

The Preliminary Study on Effects of Pollinating Insects in Canola (*Brassica napus* L.) Production

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Received (Geliş tarihi): 18.06.2020

Accepted (Kabul tarihi): 27.08.2020

ABSTRACT: Although canola (*Brassica napus* L.) plants are mainly self-pollinating, the cross pollination by insect increases yield. The study was carried out at Aegean Agricultural Research Institute (AARI), Menemen/Izmir in 2016/2017 growing season. The experimental design was a Randomized Complete Block with three replications. The treatments consisted of caged with honeybee, caged with bumblebee, caged without bees and open pollination. 96% of the collected insects from open pollination plots were honeybees. It was determined that flowering period significantly shortened (5.8 days) in plots with bee compared with caged without bee, while seed number per pod (4.6), thousand seed weight (0.25 g), seed yield (1398 kg ha⁻¹), oil yield (776 kg ha⁻¹) and oil rate (1.24%) increased. Also, the contents of erucic acid and glucosinolate level resulted in an increase by pollinator visit. It was concluded that pollination by insect affected yield, yield components and composition of fatty acid in canola. The results of this preliminary study should be supported by the research findings from multi locations and years.

Key Words: Canola, *Brassica napus* L., pollination, honeybee, *Apis mellifera* L., bumble bee, *Bombus terrestris* L., yield, yield components, fatty acids.

Kanola (*Brassica napus* L.) Üretiminde Tozlayıcı Böceklerin Etkisi Üzerine Bir Ön Çalışma

ÖZ: Kanola (*Brassica napus* L.) genellikle kendine dölenen bir bitki olmasına karşın yabancı döllenme verimi artırıcı etkiye bulunmaktadır. Deneme Ege Tarımsal Araştırma Enstitüsü Menemen/Izmir koşullarında 2016/2017 yetiştirme periyodunda yürütülmüştür. Deneme deseni olarak üç tekerrürlü Tesadüf Blokları Deneme Deseni kullanılmıştır. Bal arılı kafes, bumblebee içeren kafes, arısız kafes ve açık döllenme çalışmanın konularını oluşturmuştur. Açık dölenen parsellerden toplanan böceklerin %96'sının bal arıları olduğu saptanmıştır. Arısız parseller ile karşılaştırıldığında, tüm arılı parsellerde çiçeklenme periyodunun önemli düzeyde kısaldığı (5,8 gün) buna karşın bitkide harpupta tohum sayısı (4,6), 1000 tane ağırlığı (0,25 g) tohum verimi (1398 kg ha⁻¹), yağ verimi (776 kg ha⁻¹) ile yağ oranının (%1,24) arttığı belirlenmiştir. Ayrıca, arı ziyaretleri ile birlikte erusik asit ve glukozinat miktarının arttığı saptanmıştır. Kanolada böceklerle olan döllenmenin verim, verim komponentleri ve yağ asitleri kompozisyonu üzerine etkili olduğu sonucuna varılmıştır. Bu ön çalışmanın farklı çevrelerde yürütülerek elde edilecek araştırma bulgularıyla desteklenmesi büyük önem taşımaktadır.

Anahtar Kelimeler: Kanola, *Brassica napus* L., döllenme, bal arısı, *Apis mellifera* L., bumblebee arısı, *Bombus terrestris* L., verim, verim komponentleri, yağ asitleri.

INTRODUCTION

Canola (*Brassica napus* L.) belongs to the Brassicaceae is grown for oils in many parts of the world and Turkey. The sowing areas of canola reached 37.6 mil ha worldwide and 52.5 thousand ha in Turkey (Anonymous, 2018; Anonymous, 2019). Canola seeds, a cool climate crop, contains 2% less erucic acid and 30 $\mu\text{mol g}^{-1}$ less glucosinolates (Sabbahi *et al.*, 2005; Anonymous, 2008).

