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Elicitors-induced modulation of plant performance, essential oil composition, and iridoid biosynthesis in Valeriana jatamansi Jones cultivated under controlled vertical aeroponics

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Elicitors-induced modulation of plant performance, essential oil composition, and iridoid biosynthesis in *Valeriana jatamansi* Jones cultivated under controlled vertical aeroponics

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Abstract

The integration of aeroponic cultivation and elicitation offers an efficient strategy for enhancing biomass production and specialized metabolite accumulation in medicinal plants. In the current investigation, foliar application of fungal endophyte (FE), seaweed extract (SWE), and salicylic acid (SA) were applied to aeroponically cultivated *Valeriana jatamansi* to evaluate their effect on morphological growth, biomass yield, physiological performance, antioxidant defence, essential oil production, valepotriate accumulation, biosynthetic gene expression, and antimicrobial activity. SA treatment significantly enhances morphological growth, whereas FE promotes biomass accumulation. Elicitor treatment showed improved photochemical performance and antioxidant defence as indicated by reduced malondialdehyde and H_2O_2 levels and enhanced superoxide dismutase and catalase activities in leaf and root tissues. SWE produced the highest essential oil yield (2.50%) (v/w) and patchouli alcohol concentration (238.31 mg/L), while FE resulted in the highest relative abundance of patchouli alcohol (43.27%) in the oil. FE also markedly enhanced root valepotriate accumulation, with maximum acevaltrate (0.47%), didrovaltrate (0.53%), and valtrate (3.10%) contents. Consistently, qRT-PCR profiling revealed enhanced expression of iridoid pathway genes, including *SLS*, *IS*, *7DLGT* and *7DLS*, following elicitor treatment in leaf and root tissue. Essential oil exhibited strain-dependent antimicrobial activity, with *Bacillus subtilis* being the most susceptible strain (MIC 0.06 mg/mL). Overall, these findings demonstrate that combining aeroponic cultivation with targeted elicitation can effectively enhance biomass productivity, physiological resilience, essential oil quality, and valuable specialized metabolites in *V. jatamansi*, presenting a potential strategy for sustainable, scalable production of high-value medicinal plant products.

Keywords: *Valeriana jatamansi*, Aeroponics, Elicitors, Secondary metabolites, Fungal endophytes

Introduction

Valeriana jatamansi Jones (family: Caprifoliaceae) is an important medicinal plant widely occurring across the temperate and subtropical Himalayan areas at altitudes between 900 and 3,000 m amsl [1]. Owing to its broad spectrum of pharmacological properties, the species occupies a central position in both traditional Ayurvedic medicine and modern therapeutics. The medicinal importance of *V. jatamansi* is primarily attributed to its root and rhizome, which are rich in valepotriates, including valtrate, didrovaltrate, and acevaltrate, known for their

anticonvulsant, antispasmodic, and antidepressant activities [2, 3]. In addition, the roots and rhizomes are an important source of essential oil, a valuable natural product. The essential oil content in the roots and rhizomes varies considerably, ranging from 0.05% to 2.00% depending on genotype and environmental conditions. The oil is predominantly composed of sesquiterpenes, including patchouli alcohol, maaliol, α-guaiene, kessane, seychellene, α-patchoulene, and α-santalene [4], which exhibit diverse therapeutic properties including antibacterial, antifungal, anxiolytic, and sedative effects [2, 3, 1]. Among these constituents, patchouli alcohol has consistently been reported as the major bioactive compound [5] and extensively employed in the industry for its applications in pharmaceuticals, flavours, and perfumery [6]. The yield, chemical composition, and relative abundance of essential oil constituents in *V. jatamansi* are highly variable and determined by genetic and environmental constraints. Seasonal variation [7], population diversity [8], plant organ [9], planting material [10], genetic background [11], and altitudinal and habitat conditions [12] have all been reported to significantly affect essential oil yield and the accumulation of specialized metabolites.

The increasing industrial demand for *V. jatamansi* phytochemicals and oil extractions is currently met almost predominantly through wild harvesting and conventional field cultivation, resulting in an unsustainable demand-supply imbalance and increasing pressure on natural populations [13]. Therefore, the development of sustainable and controlled cultivation strategies is essential to ensure a continuous supply of high-quality plant biomass. In this regard, soilless cultivation technologies, particularly hydroponic and aeroponic systems, have gained recognition as potential alternatives for the commercial production of medicinal plants, offering superior biomass and phytochemical consistency [14, 15]. These systems allow precise regulation of critical environmental parameters, including nutrient availability, pH, electrical conductivity (EC), root-zone aeration, and temperature, thereby minimizing environmental variability and improving plant productivity and metabolite accumulation. Among soilless cultivation techniques, aeroponics is an advanced production system in which plant roots are hung in a closed chamber and intermittently supplied with an automated nutrient solution via high-pressure nozzles or foggers, ensuring optimal oxygen availability and efficient nutrient uptake [16, 15]. The enhanced control over the root zone environment, together with improved water and nutrient efficiency, makes aeroponics suitable for cultivating high-value medicinal plants. Thakur et al. [17] reported that aeroponically grown *Picrorhiza kurroa* exhibited increased biomass production. Additionally, Partap et al. [13] observed elevated phytochemical accumulation in aeroponically cultivated *V. jatamansi*.

Recent studies have demonstrated that integrating biostimulants and elicitors into aeroponic cultivation further enhances growth, stress tolerance and specialized metabolite production [13, 18, 19]. Seaweed extracts (SWE) and fungal endophytes (FE) are widely recognized biostimulants that enhance plant performance by modulating physiological, biochemical and molecular processes involved in growth, nutrient acquisition, stress adaptation, and specialized metabolite biosynthesis. Their effectiveness can be ascribed to the presence of diverse phytoconstituents, including phytohormones, vitamins, phenolic compounds and other signaling molecules, which collectively improve antioxidant capacity, metabolic regulation, and plant resilience under controlled cultivation conditions [3, 20]. Additionally, salicylic acid (SA) is a low-molecular-weight phenolic phytohormone, is one of the most extensively studies elicitors due to its ability to regulate plant growth, antioxidant defense and secondary metabolism, Earlier investigations have demonstrated that the administrations of biostimulant and SA under aeroponics cultivation significantly enhance biomass production, nutrient uptake, antioxidant capacity and the accumulation of valuable bioactive compound through improved nutrient assimilation, phytohormone signaling nitrogen metabolism and ROS homeostasis [21-24].

