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Research article/Araştırma makalesi

The pollination and reproduction success of *Salvia sclarea*

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Abstract

In this study, the pollinators of *Salvia sclarea* (Lamiaceae) and their behaviors, pollen viability, stigma receptivity, pollination biology and reproduction success were investigated. On observation, 8 insect taxa: *Xylocopa violacea, Vespula germanica, Megachile pilidens, Sphex spirifex, Anthocopa bidentata bidentata, Apis mellifera, Anthophora* sp., *Sphaerophoria* sp. were reported to pollinate the plant for different reasons. Stigma receptivity begins from the bud phase of the flower (protogyny) when the anthers open at the same level as the stigma, it surpasses the anthers and comes out of the upper lip of the corolla and activates the allogamy mechanism. In the bagging experiments which consisted 100 flowers 1.95 (± 1.69) ovules per flower were calculated to be fertilized while in the control group it was 3.92 (± 0.34 ovules). According to ISI index (ISI = 0,497) the plant was identified to be semi-incompatible. This species is cultivated for its medical and aromatic significance. To ensure seed production in this species, its autogamy is replaced by the allogamy mechanism. This is important in terms of the species productivity and seed output. In addition, *S. sclarea* fields are considered to contribute positively to honey production as they are pollinated by bees.

Key words: *Salvia*, pollination, *Xylocopa*

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Salvia sclarea'nın tozlaşması ve üreme başarısı

Özet

Bu çalışmada *Salvia sclarea* (Lamiaceae)'nın tozlaştırıcı böcekleri ve davranışları, polen canlılığı, stigma aktivasyonu, üretilen toplam polen ve ovül miktarı verileri üzerinden tozlaşma biyolojisi ve üreme başarısı araştırılmıştır. Gözlemler sonucu, 8 böcek taksonunun (*Xylocopa violacea,Vespula germanica, Megachile pilidens, Sphex spirifex, Anthocopa bidentata bidentata, Apis mellifera, Anthophora* sp., *Sphaerophoria* sp.) bitkiyi çeşitli amaçlarla ziyaret ettiği belirlenmiştir. Canlılık testlerinde tomurcuk safhasından itibaren stigmanın aktif olduğu (protoginik), anterlerin ise stigmayla aynı seviyeye geldikten sonra açıldığı, stigmanın anter seviyesini geçerek korolla üst dudağından dışarı çıktığı ve yabancı dölleklik mekanizmasını etkin hale getirdiği belirlenmiştir. Kapatma denemelerinde, yüz çiçekte çiçek başına ortalama 1.95 (± 1.69), kontrol grubunda ise 3.92 (± 0.34) ovülün döllendiği, bu nedenle ISI (ISI=0,497) indeksine göre, bitkinin kısmen kendine döllek olduğu saptanmıştır. Doğal bir bitki olmasına rağmen tıbbi ve aromatik öneminden dolayı büyük oranda kültüre alınarak yetiştirilen bu tür, yabancı tozlaşma mekanizmasının yanı sıra kendine dölleklik mekanizmasını da yaklaşık olarak yarı yarıya çalıştırarak tohum üretimini garantiye almaktadır. Bu durum kültüre edilen türün verimliliği ve tohum eldesi açısından önemlidir. Ayrıca arı gruplarıyla tozlaşması sebebiyle bu tarz kültür alanlarının arıcılıkta, bal üretimine olumlu katkısı olacağı düşünülmektedir.

Anahtar kelimeler: *Salvia,* tozlaşma, *Xylocopa*

1. Introduction

In nature, mutualistic relationships exist between animals and plants. Pollination, seed distribution and ant-plant protection are considered as key ecological processes for numerous terrestrial ecosystems. Therefore, a good

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understanding of the mutualistic interactions between plants and animals is vital importance with regards to the management and protection of these ecosystems. The organic features of living beings enable interaction within them and are very important in comprehending interaction of evolutionary and ecological processes (Nepi et al. 2009).

