



Full length article

Pollination system and effective pollinators of *Argania spinosa* (L. Skeels)

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ABSTRACT

This work deals with *Argania spinosa* breeding system and its associated pollinators in two different sites located in Souss valley. The main objective of this work was to study the effect of three pollination treatments on the percentage of fruit set of argan tree as well as to determine its potential pollinators. To study the breeding system, three treatments were applied for flower buds. The first treatment is an open pollination for insects and wind, the second one concerns only wind pollination, and the third one is a self-pollination. On the other hand, flower visitors were assessed to evaluate their frequency (F) and their activity rate (AR) which were exploited to calculate the index of visitation rate ($I_{VR} = F \times AR$) for each visitor. Pollen loads were also counted for the main pollinators as well as their foraging behavior. A highly significant fruit set was obtained from insect open pollination compared to the other treatments ($p < 0.001$), which ensures up to 88.05% of fruit set. However, 7.94% and 4.01% of fruit set were obtained from wind pollination and self pollination respectively. Forty three species visit argan flowers at both sites (43 insects visitors in Belfaa vs 38 in Azrarag). Highly significant frequency for Hymenoptera was recorded in Azrarag 53.2% than Belfaa (25.4%). On the other hand, a highly significant frequency for Diptera was recorded in Belfaa (61.9%) than Azrarag (36%). However, no significant difference was recorded for Coleoptera frequency in both sites ($P = 0.08$). Based on pollen loads and I_{VR} , *Apis mellifera*, Halictidae bees, and Syrphidae species were the most pollinators for the argan tree. I_{VR} computed for *A. mellifera* and their pollen loads were highly significant compared with other visitors. Breeding system of argan tree is closely dependent on insect pollination. Management of argan forest and orchards should be designed to increase and preserve insects community.

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1. Introduction

Argania spinosa is an endemic tree in Morocco. The argan forest has been declared as Biosphere Reserve in 1998. Argan tree has an essential role in the ecology, economy, and social relations of the local communities (Ait Aabd, 2019). The agro-sylvo-pastoral sys-

tem in this biosphere reserve is based on four major productions: goat, barley, wood and argan oil (Bourbouze and El Aïch, 2005). In spite of their ecological and socio-economic importance, argan tree pollination system is poorly investigated. The preliminary study conducted by Mouhaddab et al. (2016) states that argan is self-incompatible. Self-incompatible plants present high dependence on pollinators for sexual reproduction (Richards, 1997). Consequently, there is a lack of information regarding to the rate of each pollination type on fruit set and their eventual associated pollinators. According to Kevan (1999), pollination is a pivotal process in almost all productive terrestrial ecosystems including those dominated by agriculture, because they are in the front line of sustainable productivity through plant reproduction. Many studies link the importance of pollination (and other mutualisms) to plant conservation (Kearns et al., 1998; Spira, 2001; Gascoigne et al., 2009; Watts et al., 2012; Barber and Gordon, 2015; Bailey and

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Kevan, 2017). Argan is a monoecious species whose flowers, small and hermaphroditic, occur either single or grouped (inflorescence of glomerul type) on the axils of the leaves and of the shoots or on the nodes of mature wood (Perrot, 1907; Benlahbil and Bani-Aameur, 2002). The number of glomerules and flowers per glomerule depends on rainfall and temperatures recorded during the flowering year (Bani-Aameur, 2000; Bani-Aameur, 2002). The flowering peak occurred during March–April for the most Argan trees (Benlahbil, 2003). According to Gallai et al. (2009) the worldwide economic value of insect pollination was €153 billion; the same study reported that insect pollination economic value in North Africa is € 4.2 billions (Gallai et al., 2009). Bees are the dominant taxon providing crop pollination services, but birds, bats, moths, flies and other insects can also be important (Elmqvist et al., 2010). *Apis mellifera* is the most frequent floral visitor in natural habitats worldwide (Hung et al., 2018). In addition, *A. mellifera* is considered the most frequently used pollinator for the vast majority of the agriculture crops that require insect pollination (McGregor, 1976; Free, 1993).

This study focused on two main objectives. The first objective is to study argan breeding system by comparing the different rates contributed by insect pollination, self-pollination and wind pollination on argan fruit set. The second objective is to assess insects visiting argan flowers and their identification as well as assessment of visitors frequency (F), their activity rate (AR), their index of visitation rate (I_{VR}) and argane pollen loads by the main visitors were computed in order to determine the potential pollinators of *A. spinosa*.

2. Materials and methods

2.1. Study sites

This work has been conducted in two argan tree sites in the South-west of Morocco (Fig. 1). The first site is an argan orchard at experimental farm of National Institute for Agronomic Research (INRA) located in Belfaa (30.0434 N, –9.55635 W). This orchard (2

Ha) has been planted in 2010 as an experiment of argan tree domestication at a density of 150 trees/hectare. The argan trees height ranged from 3 to 5 m. The drip irrigation and organic manures were applied as needed. The second site belongs to argan forest located in the Azrarag argan forest (30.47915 N, –9.47609 W) and the trees size ranged from 2 to 6 m high. In this site, argan is a source of income for the local population. The extracted almonds from argan fruits may be sold directly or used for oil extraction and its derivative products are sold locally or exported.

2.2. Pollination treatment

To evaluate the effect of wind, insects, as well as the rate of self-pollination on *A. spinosa* fruit set, three treatments were applied in a randomized complete block design (RCBD) for flower buds of eleven trees during spring 2018. The first treatment is an open pollination to insects and wind. The second treatment concerns only wind pollination; buds flowers were bagged before anthesis with fine-mesh nylon material (width 250 μm) that excluded insects but allowed access to airborne pollen whose size varied from 17 to 32 μm (Bani-Aameur, 2002). The third one is a self pollination in which flower buds were enclosed in paper bags to stop external pollen. Nine shoots of an average size of 30 cm were selected from each tree. Three replicates of each treatment were applied to the selected shoots containing 30 glomerules for each studied tree ($n = 33$; three replicates \times 11 trees). The number of fruits set was checked periodically for each tree and treatment.

