

The Reproductive Ecology of *Boerhavia diffusa* L. and *Boerhavia erecta* L. (Nyctaginaceae)

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Abstract: *Boerhavia diffusa* and *B. erecta* occur commonly and grow well displaying vegetative, flowering, and fruiting phases either simultaneously or alternately throughout the year in various habitats. In both species, the flowers open at dawn by unfolding the petaloid perianth during which the stamens, with dehiscent anthers, and the style and stigma in a receptive state, gradually uncoil to become erect; in this process, autogamous self-pollination occurs. The flowers that failed to self- or cross-pollinate autonomously during the process of anthesis or by vector-mediation in open condition have the option to resort to autogamous self-pollination during the process of the closing of the petaloid perianth covering the sex organs within. Both species attract bees, wasps, flies, and butterflies to their flowers, but only bees and butterflies act as appropriate pollinators, while the other insects act as additional pollinators. The fruit is a one-seeded indehiscent anthocarp with mucoid secretions which is adapted for zoochory, anthropochory, ornithochory, and hydrochory. Seeds are non-dormant and germinate immediately after dispersal. Furthermore, these species also multiply asexually through the fragmentation of the rhizome. In these plant species, the functionality of sexual and asexual reproduction modes enables them to invade new habitats and form prolific populations. Since these plant species control soil erosion by covering the soil effectively, they can be considered for the eco-restoration of ecologically damaged and deteriorated habitats and ecosystems.

Keywords: *Boerhavia diffusa*, *Boerhavia erecta*, Bees, Butterflies, Zoochory, Anthropochory, Ornithochory, Hydrochory.

Introduction

The genus *Boerhavia* was named after an eighteenth-century Dutch botanist, Hermann Boerhaave, at the University of Leiden, Germany. The generic name is often written in the literature as *Boerhaavia* (Hiruma-Lima *et al.*, 2000), but Linnaeus Latinized Boerhaave's name to Boerhavius and adopted the spelling *Boerhavia*, which is the correct spelling to be used (Spellenberg, 2004). The distribution and the number of genera and species in the genus *Boerhavia* have been reported differently by different authors. Hutchinson and Dalziel (1954) reported that the genus *Boerhavia* is distributed mainly across tropical America and in small numbers in the tropics and subtropics of the Old World. An anonymous publication (1988) confirmed that the genus has forty species, which are widely distributed in China, Egypt, Pakistan, Sudan, Sri Lanka, South Africa, USA, India, and several Middle East countries. Spellenberg (2000) stated that *Boerhavia* species are widespread, and their dispersal is mostly attributed to birds and human activity. Patil & Sanjivani (2016) confirmed that this genus is widely distributed in tropical, subtropical, and the temperate regions of the world including Mexico, America, Africa, Asia, Indian Ocean Islands, the Pacific Islands, and Australia. Douglas & Spellenberg (2010) noted that there about forty species worldwide, but

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these species mostly occur in the southwestern parts of North America. Chopra (1969) and Dev (2006) pointed out that six species of *Boerhavia*, *B. diffusa*, *B. repens*, *B. chinensis*, *B. erecta*, *B. elegans* (synonym: *B. rubicunda*), and *B. reniformis* (synonym: *B. rependa*) are found in India. Dutta *et al.* (2015) documented that *Boerhavia* is represented by four species, *B. crispa*, *B. diffusa*, *B. erecta*, and *B. rubicunda* in India. Chopra (1969) and Dev (2006) confirmed that most *Boerhavia* species possess worldwide medicinal uses; hence they have occupied positions in different systems of medicine including Indian Ayurveda, Siddha and Unani, Martinican medicine, African medicine, traditional Chinese medicines, as well as Indian and Brazilian pharmacopoeia. As for the *Boerhavia* genus, the species are annual or perennial herbs with a diffuse habit and produce campanulate flowers; the fruit is a 3-5 ribbed glabrous or glandular pubescent anthocarp (Bittrich and Kuhn, 1993; Jordaan, 2000). The *Boerhavia* flowers produce a narrow perianth tube and are melittophilous. They are suited for head pollination by small bees; they are fit for both bee and fly pollination (Bittrich and Kuhn, 1993). Spellenberg (2000) reported that in *Boerhavia coccinea*, *B. intermedia*, *B. spicata*, *B. torreyana* and *B. wrightii*, individual flowers open only for a portion of a day. The flowers open at dawn and their opening begins with the opening of the corolla-like perianth and the uncoiling of stamens and styles. With the progression of the morning, the filaments and style curl and the anthers haphazardly contact the stigma affecting autogamous self-pollination. The perianth closure begins in the late morning containing the stamens and style within and wrinkles by mid-afternoon of the same day. *B. coccinea* is visited by bees of *Scolia*, *Mellisodes*, by wasps of *Holopyga rudis* and *Ammophila*, and also by calliphorid, syrphid and muscid flies. *B. intermedia*, *B. torreyana* and *B. wrightii* are visited by *Mellisodes* bees and the *Holopyga rudis* wasp and also by the tachinid and syrphid flies. *B. spicata* is visited by *Scolia* and *Mellisodes* bees,

Ammophila and *Ichneumonid* wasps, and also by calliphorid and syrphid flies (Spellenberg, 2000). *B. coccinea* is pollinated by social bees and social wasps in Caatinga in the northeastern Brazil (de Mendonca Santos *et al.*, 2010).

Bromilow (2010) noted that *Boerhavia diffusa* is native to South America, but Patil *et al.* (2016) stated that this species is found in the tropical, subtropical, and the temperate regions of the world. Struwig and Siebert (2013) mentioned that the specific epithet, *diffusa*, refers to the spreading or diffuse nature of the plant. Kirtikar and Basu (1933) reported that *Boerhaavia diffusa* is distributed throughout India and is extensively used in many parts of the country as a traditional medicine. Its utility as a drug to regenerate new cells in the body is promising and is well-known in Ayurvedic medicine. An anonymous publication (1988) confirmed that *B. diffusa* is an important medicinal herb indigenous to India. It grows well on wastelands and in fields during the rainy season. It grows in open sunlight and endures severe abiotic stresses such as UV exposure, high temperature, water, and nutrient deficiencies. In West Bengal, it is cultivated for its leaves for use in medicine. Bhardwaj and Sharma (2019) reported that *B. diffusa* has a tremendous potential of medicinal value and in fact has been traditionally used in various ailments such as syphilis, leukoderma, and blood disorders. In India, *B. diffusa* has a long history of therapeutic uses in the indigenous medicinal tribal communities and in the Ayurvedic system of medicine (Chaudhary and Dantu, 2014 and the references therein). Patil and Sanjivani (2016) stated that *B. diffusa* is extensively used by local people and medicinal practitioners for treatments of hepatitis, urinary disorders, gastrointestinal diseases, inflammations, skin problems, infectious diseases, and asthma in India. Chaturvedi (1989) reported that autogamous self-pollination is functional in *B. diffusa*, but hymenopterans and dipterans also pollinate the plant. Mwangi (2009) reported that *B. diffusa* is an important floral source

for a diversity of bee species which include *Apis mellifera*, *Ceratina* spp., *Lipotrichis* spp., *Macrogalea candida*, *Megachile* spp., *Pseudapis* spp., *Thyreus* spp. and *Xylocopa senoir* in the Arabuko Sokoke Forest, Nigeria. Mahale (2019) noted that *B. diffusa* is an important nectar source for the honeybees, *A. dorsata*, *A. cerana* and *A. florea* in the cultivated and uncultivated areas of Ahmednagar in the South-Eastern region of Western Ghats. Churi *et al.* (2020) noted that *B. diffusa* is a nectar plant for *Junonia lemonias*, *J. almana*, *Castalius risomon*, *Appias olferna*, *Eurema hecabe*, *Ypthima* spp., *Colotis amata*, *C. danae*, and *C. protractus* in South India.

