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Determination of the antibacterial effect of rose oil on watermelon bacterial fruit spot (*Acidovorax citrulli* (Schaad et al.) Schaad et al.) and its impact on watermelon seed applications

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Abstract

Rose oil, a natural product with a long history in cosmetics, has potent antimicrobial, antiseptic, antiparasitic, and antibacterial properties. A study was conducted using rose oil obtained from roses grown in the Isparta province of Turkey. The oil was extracted using the Clevenger hydrodistillation method and analyzed using Gas Chromatography Mass Selective Detector (GC-MSD). The study investigated the antibacterial effects of different concentrations of rose oil (1, 5, 10, 20, 30, 40, 50, and 100 ppm) against *Acidovorax citrulli* (*Ac*) using *in vitro* both the paper disk diffusion and the volatile effect methods. Thyme oil (*Thymbra spicata*) (100 ppm) and the antibiotic streptomycin (100 ppm) were also tested for comparison. Watermelon seeds were treated with various doses of rose oil. The study found that 50 ppm of rose oil was the most effective dose *in vitro* and completely prevented the development of *Ac*.and did not negatively affect the germination of watermelon seeds. Analysis of the rose oil contents confirmed that citronellol and geraniol, the main ingredients of rose oil, contribute to its antibacterial effect against *Ac*. In conclusion, the study suggests that rose oil has the potential to be used as a natural seed protectant against the seed-borne bacterial pathogen *Ac*.

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Gül (*Rosa damascena* Mill.) yağının karpuz bakteriyel meyve lekesi etmenine (*Acidovorax citrulli* (Schaad et al.) Schaad et al.) karşı olan antibakteriyel etkisi ile karpuz tohum uygulamalarındaki antibakteriyel etkisinin belirlenmesi

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Öz

Gül yağı uzun yıllardır kozmetik alanında kullanım alanına sahip doğal bir ürün olup aynı zamanda güçlü bir antimikrobiyal, antiseptik, antiparasitik ve antibakteriyel özelliklere sahip olan bir üründür. Bu çalışmada, Türkiye'de Isparta ilinde yetiştirilen gül petallerinden elde edilen gül yağı Clevenger hidrodistilasyon yöntemiyle elde edilmiş ve içerikleri GC-MSD (Gaz Kromatografi Kütle Seçici Dedektör) yöntemiyle analiz edilmiştir. Gül yağının farklı dozlarının (1, 5, 10, 20, 30, 40, 50 ve 100 µL/mL) antibakteriyel etkisi *Acidovorax citrulli* (*Ac*)' ye karşı *in vitro*'da kağıt disk difüzyon ve uçucu etki belirleme yöntemleriyle test edilmiştir. Gül yağının etkisini karşılaştırmak için kekik yağı (*Thymbra spicata*) (100 µL/mL) ile streptomycin (100 ug/mL) antibiyotiği kullanılmıştır. Ayrıca, gül yağının farklı dozları karpuz tohumları ile muamele edilmiştir. Yapılan çalışma sonuçlarına göre *in vitro*'da 50 µL/mL'lik dozun en etkili doz olduğu tespit edilmiştir. Yine 50 µL/mL'lik dozun karpuz tohumları ile yapılan çalışmada *Ac*' nin gelişimini tamamen engellediği ve karpuz tohumlarının çimlenmesi üzerine de olumsuz bir etkiye sahip olmadığı belirlenmiştir. Elde edilen gül yağının içerikleri GC-MSD analizi ile tespit edilmiştir. Sonuç olarak, tohumla taşınan bakteriyel patojen *Ac*' ye karşı gül yağının doğal bir tohum koruyucu olarak kullanılabilme potansiyeline sahip olduğunu ve gül yağının antibakteriyel etkisinin gül yağının ana maddesi olan citronellol ve geroniol'den kaynaklandığı düşünülmektedir.

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Introduction

Rose is a significant plant in culture, cultivated as an ornamental plant, for cut flowers, and for rose oil. There are several rose varieties used in commercial production of rose oil worldwide. These include "*Rosa damascena* Mill", "*Rosa gallica* L.", "*Rosa alba* L.", "*Rosa centifolia* L.", "*Rosa moschata* Herrm", and "*Rosa rugosa* L.". Among these, *Rosa damascena* Mill is the most preferred and most fragrant variety, from which the highest quality rose oil can be obtained. It is also known as "Isparta rose", "Kazanlık rose", "Damascus rose", "Pink oil rose", or simply "Oil Rose". Rose oil, obtained from the oil rose, is one of the most valuable and expensive essential oils in the global market (Kovatcheva et al. 2010, 2011; Topalov, 1978)

An average of 15-16 thousand tons of rose oil is produced annually in the world. Approximately 90% of the world's rose oil production is met by Turkey and Bulgaria. In addition, rose oil is also partially produced in Morocco, Iran, Afghanistan, India, China, Caucasian countries, Arabia, and Northern block countries. These countries can meet at most 10% of the world demand (Sharma and Kumar, 2016; Krussman, 1981). Turkey and Bulgaria are the leading countries in the market, producing approximately 80-90 % of rose oil (Kovatcheva et al. 2010).

