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Pollination Ecology of Rhynchosia minima (L.) DC. (Fabaceae) in the Southern Eastern Ghats, Andhra Pradesh, India

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Abstract. *Rhynchosia minima* is prostrate climbing herb. In India it flowers during September-March with peak flowering during January. The flowers are hermaphroditic, nectariferous, self-compatible and have explosive pollination mechanism adapted for pollination by 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. Seed dispersal occurs by explosive pod dehiscence. Perennial root stock resurrects back to life during rainy season. Seeds also germinate at the same time but their continued growth is subject to the availability of soil moisture content. Therefore, *R. minima* expands its population size and succeeds as a weed in water-saturated habitats only. The plant is used as medicine, animal forage and human food and hence it has the potential for exploitation commercially.

Key words: economic value**,** explosive pod dehiscence, explosive pollination mechanism, hermaphroditism, melittophily**,** *Rhynchosia minima*.

Introduction

Rhynchosia is a genus of the legume family fabaceae, tribe phaseoleae and subtribe cajaninae (LACKEY, 1981; JAYASURIYA, 2014). It consists of more than 232 species and occurs in both the eastern and western hemisphere in warm temperate and tropical regions (GREAR, 1978; JACA *et al*., 2018; SCHRIRE 2005; TURNER 2011). 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. albiflora, R. beddomei, R. cana, 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. minima* is a pantropical species but it is thought to be native to the Old World and introduced and naturalized in the New World. It is listed as least concern in bhutan (LOPEZ POVEDA, 2012). Its population is believed to be stable and no real threats are known at present. The population size of this species is not known, but recent surveys between 1980 and 2008 from throughout the range of the species in india suggest it occurs in groups of plants from 30 to 500 individuals (LOPEZ POVEDA, 2012). In australia, two varieties have been reported in *R. minima* based on

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pod hair character, var. *minima* as having pods with short fine hairs only and var. *australis* as having short fine hairs and long tubercular-based hairs on the pods (ANDREWS, 1952; STANLEY & ROSS, 1983). Later, HARDING *et al.* (1989) reported that *R. minima* is highly variable and there are four varieties in australia, var. *amaliae, australis, minima* and *tomentosa*. These authors also stated that this species with many ecotypes that vary in their adaptation and growth characteristics has the potential to exploit it as a forage plant. Of these infraspecific taxa, *R. minima* var. *minima* is studied for its pollination ecology.

Different authors noted that *R. minima* is widely used as a medicinal plant. CHUTE & TIWARI (2002) noted that in the indian state of maharashtra, the tribals of bhandara and gadchiroli district use its seed extract as an eye drop to cure conjunctivitis and inflammation. MUSTAK *et al.* (2006) mentioned that the whole plant is used for bath of the mother after delivery in pakistan. GUNDIDZA *et al.* (2009) stated that the plant is traditionally used in skin conditioning and to alleviate boils in zimbabwe. LOPEZ POVEDA (2012) described that the plant is used for medicines such as abortifacients, ecbolics, general healing, medicines to treat sickness such as haemorrhoids, heart, diarrhoea and dysentery. It is also used as food (sweets) and its seeds are used as miscellaneous poison or repellents. GILLETT *et al.* (1971) mentioned that the plant is used as animal forage; its palatability has been believed to vary widely with different ecotypes that occur in different habitats. HASSELL (1945) stated that in queensland, the plant is eaten readily by animals when young due to high palatability however, mature plant is not eaten due to fibrous and coarse nature. BEESTON (1978) listed *R. minima* as highly palatable for animals in the blackall district of central west queensland while BOYLAND (1973) mentioned this plant as moderately palatable for animals in the far south-west of queensland. BOGDAN (1977) stated that in kenya, the plant is readily eaten by cattle than sheep; however, the cattle stay away during its flowering phase due to slight emission of scent from flowers. SHUKLA *et al.* (1970) noted that this plant is palatable to sheep in india. Despite its wide use for humans and animals, this species has not been studied for its reproductive ecology in any part of the world to understand the factors that contribute to the success of this plant as a weed in widely different ecological conditions that prevail in the tropical latitudes.

Franco (1995, Pers. comm., Campinas, University of Campinas) provided floral details of *Rhynchosia* in Brazil. He reported that *Rhynchosia* 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*. It is in this context, the present study was contemplated to provide the details of pollination ecology of *R. minima* to understand the sexual reproduction with which the plant is able to propagate and occupy different ecological niches in tropical regions.

