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Floral Biology Of Selected Medicinal Plants Belongings To Lamiaceae

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Abstract: Flowering plants have developed a notable variety of floral adaptations and reproductive strategies due to natural selection influenced by pollinators. The structure and operation of sexual units influence pollen distribution and reception patterns, and, in conjunction with post-pollination mechanisms such as incompatibility systems, dictate the reproductive fitness of the plant. Reproductive biology holds significant importance in rare and endemic species because of their limited population and dispersion sizes. Lavandula officinalis, Hyssopus officinalis, Melissa officinalis, Salvia sclarea, and Salvia officinalis are the five Lamiaceae species whose floral biology and attractiveness were investigated by establishing a correlation between the morphological traits of their flowers and nectar production and pollinator visits, particularly in relation to honey bee forage preferences. While these species are farmed for therapeutic purposes, they also serve as a substantial source of pollen and nectar for honey production; thus, the study attempted to assess their melliferousness and importance for bee forage. Seven different kinds of Hymenoptera, three species of Diptera, and two species of day-flying Lepidoptera were observed visiting pollinators. The honey bee was the predominant pollinator, with flower aroma and color as the primary factors of attraction, succeeded by corolla shape and size, and to a lesser degree, nectar availability. The number of open flowers in each whorl and the length of the corolla tube were not very important. The most beautiful plants seemed S. officinalis, H. officinalis, and L. officinalis, while M. officinalis was the least attractive and S. sclarea was completely unappealing to all pollinators, with the exception of Xylocopa violacea, based on all flower characteristics that were analyzed and observations of pollinator behaviour and visits.

Keywords: Lamiaceae, honey bee, pollination, nectar production, flower morphology

Introduction

Flowers serve as useful reproductive organs for sexual reproduction. They balance multiple contradictory demands. The accessibility of resources versus the requirement for elaborate displays to attract pollinators, the production of offspring through self-pollination versus the necessity of increasing genetic variety throughout crossing, and the choice between giving pollen as a floral attractant and storing pollen for reproduction are a few examples. Each bloom can thus be seen as a species-specific compromise for managing sexual reproduction. The 'bilabiate blossom' distinguishing the Lamiaceae is architecture for nototribic (lateral) pollen implantation. It has evolved several times in analogous and indicates that distantly related plants can approach to similar functional solutions and that closely related plants can realise these solutions via varied morphological means. They concentrate on the adaptive relevance and phenotypic diversity of the Lamiaceae bilabiate bloom. Starting from the broad concept of a bilabiate flower among the angiosperms (Westerkamp and Claßen-Bockhoff, 2007), three papers focus on Salvia and its distinctive lever mechanism.

Walker & Sytsma (2007) reconstruct the various genesis of the lever-like stamen structure, indicating that even this particular characteristic has developed in parallel. By estimating the lever mechanism's usefulness as a biomechanical pollen-dispensing instrument, Reith et al. (2007) cast uncertainty on the adaptive relevance of the device in Salvia pratensis. Wester and Claßen-Bockhoff (2007) depict the morphological diversity of the Salvia flower due to the shift from bee to bird pollination, in which relative proportions and locations play a substantial effect.

Westerkamp and Claßen-Bockhoff (2007) broaden the concept of the bilabiate flower and characterize it as a universal design. The bilabiate flower is described as a (reversible) one- way architecture that, in mainly cases, offers nectar at its base, thereby becoming independent of morphological and taxonomic relationships. It is contained dorsally by a "roof" that covers the reproductive surface (pollen and stigma) and ventrally by a "floor." The proximal "alignment channel" and the distal "pollination chamber" comprise the majority of visitation paths. The precise placement of the visitor is typically defined by the small "alignment channel." The dorsal side of the reproductive structures is inexorably in contact with the floor and roof due to their constant distance from each other. Blooms that are bilabiate by nature are nototribic. Clearly, they developed to defend pollen from bees that gather pollen. In their quest to optimize pollen gathering and provide for their young, social bees frequently leave very little pollen for other bees to fertilize. Flowers conceal pollen and place it on insects in locations that are beyond their legs' reach, preserving it for its intended purpose. The best depositions take place where access is restricted, on the insect's back, particularly during flight when grooming and reloading typically take place. A compelling illustration of parallel evolution is the bilabiate flower structure, which evolved in multiple angiosperm species simultaneously. The authors outline the fundamental limitations of the bilabiate architecture before summarizing the primary trends in diversification, with a focus on the Lamiales.