One of the most important raw materials in the perfume and cosmetic industry is rose oil. Additionally, it is used in the pharmaceutical industry and food industry, including products such as liquor, cake, chewing gum, Turkish delight, jam, and additives due to its antibacterial and antioxidant properties. Rose oil is also utilized as a fragrance in soaps, detergents, shampoos, and toothpastes, and as an antiseptic raw material in medicine (Kıneci, 2005; Gökdoğan, 2011; Örmeci Kart et al., 2012). Moreover, in addition to the perfumery industry, rose oil is also used in various cosmetic fields such as rose cream and rose lotion (Dağlı, 2019).

Turkey, being one of the leading countries in the world rose products market, had an average export value of rose products of approximately 12.6 million Euros between 2016 and 2020 (Bıtrak and Hatırlı, 2022).

In order to obtain 1 kg of rose oil, 3-3.5 tons of fresh rose flowers are distilled, with an average essential oil yield of 0.028-0.033%. Additionally, by extracting 300 kg of fresh rose flowers with n-hexane, 1 kg of concrete is obtained with an average concrete yield of 0.30% (Tüik, 2022). The main fragrance components in rose oils consist of 70-85% monoterpene alcohols and 15-30% paraffins and stearoptenes. The key elements determining the quality of rose oil are monoterpene alcohols such as citronellol (25-60%), geraniol (6-26%), and nerol (3-12%) (Anaç, 1984; Başer, 1992; Bayrak and Akgül, 1994; Başer et al., 2003).
Watermelon and melon are highly demanded products in cucurbit production. Turkey ranks second after China in watermelon and melon production globally (Turkstat, 2024).

Essential oils have been widely used in various areas such as food, pharmacy, perfume, cosmetics, and in numerous studies since the 1980s due to their antimicrobial effects (Deans and Svoboda, 1990). In recent years, essential oils have found application in agriculture and the food industry due to their bactericidal, fungicidal, antiparasitic, and insecticidal properties, as well as in medical and cosmetic applications (Barata et al., 1998). It is widely acknowledged that essential oils offer more advantages than synthetic pesticides as they are environmentally friendly and do not possess residual and phytotoxic properties (Badei et al., 1996; Bishop and Thorton, 1997). The Mediterranean region significantly contributes to greenhouse watermelon and melon production in Turkey, with the Antalya province

playing a crucial role in greenhouse watermelon production (82,117 tons) and melon production (38,236 tons) (Tüik, 2023).

Product losses due to diseases and pests are a significant issue in Turkey and worldwide, particularly in the watermelon industry. Various diseases can lead to substantial losses in watermelon crops, especially during the plant's growth stages. The Watermelon Bacterial Fruit Spot disease, caused by *Acidovorax citrulli* (*Ac*) (Schaad et al., 2008), is a bacterial infection that reduces both the market value and yield of watermelon. *Ac* is responsible for epidemics in numerous countries and is included on the quarantine list. It is a seed-borne pathogen, primarily transmitted through contaminated seeds, which are the main source of infection for the spread of *Ac*. This pathogen causes diseases in many hosts of the Cucurbitaceae family, particularly watermelon, during the transfer from seed to seedling (Hopkins and Thompson, 2002). Watermelon seedlings grown from contaminated seeds can display symptoms of watermelon bacterial fruit spot 6 to 10 days after germination. High temperature, relative humidity, and severe seed contamination contribute to the rapid spread of *Ac* (Burdman and Walcott, 2012).

The following methods are used to combat the disease: various cultural measures, use of certified seeds, different chemical and biological applications to seeds, use of resistant varieties, spraying of green parts, and soil solarization. However, the disease lacks an effective chemical control, causes economic damage to the fruit, is carried by seeds, and is on the quarantine list. This highlights the importance of combating the disease. In this study, we tested the antibacterial effects of different doses (1, 5, 10, 20, 30, 40, 50, and 100 µL/mL) of rose oil against the watermelon bacterial fruit spot agent, *Ac*, using disc diffusion and vapor effect methods. We aimed to determine the potential of rose oil to be used as a natural seed protectant.

