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Bee-pollination in *Crotalaria laburnifolia*, *Crotalaria medicaginea*, *Crotalaria retusa* and *Crotalaria verrucosa* and *C. retusa* as a source of alkaloids for nymphalid butterflies

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ABSTRACT

Crotalaria laburnifolia, *C. medicaginea*, *C. retusa* and *C. verrucosa* species occur throughout the year in wet habitats but prolific growth occurs during wet season only. *C. laburnifolia*, *C. retusa* and *C. verrucosa* are large-flowered while *C. medicaginea* is small-flowered. The flowers of all species are bisexual with typical papilionaceous corolla which is golden-yellow in *C. laburnifolia*, *C. medicaginea* and *C. retusa*, and bluish-white with dark purple lines in the center of the standard petal in *C. verrucosa*. The ovary has a single carpel in *C. laburnifolia*, *C. medicaginea* and *C. retusa* but in *C. verrucosa*, the ovary has 2 or 3 apocarpous ovaries. In all four *Crotalaria* species, the flowers are protandrous, nectariferous with brush type pollination mechanism and bee-pollinated. *C. retusa* is used as alkaloid source by nymphalid butterflies, *Tirumala limniace* and *Danaus chrysippus*, the former collects alkaloids from the stem and leaf petioles while the latter from the sepals. These butterflies use alkaloids for protection against their predators and for the biosynthesis of pheromones to attract mates. The alkaloid collection activity by nymphalids indirectly controls the proliferation of *C. retusa* in natural or disturbed habitats.

Keywords: *Crotalaria*, brush-type pollination mechanism, bee-pollination, alkaloids, nymphalids.

1. INTRODUCTION

The genus *Crotalaria* has about 600 species which are distributed throughout the world of which about 500 species occur in Africa alone where they are used as green manure crops in farming systems. Some species control the proliferation of

plant nematode parasites (Werchan et al. 1989; Sy et al. 2000; Boatwright et al. 2008; Dhatchanamoorthy et al., 2021). The species of this genus produce toxic pyrrolizidine alkaloids as part of their defense against herbivores. The rhizobial partner, the symbiotic rhizobium with these species produces root nodules which trigger the biosynthesis of these alkaloids and they are then transported to different above ground parts of the plants (Irmer et al. 2015). The pyrrolizidine alkaloids include monocrotaline and spectabiline but both kinds are not produced by the same species. The alkaloids produced by *C. spectabilis*, *C. retusa*, *C. alata* and *C. quinquefolia* display high levels of toxicity while those produced by *C. australis*, *C. maxillaris*, *C. sphaerocarpa*, *C. juncea* and *C. brevidens* display lowest levels of toxicity. These alkaloids are toxic to birds and large mammals but the toxic levels depend on the production levels of these alkaloids by individual species (Williams and Molyneux 1987). In pastoral regions of Australia and Brazil, the toxic alkaloids produced by *Crotalaria* species induce fatal toxicity in sheep, cows and horses when grazed by them (Everist 1981; Nobre et al. 2005); these alkaloids cause severe hepato-toxicity and renal failure (Williams and Molyneux 1987; Brugnerotto et al. 2020). Fletcher et al. (2009) reported that *Crotalaria* species containing hepatotoxic pyrrolizidine alkaloids grow widely in pastures in northern Australia and these alkaloids sporadically poison grazing livestock. *C. goreensis*, *C. aridicola* subsp. *densifolia*, and *C. medicaginea* var. *neglecta* do not produce hepatotoxic alkaloids. *C. novae-hollandiae*, *C. ramoisissima*, *C. retusa* and *C. crispata* are abundant and produce high levels of hepatotoxic alkaloids which pose the high risk to livestock health. Further, *C. spectabilis* and *C. mitchellii* with hepatotoxic alkaloids produce high risk to livestock health. Fletcher et al. (2011) reported that in grazing regions of central-west Queensland, Australia, when horses consumed *C. medicaginea*, which produces toxic alkaloids, resulted in their death. Hall (2005) stated that *Crotalaria* species are potentially fatal to livestock due to the presence of pyrrolizidine alkaloids. *C. lanceolata*, *C. pallida* and *C. spectabilis* introduced in the United States of America have been considered to be fatal to livestock. *C. retusa* has been reported to be toxic to cattle.