Material and Methods

Study site

The study region is an integral part of Southern Eastern Ghats of Andhra Pradesh in Peninsular India. The area is located at

13°40′N latitude and 79°19′E longitude. 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. The temperature ranges from 40°C to 42°C during March to May and at other times varies from 26°C to 34°C. The rainfall varies from 975 to 1115 mm. In this area, *Rhynchosia minima* grows as a small population in open areas of soil rich in moisture and litter and as isolated individuals in open rocky areas with less soil, moisture and litter.

Flowering and floral biology

Flowering season was defined based on regular field trips made. Twenty inflorescences were tagged and followed to record the length of flowering and the number of flowers produced. 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 hand lens). This procedure was followed for counting the number of pollen grains in each anther collected. 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 average volume of nectar per flower was determined and expressed in µl based; for this ten flowers were used. 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 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.

Stigma receptivity

In visual method, the stigma physical state (wet or dry) was considered to record the commencement of receptivity. H_2O_2 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 present, the percentage of fruit set was calculated for each mode.

Flower-visitors

The flower foragers included only bees. 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 flowering 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 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 (Solomon Raju & Radha Krishna, 2017).

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 (SOLOMON RAJU & RADHA KRISHNA, 2017).

Natural fruit set, seed dispersal and seedling ecology

A sample of flowers on twenty five plants were tagged on different plants 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. Field observations were also made on fruit and seed dispersal modes, seed germination and seedling establishment to the extent possible (SOLOMON RAJU & RADHA KRISHNA, 2017).

Results

Phenology

Rhynchosia minima is a perennial prostrate, climbing herb with slender stem that grows in open areas. The plant re-grows from below ground perennial root stock and from the seed during June-August during which growth and leaf flushing occurs. The plants growing in water saturated soils are robust when compared to those growing in water stress soils (Fig. 3a,b). The leaves are trifoliate with reticulate venation. The leaflets are petiolate, ovate-rhombic, and puberulous, especially beneath. The flowering occurs during September-March with peak flowering in January. The plants wither and disappear in April. The flowers are borne in pedunculate axillary and 50-70 mm long lax racemes; individual racemes are 4-6 flowered which open over a period of 2-4 days (Fig. 3c).

Flower morphology

The flowers are pedicellate, small (5.9 ± 0.6 mm long and 6.1 ± 0.5 mm wide), yellow, odorless, papilionaceous, zygomorphic and bisexual. The calyx is green with yellow tinge and consists of 5 free, linear-lanceolate, pubescent, 3-4 mm long sepals. The corolla is bright yellow, pubescent, consists of upper standard petal, two wing petals and two keel petals. The standard petal is large (4.9 ± 0.3mm long and 5.7 ± 0.4mm wide), yellow streaked with purple veins outside and inside but prominent at the bottom of the inside mid-region which serves as nectar guide; 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 opens. The two adjacent petals (4.7 ± 0.4 mm long and 2.6 ± 0.4 mm wide), called wing petals surround the two bottom petals, called keel petals (4.4 ± 0.4mm long and 2.3 ± 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. 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, 5.6 ± 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. The distal parts of the filaments are free and contain 1 mm long uniform dithecous anthers (Fig. 3j). The ovary is sessile, green, villous, 2.3 ± 0.4 mm long and lies in the sheath of the filaments along the cylindrical part of the keel (Fig. 3l,m). It is monocarpellary and monolocular with two ovules arranged on marginal placentation (Fig. 3o). It has a long glabrous style with a capitate wet shiny stigma (Fig. 3n), both together account for a length of 3.6 ± 0.4 mm. The stigma is situated at the height of the anthers (Fig. 3j). The distal portion of free filaments and style and stigma are incurved and clamped into the keel petals.

Floral biology

Mature buds (Fig. 3f) open during 1230-1530 h with peak anthesis during 1330-1430 h (Table 1). 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 (Fig. 3g,h). 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 588.6 ± 65.98 and per flower is 5,886. The pollen-ovule ratio is 2,943:1. The pollen grains are monads, spheroidal, 17.43 ± 2.49 µm in size, powdery and tricolporate, angulaperturate with reticulate exine (Fig. 3k). 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 1.2 ± 0.03 µl of nectar with 0.38 mg of sugar. The nectar sugar concentration is 28% (Range 26-30%) consisting of sucrose, glucose and fructose with the first as dominant. 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 (Fig. 3d,e). The closed flowers remain so even during most part of the fruit development.

