

## Effects of honeybee and other bee pollination and nanocomposite fertilizers on improvement of vegetative and reproductive characteristics of different cultivars of sunflower (*Helianthus annuus*) in Karbala City, Iraq

L. Qasim Alkinani<sup>1</sup>, A. Rasekh<sup>2\*</sup> and A. Najm Almosawy<sup>3</sup>

1. Ph.D. student, Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. \*Corresponding Author: Professor, Department of Plant Protection, College of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran (a.rasekh@scu.ac.ir)
3. Professor, Department of Field Crops, College of Agriculture, University of Karbala, Karbala, Iraq

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### Abstract

A field experiment was conducted during spring 2017 to determine the effect of honeybee (*Apis mellifera* L.) pollination and four concentrations (0, 1, 1.5, and 2 ml/L) of the protic-carbon nanocomposite on vegetative (plant height and plant leaf area) and reproductive (number of seeds per head) characteristics of 6 cultivars of sunflower (*Helianthus annuus* L.). For this purpose, a plot of land (420 mm<sup>2</sup>) was selected and 6 cultivars (French E5g12, Argentine A4g13, American F13g4, Turkish C13s4, Iraqi G14s, and Iraqi Q5g14) were individually planted in 6 replicates. For non-pollination treatment, the plants were covered with muslin cloth (40 Mesh), while in pollination treatment; plants were uncovered so that, honeybees and other pollinators could easily pollinate the sunflowers. Nine honeybee hives were located nearby the field. Data were analyzed using a two-way analysis of variance to investigate the effects of insect pollination and different concentrations of nanocomposite, or the effects of insect pollination and different cultivars of sunflower. The results revealed that occurrence of pollination and increasing concentration of nanocomposite significantly influenced vegetative and reproductive characteristics of all cultivars. Moreover, unlike vegetative characteristics, in which there were no interactions between pollination and cultivar type, reproductive characteristics were influenced by the interaction of these two variables, and in most treatments, vegetative and reproductive characteristics were significantly higher in the plant treated by insect pollination than non-pollinated plants. Our results confirmed the previous findings regarding higher vegetative characteristics and production of seeds in sunflowers as a result of introduction of honeybee colonies in culture.

**Keywords:** *Apis mellefera*, cultivar of sunflower, different concentrations of nanocomposite

## Introduction

Sunflower, *Helianthus annuus* L., is considered as an important source of edible oil (Guo et al., 2017). This plant belongs to the family of Asteraceae and is ranked the third in terms of oil production in the world. Seed of this plant is also used for food (Guo et al., 2017), and its cultivation is assumed as an important economic alternative in crop rotation and provides succession of crops and intercropping in seed-producing regions (Porto et al., 2007).

Inoculation and fertilization of sunflowers are among the most important factors in its seed yield (Chandra et al., 2010). Insects can be considered as effective factors in flower pollination, among which bees are the most important group (Primack and Inouye, 1993). During the last decades, decline in abundance and diversity of wild insects has led to an increasing demand for the Western honeybee, *Apis mellifera* L. (Hymenoptera, Apidae) (Biesmeijer et al., 2006; Aizen et al., 2008), that despite commercial relevance of colony products (e.g., honey and pollen), activity of which as crop pollinator has remained as its most important economic contribution (Abrol, 2012). European honeybee (*A. mellifera*) is an important pollinator compared to other insects, because of large population of this insect and the fact that its nutrition depends on nectar and pollen of flowers (van der Sluijs and Vaage, 2016). Honeybee workers visit several plants to suck nectar and collect pollen. Workers gather pollen into pollen baskets on their back legs and carry it back to hive, where it is used as food for developing brood (Müller et al., 2006; Rojarsi et al., 2012). Pollination has an important role in enhancing performance of crops, such as bean, rapeseed, tomatoes, zucchini, and sunflower (Prasifka et al., 2018). Wittmann (2007) stated that honeybee pollination would raise farmers' income up to 50%. The sunflower head consists of many individual florets, each of which passes first through a male stage and then a female stage (Free, 1993). Although the plant is self-compatible, bees often increase sunflower seed set through cross-pollination between individual plants by moving pollen from male-stage florets to female-stage florets within the same flower head (Greenleaf and Kremen, 2006).

