



Soil-mediated Rhizobacteria Modulate SA-JA Transcriptional Antagonism in Tomato to Enhance Systemic Resistance Against a Specialist and a Generalist Herbivore

Muhammad Wajid Javed¹ · Jam Nazeer Ahmad¹ · Samina Tanwir² · Ahmad Nawaz³

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Abstract

Salicylic (SA) and jasmonic acid (JA) defense pathways antagonize each other to compromise host plant defense. Modulation of SA-JA transcriptional antagonism could enhance plant resistance against herbivores. Tomato, *Solanum lycopersicum*, was treated with phytohormonal inducers BTH (Benzothiadiazole-SA synthetic analogue) and MeJA (Methyl jasmonate-JA analogue) through foliar applications at 0, 0.5, and 1 mM, while PGP rhizobacteria (*Bacillus* sp. Mb01 and *Pseudomonas* sp. Mb05) were applied through roots using a randomized block design having three replications. RNA transcripts of SA responsive genes (*PR1a* and *PAL*) and JA eliciting genes (*PIN2* and *LOX*) were studied. Individual application of SA/JA synthetic analogues improved the levels of their respective marker genes but generated an antagonism by masking each other's expression. Conversely, individual and combined treatment with rhizobacteria modulates SA/JA antagonism to enhance the expression of SA marker genes under MeJA treatment and of JA marker genes under BTH treatment. This improvement was tested against a specialist (whitefly, *Bemisia tabaci*) and a generalist herbivore (tomato fruit borer, *Helicoverpa armigera*) to assess enhanced pest resistance. Although SA and MeJA reduced the mean larval weight, larval survival, and adult emergence of *H. armigera* along with the mean nymph population of *B. tabaci*, the results were more pronounced when inducers were applied along with rhizobacteria. Instead of separate applications of BTH/MeJA, their combined application with PGPR is recommended to improve hostplant resistance against phytophagous pests. However, investigations on other SA and JA eliciting genes, PGPR species, and pests are required to implement these findings in a broader crop -pest spectrum.

Keywords Defense signalization · Lipxygenase · Pathogenesis-related proteins · Phenyl ammonia lyase · Proteinase inhibitor

1 Introduction

Plants have evolved a complex system of defense against invading herbivores (Kameswaran et al. 2024). Salicylic acid (SA) pathway produces systemic acquired resistance (SAR), and jasmonic acid (JA) defense pathway activates induced systemic resistance (ISR) in plants (Ahmad et al. 2014; Wang et al. 2025a, b). *PR1a*-pathogenesis-related proteins and *PAL*-phenyl ammonia lyase are marker genes for the SA defense pathway, whereas *PIN2*-proteinase inhibitor2 and *LOX*-lipoxygenase are the marker genes for the JA defense pathway in plants (Veselova et al. 2024; Kaleh et al. 2025). *PAL* synthesizes SA through the phenylpropanoid pathway, while *LOX* produces JA through the oxophytodienoic acid biosynthetic pathway (Kamalanathan et al. 2023).

✉ Ahmad Nawaz
a.nawaz1@squ.edu.om
Muhammad Wajid Javed
muhammadwajidjaved@gmail.com
Jam Nazeer Ahmad
jam.ahmad@uaf.edu.pk

¹ Department of Entomology, University of Agriculture, Faisalabad 38000, Pakistan

² Department of Botany, University of Agriculture, Faisalabad 38000, Pakistan

³ Department of Plant Sciences, College of Agricultural and Marine Sciences, Sultan Qaboos University, Al-Khoud 123, Muscat, Oman

In a plant system, both pathways obscure the expression and response of each other to generate a crosstalk, which ultimately compromises host plant defense (Ahmad et al. 2013; Parvez et al. 2024; Javed et al. 2025a, c). A defense-compromised plant is the first target of a herbivore. Herbivores manipulate host plant defense for their survival and successful growth (Zarate et al. 2007; Javed et al. 2025a, d).

Tomato, *Solanum lycopersicum* L. (Solanaceae: Solanales), is the emerging model plant to explore herbivore-plant interactions. This is attacked by a specialist as well as a generalist herbivore (Morin et al. 2024; Singh et al. 2025). Whitefly, *Bemisia tabaci* (Aleyrodidae: Hemiptera), is an invasive specialist herbivore and causes heavy economic loss by direct phloem feeding and indirectly by viral transmission (Idrees et al. 2024). This pest overcomes host plant defense through manipulation of the SA-JA signaling network (Kaleh et al. 2025). The tomato fruit borer, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a generalist herbivore (~280 host plants) infesting more than 70 plant families (Singh et al. 2025). It is present in African, American, Australian, Asian, and European continents and causes an economic loss of 5 billion US dollars (Usman et al. 2013; War et al. 2015; Singh et al. 2025). The infestation of this parasite may cause a yield loss of 53% in tomatoes (Usman et al. 2013).

Both herbivores have developed a strong interaction with host plants for their survival and to struggle against plant defense (Zarate et al. 2007; Idrees et al. 2024). To encounter this problem, plants utilize their hormone-based regulatory network to function against *B. tabaci* and *H. armigera*, where SA and JA are important defense signaling molecules (War et al. 2015; Morin et al. 2024).

SA is a plant signaling molecule, which is important in producing systemic acquired resistance against pests (insects and pathogens), enhancing anti-oxidant systems, mitigating abiotic stresses, and regulating plant growth through including seed germination, flowering, and photosynthesis (Liu et al. 2026; Waseem et al. 2026). While, JA is also a naturally occurring hormones in plants, helping in root development, fruit ripening, seed growth, pollen development (War et al. 2015). Chang et al. (2008) reported the role of JA in trichome formation in tomato plants against herbivores. The JA defense pathway is regulated under *PIN2* expression, which is a well-known protein in plant tissues to inhibit the catalysis of proteolytic enzymes (Kanwal et al. 2024). In this way, it can affect food digestion in herbivores (Veselova et al. 2024). *PIN2* is elicited in plants through foliar application of synthetic analogues, e.g., methyl jasmonate (MeJA) (Ahmad et al. 2014).

Similarly, the SA pathway is elicited in response to its synthetic analogues BTH (Benzo(1,2,3)thiadiazole-7-carbothioic acid-*S*-methyl ester),

where *PR1a*-pathogenesis related protein serves as a molecular marker for resistance induction (Puthoff, 2010; Ahmad et al. 2013; Zhang et al. 2019).

Several studies reported the resistance inducing role of SA and JA to reduce pest abundance on different crops (Zarate et al. 2007; War et al. 2015; Javed et al. 2022, 2025a, c; Kanwal et al. 2024; Chen et al. 2026). For example, Zarate et al. (2007) revealed that application of SA and JA reduced the population of *B. tabaci* and improved host defense in *Arabidopsis thaliana*. War et al. (2011) and (2015) reported that SA and JA treatment efficiently reduced *Spodoptera exigua* and *H. armigera*, respectively, in groundnuts. Javed et al. (2022), (2025a, b) indicated that SA application reduced *Brevicoryne brassicae* in canola in greenhouse and field conditions while affecting several biological fitness parameters. Foliar treatment of SA and JA on maize also reduced the population of *S. frugiperda* and improved different key genes of the SA-JA defense pathway, e.g., *PR1*, *PAL*, *LOX5*, *SPI*, *WRKY28*, and *ICS*.

