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Effects of Thyme Hydrolate on Rooting of Grapevine Cuttings

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Article Info	ABSTRACT
Received: 27.02.2025 Accepted: 29.05.2025 Published: 30.06.2025	Recently, the sustainability of agricultural production, influenced by intensive agricultural practices and climate change, has become increasingly important, particularly through the adoption of environmentally friendly methods. Researchers are showing growing interest in precision agriculture techniques, and sustainable farming practices are being actively developed. Phylloxera (Daktulosphaira vitifoliae Fitch) infestations have led to the widespread adoption of grafted vines as one of the most sustainable methods in viticulture. However, some grapevine rootstocks exhibit poor rooting and grafting success. To address this issue, synthetic chemicals are often used to enhance the rooting rate of cuttings. Nevertheless, the harmful effects of these chemicals on human health and the environment have increased interest in biostimulants in recent years. This study investigates the effects of thyme hydrolate on the rooting of grapevine cuttings. To this end, cuttings from the 41 B (V. vinifera x V. berlandieri) rootstock and the 'Narince' (V. vinifera L.) table grape variety were dipped in thyme hydrolate for three seconds before being planted in a rooting medium. The untreated cuttings served as the control group. Root number, root length (cm), fresh and dry root weights (g), shoot length (cm), and shoot diameter (mm) were measured. The results showed that thyme hydrolate exhibited the highest values for root length (24.67 cm), root number (12.67), root fresh weight (4.07 g), and root dry weight (0.70 g). In contrast, the control vines of the 'Narince' showed the greatest shoot length (25.67 cm) and shoot diameter (4.32 mm). Overall, the findings indicate that thyme hydrolate is a promising, environmentally friendly approach for enhancing root development in grapevine saplings.
Keywords: Biostimulant, Grapevine Rooting, Nursery Practices, Precision Agriculture, Thyme Hydrolate.	

Kekik Hidrolatının Asma Çeliklerinde Köklenmeye Etkileri

Makale Bilgisi	ÖZET
Geliş Tarihi: 27.02.2025 Kabul Tarihi: 29.05.2025 Yayın Tarihi: 30.06.2025	Yoğun tarım faaliyetleri ve iklim değişikliğinin etkisi altındaki tarımsal üretimin çevre dostu uygulamalarla sürdürülebilirliğinin sağlanması son yıllarda önem kazanmıştır. Bu bağlamda, araştırmacıların hassas tarım tekniklerine olan ilgisi her geçen gün artmakta ve sürdürülebilir tarım yöntemleri geliştirilmektedir. Sürdürülebilirlik, gelecek nesillerin ihtiyaç duyduğu kaynakların bulunabilirliğini ve kalitesini koruyarak mevcut nesillerin gereksinimlerini karşılamak anlamına gelir. Filoksera (Daktulosphaira vitifoliae Fitch) saldırıları nedeniyle, bağcılıkta en sürdürülebilir yöntemlerden biri aşılı asma kullanmaktır. Ancak bazı asma anaçlarının köklenme ve aşı tutma kabiliyeti zayıftır. Bu nedenle çeliklerin köklenme oranını artırmak amacıyla bazı sentetik kimyasallar kullanılmaktadır. Ancak sentetik kimyasalların insan sağlığı ve çevreye verdiği zararlar nedeniyle son yıllarda biyostimulantlara ilgi artmıştır. Bu çalışmada, kekik izolatının asma çeliklerinin köklenmesi üzerine etkileri belirlenmiştir. Bu amaçla 41 B (V. vinifera x V. berlandieri) asma anacı ile 'Narince' (V. vinifera L.) sofralık üzüm çeşidinin odun çelikleri kekik hidrolatına üçer saniye batırılarak köklendirme ortanına dikilmiştir. Herhangi bir uygulamanın yapılmadığı çelikler ise kontrol grubunu oluşturmuştur. Çeliklerde kök sayısı (adet), kök uzunluğu (cm), kök yaş ağırlığı (g) ve kök kuru ağırlığı (g), sürgün uzunluğu (cm) ve sürgün çapı (mm) belirlenmiştir. Elde edilen verilere göre, kekik hidrolatı uygulaması iki genotipte de kök sayısı, kök uzunluğu, kök yaşısı (12.67 adet), kök taze (4.07 g) ve kuru (0.70 g) ağırlıkları 'Narince' çeşidinde kekik hidrolatı uygulamasında belirlenmiştir. En yüksek sürgün uzunluğu (25.67 cm) ve çapı (4.32 mm) ise 'Narince' kontrol asmalarında bulunmuştur. Genel bulgular, asma fidanı köklendirmede çevre dostu uygulama olarak kekik hidrolatını örerebileceğini göstermiştir.
Anahtar Kelimeler: Asma Köklendirme, Biyostimulant, Fidanlık Uygulamaları, Hassas Tarım, Kekik Hidrolatı.	

