



Expression profiling of PR genes and the protective effect of *Pseudomonas syringae*, *Arthrosira platensis* and *Elettaria cardamomum* in tomato (*Solanum lycopersicum*) under diverse biotic stress conditions

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Abstract

Pathogenesis-related (PR) genes play a central role in plant defense, yet their expression can vary widely under natural conditions. This study analyzed the expression patterns of two key PR genes PR1 (*SL_PR1a2*) and PR10 (*Sol_I_PR_10_4.02*), in tomato following treatments with *Arthrosira platensis*, *Elettaria cardamomum*, and their combinations with *Pseudomonas syringae*. Gene expression was quantified using qRT-PCR under both field conditions (1 day post inoculation and 1 week post inoculation) and laboratory conditions (3 days post inoculation). Bar-graph analysis revealed that *A. platensis* and *E. cardamomum* induced PR genes with treatment-specific and time-dependent effects, while *P. syringae* alone suppressed expression. Laboratory assays showed strong, dose-dependent induction by *E. cardamomum* and consistent activation by *A. platensis*. Time-course curves and heat maps demonstrated that laboratory conditions support stable and robust PR-gene induction, whereas field responses are heterogeneous and less predictable, reflecting environmental fluctuations. These findings highlight the potential of *A. platensis* and *E. cardamomum* as natural defense enhancers, particularly in controlled environments, and provide insight into optimizing biostimulant-based strategies for disease management in tomato.

Keywords Tomato · *Pseudomonas syringae* · PR1 · PR10 · *Elettaria cardamomum* · *Arthrosira platensis* · Gene expression · Plant defense

Introduction

Pathogenesis-related (PR) proteins are key components of plant immunity, accumulating in response to both biotic and abiotic stress stimuli. To date, 17 PR families (PR1–PR17) have been described, with diverse antimicrobial and signaling roles (Han et al. 2023). Among these, PR1 and PR10 are particularly important in plant-pathogen interactions. PR1 is one of the most widely used molecular markers of the salicylic acid (SA) mediated defense pathway and systemic acquired resistance (SAR), belonging to the CAP (cysteine-rich secretory proteins, antigen 5, PR-1) superfamily (Ali et al. 2018; Han et al. 2023). In tomato, several PR1 genes have been bio informatically characterized and shown to respond to both biotic and abiotic stresses, underscoring their regulatory roles in defense (Akbadak et al. 2020). PR10 proteins, by contrast, are small (~16–19 kDa), acidic proteins with ribonuclease and ligand-binding activities, implicated in signal transduction

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and direct antimicrobial functions (Sinha et al. 2020; Islam et al. 2023). A genome-wide study identified 45 PR10 genes in tomato, many of which are strongly induced under viral and *Pseudomonas syringae* stress, highlighting their contribution to defense signaling (Islam et al. 2023). PR10 expression has been detected in fruit exo-carp tissue, suggesting functional roles beyond vegetative defense (Ruan et al. 2024).

Tomato (*Solanum lycopersicum*), one of the most widely cultivated vegetable crops globally, remains highly susceptible to diverse *P. syringae* pathogens such as *P. syringae*, *Ralstonia solanacearum*, and *Xanthomonas* spp., leading to significant yield losses (Savary et al. 2019; Panno et al. 2021; Phiri et al. 2024). In some regions, such as the Mediterranean, losses caused by the bacterium *P. syringae* can reach up to 75%, while in Ontario, *P. syringae* pathogens are associated with a yield reduction of up to 60%. *Pseudomonas syringae* is a highly adaptable bacteria species that thrives in various environments, including soil, water, and plant surfaces. It is considered a significant phytopathogen due to its ability to infect a wide range of plant hosts, particularly economically important crops and fruit species (Lonjon et al. 2024).

Enhancing tomato disease resistance through sustainable, eco-friendly strategies is therefore a key research priority. Natural elicitors such as essential oils (EOs) and algae-derived biostimulants have received increasing attention for their ability to modulate plant defense pathways. Essential oils, including cardamom EO, are rich in terpenes and phenolics with strong antimicrobial and signaling activities (Goudarzvand Chegini and Abbasipour 2017). In tomato, PR1-mediated defense can also be enhanced through endogenous signaling peptides derived from PR1 cleavage, such as CAPE1, which increases resistance to *R. solanacearum* (Chen et al. 2014). Microalgae and cyanobacteria biostimulants such as *Arthrospira platensis* contain polysaccharides, proteins, and antioxidants that activate defense enzymes (PAL, chitinase, β -1,3-glucanase) and enhance resistance against stress and pathogens (Rachidi et al. 2021; Parmar et al. 2023).

The present study aimed to investigate the expression patterns of two defense-related genes, *SL_PR1a2* and *Sol_1_PR10_4.02*, in tomato plants exposed to *P. syringae* infection and natural elicitor treatments. Field experiments evaluated the effects of *E. cardamomum*, *A. platensis*, and their combinations under natural growth conditions, while a controlled laboratory assay examined the dose-dependent effect of *E. cardamomum* (cardamom essential oil) combined with *P. syringae* in fruit tissue. This dual experimental approach aimed to determine whether eco-friendly treatments can activate PR-mediated defense pathways across different tissues and to clarify how dose and tissue context influence the magnitude of the response.

Materials and methods

Plant material and growth conditions

Tomato plants (*Solanum lycopersicum* L., cv. Orbit) were cultivated under natural outdoor conditions in the area of the Botanical Garden of the Slovak University of Agriculture in Nitra, Slovakia (48°19'15" N, 18°06'30" E; 144 m a.s.l.). Plants were grown under ambient light, temperature, and humidity and maintained under standard field management practices. Young, healthy plants at a comparable growth stage were selected for the field experiment.