Boron has been known to be an essential element for growth of higher plants (Bolanos et al., 2004; Sotomayor et al., 2010). Boron is essential for a series of physiological processes, such as processes associated with plant reproduction, basic flowering, and fruiting (Blevins and Lukaszewski, 1998). The recent studies have confirmed essential processes regarding structural conservation of cell walls, functional conservation of cell membranes, and support of metabolic activities specific to boron (Bolanos et al., 2004). Vegetative tissues have relatively lower boron content than flower tissues. Although, concentration of boron in reproductive organs of plants is different for example, pollen grains of most species are naturally low in boron, while styles, stigmas, and ovaries generally have higher concentrations (Blevins and Lukaszewski, 1998). Low boron levels in flowers reduce fertility by influencing growth of the pollen tube and damaging pollen formation (Shireen et al., 2018). Low boron levels can also have post-insemination effects that affect embryogenesis, leading to seed abortion and fruit malformation (Dell and Huang, 1997). Boron also causes plants to withstand certain diseases for example, in the sunflower plants; it has been shown to cause resistance against powdery mildew caused by *Erysiphe cichoracearum* (Schutte, 1964).

Therefore, this study was done to simultaneously determine the effect of honeybee pollination and also different concentrations of Proteck Calbor nanocomposite, on some vegetative and reproductive characteristics of 6 different cultivars of sunflower.

## Material and Methods

A field experiment was performed in Educational Farm of College of Agriculture (The University of Karbala, Iraq) during spring 2017 to determine the effect of honeybee (*A. mellifera*) pollination and four concentrations (0, 1, 1.5, and 2 ml/L) of the protic-carbon nanocomposite on vegetative (plant height and plant leaf area) and reproductive (number of seeds per head) characteristics of 6 cultivars of sunflower (*H. annuus*). For this purpose, a plot of land (420 mm<sup>2</sup>; 17.5 m × 24 m) was selected and 6 cultivars (including of French E5g12, Argentine, A4g13,

American F13g4, Turkish C13s4, Iraqi G14s, and Iraqi Q5g14) were individually planted in 6 replicates. Each cultivar was planted in 15 rows (each of which was 4.0 m long with 0.5 m distance from each other). From a total of 144 cultivated plants (25 cm of distance in row and 50 cm of distance between rows) for each cultivar, 18 plants were allocated to covered and uncovered treatments to be exposed to four different concentrations of nanocomposite.

Thirty days after planting, nanocomposite was applied to the plants at the above mentioned concentrations. For non-pollination treatment, the plants were covered with muslin cloth (40 Mesh) so that, honeybees and other pollinator insects were not allowed to enter. While in pollination treatment, plants were uncovered so that honeybees and other pollinator insects could easily pollinate the sunflowers. Nine honeybee hives were located nearby. Descriptions related to developmental characteristics were provided in plants grown in all treatments, as described below:

1. Plant height (cm): Plant height was measured at a complete fertilization stage, from soil surface to lower plant node;
2. Plant leaf surface (cm): Leaf area of the plant was measured at the maximum fruiting stage by measuring the maximum leaf width;
3. Number of seeds per head: The number of grains in each sunflower disk was calculated by manual harvesting. This calculation included count of full and empty grains.

#### Statistical analysis:

Data for vegetative and reproductive characteristics of sunflower were analyzed using two-way analysis of variance (ANOVA) for the effects of insect pollination and different concentrations of nanocomposite, or effects of insect pollination and different cultivar of sunflower as independent fixed factors (SPSS

ver. 17.0, 2008). If the interaction was not significant, the data were pooled and reanalyzed using simple one-way ANOVA of the dependent variable (Seltman, 2018). In this case, the data of each cultivar, including that pollination was performed or not, were pooled and comparisons were made between different cultivars. Means were separated with the Tukey's test ( $\alpha = 0.05$ ) whenever more than two treatments were compared (SPSS ver. 17.0, 2008).

## Results and discussion

### Plant height

There were significant main effects of both pollination and different cultivar on plant height, while the 'pollination  $\times$  cultivar' interaction was not significant (Table 1). Pollination had a significant effect on plant height such that, in all treatments, with exception of two treatments (Turkish C13s4 in zero concentration and Iraqi Q5g14 in 1.5 ml/L concentration of nanocomposite), the presence and activity of honeybees led to an increase in plant height (Table 2).

Reanalysis of the pooled data showed that in samples not treated with nanocomposite (zero concentration of nanocomposite), there was no significant difference between heights of different cultivars ( $F_{5,30} = 1.835$ ,  $P = 0.136$ ), while following application of nanocomposite, differences appeared between heights of different cultivars. Accordingly, at concentration of 1 ml/L of nanocomposite, plant height in cultivar of French E5g12 ( $122.7 \pm 4.6$ ) was significantly higher than cultivar of Iraqi G14s ( $98.5 \pm 7.6$ ) ( $F_{5,30} = 2.798$ ,  $P = 0.034$ ), and at concentrations of 1.5 and 2 ml/L, similarly, the lowest plant height was observed in Iraqi G14s cultivar ( $98.5 \pm 18.6$ ,  $F_{5,30} = 5.081$ ,  $P = 0.002$ ;  $121.8 \pm 15.0$ ,  $F_{5,30} = 2.286$ ,  $P = 0.005$ , respectively).

