Diversity of insect pollinators with reference to their impact on yield production of canola (*Brassica napus* L.) in Ismailia, Egypt

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SUMMARY

A study of insect pollinators and their impact on canola yield was conducted during the 2013/2014 and 2014/2015 growing seasons. The study was carried out at an experimental farm, Faculty of Agriculture, Suez Canal University, Ismailia. The results revealed that 21 species of insect pollinators belonging to 14 families under four orders visited canola flowers. The abundance of Hymenoptera insects reached the maximum of 67.90%, followed by Diptera 14.97%, Coleoptera 13.61%, then Lepidoptera 2.26% as average of both seasons. In open pollination, *Colletes lacunatus* had the maximum percent abundance in the two seasons (30.45 and 29.34%, respectively) followed by *Apis mellifera* (12.34 and 17.73%, respectively), compared to other bees and different pollinators. Peaks of foraging activity of both *C. lacunatus* and *A. mellifera* were mainly observed from 1:00 to 3:00 pm and they corresponded to the number of flowering plants. Open pollination increased the number of pods per plant, seeds per pod, weight of 1000 seeds, yield per plant, yield per feddan (1 fed = 0.42 ha) and seed germination, compared to non-open pollination.

Keywords: Insect pollinators; Diversity; Canola; Yield; Egypt

INTRODUCTION

Canola, *Brassica napus* L, belongs to the family Brassicaceae and is cultivated in many parts of the world, standing out as an excellent economic alternative to other human consumable oils and biodiesels (Miri, 2007; Marjanović-Jeromela, 2008). In Egypt, canola

has a bright future in contributing to a reduction in oil deficiency gap between production and consumption of edible oils, particularly as it could be successfully grown during winter season on newly reclaimed land outside the old zone of the Nile valley and thus get around competition with other crops grown in the old area of cultivation (Sharaan et al., 2002).

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There are several insect pollinators visiting canola in Egypt, but the honeybee, *Apis mellifera* L. (Apidae: Hymenoptera), is the most abundant pollinator of its flowers (Sayed & Teilep, 2013; Mahmoud & Shebl, 2014). Canola has entomophilous flowers capable of both self-and cross-pollination. The out-crossing rate range is 12-47% (Becker et al., 1992) depending on cultivar. The flower of *B. napus* has a generalized open structure that almost every group of pollinating insects can feed from. The yellow color of the flower with shallow placement of visible nectar mostly attracts bees, flies, and butterflies (Kunin, 1997).

Pollination of canola can have positive effects, such as shortening of the period of flowering and raceme production, acceleration of ripening, increase in seed weight (Rosa et al., 2011; Bommarco et al., 2012), seed oil content (Bommarco et al., 2012) and seed yield. Klein et al. (2007) highlighted the importance of insect pollination for the production of fruits and seeds. Besides contributing to the preservation of natural ecosystems, bee pollination is one of the main sources for improvement of crop productivity (Mahmoud, 2012). Thus honey bees, *A. mellifera*, are considered the most important flower visitors (Delaplane & Mayer, 2000) and most efficient *B. napus* pollinators (Free, 1993; Sabbahi et al., 2005).

The objective of the current study was to investigate the diversity of insect pollinators on canola flowers, the foraging activity of some bees and impact of insect pollination on canola yield.

MATERIAL AND METHODS

Experimental design

A field experiment was conducted at the experimental farm of the Faculty of Agriculture, Suez Canal University, Ismailia, Egypt, during 2013-2014 and 2014-2015 seasons. Canola, cv. Serw 4 (the most common cultivar grown in Ismailia region) was sown on 21 October 2013 and on 20 October 2014. The soil of the experimental site was sandy soil (86.21% sand, 10.5 % silt and 3.29 clay) with pH 8.02 and EC 0.44 dsm⁻¹. Plants were thinned to one plant per hill and 20 cm distance between hills insured a density of 27.000 plants/fed. Recommended cultural and agronomic practices were applied from sowing to harvest. No insecticide was sprayed in or around the experimental fields. The experimental area was divided into two treatment areas (open pollination and caged plants). Each treatment

included four replicates. The replicates were distributed in a complete randomized block design.