2.3. Pollinators data collection and sampling

Data collection and samples were occurred on sunny and warm days (Temperature ranged from 23 to 28 °C) with little or no wind in April and May during two consecutive years (2018 & 2019). In both study sites, eight trees within 3 m of high average at a blossom peak were randomly selected. Each tree was observed during 15 min; thus, arrival and departure of insect-visiting flowers were recorded between 9.00 AM and 1.00 PM. Each site was visited four

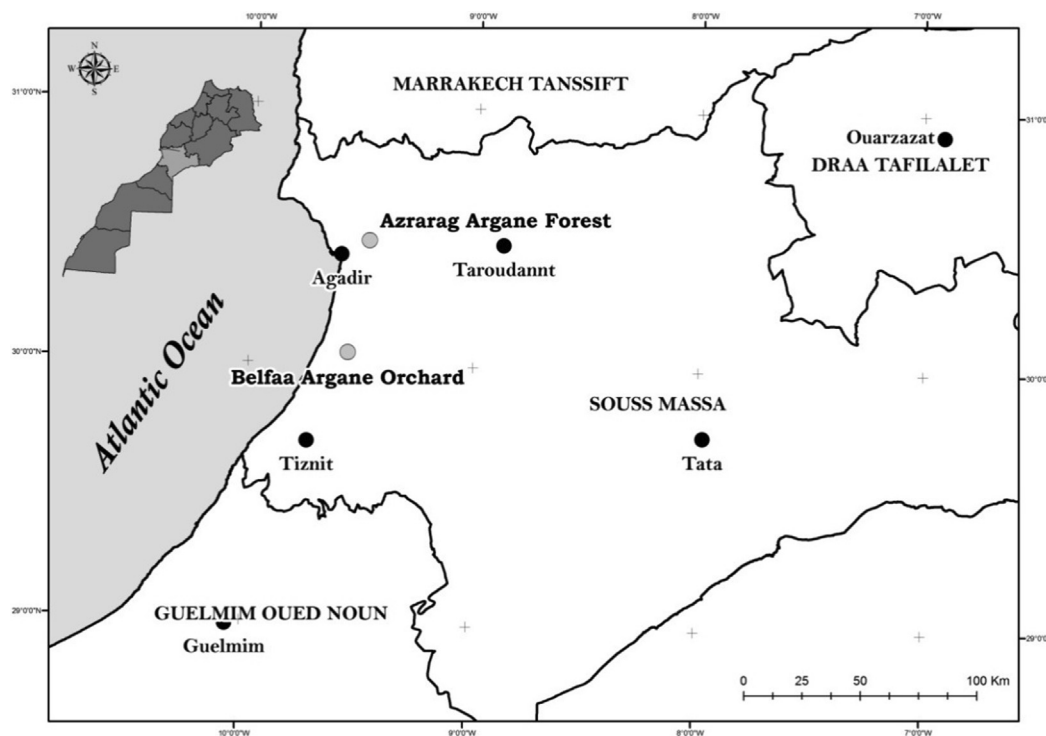


Fig. 1. Map representing the localization of the study sites (gray spot).

days. Data of 32 observations (observation during 15 min/tree) were registered in each site. Codes were attributing for the unknown species on the field. In order to assess pollen loads and identification of pollinator species, insects were captured using sweep net and mouth aspirator. Trapped insects were immediately transferred to laboratory and preserved in ethanol 70%. Unknown species were mounted and identified using stereo-microscope and specific keys to species or genus level in the Entomological Laboratory of Integrated Crop Production Unit (INRA, Agadir, Morocco). Additional samples were done to calculate Activity Rate (AR) which is the number of flowers visited per minute by visitors (we recorded number of flowers visited per minutes for at least twenty individuals of each species). Handling time was also calculated for each visitor (time spent for foraging a single flower was registered for each species ($n = 20$), and percentage frequency (F) of each visitor was calculated as mean number of each species relative to the total number of visitors in each study site ($\times 100$). In order to determine the potential pollinators, an index of visitation rate was calculated using AR and F ($I_{VR} = F \times AR$) according to Albano et al. (2009) and Talavera et al. (2001). On the other hand, the main visitors were tracked for at least three minutes to assess their behavior on argan flower (pollen and/or Nectar feed, foraging behaviors). Loaded pollen was extracted from pollinators according to the methodology adapted by Moisan-Deserres et al. (2014) and Scott et al. (2016). The specimen entire body (except scopa of bees) was placed into tubes containing 10 ml of 70% ethanol. The tubes were then vortexed for 30 s, and centrifuged at 3000 rpm for 12 min. The pellets obtained were dried at 40 °C. One ml of distilled water containing 0.5% of Tween 20 was added to dried pellets. Pollen counting was done by adding 10 μ l on hemacytometer under microscope.

2.4. Data analysis

Statistical analyses were performed using the Statistica 6.0 software. The analyzed parameters were argan fruit set resulted from each pollination treatment, the visitation rate index (I_{VR}), percentage frequency of visitors, and pollen loads. Obtained data were subjected to One-way ANOVA test at $p < 0.05$ for each taxon comparing the both studied sites separately. If significant differences were found, the parameters mean values were then compared using Tukey's HSD post hoc at 95% confidence limit.

