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Floral biology, floral volatile organic compounds and floral visitors of *Chromolaena odorata*, an invasive alien species in West Bengal, India

UJJWAL LAYEK, ALOKESH DAS, UDAY DAS♥
Department of Botany, Rampurhat College. Birbhum, Rampurhat, West Bengal 731224, India, ♥email: udaydas.vb@gmail.com

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Abstract. *Layek U, Das A, Das U. 2022. Floral biology, floral volatile organic compounds and floral visitors of* Chromolaena odorata, *an invasive alien species in West Bengal, India. Biodiversitas 23: 2118-2129. Chromolaena odorata* (L.) R.M. King & H. Rob. is a fast-growing weed native to the Neotropics and introduced in several regions of Africa, the Pacific Islands, and Southeast Asia, including West Bengal in India. It is one of the world's most widespread and troublesome invasive alien plant species (IAPS) that severely infest natural habitats and crop fields. However, extensive data documents about the reproductive ecology are unknown, especially within West Bengal. This study assesses the floral biology, floral volatile organic compounds (VOCs), and floral visitors of the weed. The weed species flowers from October to January. Florets are white to purple, with short, narrow corolla tubes and deep-seated nectar. Abundant VOCs are benzyl stearate, 2,4-decadienal, n-hexadecanoic acid, 1-hexyl-2-nitrocyclohexane, and o-decyl hydroxylamine. Flower heads were visited by numerous insect groups, though the diversity of Lepidoptera (mainly butterflies) was higher than the other insect orders. The weed is pollinated by diverse insect groups (e.g., butterflies, flies, honeybees, leafcutter bees, solitary bees, and wasps). However, vital pollination services to the weed were provided by butterflies (based on the 'approximate pollination value'). Our findings may conclude that the clustered capitula, longer stigmatic receptivity, and broad array of pollinators resulted in too much fruit set. It is one of the critical factors that support the high growth rate and invasive nature of the weed in dry habitats.

Keywords: Approximate pollination value, butterfly, invasive alien species, volatile organic compound

INTRODUCTION

The invasion of non-indigenous plants within the natural habitats has multiple impacts (both positive and negative) on the environment and wildlife. Some alien species may have positive effects, like providing food and shelter or securing ecosystem processes. But most non-indigenous plants are widely recognized as a core problem for conservation strategies (Espinosa-García and Villaseñor 2017; Heywood 2017). Biological invasion and its impacts need to be carefully considered in conservation planning, as they can affect the effectiveness of conservation efforts. Regarding the biological invasion in conservation planning following approaches may be taken: (i) avoid (highly invaded areas in conservation planning, (ii) protect (restore them to a better state through adequate management actions, or (iii) ignore (alien species are ignored, in the deficit of knowledge on how to deal with biological invasions in the planning phase). The selected approach has important implications for spatial conservation priorities and can lead to very different outcomes (Giakoumi et al. 2016). Invasive alien plant species (IAPS) may compete with native species and are considered the major drivers of biodiversity loss (Bartz and Kowarik 2019; Jones and McDermott 2018; Rai and Singh 2020). and therefore, they may alter ecosystem functioning and community structure (Gallien and Carboni 2017; Mollot et al. 2017). Native plants can act as a sink for air pollutants and contribute to carbon sequestration (Shackleton et al. 2019). Therefore, loss of plant diversity may indirectly affect human health through perturbations in the environmental quality (Jones and McDermott 2018). The spread of the IAPS can affect human health through their pollen and toxins (Plaza et al. 2018). The human health impacts of invasion are further exacerbated by the spread of vector-borne pathogens (Clow et al. 2017; Schindler et al. 2018).

Merely a single attribute does not decide the success of the invasive alien species. Plant invasion, anthropogenic disturbances, climate change, biodiversity and human health may have a complex and intricate relationship (Rai and Kim 2020). Thus, invasion ecology is increasingly being considered a trans-disciplinary subject, intimately linked with global change biology, land-use change, health science, and conservation biology (Heshmati et al. 2019). The knowledge about the ecological mechanisms of IAPS and their impacts on ecosystem services and human health is necessary for environmental management and human well-being perspective. The quest for appropriate implications for IAPS biomass management gives an impetus to mitigating the associated human health hazards. Therefore, understanding the invasion ecology of an alien species is of paramount importance for developing suitable management strategies.

Most invasive exotic plants belong to the Asteraceae family (Mugendhiran et al. 2020). Majorities of the invasive plants of Asteraceae have high allelopathic properties and have harmful effects on nature and the plant population. In addition, a prolific alien has a chance of creating its own micro-climate in the habitat, alienating the endemic species of the habitat (Mugendhiran et al. 2020).

The members of Asteraceae have a high diversity of reproductive mechanisms. In many species, florets opened in the early morning and were protandrous (Valentin-Silva et al. 2016). In the early anthesis period, secondary pollen presentation occurs during the staminate phase, following the pistillate phase. Most of the members of Asteraceae are self-compatible (Grombone-Guaratini et al. 2004). However, almost all members benefitted from cross-pollination, done mainly by butterflies (Valentin-Silva et al. 2016).

The plant *Chromolaena odorata* (L.) King & H. E. Robins (family: Asteraceae) is an alien plant species in Indian states, including West Bengal. Though the weed has some medicinal importance [e.g., antimicrobial (Stanley et al. 2014), wound healing (Sirinthipaporn and Jiravngkoorskul 2017), anti-inflammatory, and antipyretic activities (Owoyele et al. 2013)], it becoming more abundant, due to its ability to withstand dry and stresses environmental conditions (Kriticos et al. 2005), and being flagged as one of the most troublesome weeds in many regions. Therefore, it is essential to work out the weed's invasion ecology to develop sustainable management practices. Several hypotheses (like the enemy release, novel weapon, and empty niche) have been proposed to explain the invasion of IAPS in new habitats. We hypothesized that the weed became an aggressive colonizer due to its higher reproductive success led by pollination services. The current study was conducted to assess floral biology (including flowering time, floral morphology, volatile floral compounds and anthesis period) of the invasive alien species *Chromolaena odorata* in West Bengal, India. We also worked out the pollination ecology of the plant species by surveying floral visitors and determining their relative importance in pollination services.

MATERIALS AND METHODS

Plant species and study site

The study was carried out on *Chromolaena odorata* (L.) King & H. E. Robins, a member of the Asteraceae family. The weed is commonly known as banmara (in Nepal), sahp seua (in Thailand), siam weed (in Malaysia), white snakeroot in English and bhut bhairavi in Bengali. It is a fast-growing, perennial weed found in many parts of the world (Omokhua et al. 2016; Shackleton et al. 2017). It was introduced in India from tropical America during World War II, and since it has spread widely. Today, it is a dominant weed on roadsides, wastelands, and other exposed areas (Biswas et al. 2014).

