



## Evaluation Of Spatio-Temporal & Nectar Robbing Effect On Plant-Pollinator Interactions With Special Reference To Darjeeling District

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### Abstract

The consequences of nectar robbing, or the illegal collection of nectar, on plant sexual reproduction and the local population of pollinators might vary. When nectar thieves harm flowers in their quest for nectar, it may alter the behaviour of subsequent flower visits and, in turn, reduce the likelihood of successful plant reproduction. However, researchers have only looked on nectar manipulation by nectar thieves. Among the Lamiaceae family of plants, *Leucas aspera* Willd from the middle of June through the beginning of February, *L. aspera* bloomed. *L. aspera* was examined for this study in several locations around Darjeeling. We have selected 77 different insect species recorded as having visited the focus flowers of *L. aspera* in 35 different plant populations. Even while nectar stealing in *L. aspera* had no effect on plant reproduction, it did have an effect on the pollinator population. The importance of nectar robbers to plant ecology and evolution may have been underestimated in the past and should be further considered in future studies.

**Keywords:** Nectar, Flower, Visitors, Pollinator, *Leucas aspera*

### I. INTRODUCTION

Floral nectar and pollen are supplied as a reward for the biotic pollination agents. Rewards for pollination are closely correlated with certain functional groupings of pollinations, although they vary greatly in terms of their content, volume, and accessibility. Flowers that rely on animal pollination typically provide nectar as a mutualistic incentive. Nectar mostly contains the monosaccharides glucose and fructose as well as the disaccharides sucrose. Insights on the most important classes of pollinators may be gleaned from data on the nectar's chemical makeup. Traditionally, it has been believed that the ratio of disaccharides to monosaccharides in nectars—that is, the sucrose to hexose ratio—is a distinguishing compositional feature of nectars that may be reliably correlated with the kind of pollinator.

The medicinal value of *Leucas aspera* has increased its popularity. Locally, the plant is known as thumba, and historically, it has served as the primary active element in a number of different remedies. It has several uses, including curing a cold, alleviating a headache, and reducing redness. The leaves of *L. aspera* are put on bites of serpents, deadly insects and scorpion sting and also used as pesticide and mosquito repellent in rural region.

Among the multiple interactions that take place between plants and animals, biotic pollination is considered a mutualistic interaction in which plants benefit from the fertilisation of their ovules by animal pollen vectors, while animals obtain some kind of reward. However, there are exploiters of mutualisms that obtain benefits without providing a reward in return. One example is nectar robbers, which obtain nectar from a perforation or a slit in the corolla or calyx, usually without transferring pollen to the stigma. The paradigm that nectar robbing is harmful for plants began with Darwin's description of bees making holes in corolla and their probable negative effects. Indeed, empirical evidence showed that nectar robbing might damage the reproductive structures of flowers during robbing, which directly affects female plant reproductive success. In addition, the perforation made on the flowers by primary nectar robbers facilitates nectar access for secondary robbers, or may induce pollinators to act as secondary nectar robbers if nectar extraction is easier in robbed than in unrobbed flowers.

### Nectar robbing

Nectar robbing is an outcome of the ability of some floral foragers to steal nectar without effecting pollination. The phenomenon is prevalent in many taxonomically unrelated species of flowering plants, and particularly those that hold concealed nectar in a tubular or spurred corolla. The robbers may either pierce through the corolla tube, or make a hole in the calyx cup to withdraw nectar. Interestingly, the robbers may sometimes change their role to pollinators in the same or different plant species.

Nectar robbing carries an obvious negative connotation with detrimental outcomes on the fitness of plants. However, there are several instances where robbing produces partial-negative or weak-positive effects. The net positive consequence becomes apparent when the fruit-set increases in response to robbing. Although the phenomenon of nectar robbing is of common occurrence, the evidences for the types of interaction-frameworks and the key attributes that yield a range of consequences, have only recently begun to emerge.

In a plant-pollinator-robber interaction milieu, the dynamics of nectar production and pollinator's behaviour have a crucial bearing on the net outcome. Nectar robbers can directly affect the plant reproductive success either by damaging the floral parts or by acting as pollinator in the same or different floral visit. Indirectly, the robbers may benefit sexual reproduction of plants by altering the behaviour of pollinators.