Many previous investigations have reported that various biotic, abiotic elicitors and biostimulants significantly enhance plant growth and bioactive content in *V. jatamansi* under aeroponic, field, and *in vitro* conditions [25, 13, 2, 3, 26]. Despite the recognized potential of SWE and FE as powerful biostimulants and SA as a potential elicitor, their application in aeroponic systems for *V. jatamansi* has not yet been explored, representing a critical research gap. The current investigation reports the effects of foliar treatments of SWE, FE, and SA on aeroponically cultivated *V. jatamansi*. The uniqueness of the current investigations lies in investigating the roles of SWE, FE, and SA elicitation in modulating morphological parameters, physiological traits, biochemical composition, secondary metabolite production, essential oil accumulation, and antimicrobial potential under controlled vertical aeroponic conditions.

Materials and methods

Plant material, acclimatization, and aeroponic cultivation conditions

For the experimental work, *V. jatamansi* seedlings (7-8 cm in height) were procured from the Agrotechnology Division, CSIR-Institute of Himalayan Bioresource Technology (IHBT), Palampur. The experiment was conducted in a fully automated, SCADA-based (Supervisory

Control and Data Acquisition) vertical aeroponic greenhouse at CSIR-IHBT, Palampur, Himachal Pradesh, India, at an elevation of 1340 m amsl (32.109501° N, 76.535797° E) [27]. Before transfer to the aeroponic system, the seedlings were treated with 0.05% formalin solution (2 min) and 1% Bavistin (5 min) to minimize potential contamination by soil-borne microorganisms and pathogens [17]. Following sterilization, the seedlings were acclimatized to liquid culture conditions for 2 weeks in trays containing Hoagland's No. 2 Basal Salt Mixture (HiMedia, India), maintained at pH 6.8 and an EC of 0.5 mS cm^{-1}. This acclimatization step was performed to facilitate adaptation to the soilless growth environment and minimize transplant shock and mortality. After successful acclimatization, the plants were transferred to a vertical tower of an aeroponic system (Fig. 1). The experiment was conducted for a six-month duration, and data were recorded at monthly intervals. The plants were categorized into four experimental groups, comprising 384 plants each: fungal endophyte (FE), salicylic acid (SA), seaweed extract (SWE), and the untreated control. For each treatment, 384 plants were cultivated among eight aeroponic vertical towers, with a cultivation capacity of 48 plants per tower. The roots were intermittently misted with Hoagland's No. 2 Basal Salt Mixture using an automated aeroponic mist system, with a spray duration of 10 min at each irrigation cycle. The EC of the nutrient medium was progressively maintained throughout the experiment, starting at 0.5 mS cm^{-1}, increasing to 1.5 mS cm^{-1} after three months, and reaching 2.5 mS cm^{-1} two weeks before harvest. The vertical aeroponic greenhouse conditions were adjusted at 25 ± 2 °C temperature and 65-70% relative humidity, with a 16 h light/8 h dark photoperiod. The nutrient medium pH was maintained at 6.8-7.0, and its temperature at 10-12 °C using a chiller. Plants were cultivated under full-spectrum LED lighting comprising 15% blue, 2% green, 65% red, and 17% far-red wavelengths, with 82% PAR contribution, while photosynthetic photon flux density (PPFD) was maintained at 150 µmol m^{-2} s^{-1} using PAR lamps. Water and nutrient solution levels were monitored daily to compensate for losses due to plant uptake and evaporation, and the nutrient solution reservoirs were replenished weekly to maintain the required volume and nutrient availability throughout the experiment.

Isolation, characterization, and preparation of endophyte-based consortium

For the endophyte treatment, endophytic fungi were isolated from the rhizomes, leaves, and seeds of *V. jatamansi* using a modified surface sterilization method [28, 3]. The procedure was optimized to eliminate surface associated microorganisms while retaining viable endophytic fungi within plant tissues. The plant material and its collection details have been described previously [3]. In brief, the fresh plant tissue was thoroughly rinsed with distilled water to

eliminate the adhered soil and other surface debris. The plant tissues were surface sterilized with 1% sodium hypochlorite (HiMedia, India). Rhizome tissues were treated for 8 min, whereas leaf tissues and seeds were treated for 3 min. This was followed by immersion in 70% ethanol (Changshu Hongsheng Fine Chemicals, China) for 1 min. The tissues were subsequently washed 5 times with sterile distilled water and air-dried. Sterilization efficacy was checked by plating 100 µL of rinse water onto potato dextrose agar (PDA) (HiMedia, India), followed by incubation at 28 ± 2 °C and monitoring for microbial growth. The absence of growth was considered the evidence of successful surface sterilization. The sterilized tissues were then aseptically cut into approximately 0.5 cm pieces and placed on PDA supplemented with streptomycin (50 µg/mL) and chloramphenicol (50 µg/mL) (HiMedia, India) to suppress bacterial contamination and facilitate fungal recovery, followed by incubation at 28 ± 2 °C for 10-15 days and monitoring for microbial growth. The emerging colonies were examined based on their distinct morphological characteristics, and representative fungal isolates were transferred to fresh PDA plates. Actively growing hyphal tips were subcultured 2-3 times to obtain pure cultures. The purified endophytic fungal isolates were maintained on PDA and preserved in glycerol-containing stocks at −80 °C for further use.

Molecular characterization and identification of fungal endophytes

The fungal isolates were characterized at the molecular level using the internal transcribed spacer (ITS) region of the ribosomal RNA gene. Genomic DNA was isolated from approximately 300 mg of fresh fungal mycelium using a cetyltrimethylammonium bromide (CTAB)-based protocol described by Sambrook et al. [29]. The quality and integrity of the extracted DNA were checked by electrophoresis on a 0.8% agarose gel. The ITS1-5.8S-ITS2 region was amplified using the universal fungal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) following White et al. [30]. ITS amplification and sequencing were performed following Thakur et al. [3]. The resulting sequences were compared with reference sequences in the NCBI GenBank database using BLAST for taxonomic identification. Based on sequence similarity and available reference sequences, the fungal isolates were assigned to the appropriate taxonomic level.