3. Results

3.1. Pollination mode

Monitoring the growth and maturation of argan fruit allowed us to obtain results for the three treatments for each tree. The statistical analysis of the results by one way ANOVA without taking into account the heterogeneity of the trees showed a high significant difference between open pollination and the two other treatments ($n = 33$, $p < 0.0001$). However, there is no significant difference between wind and self pollination ($n = 33$, $p > 0.05$) (Fig. 2). In addition, entomophilous is the most dominating pollination mode in *A. spinosa*. In this case, 88.05% of fruit set were provided by this mode. The anemophily comes in second place, which participated by 7.94% on fruit set. However, self-pollination provides 4.01% of fruit set (Fig. 3). The obtained results were exploited to highlight the eventual effect of the three pollination treatments on fruit set for each studied tree. The result of this analysis shows that seven trees (T1, T3, T6, T7, T8, T10, and T11) showed a very highly significant difference between open pollination and the two others. On the other hand, no significant difference was recorded for the

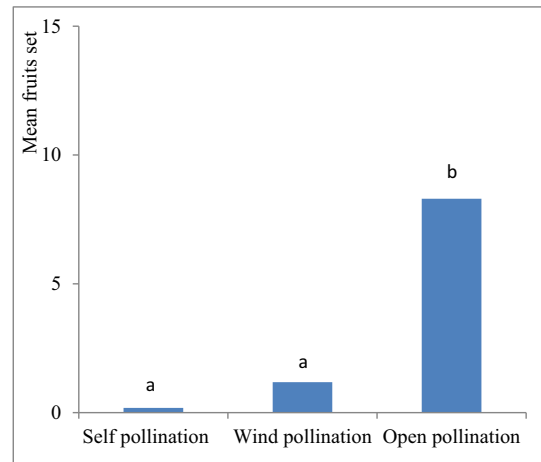


Fig. 2. Mean of fruit set obtained from 30 glomerules. Different letters denote significant difference (Tukey's HSD test, at $p < 0.05$).

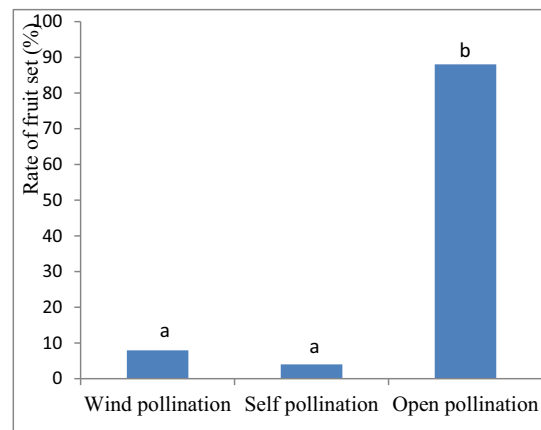


Fig. 3. Mean percentage of fruit set obtained from each pollination treatment for all studied trees (data pooled). Different letters denote significant difference (Tukey's HSD test, at $p < 0.05$).

other remaining trees (T2, T4, T5, and T9), did not show any significant difference between the three pollination treatments (Fig. 4).

3.2. Argan flower visitors

Argan flower attracted a large spectrum of insect visitors. In total, forty three species from Hymenoptera, Diptera, Coleoptera, Lepidoptera and Hemiptera visit argan flower in both studied sites. Forty three visitors taxa were recorded in Belfaa orchard vs only 38 in Azrarag forest. Diptera was the most abundant group in Belfaa within a frequency of 61.9% followed by Hymenoptera (25.4%). However, Coleoptera and Lepidoptera were less abundant and their frequencies are 9.2% and 3.5% respectively. In contrast, Hymenoptera were the most abundant taxon in Azrarag argan forest they represent 53.2% of total visitors, followed by Diptera (36%); while the last row is occupied by beetles (12.1%). No Lepidoptera have been recorded in this site. Moreover, computed frequencies for each order indicated that Hymenoptera frequency in Azrarag forest is highly significant than recorded frequency in Belfaa orchard ($p < 0.00001$). Diptera frequency observed in Azrarag forest was highly significant (61.9%) vs (36%) in Belfaa orchard ($p < 0.00001$). However, no significant difference was recorded for Coleoptera frequency in both sites ($P = 0.08$) (Fig. 5). Diptera were the most diversified visitors. This group was represented by

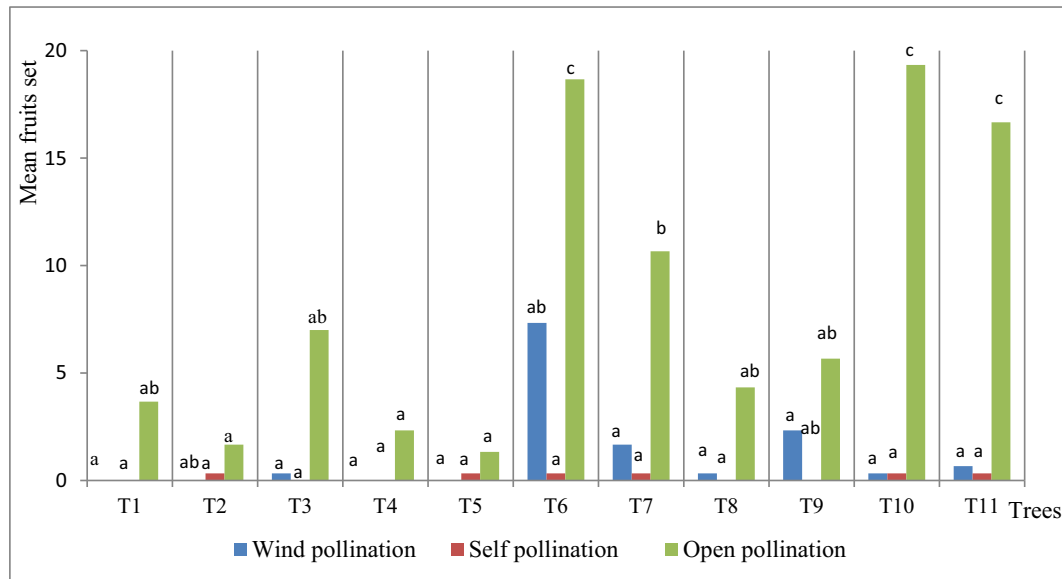


Fig. 4. Mean of fruit set from 30 glomerules per treatment by selected trees. Different letters denote significant difference (Tukey's HSD test, at $p < 0.05$).