The net consequences of nectar robbing are largely realized in the context of plant mating system. Whereas nectar robbing usually produces a negative to weak-positive effect in self-compatible systems, in self-incompatible (SI) plants increased pollen flow between the conspecifics may yield greater positive effects provided that the pollinator is not limited. Additionally, the pollinator's shorter flower-handling time and fewer floral visits per plant in a bout may also reduce the chances of stigma clogging by self-pollen. Owing to several problems in designing the experiments, these aspects have been rarely investigated in tree species. There are also relatively limited studies that represent the effect of avian nectar robbers in ornithophilous self-incompatible plant species.

### **Leucas aspera**

*Leucas aspera* is an annual herb with a branching, 15–60 cm tall stem with branches that are robust and hispid and abruptly quadrangular in shape. Flowers are white and sessile, tiny, and arranged in dense whorls at the terminal or axillary nodes; leaves are sub-sessile or briefly petiolate, linear or linearly lanceolate, obtuse, pubescent, up to 8.0 cm long and 1.25 cm wide, with whole or crenate edge; petiole 2.5-6 mm long. Mouth small, very oblique, not villous, upper part produced forward; teeth small, triangular, bristle-tipped, ciliate, the upper tooth being the largest; calyx variable, tubular, 8-13 mm long; tube curved, contracted above the nutlets; lower half usually glabrous and membranous, upper half ribbed and hispid. Upper lip is 3 mm long and densely white-woolly; lower lip is almost twice as long, with the centre lobe large, rounded, and the lateral lobes tiny and subacute. Corolla length is 1 cm. Fruit nutlets that are 2.5 mm in length and brown in colour with a smooth, angular inner face and a more rounded outer face.

The genus *Leucas* (subgenus *Thumbai*) Linn. (family Lamiaceae) is widely dispersed in India, from the Himalayas to the southern tip of Ceylon. A traditional antipyretic and pesticide, the plant has several uses. Flowers have several medicinal uses, including as a tonic, insect repellent, diuretic, expectorant, and emmenagogue. It is believed that the leaves might help with persistent skin conditions including psoriasis and rheumatoid arthritis. While treating snake bites, bruised leaves are administered topically.

- Kingdom: Plantae, Plant
- Subkingdom: Tracheobionta, Vascular plant
- Super division: Spermatophyta, Seed plant
- Division: Angiosperma
- Class: Dicotyledonae
- Sub-class: Gamopetalae
- Series: Bicarpellatae
- Order: Tubiflorae
- Family: Lamiaceae
- Genus: *Leucas*
- Species: *aspera* (Willd.) Link

*L. aspera* has several uses in rural areas, including food and supplementation. Shirangi, 1947 may have been the first person to study the phytochemistry of the *Leucas* genus through their work with *L. aspera*. In plants of the genus *Leucas*, phenolics are present in abundance. Compounds identified from *L. aspera* include leucolactone from the root portions, triterpenoid lactone, long chain compounds 1-dotriacontanol, 1- hydroxytetracontan-4-one, 32-methyltetracontane, aliphatic ketones, and longchain compounds nonatriacontane. Some aliphatic ketols were extracted from *L. aspera* shoots; they included 28-hydroxypentatriacontan-7-one, 7-hydroxy-dotriacontan-2-one, and 5-acetoxy-triacontane. Some of the identified phytocompounds of *L. aspera* include nicotine, *alpha-farnesene*, *alpha-thujene*, menthol, amyl propionate, and isoamyl propionate, all of which were found in the volatiles of the plant's leaves and flowers.

## **II. REVIEW OF RELATED STUDIES**

**Mackin, Chris et al., (2021)** We need a better knowledge of how the introduction of new interacting partners might alter plant reproduction since many plant-pollinator interactions are changing as species' ranges fluctuate. Insects called

nectar thieves are known to strip flowers of their nectar rewards without aiding in pollination. There may be consequences for the plant's reproductive success if nectar-stealing insects join the floral visitor cast. We zero in on the recent plant colonist *Digitalis purpurea*, which is seldom plundered in its native range but is subject to intensive nectar theft in its imported region. In this study, we investigate whether or not experimental nectar thieving has a negative effect on reproductive success. We evaluated the frequency of visits, length of visits, percentage of flowers visited, and rejection of inflorescences of the primary pollinators to see if their behaviour has shifted in response to nectar stealing. We measured the number of seeds produced per fruit and the amount of pollen exported as surrogates for the female and male components of reproductive output, respectively, to determine the impacts of robbery on fitness. Bumblebees' visit frequency and duration were drastically cut in half due to nectar theft. Furthermore, a reduced percentage of flowers on an inflorescence that had been stolen were visited by bumblebees. There was no difference in the export of pollen grains from flowers in the robbed treatment despite the fact that they produced considerably fewer seeds per fruit on average. One possible explanation for our observation that robbery reduces seed output is that robbers spend less time and energy pollinating flowers as a result of being harassed. Here, we consider how nectar stealing and other changes in pollinator settings can affect plant reproduction in the future.