Prior to the application of experimental treatments, all plants were uniformly sprayed with a 1% sodium hypochlorite (NaOCl) solution on 28 May 2024 in order to reduce surface microbial contamination. This treatment represented a short-term surface disinfection step and did not involve prolonged exposure to the disinfectant. After NaOCl application, plants were left under field conditions for a 24 h stabilization period before further treatments.

Preparation of *P. syringae* plant bacteria

Pseudomonas syringae strain CCM 2868 (also referred to as strain HpE940 or J.D. Ott 831) was isolated from cherry (*Prunus avium*) and belongs to the pathovar *syringae*. The bacterium was purchased from the Czech Microorganism Collection (CCM, Brno, Czech Republic). This Gram-negative *P. syringae* causes a variety of plant diseases, particularly on tomato, and is known for its ability to cause infections on a wide range of host plants (Lindeberg et al. 2009). After cultivation, the culture density of *P. syringae* was adjusted to 0.5 McFarland.

Field treatments and sampling

The field experiment evaluated tomato defense gene expression in response to *P. syringae* infection and selected natural elicitors. Treatments included *P. syringae*, *E. cardamomum* essential oil, *A. platensis*, and their respective combinations. Bacterial inoculation was performed on 29 May 2024 using a *P. syringae* suspension adjusted to 0.5 McFarland, corresponding to approximately 1.5×10^8 CFU mL⁻¹. The inoculum was applied to the foliage by spraying.

In addition to bacterial treatment, plants were treated with a 5% formulation of *E. cardamomum* essential oil or a 5% aqueous suspension of *A. platensis*. Treatments were applied individually or in combination according to the experimental design.

Six experimental treatment groups were established: untreated control, *P. syringae* alone, *A. platensis* alone, *E. cardamomum* alone, *P. syringae* + *A. platensis*, and *P. syringae* + *E. cardamomum*.

Leaf samples were collected at three time points: prior to treatment (pre-treatment, Pt), 1 day post-inoculation (1 dpi), and 1 week post-inoculation (1 wpi). Collected samples were used for downstream gene expression analyses.

Preparation of *A. platensis* applications

The cyanobacterium *Arthrospira platensis* in powder form (Spirulina: origin China) was purchased from the Slovenian company FutuNatura, which offers certified organic production SI-EKO-002, non-EU agriculture. All samples were purchased in 2022, stored in a dry environment, in the dark at laboratory temperature (± 20 °C) and hermetically sealed. To prepare a 5% water extract, 100 g powdered cyanobacteria was weighed and then poured in 1 L distilled water. The mixture was stirred for 24 h at 22 °C in the dark. After the incubation period, the extract was filtered through Whatman No. 2 filter paper and then poured into a hand-held nebulizer. This nebulizer was set for fine atomization, which produces particles with a diameter of about 20–50 μm , thus ensuring uniform coverage of the plant surface.

Preparation of *E. cardamomum*

Cardamom essential oil (*Elettaria cardamomum* Maton var. *minuscula*, family Zingiberaceae) was purchased from Hanus Ltd.; the origin of the plant material was Guatemala. The essential oil was obtained by distillation of the fruits. According to the manufacturer's information, the oil contained mainly 1,8-cineole (eucalyptol; 34%), D-limonene, neral, geraniol, and geranial. For the preparation of the application formulation, the essential oil was first mixed with 0.1% Tween 20, and the resulting mixture was homogenized by ultrasonication using an ultrasonic bath (Kraintek, Czech Republic) for 5 min at room temperature. Subsequently, the homogenized mixture was diluted with distilled water to obtain a final essential oil concentration of 5% (v/v). After dilution, the formulation was briefly homogenized again by ultrasonication to ensure uniform dispersion of the essential oil in the aqueous phase. The formulation was prepared immediately before application and used without storage. Application was carried out by foliar spraying using a hand-held sprayer adjusted for fine atomization, producing droplets with a diameter of approximately 20–50 μm , thereby ensuring uniform coverage of the leaf surface.

Plant material and experimental design

Tomato plants were divided into 6 groups of 5 plants each in three replications, (15 plants each).

Experimental groups:

- Control: Plants without any treatment.
- Bacteria: Plants treated with *P. syringae* only.
- Cyanobacteria: Plants treated with 5% water extract of *A. platensis*.
- Essential Oil: Plants treated with 5% cardamom essential oil (*E. cardamomum*).
- Bacteria + Essential oil: Plants treated with *P. syringae* followed by 5% *E. cardamomum*.
- Bacteria + Cyanobacteria: Plants treated with *P. syringae* followed by 5% water extract of *A. platensis*.

Laboratory dose–response assay

To validate the results obtained under field conditions, a laboratory in situ assay was conducted using tomato fruits. Fruits were inoculated with *P. syringae* (0.5 McFarland corresponding to approximately 1.5×10^8 CFU mL^{-1}) and subsequently treated with the essential oil applied directly to the fruit surface. For in situ application, the essential oil was dissolved in ethyl acetate, which is commonly used as a solvent for essential oils in in situ assays due to its high volatility, allowing rapid evaporation after application and minimizing prolonged solvent effects on fruit tissues. The solvent control consisted of the application of *P. syringae* in combination with 0.1% ethyl acetate. The tested treatments included essential oil applications at concentrations of 62.5, 125, 250, and 500 $\mu\text{g mL}^{-1}$, each co-applied with *P. syringae*. Samples were collected 3 days post-inoculation (3 dpi) to assess early defense responses, following previously published protocols (Herman et al. 2008; Furci et al. 2023). Each treatment was performed in three biological replicates, and qRT-PCR analysis was conducted in technical triplicates.