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INTRODUCTION

Global grape production totals 74,942,572.95 tons, cultivated across 6,730,179 hectares of land. The largest producers are China (12,672,896.21 tons), Italy (8,437,970 tons), France (6,199,950 tons), Spain (5,902,040 tons), the United States (5,372,800 tons), and Turkey (4,165,000 tons) (FAO, 2024). However, as the global population continues to grow, food demand is rising, prompting the adoption of intensive agricultural practices to meet these increasing needs.

Various methods, including chemical treatments, cultural practices, and the use of resistant varieties, are employed to combat diseases and pests (Allam *et al.*, 2022). The intensive use of chemical applications to sustainably meet the nutritional demands of the rapidly growing global population has resulted in environmental pollution. Consequently, the growing pressures of intensive agricultural practices and the challenges posed by climate change are amplifying the significance of environmentally sustainable precision agriculture techniques as a means to ensure the continued viability of agricultural production (Gayretli *et al.*, 2023). However, despite the promising advancements in organic farming in recent years, the amount of organic production remains insufficient (Ağızan *et al.*, 2024). In this context, there is a growing scholarly interest in organic applications, and sustainable agricultural methods are being consistently developed.

Sustainability involves meeting the needs of the present generation while ensuring that the resources required by future generations remain available and of adequate quality. Factors such as the growing global population, technological advancements, urbanization, and industrialization contribute to an increase in greenhouse gas emissions, the depletion of water resources, land degradation, and various health issues (Akay and Demir, 2020). In agriculture, sustainability is further constrained by challenges such as soil degradation and water scarcity, which could lead to significant food shortages in the future (Muhammed Loay *et al.*, 2022).

The most sustainable approach to combating phylloxera (*Daktulosphaira vitifoliae* Fitch) infestations, a major challenge in viticulture, is the use of grafted grapevines. However, certain vine rootstocks exhibit low rooting and grafting success rates. As a result, synthetic chemicals are often employed to enhance the rooting success of cuttings.

Auxin-derived hormones, such as indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), and indole-3-acetic acid (IAA), are currently the most commonly used chemicals to promote rooting in cuttings (Estrella-Maldonado *et al.*, 2022; Sabir *et al.*, 2004; Salis *et al.*, 2017). However, growing concerns regarding the detrimental effects of synthetic chemicals on human health and the environment have led to a rise in interest in biostimulants in recent years.

