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ONOSMA (BORAGINACEAE) POLLEN INTERACTIONS WITH *BOMBUS TERRESTRIS* (BUFF-TAILED BUMBLEBEE) SPECIES IN JORDAN

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ABSTRACT

The relationships between pollen morphology of three *Onosma* species and their interactions with their insect visitors *Bombus terrestris* during springs seasons 2012 and 2013 were studied in Jordan. Pollen morphological characteristics (type and size of pollen grains, pollen shape, symmetrical structures between pollen grains and pollen visitors, polar and equatorial measurements, P/E ratios, structures of endo- and ecto- apertures) were investigated to formulate any possible way of interactions. Certain kind of bees *Bombus terrestris* (buff-tailed bumblebee) was observed as the most abundant visitor especially on the flowers of *Onosma* in April although many other honey bees like *Apis mellifera*, *B. impatiens*, *A. dorsata* and *A. florea* were observed but in very low frequencies**.** *Onosma* species in Jordan as all boraginacean species produces a large amount of whitish polliniferous dust, thus considered as a potential source for all pollen visitor species of bees, especially *B. terrestris* known as buff-tailed bumblebee. Three *Onosma* species were investigated palynologically by LM and SEM. The pollen class was either 3 zonocolporate (*O. aleppica* Boiss and *Onosma frutescens* Lam.) or 4-zonocolporate (*O. cinerea* Schreber). Polar measurements ranges were 37-43 µm for *Onosma aleppica* Boiss, 42-45 µm for *O. cinerea* Screber and 41-44 µm for *O. frutescens* Lam. Equatorial measurement ranges were 26-34 µm for *O. aleppica* Boiss, 28-34 µm for *O. cinerea* Screber and 28-33 µm for *O. frutescens* Lam. P/E ratio ranged between 1.41 – 1.39 for *O. aleppica*, 1.49-1.33 for *O. cinerea* Schreber and 1.45-1.28 for *O. frutescens* Lam. The results of pollen outline indicated that equatorial view for all *Onosma* species was rectangular - elliptic; the polar view was triangular for all species. P/E ratio view was erect for all species examined. The most important diagonistic factor in recognition of pollen morphology of *Onosma* species was in the endoaperture and ectoaperture which showed some kind of variation reflected on their pollen visitor association. The morphology of ectoaperture structures (colpi , fastigium and margins) for the three species exhibited short colpi, absence of fastigium with indistinct margins for *O. aleppica*, long colpi, presence of fastigium and indistinct margin for *O. cinerea* Schreber and short colpi, presence of fastigium and distinct margin for *O. frutescens* Lam. The morphology of endoaperture structures (colpi, costae and margins) reflected also distinguished variation. *O. aleppica* showed short colpi, presence of costae and indistinct margin. *O. cinerea* showed short to long colpi in general, absence of costae and distinct margins.

Keywords: *Bombus terrestris*, Boraginaceae, Jordan, *Onosma*, Pollen interactions

INTRODUCTION

Onosma as genus from boraginaceae is well known and widely distributed in Jordan, which is located in unique position in the heart of the Middle East between longitudes 53^0 40' and 39^0E, and between latitudes 29^0 30' and 34^0 N. This location of Jordan gives this country extraordinary opportunity to comprise at least four main different phytogeographical elements from north to south and from west to east (Mediteranean, Irano-Teranean, Saharo-Arabian

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and Nubo-Sudanian). Surely, this leads to geobotanical variation which enriches the plant biodiversity in jordanian territories and reflects over the high spectrum of plant species. *Onosma* is widely producing different kinds of pollineferous dusts especially during spring pollination times, which interconnected large variation of insects especially buff-tailed bumblebees as pollen visitors between the wild and cultivated species (Bender, 1974; Al-Quran, 2010, 2011).