The study was conducted in Bankura (22°38'-23°38' N and 86°36'-87°46' E) and Birbhum (23°32'-24°35' N and 87°5'-88°1' E) districts of West Bengal, India during 2019-2021. These regions show hot summer (day temperature could rise to 39°C) during April-June, cold-dry winter (temperature may fall up to 7°C; relative humidity 50-70%) in December-January. Annual rainfall is very low (13.50 cm in Bankura district and 14.30 cm in Birbhum district), and the bulk of this comes in July-August. Major parts characterize by alluvium, and red lateritic soil types. Though a significant land part is used for cropping, a considerable amount is covered with thin forest. The studied plant patches occurred on road-sides, un-used playgrounds, and un-cultivated field areas. Within the study areas, predominant vegetation was of seasonal crops (e.g., *Allium cepa* L., *Brassica* spp., *Coriendrum sativum* L.), planted groves (e.g., *Acacia auriculiformis* A. Cunn. ex Benth. and *Eucalyptus* spp.), and sporadic trees (e.g., *Borassus flabellifer* L.).

Floral biology

To record the flowering period of the weed, we carried out field surveys throughout the years. Initially, the time interval between two successive surveys was longer (about 15 days) and continued until flower bud initiation was noticeable. Then we shortened the time interval to about 7 days. We counted the number of flowering twigs per individual from randomly selected 50 plants during the peak flowering period. We recorded the number of capitula per flowering twig (n = 50, selected from 50 plants), the number of florets per capitulum (n = 50, selected from 50 plants), and the longevity of florets (n = 60; selected from 60 capitula of 20 plants, 3 capitula from each plant). To determine the longevity of individual florets, we selected capitula (20 capitula from 20 plants on a sampling day) in the late afternoon, and all the opened florets were marked for discarding on the next day's selection. On the following morning (8.00-10.00 am), we observed the selected capitula, and 20 newly opened florets (one per capitulum per plant) were selected and marked by given black dots of ink on their one petal tips. Then, we observed the marked florets two times daily (morning and late afternoon) until senescence of the florets took place. Senescence was characterized by the dry out of style of the selected florets. We repeated the same process three times, and each time different capitula were chosen from these plants. We noted the morphology of florets. Pollen production per floret (10 florets from 10 capitula belonging to 10 plants) was estimated. To count the pollen grains, we took the anthers of a nearly matured, unopened floret into a glass vial. Then added 1 mL of 0.4 M sucrose solution and crushed with a glass rod to wash out the pollen grains from the anthers. Then, the solution was filtrated to remove the debris. After shaking, 10 µL of solution from a sample was taken by micropipette on a glass slide, and counted the number of pollen grains suspended in the solution. We repeated this procedure three times for each sample and estimated the average number of pollen grains in 10 µL and then the total number of pollen grains per floret multiplied by 100. For the study of pollen morphology (unit, size, shape, apertural characters, and exine ornamentation), pollen grains were processed using the acetolysis method (Erdtman 1960). A microscopic study was done with a compound light microscope (Leica DM1000), and microphotographs of pollen grains with suitable magnifications were taken with a Leica DFC295 digital camera. The number of ovules per ovary was counted directly by rupturing the ovary wall.

To test the pollen viability and stigma receptivity, six time-slots were chosen (0 h, 6 h, 24 h, 30 h, 48 h, and 54 h) after opening a floret. For this purpose, 60 florets (10

florets per time-slots from 10 plants) were selected to test pollen viability and 180 florets (30 florets per time-slots from 30 capitula of 10 plants, 3 capitula per plant) for stigma receptivity test. The flowering twigs (having the capitula in which florets were selected for pollen viability test) were bagged with nylon net to restrict visitors' activity on the capitula. The viability assessment of pollen grains was carried out by staining the pollen with 1% of 2,3,4-triphenyl tetrazolium chloride (TTC) solution. To prepare the staining solution, 0.1 g TTC and 6 g sucrose were dissolved in 10 mL distilled water. A drop of this solution was taken on a glass slide. Pollen grains were added to this solution and covered with a coverslip. After 2 hours of incubation at room temperature, observed under a light microscope, and percentages of viable pollen grains (stained orange or bright red color) were recorded. Stigma receptivity was measured using the benzidine-H_2O_2 test.

For floral volatiles analysis, six capitula (each capitulum having peripheral opened florets but not in senescence stage and a few central florets are in bud stage) were collected into a 20 ml ThermoFisher Scientific glass vial. Then, the vials were capped with the help of a crimper. The vials (containing capitula) were incubated for 3-4 h in the laboratory at room temperature. For floral volatiles analysis, we used the head-space adsorption method. The vials (containing floral volatiles) were placed into TriPlus RSH Autosampler to inject the samples into Trace 1300 Gas Chromatography (Thermo Scientific) by a splitless injector. Volatile compounds were separated in the Gas Chromatography using a TG-WAXMS column (30 m × 0.25 mm × 0.25 µm) with a carrier gas (i.e., helium) at a linear velocity of 1.2 mL/min. The temperature was programmed at 40°C for 3 min, and then increased to 250°C, gaining 6°C/min and maintaining the temperature of the transfer line at 250°C. We have taken a blank air sample (vial without capitula) and used it as a control during the analysis for background correction. The gas chromatograph (GC) was connected to an ISQ QD Single Quadrupole Mass Spectrometer. Volatile organic compounds (VOCs) were identified by the retention indices (RIs) and mass spectra compared with the reference standards listed in NIST 2017 library.

Floral visitors

We observed the visitors at different time slots (6.00-8.00 h, 8.00-10.00 h, 10.00-12.00 h, 12.00-14.00 h, 14.00-16.00 h, and 16.00-18.00 h) during peak flowering time (i.e., November-December) of the two flowering sessions (i.e., October 2019-January 2020 and October 2020-January 2021). A direct observation method was followed to encounter floral visitors. To record the number of visiting insects, one survey (i.e., plant-based sampling) period comprises 5 min and on closely situated approx 20 flowering twigs. We performed 240 surveys (10 surveys/time-slot/session for each studied district). The encountered visitors were identified (up to species level) in the field or captured (with the help of an insect net) for later identification. The entrapped insects were put into a glass vial containing a piece of cotton (which was soaked with ethyl acetate) and later on sent to entomologists (at the ZSI, Kolkata) for identification. The relative abundance (RA) of each flower-visiting species was calculated as follows (Layek et al. 2020):

$$\text{RA}\ (\%) = \frac{\text{n}i}{\text{N}} \times 100$$

where n*i* is the number of encountered individuals of the insect species *i*, and N is the total number of encountered individuals of all the flower-visiting species on the plant.

