development, h. Fruit with well developed seed, i. Healthy seeds, j-l. Explosive fruit dehiscence.



Figure 7. *Rhynchosia beddomei*: a. Infected fruits, b. & c. Fruit with insect pupa, d. & e. Pupal case, f. Fruit with exit hole drilled by adult insect, g. Seedling, h. Several naturally emerged seedlings

### **Fruiting behavior**

The fruit growth and development begins immediately after pollination and fertilization. The fruits mature within three weeks (Figure 6a). The sepals enclose the growing fruit initially and the fruit emerges out of the sepals gradually with its gradual growth and development. Fruit is green initially and brown to dark brown when ripe and dry (Figure 6b-g). It is a non-fleshy, hairy, glandular, circular to narrowly oblong,  $12 \pm 0.6$  mm long,  $5.8 \pm 0.4$  mm wide, compressed one-seeded pod. Fruit infestation was recorded and it came to be 21.55%. The infested pods showed different stages of the insect pest (unidentified) (Figure 7a-e). Each infested pod invariably contained one larva or pupa anchored to seed part. The insect pest was found to be using the floral buds for breeding because the mature pods ready for dehiscence contained exit holes through which adult insects came out (Figure 7f). But, the adult insect pest left the pod prior to the pod dehiscence.

### **Seed ecology**

Mature and dry fruits display explosive dehiscence to disperse seeds. The fruit with bi-valvate configuration dehisce elastically exposing the seeds (Figure 6h-l). The seed is reddish brown to black, compressed, sub-reniform, glabrescent,  $3.8 \pm 0.4$  mm long,  $2.6 \pm 0.5$  mm wide and shiny with a prominent strophiole. Depending on the state of seed in terms of its dryness, the seed is either scattered into the air or remains attached to the fruit wall. Seeds germinate during rainy season which starts from June onwards. Seedlings grow continually but their growth rate is subject to the availability of moisture status of the soil (Figure 7g,h).

### **Discussion**

*Rhynchosia beddomei* is a deciduous shrub which shows growth from underground root stock during rainy season. It also produces new plants from seed stock at the same time. Full leaf flushing occurs by the end of October and then onwards, floral bud initiation takes place. The flowering season is well defined and is confined to winter season when most of the plants in the area do not bloom. At this time of the year, a few low ground herbs with scattered distribution show sporadic flowering depending on the soil moisture and nutrient status. The occurrence of the shrub as a small population, near synchronous flowering at population level and display of terminal and axillary pedunculate yellow flowers against the silvery, silky and coriaceous leaflets appear quite prominent and attract appropriate foragers that effect pollination.

In *R. beddomei*, hermaphroditic sexual system is functional due to production of fertile pollen grains and functional ovary. The flowers display the near synchronous hermaphroditism or homogamy due to the occurrence of anther

dehiscence in mature bud stage and receptivity of stigma during anthesis. The stigma is placed slightly above the height of dehisced anthers but the entire length of reproductive column remains inside the keel petals even after anthesis; in this situation, there is a likelihood of the occurrence autonomous autogamy. But, hand-pollination tests indicated that autonomous autogamy does not occur despite self-compatibility but it is functional because fruiting occurred when this mode of pollination is manipulated by brushing the stigma with its own pollen. Such a situation suggests that the flowers are essentially dependent on flower foragers for fruit set through self- as well as cross-pollination. 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 out-crossing. Lloyd & Schoen (1992) reported that the stigmatic membrane and the collar of peri-stigmatic hairs are related to the breeding system in the sub-family Papilionoideae. The stigmatic membrane prevents autonomous self-pollination in certain members of the tribe Phaseoleae. The peri-stigmatic hairs present in these members facilitate maximization of out-crossing by preventing self-pollen deposition on the stigmas during the stages of anthesis. In these members, the stigmatic membrane has thick cuticle and requires a rupture which is caused by the pollinator during flower visit. Shivanna & Owens (1989) stated that the rupture of the stigmatic surface by pollinator permits the pollen to germinate in the flowers of Phaseoleae members with thick stigmatic cuticle. On the contrary, Castro & Agullo (1998) reported that in *Vigna*, a member of the tribe Phaseoleae, autonomous self-pollination may occur by spontaneous rupture of the stigmatic membrane. Similar stigmatic surface that prevents self-fertilization has also been reported in *Vicia faba* (tribe Viciae) (Lord & Heslop-Harrison 1984) and in *Medicago scutellata* (tribe Trifolieae) (Krietner & Sorensen 1985); however, in these species auto-fertile lines have been reported to have thin stigmatic cuticles allowing spontaneous disruption and self-fertilization. In *R. beddomei*, the stigmatic surface appears to have thick cuticle and does not have the mechanism of causing spontaneous rupture to facilitate autonomous self-pollination. In effect, the tripping of keel petals appears to be essential to cause rupture on the stigmatic surface by the tripping agent due to which there is more likelihood of the occurrence of either geitonogamy or xenogamy. The fruit set rates recorded in hand-pollinated geitonogamy and xenogamy also substantiate 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.

