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REPRODUCTIVE BIOLOGY OF THE HILL GLORY BOWER, *CLERODENDRUM INFORTUNATUM* L. (LAMIACEAE)

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Abstract – *Clerodendrum infortunatum* L. is a gregarious woody shrub that flowers during dry season. The flowers are strongly protandrous, herkogamous and dichogamous displaying temporal dioecy. The flowers prevent spontaneous autogamy but facilitate geitonogamy and xenogamy which together constitute the facultative xenogamous breeding system. The flowers are visited by diurnal insects, bees, ants, thrips and butterflies of which only papilionid and pierid butterflies are pollinators. The fruit is an indehiscent drupe with fleshy mericarps and each mericarp consists of one seed only. The reddish fruiting calyx exposes the ripe fleshy fruit. The birds such as *Acridotheres tristis*, *Corvus splendens*, *Corvus macrorhynchos* and *Turdoides caudatus*, and humans feed on the fleshy part of the fruit and disperse seeds. The seeds soon dry up and also disperse by wind easily due to dry conditions during summer season. Therefore, the plant is ornithochorous, anthropochorous and anemochorous. The seeds germinate and produce new plants during wet season. Further, the perennial root stock also re-sprouts at the same time and produces new growth to carry out new reproductive cycle.

Keywords: *Clerodendrum infortunatum*, dichogamy, herkogamy, facultative xenogamy, ornithochory, anthropochory, anemochory

Introduction

Clerodendrum was long referred to Verbenaceae, but its placement was corrected following a series of molecular-based studies (Olmstead et al., 1993; Wagstaff et al., 1998), such that it now falls into Lamiaceae (Harley et al., 2004), but by 2010 it had been narrowed to about 150 (Yuan et al., 2010). It is native to tropical and warm temperate regions of the world, with most of the species occurring in tropical Africa and southern Asia, but with a few in the tropical Americas and northern Australasia, and a few extending north into the temperate zone in eastern Asia (Mabberley, 2008; Florence et al., 2012). In *Clerodendrum*, many species of Malesian origin are highly ornamental, having large showy inflorescences and attractive foliage, and some are already popular in the global horticultural trade. *Clerodendrum* species have also been used medicinally for centuries in their countries of origin and rigorous scientific trials are now underway to evaluate the potential of compounds extracted from them, notably those with antipyretic and anti-inflammatory properties and antiviral activity (Kim et al., 2001; Shrivastava & Patel, 2007). The genus displays a high degree of morphological and cytological variations, and it is also a chemically diversified genus. Terpenoids such as steroids, neo-clerodane diterpenes are the major secondary metabolites. Phenolic compounds have frequently been reported among which phenyl propanoids and flavonoids predominate. A few species have been reported to have macrocyclic alkaloids and cyanogenetic glycosides (Muthu et al. 2010). This genus is widely used in various indigenous systems of medicine for the treatment of life threatening diseases such as syphilis, typhoid, cancer, jaundice and hypertension. Apart from the medicinal uses, some species are used as ornamentals and cultivated for aesthetic purposes (Florence et al., 2012). The members of this genus have an unusual pollination syndrome that avoids self-pollination. This mating system represents dichogamy by protandry and herkogamy (Yuan et al., 2010).

Different workers have studied a few species of *Clerodendrum* for their pollination aspects. Keng (1990) reported that *C.*

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laevifolium is entomophilous and ornithochorous. Shamim et al. (2010) reported that *C. viscosum* is exclusively cross-pollinated by ants, butterflies and hawk-moths. Rohitash & Jain (2010) noted that C. *splendens* with facultative xenogamous mating system is pollinated by *Xylocopa* sp., *Eumenes* sp. and *Camponotous campestris.* McMullen (2011) reported that *C. molle* exhibits facultative xenogamy and is pollinated by nocturnal and diurnal visitors. Sakamoto et al. (2012) reported that *C. trichotomum* is protandrous and is effectively pollinated by *Papilio* and *Xylocopa* species. Mizusawa et al. (2014) reported that *C. izuinsulare* is homogamous, partially self-compatible and principally pollinated by lepidopterans. Singh et al. (2012) reported that *C. serratum* with dichogamy and herkogamy avoids selfing. Rohitash (2016) reported that *C. phlomidis* with weak protandry and facultative xenogamy is pollinated by honey bees and butterflies. Primack et al. (1981) reported that *C. inerme* is strongly protandrous and visited by hawkmoths during dusk hours. Solomon Raju & Rajendra Kumar (2016) stated that *C. inerme* with dichogamy and herkogamy is pollinated by diurnal hawkmoths and also visited by bees and butterflies. The fruit is a capsule and breaks open to disperse nutlets. Birds such as *Acridotheres tristis*, *Corvus splendens*, *Corvus macrorhynchos* and *Turdoides caudatus* disperse nutlets during early winter season. Singhmura (2016) reported that *C. infortunatum* is a commonly used traditional medicinal plant for several ailments such as asthma, cough, tumors, diarrhea, skin disorder, venereal and scrofulus complaints, wounds, wormicide, post-natal complications, as vermifuge, febrifuge in malarial fever specially in children, laxative, cholagogue, for removal ascarids in anus, as antidote for colic, scorpion sting and snake bite in various regions around the globe. Rajurkar (2010) reported that *C. infortunatum* is protandrous and considered to be pollinated by moths or some allied insects. Byragi Reddy & Subba Reddi (1995) reported that *C. infortunatum* with facultative xenogamous mating system is exclusively pollinated by papilionid butterflies. Since there is limited information on the reproductive ecology of *Clerodendrum* species, the present study is contemplated to provide information on phenology, floral biology, pollination, pollinators, sexual system, breeding system, fruiting behaviour and seed dispersal of *C. infortunatum*. The information will help to understand its sexual reproduction and the reasons for its current state of distribution.