Danaine butterflies feed at withered plants of some genera of Asteraceae, Boraginaceae and Fabaceae for acquiring pyrrolizidine alkaloids (Pliske 1975; Boppre 1983). They apply a fluid from their proboscides to dissolve alkaloids in order to imbibe them. The male butterflies require these alkaloids as precursors for the biosynthesis of a pheromone component. Both male and female butterflies store alkaloids for defense against their predators (Edgar et al. 1976; 1979; Rothschild et al. 1979; Conner et al. 1981). Danaines, *Tirumala petiverana*, *Danaus chrysippus* and *Amauris ochlea* aggregate on *Heliotropium pectinatum* (Boraginaceae) and damage fresh plant tissue to obtain pyrrolizidine alkaloids (Boppre 1983). *D. plexippus* suck the exudates from the withered leaves of *Asclepias* species, and species of Boraginaceae and Asteraceae to obtain pyrrolizidine alkaloids which facilitate its mating and deterrence from its predators (Bell 2001). In certain danaid butterflies, the pyrrolizidine alkaloids have a key role in host-plant selection and as a sex pheromone (Jones and Blum 1983). Ithomiine butterflies acquire protecting chemicals from the dead or withered plant species of Boraginaceae, Asteraceae, Apocynaceae and Orchidaceae which are used mainly by the male butterflies (Lamas 1999).

In the genus *Crotalaria*, the papilionoid flower structure is adapted to melittophily or bee-pollination. The standard petal attracts pollinators while the wing petals and keel form a landing platform. Sculpturing on the wing petals creates a rough area to ease gripping and balance of the pollinator while foraging nectar (Arroyo, 1981). Further, the flowers are brush type, characterized by a rostrate keel, highly dimorphic anthers, stylar trichomes and elaborate callosities on the standard petal. This brush type pollination mechanism is a modification of the pump type pollination mechanism; it differs only in the more efficient action of pumping mechanism. The trichomes present on the apical portion of the style aid to brush the pollen out of the keel beak after anthesis facilitating the promotion of cross-pollination and delaying self-pollination (Etcheverry, 2001a,b,c, 2003; Le Roux and Ben-Erik 2012). Kundu (1964) stated that *Crotalaria* species are generally self-incompatible but self-pollination occurs only when the stigmatic surface is stimulated by insects or by other means. Tidke and Patil (2000) reported that in *C. sericea*, the glued petals aid in the occurrence of self-pollination while bee visits result in out-crossing. Etcheverry (2001b) reported that *C. stipularia* flowers are nectariferous and protandrous with stigma membraneless; the pollen/ovule ratio is indicative of facultative xenogamy. The pollination mechanism is of brush type which is triggered by bees, *Epanthidium erythrocephalum* and *Pseudocentron* sp. Wyk (2005) reported that *Crotalaria* flowers adapted for buzz-pollination by large bees, *Vegactile* and *Xylocopa* species. Kar and Datta (2018) reported that *C. spectabilis* flowers are protandrous and pollinated by butterflies, beetles and bees but the upward movement of filaments to the proximity of the stigma might facilitate the occurrence of autonomous self-pollination. Meagher et al. (2019) reported that *C. juncea* flowers are nectariferous and pollinated by *Xylocopa virginica*, *X. micans*, *Megachile sculpturalis*, *M. mendica* and *M. georgica*. Kumar et al. (2019) reported that *C. juncea* is self-compatible, herkogamous and cross-pollinating. Large bees, *Megachile lanata*, *M. bicolor*, *M. disjuncta* and *M. anthracina* and *Xylocopa fenestrata* are the effective pollinators while *Apis* bees although visit the flowers are never involved in pollination. The flowers resort to delayed autonomous self-pollination by the elongation of filaments to reach the stigma level in the absence of pollinators. Devi et al. (2022) reported that in *C. mysorensis* the floral architecture indicates that it is an outbreeder but it is a strict in-breeder due to its vespereal nature and brief flowering period.

