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## Effect of Lavender Essential Oil, Potassium phosphite and Potassium Silicate on Antioxidant Enzyme Activity and Gene Expression in Almond Plants Infected with *Pseudomonas syringae* pv. *syringae*

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### ABSTRACT

**Objective:** This study aimed to investigate the effects of lavender essential oil, potassium silicate, and potassium silicate on the activity of antioxidant enzymes (catalase, and polyphenol oxidase), and the expression of genes associated with these enzymes in almond seedlings infected with *Pseudomonas syringae* pv. *syringae*, the causal agent of bacterial canker in almonds. **Materials and Methods:** Almond leaves were treated with different concentrations of lavender essential oil (500, 1000, and 1500 ppm), potassium phosphite, and potassium silicate. The activity of catalase, and polyphenol oxidase enzymes, as well as the expression of genes related to these enzymes, were measured at various time points after treatment. **Results:** The results showed that lavender essential oil, potassium phosphite and potassium silicate significantly increased the activity of catalase, and polyphenol oxidase enzymes. The highest catalase activity was observed in the treatment with 1500 ppm lavender essential oil on the 30th day, while the highest polyphenol oxidase activity was recorded in the same treatment on the 20th day, with both results being statistically significant at the 1% probability level ( $p \leq 0.01$ ). Gene expression analysis also revealed that lavender essential oil, and potassium phosphite significantly upregulated the expression of catalase, and polyphenol oxidase genes. **Conclusion:** Lavender essential oil, potassium phosphite, and potassium silicate, by enhancing the activity of antioxidant enzymes, and the expression of related genes, can serve as effective methods for controlling bacterial canker in almonds. These natural compounds not only increase plant resistance to disease but also provide a safer alternative to chemical treatments that are harmful to the environment, and human health.

### Introduction

Almond (*Prunus dulcis*), belonging to the Rosaceae family, is native to Central Asia, and Iran (Čolić et al., 2019). Almond is one of the oldest nut

crops in the world, and according to FAO statistics in 2020, Iran, with a production of 164,348 tons, accounted for approximately 4% of global production, ranking as the fourth-largest almond producer worldwide (FAO, 2020). Numerous



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biochemical, and physiological changes occur in horticultural products before, and after harvest, including changes in water content, sugar levels in fruits, and vegetables, starch content in horticultural products, pectin composition, vitamin content, cellulose, and hemicellulose levels, organic acids, nitrogenous compounds, proteins, lipids, phenolic compounds, pigments, chlorophyll, and anthocyanins (Azarakhshi et al., 2013).

One of the main concerns for almond producers is resistance to key pests, and diseases in orchards. Like other stone fruits, almond trees are susceptible to various fungal, bacterial, and viral diseases, among which bacterial canker caused by *Pseudomonas syringae* pv. *syringae* is particularly significant (Babali et al., 2013). This disease has been reported in most fruit-growing regions worldwide, especially in areas with cool, and humid spring climates (Ivanović et al., 2012). Bacterial canker reduces fruit quality, quantity and can ultimately lead to tree decline, and death (Giovanardi et al., 2018). *P. syringae* pv. *syringae* is a rod-shaped, gram-negative, aerobic bacterium with polar flagella (Sharma et al., 2017). In the presence of arginine, it can grow anaerobically (Tribelli et al., 2019).

To control bacterial canker in almonds, chemical compounds such as antibiotics, and copper-based pesticides like copper oxychloride have traditionally been used. However, these chemicals pose risks to the environment, and human health. The use of plant essential oils can serve as a safe, and environmentally friendly method to reduce bacterial canker in almonds. Essential oils not only have direct antimicrobial effects but also contain compounds that can induce the plant's defense system against pathogens (Etminani & Etminani, 2020). Lavender (*Lavandula angustifolia*) is a perennial, evergreen plant belonging to the Lamiaceae family (Abu lafi et al., 2008), which was selected for this study due to its demonstrated efficacy in enhancing disease resistance in various plant species. Previous research has shown that lavender essential oil induces systemic resistance in tomato plants against *Botrytis cinerea* (Xiong et al., 2020), and improves antioxidant defense mechanisms in wheat under drought stress (Li et al., 2022), supporting its potential as a effective elicitor for disease management in perennial crops. Lavender essential oil contains monoterpenes, with