Materials and methods

Twenty scattered individuals of *Clerodendrum infortunatum* growing at Sunkarimetta forest block of Araku Valley Reserve Forest in Visakhapatnam district (17°15' and 18°32' N, 18°54' and 83°30' E and 900 amsl), Andhra Pradesh, India, were used for the study. Regular field visits were made to record the timings of leaf fall, leaf flushing and flowering events. The inflorescence type and the number of flowers per inflorescence were noted. Ten inflorescences prior to commencement of their flowering were tagged and followed daily to record the flowering duration. Twenty-five fresh flowers were used to record the flower type, sex, shape, colour, odour, symmetry, calyx, corolla, stamens and style. The floral configuration and rewards presentation aspects were examined in relation to the forage collection activity of insects. Anthesis was initially recorded by observing the marked inflorescences in the field. Later, the observations were made 3 to 4 times on different days in order to record accurate anthesis schedule. Similarly, the mature buds were followed to record the time of anther dehiscence. The pollen presentation pattern was also investigated by recording how anthers dehisced and the same was confirmed by observing the anthers under a 10x hand lens. The presence of nectar was determined by gently pulling a flower from its calyx and firmly pressing its base against a hard surface. The protocols provided by Dafni et al. (2005) were used for measuring the nectar volume, sugar concentration, sugar types and amino acid types. The micropipette was inserted into the flower base to extract nectar for measurement. The average of ten flowers was taken as the total volume of nectar/flower and expressed in µl. Similarly, a sample of nectar was used for measuring nectar sugar concentration at selected intervals of time; the Hand Sugar Refractometer (Erma, Japan) was used for this purpose. Nectar was spotted on Whatman No. 1 filter paper along with the standard samples of glucose, fructose and sucrose. The paper was run ascendingly in chromatography chamber 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^{o}C in an electric oven for 20 minutes for the development of spots from the nectar and the standard sugars. The developed spots were compared with the spots of the standard sugars to record the sugar types present. The sugar content/flower is expressed as the product of nectar volume and sugar concentration per unit volume, mg/µl. This was 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. Nectar was spotted on Whatman No. 1 filter paper along with the standard samples of twenty-one amino acids, namely, arginine, tyrosine, alanine, aspartic acid, butyric acid, cysteine, cystine, glutamic acid, glycine, histidine, hydroxyl-proline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan and valine. The paper was run ascendingly in chromatography chamber for 24 hours with a solvent system of n-butanol-acetic acid-water (4:1:5). The chromatogram was detected with 0.2% ninhydrin reagent and dried at 85^{o}C in an electric oven for 15 minutes for the development of spots from the nectar and the standard amino acids. The developed nectar spots were compared with the spots of the standard amino acids and recorded the amino acid types present in the nectar. Ten mature but un-dehisced anthers were collected from different individuals 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. 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. Five dehisced anthers were collected in a Petri dish and the pollen removed from these anthers was examined under microscope for recording the pollen grain features. The pollen-ovule ratio was determined as per the method described by Cruden (1977). The stigma receptivity was observed visually and by H_2O_2 test. In visual method, the stigma physical state (wet or dry) and the unfolding of its lobes were considered to record the commencement of receptivity; withering of the lobes was taken as loss of receptivity. H_2O_2 test as given in Dafni et al. (2005) was followed for noting the stigma receptivity period.

The insect species were observed visually and by using binoculars; those that could not be identified on spot were captured and later identified with the help of the identified specimens available in the Department of Environmental Sciences, Andhra University, Visakhapatnam. Butterflies were identified to species level by consulting the books of Kunte (2007) and Gunathilagaraj et al. (1998). The foraging activities of insects were recorded for 10 min at each hour for the entire day on 3 or 4 occasions depending on the possibility and the data was tabulated to use the same for further analysis, especially to understand the foraging activity rate at different times of the day. For each species, 20-30 inflorescences were selected to record the foraging visits of insects. The data thus obtained was used to calculate the percentage of foraging visits made by each category insects per day to evaluate their association and pollination role. The insects feeding on nectar and/or pollen were carefully observed to assess their role in effecting pollination. They were observed on a number of occasions for their foraging behaviour such as mode of approach, landing, probing behaviour, contact with essential organs to result in pollination, and inter-plant foraging activity in terms of cross-pollination. Ten individuals of each insect species were captured while collecting pollen and/or nectar on the flowers; the collection was done during their peak foraging activity period. The captured specimens of insects were brought to the laboratory, washed in ethyl alcohol, stained with aniline-blue on a glass slide and observed under microscope to count the number of pollen grains present and evaluate their relative pollen carryover efficiency and pollination role.

Mature flower buds of some inflorescences on different individuals were tagged and enclosed in paper bags to test different modes of pollination. The stigmas of flowers were pollinated with the pollen of the same flower manually by using a brush and bagged to test manipulated autogamy. The flowers were fine-mesh bagged as such without hand pollination to test spontaneous autogamy. The emasculated flowers were hand-pollinated with the pollen of a different flower on the same plant and bagged to test geitonogamy. The emasculated flowers were pollinated with the pollen of a different individual and bagged to test xenogamy. All these modes of pollinations were kept under regular observation until fruit set. Then, the percentage of fruit set and seed set was calculated. The flowers/ inflorescences on ten individuals were tagged prior to anthesis and followed for fruit and seed set in open-pollinations. The resulting fruit and seed output were pooled up for calculating fruit and seed set rates. Fruit maturation period, the fruit and seed characteristics were recorded. Field observations were made regularly to record fruit and seed dispersal modes. Casual observations were also made to record whether the seeds germinate immediately after they are dispersed or not.

Results

Phenology

C. infortunatum is a perennial deciduous gregarious erect shrub that grows to a height of up to 2 m (Fig. 1a). It produces blunt quadrangular branches covered with silky hairs forming dense vegetation. It has large, soft, heart-shaped, hairy leaves which emit unpleasant odour when bruised. The plant appears in late June or early July and gradually grows to reproductive maturity by late January. Then, the flowering starts and continues until April but peak flowering occurs between mid-February and mid-March (Fig. 1b). Each branch is terminated with pyramid-shaped peduncled panicles consisting of 12 to 132 pedicellate flowers which open over a period of 6 to 35 days (Fig. 1c,d). The flowers orient slightly horizontally.

Flower morphology

The flowers are large (52.2 ± 0.4 mm long and 21.1 ± 2 mm wide), sweet scented, zygomorphic and bisexual. Calyx is green, polysepalous consisting of 5 lanceolate sepals, cup-shaped, tomentose, 16.3 ± 1.5 mm long, 7.2 ± 0.4 mm wide and acuminate at the tip. Corolla is white, tubate (15.2 ± 0.6 mm), 5 lobed at the tip; each lobe 15.7 ± 1.7 mm long and

Figure 1. *Clerodendrum infortunatum:* a. Gregarious growth of the shrub, b. Individual plant in flowering phase, c. Inflorescence with mature and maturing buds, d. Fully anthesed inflorescence.

6.7 ± 0.8 mm wide, tinged pink at the base and reflexed. The stamens are 4, epipetalous, 40.8 ± 1.6 mm long and protrude out of the flower. The anthers have versatile fixation, purple-coloured, oblong, 3 mm long, dithecous and introrse. The ovary is globose with two carpels each is bi-locular due to formation of a false septum; it is characteristically 4-ovuled (Fig. 2l,m). The ovules are erect, anatropous and arranged on axile placentation. The style is slender, white, 45.7 ± 2.1 mm and ends with simple bifid slightly purple-coloured stigma. The style and stigma are spring up from the center of the flower and stand slightly below the level of anthers.