**Varma V M, Sangeetha et al., (2020)** The consequences of nectar robbing, or the illegal collection of nectar, on plant sexual reproduction and the local population of pollinators might vary. When nectar thieves harm flowers in their quest for nectar, it may alter the behaviour of subsequent flower visits and, in turn, reduce the likelihood of successful plant reproduction. However, researchers have only looked on nectar manipulation by nectar thieves. *Hoplonomia sp.* (*Halictidae*), a species of short-tongued bee, was seen stealing nectar from the zygomorphic flowers of *Leucas aspera* (*Lamiaceae*) by mutilating the prominent lower petal. We theorized that *L. aspera* flowers' mutilated bottom petal discourages appropriate pollinators, which might have an impact on the plant's ability to reproduce. As a preliminary step, we monitored the percentage of naturally-robbed flowers throughout three years in order to be sure it wasn't just a local phenomenon caused by a few of bees. The dynamics of nectar and fruit production in both unrobbed and robbed open flowers in naturally robbed populations were then analysed. There was a huge range in the percentage of flower theft from year to year and location to location. Although robbing had no effect on nectar dynamics, it did change the appearance of flowers to the point that fewer pollinators visited them and the pollinator community on robbed flowers was different. However, nectar thieving had no effect on the reproductive capacity of the plants themselves, which is a motherly role. This research is the first to demonstrate the potential ecological significance of a nectar robber's influence on flower shape.

**Varma V M, Sangeetha & Sinu, Palatty. (2019)** Robbery of nectar (also known as "nectar foraging") may have negative, neutral, or beneficial consequences on the maternal function of plant reproduction and/or on pollinators. It has been hypothesised that nectar stealing does not negatively affect the maternal function of plant reproduction in autogamous and mixed breeding plants, but additional research is needed to confirm this. In this study, we analysed the effects of natural nectar stealing on the maternal function of plant reproduction and the pollinator visiting features of *Sesamum radiatum*, an autogamous plant. Open flowers, both untouched and picked, attracted pollinators. The frequency and duration of pollinator visits to stolen flowers were also analysed. These flower species' seed banks were studied. Primary robber and legitimate pollinator *Xylocopa latipes*, exclusive primary robber *X. bryorum*, and global pollinator *Megachile disjuncta*. The majority of pollinators who visited plundered flowers did so as secondary nectar thieves. Foraging time was reduced significantly when pollinators stole nectar, which was beneficial to pollinator efficiency. The process of seed set was unaffected by robbery, suggesting that it had no influence on the plant's ability to reproduce. In both autogamous and mixed breeding plants, our research suggests that nectar thieving may not negatively impact reproductive success.

**Bergamo, Pedro & Sazima, Marlies. (2018)** Pollinator behaviour and plant fitness may be influenced by nectar-stealing insects. The principal pollinators of a certain plant species may provide a signal as to whether or not the impacts of flower theft are beneficial or negative. To better understand the mechanisms that contribute to the varying effects of nectar stealing on plant reproduction, comparative studies are essential. This study looked at how the stingless bee *Trigona spinipes* (Apidae), a common nectar robber in the Neotropics, affected the reproductive success of two flowering plant species: *Crotalaria vitellina* (*Fabaceae*), which is pollinated by bees, and *Besleria longimucronata*, which is pollinated by hummingbirds (*Gesneriaceae*). To assess the impact of robbing on plant reproductive success, we compared the seed number per fruit of robbed fruits (identified by the presence of scars in the calyx, caused by the robber) with that of intact fruits and found that robbing rates varied significantly across species (number of flowers robbed as a percentage of total flower display). Robbed flowers were more common in the hummingbird-pollinated *B. longimucronata* than in the bee-pollinated *C. vitellina*. We also discovered that robbery has a detrimental impact on seed yield for *B. longimucronata* but has no effect for *C. vitellina*. Based on prior research on pollinator behaviour, we know that hummingbirds tend to avoid nectar-robbed flowers, but bees generally do not show a preference between robbed and undamaged blooms. As we've shown, the same robber species can have varying impacts on the reproductive success of similar plant species. The result of nectar robbing, then, will rely on the dynamic between plant characteristics and pollinator actions.