Plant hormonal crosstalk may appear when the same or different natural plant hormones (i.e., salicylic acid, jasmonic acid, Absciscic acid, Auxins, ethylene) mask or enhance the relative effects of one another (Zarate et al. 2007; Ahmad et al. 2013; Idrees et al. 2024; Irfan et al. 2026). This crosstalk might sometimes be utilized by the herbivores to manipulate the plant defensive mechanisms in their own favor (War et al. 2011; Veselova et al. 2024). However, JA and SA may act to induce indirect plant protection responses as indicated in groundnuts (War et al. 2011, 2015) and tomatoes (Zhang et al. 2019). However, an antagonistic interaction of SA and JA is well documented, where several studies have reported SA-JA crosstalk to compromise host plant defense in response to herbivores (Zarate et al. 2007; War et al. 2011, 2015; Nasab et al. 2019; Zhang et al. 2019; Kanwal et al. 2024; Yan et al. 2025).

Plant hormonal crosstalk is bidirectional (Fàbregas et al. 2026). Plant growth promoting rhizobacteria (PGPRs) may help to modulate this crosstalk, directly or indirectly (Khoso et al. 2024; Wahab et al. 2024). Directly, they act as bio-fertilizers to divert JA for increasing root growth and SA for reducing pest stress (Wahab et al. 2024). Indirectly, they could induce systemic resistance in plants (Khoso et al. 2024). *Bacillus* and *Pseudomonas* are among the most important PGPR genera to induce systemic resistance in plants to suppress different insect pests (Fourneau et al. 2024; Ullah et al. 2024; Irfan et al. 2026).

Yan et al. (2025) used *B. amyloliquefaciens* to suppress *S. litura* in cowpeas. *Fictibacillus solisalsi*, *B. pumilus*, and *B. subtilis* also reduced the population of *S. litura* in maize (Cortez et al. 2024). PGPRs, when applied together with a foliar inducer, improve the efficiency of that inducer. Nasab et al. (2019) applied *P. fluorescens*, *Azotobacter*

chroococcum, and *Azospirillum brasilense* together with humic acid, where *B. brassicae* populations in canola were reduced significantly over their individual application. Kanjana et al. (2026) highlighted the role of microbial plant volatiles to potentiate the impact of PGR to improve herbivore resistance. Authors indicated the priming effect and existence of crosstalk; however, no particular study was conducted to explore its underlying molecular mechanism.

Several other researchers also reported SA-JA crosstalk or antagonism (Zhang et al. 2019; Kanwal et al. 2024; Yan et al. 2025), but no investigation was conducted to modulate this antagonism in favor of plants for enhanced pest resistance. To the best of our knowledge, no study has explored the combined application of PGPRs with BTH/MeJA against specialist and generalist herbivores. In addition, our study investigated the underlying SA-JA defense pathway in response to simultaneous PGPRs and inducers' treatments. So, the study was motivated by the research question of how the combined root treatment of PGPRs and foliar application of BTH/MeJA alter the SA-JA antagonism at the molecular level to improve herbivore resistance in tomato plants?

The study was based on the hypothesis that the combined use of certain PGPRs with phytohormonal inducers may improve pest resistance in tomato by reducing SA-JA antagonism. We integrated foliar inducers with root treatment of PGPRs with an objective to modulate SA/JA antagonism to enhance plant resistance. Both SA and JA were applied at 0-, 0.5-, and 1-mM concentrations individually and in combination with two PGPRs, i.e., *Bacillus* sp. Mb01 and *Pseudomonas* sp. Mb05 in 11 treatments. RNA transcripts of SA and JA eliciting genes, i.e., *PR1a*, *PAL*, *PIN2*, and *LOX*, were measured using real time PCR. Performance of *H. armigera* was recorded in terms of larval weight, larval survival, and adult emergence. The mean population of *B. tabaci* was recorded 3rd, 5th, and 7th days post infestation. This will help to produce more efficient pest-resilient crops under changing climates.

2 Materials and Methods

2.1 Insect Rearing

H. armigera larvae were collected from chickpea and then reared on semi-synthetic chickpea flour diet in an insect growth chamber (temperature: 25 ± 2 °C; relative humidity: $60 \pm 5\%$; photoperiod: 12 hours light and 12 hours dark period). One larva per vial (12 dram: 1.13" x 2.44") was maintained to avoid cannibalism (Kanwal et al. 2024). After pupation, each pupa was sprayed with 2.5% ethanol and

placed in a glass petri dish (dish height 25 mm, lid diameter 150 mm) with a wrap of tissue paper inside. Post emergence, 15 pairs were released per plastic jar (15 cm diameter and 30 cm depth) covered with muslin cloth. Each jar was provided with 15% honey solution-dipped cotton swabs for adult feeding. Tissue paper was provided as an egg-laying substance, which was removed after egg laying (Saleem et al. 2024). Neonates were shifted into individual plastic vials with a piece of artificial diet for further rearing. *H. armigera* 3rd instar larvae were used in the bioassay and gene expression experiments. The 3rd instar was chosen as it be a standard larval stage for studying the toxic impacts of synthetic pesticides.

Middle East Asia Minor whiteflies (*Bemisia tabaci*) (Crossley and Snyder 2020) were collected from different tomato fields. They were nourished on tomato host plants (*Solanum lycopersicum* cv. Ailsa Craig) for more than three years. The Dinsdale et al. (2010) method was used to confirm species purity based on mitochondrial CO1 (cytochrome oxidase) activities. The climatic regimes, 25 ± 2 °C temperature, $65 \pm 5\%$ relative humidity, and 14 light: 10 dark photoperiods were maintained. Bioassay was followed with 4th instar (red-eyed nymphs) of whiteflies, as 4th instar was the final stage before pupation and was easy to identify due to red eyes. Any impact on this stage would have a significant impact on the whitefly population.

2.2 Plant Cultivation

Tomato plants were grown from cultivar Ailsa Craig (*Solanum lycopersicum* L.) procured from University of California, Davis, USA (Tomato Genetics Resource Centre) and maintained at 22 °C night and 28 °C Day ($65 \pm 5\%$ RH). Seedlings were transferred to pots (7 kg: 18 cm diameter and 20 cm height) containing organic matter and sand (80%, 20%) (Ahmad et al. 2014). Irrigation, weeding, and other agronomic practices were followed regularly. About 60-days old plants were used in the insect bioassay experiment. Pots were randomized regularly to avoid any positional effect.

2.3 Preparation of Phytohormonal Inducers

Phytohormonal inducers were screened initially at 0, 0.5, 1-, 1.5-, and 2-mM concentration, where the first three concentrations showed better results. Higher concentrations revealed phytotoxic reactions, e.g., necrosis, stunted growth, leaf burning, and twisting. The 0.5 and 1 mM did not only show a higher defense gene expression but also had no toxic impacts on plants. BTH (2,1,3-Benzothiadiazole, 98%, Sigma-Aldrich,

USA) (Table S1) 0.5- and 1-mM concentrations were produced by dissolving 68.085 and 136.17 mg in 1 L distilled water. Both concentrations of MeJA [3-Oxo-2-(2-pentenyl) cyclopentaneacetic acid, 95%, Merck-Sigma-Aldrich, USA] were solubilized in a solution of 1% ethanol containing 0.1% triton X100 (Ahmad et al. 2014) to form 0.5 (112.115 mg) and 1 mM (224.30 mg) treatments. Handheld sprayer (1 L-Top gun) was used to spray each plant at the rate of 30 ml of solution. The 0 mM BTH was sprayed with distilled water (H₂O) while 0 mM MeJA was treated with 1% ethyl alcohol in 0.1% triton X100 (ethOH) (Ahmad et al. 2013).

