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EFFECTS OF BIOTARM ON GERMINATION IN AYVALIK OLIVE TYPE AND WILD OLIVE SEEDS

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Abstract: The possibility of germination of a olive seed is either non-existent or very low even in suitable conditions unless there is any supporting factor. Olive seeds (*Olea europaea* L.) are characterized by a particularly low germination percentage. This study was planned to determine the effect of Biotarm on germination level in the greenhouse by using the seeds of olive fruits obtained from the olive seedlings and Ayvalık olive variety in Kozan/Adana region at the end of October. In the study in order to evaluate seed germination, 1% and 2.5% Biotarm activator were used in the control group. Germination was observed approximately 90-110 days after seed sowing. At the end of 6.5-7 months, the plants that were of suitable size and ready for planting were uprooted and measured. As a result of the study; when the germination percentages and root structures of Ayvalık olive variety and seedling seeds were evaluated, it was determined that the control group without any treatment remained at a low level. At the same time, the Biotarm treatments did not differ significantly by dose in terms of promoting germination. The germination rate of wild olive seeds was found to be higher in all applications compared to the Ayvalık variety. This was interpreted as the ability to show stronger emergence due to due to their wild-type characteristics. In terms of treatments, the highest germination rate was determined in the 2.5% Biotarm (45%) and the lowest value in the Control (31.99%). The seed germination studies should be continued within this scope.

Keywords: Olive, Seedling, Seed, Germination, Activator

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1. Introduction

Propagation of olive trees (*Olea europaea* L.) occurs in the form of grafting and cutting when in commercial cultivation. However, the generative (seed) propagation becomes also important, especially in providing rootstock. The possibility of germination of olive seeds is very low even under suitable conditions unless supporting factors are present. In determining the germination feature of a seed, the strength of germination and the germination percentage should be evaluated (Kaşka and Yılmaz, 1987). The germination power of wild olive seeds is lower than cultivated varieties and according to Usanmaz (1972), the germination rates of seedlings (seeds obtained from wild olive plants) were between 10.1-17.2%, while this rate in yoz (seeds of cultivated varieties) was between 9.6-34.3%.

The olive tree, *Olea europaea* L., is characterized by a particularly low germination rate and percentage. Germination inhibiting substances found in the woody part surrounding the seed and in the seed coat, endosperm and the embryo itself may delay germination by one or three years (Sotomayor-León and Cabellero, 1990) and in many varieties the germination is irregular and the rate may not exceed 10% (Acebedo et al., 1997). Various morphological and physiological factors in the seed and fruit flesh can significantly delay germination (Scaramuzzi and Baldini 1963; Lagarda et al. 1983; Germanà et al. 2014). The major obstacle to olive seed germination is the stony endocarp and the dormancy period which is originate from seed coat, endosperm and the embryo itself (Lagarda et al. 1983). Additionally, standardized propagation by seed is also important for the production of rootstocks for grafting difficult-to-root varieties. At the same time, rapid seed germination is also necessary for breeding studies and hybridizations (Germanà et al. 2014). The average germination time of olive seeds varies between 81 and 300 days while the germination time of kernels varies between 84 and 550 days (Ruby 1916; Loussert and Brousse 1978; Hannahi et al. 2011). In olive seed germination physiological and mechanical dormancy is observed. The mean time for germination ranged from 81 to 300 days for seeds and from 84 to 550 days for stones/endocarps (Ruby 1916;

Loussert and Brousse 1978).

In a study, the optimum germination temperature of Manzanillo olive seeds were reported to be 15 °C in seeds with kernel while it was 25 °C in only embryo sowing and the best date to take the seed was 30th October. In the study, the researchers recorded the best temperature after germination was 25 °C (Lagarda et al. 1983). Sotomayor-León and Cabellero (1990) emphasized that in order to eliminate dormancy in the germination of Arbequina, Oveslati, Galega, Picual and Manzanilla olive varieties, the seeds should be stratified at 15 °C for 1 month and that it would be more effective to separate the embryo from the seed before sowing. In another study, De la Rosa et al. (2006) reported that the harvesting period between July and November of Arbequina, Empeltre, Koroneiki, Manzanilla de Sevilla and Picual seeds showed differences and the earliest harvest date had the highest germination percentage (80-90%). Yüce (1979) reported similar results and the germination percentage was found to increase in early harvest because the seed enters dormancy at the black maturity stage and in the in the presence of GA_3 and IAA. In another study, Diana and Gaetani (1979) reported that soaking seeds in 10% sulphuric acid of Carolea, Dolce di Rossano and Olivastro olive varieties shortened the germination time and increased the germination percentage. Hosseinpour (1998) grew the seeds of wild olives (*Olea ferruginea* Royle) grown in Iran, in sand after their shells were cracked and kept in water for 5 days. It was observed that the seeds germinated at a rate of 62% in about 30 days (Gözel, 2006).