Boerhavia erecta is native to the United States, Mexico, Central America, and western South America but is now cosmopolitan in tropical and subtropical regions of the world (Chen and Wu, 2007). The specific epithet, *erecta*, refers to the erection habits or upright inflorescences (Struwig and Siebert 2013). This herb is used in traditional medicine and as food in West and East Africa, where its leaves are eaten as a vegetable or are used in sauces (Schmelzer, 2006). Its roots are used for the treatment of various human diseases in traditional medicine (Nisha *et al.*, 2018). The plant powder is smoked as a cigarette once a day for one month to get relief from asthma (Shanmugam *et al.*, 2012). Despite the availability of the *Boerhavia* species and their medicinal and edible value, they have not been systematically investigated for any aspect of their reproductive ecology. Therefore, the present study was conducted to provide the details on the reproductive ecology of *Boerhavia diffusa* L. and *Boerhavia erecta* L. (Family: Nyctaginaceae; tribe: Nyctagineae) and to discuss relevant works on these two plant species published by others.

Materials and Methods

Study area

The wild pockets of *Boerhavia diffusa* and *Boerhavia erecta* in the scrub jungle

deciduous ecosystem located on the outskirts of Visakhapatnam city (Latitude 17.8909°N, Longitude 83.1908°E, altitude 209 amsl), Andhra Pradesh, India, were selected for the study between May 2018 and December 2019.

Floral morphology and biology

Twenty mature buds, four on five different plants of each plant species were marked to record anthesis and the anther dehiscence schedule. The anther dehiscence mode was also recorded by using a 10x hand lens. The flower morphological details such as flower sex, shape, size, colour, odour, perianth, stamens and ovary were recorded. The stamens were described regarding their number, origin, position, and whether they were exposed or hidden during the flower stage. Based on variation in the stamen number, the percentage of one-, two- and three-stamened flowers in each species was calculated by using 90 flowers collected randomly from different plants. The ovary details and its concealment by the lower part of perianth were carefully observed. The movements of sex organs, during the time of flower-opening and closing of the perianth, were carefully observed to find out whether spontaneous autogamy occurs or not. In case of occurrence, observations of whether it was absolute or not in one-, two- and three-stamened flowers were also made. For each plant species, ten mature buds with un-dehiscid anthers were collected from different plants and were kept in a Petri dish. A single anther was taken out each time and was placed on a clean microscope slide (75 x 25 mm). These were crushed with a glass rod, and a small drop of lactophenol-aniline-blue was added to disperse the pollen grains equally to the fixed area on the slide and the pollen grains were counted under a compound microscope (40x objective, 10x eye piece). This procedure was followed for counting the number of pollen grains in one-, two, and three-stamened flowers. Based on the pollen counts of each anther, the mean number of pollen grains produced per anther

was calculated. 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. At the same time, the pollen grains' characteristics were also recorded. Furthermore, the pollen-ovule ratio was calculated separately for one-three stamened flowers using the method followed by Cruden (1977). The protocols described by Mondal *et al.* (2009) were followed for the analysis and identification of amino acid types in the pollen of both plant species. The method described by Sadasivam and Manickam (1997) was followed for protein extraction from the pollen samples, while the method of Lowry *et al.* (1951) was followed for estimating the protein content in the samples. The Hydrogen peroxidase test described by Dafni *et al.* (2005) was used to record the commencement and duration of stigma receptivity.

Fruit set in spontaneous autogamy and open-pollinations

Eighty mature buds, eight from ten plants of each species were bagged and observed for three weeks to record the fruit set rate in spontaneous autogamy. Fifty flowers, five from ten plants of each species were tagged prior to anthesis and were observed for fruit set rate in open-pollinations.

Flower-visitors and pollination

Flowers were observed from morning to evening for four days to record flower-visitors to each plant species. The flower-visiting bees, wasps, and flies were identified according to the Zoological Survey of India, Calcutta, India, while the flower-visiting butterflies were identified with the help of the Field Guide of Butterflies book by Gunathilagaraj *et al.* (1998). The approach of flower visitors to flowers, flower-probing, the collected forage, and the contact between their body parts with the stigma and stamens, were carefully observed by standing close to the flowering patch and by using a field binocular to record their pollination role. The

number of foraging visits made by the flower visitors was recorded at each hour for ten minutes from morning to evening for four days at fifty plants flowering profusely. The data collected on the foraging visits of these insects were tabulated and the mean number of foraging visits at each hour was calculated to know the foraging pattern of insects through time. The same data were also used to calculate the percentage of foraging visits made by each category of insects separately.

Fruit and seed aspects

Forty mature buds were tagged and observed for a period of three weeks to record fruit growth, development and maturation period. The fruit dehiscence mode and seed dispersal aspects were observed carefully. Fruit and seed morphological characteristics were described to understand fruit/seed dispersal modes. Field observations were made throughout the year on fruit/seed dispersal agents, seed germination, and the production of new plants.

Results

The plant and flowering phenology

Boerhavia diffusa is a perennial prostrate creeping herb (Figure 1a), while *B. erecta* is a perennial erect herb; the former grows well in wet and semi-wet areas, while the latter grows well in semi-wet and sandy soils. They form extensive mats of populations in open areas where ground flora is almost absent. *B. diffusa* dominates in areas where other low ground herbaceous species grow simultaneously but *B. erecta* has a scattered occurrence in areas occupied with several low ground mat-forming herbaceous species. In both, the stem is slender, woody towards the base, decumbent, greenish to purplish, and is many-branched from a tap root, but the branches are highly diffused with a prostrate form in *B. diffusa*, while they are slightly diffused with an erect form in *B. erecta*. Leaves are petiolated, arranged oppositely in unequal pairs, broadly ovate, entire, and



Figure 1. *Boerhavia diffusa*: a. Habit, b-d. Different stages of buds, e-g. Flowers, h-j. Autogamy taking place during anthesis due to gradual uncoiling of style during which the capitate stigma gets coated with pollen from dehiscent anthers, k. Ovule, l. & m. Early stages of seed development, n. Fruits.

smooth. The plants of both species grow throughout the year if the soil is moderately wet, but they show prolific growth during the rainy season with a profuse flowering between July and September (Figure 3a). In both species, the perennial underground tap root sprouts to produce new growth and repeats its sexual cycle if the soil has sufficient moisture and nutrients. The inflorescence is a cymose panicle borne in axillary and terminal positions in both species. In *B. diffusa*, the inflorescence has a long peduncle composed of several cymes and each cyme produces 8.3 ± 3.01 pedicellate flowers. In *B. erecta*, the inflorescence has long primary and short secondary peduncles, and both types are covered with minute hairs. The secondary peduncles consist of two to four pedicellate flowers (Figure 3b). In both species, all flowers of individual cymes open on the same day.