2.4 Isolation of PGPRs

Samples of rhizosphere were collected from tomato fields in the University of Agriculture, Faisalabad (UAF). The plant rhizosphere was pulled out with intact plant roots and then covered with plastic bags to store at −4 °C. PGPR extraction was done in the Microbiology Lab of the Department of Agronomy, UAF, using Luria Bertany agar medium (Luria and Burrous 1957). The rhizosphere (12 g) along with distilled water (100 ml) were taken in a conical flask and shaken for 10 min on a rotary shaker. 1 ml suspension was taken into another 10 ml tube and shaken again for 3 min. Then, serial dilutions (10^{-7}) were produced using distilled water. Then 0.1 ml aliquot was spread on LB agar media, which was incubated at 28 °C for 3 days. Bacterial colonies were noticed over the streaks. The well-produced colonies were picked, restreaked, and incubated on fresh LB media plates. The procedure was repeated three times. A single colony culture was coded and stored for later use (Akhtar et al. 2016).

2.5 PGPR Treatment

For rhizobacteria, *Bacillus* sp. Mb01 and *Pseudomonas* sp. Mb05 suspensions were prepared at an optical density of 0.2 (650 nm; 2.6×10^7 CFU mL⁻¹) (Kurabachew and Wydra 2014). Before planting, the roots of seedlings were submerged in the bacterial solution (50 ml) for the whole night, and later on, the bacterial solution was diluted to 200 ml and applied to the pot's soil (Valenzuela-Soto et al. 2010). Untreated control plants were applied with sterile water. In combined treatments, phytohormones were applied to leaves and PGPRs through roots. Only the best performing treatments were followed in combined treatments, i.e., 1 mM BTH and 0.5 mM MeJA from inducers and PGPR2 (*Pseudomonas* sp. Mb05) from PGPR treatments (Table S2).

2.6 Insect Performance Experiment

2.6.1 *Helicoverpa armigera*

Six larvae per plant (on top, middle, and lower leaves) were released on each plant, which was covered with a wooden cage (60 × 60 × 90 cm³). For insect performance experiments, plants were treated with phytohormonal inducers and PGPRs a day before larval infestation. The weight of each larva was recorded on an electronic weighing scale before releasing it on each plant. Later on, the larvae were allowed to feed for 3 days and were taken again to measure larval weight. Then larvae were placed on the same plants and allowed to feed for 5 and 7 days, and larval weights were measured for 5th and 7th days post infestation (DPI). In addition, larval survival was also recorded till they transformed into pupae. Adult emergence out of pupae was also recorded on treated and control plants. Each reading was taken from three independent technical replications.

2.6.2 *Bemisia tabaci*

After treatment, the test plants were infested with 80–100 whiteflies per plant via gentle aspiration. Adults were removed after 3 days of introduction to have sufficient egg laying. Later on, nymphs that emerged from eggs ($n=7$ per replication) were allowed to transform into 4th instar (red-eyed nymphs). Mean populations of 4th instar were recorded at 3, 5, and 7 DPIs. Three technical replications were tested for each treatment.

2.7 Molecular Studies

2.7.1 Leaf Sampling

For gene expression analysis, non-infested plants were treated with phytohormonal inducers for 24 h to induce systemic resistance (SAR and ISR) in plants. PGPR was applied through the root drenching method. Later on, leaf samples were taken and frozen in liquid nitrogen, and stored at −80 °C until further use. Leaves were collected from 5 plants to form one biological replicate. Three replications were followed independently in each experiment. Correspondingly, leaves from respective positions on control plants were also plucked, frozen, and stored.

2.7.2 Total RNA Isolation

Total RNA from leaves of control and treated tomato plants was extracted using TriZol LS-Reagent® (Invitrogen-Carlsbad, MI, USA). Concisely, collected leaf samples (0.3 g)

were finely pulverized in liquid nitrogen. The crushed material was mixed properly in TriZol LS-Reagent®, and isolation was performed following the manufacturer's protocol. The nucleic acids were dissolved in sterile deionized water (15 µL). RNAs (10 µg) from leaf portions were treated with RQ1 RNase-Free DNase (15 units) (Promega, Madison, WI, USA) for 1 h (37 °C) in the presence of 1X reaction buffer (Promega, Madison, WI, USA), keeping the final volume of 50 µL. The nucleic acid was quantified by spectrophotometry at $\lambda=260$ nm.

2.7.3 cDNA Synthesis

The cDNA was synthesized using the cDNA synthesis kit. The procedure was followed from Ahmad et al. (2013). The cDNA was synthesized from RQ1-DNase-treated RNA (1 µg) from treated and non-treated leaves through oligodT18 primers (5 µM) and Superscript-II Reverse Transcriptase (200 units) (Invitrogen, Carlsbad, USA) in a reaction mix (25 µL) having RNase Out (40 units) (Invitrogen), DTT (10 mM), and dNTPs (20 µM). Before the addition of reverse transcriptase and other ingredients, RNA+oligodT18 was denatured for 5 min (65 °C). A control treatment having no reverse transcriptase was also followed to verify DNase treatment efficacy.

2.7.4 Real Time PCR

The nucleotide sequence of the desired DNA of defense genes (*PR1a*, *PAL*, *LOX*, and *PIN2*) was determined from Gene-Bank (<http://www.ncbi.nlm.nih.gov/>). The primer sequence designs were ordered from the company MWG Biotech AG (Ebersberg, Germany). The primers of defensive genes of demand (Table S3) were used for amplification through a gradient thermal cycler (Peqlab, Germany). Real Time PCR cycle was comprised of 45 steps of denaturation at 95 °C for 15 min (each step of 20 s), 45 s annealing step at different ranges of annealing temperatures, and 45 s elongation step (72 °C) for 80 cycles. Each sample was followed by three replications. Primers' efficiencies were estimated using CFX 96 Bio-Rad software through serial dilutions of PCR amplicons in ten-fold (no dilution, 0.1, 0.01, 0.001, and 0.001%), which were recorded between 96 and 110%.

2.7.5 Relative Gene Expression Measurement

Values for relative gene expression (RGE) were calculated using the method of Ahmad et al. (2013). RGE value of less than 1 implies gene inhibition or down-regulation, whereas values greater than 1 show gene activation or up-regulation compared to control genes.

2.8 Statistical Data Analysis

Data were initially tested for assumptions of ANOVA for normal distribution using the Shapiro-Wilk test and for homoscedasticity using Bartlett's test, respectively, for each dataset. Values from three independent replications were averaged, and standard errors (SE) were calculated. They were log transformed using $\log(x)$, where necessary. The statistical significance of gene expressions, larval survival, and percentage of adult emergence in MeJA, BTH, PGPRs treated and non-treated plants was subjected to one-way analysis of variance, and means were compared using the Tukey HSD test ($\alpha=0.05$). However, the data on the mean larval weight of *H. armigera* and population emergence of *B. tabaci* was analyzed using repeated-measure (RM) two-way ANOVA. Sphericity was also tested as an additional assumption of RM ANOVA. Statistical values for fixed and random factors, i.e., treatment, time, and subject, respectively, were calculated along with interaction effects. GenStat, Edition 21 st (VSN International Limited, England & Wales) was used for data analyses.

3 Results

3.1 Responses of Salicylic Acid Marker Genes

Relative expressions of *PR1a* and *PAL* RNA transcripts showed statistically significant improvement in response to inducers and PGPRs application (*PR1a*: $F=562.3$, $p<0.001$; *PAL*: $F=721.7$, $p<0.001$) (Figs. 1 and 2). Application of 0.5 and 1 mM BTH upregulated the expression of RNA transcripts for *PR1a* to 2.99 and 3.63 folds over the control (*EF1a*), while the same levels of MeJA downregulated the expression by 0.77 and 0.79 folds (Fig. 1). Root treatment of *Bacillus* sp. Mb-01 (PGPR1) upregulated *PR1a* by 1.93 folds and *Pseudomonas* sp. Mb-05 (PGPR2) to 2.23 folds. When *Pseudomonas* sp. Mb-05 was applied in combination with either 1 mM BTH or 0.5 mM MeJA; it upregulated the expression of *PR1a* by 3.05 and 1.17 folds, respectively, which was downregulated to 0.77 under the individual application of MeJA (Fig. 1).