Onosma genus has unique potential in production of a vast quantities of nectar-pollen complex secreted from a certain secretory cells mixed with white pollineferous material attracting different kinds of hymenopteran species. The investigation of pollen morphology of *Onosma* is considered very important morphological evidence to investigate some aspects related to pollen class, endoaperture, ectoaperture structures, polar and equatorial measurements, P/E ratio and pollen outline shape and structure, to see whether some palynological variations among the examined species exist and rely on for such characterizations and differentiation among the *Onosma* species having the role for such attraction between the pollen grains and their visitors especially *Bombus terrestris* (Zohary and Feibrun-Dothan, 1962-1986; Khayyat and Mursi, 1981; Peter, 1994; Punt *et al*., 1994; Adam, 2001; Prance, 2001).

The floral parts of *Onosma* species consist of gametophytic male structures represented by androecium and gametophytic female structures represented by gynoecium located in the central part of the flowers surrounded externally by perianth parts represented by calyx and corolla. This kind of floral arrangement is typically found as general morphological shape of this family enabling the flowers of *Onosma* to attract certain types of buff-tailed bumblebees (*B. terrestris*) during the pollination times. Also the inflorescence of *Onosma* is similar to other boraginacean and composed of many simple clusters of several flowers. The presence of colored lobulated external purple glandular trichomes and hairs in form of papillae near the base of floral parts especially corolla, is considered the source of nectar production and has potential to release high quantities of nectar-pollen substrate to facilitate this kind of pollen visitors' attraction (Zohary, 1973; Anderson and Gensel, 1976; Boulos, 1979; Al-Quran, 2005).

All the palynological terms and definitions used in general in such studies are related to the structures seen by LM and ultrastructural parts of the pollen grains seen by SEM and are focusing in the ectoaperture and endoaperture of pollen grains. These structures are considered as a complex structures especially which deals with colpi, costae, fastigia. These structures are vary from species to species. These structures which are similar to echinae and micro-echinae in form of spine-like projections giving the pollen exine an ornamental view may be investigated to see whether they can depend upon as an important distinguishing complimentary characters 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).

It is obvious from the previous studies that the lack of any pollen insect associations leads to understand the image between the pollen grains of *Onosma* and its pollen visitors like buff-tailed bumblebees, which was the most intrinsic factor to initiate this kind of investigation and in addition to that, these species are producing certain kinds of pollen grains visited by a different types of insects representing different orders and classes. Absolutely the floral structures of *Onosma* species with their pollen grains are considered to have the potentiality to form this kind of specialized intimate relationship with pollen visitors to reinforce this association with pollen and nectar as rewards for the visitors. Doubtless, there was a correlations representing similarities which led to some kind of superimposing among the anther structures as the source of pollen grains, corolla bases as the source of nectar and the pollen visitors attracted. So these pollen morphological characteristics and the pollen-nectar as rewards to pollen visitors form what is similar to blooming time. All previous works are stressing on the seasonality followed by the different pollen visitors of insects as the ultimate factor of initiation reflecting different observed patterns of visitor distribution among the examines species of *Onosma* for the collection of pollen-nectar as potential rewards introduced by these plant flowers (Al-Eisawi, 1977; Boulos, 1977; Boulos and Lehham, 1978; Thorp, 1979; Stickel *et al*., 2000; Adailkan and Guathaman, 2001).

The formulation of pollen-insect association between the insects and the pollen morphology of *Onosma* species help to explore their interactions with insect visitors *B. terestris* (buff-tailed bomble bees) and surely reflect some kind of specialized structural adaptations. Pollen morphology traits regarding ectoaperture, endoaperture morphology of pollen grains, fastigia, colpi of pollen grains, margins of polar and equatorial measurements, pollen type, size of pollen grains, pollen shape, symmetrical structures between pollen grains and the pollen visitors, and therefore P/E ratio are playing a major rules to formulate any possible way of interactions. Buff-tailed bomble bees (*B. terrestris*) was the most observed and abundant especially on the flowers of *Onosma* in April.

Four main objectives of this study were: (1) to speculate whether *Onosma* plant flower having some kind of particular morphological and palynological characteristics to attract certain groups of pollen visitors over others; (2) to speculate whether the understanding of flower rewards in form of nectar-pollen complex is considered as the motivating factor for that may play the major role of such pollen visitor attractions; (3) to speculate whether the attractions between the pollen visitors with the pollen grains of flowering *Onosma* particularly in the field area have a clear investigated image concerning this kind of association.