In the flowers, the stigma is free from pollen on the first day but the staminal filaments grow substantially to reach the level of the stigma to resort to autonomous self-pollination.

With this backdrop, the present study is aimed at reporting on floral features, pollination mechanism and pollinators of *Crotalaria laburnifolia* L., *C. medicaginea* Lam., *C. retusa* L. and *C. verrucosa* L (Fabaceae). Further, the use of *C. retusa* as a source of pyrrolizidine alkaloids for danaid butterflies as chemical defense against its herbivores has also been examined. The findings of this study are evaluated in the light of relevant information available on *Crotalaria* genus.

2. MATERIALS AND METHODS

Crotalaria laburnifolia, *C. verrucosa* growing wild on Andhra University campus, *C. medicaginea* in open habitat of Kailasagiri in Visakhapatnam District and *C. retusa* at a wild patch near Coringa, East Godavari District, Andhra Pradesh, India, were selected for field study during July-November 2021. The vegetative growth and sexual reproduction events were carefully observed. Twenty flowers from each plant species were examined to note the floral traits. The flower-opening schedule, anther dehiscence timing and stigma receptivity of each plant species was recorded. Different stages of buds and flowers were observed to record nectar secretion levels if nectar is secreted. Field observations on flower visitors were made day-long day to record their scientific names, the foraging behavior and forage sought. Further, field observations were also made on butterflies whether they use any of these plant species as source of alkaloids. All recorded observations and descriptions were systematically discussed with the concerned works.

3. RESULTS

Crotalaria laburnifolia and *C. retusa* are perennial bushy shrubs while *C. verrucosa* is an annual erect herb and *C. medicaginea* is a perennial prostrate herb which has sparse growth and flowering in dry rocky habitats (Figure 2a) and profuse growth and flowering in semi-wet and wet habitats (Figure 2b). All four species have branched and glabrous or slightly pubescent stems. Leaves are petiolate in all these species; they are simple in *C. retusa* and *C. verrucosa* while they are trifoliate in *C. laburnifolia* and *C. medicaginea*. They are oblanceolate in *C. retusa*, elliptic with rounded apex in *C. laburnifolia*, ovate with wavy margins in *C. verrucosa* and obovate-oblong in *C. medicaginea*. All four species show prolific vegetative growth during rainy season and then display flowering from late October to mid-February. They extend their growth, flowering and fruiting if the habitat is wet. The inflorescence is a lax-many-flowered terminal raceme in *C. laburnifolia* (Figure 1a,b), terminal as well as leaf-opposed 2-9 flowered raceme in *C. medicaginea* (Figure 2c), terminal erect raceme with about 25-27 buds in *C. retusa*, 10-14 flowered terminal or lateral raceme in *C. verrucosa* (Figure 4a-c). In all these species, the buds mature and produce flowers in acropetal succession.

The floral description given here relates to all four plant species unless and otherwise specified. The flowers are showy, hermaphroditic and zygomorphic with five sepals united into a small tube. The corolla is typically papilionaceous but its color is different in these species, it is golden-yellow in *C. laburnifolia*, *C. medicaginea* and *C. retusa* while it is bluish white in *C. verrucosa*; in the last species, the standard petal has dark purple lines in the center. The stamens are 10, monadelphous with dimorphic 2-celled introrse anthers with fertile pollen (Figure 4h) but 5 anthers are dorsifixed while 5 others are basifixed in all four plant species (Figure 4f,g). In *C. laburnifolia*, *C. medicaginea* and *C. retusa*, the ovary is green, monocarpellary and monolocular stalked ovary; the ovules per ovary are 20-22 in *C. laburnifolia*, many-ovuled in *C. medicaginea* and 16-19 ovules in *C. retusa*. In *C. verrucosa*, the ovary consists of bicarpellary or tricarpellary apocarpous ovaries and each ovary with 6 or 7 ovules. The style is long with trichomes or hairs towards the apical portion in all four species (Figure 4i-l). Fruit is characteristically a pod. It is oblong-clavate and glabrous in *C. laburnifolia*, spherical and velvety in *C. medicaginea*, linear-oblong and glabrous in *C. retusa*, and oblong and glabrous in *C. verrucosa*; but the pods are dehiscent, inflated and the seed number per pod varies with each plant species according to the pollination and fertilization rate (Figure 5h). The seeds gradually become loose and rattle when pods mature due to shaking by wind or by other means in all four plant species.

