Alinteri J. of Agr. Sci. (2021) 36(1): 362-367
e-ISSN: 2587-2249
info@alinteridergisi.com

http://dergipark.gov.tr/alinterizbd
http://www.alinteridergisi.com/
DOI:10.47059/alinteri/V36I1/AJAS21054

RESEARCH ARTICLE

Increasing *Carthamus Tinctorius* L. Yield by Managed Bees

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ARTICLE INFO

Article History:
Received: 18.03.2021
Accepted: 20.04.2021
Available Online: 21.06.2021

Keywords:
Safflower
Bombus Terrestris
Apis Mellifera
Seed Quality
Pollination

ABSTRACT

Carthamus tinctorius L. (safflower) is a drought-tolerant plant that has been cultivated for its oil, carthamin pigment, and edible parts. Seed production by safflower is related to its pollination success. The aim of this study was to evaluate the effects of *Apis mellifera L.* (honey bee) and *Bombus terrestris L.* (bumble bee) pollination on safflower seed quality. Experiments with five treatments were prepared, four of which consisted of cages with either honey bees, bumble bees, or honey bees and bumble bees together in them, or without any insects, plus one open-pollinated plot treatment. To determine seed quality in the different treatments, the total seed yield, 1000 grain weight of seeds, number of seeds per capitulum, total oil content, fatty acid composition, and total oil yield in each treatment was determined. Total seed and oil yield was increased with bee pollination, whereas no significant difference was found in the total oil content and 1000 grain weight of seeds among treatments. Significant differences were observed among treatments in their seeds' fatty acid compositions, specifically in their content of oleic acid, linoleic acid, and linolenic acid. As a result, it was found that pollination by bees can positively affect the seed characteristics of safflower, while also increasing the total seed yield. We thus recommend using commercial bees in the cultivation of safflower.

Please cite this paper as follows:

Özenirler, C. and Sorkun, K. (2021). Increasing *Carthamus Tinctorius* L. Yield by Managed Bees. *Alinteri Journal of Agriculture Sciences, 36*(1): 362-367. doi: 10.47059/alinteri/V36I1/AJAS21054

Introduction

Carthamus tinctorius L. (safflower) (Asteraceae) has been cultivated for its oil, carthamin pigment, and edible parts, such as its leaves and shoots (Geçgel et al., 2007), for centuries. It is a drought-tolerant plant that can be cultivated in Asia, North America, and South America (Weiss, 1983; Mihaela et al., 2013). The multi-purpose usage of this plant has increased the demand for its agricultural production (Anjani and Yadav, 2017), and its production is thus increasing almost daily. The world's annual production of safflower seed was 326,997 tons in 1961 and 948,516 tons in 2016 (FAOSTAT, 2016). The drought tolerance and high oil content of safflower are two of this plant's other important characteristics that have led to several studies being done on it.

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Although safflower has been considered a predominantly autogamous plant (Knowles, 1969; Patil et al., 1991; Nabloussi et al., 2013), it was reported that the presence of abundant insect pollinators could increase its outcrossing rate from 10 to 50% (Dajue and Mündel, 1996; Singh and Nimbkar, 2006; Rudolphi et al., 2008; Nabloussi et al., 2013). Outcrossing is an important factor that influences the seed quality of safflower (Knowles, 1969; Kumari and Pandey, 2005). Boch (1961) compared the seed production between safflowers in cages and those in open fields, and found that the seed production of plants in open fields was almost twice that of those in cages (Boch, 1961). According to Abrol (2009), the total seed yield of safflower can increase by 4-114% with cross-pollination (Abrol, 2009).

There are many safflower cultivars and genetically selected lines that are planted in Asia, North America, and South America (Mihaela et al., 2013). Basically, these cultivars can be classified as being either an oleic and linoleic type by using their fatty acid composition (Khan et al., 2009) as a key character. Standard safflower oil contains

about 6-8% palmitic acid, 2-3% stearic acid, 16-20% oleic acid, and 71-75% linoleic acid. In addition, very low levels of miristic (0.24%) and behenic (0.43%) acids are present (Baydar, 2000; Geçgel et al., 2007). The 'Remzibey-05' line, which was registered in Turkey in 2005, is one of the most frequently cultivated safflower lines. This line is an oleic-linoleic type, with an oleic acid composition of 35-60% (Babaoğlu and Güzel, 2015).