Salvia, which has about a thousand species, demonstrates parallel evolution as well. Moreover, the family's most recognizable staminal lever mechanism sets it apart from the others. It is also the largest group. By tying stamen morphology to the Mentheae phylogeny, Walker and Sytsma (2007) not only confirm this viewpoint but also provide multiple antecedents for the lever-like architecture in the second article. Drawing on a previous study that elucidates Salvia's polyphyly (Walker et al., 2004), they identify nine distinct stamen morphologies within the three different species of Salvia. The authors investigate the root cause of morphological homology in light of the different origins of lever-like stamens and come to the conclusion that even complex structures can be duplicated in response to comparable genetic canalizations and selective regimes. The latter is seen as a significant innovation driving adaptive radiation, since major speciation occurred only in those lineages using a staminal lever mechanism (Claßen-Bockhoff et al., 2004).

Through qualitative and semi-quantitative analysis, they rebuild stress and strains by examining the relationship between flowers and honey bees. In summary, the forces involved in pollen transfer are influenced by two factors: (1) the upper lever arm's physical characteristics, such as length and elasticity, and (2) the bee's spatial arrangement. All of the flower's components are synorganized that is, they work together making the entire bloom a biomechanical structure. The subject of the staminal levers' functional importance in ornithophilous species arises from their presumed use in bee-pollinated Salvia varieties for pollen distribution. A survey of the floral structure in 186 ornithophilous Salvia varieties is presented in the fourth article by Wester and Claßen-Bockhoff (2007). The discovery of 63 individuals that transmit pollen without a working lever mechanism instead of being diminished or immobile due to stiff joints or spatial constrictions is their most unexpected finding. Rather, the pollen is widely available and situated quite a distance away from the nectar reward in this species. The adaptation to birds results in the concomitant reduction of the staminal lever apparatus. By lowering the landing area and lengthening and constricting the nectar channel using various morphological techniques, bee exclusion is achieved. In order to safely deposit pollen on the feathered head of the bird, the separation among nectar and pollen must be increased. In Salvia species that are pollinated by birds, two floral building lines have developed. As an example of how even well-established constructs can become obsolete during the course of evolution, one preserves the lever mechanism and incorporates it into the bird-flower syndrome, while the other entirely reorganizes the pollination mechanism. In addition to being a useful tool for adapting to a particular Pollinator guild, variable floral structural proportions can also have an immediate impact on the breeding system. The relative positions of the stigmatic tissues and pollen sacs in self- fertile individuals influence the degree of xenogamy and geitonogamy associated with it. The diminishment of floral traits correlates to male sterility in both of the species. Intermediate plants with varying degrees of pollen abortion also exhibit this. In contrast to the male sterile blooms, these are thought to be the outcome of a delayed degeneration of one of the phases of microsporogenesis. The findings clarify how floral characteristics that benefit from self-pollination and pollen discounting avoidance, such as bloom size and style length.

Materials and methods

The following five grown Lamiaceae species were examined: Melissa officinalis L., Salvia officinalis L., Hyssopus officinalis L., and Salvia sclarea L., a few medicinal and fragrant plants are grown in natural habitat, studies of nectar production and flower visits were carried out during flowering season. Additionally established were the flower biomass, flowering duration, flower longevity, number of whorls per inflorescence, and total number of flowers per plant. Flowers selected at random were marked at the bud stage and monitored until the first signs of ageing. The literature included information on the quantitative and qualitative makeup of the essential oil that was extracted from various plant sections.

