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Pollen Viability and Germination Levels with Amount of Pollen Production of Some Important Olive Cultivars in Türkiye

Cansu Dölek[1,a,*], Mücahit Taha Özkaya[2,b], Sinan Eti[3,c], Şenay Karabıyık[3,d], Neslihan Taskin Madrigal Fletcher[4,e]

[1] *Department of Horticulture, Kozan Vocational High School, Cukurova University, Adana, Türkiye*
[2] *Department of Horticulture, Faculty of Agriculture, Ankara University, Adana, Türkiye*
[3] *Department of Horticulture, Faculty of Agriculture, Cukurova University, Adana, Türkiye*
[4] *Independent Researcher, Albany School of English, Cordoba, Spain*
[*] *Corresponding author*

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ABSTRACT

This research was carried out to determine the pollination abilities of Domat, Gemlik and Sarı Ulak olive cultivars. Pollen viability rate, germination rate and amount of pollen production were examined during the periods when 25%, 50% and 75% of the flowers were opened. According to the results obtained from three years' experimental study, the highest percentages of pollen viability were found in Gemlik (81.51%) and Domat (81.50%), whereas Sarı Ulak cultivar (77.41%) showed the lowest viability. In pollen germination percentages, the highest values on the basis of cultivars were determined 59.85% (Domat), 59.74% (Gemlik) and 52.12% (Sarı Ulak). The highest amount of pollen production in a flower was found in the Sarı Ulak cultivar (329,046) while it was followed by Domat (319,693) and Gemlik (306,385). Despite the high pollen production in Sarı Ulak cultivar, the pollen viability and germination rates were found to be low. In Gemlik and Domat cultivars, all values were obtained relatively near. In addition, it had been noted that the values related to pollen viability, germination and production amount were higher during 50% and 75% flowering periods than 25%.

[a] *cansudolek.90@gmail.com* *https://orcid.org/0000-0001-7628-0676*
[b] *ozkaya@agri.ankara.edu.tr* *https://orcid.org/0000-0002-6571-5985*
[c] *seti@cu.edu.tr* *https://orcid.org/0000-0001-7705-0856*
[d] *skarabiyik@cu.edu.tr* *https://orcid.org/0000-0001-8579-6228*
[e] *neslihantaskin@hotmail.com* *https://orcid.org/0009-0003-4197-1874*

Introduction

The Oleacea family, which is a species of the genus Olea (*Olea europaea* L.), with a homeland of Upper Mesopotamia, including Southeast Anatolian Region and Southern Asia Minor. Olives spreaded from here to the whole world mainly from three branches: the first via Egypt Tunisia and Morocco; the second throughout Anatolia, the Aegean Islands, Greece, Italy, Spain and the third also via Iran, Pakistan and China (Özkaya et al., 2010). The spreading area of the olive tree is located between latitude of 30° and 45° in both northern and southern hemispheres. Olives are Mediterranean climate plants characterized by dry and hot summers (Pansiot and Rebour, 1964). Olive trees have andromonoecious inflorescences containing both hermaphrodite (perfect) and staminate (male) flowers together (Cuevas and Polito, 2004).

Cuevas and Rollo (1990) have noted that the excellent flower ratio in olive trees with low flower density is higher than the trees with a large number of flowers. However, in some cases there were plenty of male flowers Martins et al. (2006) and the lack of sufficient hermaphrodite flowers decreased yield (Mete and Mısırlı, 2009).

According to Cuevas et al. (2001), the pollenizer cultivar should be compatible and synchronized with the main cultivar while it should bloom regularly. Considering the function of pollens, it is convenient to choose efficient pollenizers with high viability and commercial value especially for table and olive oil).

