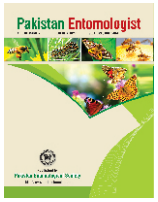
Pakistan Entomologist

Journal homepage: www.pakentomol.com



INVESTIGATIING THE ASSOCIATION OF MORPHOMETRIC POLLEN TRAITS OF VARIOUS *ECHIUM* SPECIES WITH POLLEN-VISITOR INSECT, *APIS MELLIFERA* IN JORDAN

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ARTICLE INFORMATION

Received: April 4, 2014

Received in revised form: November 6, 2014

Accepted: December 17, 2014

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ABSTRACT

Three *Echium* (purple bugloss) species were investigated palynologically by (LM) and (SEM). The three *Echium* species have 3- or 4-zonocolporate pollen class. Polar measurements ranged between 36-45 µm. *E. angustifolium* Miller had the smallest polar measurements (36–42 µm), While *E. glomeratum* Poiret and *E. judaeum* Lacaita had polar measurements in the range of 40–45 µm and 41–44 µm, respectively. Equatorial measurements ranged between 28-35 µm for *E. angustifolium*, 29-35 µm for *E. glomeratum* and 29-34 µm for *E. judaeum*. P/E ratio ranged between 1.5–1.42 for *E. angustifolium*, 1.42–1.30 for *E. glomeratum* and 1.43–1.25 for *E. judaeum*. *The results regarding* pollen outline indicated that the equatorial view for all *Echium* species was rectangular-elliptic; whereas, the polar view was triangular. P/E ratio was erect for all species examined. The morphology of ectoaperture structures (colpi, fastigium and margins) had reflected no variation. The three species exhibited short colpi with distinct to indistinct margins. The morphology of endoaperture structures (colpi, costae and margins) had reflected distinguished variation. *Echium* species had short colpi in general. Costae and margins showed some kind of variations. Honey bees (*Apis mellifera*) was the most observed and abundant especially on the flowers of *Echium* species in April due to presence of certain pollen-insect associations. Pollen grains of *Echium* species are considered to have a specialized relationship with some pollen visitors although having pollen and nectar as rewards for all pollen visitors. This kind of interaction between *Echium* species with specific pollen visitors (*A. mellifera*) found associated with morphometric characteristics of pollens, pollen-nectar as rewards to pollen visitors. Seasonality is considered as the ultimate factor reflecting different observed patterns of visitor distribution among plant flowers for the collection of pollen-nectar since *Echium* is producing a large amount of nectar and pollinferous material.

Keywords: Echium species, Honey bees, Jordan, Morphometric traits of pollens, Pollen-insect's interactions

INTRODUCTION

Jordan is located in unique position in the heart of the Middle East between longitudes 53^0 40' and 39^0 E, and between latitudes 29^0 30' and 34^0 N. This location of Jordan gives this country the opportunity to comprise at least four main different phytogeographical elements from north to south and from west to east (Mediterranean, Irano-teranean, Saharo-arabian and Nubo-sudanian). This kind of geographical variation also leads to geobotanical variation which makes the plant biodiversity in Jordanian territories ultimately high and reflects high spectrum of plant species widely producing different kinds of pollineferous dusts especially during spring pollination times as well as interconnects large variation of insects as pollen visitors between the wild and cultivated species, especially of Echium (purple bugloss) species (Bender, 1974; Karim and Al-Quran, 1986, 1988).

Echium as genus from Boraginaceae is widely distributed in

Cite this article as: Al-Quran, S., 2014. Investigatiing the association of morphometric pollen traits of various *Echium* species with pollen-visitor insect, *Apis mellifera* in Jordan. Pak. Entomol., 36(2):83-87.

Jordan and is well known for its ability to produce a vast quantities of nectar secreted from a certain secretory cells and white pollineferous material consisting pollen grains. Pollen morphology of *Echium* is considered an important morphological evidence for investigating various pollen characters including pollen class, polar and equatorial measurements, (P/E) ratio, pollen outline, endoaperture and ectoaperture structures. Such morphological characterizations and differentiation help to determine whether some palynological variations among the examined species exist (Zohary and Feibrun-Dothan, 1962-1986; Adam, 2001; Khayyat and Mursi, 1981; Peter, 1994; Prance, 2001; Punt *et al*., 1994).