Material and Method

Plant Material

Rosa damascena Mill, also known as Isparta Rose, Damascus Rose, and Pink Oil Rose, is grown as plant material in the province of Isparta. It is collected from rose gardens in the early morning hours, typically between 7:30-8:00 on June in 2023 year. The rose flowers are used (Figure 1).

Figure 1. Isparta Rose, Damascus Rose, Pink Oil Rose (*Rosa damascena* Mill.)

Extraction of Rose Oil

To obtain rose oil, 1 kg of fresh rose flowers were put into a 5 L flask with a Clevenger hydro-distillation unit, and 3 L of pure water was added (Figure-2A). Distillation was carried out for 6 hours after the water in the distillation flask started to boil (Figure-2B). At the end of distillation, the rose oil that

accumulated on the water and the rose water that accumulated under the oil were collected. Petroleum ether was added twice, and the extract was separated from the water. Petroleum ether in the extract was removed with a rotary evaporator at 45°C. Finally, the oils were stored in the refrigerator at $+4^0$C, protected from light until used.

Figure 2. (A) Rose plant **(B)** Clevenger tool used to obtain rose oil

Determination of the Content of Rose Oil by GC-MSD

1 µL of a sample from the upper phase containing rose essential oil was injected into the GC/MSD device, and the results were analyzed using Gas Chromatography-Mass Selective Detector (GC-MSD).(Agilent, Hewlett Packard) (GC 7980 - MSD 5975). The following parameters used in the analysis:
- Heating rate: 70°C/min up to 250°C for 10 minutes
- Injection volume: 1 µl
- Inlet type: S/SL
- Split ratio: 1:50
- Column: Agilent 19091N-116, 260°C, dimensions 60 m x 320 mm x 0.25 mm
- MS source temperature: 230°C
- MS Quad temperature: 150°C
- Constant flow: 0.70 ml/min
- Helium was used as the carrier gas at a flow rate of 0.7 ml/min.

The analysis conditions were set according to Basım and Basım (2018). Essential oil components were analyzed using the ChemStation program and NIST 0.5 AL.

Bacterial Culture

The plant pathogenic bacterium *Acidovorax citrulli* (*Ac*)used in the study was obtained from the culture collection of Akdeniz University, Faculty of Agriculture, Department of Plant Protection. The most virulent strain of *Ac*, which had been stored in culture stocks in 2 ml sterile tubes with screw caps at -80°C, was used in the study.

Paper Disc Diffusion Method

The bacterial stock culture was shaken in Nutrient Broth (Acumedia Manufacturers, Inc., Maryland, USA) medium at 27°C in a shaker for 24 hours. The bacterial cell concentration was adjusted to 10^8 cfu/ml. Then, 100 µl of this bacterial suspension was taken and inoculated into 8 cm diameter petri dishes containing Nutrient Agar (Merck) (NA) medium. To determine the antibacterial activity of rose oil, the paper disc diffusion method was used. Sterilized paper discs (5 mm) (Schleichen Schuell #5891) were placed at different distances from each other in petri dishes inoculated with bacteria. Different doses of rose oil (1, 5, 10, 20, 30, 40, 50, 100 µL /mL) were tested against *Ac*. Sterile distilled water was used as a control. Additionally, the effectiveness of rose oil was compared with *Thymbra spicata* essential oil (100 µL/mL) and Streptomycin (100 µL/mL). The petri dishes were then incubated at 27°C for 48. After 48 hours, the inhibition zones formed around the paper discs were measured (Basım and Basım, 2000, Basım and Basım, 2003, 2004). The tests were conducted in three repetitions in a completely randomized plot design.

Determination of the volatile effect of rose oil

The bacterial stock culture was shaken in Nutrient Broth (Acumedia Manufacturers, Inc., Maryland, USA) medium at 27°C in a shaker for 24 hours. The bacterial cell concentration was adjusted to 10^8 cfu/ml. Next, 100 µl of this bacterial suspension diluted 10^{-4} was taken and inoculated into 8 cm diameter petri dishes containing NA (Nutrient Agar) medium. The bacteria were spread on the medium using a sterile glass spreader. After inoculation, the media were left to dry for 15-20 minutes. Then, different doses (1, 5, 10, 20, 30, 40, 50, 100 µL/mL) of rose oil were applied to the lids to detect the vapor upside down. After the applications, the lids of the petri dishes were covered with transparent parafilm and incubated at 27°C for 48 hours (Basım and Basım, 2000, Basım and Basım, 2003, 2004). Only sterile water was added to the lid of the control petri dishes. *T. spicata* essential oil (100 µL/mL) was used for comparison.