RNA extraction and cDNA synthesis

Total RNA was extracted from both leaf and fruit tissues using the RNeasy Plant Mini Kit (Qiagen, Germany). RNA purity was confirmed ($A_{260}/A_{280} = 1.8\text{--}2.0$) using a Nano Drop spectrophotometer (Thermo Fisher Scientific, USA). First-strand cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific) according to the manufacturer's instructions.

Table 1 Characteristics of primers used as target genes in qRT-PCR analysis

Primers	Sequence (5' → 3')	Amplicon Size (bp)
SL_PR1a2	F: GACTTGGAATCCCGAGCACA R: GTTTGGCTTTTCCGACACCC	150
Sol_1_PR10_4.02	F: GCTGGAGATGGAGGTTGTGT R: TGGCTTTCCTTTGCCTACA	103

Quantitative real-time PCR (qRT-PCR)

Relative transcript levels of *SL_PR1a2* and *Sol_1_PR10_4.02* (Table 1) were quantified by qRT-PCR using EF1 α as the reference gene (Table 2) (Zheng et al. 2016). Reactions (10 μ L) were performed using Elizyme SYBR Green qPCR Master Mix (Elisabeth Pharmacon, Czech Republic) with cycling conditions: 95 °C for 2 min, followed by 40 cycles of 95 °C for 5 s and 63 °C for 20 s. Melt-curve analysis confirmed primer specificity.

Data analysis

Relative expression levels were calculated using the Pfaffl method (Pfaffl 2001) to account for gene-specific PCR efficiencies. Statistical analyses were performed in R version 4.2.2 using the stats, agricolae, multcompView, and tidyverse packages. Custom R scripts were used for data import, organization, execution of one-way ANOVA models, post hoc Tukey's HSD tests, and generation of heatmaps and time-course visualizations.

Data from three biological replicates were used directly in all analyses and were not averaged prior to ANOVA. For field data (Field_data.csv), a factorial experimental design with two fixed factors (treatment and time: 1 dpi and 1 wpi) was used, and a two-way ANOVA was conducted to assess main and interaction effects. For laboratory data (lab_data.csv), a completely randomized design was applied, and one-way ANOVA was performed at 3 dpi. For individual gene responses (PR1 and PR10), separate one-way ANOVAs were conducted at 3 dpi.

When significant effects were detected, Tukey's HSD test was applied, and groupings were generated using the agricolae package. Bar graphs showing log₂ fold change (log₂FC) were prepared using GraphPad Prism, while heatmaps and time-course plots were generated using ggplot2, pheatmap, RColorBrewer, patchwork, and magick. Results are

presented as mean $\pm$ standard error (SE) of three biological replicates. Statistical significance was set at $P < 0.05$.

Results

Treatment of leaf infection

Different impact of various treatments on leaf infection by *P. syringae* was observed. The control group exhibited a low level of infection, with 11.33% of leaves showing signs of infestation. In contrast, the group treated with *P. syringae* alone had a significantly higher infection rate of 62.00% (Table 3). These findings suggest that while *P. syringae* alone induces a high infection rate, both *A. platensis* and *E. cardamomum* treatments can effectively reduce the infection. The combination treatments of *P. syringae* with either *A. platensis* or *E. cardamomum* showed intermediate effects compared to the *P. syringae*-only treatment.

Gene expression analysis using qRT-PCR

In field conditions, PR1 and PR10 expression (log₂ fold change relative to pre-treatment baseline = 0) showed different responses to treatments at 1 day post-inoculation (1 dpi) (Fig. 1a, c) and 1 week post inoculation (1 wpi) (Fig. 1 b,c). Under control the genes remained nearly baseline (PR1 –0.1 to 0.3; PR10 0.5 to –0.2). With *P. syringae* alone, PR1 was strongly suppressed at both 1 dpi and 1 wpi (–0.4, –3.0), while PR10 first dropped (–1.0) then partially recovered (+0.5). *A. platensis* treatment induced

Table 3 Impact of treatments on the percentage of infected tomato leaves

Group	Number of infested leaves	Total number of leaves	% leaf infection
Control	17 $\pm$ 1.53 ^{ab}	150	11.33 $\pm$ 3.06 ^{ab}
<i>P. syringae</i>	93 $\pm$ 3.00 ^c	150	62.00 $\pm$ 6.00 ^c
<i>A. platensis</i>	10 $\pm$ 0.58 ^a	150	6.66 $\pm$ 1.15 ^a
<i>E. cardamomum</i>	5 $\pm$ 1.15 ^a	150	3.33 $\pm$ 2.31 ^a
<i>P. syringae</i> + <i>E. cardamomum</i>	34 $\pm$ 6.66 ^b	150	22.67 $\pm$ 13.32 ^b
<i>P. syringae</i> + <i>A. platensis</i>	40 $\pm$ 4.16 ^b	150	26.67 $\pm$ 8.33 ^b

Data are the mean ($\pm$ SD) of 3 samples. Different letters (^{a,b,c,d}) indicate significant differences (Tukey, $p \leq 0.05$).