**Bronstein, Judith et al., (2017)** How do animals forage when food supplies fluctuate? The idea of floral constancy has offered a framework for elucidating the reasons why animals foraging at flowers visit some types of flowers but not others. In this article, we use this concept to its fullest extent by analysing how flower-viewing tourists handle flowers. Animals that "rob" flowers of their nectar by biting holes in them often do not help with pollination. There are several foragers who can alternate between stealing nectar and visiting flowers legally. We provide evidence that individuals may, in theory, alter foraging strategies but often do not. We investigate whether people show strategy constancy, or a preference for one method of visiting flowers over another. We next extrapolate floral constancy hypotheses to determine when and why floral site-seekers could display strategy consistency. The ecological and evolutionary effects of nectar stealing on plants and their interactions with pollinators are an attractive area for further study.

**Fumero-Caban, Jose & Melendez-Ackerman, Elvia. (2013)** While nectar robbing is suggestive of a detrimental fitness effect, the occurrence of nectar robbing does not prove it. Using a combination of census data and field studies, we were able to deduce that nectar stealing has a wide-ranging and complicated influence on nectar production rates, pollinator behaviour, pollen export, and female reproductive success in *Pitcairnia angustifolia*. At least four species of animals, including both a robber-like pollinator and a secondary robber, visited the blooms in their native habitat. The percentage of nectar stolen by insects is 40-100 percent in nature. Fruit set was unrelated to nectar robbing variation in any year, whereas seed set was only slightly positively correlated with this natural factor in one year. When long-billed hummingbirds were exposed to flowers that had been intentionally robbed of nectar, they still fed like secondary nectar thieves, and nectar output and concentration were unaffected. No significant difference was seen between robbed and unrobbed flower patches in terms of the number of stigmas treated with pollen dye analogues or the average distance these analogues travelled, however the maximum distance these analogues travelled was greater when nectar robbery was not prohibited. Overall, the frequency of nectar robbing within a plant was unaffected by the proportion of robbed flowers on an inflorescence, and may have even been weakly positively affected by it (i.e. positive correlation between nectar robbing and fruit set in 2002).

**Shrishail K. Kulloli et al., (2011)** Three species, *Leonotis nepetifolia* (L.) R. Br., *Leucas aspera* (Willd.) Link, and *Orthosiphon thymiflorus* (Roth) Sleesen, were evaluated for nectar dynamics and pollination in their natural environments. The nectar dynamics and pollinator preferences of these essential *Lamiaceae* medicinal plants are poorly understood. Many animals, including honey bees, butterflies, flies, ants, birds, and hawk moths, forage on flowers. In all species, sucrose-glucose and fructose predominate in the nectar. The vast majority of the visits (96%) were due to insects. Its nectar exhibits considerable temporal variations in total sugar volume and concentration. In terms of removal by visitors and evaporation or condensation, these changes are halted. The majority of insects and birds' foraging behaviour suggested that they are actual pollen-transfer agents. However, the sporadic appearances of butterflies and sunbirds indicate that they only utilize these plants as a temporary source of food. Honey bees may be the primary pollinator of these species since they accounted for roughly 66% of visits in all three species.

**Prajapati et al., (2010)** *Leucas aspera*, often known as "Thumbai," is found all over India, from the Himalayas to Ceylon. The plant has historically been employed as a pesticide and antipyretic. It has been demonstrated to have a variety of pharmacological effects in terms of medicine, including antifungal, antioxidant, antibacterial, antinociceptive, and cytotoxic activity. The existence of numerous phytochemical components, including triterpenoids, oleanolic acid, ursolic acid, and b-sitosterol, as well as nicotine, sterols, glucoside, diterpenes, and phenolic compounds (4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)-phenol, is also revealed by research. These investigations show that *L. aspera* is a source of chemical compounds that have medicinal activity and a variety of pharmacological effects, which supports the development of new therapeutic applications for this plant.

### III.MATERIAL AND METHODS

#### Study site

We made some random observations of *L. aspera* in several locations around Darjeeling.