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 (Fig. 3i). 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 visit 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 are rare and the temperatures are usually low (21-26°C). 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, 16% in hand-pollinated autogamy, 50% in geitonogamy, 88% in xenogamy and 46% in open-pollination (Table 2).

Table 1. Anthesis as a function of time in *Rhynchosia minima.*

Time (h)	No. of flowers anthesed	Percentage of Anthesis
1130	0	0
1230	11	15
1330	25	34
1430	29	40
1530	8	11
1630	0	0

No. of mature buds tagged: 73

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

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	8	16
Geitonogamy	50	25	50
Xenogamy	50	44	88
Open-pollination	351	160	46

Bee pollinators and pollination

The flowers were exclusively foraged by three species of bees for both nectar and pollen. The foraging activity began from 1100 h onwards and ceased at 1700 h (Table 3). The bees started probing the mature buds with partial opening of standard petal and showed peak foraging activity during 1200-1300 h (Fig. 1). The bees belonged to only one order, Hymenoptera, one family, Apidae and two sub-families, Apinae, and Nomiinae. One bee species belonged to Apinae and two species to Nomiinae (Fig. 3p,q). More individuals of *A. florea* and a few individuals of *Ceratina* sp. and *Nomia* sp. were recorded at the flowers. Individually, *A. florea* made 35%, *Ceratina* sp. 33% and *Nomia* sp. 32% of total foraging visits (Fig. 2). The body washings of foraging bees showed variation in the pollen carrying capacity; the average pollen recorded on *A. florea* was 174.2, *Ceratina* sp. 134.3 and *Nomia* sp. 106.4 (Table 3). The flowers were visited several times by bees but new visits lasted shorter than the first one. With respect to their behavior, the bees landed on the wing petals and the keel, with their head near the standard petal. 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). To collect pollen, the bees took "U" turn after nectar collection and proceeded to the stamens to collect pollen.

Fruiting behavior

The fruit growth and development begins immediately after pollination and fertilization. The fruits mature within three weeks (Fig. 4a,b, e-h). 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. It is a non-fleshy, hairy, oblong, 13.7 ± 1.0 mm long, 3.9 ± 0.2 mm wide, compressed, rounded and apiculated pod with the remains of the style at the apex and narrowing towards the base. The pods produced mostly two seeds but rarely one seed; it is compressed between two seeds. In certain 2-seeded pods, one seed is usually healthy and the other is aborted (Fig. 4i).

Seed ecology

Mature and dry fruits display explosive dehiscence to disperse seeds. The pod with bi-valvate configuration dehisce elastically ejecting the seeds (Fig. 4c,d). The seed is greyish to brown, compressed, reniform, finely pubescent, shortly beaked, 2.9 ± 0.2 mm long, 1.9 ± 0.2 mm wide and shiny without strophiole (Fig. 4j). Seeds germinate during rainy season which starts from June to August. Seedlings grow continually but their growth rate is subject to the availability of moisture status of the soil. In areas where soil is saturated with moisture and contains litter, seeds continue growth and produce mature plants within two months and subsequently commence flowering and fruiting.