**Table1. Two-way ANOVA of the effects of pollination (performed or non-performed) and cultivar (six different cultivars) on vegetative and reproductive characteristics of sunflower plant.**

	d.f.	Height		Leaf area		Number of seeds	
		<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Pollination	1	49.9	< 0.001	22.2	< 0.001	54.3	< 0.001
Cultivar	5	8.1	< 0.001	7.8	< 0.001	6.0	< 0.001
Pollination*Cultivar	5	2.2	0.061	0.314	0.9	5.5	< 0.001
Residual d.f.	132						

**Table 2. Mean ( $\pm$ SE) height of 6 different sunflower (*Helianthus annuus*) cultivars, treated with different concentrations of nanocomposite exposed or not exposed to honeybee pollination.**

Concentration of nanocomposite (ml/L)	Pollination by honeybee	Cultivar						F	df	P
		French E5g12	Argentine A4g13	American F13g4	Turkish C13s4	Iraqi G14s	Iraqi Q5g14			
0 ml/L (Control)	Non-performed	85.8 $\pm$ 3.1 Bbc	95.3 $\pm$ 2.0 Bab	100.7 $\pm$ 3.1 Ba	90.8 $\pm$ 4.5 Aab	74.2 $\pm$ 0.2 Bc	84.3 $\pm$ 2.7 Abc	10.3	5,12	0.001
	Performed	111.7 $\pm$ 4.6 Aa	115.5 $\pm$ 2.8 Aa	115.0 $\pm$ 3.8 Aa	101.3 $\pm$ 2.0 Aa	105.8 $\pm$ 0.3 Aa	101.2 $\pm$ 6.1 Aa	3.0	5,12	0.06
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	21.6; 0.01	34.5; 0.005	8.6; 0.04	4.5; 0.1	722; <0.001	6.3; 0.07			
1.0 ml/L	Non-performed	108.3 $\pm$ 1.9 Ba	108.7 $\pm$ 5.8 Ba	112.8 $\pm$ 2.7 Ba	105.5 $\pm$ 1.0 Ba	82.0 $\pm$ 0.6 Bb	105.3 $\pm$ 3.3 Ba	12.8	5,12	<0.001
	Performed	131.5 $\pm$ 3.2 Aa	130.0 $\pm$ 2.2 Aab	132.5 $\pm$ 1.5 Aa	122.7 $\pm$ 6.2 Aab	115.0 $\pm$ 4.1 Ab	115.0 $\pm$ 0.8 Ab	5.4	5,12	0.008
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	38.7; 0.003	11.9; 0.03	39.6; 0.003	7.5; 0.05	63.7; 0.001	7.9; 0.05			
1.5 ml/L	Non-performed	127.3 $\pm$ 2.3 Ba	118.2 $\pm$ 2.3 Bb	123.2 $\pm$ 1.9 Bab	116.7 $\pm$ 1.4 Bb	82.7 $\pm$ 0.9 Bc	116.7 $\pm$ 2.5 Ab	66.3	5,12	<0.001
	Performed	141.8 $\pm$ 1.6 Aa	138.5 $\pm$ 3.3 Aab	141.0 $\pm$ 5.5 Aa	123.5 $\pm$ 0.9 Abc	123.7 $\pm$ 6.2 Abc	121.7 $\pm$ 1.5 Ac	6.6	5,12	0.003
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	21.6; 0.007	25.9; 0.007	9.3; 0.04	17.9; 0.01	43.2; 0.003	3.1; 0.16			
2.0 ml/L	Non-performed	132.8 $\pm$ 2.6 Bab	142.7 $\pm$ 3.9 Ba	135.8 $\pm$ 1.2 Ba	120.7 $\pm$ 0.4 Bb	92.7 $\pm$ 5.2 Bc	120.3 $\pm$ 1.4 Bb	35.3	5,12	<0.001
	Performed	148.2 $\pm$ 4.9 Abc	171.5 $\pm$ 2.3 Ab	220.5 $\pm$ 2.2 Aa	133.8 $\pm$ 1.8 Ac	150.8 $\pm$ 9.9 Abc	133.7 $\pm$ 3.0 Ac	21.6	5,12	<0.001
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	7.6; 0.05	40.0; 0.003	1157; <0.01	48.8; 0.002	12.0; 0.03	16.1; 0.02			

Values bearing the same upper case letters were not significantly different between pollination occurring within a cultivar and a certain concentration of nanocomposite, values bearing the same lower case letters were not significantly different between different cultivars within the same state of pollination and a certain concentration of nanocomposite (ANOVA Two-way followed by Tukey test,  $P > 0.05$ ).