The oil yield and fatty acid composition of safflower can be affected by cultivar type, weather conditions, morphology, physiology, and plant management during plant growth (e.g., density, irrigation, planting time, and fertilization) (Sabzalian et al., 2008; Saeidi et al., 2018).

C. tinctorius is a forage plant for many members of the insect orders Hymenoptera, Lepidoptera, Diptera, Coleoptera, and Odonata due to the large reserves nectar or/and pollen resources it harbors (Pandey and Kumari, 2007; Shao et al., 2012). However, 95% of the insect visitors to safflower were previously recorded to be Hymenoptera (Boch, 1961). *Apis cerena* Fabricius, *A. indica* Fabricius, *A. dorsata* Fabricius, and *A. mellifera* L. were the most abundant foragers observed on safflower blossoms, with lower abundances of other bees, such as species of bumble bees and solitary bees, also being observed (Pandey and Kumari, 2007).

There have been several studies done on the interactions between pollinator insects and oilseed plants, especially on rapeseed (*Brassica napus* L.) (Free and Nuttall, 1968; Steffan-Dewenter, 2003; Bommarco et al., 2012; Lindström et al., 2016) and sunflower (*Helianthus annuus* L.) (Delaude et al., 1979; Degrandi-Hoffman and Chambers, 2006; Öz et al., 2009; Said et al., 2017). Although safflower is an important source of edible oil, and is also important for biodiesel production in drought conditions (Emongor, 2010), the number and extent of studies that have focused on the pollination ecology of safflower (Boch, 1961; Eckert, 1962; Rubis et al., 1966; Barbier et al., 1976; Langridge and Goodman, 1980; Deshmukh et al.,1985; Singh et al., 2000; Abrol, 2009; Cresswell, 2010; Nabloussi et al., 2013; Navatha and Sreedevi, 2015;) is still insufficient.

A. mellifera and *Bombus terrestris* L. are two of the manageable bee species that are used for agricultural production (Bosch and Kemp, 2002). The production of more than 90 crops depends on managed bees, whether for setting seeds or increasing their yield (Allen-Wardell et al., 1998; Abrol, 2011).

Accordingly, this study was carried out to investigate the effects of honey bees and bumble bees on the seed yield and quality of *C. tinctorius* (safflower) of the 'Remzibey-05' line.

Materials and Methods

Seeds were provided by the Directorate of Trakya Agricultural Research Institute, Republic of Turkey Ministry of Food Agriculture and Livestock. The treatment field was located at the Beytepe Campus of Hacettepe University in Ankara, Turkey (39°52'05.93" N; 32°43'47.94" E; 1042 m above sea level). Seeds were sown between April 16, 2015 and April 18, 2015. Although safflower is typically cultivated in rows, it is considered the best practice to keep an empty row between the planted rows for the sake of weed control. For this purpose, the recommended interval between rows is 15-20 cm. When the row spacing is 15-20 cm, 4-6 kg of seed should be used per decare (i.e. per 1000 m^2). Five 90 m^2 fields were prepared for sowing, and each was sown by hand with 110 g of seeds. No irrigation was applied during the trials. The blooming period started on July 15, 2015 and finished on July 27, 2015. Before the blooming period, four of the 90 m^2 fields were surrounded with teflon netting to prepare approximately 270 m^3 cages. The netting allowed water, air, and sunlight to pass, while preventing pollination by all other insects aside from the managed bees included in specific treatment cages. The cage size was 5 m × 18 m × 3 m, and the screen mesh size was 0.1 mm × 0.1 mm.

Apis mellifera L. colonies were provided by the Development Foundation of Turkey, which is one of the official colony breeding companies in Turkey and was founded in 1978. The honey bee colonies each contained about 150 workers, brood, and a queen. *Bombus terrestris* L. commercial colonies were provided by Koppert Biological Systems-Turkey. The bumble bee colonies each contained about 80 workers, brood, and a queen. Bee colonies were introduced into the cages two days after (July 17, 2015) the blooming period started. There were three honey bee hives located just 20 m away from the treatment fields.