MATERIALS AND METHODS

The collected fresh polliniferous material from different sites representing the different jordanian territories, belonging to three *Onosma* species (*Onosma aleppica*, *Onosma cinerea* Schreber and *Onosma frutescens* Lam.) by using field collecting tools (field viales and small brushes). This material was acetolysed typically according to Erdtman (1960) standard method. So twenty five (25) randomly chosen acetolysed pollen grains of *Onosma* species from five (5) different slides were prepared for pollen measurements by LM. All palynological measurements related to the pollen morphological structures with their statistical calculations were estimated for the morphometric data. All the terminologies adopted were based on Punt *et al*. (1994). All

LM micrographs and measurements were taken by using Nikon HFX-11 microscope and ocular micrometer scale by glycerin Jelly method. For SEM studies, the treated pollen grains were coated by carbon layer first andthen by gold layer to increase the conduction and electron yield rates. The micrographs of pollens were then taken by SEM.

All observations were taken in the area of study of Ajlun in northern heights of Jordan from April 2011 to April 2012 during the booming syndrome The methodology used for recording data from plant species *Onosma* was according to visitors and pollen counts, photographic records and specimens collection with entomological nets.

This study of plant-insect interaction was conducted in the plant communities mainly dominated by certain types of boraginacean plants especially *Onosma* species and most of them are commonly known as entomophilous plant species. Each *Onosma* species studied and visited regularly by pollen visitors showed some kind of correlation between flowering phenology and insect censuses with buff-tailed bumble bees (*Bombus terrestris*). The pollen grains were collected and photographed by LM and SEM.

RESULTS AND DISCUSSION

Plates of all LM and SEM micrographs taken for the pollen grains of *Onosma* species were prepared and reported with their full captions (Fig. 1, 2 and 3). All palynological data concerning the observations and measurements of the pollen grains of *Onosma* species were constructed (Table 1 and 2). The data were collected in two main categories: (1) measurements concerning the pollen morphology of the examined *Onosma* pollen grains (2) all observations collected from the field related to pollen-insect interactions concerning the pollen of *Onosma* species and buff-tailed bomble bees as pollen visitors. Related to the first category; the pollen class of the examined *Onosma* species was either 3- zonocolporate (*O. aleppica* and *O. frutescens*) or 4-zonocolporate (*O. cinerea*). Polar measurements ranged from 37-43 µm for *O. aleppica*, 42-45 µm for *O. cinerea* and 41-44 µm for *O. frutescens*. Equatorial measurement ranged from 26-34 µm for *O. aleppica*, 28-34 µm for *O. cinerea* and 28-33 µm for *O. frutescens*. P/E ratio ranged between 1.41 – 1.39 for *O. aleppica*, 1.49-1.33 for *O. cinerea* and (1.45-1.28) for . *frutescens* (Table 1). The morphology of ectoaperture structures (colpi , fastigium and margins) exhibited short colpi, absence of fastigium with indistinct margins for *O. aleppica*, long colpi, presence of fastigium and indistinct margin for *O. cinerea* and short colpi, presence of fastigium and distinct margin of *O. frutescens* (Table 2). The morphology of endoaperture structures (colpi, costae and margins) also reflected distinguished variation. Among three plant species, *O. aleppica* showed short colpi, presence of costae and indistinct margin, *O. cinerea* showed short to long colpi in general, absence of costae and indistinct margins, whereas, *O. frutescens* exhibited short colpi, presence of costae and distinct margin (Table 2). Pollen outline were also recognized. The equatorial view was rectangular - elliptic; the polar view was triangular and P/E ratio view was erect for all *Onosma* species (Table 2). The most important diagonistic factor in recognition of pollen morphology of *Onosma* species is in the endoaperture and ectoaperture which showed some kind of variation reflected on their pollen visitor association.