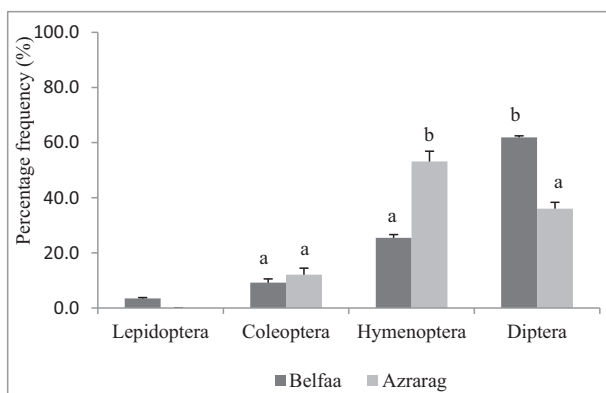


Fig. 5. Percentage frequency of main pollinators groups in two localities. Statistical analysis was performed for each taxon comparing the both studied sites. Different letters denote significant difference (Tukey's HSD test, at $p < 0.05$).

twenty two species belonging to thirteen families. Syrphidae and Calliphoridae were the most diverse families represented by five and four species respectively. Hymenoptera was the second important group, represented by eleven species belonging to seven families. Four species belonging to Halictidae, two Apidae (*Apis mellifera* and *Bombus terrestris*), one Braconidae (*Psytalia concolor*), and four ants (Formicidae) belonging to three subfamilies. While Coleoptera and Lepidoptera were less abundant and diversified visitors. Seven species of Coleoptera were recorded in both sites, and only one species of Lepidoptera (*Amata alicia*) was identified in Belfaa (Table 1).

3.2.1. Activity rate and visitation rate index

Activity rate means the number of flower visited by each taxon during one minute. A high activity rate was computed only for visitors belonging to Apidae, Halictidae and Syrphidae. Therefore, the highest AR was computed for *A. mellifera* (9.50 ± 1.79). The AR computed for Halictidae bees ranged from 3.70 ± 1.21 computed for *Nomioides* sp. to 6.10 ± 1.21 for *Lasioglossum sp1*. Among Diptera, the high AR was observed for Syrphidae. Within Syrphidae family, the high AR was computed for *Eristalis tenax* (6.10 ± 1.33), and the low value was computed for *Eristalinus taeniops* (4.85 ± 1.31). How-

ever, the AR recorded for the other Diptera visitors was low. On the other hand, all coleopteran visitors had a very low AR (< 0.1). The activity rate recorded by *Amata alicia*, the only Lepidoptera visitors, was relatively low (2.15 ± 1.09). Visitation rate index is a result of multiplication of two parameters (percentage frequency of each visitor and their activity rate). The high I_{VR} was computed for Hymenoptera order. The highest I_{VR} was computed for *A. mellifera* (Apidae) in Azrarag Argan forest (244.41 ± 41.14) and in Belfaa orchard (139.39 ± 11.47). Comparing I_{VR} computed for Hymenoptera species in both sites, a significant difference was found for *A. mellifera* ($P < 0.01$), *Lasioglossum sp1* ($P < 0.00001$) and for *Lasioglossum sp2* ($P < 0.001$). However, no significant difference was found for *Nomioides* sp. (Fig. 6). Among Diptera, the highest I_{VR} was computed for *Eristalis tenax* in Azrarag forest (23.59 ± 0.99), and for *Eristalinus taeniops* in Belfaa orchard (17.09 ± 3.12) (Table 1). Comparing I_{VR} calculated for the main Diptera visitors, a significant difference was reported between two sites for *E. tenax* ($P < 0.01$), *E. taeniops* ($P < 0.01$), and for *Lucilia* sp. ($P < 0.01$). No significant difference was observed for the other Syrphidae (Fig. 7). On the other hand, the low I_{VR} was computed for the other Diptera as well as for beetles in both sites. Their I_{VR} doesn't exceed 0.5 (Table 1).

3.2.2. Visitors behavior foraging

Most insect visitors start their foraging activity early during warm days. During foggy and cold mornings foraging activity decreases for several species. But as soon as the sun reappears, the visitors restart their normal activity. Hymenoptera were the most active visitors, their visit consist on short foraging which alternated between flower foraging and flying moment for researching further flower. Except *A. mellifera* that visit argan fruits probably for harvesting the latex, the other Hymenopterans visit only flower. In general a short handling time was recorded for Hymenopterans visitors, and the shortest handling time was registered for *A. mellifera* ($6.4 \text{ s} \pm 3.4$); while, the longest was observed for *Nomioides* sp (20.4 ± 5.3) (Table 1). After visiting a few flowers on the same argan tree that rarely exceeds 10 flowers, Halictidae and *A. mellifera* leave the tree to forage flowers of another argan-tree. Except ants that collect nectar only, several Hymenopterans visitors, foraged for collecting pollen and nectar. *Tapinoma magnum*, generally nesting under trees is very abundant; a long thread of workers was observed on some trees. However, *Camponotus*

Table 1

Argan flower visitors, and their mean (\pm SD) of activity rate (AR), Handling time, Frequency Percentage (F) and Index of Visitation Rate (I_{VR}) recorded in Belfaa orchard and Azrarag forest.