The most suitable periods for seed planting were determined as 1 October - 15 November for the Memecik variety and 1 October - 1 November for the Ayvalık variety. It has been stated that there is no need for any seed waiting time or folding for seedlings. It has been stated that the ripening period does not change the germination time according to the varieties.

This study was planned to investigate seed germination rates in simpler environments that could adapt to production conditions without increasing costs, in addition to in vitro studies. The study determined that germination rates could change even without laboratory conditions. It was observed that germination percentage could be increased with a different indicator application under greenhouse conditions, and it is considered that this could serve as an example for other future studies.

2. Materials and Methods

This study was planned to determine the germination level in the greenhouse using the seeds of olive fruits obtained from the olive seedlings and Ayvalık olive varieties in the Kozan region. To prevent any risk of contamination in the greenhouse, fungicide was sprayed and the greenhouse was kept closed for 5 days. Before planting the seeds, the crates were sprayed with fungicide and filled with finely sifted sand. Fruits were taken from healthy trees at the end of October and the seeds were extracted. The obtained seeds were washed and separated from the fruit residues and dried for one week. The seeds were planted without being broken.

In the study, 1% and 2.5% Biotarm activator was used to enhance seed germination and a control group was formed that left untreated. The doses were determined according to the previous study by Güler et al. (2017).

As Biotarm is an organic solution, it allows plants to be nourished through their roots and/or leaves. It has positive effects on plant health in olive trees experiencing abiotic and biotic stress. It promotes adventitious root formation. Biotarm contains free biocatalysts, minerals, organic stabilizers, biological surfactants, H_2O, and emulsifiers.

The study was conducted in three replications using 30 olive seeds for each application. The seeds were sown in the boxes in the greenhouse at a depth of 3 cm. To ensure germination success, the temperature under cover was kept at 20-25 °C and 75-80% humidity. Biotarm applications continued for 15-day periods until germination was observed. Biotarm was applied to the root zone by spraying. Regular weeding and fungicide treatments were carried out to get healthy seedlings. Since fungal agents can develop in the constantly humid greenhouse environment, they were sprayed with a fungicide.

In the study, the duration of germination was determined and at the end of seven months that the plants became to suitable size to plant, they were removed and root length (measured from the collar to the tip of the primary root), shoot length (measured from soil surface to the apical meristem), and number of true leaves were recorded for each seedling. The obtained results were evaluated using the JMP 13.0.2 statistical software based on a factorial experimental design, considering seed origin and treatment doses as the main factors. Arc-sin transformation was applied to percentage data before analysis.

3. Results

3.1. Germination Duration and Percentage

Germination first began in the seedling group, and emergence was detected on the 90th day after sowing.. Germination in the Ayvalık variety was observed within the following week. At the end of the 4-month period, germination of the seeds was completed (Figure 1).

When germination percentage was evaluated, seed origin and treatment means were found to be significant at $P<0.001$. The highest germination rate was obtained from the wild seedling group (42.38%), while the lowest was observed in the Ayvalık variety. In terms of treatment means, the highest values were obtained from 1% and 2.5% Biotarm treatments (41.11% and 45%), while the lowest values was found in the Control (31.99%). The variety x treatment interaction were found to be non-significant (Table 1).

Figure 1. Planting seeds in boxes inside the greenhouse and the beginning of germination.

Table 1. Germination percentage values of germinating plants (%)

Treatments	Seed Origin		
	Wild seed	Ayvalık	Treatment average
Control	35.22	28.78	31.99 B
%1 Biotarm	43.07	39.14	41.11 A
%2,5 Biotarm	48.84	41.15	45.00 A
Seed Origin Average	42.38 a	36.36 b	

$P_{seed\ origin}$= ***, $P_{treatment}$= ***, $P_{seed\ originxtreatment}$= N.S., N.S.= not significant, ***= P<0.001.

Table 2. Root length values of germinated plants (cm)

Treatments	Seed Origin		
	Wild seed	Ayvalık	Treatment average
Control	10.08 b	8.88 b	9.48 B
%1 Biotarm	11.80 a	13.37 a	12.58 A
%2,5 Biotarm	12.23 a	12.40 a	12.32 A
Seed Origin Average	11.37	11.55	

$P_{seed\ origin}$= N.S., $P_{treatment}$= ***, $P_{seed\ originxtreatment}$= *, N.S.= Not Significant, ***= P<0.001, *= P<0.05.

Figure 2. Root and shoot length in germinated plants. Left: Wild Sees, Right: Ayvalık (Control, 1%, 2.5% Biotarm, respectively).