#### Effect of nectar robbing on pollinators

For five years, researchers in Darjeeling district monitored 35 sites to see how nectar thieving affected the number of visitors and the kind of people that came to see the sites (2017-21). A total of three different kinds of flowers were spotted in each location, all within a 10-square-meter area: those that had been naturally stolen, those that had not been robbed, and those that had been chopped by hand. *Hoplonomia sp.* rips the bottom petal of *L. aspera* in two, therefore it was important to verify that pollinators avoided the robbed flowers because of the damage rather than because of any probable chemical aroma that the robber may employ to mark the visited flowers. A pair of sterile forceps was used to cut apart the base of each flower petal, creating a fissure similar to those formed by *Hoplonomia sp.* There were a total of 2,698 flowers that had been stolen, 11,348 flowers that had not been stolen, and 1,609 flowers that had been manually cut, with an average of 19, 68, and 40 flowers per site, every year.

Planned flower watches were conducted to collect data on the variety, frequency, and feeding habits of the floral visits. From 07:00 h to 08:00 h, we monitored all three types of flowers, noting the species of visitor, the frequency with which they came, and the sort of floral resource they sought. Visitation rates per flower-h were computed for all visitors (total visits of all visitors/the number of flowers viewed), for each visitor taxon (total visits of a given species/the number of flowers viewed), and for five insect functional groups described below (total visits of insects of a functional group/the number of flowers viewed) in each flower category. The usual quantitative measure of the visiting rate utilized in all the statistical studies was the visitation rate per flower-h. To analyses visitors' foraging habits, we kept track of the floral resource they collected (pollen or nectar) and the sort of visit they made (legal, primary robbing, and secondary robbing). In this section of the research, it is unclear whether or if real pollinators visited the flowers that were stolen either spontaneously or by hand. However, robber species were not permitted to enter the central unrobbed blossoms. During the observations, we would either net or chase away any robber species that came too close to the blooms we wanted to keep unmolested. Robbed flowers were separated from the group of unrobbed flowers and discarded if they were unable to deter the thief from visiting the focus unrobbed flowers. There was a visit if a visitor landed on the bloom and foraged for nectar and/or pollen. There was technically a "no visit" tally if a visitor came close to the bloom but did not land on the bottom petal. Secondary nectar robbing visits were defined as any further visits by pollinators or *Hoplonomia* sp. to previously plundered flowers. Bees, butterflies, moths, and other insects are all legitimate visitors to flowers. The size of the bee in relation to the flower's bottom petal was used to further categorise bees into two sizes: giant and tiny. Bees were categorized as tiny or large depending on their size relative to the length of the bottom petal (9 mm). Some occasional pollinators, like ants and tiny bees, especially stingless bees, scavenged for nectar in the open calyxes and corolla tubes of dead flowers. These kinds of visits were left out of the calculations.

### Data analysis

We used a generalised linear model with a binomial distribution for the error type to examine if the percentage of stolen flowers varied over time of day, location, and year. The proportion of stolen flowers per site was utilised as the response variable, with year, site, and diurnal time serving as the fixed categorical factors in the models. Type III ANOVA was used, which can be found in the R-package "car," to examine the significance of the model's explanatory variables. Repeated-measures ANOVA in R's ez package was used to assess the variance in nectar replenishment between flowers that had been robbed and those that hadn't at various intervals of time (Easy analysis and visualisation of factorial experiments).

We first used the Analysis of Similarity (ANOSIM) function in the vegan R package to determine if the pollinator community of naturally robbed and manually clipped flowers was distinct or alike before investigating the influence of nectar robbery on individual characteristics of the bee population. We built the dissimilarity matrix for several locations using the frequency with which pollinators visited flowers that had been both naturally stolen and humanly harvested. Statistical analysis of the similarity between the flower-visitor communities of wild and cultivated flowers (ANOSIM:  $R=0.06$ ) supported this hypothesis. Robbed and unrobbed flowers from both human and natural sources were combined into a single category for all statistical analyses. It also helped even out the ratio of naturally stolen blooms to untaken blossoms among populations of plants.

The impact of nectar robbery on the frequency and wealth of genuine visits was examined using a different set of generalized linear mixed models (GLMMs). These models employ the quantity of blooms seen in plant populations as a covariate as a fixed factor, and the years and site IDs as random factors in a nested form. To determine which functional group(s) are most impacted by nectar robbing, we used the species richness and visitation rate of all floral visitors and visits from various functional groups as response variables in multiple models. Error types were modeled using a Gaussian distribution for the information on the number of visitors, and a Poisson distribution with log as a link function for the information on the number of different species. Further, the quantitative interaction matrix found in the R-package bipartite was used to analyze the network of interactions generated by bee pollinators and the flower type (robbed and unrobbed).