Compatibility assessment of fungal endophytes

The compatibility of the eight selected fungal endophytes was assessed using a dual-culture assay prior to the preparation of the consortium [31]. Briefly, actively growing cultures of each

fungal isolate were maintained on PDA, and 5-mm agar plugs were excised from the actively growing margins of 5-7 day old cultures using a sterile cork borer. Four fungal discs from different isolates were placed on a 90 mm PDA plate at approximately equal distances from one another and cultured at 28 ± 2 °C for 7 days. All combinations of the eight fungal isolates were tested. The plates were monitored regularly for fungal growth and interaction between the isolates. Compatibility was assessed by the absence of a distinct inhibition zone, normal radial growth, and the capacity to grow in close proximity without visible antagonistic effects. Based on these observations, compatible isolates were considered suitable for subsequent consortium preparation.

Preparation of endophyte-based consortium

For consortium preparation, agar plugs containing actively growing mycelia were then transferred to a 250 mL flask comprising 100 mL of potato dextrose broth (PDB). After that, incubation was done under static conditions at 28°C for 10 days. The fungal biomass was separated from the culture broth using a sterile filter paper (Filterking, India) and suspended in 0.85% NaCl. The suspension was shaken for 2 h to facilitate spore release, then filtered to remove mycelial fragments, yielding a suitable spore suspension for application. For consortium preparation, individual spore suspensions of all selected fungal isolates were adjusted to 1×10^8 spores mL^{-1} in sterile 0.85% NaCl and mixed in equal proportions to prepare the endophyte-based consortium (Fig. S1a, c, d). Sterile 0.85% NaCl, without fungal inoculum, was used for the uninoculated control plants. To confirm the successful colonization, endophytic fungi were reisolated from the leaves of *V. jatamansi* plants at 60 days post-inoculation.

Elicitor selection, preparation, and screening

In addition to the endophyte-based consortium, SA and SWE were evaluated as hormonal and algae derived elicitors, respectively. Analytical grade SA was procured from HiMedia Laboratories (Mumbai, India), while a *Kappaphycus alvarezii* based seaweed biostimulant was obtained from Aquagri Processing Private Limited (New Delhi, India). The SA and SWE powders were dissolved separately in sterilized deionized distilled water using a magnetic stirring hot plate at 200 rpm and 35 °C. A stock solution of SA (10 mg/mL) and SWE (10 gm/L) was prepared and then filter-sterilized using a syringe filter. The sterile stock solution was kept at 4 °C. Initial screening trials were performed to assess the optimal concentrations of SA and SWE. Various concentrations of SA (25, 50, 100, 200, and 400 µM) and SWE (0.5, 1, 2, 4, and

8 gm/L) were tested to determine the optimum concentration. Based on the shoot length after 1 month, SA (100 µM) and SWE (4 gm/L) were selected as the optimal concentrations for subsequent experiments (Fig. S2a, b).

Foliar application of elicitors

Following the optimization of SA and SWE concentrations and preparation of the compatible endophyte-based consortium (Fig. S1e), the three elicitor treatments were evaluated in *V. jatamansi* plants cultivated in a vertical aeroponic system. SA (100 µM), SWE (4 gm/L), and the FE (1×10^8 spores mL^{-1}; 10 ml/plant) were applied to the foliage by foliar spraying. Tween 20 (0.01%, v/v) was supplemented to the treatment solutions as a surfactant to facilitate uniform foliar application. The plants were subsequently cultivated under aeroponic conditions to further assess morphological, physiological, biochemical, essential oil, and specialized metabolite responses.

Morphological and growth biomass analysis

Morphological growth and biomass yield assessments were recorded at monthly intervals. For biomass yield estimation, leaf and root tissues were harvested, oven-dried at 35 °C for one week, and weighed on a digital weighing balance (Aczet, India).

Chlorophyll fluorescence imaging

Chlorophyll fluorescence imaging was carried out in the morning (9:30-11:30 AM) using a closed FluorCAM FC 800-C (Photon Systems Instruments, Brno, Czech Republic) instrument. Before imaging, leaves were dark adapted for 20 min to ensure accurate fluorescence measurements. Several fluorescence parameters were evaluated, including the maximum quantum efficiency of PSII (QYmax), effective quantum yield of PSII (ϕPSII), non-photochemical quenching (NPQ), photochemical quenching coefficients based on the puddle (qP) and lake (qL) models, non-photochemical quenching coefficient (qN), and the relative fluorescence decrease ratio (Rfd) under steady state conditions. The FluorCam 7 software was employed for instrument control, image acquisition, data analysis, and visualization of fluorescence signals. Fluorescence images were represented in pseudo-colours, where blue indicated lower fluorescence intensity, and red indicated higher fluorescence intensity. Individual fluorescence parameters were displayed using specific colour scales [2].

Malondialdehyde quantification

Malondialdehyde (MDA) content was estimated using the thiobarbituric acid (TBA) reaction, following the method of Li et al. [32]. 100 mg of leaf and root tissues were pulverized in liquid nitrogen. The powdered material was mixed with an extract encompassing 0.5% TBA and 20% trichloroacetic acid (TCA), along with 0.25 mL of 175 mM NaCl, and the final volume was adjusted to 2 mL with 50 mM Tris-Cl buffer, pH 8.0. The reaction mixture was incubated at 95 °C for 5 min, then rapidly cooled on ice. After centrifugation at 14,000×g for 5 min at 4 °C, the absorbance of the resulting supernatant was recorded at 532 nm and corrected for non-specific absorbance at 600 nm. MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and expressed as µmol g^{-1} fresh weight.

The final concentration was determined using:

$$\text{MDA (µmol g}^{-1}\text{ FW)} = 6.45 \times (OD_{532}\text{-}OD_{600}) - (0.56 \times OD_{450})$$

Hydrogen peroxide quantification

Hydrogen Peroxide (H_2O_2) activity was estimated following the method of Das et al. [33]. Leaf and root tissues (100 mg) were homogenized in 2 mL precooled sodium phosphate buffer (pH 7.0). After that, centrifuging was done at 10,000 × g for 20 min. The resulting supernatant was subjected to H_2O_2 quantification. For the assay, 2 mL of the supernatant, diluted with 10 mM sodium phosphate buffer, was mixed with 2 mL of 5% potassium dichromate and glacial acetic acid (1:3 w/v). The mixture was filtered, and the absorbance was measured at 570 nm. H_2O_2 concentration was reported as micromole of g^{-1} fresh weight.