Floral biology

The mature buds with coiled stamens and pistil inside open during 0500-0700 h by the splitting of petal lobes (Fig. 2a). Petals expand and reflex gradually; then the stamens, style and stigma extend beyond the rim of the corolla tube. During anthesis, the coiled stamens and pistil gradually uncoil; the latter uncoils first and stands nearly erect parallel to the central axis of the flower but the stigma lobes remain fused and stand erect on the day of anthesis. The stamens gradually uncoil, stand nearly erect and they are spatially separated from the pistil without any contact at any point of time during the flower life. The uncoiling of both stamens and pistil takes place within a span of about two hours. The anthers dehisce one hour after anthesis by longitudinal slits exposing the creamy white pollen. After the pollen is shed, the stamens gradually bend downward and orient either to one side or to both sides of the flower towards the end of the day. On the 2nd day morning, the stigma attains receptivity by stretching out its lobes and taking erect position; the receptivity ceases by the evening of 3rd of anthesis (Fig. 2b-j). The corolla, stamens and stigma wither and fall off together on 4th or 5th day. The flowers are morphologically hermaphroditic but functionally staminate on day 1 and pistillate on day 2 and 3, the situation of which totally prevents the occurrence of spontaneous autogamy but facilitates the occurrence of geitonogamy. Such a floral sexual function is the characteristic of temporal dioecy.

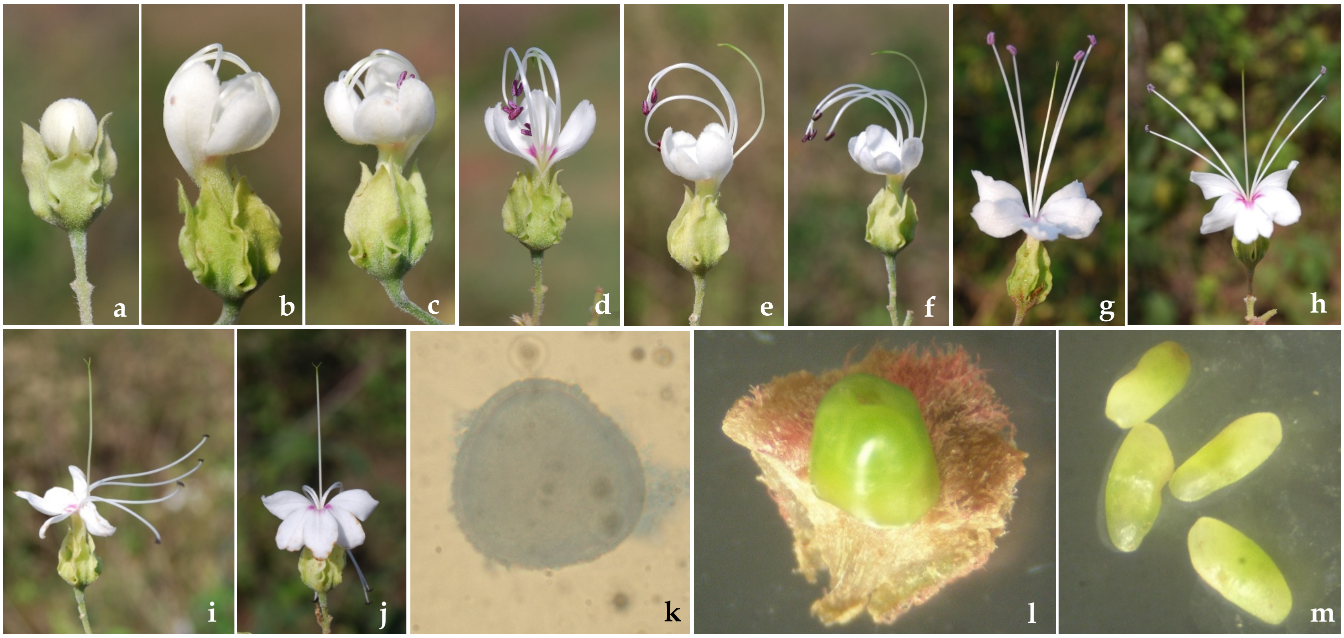

Figure 2. *Clerodendrum infortunatum:* a. Mature bud, b-f. Different stages of anthesis with spatial separation of curved stamens and pistil (undehisced anthers and unreceptive stigma), g. Flower with near erect position of stamens with dehisced anthers and unreceptive stigma, h. Flower with splayed dehisced anthers and completely erect position of unreceptive stigma, i. Flower with receptive stigma (unfolding of stigmatic lobes) and downward position of stamens far away from the stigma, j. Flower with completely recurved stamens and erect receptive stigma, k. Pollen grains, l. Ovary, m. Ovules.

The pollen output per anther is 1041 ± 201.90. The total pollen productivity in individual flowers is 4165 ± 807.59. The pollen-ovule ratio is 1041: 1. The pollen grains are spheroidal, tricolporate, prolate, reticulate-rugulate with spinules widely distributed and 47.3 ± 0.65 µm long and 52.2 ± 0.8 µm wide (Fig. 2k). Nectar is secreted around the ovary inside the corolla base during post-anthesis period throughout the sexually active phase of the flower. Its secretion is gradual from anthesis onwards, reaches its peak after three hours and decreases gradually thereafter each day. The total volume of nectar produced is 5.7 ± 0.67 µl on day 1, 2.4 ± 0.54 µl on day 2 and 0.78 ± 0.21 µl on day 3. A flower produces a mean volume of 8.88 µl during its lifespan. The corolla tube is half filled with nectar on day 1, filled to 1/4th on day 2 and is almost at the corolla base on day 3. However, the flowers that did not receive any visits from the foragers had accumulated nectar due to its continuous secretion and accordingly the corolla tube is mostly filled with nectar. The nectar sugar concentration varied from 13-32% (23.2 ± 6.64) on day 1, 12-27% (19.9 ± 5.72) on day 2 and 10-17% (13.5 ± 2.41) on day 3. The overall average sugar concentration in the nectar is 18.66%. The sugars present in the nectar include sucrose, glucose and fructose; the first sugar is the most dominant. The total sugar content in the nectar of a flower is 1.81 mg. The nectar also includes the essential amino acids such as arginine, histidine, isoleucine, lysine, methionine, threonine and tryptophan, and the non-essential amino acids such as alanine, aspartic acid, aminobutyric acid, cysteine, cystine, glutamic acid, glycine, proline and serine. The petiole and calyx have extra-floral nectaries which produce nectar in minute volume. This nectar is an additional source for the foragers. The calyx is persistent and gradually bulges enclosing the fruit in case of fertilized flowers.