Observation and identification of insect pollinators

Plants were examined weekly during the flowering period for collection and identification of different insect pollinators. Three methods were used to collect insect pollinators from the canola plants. A sweep net measuring 40 cm in diameter was used to collect insects flying over the plots and pan traps containing 4% formalin solution were used to trap insects walking on the floor in addition to visual observation. Observation of pollinators visiting canola inflorescences was recorded whenever possible before specimens were collected and preserved for identification. The collected pollinators were killed in a killing bottle and transferred to the laboratory. Large insects were pinned, labeled and preserved in a collection box. Smaller insects were mounted, labeled and preserved too. Insects were identified to species where possible by using published systematic keys and direct comparison with museum specimens housed at the Department of Plant Protection, Ismailia.

Foraging activity of bees

Experimental observation was done during four periods of the day: 9:00-11:00 am, 11:00 am-1:00 pm, 1:00-3:00 pm and 3:00-5:00 pm, starting from initial flowering until flower withering. The number of pollinators visiting each square meter area were counted for five minutes in each period. Four spots of 1 m² area were selected randomly, and the number of A. mellifera and Colletes lacunatus Dours was counted for five minutes by using electronic stopwatch, voice recorder and digital video camera. The data were then interpreted and analyzed to assess the most favourable period of the day for bee species to visit canola inflorescences and the most dominant species in each day and at particular times of day.

Effects of insect pollination on canola yield

Before flowering began, 100 canola plants were caged by insect screen to prevent pollinators' access to inflorescences, and 100 plants were exposed to pollinators. Plants were harvested at the end of the fruiting period. The number of pods per plant and seeds per pod were counted, and the weight of 1000 seeds, yield per plant, yield per fed and germination percentage recorded.

Statistical analysis

Data obtained in the present study were subjected to an analysis of variance (ANOVA) with the honestly significant difference value calculated as Tukey's statistic at $P \le 0.05$. (SAS Institute 2004).

RESULTS

Diversity and abundance of insect pollinators

A total number of 1853 and 2026 insect pollinators associated with *Brassica napus* were observed over the 2013/2014 and 2014/2015 growing seasons. They belonged to 21 species in 4 orders (Tables 1, 2 and 3). Among the four orders, Hymenoptera and Diptera shared the maximum number of species (eight and six species, respectively),

followed by Coleoptera (four species) and Lepidoptera (three species). The abundance of Hymenoptera reached the maximum of 66.39 and 65.41% (average 65.90%), and was followed by Diptera 16.83 and 18.07% (average 17.45%), Coleoptera 14.30 and 12.17% (average 13.23%), and Lepidoptera 3.02 and 4.23% (average 3.62%) throughout the 2013/2014 and 2014/2015 growing seasons, respectively (Figure 1). Out of Hymenoptera species, two species of inflorescence visiting insects showed high abundance, i.e. C. lacunatus (30.45 and 28.97%), and A. mellifera (12.34 and 17.67%). All species of Hymenoptera visitors were observed as both pollen and nectar foragers, whereas all Diptera and Lepidoptera species were observed as nectar foragers and only accidentally transferred pollen. The three species of Coleoptera were casual visitors of canola inflorescences and were not participating in nectar or pollen foraging (Table 1).

Table 1. Species of insect pollinators visiting canola inflorescences during 2013/2014 and 2014/2015 seasons

Order	Family	Species	Common name	Forage source*
	Apidae	Apis mellifera L.	Honey bee	P/N
		Andrena ovatula Kirby	Sand bee	P/N
	Andrenidae	Andrena mariana Warncke	Sand bee	P/N
		Andrena savignyi Spinola	Sand bee	P/N
Hymenoptera		Andrena fuscosa Erichson	Sand bee	P/N
	Colletidae	Colletes lacunatus Dours	Plasterer bee	P/N
	Megachilidae	Osmia latreillei Spinola	Mason bee	P/N
	Halictidae	Nomioides sp.	Sweat bee	P/N
	Nititulidae	Glischrochilus guadrisignatus (Say)	Four spotted sap beetle	С
Coleoptera	Scarabaeidae	Tropinata squalida (Scop.)	Hairy rose beetle	С
	0	Coccinella undecimpunctata L.	Eleven spotted lady beetle	С
	Coccinellidae	Coccinella septempunctata L.	Seven spotted lady beetle	C
		Syrphus corolla Fabricius	Hover fly	N
	Syrphidae	Eristalis sp.	Hover fly	N
		Sarcophaga sp.	Flesh fly	N
Diptera	Sarcophagidae	Wohlfahrtia sp.	Flesh fly	N
		Musca domestica L.	House fly	N
	Muscidae	Fannia canicularis L.	Little house fly	N
	Pieridae	Pieris rapae (L.)	Cabbage white butterfly	N
Lepidoptera	Nymphalidae	Danaus chrysippus (L.)	Plain tiger	N
	Lycaenidae	Polyommatus baeticus (L.)	Bean butterfly	N