Superoxide dismutase quantification

Superoxide dismutase (SOD) activity was calculated following the protocol established by Vuleta et al. [34]. Fresh tissues were homogenized in 50 mM phosphate cold extraction buffer (pH 7.8). After that, centrifugation was done at 10,000 × g for 15-20 min at 4°C. The reaction mixture (1 mL) included 50 mM sodium phosphate buffer, 12 mM methionine, 1.72 mM nitroblue tetrazolium chloride (NBT), 1% Triton X-100, 30 µL of riboflavin and 100 µL of enzyme extract. Reaction mixtures were illuminated for 30 min, and absorbance was measured at 560 nm using a UV/Vis spectrophotometer (Thermo Scientific, USA). SOD activity was reported as µmol g^{-1} fresh weight.

Catalase activity

Catalase (CAT) activity was calculated using the protocol of Hasanuzzaman et al. [35]. The assay mixture consisted of 0.2 mL of enzyme extract, 1.5 mL of 100 mM sodium phosphate

buffer (pH 7.0), 0.4 mL of distilled water, and 1 mL of 30 mM H_2O_2. The decrease in absorbance was recorded at 240 nm, 15 second intervals for 1 min using a UV/Vis spectrophotometer (Thermo Scientific, USA). CAT activity was reported in units of micromoles g^{-1} fresh weight.

Extraction of essential oil from root tissue

The essential oil from the dried root tissue was extracted by the hydro-distillation method using a Clevenger-type apparatus. All the samples were distilled for 6 h. After recording the extracted oil reading, the oil concentrations (%) in the roots were calculated from the fresh samples (100 gm each). Then, the extracted oil samples were dehydrated using anhydrous Na_2SO_4 and subsequently kept at 4°C for a qualitative study [4].

GC-MS analysis of essential oil

The extracted oil was analyzed for patchouli alcohol quantification using an Agilent 8890 GC coupled with a 5977 GC-MS system (Agilent, USA), equipped with an HP-5ms Ultra Inert fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The oven temperature was initially maintained at 70 °C for 3 min, increased to 220 °C at 4 °C/min, and held for 4.5 min, resulting in a total run time of 45 min. The injector was operated in split mode at 240 °C with a 10:1 split ratio, using helium as the carrier gas at a flow rate of 1.0 mL/min. An injection volume of 1.0 μL was introduced using an Agilent 7693A autosampler. Patchouli alcohol was quantified using calibration curves generated from peak area and standard concentration (0.0667-1000 mg/mL). Volatile constituents were identified based on retention times and relative retention indices calculated against an n-alkane mixture (C9-C24), with mass spectra matched against the NIST and Wiley GC-MS databases. Compound identifications followed the approaches of Stein [36] and Adams [37].

Antimicrobial activity

The antimicrobial activity of *V. jatamansi* essential oil was evaluated against Gram-positive bacteria (*Bacillus subtilis* MTCC121 and *Staphylococcus aureus* MTCC96) and Gram-negative bacteria (*Escherichia coli* MTCC43 and *Salmonella typhimurium* MTCC733) using the broth microdilution method. Sample stock solutions were prepared in ethanol at 5.0 mg/mL, and the assay was conducted in sterile 96-well microtitre plates following Aggarwal et al. [38]. Briefly, 170 μL of nutrient broth and 20 μL of the sample were added to each well (final concentration: 0.50 mg/mL). After incubation at 37 °C for 1 h to facilitate ethanol evaporation,

10 µL of bacterial culture was added. Based on the initial screening after 8 h of incubation, the samples showing≥ 95% inhibition were used to determine the minimum inhibitory concentration (MIC). Furthermore, the minimum bactericidal concentration (MBC) was assessed following the methods of Aggarwal et al. [38].

Secondary metabolites analysis

Dried leaf and root tissues (100 mg) were extracted with 5 mL of methanol by overnight shaking and sonication (45 °C, 30 min). The extracts were centrifuged (1807 × g, 10 min, 4 °C) and filtered through 0.22-µm syringe filters (Moxcare, Ambala, India). Valtrate, acevaltrate, and didrovaltrate were quantified by the ACQUITY™ UPLC H-Class system using 1 mg/mL methanolic standards, following Kumar et al. [1].

RNA extraction and quantitative real-time PCR profiling

The expression profile of the iridoid biosynthetic pathway genes, including *GES* (*geraniol synthase*), *G10H* (*geraniol 10-hydroxylase*), *IS* (*iridoid synthase*), *7DLS* (*7-deoxyloganetic acid synthase*), *7DLGT* (*7-deoxyloganetic acid glucosyl transferase*), and *SLS* (*secologanin synthase*), was determined through quantitative real-time PCR. In brief, 100 mg of tissue was ground in liquid nitrogen, and total RNA was extracted using TRIzol (Sigma-Aldrich, India), followed by chloroform phase separation and 2-propanol precipitation. The RNA pellet was washed with 70% ethanol, air-dried, and quantified using a Nanodrop spectrophotometer. cDNA was synthesized and normalized to 100 ng/µL for qRT-PCR (QuantStudio 3, Thermo Scientific). Gene-specific primers were designed following Shuang and Chenshu [39]. Amplification was performed at 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min [2, 3]. Reactions were performed in triplicate, and relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method with *GAPDH* as the reference gene.

Statistical analysis

The data were statistically analyzed using SPSS software (version 25.0.0; IBM Corp., Chicago, USA). Data were subjected to one-way analysis of variance (ANOVA), followed by Duncan's multiple range test (DMRT) at $P \leq 0.05$. Results are presented as mean ± standard error (SE) of three biological replicates (n = 3), except for morphological parameters, which were evaluated using 15 replicates (n = 15).

Results

Isolation and molecular identification of fungal endophytes

The endophytic fungal community selected for the present study comprised isolates representing three plant tissues of *V. jatamansi*. Four isolates were rhizome-associated, three were leaf-associated, and one was seed-associated, including *Fusarium oxysporum* VRzFF, *Penicillium sajarovii* VRzFG, *P. raistrickii* VRzFK, *P. sajarovii* VRzFL, *Colletotrichum higginsianum* VLF12, *Paraphoma chrysanthemicola* VLF15, and *Colletotrichum* sp. VLF16, and *Penicillium chrysogenum* VSF19. Rhizome-associated endophytes were isolated and molecularly characterized in our previous study [3], while leaf-associated endophytes were identified in the present study using ITS sequence analysis. The resulting sequences were deposited in the NCBI GenBank database, and the corresponding accession numbers are provided in Table S1.

