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Temporal variation in flower visiting insects of *Calotropis gigantea* in the Southern Province of Sri Lanka

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Abstract *Calotropis gigantea* (Giant milkweed) is a medicinal plant native to a few Asian countries including Sri Lanka. However, the plant is considered invasive in countries such as Australia, the USA and Brazil. It produces flowers throughout the year leading to massive fruit production, and pollination may directly influence the reproductive output of the plant. Knowledge of the insect flower visitors of *C. gigantea* is essential to cultivate them for experimental purposes, to conserve them in their native range, or to eradicate them in their introduced range. Though the plant is commonly distributed in Sri Lanka, information on its flower visitors is rare. Therefore, the objective of this study was to identify the flower-visiting insects of *C. gigantea* and their temporal abundance. Monthly field visits were made to eleven sites in Southern Province from August 2015 to August 2016. During sampling, flower visitor species, their abundance, and the number of *Calotropis* fruits in the selected plants were recorded. *Danaus chrysippus*, *Xylocopa caerulea*, *Xylocopa fenestrate* and *Apis cerana* were identified as flower visitors of *C. gigantea*. The most common was *Xylocopa* spp. and it was recorded in all sites, and during all studied months while *Danaus chrysippus* was uncommon. The abundance of *Xylocopa* spp. had a positive correlation with the abundance of *C. gigantea* fruits ($r = 0.094$, $p<0.001$) suggesting its likely role in the pollination of the latter. Monthly rainfall had a strong correlation ($r = 0.806$, $p = 0.002$) with the flower-visiting insect abundance *of C. gigantea* in the Matara district. Mean flower-visitor abundance was comparatively high in sites having the least anthropological impact.

Keywords: *Calotropis gigantea*, flower visitors, Sri Lanka

1 Introduction

Calotropis spp. are native to tropical and subtropical regions of Africa and Asia (Vitelli *et al.* 2008) and belong to the family Apocynaceae. The plant is rich with different phytochemical properties including purgative, anthelmintic (Singh *et al.* 2013), bactericidal, vermicidal, analgesic, anticonvulsant, anxiolytic, sedative, wound healing, anti-diabetic (Singh *et al.* 2013), antipyretic, larvicidal, anti-arthritic, anti-hyperlipidemic, anti-inflammatory, anticancer (Kumar and Kumar 2015) diaphoretic, carminative and expectorant (Kumar and Kumar 2015). Different plant parts of *C. gigantea* are used to cure a wide range of diseases including bronchial asthma, cholera, convulsions, pneumonia, ringworm infection, smallpox infection, toothache, epilepsy, fever, leprosy, skin diseases, and epilepsy (Kumar and Kumar 2015).

Despite its medicinal value, *Calotropis* is an emerging problem in countries such as Australia, the USA, Mexico, the Caribbean islands, and Brazil as it is invasive (Dhileepan 2014). *Calotropis* spp. produce flowers throughout the year (El- Tantawy 2000) leading to enormous fruit production throughout the year (Hassan *et al.* 2015). In most cases, a well-grown *Calotropis* tree monthly produces numerous fruits, and a single fruit contains (433.2 ±19) many seeds (Menge *et al*. 2016). The seeds disperse through the wind, water as well as animals (Kaur *et al.* 2021). Numerous seed production and successful dispersal of seeds are key features of *Calotropis* invasiveness (Menge *et al.* 2016). As chemical and mechanical methods are not fruitful in controlling *Calotropis* spp., scientists pay more attention to biological control methods (Dhileepan 2014). To control the invasiveness of *Calotropi*s, it is important to study phytophagous insects which feed on the plant. Also, it is important to study flower visitors of *Calotropis* as some of them directly influence pollination.

Therefore, scientists need to pay attention to studying common insect flower visitors of *C. gigantea* that are responsible for the massive seed production of the plant throughout the year (Wijeweera *et al*. 2022). *Calotropis* flower is an attractive source of nectar for flower visitors and few of them are co-evolved with the *Calotropis* flower (Salau and Nasiru 2015). Most of the reported flower visitors of *Calotropis* are limited to *C. procera* plant, and belonged to the order Hymenoptera including carpenter bees, honeybees, ants, and wasps. In addition, there are few species belonging to the order Diptera and Lepidoptera (Zafar *et al*. 2018). Except for a few known records related to *C. gigantea* (Karunaratne *et al*. 2005, Jayasinghe *et al*. 2013, Perera and Wickramasinghe 2014, Wijeweera *et al*. 2022), no detailed study on its associated fauna is reported from Sri Lanka. The knowledge of flower visitors of *Calotropis* spp. is highly important for seed production, growing them for experimental purposes, conserving the plants in their native range, and controlling their invasiveness in the introduced range. Therefore, the present study is aimed to examine the abundance and temporal variation of insect flower visitors associated with *C. gigantea* plants in selected sites of the Southern Province in Sri Lanka.