With respect to the second category; the insect-plant initiation of interactions is referred to the pollen morphology of studied species (long or short colpi, distinct or indistinct endoapertures and ectoapertures, presence or absence of fastigia and costae). Also *Onosma* species in Jordan as well as for all boraginacean pollen grains produces a large amount of whitish polliniferous dust and constitutes a potential source of pollen for different species of buff-tailed bumble bees, providing an interesting field for attraction of such insects. Depending on the previous palynological results and measurements obtained from pollen grains of *Onosma* studied, it obviously cannot be relied on delimitations of *Onosma* species at species level, but surely can be relied on to explain the intimate relationship between the morphology of pollen grains and pollen visitors of *B. terrestris*. To explain this point clearly, it is very important to reconize the presence of such differences between the pollen grains related to polar (P) and equatorial measurements of pollen grains exhibited by an ecto- and endo- apertures morphology which is playing the role of variations in pollen-visitor attraction among the different species of *Onosma*. This was seen clearly in most of the pollen-insect communities studied where the presence of specialized and intimate insect-flower associations refers to complicated *Onosma* flower morphologies which hinders or facilitates this kind of visitation depending on the species visited by wide range of other insect groups. So it is clear that *B. terrestris* colonies are active approximately in all over the year seasons extending from winter to summer to late autumn to collect as large possible quantities of nectar as they can. So these foragers appeared in the study area early in the season on plants that show high densities and high nectar productions especially on *Onosma* flowers.

In more details, the investigated pollen grains of *Onosma* were indicating the presence or absence of some morphological characters especially in the pollen ornamentations. The size of the pollen grains and the occurrence of pollen ecto-and endo-apertures of some species of *Onosma* are highly related to the shedding process of these grains inside of the poricidal anthers during anthesis process. The shedding process is executed through pollen visitor vibrations or "buzz pollination" by visiting buff-tailed bomble bees. Exactly relying on the same principle of expression, larger pollen grains of *Onosma* species with high complex pollen endoaperture and ectoaperture ornamentations block the anther anthesis to expel the pollen grains. So in this way, it is worthy to assure the presence of close relationship between the type and size of pollen morphology and the pollination syndrome by "buzz pollination" reinforcing the observations collected from the fields. It is obvious that small sized pollen grains with little ornamentation will be expelled more easily from the poricidal anthers during the vibration of the honey bees (Erickson, 1975; Edmond, 1984; Adam, 2001; Al-Quran, 2004a, 2004b; Buchman, 1986; Thorp, 1979).

The observations and data collected showed the presence of other types of insects like honey bees (Apis mellifera), *B. impatiens*, *Apis dorsata* and *A. florea* and wasps from *Pterygophorus* species but in low frequencies. All the pollen visitors observed used the "buzz pollination" procedure

during their foraging behaviour. The previous published studies report that "buzz pollination syndrome" requires hymenopteran species with a specific behavior for pollen removal which is typically applied to the bees from *A. mellifera* and *B. terrestris*. It is important to demonstrate the importance of this kind of association between the partners to improve the apiculture management in honey. So the previous researchers were trying to provide evidences of such pollen collection strategy from pollen grains of *Onosma* species by such kind of bees (Buchman, 1986; Adam, 2001). There was a lot of previous workings investigating on similar aspects of interrelationship of *Vespa orientalis* L. on the pollen grains of *Anchusa* species. The results obtained from the previous workings approved these results obtained since they showed that the collection of pollen grains by the pollen visitors due to such pollen characteristics related to the ornamentations of pollen, endo-and ecto-apertures and these results consequently are clarifying the importance of the palynological data to understand. (Pyle *et al*., 1991; Rates, 2001; Peter, 1994; Ricklefs, 2004; Anderson and Gensel, 1976; Friedman *et al*., 1986; Joud *et al*., 2001; Eddouks *et al*., 2002).