Nectar production

The quantity of nectar secreted daily by each flower, the rate at which nectar is secreted, and the dynamics of nectar secretion throughout the day and season were all investigated in the context of nectar output. During the day, nectar was harvested using the microcapillary method outlined in McKenna and Thomson (1988) at three-hour intervals. To keep flower visitors out, inflorescences were wrapped with tiny mesh for 12 hours before nectar was removed (starting from the previous day's evening) and in between daily measurements. The formula for calculating the nectar volume (measured in µl per flower; n = 5) was ◯V = Σ(r2π H)/flower number ± SE (µl/flower), where r is the capillary glass tube's radius (mm) and H is the nectar amplitude in the tube (mm).

Pollinator observation

At three-hour intervals during the day during nectar sampling, insects observed visiting the flowers were counted every fifteen days. This process was often repeated three times in quick succession to provide a mean insect count per plant at each sample time. The number of flower visitors and how often they arrived (every 10 minutes per plant) were captured on camera. As recommended by Dafni et al. (1988), the percentage of visited flowers was measured on three randomly selected plants of each species with respect to the total number of open flowers per plant.

Results

Flower biology

Among the Lamiaceae species under investigation are M. officinalis, a perennial herbaceous herb; S. sclarea, a biennial; and H., L., and S. officinalis, perennial evergreen woody-based sub-shrubs. The 2-lipped corollas and sepals are varying degrees of unity in all flowers. Pollinating insects land on the upper lip, which frequently has two lobes and forms a hood around the base of the lip. While the upper lip is inverted in the other three species, it is more developed in S. sclarea and L. officinalis. With the exception of H. officinalis, which possesses prominent, out-of-the-flower fertile portions, sterile stamens (2 or 4), and pistil are primarily overshadowed. A comparative examination of the morphometric features of flowers showed that S. sclarea is the largest flower, followed in order of decreasing floral size by S. officinalis, M. officinalis, H. officinalis, and L. officinalis (Table 1). S. sclarea has the longest inflorescence (38.1 cm) and L. officinalis has the smallest (7.2 cm), which is composed of blooms that occur in many clusters of six on the stalk. S. sclarea also exhibited the greatest values for floral biomass, calyx length, corolla height, and flower length. H. Officinalis is thought to be the plant with the greatest number of produced flowers among the species examined, taking into account the average number of open flowers per plant at full blooming stage, flower longevity, and the flowering duration. The majority of species have variable flower colors. Three kinds of H. officinalis coexist with varying shades of pink and blue as well as occasionally white corollas. L. officinalis and S. officinalis have distinct blue undertones, whereas the corolla of M. officinalis is creamy to white, that of S. sclarea is white to pale lilac, and so on.

Table 1. Morphometric characteristics of flower and inflorescence of five Lamiaceae species.

	H. officinalis	L. officinalis	M. officinalis	S. officinalis	S. sclarea
ver length (mm)	12.50 ± 0.79	11.20 ± 0.91	15.40 ± 0.42	21.30 ± 1.57	24.10 ± 0.65
Calyx length (mm)	6.40 ± 0.65	4.74 ± 0.50	8.50 ± 0.35	11.44 ± 0.26	14.08 ± 0.43
Corolla tube	6.76 ± 0.51	7.50 ± 0.61	10.50 ± 0.5	11.00 ± 0.35	9.00 ± 0.14
length (mm)					
Corolla width (mm)	5.80 ± 1.44	7.60 ± 0.65	7.66 ±0.7603	9.30 ± 0.44	8.08 ± 0.24
Corolla height (mm)	7.96 ± 1.13	8.50 ± 0.93	7.30 ± 0.27	15.00 ± 1.17	20.00 ± 0.61
Diameter of corolla tube opening (mm)	2.02 ± 0.47	1.68 ± 0.25	2 ± 0.35	3.7 ± 0.67	2.16 ± 0.23
Floral biomass (g)	0.0065 ± 0.0005	0.0111 ± 0.0007	0.0054 ± 0.0006	0.0652 ± 0.0052	0.0697 ± 0.0077
Inflorescence length (mm)	203.2 ± 76.24	72 ± 23.61	357 ± 134.59	199 ± 53.82	381 ± 122.83
Whorls per inflorescence	19.2 ± 1.10	5.6 ± 0.56	10.8 ± 0.45	7.8 ± 2.28	7.4 ± 0.89
Total number of flowers per whorl	28.4 ± 2.30	7.4 ± 1.67	27 ± 0	10.8 ± 1.79	6 ± 0
Number of open flowers per whorl	3.6 ± 1.14	3 ± 1	2.4 ± 1.14	2.6 ± 0.89	3.2 ± 1.92