Besides, the highest upregulation of *PAL* RNA transcripts was recorded in 1 mM BTH (3.41 folds) (Fig. 2). The 0.5 mM BTH also upregulated its expression by 2.39 folds over the control *EF1a* gene. Conversely, application of MeJA again suppressed and downregulated the *PAL* expression to 0.95 folds (0.5 mM) and further to 0.69 (1 mM). Conversely, PGPR root priming upregulated its expression by 2.13 folds (Mb-01) and 2.27 (Mb-05). Application of rhizobacteria along with phytohormonal inducers improved SA

Fig. 1 Transcriptional gene expression (mean $\pm$ SE, $n=3$) of *PR1a* (acidic-Pathogenesis related protein) in response to the application of phytohormonal inducers (BTH: Benzothiadiazole and MeJA: Methyl Jasmonate) and rhizobacteria (PGPR1: *Bacillus* sp. Mb-01 and PGPR2: *Pseudomonas* sp. Mb-05). Control represents the expression of *EF1a* (Elongation Factor-1 alpha). ANOVA results were significant following the Tukey test (**: $p<0.01$, ***: $p<0.001$)

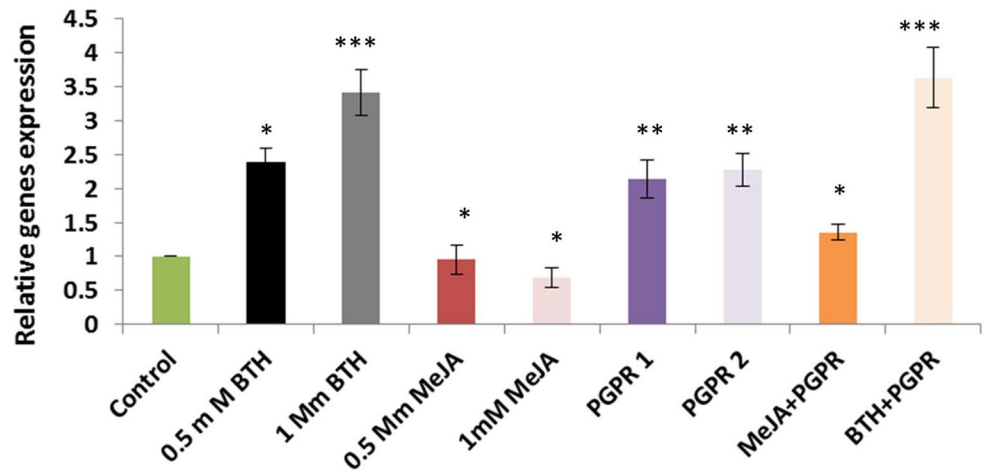


Fig. 2 *PAL* (Phenyl Ammonia Lyase) RNA transcripts relative expression (mean $\pm$ SE, $n=3$) in comparison to the control *EF1a* gene when tomato plants were treated with BTH: Benzothiadiazole, MeJA: Methyl Jasmonate, and rhizobacteria (PGPR1: *Bacillus* sp. Mb-01 and PGPR2: *Pseudomonas* sp. Mb-05). ANOVA results were statistically significant as *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$

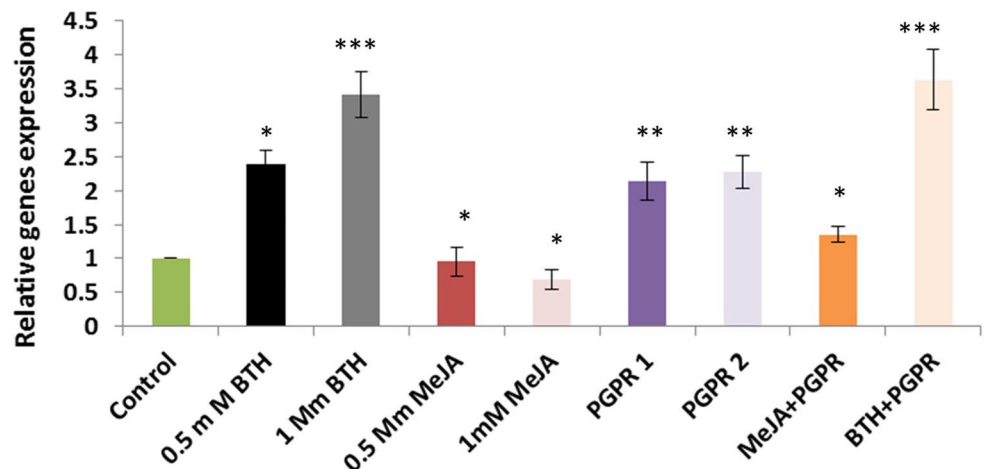
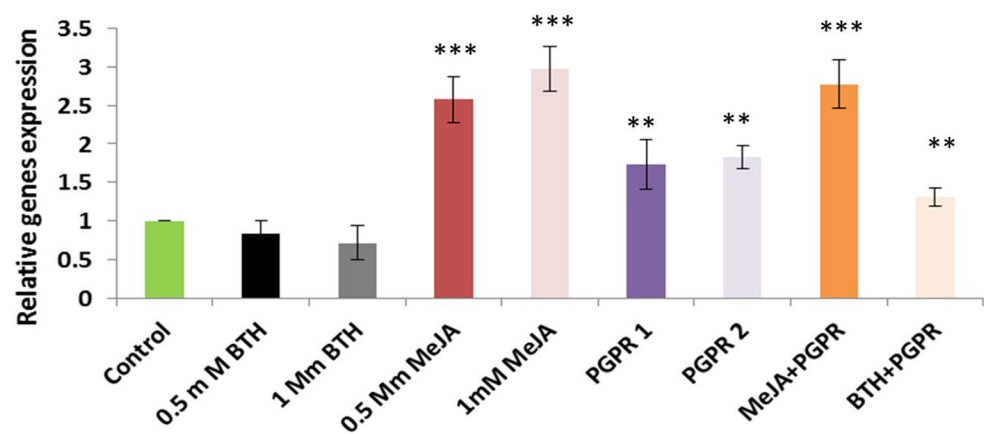


Fig. 3 RNA transcripts expression (mean $\pm$ SE, $n=3$) of *PIN2* (Proteinase inhibitor) in response to Benzothiadiazole (BTH), Methyl Jasmonate (MeJA), and rhizobacteria (PGPR1: *Bacillus* sp. Mb-01 and PGPR2: *Pseudomonas* sp. Mb-05) over the control *EF1a* gene. Results were significant (**: $p<0.01$, ***: $p<0.001$)



defense further and enhanced *PAL* expression to 3.62 folds (BTH) and 1.35 folds (MeJA) (Fig. 2).

3.2 Jasmonic Acid Marker Genes

PIN2 expression was affected significantly ($F=901.5$, $p<0.001$) under the inducers as well as PGPR treatments (Fig. 3). Both concentrations of MeJA upregulated *PIN2* expression (0.5

mM: 2.97, 1 mM: 2.57 folds) while BTH downregulated it (0.5 mM: 0.77, 1 mM: 0.64 folds). Treatment of PGPRs Mb-01 and Mb-05 upregulated the gene expression by 1.73 and 1.83 folds. Simultaneous application of bacterium with inducers provided promising results and modulated the hormonal antagonism to improve *PIN2* expression (1.12 folds) under BTH treatment. Its expression was enhanced substantially (2.77 folds) when the bacterium was applied in combination with MeJA (Fig. 3).