With their stamens having a unique lever mechanism and their interaction with pollinators, the species in the *Salvia* genus are an ideal model for studying plant-insect interaction (Will and Claßen-Bockhoff, 2014). *Salvia* genus has approximately 900-1000 species around the world. Of these, about 30 grow in Cape Town, South Africa, 250 in Western Asia-Mediterranean region, 90 in Southwestern Asia and 20 (Section Audibertia) in Northern America. Of the *Calosphace* subspecies, 300 grow in Central America, 150 in the north of Southern America and 60 around Argentina, Paraguay, Chile and Uruguay in South America (Walker et al., 2004). Spread over several continents, this genus differs in both its reproduction mechanism and phenotype.

In Turkey, there are 106 taxa in genus *Salvia* (Güner et al., 2012). Of these species, 57 (53.77%) are endemic, 4 (3.77%) are rare and non-endemic while 45 (42.46%) are widespread locally and globally (Kahraman et al., 2012).

The leaves of *Salvia* species have been used in the treatment of cough, digestive troubles and pyorrhea in traditional folk medicine for a long time. They have various biological activities such as antibacterial, antifungal, antiviral, antiseptic and anticancer properties (Şenkal vd., 2012). The essential oil obtained from these species is utilized in different industries particularly food, beverage, perfumery, cosmetics and pharmaceutics. Also used in the nutrition of some wild and domestic animals. The prevention of lipid oxidation is important for the extension of the shelf life of animal products (Gülçin et al., 2004; Önenç ve Açıkgöz, 2005).

It is important to understand the pollination biology and reproductive success for the improvement of cultured varieties. It is a widespread belief that flowers integrate their organs with their pollinators for effective pollination. *Salvia* species have a special stamen type (Hildebrand, 1865; Zhang et al., 2010). Each flower has a special 2-stamen-lever mechanism positioned at the entrance of corolla tube with fertile anthers under the upper lip of the corolla. There have been numerous researches on this special mechanism in *Salvia* (Trapp, 1956; Zhang et al., 2010). Pollination and functional ecological studies have investigated the importance of this lever mechanism (Grant, 1950; Grant and Grant, 1964; Claßen-Bockhoff et al., 2004; Reith et al., 2007; Wester and Claßen-Bockhoff, 2007; Zhang et al., 2010). However, very little is known about this lever mechanism and its interaction with pollinators (Zhang et al., 2010; Kahraman et al., 2009).

This study reveals the pollination biology of *S. sclarea* L. and contributes to improving the productivity of this economically important species.

2. Materials and methods

Research was carried out in the study plots of Ege University, Ödemiş Vocational School of Higher Education on 240 pieces a year old plants sowed in 4 plots of 60 plants each with a spacing of 60 cm × 30 cm within rows and 3 m × 3 m between plants on each row. On observation, data on pollination were collected for 2 days between 9.30 and 16.30 hr. Minimum and maximum temperatures (°C) and wind speed (m/sec) were measured every half hour with a thermometer and handheld anemometer, respectively while nectar density was recorded with an Eclipse handheld refractometer 0-50 °Brix at one-hour intervals. The amount of sucrose was determined by applying Kakutani's (1993) formula on the collected density data. To study the pollination mechanism, 100 flowers before anthesis were covered with an air permeable filter paper. An average number of nutlets were calculated from 100 unprocessed flowers which were as the control group. One week later, the covered and control group flowers were picked and pollinated ovules were examined under a stereomicroscope. To identify ISI index, Zapata and Arroyo (1978) equation were used. For stigma and pollen viability, DAB-UREA test and MTT test were applied, respectively applied on 30 flowers (Dafni, 2007). Anthers were prepared by Wodehouse's method (1965) and pollens were counted directly with using microscope slide-grids. For the identification of pollens reaching the stigma, concentrated H_2SO_4 was dripped on 30 stigma and pollens were counted under the Olympus CX21 binocular microscope. Each ovule was fertilized by apollen grains, for that reason we approve that the numbers of fertilized ovules are equal to pollen grains reaching to ovule. All pollen and ovule measurements were given by calculating mean and standard deviations.

