

## Effects of classical and organomineral fertilizer applications on pollen quality and quantity in gemlik olive cultivar

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### Abstract

Olive is one of the important plants worldwide in terms of economic and cultural conditions. It is used in fresh consumption, olive oil, cosmetic and pharmaceutical fields and unique numinous plants. Olive can maintain its life for many years even in barren conditions but for an economic cultivation, fertile and preferred cultivars should be used in orchards and optimum maintenance conditions should be provided. At the same time the climate change is also convert the usual behavior of the plants. This study aims to determine the effects of classic and organomineral fertilizers on pollen quality and quantity parameters. In this study, classical fertilizers like Urea+MgSO<sub>4</sub> and KNO<sub>3</sub>+H<sub>3</sub>BO<sub>3</sub>+ZnSO<sub>4</sub> with Raykat Start, Raykat Growth and Fitomare organomineral fertilizers were sprayed foliarly before flowering on Gemlik olive cultivar and pollen studies were conducted on the flowers formed at full flowering. Within the study, effects of the treatments were evaluated on pollen viability and germination levels, the amount of pollen produced in one flower and the normally developed pollen ratio. As a result of this study, fertilization treatments positively affected pollen quality and quantity with regard to control treatment. In this context, the pollen viability level differed between 74.15% and 89.92%, the pollen germination level between 45.48% and 70.35% and pollen per flower was between 307.238 and 446.761. The lowest data were obtained from control treatments, while the highest were from Raykat group fertilizers. Especially, the 54% increase in pollen germination level with Raykat growth fertilization was one of the most important results of this study. In conclusion, it was determined that the foliar spray of organomineral fertilizers enhanced the pollen properties. Raykat Start, Raykat Growth and Fitomare organomineral fertilizers used in this study significantly increased pollen quality and quantity.

**Keywords:** *Olea europea*, Fertilization, Pollen, Climate change, Fruit set

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## INTRODUCTION

Olive cultivation in the World is economically carried out in countries bordering the Mediterranean or in the microclimate regions of continents and countries with a Mediterranean climate (Polat and Tunalıoğlu, 2012). Olive (*Olea europea* L.) is a multifunctional Mediterranean plant with an extraordinary history and tradition that can live for many years and has an important role in table and oil production (Sales et al., 2020). Although the olive tree is drought tolerant, it is one of the agricultural products that will be significantly affected by global climate changes. It is necessary to determine the precautions to take for olive plantations in order not to be affected by increasing climate change problems (Sevim et al., 2022). Within the scope of these precautions; determining the effects of maintenance conditions, the plant's water use efficiency and effective fertilization methods on vegetative and generative production are among the primary factors.

In recent years, problems related to fruit set problems in olive varieties have become widespread. Since low yield is mostly due to pistil and stamen development problems, it is necessary to know the obstacles that may be

experienced in the development of pollen characteristics and ovule and to develop solutions for these problems (Ateyyeh et al., 2000). Within the scope of researching these problems pollen viability of olive varieties (Mete et al., 2015), pollen germination (Atteyyeh et al., 2000; Acarsoy et al., 2011; Dölek-Gencer et al., 2023), pollen tube development (Selak et al., 2013), studies on pistil and ovule situations (Rapaport et al., 2022) were increased in recent years.

Nutrition in the olive plant enables the plant to have a better physiology and therefore improves the pollen properties by allowing the plant to produce stronger pollen and pollinate better (Sharafi and Raina, 2021). Although pollen is an anatomically simpler structure compared to other differentiated tissues and plant organs, it is responsible for carrying the male gamete to the pistil of the flower (Patel and Mankad, 2014). For this reason, pollen is a structure that must have high performance to ensure a successful fruit set in species that do not form fruit parthenocarpically.

Determining the effects of different treatments on olive pollen quality and quantity in Mediterranean climate conditions, where the effects of climate change are seen at high levels, is of great importance in protecting fruit sets against climate change. In this study, it was aimed to determine the effect of recently produced organomineral fertilizers and classical fertilizers on pollen viability and germination levels and pollen production amount in the Gemlik olive variety.

## MATERIALS AND METHODS

### Materials

This study was conducted in an olive orchard at 400 m above sea level in the Düziçi district of Osmaniye province during the 2019-2020 growing season. In the experiment, 13-year-old Gemlik olive variety was used, which was planted at 6x6 m intervals. Routine maintenance conditions were carried out in optimum and disease and pest treatments were made with the instructions so that the pollen was not affected.