Fruit set (binary variable: 0 = no fruit; 1 = formed fruit) and seed set (number of seeds produced out of four ovules total) were examined in caged and unrobbed open flowers to examine the effect of caging (autonomous selfing) on the maternal role of plant reproduction. We compared the fruit set and seed set of open unrobbed flowers with those of open robbed blooms to examine the impact of nectar robbery on the maternal function of plant reproduction. Both the effect of caging and the effect of nectar robbing on fruit set and seed set were analyzed using a modified linear mixed model and a linear mixed model, respectively. We used a binomial distribution to account for errors when the fruit set was the response variable. If the seed pool was the response variable, the models employed the  $\log x+0.5$  number of seeds as the response variable and a Gaussian distribution. The random variable in both models was the presence or absence of plants inside certain areas. lme4 and lmerTest, two R packages, were used to run the linear and extended linear mixed models, respectively. R version 3.2.5 was utilized for all statistical testing.

## IV. RESULTS

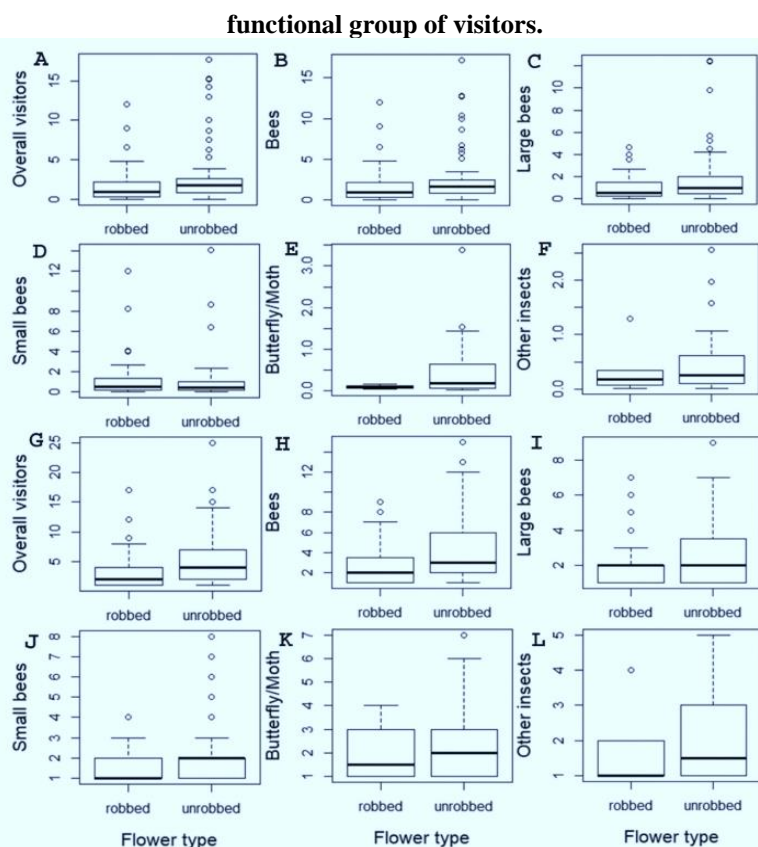
There were 77 different insect species recorded as having visited the focus flowers of *L. aspera* in 35 different plant populations. Twenty-four species of big bees and eleven kinds of little bees, twenty-five species of butterflies and

moths, and sixteen species of other insects are among the visitors. We counted 42 types of insects that visited the untouched flowers and 26 species that visited the plundered ones.

Unrobbed flowers had considerably greater visiting rates from all visitors, bees, and big bees, but not from other functional groups (Table 1; Figure 1). Besides attracting a more diverse array of pollinators, unharvested blossoms saw a boost in visitor species richness across the board (Table 1; Figure 1), with the exception of butterflies/moths, tiny bees, and other insects (Table 1; Figure 1). The community structure and composition of flower-visiting bees were shown to change due to nectar stealing, as evidenced by the interaction network.