Five treatment plots were established, which consisted of four cages with either honey bees and bumble bees caged together (T1), bumble bees only (T2), honey bees only (T3), or without any insects (T4), and one open-pollinated plot (T5).

The plants were harvested by hand on September 11, 2015. Seeds were cleaned with a Röber brand D-4950 MINDEN model seed cleaning machine and made ready for further analyses at the Republic of Turkey Ministry of Food Agriculture and Livestock's Seed Gene Center.

Measured Yield Components of Safflower

Seed quality was evaluated in terms of six characters:

Total seed yield: After the harvest of all plants, all seeds produced were weighed for each treatment plot. The data were transformed from m^2/g to kg/ha.

Number of seeds per capitulum: The capitula of 30 plants from each treatment plot were harvested separately to investigate the number of seeds present in each capitulum. The sampled plants were chosen randomly from each plot, and the results are presented herein as the mean and standard deviation per treatment.

Weight of 1000 seeds: Seeds were chosen randomly from the total harvest (i.e. five samples of 1000 seeds from the total harvest were taken per plot). The results are presented herein as the mean and standard deviation per treatment.

Fatty acid composition and total oil content: These analyses were conducted with 10 randomly chosen samples taken from each treatment plot. Each sample had a mass of

50 g. The analyses were conducted in the Republic of Turkey Ministry of Agriculture's Food Analysis Laboratories. Fatty acid compositions were analyzed with the AOAC 17 ed.2002,969.3341.1.28-TUPAC7 1987 method, and the total oil content of the seeds was evaluated with the TS EN ISO 659 method. The results are presented herein as the mean and standard deviation per treatment.

Oil yield (g/ha): Oil yield (g/ha) was calculated using the formula: *oil yield = %oil × seed yield* (Saeidi et al., 2018).

Statistical Analyses

The results were analyzed in R software (Fox et al., 2009). One-way analysis of variance (ANOVA) was used to compare results among treatments, and was then followed by Tukey's test to determine the statistical significance of the multiple comparisons among different treatment groups' means.

Results and Discussion

Total Seed Yield

The highest safflower seed yield was found in the caged bumble bee treatment (T2) (173.2 kg/ha), followed by the open-pollinated (154.6 kg/ha) (T5), caged honey bees and bumble bees together (152.4 kg/ha) (T1), caged honey bee (114.7 kg/ha) (T3), and caged without any insects (90.2 kg/ha) (T4) treatments (Table 1).

Table 1. Total seed and oil yield and mean ± standard deviation of number of seeds per capitulum, weight of 1000 seeds, and total oil content for plots in five treatment groups. T1: caged honey bees with bumble bees (HB+BB); T2: caged bumble bees (BB); T3: caged honey bees (HB); T4: caged without any insects (WB); T5: open-pollinated (OP).

Group	Total seed yield (kg/ha)	Number of seeds per capitulum	Weight of 1000 seeds (g)	Total oil content (%)	Oil yield (g/ha)
T1 (HB+BB)	152.4	46.1 ± 12.66	27.68 ± 0.7	28.517 ± 2.11	4345.99
T2 (BB)	173.2	43.7 ± 9.5	28.3 ± 0.5	29.295 ± 1.43	5073.89
T3 (HB)	114.7	48 ± 10.1	27.64 ± 0.6	29.185 ± 2.39	3347.51
T4 (WB)	90.2	43.06 ± 7.7	29.02 ± 0.1	28.605 ± 1.84	2580.17
T5 (OP)	154.6	49.03 ± 9.11	29.56 ± 0.5	27.577 ± 1.01	4263.40

Some expectations from previous research suggested that increasing the number of pollinator species should cause much higher rates of pollination (Brittain et al., 2013). However, in our study we found that the competition between honey bees and bumble bees in caged conditions negatively impacted overall pollination rates.