3.2. Root Length

When plants were evaluated in terms of root length, seed origin means were not statistically significant, whereas treatment means were significant at P=0.001. The highest values were found similarly in the 1% and 2.5% Biotarm treatments (12.58 cm and 12.32 cm) and the lowest values were determined in the Control treatment (8.88 cm). The variety x treatment was significant at P<0.05. The highest value was seen in the Ayvalık x 1% Biotarm group at 13.37 cm and the lowest as 8.88 cm in the Ayvalık x Control interaction (Table 2, Figure 2).

3.3. Shoot Length

The shoot length data of germinated plants are given in Table 3. In terms of shoot length, no differences were observed between variety means or the variety × treatment interaction, while differences between treatment means were significant at P<0.001. The highest shoot length was obtained from the 2.5% Biotarm treatment (11.65 cm), while the lowest was measured in the Control (8.98 cm) (Table 3, Figure 2).

3.4. Leaf Number

In terms of leaf number data, while there was no difference between treatment averages and seed origin x

treatment interactions, the variety averages was found to be significant according to P<0.01. The highest number of leaves was found in the Ayvalık variety (11.93 leaves), while the lowest was observed in the seedling group (10.33 leaves) (Table 4).

Table 3. Shoot length values of germinated plants (cm)

Treatments	Seed Origin		Treatment average
	Wild seed	Ayvalık	
Control	9.39	8.56	8.98 C
%1 Biotarm	10.62	10.94	10.78 B
%2,5 Biotarm	11.95	11.35	11.65 A
Seed Origin Average	10.65	10.28	

$P_{seed\ origin}$= N.S., $P_{treatment}$= ***, $P_{seed\ originxtreatment}$= N.S., N.S.= not significant, ***= P<0.001.

Table 4. Leaf number values of germinated plants (number)

Treatments	Seed Origin		Treatment average
	Wild seed	Ayvalık	
Control	10.27	10.88	10.58
%1 Biotarm	10	11.97	10.99
%2,5 Biotarm	10.73	11.93	11.33
Seed Origin Average	10.33 b	11.6 a	

$P_{seed\ origin}$= **, $P_{treatments}$= N.S., $P_{seed\ originxtreatment}$= N.S., N.S.= not significant, **= P<0.01.

4. Discussion

While vegetative propagation methods are economically important in seedling production, rootstock production using seeds stands out in terms of resistance to global climate change and soil factors. In particularly, wild olive plants have high adaptation to natural conditions, which has led to the olive tree to be described as very long-lived and even immortal. An olive plant grown from seed can re-sprout after drying caused by various factors, provided that its roots remain healthy, due to its strong root system.. For this reason, grafting commercial cultivation onto seed grown rootstocks provides additional advantages compared to vegetative propagation methods. Seed germination varies depending on temperature (15-25 °C), dormancy status (storage at 5-10 °C for 4-14 days), fruit maturity level (with early harvest), scarification (abrasion, cracking, subjecting to water and chemical treatment), and medium (sand or 1:1 sand:peat).

In recent studies it was reported that the endocarp has a negative effect on seed germination of 'Manzanillo' olive variety in both stratified and unstratified seeds (Crisosto and Sutter, 1985). In olive seeds the high rates of germination were obtained only when the endocarp was removed completely or when the root tip was removed. At the same time, the germination percentage of seeds with endocarp does not increase with stratification, but stratification for 30 days at 15 °C has been shown to break the dormancy of the intact seed (Istambouli and Neville, 1977; Crisosto and Sutter 1985). In another study, Morales-Sillero et al. (2012) reported that the highest rates in terms of germination abilities of Hojiblanca, Manzanilla Caceren˜a, Manzanilla de Sevilla, Toffahi and Uovo di Piccione olive cultivars were obtained with the 30-day stratification procedure at 14 °C. After 18 days, increasing the temperature to 25 °C improved root length and reduced the average seedling emergence time. Seedlings that grow faster in the early stages, expressed as primary shoot height and diameter, tend to overcome the juvenile sterility period earlier. In the present study, the temperature inside the greenhouse was kept around 25 °C and attention was also paid to the high humidity rate. The germination percentage of wild olive (*Olea europaea* L. var. sylvestris) seeds and embryos increased with the harvest in October, these percentages decreased in November and none of the seeds and embryos harvested in mid-December germinated. In the germination of wild seeds, the germination percentage in the Control group was 20%. In this study, the optimum conditions to achieve a germination rate greater than 80% were to use isolated embryos instead of seeds, to harvest the seeds in mid-to-late October, for five to thirteen days before planting the seeds (47% and 94% germination rates, respectively) (Hannahi et al., 2011). Another study recommended to keep the seeds at 4°C in order to confirm the effectiveness of cold treatment on wild seed germination as reported by Wallali (1971).