Fig. 4 Relative gene expression (mean $\pm$ SE, $n=3$) of *LOX* (Lipoxygenase) RNA transcripts under BTH (Benzothiadiazole), MeJA (Methyl Jasmonate), and PGPR treatments (*Bacillus* sp. Mb-01 and *Pseudomonas* sp. Mb-05). *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$ indicated statistically significant response

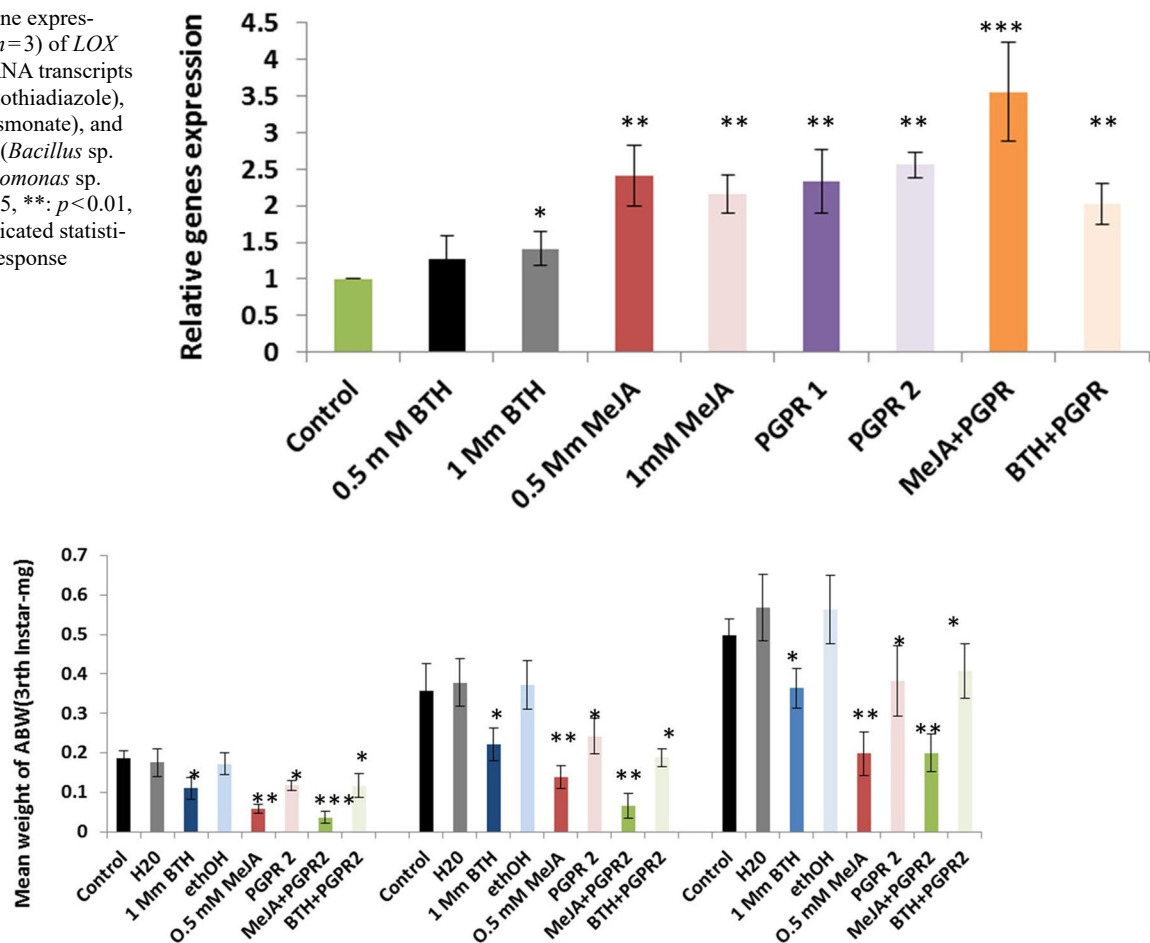
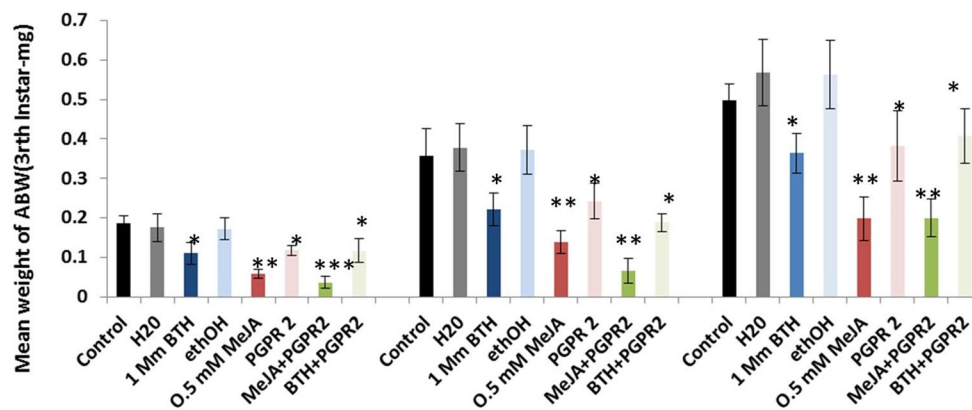


Fig. 5 Mean $\pm$ SE larval weight (mg) of ABW (American Bollworm, i.e., *Helicoverpa armigera*) 3rd instar ($n=18$) on tomato plants under different phytohormonal and PGPR inducers where H₂O is distilled water, ethOH: 1% ethanol and 0.1% triton X100, BTH: Benzothiadiazole, MeJA: Methyl Jasmonate, and PGPR2: *Pseudomonas* sp. Mb-05. Larval weight gain was recorded at 3-, 5-, and 7-days post infestation (left to right) where repeated measure Two-way ANOVA results were statistically significant (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$)



Expressions of *LOX* RNA transcripts were also affected significantly in response to applied treatments ($F=1752.3$, $p<0.001$) (Fig. 4). The 0.5 mM MeJA revealed the highest upregulation (2.51 folds), followed by 1 mM (2.41 folds). BTH also improved its expression slightly in 0.5 mM (1.27 folds) and a bit further in 1 mM (1.41 folds). Mb-01 as well as Mb-05 upregulated *PIN2* expression by 2.33 and 2.56 folds, respectively, and the results were outstanding when Mb-05 was applied with MeJA (3.55 folds) (Fig. 4).

3.3 Insect Performance Assay

Effects of PGPR-modulated antagonism were investigated on the performance of a generalist herbivore *H. armigera* in terms of gain in larval weight, larval survival, and emergence of healthy adults. Effects of time ($F=327.7$, $p<0.001$), treatments ($F=364.3$, $p<0.001$), time $\times$ treatment ($F=104.2$, $p<0.001$) were statistically significant (Fig. 5). After the

3rd DPI, the lowest mean larval weight was shown when MeJA was applied in combination with Mb-05, followed by 0.5 mM MeJA. Interestingly, the Mb-05 combination with BTH did not cause a significant reduction in larval weight, and the results were similar to the individual application of 1 mM BTH. Application of distilled water and ethanol also caused a slight reduction in larval weight gains.