In sustainable agriculture, minimizing the use of synthetic chemicals and adopting alternative products is an effective strategy for ensuring the renewal of natural resources (Küçük *et al.*, 2024; Özyiğit, 2021). Plant extracts, in particular, have attracted considerable research attention in recent years due to their rich content of bioactive compounds. These compounds typically include organic substances such as polyphenols, vitamins, plant hormones, and amino acids, as well as both macro- and microelements (Karaşahin, 2022). Plant extracts have demonstrated positive effects on various plant traits, including seed germination (Ben-Jabeur *et al.*, 2019), rooting of cuttings (Gayretli and Sabır, 2024), physiological (Lucini *et al.*, 2018) and vegetative (Chaouch *et al.*, 2023; El-Hefny and Hussien, 2025; Solgi *et al.*, 2025) properties. Thyme (*Thymus spp.* L.), a member of the Lamiaceae family, has been increasingly studied in recent years for its biostimulant properties (Ben-Jabeur *et al.*, 2015; Beni *et al.*, 2014; Bînzari *et al.*, 2022).

Water-soluble extracts of thyme are primarily composed of amino acids, aliphatic compounds, vitamins such as ascorbic acid (vitamin C), organic acids like citric and formic acids, carbohydrates including sucrose and glucose, as well as aromatic and phenolic compounds. Thyme oil and hydrolate have been applied to various plant species, where they have been shown to promote plant growth (Ben-Jabeur *et al.*, 2015; Ben-Jabeur *et al.*, 2019; Beni *et al.*, 2020; Chaouch *et al.*, 2023; Gayretli and Sabır, 2024; Karaşahin, 2022; Sokmen *et al.*, 2004).

Over the past 20 years, grapevine sapling production has doubled, increasing from 2 million to approximately 4 million. Similarly, the production of fruit saplings has experienced a significant rise, growing from 3 million to 112 million (BÜGEM, 2024). The overall findings will contribute not only to the production of grapevine saplings but also to the cultivation of other fruit saplings propagated through vegetative methods using organic practices.

This study investigates the effects of thyme hydrolate on the rooting of grapevine cuttings as an alternative to synthetic chemicals in grapevine sapling production. For this purpose, rootless cuttings of the 41 B (V. vinifera \times V. berlandieri) grapevine rootstock, known for its low rooting capacity, and the Narince (V. vinifera L.) table grape cultivar, a V. vinifera L. species with high rooting ability, were used.

MATERIALS AND METHODS

This study was carried out in 2024 at the Research and Application Greenhouse of the Department of Horticulture, Faculty of Agriculture, Selçuk University (38°01.814' N, 032°30.546' E, and an altitude of 1158 meters).

Material

In this study, two grapevine genotypes with varying rooting abilities (one with low rooting potential and the other with high rooting potential) were utilized to assess the effects of thyme hydrolate on rooting. For this purpose, rootless cuttings of 41 B (*V. vinifera* x *V. berlandieri*) grapevine rootstock and the 'Narince' (*V. vinifera* L.) table grape variety were pruned in March and stored under cold conditions (+4°C and 80% humidity) until the start of the experiment. Thyme hydrolate was obtained from *Thymus vulgaris*, a species within the *Thymus* genus. Sterile agricultural perlite (with particle sizes larger than 3 mm in diameter) was used as the rooting medium for the cuttings.

Research Design

In the experiment, the 'Narince' variety and 41 B rootstock were designated as the main plots. Each main plot was further divided into two subplots, representing the control and thyme hydrolate treatments. Each treatment was replicated three times, with 20 cuttings used in each replication.

Measurements and Analysis

The number of roots was determined by counting the primary roots that emerged from the basal part of the cuttings. The lengths of the roots originating from the basal portion of the cuttings were measured using a ruler, and the root length (cm) was recorded. The fresh weight of the roots was determined by cutting the cuttings at the root collar and weighing them (g). Subsequently, the roots were dried at 65°C for 48 hours, and their dry weight was measured (g). The length of the shoots on the cuttings was measured using a ruler (cm). The shoot diameter was determined (mm) using a digital caliper at the midpoint between the 2nd and 3rd nodes of the same shoots, measured in two directions (Gayretli and Sabir, 2024).

To obtain thyme hydrolate for the experiment, water and volatile oil were evaporated, concentrated in the cooler, and collected in a collection container (Kürkçüoğlu, 1995).