3. Results

3.1. Insect Species that Visit Flowers and Their Behaviors

8 insects taxa (*Xylocopa violacea* L., *Vespula germanica* Fabricius, *Megachile pilidens* Alfken, *Sphex spirifex* L., *Anthocopa bidentata bidentata* Morawitz, *Apis mellifera* L., *Anthophora* sp., *Sphaerophoria* sp.) have been observed to visit the *S. sclarea* for various purposes. *X. violacea, M. pilidens, A. bidentata bidentata, A. mellifera,* and *S. spirifex* are the most frequent visitors of the plant (Figure 1). Megachilidae, *A. bidentata bidentata, Sphaerophoria* sp., *M. pilidens* and *S. spirifex* visit the plant for nectar; however, it can't activate the lever mechanism of the stamens (Figure 2a, b and c). It was identified that *V. germanica* visits the flowers to hunt insects (Figure 2d).

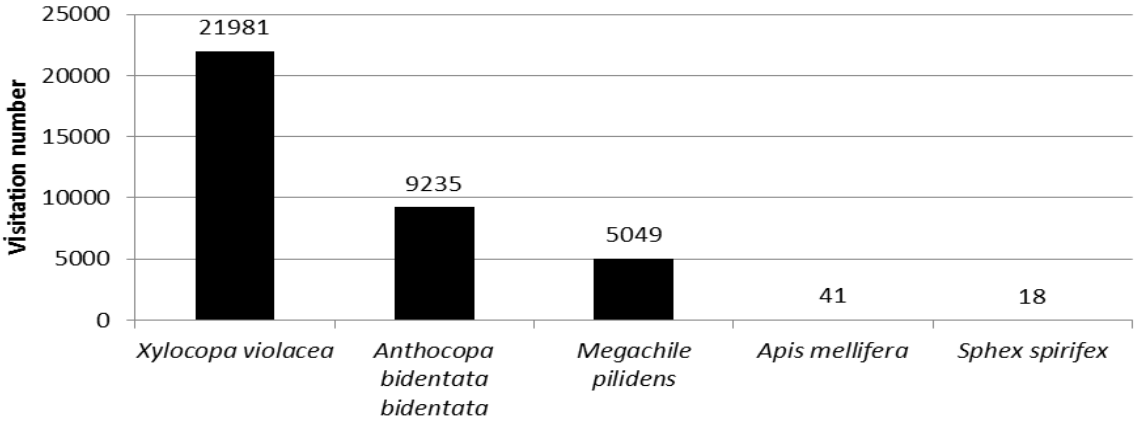

Figure 1. Most frequent visitors and number of visits

Figure 2. Insect species which cannot activate the lever mechanism of stamens a) *A. bidentata bidentata* (b) *M. pilidens* (c) *A. mellifera* (d) *S. spirifex*

3.2. The role of X. violacea in Pollination

X. violacea comes in contact with the two sterile thecaes below when it reaches the corolla tube to collect nectar upon landing on the lower lip of the corolla. This contact enables the two fertile thecaes to touch the head by means of the lever mechanism of the stamens (Figure 3a). On pushing their bodies forward to collect the nectar around the ovary, fertile thecae load the pollens with a sweeping motion unto the bee’s thorax (Figure 3b and c). Finally, the bee raises its abdomen up and thus the thecae in the thorax continues the sweeping motion towards the tip of the abdomen. Pollens are loaded into all segments except the first and last three segments and in this stage, the upper lip of the corolla comes down and moves the stylus. When the stylus moves, the stigma touches the third segment of the bee’s abdomen and the pollens are loaded unto the stigma. (Figure 3d, e and f). The other insect taxa except *X. violacea* either cannot come in contact with the stamens as they are too short to reach the filament or even if they manage to lower the fertile stamens above, they cannot move the lever mechanism sufficiently due to the lack of force required to push the stamens’ sterile thecae below (Figure 3c and d).