Flower morphology

In both species, the flowers are actinomorphic and bisexual. They are 3-4 mm long in *B. diffusa* and 2-3 mm long in *B. erecta*. In both species, the perianth is gamocorolline representing the calyx and corolla. The lower part of the perianth is sepaloid, green, glabrous, clavate, tubular, and five-ribbed enclosing the ovary. The upper part of the perianth is petaloid, funnel-shaped extending at the rim into five lobes and each lobe is bifid and glabrous. The petaloid perianth, however, is red to violet in *B. diffusa* and is white with pink-stripes in *B. erecta*. In both species, the stamens vary in number from one to three. In *B. diffusa*, the three-stamened flowers account for 74%, the two-stamened ones, for 21%, and the one-stamened for 5%. In *B. erecta*, the corresponding percentages are 67%, 29% and 4% respectively. In both species, the stamens are free, slender, exserted, and their

filaments are filiform and fixed at the base of the petaloid part of the perianth; anthers are creamy white, two-celled, monomorphic, and dorsifixed. The stamens are 3 mm long in *B. diffusa* and 1.5-2 mm long in *B. erecta*. In both species, the ovary has one carpel with one locule consisting of a solitary ovule in basal placentation (Figure 1k, 3i). The style is slender, terminal, filiform, and glabrous; it is light purple and 2 mm long in *B. diffusa*, while it is white and 1.3-1.5 mm long in *B. erecta*. In both species, the stigma

reaches slightly above the top of the petaloid perianth; it is 1.5 mm long, light purple and peltate in *B. diffusa*, while it is 1 mm long, white and discoid in *B. erecta* (Figure 3h).

Floral biology

In both species, the buds mature slowly and open between 6:00- and 6:30 am (Figure 1b-g, 3c-e), and anther dehiscence occurs by longitudinal-lateral slits simultaneously. Their opening begins with the opening of

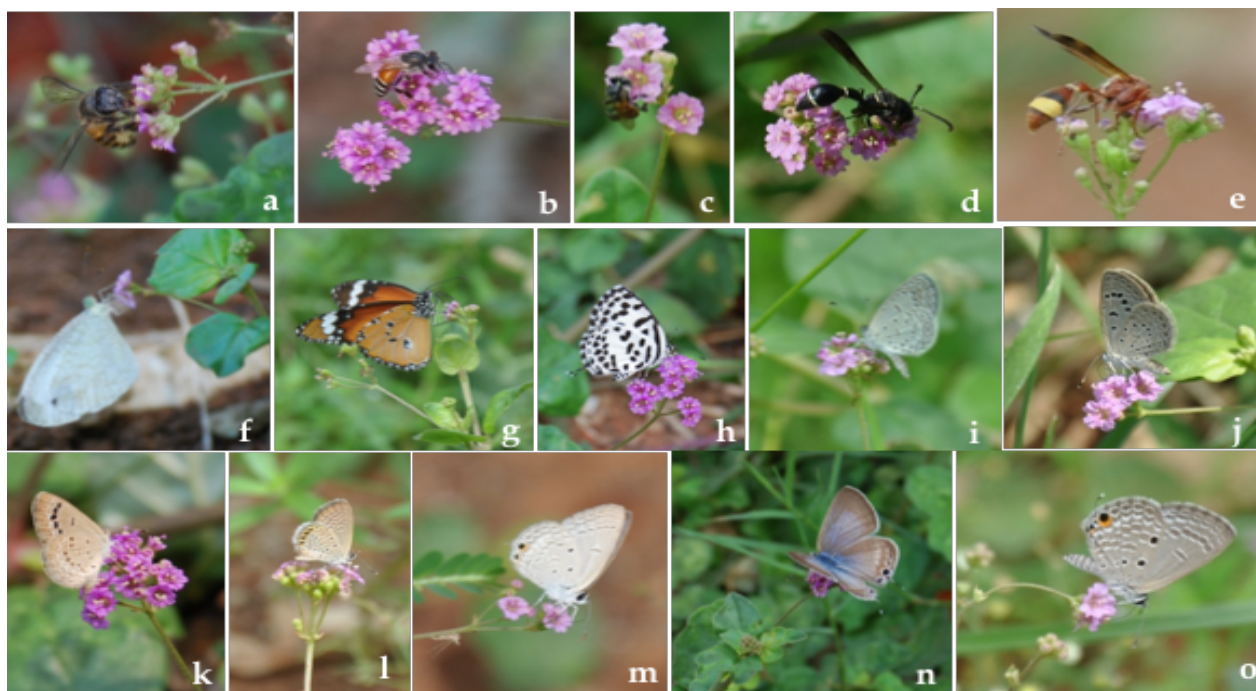


Figure 2. *Boerhavia diffusa*: a. *Apis cerana*, b. *Apis florea*, c. *Trigona iridipennis*, d. *Rhynchium* sp., e. *Polistes* sp., f. Pierid, *Leptosia nina*, g. Nymphalid, *Danaus chrysippus*, h-o. Lycaenids – h. *Castalius rosimon*, i. *Zizula hylax*, j. *Zizeeria karasandra*, k. *Zizina otis*, l. *Freyeria trochylus*, m. *Eucheris lacturnus*, n. *Everes lacturnus*, o. *Chilades pandava*.

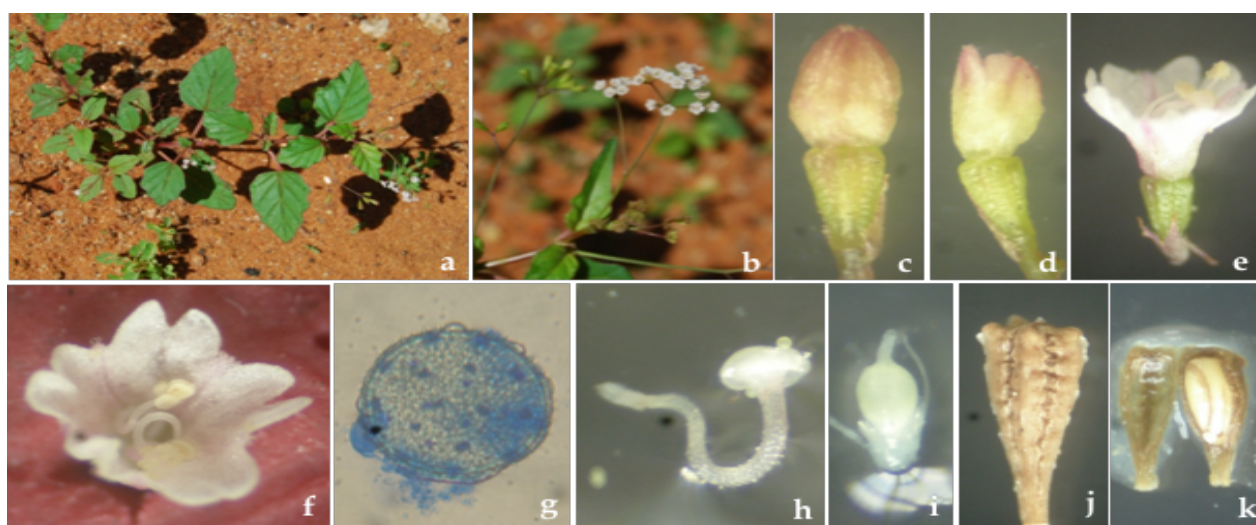


Figure 3. *Boerhavia erecta*: a. Twig in flowering phase, b. Flowering inflorescence, c. Mature bud, d. Anthesing bud, e. Flower, f. Position of stamens in open flower, g. Pollen grain, h. Style with discoid stigma, i. Ovule, j. Fruit, k. Seed.