In the study, 5 different fertilizer applications consisting of classical and new organomineral fertilizers with a control treatment were applied foliarly before flowering. The applications are arranged as follows.

1. Urea+MgSO<sub>4</sub> (Urea+Mg): 0.5% urea and 0.5% magnesium sulfate were mixed.
2. KNO<sub>3</sub>+H<sub>3</sub>BO<sub>3</sub>+ZnSO<sub>4</sub> (K+B+Zn): 0.5% doses of all three fertilizers were mixed.
3. Raykat Start (R-Start): 300cc Raykat Start was added to 100lt of water.
4. Raykat Growth (R-Growth): 300 cc Raykart Growth was added to 100 lt of water.
5. Fitomare: 300 cc Fitomare was added to 100 lt of water.
6. Control: Water application was made simultaneously with other applications.

### Methods

Treatments were arranged in 3 replications with one tree in each replication. Ferti-Vant organic content surfactant was used with the treatments. All treatments were made in the morning before flowering as a single dose by using an automatic sprayer.

As a result of the treatments made in the experiment, pollen viability and germination levels and pollen production were determined. In this context, in order to obtain pollen, branches containing buds that had not yet opened but were about to bloom were cut and placed in water under laboratory conditions. In this way, the flowers were opened and released their pollens. Obtained pollen was used in pollen viability and germination tests. Flower buds that will open within one day were used for pollen production.

*In vitro* pollen viability tests: Pollen viability tests were made by 1% 2,3,5 TTC (Triphenyl Tetrazolium Chloride) solution prepared according to Norton (1966). In this context, 3 slides were prepared for each treatment and at least 100 pollen counts were made on each slide. An Olympus BX 51 light microscope was used for counts. During counting, pollens stained dark red were considered "viable" light red or pinkies were considered "semi-viable," and colorless ones were considered "non-viable." During calculation, 50% of the semi-viable pollen was assumed to be viable, along with the separate rates of each group and this value was added to the viable pollen and the "viability rate" was determined by calculation.

*In vitro* pollen germination tests: For pollen germination experiments, the "agar in petri method was used" with a germination medium containing 1% agar + 15% sucrose added to 100 ppm Boric acid solution (Ilgin et al., 2007; Dölek-Gencer et al., 2023). Counts were made under an Olympus BX51 light microscope. During counting, the pollens with pollen tubes longer than their own diameter were considered as germinated and the effect of the treatments on the pollen germination rate was determined. In pollen germination experiments, 3 petri dishes were prepared for each application and at least 100 pollen counts were counted in each petri dish.

Determination of Pollen Production: The amount of pollen production in the flowers obtained as a result of the applications was determined by the "hemocytometric method". For this purpose, thirty flowers were taken from the branches taken at full bloom and brought to the laboratory, from flower buds that had not yet opened but were about to open. Three replicates were formed with 10 flowers for each. Individual anthers of flowers taken from each replicate were separated from their filaments and placed in small plastic boxes. The prepared boxes with anthers were kept for 15 days for the anthers to dry. In order to perform pollen counts, pollens were prepared as explained by Eti (1990) and Eti et al. (1996). The pollen counts were made under an Olympus BX51 microscope.

The amount of pollen in a flower was calculated using the calculation as stated by Eti (1990). During the counting, normally developed pollen rates were also determined by counting the amount of pollen that did not show normal development or malformed shape and the amount of pollen that developed normally (Anvari, 1977).

Analysis of variance was applied to the data obtained in the study using the JMP statistical package program and the differences were determined using the LSD test. The arc-sin transformation was applied to the calculated percentage values.

## RESULTS AND DISCUSSION

In this study, pollen viability and germination levels, pollen production amount and normally developed pollen rates were determined in the flowers obtained from 5 different classical and organomineral fertilizer treatments on the Gemlik olive variety.

### Pollen Viability Rate

The viable, semi-viable and non-viable pollen ratios with pollen viability rates determined from 6 different treatments are given in Table 1. The table shows that the effect of the treatments on the pollen viability levels was statistically significant. In terms of viable pollen rates; R-Start, K+B+Zn and R-Growth fertilizers had the highest viability levels (85.71%, 78.84% and 77.81%, respectively), while Fitomare and Control applications had the lowest rates (60.90% and 64.35%, respectively).