In all four *Crotalaria* species, the mature buds are usually open in the afternoon hours but a few buds show early anthesis before noon. The flowers are protandrous and anther dehiscence occurs in mature bud by longitudinal slits while the stigma becomes receptive shortly after anther dehiscence. Nectar secretion process is almost complete by the time of anthesis and its location can be seen through nectar windows (Figure 4d,e). The floral sex organs remain inside the keel petals which are escorted by wing petals even after the unfolding of the standard petal. The sex organs come out of the keel petals only when the forager lands on the keel petals to probe for nectar.



Figure 1. *Crotalaria laburnifolia*: a. Twig with inflorescence, b. Papilionaceous flower, c. & d. *Ceratina* sp. probing the keel petals for pollen collection, e. & f. *Xylocopa latipes*, g. *Xylocopa pubescens*, h. *Nomia* sp. attempting to collect sap from the calyx.



Figure 2. *Crotalaria medicaginea*: a. Habit - sparse growth and flowering in dry, rocky areas. b. Profuse growth and flowering, c. Flower, d. *Apis florea* probing for pollen, e. *Pithitis smaragdina* probing mature buds for nectar collection, f. *Pithitis smaragdina* probing for pollen collection.



Figure 3. *Crotalaria retusa*: a-d. Nymphalid butterflies - a. & b. Aggregation of *Tirumala limniace* individuals for collection of sap from fresh leaves, c. & d. *Danaus chrysippus* individuals collecting sap from calyx.

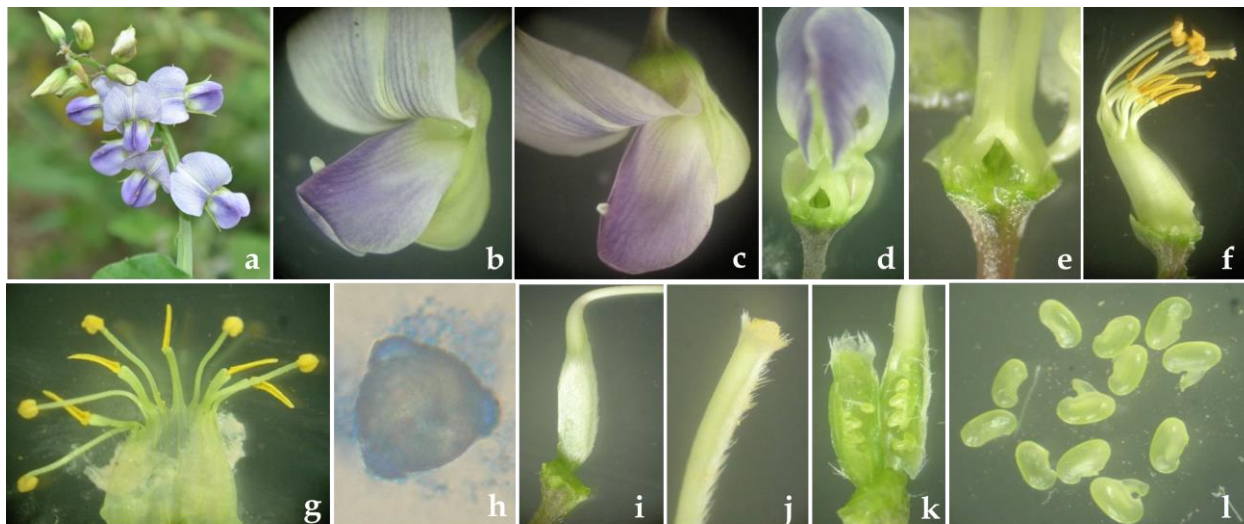


Figure 4. *Crotalaria verrucosa*: a. Flowering inflorescence, b. & c. Flower, d. & e. Nectar windows, f. & g. Monoadelphous with dimorphic stamens, h. Pollen grain, i. Pistil, j. Stigma, k. Ovary with ovules, l. ovules.