Breeding systems

The results of breeding systems indicate that the flowers are self-compatible and self-pollinating. The fruit set is absent in spontaneous and facilitated autogamy, 45% in geitonogamy, 67% in xenogamy and 31% in open pollinations. Seed set rate is 59% in geitonogamy, 81% in xenogamy and 70% in open pollinations (Table 1).

Table 1. Results of breeding systems in *Clerodendrum infortunatum*

Mode of breeding system	Number of flowers sampled	Number of flowers set fruit	Fruit set (%)	Seed set (%)
Spontaneous Autogamy	25	0	0	0
Facilitated Autogamy	25	0	0	0
Geitonogamy	65	29	45	59
Xenogamy	76	51	67	81
Open pollination	231	72	31	70

Foraging activity and pollination

The flowers are specialized and the stamens and stigma are exposed when the petals unfold and reflex. They were foraged by bees, ants and butterflies during day-time (Table 2). The bees were *Apis dorsata* (Fig. 3a,b), *A. cerana* (Fig. 3c,d), *A. florea* (Fig. 3e), *Xylocopa latipes* (Fig. 3f) and *Halictus* sp (Fig. 3g,h). The ant was *Camponotus* sp (Fig. 3i). The butterflies were *Pachliopta aristolochiae* (Fig. 4a), *Papilio demoleus* (Fig. 4d), *P. polytes* (Fig. 4b,c) (Papilionidae), *Delias eucharis* (Fig. 4e) (Pieridae), *Ypthima huebneri* (Fig. 4f), *Acraea violae* (Fig. 4g,h), *Junonia lemonias* and *Danaus chrysippus* (Nymphalidae). The bees and ants foraged during day time from 0900 to 1700 h with more activity during 1100-1200 h while butterflies foraged during 0900-1600 h with more activity during 1000-1100 h (Fig. 5, 6). Among bees, *Apis* bees visited the flowers very frequently than the other bees and also the ants. Among butterflies, papilionid and pierid butterflies frequented the flowers regularly and consistently during the flowering season. The bees made 42%, ants 4%

Table 2. List of insect foragers on *Clerodendrum infortunatum*

Order	Family	Genus	Species	Common Name	Forage Sought
Hymenoptera	Apidae	*Apis*	*dorsata* F.	Rock Honey Bee	Nectar
		Apis	*cerana* F.	Indian Honey Bee	Nectar
		Apis	*florea* F.	Dwarf Honey Bee	Nectar
	Anthophoridae	*Xylocopa*	*latipes* L.	Carpenter Bee	Nectar
	Halictidae	*Halictus*	sp.	Sweat Bee	Pollen
	Formicidae	*Camponotus*	sp.	Carpenter Ant	Nectar
Lepidoptera	Papilionidae	*Pachliopta*	*aristolochiae* F.	Common Rose	Nectar
		Papilio	*demoleus* L.	Lime Butterfly	Nectar
		Papilio	*polytes* L.	Common Mormon	Nectar
	Pieridae	*Delias*	*eucharis* Drury.	Common Jezebel	Nectar
	Nymphalidae	*Ypthima*	*huebneri* Kirby	Common Four Ring	Nectar
		Acraea	*violae* F.	Twany Coster	Nectar
		Junonia	*lemonias* L.	Lemon Pansy	Nectar
		Danaus	*chrysippus* L.	Plain Tiger	Nectar

Figure 3. *Clerodendrum infortunatum*: a. & b. *Apis dorsata*, c. & d. *Apis cerana*, e. *Apis florea*, f. *Xylocopa latipes*, g. & h. *Halictus* sp., i. *Camponotus* sp.

and butterflies 54% of total foraging visits (Fig. 7). The body washings of these foragers collected from the flowers during peak foraging activity revealed the presence of pollen only on the wings and body of papilionid and pierid butterflies. The mean number of pollen grains recorded on these butterflies varied from 32 to 49 (Table 3). The bees and ants did not carry any pollen on any part of their body.

Among bees, *Halictus* sp. foraged for pollen only while all others for nectar only. *Halictus* bees being small-bodied with short tongue was found to be unable to access the location of nectar and hence it simply concentrated on collecting pollen from the dehisced anthers which are situated away from the stigma. In consequence, these bees acted as mere foragers but not as pollinators. Similarly, all other bees with varied body sizes and tongue lengths approached the flowers in upright position, landed on the flowers and probed the corolla tube skilfully to collect nectar with ease. They never attempted to collect pollen from the anthers. During nectar collection, these bees never had any contact with either the stamens or the stigma and hence they proved to be mere nectar foragers-cum-thieves as they did not have any role in pollination. They did not show any bias to the functional sex phase of the flowers for visitation. Further, these bees never attempted to collect nectar from extra-floral nectaries.

Table 3. Pollen recorded in the body washings of butterflies on *Clerodendrum infortunatum*

Butterfly Species	**Sample size (N)**	**Number of pollen grains**		
		Range	**Mean**	**S.D**
Pachliopta aristolochiae	10	25-61	49.3	9.59
Papilio demoleus	10	13-46	32.4	9.33
Papilio polytes	10	18-51	40.7	10.13
Delias eucharis	10	12-40	32.3	8.48
Ypthima huebneri	10	0	0	0
Acraea violae	10	0	0	0
Junonia lemonias	10	0	0	0
Danaus chrysippus	10	0	0	0

The ant, *Camponotus* was a resident forager. It was found crawling on the plant and alternately foraging for nectar from both extra-floral nectaries and flowers. It collected nectar from 1-3 days old flowers without any discrimination. It never made any attempts to crawl towards the anthers to collect pollen. Further, it never had any contact with either the anthers or the stigma while crawling all over the plant and hence it served as mere nectar forager-cum-thieve as it did not have any role in pollination.