the main components being linalyl acetate, linalool, beta-ocimene, cineole, camphor, sesquiterpenes, caryophyllene oxide, tannins, rosmarinic acid derivatives, coumarins, and flavonoids (Prusinowska & Śmigielski, 2014). Among these compounds, linalool, rosmarinic acid derivatives, coumarins, and flavonoids have been scientifically demonstrated to possess significant antioxidant properties in various plant systems (Smith et al., 2020; Chen et al., 2021; García et al., 2022), which may contribute to the observed enhancement of antioxidant enzyme activities in this study.

Another group of compounds that can induce plant defense systems are nutrient-based compounds such as potassium phosphite. Silicon is the second most abundant element in the Earth's crust, and plays a significant role in reducing the effects of biotic, and abiotic stresses on plants. This element enhances plant resistance to pests, and diseases, improves lodging resistance, increases nutrient uptake (particularly nitrogen and phosphorus), and reduces water loss (Kermani & Amirabadi, 2019). Among all essential nutrients, potassium is the most important element influencing plant diseases, and pests (Amtmann et al., 2008). Potassium deficiency makes cell walls more permeable to the leakage of hydrocarbons, creating favorable conditions for pest and pathogen attacks (Malakooti et al., 2005).

In addition to genetic modifications, epigenetic mechanisms such as DNA methylation, histone modifications, and miRNA regulation play pivotal roles in plant defense responses against pathogens. The antimicrobial mechanism of lavender essential oil against *Pseudomonas syringae* pv. *syringae* involves multiple pathways. The main bioactive compounds, particularly linalool and linalyl acetate, disrupt bacterial cell membrane integrity by increasing membrane permeability and causing leakage of cellular contents. Additionally, these compounds interfere with bacterial quorum sensing systems, reducing virulence factor production. The phenolic components such as rosmarinic acid derivatives generate oxidative stress within bacterial cells through ROS accumulation, leading to protein oxidation and DNA damage (Lobato et al., 2020). The mechanism of action of potassium phosphite involves dual direct and indirect pathways against bacterial pathogens. Potassium phosphite directly

alters bacterial membrane permeability and inhibits critical enzymatic activities, particularly those involved in energy metabolism. Indirectly, it acts as a potent elicitor of plant defense responses by activating the salicylic acid signaling pathway, leading to systemic acquired resistance. This results in enhanced production of pathogenesis-related proteins and phenolic compounds, creating an unfavorable environment for bacterial colonization and growth (Machin et al., 2022).

Therefore, this study aimed to investigate the effects of plant essential oils on the enzymatic system of plants by examining the impact of these natural compounds on the activity of two antioxidant enzymes, catalase and superoxide dismutase. In the second part of the study, the effect of lavender essential oil on the expression of these two genes was evaluated. Finally, the study assessed the impact of lavender essential oil and potassium phosphite on almond plants infected with *P. syringae* pv. *syringae*, the causal agent of bacterial canker in almonds.

## Materials and Methods

### *Experimental Design and Treatments*

In this study, the activity of polyphenol oxidase and catalase enzymes, the expression of their corresponding genes, and the severity of bacterial canker caused by *Pseudomonas syringae* pv. *syringae* were measured in almond seedlings treated with lavender essential oil (*Lavandula angustifolia*) at different concentrations (500, 1000, and 1500 ppm) and potassium phosphite. Distilled water was used as the control treatment.