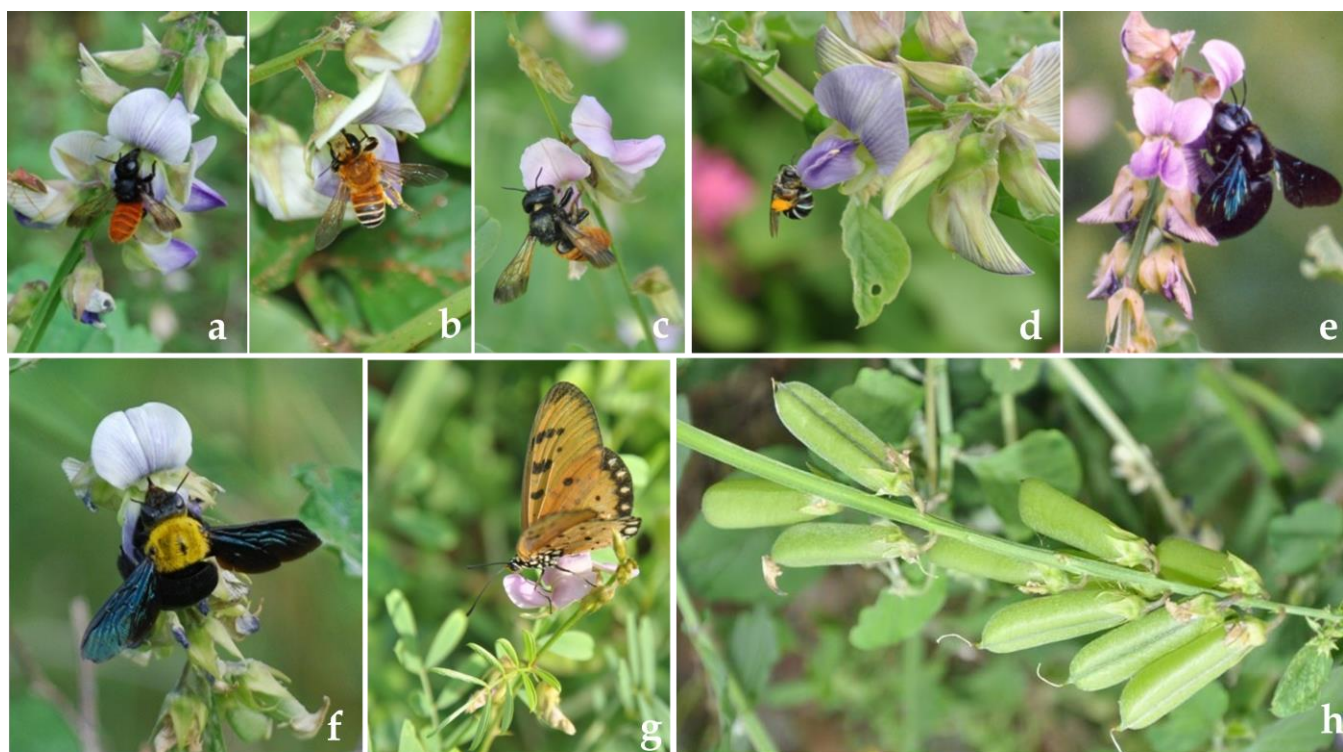


Figure 5. *Crotalaria verrucosa*: a. & b. *Megachile* sp. probing the flower for nectar collection, c. *Lasioglossum* sp. probing the flower for nectar collection, d. *Nomia* sp. probing the keel petals for pollen collection, e. *Xylocopa latipes* probing the flower for nectar collection, f. *Xylocopa pubescens* probing the flower nectar collection, g. Nymphalid butterfly, *Acrae violae* probing the flower for nectar collection, h. Fruits.