All butterflies recorded were found to collect only nectar. They approached the horizontally or slightly vertically oriented flowers in upright position, landed and/or hovered at the flowers and collected nectar. While collecting nectar, they constantly fluttered their wings in vertical position. They visited the fresh and old flowers without any discrimination for nectar collection. During nectar collection by papilionid and pierid butterflies, their wings, rear body and legs had contact with the

Figure 4. *Clerodendrum infortunatum:* a-d. Papilionids – a. *Pachliopta aristolochiae,* b. *Papilio polytes* (female), c. *Papilio polytes* (male), d. *Papilio demoleus*, e. Pierid, *Delias eucharis*, f-h. Nymphalids – f. *Ypthima huebneri*, g. & h. *Acraea violae*.

anthers and the stigma facilitating the occurrence of pollination. Because, their large body and long proboscis facilitated contact with the stamens and stigma situated far away from the throat of the corolla tube during search for nectar. The proboscis length is 17 mm in *P. aristolochiae*, 16 mm in *P. demoleus*, 18 mm in *P. polytes* and 14 mm in *D. eucharis*. The nymphalid butterflies that visited the flowers were small with 10-12 mm long proboscis and did not have any contact with the stamens or the stigma during approach or landing or nectar collection. They were considered to be legitimate nectar thieves.

The flowers were pollinated exclusively by papilionid and pierid butterflies while nymphalid butterflies, bees and ants were visitors. The pollinator butterflies visited the flowers in quick succession from the same or different inflorescences of the same or different plants situated nearby or at a distance. Such a foraging activity coupled with their pollination role and pollen carrying ability was considered as important in promoting out-crossing in addition to self-pollination through geitonogamy. As papilionids and perids are swift and long-distance fliers, they could promote out-crossing among different populations distributed in the study area.

In addition to bees, ants and butterflies, thrips were also found to be using the floral buds for breeding and flowers as sources of nectar. Thrips came out from the floral buds during anthesis and were found feeding on the nectar. As resident foragers, they confined their foraging activity to the corolla part of the flowers only. In effect, they never had any contact with the stamens and stigma due to which they stood as mere nectar foragers without effecting pollination. The depeleted or exhausted state of standing crop of nectar by non-pollinator insects was found to be compelling the pollinator butterflies to pay multiple visits to the same plants either in sequence or at random to quench their thirst for nectar. Such repeated visitation by pollinator butterflies at population or habitat level was considered to be promoting out-crossing manifold than selfing through geitonogamy.

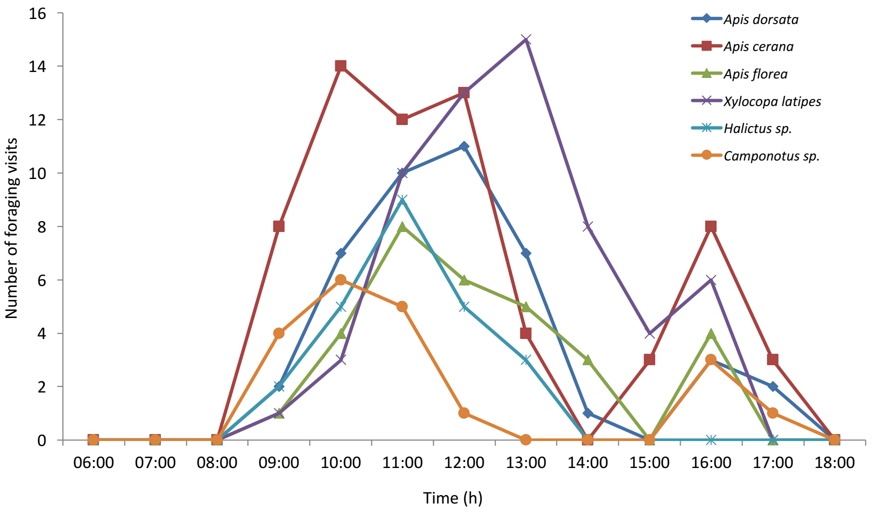

Figure 5. Hourly foraging activity of bees and ant on *Clerodendrum infortunatum*

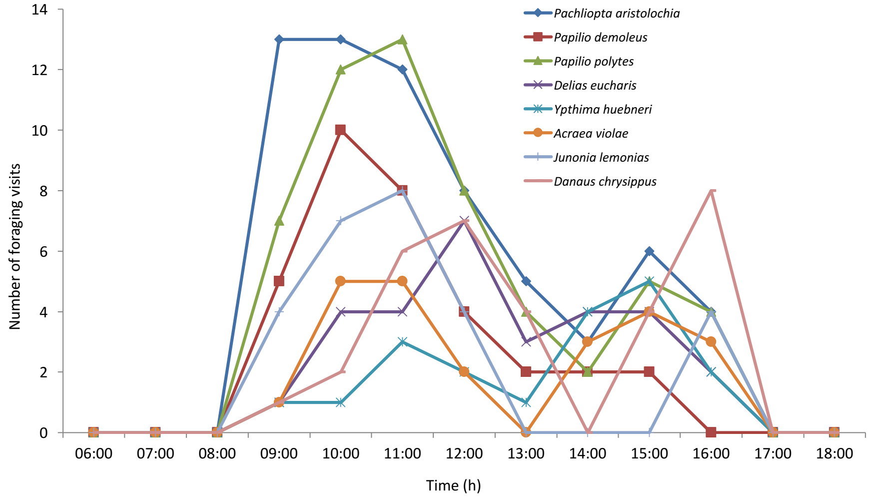

Figure 6. Hourly foraging activity of butterflies on *Clerodendrum infortunatum*

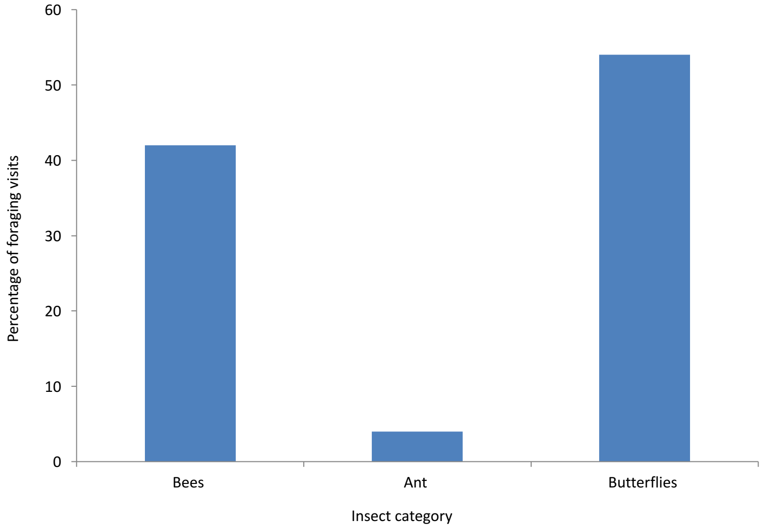

Figure 7. Percentage of foraging visits of different categories of insects on *Clerodendrum infortunatum*