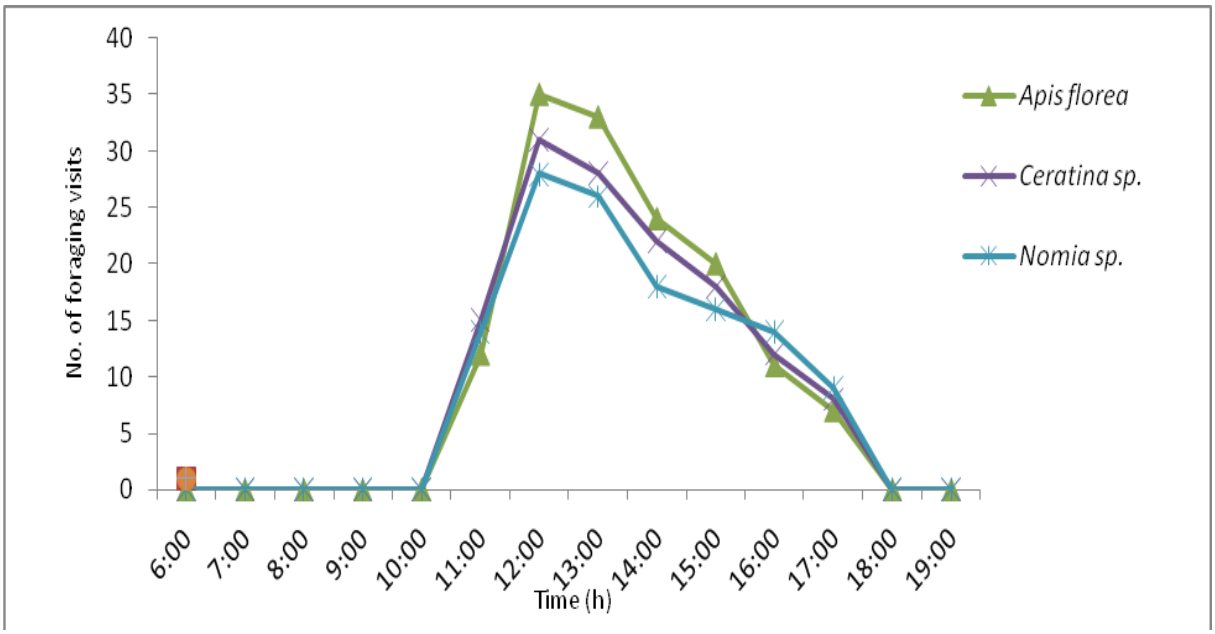

Fig. 1. Hourly foraging activity of bees on *Rhynchosia minima*.

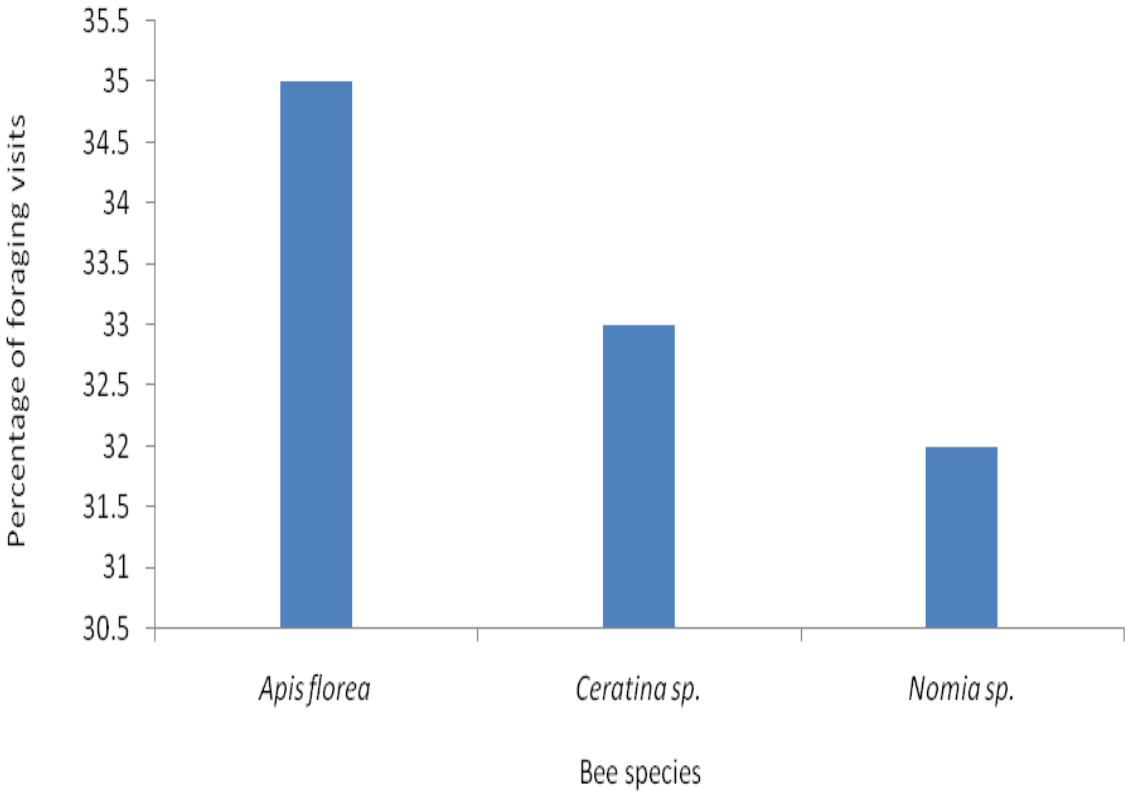

Fig. 2. Percentage of foraging visits of individuals bees on *Rhynchosia minima*.