the upper petaloid perianth and the uncoiling of stamens, style and stigma (Figure 3f). As the anthers present pollen during anthesis, the style in the process of uncoiling enables the peltate stigma to haphazardly contact the anthers. Then, the pollen from the anthers transfers to the stigma, and as a result autogamous self-pollination occurs (Figure 1h-j). The occurrence of this autogamous pollination was found to be certain in the three-stamened flowers but was not certain in neither the two-stamened nor in the one-stamened flowers. In the three-stamened flowers, the stigma during the process of uncoiling surely contacts the anthers due to the presence of a narrow space between them. Whilst in the two-stamened flowers, the additional space, created by the absence of the third stamen, reduces the chances for the uncoiling stigma to contact the anthers which results in autonomous autogamy. Similarly, in the one-stamened flowers, the chances for the uncoiling stigma to contact the anthers are further reduced and the autonomous autogamy occurs only when the stigma uncoils facing the solitary anther, and upon contact with the latter simultaneously. After the complete opening of the petaloid perianth, the stamens, style and stigma become erect and spatially separated; the anthers are placed at or slightly above the perianth rim, while the stigma stands above

the top of the perianth. The petaloid perianth remains open until 1400 h, and thereafter the perianth lobes gradually close back by 1600 h. Prior to the initiation of perianth closure, the stamens and terminal part of the style and stigma coil downwards to enable the perianth lobes to close back. In this process, if the anthers still have pollen and are still facing the coiling stigma, then, autogamous self-pollination occurs. The chances for the occurrence of autogamous self-pollination during perianth closure are much higher in the three-stamened flowers than in the two- and one-stamened flowers. The color of the pollen grains is creamy white, spheroidal and pantoporate with spinulose thick exine and tubuliferous tectum; they are 58.1 μm in size in *B. diffusa* and 63.13 μm in size in *B. erecta* (Figure 3g). In both species, the pollen production rate per anther is constant in the one-, the two-, and the three-stamened flowers; it is 119.8 ± 7.02 in *B. diffusa* and 112.6 ± 9.74 in *B. erecta*. In *B. diffusa*, the pollen/ovule ratio was 120:1 in the 1-stamened flowers, 240:1 in the two-stamened flowers and 360:1 in the three-stamened flowers. In *B. erecta*, the pollen/ovule ratio was 113:1 in the one-stamened flowers, 226:1 in the two-stamened flowers and 339:1 in the three-stamened flowers. In both species, the pollen contains five essential amino acids and six non-essential

Table 1. Essential amino acids present in the pollen of *Boerhavia diffusa* and *Boerhavia erecta*

Essential amino acids			Non-essential amino acids		
Amino acid type	<i>Boerhavia diffusa</i>	<i>Boerhavia erecta</i>	Amino acid type	<i>Boerhavia diffusa</i>	<i>Boerhavia erecta</i>
Threonine	+	+	Alanine	+	+
Valine	+	+	Amino butyric acid	-	-
Methionine	-	-	Aspartic acid	-	-
Leucine	+	+	Cysteine	+	+
Iso leucine	+	+	Cystine	+	+
Lysine	+	+	Glutamic acid	+	+
Phenyl alanine	-	-	Glycine	-	-
Histidine	-	-	Hydroxy proline	+	+
Arginine	-	-	Proline	+	+
Tryptophan	-	-	Serine	-	-
			Tyrosine	-	-

amino acids. The essential amino acids are threonine, valine, leucine, isoleucine, and lysine. The nonessential amino acids include alanine, cysteine, cystine, glutamic acid, hydroxy proline, and proline (Table 1).

The total protein content per 1 mg of pollen is 190.45 µg in *B. diffusa* and 176.85 µg in *B. erecta*. In both species, the nectariferous tissue is continuous at the base of the filaments and expands close to the base of the free parts of filaments. Nectar is produced at the base of petaloid perianth just above the ovary; it is secreted in trace amount only. The stigma is receptive at the time of the unfolding of the petaloid perianth and remain receptive until the evening of the same day. The petaloid perianth together with sex organs falls off by the afternoon of the next day.

Foraging activity and pollination

The flowers *B. diffusa* and *B. erecta* were indiscriminately foraged by different insect categories during day light hours. Both species were foraged by bees, wasps, and butterflies, but *B. erecta* was also foraged by the fly *Musca* sp (Figure 4h,i) (Table 2). The bees were *Apis cerana* (Figure 2a, 4a), *A. florea* (Figure 2b), *Trigona iridipennis* (Figure 2c, 4b), and *Ceratina* sp. (Figure 4c) (Apidae); the first three species were common to both plant species, while the last bee species confined its visits to *B. erecta* only. The wasps included *Rhynchium* sp. (Figure 2d) and *Polistes* sp. (Figure 2e) (Vespidae), *Campsomeris annulata* (Scoliidae) (Figure 4d,e) and *Sphex* sp. (Sphecidae) (Figure 4f,g); the first two species visited the *B. diffusa* flowers only, while the other two species confined their visits to *B. erecta*. In both species, all insect foragers began their foraging activity at 0700 h, and gradually increased their activity towards noon paying maximum visits at 1000-1200 h; their foraging activity decreased towards evening. Their foraging activity ceased for the day at 1400 h in *B. diffusa* and at 1300 h in *B. erecta* (Figure 5,7). The butterflies included *Leptosia nina* (Pieridae) (Figure 2f), *Danaus chrysippus* (Nymphalidae)

(Figure 2g), and *Castalius rosimon* (Figure 2h), *Pseudozizeeria maha*, *Zizula hylax* (Figure 2i, 4k), *Zizeeria karsandra* (Figure 2j, 4m), *Zizina otis* (Figure 2k, 4l), *Freyeria trochylus* (Figure 2l, 4n), *Euchrysops cnejus* (Figure 2m), *Everes lacturnus* (Figure 2n), *Jamides celeno*, *Chilades pandava* (Figure 2o), *C. laius* (Figure 4o) and *Leptotes plinius* (Figure 4j) (Lycaenidae). Of these, the pierid and nymphalid butterflies visited the *B. diffusa* flowers only. Among the lycaenid butterflies, *C. rosimon*, *P. maha* and *C. pandava* confined their visits to the *B. diffusa* flowers, while *C. laius* and *L. plinius* confined their visits to the *B. erecta* flowers only. All other lycaenids visited both of the *B. diffusa* and *B. erecta* flowers. In both plant species, the butterflies began their foraging activity at 0700 h, gradually increasing their flower visiting rate and paying maximum visits between 1000-1100 h. They then decreased their visits and terminated the foraging activity at 1300 h (Figure 6,8). In the *B. diffusa*, bees made 24%, wasps 16% and butterflies 60% of the total foraging visits. In *B. erecta*, bees made 43%, wasps 9% and butterflies 48% of the total foraging visits. In both plant species, the forage collected by bees included both pollen and nectar, while that collected by the wasps, the fly, and the butterflies included only nectar. Bees and butterflies were regular and consistent foragers, while wasps and the fly were irregular and inconsistent foragers even during the phase of the prolific flowering of the plant. Among butterflies, the nymphalid butterfly, *D. chrysippus* was an occasional forager on *B. diffusa* flowers.

