# The effects of pollen sources and foliar application of zinc and boron on fruit set and fruit traits of three hazelnut cultivars

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#### Summary

The productivity of plants is generally influenced by the environment, the physiology of plant species and their management, species genetics, and their interactions. The present research aimed to assess the effects of various pollen sources ('Boliba', 'Gerche', and 'Daviana') on physical and chemical traits of nuts in some dominant cultivars ('Gerde-Eshkevarat', 'Fertile', and 'Segorbe') in Iran's hazelnut production industry. The effects of the application of micronutrients B as borax and Zn as zinc sulfate on improving the productivity of vegetative and reproductive processes, and then the interactive effect of these factors on hazelnut and kernel yield and quality were evaluated. The results showed that there was dichogamy in all studied cultivars and all cultivars were protandrous. The blooming time of male and female flowers was different among cultivars. After the nuts were harvested, nut and kernel traits were assessed. The highest weight of nuts with green husk (7.1 g) was related to 'Fertile'  $\times$ 'Gerche'  $\times$  borax + zinc sulfate' and the lowest (2.9 g) to the treatment of 'Segorbe'  $\times$  'Daviana'  $\times$  borax + zinc. The results indicated that the effect of the pollinizer parent was significant on hazelnut kernel and nut traits. The highest nut and kernel dimensions were obtained from 'Fertile'. The local variety ('Gerde-Eshkevarat') produced the widest kernels. In conclusion, among the assessed cultivars, the foliar application of zinc and boron had a significant effect on the quality (oil, Zn and B) of the hazelnuts.

#### Key words

Corylus avellana L., pollinizer, micronutrient, oil, zinc

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# Introduction

In recent years, nuts have found a special place in people's food regime due to their potential health benefits. Hazelnuts (Corylus avellana L.) are one of the main nuts in the world (Fattahi et al., 2014). Hazelnut is a monoecious, protandrous, anemophilous (wind pollinated) and sporophytic self-incompatible crop (Mehlenbacher and Smith, 1991; Baldwin, 2015). The main use of hazelnut kernels in food industries needs the production of cultivars with the least defects in morphological and chemical attributes (Boccacci et al., 2013). Commercial hazelnut cultivars differ both in pollen compatibility and the traits of fruit, kernel, and kernel composition. Some traits are, however, influenced by environmental conditions. The average hazelnut yield is low in Iran. It is reportedly about 889 kg ha-1 whereas its average global yield is 1496 kg ha<sup>-1</sup> ranging from a maximum of 2461 kg ha<sup>-1</sup> for Armenia to a minimum of 200 kg ha<sup>-1</sup> for Ukraine (FAOSTAT, 2017). The enhancement of yield requires the improvement of fruit set and adequate fruit development. To achieve this goal, all processes related to flower bud formation, flowering, fertilization, fruit set, and finally fruit formation until harvest time should happen at a certain level (Sotomayor et al., 2002).

Various researchers have reported the effect of specific parent pollen on the characteristics of kernels (xenia) or nuts (metaxenia) in several nut crops. Xenia and metaxenia have been observed in chestnuts (Pontikis, 1977; Crane and Iwakiri, 1980), pecan (Marquard, 1988), and almond (Vezvaie and Jackson, 1995; Kumar and Das, 1996). Cross-pollination of hazelnuts increased their nut and kernel weight and reduced blank fruit set (Javadi and Abedi Gheshlaghi, 2006; Fattahi et al., 2014). During rapid nut growth and development in which the competition of reproductive organs and roots for nutrient uptake reduces the activity of the roots, nutrient uptake decreases. This competition can be controlled by foliar application of nutrients in a timely manner (Andrade et al., 2009). Boron (B) is an essential nutrient that is accompanied by the vegetative and reproductive growth of plants and is involved in the antioxidant systems of vascular plants. Since B is involved in several reproductive processes, such as flowering, pollen tube growth, and fruit maturing, it has been reported to play a more essential role in fertility than in vegetative structures (Christensen et al., 2016). Zinc (Zn) is another micronutrient related to fruit set and fertilization. Its role in flowering is related to the synthesis of tryptophan as the precursor of auxin synthesis and flowering enhancement (Sotomayor et al., 2002). It is also involved in the process of mobilization of metabolites to buds and/or the related locations. Zn deficiency causes the leaves to be small and thin and the internodes to be short, and this finally results in the withering of twigs (Pandit et al., 2015). The present study aimed to assess the effect of different pollen sources on the physical and chemical traits of fruit in some dominant Iranian hazelnut cultivars. This assessment was performed from two perspectives. The first perspective dealt with the impacts of pollen sources on the physical and chemical traits of fruits, which is of crucial importance for the production of hazelnut considering its two famous phenomena, i.e. self-incompatibility and heterogamy. The second perspective addressed the application of micronutrients involved in improving reproductive and vegetative processes and their interactive effects on hazelnut fruit and kernel yield and quality with the aim of achieving more optimal nuts and kernels with higher content of nutrients (oil, protein, etc.) and lower levels of adverse traits.