Entomophilous flowers capable of both self- and cross- pollination can be seen in the canola (Treu and Emberlin 2000). Although canola can be considered as self-compatible, it has a certain degree of incompatibility in some cultivars (Ockendon, 1972; Gowers, 1981). Therefore, pollen activity between plants by insects was necessary for optimal pollination (Manning and Wallis, 2005). The stigma of canola exceeds the height of the anthers, so the pollen of the flower itself to fall on the stigma is not possible. This can trigger cross-pollination (Witter *et al.*, 2014).

The flowers of canola have a nectar contains high concentrations of sugars and an attractive colour and structure for particularly bees. It was accepted that honeybees (*Apis mellifera*) and bumble bees (*Bombus* spp.) are played a major role in the pollen transfer (Anonymous, 2008; Witter *et al.*, 2015). Bee pollination has important benefits; (1) uniform flowering and earliness, (2) maximize seed set (3) increasing seed weight per plant of 13% - 50% (Sabbahi *et al.*, 2005; Abrol, 2007).

Abrol (2007) revealed that an adequate pollination process with bee visit ensures the reproductive cycle and increase their productivity indices in Brassicaceae. Korkmaz (2003) emphasized the there is a significant increase in seed yield of forage rape (*Brassica napus* L. Metzg.) with the contribution of insect pollination. Similarly, Oz *et al.* (2008) found that honeybee pollination increased the seed yield significantly but not protein and oil percentage in seeds of winter rapeseed. The important increases observed in 31.9% of seeds per plant (Mussury and Fernandes, 2000), 34.5% of seed weight (Williams *et al.*,

1986; Adegas and Nogueira-Couto, 1992), 15.54% of the number of pods per plant and 33.5% of seed yield per plant (Harrad *et al.*, 2015), 30.4% of thousand kernel weight (Kamel *et al.*, 2015) when compared to autogamy conditions.

Generally, many studies on pollination with bees in canola have focused on the yield and yield components such as the rate of fruit set and the number of seeds per pod. A limited number of researches have been conducted on the change of fatty acid composition and glucosinolate amount. Therefore, this study was designed to investigate the effect of bee pollination on yield, yield components, fatty acid composition, protein and glucosinolates amount of canola.

MATERIAL and METHODS

A field experiment was conducted at the Aegean Agricultural Research Institute (AARI), Menemen-Izmir, Turkey (38°56' N 27°05' E). ETAE-K-23.1, canola advanced line improved by AARI was used as material. The experimental soil was loamy characteristics. Climatic data (monthly mean temperature and precipitation) was gathered between October 2016 and June 2017 (Figure 1). The mean temperatures of experimental year recorded higher than long period except, December and January. The monthly precipitation (mm) from October to June shows that December and January exceed long-term precipitation (Anonymous, 2017).

Canola was sown on 26 November 2016. The experiment was arranged as Randomized Complete Block Design with 3 replications, where the treatments studied were (A) caged without bee, (B) caged with honey bee, (C) caged with bombus and (D) open pollination plots. Insect-proof cage was used to prevent the penetration of insect in A, B and C plots (Figure 2). Plants in open pollinated plots examined weekly during the flowering period for identification of different insect pollinators. In the plots with honey bee, 1 hive with 4-5 frames, the queen bee of the same age and the same hatching areas for A were placed before flowering stage of canola. Similarly, 2 bombus colonies for B were used.

The plot size for trials was 8.75 m², which consisted of 5 rows of 5 m length. The distance between and within rows were 0.35 m and 0.05 m, respectively. The plot area at harvest was 5.25 square meters. The compose fertilizer (15.15.15) of 500 kg ha⁻¹ was applied before sowing, and 250 kg ha⁻¹ of ammonium sulfate was given at the bolting stage. No insecticide was sprayed in both trials and around the experiment. All agronomical practices were applied according to recommendation for Aegean Region canola growing.