On the 5th DPI, results were more suppressive in Mb05+MeJA, followed by 0.5 mM MeJA, Mb05+BTH, and 1 mM BTH. While on the 7th DPI, the results of MeJA were similar to those of Mb05+MeJA. Individual application of 1 mM BTH appeared a bit suppressive; however, its combined application with Mb-05 did not produce any additional benefit (Fig. 5).

Later on, those larvae were allowed to feed, and their survival was noted, which showed a statistically significant response ($F=117.4$, $p<0.001$) (Fig. 6). Individual application of BTH and rhizobacteria reduced the larval survival. Combined treatments also reduced larval survival a little

Fig. 6 Mean survival $\pm$ SE of 3rd larval instar (%) ($n=18$) of *Helicoverpa armigera* on treated and non-treated plants (H₂O: Distilled water, ethOH: 1% ethanol and 0.1% triton X100, BTH: Benzothiadiazole, MeJA: Methyl Jasmonate, and PGPR2: *Pseudomonas* sp. Mb-05). ANOVA results were statistically significant followed by Tukey test (*: $p<0.05$, **: $p<0.01$, ***: $p<0.001$)

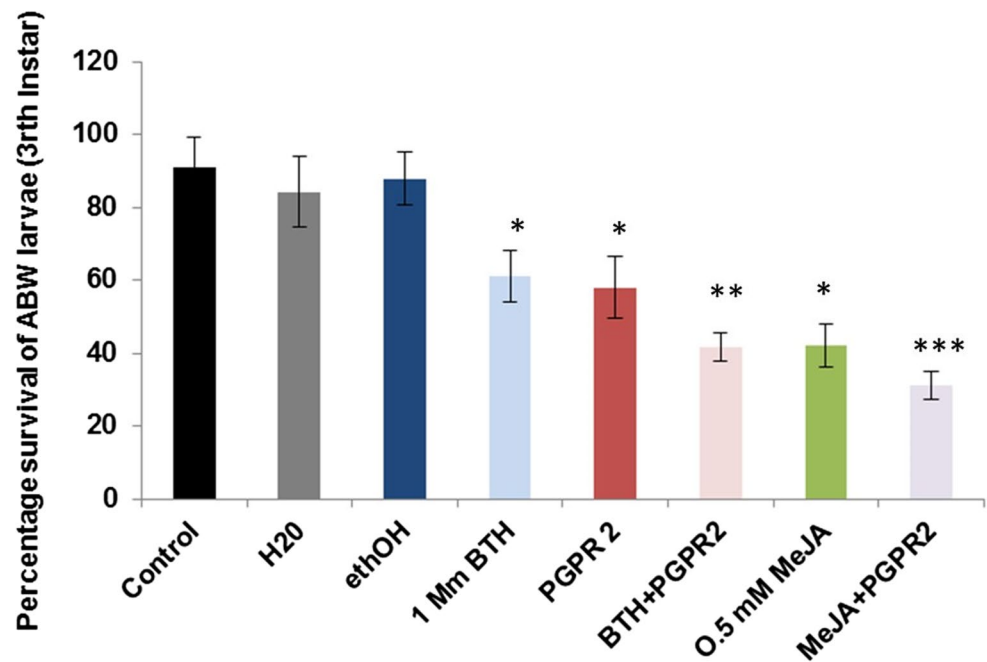
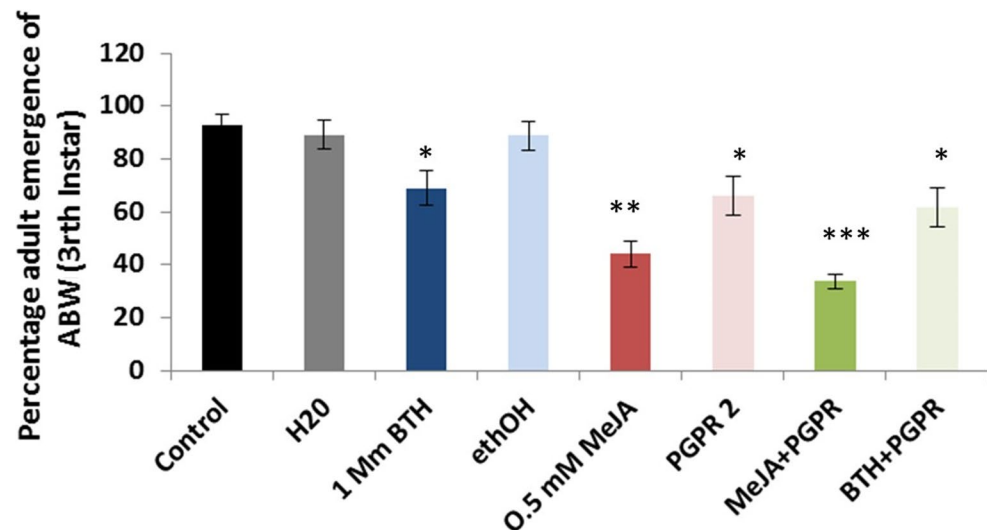


Fig. 7 Percentage adult emergence $\pm$ SE ($n=18$) of *Helicoverpa armigera* moths under H₂O: Distilled water, ethOH: 1% ethanol and 0.1% triton X100, BTH: Benzothiadiazole, MeJA: Methyl Jasmonate, and PGPR2: *Pseudomonas* sp. Mb-05 treatments. ANOVA results following Tukey test were significant at *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$



further, where the best results were obtained in MeJA+Mb05 treatment (Fig. 6).

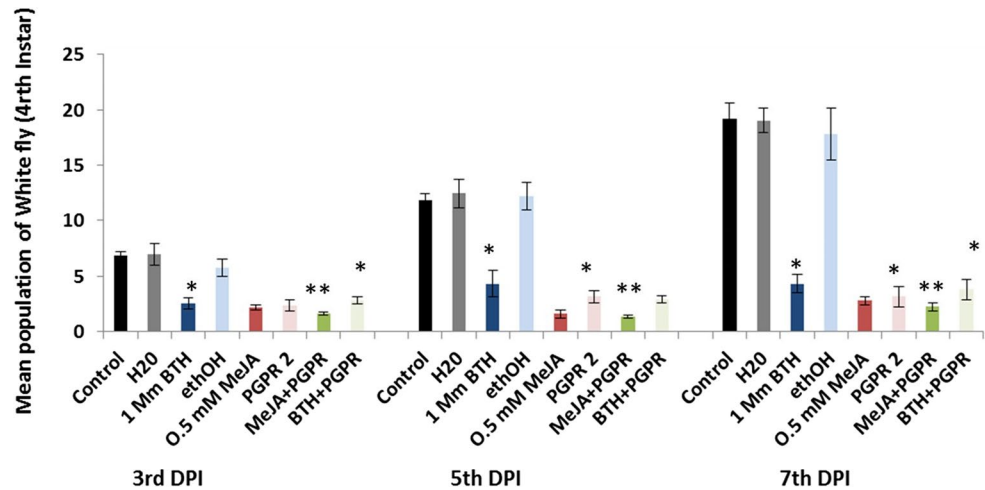
When those larvae were pupated, and adults began to emerge, the individual and combined application of MeJA showed a statistically significant ($F=195.9$, $p<0.001$) impact on adult emergence (Fig. 7). BTH and rhizobacteria, although reduced the adult emergence significantly, but the results were more additive when BTH was applied following rhizobacterium application. The lowest response was recorded in MeJA individual and combined treatments (Fig. 7).