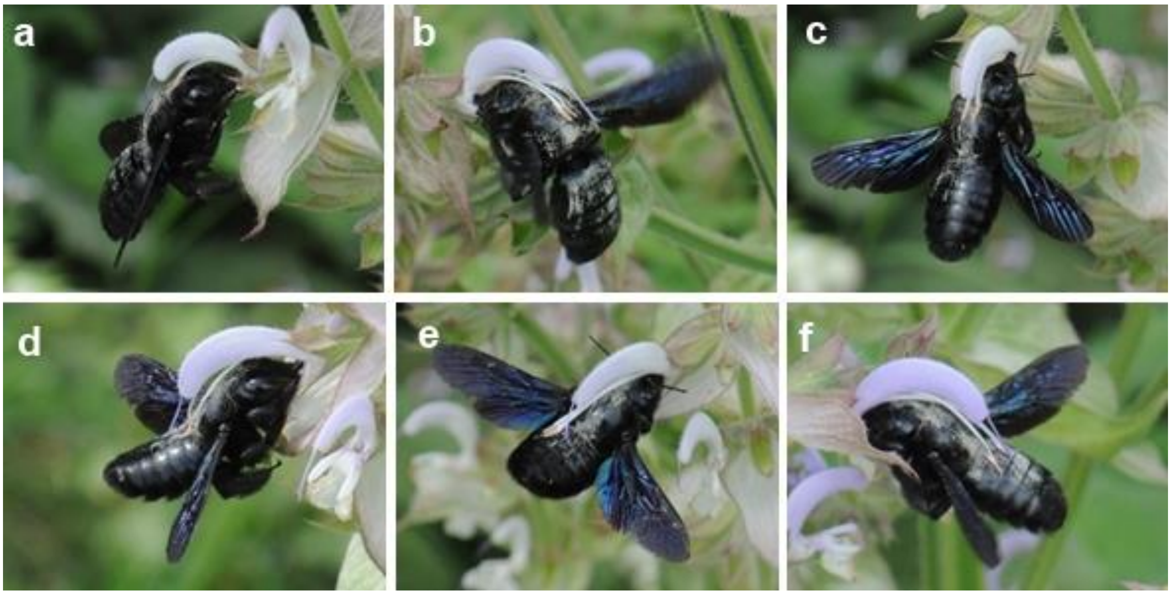

Figure 3. Pollination behavior of *X. violacea* on *S. sclarea*

3.3. Pollination activity

The visits of *X. violacea*, the only pollinator of *S. sclarea,* vary depending on temperature, wind and the amount of sucrose in the nectar.

3.3.1. Temperature: *X. violacea* makes the most frequent visits during the morning hours at 35.5ºC. As temperature increases, the number of visits decreases. From 16.00 hours, temperature decreases thereby increasing wind speed which reduces the pollinator's activities (Figure 4a).
3.3.2. Wind: At the morning hours, when the wind speed is below 1 m/ sec, *X. violacea* makes the most frequent visits. Between 10.30 and 11.30 when the wind speed reaches over 2 m/ sec, the number of the visits was reduced. Between 12.30 and 13.30, an increase in wind speed of over 3m/sec further was reduced the number of visits. Between 15.30 and 16.00, the wind of over 5m/sec further was reduced the frequent of *X. violacea*'s visits (Figure 4b).
3.3.3. Nectar: The concentration of nectar ranges from 23% to 50%. *X. violacea* mades the most frequent visits between 09.30 and 10.30 when the amount of sucrose in nectar was 0.3 mg and the least frequent visits between 15.30 and 16.30. Between 10.30-12.30 and 13.30-15.30, the amount of sucrose in nectar reached up to 0.5 mg while between 13.30 and 14.30, the amount of sucrose in nectar was measured as 0.4 mg (Figure 4c).