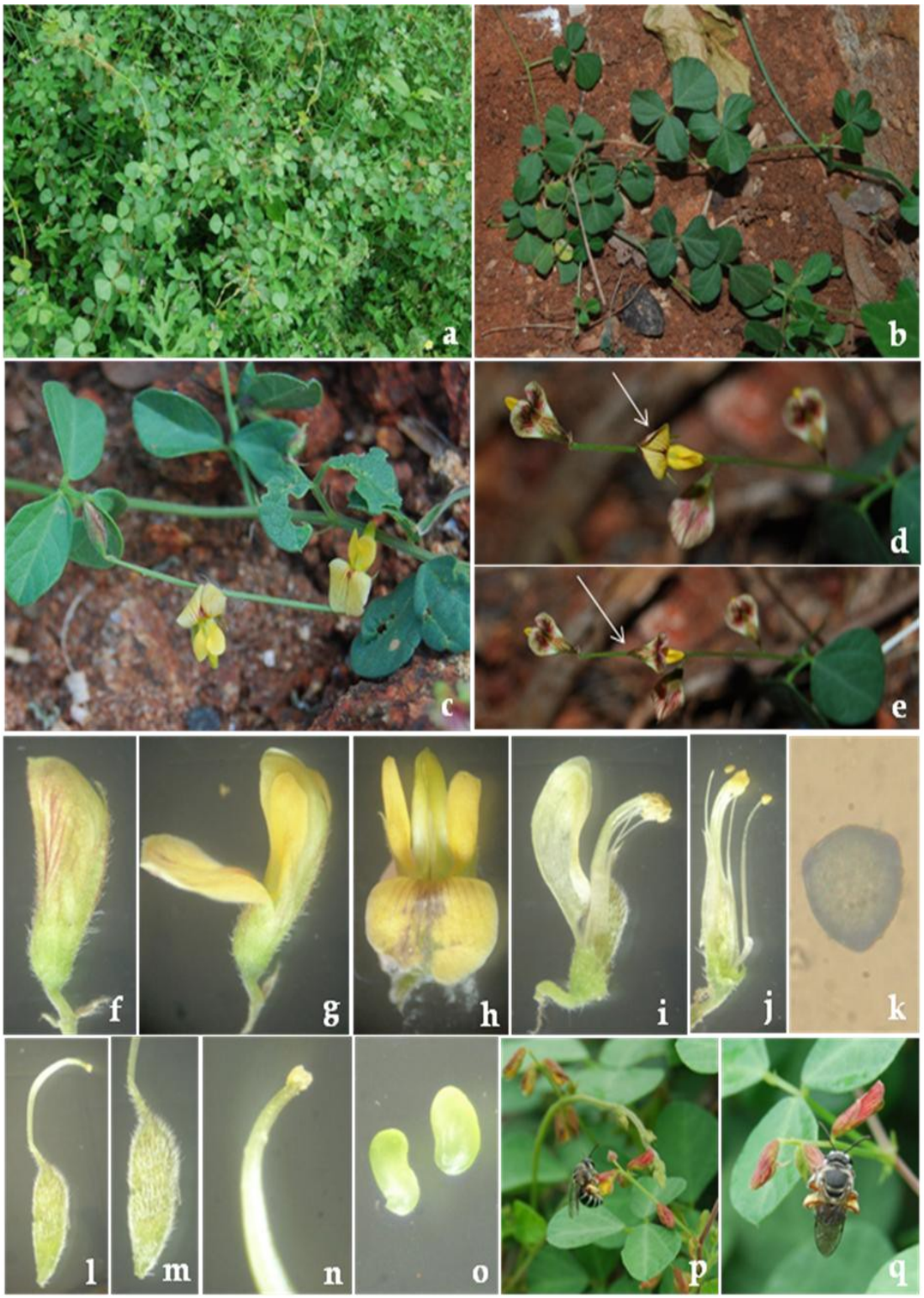

Fig. 3. *Rhynchosia minima*: a. Luxurious growth in water-saturated soil, b. Individual plant in water-stress soils, c. Flowers, d-e. Closure of standard petal covering the wing and keel petals, f. Mature bud, g. & h. Flower with stamens and stigma housed in keel petals, I. Explosive release of stamens and stigma from keel petals, j. Stamens and stigma, k. Pollen grain, l. Pistil, m. Pubescent ovary, n. Capitate stigma, o. Ovules, p. & q. *Nomia* bees.