S. sclarea was not at all appealing to honey bees, even though it was the most scented and rich in nectar. The entire plants of S. officinalis, L. officinalis, H. officinalis, and crushed leaves of M. officinalis smell pleasant to human senses, in contrast to the intense and heavy perfume of S. sclarea. L. officinalis has the longest floral lifespan (5-8 days), which is about comparable to that of H. officinalis, while M. officinalis has the shortest (no more than 20 hours). Without a doubt, if pollination took place in the meantime, the flower's lifespan was reduced. In the second part of May, S. officinalis was the first plant to flower in terms of flowering dynamics. About two weeks later, in June, other plants began to bloom in the following order: H. officinalis, L. officinalis, S. sclarea, and M. officinalis. After fifteen days, these plants reached full bloom. All species typically flower for two to five months, although under ideal weather, they might flower constantly or sporadically until the start of fall.

Comparative analysis of nectar production

The largest overall daily nectar volume per flower and nectar secretion rate were recorded in

S. sclarea (average 2.288 ± 0.76 µl; 0.152 ± 0.04 µl/h), and the lowest in M. officinalis (average 0.236 ± 0.17 µl; 0.015 ± 0.01 µl/h). Based on comparative research of the diurnal pattern of nectar discharge in the specified microclimatic habitat circumstances, we discovered that the flowers of all investigated species released nectar continuously during the day, with more or less pronounced decreasing tendency towards the evening. Two models of diurnal dynamics of nectar secretion, including one (H. officinalis, L. officinalis, S. officinalis and S. sclarea) and two secretory peaks (M. officinalis), have been established. Given the seasonal dynamics of nectar secretion, variability of nectar amount per flower and discontinuity in the nectar production, predominantly based on the local microclimate, were ascertained. The five species provided bee pasture in our trial field continuously from mid- May to September. The imposed plantation growing conditions restricted the flowering time which ordinarily under favourable conditions may be extended to October (particularly in the case of L. officinalis and H. officinalis). In addition, planned cutting of these herbs intended for medicinal purpose, interrupts the continuity of flowering, which is reflected mostly in the bee visitation.