Table 2 Characteristics of primers used as reference gene in qRT-PCR analysis

Gene	Accession No	Sequence (5' → 3')	Amplicon Size (bp)	Reference
EF1 α	X14449	F: ACAGGCGTTCAGGTAAGGAA R: GAGGGTATTCAGCAAAGGTCTC	120	(Zheng et al. 2016)

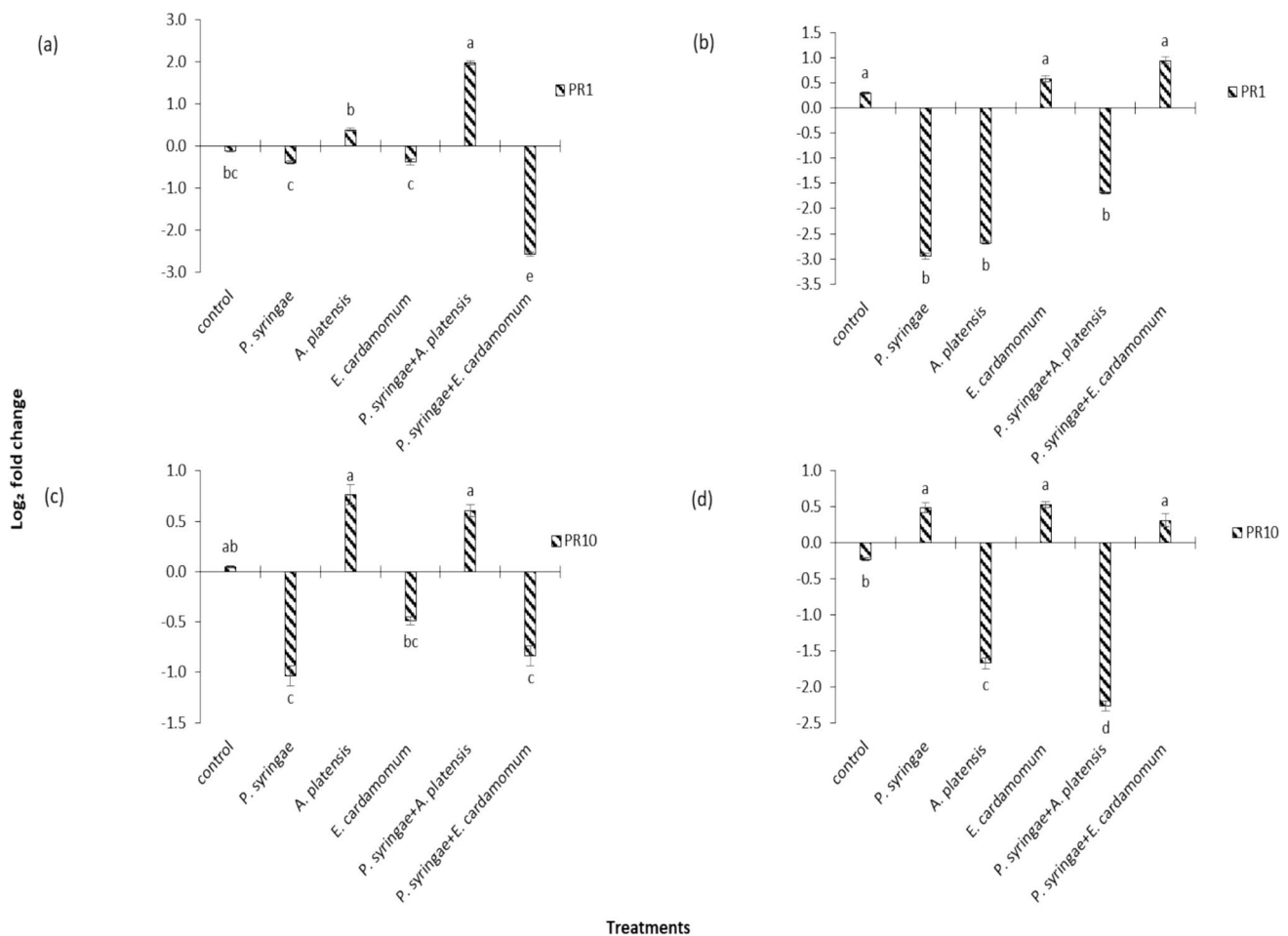


Fig. 1 Expression profiles of PR1 and PR10 genes in tomato plants following bacterial inoculation at two time points. **(a)** PR1 expression at 1 day post-inoculation (1dpi). **(b)** PR1 expression at 1 week post-inoculation (1wpi). **(c)** PR10 expression at 1dpi. **(d)** PR10 expression at 1wpi. Error bars show SE ($n=3$). Data represent mean log₂ fold change ($\pm$ SE) of three biological replicates. Bars above the zero line indicate gene upregulation, whereas negative values indicate downregulation relative to the untreated control. Different letters indicate significant differences (Tukey, $p < 0.05$). Two-way ANOVA for PR1

revealed significant effects of treatment ($F_{5,24} = 20.22$, $p < 0.012$), time ($F_{1,24} = 30.52$, $p < 0.011$), and their interaction ($F_{5,24} = 69.08$, $p < 0.001$) on PR1 expression. Levene's test confirmed homogeneity of variance ($F = 0.67$, $p = 0.749$). Two-way ANOVA also showed significant effects of treatment ($F_{5,24} = 9.40$, $p < 0.015$), time ($F_{1,24} = 14.18$, $p < 0.001$), and their interaction ($F_{5,24} = 95.57$, $p < 0.016$). Levene's test confirmed homogeneity of variance ($F = 0.80$, $p = 0.640$).

both genes at 1 dpi (+0.4, +0.8), but both were repressed at 1 wpi (−2.7 to −1.7). *E. cardamomum* alone had mild down regulation at 1 dpi (−0.4, −0.5), followed by modest upregulation at 1 wpi (+0.6, +0.5). Combination treatments showed complex dynamics: *P. syringae* + *A. platensis* gave strong early induction (PR1 +2.0; PR10 +0.6) then suppression at 1 wpi (−1.7, −2.3), while *P. syringae* + *E. cardamomum* was repressed at 1 dpi (−2.6, −0.8) then modestly positive at 1 wpi (+0.9, +0.3).