Taxon	AR (Mean \pm SD)	Handling time(S) (Mean \pm SD)	Purpose of visit	Azrarag		Belfaa		
family	Species			F (%)	I_{VR}	F (%)	I_{VR}	
Apidae	<i>Apis mellifera</i>	9.50 \pm 1.79	6.4 \pm 3.4	N/P	25.73 \pm 4.33	244.41 \pm 41.14	14.67 \pm 1.21	139.39 \pm 11.47
Halictidae	<i>Lasioglossum sp 1</i>	6.10 \pm 1.21	12.3 \pm 4.4	N/P	12.17 \pm 1.02	74.24 \pm 6.22	2.66 \pm 0.25	16.25 \pm 1.55
	<i>Lasioglossum sp 2</i>	5.10 \pm 1.17	13.1 \pm 4.5	N/P	11.02 \pm 2.17	56.21 \pm 11.08	3.01 \pm 0.33	15.37 \pm 1.67
	<i>Nomioides sp.</i>	3.70 \pm 1.21	20.4 \pm 5.3	N/P	2.14 \pm 1.39	7.91 \pm 5.13	1.65 \pm 0.13	6.10 \pm 0.46
Apidae	<i>Bombus terrestris</i>	---	---	N/P	0.36 \pm 0.41	---	0.11 \pm 0.13	---
Halictidae	<i>Halictus sp.</i>	---	---	N/P	0.00 \pm 0.00	---	0.05 \pm 0.11	---
Braconidae	<i>Psytalia concolor</i>	---	---	N	1.74 \pm 0.60	---	2.72 \pm 0.94	---
Dolichoderinae	<i>Tapinoma magnum</i>	---	---	N	---	---	---	---
Formicinae	<i>Camponotus sp.</i>	---	---	N	---	---	---	---
	<i>Camponotus brullei</i>	---	---	N	---	---	---	---
Myrmicinae	<i>Pheidole pallidula</i>	---	---	N	---	---	---	---
Syrphidae	<i>Eristalis sp.</i>	5.55 \pm 1.36	11.7 \pm 4.7	N	1.59 \pm 0.70	8.82 \pm 3.88	2.08 \pm 0.57	11.53 \pm 3.18
	<i>Eristalis tenax</i>	6.10 \pm 1.33	12.5 \pm 4.4	N	3.87 \pm 0.16	23.59 \pm 0.99	2.57 \pm 0.64	15.67 \pm 3.92
	<i>Eristalinus taeniops</i>	4.85 \pm 1.31	10.4 \pm 3.9	N	2.15 \pm 0.72	10.41 \pm 3.48	3.52 \pm 0.64	17.09 \pm 3.12
	<i>Simosyrphus aegyptius</i>	5.95 \pm 1.36	---	N	0.90 \pm 0.43	5.35 \pm 2.54	1.05 \pm 0.54	6.22 \pm 3.18
	<i>Syritta pipiens</i>	5.65 \pm 1.27	---	N	1.75 \pm 0.63	9.89 \pm 3.56	1.34 \pm 0.66	7.59 \pm 3.74
Calliphoridae	<i>Lucilia sp.</i>	1.90 \pm 1.65	9.5 \pm 4.1	N	3.17 \pm 0.90	6.01 \pm 1.72	6.22 \pm 0.73	11.81 \pm 1.38
	<i>Calliphora vomitoria</i>	2.35 \pm 1.42	12.1 \pm 4.7	N	0.33 \pm 0.38	0.78 \pm 0.90	1.58 \pm 0.07	3.72 \pm 0.17
	<i>Calliphora vicina</i>	2.70 \pm 1.84	12.2 \pm 5.6	N	0.51 \pm 0.34	1.39 \pm 0.93	1.06 \pm 0.21	2.87 \pm 0.56
	<i>Pollenia sp.</i>	1.20 \pm 1.11	---	N	0.68 \pm 0.54	0.82 \pm 0.65	1.31 \pm 0.30	1.57 \pm 0.36
tachinidae	<i>Admontia sp.</i>	1.90 \pm 1.41	7.0 \pm 3.6	N	1.58 \pm 0.81	2.99 \pm 1.53	3.47 \pm 0.62	6.58 \pm 1.19
Sarcophagidae	<i>Sarcophaga sp.</i>	1.70 \pm 1.42	---	N	0.00	0.00	1.58 \pm 0.07	2.69 \pm 0.13
	<i>Sarcophaga carnaria</i>	2.15 \pm 1.73	11.2 \pm 6.0	N	1.25 \pm 0.39	2.68 \pm 0.85	1.09 \pm 0.38	2.34 \pm 0.82
Anthomyiidae	<i>Anthomyia pluvialis</i>	1.30 \pm 1.26	---	N	1.77 \pm 0.47	2.31 \pm 0.61	1.73 \pm 0.50	2.25 \pm 0.52
	<i>Anthomyia sp.</i>	1.25 \pm 1.16	---	N	2.07 \pm 1.54	2.5 \pm 1.92	1.29 \pm 0.30	1.61 \pm 1.03
Rhiniidae	<i>Rhinia sp.</i>	<0.10	21.4 \pm 17.9	N	1.02 \pm 0.84	0.10 \pm 0.08	2.62 \pm 0.55	0.26 \pm 0.06
Muscidae	<i>Musca domestica</i>	1.50	11.6 \pm 6.9	N	2.49 \pm 0.79	3.73 \pm 1.18	7.32 \pm 0.38	10.97 \pm 0.56
Bombyliidae	<i>Geron sp.</i>	<0.50	---	N	2.46 \pm 0.70	0.25 \pm 0.07	2.45 \pm 0.29	0.24 \pm 0.03
Bibionidae	<i>Biblio sp.</i>	<0.50	---	N	0.00	0.0	0.91 \pm 0.18	0.09 \pm 0.02
Stratiomyidae	<i>Microchrysa sp.</i>	<0.50	---	N	1.56 \pm 1.23	0.16 \pm 0.12	1.80 \pm 0.51	0.18 \pm 0.05
Tephritidae	<i>Ceratitis capitata</i>	<0.50	---	N	2.63 \pm 1.03	0.26 \pm 0.10	3.66 \pm 1.08	0.37 \pm 0.11
Chloropidae	<i>Thaumatomyia sp.</i>	1.20 \pm 1.36	---	N	3.92 \pm 1.14	4.71 \pm 1.37	3.26 \pm 1.07	3.91 \pm 1.29
Diptera sp	<i>Non identified</i>	<0.10	>60	N	0.00	0.00	5.14 \pm 0.77	0.51 \pm 0.08
Drosophilidae	<i>Drosophilidae sp.</i>	<0.50	---	N	0.00	0.00	0.84 \pm 0.21	0.42 \pm 0.11
Miridae	<i>Dryophilocoris sp.</i>	---	---	N	---	---	---	---
Carabidae	<i>Calathus sp.</i>	<0.10	>180	P/PT	2.99 \pm 1.53	0.30 \pm 0.15	2.57 \pm 0.64	0.26 \pm 0.03
Dermestidae	<i>Anthrenus sp.</i>	<0.10	>180	P	6.64 \pm 1.25	0.66 \pm 0.13	3.78 \pm 0.25	0.38 \pm 0.08
Scarabaeidae	<i>Oxythyrea funesta</i>	<0.10	>180	P	0.89 \pm 0.66	0.09 \pm 0.07	0.28 \pm 0.20	0.03 \pm 0.02
Mordeliidae	<i>Mordella bipunctata</i>	<0.10	>120	P	0.16 \pm 0.33	0.02 \pm 0.03	0.28 \pm 0.09	0.03 \pm 0.01
Cantharidae	<i>Rhagonycha sp.</i>	<0.10	>120	P	0.00	0.00 \pm 0.00	0.27 \pm 0.20	0.03 \pm 0.02
Malachiidae	<i>Attalus sp.</i>	<0.10	>120	P	0.87 \pm 0.30	0.09 \pm 0.03	0.34 \pm 0.12	0.03 \pm 0.01
Chrysomelidae	<i>Bruchidius sp.</i>	<0.10	>120	P	0.36 \pm 0.41	0.04 \pm 0.04	0.51 \pm 0.08	0.05 \pm 0.01
Arctiidae	<i>Amata alicia</i>	2.15 \pm 1.09	27.4 \pm 19.0	N	0.00	0.00	3.40 \pm 0.29	7.31 \pm 0.62