To estimate the flower visitation rate of the visitors, we considered the number of flowering twigs (having several closely situated capitula) visited in a 1-minute duration. Here, we evaluated the number of visited twigs instead of florets or capitula. Because the florets are very small and capitula are very close in association, most visitors touch more than one capitulum in a single visit. Generally, we recorded the data (number of visited twigs/min) 30 times for an insect species. For insect species that spent much time on a twig, we counted the number of twigs visited in 10 minutes duration instead of 1-minute and then divided by 10 to convert the value into a 1-minute duration. For these visitors, we recorded the data (number of visited twigs/10 min) 10 times for an insect species.

To estimate the number of pollen grains of the plant species carried by a visitor species, we entrapped the visitors ($n \geq 5$ for each dominant species; we did not consider the insect species for which sample size was less than 5) with an insect net. With the closed observation of the captured bee species with 10 x hand lens, we noticed whether stacked pollen loads were there or not. We considered the body surface pollen grains (excluding stacked pollen loads on corbiculae, scopae, or other parts) and stacked pollen loads (in the case of carpenter bees, honeybees, leafcutter bees, and solitary bees) in different ways. To separately count the stacked pollen grains, the hind legs of these visitors were amputated and put into a glass vial separately. To count stacked pollen grains on the abdomen (in the case of leafcutter bees), we estimated the total body surface pollen grains content of pollen (or mixed) forager, then deduced the average number of body surface pollen grains of a nectar forager (i.e., the forager which was without stack pollen load). Here, the ratio of pollen forager to total forager was also considered in calculating the average number of pollen grains in stacked pollen loads. To estimate body surface pollen grains content, we added 1.0-6.0 mL (depending on the body size of the captured visitor) of 0.4 M sucrose solution in the vials containing insects' bodies and shaken vigorously to wash out the pollen grains from their body. Then, we counted the pollen grains by the method mentioned above (for the pollen count of a floret). To count the pollen grains content in stack pollen loads, we added 1.0 mL sucrose solution to the stacked pollen loads (on hind legs or abdomen). After shaking the vial containing pollen loads, 1 µL of each sample was charged into the counting chamber of a hemocytometer. Then, we counted the number of pollen grains (present within the large square for WBC) with the help of a light microscope at 10 × 15

magnification. The total number of pollen grains per sample was estimated from the mean value (average number of pollen grains counted in WBC chamber) multiplied by 10000 (because the stock volume of a pollen sample was 1 mL and the volume of a large square for WBC is 0.1 μL). Here we considered only the weed pollen grains and ignored other pollen types, if present.

To guess the importance of the floral visitors as pollinators of the plant species, we calculated an 'approximate pollination value (APV)' for each flower-visiting species (according to Layek et al. 2022) as follows:

$$APV = RA \times VR \times PCV$$

Here, RA is the relative abundance of a flower-visiting species, VR is the visitation rate, and PCV is the pollen carrying value of the insect species. The 'pollen carrying value (PCV)' of floral visitors is derived from the summation of two components: (i) PCV 1 (i.e., body surface pollen content excluding stack pollen loads on corbiculae or scopae or abdomen; ranging from 0 to 5) and (ii) PCV 2 (i.e., stack pollen content on corbiculae or scopae or abdomen; ranging from 0 to 3) (Table 1).

Data analysis

The richness of the visitor community was calculated using an abundance-based coverage estimator. The richness of each insect order was calculated using Margalef's Index, D [D = (S − 1)/ln N; S is the number of species and N is the total number of individuals in the sample). The diversity of floral visitors was estimated using the Maximum Likehood Estimator (MLE) of the Shannon Diversity Index. Descriptive analyses of the data were carried out to obtain mean and standard deviation. We performed 'Shapiro-Wilk' and 'Kolmogorov-Smirnov' tests to check whether the data are normally distributed or not. In the case of normally distributed data (e.g., flowering twigs visitation rate of different insect species) we followed a parametric test 'One-way ANOVA' and $P \leq 0.05$ was considered statistically significant. Above statistical analyses were performed using SPSS (16.0) statistical packages and Microsoft Excel.

RESULTS AND DISCUSSION

Floral biology

The flowering of *Chromolaena odorata* occurs in October-January, with masses of flowers produced in its peak time (November-December). The number of flowering twigs per plant varied (ranges: 4-45; average: 24.72 ± 9.34) and mainly depended on the plant size and the number of branches. The flowering twigs consist of corymbs of cylindrical capitula and arise at the terminal part of the branches. The number of capitula per flowering twig was 16.18 ± 3.51. A capitulum consists of 18-30 tubular florets, with an average number of 24.20 ± 2.34. The maturation of florets within a capitulum takes place in a centripetal manner, i.e., peripheral florets matured earlier (Figure 1). The life span of florets is 2-3 days. Senescence began with the dried-out of style and became dark brown, followed by other floral parts (Figure 2). The florets open throughout the daytime, peaking at 7.00-10.00 h. They are very small, white to light purple, zygomorphic, and bisexual. The calyx is reduced to pappus. The tubular corolla is with five teeth at the tip. The stamens are five, epipetalous, arise from the base of the corolla. Filaments are free, and anthers are united, representing syngenesium condition. Anthers are dithecous, and the connective is prolonged into a hood. Their bases produce hairy outgrowths that act as a protective envelope for the nectaries. The narrow anthers form a hollow space, where pollen is liberated. The number of pollen grains per floret was 1016.67 ± 196.42. The pollen grains are very small, spheroidal (20 in diameter), trizonocolporate, with echinate exine ornamentation (Figure 3). The ovary is bicarpellary, syncarpous, and unilocular with a single basal ovule. The style is forked into two parts. The receptive surfaces of the stylar branches stay closed as they grow through the hollow space. Then, the style stretches out beyond the anthers spreads out its branches to receive pollen.

From GC-MS analysis of floral volatile organic compounds (VOCs), we identified 20 compounds (Table 2, Figure 4). Among them, retention time was lower in alpha-pinene, cyclohexanemethyl propanoate, alpha-ocimene, 1-pentanol, geijerene, cis-hept-2-enal; and higher in o-decyl hydroxylamine, benzyl stearate, n-hexadecanoic acid, 1-hexa-2-nitocyclohexane. Relative abundance was higher in benzyl stearate, 2,4-decadienal, 1-hexa-2-nitocyclohexane and n-hexadecanoic acid.