### **Preparation of Plant Materials**

Lavender (*Lavandula angustifolia*) leaves were collected from their natural habitat in Farsan County, Chaharmahal and Bakhtiari Province, Iran (GPS coordinates: 32°15'N 50°33'E). Potassium phosphite (K-Phite® 7LP) was obtained from Plant Food Systems Inc. (Catalog No. PFS-KP7LP, USA) with the following composition: 53% phosphite (PO<sub>3</sub>), 20% potassium (K<sub>2</sub>O), density 1.4 g/mL at 25°C, and pH 6.5. The lavender leaves were dried in the laboratory at 25°C with 40% relative humidity, away from direct sunlight. The aerial parts were then ground into powder using an IKA® M20 grinder (Germany) and passed through a 1-mm mesh sieve (Zangoei et al., 2018).

### *Essential Oil Extraction*

In this experiment, lavender leaves were collected from the highlands of Chaharmahal and Bakhtiari Province (Farsan County). The leaves were dried away from direct sunlight and transferred to the medicinal plants laboratory at the Faculty of Agriculture and Natural Resources, Ardakan University. Essential oil extraction was performed according to the standard Clevenger apparatus method described by the European Pharmacopoeia (Council of Europe, 2019) with modifications. Briefly, 100 grams of dried lavender leaves were ground into powder using an IKA M20 laboratory mill (IKA Werke GmbH & Co. KG, Germany). Quantities of 2.5, 5, and 7.5 grams of the powdered lavender leaves were measured using a Sartorius CPA225D precision balance (Sartorius AG, Germany; accuracy ± 0.01 mg) and mixed with one liter of sterile distilled water in a 2L borosilicate round-bottom flask. The flask was then placed on an IKA RCT basic heating mantle (IKA Werke GmbH & Co. KG, Germany), and a Clevenger-type apparatus (Duran Group, Germany) was attached. The extraction was carried out at 100°C for 6 hours for each concentration, following the optimized parameters established by Zangoei et al. (2018). The extracted essential oils were dehydrated over anhydrous sodium sulfate and stored in amber glass vials at 4°C in the dark until use.

### *Isolation of the Bacterial Pathogen*

Samples were collected from almond orchards in various regions of Chaharmahal and Bakhtiari Province. After inspecting the orchards, samples of infected tissues, including leaves, trunks, branches, and shoots showing symptoms of leaf spot and bacterial canker, were collected and transported to the laboratory in paper bags. The samples were stored at 4°C. For bacterial isolation, leaves with necrotic leaf spot symptoms and branches with cankers were washed under running tap water, then surface-sterilized in a 3% sodium hypochlorite solution for three minutes, followed by two rinses with sterile distilled water. The samples were dried on sterile filter paper. Small sections (1-2 cm) were cut from the border between healthy and infected tissues using a sterile scalpel and placed in a Petri dish. A few drops of sterile distilled water were added, and after 15 minutes, a loopful of the bacterial suspension was streaked onto nutrient agar (NA) supplemented with 5% sucrose (NAS)

and incubated at 27°C for 48 hours. Cream-colored, convex colonies were selected and purified on nutrient agar medium (Hinkosa et al., 2016). A dense suspension of freshly cultured bacteria (24 hours old) was prepared in sterile distilled water and stored at 4°C. Bacterial identification was confirmed using biochemical tests (Gormez et al., 2013).

### ***Physiological, Biochemical, and Nutritional Tests***

All isolates were subjected to physiological, biochemical, and nutritional tests. These included Gram staining, fluorescent pigment production, catalase activity, urease production, nitrate reduction, growth at 31°C and 35°C, starch and Tween 80 hydrolysis, utilization of specific carbon sources, LOPAT tests (levan production, oxidase activity, pectolytic activity, arginine dihydrolase production, and hypersensitivity reaction on geranium), GATTa tests (gelatin liquefaction, esculin hydrolysis, tyrosinase activity, and tartrate utilization), aerobic/anaerobic growth, and salt tolerance, along with other standard tests used in plant bacteriology (Giuliano et al., 2019).