The inflorescence of *Echium* species is similar to other boraginacean species. It is composed of many simple clusters of several flowers. The floral parts of this family consisting of calyx, corolla, androecium parts and gynoecium parts are typical features which enable the flowers of *Echium* genus to attract certain types of honey bees (*Apis mellifera*) during the pollination times. The presence of lobulated external purple hairs near the base of floral parts especially corolla, facilitate pollen visitors' attraction (Zohary, 1973; Anderson and Gensel, 1976; Boulos,1977; Al-Quran, 2005).

The relationships and ietraction between pollen morphology of *Echium* species and insect visitors, *A. mellifera* (honey bees) is surely reflecting some kind of specialized adaptation of association. Pollen morphology traits like pollen type, size of pollen grains, pollen shape, symmetrical structures between pollen grains and the pollen visitors, ectoaperture and endoaperture morphology of pollen grains, fastigia, colpi of pollen grains, margins of polar and equatorial measurements and P/E ratio play a major role in formulating any possible way of interactions. Honey bees (*A. mellifera*) is the most observed and abundant insect visitor especially on the flowers of *Echium* species in April. The lack of pollen-insect associations between the pollen grains of *Echium* and some of its pollen visitors is the most intrinsic factor to initiate this kind of investigation. The pollen grains of *Echium* are considered to have a specialized narrower and intimate spectra of insect visitors although having ample quantity of pollen and nectar as a source of attraction for other visitors. It seems that there exists an association among pollen visitors, pollen morphological characteristics, pollen-nectar and seasonality. The seasonality is also considered as a crucial factor reflecting different patterns of visitor distribution among plant flowers for the collection of pollen-nectar (Boulos and Al-Eisawi, 1977; Boulos and Lehham, 1977; Thorp, 1979; Stickel *et al*., 2000; Adailkan and Guathaman, 2001).

The palynological terms and expressions related to the structures and ultrastructural parts pollen grains seen by LM and SEM, respectively mainly focus on the ectoaperture and endoaperture of pollen grains. These structures are considered as a complex structures which deals with colpi, costae, fastigia and vary from species to species. For example, these structures are in form of spine-like projections in echinae and micro-echinae are considered as an important distinguishing character in delimitation among the species of the same genus or the subspecies of the same species (Sharma, 1974; Samways, 1990; Rodriguez *et al*., 1998; Al-Quran, 2010, 2011).

The objectives of this study in addition to what is mentioned above are: (1) to have a clear investigated image concerning the attractions between pollen visitors and the pollen grains of flowering *Echium* in a particular field area to identify subjected controlled factors controlling this association, (2) to understand the overlapping between the flowering periods of the attracted plant species and the collection activity periods of the pollen visitors in that area which is considered the most active role which plays to formulate such such kind of intimate relationship amongst the partners. (3) is *Echium* plant flower presents some kind of particular morphological and palynological characteristics to attract certain groups of pollen visitors over others. (4) understanding the flower rewards in form of nectar-pollen complex which is considered as the motivating factor that may play the majority in terms of such pollen visitor attractions. (5) are the flower shape, colour and odour playing inconspicuous role in formulating the inter-relationship between the pollen morphology and pollen visitors. It is helpful also in this regard to give finally an illustrated picture relating between the pollen grains of *Echium* species and the different honey bees as pollen visitors and collectors.

MATERIALS AND METHODS

Plant species

Three plant species including *E. angustifolium E. glomeratum* and *E. judaeum* were selected for this study because boraginacean plants especially *Echium* species are mainly dominated and most of them are entomophilous. This study was carried out in Ajlun area of northern heights of Jordan from April 2011 to April 2012 during the booming syndrome.

Insect species

Each *Echium* species show a close association with *Apis mellifera* (honey bee). That's why, *A. mellifera* was surveyed using net sweeping method. The specimen of *A. mellifera* were collected with entomological nets, brought into laboratory, identified and counted. The insect population data was then correlated and regressed for determining the type and nature of association between flowering phenology/pollen-traits and insect censuses.