Study of flower visitors

Three types of pollination were portrayed with regard to insect classes: melitophily, myophily, and psychophily. The honey bee, bumble bees (Bombus terrestris / lucorum L., Bombus hortorum L., Bombus pascuorum Scopoli, and B. lapidarius L.), and carpenter bees (Xylocopa violacea L.) were among the bee visitors. These bees actively collected nectar and pollen from S. sclarea, L. officinalis, and H. officinalis. Honey bees contributed to almost all of the documented insect visits, next to bumble bees. Other insects, making an average of less than two visits per plant in 10 minutes, were flies, butterflies and solitary bees. Bee flies and hover flies, which gather nectar and pollen, were among the visiting flies. L. officinalis attracted two of the largest numbers of common hover flies (Syrphidae), which were identified as Eristalis tenax L. and Episyrphus balteatus De Geer, also known as Mačukanović-Jocić, Stevanović, Mladenović, and Jocić. The corolla tubes of L. officinalis and H. officinalis were seen to be exploited by the bee fly Bombylius important L. (Bombyliidae). Many cabbage white butterflies (Pieris brassicae L., Pieridae) were typically seen together on the same plant in plantation conditions with abundant nectar supplies. The individuals stayed in the region for a considerable amount of time, feeding slowly. While Macroglossum stellatarum L. (Sphingidae) adults were seen to be highly active during the day, the majority of specimens were typically recorded between 12.00 and 19.00 hours. (Table 2). Table 2 contains a list of the most common visitors to each species along with visitor counts. After comparing the average frequency of bee inspections to the species under study, it was found that M. officinalis flowers received the fewest visits while H. Officinalis blooms received the most (Table 2). For S. officinalis, H. officinalis, and L. officinalis, honey bees visited flowers continuously and in large numbers during the day; for M. officinalis, they visited flowers sporadically and discontinuously, clearly showing no interest in the plant; and, lastly, for S. sclarea, honey bees never visited flowers.

Table 2. Flower visitors and their number in 10 min per plant during nectar collecting days. Data represent average values for the investigation period.

Plant	Flower visitor	Frequency of visitors (No/10 min/plant)	% of visited flowers
H. officinalis	Bombus terrestris./	6.17	17.12
	B. lapidarius	5.16	8.12
	Apis mellifera	30.28	43.67
	Pieris brassicae	Sporadically	-
L. officinalis	Bombus terrestris/ lucorum	4.23	16.23
	Bombus pascuorum	3.10	10.29
	Apis mellifera	18.33	61.12
	Pieris brassicae	Sporadically	-
	Macroglossum stellatarum	1.30	7.11
	Episyrphus balteatus	1.90	0.50
	Eristalis tenax	1.90	0.27
	Bombylius major	1.97	4.23
M. officinalis	Apis mellifera	1.40	0.02
S. officinalis	Bombus terrestris/ lucorum	Sporadically	-
	Apis mellifera	14.93	18.22
	Bombus pascuorum	14.50	17.23
	Bombus hortorum	3.15	6.12
S. sclarea	Xylocopa violacea	0.13	2.54

Statistical analysis

According to DCA, a few evaluated flower characteristics were important for differentiating between species. The length of the calyx, the number of whorls per inflorescence, and the overall quantity of flowers per whorl were the most significant variables in relating differences between species across the initial canonical dimension. Most factors, including flower length, corolla width, number of whorls per inflorescence, total number of flowers per whorl, flower lifespan, and corolla tube length and number of whorls per inflorescence, respectively, contributed to the discrimination of species by the second and third canonical axes. When species were distinguished based on multivariate mean values (also known as "centroids"), S. officinalis and S. sclarea were shown to be distinct from the other three species along the first canonical axis. This position may be explained by the lever biomechanism present in a certain floral structure of Salvia species, which is followed by a similar flower biomass and size. Discrimination along the second discriminant axis revealed some groupings with M. and H. officinalis, as well as S. officianalis, S. sclarea, and L. officinalis. Regarding the third canonical axis and species discrimination, no noteworthy groupings of species were found. Out of all the flower variables tested, including flower length, calyx length, corolla tube opening diameter, inflorescence length, number of whorls per inflorescence, and longest flower lifespan, it appears that only L. officinalis was the most distant from the others. This is likely due to the plant having lower values for the majority of these variables. All of the measured floral attributes ($p < 0.001$), with the exception of the character representing the number of open flowers per whorl, demonstrated statistically significant differences between the species when investigated with univariate statistics and analysis of variance (ANOVA).