## Materials and Methods

The study was carried out as a split-factorial experiment with three factors based on a randomized complete block design with three replications in the Eshkevarat region of Guilan Province, Iran. The first factor was assigned to selected commercial cultivars as maternal parent ('Gerde-Eshkevarat', 'Fertile', and 'Segorbe'), the second factor was assigned to pollen at four levels (selfpollination and the pollens of 'Gerche', 'Boliba', and 'Daviana'), and the third factor was devoted to the foliar application of two micronutrients including boron (B) and zinc (Zn) at four levels (control, B, Zn, and B + Zn). So, 16 trees from each cultivar in three replications (branches) were selected from different geographical directions, and their branches were packaged after discarding their catkins. In the self-pollination treatment, the catkins were not removed from the packaged branches. To prepare pollen, catkin-containing branches of pollinizers were placed in water during catkin elongation and before pollen shedding for the pollen to shed on a paper. Then, the pollens of the cultivars were collected separately and were stored in lidded containers at -18°C to preserve their vigor until pollination time and to avoid losing their germination potential. When female flower clusters started to open, the number of female flowers was recorded. After redcolored stigmas appeared, they were pollinated with the pollen of 'Boliba', 'Gerche', and Davian by hand. At the end of the effective pollination period, the pockets were removed from the branches. The foliar application to the trees was performed by hand and using a fogging system. During the research, all treatments were uniformly and equally subjected to cultivation operations. The spray treatments including control, boron (B) as borax (3000 mg·L<sup>-1</sup>), zinc (Zn) as zinc sulfate (3000 mg·L<sup>-1</sup>), and B + Zn at the same rates were performed in spring (May) during the peak leaf activity period (Paula et al., 2003).

At maturity and coloring period of hazelnuts, the nuts of the individual branches were harvested separately to determine their quantitative (nut number per cluster, nut formation percentage, total nut weight, twin kernels, blank nuts) and qualitative parameters (oil, and fruit Zn and B contents). The oil content was measured by the Soxhlet method. After de-husking, the kernels were ground. The oil of the powder was extracted by the Soxhlet method (at 45°C with dry diethyl ether solvent). The solvent of the extracted oil was separated in an oven at vacuum at 41°C, and the oil content was measured (Dieffenbacher and Pocklington, 1987). To determine fruit B content, B extract was prepared by the method of digestion with dry combustion. An amount of 1 g of sample was placed in a digestion flask, where 100 mL of CaO was added and the mixture was heated on a hot plate at 200°C. After cooling, sample was heated at 500°C for 1.5 h. Subsequently, 10 mL of H<sub>2</sub>SO<sub>4</sub> (0.5 M) was added into the digestion set. Its absorbance was read at 540 nm with a spectrophotometer (Emami, 1996). To find out Zn content, hazelnuts were oven-dried at 75°C for 24 hours. Then, they were ground. An amount of 2 g of the dried samples was poured into a Chinese container and was heated in an electrical furnace up to 550°C for 48 hours to get fully dried. After they were cooled, 10 mL of hydrochloric acid (2N) was added, and after the reaction was completed, the sample was placed in a water

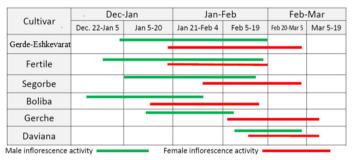
bath at 80°C to have the first steam flow out. The resulting solution was filtrated and double-distilled water was addeded to volume of 25 cm<sup>3</sup>. Zinc content was measured by an atomic absorption device (Emami, 1996). Data were statistically analyzed by the MSTATC and SPSS software packages. The means of the treatments were also compared by the LSD test at  $P \le 0.05$  significance level.

# **Results and Discussion**

# The period of pollen release and the receipt of female flower

During the trial, the activity periods of male and female flowers were recorded to check the overlap of blooming among cultivars and dichogamy phenamenon. To check the activity period of male flowers, the initiation and termination of pollen release were recorded. Female flower anthesis was supposed to commence when red-color stigmas appeared and it was supposed to be terminated when the stigmas got black (Fig 1). Accordingly, dichogamy was observed in all cultivars. All cultivars were protandrous and their pollen release started before the acceptance by female flowers.

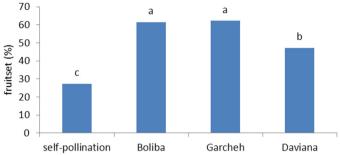
However, dichogamy is influenced by climatic and geographical conditions and it is possible to vary by year. The shortest overlap period, i.e. the most severe dichogamy, was observed in 'Gerche'. Also, the local variety ('Gerde-Eshkevarat') had the longest overlap time of male and female flowers. Various factors influence flowering time, e.g. cultivar, chilling requirement, growth conditions, and age (Moore and Janick, 1983).