Under controlled laboratory conditions, the expression of PR1 and PR10 was strongly influenced by *E. cardamomum* concentration (Fig. 2. a,b). qRT-PCR quantification showed high linearity for both genes (PR1: $y = 6.8362x + 33.274$, $R^2 = 0.994$; PR10:

$y = 4.9375x + 25.911$, $R^2 = 0.925$), confirming reliable amplification efficiency. PR1 exhibited marked induction at low and high EO concentrations, with the strongest expression detected at 6.25 and $5.00 \times 10^2 \mu\text{g mL}^{-1}$, where log₂ fold change values were substantially elevated relative to the control. In contrast, mid-range EO concentrations (125, 250 $\mu\text{g mL}^{-1}$) resulted in lower PR1 activation. PR10 followed a similar but less pronounced trend, showing moderate induction at 6.25, 125 $\mu\text{g mL}^{-1}$ and relatively lower expression at 250 $\mu\text{g mL}^{-1}$. At the highest EO concentration (500 $\mu\text{g mL}^{-1}$), PR10 expression increased again but remained lower in amplitude compared with PR1. Overall, the laboratory data indicate a biphasic, dose-responsive pattern, where both PR genes respond

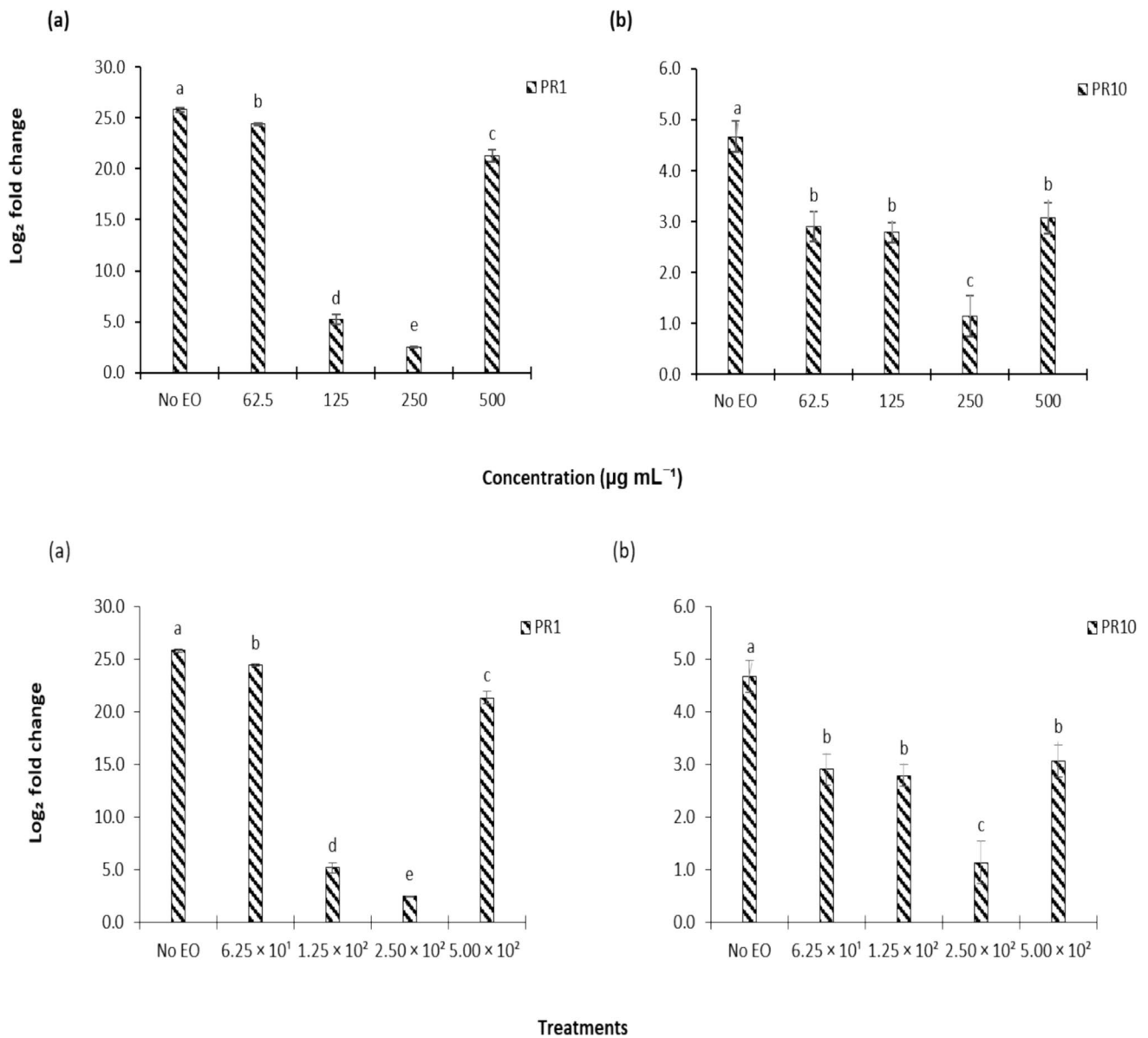


Fig. 2 Log₂ fold change in *SL-PR1a2* (PR1) (a), and *Sol-1-PR10_4.02* (PR10) (b), expression in tomato fruits treated with *E. cardamomum* at different concentrations (6.25, 125, 250, 500 µg mL⁻¹) and *P. syringae*.

Control (no *E. cardamomum*, no *P. syringae*) served as baseline. Error bars show ± SE (n=3). Different letters indicate significant differences (Tukey, $p \leq 0.05$)

strongly to EO at optimal low and high concentrations, demonstrating clearer and more consistent activation than under field conditions.