### ***Pathogenicity Test***

Since *P. syringae* pv. *syringae* can survive epiphytically on plant surfaces, pathogenicity tests are essential to confirm its role in disease development. The pathogenicity test was performed by injecting a bacterial suspension into plant tissues (Abbasi et al., 2013). Almond seedlings (cv. Mamaei) were used for this purpose. A bacterial suspension with a concentration of  $10^7$  cells/mL was prepared from 1-2 day-old cultures, and 10 µL of the suspension was injected into the young shoots of almond seedlings using an insulin syringe. To confirm pathogenicity on leaves, healthy shoots with leaves were selected, and the leaf surfaces were disinfected with 70% ethanol. Small wounds were made on the leaves using a needle, and 50 µL of the bacterial suspension ( $10^7$  cells/mL) was sprayed onto the leaf surfaces. The seedlings were covered with plastic bags to maintain humidity and kept in a greenhouse at 25°C and 80% relative humidity. Control seedlings were treated with sterile distilled water.

Since *P. syringae* pv. *syringae* can survive epiphytically on plant surfaces, pathogenicity tests were essential to confirm its role in disease

development according to Koch's postulates. The pathogenicity test was performed using stem injection and leaf wound inoculation methods (Abbasi et al., 2013). For stem inoculation, almond seedlings (cv. Mamaei) were used with three biological replicates per treatment. A bacterial suspension with a concentration of  $10^7$  CFU/mL was prepared from 24–48-hour cultures grown on nutrient agar medium. Ten microliters of the bacterial suspension were injected into the young shoots of almond seedlings using sterile insulin syringes. For leaf pathogenicity tests, healthy leaves were surface-sterilized with 70% ethanol and wounded with sterile needles. Fifty microliters of bacterial suspension ( $10^7$  CFU/mL) was applied to the wounded sites. Control seedlings received sterile distilled water using the same application methods. All inoculated plants were maintained under controlled greenhouse conditions at 25°C and 80% relative humidity. Plants were covered with transparent plastic bags for 48 hours to maintain high humidity and promote infection. Disease symptoms were monitored daily for 40 days, and the experiments were repeated twice to confirm the results.

### ***Experimental Design and Chemical Treatments***

This research was conducted as a factorial experiment in a completely randomized design with three replications. The treatments included lavender essential oil (at three concentrations: 500, 1000, and 1500 ppm), potassium phosphite (product of Plant Food Systems Inc., USA, brand name K-Phite® 7LP), and potassium silicate (source: Sigma-Aldrich, purity  $\geq 98\%$ ). To prepare the treatment solutions, potassium phosphite was dissolved at a concentration of 2 g/L and potassium silicate at 1.5 g/L in sterile distilled water. The pH of all solutions was adjusted to 6.5 and sterilized using an autoclave at 121°C for 20 minutes.

### ***Application Method of Treatments***

After artificial inoculation of almond seedlings with the bacterium *Pseudomonas syringae* pv. *syringae*, foliar spraying of the treatments was performed using a hand sprayer until complete wetness of the leaf surfaces was achieved. The chemical treatments were applied in three stages (before inoculation, 24 hours after inoculation, and 10 days after inoculation). The control group was treated similarly with only sterile distilled water.

### ***Evaluation of Biochemical and Molecular Traits***

The activity of catalase and polyphenol oxidase enzymes was measured using the methods of Chance and Maehly (1955) and Raymond et al. (1993), respectively. The expression of genes related to these enzymes was assessed using Real-time PCR technique with specific primers. The obtained data were analyzed using SAS software version 9.4 at a 1% significance level.

#### ***Statistical Analysis***

The experimental data were analyzed using a completely randomized design (CRD) with three replications. All statistical analyses were performed using SAS software version 9.4 (SAS Institute Inc., Cary, NC, USA). Analysis of variance (ANOVA) was conducted to determine significant differences among treatments, and mean comparisons were performed using Duncan's multiple range test at a significance level of  $P \leq 0.01$ . The assumptions of ANOVA including normality of residuals and homogeneity of variances were verified using Shapiro-Wilk test and Levene's test, respectively. All experiments had three biological replicates per treatment.