In both plant species, the flowers, with the small petaloid tube consisting of nectar and situated just above the ovary, were found to be promiscuous for visitation by different insects. The nectar although seated at the ovary base was found to be accessible by small-tongued bees, wasps, and butterflies with small proboscis. The placement of anthers at or slightly above the rim of the petaloid perianth was found to be facilitating easy access for the pollen-collecting bees. The production of minute nectar and a small

Table 2. List of insect foragers on *Boerhavia diffusa* and *Boerhavia erecta*

Order	Family	Insect species	<i>B. diffusa</i>	<i>B. erecta</i>	Forage sought
Hymenoptera	Apidae	<i>Apis cerana</i> F.	+	+	Pollen and Nectar
		<i>Apis florea</i> F.	+	+	Pollen and Nectar
		<i>Trigona iridipennis</i> Smith	+	+	Pollen and Nectar
		<i>Ceratina</i> sp.	-	+	Pollen and Nectar
	Scoliidae	<i>Campsomeris annulata</i> F.	-	+	Nectar
	Sphecidae	<i>Sphex</i> sp.	-	+	Nectar
	Vespidae	<i>Rhynchium</i> sp.	+	-	Nectar
		<i>Polistes</i> sp.	+	-	Nectar
Diptera	Muscidae	<i>Musca</i> sp.	-	+	nectar
Lepidoptera	Pieridae	<i>Leptosia nina</i> F.	+	-	Nectar
	Nymphalidae	<i>Danaus chrysippus</i> L.	+	-	Nectar
		<i>Euchrysops cnejus</i> F.	+	-	Nectar
	Lycaenidae	<i>Castalius rosimon</i> F.	+	-	Nectar
		<i>Leptotes plinius</i> F.	-	+	
		<i>Zizula hylax</i> F.	+	+	Nectar
		<i>Pseudozizeeria maha</i> Kollar	+	-	Nectar
		<i>Zizeeria karsandra</i> Moore	+	+	Nectar
		<i>Zizina otis</i> F.	+	+	Nectar
		<i>Freyeria trochylus</i> Freyer	+	+	Nectar
		<i>Everes lacturnus</i> Godart	+	+	Nectar
		<i>Chilades pandava</i> Horsfield	+	-	Nectar
		<i>Chilades laius</i> Stoll.	-	+	
		<i>Jamides celeno</i> Cr.	+	+	Nectar

quantity of pollen by individual flowers was found to drive both pollen- and nectar-collecting insects to visit as many flowers as available in the flowering patch to meet their requirement for the day. In this process, these insects were found to make multiple visits to most of the flowers, and such a foraging behavior was considered to be promoting geitonogamy and xenogamy. Being small-bodied, bees and flies approached the flowers in an upright manner, landing on the flat-topped umbels and inserting their tongues into the campanulate petaloid perianth of individual flowers to collect nectar. During the same visit, they also moved towards the anthers which are situated just above the perianth and collected pollen grains. All the three bee species loaded pollen into their corbiculae, and the pollen was also deposited on their ventral side and forehead. The ventral side of the bee

body had contact with the peltate stigma and anthers during nectar and pollen collection and such a contact affected self- and/or cross-pollination. Being large-bodied, wasps approached the flowers in an upright way, landed on the same or adjacent umbels, and then inserted their straw-like tongues into the base of petaloid perianth to collect nectar; in doing so, they brushed the stigma and anthers with their ventral surface affecting self- and/or cross-pollination. Butterflies also approached the flowers in their upright position, landing on single or multiple umbels and inserting their proboscis to collect nectar. During that, they brushed the stigma and the anthers of the visited flower and also of the flowers of adjacent umbels with their proboscis, forehead, thorax, and abdomen. Such foraging behavior, used to collect nectar, was found to be affecting self- and/or cross-pollination. Furthermore,

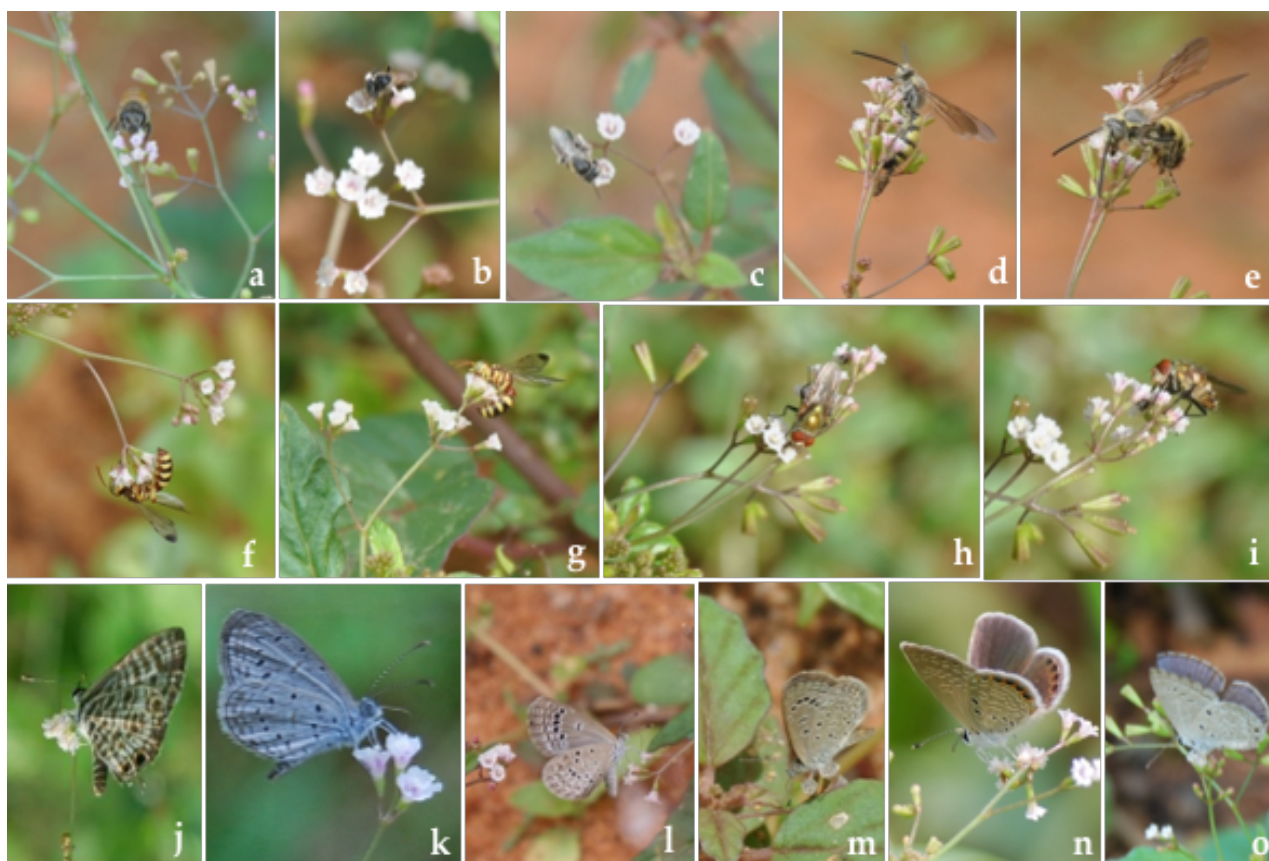


Figure 4. *Boerhavia erecta*: a. *Apis cerana* collecting nectar, b. *Trigona iridipennis* collecting nectar, b. *Ceratina* sp. collecting pollen, d. & e. *Campsomeris annulata* collecting nectar, f. & g. *Sphex* sp. collecting nectar, h. & i. *Musca* sp. collecting nectar, j-o: Lycaenid butterflies collecting nectar: j. *Leptotes plinius* k. *Zizula hylax*, l. *Zizina otis*, m. *Zizeeria karsandra*, n. *Freyeria trochylus*, o. *Chilades laius*.