C. laburnifolia flowers were visited and pollinated by *Ceratina* sp. (Figure 1c,d), *Nomia* sp., *Xylocopa latipes* (Figure 1e,f) and *X. pubescens* (Figure 1g); the first two bee species collected only pollen while the other bee species collected only nectar. The pollen collecting bees were small in size to press the keel and wing petals downwards to collect nectar. They simply landed on the keel and wing petals and gradually probed for the concealed anthers to collect pollen and in this process they were able to carry out pollination. *Nomia* bees were also found to make attempts to collect sap from the calyx (Figure 1h) but it was not clear whether they were really resorting to suck sap or not. The *Xylocopa* bees probed the flowers from the front, pressed the keel and wing downwards and slightly lifted the standard petal upwards to access the nectar placed in concealed state at the corolla base. In so doing, the sex organs ejected out of the keel petals forcibly releasing the brush-like stigma due to the presence of trichomes on its apical portion, which simultaneously brushed the ventral side of the pollen-laden probing bee due to which the stigma is coated with pollen. At the same time, the pollen from the dehisced anthers ejected pollen due to which the ventral side of the bee got sprinkled with pollen. Therefore, the foraging visits of these bees to the flowers resulted in effecting pollination, mostly out-crossing, and also in the occurrence of pollen transfer unto the bee for pollination in their subsequent visits to other conspecific flowers.

C. medicaginea flowers being small in size were visited by *Apis florea* (Figure 2d) and *Pithitis smaragdina* only (Figure 2e,f); these bees probed the flowers from the side of standard petal and of keel and wing petals in which the sex organs are concealed. In this process, they were able to depress the keel petals together with wing petals to access and collect nectar and also they probed the keel petals for collecting pollen; these two probing modes resulted in effecting self- and/or cross-pollination. Further, these bees, especially *P. smaragdina* even made attempts to probe mature buds for nectar as well as pollen.

C. retusa flowers were foraged exclusively by *Xylocopa latipes* and *X. pubescens* for nectar collection for which they employed the foraging behavior that was employed for nectar collection from *C. laburnifolia* flowers; they acted as pollinators. The Nymphalid butterflies, *Tirumala limniace* and *Danaus chrysippus* visited the plant regularly day-long for the collection of alkaloids. Several individuals of *T. limniace* visited the plant to scratch the stem and leaf petioles of the living plant to obtain the alkaloids (Figure 3a,b) while *D. chrysippus* visited the flowers and scratched the sepals to obtain alkaloids (Figure 3c,d). The stem, leaf petioles and sepals scratched by the respective butterflies gradually withered. Both butterflies occasionally visited the flowers, especially the ones which were already probed by carpenter bees. In such flowers, the standard petal is a bit loose which most likely facilitates the

probing butterflies to insert their proboscis with ease through the space between the standard petal and keel and wing petals to access and collect nectar. It is not clear whether the butterflies could get nectar from the flowers which were never visited by carpenter bees.

C. verrucosa flowers were foraged by bees such as *Megachile* sp. (Figure 5a,b), *Lasioglossum* sp. (Figure 5c), *Nomia* sp. (Figure 5d), *Xylocopa latipes* (Figure 5e) and *X. pubescens* (Figure 5f), and also by the nymphalid butterfly, *Acrae violae* (Figure 5g). All bee species were consistent foragers while the butterfly was an occasional forager; all these foragers except *Nomia* sp. were exclusive nectar collectors while *Nomia* sp. was a pollen collector. All bee species seeking nectar foraged legitimately, landed on the keel and wing petals and probed the flowers in the manner that *Xylocopa* bees did for nectar collection from the flowers of *C. laburnifolia* and *C. retusa* and this manner of flower probing resulted in the occurrence of either self- or cross-pollination. The butterfly also probed the flowers in the same way for nectar collection but it has no role in effecting pollination since the sex organs never came out of the keel petals while it is probing the flower for nectar.

4. DISCUSSION

In Fabaceae, most self-compatible species do not display the spontaneous germination of self-pollen because the stigma is lined by a membrane; this membrane is to be scratched to enable the self- or cross-pollen to germinate. This scratching is attained during the process of exposure of floral sex organs by a legitimate visitor (Endress 1996). Jacobi et al. (2005) reported that *Crotalaria pallida*, *C. lanceolata* and *C. retusa* are self-compatible and have the ability to spontaneously self-pollinate due to absence of a membrane in the stigma and this is an important characteristic of the tribe Crotalariaeae. In this study, all four *Crotalaria* species appear to be self-compatible and lack the stigmatic membrane that prevents autonomous self-pollination; these floral functional traits facilitate the occurrence of spontaneous self-pollination which is substantiated by high pod and seed set rate.