In our study, the highest germination rate was found in Seedling plants (42.38%), while the lowest germination rate was observed in Ayvalık variety (36.36%). In terms of treatemnts, the highest germination rate was determined in the 2.5% Biotarm application (45%), while the lowest was observed in the Control (31.99%). While the highest root length values were detected in the Ayvalık 1% Biotarm application with 13.37 cm, the lowest values were seen in the Ayvalık Control group with 8.88 cm. When no application was made, the rooting percentage and root structures were found to be lower for both varieties.

Rostami and Shasavar (2009) observed the lowest germination percentage in control applications in their studies. The highest germination percentage of Koroneiki variety (up to 73%) was obtained after 6 hours of 97% sulfuric acid solution applications. For Arbequina variety, the best result was observed after 9 hours of 97% sulfuric acid application (69.5%). This situation shows us that when excessive amounts of chemicals are applied, there are decreases in germination or non-emergence situations in seeds. In our study, however, germination rates increased with the use of a naturally-derived indicator, and such adverse effects were eliminated. A similar study was conducted by Mirzajani Fathkouhi et al.

(2022) on Arbequina, Balidi, and Mari varieties, and it was determined that the application of 9 hours sulfuric acid + 1000 hours cold stratification on Mari variety seeds provided the highest germination rate. Additionally, the lowest germination number (1 seed) was observed in the group without any application. In the study, while the lowest values in terms of germination rate and root length were observed in the Control group, an increase in these characteristics was determined with the applied treatment.

Gözel (2006) found the highest germination rates in Kilis oily and Nizip oily olive variety seeds under protected cultivation in 10% H_2SO_4 (30 min) + 300 ppm GA_3 (24 hours), 10% H_2SO_4 (30 min) + 400 ppm GA_3 (24 hours), and Endocarp cracking applications. Abu-Qaoud (2005) obtained the highest germination percentage (60%) in Arbequina olive variety from 0.1 N H_2SO_4 application for 24 hours, 115 days after seed sowing. Hopkins and Hüner (2004) stated that treatments NaOH 1 N for 24 h dips, GA_3 for 24 h dips found with the best treatments for seed germination and at significant level. Halder et al. (2023) reported that in Indian olive (*Elaeocarpus floribundus*), maximum germination was recorded in the seeds treated with Benzyl Adenine 100 ppm followed by H_2SO_4 5%, whereas highest speed of germination (56.69%) was noticed in H_2SO_4 5% followed by Benzyl Adenine 100 ppm. The maximum average height of the seedlings (12.26 cm) and average number of leaves (6.86) was recorded in Benzyl Adenine treatment 100 ppm followed by H_2SO_4 5%. Completion of germination took about 150 days from seed sowing. Cytokinins regulate growth in various ways in different plants and affect germination rate (Graeber et al. 2012; El-Ghamery and Mousa, 2017). In the study, similar to the findings of the above researchers, germination was completed after 110 days. The average number of leaves was 10.96. Shoot lengths showed an increase with the applied treatments. The 2.5% Biotarm application showed the highest shoot length with 11.65 cm, while plants with the least shoot length were measured in the Control application (8.98 cm). While leaf number ratios did not show much variation between applications, 11.93 leaves were found in the Ayvalık olive group and 10.33 leaves in the wild seed group. This situation showed progress inversely proportional to shoot length depending on the growth rate of the varieties. In terms of Ayvalık variety characteristics, internodes are longer, while in wild seed plants, internodes are closer to each other.

When the germination percentages and root structures of Ayvalık olive variety and wild seeds were evaluated, it was determined that the control group without any application remained at a low level. It was determined that differences Biotarm doses in promoting germination were limited.

5. Conclusion

As a result of the study; germination first started in the seedling group and was detected on the 90th day after sowing. Germination was also observed in the Ayvalık variety within the following week. A total period of 200-210 days was required for seed germination to be completed and for the plants to be transferred into bags. The germination rate of wild seeds was found to be higher in all treatments compared to the Ayvalık variety and this was interpreted as the ability to show stronger emergence due to its wild nature. For this reason, root lengths and densities were also found to be higher. The highest germination rate was determined in the 2.5% Biotarm application (45%) and the lowest values in the Control treatment (31.99%).

It is planned to continue studies with applications involving harvesting at different maturity stages, storage in cold conditions at 5-10 °C, and sowing in seed and embryo forms. It was observed that Biotarm application increased germination rate by approximately 15% in olive seed sowing, and higher rates are expected with this application. In this way, the extent to which germination increases according to variety will be determined. Biotarm application also caused an increase in shoot and root length, along with increased root density. With the application, germination time was shortened and the obtained plants were found to be more vigorous. The high number of fibrous roots is also thought to provide convenience for soil attachment and easy root establishment.

Author Contributions

The percentages of the authors' contributions are presented below. All authors reviewed and approved the final version of the manuscript.

	C.D.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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