 $\textbf{Table 2.} \ Species \ and \ individual \ number \ of \ insect \ pollinators \ on \ canola \ flowers \ during \ 2013/2014 \ season$

Species	1*	2	3	4	5	6	7	8	9	Total	%	Order
Colletes lacunatus Dours	18	44	55	84	92	88	87	56	36	560	30.45	
Apis mellifera L.	4	12	17	25	29	37	44	39	20	227	12.34	Hymenoptera 66.39
Andrena ovatula Kirby	4	20	30	36	31	34	20	17	7	199	10.82	
Osmia latreillei Spinola	1	3	6	7	17	10	9	4	4	61	3.31	
Nomioides sp.	0	6	26	37	33	42	16	10	4	174	9.46	
Glischrochilus guadrisignatus (Say)	0	0	3	8	2	3	6	4	4	30	1.63	
Tropinata squalida (Scop.)	0	0	0	7	17	22	18	3	1	68	3.69	Coleoptera 14.30
Coccinella undecimpunctata L.	3	6	6	7	7	18	17	12	10	86	4.67	
Coccinella septempunctata L.	4	5	9	10	13	12	13	10	3	79	4.29	
Syrphus corolla Fabricius	5	7	14	15	20	29	21	10	5	126	6.85	
Eristalis sp.	3	6	9	11	13	9	8	4	3	66	3.58	Diptera
Musca domestica L.	5	7	10	13	11	15	14	10	4	89	4.62	16.83
Fannia canicularis L.	2	5	2	6	4	9	0	1	3	32	1.74	
Pieris rapae (L.)	0	0	0	5	0	3	4	9	3	24	0.76	Lepidoptera 3.02
Danaus chrysippus (L.)	1	2	0	0	0	4	0	5	2	14	0.76	
Polyommatus baeticus (L.)	0	0	0	3	7	0	2	4	2	18	0.97	

^{*} Weeks of flowering period

Table 3. Species and individual number of insect pollinators on canola flowers during 2014/2015 season

Species	1*	2	3	4	5	6	7	8	9	Total	%	Order
Colletes lacunatus Dours	15	34	65	90	86	104	90	71	32	587	28.97	
Apis mellifera L.	7	22	34	59	67	70	55	27	17	358	17.67	Hymenoptera 65.41
Andrena mariana Warncke	0	3	6	11	16	14	5	5	3	63	3.10	
Andrena savignyi Spinola	1	4	7	7	5	10	6	4	1	45	2.22	
Andrena fuscosa Erichson	0	3	3	5	6	8	4	2	2	33	1.62	
Andrena ovatula Kirby	2	11	25	29	37	31	20	8	3	166	8.19	
<i>Osmia latreillei</i> Spinola	0	0	0	7	5	5	7	2	1	27	1.33	
Nomioides sp.	0	0	2	9	10	5	9	7	5	47	2.31	
Glischrochilus guadrisignatus (Say)	3	3	5	11	7	3	9	8	5	54	2.66	
Tropinata squalida (Scop.)	0	0	0	0	10	13	20	14	7	64	3.15	Coleoptera 12.17
Coccinella undecimpunctata L.	0	4	8	10	10	11	13	7	9	72	3.55	
Coccinella septempunctata L.	1	5	4	12	8	5	9	9	4	57	2.81	
Syrphus corolla Fabricius	5	7	14	15	20	29	21	10	5	126	6.21	
Eristalis sp.	3	6	9	11	13	9	8	4	3	66	3.25	
Musca domestica L.	5	7	10	13	11	15	14	10	4	89	4.39	Diptera
Fannia canicularis L.	2	5	2	6	4	9	0	1	3	32	1.57	18.07
Sarcophaga sp.	0	0	0	3	4	0	4	3	4	18	0.88	
Wohlfahrtia sp.	1	1	5	4	7	8	5	3	2	36	1.77	
Pieris rapae (L.)	1	2	4	5	3	2	3	4	2	26	1.28	Lepidoptera 4.23
Danaus chrysippus (L.)	2	4	4	4	3	3	3	2	3	28	1.38	
Polyommatus baeticus (L.)	1	2	3	6	4	4	7	3	2	32	1.57	