Table 1. Pollen carrying value (PCV = PCV 1 + PCV 2) of floral visitors

Body surface pollen content (excluding stack pollen loads)	**Value of PCV 1**
0	0
<100	0.5
100-200	1
>200-500	1.5
>500-1000	2
>1000-2000	2.5
>2000-5000	3
>5000-10000	3.5
>10000-20000	4
>20000-50000	4.5
>50000	5
Stacked pollen loads on corbiculae, scopae, or abdomen	**Value of PCV 2**
0	0
<1000	0.5
1000-5000	1
>5000-10000	1.5
>10000-20000	2
>20000-50000	2.5
>50000	3

Figure 1. A capitulum showing the maturation of florets in a centripetal manner. Scale bar = 5 mm

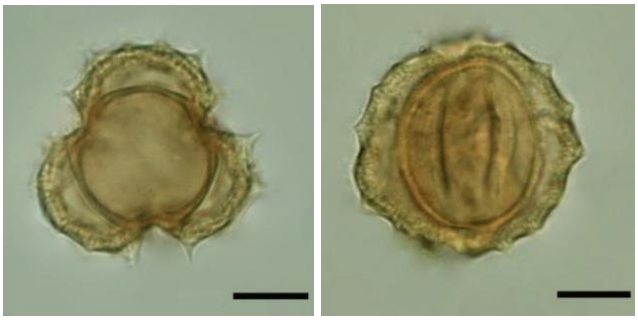
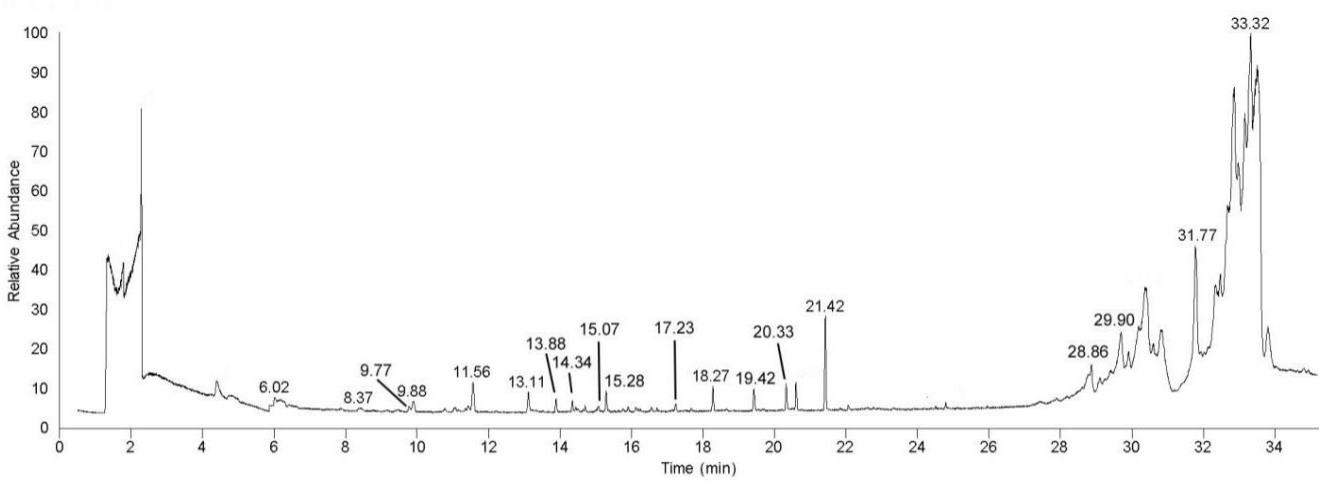


Figure 3. Pollen grains of *Chromolaena odorata*. A. Polar view; B. Equatorial view. Scale bars = 5 µm

Figure 2. Individual florets of different stages (A-E). Scale bar = 5 mm



6.02- alpha-pinene; **8.37-** cyclohexanemethyl propanoate; **9.77-** alpha-ocimene; **9.88-** 1-pentanol; **11.56-** cis-hept-2-enal; **13.11-** nonanal; **13.88-** 5-methyl-1-heptanol; **14.34-** 1-octen-3-ol; **15.07-** alpha-copaene; **15.28-** 4-bromo-1-cyclohexene; **17.23-** caryophyllene; **18.27-** trans-2-decenal; **19.42-** germacrene D; **20.33-** 2- undecenal; **21.42-** 2,4-decadienal; **28.86-** o-decyl hydroxylamine; **29.90-** benzyl stearate; **31.77-** n-hexadecanoic acid; **33.32-** 1-hexa-2-nitocyclohexane.

Figure 4. Floral volatile organic compounds

The percentage of viable pollen was the highest (69.14 ± 6.95) during the opening of florets, then gradually decreased (Table 3). The receptivity of stigma was higher during floret's opening time to 6 hours post-opening. Then gradually decreased and given positive result (showed receptivity) up to 3rd day morning (48 h after floret opening).

Floral visitors

A total of 49 insect species were identified as floral visitors of *Chromolaena odorata* (Table 4; Figure 5-7). Margalef's index of visitor community was 68.30 ± 0.76, and Shannon's diversity index was 3.72 ± 0.02. Insect order wise Margalef's index and Shannon diversity index was higher in Lepidoptera (Margalef's index: 5.17 ± 0.05; Shannon diversity index: 3.07 ± 0.04) and Hymenoptera (Margalef's index: 4.93 ± 0.05; Shannon diversity index: 2.98 ± 0.04). Within the visitor's spectrum, the order Lepidoptera is represented by 27 species, followed by Hymenoptera (13 species), Diptera (5 species), and Hemiptera (4 species). Dominant insect families were Nymphalidae and Pieridae of Lepidoptera and Apidae of Hymenoptera. Throughout the daytime, most abundant visitors were *Rapala monea* (RA = 7.18%), *Danaus chrysippus* (RA = 5.53%), *Mycalesis perseus* (RA = 4.84%), *Eurema hecabe* (RA = 4.80%), *Junonia atlites* (RA = 4.72%), *Euploea core* (RA = 4.68%), *Appias libythea* (RA = 4.60%), *Suastus gremius* (RA = 4.40%), and *Rapala varuna* (RA = 4.16%). According to hours of the day, the numbers of encountered floral visitors were higher during 10.00-14.00 h and lowered in early-morning (6.00-8.00 h) and late-afternoon (16.00-18.00 h). Regarding the insect orders, the numbers of Dipteran flies were higher in the morning (8.00-10.00 h) and then gradually decreased (Figure 8). Hemipteran members showed almost equal numbers throughout the day hours. The abundance of Hymenopteran members increased during 8.00-12.00 h and lowered in the early morning and late afternoon. The numbers of encountered butterflies were higher during 10.00-14.00 h. Though, hawk moths (*Macroglossum gyrans* and *Cephonodes hylas*) showed foraging activity during the evening. As floral resources, bugs, butterflies, moths, and wasps collected nectar from the florets. Honeybees, leafcutter bees, and solitary bees collected both nectar and pollen from the plant species.