Edmonds (1984) documented that morphological variations in pollen grains of the genus *Solanum* (Solanaceae) reflected no any significant morphological variations, because they refered only to exine ornamentation level related to the certain structures of ecto-and endo-apertures morphology, similar to what is happening in *Anchusa* species.The variation in ornamentation of ecto-and endo-apertures morphology of pollen grains is considered the crucial factor determining the phylogenetic relationships among the species in addition to genomic combinations, isolation and speciation. This result is confirmed by Al-Quran (2004a) through his study of pollen grains of 11 species of *Hyoscyamus* collected from different parts of Jordan. Other researches (Erickson, 1975; Thorp, 1979; Buchmann, 1986) further demonstrated the presence of certain electrostatic forces facilitating the attachment of the pollen to the body of the insect at the moment of pollination by vibration through the blooming syndrome, which facilitates finally its transference to the stigma.

Table 1

Pollen class, measurements of polar, equatorial views and P/E ratio of different *Onosma* species.

Plant Species	Pollen class	P (µm)	E (µm)	P/E
Onosma aleppica Boiss	3 - Zonocolporate	37 - 43	26 - 34	1.41 – 1.39
Onosma cinerea Schreber	4 - Zonocolporate	42 - 45	28 - 34	1.49 – 1.33
Onosma frutescens Lam.	3 - Zonocolporate	41 - 44	28 - 33	1.45 – 1.28

P = polar; E = equatorial; P/E = polar- equatorial ratio

Table 2

Outline views, ectoaperture and endoaperture structures of different *Onosma species*.

Plant Species	Outline			Ectoaperture			Endoaperture		
	E view	P view	P/E view	Colpi	Fastigiu	Margin	Colpi	Costae	Margin
Onosma aleppica Boiss	rectangularelliptic	triangular	Erect	s	-	ind.	s	+	ind.
Onosma cinerea Schreber	elliptic	triangular	Erect	I	+	ind.	s-I	-	ind.
Onosma frutescens Lam.	elliptic	triangular	Erect	s	+	d.	s	+	d.

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

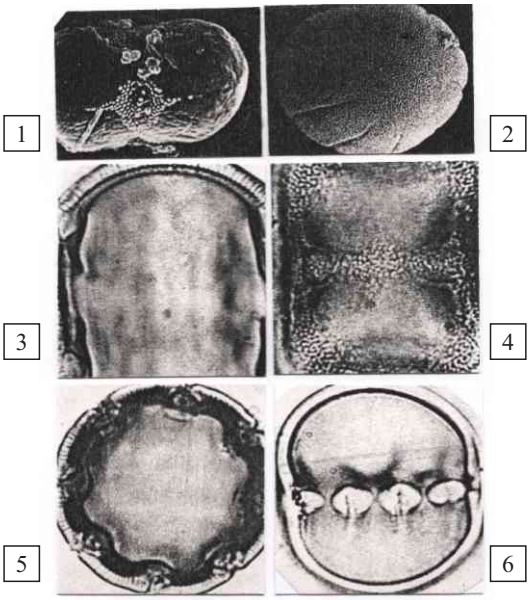

Fig . 1

Plates representing SEM micrograph of pollen grains of *Anchusa aegyptiaca* (1800 X; polar view) (1) and *Anchusa aegyptiaca* (1900 X; polar view) (2) while LM micrograph of pollen grains of *Anchusa itaica* (1000 X; equatorial view) (3), *Anchusa strigosa* (1100X; equatorial view) (4), *.Anchusa itaica* (1200X; polar view) (5) and *Anchusa strigosa* (1160X; equatorial view) (6).