Fruiting behavior

Pollinated and fertilized flowers initiate fruit development immediately and take 20-25 days to produce mature fruits. Fruit is nearly a globose indehiscent drupe, 8.97 ± 0.91 mm long, green initially, bluish-black or black upon ripening and enclosed in the red accrescent fruiting calyx (Fig. 8a-e, g). Gradually, the calyx opens up and exposes the fruit (Fig. 8f). The drupe consists of four somewhat fleshy mericarps, each with one seed and enclosed by the outer fleshy layer (Fig. 8h). They are oval-shaped, light purple, 4.23 ± 0.97 mm long; the endocarp that encompasses the seeds has a clear set of longitudinal to horizontal ridges. Fruit dispersal occurs during April-May. The bluish-purple fleshy fruits against red fruiting calyx are quite attractive to birds. The birds such as *Acridotheres tristis* (Indian Myna), *Corvus splendens* (House Crow), *Corvus macrorhynchos* (Jungle Crow) and *Turdoides caudatus* (Common Babbler) were found to feed on the pulp of the fruits and in the process disperse them to different places. The fruiting calyx remains attached to the mother plant even after fruit dispersal. The dry seeds free from the fleshy part of the fruit also disperse easily by wind

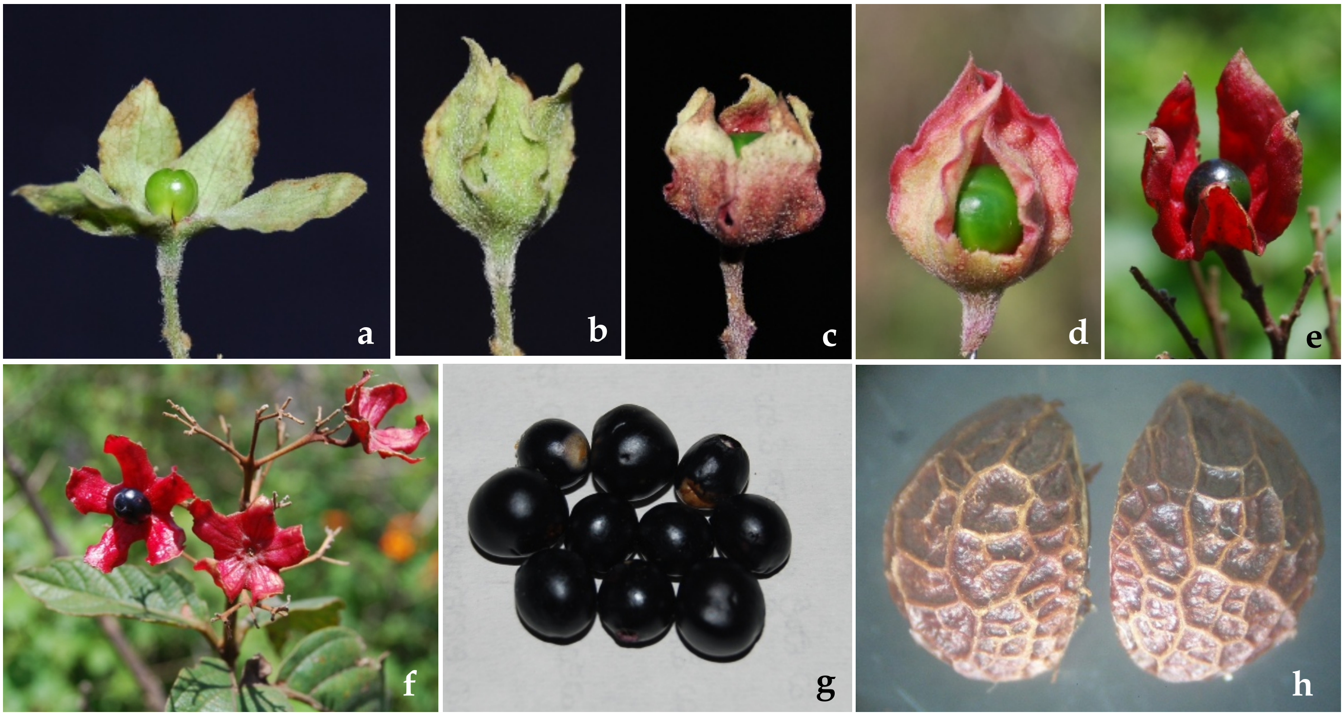

Figure 8. *Clerodendrum infortunatum:* a. Growing fruit inside the persistent calyx. b-e. Different stages of fruit development and colour change in fruiting calyx from green to scarlet, f. Exposure of ripe fruit by the complete unfolding of fruiting calyx (left side) and fruit drop and retention of fruiting calyx with the parent plant (right side), g. Ripe bluish black drupaceous fruits, h. Pyrene or schizocarp seeds.

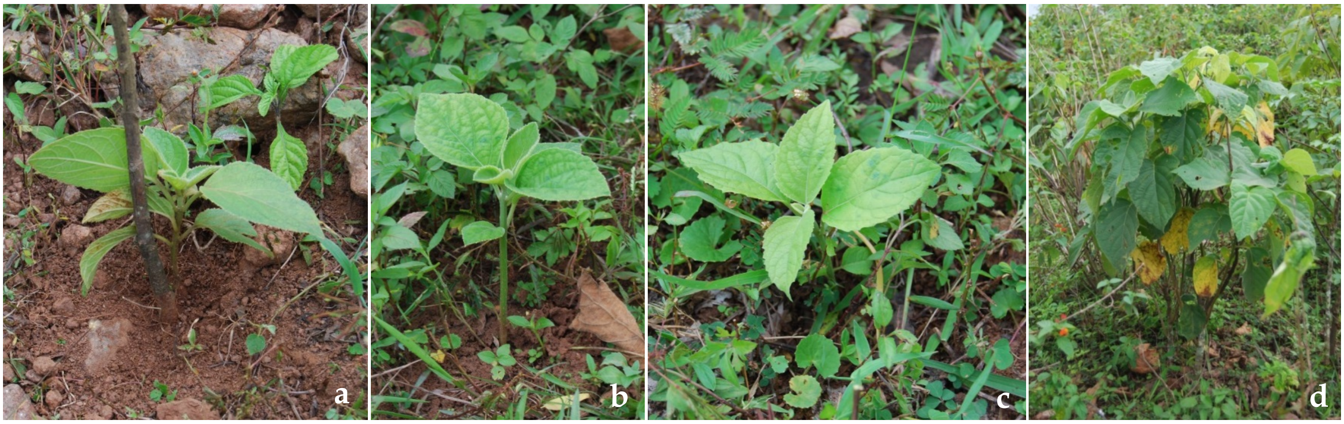

Figure 9. *Clerodendrum infortunatum:* a. New growth from the underground root stock, b. & c. Saplings, d. Mature plant.

due to dry season. Locals eat the fleshy part of the fruit and throw the nutlets and in this way humans also take part in the dispersal of seeds. Hence, ornithochory (bird dispersal), anemochory (wind dispersal) and anthropochory (dispersal by humans) are functional in this species. Schizocarp seeds germinate during rainy season soon after the occurrence of first monsoon rains in June. The seedlings grow and produce new plants if there is enough moisture in the soil, otherwise they soon perish (Fig. 9b-d). The perennial underground root stock of plants also sprouts and produces new growth and carries out new reproductive cycle (Fig. 9a).