Martins et al. (2006) examined the flower structures of Morisca olive cultivar grown in 5 different experimental orchards. It was determined that the total number of flowers, the number of perfect flowers and the quality of pollen were low, which showed that the observed deficiencies were due to characteristics ofcultivars, but not a serious limitation for fruit production. In another study, the authors examined on flower quality of Sikitita, Picual and Arbequina cultivars (Moreno-Aliasa et al., 2013). The

results of this study showed that, Arbequina (pollenizer) cultivar generally offered higher values than Picual (main) cultivar while Sikitita cultivar showed intermediate values compared to its parents in terms of the number and percentage of hermaphrodite flowers. In this case, it is revealed once again that the genetic characteristics of the cultivars have a significant effect on the production of healthy flowers.

In many plants, fruit set is closely related to the viability and germination levels of the pollenizer plant. In addition to the pollination biology studies to be carried out under natural conditions to determine whether pollen can be used as an appropriate pollenizer in fertilization and different hybridization combinations. It has also been stated that the results obtained from the *in vitro* studies are also important (Paydaş et al., 1996).

Using agar + sucrose mixtures in *in vitro* pollen germination tests has advantages such as easy intake of different carbohydrates, constant relative air humidity and aerobic conditions on the agar surface. It was stated as well that it might cause pollen to lose its germination ability due to some disadvantages such as suitable conditions for fungal infections and unadjusted temperature of agar. On the other hand it has been stated that, pollen viability tests give more positive results than the pollen germination tests performed under *in vitro* conditions. It is due to the reasons such as not including variable environmental factors likehumidity, temperature and nutrient properties (Eti, 1991).

In this study, it was aimed to determine the characteristics of pollen quality and amount of pollen production of Domat, Gemlik and Sarı Ulak olive cultivars which are used as main cultivars and pollenizers in the orchards.

Materials and methods

This study was carried out in an olive orchard in Tarsus district of Mersin province. Domat, Gemlik and Sarı Ulak olive cultivars were used as main cultivars to evaluate the pollen viability and germination levelswith the amount of pollen production.

Pollens were collected at 25% (BBCH=63), 50% (BBCH=65) and 75% (BBCH=67) flowering periods and transfered to the laboratory. According to Sanz-Cortés et al. (2002), flowering periods were indicated by BBCH codes (Dölek and Özkaya, 2021).

Viability tests were performed with 1% 2,3,5 Triphenyl Tetrazolium Chloride (TTC) added to 60% sucrose solution (Norton, 1966). In order to determine pollen viability, pollens were counted in 3 randomly selected areas on 3 replicates for each cultivar. This test was repeated when 25%, 50% and 75% of the flowers bloomed for each cultivar. Pollen staining with TTC took 3-4 hours and counts were carried out under a light microscope. During the examination, pollens stained dark red were evaluated as “absolutely viable”, those stained light red and pink as “semi-viable”, and those did not stained as “non-viable” (Figure 1.a). Theoretically, 50% of the pollen determined as semi-viable was assumed to be viable, half of this value was added to the absolutely viablepollen, and thus the amount of 'viable' pollen was found by calculation (Karabıyık and Eti, 2015).

Pollen germination tests were carried out using the " agar in petri " method at 25°C with 1% agar + 15% sucrose + 100 ppm H_3BO_3 (Mete, 2009). Pollens were spreaded to the medium and were counted after 4-5 hours. Pollen germination levels were determined by counting 3 randomly selected areas in 3 petri dishes for each different season of each cultivar (Figure 1.b).

“Hemacytometric Method” was used to determine the amount of pollen production in a flower of the cultivarsused in the experiment (Eti 1990). For this purpose, anthers of 10 flowers were taken at bloom stage and placed in small plastic boxes with 3 replications. Anthers were allowed to dry for about 2 weeks and the preparates were prepared according to Eti (1990) and counted on the hemocytometric microscope slide (thoma slide) (Figure 2.a). Ocular micrometer was used in microscope examinations (Figure 2.b). During the counting, besides the normally developed pollen, amount of morphologically abnormal pollens were also determined.