Schrire (1989) stated that the ecological and evolutionary success of Leguminosae has been related to biotic pollination mechanisms. The three sub-

families within this family have achieved a characteristic floral architecture, in which plants within the sub-family Papilionoideae have developed the most complex floral mechanisms. Plants within the Papilionoideae have zygomorphic flowers that are mainly bee-pollinated (Westerkamp 1997); although bird pollination and bat pollination have also been recorded within the subfamily (Ortega-Olivencia et al. 2005). In bee-pollinated flowers of Papilionoideae, it is assumed that each part of the corolla is specialized for a particular role in pollinator attraction and the success of pollination. The flag or standard petal attracts pollinators; the keel protects androecium and gynoecium and, together with the wings, provides a platform for the insects to land on. The wings also operate as levers that raise or lower the keel (Stirton 1981). The flowers typical of pollination by the bee family Apidae are zygomorphic, bright yellow or blue with nectar guides, and frequently with hidden rewards such as those in the Lamiaceae, Scrophulariaceae, Fabaceae and Orchidaceae (Faegri & van der Pijl 1979). In the present study, the Fabaceae member, *R. beddomei* has papilionaceous corolla with flag, wing and keel petals; 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.

Within the sub-family Papilionoideae, primary and secondary pollen presentations have been reported. In plants with primary pollen presentation, pollen is delivered directly from the anthers to the vector's body. In plants with secondary pollen presentation, pollen grains are delivered first on a floral part such as the keel petals in Papilionoideae and then on the body of the vector implying an accurate delivery of pollen on the vector's body (Howell et al. 1993). These two pollen presentation patterns are associated with the four types of basic pollination mechanisms - valvular, pump, explosive and brush, all of them are associated with a particular floral architecture and kinetics. In the valvular type, pollen presentation is primary, whereas in the other three mechanisms, it is secondary (Yeo 1993). In the explosive mechanism, commonly only one pollination event occurs and it has evolved independently in several tribes (Small 1988), while in the other three mechanisms, repeated visitation is possible (Westerkamp 1997). In the present study, *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 pollen delivery is the representative of primary pollen presentation associated with explosive pollination mechanism. In the flowers, the staminal column is held under pressure within the keel, and when the tension is released by the forager, the same column snaps forward against the standard petal causing all the pollen to be instantly released. The reproductive column remains

exposed and does not return back to its original state but the keel petals return back partially covering the stamens and stigma. The efficiency of explosive pollination mechanism depends on the ambient weather conditions, especially temperature and relative humidity. Since *R. beddomei* flowers during winter season, it accordingly commences anthesis from noon onwards by which time the ambient air will be relatively dry and hence is conducive for the efficient functioning of the explosive pollination mechanism. Further, the bees also commence their foraging activity from noon onwards and continue forage collection until the flowers close back. The concealment of the stamens within the keel petals until it is tripped is an advantage for the plant to secure pollen from unusual rains and ambient moisture conditions during winter season (Peter et al. 2004).