(N: nectar; P: pollen; PT: petal tissue).

brullei, *Camponotus sp.* and *Pheidole pallidula* are less abundant. Unlike Hymenoptera, Diptera (except Syrphidae) visitors spend a long time on the same tree. Their foraging activity is discontinued;

their flower foraging is alternated with a rest period on the leaf or the stem. During this rest, the individual may stand still or make mating parry movements. Among Diptera, handling time varied according to the species; thus, a short handling time was recorded

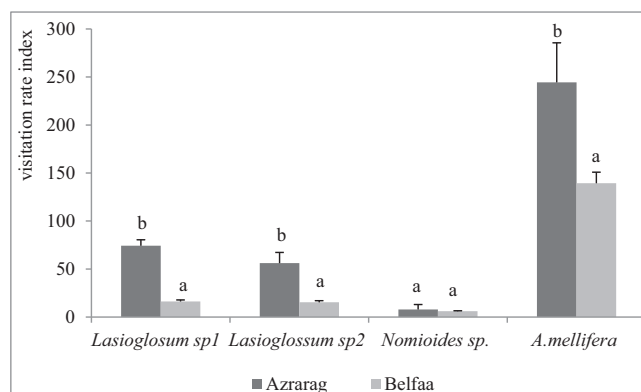


Fig. 6. Visitation Rate Index recorded for main hymenopteran pollinators in the two studied sites. Statistical analysis was performed for each taxon comparing the both studied sites. Different letters denote significant difference (Tukey's HSD test, at $p < 0.05$).

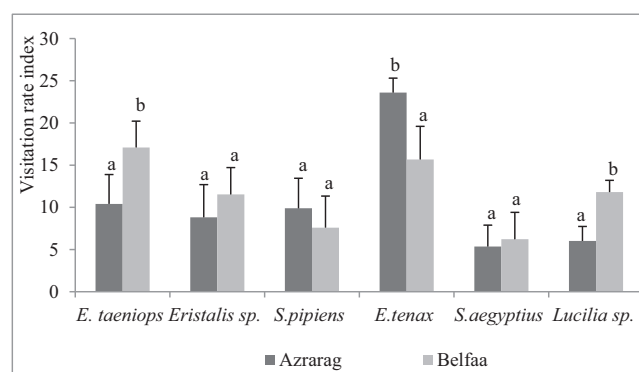


Fig. 7. Visitation Rate Index recorded for main dipteran pollinators in both studied sites. Statistical analysis was performed for each taxon comparing the both studied sites. Different letters denote significant difference (Tukey's HSD test, at $p < 0.05$).

for species belonging to Calliphoridae, Tachinidae, Syrphidae. However, a longest handling time was recorded for Rhiniidae (21.4 s \pm 17.9 s) (Table 1). All beetles collected on argan flower are pollen feeders. They were less active and rarely showed on flight action. Once installed on the flower, they start to feed on pollen. Therefore, beetles handling time was very long. It exceeds 120 s for *Mordella bipunctata*, *Rhagonycha* sp., *Attalus* sp. and *Bruchidius* sp., while it is over 180 s for *Calathus* sp., *Anthrenus* sp., and *Oxythyrea funesta*. The only Lepidoptera species, *Amata alicia* collected on argan flowers spent a long time on the same tree (>15 min). Their activity consists on slow movement between the branches of the same tree. Open dish and emerged pistil of flower during bloom period allows for all visitors to contact the stigma and anthers whatever the shape and size of their body. Except for Diptera mating competition, no competition between other pollinators was shown.