The flower visitation rate (here, the number of flowering twigs visited per minute) was significantly varied from species to species ($F_{41,\ 958}$ = 31.64, P = 8.5E-149). In general, the visitation rate was higher in Hymenopteran members than in the Dipteran, Hemipteran, and Lepidopteran members (except for the hawk moths, they hovered at the florets, inserted the proboscis, and collected nectar in quick succession).

All the floral visitors carried pollen grains of *Chromolaena odorata* on their body surfaces. In this regard, we are assuming that all these visitors acted as pollinators for the plant species. However, pollen content varied from species to species. Members of Apidae and Megachilidae carried more pollen compared to other insect families. The combined parameter 'approximate pollination value (APV)' was higher in *Apis cerana*, *Apis dorsata*, *Danaus chrysippus*, *Euploea core*, *Halictus acrocephalus*, *Junonia atlites*, *Megachile disjuncta*, and *Megachile lanata*. In insect group-wise consideration, APV was much higher in butterflies than the flies, honeybees, leafcutter bees, solitary bees, and wasps. For this, we can say that the flowers of the weed species are mainly butterfly-pollinated. During forage, the butterflies stretch out their proboscises to reach the floret base to access nectar; while doing so, the proboscis invariably contacts the stigmatic surfaces of stylar branches and affects pollination.

Table 2. Volatile organic compounds (VOCs) emitted by florets of *Chromolaena odorata*

VOCs	Empirical formula	Molecular weight	Retention time (min)
alpha-pinene	$C_{10}H_{16}$	136	6.02
cyclohexanemethyl propanoate	$C_{10}H_{18}O_2$	170	8.37
alpha-ocimene	$C_{10}H_{16}$	136	9.77
1-pentanol	$C_5H_{12}O$	88	9.88
geijerene	$C_{12}H_{18}$	162	11.44
cis-hept-2-enal	$C_7H_{12}O$	112	11.56
nonanal	$C_9H_{18}O$	142	13.11
5-methyl-1-heptanol	$C_8H_{18}O$	130	13.88
1-octen-3-ol	C_8H1_6O	128	14.34
alpha-copaene	$C_{15}H_{24}$	204	15.07
4-bromo-1-cyclohexene	C_6H_9Br	160	15.28
caryophyllene	$C_{15}H_{24}$	204	17.23
trans-2-decenal	$C_{10}H_{18}O$	154	18.27
germacrene D	$C_{15}H_{24}$	204	19.42
2- undecenal	$C_{11}H_{20}O$	168	20.33
2,4-decadienal	$C_{10}H_{16}O$	152	21.42
o-decyl hydroxylamine	$C_{10}H_{23}NO$	173	28.86
benzyl stearate	$C_{25}H_{42}O_2$	374	29.90
n-hexadecanoic acid	$C_{16}H_{32}O_2$	256	31.77
1-hexa-2-nitocyclohexane	$C_{12}H_{23}NO_2$	213	33.32

Table 3. Pollen viability and stigma receptivity of *Chromolaena odorata*

Parameter	Time hours after opening of floret					
	0 h	6 h	24 h	30 h	48 h	54 h
Pollen viability (%)	69.14 ± 6.95	65.38 ± 7.19	55.69 ± 7.14	48.64 ± 6.00	36.62 ± 5.20	29.82 ± 5.00
Stigma receptivity (%)	70 ± 10	80 ± 10	53.33 ± 5.77	40 ± 10	16.67 ± 5.77	0

Table 4. Floral visitors of *Chromolaena odorata* in Bankura and Birbhum districts, West Bengal, India

Order/Family	Insect species	Relative abundance	Floral resources	Visitation rate	Pollen carrying value	APV
Diptera						
Calliphoridae	*Lucilia* sp.	0.36	P	-	0.5 + 0	-
Ephydridae	*Psilopa nitidula*	0.20	P	-	0.5 + 0	-
Rhiniidae	*Stomorhina discolor*	0.32	P	-	0.5 + 0	-
Syrphidae	*Eristalinus megacephalus*	0.32	P	0.31	0.5 + 0	0.05
	Syritta pipiens	1.25	P	0.42	0.5 + 0	0.26
Hemiptera						
Alydidae	*Leptocoriza acuta*	0.52	N	-	0.5 + 0	-
Lygaeidae	*Graptostethus servus*	0.40	N	-	0.5 + 0	-
Pentatomidae	*Agonoscelis nubilis*	0.69	N	-	0.5 + 0	-
	Chinavia hilaris	0.44	N	-	0.5 + 0	-
Hymenoptera						
Apidae	*Apis cerana*	0.77	N+P	2.07	2 + 2	6.38
	Apis dorsata	0.56	N+P	2.23	2 + 2.5	5.62
	Apis florea	0.36	N+P	1.80	1.5 + 2	1.62
	Ceratina binghami	0.93	N+P	1.60	1.5 + 1	3.72
	Thyreus nitidulus	0.52	N+P	2.30	2 + 0	2.39
Chalcididae	*Brachymeria* sp.	1.49	N	0.90	1 + 0	1.34
Chrysididae	*Chrysis angolensis*	0.28	N	2.40	-	-
Halictidae	*Halictus acrocephalus*	1.78	N+P	1.53	1.5 + 1	6.81
Megachilidae	*Megachile disjuncta*	0.85	N+P	2.43	2.5 + 0	5.16
	Megachile lanata	1.41	N+P	2.33	2.5 + 0	8.21
Scoliidae	*Scolia* sp.	0.24	N	2.10	-	-
Vespidae	*Ancistrocerus catskill*	1.49	N	1.43	1 + 0	2.13
	Phimenes flavopictus	1.69	N	1.60	1 + 0	2.70
Lepidoptera						
Hesperiidae	*Suastus gremius*	4.40	N	0.26	1 + 0	1.16
	Telicota colon	3.39	N	0.28	0.5 + 0	0.47
Lycaenidae	*Anthene lycaenina*	1.90	N	0.31	0.5 + 0	0.29
	Catochrysops strato	3.75	N	0.28	1 + 0	1.05
	Rapala manea	7.18	N	0.13	1.5 + 0	1.40
	Rapala varuna	4.16	N	0.15	1.5 + 0	0.94
Nymphalidae	*Danaus chrysippus*	5.53	N	1.30	1 + 0	7.20
	Danaus genutia	1.78	N	1.37	1 + 0	2.44
	Elymnias hypermnestra	1.09	N	0.11	1 + 0	0.12
	Euploea core	4.68	N	1.40	1.5 + 0	9.83
	Junonia almana	1.86	N	0.22	1.5 + 0	0.61
	Junonia atlites	4.72	N	1.43	1.5 + 0	10.12
	Junonia iphita	1.82	N	1.30	1.5 + 0	3.55
	Mycalesis perseus	4.84	N	0.16	1 + 0	0.77
	Neptis hylas	3.51	N	1.37	1 + 0	4.81
	Phalanta phalantha	1.29	N	0.21	1 + 0	0.27
	Tirumala limniace	1.65	N	1.37	1.5 + 0	3.39
Papilionidae	*Pachliopta hector*	2.95	N	2.23	0.5 + 0	3.29
	Papilio polytes	1.90	N	2.03	0.5 + 0	1.93
Pieridae	*Appias libythea*	4.60	N	1.27	1 + 0	5.84
	Catopsilia pomona	3.39	N	1.23	1 + 0	4.17
	Eurema blanda	2.91	N	1.13	0.5 + 0	1.64
	Eurema hecabe	4.80	N	1.07	0.5 + 0	2.57
	Leptosia nina	1.45	N	1.20	0.5 + 0	0.87
	Pareronia hippia	3.29	N	1.23	1 + 0	4.05
Sphingidae	*Cephonodes hylas*	0.12	N	3.07	-	-
	Macroglossum gyrans	0.16	N	3.43	-	-