Collection of polliniferous material and preparation of their slides for LM and SEM studies

Fresh polliniferous material was collected from three *Echium* species (*E. angustifolium* Miller, *E. glomeratum* Poiret and *E. judaeum* Lacaita) by using field collecting tools (field vials and small brushes) from different sites representing the different Jordanian territories. The collected material was acetolysis by the standard method described by Erdtman (1960). For preparation of slide, twenty five randomly chosen acetolysed pollen grains of each *Echium* species taken and five different slides (each carrying five pollen grains) were prepared to collect morphometric data of the pollen grains like pollen class, measurements of polar (P) and equatorial (E) views, P/E ratio, polar and equatorial outline views and ectoaperture as well as endoaperture structures (colpi, costae

and fastigia) by (LM). These morphometric data of pollens were analyzed statistically by for calculations of their arithmetic means and standard deviation. All the terminologies adopted were based on Punt *et al*. (1994). All the LM micrographs and measurements were recorded with Nikon HFX-11 microscope and ocular micrometer scale by using glycerin Jelly method. For SEM studies, the acetolysed pollen grains were coated by carbon layer first, then by gold layer to increase the conduction and electron yield rates. The micrographs were taken by SEM after dual coating of the pollen grains.

RESULTS AND DISCUSSION

The LM and SEM micrographs concerning the pollen grains of *Echium* (purple bugloss) species were present in Figure 1, 2 and 3with their full captions while palynological data concerning the traits and morphometric measurements of the pollen grains of *Echium* species were presented in Table 1 and 2. These results show that *Echium* pollen grains belonged to pollen class 3-zonocolporate for *E. angustifolia* and *E. glomeratum*, while associated with pollen class 4-zonocolporate for *E. judaeum* Lacaita. The polar measurements of pollens ranged from 36 to 42 µm for *E. angustifolia*, 40 to 45 µm for *E. glomeratum* and 41 to 44 µm for *E. judaeum*. The equatorial measurements of pollens were found in the range of 28-35 µm for *E. angustifolia*, 29-35 µm for *E. glomeratum* and 29-34 µm for *E. judaeum* (Table 1). The outline equatorial view of the studied species was elliptic for *E. angustifolia* and *E. judaeum* , while rectangular for *E. glomeratum*. The outline polar view was triangular, while P/E ratio was erect for all *Echium* species (Table 2). The morphology of ectoaperture structures (colpi , fastigium and margins) exhibited no variation amongst the *Echium* species. The three species exhibited short colpi with indistinct margins except for *E. angustifolia* (distinct margin). The morphology of endoaperture structures (colpi, costae and margins) have reflected distinguished variation in colpi length. The colpi were short for *E. angustifolia* and *E. judaeum*, while long for *E. glomeratum*. All *Echium* species had indistinct endoaperture margins. The endoaperture costae were present in pollen grains of *E. angustifolia* and *E. judaeum,* while absent in pollen grains of *E. glomeratum* (Table 2). *Echium* species as well as all boraginacean plants in Jordan produce a large amount of whitish polliniferous dust which constitutes a potential source of pollen grains and provides an interesting source of attraction for different species of honey bees. Insect-plant intimate interactions is primarily associated with the pollen morphology of studied species (long or short colpi, distinct or indistinct endoapertures and ectoapertures, presence or absence of fastigia and costae). The difference between polar (P) and equatorial measurements of pollen grains exhibited by an ecto- and endo- apertures morphology plays important role in pollen-visitor attraction.