In terms of semi-viable pollen, it was noted that Fitomare treatment had the highest semi-viable pollen rate. In the study, it was determined that the non-viable pollen rates indicating the amount of poor-quality pollen, varied between 3.59% (K+B+Zn) and 16.05% (Control).

The pollen viability levels, which were theoretically determined considering that half of the semi-viable pollen was alive, in parallel with the viable pollen rates. In this context, it was determined that the highest pollen viability rates were obtained from R-Start (89.92%) and K+B+Zn (87.63%) treatments. The lowest viability level was seen in the control application with a rate of 74.15%.

In a study made by Acarsoy et al. (2011) on Domat olive variety, it was reported that as a result of 9 different fertilizer applications also containing Boron and Potassium, the combined application of Liquid Boron + Urea + KNO<sub>3</sub> showed the highest values in terms of pollen vitality levels (92.20%). In another study, the pollen viability level of the Gemlik olive variety in Tarsus conditions was 81.51% (Dölek Gencer et al., 2023). In this study, it was determined that pollen viability levels were sufficient in both fertilization and control treatments. Studies have shown that pollen viability levels may change depending on genetics, ecology and years (Ferri et al., 2008; Mete et al., 2015). At the same time, nutrition and maintenance conditions are also important in this change (Acarsoy et al., 2011; Karataş, 2022).

Table 1. Effects of foliar fertilizers on pollen viability levels in Gemlik olive cultivar (%)<sup>1</sup>

Treatments	Viable (A)	Semi-viable (B)	Non-viable (C)	Viability (A+B/2)
Urea+Mg	76.95 ab <sup>2</sup>	14.79 bc	8.26 abc	84.35 ab
K+B+Zn	78.84 a	17.57 b	3.59 c	87.63 a
Raykat Start	85.71 a	8.41 c	5.88 bc	89.92 a
Raykat Growth	77.81 a	12.05 bc	10.14 ab	83.84 abc
Fitomare	60.90 c	31.19 a	7.91 bc	76.50 bc
Control	64.35 bc	19.59 b	16.05 a	74.15 c
P	**	**	*	*

(1): Data were analyzed after arc-sin transformation

(2): Different letters in the same column are statistically important \*means P<0.05; \*\* means P<0.01

### Pollen Germination Level

The effect of fertilizers applied in the experiment on the pollen germination level is given in Table 2. It was determined that pollen germination levels were statistically affected by the treatments at a level of 1%. In this context, it was determined that R-Growth significantly affected pollen germination compared to other treatments as 70.35%, followed by Fitomare (59.42%), Urea+Mg (57.89%), K+B+Zn (52.25%) and R-Start (50.67%) treatments, respectively. The lowest germination level was obtained from the Control application at 45.48%.

Pollen germination and pollen tube development on stigma in fruit trees are the most important features in terms of pollen quality. A high level of pollen germination and rapid pollen tube development are required for effective pollination. Otherwise, there may be problems in pollen tubes reaching the end of the ovule's lifespan (Sharafi., 2011).

In a study conducted under Izmir conditions, it was reported that the application of Liquid Boron + Urea + KNO<sub>3</sub> through 9 different fertilizers on the Domat olive variety showed the highest values (52.80%) in terms of pollen germination level (Acarsoy et al., 2011). In another study, Spinardi and Bassi (2012) stated that the use of Boron in olives could increase germination by up to 6.3 times, reaching 48% in varieties with low pollen germination levels.

Studies showed that fertilizer treatments positively affect fruit set (Gündeşli and Nikpeyma, 2016), and boron-containing fertilizers are especially important in terms of pollen (Nyamora et al., 2000; Acarsoy et al., 2011;

Sarıdaş et al., 2021). Dell and Huang (1997) reported that the amount of boron contained in the plant is closely related to events such as fertilization, seed set and germination, while the low levels of boron in flowers show their primary effects on fertilization by reducing microspore formation and pollen tube development. In this context, it is thought that fertilizing the plant with boron-containing fertilizers will directly affect fruit set by increasing pollen germination level. Considering that Raykat group fertilizers, among the boron-containing organomineral fertilizers used in the study, contain 0.03% Boron and Fitomare contains 0.35% Boron, which directly shows the reason for the increase in pollen germination with the use of these fertilizers.