Discussion

The majority of the observed pollinators were quite rare, according to our analysis of the five Lamiaceae species' melliferous potential and attraction. This suggests that the flowers are mostly attractive to hymenopterans in the Apidae family. Notably regulars were bumblebee and honey bee species, especially to the blue-flowered species with significant nectar potential. Butterflies, small and large hover flies, solitary bees, and bee flies were among the less common visitors to the flowers. According to Herrera (1988) and López-Pujol et al. (2004), and other sources, Syrphidae and Bombyliidae are generally generalists in their floral preferences and have been identified as pollinators or frequent visitors of thousands of kinds of flowering plants, including Lamiaceae. Throughout the duration of the study, butterflies made comparatively few intermittent daylight visits. Though Kelber (2003) concluded that adults, with their high energy demands, prefer flowers with lots of nectar, the most frequent visitor was the diurnal humming bird hawk moth Macroglossum stellatarum, especially found on L. officinalis whose flowers were not the richest in nectar among the investigated species. It is clear that other attractants, rather than the quantity of nectar controlled this moth's flower choices given that, according to Kelber (2002), it is able to correlate flower color, size, and pattern with the presence or lack of food. However, butterflies and flies are not necessary for the pollination of these plants. The Bombus species, which use the length of their proboscis as a physical indicator of how they use resources, were observed collecting nectar from species with blue flowers. The relationship between the length of the proboscis and the amount of time bees spend on flowers with varying corolla tubes has already been extensively studied (Herrera, 1989; Inouye, 2004).

Pollinating flowers like H. officinalis and L. officinalis with shorter corolla tubes are the fairly common bees, Bombus terrestris and B. lucorum, whose workers are nearly identical. With a tongue that was relatively short, B. lapidarius liked the inflorescences of H. officinalis, which were composed of numerous little flowers, but B. pascuorum, whose tongue was medium-length, was frequently observed feeding on S. and L. officinalis. These findings corroborate the theory put forth by Terletskaya et al. (2023) that the presence of moderately long flowers on other Lamiaceae species, like Stachys slyvatica, Lamium album, and Salvia pratensis (with mean corolla depths ranging from 8.5 to 11.37 mm), by bees indicates that these plants and their pollinators are morphologically compatible.

Flower appearance was recently studied in relation to honey bee attraction as the most frequent and most effective pollinator seen, which is consistent with predictions that the five investigated species are primarily bee-pollinated. This analysis revealed that the honey bee never visited S. sclarea, whose only pollinator was the solitary bee X. violacea, despite the fact that it is polylectic and cannot specialize on a particular species (Schmidt and Johnson, 1984). Instead, it must learn how to handle the actual blossoms. A morphological barrier to honey bee pollination is the large blooms of S. sclarea, which have high-positioned fertile sections beneath the activated upper corolla lip and a saggy incavation of the lower lip. Honey bees' foraging decisions are influenced by a variety of elements, including color and floral scent. Given that the corolla tube has the greatest opening and the lower lip serves as a landing platform, it has been found that S. officinalis flowers are most suited for honey bee pollination. The size of the corolla tube, the average 2 mm diameter of the opening, the sigmoid corolla, the lower lip's nonconformity toward landing, and the ease with which the calyx and corolla separate upon light contact make the M. officinalis flower the least accessible from the viewpoint of the visitor. With the shortest corolla tube (average 6.76 mm) and nectar storage at a depth appropriate for the dimension of the proboscis, the flower of H. officinalis is particularly adapted to honey bee pollination based on its shape. Compared to L. officinalis, this species secretes less nectar per flower, but because of the long, dense inflorescence that the flowers form, which lowers the cost of interflowed transit for foraging insects, the species is more profitable. Although there is no proven link between the measurement of the corolla canal and how attractive a flower is to honey bees, this characteristic may be important. Some people believe that honey bees never visit flowers with corolla tubes longer than 10 mm because they cannot reach the nectar with their 6.5 mm (6.2 - 6.7 mm) long proboscis (Wolff, 2006; MacInnis et al., 2023). H. officinalis and L. officinalis should be the most appealing, according to a comparison of the lengths of their probosci and corolla tubes. Nonetheless, a difference in their attraction level was noted, indicating that in the same amount of time, about 30 honey bees visited one plant of H. officinalis and 18 visited one plant of L. officinalis. Conversely, M. officinalis and S. officinalis both have identical corolla tube lengths (11 and 10.5 mm), but the former's blooms were much more gorgeous than the latter's. When considered in isolation, flower size had no discernible effect on bee attraction; however, it naturally did when combined with other features like color, shape, and the spacing between floral units. This characteristic could possibly elucidate why colored objects register larger in the honey bee's area of vision. Nectar guidelines and nectar features are appeal variables in addition to floral structure, color, and scent (Harborne, 1988).