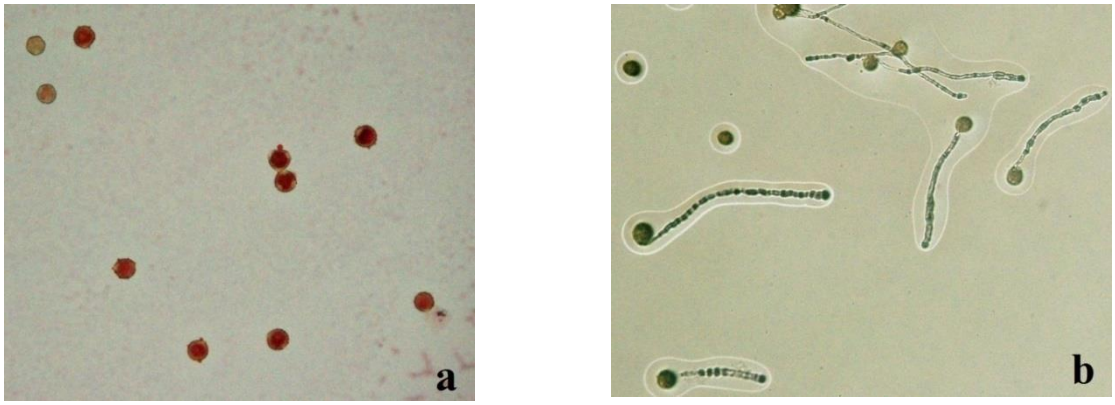

Figure 1. Visuals of pollen viability (a) and germination (b) tests

Figure 2. Visuals of hemocytometric microscope slide (a) and the pollens that appear on the ocular micrometer (b)

Table 1. Pollen viability levels of olives in terms of years, flowering periods and cultivars (%)[1,2]

Years	Cultivars	Flowering Periods			
		25% (BBCH=63)	50% (BBCH=65)	75% (BBCH=67)	Cultivar Average
2017	Domat	73.30	76.91	77.50	75.90AB
	Gemlik	81.44	79.08	84.02	81.51 A
	Sarı Ulak	69.29	75.68	74.84	73.27 B
	Period Average	74.68	77.22	78.79	
	$LSD_{cultivar}$:4.038* LSD_{period}: N. S. $LSD_{cultivar\ x\ period}$: N. S.				
2018	Domat	77.33	83.38	83.77	81.50 A
	Gemlik	76.30	80.92	79.92	79.04AB
	Sarı Ulak	78.01	80.02	74.14	77.41 B
	Period Average	77.20 B	81.44 A	79.29 AB	
	$LSD_{Cultivar}$: 2.284 ** LSD_{Period}: 2.284 ** $LSD_{Cultivar\ x\ Period}$: N. S.				
2019	Domat	70.92 cd	82.22 a	65.96 de	73.03 A
	Gemlik	67.24 de	76.40 b	62.21 e	68.61 B
	Sarı Ulak	63.95 e	74.68 bc	70.85 cd	69.83 B
	Period Average	67.37 B	77.76 A	66.34 B	
	$LSD_{Cultivar}$:1.938 ** LSD_{Period}:1.938 *** $LSD_{Cultivar\ x\ Period}$: 3.357 ***				

[1]Values were analysed after arc-sin transformation; [2]Differences between averages shown in separate letters in the same column were found to be statistically significant; N.S.: Not significant; **, P<0.01; ***P<0.001.

Two factor randomized complete block design with 3 replications was conducted for pollen studies. Arc-sin transformation was used in percent values before analyses. JMP 8.0.1 programme was used for Anova test and the results were compared with LSD (5%).

Results and Discussion

Pollen Viability Level

As a result of the pollen viability tests conducted in 2017, the highest value among the cultivars was obtained from Gemlik cultivar with 81.51%, followed by Domat (75.90%) and Sarı Ulak (73.27%), respectively. It was observed that the values of the period averages ranged from 74.68% (25% flowering) to 78.79% (75% flowering) (Table 1).