2 Methods

2.1 Study sites

To study the flower visitors of *C. gigantea*, eleven sites from the Southern province (three from the Matara district, four from Hambantota, and four from the Galle districts) were selected (Table 1). The sites were selected randomly and they were located more than 3 km away from each other. From each study site, six mature *C. gigantea* plants of approximately similar height were selected (above 1 m). The stems of the selected plants were numbered with white paint. Sampling was done within 3 hours at each site between 6.00 am and 6.00 pm, while allocating 20-30 minutes per selected plant (Wijeweera *et al.* 2021). Each site was sampled monthly from August 2015 to July 2016. During sampling, flower visitor species, their abundance, and the number of *Calotropis* fruits in each selected *Calotropis* plant were recorded. The flower visitor abundance was obtained by counting all the individuals of flower visitor species per plant during the observation time.

In addition, the habitat characteristics of the sites were recorded (Table 1). The land within hundred meters of the seashore was considered a coastal region while the rest of the land was an inland region (Table 1). Sites located in towns were categorized as "urban sites" while sites located in villages were "rural sites". Similarly, sites having obvious anthropogenic influence (active cemeteries, garbage dumping sites, road edges, and railway edges) were categorized as "disturbed sites" while those having no obvious anthropogenic effects were included in the category of "undisturbed site" (Table 1).

Table 1. Location and physical nature of selected sites in the southern province, Sri Lanka.

Site	Location	District	Coastal/ Inland	Rural/ Urban	Disturbed/ Undisturbed	Special remarks on site
Kamburugamuwa	5^0 56' N; 80^0 29'E	Matara	Coastal	Rural	Undisturbed	Abandon land
Devinuwara	5^0 55' N; 80^0 34'E	Matara	Coastal	Urban	Disturbed	Bridge construction site
Palena	5^0 56' N;80^0 29' E	Matara	Inland	Rural	Disturbed	Railway edges
Dadalla	6^0 2' N; 80^0 11' E	Galle	Coastal	Urban	Undisturbed	Abandon old crematory
Thalpe	5^0 59'N; 80^0 16' E	Galle	Coastal	Urban	Disturbed	Active cemetery
Habaraduwa	5^0 59' N 80^0 18' E	Galle	Inland	Rural	Undisturbed	Abandon land
Kathaluwa	$5^0$58'N; 80^0 20'E	Galle	Coastal	Urban	Disturbed	Beach armoring
Kalametiya	6^0 6' N; 80^0 55' E	Hambantota	Inland	Rural	Undisturbed	Bird Sanctuary
Medilla	6^0 2'N; 80^0 48' E	Hambantota	Coastal	Urban	Disturbed	Garbage dumping site
Tangalle	6^0 1'N; 80^0 47'E	Hambantota	Coastal	Urban	Disturbed	Road edges
Nalagama	6^0 4'N; 80^0 47'E	Hambantota	Inland	Rural	Disturbed	Crop field

2.2 Preservation and identification of flower visitors

Samples of live insects vising *C. gigantea* flowers were collected into glass specimen bottles. Insects were directly captured during their feeding on *Calotropis* flowers by using an insect net and a butterfly net. The specimens were transported to the Department of Zoology, University of Ruhuna, preserved, pinned and kept in a collection. Specimens were identified by the taxonomists of the Entomology Division, The Horticultural Crop Research and Development Institute (HORDI), Gannoruwa, Sri Lanka.