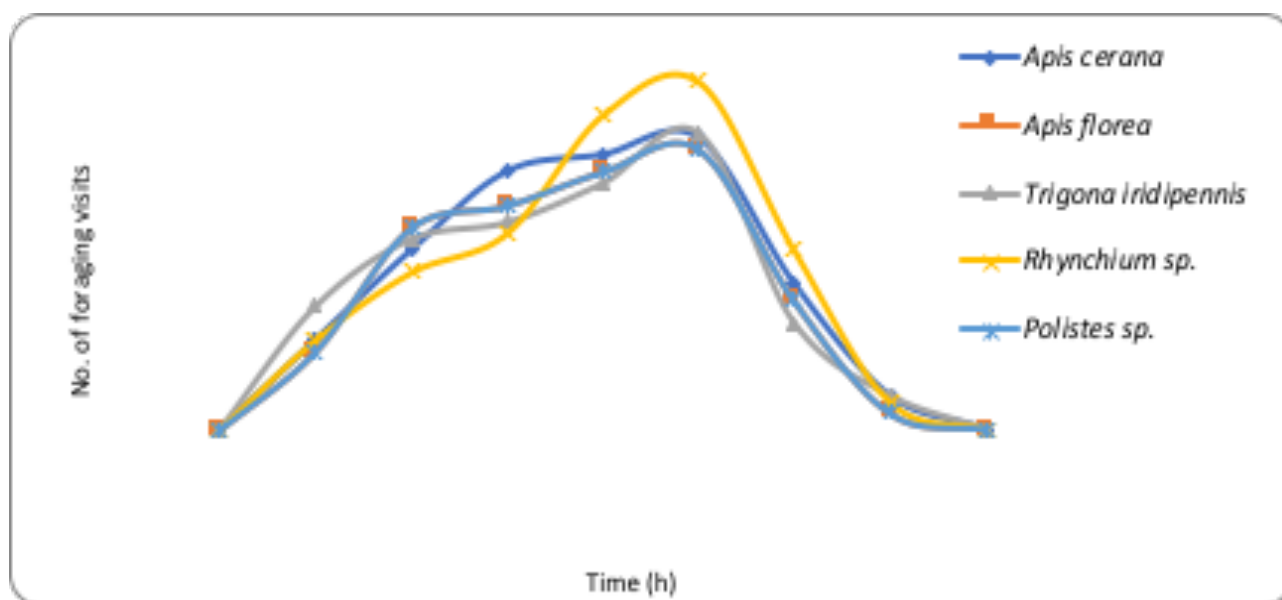


Figure 5. Hourly foraging activity of bees and wasps on *Boerhavia diffusa*

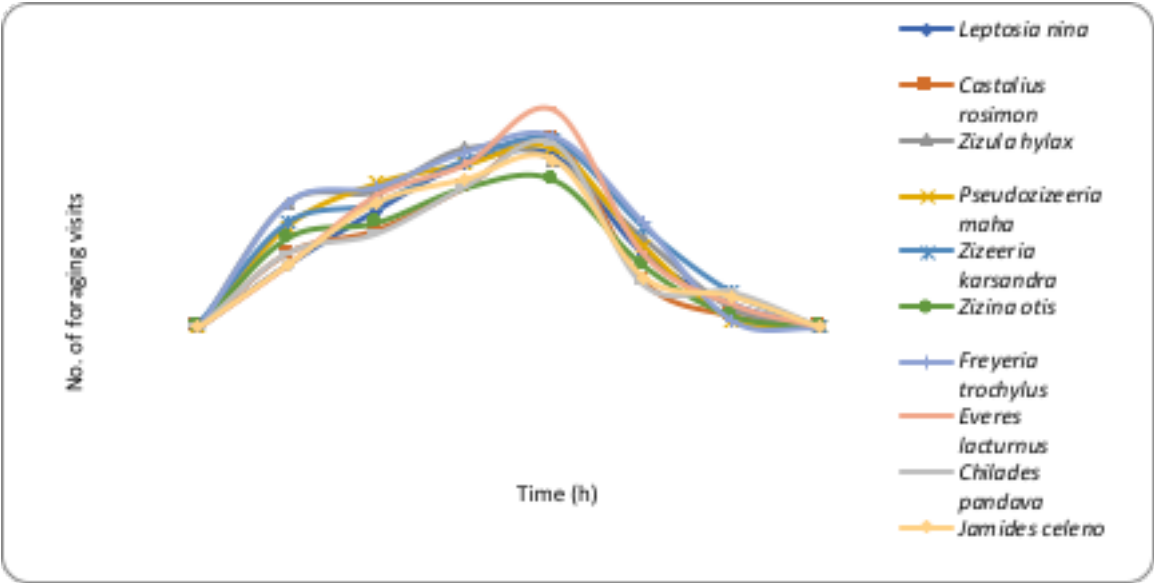


Figure 6. Hourly foraging visits of butterflies on *Boerhavia diffusa*

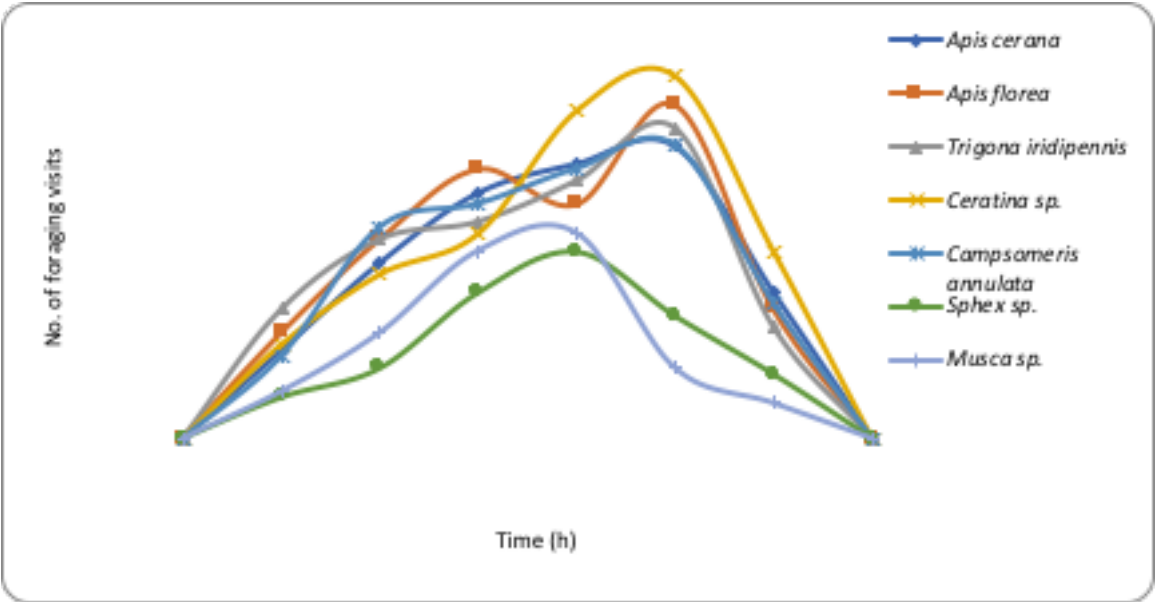


Figure 7. Hourly foraging visits of bees and wasps on *Boerhavia erecta*

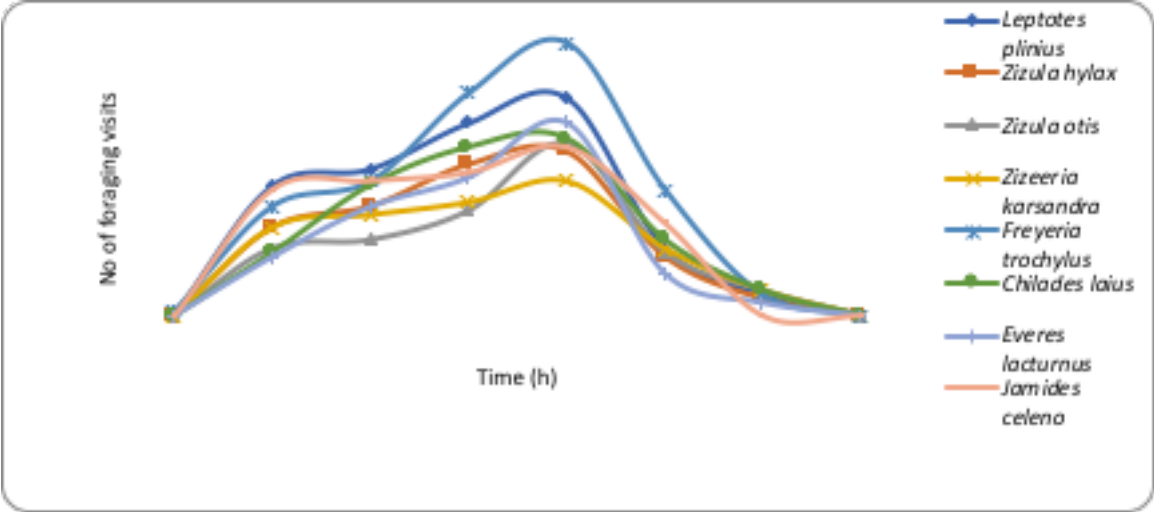


Figure 8. Hourly foraging visits of butterflies on *Boerhavia erecta*

the butterflies also collected nectar without landing on the umbels, but while holding the flowers with their legs in a hanging mode. In this foraging behavior, only the proboscis and/or their forehead had contact with the stigma and anthers which resulted in pollination. Therefore, being consistent and regular foragers to the plant, bees and butterflies were treated as the actual pollinators and wasps as additional pollinators.

Fruiting ecology and seed dispersal

In both species, the fertilized flowers produce fruits within one to two weeks. Fruit set rate in the bagged flowers was 78% in *B. diffusa* and 81% in *B. erecta*. In open-pollinations, fruit set rate was 92% in *B. diffusa* and 89% in *B. erecta*. In both, fruit is a one-seeded indehiscent anthocarp in which the lower part of the perianth remains attached to the fruit (Figure 1n, 3j). It is simple, sessile, 3 mm long, ellipsoid clavate-shaped, sticky, and pentangular with a slight indentation at the apex and is covered with glandular hairs. Furthermore, it secretes a hygroscopic mucoidal substance produced in the columnar cells of the ribs. Seeds are minute, five-sided, wedge-shaped, white and truncate at top (Figure 1l,m, 3k); they are 2.5 mm long, 1.5 mm wide in *B. diffusa*, and 1.5-1.8 mm long and 1.2 mm wide in *B. erecta*. In both species, the sticky fruit with the mucoidal secretion appeared to be an adaptive trait to facilitate dispersal upon contacting with animals and humans because the fruit readily adheres to the objects they touch and detaches itself from the plant. The fruits of both species were also dispersed by birds, namely, *Acridotheres tristis* (Indian Myna), *Turdoides caudatus* (Common Babbler) and *Turdoides striatus* (Jungle Babbler). Furthermore, the fruits were found to be dispersed through rainwater. The seeds were non-dormant and germinate almost immediately upon the decomposition of perianth residue and fruit pericarp in the vicinity of parental sites provided that the soil has moisture. In addition to seed propagation, both species also multiply by

the fragmentation of the rhizome to expand their population size. The function of both sexual and asexual modes enables them to propagate continually and display vegetative phase, flowering and fruiting phases either simultaneously or alternately throughout the year in the habitats where the soil is continually moist or wet.

Discussion