Compatibility of fungal endophytes

The compatibility of the eight selected fungal endophytes was evaluated before consortium preparation. All tested isolates showed normal growth when co-cultured, and no clear inhibition zones or pronounced antagonistic interactions were observed between the fungal colonies (Fig. S1b). The absence of visible growth inhibition indicated that the selected endophytes were compatible with one another and, therefore, suitable for developing the fungal consortium.

Effect of elicitors on morphological traits and biomass yield

Elicitor treatments markedly affected the morphological characteristics and growth biomass yield. Shoot length analysis showed that SA treated plants exhibited a 1.18 fold increase over the control, followed by FE treatment at 1.12 fold and SWE treatment at 1.04 fold (Fig. 2a). Leaf length analysis revealed a slight increase in SA-treated plants, 1.03 fold compared to the control, whereas SWE and FE treatments resulted in 0.98 fold and 0.95 fold changes, respectively, indicating marginal reductions in leaf length relative to the control (Fig. 2b). Furthermore, maximum leaf width was recorded in SA treated plants, showing a 1.00 fold than the control. Conversely, SWE and FE treated plants exhibited 0.98 fold and 0.97 fold changes, respectively, relative to the control (Fig. 2c). Root length analysis revealed that SA treatment resulted in the highest enhancement, exhibiting a 1.46 fold increase compared with the control, followed by FE treatment, 1.35 fold and SWE treatment, 1.10 fold (Fig. 2d).

Leaf fresh weight (FW) was highest in FE treated plants, showing a 1.29 fold increase over the control, followed by SA treatment, 1.17 fold and SWE treatment, 1.03 fold (Fig. 3a). Similarly, leaf dry weight (DW) was markedly enhanced in FE treated plants, showing a 1.42 fold increase

relative to the control, whereas SA treatment resulted in a 1.07 fold increase. In contrast, SWE treatment showed a slight reduction in leaf DW, with a 0.92 fold change relative to the control (Fig. 3c). Root FW showed the maximum increase in FE treated plants, exhibiting a 1.51 fold enhancement over the control, followed by SA treated plants, 1.41 fold and SWE treated plants, 1.36 fold (Fig. 3b). Similarly, DW increased by 1.30, 1.15, and 1.10 fold in FE, SA, and SWE treated plants, respectively, compared with the control (Fig. 3d).

Effect of elicitors on physiological characteristics

Chlorophyll fluorescence analysis was conducted to assess the physiological performance of *V. jatamansi* (Fig. S3). The QY_max value exhibited notable similarity across all treatment and control conditions, value ranged from a 0.74-0.81 in both treated and control plants, indicating superior overall photosynthetic efficiency (Fig. 4a). The ϕPSII values were higher in FE treated plants, suggesting more efficient light energy utilization than in SA, SWE, and control plants (Fig. 4b). NPQ values were similar among elicitor treated and control plants during the initial growth stages (Fig. 4c). Although SA treated plants exhibited slightly lower NPQ values at later stages, the overall comparable NPQ levels among treatments suggest similar photoprotective dissipation of excess light energy. qN values ranging from 0.52 in control to 0.78 in SWE treated plants, indicating moderate to high photoprotective capacity and efficient dissipation of excess light energy in both treated and control plants (Fig. 4d). Compared with FE treated plants, SA, SWE, and control treated plants showed slightly lower qP (Fig. 5a) and qL values, particularly during the 2^{nd}, 3^{rd}, and 4^{th} months, indicating lower PSII photochemical efficiency and reduced light-use efficiency (Fig. 5b). Rfd value showed enhanced photosynthetic vitality and improved physiological performance in elicitor-treated and control plants (Fig. 5c).

Effect of elicitors on MDA content

Malondialdehyde (MDA) content, a reliable marker of oxidative damage to membrane lipids, was markedly decreased in elicitor treated plants compared to control plants. Among the treatments, SWE was the most effective in reducing MDA content by 1.40-3.57 fold and 2.10-4.14 fold in leaf and root tissue, respectively, compared to the control. FE also significantly reduced MDA accumulation with a 1.40-1.80-fold reduction in leaf and a 1.06-2.17-fold reduction in root compared to the control. Likewise, SA treatment decreased MDA content in leaf tissue by 1.28-1.40 fold and in root tissue by 1.28-2.00 fold compared to the control (Fig.

6a,b). Overall, lipid peroxidation was most effectively reduced by SWE, followed by FE and SA treatments.

Effect of elicitors on H_2O_2 content

Hydrogen peroxide (H_2O_2) is a key indicator of oxidative stress in plants, and a reduced H_2O_2 level reflects enhanced antioxidant defence and improved cellular protection. H_2O_2 content exhibited treatment and tissue specific variations throughout the experimental period. In leaf tissues, SWE treatment reduced H_2O_2 content by 1.45, 1.30, 1.03, and 1.20 fold during the 1st, 4th, 5th, and 6th months, respectively, whereas a slight increase of 1.17 and 1.05 fold was observed during the 2nd and 3rd months compared with the control (Fig. 6c). In root tissues, SWE consistently decreased H_2O_2 content by 1.00-1.36 fold relative to the control (Fig. 6d). In FE treatment in leaf tissues, H_2O_2 content was found to be decreased by 1.39, 1.25, 1.08, and 1.05 fold during the 1st, 4th, 5th, and 6th months, respectively, while increases of 1.51- and 1.02-fold were recorded during the 2nd and 3rd months compared with the control. In root tissues, FE treatment showed elevated H_2O_2 content by 1.41 and 1.07 fold during the 1st and 4th months, respectively, whereas reductions of 1.40, 1.07, 1.23, and 1.06 fold were observed during the 2nd, 3rd, 5th, and 6th months, respectively, compared with the control. Similarly, SA treatment altered H_2O_2 levels over time. In leaf tissues, H_2O_2 content decreased by 1.48, 1.11, and 1.15 fold during the 1st, 4th, and 6th months, respectively, while increases of 1.51, 1.01, and 1.30 fold were observed during the 2nd, 3rd, and 5th months, respectively, compared with the control. In root tissues, SA consistently reduced H_2O_2 content by 1.07-1.22 fold relative to the control. Overall, SWE was the most effective in reducing H_2O_2 accumulation, followed by FE and SA, indicating the improved regulation of oxidative stress.