**Table 1: Model results show the effect of nectar robbing on visitation rate and species richness of different**

Response variable	Estimate ± SE	N	t-value	p-value
<b>Visitation rate</b>				
Overall visitors	2.51±0.97	122	2.6	0.01
All bees	1.9±0.86	122	2.2	0.029
Large bees	1.57±0.71	105	2.2	0.03
Small bees	0.01±0.88	94	0.02	0.9
Butterflies & Moths	-0.32±0.24	35	-1.32	0.3
Other Insects	0.61±0.64	26	0.94	0.35
<b>Species richness</b>				
Overall visitors	1.24±0.21	122	6.03	<0.00005
All bees	0.88±0.21	122	4.11	<0.0003
Large bees	0.56±0.27	105	2.13	0.03
Small bees	0.46±0.31	94	1.49	0.12
Butterflies & Moths	1.09±1.78	35	0.61	0.54
Other Insects	0.53±0.89	26	0.59	0.55



**Figure 1: Visitation rate (visits/flower-h) (A-F) and Visitor species richness (G-L) of overall visitors and pollinators of different functional groups per site in robbed and unrobbed flowers of *Leucas aspera*.**

**Spatio-temporal effect on pollinators**

There was no relationship found between years, location, or interplay of the two in terms of the frequency with which visitors, bees, and other pollinators stopped by a flower garden. Although butterfly and moth occurrence rates changed

depending on location, year, and the interplay of these two factors, butterfly and moth diversity remained constant (Table 2). Among *T. rhomboidea*, there were significant differences in visitor richness across sites ( $\chi^2=14.6$ , d.f.=5,  $p=0.01$ ) and populations ( $\chi^2=51.9$ , d.f.=33,  $p=0.02$ ), and a lesser degree of variation between years ( $\chi^2=8.37$ , d.f.=4,  $p=0.07$ ). Similarly, there were population-specific differences in the abundance of visitors in *S. acuta* ( $\chi^2=47.87$ , d.f.=29,  $p=0.01$ ) and *L. aspera* ( $\chi^2=54.56$ , d.f.=35,  $p=0.01$ ). There was a significant year-to-year variation in the abundance of visitors to both *H. suaveolens* ( $\chi^2=7.66$ , d.f.=3,  $p=0.05$ ) and *M. pudica* ( $\chi^2=14.5$ , d.f.=3,  $p=0.002$ ). There was no correlation between location, population size, or number of visitors in the study of other plant species.

**Table 2: Spatio-temporal effect on visitation rate and richness of various floral visitors**

Overall visitors				Bees		Butterflies/moths		Other insects	
Response	Effect	DF	F/ $\chi^2$	DF	F/ $\chi^2$	DF	F/ $\chi^2$	DF	F/ $\chi^2$
Visit rate	Location (L)	5,144	0.74	5,142	0.74	5,77	3.34**	5,73	0.88
	Year (Y)	4,145	1.89	4,143	1.05	4,78	5.16***	4,72	1.05
	L*Y	20,143	0.91	20,141	2.06	19,76	2.06*	14,71	0.5
Richness	Location (L)	5	0.67	5	2.27	5	3.31	5	0.33
	Year (Y)	4	8.22	4	1.02	4	3.58	4	0.21
	L*Y	20	0.51	11	13.44	19	4.82	14	2.04

\* $p<0.05$ , \*\* $p<0.005$ , \*\*\* $p<0.0005$

One stingless bee species was among the 56 species of solitary bees, 3 species of honey bees, and 12 plant species included in the collection. However, not all 12 plant species were visited by every species. Therefore, we categorised the frequency of visits by bee species as follows: *solitary bees*, *T. iridipennis*, *Apis spp.*, and three kinds of *Apis bees*. We then looked at whether there was a difference in the frequency of their visits across time and space. Unlike the frequency of visits by stingless bees, honey bees and solitary bees did not differ in frequency based on latitude, year, or the relationship of latitude and year (Table 3).

**Table 3: Spatio-temporal effect on visitation rate of *Apis spp.*, *solitary bees*, *stingless bee* and visitor richness of *solitary bees***

<i>Apis spp.</i>				<i>Solitary bees</i>		<i>Stingless bee</i>	
Response	Effect	DF	F/ $\chi^2$	DF	F/ $\chi^2$	DF	F/ $\chi^2$
Visit rate	Location (L)	5,151	0.8	5,149	0.75	5,146	2.89*
	Year (Y)	4,150	0.1	4,148	1.55	4,148	1.52
	L*Y	20,150	0.9	20,148	0.96	20,148	2.08***
Visitor richness	Location (L)	-	-	5	2.49	-	-
	Year (Y)	-	-	4	2.38	-	-
	L*Y	-	-	20	21.2	-	-

\* $p<0.05$ , \*\*\* $p<0.0005$

## V. CONCLUSION

The tube that holds the corolla of *L. aspera* is barely 5.7 mm long. Thus, the nectar was only reachable by the largest long-tongued bees, and even the majority of the smaller long-tongued bees had a hard time getting to it (*Apis florea*, *Trigona iridipennis*, *Braunsapis sp.*, *Ceratina hieroglyphica*, and *C. unimaculata*). In other plant systems, these bees were known as main nectar robbers, but in *L. aspera*, they may be pollen foragers or nectar foragers of open calyxes and shed flowers. Those five and three short-tongued bees (*Halictus sp.3*, *Halictus sp.5*, *Seladonia sp.*) were only able to gain access to the nectar by stealing it. Consequently, its capacity to engage in secondary nectar stealing had no unfavorable effects on the visiting patterns of tiny bees.