3.2.3. Pollen loads.

Pollen loads varied between argan flower visitors. The highest number of argan pollen was carried by *A. mellifera* (29689 \pm 10035) followed by Halictidae bees; *Lasioglossum* sp2 (11167 \pm 2020) and *Lasioglossum* sp1 (10833 \pm 1258). While the third position was occupied by hoverflies *E. tenax* (10500 \pm 1500) and *E. taeniops* (8000 \pm 1000). On the other hand, ants species as well as the several Diptera visitors and Hemiptera were poor pollen loads. The main beetle's visitors *Anthrenus* sp. and *Calathus* sp. loaded a high number of argan pollen than other Coleoptera; their pollen loads were 1044 and 6600 respectively (Fig. 8). *A. mellifera* carried a highly significant number of argan pollen than the other visitors ($p < 0.0001$) (Fig. 8).

4. Discussion

Open pollination treatment showed the importance of insect on *A. spinosa* pollination system. Our field experiments shows that 88.05% fruit set were obtained from insect pollination, vs 7.94% from anemophilous pollination and 4.01% from self pollination.

The obtained results showed that *A. spinosa* is an outcrossing and self incompatible species. Outcomes from our pollination experiments survey coincide with finding of Mouhaddab et al. (2016) study. Self incompatibility mechanism of *A. spinosa* not studied yet and therefore, no data are available for now. Nerd et al. (1998) study states that some degree of postzygotic discrimination probably occurs in argan. In contrast, Benlahbil et al. (2015) report a very low germination rate of pollen grains per stigma after self pollination, as well as a low rate of pollen tubes traveled 100% of the style length; as a result, self incompatibility barriers occur in stigma and style. Low fruit set obtained from wind pollination treatments may be explained by limited pollen spread by wind. Previous studies indicate that argan pollens spread over a short distance under the wind action (Nerd et al., 1998; Mouhaddab et al., 2016).

Despite argan's flowers are small with no attractive color - greenish to yellowish-, the spectrum of argan's flowers visitors is quite large, including members from five orders; Diptera, Hymenoptera, Coleoptera, Lepidoptera and Hemiptera. Some factors such as the high number of flowers, the availability and accessibility of pollen and nectar, as well as unspecialized flower traits and probably the soft scent emitted by these small flowers probably allowed to attract this wide spectrum of visitors. Diptera foraged on argan flower were the most diverse visitors in both studied sites and most abundant in Belfaa site. Inouye et al. (2015) study reported that Diptera are the most common visitors to flowers from a variety of plant families. Pollen and nectar were the main reward to argan flower visitors. Our field observations allowed to determine each visitors rewards. Therefore, all coleopteran feeds on pollen, except *Calathus* sp. that feed on petal too. Several Hymenoptera visitors' rewards are Pollen and nectar. However, Lepidoptera and several Diptera foraged for nectar (Table 1). According to Kevan (1999), pollen provides protein, lipid, carbohydrate, vitamins and minerals in various amounts depending on the plant species. Nectar is mostly a sugary solution and provides energy for locomotion, mostly flight. Argan flowers are hermaphrodites, therefore whatever the purpose of visit (pollen and/or nectar

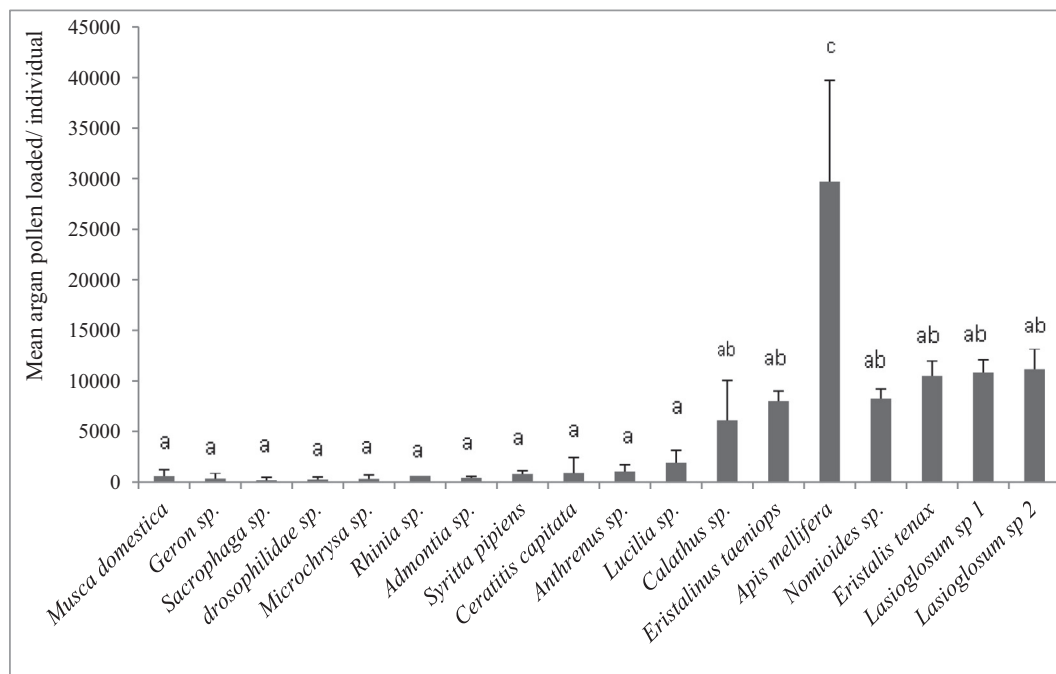


Fig. 8. Mean (+SE) argan pollen loads by main argan flower visitors sampled in Belfaa argan orchard and Azrararg argan Forest. Different letters denote significant differences (Tukey's HSD test, at $p < 0.05$).