Note: APV: approximate pollination value; N: nectar; P: pollen

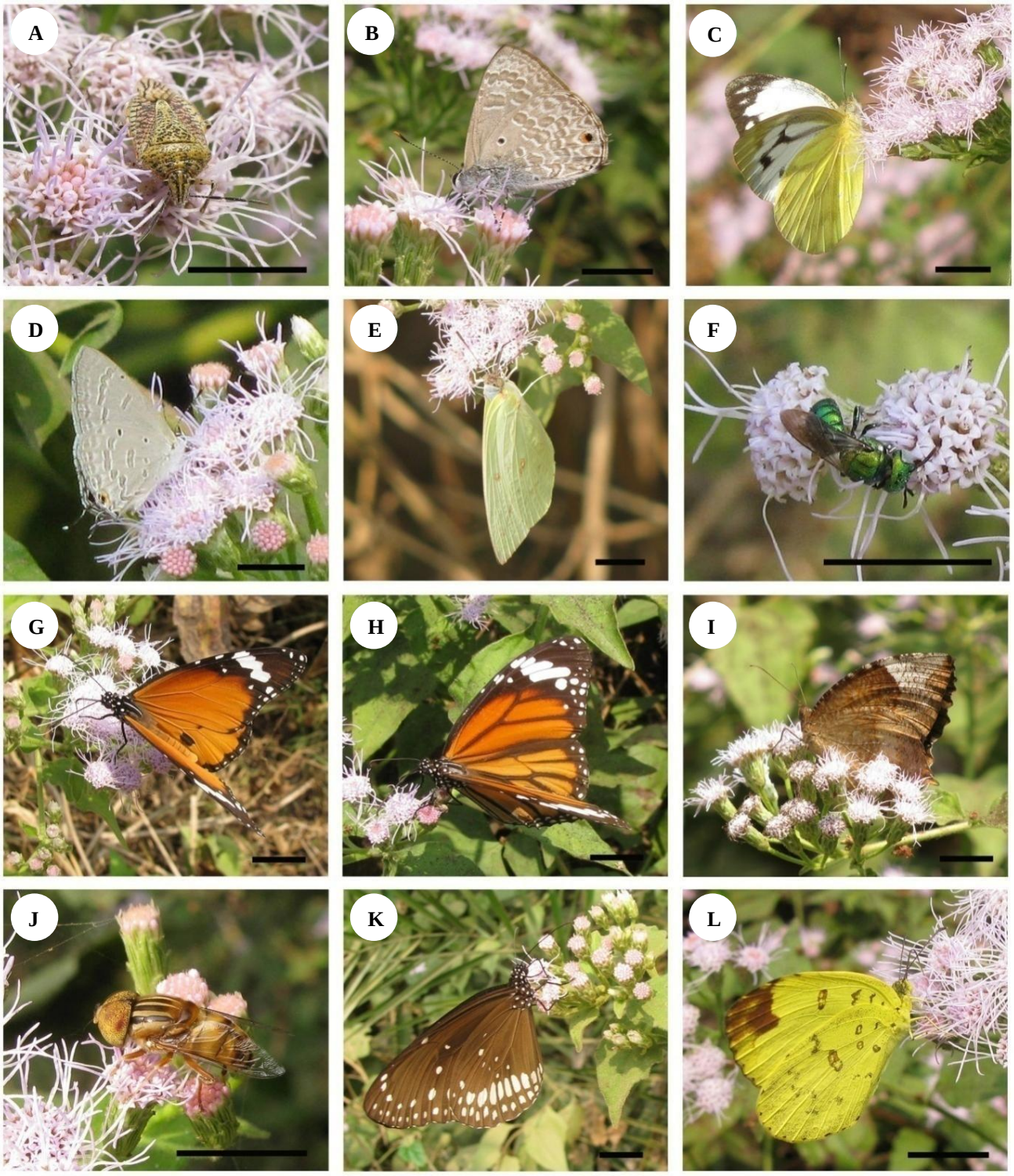


Figure 5. Floral visitors of *Chromolaena odorata.* A. *Agonoscelis nubilis,* B. *Anthene lycaenina,* C. *Appias libythea,* D. *Catochrysops strato,* E. *Catopsilia pomona,* F. *Ceratina binghami,* G. *Danaus chrysippus,* H. *Danaus genutia,* I. *Elymnias hypermnestra,* J. *Eristalinus megacephalus,* K. *Euploea core,* L. *Eurema blanda.* Scale bar = 10 mm