Number of Seeds Per Capitulum

The number of seeds per capitulum in each treatment was found to be: T1 (caged both) = 46.1 ± 12.66; T2 (caged bumble bee) = 43.7 ± 9.5; T3 (caged honey bee) = 48 ± 10.1; T4 (no bee) = 43.06 ± 7.7; and T5 (open) = 49.03 ± 9.11 (Table 1).

There were no statistically significant differences found among treatment means ($F(4,145) = 2.063$, $p = 0.0886$).

Weight of 1000 Seeds

The weight of 1000 seeds for each treatment plot (n = 5 samples per treatment) were found to be: T1 (caged both) = 27.68 ± 0.7 g; T2 (caged bumble bee) = 28.3 ± 0.5 g; T3 (caged honey bee) = 27.64 ± 0.6 g; T4 (no bee) = 29.02 ± 0.1 g; T5 (open) = 29.56 ± 0.5 g (Table 1).

A one-way ANOVA was conducted to determine if the weight of 1000 seeds significantly differed among treatments with different pollinators. The results were found to be significantly different among treatments ($F(4,20) = 11.22$, $p = 6.13\times10^{-5}$). Tukey's post-hoc test revealed that the weight of 1000 seeds was significantly different among all treatment groups, except between T3 and T1 ($p = 0.998$). Only the 95% confidence interval for the difference between T3 and T1 contained 0. Thus, the efficiency for the different pollinator treatments to improve the weight of 1000 seeds from each plot can be summarized in the following order: T5 > T4 > T2 > T3 = T1.

Fatty Acid Composition and Total Oil Content

The fatty acid composition of the seeds was evaluated according to the AOAC 17 ed.2002,969.3341.1.28-TUPAC7 1987 method, and in total 21 different fatty acids were investigated. Seven of these (caproic acid (C6:0), caprylic acid (C8:0), capric acid (C10:0), lauric acid (C12:0), interdic acid (C20:0), erucic acid (C22:1), and docosadienoic acid (C22:2) were not detected in the safflower seed oil in any treatment.

The remaining 14 fatty acids that were investigated were detected in all of the samples evaluated (Table 2). A one-way ANOVA was conducted to determine whether the fatty acid composition differed among treatment groups with different pollinators.

Table 2. Mean ± standard deviation of the relative content of different fatty acids in safflower seed in five plots in different treatment groups. T1: caged honey bees with bumble bees (HB+BB); T2: caged bumble bees (BB); T3: caged honey bees (HB); T4: caged without any insects (WB); T5: open-pollinated (OP). * indicates significant differences among groups at a 5 % probability level.

	Treatment groups				
Fatty acid composition (% m:m methyl esters)	T1 (HB+BB)	T2 (BB)	T3 (HB)	T4 (WB)	T5 (OP)
Myristic acid (C14:0)	0.111±0.02	0.12±0.04	0.107±0.02	0.199±0.27	0.105±0.01
Palmitic acid (C16:0)	6.628±0.92	6.777±0.68	6.026±0.05	6.639±0.85	6.382±0.24
Palmitoleic acid (C16:1)	0.102±0.03	0.094±0.02	0.085±0.01	0.1±0.03	0.104±0.01
Margaric acid (C17:0) (= heptadecanoic acid)	0.047±0.02	0.041±0.02	0.032±0	0.035±0.02	0.033±0.01
Heptadecenoic acid (C17:1)	0.027±0.01	0.027±0	0.026±0.01	0.026±0.01	0.032±0.01
Stearic acid (C18:0)	2.266±0.33	2.569±0.94	2.12±0.08	2.415±0.52	2.223±0.46
Oleic acid (C18:1)*	42.023±4.46	39.629±1.4	37.985±1.69	40.645±3.97	43.111±1.67
Linoleic acid (C18:2)*	47.282±5.94	49.273±2.32	51.71±0.75	48.539±5.55	46.459±1.5
Linolenic acid (C18:3)*	0.11±0.03	0.1±0.02	0.124±0.01	0.103±0.02	0.096±0.01
Arachidonic acid (C20:0)	0.428±0.06	0.434±0.03	0.422±0.02	0.446±0.06	0.471±0.05
Gadoleic acid/ecosenic acid (C20:1)	0.214±0.02	0.213±0.01	0.207±0.03	0.221±0.03	0.224±0.03
Behenic acid (C22:0)	0.343±0.06	0.336±0.03	0.332±0.05	0.309±0.13	0.36±0.04
Lignoceric acid (C24:0)	0.1±0.02	0.114±0.03	0.138±0.04	0.123±0.07	0.133±0.02
Nervonic acid (C24:1)	0.146±0.05	0.157±0.03	0.188±0.06	0.153±0.05	0.152±0.02