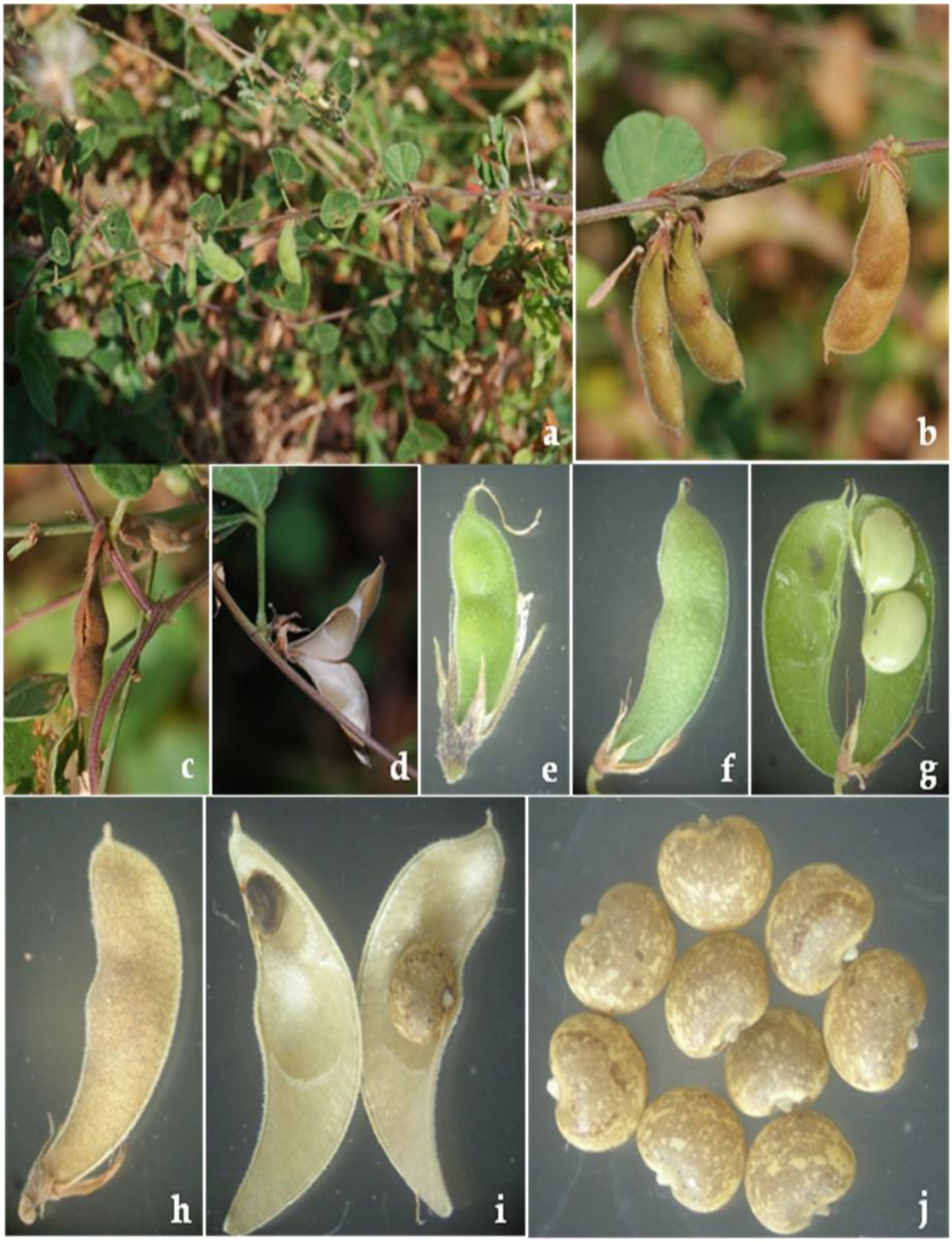

Fig. 4. *Rhynchosia minima*: a. Fruiting branch with maturing and mature pods, b. Maturing pods, c. Mature and dry pods partly split for seed release, d. Explosive twisting of pod for seed release , e-h. Different stages of pod development, I. Pod with one healthy seed and one aborted seed, j. Healthy seeds.