As a result of the pollen viability tests conducted in 2018, the highest level of viability belonged to the Domat cultivar (81.50%), while the lowest level of viability was determined in the Sarı Ulak cultivar (77.41%). The highest value in terms of period averages was determined to be 50% flowering (81.44%), followed by 75% flowering (79.29%) and 25% flowering (77.2%), respectively (Table 1).

In 2019, the highest level of pollen viability in terms of cultivar averages was determined in the Domat cultivar (73.03%), while other cultivars showed close values. The highest value in the period averages was 50% flowering (77.76%). In the cultivar and period interaction, the level of pollen viability ranged from the lowest 62.21% (Gemlik-75% flowering) and the highest 82.22% (Domat-50% flowering) (Table 1).

According to the results of the three-years trial, the highest values in terms of pollen viability levels were determined in Gemlik in 2017, Domat in 2018 and 2019; the lowest values were found in Sarı Ulak in 2017 and 2018 while it was lowest in Gemlik and Sarı Ulak cultivars in 2019. The highest values in the period averages were 75% flowering in 2017 and 50% in 2018 and 2019. On the contrary, the lowest viability values were detected in 25% flowering in 2017 and 2018 while it was 25% and 75% flowering in 2019.

Martins et al. (2006) conducted a study on Morisca olive cultivars grown in 5 different experimental orchards and it was determined that pollen viability was very low (between 3.84% and 11.04%) in all orchards. In another study by Palasciano et al. (2008) on olives; it was determined that the cultivar with the highest pollen viability (97.6%) was Cipressino, and the cultivar with the lowest (48.0%) in this respect was Cellina di Nardo.

Mete and Mısırlı (2009) reported that the highest pollen viability rate was found in Edincik Su cultivar with 69.07% and the lowest rate was in Eşek olives with 8.93% in the TTC test applied as part of their experiment in different

olive cultivars. In the second year of the trial, The Eğri Burun and Kilis Yağlık cultivars have showed high values with a viability level of 90.68% and 88.82%, respectively, while Eşek olives have ranked last with a viability rate of 18.07%.

In a study conducted in Chott Mariem (Tunusia), pollen viability ratio of Meski and Gerbou olive cultivars was found as 60.50% and 88.85%, respectively in on year, while in off year the ratios were 97.47% and 62.38%, respectively (Methamem et al., 2015). Sanchez-Estrada and Cuevas (2019) studied pollen viability levels in Northern Mexico in 2016 and 2017. The authors found that the pollen viability levels were 68.9%, 84.8% and 70.1% in 2016;82.9%, 78.3% and 73.7% in 2017 for Manzanilla, Barouni and Sevillano cultivars, respectively. Mete et al. (2015) have reported that pollen viability rates ranged from 46.43% (Saurani) to 88.65% (Sarı Ulak) in the first year and 73.69% (Memecik) to 94.04% (Edincik Su) in the second year. The results of recent studies found to be parallel with our study.

Pollen Germination Level

As a result of pollen germination tests conducted in 2017, the highest values were found in Domat (46.26%) and Gemlik (45.36%), while the value of Sarı Ulak cultivar (34.17%) was found to be lower. Germination values between the period averages are 50% flowering (54.08%), 75% flowering (42.88%) and 25% flowering (28.84%), respectively, from high to low. In terms of cultivar and period interaction, pollen germination values have ranged from 15.13% (Sarı Ulak 25% flowering) to 55.95% (Domat 50% flowering) (Table 2).

In 2018, the values of cultivar averages changed between 49.86% (Sarı Ulak) and 59.35% (Domat). In terms of cultivar and period interaction, the highest germination value was found during the 50% flowering period (66.75%) of the Domat cultivar and the lowest value in this respect was found during the 25% flowering period of Sarı Ulak (39.47%) (Table 2).

In 2019, pollen germination levels of the cultivars were determined the highest in Domat (59.85%) and Gemlik (59.74%) cultivars, and the lowest value was determined in Sarı Ulak (52.12%) cultivar. In cultivar and period interaction, pollen germination levels have ranged from 42.03% (Sarı Ulak-75% flowering) to 69.40% (Sarı Ulak-50% flowering) (Table 2).