**Figure 1.** Flower periods of the studied hazelnut cultivars and their overlaps with blooms

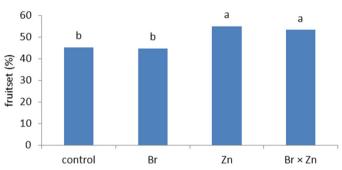
#### Fruit set

Different cultivars of plants differ in flower and fruit traits significantly depending on their genetic nature and ecological conditions. Based on the results, fruit set percentage, as the most important yield component, was significantly influenced by the pollen source. It was the lowest in self-pollinated plants in all studied cultivars. The treatments exhibited significant differences induced by pollinizer cultivars. Fattahi et al. (2014) reported that the use of pollinizer increased final nut set in the studied cultivars ('Segorbe', 'Rond', 'Fertile', 'Negret') so that nut development percentage varied from 6.3 to 11.4 in the self-pollination treatment, whereas it was 66 - 82% in different cultivars when pollinizers were applied. Obviously, successful fruit set and fruit growth depend on pollination and the subsequent fertilization undeniably. The comparison of means (Fig 2) revealed that the highest fruit set percentage was obtained from the plants treated with the pollinizer 'Gerche', and 'Boliba'. The lowest was 27.23% for the self-pollinated plants.



**Figure 2.** The effect of pollinizer on fruit set percentage (columns with different letters indicate significant differences according to LSD test at  $P \le 0.05$ )

The analysis of variance (ANOVA) showed that the simple effect of foliar application of micronutrients (Table 1) was significant on fruit set. Based on means comparison, the highest fruit set percentage was related to the treatment of Zn, and B + Zn, but they differed from the control significantly (Fig 3). Solar and Stampar (2001) reported that crop yield was higher when Zn and B were applied.



**Figure 3.** The effect of micronutrients on fruit set percentage (columns with different letters indicate significant differences according to LSD test at  $P \le 0.05$ )

According to the results (Table 2), the interaction of 'pollinizer cultivar × foliar application' was significant for fruit set. The highest percentage of fruit set (78.7%) was obtained from 'pollinizer 'Gerche'  $\times$  zinc sulfate'. The fruit set significantly varied among cultivars from 14.43 to 38.24% under the self-pollination treatment and from 33.8 to 65.83% under the pollinizer treatments. It even reached as high as 78.80% with foliar application depending on pollen type and cultivar. Javadi and Abedi (2006) reported that the cross pollination influenced fruit set significantly. Fattahi et al. (2014) stated that the application of pollinizer improved the final nut set in their studied cultivars ('Segorbe', 'Rond', 'Fertile', and 'Negret') significantly. A study on the self-compatible cultivars of almond revealed that the effect of pollen type was significant on nut formation. The rate of fruit set was higher in free pollination than in self-pollination so that self-pollination decreased nut formation significantly (Momenpour et al., 2011). Similarly, the use of pollinizers ('Boliba', 'Gerche', and 'Daviana') more than doubled nut formation versus self-pollination. In most treatments, the application of B + Zn improved nut formation.

Table 1. Analysis of va	ariance for the effect of ex	perimental treatments on	the recorded traits of hazelnuts
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Source of variability	16	Means of squares							
	df	Fruit set	Nut	Total nut weight	Kernel weight	Oil	Zn	В	
Replication	2	43.130 <sup>ns†</sup>	0.194 <sup>ns</sup>	0.448 <sup>ns</sup>	0.005**	0.96 <sup>ns</sup>	0.25*	2.31 <sup>ns</sup>	
Maternal cultivar (A)	2	651.248*	0.549 <sup>ns</sup>	57.863**	1.229**	426.84**	11.66**	307.34**	
Error	4	65.538	0.497	0.410	0.009	4.59	0.02	0.54	
Pollinizer cultivar (B)	3	9638.015**	6.359	0.227 <sup>ns</sup>	0.19 <sup>ns</sup>	40.37**	0.83**	115.41**	
AB	6	1422.213**	1.262*	0.332 <sup>ns</sup>	0.033**	220.89**	0.59**	208.01**	
Micronutrients (C)	3	1043.751*	0.507 <sup>ns</sup>	2.286**	0.117**	302.30**	2.62**	438.89**	
AC	6	757.320*	1.076*	1.797**	0.113**	19.52**	0.64**	89.43**	
BC	9	763.246*	1.359**	0.847	0.058**	26.36**	0.59**	82.51**	
ABC	18	1163.985**	0.900*	1.007**	0.040**	25.98**	0.30**	44.98**	
Error	90	295.971	0.485	0.346	0.010	2.97	0.08	1.49	
CV (%)		34.76	33.53	12.97	9.87	3.29	6.71	4.62	

Note: ns - non-significant, \* - significant at  $P \le 0.05$ , \*\*- significant at  $P \le 0.01$ 

<b>Table 2.</b> Means comparison for	for the interactive effect	of pollinizer × foliar app	plication on traits of hazelnuts