Table 3. Pollen recorded in the body washings of bee foragers on *Rhynchosia minima.*

Bee species	Sample size (N)	Number of pollen grains		
		Range	Mean	S.D
Apis florea	10	79-276	174.2	72.05
Ceratina sp.	10	61-183	134.3	38.21
Nomia sp.	10	43-147	106.4	32.43

Discussion

Rhynchosia minima grows in diverse habitats of tropical regions of the world. LOPEZ POVEDA (2012) documented that this species occurs in grassland, grassland with scattered trees, woody bush land, ruderal land, roadside, grazed and human disturbed land, plain land and sandy black soil. HARDING *et al.* (1989) also stated that this species occurs in a variety of habitats but most often on self-mulching heavy clay soils, from sands and sandy loams. With its ability to grow in different habitats, it appears to have evolved certain characters that are adaptive to the habitat(s) where it occurs. Such evolved characters might have led to the origin of different ecotypes or varieties in this species. In australia, var. *minima* and var. *australis* have been reported in this species complex based on pod hair character (STANLEY & ROSS, 1983; ANDREWS, 1952). Later in the same country, HARDING *et al.* (1989) described four varieties - *amaliae, australis, minima* and *tomentosa* in this species complex based on variations in their adaptation and growth characteristics. In the present study, *R. minima* has been found to grow in water-saturated and water stress soils. It grows as a population in water-saturated soils while as scattered or isolated individuals in soils experiencing water stress and rocky areas with little soil content. *R. minima* did not show any notable variations in their growth or morphological characters but the plants growing in moist soils are comparatively robust to those growing in drought or rocky areas. However, further work in this line in different habitats in India may reveal the existence of ecotypes or varieties in this species.

Rhynchosia minima is a prostrate, climbing herb which grows from perennial root stock during rainy season. It also produces new plants from seed stock at the same time. Full leaf flushing is complete by the end of August and floral bud initiation takes place in October. The flowering season is well defined and is confined to north-east monsoon and winter seasons. Individual plants produce a small number of flowers during their life time due to production of a few inflorescences and each of which producing a maximum of only six flowers. The plant during flowering phase does not attract many flower foragers although different categories of insects exist in the vicinity; the prostrate habit, display of a few flowers at ground level and dull banner petal (standard petal) appear to be responsible for the non-receipt of visits by many insects.

In *R. minima*, 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 entire reproductive column stays 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. 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 vicieae) (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. minima*, 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 six 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; LPWG, 2017) although bird pollination and bat pollination have also been recorded (ORTEGA-OLIVENCIA *et al.,* 2005). In bee-pollinated flowers of Papilionoideae, 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. minima* 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 reproductive column. The flowers are typical of pollination by bees since they are zygomorphic, standard petal 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. minima* 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. minima* 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 around noon time 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 the flowering season of this plant (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. minima* 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. minima* with melittophilous pollination syndrome also produces sucrose-rich nectar which is utilized exclusively by long-tongued bees. *Apis, ceratina* and *nomia* bees recorded on this herb have been documented as long-tongued bees (CRUDEN *et al.*, 1983; ROUBIK, 1992; ROUBIK, 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. minima* produce a small volume of nectar with 28% sugar concentration. Further, the energy yield from nectar appears to be in tune with the requirement of energy by bees. Therefore, *R. minima* 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 of *R. minima* never received visits from other categories of insects.

In *R. minima*, 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. The bees visiting the flowers seem to be efficient in tripping the flowers because the flower size and petal strength are commensurate with the bee size and the force employed to depress the wing petals to access the nectar. 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. minima* serves also as a pollen source for foraging bees.

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. minima* shows highest p/o ratio when compared to that of facultative xenogamy used by CRUDEN (1977). The highest P/O ratio in this plant species appears to be a consequence of pollen collection activity by bees. Therefore, it is inevitable for *R. minima* 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.