The results showed that the interaction between nanocomposite and sunflower cultivars had a significant effect on plant height. The highest increase was observed in the plants treated with 2 ml/L of nanocomposite (60.85%) with a height of 187.82 cm and the lowest increase in plant height was observed at zero concentration of nanocomposite with a height of 116.76 cm.

Our results are consistent with the study by Eagleton et al. (1988) who reported a significant difference in plant height between different sunflower cultivars.

The results of previous studies have shown that average number of bees per blooming head was significantly different, which is probably due to differences in nectar production and/or accessibility of nectar to bees (Dag et al., 2002). Accordingly, the differences observed between cultivars may be related to intensity of honeybee activity on certain cultivar.

#### **Leaf surface of the plant**

There were significant main effects regarding both pollination and different cultivars on leaf surface, while the 'pollination × cultivar' interaction was not significant (Table 1).

Pollination had a significant effect on leaf surface of the plant (Table 3). While at 2 ml/L concentration of nanocomposite, the presence and activity of honeybees led to an increase in leaf surface of all 6 sunflower cultivars, in other concentrations, pollination had a significant positive effect on some cultivars (Argentine A4g13, Turkish C13s4, and Iraqi Q5g14 in zero concentration; Argentine A4g13, and Iraqi G14s in 1 ml/L concentration; Iraqi G14s, and Iraqi Q5g14 in 1.5 concentration of nanocomposite) (Table 3).

Similar to the results of the present study, in a previous experiment, in which additional hand pollination was applied for *Primula veris*, as a perennial spring-flowering rosette species, a higher net photosynthetic capacity was observed in leaves after hand pollination, which led to the increased leaf growth (Lehtilä and Syrjänen, 1995).

Statistical analysis showed a significant increase in leaf area in sunflower plants pollinated with insects (honeybees and other pollinators) and treated with nanocomposite

(Table 3). The highest leaf area was observed in plants treated with 2 ml of nanocomposite by 89% with a width of 17.38 cm and the lowest leaf width was observed at concentration of nanocomposite by 9.16 cm.

Reanalysis of the pooled data showed significant difference between leaf surface of different cultivars in control ( $F_{5,30} = 7.99$ ,  $P < 0.001$ ) and all samples treated with concentrations of nanocomposite (1 ml/L:  $F_{5,30} = 8.035$ ,  $P < 0.001$ ; 1.5 ml/L:  $F_{5,30} = 4.905$ ,  $P < 0.001$ ; 2 ml/L:  $F_{5,30} = 7.344$ ,  $P < 0.001$ ). The Argentine A4g13 cultivar had the lowest leaf area, in all concentrations ( $6.67 \pm 0.9$ ;  $9.5 \pm 0.78$ ;  $11.25 \pm 0.79$ ;  $12.5 \pm 0.47$ , respectively) and the Iraqi G14s had the highest leaf area, in all concentrations ( $11.4 \pm 0.37$ ;  $14.5 \pm 0.48$ ;  $17 \pm 0.61$ ;  $21.6 \pm 1.4$ , respectively), with no significant difference with American F13g4 and French E5g12 cultivars.

#### **Number of seeds per head**

There were significant main effects regarding both pollination and different cultivars on number of seeds per head, and the 'pollination × cultivar' interaction was significant (Table 1).

In all treatments, with exception of treatments from the French E5g12 cultivar, pollination, the presence, and activity of honeybees led to an increase in the number of seeds (Table 4).

Unlike vegetative characteristics, in which there were no interactions between pollination and cultivar type, reproductive characteristics (number of seeds) were influenced by the interaction of these two variables. Despite the fact that in most of the treatments in the present study, occurrence of pollination led to an increase in the number of seeds however, in a comparison between cultivars, it was found that pollination had different additive effects, and in some cultivars, a greater increase was observed in the number of seeds compared to some other cultivars.

Based on statistical analysis, treatment with nanocomposite significantly increased the number of seeds such that, the highest increase was observed in plants treated with 2 ml of fertilizer with an average of 1008.9 seeds. The non-treated plants had the lowest number with average of 346.93 seeds.