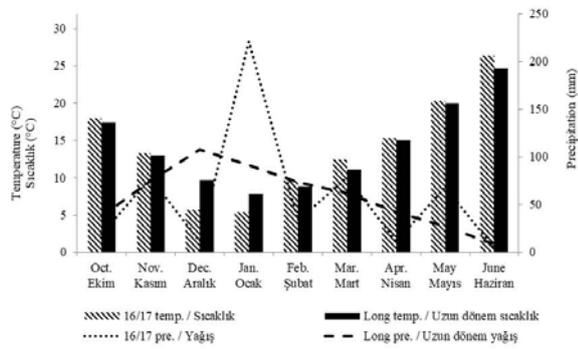


Figure 1. Monthly average temperature (°C) and amount (/depth) of rainfall in Menemen in 2016-17 and longterm.
Şekil 1. Menemen'e ait 2016-17 yılı ve uzun dönem aylık sıcaklık (°C) ortalamaları ve yağış miktarı.



Figure 2. Experimental parcels with cages and open-pollinated.
Şekil 2. Kafesli ve açık tozlanan deneme parselleri.

The flowering period (from emergence to 75% flowering; day), plant height (cm), number of branches per plant, number of pods per plant and number of seeds per pod were recorded in randomized ten plants of each plots. Seed yield (kg ha⁻¹) was determined based on 5% moisture level with all plants in harvest area of each plot. Harvested material with 5% moisture was used for thousand kernel weight (g), oil (by NMR) and

protein analysis (by Kjeldahl). Oil content and protein content were calculated according to methods recommended by Ganlund and Zimmerman (1975) and Anonymous (1977), respectively. Fatty acid contents (%) such as oleic, linoleic, linolenic, palmitic, stearic, erucic acid and glucosinolate (µmol g⁻¹) level were determined (Christie, 1973; Daun and McGregor, 1983).

The data of yield, yield components and quality parameters were subjected to analysis of variance (ANOVA) using Micro Computer Statistical Program (MSTAT) (Russell, 1986). The differences between the means were compared by the least significant difference (LSD) at the 5% level according to Steel and Torrie (1980).

RESULTS and DISCUSSION

Although the pesticides used in the intensive agriculture of Aegean region cause a reducing in the insect population, blooming canola parcels are remarkably attractive to insects due to flowers with high concentrated sugars and yellow petals. Figure 3 showed the visiting insect species at the blooming stage of canola in open pollination plots. It was identified that these pollinators were honey bee, bombus and other insects belonged in three orders, Hymenoptera, Diptera and Coleoptera. In our observations, the numbers of insects collected were 454 honey bees, 4 bombus, 4 insects from Coleoptera, 4 from other Hymenoptera and 7 from Diptera. The most of the collected insects was honeybees of 96% parallel to Kotowski (2005) who was found that the dominant pollinating insect was always honeybees (89%).

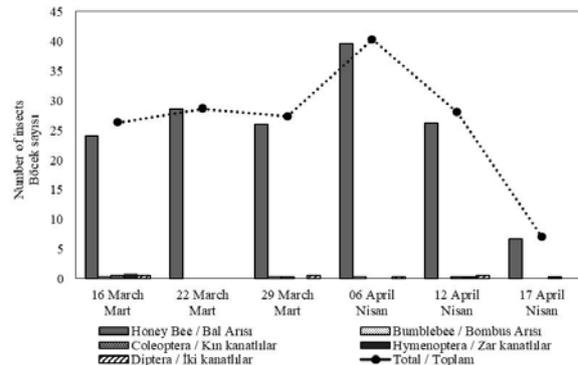


Figure 3. The average number of insects visiting open pollinated plots for weekly.
Şekil 3. Açık tozlanan parselleri ziyaret eden böceklerin haftalık ortalama değerleri.