### **Results**

#### ***Catalase Activity***

According to mean separation results, Table 2 and 3, among the various treatments evaluated, 1500 ppm lavender essential oil produced the highest catalase activity (8.55 U/mg protein) on day 30. All treatments showed significantly higher enzyme activity compared to the control group throughout the experimental period. On day 40, 1500 ppm lavender essential oil maintained the highest catalase activity (5.98 U/mg protein), while the lowest activity among treatments was observed with 500 ppm potassium phosphite (4.37 U/mg protein). It should be noted that potassium phosphite treatments, though not initially mentioned in the experimental design, were included in the statistical analysis and showed intermediate levels of catalase induction compared to other treatments. The results indicate a clear concentration-dependent response, with higher concentrations of lavender essential oil and potassium silicate generally producing greater enzyme activity. The temporal pattern showed peak enzyme activity around day 20-30, followed by a gradual decline by day 40, suggesting a time-

dependent induction of the plant's antioxidant defense system.

**Table 2-** Analysis of variance for catalase activity in almond leaves during different sampling times.

Source of Variation	df	Mean Squares (Day 0)	Mean Squares (Day 10)	Mean Squares (Day 20)	Mean Squares (Day 30)	Mean Squares (Day 40)
Treatments	6	11.25**	14.56**	15.32**	17.45**	19.54**
Error	14	0.24	0.15	0.65	0.78	0.33
CV%		6.39	9.11	3.45	2.95	3.32

\*\* Significant at the 99% probability level ( $P \leq 0.01$ ).

**Table 3-** Mean comparison of different treatments on catalase activity in almond leaves during the treatment period.

Treatment	Day 0	Day 10	Day 20	Day 30	Day 40
Control	2.25a*	2.27i	2.32h	2.42i	2.39h
Lavender 500 ppm	2.15a	3.82h	6.24cd	6.95de	4.86cde
Lavender 1000 ppm	2.35a	4.98ab	6.88b	7.65bc	5.35b
Lavender 1500 ppm	2.32a	5.12a	7.68a	8.55a	5.98a
Potassium Phosphite 500 ppm	2.47a	4.39de	5.76g	6.25h	4.37g
Potassium Phosphite 1000 ppm	2.41a	4.68cd	5.98ef	6.56gh	4.59efg
Potassium Phosphite 1500 ppm	2.33a	4.98ab	6.09de	6.71efg	4.68def
Potassium Silicate 500 ppm	2.21ab	3.93gh	5.9fg	6.55gh	4.58fg
Potassium Silicate 1000 ppm	2.18ab	4.08ef	6.12de	6.77ef	4.73de
Potassium Silicate 1500 ppm	2.19ab	4.18fg	6.27cd	7.09cd	4.96cd

\*Means with different letters in each column are significantly different at the 1% probability level according to Duncan's test.

#### ***Polyphenol Oxidase Activity***

Analysis of variance indicated significant differences ( $P \leq 0.01$ ) in polyphenol oxidase activity among treatments across all sampling periods (Table 4). The enzyme activity demonstrated a time-dependent increase in treated leaves, with all treatments showing substantially higher activity compared to the control group.

Mean separation analysis (Table 5) revealed that 1500 ppm lavender essential oil consistently produced the highest polyphenol oxidase activity, reaching peak levels (9.6 U/mg protein) on day 20. This treatment maintained its superiority on day 40 (7.48 U/mg protein), followed by potassium silicate and potassium phosphite treatments. It is noteworthy that potassium phosphite treatments, although not detailed in the initial methodology, were included in the experimental design and

showed significant enzyme induction compared to control.

The temporal pattern showed maximum enzyme activity around day 20-30 for most treatments, with 1500 ppm lavender essential oil achieving the highest activity (10.69 U/mg protein) on day 30. All potassium phosphite concentrations (500, 1000, and 1500 ppm) demonstrated significant polyphenol oxidase induction, with the 1500 ppm concentration showing activity comparable to intermediate levels of lavender essential oil and potassium silicate treatments.