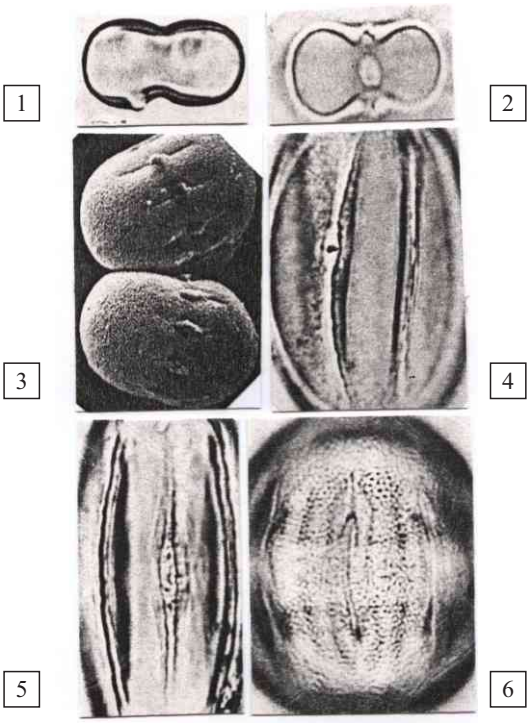

Fig . 2

Plates representing LM micrograph of pollen grains of *Anchusa aegyptiaca* (1100 X; polar view) (1), *Anchusa itaica* (1200 X; polar view) (2), *Anchusa aegyptiaca* (1100 X; equatorial view) (4), *Anchusa itaica* (1000 X; ornamentation in focus) (5) and *Anchusa aegyptiaca* (1100X; ornamentation in focus) (6) while SEM micrograph pollen grains of *Anchusa strigosa* (1700 X; equatorial view) (3).

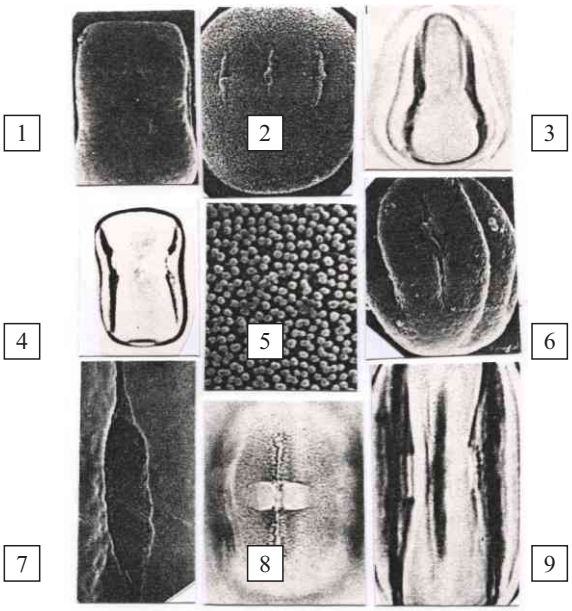

Fig. 3

Plates representing SEM micrograph of pollen grains of *Anchusa itaica* (1900 X; polar view) (1), *Anchusa itaica* (1900 X; with ornamentation) (2), *Hyoscyamus reticulatus* (2220 X; endoaperture colpi) (5), *Anchusa aegyptiaca* (L.) (2500 X; 3-Zonocolporate with colpi ends) (6) and *Anchusa itaica* (1250 X; equatorial view) (7) while LM micrograph of pollen grains of *Anchusa itaica* (1100 X; equatorial view) (3), *Anchusa aegyptiaca* (1000 X; 3 –Zonocolporate) (4), *Anchusa aegyptiaca* (L.) (1030X; equatorial view) (4) and *Anchusa strigosa* (1100X; polar view) (9).

CONCLUSION

The previous results obtained by the researcher have showed the presence of four different aspects that must be taken in consideration to reach a better understanding of the linkage between pollen morphology of *Onosma* and the pollen visitor (*B. terrestris*): (1) phenology construction between the partners (2) morphology of the pollen visitors, (2) pollen morphology and (3) nectar-pollen rewards given by the flower to the pollen visitor. In other words, the better understanding of importance between pollen morphology, floral rewards and flowering time of each plant species is very important to interpret why certain insects are attracted to a certain flowers but not to others. These kind of studies along with other complementary studies should be intensified further to have a better understanding of the existing relationships between the pollinators and the attracted species to justify the occurrence of pollination syndrome by vibration found in the observed bees in form of "buzz pollination".

Finally, the main conclusion is that small pollen grain, ornamentation aspects in ecto-and endo-apertures and psilated exine all together give the outline of pollen

morphology that allow pollen grain access by small sized bees as observed with buff-tailed bumble bees species.

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