Even while nectar stealing in *L. aspera* had no effect on plant reproduction, it did have an effect on the pollinator population. To the best of our knowledge, this is the first demonstration of how a thief may significantly alter the morphology of flowers, changing their look and making it more difficult for authorised visitors to enjoy them. The field of nectar theft has been studied for half a century, yet scientists are still left with many unanswered problems.

## REFERENCES: -

- Mackin, Chris & Goulson, Dave & Castellanos, Maria. (2021). Novel nectar robbing negatively affects reproduction in *Digitalis purpurea*. *Ecology and Evolution*. 11. 10.1002/ece3.8068.
- Varma V M, Sangeetha & Tp, Rajesh & Kandoth, Manoj & G., Asha & Jobiraj, T. & Sinu, Palatty & Thayyullathil, Jobiraj. (2020). Nectar robbers deter legitimate pollinators by mutilating flowers. *Oikos*. 129. 10.1111/oik.06988.
- Varma V M, Sangeetha & Sinu, Palatty. (2019). Nectar robbing in bellflower (*Sesamum radiatum*) benefited pollinators but unaffected maternal function of plant reproduction. *Scientific Reports*. 9. 8357. 10.1038/s41598-019-44741-y.

4. Bergamo, Pedro & Sazima, Marlies. (2018). Differential Outcomes of Nectar Robbing on the Reproductive Success of a Melittophilous and an Ornithophilous Species. *International Journal of Plant Sciences*. 179. 000-000. 10.1086/696234.
5. Bronstein, Judith & Barker, Jessica & Lichtenberg, Elinor & Richardson, Leif & Irwin, Rebecca. (2017). The behavioral ecology of nectar robbing: Why be tactic constant?. *Current Opinion in Insect Science*. 21. 10.1016/j.cois.2017.05.013.
6. Fumero-Caban, Jose & Melendez-Ackerman, Elvia. (2013). Effects of nectar robbing on pollinator behavior and plant reproductive success of *Pitcairnia angustifolia* (Bromeliaceae). *Plant Species Biology*. 28. 10.1111/j.1442-1984.2012.00388.x.
7. Erancheri P, Kumar SP. Insect diversity and pollination biology of *Leucas chinensis* (Lamiaceae). *Annals of Plant Sciences* 2013; 02(07):230-233
8. A.K.M. Mominul Islam and Hisashi Kato-Noguchi, Allelopathic potentiality of medicinal plant *Leucas aspera*, *International Journal of Sustainable Agriculture*, 4 (1), 2012, 1-7.
9. R. Srinivasan, B. Ravali, P. Suvarchala, A. Honey, A. Tejaswini And P. Neeraja, *Leucas aspera* - Medicial Plant: A review, *International Journal of Pharma and Bio Sciences*, 2(1), 2011, 153-159.
10. Shrishail K. Kulloli<sup>1</sup>, Arun N. Chandore, and Makarand M. Aitawade, Nectar dynamics and pollination studies in three species of Lamiaceae, *Current Science*, 100(4), 25, 2011, 509-516.
11. Rai V, Agarwaal M, Agnihotri AK, Khatoon S, Rawat AK, Mehrotra S. Pharmacognostical evaluation of *Leucas aspera* Link. *Nat Prod Sci* 2005;11:109-14.
12. Prajapati, M. S., Patel, J. B., Modi, K., & Shah, M. B. (2010). *Leucas aspera*: A review. *Pharmacognosy reviews*, 4(7), 85–87. <https://doi.org/10.4103/0973-7847.65330>
13. Shrishail K. Kulloli, Arun N. Chandore and Makarand M. Aitawade (2011) “Nectar dynamics and pollination studies in three species of Lamiaceae” *CURRENT SCIENCE*, VOL. 100, NO. 4, 25 FEBRUARY 2011