The results regarding the traits of pollen grains of *Echium* species indicated the presence or absence of some morphological pollen ornamentations and variation in the size of the pollen grains. The occurrence of the little complex ornamentation in some species of *Echium* is highly related with the expulsion process of these grains inside of the poricidal anthers during anthesis process. This pollen grains expulsion process may be the crucial factor and play vital role in the execution anthesis through pollen visitor vibrations or "buzz pollination" by visiting honey bees. Depending on the same principle of expulsion, the large pollen grains of *Echium* species with high complex pollen endoaperture and ectoaperture ornamentations could form deposits that block the process of anthesis and hinder the expulsion of the pollen grains. These facts provide hypothesis about the presence of close relationship between the type and size of pollen morphology and the pollination syndrome by "buzz pollination". It is obvious that small sized pollen grains with little ornamentation are expelled more easily from the poricidal anthers during the vibration of the honey bees and vice versa (Erickson,1975; Thorp, 1979; Buchman, 1986; Adam, 2001; Al-Quran, 2004a,b).

A lot of previous workers investigated the interrelationship between *Vespa orientalis* L. and the pollen grains traits of *Anchusa* species. They reported that the collection of pollen grains by the pollen visitors were highly associated with pollen characteristics like ornamentations of pollen endo- and ecto- apertures. Their results also clarify the importance of the palynological data in better understanding of association between morphology of pollen grains and the visitors (Anderson and Gensel, 1976; Friedman *et al*., 1986; Pyle *et al*., 1991; Peter, 1994; Joud *et al*., 2001; Rates, 2001; Eddouks *et al*., 2002; Ricklefs, 2004). So the variation in ornamentation character of ecto- and endo- apertures morphology of pollen grains is due to phylogenetic relationships among the species (Al-Quran, 2004a).

The observations showed that the pollen visitors used the "buzz pollination" procedure during the foraging behaviour. All previous published studies report that "buzz pollination syndrome" requires bees with a specific behaviour for pollen removal which is typically applied to the bees from *Apis mellifera* species. The previous studies also provide evidences of such pollen collection strategy from pollen grains of *Anchusa* species by bees (Buchman, 1986; Adam, 2001). Other researches (Buchmann 1986; Erickson 1975; Thorp, 1979) demonstrated the presence of electrostatic forces, which facilitate the attachment of the pollen to the body of the insect at the moment of pollination by vibration, which facilitates finally its transference to the stigma (Buchman, 1986).

A. mellifera colonies are active foragers appeared in the study area early in the season on plants that show high densities and high nectar productions.

CONCLUSION

It is concluded that morphology of pollen visitors, pollen morphology, floral rewards in form of nectar-pollen and phenology construction are four aspects that must be considered for better understanding of the linkage among insect visitors, pollen morphology, floral rewards and flowering time of each plant species. These aspects also help to interpret concretely the reason of why does certain insects visit certain flowers? These results also indicate that flowers with similar reward composition tend to attract similar groups of visitors. The pollen diagnostic criteria as palynological evidence in taxonomy and delimitation among the three *Echium* species is not suitable criteria because some of the

Table 1

Pollen class, measurements of polar (P) and equatorial (E) views and P/E ratio of various *Echium* species.

Plant species	Pollen class	P (µm)	E (µm)	P/E
Echium angustifolium Miller	3-Zonocolporate	36-42	28-35	1.5-1.42
Echium glomeratum Poiret	3-Zonocolporate	40-45	29-35	1.42-1.30
Echium judaeum Lacaita	4-Zonocolporate	41-44	29-34	1.43-1.25

P = Polar; E = Equatorial

Table 2

Outline views, ectoaperture and endoaperture structures of various *Echium* species.

Plant species	Outline views			Ectoaperture			Endoaperture		
	E view	P view	P/E view	colpi	fastigium	margin	colpi	costae	margin
Echium angustifolium Miller	elliptic	triangular	Erect	s	+	d.	s	+	ind.
Echium glomeratum Poiret	rectangular	triangular	Erect	s	+	ind.	l	-	ind.
Echium judaeum Lacaita	elliptic	triangular	Erect	s	+	ind.	s	+	ind.

P = polar; E = equatorial; + = present; - = abscent; ind = indistinct; d = distinct; s = short; l = long

pollen structures and measurements seen by LM and SEM in this study can't be depend upon in this kind of delimitation. However, the morphological variations of pollen grains explain why do some pollen visitors are attracted to certain plant pollens more than the others. This kind of studies and other complementary studies should be intensified to have a better understanding of the existing relationships between the pollinators and all *Echium* species.

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