Application of phytohormonal inducers and PGPRs also manipulated SA-JA antagonism against a specialist herbivore (*B. tabaci*), where statistically significant impacts were shown due to time ($F=135.7$, $p<0.01$), treatments

($F=251.7$, $p<0.001$), time $\times$ treatments ($F=89.1$, $p<0.01$) (Fig. 8). Three-days post infestation, 1 mM BTH and 0.5 mM MeJA reduced the mean nymph population equally; however, the response of MeJA was more effective, followed by Mb-05 treatment.

On the other hand, at 5th DPI, results of 0 mM BTH and MeJA, i.e., distilled water and 1% ethanol, were similar to the control. The MeJA+Mb-05 reduced the nymph population the most, followed by MeJA, BTH+Mb-05, BTH, and Mb-05. Similarly, the mean population began to improve at 7th DPI as the resistance induced by phytohormonal inducers began to reduce. However, BTH was still effective to reduce *B. tabaci* population in individual and combined applications with Mb-05. The 0.5 mM MeJA was the most efficient treatment, followed by MeJA+Mb-05 (Fig. 8).

Fig. 8 Mean population $\pm$ SE ($n=21$) of *Bemisia tabaci* nymphs (4th instar) on tomato plants under H₂O: Distilled water, ethOH: 1% ethanol and 0.1% triton X100, BTH: Benzothiadiazole, MeJA: Methyl Jasmonate, and PGPR2: *Pseudomonas* sp. Mb-05. Responses were observed at 3-, 5-, and 7-days post infestation where repeated measure two way-ANOVA results were statistically significant at *: $p<0.05$, **: $p<0.01$



4 Discussion

Treatment of phytohormonal inducers activated *PR1a*, *PIN2*, *PAL*, and *LOX* transcriptional factors to elicit the SA and JA defense pathway. These findings were in line with Zarate et al. (2007), where exogenous application of SA and MeJA in *A. thaliana* activated the SA (*PR1*, *PR5*, *BGL2*) and JA transcripts (*PDF1.2*, *VSP1*). Zhang et al. (2013) also revealed the activation of *PR1*, *EDS1* (SA-eliciting genes), and *OPR3*, *LOX2* (JA-responsive genes) in *A. thaliana* in response to exogenous phytohormonal inducer treatment.

Gene activation in response to exogenous application of BTH and MeJA was governed by an intricate pattern of molecular systems biology, which fine tunes metabolic products for SA/JA biosynthesis (Jafarbeigi et al. 2021). Ullah et al. (2022) revealed the stimulation of plant molecular systems to activate *PR1*, *PR2*, *WRKY 18*, and *WRKY 89* transcriptional factors in response to foliar application of BTH, MeSA, and MeJA in poplar. In another study on poplar, exogenous application of BTH and MeJA enhanced the expression of some other SA/JA eliciting genes *NPR1* (non-expressor of pathogenesis-related proteins) and *JAZ10a* (Jasmonate ZIM-domain encoding gene), along with *PR1*, *PR2*, *PR3*, *WRKY 18*, and *WRKY 89* through the modulation of plant systems biology.

Expression of defense genes is also mediated by the elicitation of different proteins. Alkahtani et al. (2011) confirmed this in a study where application of SA and Bion (commercial formulation of BTH) effectively activated pathogenesis related proteins (β -1, 3-glucanase, chitinase, polyphenol oxidase, and peroxidase) in cucumber, which led to activating transcriptional factors.

A gene expression is up- or downregulated due to a variation in its localized pattern in the plasma membrane. Sun et al. (2011) revealed that application of MeJA altered the pattern of *PIN2* and other transcriptional factors by changing

their localized concentrations in the plasma membrane and endocytosis mechanism. This intracellular movement of mRNA in regulating gene expression is controlled by internal and external signals, including other plant hormones, e.g., auxins (Monte et al. 2019).

BTH/MeJA upregulated the expression of their concerned transcripts and suppressed the other. SA-mediated suppression of JA-responsive genes might be linked with the upregulation of JAZ proteins, e.g., *MpJAZ*, which are the negative regulators of JA biosynthesis (Monte et al. 2019). This also limits the entrance of the cell nucleus or transport of JA-transcripts by affecting jasmonoyl-isoleucine (JA-Ile), which is an important JA transporter in *A. thaliana* (Li et al. 2017). A similar mechanism was thought to be adopted by JA to suppress SA expression (Ullah et al. 2022).

Individual application of both PGPRs was encouraging to simultaneously activate SA and JA marker genes. This was in agreement with Samain et al. (2022), who demonstrated the simultaneous elicitation of SA/JA marker genes (*PR1*, *TaCHI*, *TaLOX*, *TaCAT*, and *TaPOX*) in wheat in response to the application of *Paenibacillus* sp. B2 and *Arthrobacter* spp. AA. Arkhipov et al. (2023) also indicated the instantaneous activation of SA/JA pathways through the expression of *PAL1*, *NPR1*, *LOX1*, and *PR1* transcriptional factors in *Bacillus velezensis*-inoculated tomatoes.

Rhizobacteria application in combination with BTH and MeJA elicited both SA (*PR1a* and *PAL*) and JA transcriptional markers (*PIN2* and *LOX*), simultaneously. This was agreed with the results of Jafarbeigi et al. (2021), where *ASR1*, *PIN2*, and *PAL* were activated when SA was applied in combination with *Trichoderma harzianum* in tomato and eggplant. Kamalanathan et al. (2023) also showed that tomato root priming with *Bacillus velezensis* VB7 against root knot nematodes improved the expression of both SA and JA transcripts, e.g., *PR*, *PAL*, and *LOX*.

The potential mechanism behind rhizobacteria-mediated modulation of SA-JA antagonism might be governed by

transcriptional control over SA/JA biosynthesis. This may happen through post-transcriptional processes, gene localization, DNA binding, and/or restriction of transcriptional factor-degrading agents (Caarls et al. 2015).

Application of BTH and MeJA in combination with isolated rhizobacteria enhanced systemic resistance in tomato. *H. armigera* survived well on the controlled plants through degradation, excretion, sequestration, and detoxification of harmful plant chemicals (Cortez et al. 2024). However, BTH and MeJA reduced larval weight, larval survival, and adult emergence of *H. armigera*. This was in line with the results of War et al. (2015) where foliar application of SA and JA reduced *H. armigera* larval weight, survival, and plant damage in groundnuts. Reduction in larval weight and survival may be attributed to the higher activities of PAL, LOX, PIN, PPO, POD, CAT, Phenolics, and tannins in host-plants (War et al. 2015). Upregulation of protein kinases might be another reason which controlled the process of amino acid phosphorylation to turn a protein on or off (War and Sharma 2014).

Gordy et al. (2015) also indicated BTH and MeJA-mediated reduction in larval performance of *S. frugiperda* in cotton, maize, rice, and soybean, where MeJA was more effective than BTH. Our findings agreed with this, where MeJA performed well over BTH. Chang et al. (2008) associated this with the increased production of trichomes in JA treatments, which ultimately limited plant herbivory. Scott et al. (2010) stated another reason where inducers, specifically MeJA decreased larval weight, survival, and adult performance by affecting larval feeding at different phases, i.e., pre/post ingestion and post digestion. This was also confirmed in the experiments of Scott et al. (2010) where *Trichoplusia ni* had 25% less food consumption and 62% higher larval gut damage while feeding on Prosystemin tomato (an over-expressor of JA).

Kanwal et al. (2024) also revealed lower survival, adult emergence, and larval weight of *S. frugiperda* on BTH and MeJA-treated plants of maize. SA/JA produces defense against insects through alternation in cellular metabolism and lignification of cell walls to make plant tissues less digestible (Akbar et al. 2012; Waseem et al. 2026). These phyto-inducers could also regulate polyphenol oxidase (PPO) to lower the hostplant's nutritional quality. PPO produced highly toxic quinones, which interacted with the nucleophilic chain of amino acids to reduce the availability of digestible proteins (Bhonwong et al. 2009).