DISCUSSION

Rajurkar (2010) reported that *C. infortunatum* is a common roadside shrub in North India and flowers during February-May. In the study area also, *C. infortunatum* flowers during the same period suggesting that it is a dry season bloomer. It is a gregarious shrub and its flowers are important for certain insects because of dearth of floral resources at this time. The plant is a prolific weed but it is not a menace to local or native herbs or woody shrubs because of its flowering during dry season when most of the herbs disappear and woody shrubs shed foliage and stay out of flowering. *C. infortunatum* produces typical multi-flowered panicles at the ends of the branches which are quite prominent. The white flowers against bright green foliage are well displayed. Primack et al. (1981) reported that the flowers within the inflorescence of *Clerodendrum inerme* generally show the same developmental stage, so that the flowers in the same inflorescence are unlikely to pollinate each other. On the contrary, the flowers of individual panicles of *C. infortunatum* show different developmental stages and open within a week or more than a month depending on the number of flowers produced by the inflorescence. This difference in developmental stages of flower buds within the panicle is most likely to facilitate selfing if pollinators are available.

The flowers open during evening hours in *C. molle* (McMullen, 2011), *C. inerme* (Solomon Raju & Rajendra Kumar, 2016), during night time in *C. phlomidis* (Rohitash, 2016) and during morning hours in *C. infortunatum* (Byragi Reddy & Subba Reddi, 1995). The present study noted that *C. infortunatum* produces flowers during early morning hours. Primack et al. (1981) reported that the flowers of *C. inerme* are fragrant and the calyx cup is covered with an irregular series of raised elliptical glands which apparently function as extra-floral nectaries and the ants commonly feed at them. Solomon Raju & Rajendra Kumar (2016) reported that *C. inerme* does not show such glands on the calyx cup and also the ants do not visit the flowers. Rajurkar (2010) reported that *C. infortunatum* produces extra-floral nectaries on the petiole and calyx. The present study substantiates this finding and also adds that ants collect nectar from these nectaries in addition to floral nectar.

Rajurkar (2010) reported that *C. infortunatum* exhibits protandry. Byragi Reddy & Subba Reddi (1995) reported that *C. infortunatum* is protandrous, prevents spontaneous autogamy but facilitates geitonogamy and xenogamy. The present study showed that *C. infortunatum* is strongly protandrous, herkogamous and dichogamous with the staminate phase on the day of anthesis and pistillate phase on 2nd and 3rd day of flower life. Such a sexual expression by its hermaphroditic flowers characterizes the functioning of temporal dioecy. In consequence, the flowers prevent the occurrence of spontaneous and facilitated autogamy but facilitate the occurrence of geitonogamy and xenogamy which together constitute the facultative xenogamous breeding system. The pollen-ovule ratio also substantiates the function of this breeding system and the ratio falls in the range of pollen-ovule ratio (244.7-2,588) for facultative xenogamy provided by Cruden (1977). Although geitonogamy is self-fertilization, in that the pollen fertilizing the ovules originates from the same parent plant, different parts of the branches of the plant may diverge genetically through somatic mutation (Roubik, 1995). Such a genetic divergence was experimentally proved in *Byrsonima crassifolia* in which one of five trees used in experiments was initially found to be self-incompatible produced fruits through self-pollination when the flowering period was nearly over and in *Pscidia carthagenensis* in which one-day old artificially self-pollinated flowers did not set fruit but 2-day old flowers set some fruits (Baker et al., 1983). In *C. infortunatum*, fruit set through geitonogamy indicates that the plant is self-compatible despite strong protandry and fruit set through xenogamy is an indication of cross-compatibility. Geitonogamic mode of pollination renders the plant to set fruit in isolated colonizing plants. As a result, certain floral mechanisms which promote out-crossing with its associated genetic advantages can be expected in established populations (Primack et al., 1981). In *C. infortunatum,* the protandry together with different positions of stamens and style in different sets of flowers would allow fruit set in isolated or congregated individuals while totally preventing selfing within individual flowers. Such a dual breeding system is advantageous for *C. infortunatum* to colonize new habitats. Byragi Reddy & Subba Reddi (1995) described the changing positions of stamens and style during flower life. These authors describe that the stamens and style appear twisted and curled upward in bud condition. After the flower is open, the stamens uncoil after the style. The stamens become linear and face the horizontal corolla tube, while the style with the closely pressed stigmatic lobes is bent down towards the lower lip. By the evening of the same day, the stamens bend sideways, two stamens to each side. On the 2nd morning, the style straightens to occupy the position taken by the stamens previously and the stigmatic lobes spread out to receive pollen. The present study showed that *C. infortunatum* flowers first display the erect position of the style with the stigmatic lobes fused parallel to the central axis of the flower one hour after anthesis. Again, after one hour, the stamens uncoil and stand nearly erect without any physical contact with the style or stigma. But, the stamens dehisce at the end of one hour after anthesis; the nearly erect stamens gradually bend downwards and orient either to one side or to both sides of the flower by the evening of the day of anthesis. The stigmatic lobes spread out attaining receptivity on the morning of the 2nd day and stay receptive until the evening of the 3rd day. Therefore, this study refutes the description provided by Byragi Reddy and Subba Reddi (1995) regarding the positions of stamens and style during flower life to avoid spontaneous autogamy.