The differences among the four treatments were significant for flowering period (FP), plant height (PH), yield, seed number per pod (SN/P), thousand kernel weight (TKW), oil rate (OR) and oil yield (OY) (Table 1 and 2). Among the treatments, flowering period ranged from 46.33 days (caged with honey bee and caged with bombus) to 52.33 days (caged without bee). It was clearly seen that flower visits by bees reduced flowering period significantly (approximately 11.47%) when the caged without bee was compared to caged plots with bee and bombus and open pollination. This result is consistent with studies demonstrating that insect-pollinated canola flowers are shorter-lived (Mesquida *et al.*, 1988; Adamidis *et al.*, 2019). The recorded values in caged without bee plots were 2881 kg ha⁻¹ for yield, 3.22 g for TKW, 50.87% for OR and 1467 kg ha⁻¹ for OY. The lowest values for caged without bee plots revealed positive effects of the pollinator visitors on yield, SN/P, TKW, OR and OY. The significant highest yields of the open pollination (4963 kg ha⁻¹) compared to caged with honeybee (3605 kg ha⁻¹) and bombus (4269 kg ha⁻¹) indicated that open visit of honey bees (Figure 3) is the most important treatments to increase the yield.

The results of studies conducted by Bommarco *et al.* (2012) and Lindström *et al.* (2016) confirmed that insect pollination increased canola yield. Table 2 showed that the protein content of seed varied between 23.02% (open pollination) and 24.61% (caged with bombus).

Open pollination increased pod number by 6.68%, seed number per pod by 10.10%, thousand kernel weight by 13.35%, oil rate by 4.63% and oil yield by 80.09% compared with caged without bee. Similar findings were documented for canola (Sabbahi *et al.*, 2005; Manning and Wallis, 2005; Munawar *et al.*, 2009; Harrad *et al.*, 2015; Kamel *et al.*, 2015). The statistically lowest plant height and partially branch number obtained from open pollination compared with caged plots revealed that cage material can show a shading effect on plant that prevents light quality and promotes vegetative growth. This result is in general agreement with Harrad *et al.* (2015) who stated that the average plant height of rapeseed and faba bean under insect-proof cage were significantly higher than those growing outside the cage.

Table 1. Mean values of flowering period (FP), plant height (PH), branch number (BN), pod number (PN) and seed number per pod (SN/P).

Çizelge 1. Çiçeklenme periyodu (ÇP), bitki boyu (BB), yan dal sayısı (YDS), harnup sayısı (HS) ve harnupta tohum sayısı (TS/H)'na ilişkin ortalama değerler.

Treatments Uygulamalar	FP ÇP (day/gün)	PH BB (cm)	BN YDS (no./adet)	PN HS (no./adet)	SN/P TS/H (no./adet)
Caged with honey bee / Bal arı kafes	46.33 b	195,97 a	8,20	415,5	28,9 b
Caged with bombus / Bombuslu kafes	46.33 b	198,83 a	8,13	425,7	29,8 ab
Caged without bee / Arısız kafes	52.33 a	197,83 a	7,87	405,5	27,7 c
Open pollination / Açık tozlaşma	47.00 b	185,73 b	7,87	432,6	30,5 a
LSD ($\alpha:0.05$)	1.00	5,37	ÖD	ÖD	1,12
CV (%)	1.04	1.38	14.11	4.29	1.90

Same letters in a column are not significantly different at the 0.05 probability levels.

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0.05$) yoktur.

Table 2. Mean values of yield, thousand kernel weight (TKW), oil rate (OR), oil yield (OY) and protein rate (PRO).

Çizelge 2. Verim, bin dane ağırlığı (BDA), yağ oranı (YO), yağ verimi (YV) ve protein oranına ilişkin ortalama değerler.

Treatments Uygulamalar	Yield Verim (kg ha ⁻¹)	TKW BDA (g)	OR YO (%)	OY YV (kg ha ⁻¹)	PRO Protein (%)
Caged with honey bee / Bal arı kafes	3605 c	3.33 b	51.73 ab	1867 b	23.25
Caged with bombus / Bombuslu kafes	4269 b	3.45 ab	51.37 b	2192 b	24.61
Caged without bee / Arısız kafes	2881 d	3.22 b	50.87 b	1467 c	24.59
Open pollination / Açık tozlaşma	4963 a	3.65 a	53.23 a	2642 a	23.02
LSD ($\alpha:0.05$)	611.10	0.26	1.55	333.1	ÖD
CV (%)	7.78	3.86	1.50	8.16	3.66

Same letters in a column are not significantly different at the 0.05 probability levels.