2.3 Statistical analysis

Statistical analysis was done using one-way ANOVA and two-way ANOVA comparing flower visitor abundance between sites and months. As mean abundance was normally distributed, the parametric test of ANOVA was applied. Correlation analysis was used to test any associations between mean pollinator abundance with respect to rainfall. For that, monthly rainfall data related to three districts of Southern Province (during the sampling period) was obtained from Sri Lankan Meteorological Department (Source data: Appendix). The mean value of the monthly rainfall in each district was considered the independent variable. Also, monthly mean pollinator abundance in all sites of each district was taken as the dependent variable. Similarly, correlations were tested with respect to the monthly mean pollinator abundance and monthly fruit production in all sites during the study period. Here, the mean pollinator abundance was the dependent variable while mean fruit production was the independent variable. Statistical analysis was done using Minitab 17 statistical software.

3 Results and discussion

3.1 Flower visiting insects of *Calotropis gigantea* in Southern Province

Four species of insect flower visitors belonging to three families were recorded (Table 2) of which, *Xylocopa caerulea* and *Xylocopa fenestrate* were the most common insects recorded at every site throughout the year. Even a few decades ago, they were the most common flower visitors of *Calotropis* in Sri Lanka (Wanntorp 1974). Two different species have been recorded from India, i.e., *X. pubescens* and *X. sulcatipes*. They are leading flower visitors of the plant in the Indian subcontinent. *Xylocopa* spp. act as major flower visitors of *Calotropis* in their native range, Asia and Africa (Zafar *et al*. 2018). However, out of their native range, these species appear to be minor flower visitors of *Calotropis* (Menge 2017). *Xylocopa* spp. forage between dawn and dusk. However, they occasionally visit flowers at night under the moonlight (Somanathan and Borges 2001).

Table 2. Flower visiting insects associated with *C. gigantea* in Southern Province, Sri Lanka.

Order	Family	Common name	Scientific name
Hymenoptera	Anthophoridae	Carpenter bee	*Xylocopa caerulea* Fabricius
			Xylocopa fenestrate Fabricius
Lepidoptera	Papilionidae	The plain tiger	*Danaus chrysippus* Linnaeus
Hymenoptera	Apidae	Honeybee	*Apis cerana* Fabricius

In the present study, only one butterfly species, *Danaus chrysippus* visited *Calotropis* to gain nectar as well as to lay eggs. The same species is recorded in *C. procera* in India and Saudi Arabia (Ismail 2014, Zafar *et al.* 2018). Even though several bee species visit *Calotropis* flowers in several regions of the world, the present study recorded only *Apis cerana*. Also, it is not recorded on *Calotropis* in India, instead, a few other species (*A. dorsata*, *A. florae* and *A. mellifera*) of the same genus were recorded (Sudan 2013, Zafar *et al.* 2018). Furthermore, Zafar *et al*. (2018) recorded three additional bee species, i.e. *Trigona iridipennis*, *Anthophora cingulata*, and *Anthophora bicincta* from India. The Indian bee species only were recorded from *C. procera* except for the bee *Trigona sp*. (Zafar *et al*. 2018). These studies have revealed that *Xylocopa* spp. act as pollinators of *Calotropis* while gaining nectar as well as pollen. *Danaus chrysippus* and the above-mentioned bee species only visit to gain nectar and do not pollinate the flowers (Zafar *et al*. 2018).

3.2 Temporal variation of flower visitors in Southern Province

Mean flower visitor abundance/ species-wise flower visitor abundance (per district) with respect to studied months are illustrated in Figure 1. In all three districts of the Southern province, a combination of flower visitors (*D. chrysippus*, *X. caerulea*, *X. fenestrate* and *A. cerana*) was recorded throughout the year. It was statistically also proved that total flower visitor abundance was not significantly different with respect to the month of the year (Table 3). However, there is a significant difference in mean flower visitor abundance among study sites (Table 3).

Danaus chrysippus was recorded in all three districts of Southern Province but only during 3 months of the year (May, July, and November). Also, *D. chrysippus* was recorded from the Matara district throughout the year except in September and February. Apparently, a higher mean abundance (1-0.67) of *D. chrysippus* was recorded in all districts during May (Figure 1). *A. cerana* was recorded in all districts with low (mean) abundance (1.25-0) throughout the year except April where *A. cerana* was more abundant (8. 33) in the Matara district. During December, no *A. cerana* was recorded from all districts (Figure 1). *Xylocopa* spp. were recorded in Galle and Matara districts throughout the year (Figure 1). In the Hambantota district, it was found in eleven months except in November. Comparatively, a higher mean abundance (5.33-0.75) of *Xylocopa* spp. were observed from February to July (Figure 1). There was a significant difference in the mean abundance of *Xylocopa* spp. with respect to sampling sites (Table 3).