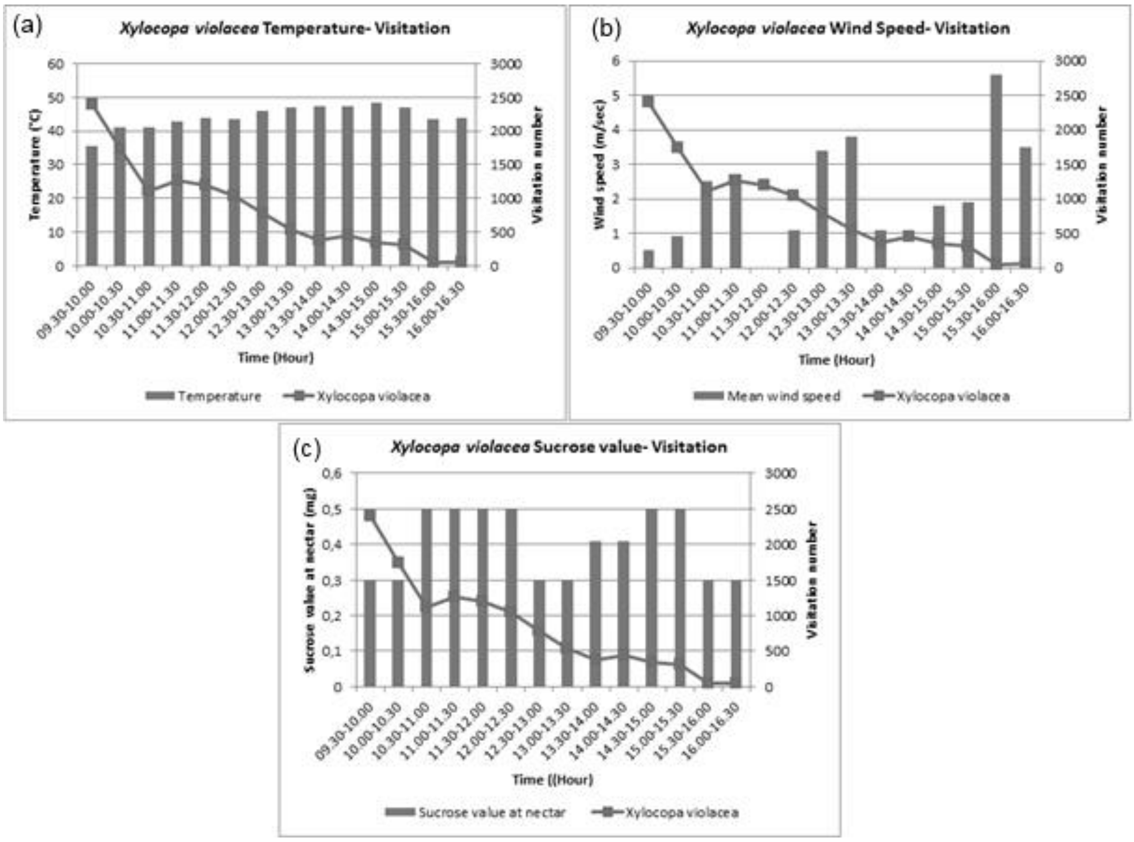

Figure 4. (a) Temperature (b) wind and (c) sucrose value dependent visitation of *X. violacea*

3.4. Pollination Success

The viability tests before anthesis have been shown that the pollens of the plant are alive and the side of stigma that allows pollens is active. A flower produces 132402 (±1260.56) pollen grains. Of these, 81% are viable. Since *X. violacea* is the only defined pollinator of the *S. sclarea*, 0.71% of the pollens reach the stigma after flowering. The pollens that reach the stigma, 5.62% pass through the stylus, reach the ovaries and carry out pollination. While the pollination success was 98% for the group that the covering test was not applied, this rate is 48.75% for the covered group. Each flower potentially produce 4 nutlets but the fertilized nutlet numbers are calculated 3.9 (±0.34) in control group and 1.95 (±1.69) in covered group. While 0.3% of the pollens were produced by the flowers left uncovered reached the ovaries, only 0.1% was recorded for the covered group. The ISI was determined using the Zapata and Arroyo's (1978) equation thus:

ISI = Fruit set from self-pollination / fruit set from cross-pollination

In accordance with this equation, the value was categorized into:

> 1 : Self incompatible

> 0.2 : Partly self incompatible
< 0.2 : Highly self incompatible
0 : Totally self incompatible
ISI = 1.95 / 3.92 = 0.49 According to these data, *S. sclarea* is partly self-incompatible.