Statistical Analysis

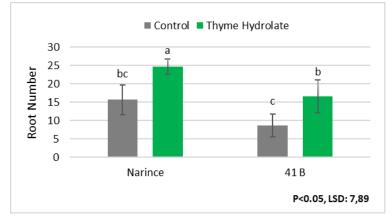
The data obtained were analyzed using the JMP statistical program (SAS Institute Inc., Cary, NC, USA, version 5.0.1). Significant differences between the means were determined using the LSD test at a significance level of P < 0.05, based on the genotype × treatment interaction.

RESULTS

Thyme hydrolate application significantly increased the number of roots in both grapevine genotypes (Fig. 1). The highest root count was observed in the 'Narince' with the thyme hydrolate treatment, followed by the TH application in the 41B rootstock (24.67 and 16.67, respectively). The lowest root number was recorded in the control group of the 41B rootstock (8.67). As anticipated, the 'Narince' variety exhibited a higher root count than the 41B rootstock in both treatments.

Figure 1

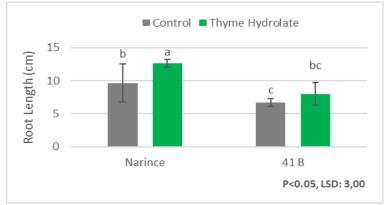
Effects of thyme hydrolate application on the root number of 41 B rootstock and Narince variety cuttings. Mean values with different letters indicate statistically significant differences (P < 0.05).



Thyme hydrolate significantly influenced the root length of the grapevine genotypes, as shown in Figure 2. The longest roots were observed in the thyme hydrolate-treated 'Narince' variety (12.67 cm), while the shortest roots were found in the control treatment of the 41B rootstock (6.67 cm). The positive effects of thyme hydrolate on both root length and root number were evident.

Figure 2

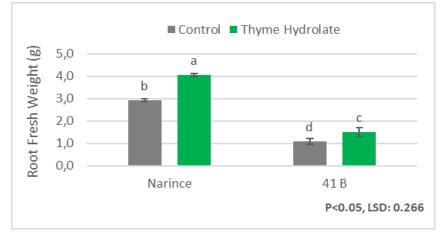
*Effects of thyme hydrolate application on root length of 41 B rootstock and Narince variety cuttings. Mean values with different letters indicate statistically significant differences (*P<0.05)*.*



Thyme hydrolate application significantly increased the fresh weight of the roots (Fig. 3). The highest root fresh weight was observed in the thyme hydrolate-treated 'Narince' variety (4.07 g). In contrast, the lowest fresh weights were recorded in the control (1.10 g) and thyme hydrolate (1.50 g) treatments of the 41B rootstock, respectively.

Figure 3

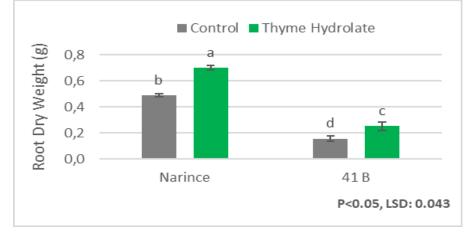
Effects of thyme hydrolate application on root fresh weight of 41 B rootstock and Narince variety cuttings. Mean values with different letters indicate statistically significant differences (P < 0.05).



As shown in Figure 4, thyme hydrolate application had a significant effect on root dry weight. The highest root dry weight was observed in the thyme hydrolate-treated 'Narince' variety (0.70 g), while the lowest values were recorded in the control treatment of the 41B rootstock (0.16 g). Thyme hydrolate application increased dry matter accumulation in the roots of both genotypes.

Figure 4

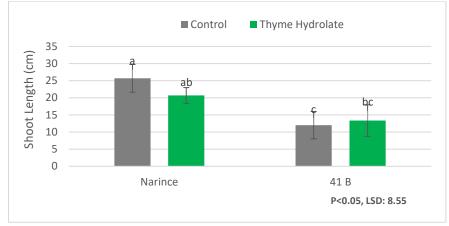
Effects of thyme hydrolate application on root dry weight in cuttings of 41 B rootstock and Narince variety. Mean values with different letters indicate statistically significant differences (P < 0.05).