Effect of elicitors on SOD activity

SOD activity was markedly influenced by elicitor treatment. Among the elicitor treatments, the highest increase in SOD activity was observed with SWE, with 1.00-4.00 fold increases in leaf tissue and 1.33-4.00 fold increases in root tissue compared to the control (Fig. 7a,b). FE treatment also significantly increased SOD activity, which was 1.00-3.00 fold higher in leaves and 2.00-4.00 fold higher in roots than the control. Similarly, SA treatment increased SOD activity in leaf tissues by 1.33-4.00 fold and in root tissues by 1.00-2.50 fold as compared with control plants. Overall, SWE treatment increased SOD activity, followed by FE and SA, indicating a more potent antioxidant defence response in treated plants.

Effect of elicitors on CAT activity

Catalase (CAT) is an important indicator of the enzymatic antioxidant defence system, exhibiting marked variation among the elicitor-treated and control plants. In leaf tissue, SWE resulted in a 3.21-11.07 fold increase in CAT activity, while root tissue exhibited a 1.02-4.07 fold increase as compared to the control (Fig. 7c). FE treatment also significantly enhanced CAT activity, showing a 2.18-9.83 fold increase in leaf tissue and in root tissue CAT activity increase by 1.33, 1.39, 3.56 and 1.97 fold during the 1st, 2nd, 4th, and 6th months, respectively, whereas 1.08 and 1.20 fold reductions were observed during the 3rd and 5th months than the control. Similarly, SA treatment enhances CAT activity by 1.44-13.45 fold in leaf tissue relative to the control. In root tissue, CAT activity was increased by 1.15, 1.41, 1.55, 1.00 and 1.43 fold during 1st, 2nd, 4th, 5th and 6th months, respectively, while a 1.26-fold reduction was observed during the 3rd month compared to the control (Fig. 7d). The greater CAT activity in leaves is likely associated with higher photosynthetic activity, which generates large amounts of ROS, thereby requiring a more robust antioxidant defence system to maintain cellular redox homeostasis.

Effect of elicitors on essential oil yield and patchouli alcohol concentration

Patchouli alcohol is recognized as a principal marker compound in *V. jatamansi* essential oil and is widely associated with its characteristic aroma and pharmacological properties. The essential oil yield of roots varied significantly across treatments. The maximum essential oil yield (v/w) was recorded in SWE treated plants (2.50%), followed by SA treated plants (1.90%) and FE-treated plants (1.65%), whereas control plants exhibited the lowest oil content (1.50%). Compared with the control, essential oil content increased by 66.7%, 26.7%, and 10.0% in SWE, SA, and FE treated plants, respectively (Fig. S4a). GC-MS analysis revealed significant variation in patchouli alcohol concentration among different elicitor treatments (Fig. S4b). The highest patchouli alcohol concentration was recorded in the SWE treated plants (238.31 mg/L), followed by SA (205.11 mg/L), FE-treated plants (128.20 mg/L) and the control plants (92.54 mg/L).

Effect of elicitors on essential oil compositions

GC-MS analysis identified 22 volatile compounds in control plants, 21 compounds in SWE treated plants, 19 compounds in SA treated plants, and 16 compounds in FE treated plants (Table 1). Patchouli alcohol was the most abundant constituent across all treatments, accounting for (43.27%) in FE treated plants, followed by control (40.32%), SWE treated (39.50%), and SA treated plants (35.59%). δ-Guaiene was the second most abundant volatile

compound, showing the highest relative abundance in control plants (8.62%), followed by SWE treated (7.95%), SA treated (7.45%), and FE treated plants (4.97%). In contrast, Maaliol showed the highest accumulation in FE treated plants (7.76%), whereas lower levels were observed in control (4.99%), SA treated (4.66%), and SWE treated (4.39%) plants. Several other volatile constituents were detected across the different elicitor treatments these included α-Fenchene (1.27-1.99%), β-Patchoulene (0.54-2.23%), β-Elemene (1.07-1.47%), α-Santalene (1.32-2.10%), β-Gurjunene (2.11-2.88%), α-Guaiaene (5.21-5.33%), α-Guajene (2.70-4.70%), Aromadendrene (4.71-6.83%), Seychellene (3.34-4.12%), α-Guaiene (2.94-4.79%), α-Patchoulene (2.31-3.71%), α-Gurjunene (0.29-0.79%), Italicene (1.07-1.23%), β-Selinene (0.30-2.29%), α-Selinene (1.49-2.65%), α-Humulene (2.39-2.58%), α-Maaliene (1.89-2.11%), Kessane (0.62-0.69%), Viridiflorene (1.00-1.29%), and Longifolenaldehyde (0.94-1.83%). These compounds were present in varying proportions in the elicitor treated and control plants, contributing to the overall volatile profile of *V. jatamansi*.

Effect of elicitors on antimicrobial activity of essential oil

The antimicrobial activities of four distinct *V. jatamansi* essential oils were tested against standard Gram-negative and Gram-positive bacterial strains using the broth microdilution assay (Fig. S5). At 0.50 mg/mL, the extracts exhibited variable, strain-dependent antimicrobial potential after 8 h of incubation at 37 °C compared with the positive control (tetracycline). (Table S2). In general, Gram-positive strains showed higher susceptibility as compared to Gram-negative strains. All extracts exhibited strong inhibition (>99%) against *B. subtilis*, while *S. aureus* inhibition remained ≥ 76.27 %. Conversely, Gram-negative bacteria showed greater resilience, with growth inhibition ≥58.42% in *S. typhimurium*. *E. coli* proved to be the least susceptible organism tested, with an inhibition of 26.56 ± 14.96%. In addition, sample SWE exhibited broad-spectrum efficacy by demonstrating significant inhibition (≥ 96.85%) against *S. typhimurium*, *B. subtilis*, and *S. aureus*. Based on these screening trends, the MIC was estimated for the active samples across a concentration range of 0.03-0.50 mg/mL. Concentration-dependent profiling showed that all four extracts effectively repressed the growth of *B. subtilis*, establishing an MIC of 0.06 mg/mL, below which inhibition reduced to <95% (Table S3). For the broad-spectrum extract SWE, the MIC was found to be 0.06 mg/mL against *S. aureus*, while *S. typhimurium* exhibited the highest MIC of 0.25 mg/mL (Table S4). However, upon subculturing the broth from these wells onto agar plates to determine MBC, persistent bacterial growth was observed after 24 hours of incubation.