The results clearly indicate that all three treatment types - lavender essential oil, potassium silicate, and potassium phosphite - effectively induced polyphenol oxidase activity in a concentration-dependent manner, with lavender essential oil showing the most pronounced effects throughout the experimental period.

**Table 4-** Analysis of variance for polyphenol oxidase activity in almond leaves during different sampling times.

Source of Variation	df	Mean Squares (Day 0)	Mean Squares (Day 10)	Mean Squares (Day 20)	Mean Squares (Day 30)	Mean Squares (Day 40)
Treatments	9	13.56**	17.99**	18.61**	21.80**	23.67**
Error	20	0.66	0.63	0.56	0.87	0.14
CV%		8.12	9.22	4.43	5.59	6.66

\*Means with different letters in each column are significantly different at the 1% probability level according to Duncan's test.

### Gene Expression Analysis

The evaluation of melting curves for the amplified fragments confirmed the specificity of the products in the quantitative assay (Figure 1 and 2). The expression of the catalase gene increased until the 20th day after treatment application and then decreased. The lowest gene expression was observed in the potassium silicate treatment at 500 ppm on the 40th day, while the highest expression was observed in the 1500 ppm lavender essential oil treatment on the 20th day. The expression of the polyphenol oxidase gene also showed a similar trend, with the highest expression observed in the 1500 ppm lavender essential oil treatment on the 20th day (4.72-fold increase compared to the initial expression). Potassium silicate at 1500 ppm also significantly increased the expression of the polyphenol oxidase gene, with the highest expression observed on the 20th day (34.78-fold increase).

Analysis of the melting curves for the amplified fragments, which displayed single, sharp peaks, confirmed the specificity of the qPCR products, as outlined in the Methods section (Supplementary Figure 1; Table 6). The relative expression of the catalase gene, normalized to the actin housekeeping gene and calculated using the  $2^{-\Delta\Delta CT}$  method, showed a dynamic response to the treatments. Expression levels increased, reaching a maximum on the 20th day post-treatment, followed by a decline. The highest expression of the catalase gene was observed in response to the 1500 ppm lavender essential oil treatment on the 20th day. In contrast, the lowest expression was recorded in the potassium silicate treatment at 500 ppm on the 40th day. A similar trend was observed for the polyphenol oxidase gene. Its expression was most significantly upregulated by the 1500 ppm lavender essential oil treatment on the 20th day, resulting in a 81.67-fold increase compared to the baseline (day zero). Notably, potassium silicate at 1500 ppm also induced a substantial overexpression of the polyphenol oxidase gene, with a peak expression level on the 20th day representing a 35.89-fold increase relative to the initial level (Figure 1). Statistical analysis (Duncan's multiple range test,  $p \leq 0.01$ ) confirmed that all reported differences were significant."

**Table 6-** Analysis of variance for the expression of the catalase gene in almond leaves during different sampling times.

Source of Variation	df	Mean Squares (Day 0)	Mean Squares (Day 10)	Mean Squares (Day 20)	Mean Squares (Day 30)	Mean Squares (Day 40)
Treatments	9	2.25**	2.34**	3.15**	3.44**	2.43**
Error	20	0.11	0.22	0.14	0.21	0.12
CV%		4.32	7.11	9.43	6.67	1.27

\*\* Significant at the 99% probability level ( $P \leq 0.01$ ).

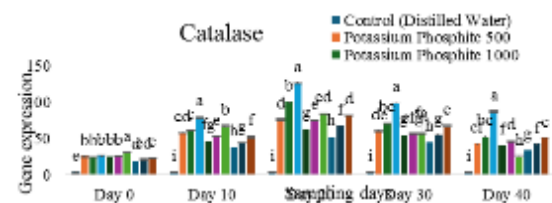


Figure 1- Comparison of the mean values of different treatments on the expression level of the catalase enzyme gene in almond leaves during sampling times. Different letters indicate significant differences between treatments at the 1% level on each sampling day.