Treatment	Nut formation (%)	Nut number per cluster	Total nut weight (g)	Kernel weight (g)	
Self-pollination × control	26.80 ij <sup>†</sup>	2.44 abc	4.23 de	0.89 gh	
Self-pollination × B	20.65 j	2.00 cde	4.26 de	0.97 efg	
Self-pollination × Zn	30.38 hij	2.33 bcd	4.22 de	1.02 cdef	
Self-pollination $\times$ B + Zn	30.07 ghij	2.77 ab	5.03 a	1.07 abcd	
'Boliba'× control	69.38 ab	2.33 bcd	4.26 de	1.00 cdef	
Boliba' × B	46.14 efgh	2.33 bcd	4.84 abc	1.05 bcde	
Boliba'× Zn	63.76 abcd	2.22 bcd	4.29 cde	0.95 fg	
Boliba'× B + Zn	66.24 abc	3.00 a	4.64 a-d	0.93 fgh	
Gerche'× control	44.39 efgh	1.44 ef	4.08 e	0.84 h	
Gerche' × B	58.43 bcde	2.00 cde	5.13 a	1.12 ab	
Gerche'× Zn	78.70 a	2.33 bcd	4.31 cde	0.96 efg	
Gerche' $\times$ B + Zn	67.16 abc	1.55 ef	4.93 ab	1.16 a	
Daviana'× control	40.00 fghi	2.00 cde	4.45 bcd	1.02 cdef	
Daviana' × B	53.15 cdef	1.33 f	4.82 abc	1.08 abc	
Daviana' × Zn	47.01 efg	1.77 def	4.71 a-d	0.98 defg	
Daviana' $\times$ B + Zn	48.52 def	1.33 f	4.33 cde	1.02 cdef	

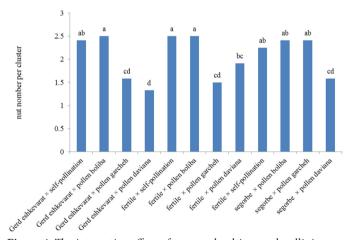
Note: means followed by different letters in the column indicate significant differences according to LSD test at  $P \le 0.05$ 

However, it seems that the selection of an appropriate pollinizer is very important. In our trial, the pollinizer 'Gerche' outperformed 'Boliba' and 'Daviana' significantly in improving the percentage of fruit set in the studied maternal cultivars.

The trilateral impact of 'maternal cultivar  $\times$  pollinizer  $\times$  foliar application of micronutrients' was significant for fruit set percentage (Table 1). 'Segorbe'  $\times$  pollinizer 'Boliba'  $\times$  no foliar application' had the highest fruit set percentage (93.3%) (Table 3). The lowest fruit set was observed in the interaction of 'Gerde-Eshkevarat'  $\times$  self-pollination  $\times$  no foliar application'. These results imply that the selection of a good pollinizer for maternal cultivars like 'Segorbe' is more effective than the foliar application of Zn and B. The self-pollinated 'Segorbe' plants, the foliar application of B and Zn increased nut formation by about 7%.

# Nut number per cluster

Nut number per cluster, which is an effective factor in the yield of hazel trees, is highly heritable. Mehlenbacher (2000) found its heritability to be  $h^2 = 0.67$ . This means that this trait is about 70% influenced by genetics, while other factors, including environmental factors and agronomic operations, account for 30% of this trait. The results showed that this trait ranged from 1.3 - 3 nuts/cluster when influenced by the pollen source (Fig 4).



**Figure 4.** The interactive effect of maternal cultivar and pollinizer on nut number per cluster in the studied hazel cultivars (columns with different letters indicate significant differences according to LSD test at  $P \le 0.05$ )

The results (Table 1) indicated that nut number per cluster was significantly influenced by the interaction of maternal and pollinizer cultivars. The highest number of nuts (2.5) was obtained from 'local variety  $\times$  'Boliba" and 'Fertile'  $\times$  'Boliba" and the lowest (1.3) from 'local variety  $\times$  'Daviana" (Fig 4). These results reflect the high dependence of this trait on cultivar and pollen source. Nonetheless, the combined application of micronutrients had a positive effect on this trait in most treatments so that the interaction of pollinizer and foliar application was significant for this trait (Table 1). The comparison of means (Table 2) revealed that the pollinizer 'Boliba' sprayed with borax and zinc sulfate produced the highest number of fruits per cluster (3 nuts). It appears that the positive effect of foliar application of B and Zn on extending survival and increasing final fruit formation was rooted in their positive impact on photosynthesis and their contribution to the nutritional balance of the trees. Zinc is a micronutrient that is related to fruit set and fertility (Sotomayor et al., 2002).

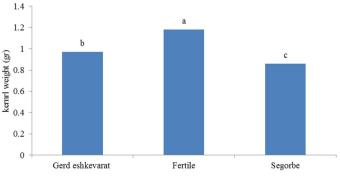
The results revealed the significant impact of pollen source on nut number. The highest number of nuts per cluster (3.6 nuts) was obtained from the interaction of 'Fertile' × 'Boliba' × borax + zinc sulfate'. The lowest (1 nut) was obtained from 'Gerde-Eshkevarat' × 'Gerche' × borax + zinc sulfate', 'Fertile' × 'Daviana' × borax', and 'Segorbe' × 'Daviana' × borax + zinc sulfate', showing higher significance of pollen source than micronutrient (Table 3). Despite different responses originating from plant genetics and/or pollen source, the exhibition of various metaxenia impacts is significant. Kardoush and Ayman (2009) argue that artificial pollination is an effective method for pistachios so that it can substitute natural pollination and ensure high yields and quality of nuts. Artificial pollination may have positive or negative impacts on nut number per cluster. Likewise, artificial (manual) pollination in the present study outperformed self-pollination in most cultivars.