When the results of the three-year trials were evaluated together, it was determined that the highest values in terms of pollen germination levels were in Domat and Gemlik cultivars in all three trial years, while the lowest values were obtained in Sarı Ulak cultivar. In terms of different flowering periods, the highest pollen germination values were found during 50% flowering periods in all three trial years, followed by 75% and 25% flowering periods respectively.

Yalcinkaya et al. (2002) determined that, in the first year of their experimentsthe pollen germination rate of used olive cultivars were 58.60% for Edincik Su and 30.00% for Uslu; while in the second year the rates were 48.00% for Edincik Su and 42.70% for Uslu. Ersoy et al. (1998) have been reported that the pollen germination rate was between 3.60% and 11.00% in Memecik olive cultivar, 2.30% to 11.00% in the Ayvalik, 0.00% to 5.70% in the Gemlik and 0.00% to 11.90% in Uslu cultivar. In another study, Ferri et al. (2008) have been reported that the viability and germination levels of olive pollens may vary depending on genetic and environmental factors. Koulouris et al. (2009) have observed the effect of heat on pollen germination and pollen tube growth in four olive cultivars and found that different cultivars had different optimum pollen germination temperatures, but pollen germination in all cultivars increased at 25^0C. In this study, temperatures were ranged between 15 and 25^0C during the flowering period and pollen viability and germination tests under laboratory conditions were also performed at 25^0C in order to achieve ideal results.

Table 2. Pollen viability levels of olives in terms of years, flowering periods and cultivars (%)[1,2]

Years	Cultivars	Flowering Periods			
		25% (BBCH=63)	50% (BBCH=65)	75% (BBCH=67)	Cultivar Average
2017	Domat	27.46 bc	55.95 a	55.36 a	46.26 A
	Gemlik	43.92 ab	50.80 a	41.37 ab	45.36 A
	Sarı Ulak	15.13 c	55.49 a	31.90 b	34.17 B
	period average	28.84 C	54.08 A	42.88 B	
	$LSD_{Cultivar}$: 5.852 ** LSD_{Period}: 5.852 *** $LSD_{Cultivar\ x\ Period}$: 10.136 **				
2018	Domat	51.48 bcd	66.75 a	59.83 abc	59.35
	Gemlik	56.03 abc	49.61 bcd	63.11 ab	56.25
	Sarı Ulak	39.47 d	62.10 abc	48.02 cd	49.86
	period average	48.99 B	59.49 A	56.99 AB	
	$LSD_{Cultivar}$: Ö.D. LSD_{Period}: 4.825 ** $LSD_{Cultivar\ x\ Period}$: 8.356 **				
2019	Domat	53.24 bc	63.28 ab	63.04 ab	59.85 A
	Gemlik	60.22 ab	65.07 a	53.91 bc	59.74 A
	Sarı Ulak	44.93 cd	69.40 a	42.03 d	52.12 B
	period average	52.80 B	65.92 A	52.99 B	
	$LSD_{Cultivar}$: 3.494 ** LSD_{Period}: 3.494 *** $LSD_{Cultivar\ x\ Period}$: 6.052 **				

[1]Values were analysed after arc-sin transformation; [2]Differences between averages shown in separate letters in the same column were found to be statistically significant; **, P<0.01; ***P<0.001.

Table 3. Amount of pollen production of olives in terms of years, flowering periods and cultivars (Quantity) [1]