Overall, no significant differences were found in the relative content of 11 fatty acids among treatments by one-way ANOVA. These fatty acids were: myristic acid (C14:0) ($F(4, 45) = 0.99$, $p = 0.423$), palmitic acid (C16:0) ($F(4, 45) = 1.989$, $p = 0.112$), palmitoleic acid (C16:1) ($F(4, 45) = 1.421$, $p = 0.243$), margaric acid (C17:0) ($F(4, 45) = 1.268$, $p = 0.296$), heptadecenoic acid (C17:1) ($F(4, 45) = 0.991$, $p = 0.422$), stearic acid (C18:0) ($F(4, 45) = 0.979$, $p = 0.428$), arachidonic acid (C20:0) ($F(4, 45) = 1.81$, $p = 0.144$), gadoleic acid/ecosenic acid (C20:1) ($F(4, 45) = 0.58$, $p = 0.676$), behenic acid (C22:0) ($F(4, 45) = 1.33$, $p = 0.273$), lignoceric acid (C24:0) ($F(4, 45) = 1.42$, $p = 0.242$), and nervonic acid (C24:1) ($F(4, 45) = 0.66$, $p = 0.626$).

On the other hand, the relative content of oleic acid (C18:1) ($F(4, 45) = 4.413$, $p = 0.00429$), linoleic acid (C18:2) ($F(4, 45) = 2.618$, $p = 0.0474$), and linolenic acid (C18:3) ($F(4, 45) = 3.073$, $p = 0.0254$) were found to significantly differ among treatment groups. The highest linoleic acid content (51.71%) was found in T3, whereas the lowest (46.45%) was found in T5 (Table 2).

A one-way ANOVA was conducted to determine whether total oil content significantly differed among treatments with different pollinators. Based on this, there were found to be no significant differences among treatment group means ($F(4, 45) = 1.332$, $p = 0.273$).

Oil Yield (g/ha)

The highest safflower oil yield was found in the caged bumble bee treatment (T2) (5073.89 g/ha), followed by the caged honey bees and bumble bees together (4345.99 g/ha) (T1), open-pollinated (4263.40 g/ha) (T5), caged honey bee (3347.51g/ha) (T3), and caged without any insects (2580.17 g/ha) (T4) treatments (Table 1).

The total seed yield was higher in the managed bee treatment groups compared to that in the treatment without bees and the open-pollinated plot. According to our results, in the open field where the honey bee colonies were located, the total seed yield and oil yield were higher than that in the other plots except the T2 (BB) ones.

Conclusion

The importance of safflower as an oilseed crop has increased in recent decades, especially due to the increasing global production of biofuels (Dordas and Sioulas, 2008; Koutroubas and Papakosta, 2010) and safflower's excellent adaptability to drought conditions (Kurhade et al., 2016). Our results showed that seed set rate was much higher in the treatments with bees than in those without them.

Safflower is a highly melliferous plant (Zanetti et al., 2013), and according to our results the use of managed pollinators could increase its total seed yield. As was mentioned earlier, safflower is an open-field cultivated crop. We thus recommend the use of commercial bees in the cultivation of safflower.

Acknowledgements

This study is a part of Çiğdem Özenirler's PhD thesis, and was supported by Hacettepe University Research Foundation Project No:014B0460100.

Compliance with Ethical Standards

Authors' Contributions

CO: Designed the study and interpreted data, performed the field and laboratory work and drafted the paper
KS: Designed the study and interpreted data

Conflict of Interest

The authors declare that there is no conflict of interest.

Statement on the Welfare of Animals

For this type of study, formal consent is not required.

Statement of Human Rights

This study does not involve human participants.

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