Seed inoculation of A. citrulli and determination of the antibacterial effect of rose oil

One hundred watermelon seeds were placed in sterile 100 cm3 glass jars. They were treated with 1ml of $1x10^8$ cfu/ml *Ac*. Essential rose oils at concentrations of 1, 5, 10, 20, 30, 40, 50, and 100 µL/mL were placed on the inner central surface of the sterile lids of the glass jars. The lids were then closed, the surroundings were covered with parafilm, and the jars were incubated at 27°C for 48 hours. After 48 hours, isolations were made from the watermelon seeds taken from the jars, and it was evaluated whether the bacteria grew on the Nutrient Agar medium. Sterile water was applied to control jars.

Statistical Analysis

Standard analysis of variance (ANOVA) was carried out using the statistical computer software program SPSS 10.0. Significance was determined according to Duncan's multiple range test ($p < 0.01$).

Results and Discussion

Determination of the Content of Rose Oil by GC-MSD

The main components of rose oil are listed in Table 1. The major compounds in the rose oil include Citronellol (33.47%), Geraniol (18.87%), Nerol (11.14%), Eugenol (2.04%), Linalool (0.51%), beta-pinene (0.31%), and trans-rose oxide (0.13%). Cutler (2003) found similar results in their studies,

identifying the principal constituents of the rose oil as citronellol (34–55%), geraniol (around 14%), and nerol (around 7%). Rose oil has a complex composition, containing over 200 components from different classes of chemical compounds, which contributes to its multifaceted biological effects (Gochev et al., 2010). Although the main ingredients of rose oil have been determined in many studies, the ratios of oil contents may vary based on factors such as the plant's harvest time, climatic data, geographical location, environmental factors, extraction method, and extraction conditions (Yeşil Çeliktaş et al., 2007). Rose oil has been claimed to have various medicinal properties, including anti-inflammatory, anti-infective, and wound healing activities, and has been used for relieving headache, hemorrhoids, inflammatory conditions of the gastrointestinal tract, and muscular pain (Mohebitabar et al., 2017). The therapeutic efficacy of rose oils and extracts, as well as their ingredients, encompasses antidepressant effects, psychological relaxation, improvement of sexual dysfunction, antioxidant, antimicrobial, antifungal, probiotic, and antipyretic effects, smooth muscle relaxation, lipid-lowering content, and antiulcerogenic effects (Mahboubi 2016; Gochev et al., 2010; Erdoğan et al., 2017).

Table 1. ***Mainly components of rose oil detected by GC-MSD (%)***

Chemical components	RI	(%)
Citronellol	1550	33.47
Geraniol	1420	18.87
Nerol	1300	11.14
Eugenol	1275	2.04
Linalool	1100	0.51
β-pinene	980	0.31
Trans-rose oxide	825	0.13

RI: Retention index

The study found that the 50 μL/mL dose of rose (*Rosa damascena* Mill.) oil was the most effective in combating bacteria (refer to Table 2). When comparing the volatile effect of rose essential oil against *Ac* to the control, the 1 ppm dose was found to be ineffective, while the 5, 10, 20, 30, and 40 μL/mL doses were effective. On the other hand, the 50 and 100 μL/mL doses were found to be the most effective against *Ac*.

Table 2. **Inhibition zones of different doses of rose oil against *Acidovorax citrulli***

Treatments	Doses (μL/mL)	Inhibition zones* (mm)
Control (Sterile water)	-	0
Rose oil	1	0 a
Rose oil	5	10 b
Rose oil	10	16 c
Rose oil	20	24 d
Rose oil	30	27 d
Rose oil	40	30 e
Rose oil	50	34 e
Rose oil	100	36 f
Tymbra spicata	100	35 e
Streptomycin	100	15 c

* Values expressed are mean of the three replicates. Values given separately for rose oil within each row followed by different letters are significantly different at $p < 0.01$.

Table 3. Volatile effect of different concentrations of rose oil against *Ac*

Treatments	Doses (μL/mL)	Number of colonies* (cfu/ml)	Inhibition Rate (%)
Control (Sterile water)	-	1650 a	00.00
Rose oil	1	995 b	39.70
Rose oil	5	725 c	56.06
Rose oil	10	436 d	73.58
Rose oil	20	278 e	83.15
Rose oil	30	97 f	94.12
Rose oil	40	43 g	97.39
Rose oil	50	5 h	99.70
Rose oil	100	0 i	100.00
Tymbra spicata	100	180 e	89.09

*Values expressed are mean of the three replicates, * Values given separately for rose oil within each row followed by different letters are significantly different at $p < 0.01$.