**Table 3. Mean ( $\pm$ SE) leaf area of 6 different sunflower (*Helianthus annuus*) cultivars, treated with different concentrations of nanocomposite exposed or not exposed to honeybee pollination.**

Concentration of nanocomposite (ml/L)	Pollination by honeybee	Cultivar						<i>F</i>	<i>df</i>	<i>P</i>
		French E5g12	Argentine A4g13	American F13g4	Turkish C13s4	Iraqi G14s	Iraqi Q5g14			
0 ml/L (control)	Non-performed	9.3 $\pm$ 0.7 Aa	4.8 $\pm$ 0.3 Bb	10.6 $\pm$ 0.3 Aa	6.2 $\pm$ 0.3 Bb	11.0 $\pm$ 0.6 Aa	5.0 $\pm$ 0.5 Bb	34.5	5,12	<0.001
	Performed	11.0 $\pm$ 0.0 Aa	8.6 $\pm$ 0.4 Ab	11.8 $\pm$ 0.4 <sup>Aa</sup>	8.7 $\pm$ 0.3 Ab	11.8 $\pm$ 0.4 <sup>Aa</sup>	10.5 $\pm$ 0.3 <sup>Aa</sup>	18.1	5,12	<0.001
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	5.3; 0.08	68.3; 0.001	5.5; 0.08	28.1; 0.006	1.3; 0.315	90.8; 0.001			
1.0 ml/L	Non-performed	11.3 $\pm$ 0.7 Ab	8.3 $\pm$ 0.4 Bc	12.3 $\pm$ 0.2 Aab	8.2 $\pm$ 0.2 Ac	13.5 $\pm$ 0.3 Ba	9.3 $\pm$ 0.6 Ac	24.6	5,12	<0.001
	Performed	12.7 $\pm$ 0.4 Ab	12.3 $\pm$ 0.2 Ab	12.7 $\pm$ 0.4 Ab	10.8 $\pm$ 1.1 Ab	15.5 $\pm$ 0.3 Aa	11.7 $\pm$ 0.9 Ab	6.1	5,12	0.005
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	2.5; 0.19	77.5; 0.001	0.5; 0.52	5.8; 0.07	24.0; 0.008	4.8; 0.09			
1.5 ml/L	Non-performed	12.8 $\pm$ 0.9 Aabc	12.9 $\pm$ 0.9 Aabc	14.8 $\pm$ 0.2 Aab	10.7 $\pm$ 1.7 Ac	15.7 $\pm$ 0.2 Ba	11.5 $\pm$ 0.5 Bbc	4.7	5,12	0.013
	Performed	16.5 $\pm$ 2.1 Aab	15.5 $\pm$ 0.6 Aab	16.5 $\pm$ 2.1 Aab	11.8 $\pm$ 0.2 Ab	18.3 $\pm$ 0.2 Aa	14.8 $\pm$ 0.2 Aab	3.2	5,12	0.048
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	2.6; 0.18	6.1; 0.07	0.64; 0.47	0.49; 0.52	128; <0.001	40.0; 0.003			
2.0 ml/L	Non-performed	16.3 $\pm$ 0.3 Bb	16.7 $\pm$ 0.4 Bb	18.5 $\pm$ 0.6 Ba	11.5 $\pm$ 0.0 Bc	18.5 $\pm$ 0.6 Ba	12.5 $\pm$ 0.3 Bc	51.0	5,12	<0.001
	Performed	25.5 $\pm$ 1.0 Aa	19.3 $\pm$ 0.4 Ab	24.7 $\pm$ 0.6 Aa	13.5 $\pm$ 0.3 Ac	23.5 $\pm$ 1.2 Aa	18.7 $\pm$ 0.9 Ab	32.7	5,12	<0.001
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	70.3; 0.001	18.3; 0.013	54.8; 0.002	48.0; 0.002	15.0; 0.018	44.2; 0.003			

Values bearing the same upper case letters were not significantly different between pollination occurring within a cultivar and a certain concentration of nanocomposite, values bearing the same lower case letters were not significantly different between different cultivars within the same state of pollination and a certain concentration of nanocomposite (ANOVA Two-way followed by Tukey test,  $P > 0.05$ ).

**Table 4. Mean ( $\pm$ SE) number of seeds of 6 different sunflower (*Helianthus annuus*) cultivars, treated with different concentrations of nanocomposite exposed or not exposed to honeybee pollination.**