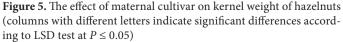
## Total nut weight

Based on the ANOVA (Table 1), the interactive effect of maternal cultivar, pollinizer cultivar, and micronutrients was significant on the weight of nuts with green husk. The highest weight of nuts with green husk (7.1 g) was related to 'Fertile' × 'Gerche' × borax + zinc sulfate' and the lowest (2.9 g) to the treatment of 'Segorbe' × 'Daviana' × borax + zinc' (Table 3). The difference is higher than 300%, reflecting the very desirable effect of pollen source on total nut weight. Hazelnut weight is highly heritable ( $h^2 = 0.84$ ) and it is very influential on yield (Yao and Mehlenbacher, 2000). This trait was found in our study to be significantly different among cultivars. McCluskey et al. (2001) reported that the nut weight of Barcelona ('Fertile') was in the range of 1-3 g, which is consistent with our results. 'Fertile' nut weight was also reported to be 3.33 g by Valentini et al. (2006), supporting our results.

# Kernel weight

The results showed that cultivar and pollinizer and their interaction had significant influence on kernel weight. The application of micronutrients, also, increased kernel weight versus the control. The highest kernel weight (1.1 g) was produced by 'Fertile' and the lowest (0.86 g) by 'Segorbe' (Fig 5). Kernel weight as influenced by the treatments varied in the range of 0.6 - 1.4 g.