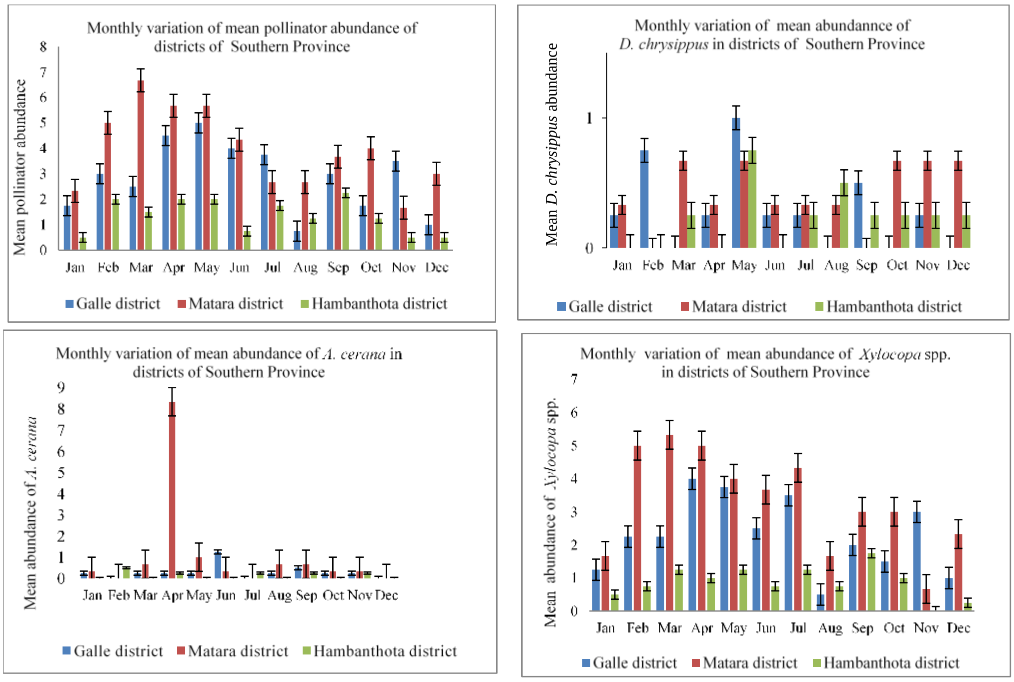

Fig 1. Flower visiting insect abundance of *C. gigantea* in Southern Province, Sri Lanka from August 2015 to July 2016.
(Note: the graph depicts the months Jan-July in 2016 first, followed by Aug-Dec in 2015)

Table 3. ANOVA analysis on mean abundance of flower visiting insects with respect to study sites and months.

Variable combination		One-way ANOVA		Two-way ANOVA	
		P value	F value	P value	F value
Mean flower visitor abundance (total)	Vs. Month	0.351	1.12	0.162	1.47
	Vs. Site	< 0.001	4.53	< 0.001	4.71
Mean *D. chrysippus* abundance	Vs. Month	0.241	1.30	0.213	1.35
	Vs. Site	0.160	1.47	0.145	1.51
Mean *A. cerana* abundance	Vs. Month	0.573	0.86	0.526	0.91
	Vs. Site	0.081	1.73	1.72	0.085

Comparatively, a higher mean abundance of pollinators was recorded in Kamburugamuwa site which was an undisturbed and rural site (Table 4). *Xylocopa* spp. were high in undisturbed sites of Kamburugamuwa and Dadalla. In contrast, they were low in Nalagama and Tangalle sites with respect to other sites. Nalagama site was very close to paddy fields which were continuously exposed to agrochemicals. Tangalle site was located near the main road where *Calotropis* plants were covered with dust, vehicle smoke, and tar-dust particles. According to Calderone (2012), pesticides, herbicides (Watanabe 2014), habitat alteration, and pollution are considered major risk factors for flower visitors. These factors might be the reason for the low flower visitor abundance in the above sites.

Table 4: Descriptive statistics (mean/SD) of mean flower visiting insect abundance with respect to site characters.