Based on our subjective evaluation of scent strength and the available literature, every species under investigation is very fragrant. The most significant element in the biochemical communication (attractant or repellent) between plants and animals is thought to be the scent that surrounds the plant, which is derived from volatile essential oils (Harborne, 1988). Still, some Lamiaceae flowers, such as Melissa's, have no perfume. For this reason, as previously noted (Beker et al., 1989), the aroma of the entire plant draws over a greater distance, and the subtle scent of the corolla then goes directly to the nectar. According to some previous studies, the dried leaves of M. officinalis contain 0.32% essential oil, primarily composed of aromatic (citral) and polyphenolic constituents, and the corolla of the plant contains a small amount (0.002%) of essential oil with a complex composition, with β-cariofilen dominating (57.2%) (Carnat et al., 1998). According to Dafni et al. (1988), M. officinalis is a poor food supplier of honey bees. Our data, on the contrary hand, clearly contradict Ara et al., (2021)'s description of the plant as a very good melliferous plant, which highlights its low nectar output as well. A pollinator also receives a visual signal at lesser distances. For honey bees, not all colors are equally appealing. Brightly colored petals on bee blossoms give the impression that they are blue or yellow to the human sight (Da et al., 2005). Although Harborne (1988) noted that the bees are sensitive to UV-absorbing flavones and flavonols present in white corollas, playing a crucial role in bringing about the fascinating pollinating guide patterns, honey bees very rarely or never visited the mostly white colored flowers of M. officinalis and S. sclarea. However, there was strong evidence that the color of the corollas played an integral part in attraction (S. officinalis and M. officinalis). Honey guides, which can be seen or unseen on the petals of a flower, direct a bee toward the nectar reservoir as it lands there (Harborne, 1988). Seldom has the function of floral guides in pollinator direction been studied experimentally (Waser and Price, 1985).

The current study solely examined S. officinalis flowers for their distinctive color patterns, which serve as visual honey cues. These patterns are the result of blue pigments that are distributed differently throughout the tubular corolla, making it simpler for bees to identify the species. The remaining species' flowers include patterns that reflect light in the UV spectrum, creating "nectar guides" that are invisible to humans but help bees find sources of pollen or nectar. Because of their inclination towards shorter wavelengths of light, bees are able to perceive flowers in UV light in addition to their preferred colors of yellow and blue (Harborne, 1988). While most flowers share the characteristic of UV fluorescence, some components of the flower, like the style, pollen grains, nectar glands, stamens (anthers), and nectar, may only exhibit this phenomenon momentarily (Thorp et al., 1975; Mačukanović- Jocić et al., 2007). As reported for Ocimum basilicum and many other Lamiaceae flowers (Mačukanović-Jocić et al., 2007; Iriel and Lagorio, 2010), the investigated flowers have strong UV reflectance of their stamens (anthers), exhibiting a beautiful UV autofluorescence that may be used to attract bees. The results we achieved indicate that foraging efficiency would be enhanced by highly positioned anthers beneath the top corolla lip, while lower lip- positioned anthers like those found in H. officinalis flowers control where visitors land. The combination of an unappealing floral color, an unpleasant smell, and morphological incompatibility the lack of co-adaptation to honey bee pollination makes S. sclarea flowers unsightly. Despite being polylectic, the lone carpenter bee coevolved with S. sclarea and is adapted for pollinating it.