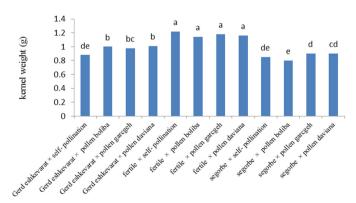
Treatment	Fruit set (%)	Nut No/ cluster	Total nut weight (g)	Kernel weight (g)	Oil (%)	Zn (mg·kg <sup>-1</sup> )	B (mg⋅kg <sup>-1</sup> )
'Gerde-Eshkevarat' $\times$ self-pollination $\times$ no foliar application	11.87 q	2.00 c-f	3.80 j-o	0.83 o-t	44.77 q†	3.80 n-q	23.27 qrs
'Gerde-Eshkevarat' $\times$ self-pollination $\times$ B	12.54 q	2.33 b-e	4.10 h-l	0.81 p-u	57.83 c-f	2.84 v	28.60 jkl
'Gerde-Eshkevarat' $\times$ self-pollination $\times$ Zn	21.06opq	2.33 b-e	3.73 ј-о	0.88 m-s	48.54 op	3.60 p-t	21.93 s-v
'Gerde-Eshkevarat' $\times$ self-pollination $\times$ B + Zn	12.25 q	3.00 abc	4.40 g-j	1.00 i-n	53.88 hij	3.90 l-p	28.27 jkl
'Gerde-Eshkevarat' × 'Boliba' × no foliar application	51.84e-m	2.33 b-e	3.96 i-n	0.98 j-o	48.65 nop	2.59 v	21.10 u-x
'Gerde-Eshkevarat' × 'Boliba' × B	70.00 a-g	3.00 abc	4.36 g-k	1.06 e-l	50.21 l-o	4.20 h-l	26.10 mn
'Gerde-Eshkevarat' × 'Boliba' × Zn	50.71 e-n	2.66 a-e	4.23 h-l	1.02 h-m	56.51 ef	3.23 u	21.67 t-w
'Gerde-Eshkevarat' × 'Boliba' × B + Zn	43.18 g-o	2.00 c-f	3.91 j-n	0.94 l-r	54.43 ghi	3.75 o-r	23.13 qrs
'Gerde-Eshkevarat' × 'Gerche' × no foliar application	38.73 i-q	1.00 f	4.01 j-m	0.93 l-s	47.88 p	3.83 n-q	21.27 u-x
'Gerde-Eshkevarat' × 'Gerche' × B	47.35 g-o	2.33 b-e	3.99 i-n	1.00 i-n	53.57 ij	3.83 n-q	29.03 hij
'Gerde-Eshkevarat' × 'Gerche' × Zn	88.89 abc	2.00 c-f	4.10 h-l	1.01h-n	50.60 k-n	4.03 j-o	20.58 v-y
'Gerde-Eshkevarat' × 'Gerche' × B + Zn	88.33 abc	1.00 f	3.83 j-o	1.00i-n	53.09 ij	3.53 q-u	24.70 nop
'Gerde-Eshkevarat' × 'Daviana' × no foliar application	56.67 d-l	1.33 ef	3.11mno	0.96 k-p	50.89 kl	3.53 q-u	20.20 xy
'Gerde-Eshkevarat' × 'Daviana' × B	63.89 b-j	1.33 ef	5.30 d-g	1.18 c-g	61.70 b	3.35 tu	28.17 ijk
'Gerde-Eshkevarat' × 'Daviana' × Zn	70.00 a-g	1.33 ef	4.23 h-l	0.96 k-p	59.68 c	3.77 opq	22.97 q-t
'Gerde-Eshkevarat' × 'Daviana' × B + Zn	43.33 g-o	1.33 ef	3.71 ј-о	0.96 k-p	65.79 a	3.43 stu	24.25 opq
'Fertile' $\times$ self-pollination $\times$ no foliar application	32.2 l-q	2.33 b-e	5.53 c-f	1.13 с-ј	55.85 fgh	4.11 i-n	23.75 pqr
'Fertile' × self-pollination × B	35.55 k-q	2.33 b-e	5.38 def	1.13 с-ј	56.13 efg	4.11 i-n	24.00 pq
'Fertile' × self-pollination × Zn	47.69 g-o	3.00 abc	5.50 c-f	1.23 cde	56.04 efg	4.80 bc	24.00 pq
'Fertile' × self-pollination × B + Zn	37.52 i-q	2.33 b-e	6.91 ab	1.41 ab	57.15 def	4.17 h-m	28.50 jkl
'Fertile' $\times$ 'Boliba' $\times$ no foliar application	62.98 c-k	2.00 c-f	5.36 def	0.99 i-o	55.95 efg	4.38 e-i	18.75 z
'Fertile' × 'Boliba' × B	45.00 f-o	2.33 b-e	5.93 cd	1.16 c-h	57.90 cde	4.63 b-e	21.63 t-w
'Fertile' × 'Boliba' × Zn	91.67 ab	2.00 c-f	5.59 c-f	1.23 cd	58.76 cd	4.68 b-e	30.71 g
'Fertile' × 'Boliba' × B + Zn	66.67 a-h	3.66 a	6.13 bcd	1.20 c-f	58.54 cd	5.36 a	25.47 no
'Fertile' × Gerche × no foliar application	64.45 b-i	1.33 ef	4.90 f-i	1.01 h-n	52.10 jkl	3.73 o-s	18.70 z
'Fertile' $\times$ Gerche $\times$ B	64.44 b-i	1.33 ef	6.18 bcd	1.15 c-i	57.43 def	4.27 g-k	31.34 fg
'Fertile' × Gerche × Zn	77.78 a-e	1.66 def	5.40 c-f	1.09 d-l	52.28 jk	4.73 bcd	18.67 z
'Fertile' × Gerche × B + Zn	32.59 l-q	1.66 def	7.16 a	1.49 a	57.71 c-f	4.31 f-j	30.17 ghi
'Fertile' × 'Daviana' × no foliar application	41.67 h-p	2.66 a-d	5.90 cde	1.10 d-k	03 q	3.87 m-p	30.25 gh
'Fertile' × 'Daviana' × B	42.78 g-o	1.00 f	5.50 c-f	1.07 e-l	48.58 op	3.80 n-q	37.48 c
'Fertile' × 'Daviana' × Zn	43.18 g-o	2.33 b-e	4.95 e-h	1.20 c-f	48.88 m-p	4.56 c-g	34.33 d
'Fertile'בDaviana'×B + Zn	72.22 a-f	1.66 def	6.34 abc	1.27 bc	56.44 ef	3.98 k-o	20.35wxy
'Segorbe' $\times$ self-pollination $\times$ no foliar application	36.31 j-q	3.00 abc	3.37 l-o	0.70 tuv	35.30 r	3.3 o-s	20.25wxy
'Segorbe' × self-pollination × B	13.86 pq	1.33 ef	3.30 l-o	0.96 k-p	48.15 p	4.71 bcd	33.00 de
'Segorbe' × self-pollination × Zn	22.41opq	0.66def	3.43 k-o	0.95 k-q	47.70 p	4.95 b	31.20 fg
'Segorbe' × self-pollination × B + Zn	43.45 g-o	3.00 abc	3.79 j-o	0.80 q-u	48.80 nop	4.80 bc	32.50 ef
'Segorbe' × 'Boliba' × no foliar application	93.33 a	2.66 a-d	3.46 j-o	1.02 g-m	47.56 p	4.11 i-n	22.50 r-u
'Segorbe' × 'Boliba' × B	23.41 n-q	1.66 def	4.22 h-l	0.93 l-s	48.40 op	5.53 a	44.25 a
'Segorbe' × 'Boliba' × Zn	48.89 f-o	2.00 c-f	3.05 no	0.60 v	47.92 p	4.82 bc	41.25 b
'Segorbe' $\times$ 'Boliba' $\times$ B + Zn	88.89 abc	3.33 ab	3.87 j-o	0.66 uv	48.19 p	4.73 bcd	40.25 b