Site	Site characteristics	*D. chrysippus*		*A. cerana*		*Xylocopa* spp.	
		Mean	SD	Mean	SD	Mean	SD
Kamburugamuwa	Coastal, Rural, Undisturbed	0.583	0.193	0.667	0.414	5.00	1.05
Devinuwara	Coastal, Urban, Disturbed	0.417	0.149	0.250	0.179	1.333	0.310
Palena	Inland, Rural, Disturbed	0.250	0.131	2.25	1.46	3.583	0.830
Dadalla	Coastal, Urban, Undisturbed	0.417	0.149	0.417	0.149	4.250	0.719
Thalpe	Coastal, Urban, Disturbed	0.500	0.230	0.610	0.337	2.500	0.379
Habaraduwa	Inland, Rural, Undisturbed	0.000	0.000	0.083	0.025	1.417	0.358
Kathaluwa	Coastal, Urban, Disturbed	0.250	0.131	0.167	0.112	1.000	0.408
Kalametiya	Inland, Rural, Undisturbed	0.250	0.131	0.083	0.025	1.250	0.392
Medilla	Coastal, Urban, Disturbed	0.167	0.112	0.083	0.025	1.583	0.417
Tangalle	Coastal, Urban, Disturbed	0.417	0.193	0.167	0.112	0.833	0.297
Nalagama	Inland, Rural, Disturbed	0.083	0.025	0.167	0.112	0.083	0.025

Furthermore, correlations were obtained for *C. gigantea* fruit abundance with respect to flower visitor abundance. As *Calotropis* flowers are loosely attached by the petiole to the flower bunch, light press, mechanical damage, or touch can cause the detachment of flowers from the tree. As we need to avoid human influence on flower detachment, flower count was not taken. Instead of that, the fruits were counted as it was an outcome of pollinated flowers.

Table 5. Correlation between the mean abundance of flower visitor species and *Calotropis gigantea* fruit production (mean abundance of fruits) of Southern province, Sri Lanka.

Mean abundance of flower visitor species	Mean abundance of *Calotropis* fruits	
	Correlation coefficient	P value
D. chrysippus	0.007	0.968
A. cerana	0.249	0.143
Xylocopa spp.	**0.094**	**<0.001**

For the analysis, mean pollinator abundance/fruit abundance in all sites throughout the study period (12 months) was considered. Statistically, *C. gigantea* fruit abundance is correlated with the abundance of *Xylocopa* spp. ($r = 0.094$, $p<0.001$) indicating that they play a major role in the pollination of *Calotropis* flowers (Table 5). The patchy distribution might cause a low r-value. The body structure of *Xylocopa* spp. are specially co-evolved with floral morphology (Zafar *et al.* 2018) leading to successful pollination which might significantly influence on fruit production.

In addition, there was no correlation between monthly rainfall and mean flower visitor abundance in the study sites of Galle and Hambantota indicating the amount of rainfall has no effect on the abundance. However, in the Matara district rainfall had an influence on mean flower visitor abundance (Table 6).

Table 6. Correlation between the mean abundance of flower visitors and monthly rainfall in three districts of Southern province, Sri Lanka.

Monthly rainfall (mm)	Mean abundance of flower visitors	
	Correlation coefficient	P value
In Galle district	0.041	0.900
In Matara district	**0.806**	**0.002**
In Hambantota district	- 0.237	0.458

Studies showed that flower visitors vary according to abiotic factors like temperature, wind velocity and intensity of solar radiation (Herrera 1990). Normally flower visitors such as honeybees visit in the mornings, from 7 am to 11 am (Bukero *et al.* 2015) while the Carpenter bees visit the flowers in the mornings (9-11 am) and in the afternoons (3-5 pm) (Ali and Ali 1989). Personal observations also recorded that flower visitor abundance was high on warm, sunny days than on rainy and windy days.

4 Conclusions

As *C. gigantea* produces flowers throughout the year, flower visitors are also observed throughout the year. *D. chrysippus*, *X. caerulea*, *X. fenestrate* and *A. cerana* were identified as flower visiting insects of *C. gigantea* while *Xylocopa* spp. are the most common. As the major pollinator, *Xylocopa* spp. has a correlation ($r = 0.094$, $p<0.001$) with *Calotropis* fruit production. More insects visited the flowers from February to May (warmer months) than in January (cooler months). The highest flower visitor abundance was recorded in Matara district, and the rainfall of the district significantly influence ($r = 0.806$, $p = 0.002$) on flower visitor abundance. The flower visitor abundance significantly varied with study sites, and anthropological activities on study sites highly influenced on the abundance of *Xylocopa* spp.

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