War and Sharma (2014) also found lower weight gain in *H. armigera* larvae when fed on SA/JA treated plants. Lower contents of detoxification enzymes in the midgut, reduced activities of trypsin and serine protease, higher glutathione S-transferase, and lower esterase were recorded behind this reduced herbivore performance (War and Sharma 2014). In

addition, reduced larval survival and adult emergence were associated with mitochondrial damage, reduced respiration, and lowered cytochrome activities (Akbar et al. 2012). Decrease in energy reserves, especially pupal proteins, may also be another reason for lower adult emergence in phyto-hormonal inducer-treated plants (Yang et al. 2013).

Application of PGPR isolates affected larval performance and adult emergence more significantly. Zebelo et al. (2016) stated that *Pseudomonas fluorescens* caused significant larval mortalities in *S. exigua* in *A. thaliana* and cotton. Larval weight was also reduced in rhizobium-inoculated maize (Cortez et al. 2024). This may be linked with a reduced nutritional index due to activation of insect-toxic compounds in plants, e.g., caryophyllene, linalool, nerolidol, and terpenoid (Palermo et al. 2022; Cortez et al. 2024). This was also revealed when *Bacillus amylo-liquefaciens* strain GB03 reduced nutritional indices of *S. frugiperda* in *Ocimum basilicum* along with a significant reduction in larval and pupae weights (Palermo et al. 2022). Activation of SA-JA pathways is another reason. Yan et al. (2025) indicated the activation of SA/JA signaling pathways when *Bacillus amyloliquefaciens* was inoculated in cowpeas against *S. litura*. *Fictibacillus solisalsi*, *B. pumilus*, and *B. subtilis* also modulated hostplant defense in maize against *S. litura* (Cortez et al. 2024).

Exogenous inducers' treatments also reduced the performance of a specialist herbivore, *B. tabaci*. Foliar applications of SA and MeJA reduced the survival of *B. tabaci* nymphs while feeding on *A. thaliana* (Zarate et al. 2007). Javed et al. (2025a, b, c, d) also showed a reduced survival of nymphs and adults of a specialist herbivore, *Brevicoryne brassicae*, in SA-treated canola. This may be attributed to higher phenolic content and a low nutritional profile of host-plants. A significant decrease in nymph survival was related to low nymph vitality, reduced intrinsic rate, delayed reproductive period, and lower reproduction indices (Javed et al. 2022, 2025a, c). Nymph development time might also be delayed. de Freitas et al. (2019) stated that the stink bug (*Oebalus pugnax*) took more time to develop in SA and MeJA-treated plants. Insects might be fed less on treated plants due to deterrent volatiles, e.g., methyl salicylate (Liu et al. 2026).

A reduction in nymph survival may also be associated with a reduction in detoxification enzymes in the insect gut. This was confirmed that SA-treated tomato plants showed significantly lower population of mites (*Tetranychus urticae*) due to reduced contents of detoxification enzymes such as α -amylase, glutathione S-transferase, esterase, and aminopeptidase. Energy reserves, glycogen, lipid, and proteins were also lowered (Homayoonzadeh et al. 2020). Wu et al. (2025) indicated the upregulation of protease inhibitor 8 (*PIN8*) and terpene synthase 10 (*TrPS10*) to reduce the *B.*

tabaci population. While Wang et al. (2025a, b) indicated higher activities of PAL and lignin biosynthesis that resulted in reduced nymph survival and lower aphid population in MeJA-treated cucumber. Biochemical modification was also another contributory factor. Aslam et al. (2022) expressed MeJA to be effective in controlling aphids by modulating biochemical defense (SOD, POD, PPO, CAT) in wheat.

The reduction in whitefly population was least pronounced at 7 DPI, which may suggest that the induced resistance is diminishing over time. This has potential implications for resistance induction in the field and sustainable pest management strategies, as indicated in previous findings of Javed et al. (2025a, b). For sustainable pest management, multiple applications of phytohormonal inducers are required to maintain a durable level of induced resistance.

Whiteflies' nymph population was also affected in rhizobacterial treatments. Serteayn et al. (2020) indicated a lower nymph survival in pea aphids in *B. amyloliquefaciens*-inoculated broad beans. Isolates of *Pseudomonas* sp. also reduced nymph population and adult survival of *B. tabaci* in tomato (Qessaoui et al. 2020). In our integrated experiments, PGPR combination with phyto-inducers produced better results. This agrees with the findings of Nasab et al. (2019), who showed better performance of humic acid when combined with rhizobacteria (*P. fluorescens*, *Azotobacter chroococcum*, and *Azospirillum brasilense*) against *B. brassicae* in canola. Similarly, SA potential to control *B. tabaci* in tomato was also improved when it was applied in combination with *Trichoderma* (Jafarbeigi et al. 2020).

In short, the combined application of hormonal inducers and PGPRs modulated SA-JA antagonism to enhance pest resistance in tomato. Effector-triggered immunity (ETI) might be a contributing factor while modulating SA-JA crosstalk and antagonism under PGPR priming (Irfan et al. 2026). Liu et al. (2016) revealed the elicitation of SA-mediated JA pathway in *A. thaliana* through activation of *NPR3* and *NPR4* transcriptional factors. Similarly, some other examples are available in plant-pathogen interactions (Ding et al. 2011; Wang et al. 2012; Ullah et al. 2022) along with an aphid (*B. brassicae*) feeding, which improved SA and JA responsive genes to modulate SA-JA crosstalk through ETI (Chen et al. 2026).

Although our results indicated promising outcomes of SA-JA modulated antagonism for enhancing pest resistance, which was context-dependent, where combined treatments may not necessarily perform well over individual treatments. Field studies are required to validate these findings in a diverse agro-ecological and broad crop-pest system. To strengthen the mechanistic claim, tomato mutants or transgenic lines impaired in SA/JA signaling should be considered as a future step in this research. Also, endogenous hormone levels should be quantified. In addition, key

regulatory components of SA-JA signaling, such as *NPR1*, *JAZ* proteins, or *COII*, should also be assessed.

5 Conclusion

Both generalist and specialist herbivores manipulate plant defense for their own survival, leading to a salicylic-jasmonic acid crosstalk. Rhizobacteria modulate such antagonism to enhance pest resistance against a generalist (*Helicoverpa armigera*) and a specialist herbivore (*Bemisia tabaci*). Individual applications of benzothiadiazole and methyl jasmonate upregulated their respective RNA transcripts but downregulated/suppressed the others. Foliar application of phytohormonal inducers followed by PGPR root drenching with *Bacillus* sp. Mb01 and *Pseudomonas* sp. Mb05 modulated the antagonism through the elicitation of RNA transcripts of both defense pathways. This moderation was context-dependent, where the combined application of PGPR may not be performing well over individual treatment. However, enhanced pest resistance, i.e., reduced survival, larval weight, and adult emergence, was recorded. This will help in improving the role of plant hormonal inducers to improve pest resistance against both generalist and specialist herbivores.

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Data Availability Data associated with this manuscript could be made available on a suitable request from J.N.A.

Declarations

Ethics Approval and Consent to Participate Not Applicable.

Consent for Publication All authors have unanimously permitted to publish this article.

Generative AI No AI was used in the preparation of this manuscript.

Clinical Trial Information Not Applicable.

Competing interests Authors have no known financial, commercial, or other competing interests in the present research.

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