One-way ANOVA showed a highly significant effect of EO treatment on PR1 expression at 3dpi ($F_{4,10} = 3815$, $p < 0.001$). Levene's test confirmed homogeneity of variances ($F = 0.26$, $p = 0.898$). Post-hoc analysis using Tukey's HSD revealed significant differences among all treatments. ANOVA indicated a significant effect of EO treatment on PR10 expression at 3dpi ($F_{4,10} = 21.93$, $p < 0.003$).

Levene's test confirmed homogeneity of variances ($F = 0.30$, $p = 0.873$).

Time-course expression patterns and heatmap

To further visualize dynamic gene regulation, time-course line graphs were generated for PR1 and PR10 under both field and laboratory conditions (Fig. 3). These plots highlight not only the magnitude of induction/

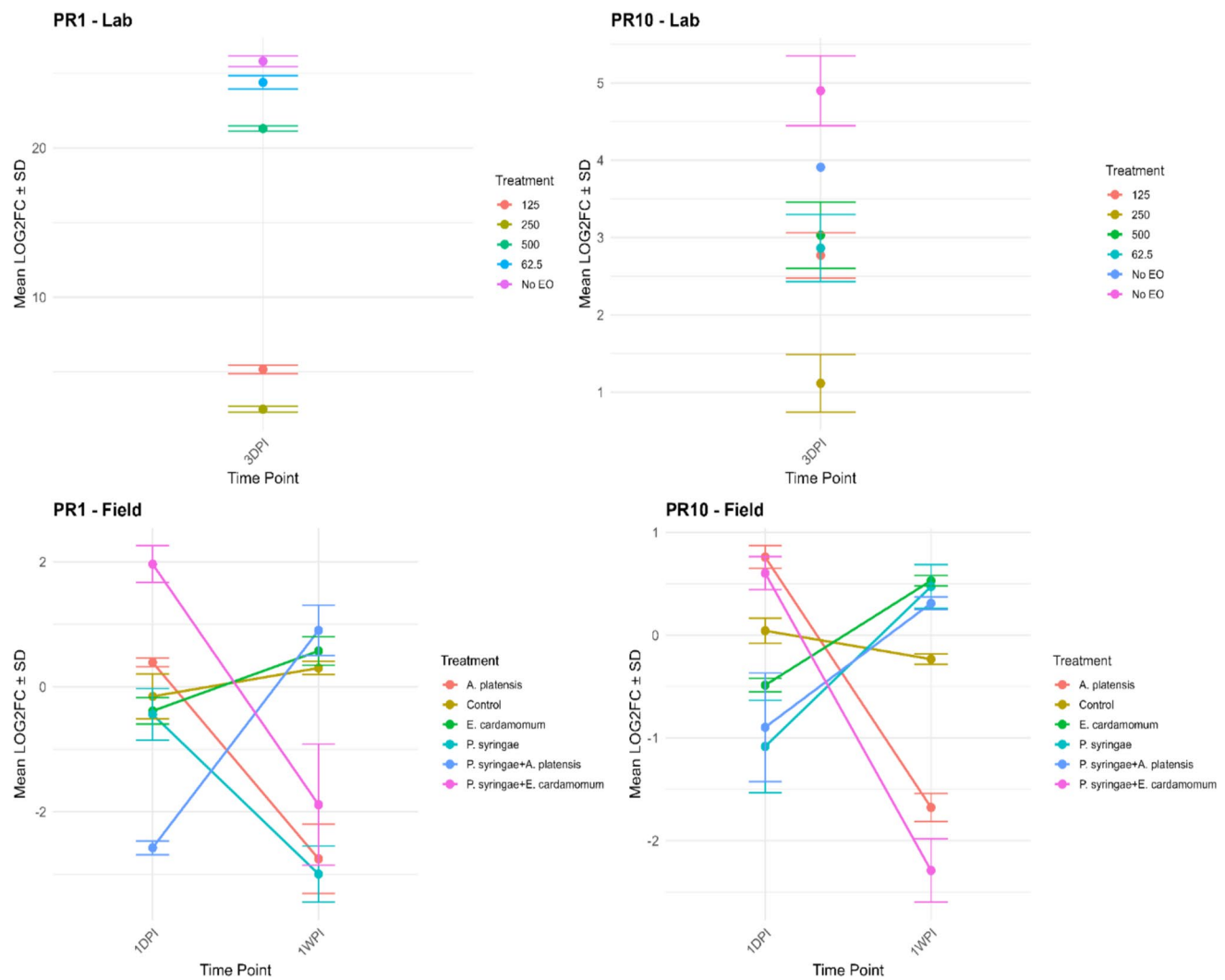


Fig. 3 Time-course expression profiles of PR1 and PR10 under laboratory (top two line graphs) and field conditions (bottom two line graphs)

suppression but also the direction and trajectory of gene responses.

PR1 and PR10 expression under field conditions at 1 dpi and 1 wpi, illustrating variable responses influenced by pathogen challenge and environmental factors (Fig. 3, bottom graphs). Expression values represent $\log_2$ fold-change relative to the pre-treatment baseline (0). PR1 and PR10 expression at 3 dpi under laboratory conditions, showing dose-dependent induction by *E. cardamomum* and consistent activation by *A. platensis* (Fig. 3, top graphs). Under field conditions, expression patterns at 1 dpi and 1 wpi were more variable. PR1 and PR10 often showed early induction (1 dpi) followed by strong suppression at 1 wpi in pathogen-stressed plants, whereas *A. platensis* and *E. cardamomum* alone produced mild increases at 1 dpi but inconsistent responses later. This demonstrates that natural environmental fluctuations reduce the stability and amplitude of PR-gene responses compared to laboratory settings.