Years	Cultivars	Flowering Periods			
		25% (BBCH=63)	50% (BBCH=65)	75% (BBCH=67)	Cultivar Average
2017	Domat	291,635 bc	339,887 a	327,558 ab	319,693 AB
	Gemlik	320,544 ab	279,732 c	297,587 bc	299,287 B
	Sarı Ulak	314,167 abc	323,520 ab	349,452 a	329,046 A
	Period Average	308,782	314,379	324,866	
	$LSD_{Cultivar}$: 22,028,4 ** LSD_{Period}: N. S. $LSD_{Cultivar\ x\ Period}$: 38,154,4 **				
2018	Domat	187,468	260,684	307,625	251,926 A
	Gemlik	157,060	206,510	263,193	208,921 B
	Sarı Ulak	186,287	252,713	303,639	247,547 A
	Period Average	176,939 C	239,969 B	291,486 A	
	$LSD_{Cultivar}$: 14,481,3*** LSD_{Period}: 14,481,3*** $LSD_{Cultivar\ x\ Period}$: N. S.				
2019	Domat	283,770 de	297,941 cde	267,651 e	283,121 C
	Gemlik	270,662 e	329,826 bc	318,666 c	306,385 B
	Sarı Ulak	382,080 a	363,481 ab	308,924 cd	351,496 A
	Period Average	312,172 AB	330,417 A	298,414 B	
	$LSD_{Cultivar}$: 20,046,9*** LSD_{Period}: 20,046,9** $LSD_{Cultivar\ x\ Period}$: 34.772,211***				

[1]The differences between the averages shown in separate letters in the same column were found to be statistically significant; N.S.:Not significant; **, P<0.01; *** P<0.001.

Mete and Mısırlı (2009) determined that the highest pollen germination rate among the examined olive cultivars were in Edincik Su (45.00%) and Erkence (42.82%) cultivars in the first year, and the lowest rate was in Eşek olive cultivars (288%). In the second year, the highest rate of pollen germination were in Domat (6791%), Edincik Su (66.79%), Uslu (65.14%) and Kilis Yağlık (64.18%) olive cultivars and the lowest was found in Eşek olive and Memecik cultivars with rates of 5.30% and 5.52%, respectively. In our study, pollen germination levels similarly ranged from 34.17% (Sarı Ulak cultivar in 2017) to 59.85% (Domat cultivar in 2019). The pollen germination values obtained by the previous researchers are in line with the results we obtained.

Amount of Pollen Production and Normally Developed Pollen Ratios

As a result of the pollen counts carried out in 2017, the highest value was obtained in Sarı Ulak cultivar (329,046), followed by Domat (319,693) and Gemlik (299,287), respectively. In addition, it was determined that the amount of pollen production increased during flowering period. In the cultivar and period interaction, the values have ranged from 279,732 (Gemlik- 50% flowering) to 349,452 (Sarı Ulak - 75% flowering) (Table 3).

In 2018, amount of pollen production per flower was found to be close to each other for Domat (251,926) and Sarı Ulak (247,547) cultivars, while Gemlik cultivar (208,921) showed lower values. When the periods averages were examined, the highest amount of pollen production per flower was determined in 75% flowering period (291,486), while other values were arrayed 50% flowering (239,969) and 25% flowering (176,939) (Table 3).

In 2019, the highest amounts of pollen per flower in terms of cultivar averages were obtained from Sarı Ulak (351,496) cultivar. This was followed by Gemlik cultivar (306,385), while the lowest values were obtained in the Domat (283,121). It was determined that in the period average, the highest value was 50% flowering (330,417), followed by 25% (312,172) and 75% (298,414) flowering periods. In terms of cultivar and period interaction, amount of pollen production have ranged from 270,662 (Gemlik 25% - flowering) and 267,651 (Domat - 75% flowering) to 382,080 (Sarı Ulak - 25% flowering) (Table 3).

In the 3 years of the trials conducted together, it was noted that Sarı Ulak cultivar had a higher pollen production than the other two olive cultivars. While pollen production increased during flowering period in 2017 and 2018, the highest amount of pollen was found during 50% flowering period in 2019.

According to Seifi et al. (2015), a mature olive tree produces about 500,000 flowers. The number of flowers and the distribution of flowers on the inflorescences are special for each cultivars, but may vary from year to year. Palasciano et al. (2008) reported that 222,516, 180,361 and 176,088 pollens were produced per flower in Arbequina, Arbosana and Pasola olive cultivars, respectively. The authors also reported that pollen can be produced between 1,000,000 and 4,000,000 in each infloresence varying by cultivars. In our study, the amount of pollen production were between 208,921 (Gemlik, in 2018) and 351,496 (Sarı Ulak, in 2019). Our results showed that the amounts of pollen production were sufficient in all three studied olive cultivars.