Effect of elicitors on valepotriate content

Elicitor treatment significantly influenced valepotriate accumulation in *V. jatamansi* cultivated under aeroponic conditions. Among the tested elicitors, FE treatment consistently produced the maximum accumulation of acevaltrate, didrovaltrate and valtrate. Acevaltrate content in leaf tissue ranged from 0.02-0.04% in the control, 0.02-0.05 under SA, 0.01-0.18% under FE, and 0.01-0.10% under SWE treatments (Fig. 8a). The maximum leaf acevaltrate (0.18%) was found in the FE treatment during the 4th month of cultivation. In root tissue, acevaltrate content ranged from 0.07-0.26% (control), 0.06-0.29% (SA), 0.08-0.047% (FE) and 0.10-0.46% (SWE), with the maximum accumulation of (0.47%) observed in FE-treated plants during the 6th month (Fig. 8b). A similar trend was observed for didrovaltrate accumulation. In leaf tissue, didrovaltrate content varied from 0.04-0.09% in the control, 0.03-0.08% in SA, 0.02-0.23% in FE and 0.03-0.14% in SWE treatment (Fig. 8c). The highest concentration (0.23%) was detected in FE-treated leaves during the 2nd month of cultivation. In roots, didrovaltrate content ranged from 0.06-0.32% (control), 0.06-0.46% (SA), 0.08-0.53% (FE) and 0.04-0.44% (SWE). The maximum root didrovaltrate content (0.53%) was also recorded under FE treatment during the 4th month (Fig. 8d). Valtrate was the predominant valepotriate detected in both tissues. In leaves, valtrate content ranged from 0.15-0.46% in the control, 0.15-0.37% in SA, 0.26-1.11% in FE and 0.17-0.74% in SWE treatments (Fig. 8e). FE treatment resulted in the highest leaf valtrate content (1.11%) during the 4th month of cultivation. Similarly, root valtrate content ranged from 1.38-2.38% (control), 1-29-2.31% (SA), 1.51-3.10% (FE), and 1.07-2.42% (SWE) (Fig. 8f). The maximum valtrate accumulation (3.10%) was observed in FE treated roots during the 4th month. Overall, FE treatment markedly enhances the biosynthesis of all three valepotriates compared with the control, SA and SWE treatment, indicating that FE elicitation is the most effective strategy for promoting valepotriate production on aeroponically cultivated *V. jatamansi*.

Effect of elicitors on iridoid biosynthesis genes

Gene expression profiling of iridoid biosynthesis was evaluated to assess the effects of different elicitors in vertically aeroponically grown *V. jatamansi*. In leaf tissue, *7DLS* showed increased expression under SA, FE, and SWE, with higher expression under SA at 2nd, 4th, and 6th months, under SWE at 3-6th months (Fig. 9). *GES* was strongly upregulated by SA and FE, particularly at 3rd, 5th, and 6th months, whereas SWE generally exhibited reduced expression. *SLS* was predominantly induced by FE throughout the sampling period, with additional enhancement under SA and SWE at specific months. *IS* was strongly upregulated by SA and SWE

specifically in the later months, whereas FE showed elevated expression at the 3rd and 6th months. *7DLGT* exhibited pronounced upregulation under FE and SWE, especially at 2-4th and 6th months, whereas SA generally showed reduced expression. *G10H* was predominantly upregulated by FE and SWE, whereas SA showed comparatively lower expression. In root tissue, *7DLS* showed elevated expression under SA, FE and SWE, particularly during later sampling months (Fig. 10). *GES* was predominantly unregulated by FE during 1-3rd months, with additional induction by SA and SWE during the 1st month. *SLS* exhibited strong upregulation under FE throughout most sampling periods, while SA and SWE also enhanced their expression during the specific months. *IS* showed increased expression under SA, FE and SWE at specific months. *7DLGT* was strongly upregulated by SA, FE, and SWE, particularly in the later months. *G10H* was predominantly upregulated by SWE, followed by FE and SA at specific months. Overall, elicitor application induces distinct tissue and treatment specific expression patterns in *V. jatamansi*. In leaves, SA predominantly upregulates *7DLS, GES,* and *IS,* whereas FE enhances *SLS, 7DLGT* and *G10H,* and SWE strongly induces *7DLS, IS, 7DLGT* and *G10H* at specific months. In roots, *IS, 7DLGT* and *G10H* showed prominent upregulation, and *7DLS, GES,* and *IS* exhibited month- and elicitor-dependent variations. Collectively, these findings indicate differential transcriptional regulation of specialized metabolite biosynthetic genes in leaf and root tissues.

Discussion

Aeroponic farming has gained widespread recognition among researchers owing to enhanced aeration, efficient nutrient uptake, and precise nutrient management, thereby promoting plant growth and phytochemical production [13, 16]. Owing to climatic constraints, variability in phytochemical composition, and increasing anthropogenic pressure on natural populations, aeroponic cultivation has emerged as a promising alternative for producing medicinal plants [40]. This cultivation system offers several advantages, including enhanced and uniform biomass production, improved plant growth and performance, and increased accumulation of secondary metabolites. Furthermore, the integration of elicitation and aeroponics represents a promising cultivation strategy, offering precise nutrient management and enhancing plant productivity and secondary metabolites [41, 42, 24]. Elicitation is a potential biotechnological strategy for boosting phytochemicals production by triggering plant defence responses. Elicitors act as signalling molecules that activate plant defence and the immune system and regulate the transcription of biosynthetic genes, thereby promoting the accumulation of stress-responsive phytochemicals that support plant adaptation and defence [16]. In the present

investigation, biotic elicitors (FE and SWE) and an abiotic elicitor (SA) were applied as foliar sprays to *V. jatamansi* plants cultivated in an aeroponic system to investigate their influence on plant growth, physiological and biochemical attributes, and the accumulation of specialized metabolites and volatile compounds.