Statistically significant differences were observed in the response of shoot length to thyme hydrolate treatment (Fig. 5). The longest shoot length was recorded in the control (25.67 cm) and thyme hydrolate (20.67 cm) treatments for the 'Narince' variety. The shortest shoot length was observed in the control treatment of the 41B rootstock (12 cm). The reduction in shoot length due to thyme hydrolate application in the 'Narince' variety was particularly notable. Furthermore, while thyme hydrolate had contrasting effects on shoot length and root number in the 'Narince' variety, this effect was not observed in the 41B rootstock

Figure 5

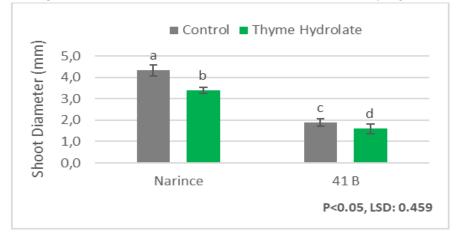
Effects of thyme hydrolate application on shoot length in cuttings of 41 B rootstock and Narince variety. Mean values with different letters indicate statistically significant differences (P<0.05).



Thyme hydrolate application significantly reduced the shoot diameter of the genotypes (Fig. 6). The largest shoot diameter was recorded in the control vines of the 'Narince' variety (4.32 mm), followed by the thyme hydrolate treatment (3.39 mm). The smallest shoot diameter was observed in the thyme hydrolate-treated 41 B rootstock (1.59 mm). Interestingly, thyme hydrolate application decreased shoot diameter in both genotypes, which contrasts with its positive effects on rooting parameters.

Figure 6

Effects of thyme hydrolate application on shoot diameter of 41 B rootstock and Narince variety cuttings. Mean values with different letters indicate statistically significant differences (P<0.05).



DISCUSSION

Plant extracts possess biostimulant properties due to the bioactive compounds they contain, including hormones, vitamins, and macro- and micronutrient elements (Karaşahin, 2022). In addition to their applications in human (Alp, 2020; Gökbaş, 2021) and animal health (Çölçimen *et al.*, 2020), plant extracts have recently been increasingly utilized as biostimulants to ensure sustainability in agriculture. This has led to extensive research into their practical use in agricultural practices (Godlewska *et al.*, 2021). Previous studies have demonstrated that plant extracts impact the levels of polyphenols, hormones (Lucini *et al.*, 2018), organic acids and sugars (Abou Chehade *et al.*, 2018). These primary and secondary metabolites regulate essential biological activities that influence both physiological responses (Barrajón-Catalán *et al.*, 2014) and phenotype of plants (Godlewska *et al.*, 2021).

Posmyk and Szafrańska (2016) emphasized that plant extracts function differently from fertilizers

or phytohormones, possessing biostimulant properties that promote plant growth. The use of natural, readily available, and cost-effective resources in plant cultivation is essential (Jang and Kuk, 2019). In this context, plant extracts hold significant potential for supporting sustainable agriculture.

The biostimulant effects of plant extracts have been studied across various plant species. For instance, a 5% root extract of *Gypsophila perfoliata* L. was found to enhance both root and shoot lengths in Hungarian vetch (Işık *et al.*, 2024). Mallow extract has been shown to increase plant height and fresh weight in lavender, as reported by (El-Hefny and Hussien, 2025). Furthermore, extract derived from the zahter plant (at concentrations of 10% and 20%) enhanced root fresh weight, root dry weight, root length, and plant height in rosemary (Solgi *et al.*, 2025). Thyme oil was found to enhance antioxidant enzyme activity and improve resistance to anthracnose disease in avocados (Bill *et al.*, 2014). Additionally, Sabır (2024) highlighted that plant oil extracts significantly enhanced callus formation and shoot growth at the grafting point of grapevines.