feed) the visitors may touch both anthers and the stigma. Therefore, pollinator efficiency is determined by its I_{VR} that reflect activity forager and frequency of visitor with pollen load. Due to their very low activity rate (<0.1) as well as the low frequency for several species, I_{VR} computed for all visitors among Coleoptera group was very low, furthermore, all members of this group remain in the same flower for a long period (long handling time). Despite their relatively high frequency, *Anthrenus* sp. showed a low I_{VR} . Therefore, coleopterans pollination effectiveness on argan is very low. The same finding was reported elsewhere studying pollination of other plants (Albano et al., 2009; Talavera et al., 2001; Li et al., 2014; Adgaba et al., 2016). Moreover, Proctor et al. (1996) showed that beetles are poor pollinators, more often behaving as pollen feeder's but occasionally accidental pollinators. Due to their low frequency as well as their low AR, the I_{VR} computed for *A. alicia* was low. Thus, it can't gather with potential pollinators of Argane. In both sites, *A. mellifera* was the most frequent argan flower visitors, its I_{VR} was highly significant compared to other visitors. Its high I_{VR} resulted in high AR and frequency (F). The high AR assessed for *A. mellifera*, may be explained by its forager behavior ability and its short handling time. According to Eckert et al. (1994), individual foragers from high brood colonies collected larger pollen loads and nectar load, as well as the longer foraging trips. Moreover, Beekman and Ratnieks (2001) found a mean foraging distance of 5.5 km during August. On the other hand, the high number of argan flower could minimizing the time needed to find further flower. Abou-Shaara (2014) states that the availability of suitable plant resources has a great impact on foraging activity, and forager bees have a preference for some resources over others. At ambient temperatures of about 20 °C, the highest activity was recorded (Tan et al., 2012). *A. mellifera* was reported as the most important pollinator across the natural systems studies (Hung et al., 2018), as well as, the most pollinator used in managed pollination (McGregor, 1976; Free, 1993; Klein et al., 2007). Due to their relatively high AR and their frequency, the two *Lasioglossum* bees showed the second high I_{VR} in Azrarag Argan forest. Except, *Nomioides* sp and *Halictus* sp., frequencies recorded for other bees including *A. mellifera* in Azrarag which is a natural argan forest were highly significant than Belfaa orchard. This difference may explained either by the negative effect of intensive agricultural practices applied in Belfaa region, which is known by its negative impact on pollinator diversity and abundance (Klein et al., 2003a; 2003b), or by the effect of pollinators competition and attraction between the plants having the same period of blooming as the argan tree (orange, cactus, annual herbaceous plants). According to previous researches, plants with similar flowering period may serve as competing pollinators (Motten, 1986; Campbell and Motten, 1985). *Bombus terrestris* and *Halictus* sp. are rather accidental visitors due to their discontinued foraging activity. Despite a high abundance observed for some ant species (e.g *Tapinoma magnum*), the ants observed on argan flower are not pollinators. Their examination under stereomicroscope show that they are a few pollen loading organisms (<10 pollen). Furthermore, their limited movement is limiting factor for pollination as state Proctor and Yeo (1972). Ant pollination was documented and known as rare pollinators. According to Claessens and Seifert (2018), there are less than twenty 20 known cases of ant pollination, and only some plant with special traits may be pollinated (e.g Peakall et al., 1990). In addition, ant's meta-pleural glands secretions affect pollen viability (Beattie et al., 1984; 1985; Hull and Beattie, 1988; Wagner, 2000; Galen and Butchart, 2003; Dutton and Frederickson, 2012). Among Diptera visitors, the highest I_{VR} was computed for Syrphidae followed by Calliphoridae and Muscidae. Syrphidae were reported as the most significant anthophilous Diptera (Jauker & wolters, 2008). The highest I_{VR} was determined for *E. tenax* ($I_{VR(Azrarag/Belfaa)} = 23.59 \pm 0.99/15.67 \pm 3.92$), followed by *E. taeniops* ($I_{VR(Azrarag/Belfaa)} = 3,52 \pm 0,64/17.09 \pm 3.12$).

E. tenax has been reported as an efficient pollinator (Solomon and Kendall, 1970; Talavera et al. 2001). Due to their low frequency in both sites *Simosyrphus aegyptius* had a lower I_{VR} . For the other Diptera, their low Visitation Rate Index (I_{VR}) is due either to their lower activity rate and their low frequency. As a result, the lowest I_{VR} was observed for species within lower AR; Rhiniidae ($I_{VR(Azrarag/Belfaa)} = 0.10 \pm 0.08/0.26 \pm 0.06$), Bombyliidae ($I_{VR} = 0.25 \pm 0.07/0.24 \pm 0.03$), Bibionidae ($I_{VR} = 0.0/0.09 \pm 0.02$), Stratiomyidae ($I_{VR} = 0.16 \pm 0.12/0.18 \pm 0.05$) and Tephritidae ($I_{VR} = 0.26 \pm 0.10/0.37 \pm 0.11$). Lower foraging rate is reported for Diptera because of their greater handling time (Herrera, 1989; Kearns and Inouye, 1993).

Based on I_{VR} and pollen load, the most potential pollinators for *A. spinosa* can be classified in terms of their decreasing importance as follow, honey bees (*A. mellifera*), wild bees Halictidae (*Lasioglossum* sp1 and *Lasioglossum* sp2), hoverfly (*E. tenax*, *E. taeniops* and *Eristalis* sp.) and Calliphoridae (*Lucilia* sp.).

5. Conclusion

This study highlights the breeding system of *A. spinosa*, as well as the rate of each type of pollination on fruit set. The main outcomes of this study was the importance of entomophilous pollination for argan tree, as well as, the establishment of an exhaustive list of their associated visitors and their potential pollinators. These results constitute an important tools that will contribute to maintain argan endemic population while preserving their genetic diversity, as well as to increasing yield and quality of oil through pollination management.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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