Normally developed pollen ratios of cultivars were given in Table 4. The values obtained from the cultivars examined were found to be very close to each other with over 96% for three years. Likewise, during the flowering period, the values gave approximate values increasing by proceeding periods.

In addition to the total amount of pollen produced in the flowers of a cultivar, it is of great importance that the ratio of pollen which in terms of morphological normally developed is high. Anvari (1977) stated that pollen that are not morphologically homogeneous, which shown deviations from the size and shape characteristics specific to the cultivar, are very unlikely to germinate. The researcher have also stated that as the normally developed pollen rate approach to 100%, the pollination potential of the cultivar will be high.

Table 4. Normally developed pollen ratios of olives in terms of years, flowering periods and cultivars (%)[1]

Years	Cultivars	Flowering Periods			
		25% (BBCH=63)	50% (BBCH=65)	75% (BBCH=67)	Cultivar Average
2017	Domat	98.41	98.80	99.10	98.77
	Gemlik	98.68	99.01	99.00	98.90
	Sarı Ulak	98.19	98.44	98.78	98.47
	Period Average	98.43	98.75	98.96	
	$LSD_{Cultivar}$: N. S. LSD_{Period}: N. S. $LSD_{Cultivar\ x\ Period}$: N. S.				
2018	Domat	95.83	96.67	97.85	96.78
	Gemlik	96.13	96.95	97.72	96.93
	Sarı Ulak	96.82	97.95	98.09	97.62
	Period Average	96.26 B	97.19 A	97.89 A	
	$LSD_{Cultivar}$: N. S. LSD_{Period}: 1.390^{***} $LSD_{Cultivar\ x\ Period}$: N. S.				
2019	Domat	96.02	98.15	97.27	97.15
	Gemlik	95.38	97.28	97.04	96.57
	Sarı Ulak	94.19	97.68	97.37	96.41
	Period Average	95.20 B	97.70 A	97.23 A	
	$LSD_{Cultivar}$: N. S. LSD_{Period}: 0.903^{***} $LSD_{Cultivar\ x\ Period}$: N. S.				

Values were analysed after arc-sin transformation; [2]Differences between averages shown in separate letters in the same column were found to be statistically significant; **, P<0.01; *** P<0.001; N. S.:Not significant; *** P <0.001.

Conclusion

As a result, the highest values obtained on the basis of cultivars (81.51% and 81.50%) in terms of three-years pollen viability percentages were found in Gemlik and Domat cultivars, while a lower value was reached in Sarı Ulak cultivar (77.41%). The same results were observed in the percentages of pollen germination, and the highest values for cultivars were determined respectively; Domat as 59.85%, Gemlik as 59.74% and Sarı Ulak as 52.12%. The results of amount of pollen production have been found to be highest in Sarı Ulak cultivar (329,046), followed by Domat (319,693) and Gemlik (306,385) cultivars. In addition, normally developed pollen rates were found to be over 96% in all three studied cultivars. In addition, it was determined that the values related to pollen viability, germination and amont of pollen production in all cultivars and in each year were higher during the 50% and 75% flowering periods compared to the 25%.

As a result, it can be indicated that pollen in Gemlik and Domat cultivars generally obtain closer values to each other in terms of pollen viability, germination and production quantities. Although the Sarı Ulak cultivar has a high pollen production compared to the other two cultivars, it was found that the pollen viability and germination rates were slightly lower. However, it can be said that all three olive cultivars examined in the trial showed high values in terms of pollen quality and quantity results and were sufficient in terms of pollination potential. Studies on pollen are guiding studies for fertilization biology and fruit set. Therefore, it is important and can be recommended to examine the related pollen properties in researches.

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