In grapevines, it has been reported that fenugreek (*Trigonella foenum-graecum* L.) extract increases the mineral content in grapevine leaves (Ebrahim, 2017). Soybean (*Glycine max*) extract has been shown to reduce the symptoms of downy mildew disease in grapevines through its antimicrobial effect (Lachhab *et al.*, 2014). Additionally, different doses of thyme hydrolate have been found to promote root and shoot development in grapevines (Gayretli and Sabır, 2024), while thyme oil reduces weight loss in grapes during storage (Abdolahi *et al.*, 2010). The literature review revealed a lack of sufficient studies on the effects of plant extracts on grapevines. Furthermore, research investigating the biostimulant properties of thyme remains limited.

Thyme extracts are particularly rich in ascorbic acid and various amino acids, both of which are known to exhibit biostimulant effects (Posmyk and Szafrańska, 2016). Additionally, thyme extracts may contain aliphatic compounds, vitamins, organic acids, carbohydrates, aromatic and phenolic compounds, as well as essential elements such as Fe, Cu, Zn, Mn, B, and Al (Broadley *et al.*, 2012). These components enable thyme extracts to activate the enzymatic systems within plant cells. Notably, compounds like carvacrol, eugenol, and thymol possess antimicrobial properties and play a role in enhancing plant defense mechanisms against pathogenic microorganisms (Gurjar *et al.*, 2012).

Thyme extracts act as biostimulants, protecting plants from oxidative stress through their nutrient content and antioxidant properties. Oxidative stress arises from the accumulation of reactive oxygen and nitrogen species, including hydrogen peroxide (Şahin, 2017), singlet oxygen (Lal *et al.*, 2019), hydroxyl (Karabulut and Gülay, 2016), superoxide (Mittler *et al.*, 2022), and peroxynitrite (Can, 2015). Oxidative stress results from an imbalance between reactive oxygen species and antioxidants in living organisms. This imbalance can give rise to cellular damage, affecting proteins, lipids, and DNA by disrupting normal cellular functions. (Temiz and Daye, 2024).

Reactive oxygen species lead to the inactivation of genes involved in polar auxin transport and auxin signaling. Furthermore, it has been suggested that thyme oil, owing to its antioxidant properties, can help protect hormonal functions (Iglesias *et al.*, 2010). The role of thyme oil in protecting phytohormones can be attributed to its high content of natural phenolic compounds, such as δ -terpinene and carvacrol, which are the primary bioactive components in the essential oils of the *Lamiaceae* family (Tohidi *et al.*, 2017). Dash *et al.* (2021) reported that carvacrol, a key component in thyme extract, is the most effective antioxidant, and it also possesses antibacterial, antimicrobial, antifungal, and antiviral properties. According to Bînzari *et al.* (2022), thyme oil does not inhibit cell proliferation in root meristems.

Ben-Jabeur *et al.* (2019) found that in wheat seeds coated with thyme oil, the levels of ABA decreased, while the levels of phenolic compounds increased, thereby promoting rooting. It has been

reported that polyphenols prevent the enzymatic degradation of endogenous auxin during the rooting process and act as cofactors for auxin (James and Thurbon, 1981).

Thyme oil was applied to the root medium of tomatoes grown in a hydroponic system, and after two days, the accumulation of phenolic compounds in the roots and, after five days, in the leaves was found to be higher compared to the control plants (Ben-Jabeur *et al.*, 2015). The researchers also reported that thyme oil enhanced peroxidase enzyme activity.

Although it has not been conclusively demonstrated that thyme oil exerts effects identical to those of phytohormones, it interacts with plant receptors or signaling pathways, eliciting responses comparable to those triggered by natural phytohormones. The application of thyme oil to strawberries has been shown to enhance shoot length, increase root number, promote root elongation, and facilitate earlier rooting (Chaouch *et al.*, 2023).