Under laboratory conditions (Fig. 3, top two line graphs), both PR1 and PR10 showed a clear induction at 3 dpi, but the magnitude and pattern differed between treatments. *E. cardamomum* produced a dose-responsive increase, with high concentrations generating the strongest PR1 activation. *A. platensis* also enhanced both PR1 and PR10, confirming that laboratory-controlled conditions favor rapid and stable gene activation within 72 h post-treatment.

Heatmap visualization strengthened these observations (Fig. 4). The laboratory heatmap (left panel) displayed more uniform and strong induction patterns for both genes, whereas the field heatmap (right panel) showed greater heterogeneity between treatments and time points. Together, the time-course graphs and heatmaps confirm that gene expression responses are more consistent and strongly expressed under controlled laboratory conditions, while field responses display broader variability due to environmental stress interactions.

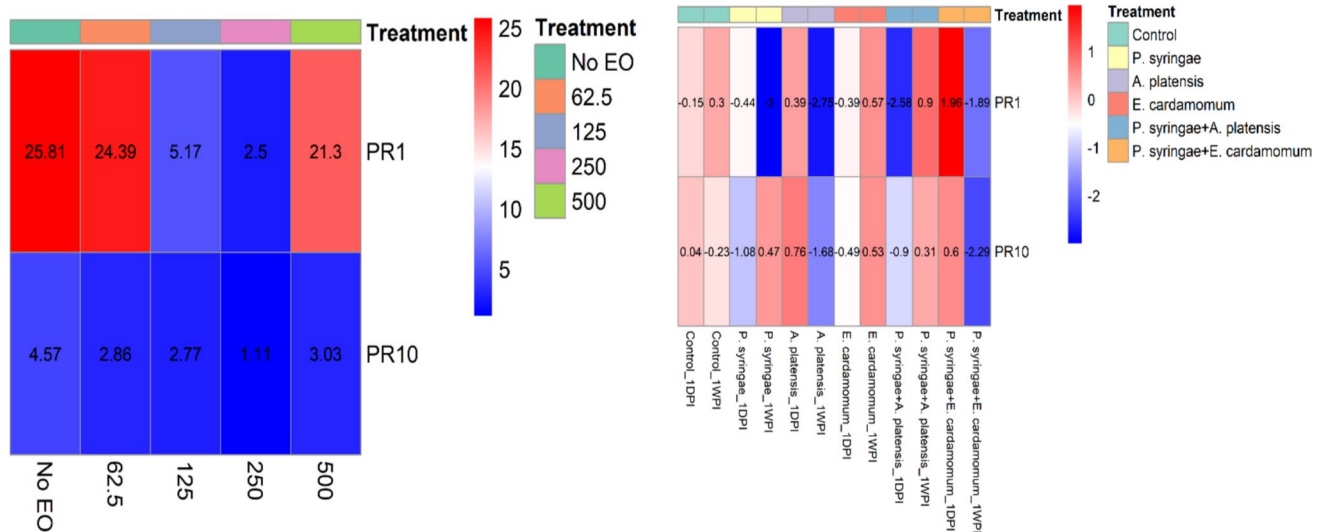


Fig. 4 Heatmap visualization of PR1 and PR10 expression under laboratory and field conditions. Left panel: Laboratory heatmap showing consistent induction patterns for both PR genes at 3 dpi across tested treatments. Right panel: Field heatmap displaying heterogeneous

expression at 1 dpi and 1 wpi, reflecting the combined effects of treatment and environmental variability. Color intensity represents scaled $\log_2$ fold-change values

Discussion

In present study, treatment of tomato plants with a 5% water extract of *A. platensis* led to a reduction in the infection severity caused by *P. syringae*, consistent with the protective effects reported in previous studies. However, the effect was partial, and the combination of *P. syringae* with *A. platensis* extract did not fully suppress disease symptoms. Compared to this, the *E. cardamomum* showed a stronger antibacterial effect, reducing infection rates more significantly. These findings support the hypothesis that cyanobacteria and essential oils can enhance plant resistance but highlight the need for their combined or integrated use for more effective biocontrol of phyto-pathogens. Overall, this research supports the growing body of evidence that bio-based treatments can contribute to sustainable plant protection strategies.

Due to the growing concerns about the environmental and health impacts of synthetic pesticides, there is increasing interest in effective and eco-friendly alternatives (Mexicano-Santoyo et al. 2024). Algae, cyanobacteria, and essential oils represent promising sources of natural bioactive compounds with antimicrobial properties. Extracts from *Sargassum wightii* were effective against *P. syringae* and *Xanthomonas oryzae* pv. *oryzae*, which are responsible for different crop diseases (Arun Kumar et al. 2005; Kumar et al. 2008). Soaking cotton seeds in *S. wightii* extract or spraying seedlings with it enhanced resistance to *Xanthomonas campestris* pv. *malvacearum*, leading to a reduction in disease incidence (Raghavendra et al. 2007). *Ascophyllum nodosum* extracts decreased disease severity in plants affected by *P. syringae*

and fungal pathogens (Jayaraj et al. 2008). Previous research showed that essential oils like *Mentha pulegium*, *Thymus vulgaris*, and *Eucalyptus globulus* have antibacterial effects against *P. syringae*, supporting their use as bio-pesticides. In contrast, *Cedrus atlantica* showed no effect. These findings are consistent with the results of the present study, where essential oil treatments reduced infection and modulated defense gene expression in tomato plants (Sabir et al. 2017).