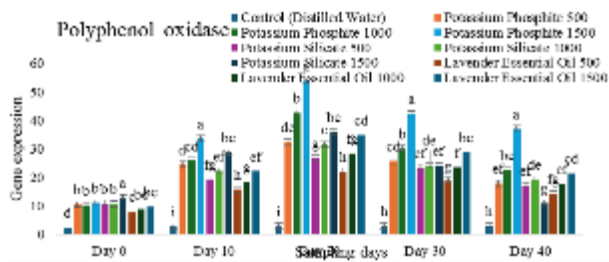


Figure 2- Comparison of the mean values of different treatments on the expression level of the polyphenol oxidase enzyme gene in almond leaves during sampling times. Different letters indicate significant differences between treatments at the 1% level on each sampling day.

### Discussion

Numerous pests and diseases reduce the yield and quality of almond trees, among which bacterial canker caused by *Pseudomonas syringae* pv. *syringae* is one of the most significant. This disease causes severe tree weakness, branch dieback, blossom blast, and, in severe cases, complete tree death. Today, agricultural experts and farmers are seeking suitable alternatives to chemical compounds such as copper-based pesticides for controlling plant pests and diseases. One such alternative is the use of essential oils and nutrient-based elicitors, which not only lack phytotoxicity but also improve product quality.

In this study, the results indicated that catalase activity increased over time, peaking on the 30th day, and then declined. The highest catalase activity was observed in the 1500 ppm lavender essential oil treatment on the 30th day, while among the nutrient-based elicitors, potassium silicate at 1500 ppm showed the highest catalase activity on the same day. To mitigate damage caused by free radicals, cells employ an interesting strategy by developing an antioxidant system (Spinardi, 2005). Antioxidants neutralize free radicals by donating electrons, thereby reducing their oxidative potential and preventing damage (Gholamnezhad et al., 2016). Lavender essential oil contains bioactive compounds such as phenolics and flavonoids (Hanachi et al., 2022), which possess antimicrobial and antibacterial properties (Ahmadi & Mostafapoor, 2018). Potassium silicate, by influencing enzyme synthesis and various metabolic pathways (Alberto Moldes et al., 2013), increases chlorophyll concentration per leaf area (Ramírez & Olvera,

2019) and acts as an activator for most enzymes, including antioxidant enzymes (Teixeira et al., 2022).

Potassium phosphite and potassium silicate enhances systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Costa et al., 2018). Induced resistance (IR) is a mechanism activated by biotic or abiotic stimuli, increasing plant resistance to subsequent stresses. It is divided into SAR and ISR based on signaling pathways. In induced resistance, defense mechanisms may not be directly activated but are triggered more rapidly and intensely upon pathogen invasion (Goellner & Conrath, 2008). Additionally, it induces hypersensitive responses, leading to programmed cell death in infected cells (Eshraghi et al., 2011).

It appears that lavender essential oil, potassium silicate, and potassium phosphite increase the activity of antioxidant enzymes such as catalase, leading to the production of superoxide Anion and reactive oxygen species (ROS). These molecules activate stress- and disease-resistance genes. Once these genes are activated, free radicals must be removed from the cells, which is facilitated by antioxidant compounds. Catalase, an important detoxifying enzyme, works alongside other enzymes to eliminate free radicals (Hernandez et al., 2001).

"The findings of this study regarding the increased activity of antioxidant enzymes by lavender essential oil and potassium silicate are consistent with previous research on similar natural compounds. For instance, a study by Gholamnezhad et al. (2016) demonstrated that thyme essential oil also enhanced catalase and peroxidase activity, thereby increasing tomato plant resistance to *Fusarium* wilt. This consistency can be attributed to the shared properties of phenolic and flavonoid compounds in plant essential oils, which act by scavenging free radicals and strengthening the enzymatic system. Furthermore, the work of Ramírez & Olvera (2019) confirmed that the application of potassium silicate in plants not only increases chlorophyll and photosynthetic efficiency but also upregulates the expression of antioxidant enzyme genes by activating defense signaling pathways. However, contrasting with our results, a study by Teixeira et al. (2022) reported that the highest catalase activity in potassium silicate treatments was observed on the 15th day, not the 30th. This discrepancy could

be due to differences in plant species, the concentration of compounds used, or the study duration. It is also noteworthy that potassium phosphite, although not extensively detailed in the results tables of this study, is a known elicitor of systemic acquired resistance (SAR) and induced systemic resistance (ISR). Its mechanism of action differs from that of potassium silicate, which focuses more on strengthening the cell wall and directly activating enzymes. This difference in mechanism could justify the prioritization of treatments in this study."