Treatment	Fruit set (%)	Nut No/ cluster	Total nut weight (g)	Kernel weight (g)	Oil (%)	Zn (mg·kg <sup>-1</sup> )	B (mg·kg <sup>-1</sup> )
'Segorbe' × 'Gerche' × no foliar application	30.00 l-q	2.00 c-f	3.35 l-o	0.60 v	48.69 nop	3.90 l-p	19.23 yz
'Segorbe' × 'Gerche' × B	63.49 c-i	2.33 b-e	5.23 d-g	1.23 cde	52.53 ijk	4.45 d-h	27.25 lm
'Segorbe' × 'Gerche' × Zn	69.44 a-h	3.33 ab	3.43 k-o	0.78 r-u	57.12 def	4.73 bcd	23.73 pqr
'Segorbe' × 'Gerche' × B + Zn	80.56 a-d	2.00 c-f	3.80 j-o	1.00 i-n	53.36 ij	4.17 h-m	22.50 r-u
'Segorbe' $\times$ 'Daviana' $\times$ no foliar application	21.00opq	2.00 c-f	4.35 g-k	1.00 i-n	47.71 p	3.43 r-u	19.97 xyz
'Segorbe' × 'Daviana' × B	52.78d-m	1.66 def	3.66 j-o	1.00 i-n	47.71 ij	4.58 c-g	27.50 klm
'Segorbe' × 'Daviana' × Zn	27.86m-q	1.66 def	4.96 e-h	0.77 stu	53.58 op	41.62 c-f	37.00 c
'Segorbe' × 'Daviana' × B + Zn	30.00 l-q	1.00 f	2.93 o	0.85 n-t	50.82 klm	4.65 b-e	23.90 pqr

Note: means followed by different letters in the column indicate significant differences according to LSD test at  $P \le 0.05$ 

Among the interactions between pollinizer and foliar application, the highest kernel weight (1.1) was related to 'pollinizer 'Gerche'  $\times$  borax + zinc' and the lowest (0.8 g) to 'pollinizer 'Gerche'  $\times$ control (no spray)'. Means comparison for the trilateral effect revealed that 'Fertile'  $\times$  pollinizer 'Gerche'  $\times$  borax + zinc sulfate' produced the highest kernel weight and 'Segorbe'  $\times$  'Gerche'  $\times$ no foliar application' produced the lowest one (Table 3). Kernel weight is a major parameter determining crop yield and predicting a cultivar's efficiency. The heritability of this trait is 0.67 (Yao and Mehlenbacher, 2000).

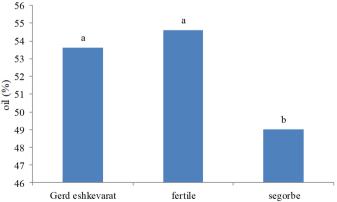
The application of micronutrients increased kernel weight significantly versus the control (no foliar application). A study on 13 hazel cultivars reported that nut weight, kernel weight, and kernel percentage differed among the cultivars significantly. The mean nut weight varied from 2.4 g for 'Segorbe' to 4 g for 'Ennis' (Baldwin, 2015). Abu-Zahra et al. (2007) found that artificial pollination had positive impact on nut number so that it increased not only the number of nuts per cluster and crop yield but also nut size and kernel dry weight. In the present study too, the use of pollinizer had diverse but significant impacts on hazelnut kernel weight when compared to self-pollination. 'Gerde-Eshkevarat' under all applied pollinizers increased kernel weight versus selfpollination, but in 'Fertile', no significant difference was observed in kernel weight between pollen sources and self-pollination (Fig 6).



**Figure 6.** The interactive effect of maternal cultivar and pollinizer on kernel weight in the studied hazel cultivars (columns with different letters indicate significant differences according to LSD test at  $P \le 0.05$ )

# Oil

The results (Table 1) revealed the significant simple and interactive effects of maternal cultivar, pollinizer, and foliar application of micronutrients on nut oil content. 'Fertile' had the highest oil content. 'Segorbe' had the lowest oil percentage (49.02%) (Fig 7). These results are in agreement with Balta et al. (2006) and Tsantili et al. (2010). Cross-pollination resulted in significant differences in oil content. Golzari et al. (2016) reported that various pollens were influential on the total oil content of walnuts. In the present study, all levels of micronutrients increased the oil content of hazelnuts in comparison to the control. The lowest oil percentage was observed in plants without any micronutrients. Bybordi and Malakouti (2005) reported the highest oil content in almonds treated with 4 mg kg<sup>-1</sup> zinc and no N application. The role of B and Zn in increasing the oil and protein content of almond nuts may be associated with their positive impact on the composition of nucleic acid and pyrimidine and some cell reactions such as starch biosynthesis. Based on our results, 'Gerde-Eshkevarat' × 'Daviana' × borax + zinc' produced the highest nut oil content and 'Segorbe'  $\times$  self-pollination  $\times$  no foliar application' produced the lowest one (Table 4). These results point to the significant effect of paternal cultivar on hazelnut oil. High fatty acid and protein contents are major characteristics in assessing nut and kernel quality of hazels.



**Figure 7.** The effect of maternal cultivar on oil percentage of hazelnuts (columns with different letters indicate significant differences according to LSD test at  $P \le 0.05$ )

The concurrent use of B and Zn at the same concentrations of 3000 mg·L<sup>-1</sup> was more effective than the other treatments in increasing hazelnut oil content. Bybordi et al. (2006) reported that Zn application affected nut oil percentage in almond and the highest oil content was observed in the plants treated with Zn.