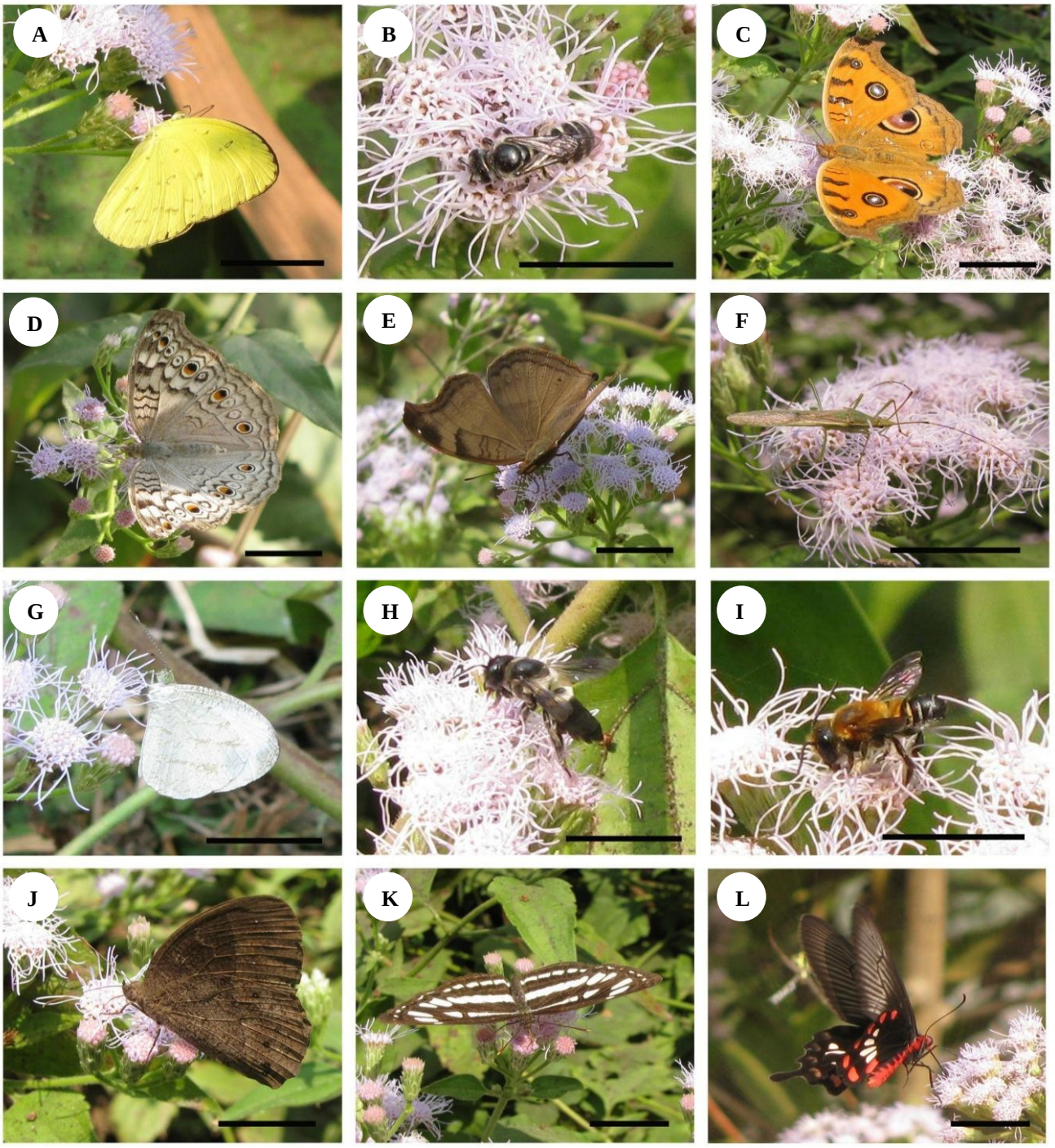


Figure 6. Floral visitors of *Chromolaena odorata*. A. *Eurema hecabe*, B. *Halictus acrocephalus*, C. *Junonia almana*, D. *Junonia atlites*, E. *Junonia iphita*, F. *Leptocoriza acuta*, G. *Leptosia nina*, H. *Megachile disjuncta*, I. *Megachile lanata*, J. *Mycalesis perseus*, K. *Neptis hylas*, L. *Pachliopta hector*. Scale bar = 10 mm