^{*} Weeks of flowering period

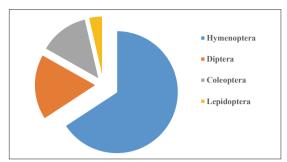
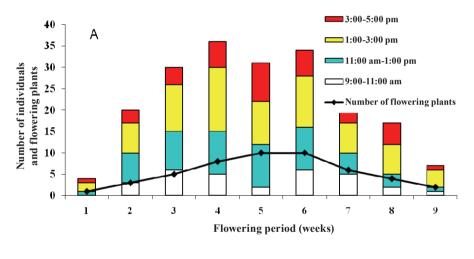


Figure 1. The mean percentage of four orders of insects visiting canola inflorescences throughout the two growing seasons

Foraging activity of *C. lacunatus* and *A. mellifera* on canola inflorescences

The foraging activity of *C. lacunatus* on canola inflorescences was observed from 9:00 am to 5:00 pm beginning with three days after flowering began

to sixty-three days of flowering over the 2013/2014 and 2014/2015 growing seasons. Foraging activity during the first week started at 9:00-11:00 am with 0.0 bees/m²/5 min and a maximum was attained at 1:00-3:00 pm with 2.0 bees/m²/5 min in the season of 2013/2014, while no foraging was observed in the first week during the second season 2014/2015 (Figure 2). A similar trend was observed throughout the nine weeks of flowering. In the remaining eight weeks, bee activity started from 9:00-11:00 am with 3.0, 6.0, 5.0, 2.0, 6.0, 5.0, 2.0 and $1.0 \text{ bees/m}^2/5 \text{ min in the season}$ of 2013/2014, and 1.0, 6.0, 5.0, 8.0, 7.0, 3.0, 2.0 and 0.0 in the season of 2014/2015, respectively. A peak bee activity was noticed at 1:00-3:00 pm with 2.0, 7.0, 11.0, 15.0, 10.0, 12.0, 7.0, 7.0 and 4.0 bees/ $m^2/5$ min in the season of 2013/2014 and 4.0, 10.0, 15.0, 13.0, 20.0, 4.0, 4.0 and 2.0 in the season of 2014/2015, respectively. Bee activity was noticed to decline as the day advanced.



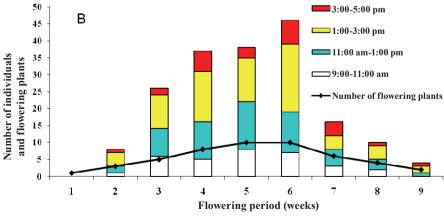
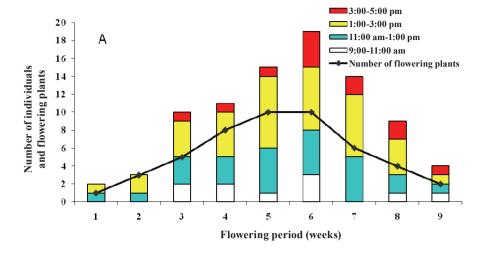


Figure 2. Foraging activity of *Colletes lacunatus* on canola inflorescences during the growing seasons of 2013/2014 (A) and 2014/2015 (B)



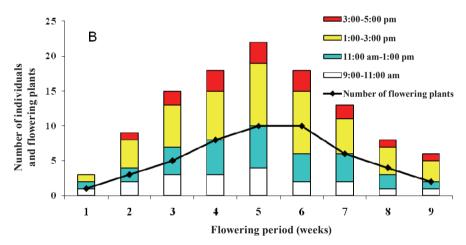


Figure 3. Foraging activity of *Apis mellifera* on canola inflorescences during the growing seasons of 2013/2014 (A) and 2014/2015 (B)

The results showed that the foraging activity of *C. lacunatus* at different times of observation over the flowering season of 2013/2014 was lower than it was in the flowering season of 2014/2015.

The foraging activity of *A. mellifera* increased from 11:0 am-1:0 pm and the highest foraging occurred at 1:0-3:0 pm and subsequently decreased in both growing seasons of canola (Figure 3). Canola inflorescences were blooming for 55 to 65 days, and the peak flowering occurred 25-45 days after the beginning of flowering, and the number of *C. lacunatus* and *A. mellifera* was relatively consistent with the density of inflorescences, but the number of species increased with the increasing number of flowering plants (Figures 2 and 3).