Begum et al. (2014) mentioned that *C. infortunatum* is an important source of nectar for certain butterflies such as the Papilionid, *Papilio polytes,* the Nymphalids, *Danaus chrysippus,* Pierids, *Eurema blanda, E. hecabe* and the Lycaenid*, Zizina otis*. Byragi Reddy & Subba Reddi (1995) reported that *C. infortunatum* is exclusively pollinated by papilionoid butterflies, *Papilio polytes, P. polymnestor* and *Atrophaneura hector*. Meera Bai (2014) has also reported that *C. infortunatum* flowers are adapted for pollination exclusively by butterflies due to non-promiscuity of floral rewards to other foragers. The present study showed that *C. infortunatum* is visited by bees, ants, thrips and butterflies during day time, but pollinated by only papilionid and pierid butterflies. The foraging activity pattern is in accordance with the pattern of standing crop of nectar. The white odoriferous flowers with tubular corolla presenting stamens and style far away from the corolla rim are appropriate for pollination by butterflies (Faegri & van der Pijl 1979). Lepidopteran-pollinated flowers are rich in nectar volume and sucrose-rich with the sugar concentration ranging from 15 to 25% (Cruden et al., 1983). In *C. infortunatum*, the nectar is secreted continuously for three consecutive days in individual flowers; it is sucrose-rich with a mean sugar concentration of 18%. Further, it is a source of certain essential and non-essential amino acids. These nectar characteristics suggest that this plant is adapted for pollination by lepidopterans.

In *C. infortunatum*, the corolla throat tinged with pink colour appears to be useful as nectar guide to direct the nectar seeking insects upon landing on the flower(s). Except *Halictus* bees, all other visiting bees collect nectar legitimately from the corolla tube with ease from day 1 and 2 flowers in which the nectar is presented or accumulated to half or 1/4 of the corolla tube. Day 3 flowers present minute to traces of nectar and hence they do not reward the bees if attempted or probed for nectar. *Halictus* bees being small-bodied with a short tongue are unable to access nectar and hence they concentrated on pollen collection only. Further, nymphalid butterflies collect nectar legitimately without any difficulty. But, all bees except *Halictus* sp. and nymphalid butterflies while collecting nectar do not contact the stamens and stigma and hence they act as mere flower visitors or legitimate nectar robbers. Ants and thrips as resident foragers do not contact with the stamens and stigma while collecting nectar from the corolla and/or extra-floral nectaries and hence they act as mere foragers or legitimate nectar robbers. The papilionid and pierid butterflies with long proboscis insert only part of their proboscis to access nectar in the flowers and in so doing, their large wings, rear body and legs invariably contact the stamens and stigma and in effect pollination occurs. But, mostly the wings contact the sex organs of flowers during which pollination occurs simultaneously; this is further substantiated by the body washings of these butterflies in which pollen is recorded. This mode of pollination is referred to as pterigotribic pollination, *sensu* Byragi Reddy & Subba Reddi (1995). The flowers robbed of nectar indirectly intensify the foraging visits by legitimate and pollinator papilionid butterflies (*Pachliopta aristolochiae, Papilio demoleus, P. polytes*), and the pierid butterfly, *Delias eucharis*. The paniculate inflorescence presenting numerous flowers in close proximity to each other is advantageous for these butterflies to reduce flight time as well as search time for flowers and hence is energetically profitable for them. Additionally, these butterflies quickly move from plant and plant and also from population to population in search of more nectar to quench their thirst during dry season. Such a foraging activity promotes cross-pollination in addition to self-pollination via geitonogamy.

Wheeler et al. (1992) noted that fruit or seed dispersal occurs in *Clerodendrum* genus through birds. Keng (1990) reported that *C. laevifolium* fruits are probably dispersed by birds. Lorence & Flynn (1997) stated that *C. macrostegium* is spread by fruit eating birds. Solomon Raju & Rajendra Kumar (2016) stated that *C. inerme* fruits are dispersed by birds such as *Acridotheres tristis*, *Corvus splendens*, *Corvus macrorhynchos* and *Turdoides caudatus*. There are no previous studies that documented fruit or seed dispersal in *C. infortunatum*. The present study reports that *C. infortunatum* produces fruits within a month after pollination and fertilization. The fruit is an indehiscent drupe with fleshy mericarps and each mericarp consists of one seed. The fruiting calyx unfolds and exposes the ripe fleshy fruit. The calyx which is green during flower life turns red during fruiting phase and advertises the presence of bluish-purple fruit. In *C. infortunatum*, the reddening of calyx after fruit maturity has nothing to do with intricate pollination mechanism as stated by Rajurkar (2010). The birds such as *Acridotheres tristis* (Indian Myna), *Corvus splendens* (House Crow), *Corvus macrorhynchos* (Jungle Crow) and *Turdoides caudatus* (Common Babbler) are attracted to the fruits displayed against reddish calyx. They use the fruits of *C. infortunatum* as food source. These birds and humans eat the fleshy part of the fruit either at the parent plant or far away and drop the seeds without any damage. The seeds soon dry up and also disperse by wind easily due to dry conditions during summer season. Fruit or seed dispersal is quite effective during summer season. Therefore, *C. infortunatum* is ornithochorous, anthropochorous and anemochorous. Casual field observations indicated that the seeds of this plant germinate and produce new plants during wet season. The seed germination and seedling establishment rate depends on the moisture state of the soil. Further, the perennial root stock also re-sprouts at the same time and produces new growth to carry out new reproductive cycle.

Clerodendrum infortunatum is widely used as a medicinal plant for several ailments such as asthma, cough, tumors, diarrhea, skin disorder, venereal and scrofulus complaints, wounds, wormicide, post-natal complications, as vermifuge, febrifuge in malarial fever specially in children, laxative, cholagogue, for removal ascarids in anus, as antidote for colic, scorpion sting and snake bite in various regions around the globe (Singhmura, 2016). Since this shrub is wide spread, it can be used in traditional medicine without affecting the surviving of wild populations. Further, it is not a menace to local plant species since it appears prominently in vegetative and flowering phase during summer season when most of the local plants disappear or go leafless. It is a prized shrub for certain local insects, which use it as a principal source of nectar during summer season. Therefore, *C. infortunatum* plays an important ecological role in the areas of its distribution and provides the necessary forage for the visiting insect fauna, especially for papilionid butterflies.

CONCLUSION

Clerodendrum infortunatum L. with gregarious woody shrub habit flowers during dry season. It is temporally dioecious which prevents autogamy but facilitates geitonogamy and xenogamy representing facultative xenogamous breeding system. The flowers are visited by different diurnal insects of which only papilionid and pierid butterflies are pollinators. The fruit is an indehiscent drupe consisting of fleshy mericarps and each mericarp has only one seed. The reddish fruiting calyx exposes the ripe fleshy fruit for dispersal agents which include birds, humans and wind. The seeds germinate and produce new plants during wet season. Further, the perennial root stock also re-sprouts at the same time and produces new growth to carry out new reproductive cycle. The study shows that the plant plays an important ecological role in the areas of its distribution and provides the necessary forage for the visiting insect fauna.

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