The genus *Boerhavia* represents four species, *B. crispa*, *B. rubicunda*, *B. diffusa* and *B. erecta* in India (Dutta *et al.*, 2015), of which *B. diffusa* and *B. erecta* are widespread and distributed throughout the country (Kirtikar and Basu, 1933; Chen and Wu, 2007). *B. diffusa* grows well in open sunlight and endures severe abiotic stresses such as UV exposure, high temperature, water and nutrient deficiencies (Anonymous, 1988). The present study shows that both *B. diffusa* and *B. erecta* occur commonly and grow well displaying vegetative, flowering and fruiting phases either simultaneously or alternately throughout the year in various habitats with wet to semi-dry soils. However, they show prolific growth, flowering and fruiting during the rainy season; they carpet the soils with their profuse growth in open habitats exposed to direct sunlight, high temperature, water and nutrient deficiencies. However, *B. diffusa* is widespread in wet to semi-set soils, while *B. erecta* is widespread in semi-wet and well-aerated sandy soils indicating that these species have different preferred habitats for their propagation and population expansion. These species can be easily identified through habit, inflorescence, and floral color characters in the field. In *B. diffusa*, the branches are prostrate and diffused with a long peduncle comprising of several cymes of pedicellate flowers with a red to violet petaloid perianth. In *B. erecta*, the branches are slightly erect spreading with long primary and short secondary peduncles; the latter type comprises several cymes of pedicellate flowers with a white petaloid perianth with pink stripes. In both species, all flowers in a cyme open on the same day

and at the same time making them attractive to their pollinating insects.

Spellenberg (2000) reported that in *Boerhavia coccinea*, *B. intermedia*, *B. spicata*, *B. torreyana*, and *B. wrightii*, individual flowers open only during a portion of a day. The flowers open at dawn and their opening begins with the opening of the corolla-like perianth and the uncoiling of stamens and styles. As the morning progresses, the filaments and style curl, and the anthers contact the stigma affecting autogamous self-pollination. The perianth closure begins in the late morning containing the stamens and style within and wrinkles by mid-afternoon of the same day. Chaturvedi (1989) reported similar autogamous self-pollination and a perianth closing function in *B. diffusa*. In the present study, the flowers of *B. diffusa* and *B. erecta* open at dawn by unfolding the petaloid perianth during which the stamens with dehiscent anthers, and the style and stigma in a receptive state gradually uncoil; in this process, the stigma brushes the anthers, and then the pollen grains transfer to the stigmatic surface resulting in autogamous self-pollination. The occurrence of autogamy by the movement of sex organs during flower-opening is almost certain in the three-stamened flowers as there is a very narrow gap between anthers and the style and stigma, but it is not so in either of the two- and the one-stamened flowers as there is a wider gap between the sex organs because of non-production of one stamen in two-stamened flowers and two stamens in one-stamened flowers. Most of the flowers produced by both plant species possess three stamens that facilitate autogamous selfing. However, the production of the two- and one-stamened flowers in both species appears to be an evolved trait to regulate autogamous selfing in order to facilitate vector-mediated autogamy, geitonogamy, or xenogamy during the open state of the flowers. Furthermore, the production of nectar and the vividly colored petaloid perianth are additional adaptations to attract insect pollinators to mediate pollen transfer within and between conspecific plants. The