Concentration of nanocomposite (ml/L)	Pollination by honeybee	Cultivar						F	df	P
		French E5g12	Argentine A4g13	American F13g4	Turkish C13s4	Iraqi G14s	Iraqi Q5g14			
0 ml/L (control)	Non-performed	141 $\pm$ 24.8 Bb	202.8 $\pm$ 14.4 Bab	184 $\pm$ 22 Bb	258.3 $\pm$ 8.3 Ba	4.3 $\pm$ 2.3 Bc	272 $\pm$ 19.6 Ba	32.3	5,12	<0.001
	Performed	322 $\pm$ 25.6 Ac	559 $\pm$ 85.2 Aab	483 $\pm$ 43.9 Aabc	301 $\pm$ 0.9 Ac	664 $\pm$ 30.5 Aa	428 $\pm$ 44 Abc	9.2	5,12	0.001
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	25.8; 0.007	17.0; 0.015	37.1; 0.004	26.7; 0.007	466; <0.001	10.4; 0.032			
1.0 ml/L	Non-performed	423 $\pm$ 72.4 Abc	667.0 $\pm$ 53.5 Aa	535 $\pm$ 17.4 Bab	389 $\pm$ 0.8 Bbc	11.3 $\pm$ 3.2 Bd	360 $\pm$ 22.6 Bc	32.7	5,12	<0.001
	Performed	533 $\pm$ 100.8 Ab	772 $\pm$ 80.3 Aab	644 $\pm$ 16 Ab	538 $\pm$ 10.8 Ab	949 $\pm$ 22.2 Aa	616 $\pm$ 61.6 Ab	6.4	5,12	0.004
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	0.78; 0.43	1.2; 0.34	21.3; 0.01	8.0; 0.047	998; <0.001	15.2; 0.02			
1.5 ml/L	Non-performed	666.2 $\pm$ 73.2 Ab	882.2 $\pm$ 38.1 Aa	686 $\pm$ 33.6 Ab	458.3 $\pm$ 69.3 Bc	19.8 $\pm$ 2.5 Bd	445 $\pm$ 23.8 Bc	52.1	5,12	<0.001
	Performed	886 $\pm$ 137 Ab	1277 $\pm$ 90.8 Aa	803 $\pm$ 27.5 Ab	863 $\pm$ 10.8 Ab	985 $\pm$ 61 Ab	772 $\pm$ 10.8 Ab	6.5	5,12	0.004
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	2.01; 0.23	16.2; 0.016	7.2; 0.045	95.3; 0.001	246; <0.001	156; <0.001			
2.0 ml/L	Non-performed	923 $\pm$ 48.8 Ab	1157 $\pm$ 91.5 Ba	790 $\pm$ 0.58 Bbc	646.5 $\pm$ 6.3 Bc	37 $\pm$ 2.8 Bd	612 $\pm$ 23.9 Bc	75.7	5,12	<0.001
	Performed	1387 $\pm$ 99.7 Ab	2097 $\pm$ 310 Aa	987 $\pm$ 64.9 Ab	980 $\pm$ 7.6 Ab	1431 $\pm$ 36.8 Ab	920.2 $\pm$ 58 Ab	10.4	5,12	<0.001
	<i>F</i> <sub>1,4</sub> ; <i>P</i>	0.063; 0.814	8.1; 0.046	27.8; 0.006	1130; <0.001	999; <0.001	23.8; 0.008			

Values bearing the same upper case letters were not significantly different between pollination occurring within a cultivar and a certain concentration of nanocomposite, values bearing the same lower case letters were not significantly different between different cultivars within the same state of pollination and a certain concentration of nanocomposite (ANOVA Two-way followed by Tukey test,  $P > 0.05$ ).

These results are in agreement with the finding of the study by Moreti et al. (1996) who reported that the number of seed was significantly higher in the plant visited by insect than those protected by cages where insects were excluded. Similarly, Freund et al. (1982) founded that the number of seeds in the pollinated sunflowers was equal to 817 per disc, while in the covered discs; it was equal to 667.8 seeds per disc. The results of this study also showed that weight of 1,000 seeds was equal to 52.1 g in the pollinated plants in comparison with 19.9 g for plants with coated discs.

Results of another study demonstrated that patterns of pollination i.e., pollination with honeybee and open pollination had the highest seed set ratios (80 and 79%, respectively), while only 45.2% of seed set was reported in the control, where plants were kept in closed system and away from insect pollination. Similar types of trends were reported in mean weight of seeds per head and 100-seed weight, which superior values belonged to open and honeybee pollination (Elmhoud Altayeb and Abdalla Nagi, 2015).

Although, using honeybee requires movement of large numbers of colonies within short periods of time, but our findings like many other results

demonstrated economic importance of honeybee pollination for ecosystem services and agricultural crops. However, based on the results of some studies, it should be noted that behavioral interactions between wild and native bees increase pollination efficiency of honeybees on hybrid sunflower up to 5- folds, (Sarah et al., 2006) therefore, the positive effects of pollination may not just be attributed to honeybee activity.

Clearly, the honeybees played a very important role in increasing vegetative and reproductive characteristics of 6 cultivars of sunflower plant, *H. annuus* in open pollinated plant population. So, honeybee hives are recommended to be kept in field of sunflower plantation. In conclusion, our results indicated a significant increase in plant height, plant leaf area, and number of seeds per head in the plants pollinated with honeybees compared to those kept in the cage without any contact with honeybees and other pollinators. This could be attributed to the effect of honeybees as efficient pollinators of the sunflower plant.