Due to their similar origins in southern Europe, these two species have evolved a mutualistic connection that ensures the survival of both parties. As far as co-adaptation is concerned, the female of X. violacea could be called a Xylocopa-flower since Kugler (1972) noted that it was the sole viable pollinator of S. sclarea. Furthermore, S. sclarea's flowering phenophase follows the life cycle of Xylocopa. While Kugler (1972) claims that Xylocopa flowers' distinctive features are also suited for bumble bee pollination, no bumble bees visited the plant during the time we studied it. Odor emission and corolla color alone, however, are insufficient to encourage recurrent insect visits because nectar and pollen serve as vital food sources. The correlation among nectar production and insect visits is supported by our research, which also supports the findings of Paton et al., (2004) and Huelsenbeck et al. (2001). These authors proposed that the energy demands of consumers are reflected in the quantitative and qualitative characteristics of floral nectar, meaning that flowers pollinated by large visitors should have a higher potential for nectar compared to those pollinated by small ones. Given the complexity of the phenomena, it is inappropriate to consider flower attractiveness in isolation from pollinator behaviour linked to forage. In contrast to the majority of other bee species, honey bees exhibit distinct behavioural methods when selecting melliferous plants. The choice of this polylectic insect to visit only one type of plant at a time, in spite of the vast simultaneous blossoming of other species growing in the same area, was one of their most clear observed behavioural norms during their food quest. For instance, despite the presence of numerous other melliferous species nearby, honey bees were seen to be steadfastly visiting the few accessible open L. officinalis blooms during the day. The fundamental requirement for fulfilling the pollination mission is to concentrate on one species while disregarding the others. Despite the fact that the pollinator's devotion is vital to the plant, Gegear and Laverty (2005) noted that the value to the animal remains unclear. The goal of animals is to obtain food as quickly as possible, not to spread pollen.

Most likely, in order to pollinate a particular flower type with the least amount of time and energy wasted, a pollinator must investigate and learn how to approach it. Those that tackle procedures with unwavering repetition and efficiency receive the highest rewards. By rejecting more emptied blossoms and accepting fewer emptied flowers than one would anticipate from random foraging, honey bees boost the efficiency of their foraging efforts. According to Wetherwax (1986), honey bees exhibit discriminating behaviour when foraging, receiving reinforcement from the aroma or nectar smell left by earlier visitors to the bloom. This finding is consistent with her findings. The fact that visits intended to facilitate primary pollen hunting may be ineffective for flowers and frequently fail to result in pollination is the reason for the growing number of reports on pollination issues with this insect (Robinson et al., 1989), particularly in monoculture. There is no precise and unambiguous relationship between a single flower feature or a combination of floral traits and honey bee visits, as has been noted previously (Dafni et al., 1988). In other words, as was demonstrated in S. sclarea, more nectar per flower and larger flowers did not equate to more visits. Aside from variations in floral yield, the most visited species, H. officinalis and L. officinalis, had very small flowers with little nectar attached. Moreover, interactions with attractive or repulsive floral traits may result in behavioural variations during foraging. The link between foraging, pollination, and floral visiting deserves more attention. The most visited species, such L. officinalis, H. officinalis, and S. officinalis, have the potential to be excellent forage plants that beekeepers might plant to boost local honey bee populations.

Future Directions

These publications will serve as a basis for further research projects by the authors and others, which will further clarify the biology of pollination, evolutionary history, and classification of five species. In the future, new insights into this five species evolution will be possible thanks to the combination of enhanced phylogenetic resolution obtained from next-generation sequencing technologies, extensive and thorough morphometric analysis of all plants and pollinators, field-based pollinator observations, and taxonomic clarifications. The observations become helpful in understanding the connections amongst plants and pollinators under various climatic circumstances because of this pollination and breeding behaviour. As a result, attempts to conserve the pollinator fauna are aided in developing, ensuring the continuation of these priceless medicinal, ecological, and sociocultural plants.

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