flowers that failed to self- or cross-pollinate autonomously during the process of anthesis or by vector-mediation in open condition have the option to resort to autogamous self-pollination by the brushing of the inward coiling of the stigma and style against the anthers bending inwards simultaneously, but this mode of pollination is subject to the availability of pollen in the anthers at that time. Moreover, the inward coiling of the sex organs is compulsory for the perianth to close back completely. The *B. diffusa* and *B. erecta* flowers govern themselves in autogamous self-pollination during the opening and closing process of the perianth lobes, while they facilitate vector-mediated self- and cross-pollination during the open condition of the perianth lobes. The flowers require only a few pollen grains for pollen and subsequent fertilization since the ovary contains only a single ovule. The movement of sex organs during the closing process of the perianth lobes and the duration of the open state of the perianth provide ample opportunities for the occurrence of self- or cross-pollination. In both plant species, pollen/ovule ratios indicate the functionality of facultative autogamy (Cruden, 1977); however, their ratios are higher than the ratios prescribed for facultative autogamy by Cruden (1977) suggesting that the high ratio is required to promote cross-pollination by insect pollinators and also to compensate for pollen loss resulting during the pollen collection activity of bee pollinators. Despite the functionality of spontaneous and vector-mediated pollination, the fruit set rate is not 100% in both plant species. Therefore, *B. diffusa* and *B. erecta* with a facultative autogamous mating system regulate spontaneous self-pollination and allow a certain percentage of cross-pollination in order to maintain genetic variation which enables them to adapt to different habitats, and to produce seed prolifically and expand their distribution range from plain lands to hill tops (Adahl *et al.*, 2006).

Bittrich and Kuhn (1993) reported that the *Boerhavia* flowers are melittophilous and are suited for head pollination by small bees;

they are fit for both bee and fly pollination. Spellenberg (2000) reported that *B. coccinea* is visited the bees of *Scolia*, *Mellisodes*, the wasps of *Holopyga rudis* and *Ammophila*, and also by the calliphorid, syrphid and muscid flies in New Mexico, United States of America. de Mendonca Santos *et al.* (2010) reported that *B. coccinea* is pollinated by social bees and social wasps in Caatinga in the northeastern Brazil (de Mendonca Santos *et al.*, 2010). Spellenberg (2000) also reported that *B. intermedia*, *B. torreyana*, *B. wrightii* and *B. spicata* are visited by bees, wasps, and flies. Chaturvedi (1989) reported that *B. diffusa* is pollinated by bees, flies, ants, and butterflies in North India. In this study, both *B. diffusa* and *B. erecta*, with a minute volume of nectar and pollen consisting of some essential and nonessential amino acids and protein content, attract bees, wasps, flies, and butterflies to their flowers. However, only bees and butterflies act as actual pollinators due to their regularity and consistency in their visits, while other insects act as additional pollinators. As the flowers are very small with a minute volume of nectar and a small amount of pollen, the insects are compelled to visit as many flowers as available in the habitat and in this process, they promote cross-pollination. In both plant species, the pollen grains are large and spinulose; these are the characteristics of insect-pollinated species (Nowicke, 1970). Therefore, both *B. diffusa* and *B. erecta* are entomophilous involving bees and lycaenid butterflies as principal pollinators.

Struwig and Siebert (2013) reported that the shape of the anthocarp, presence of ribs or wings, and the presence or absence and the position of glandular hairs are diagnostics of the *Boerhavia* species in South Africa. In this study also, anthocarp fruit characteristics can be used as diagnostic characters to differentiate *B. diffusa* from *B. erecta*. In these species, the upper part of the perianth is petaloid, while the lower part of the perianth is sepaloid. The petaloid perianth detaches by abscission after fertilization, while the sepaloid perianth containing the ovary enlarges and develops into a protective

structure around the fruit which is termed as the anthocarp (Vanvinckenroye *et al.*, 1993; Hickey and King, 2000). In both species, the one-seeded indehiscent anthocarps secrete mucoidal substance in their columnar cells in the ribs, and this secretion enables them to be sticky and adhere to the objects which contact them. This character is an adaptive trait to facilitate the dispersal of anthocarps by animals, humans, and birds. Additionally, the anthocarps also disperse through rainwater. Therefore, both *B. diffusa* and *B. erecta* are zoochorous, anthropochorous, ornithochorous, and hydrochorous. These multiple dispersal modes ensure these plant species to migrate and spread across various uncultivated and cultivated habitats in tropical and sub-tropical areas around the world.

In this study, it was found that both *B. diffusa* and *B. erecta* propagate predominantly by seeds. Since seeds are non-dormant, they immediately germinate as soon as they are dispersed and produce several batches of populations if the soil has sufficient moisture and nutrients. Additionally, these species also multiply asexually through the fragmentation of the rhizome. The dual modes of regeneration enable these plant species to occupy diverse habitats and become invasive. In these plant species, the characters such as year-long flowering and fruiting, spontaneous autogamy, entomophily, the production of non-dormant seeds, the function of multiple seed dispersal modes and propagation by the fragmentation of the rhizome constitute weed characteristics and hence *B. diffusa* and *B. erecta* are treated as prolific and invasive weeds (Baker, 1965; 1974).

Mwangi (2009) reported that *B. diffusa* is an important floral source for a diversity of bee species in the Arabuko Sokoke Forest, Nigeria. Mahale (2019) noted that *B. diffusa* is an important nectar source for honeybees in the cultivated and uncultivated areas of the Ahmednagar in South-Eastern region of Western Ghats. Churi *et al.* (2020) noted that *B. diffusa* is a nectar plant for *Junonia lemonias*, *J. almana*, *Castalius risomon*,

Appias olferna, *Eurema hecabe*, *Ypthima* spp., *Colotis amata*, *C. danae*, and *C. protractus* in South India. The present study adds that both *B. diffusa* and *B. erecta* are important pollen and nectar sources for bees, and constitute nectar sources for wasps, flies, and butterflies in all areas where these plant species occur.

Conclusions

The *Boerhavia diffusa* and *Boerhavia erecta* flowers open at dawn by unfolding the petaloid perianth during which the stamens with dehiscent anthers, and the style and stigma in a receptive state gradually uncoil to become erect; in this process, autogamous self-pollination occurs. This mode of autogamy is almost certain in the three-stamened flowers, but it is not so in neither of the two- and the one-stamened flowers. The production of nectar and vividly colored petaloid perianth are additional adaptations for insect pollination. The flowers that fail to self- or cross-pollinate autonomously during the process of anthesis or by vector-mediation in the open condition have the option to resort to autogamous self-pollination during the process of the closing of the petaloid perianth covering the sex organs within. Despite the functionality of the spontaneous and vector-mediated pollination, the fruit set rate is not 100% in both plant species which means that they regulate spontaneous self-pollination and allow a certain percentage of cross-pollination to maintain genetic variation which for sure makes them adapt to different habitats, enables them to produce seed prolifically, and which expands their distribution range. In both species, bees and butterflies act as actual pollinators, while the other insects act as additional pollinators. In both species, zoochory, anthropochory, ornithochory, and hydrochory are functional. Seeds are non-dormant, germinate immediately after dispersal and produce several batches of populations provided that the soil environment is favorable. Moreover, these species also multiply asexually through the fragmentation of the rhizome. The year-

long flowering and fruiting, the spontaneous autogamy, entomophily, the production of non-dormant seeds, the function of multiple seed dispersal modes and the propagation by the fragmentation of rhizome make them prolific and invasive weeds. The study suggests that both plant species are ideal pioneer species and can be useful in the restoration of ecologically disturbed and damaged habitats or ecosystems because they have the ability to grow in habitats with semi-wet and sandy soils. Furthermore, by establishing huge populations in such habitats these plant species play an important role in controlling soil erosion, and in the sequestration of carbon dioxide in addition to supporting certain local insect fauna by providing pollen and/or nectar during flowering period.

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