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#### **REFERENCES**

- Abrol, D.P. 2012. Pollination biology. Biodiversity conservation and agricultural production, Springer.
- Aizen, M.A., Garibaldi, L.A., Cunningham, S.A., and Klein, A.M. 2008. Long-term global trends in crop yield and production reveal no current pollination shortage but increasing pollinator dependency. *Current Biology*, 18: 1572-1575.
- Biesmeijer, J.C., Roberts, S.P.M., Reemer, M., Ohlemuller, R., Edwards, M., and Peeters, T. 2006. Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. *Science*, 313: 351-354.
- Blevins, D., and M. Lukaszewski. 1998. Boron in plant structure and function. *Annual Reviews of Plant Physiology and Plant Molecular Biology*, 49:481-500.
- Bolanos, L., Lukaszewski, K., Bonilla, I., and Blevins, D. 2004. Why boron? *Plant Physiology and Biochemistry*, 42: 907-912.
- Chandra, B.S., Sudheer Kumar, S., Ranganatha, A., and Dudhe, M.Y. 2010. Inheritance of the fertility restoration for different CMS sources in sunflower (*Helianthus annuus* L.). *SABRAO Journal of Breeding and Genetics*, 42(1): 46-50.



- Dag, A., Lior, E., and Afik, O. 2002. Pollination of confection sunflowers (*Helianthus annuus* L.) by honey bees (*Apis mellifera* L.). American Bee Journal, 142(6): 443-445.
- Dell, B., and Huang, L. 1997. Physiological response of plants to low boron. Plant and Soil. 193:103-120.
- Eagleton, G., Sandover, S., and Dickson. 1988. Research report sunflower (1981-1986). Western Australia. pp.182.
- Elmhoud Altayeb, O.A., and Abdalla Nagi, S.K. 2015. Efficiency of honeybees (*Apis mellifera*) on the production of sunflower (*Helianthus annuus* L.) seeds in the Sudan. Journal of Experimental Biology and Agricultural Sciences, 3(2): 191-195.
- Free, J.B. 1993. Insect Pollination of Crops. Academic Press, London, UK. with permission from Elsevier.
- Freund, D.E., and Furgula, B. 1982. Effect of pollination by insects on the seed set and yield of ten oil seed sunflower cultivars. American Bee Journal, 122(4): 648-652.
- Greenleaf, S.S., and Kremen, C. 2006. Wild bees enhance honey bees pollination of hybrid sunflower. Proceedings of the National Academy of Sciences of the United States of America, 103: 13890-13895.
- Guo, S., Ge, Y., and Na Jom, K. 2017. A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (*Helianthus annuus* L.). Chemistry Central journal, 11(1): 95.
- Lehtilä, K., and Syrjänen, K. 1995. Positive Effects of Pollination on Subsequent Size, Reproduction, and Survival of *Primula Veris*. Ecology, 76(4): 1084-1098.
- Moreti, A.C., Silva R.M.B., Silva, E.C.A., Alves, M.L.T.M.F., and Otsuk, I.P. 1996. Increase of sunflower (*Helianthus annuus*) seed production by pollinating insect action. Scientia Agricola, 53: 280-284 (In Portuguese, with abstract in English).
- Müller, A., Diener, S., Schnyder, S., Stutz, K., Sedivy, C., and Dorn, S. 2006. Quantitative pollen requirements of solitary bees: implications for bee conservation and the evolution of bee-flower relationships. Biological Conservation, 130: 604-615.
- Porto, W.S., Carvalho, C.G.P., and Pinto, R.J.B. 2007. Adaptability and stability as selection criteria for sunflower genotypes. Pesquisa Agropecuária Brasileira, 42: 491-499 (In Portuguese with abstract in English).
- Prasifka, J.R., Mallinger, R.E., Portlas, Z.M., Hulke, B.S., Fugate, K.K., Paradis, T., Hampton, M.E., and Carter, C.J. 2018. Using nectar-related traits to enhance crop-pollinator interactions. Frontiers in plant science, 9: 8-12.
- Primack, R.B., and Inouye, D.W. 1993. Factors affecting pollinator visitation rates: A biogeographic comparison. Current Science, 65(3): 257-262.
- Rojarsi, M., Kanakadurga, k., Durga Rani, V., and Anuradha, Ch. 2012. Honey bees-potential pollinators in hybrid seed production of sunflower. International Journal of Applied Biology and Pharmaceutical technology. 3(2): 216-221.

Sarah, S., Greenleaf, and Claire Kremen. 2006 . Wild bees enhance honey bees' pollination of hybrid sunflower. PNAS, 103(37): 13890-13895.

Schutte, K.H. 1964. The biology of the trace elements; their role in nutrition. Crosby Lock-wood and Son Ltd., London.