Percival (1961) stated that plants with deep-tubed flowers tend to produce sucrose-rich nectar, whereas those with open or shallow-tubed flowers tend to be hexose-rich. Baker & Baker (1983) stated that flowers with long corolla tube possess more sucrose in their nectar while those with short tubes possess more hexoses in their nectar. In the present study, *R. beddomei* with short corolla tube presents sucrose-rich nectar because the nectar is perfectly concealed and hence is not exposed for the breakdown of sucrose into hexoses. Concealment of nectar in this species is adaptive to protect against microorganisms, particularly yeasts, whose metabolic activities dramatically change nectar chemistry and the plant gains a benefit from keeping the nectar as sterile as possible to maintain control over its chemical composition in order to maximize pollination rate by attracting appropriate pollinators (Herrera et al. 2008). Honey bees prefer the flowers with sucrose as chief constituent of nectar (Kevan 1995). The flowers pollinated by long-tongued bees produce sucrose-rich nectar (Baker & Baker 1990). In line with this, *R. beddomei* with melittophilous pollination syndrome also produces sucrose-rich nectar which is utilized exclusively by long-tongued bees. *Apis*, *Ceratina*, *Nomia* and *Xylocopa* bees recorded on this shrub have been documented as long-tongued bees (Cruden et al. 1983; Roubik 1992; 2006). Bee-flowers tend to produce small volume of nectar with higher sugar concentration than the nectar of flowers pollinated by other animals (Opler 1983; Cruden et al. 1983). Honey bees prefer sugar concentration of 20 to 40% in the nectar (Waller 1972). On the contrary, Baker & Baker (1983) noted that honey bees prefer sugar concentration of 30 and 50% in the nectar. The honey bees have the ability to regurgitate liquid onto concentrated or even crystallized nectar, in this way, reduce its concentration so that it may be imbibed. The preferred sugar concentrations of nectar by other categories of bees have not been found in the literature. But, Pyke & Waser (1981) stated that the nectar sugar concentration of flowers pollinated by bees is generally higher than that of those pollinated by butterflies and hummingbirds; bee-pollinated flowers tend to produce nectar with sugar concentration more than 35% while

butterfly or hummingbird pollinated flowers tend to produce nectar with sugar concentration ranged between 20 and 25%. In line with these reports, the present study shows that the flowers of *R. beddomei* produce small volume of nectar with 30% sugar concentration. Further, the energy yield from nectar appears to be in tune with the requirement of energy by bees in general and carpenter bees in particular due to their larger body size. In case of carpenter bee visits to *R. beddomei* is further substantiated by the reports of Baker (1975) and Heinrich & Raven (1972) that these bees, being large in size and requiring high energy reach the floral reward only if the energetic reward is proportional to the energy expended. 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. Accordingly, the flowers never received visits from other categories of insects.

In *R. beddomei*, the keel tripping process is not self-activated to effect pollination. The flowers depend on bees for tripping of the keel petals to enable the working of explosive pollination mechanism. The flowers that were not tripped by external agents subsequently fall off. This situation explains that the plant is obligately dependent on bees for pollination. Of the bees, carpenter bees and the rock honey bee being large in size are more efficient in tripping the flowers than other bees. Carpenter bees are also more efficient in lifting the flag petal to access the nectar situated at the flower base. Since these bees collect only nectar and more efficient tripping the flowers to effect pollination, they are classified as principal pollinators. All other bees although trip the flowers and effect pollination are treated as next-rank pollinators because they reduce the availability of pollen by pollen collection. The scarcity or non-availability of reliable floral resources during winter season in the study area further enforces fidelity to *R. beddomei* by bees, in particular pollen collecting ones due to which the pollen availability for pollination gets very much reduced. Mishra & Rajesh Kumar (1997) reported that the pollen has great importance for a bee colony as pollen provides proteins, which are essential for worker honey bees to secrete glandular food (royal jelly) for rearing brood. Availability of enough pollen directly helps in more brood rearing, which ultimately leads to gradual colony build up. *R. beddomei* is a promising source of pollen for honey bees and other bees during winter season.

Cruden (1977) used the pollen-ovule (P/O) ratios as indicators of breeding systems 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. Several workers followed these P/O ratios to classify breeding systems of the plant species studied by them. Arroyo (1981) stated that the P/O varies according to the pollination mechanism within Papilionoideae. These