The expression profiles of *Sol_1_PR_10_4.02* and *SL_PR1a2* in tomato demonstrated distinct yet complementary defense responses to *P. syringae*, *A. platensis*, *E. cardamomum*, and their combinations. In field grown plants, both genes showed transient activation followed by suppression or delayed recovery, with the strongest induction observed in *P. syringae* combined with *E. cardamomum* or *A. platensis*, particularly for *SL_PR1a2*. Laboratory validation using fruit tissues further demonstrated dose-dependent induction of defense genes in response to cardamom essential oil, with *SL_PR1a2* showing higher sensitivity even at lower essential oil concentrations. The relatively stable expression observed in control plants indicates that PR gene activation is primarily stress-triggered, consistent with the role of pathogenesis-related genes as inducible defense markers (van Loon 1985). Under *P. syringae* treatment, PR gene expression remained stable initially but increased over time, indicating a delayed defence response. This pattern is consistent with previous reports describing time-dependent PR10 induction following bacterial challenge (Chun and Chandrasekaran 2019; Carreras et al. 2024). The delayed response may be attributed to the role of PR10 proteins, which enhance plant defence through ribonuclease activity (Choi et al. 2012).

Treatment with *A. platensis* elicited early induction of PR gene expression followed by gradual suppression, potentially reflecting feedback regulation within defense signaling networks. Similar transient PR10 activation patterns have been observed during fungal infections (Pulla et al. 2010). Early synthesis of PR proteins during pathogen exposure followed by transcriptional downregulation may represent a regulatory strategy to balance defense activation with metabolic cost, a phenomenon associated with systemic acquired resistance regulation (Lee et al. 2012).

The response of defense genes to essential oil concentration shows a non-linear pattern. Moderate elicitor concentrations tended to produce stronger defense activation, whereas excessive concentrations may induce transient suppression, possibly due to receptor saturation or mild phytotoxic stress (Eisfeld et al. 2022). The early induction of PR1, PR2, and PR5 following salicylic acid treatment and nematode infection is consistent with the strong *SL-PR1a2* activation observed in the *E. cardamomum* + *P. syringae* treatment, supporting the role of PR1 as a marker of salicylic acid-mediated signaling pathways (Molinari et al. 2014).

Essential oils like *E. cardamomum* modulate defense gene expression through multiple mechanisms. At moderate doses, they act as signaling elicitors, while at high concentrations, they can transiently suppress or inhibit gene expression due to phytotoxic stress (Jebur et al. 2025). Similar regulatory effects have been reported for thyme and rose essential oils, which enhanced PR gene expression and reduced disease severity in tomato plants (Saltos-Rezabala et al. 2022; Kaneko et al. 2024). These studies support our observation that *E. cardamomum*, when paired with *P. syringae*, effectively induces PR-mediated defense in a concentration-dependent manner.

Elettaria cardamomum treatment caused fluctuations in PR gene expression, likely due to its effects on membrane permeability and stress signalling pathways. When combined with *P. syringae*, the *E. cardamomum* showed a delayed upregulation, indicating a synergistic effect. These observations are consistent with reports that plant-derived essential oils can prime the plant immune system, enhancing defence responses. Similarly, combined treatment with *P. syringae* and *A. platensis* resulted in early activation of PR gene expression, followed by suppression, resembling findings in strawberry plants, where PR10 genes were induced early and subsequently downregulated (Besbes et al. 2019). Fluctuations in PR10 expression have also been associated with oxidative stress responses in plums during pathogen infection, which may help explain the temporal patterns observed in our study (El-kereamy et al. 2009). Overall, the results indicate that PR gene expression is dynamic and stress-dependent, with an early induction linked to pathogen recognition and later suppression indicative of a transition to systemic acquired resistance. Our field data further revealed

early activation of *PR10* and *PR1* homologues followed by a decline, which is consistent with transcriptomic evidence showing temporal modulation of defense pathways during pathogen invasion. For example, *Fusarium oxysporum* infection triggers early enrichment of phenylpropanoid and flavonoid biosynthesis genes, followed by regulatory downscaling as defense responses stabilize (Li et al. 2023). Microbial volatiles stimulate early defense activation in tomato, accompanied by later normalization of expression levels (Thangaraj et al. 2023).

Overall, the findings suggest that *E. cardamomum* essential oil can modulate PR-mediated defense signaling in tomato plants in a concentration and tissue-dependent manner. The combined application of essential oil and pathogen challenge may enhance defense gene activation, although the magnitude and duration of response appear to depend on treatment conditions. Despite the promising findings, this study has certain limitations that should be acknowledged. The analysis was restricted to a subset of defense-related genes, which may not capture the full complexity of plant immune responses. Transcriptional data were not complemented by protein or metabolite validation, which limits direct inference of functional outcomes. Future studies should incorporate protein and metabolite analyses to better link gene expression to defense activity, and assess the durability and consistency of elicitor-induced resistance under diverse environmental and field conditions to support practical applications.

Conclusion

This study demonstrates that PR1 and PR10 gene expression in tomato is modulated by treatments with *A. platensis* and *E. cardamomum*, with response patterns influenced by concentration, timing, and environmental conditions. Laboratory experiments showed more stable and dose-dependent induction, whereas field responses were more variable. Combined pathogen–elicitor treatments produced dynamic regulatory responses characterized by early activation followed by transcriptional downregulation. Overall, the results suggest that natural elicitors may enhance tomato defense signaling, although their effectiveness is more reliable under controlled conditions. Further studies are needed to optimize application strategies for practical crop protection.

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Experimental layout and design; Resources; Data interpretation; Funding acquisition; Supervision. A.J. (Ali Jabran) – Writing – Review & Editing. O.P. (Oleg Paulen) – Experiment organization; Writing – Review & Editing. Z.B. (Zhaojun Ban) – Writing – Review & Editing.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article. Any further information or data can be made available from the corresponding author Jana Žiarovská upon reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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