#### Zinc nut content

ANOVA (Table 1) showed that hazelnut Zn content was significantly affected by the simple and interactive effects of maternal cultivar, pollinizer, and micronutrients. 'Segorbe' had the highest nut Zn content and 'Gerde-Eshkevarat' had the lowest one. The results revealed the difference between the cultivars in Zn content, which is consistent with the results of Hosseinpour et al. (2013) and Ozdemir et al. (2001). We found that the pollen source affected nut Zn content significantly. The pollinizer 'Daviana' reduced nut Zn content by 0.17 mg·kg<sup>-1</sup> versus self-pollination. But, the pollinizer 'Boliba' increased nut Zn content by 0.43 mg·kg<sup>-1</sup> versus self-pollination. We also observed that the foliar application of Zn increased nut Zn content so that all levels of micronutrients had a statistically positive effect on nut Zn content compared to the control (no foliar application). These findings are in agreement with Cakmak (2008) and Saadati et al. (2010).

Means comparison showed that 'Segorbe' × pollinizer 'Boliba'  $\times$  borax' and 'Fertile'  $\times$  pollinizer 'Boliba'  $\times$  borax + zinc' had the highest nut Zn content of 5.53 mg·kg<sup>-1</sup>, whereas the lowest one of 2.59 mg·kg<sup>-1</sup> was observed in the treatment of 'Gerde-Eshkavart  $\times$  self-pollination  $\times$  borax + zinc sulfate' and 'Gerde-Eshkevarat'  $\times$  self-pollination  $\times$  borax'. Based on the results, it seems that the pollinizer is more effective on the nut Zn content than the micronutrient application (Table 3). Saadati et al. (2016) stated that the application of zinc sulfate and boric acid increased fruit set and leaf and fruit B content in olive. However, Zn was more influential on the qualitative traits, especially fruit size. Cakmak (2008) reported that Zn fertilization increased Zn content in seeds and improved seed vigor. Although plants have a slight Zn demand, if the nutrient is not adequately available, the plants will suffer from physiological stresses caused by the inefficiency of various enzymatic systems (Bybordi et al., 2010).

#### Boron nut content

It was revealed by ANOVA (Table 1) that the simple and interactive effects of maternal plants, pollinizers, and micronutrients were significant on hazelnut B content. The highest B content (29.14 mg·kg<sup>-1</sup>) was related to 'Segorbe' and the lowest to 'Gerde-Eshkevarat'. These results reflect the differences of the cultivars in B content, which is in line with the results of Paula et al. (2003). We found that the same pollen type, e.g. 'Boliba', on two different maternal cultivars provoked quite different responses in terms of B content. This difference was considerable between the superior treatment and the inferior treatment, amounting to 14 mg·kg<sup>-1</sup>. In addition, all micronutrient levels had a positive impact on hazelnut B content compared to the control (no foliar application). These results are in agreement with other studies (Ghaderi et al., 2003; Saadati et al., 2016). The positive response to these treatments may be associated with the involvement of B and Zn in plant metabolism due to the foliar application in spring when hazel leaves were most active, which finally increased their productivity and nut quality (Paula et al., 2003). The comparison of means (Table 3) indicated that the treatment combination of 'Segorbe' × pollinizer 'Boliba' × borax' produced the highest nut B content and the treatment of 'Fertile' × pollinizer 'Gerche' × no foliar application' produced the lowest one of 18.70 mg·kg<sup>-1</sup>. Saadati et al. (2013) found that the application of boric acid and zinc sulfate increased leaf B content significantly. Also, the treatment of boric acid increased olive fruit B content. The researchers reported that B can be allocated to younger parts of the plants despite the demand by growing sinks. According to Abdel-Karim et al. (2015), foliar application of B and Zn on avocados enhanced the quantitative and qualitative traits of the fruits. They reported that the foliar application of B was effective in most treatments. Furthermore, the foliar application of B and Zn at the same ratios (1 g·L<sup>-1</sup> Zn + 1 g·L<sup>-1</sup> B) had positive synergistic effects.

# Conclusion

The effect of pollen sources on nut and kernel properties has been documented for several nut fruit species. These effects have been attributed to the impact of cross-pollination, not breeding. Our results for hazelnuts confirmed the significant effect of pollinizer on physical and chemical characteristics of nuts and kernels. Based on the results of this assessment, the nut yield components of maternal cultivars were significantly influenced by pollen sources so that nut formation percentage was lower in the self-pollinated cultivars and it was affected by the pollinizer cultivar. These results underline the need for an appropriate pollinizer. The results, also, confirmed the dependence of nut number per cluster on cultivar and pollen source. However, the simultaneous application of micronutrients had a positive impact on this trait in most treatments. The interaction of the pollen sources and maternal cultivars was significant for nut and kernel weight. Cross-pollination caused significant variations in hazelnut oil content when compared to self-pollination. The results revealed that the foliar application of zinc sulfate and borax improved hazelnut quality significantly. In most treatments, the application of zinc sulfate was more influential on nut formation percentage than borax. Furthermore, the application of Zn and B increased the weight of nuts with husk and kernel weight. Further studies on pollen compatible cultivars are necessary for a similar area to find the overlapping time of pollination.

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