When the volatile effect of rose essential oil against *Ac* bacteria was compared to the control group, it was observed that the 1 ppm dose was ineffective, while doses of 5, 10, 20, 30, and 40 μL/mL were effective. On the other hand, doses of 50 and 100 μL/mL were found to be the most effective against the tested *Ac* bacteria (refer to Table 3). The essential oil of *T. spicata* at a concentration of 100 μL/mL exhibited a strong antibacterial effect. It's noteworthy that rose oil at a concentration of 100 μL/mL demonstrated a stronger antibacterial effect than both streptomycin and *T. spicata* (as shown in Table 2 and Table 3).

The primary components of rose oil were determined to be Citronellol (33.47%), Geraniol (18.87%), Nerol (11.14%), Eugenol (2.04%), Linalool (0.51%), beta-pinene (0.31%), and trans-rose oxide (0.13%) based on Gas Chromatography-Mass Selective Detector (GC-MSD) analysis.

Additionally, it was revealed that rose oil did not have a detrimental effect on the germination of watermelon seeds (refer to Table 4). These results indicate that rose oil has the potential to be utilized as a natural seed protectant against the seed-borne bacterial pathogen *Ac.*

Table 4. Antibacterial effects of different concentrations of rose oil on watermelon seeds

Treatments	Doses (μL/mL)	Germination rate* (%)
Control (Sterile water)	-	100
Rose oil	1	97
Rose oil	5	97
Rose oil	10	96
Rose oil	20	95
Rose oil	30	93
Rose oil	40	91
Rose oil	50	90
Rose oil	100	0
Tymbra spicata	100	0
Streptomycin	100	97

*Values expressed are mean of the three replicates

The essential oil of the rose plant can be an important part of Integrated Pest Management for controlling bacterial seed disease. No symptoms of phytotoxicity on watermelon seeds were observed with the use of rose oil from the *R. damascena* plant. It is believed to be safe for the environment and humans because it is commonly used in cosmetic products. Currently, the rose oil from *R. damascena* is highly valued in the cosmetics industry. These findings support the practical use of *R. damascena* essential oil as a natural bactericide for controlling watermelon bacterial fruit spot disease caused by certain bacteria, as well as other diseases caused by various plant pathogenic bacteria (Basım and Basım, 2018, 2012, 2004, 2003, 2001, 2000; Basım, 2012). Research has shown that rose oil has a significant antibacterial and antifungal effect on economically important plant pathogenic bacteria and fungi

The results may contribute to develop effective and environmentally friendly control agents for plant disease control. Rose oil as a potential natural bactericide and fungicide for plant bacterial and fungal disease control in agriculture.

Large amounts of rose material are necessary for rose oil extraction, making it a very expensive essential oil. Therefore, using rose oil as an alternative to pesticides for controlling bacterial and fungal diseases is not feasible, as it would significantly increase agricultural inputs. However, citronellol and geraniol, two important components of rose oil, have significant control over bacterial diseases. These compounds can be included in plant disease control programs as they can be synthetically produced. Citronellol and geraniol are the major compounds of *R. damascena* oil, and they can be obtained from different plant species such as *Cymbopogon nardus* and *Pelargonium geranium* or be synthetically produced ((Jain et al., 2001; Mahalwal and Ali, 2002). Additionally, *R. damascena* has several pharmacological properties including anti-HIV, antibacterial, antioxidant, antitussive, hypnotic, antidiabetic, and a relaxing effect on tracheal chains (Boskabady et al., 2011, Yan et al. 2023, Lee et al. 2023, Antoniadou et al., 2024).

Conclusions

In this study, rose oil has demonstrated antimicrobial activity against *Ac*. The main components of rose oil, such as citronellal, geraniol, and nerol, have previously shown antibacterial effects. Rose oil is a volatile oil obtained through the distillation of fresh flowers of *R. damascena*. The identified compounds in rose oil include citronellol (33.47%), geraniol (18.87%), nerol (11.14%), eugenol (2.04%), linalool (0.51%), beta-pinene (0.31%), and trans-rose oxide (0.13%) as its major components. *In vitro* antibacterial activities of the essential oil from *R. damascena* have been demonstrated through disc

diffusion and volatile effect testing against *Ac*. Our work has also shown that *R. damascena* exhibits antibacterial activity against *Ac*. The results suggest that essential oils, particularly thyme and rose oils, have the potential for use as antimicrobials. This study has found that plant essential oils may provide alternative treatment options and may be used for the elimination of certain bacteria.

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