TRAN & CAVANAGH (1984) reported that in leguminosae, seeds of many taxa exhibit

physical (exogenous) dormancy due to the presence of a water impermeable seed coat. With this dormancy, they remain viable for long period of time. ALI *et al.* (2012) reported such physical dormancy in *Rhynchosia capitata* due to which this species is successful as a weed. SHAUKAT & BURHAN (2000) described seed characteristics and the factors regulating germination of *r. minima* in pakistan; it exhibits differential success in different habitats with different micro-climates. RANGASWAMY & NANDAKUMAR (1985) reported that the seed coat of *Rhynchosia minima* is composed of three gradative barriers to water uptake a surface deposit of waxy material interfused with a lipoidal substance, β-sitosterol; a subjacent 3-µm adcrustation of hemicellulose-cellulose complex; and a layer of palisade cells in which the secondary walls are impregnated with arabinan and the lumen contains tannin and phenolic compounds. The micropyle and hilum function as hygroscopic valves when seed coat breaks open. In this study, a few seedlings have been sighted and this is not in tune with the abundant seed production through open-pollinations by *r. minima*; hence this species appears to have physical dormancy as reported by RANGASWAMY & NANDAKUMAR (1985). Many seeds germinate in the vicinity of the parental plants in areas of water-saturated soils during rainy season but their growth suppressed when long dry spells continued during this season. In areas of water stress, a few seeds germinated here and there but they soon perished due to water stress as a consequence of long dry spells. In both water-saturated and water stress habitats, the perennial root stock of this species seasonally resurrects and produces new growth during rainy season. The explosive pod dehiscence results in the settlement of seeds mostly in the parental sites but rain water may disperse them to new places during rainy season. Seed dormancy seems to be regulating the proliferation of *R. minima*. It is successful in building up populations only in areas where soil is water saturated and hence there it becomes a successful weed in farmlands but certainly not in areas where soil is deficient in moisture during rainy season. Therefore, *R. minima* is not a very widespread species because it is habitat-specific and requires sufficient moisture and nutrient content in the soils to establish populations. Nevertheless, *r. minima* although not successful in establishing populations in water-stress soils is a hardy plant and tolerant of drought as stated by HARDING *et al.* (1989).

R. minima is widely used as a medicinal plant. It is used to cure eye conjunctivitis and inflammation (CHUTE & TIWARI, 2002), body care (MUSTAK *et al.*, 2006), skin conditioning and boils (GUNDIDZA *et al.*, 2009), haemorrhoids, heart, diarrhoea and dysentery (LOPEZ POVEDA, 2012). It is also used as animal forage (HASSELL, 1945; SHUKLA *et al.*, 1970; GILLETT *et al.*, 1971; BOYLAND, 1973; BEESTON, 1978; BOGDAN, 1977). Further, LOPEZ POVEDA (2012) noted that the seed of this species is used as food, especially in making sweets. In this connection, it is pertinent to mention the report of HARDING *et al.* (1989) that this species is likely a potential grain crop because it is a large seed producer. Since significant variations exist in agronomic attributes such as days to flowering, pod indehiscence and seed size of *R. minima* growing in different habitats, there is a huge potential to identify and select desirable genotypes to improve grain production in this species. Therefore, further studies are suggested to exploit the varieties or ecotypes of *R. minima* for medicine, animal forage and human food.

REMANANDAN (1981) stated that *Rhynchosia*, being generically 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.

In this study, the seeds of *R. minima* have not been infested by any pod-borer or pod-fly both in water-saturated and water-stress habitats suggesting that it has physiological resistance against insect pests. Therefore, intensive and extensive research is suggested to identify and select desirable genotypes of *R. minima* that give physiological resistance against pod or seed pests in order to use them for crop improvement in pigeon pea.

Conclusion

Rhynchosia minima is a winter blooming prostrate climbing herb in India. It is hermaphroditic, self-compatible and equipped with explosive pollination mechanism adapted for melittophily. It is essentially vector-dependent for both self- and cross-pollination. Mature pods dehisce explosively to disperse seeds which germinate during wet season. The seedlings grow successfully only in soils with sufficient moisture or else they subsequently perish. Therefore, this plant grows as a successful weed in farm lands only where soil is wet and nutrient-rich. Previous reports indicate that it has medicinal, animal forage and human food values and hence there is a potential for its exploitation commercially.

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