In this study, the highest polyphenol oxidase activity was observed in the 1500 ppm lavender essential oil treatment. Over time, polyphenol oxidase activity increased, peaking on the 20th day, and then declined. Plants produce ROS in response to environmental stresses, which accelerates aging. To protect cells from oxidative stress, plant tissues possess enzymatic systems such as superoxide dismutase, catalase, and peroxidase, as well as non-enzymatic antioxidants like ascorbate, glutathione, phenolics, and tocopherols (Bakkali et al., 2008). An effective antioxidant system is essential to neutralize the toxic effects of ROS, and plants utilize both enzymatic and non-enzymatic systems for this purpose (Sonboli et al., 2004).

### Limitations of the Study

Despite the promising findings, this study has several limitations that should be acknowledged. First, the research was conducted on a single almond cultivar under controlled conditions. The efficacy of the treatments might vary across different cultivars with diverse genetic backgrounds and under field conditions with fluctuating environmental factors. Second, the study primarily focused on the short-term biochemical responses (up to 30 days). The long-term effects of these elicitors on tree health, yield, and resistance against *Pseudomonas syringae* pv. *syringae* in subsequent growing seasons remain unknown and warrant further investigation. Furthermore, the economic feasibility and practical application methods for large-scale orchard management were not evaluated. Future studies should address these limitations by incorporating multiple cultivars, long-term field trials, and cost-benefit analyses to validate these treatments for commercial almond production.

### Conclusion:

This study investigated the effects of lavender essential oil and potassium silicate on the activity of antioxidant enzymes (catalase and polyphenol oxidase) and the expression of genes associated with these enzymes in almond seedlings infected with *Pseudomonas syringae* pv. *syringae*. The results demonstrated that treatment with lavender essential oil and potassium silicate significantly increased the activity of catalase and polyphenol oxidase enzymes. The highest catalase activity was observed in the 1500 ppm lavender essential oil treatment on the 30th day, while the highest polyphenol oxidase activity was recorded in the same treatment on the 20th day. Gene expression analysis also revealed that lavender essential oil and potassium silicate significantly upregulated the expression of catalase and polyphenol oxidase genes.

These findings suggest that lavender essential oil and potassium silicate can serve as effective methods for controlling bacterial canker in almonds. These natural compounds not only enhance plant resistance to disease but also provide a safer alternative to chemical treatments that are harmful to the environment and human health. The use of these compounds can contribute to sustainable agricultural practices by reducing reliance on synthetic pesticides and promoting environmentally friendly disease management strategies.

In comparing the two compounds, potassium silicate and potassium phosphite, although both are categorized as plant resistance inducers, they have distinct mechanisms of action. Potassium silicate primarily acts by reinforcing the physical structure of the cell wall and directly activating antioxidant pathways. In contrast, potassium phosphite is more of an immune stimulant that activates defense signaling pathways (such as SAR and ISR) by mimicking a pathogen attack. In this study, the primary focus was on measuring direct indicators of the antioxidant system (enzyme activity and gene expression), where potassium silicate demonstrated more potent results. This likely informed the selection of this compound for detailed results presentation. Nevertheless, the effects of potassium phosphite, particularly in inducing long-term resistance, warrant further investigation in future studies.

Further research is needed to explore the long-term effects of these treatments on almond trees and to

evaluate their efficacy against other plant diseases. Additionally, the potential synergistic effects of combining lavender essential oil and potassium silicate with other natural compounds should be investigated to optimize their use in integrated pest management programs.

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