Table 2. Effects of foliar fertilizers on pollen germination levels in Gemlik olive cultivar (%)<sup>1</sup>

Treatments	Pollen germination
Urea+Mg	57.89 b <sup>2</sup>
K+B+Zn	52.25 bc
Raykat Start	50.67 bc
Raykat Growth	70.35 a
Fitomare	59.42 b
Control	45.48 c
P	**

(1): Data were analyzed after arc-sin transformation

(2): Different letters in the same column are statistically important \*\* means P<0.01

### Amount of Pollen Production

As a result of fertilization, the amount of pollen in a flower and the values of the normally developed pollen ratio are given in Table 3. Although the differences between the treatments in terms of the amount of pollen in a flower were not statistically significant, it was determined that higher values were obtained in all fertilization applications compared to the Control application. In this context, the highest pollen production was determined to be in the R-Start (446.761 number), followed by the Fitomare (379.458 number). In control, it was determined that 307.238 pollen were produced in one flower which was noted that this value was the lowest amount of pollen production among the treatments.

It was determined that the values in terms of normally developed pollen rate were very close, ranging between 97.08% and 99.12% and the differences between the treatments were not statistically significant.

In a study conducted in Tarsus conditions, it was reported that the amount of pollen production in the Gemlik variety was 306.385 number and the amount of pollen production was found to be higher in the full-flowering period compared to the first-flowering and end-blooming periods (Dölek-Gencer et al., 2023). Rojo et al. (2015) determined that the amount of pollen in one olive flower was around  $1.10 \times 10^5$ . Researchers also pointed out that the amount of pollen production varies according to inflorescence structure, flowering and alternate bearing features. Although previous studies have not determined the effect of fertilization on the amount of pollen production in olives, in strawberries (Sarıdaş et al., 2021) and lemons (Karataş, 2022), the increase in pollen production was proved by using boron-containing fertilizers.

In addition to the amount of pollen produced in the flowers of a variety, the high rate of normally developed pollen is also of great importance. Eti (1992) reported that having a normally developed pollen ratio above 80-90% is important for pollen quality. Karataş (2022) reported that pruning, irrigation and Phosphorus, Zinc and Boron fertilization in lemon plants increased pollen production and the amount of normally developed pollen.

Previous studies have shown that boron fertilization treatments increase the quality and quantity of pollen, and at the same time it positively affects fruit set (Nyomora et al., 2000; Spinardi and Bassi, 2012; Sarıdaş et al., 2021; Karataş, 2022). In this study, it was determined that fertilization generally increased the quality rates and pollen production. Organomineral fertilizers, in particular, enable the production of higher quality and greater quantity of pollen compared to other treatments. In addition, in another part of the same project it has been proven that Raykat Growth organomineral fertilizer treatment stands out in terms of yield per tree, fruit quality and olive oil content (Çelik et al., 2023a; Çelik et al., 2023b).

Table 3. Effects of foliar fertilizers on pollen production per flower and normally developed pollen ratio in Gemlik olive cultivar

Treatments	Pollen Production Per Flower (number)	Normally Developed Pollen Ratio (%) <sup>1</sup>
Urea+Mg	354 698	98.65
K+B+Zn	345 391	98.27
Raykat Start	446 761	99.12
Raykat Growth	347 514	98.45
Fitomare	379 458	97.08
Control	307 238	97.78
P	N.S. <sup>2</sup>	N.S.

(1): Data were analyzed after arc-sin transformation

(2): N.S. means non-significant

## CONCLUSION

Olive cultivation has been greatly affected by climate change in recent years. Climate change affects the reproductive organs the most and this directly causes a decrease in fruit set. Considering that drought and heat stress will increase in the future, it is thought that additional precautions must be taken immediately for plants to have a stronger structure, so that plant reproductive organs do not succumb to these disadvantages and complete fruit set in a healthy way. In this study which was planned based on this idea, it was determined that different organomineral and classical fertilizer treatments positively affected the quality and quantity of pollen in Gemlik olive variety compared to the control. Among the fertilizers used, Raykat Growth, Raykat Start and Fitomare organomineral fertilizers during the flowering period significantly affected the pollen properties.

## Compliance with Ethical Standards

### Peer-review

Externally peer-reviewed.

### Declaration of Interests

All authors declare that they have no conflicts of interest.

### Author contribution

Şenay Karabiyik (Ş.K.), Olcay Çelik (O.Ç.), Mehmet Ali Sarıdaş (M.A.S) and Sevgi Paydaş Kargı (S.P.K) carried out experimental part of the study. Ş.K. done laboratory tests, statistical analysis and wrote the paper. O.Ç. carried out the field studies, M.A.S. and S.P.K. reviewed the manuscript.

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