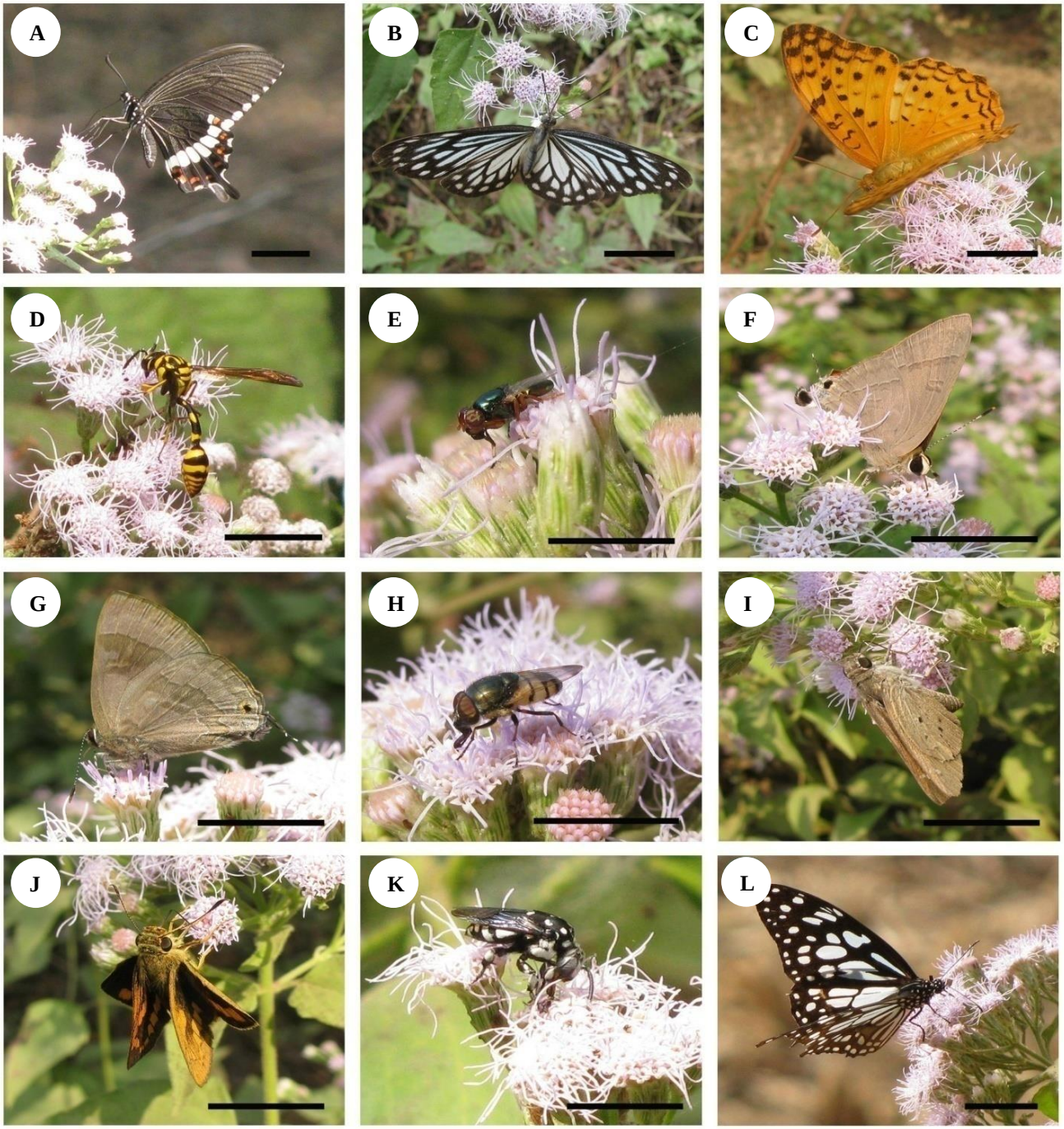


Figure 7. Floral visitors of *Chromolaena odorata.* A. *Papilio polytes*, B. *Pareronia hippia*, C. *Phalanta phalantha*, D. *Phimenes flavopictus*, E. *Psilopa nitidula*, F. *Rapala manea*, G. *Rapala varuna*, H. *Stomorhina discolor*, I. *Suastus gremius*, J. *Telicota colon*, K. *Thyreus nitidulus*, L. *Tirumala limniace*. Scale bar = 10 mm.

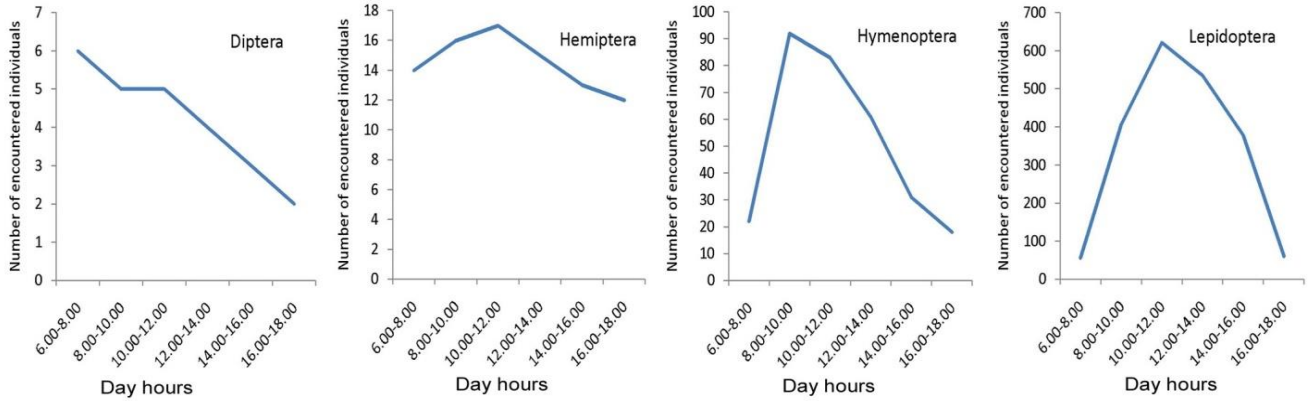


Figure 8. Daytime hours wise the number of encountered individuals of different insect orders

Discussion

The capitula of the weed (*Chromolaena odorata*) are flat-topped, which provides a good landing platform for the visitors. Florets are white to light purple, with narrow tubular corolla and deep-seated nectar. These attributes remain attractive to diverse insect groups like flies, honeybees, leafcutter bees, moths, solitary bees, and wasps. The butterfly is the most dominant (based on abundance and species composition) group. The dominance of butterflies on the plant species was also reported from outside West Bengal (Lakshmi and Raju 2011; Shihan and Kabir 2015). In general, the nectar of the members of the Asteraceae family has high hexose sugars and amino acid content (Galetto and Bunardello 2003; Venjakob et al. 2022; Wist and Davis 2008) and remains attractive to butterflies. Though many insect species remain common to the visitor's spectra of the weed recognized from different geographical regions (Lakshmi and Raju 2011; Shihan and Kabir 2015), the current visitor's spectrum was slightly different from the other spectra. Insect communities associated with a cosmopolitan weed depend the availability of a particular insect species within the habitat and on the availability of other blooming species (Ghazoul 2004; Potts et al. 2003; Wilson and Jamieson 2019). In this study, a few insect species (viz. *Danaus chrysippus*, *Mycalesis perseus*, *Rapala varuna*, *Suastus gremius*, *Tirumala limniace*) are newly reported as floral visitors of the plant species. Mildly scented florets emitted a large number of volatile organic compounds. A few of them (e.g., alpha-copaene, alpha-ocimene, caryophyllene, germacrene D) were also reported as volatile floral compounds from other plant species like *Anacardium occidentale* (Layek et al. 2021a), *Foeniculum vulgare* (Layek et al. 2021b). These volatile compounds may play an important role in the attraction of floral visitors. As the florets borne in cylindrical capitulum and numerous capitula are clustered, these features remain energetically profitable to the visitors because such arrangement reduces search and flight time. The florets secrete a trace amount of nectar (Lakshmi and Raju 2011). The floral visitors in quest of nectar visit as many florets and capitula as possible in a single foraging bout. Furthermore, patchy distribution of the plant with numerous flowering twigs may enhance the visitation rate (Akter et al. 2017), facilitates frequent movement of floral visitors among different individuals and promotes cross-pollination. Due to the close association of capitula and having many compact florets in each capitulum, direct measurement of pollination efficiency [e.g., single-visit pollination efficiency index (Spears 1983)] of the floral visitors is much more difficult. We estimated the 'approximate pollination value (APV)' for the insect visitors as an alternative. The combined parameter is associated with relative abundance, visitation rate, and pollen carrying value of the floral visitors. We think each of these parameters has been linked with the pollination efficiency of a floral visitor. Therefore, the estimated APV of the floral visitors might be a good indicator of their importance in the pollination services of the weed. In this sense, *Danaus chrysippus*, *Euploea core*, *Junonia atlites* remain the most effective pollinators of the weed. Considering insect groups, butterflies provided vital pollination services to the weed. Understanding pollinating insects can help plant sustainability and be beneficial for humankind (Mudiana and Ariyanti 2021).

As the flowers of *Chromolaena odorata* remain attractive to diverse insect groups, including butterflies, it may act as a provisioning post. Its abundant growth might also be depriving the pollination services to co-blooming native plants, which are mainly butterfly pollinated. In general, plant-animal interactions depend on the relative abundance of biotic species and environmental factors. Irrespective of the plant and animal species within the habitat, an invasive plant can reconstruct the plant-animal interactions by altering the resource availability to the visitors (Ghazoul 2004; Stout and Tiedeken 2017). The weed *Chromolaena odorata* may potentially change the topography of native interaction webs in its associated habitats. Thus, the current study is valuable in determining the impacts of the invasive alien plant species on the insect community residing in the habitat.

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