Rockinger (2016) reported that *Crotalaria* species display piston-type pollination mechanism and the weight of bee inserting its proboscis to reach the base of the staminal tube to collect nectar exerts much force on the keel petals and in consequence this force triggers the hairy style to emerge due to which the abdomen of the bee gets brushed by the pollen. Etcheverry (2001a,b,c, 2003) and Le Roux and Ben-Erik (2012) reported that *Crotalaria* flowers of brush type characterized by a rostrate keel, highly dimorphic anthers, stylar trichomes and elaborate callosities on the standard petal. This brush type pollination mechanism is a modification of the pump type pollination mechanism; it differs only in the more efficient action of pumping mechanism. The trichomes present on the apical portion of the style aid to brush the pollen out of the keel beak after anthesis facilitating the promotion of cross-pollination and delaying self-pollination. In the present study also, all four *Crotalaria* species exhibit the same floral characters and brush type pollination mechanism. The stylar trichomes play an important role in brushing the abdomen of the probing forager or bee with pollen.

In all four *Crotalaria* species, the flowers with typical papilionaceous floral configuration are well adapted for specialist pollinators. The stamens and stigma are concealed in keel petals which are enclosed outside by wing petals. The specialist pollinators upon landing press the keel and wing petals downward and the standard petal upwards to collect deeply seated and concealed nectar. As a result, the keel petals violently release the stamens and stigma during which pollen deposition on the stigma and pollen transfer unto the body of the probing pollinator occur spontaneously. Majinda et al. (2001) reported that *C. pallida*, *C. lanceolata* and *C. retusa* are adapted for pollination exclusively by large carpenter bees, *Xylocopa frontalis* and *X. grisea*. Jacobi et al. (2005) documented that the legitimate pollinators of *Crotalaria pallida*, *C. lanceolata* and *C. retusa* are *Xylocopa frontalis* and *Xylocopa grisea*. In the present study, the floral configuration of all four *Crotalaria* species indicates that it is adapted for specialist pollinators. *C. laburnifolia*, *C. retusa* and *C. verrucosa* flowers being large in size are efficiently pollinated by *Xylocopa latipes* and *X. pubescens*. *C. laburnifolia* is also pollinated by *Ceratina* and *Nomia* bees, and *C. verrucosa* by *Megachile*, *Lasioglossum* and *Nomia* bees. *C. medicaginea* flowers being small in size are pollinated by bees, *Apis florea* and *Pithitis smaragdina*. Therefore, *Crotalaria* species in general and the species now studied in particular display specialist flowers with specialized pollination adapted for specialized pollinators according to the flower size and other floral traits.

Pliske (1975) and Boppre (1983) documented that danaine butterflies feed at withered plants of some genera of Asteraceae, Boraginaceae and Fabaceae for acquiring pyrrolizidine alkaloids. Boppre (1983) reported that danaines, *Tirumala petiverana*, *Danaus chrysippus* and *Amauris ochlea* aggregate on *Heliotropium pectinatum* (Boraginaceae) and damage fresh plant tissue to obtain pyrrolizidine alkaloids. Bell (2001) reported that *D. plexippus* suck the exudates from the withered leaves of *Asclepias* species, and species of Boraginaceae and Asteraceae to obtain pyrrolizidine alkaloids which facilitate its mating and deterrence from its predators. Different authors noted that both male and female butterflies store alkaloids for defense against their predators (Edgar et al. 1976; 1979; Rothschild et al. 1979; Conner et al. 1981). Imer et al. (2015) reported that *Crotalaria* species produce toxic