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0.05$) yoktur.

Table 3. Mean values of palmitic acid (PAL), stearic acid (STR), oleic acid (OLE), linoleic acid (LIN), linolenic acid (LNL), erucic acid (ERU) and glucosinolate level (GLU).

Çizelge 3. Palmitik asit (PAL), stearik asit (STR), oleik asit (OLE), linoleik asit (LIN), linolenik asit (LNL), erusik asit (ERU), ve glukozinolat miktarına (GLU) ilişkin ortalama değerler.

Treatments Uygulamalar	Fatty Acids (%) / Yağ asitleri (%)						Glucosinolate Glukozinolat ($\mu\text{mol g}^{-1}$)
	Palmitic Palmitik	Stearic Stearik	Oleic Oleik	Linoleic Linoleik	Linolenic Linolenik	Erucic Erusik	
Caged with honey bee/ Bal arılı kafes	4.00 a	1.75 b	62.94	18.76	7.55	0.83 ab	4.59
Caged with bombus / Bombuslu kafes	3.97 a	1.77 b	64.00	18.90	7.60	0.63 bc	5.41
Caged without bee / Arısız kafes	4.07 a	1.77 b	65.61	18.99	7.45	0.48 c	4.96
Open pollination / Açık tozlaşma	3.82 b	1.96 a	64.79	17.66	7.48	0.94 a	5.72
LSD ($\alpha:0.05$)	0.13	0.11	ÖD	ÖD	ÖD	0.29	ÖD
CV (%)	1.58	3.11	2.45	2.98	3.18	20.15	13.94

Same letters in a column are not significantly different at the 0.05 probability levels.

Aynı harfle gösterilen ortalamalar arasında önemli fark ($P \leq 0.05$) yoktur.

The differences among treatments were non-significant for unsaturated fatty acids such as oleic, linoleic and linolenic acid, glucosinolate level and protein content. As expected, oleic acid is the predominant monounsaturated fatty acid, and its content varied from 62.94% to 65.61% in our study (Table 3). Linoleic and linolenic acids changed 17.66% - 18.99% and 7.45% - 7.66%, respectively. Also, the content of erucic acid ranging from 0.48 to 0.94% and the amount of glucosinolate from 5.59 to 5.72 $\mu\text{mol g}^{-1}$ are within safe limits for human health. However, significantly lower erucic acid content recorded in caged without bee (0.48%). It was clearly shown the significant differences among four treatments for saturated fatty acids such as palmitic and stearic, and unsaturated such as erucic acid. Stearic and erucic acids of open pollination increased significantly, whereas palmitic acid is reduced. Although Xie *et al.* (2011) stated that honeybee pollination has no significant effect on the fatty acid composition of the oil of rapeseed, it has been demonstrated the amount and rates of oleic and linoleic can vary depending on self-pollination or cross pollination (Brittain *et al.*, 2014). The higher values of open pollination especially compared to caged without bee indicated that cross pollination by bee triggered the synthesis of erucic acid and glucosinolate. In this case, it could be speculated

that the chances in flowering period and yield components can affect the erucic acid content and glucosinolate amount.

CONCLUSION

As a result, it can be said that canola is capable of forming more pod number per plant, seed number per pod and seed weight which ultimately bring with the increase in seed and oil yield in the presence of the pollinating bees. Honeybee (*Apis mellifera*) appear to be the most visited pollinator to canola flower. Also, the effect of pollinator visit on saturated fatty acids and erucic acid were found considerable important. It was recommended that honeybee colonies should be introduced to increase the seed yield of canola. Conducting this research in different ecologies and years is important in terms of supporting the findings obtained from this preliminary study.

ACKNOWLEDGEMENTS

This research article is part of the master thesis conducted by Mehmet ALDEMİR at Aydın Adnan Menderes University, Institute of Natural and Applied Science Field Crops Department. The authors would like to thank Aegean Agricultural Research Institute for providing research facilities.

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