4. Conclusions and discussion

Salvia species attract a lot of different pollinator with respect to their origins. While South African species are pollinated by birds and long-tongued flies, South European and North African species are pollinated by bees and short-tongued flies (Claßen-Bockhoff, 2004). It was pointed out that short-tongued flies and bees with medium sized proboscis are effective visitors of flowers (Kugler1970). Of these groups, bees were placed by Vogel (1954) in the melittoid group. In our study, bees and flies, the main pollinator group in South Europe and North Africa, were identified as the visitors of flowers. Seven of the flower visitors of S. *sclarea* are members of Hymenoptera and one of them, a member of Diptera. All the effective visitors belong to Hymenoptera as 60.51% of the total visits are made by *X. violacea*, 25.42% by *A. bidentate bidentata*, 13.9% by *M. pilidens*, 0.12% by *A. mellifera* and 0.4% by *S. spirifex.* However, these pollinators were successful at only one kind of pollination. *Salvia* species have several different kinds of lever mechanisms. The difference in the upper arm of the lever carrying the productive thecae in this mechanism determines the position of the pollinator to load pollens (Grant, 1994). Most visitors of *S. sclarea* can't perform pollen loading on their thorax and abdomen using this leverage mechanism. Pollens can only be loaded onto *X. violacea* when it moves the lever mechanism of the stamens. *X. violacea* was reported to be the only effective pollinator of *S. sclarea* in previous studies (Macukanovic-Jocic et al., 2011). Ott et al. (2016) also reported that *Xylocopa* was the only active pollinator in *Salvia alpina*. This result emphasizes the importance of *X. violacea* in achieving a healthy population of *S. sclarea.* A similar situation has also been reported in *Salvia glutinosa*. Pittioni (1942) classified *S. glutinosa* as a "stenobomboid bumble bee food plant", the lever mechanism of which hits bumble bees on the back when foraging for nectar. Honey bees don't reach the nectar because of the shorter proboscis, and only try to collect pollen from these flowers but doesn't pollinate it.

The frequent of visits by *X. violacea* decreases with increasing wind speed thus; wind is a negative factor in the pollination biology of *S. sclarea.*

The frequent of *X. violacea* visits between 09:30-10:30 when nectar concentration is 0.3 mg and 15:30-16:30 when it is 0.5 mg are inconsistent. In the morning hours the visits by *X. violacea* are at the maximum but decrease to the minimum between 15.30 and 16.30 (Figure 4C). This result shows that the amount of sucrose in the nectar does not have a direct influence on frequent of *X. violacea* visits.

Controlled pollination experiments showed that *S. sclarea* is self-compatibile. Under natural conditions, half of the seed reproduction is due to autogamy, while half is pollinator activity dependent. With the activity of *X. violacea*, seed production success in *S. sclarea* is doubled. Similar results were found in *S. scleroides* and *S. verbenaca*, allowing this species to self-fertilize if no outcrossing pollen arrives to the stigmas (Jorge et al. 2015; Navarro 1997). Allogamy enables a larger gene pool of seeds, which is of great importance in terms of obtaining a healthy population in reproduction studies (Allard et al. 1968; Brown 1979; Hamrick et al. 1979; Clegg 1980; Loveless and Hamrick 1984; Van Delden 1985; Van Dijk et al. 1988).

Results indicate that, *S. sclarea* can be used to pollination dependent agriculture for attract pollinators and also nectar dependent honey production. However; for seed production and developing variety of *S. sclarea* needed to support *X. violacea* populations.

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