pyrrolizidine alkaloids as part of their defense against herbivores. Prasad et al. (2008) reported that danaid butterflies form large aggregations on *Crotalaria retusa* during their flowering period and obtain alkaloids from the sap that emerges from withering terminal branches and stems. Johnston and Johnston (1980) reported that *Danaus* and *Euploea* scratch leaves of *Crotalaria retusa* and imbibe the sap which oozes out. Meinwald (1990) reported that *Danaus chrysippus* uses the pyrrolizidine alkaloids acquired from *C. retusa* to protect from its predators. Hall (2005) stated that the butterfly *Tirumala limniace* plays a beneficial role by causing withering of plants of *C. retusa* before flowering and causing the abortion of the developing pods. Mathew and Anto (2007) reported that *T. limniace* uses the pyrrolizidine alkaloids to deter its predators, the garden lizards. In this study, only *C. retusa* attracted the danaine butterflies, *Tirumala limniace* and *Danaus chrysippus*, the former scratched the stem and leaf petioles of healthy plants while the latter scratched the sepals to acquire alkaloids. As a result, the parts scratched by both butterfly species gradually withered which indicates that their alkaloid collection activity affects the growth and reproduction which in turn controls the proliferation of this plant species. The alkaloids acquired by butterflies from this plant species would certainly enable them to defend against their predators and also use them in the biosynthesis of pheromones which are essential in their courtship. It is not known whether livestock that feed on the foliage and pods of *C. retusa* would be affected or not. Nicolson (2007) stated that pyrrolizidine alkaloids in nectar are inhibitory to generalist-feeding butterflies but attractive to specialist feeders. Some plant species produce mildly toxic or narcotic levels of chemical constituents in their nectars. After imbibing these narcotic substances, pollinator insects become naturally intoxicated, and exhibit more “sluggish” behavior which increases the chances of successful pollination. Klinkhamer and De Jong (1993) noted that the intoxication of pollinators by nectar could lead to undesirable levels of geitonogamous self-pollination. In this study, the danaine butterflies visiting the flowers that were already visited by nectar-seeking *Xylocopa* bees could get nectar or not; the butterflies have not been found to display any sluggish behavior which implies that there is a rare change for them to access nectar location. These danaine butterflies have never utilized the other *Crotalaria* species investigated as sources of alkaloids during the study period. Therefore, the study recommends extended field studies on other *Crotalaria* species occurring in different locations to know whether any interaction between danaines and *C. laburnifolia*, *C. medicaginea* and *C. verrucosa* exists or not.

5. CONCLUSION

All four *Crotalaria* species grow throughout the year in wet areas but prolific growth occurs during wet season only. *Crotalaria laburnifolia*, *C. retusa* and *C. verrucosa* are large-flowered while *C. medicaginea* is small-flowered. The flowers are bisexual with typical papilionaceous corolla in all these species. But, the corolla is golden-yellow in *C. laburnifolia*, *C. medicaginea* and *C. retusa* while it is bluish-white with dark purple lines in the center of the standard petal in *C. verrucosa*. The ovary has a single carpel with a single locule in *C. laburnifolia*, *C. laburnifolia*, *C. medicaginea* and *C. retusa* while the ovary has 2 or 3 apocarpous ovaries. The mature pods rattle when shaken by wind or by other means and dehisce to disperse the seeds.

All four *Crotalaria* species are protandrous, nectariferous and display brush type pollination mechanism which requires tripping by flower-visiting insects. *C. laburnifolia* flowers were pollinated by *Ceratina* sp., *Nomia* sp., *Xylocopa latipes* and *X. pubescens*, *C. medicaginea* by *Apis florea* and *Pithitis smaragdina*, *C. retusa* by *Xylocopa latipes* and *X. pubescens*, and *C. verrucosa* by *Megachile* sp., *Lasioglossum* sp., *Nomia* sp., *Xylocopa latipes*, *X. pubescens*, and nymphalid butterfly, *Acrae violae*. The nymphalid butterflies, *Tirumala limniace* and *Danaus chrysippus* use *C. retusa* as a source of alkaloids; the former acquires them by scratching the stem and leaf petioles while the latter by scratching the sepals. The plant parts used for acquiring alkaloids subsequently wither away. These butterflies use alkaloids for protection against their predators and for the biosynthesis of pheromones to attract mates. Therefore, the nymphalids indirectly control the proliferation of *C. retusa* in natural or disturbed habitats.

Authors' contributions

All authors contributed equally.

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Conflicts of interests

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

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