Based on this information, it is suggested that the components present in the thyme hydrolate used in our study exhibited biostimulant and nutritional effects, minimizing cellular damage during the rooting phase due to its antioxidant properties, and promoting root and shoot development in the cuttings. In the present study, the root number, root length, and both fresh and dry root weights were found to be lower in the 41 B grapevine rootstock compared to the 'Narince' variety. It is well-known that the 41 B rootstock has a weak rooting ability, a characteristic inherited from one of its parent species, *V. berlandieri*. Consequently, a lower rooting rate is expected in the 41 B rootstock. In contrast, the 'Narince' variety exhibits a high rooting ability, as it belongs to the *V. vinifera* species. Our results confirm this expectation.

Gayretli and Sabır (2024) investigated the effects of different doses of thyme hydrolate on rooting in grapevine cuttings. Their study found that thyme hydrolate increased both root fresh and dry weights in the 'Ekşi Kara' variety. However, the same researchers reported that doses of 60% and 90% of thyme hydrolate negatively affected shoot length and diameter in the 110 R rootstock.

In this study, the application of thyme hydrolate increased root number, root length, and both root fresh and dry weights, while it decreased shoot diameter in both genotypes. These results are consistent with the findings of Gayretli and Sabir (2024). Additionally, it is believed that thyme isolate may have stimulated auxin production and, consequently, enhanced rooting by reducing ABA accumulation in the cuttings, as reported by Ben-Jabeur *et al.* (2019) and Chaouch *et al.* (2023), and increasing the concentration of phenolic compounds (Ben-Jabeur *et al.*, 2015).

CONCLUSION AND SUGGESTIONS

Environmental sustainability and resource management are among the most pressing issues in today's world. Sustainable agriculture seeks to reduce the reliance on chemical fertilizers and pesticides, which cause irreversible harm to human health and the environment. As a result, there has been a growing interest in research on alternative and sustainable production methods.

In the present study, thyme hydrolate likely inhibited ABA synthesis, promoted auxin production, and facilitated the protection of the produced auxin, possibly due to its bioactive compounds and antioxidants such as phenolic acids and flavanols, as reported in the literature. As a result, thyme hydrolate led to an increase in the root number of cuttings. Additionally, it had a positive effect on both root fresh and dry weight. Therefore, thyme hydrolate can be recommended as an environmentally friendly, sustainable approach for enhancing rooting in grapevine sapling production.

Further studies are needed to investigate the effects of different doses of thyme hydrolate on both rooting and graft retention across various genotypes. These studies will offer more comprehensive insights into the use of environmentally friendly, biostimulant plant extracts. Proper application of plant

extracts, particularly in *V. berlandieri* hybrids (such as 41B and 110 R) grapevine rootstocks, could enhance rooting and graft success. Furthermore, it has been concluded that thyme hydrolate can be utilized in organic sapling production through organic methods. Therefore, thyme hydrolate presents a valuable alternative biostimulant for environmentally friendly, cost-effective, and sustainable agriculture by reducing the reliance on synthetic chemicals in grapevine rooting.

The results of this study could contribute not only to grapevine sapling production but also to the organic cultivation of other fruit saplings propagated vegetatively.

Author Contributions

Design: Yazar 1 (%100) Data Collection: Yazar 1 (%100) Analysis or Interpretation: Yazar 1 (%100) Literature Search: Yazar 1 (%100) Writing: Yazar 1 (%100)

Funding

No.

Conflict of Interest

The authors have declared no conflict of interest.

Sustainable Development Goals (SDG)

Sustainable Development Goals: 3 Health and Quality of Life Sustainable Development Goals: 6 Clean Water and Sanitation Sustainable Development Goals: 12 Responsible Production and Consumption Sustainable Development Goals: 13 Climate Action

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