authors suggested that the plants with explosive mechanism have a low P/O because a single pollinator visit is needed for efficient transference of pollen; this low P/O is a consequence of the highly specialized, irreversible pollination mechanism, which allows only one effective exchange of pollen with pollinators. Small (1988) stated that *Medicago* species of the tribe Trifolieae with explosive pollination mechanism displays the lowest pollen-ovule ratios. Lopez et al. (1999) recorded explosive pollination mechanism with highest pollen-ovule ratios in certain genera of the Fabaceae such as *Cytisus*, *Pterospartum*, *Teline*, *Ulex*, *Stauracanthus* and *Cytisophyllum*. Etcheverry et al. (2011) stated that the Fabaceae plants which they studied with explosive pollination mechanism had intermediate pollen-ovule ratios. These authors mentioned that *Rhynchosia edulis* and *R. senna var. texana* have valvular pollination mechanism with primary pollen presentation. Both the species are classified as obligate xenogamous based on P/O ratio but *R. edulis* has been found to be facultative xenogamous in hand-pollination tests. Craufurd & Prins (1979) reported that *R. sublobata* is self-compatible and facultative xenogamous in hand-pollination tests; it is pollinated by *Xylocopa* bees. In the present study, *R. beddomei* shows highest P/O ratio even when compared to that of xenogamy used by Cruden (1977). It seems that the P/O is not always a good indicator of breeding system. The highest P/O ratio in this plant species appears to be a consequence of pollen collection activity by bees other than carpenter bees and the beetle, *Popillia impressipyga*. Therefore, it is inevitable for *R. beddomei* to produce high P/O to compensate the pollen loss caused by pollen collectors and 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). Most bruchids are oligophagous; their host range is limited to restricted plant taxa, typically tribes and sub-tribes for species utilizing the legume subfamily Faboideae (Tuda et al. 2005). The ability to use dry beans as a food resource is widespread in this family. While the hardness of dried seeds *per se* can serve as a deterrent against bruchid beetles, the loss of toxic chemicals during post-maturity drying processes may also increase survival of such granivores. The genus *Callosobruchus* (Bruchinae) includes approximately 20 species. As larvae, the species of this genus utilize seeds of legumes of the tribe Phaseoleae (Fabaceae) such as *Vigna*, *Cajanus*, *Rhynchosia acuminatifolia* and *Phaseolus*. *C. maculatus* utilizes the seeds of *V. unguiculata* in India. Phaseoleae species lack a toxic secondary compound L-canavanine, observed in other Faboideae (Bisby et al. 1994), and hence the host range of *Callosobruchus* is limited to Phaseoleae. In support of this, bioassay tests also indicated lethal effects of canavanine on the larvae of *C. maculatus* (Oliveira et al. 1999). In *R. sublobata*, the bruchid beetle infests the seeds by using the latter for its breeding (Craufurd

& Prins 1979). In the present study, an unknown insect has been found to infest the seeds of *R. beddomei* for its breeding. This insect appears to be using the floral buds for its breeding and in effect the adults emerge out from the exit hole on the fruits (pods) while the latter are still attached to the plant. Since *R. beddomei* belongs to the tribe Phaseoleae, it is not unreasonable to suggest that the insect infesting this plant is certainly a bruchid beetle and is a serious pest which is affecting the regeneration rate.

In Leguminosae, seeds of many taxa exhibit physical dormancy due to the presence of a water impermeable seed coat and imparts survival value in that impermeable seeds are capable of remaining dormant but viable for long periods of time (Tran & Cavanagh 1984). Shaukat & Burhan (2000) reported on fecundity, seed characteristics and factors regulating germination of *Rhynchosia minima* in Pakistan. It exhibits differential success in different habitats with different micro-climates. Ali et al. (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 indicated that seeds germinate in the vicinity of the parental plants during rainy season. But their growth soon suppressed due to intermittent rains and long dry spells within rainy season coupled with rocky terrain with less soil and poor in nutrient and moisture content. Field studies were carried out in this region for several years in connection with pollination ecology on other plant species. Since then, casual observations were made continually on the population size of this plant; there was no change in its population size despite seedling production every year. The perennial root stock seasonally resurrects and produces new growth. In effect, the same population exists. Further, the seeds do not disperse far away from the parental site despite the explosive break-up of pods because the seeds are usually attached to the fruit wall even after fruit dehiscence. Therefore, *R. beddomei* despite having specialized pollination mechanism with primary pollen presentation adapted to bee pollinators is unable to populate itself due to several limitations during growth season.

Different workers stated that *R. beddomei* has medicinal value. Its leaves are used for treating various diseases by external application (Madhav Chetty et al. 2008; Rama Rao & Henry 1996; Bakshu & Venkata Raju 2001). Leaf cut during leaf flushing time for medicinal purposes by locals certainly affects the vegetative growth, flowering and fruiting rate of this shrub. Its use for traditional medicine is to be regulated in order to conserve and manage the existing population. Further, studies on seed dormancy, seed germination and seedling establishment rates of this shrub are needed to understand its regeneration ecology and subsequently to plan measures for the expansion of its population size.

Remanandan (1981) stated that *Rhynchosia*, being closely related to the genus *Cajanus*, some of its species can be used to provide substantial contributions towards crop improvement in pigeon pea. Furthermore, some species of *Rhynchosia* have been experimented in India to provide physiological resistance against insect pests such as pod-borer and pod-fly in pigeon pea. Craufurd & Prins (1979) considered *R. capitata* as a promising source of fodder for the cattle during dry season in Zambia. In view of these economic and commercial values, full-fledged investigations on all species of this genus in India are suggested to utilize them in the best possible manner to improve pigeon pea yield and to use as fodder for cattle in areas where fodder is a problem, especially during dry season.

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