Yield of B. napus related to insect pollination

The diversity of insect pollinators affected the yield production of canola, the number of pods, seeds per pod, weight of 1000 seeds, yield per plant, yield per fed and seed germination.

When plants were exposed to pollinators, the number of pods per plant, seeds per pod, weight of 1000 seeds, yield per plant and yield per fed were higher (2117.7 pods, 24.6 seeds, 3.13 g seeds, 32.0 g seeds, and 677.6 kg seeds) than those of caged plants (1315.4 pods, 18.2 seeds, 2.4 g seeds 18.9 g seeds and 248.64 kg seeds, respectively). Also, insect pollination increased canola seed germination. Seed germination was higher in noncaged plants (95.2%) than in caged plants (87.8%).

Table 4. Effects of open pollination and caging of plants on some qualitative and quantitative parameters

Parameters	Open	Caged
Number of pods per plant	2117.70 a	1315.40 b
Seeds per pod	24.60 a	18.20 b
Weight of 1000 seeds	3.13 a	2.40 b
Yield per plant	32.00 a	18.90 b
Yield per fed	677.60 a	248.64 b
Germination %	95.20 a	87.80 b

Means marked with the same letter (row-wise) are not significantly different (Tukey' HSD; P≤0.05)

DISCUSSION

The aim of this study was to measure the diversity and foraging activity of important insect pollinators to help improve pollination of canola as an important oilseed crop. Our results indicate that insect diversity and foraging activity on canola may help identify the most efficient pollinators for enhancing crop yield. Apis and non-Apis bees in particular play a significant role in enhancing the productivity of canola. Our results showed that Hymenoptera (66.39 and 69.42%) were the main pollinators of B. napus. All other pollinators, i.e. Coleopteran, Diptera, and Lepidoptera, were less important (33.6 and 28.09%). Furthermore, all species of Hymenoptera were observed as both pollen and nectar foragers, while species of the other orders were nectar-only foragers or accidental pollen transferers (Bhowmik et al., 2014). Two species of bees Colletes lacunatus and Apis mellifera were found to dominate in the two seasons. B. napus is generally visited by a variety of pollinating insects worldwide, including honeybees, solitary bees and hoverflies (Ali et al., 2011; Arthur et al., 2010), as well as Andrena spp., Osmia spp., and Lasioglossum spp. (Woodcock et al., 2013). Pollination of B. napus occurs through a combination of wind and insect visitors with considerable autogamy apparent. Field and cage studies have shown positive effects of insect pollination on pod set and seed set (Stanley et al., 2013), with associated benefits to the yield and quality of production (Bommarco et al., 2012).

This study provides baseline information to underpin pollination service management strategies for safeguarding canola production in Egypt in the future, so that enhancement of insect pollination as part of crop management should be considered by farmers.

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Diverzitet insekata oprašivača i njihov uticaj na prinos uljane repice (*Brassica napus* L.) u Ismailiji, Egipat

REZIME

Ispitivani su insekti oprašivači i njihov uticaj na prinos uljane repice tokom sezona 2013/2014 i 2014/2015. Ogled je izveden na eksperimentalnom imanju Poljoprivrednog fakultet Univerziteta Suez Canal, Ismailia. Rezultati ispitivanja pokazali su prisustvo 21 vrste insekata oprašivača cveta uljane repice, koje spadaju u 14 familija i četiri reda. Zabeleženo je maksimalno prisustvo insekata iz roda Hymenoptera, sa prosečnih 67.90% u obe godine, a sledili su rodovi Diptera sa 14.97%, Coleoptera sa 13.61% i Lepidoptera sa 2.26%. U oglednoj varijanti sa otvorenim pristupom, vrsta *Colletes lacunatus* je bila najprisutnija tokom dve sezone (30.45 i 29.34%, respektivno), a zatim *Apis mellifera* (12.34 i 17.73%, respektivno), u odnosu na ostale pčele i oprašivače. Najveća aktivnost prikupljanja kod vrsta *C. lacunatus* i *A. mellifera* odvijala se u vremenskom intervalu od 13:00 do 15:00 časova i odgovarala je broju procvetalih biljaka. Otvoreni pristup biljkama povećao je broj mahuna po biljci, broj semena po mahuni, težinu 1000 semena, prinos po biljci, prinos po fedanu (1 fedan = 0.42 ha), kao i klijanje semena, u poređenju sa varijantom ogleda sa biljkama u kavezima.

Ključne reči: Insekti oprašivači; Diverzitet; Uljana repica; Prinos; Egipat