Seltman, H.J. 2018. Experimental design and analysis. Chapter 11: Two-way Aova; available at: <http://www.stat.cmu.edu/~hseltman/309/Book/Book.pdf>

Shireen, F., Nawaz, M.A., Chen, C., Zhang, Q., Zhang, Z., Sohail, H., Sun, J., Cao, H., Huang, Y., and Bie, Z. 2018. Boron: functions and approaches to enhance its availability in plants for sustainable agriculture. International Journal of Molecular Sciences, 19(7): 1856.

Sotomayor, C., Norambuena, P., and Ruiz, R. 2010. Boron dynamics related to fruit growth and seed production in kiwifruit (*Actinida deliciosa*, cv. Hayward). Ciencia e Investigación Agraria, 37(1): 133-141.

SPSS Inc. Released 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.

Van der Sluijs, J.P., and Vaage, N.S. 2016. Pollinators and global food security: the need for holistic global stewardship. Food Ethics, 1: 75-91.

Wittmann, D. 2007. Bee pollinators and economic importance of pollination in crop production, case of kakamage, Westera Kenya. Department of entomology and wildlife. University of cape coast, ULB. 93P.



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## تأثیر گرده‌افشانی زنبورعسل و سایر گرده‌افشان‌ها و کاربرد غلظت‌های مختلف نانو کمپوزیت در بهبود ویژگی‌های رویشی و زایشی ارقام مختلف آفتابگردان (*Helianthus annuus*) در کربلا، عراق

لینا قاسم الکنانی<sup>۱</sup>، آرش راسخ<sup>۲\*</sup> و احمد نجم الموسوی<sup>۳</sup>

- ۱- دانشجوی دکتری تخصصی، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، اهواز، ایران
- ۲- \*نویسنده مسوول: استاد، گروه گیاه‌پزشکی، دانشکده کشاورزی، دانشگاه شهید چمران اهواز، اهواز، ایران (a.rasekh@scu.ac.ir)
- ۳- استاد، گروه محصولات زراعی، دانشکده کشاورزی، دانشگاه کربلا، کربلا، عراق

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### چکیده

در یک مطالعه مزرعه‌ای در بهار ۱۳۹۶، اثر گرده‌افشانی زنبورعسل *Aphis mellifera* و همچنین چهار غلظت کود نانو کمپوزیت (صفر، ۱، ۱/۵ و ۲ میلی لیتر/لیتر) روی ویژگی‌های رویشی (ارتفاع گیاه و سطح برگ) و زایشی (تعداد دانه) شش کولتیوار آفتابگردان (فرانسوی E5g12، آرژانتینی A4g13، آمریکایی F13g4، ترکی C13s4، عراقی G14s و عراقی Q5g14) بررسی شد. به این منظور، قطعه زمینی به مساحت ۴۲۰ مترمربع انتخاب و هر کولتیوار در شش تکرار کاشته شد. برای پوشاندن نیمی از تکرارها از پارچه موسین (۴۰ مش) به شکل قوسی برای جلوگیری از ورود زنبورها و سایر حشرات گرده‌افشان استفاده شد. نه کندوی زنبورعسل با جمعیت مناسب به منظور گرده‌افشانی در مجاورت مزرعه مستقر شد. از تجزیه واریانس دوطرفه برای تعیین اختلاف آماری بین متغیرهای مستقل از یک طرف بین وقوع گرده‌افشانی و انواع کولتیواتور آفتابگردان و از طرف دیگر بین گرده‌افشانی و غلظت‌های مختلف کود نانو کمپوزیت استفاده شد. نتایج نشان داد که وقوع گرده‌افشانی و افزایش غلظت کود نانو کمپوزیت منجر به افزایش معنی‌دار ویژگی‌های رویشی و زایشی در تمام کولتیوارها شد. علاوه بر این، برخلاف ویژگی‌های رویشی که برهم‌کنشی بین گرده‌افشانی و نوع کولتیوار وجود نداشت، ویژگی زایشی (تعداد دانه) تحت تأثیر برهم‌کنش این دو متغیر مستقل قرار داشته و در اکثر تیمارها، ویژگی‌های رویشی و زایشی در گیاهان گرده‌افشانی شده بیش‌تر از گیاهانی بود که از گرده‌افشانی با حشرات محروم شده بودند. نتایج این مطالعه افزایش ویژگی‌های رویشی و زایشی مورد مطالعه را همراه با به‌کارگیری کندوی زنبورعسل در مزارع آفتابگردان تأیید می‌نماید.

کلیدواژه‌ها: *Apis mellifera* کولتیوار آفتابگردان، غلظت‌های مختلف نانو کمپوزیت