Elicitor-mediated modulation of morphological growth and biomass accumulation

In our study, among the different elicitor treatments, SA treated plants exhibited the maximum vegetative growth, with the highest shoot length (25.26 cm), leaf length (11.17 cm), leaf width (8.75 cm), and root length (13.29 cm). The findings of our studies are comparable with earlier reports. Partap et al. [13] also reported that the application of 0.5 mg/L yeast extract significantly enhanced plant height, root length, and root number in aeroponically cultivated *V. jatamansi*. Recently, Vinay et al. [43] demonstrated that the administration of SA (50 µM) enhances plant height and the shootlet numbers in hydroponically grown *Bacopa monnieri*. In *V. officinalis*, *Withania coagulans*, *Echinacea purpurea*, and *Cichorium intybus*, treatment with SA (150 mg/L) facilitated the maximum plant height (130.6 cm) and root length (67.5 cm), demonstrating the growth-promoting effect of SA under aeroponic cultivation [44]. Among the different elicitor treatments, FE treatment significantly enhanced biomass accumulation, resulting in the highest leaf FW (31.77 gm), leaf DW (3.46 gm), root FW (24.06 gm), and root DW (2.88 gm) compared with the other treatments. Similarly, Da Silva Oliveira et al. [45] demonstrated that inoculation of hydroponically grown lettuce with *Bacillus subtilis* (15.6×10^3 CFU mL^{-1}) significantly improved biomass accumulation, increasing root FW, root DW, and shoot DW by 34%, 51%, and 40%, respectively, relative to non-inoculated plants. In another study, inoculating *Saccharum* spp. with *B. subtilis* enhances FW and DW by improving photosynthetic efficiency, carbon uptake, and root nutrient uptake [46]. Beneficial microorganisms, like endophytes, inhabit plant rhizospheres and enhance plant growth as well as phytochemical accumulations [23, 47]. These microorganisms promote plant growth through biological N_2 fixation, phytohormone synthesis [22], enhanced nutrient uptake, and mitigation of salinity stress [48]. Collectively, these mechanisms improve plant growth, biomass accumulation, and overall plant vigour.

Elicitor-induced regulation of chlorophyll fluorescence and photochemical efficiency

Chlorophyll fluorescence analysis provides a sensitive, non-destructive assessment of the functional status of photosystem II (PSII) and the efficiency of light energy utilization for photosynthesis. In the present study, different elicitor applications distinctly altered PSII

photochemistry and energy partitioning in *V. jatamansi* cultivated under aeroponic conditions. The maximum quantum efficiency of PSII (QY_max) remained consistently high (0.67-0.82), demonstrating the sustained functional integrity of PSII under aeroponic cultivation and elicitor application. Similar observation was claimed by Sobczak et al. [24], who reported similar PSII values (0.71-0.74) in aeroponically grown *Capsicum annuum* following exposure to different EC regimes and exogenous SA treatments. In contrast, the effective quantum yield (ΦPSII/qP) was markedly enhanced by FE treatment, particularly during 2-4 months, suggesting the improved photochemical efficiency and more effective utilization of absorbed light for electron transport. This response likely reflects enhanced nutrient acquisition and metabolic activity mediated by endophyte microorganisms. The higher qP values observed in FE-treated plants indicate a greater proportion of open PSII reaction centres, supporting efficient photochemical electron transport. Likewise, Yihui et al. [49] reported increased qP and ΦPSII in *Gaeumannomyces cylindrosporus* inoculated maize, demonstrating enhanced PSII photochemical efficiency following microbial inoculation. NPQ was generally high in SWE-treated plants during the initial growth period, indicating enhanced dissipation of excess excitation energy as heat and improved photoprotection against oxidative stress. Comparable observations were obtained by Robles-Carnero et al. [50] in *Ulva compressa* under high-temperature stress. Likewise, slightly elevated qN values under SWE suggest activation of the protective energy dissipation mechanism. The higher Rfd value observed in SWE and FE plants further reflects improved photosynthetic performance and metabolic activity. Previous studies also reported that integrating aeroponic cultivation with elicitation enhanced the photosynthetic rate in *V. jatamansi* by increasing chlorophyll content, improving nutrient and CO_2 availability, and improving root-zone aeration [13]. Overall, FE enhanced photochemical efficiency, whereas SWE primarily improved photoprotection, with SA showing intermediate effects. The maintenance of high QY_max alongside increased ΦPSII, qP and Rfd indicates improved photosynthetic performance without compromising PSII integrity.

Elicitor-mediated modulation of antioxidant enzyme activity

In the present investigation, elicitor treatment significantly improved antioxidant defence, as evidenced by reduced MDA and H_2O_2 content and increased CAT and SOD activities, although their responses varied by tissue type and sampling month. The significant reduction in MDA and H_2O_2 content demonstrates that elicitor treatment effectively alleviated oxidative stress by limiting ROS accumulation and membrane lipid peroxidation. SWE treatment exhibited the strongest protective effect, reducing MDA by 1.40-3.57 fold in leaf tissue and 2.10-4.14 fold

in root tissue, while H_2O_2 content also declined throughout most of the cultivation period in both tissues. The enhanced protection is likely attributable to the diverse bioactive constituents of SWE, including phytohormones, polysaccharides, phenolics, vitamins, and folic acid, which enhance antioxidant metabolism, facilitate ROS detoxification, and preserve membrane integrity [20]. FE and SA treatments also significantly reduced MDA and H_2O_2 content, suggesting that microbial colonization improves stress tolerance through enhanced nutrient acquisition and the induction of systemic defence responses. These observations agree with earlier studies by Manikandan et al. [51], who reported that inoculation with Endophytic *Pantoea agglomerans* alleviates oxidative stress by reducing MDA and H_2O_2 levels through enhanced antioxidant defence, thereby improving host tolerance to environmental stress in hydroponically grown *Calotropis procera*. Interestingly, transient increases in H_2O_2 were observed during the 2nd and 3rd months in leaf tissue under all elicitor treatments and during specific months in FE treated roots. Such temporary increases are characteristic of elicitor-induced oxidative burst, where controlled H_2O_2 production functions as a signalling molecule to activate defence related antioxidant metabolism and specialized metabolites biosynthesis rather than causing oxidative damage. As cultivation progressed, H_2O_2 concentration declined in subsequent months, suggesting that ROS generation was effectively balanced by enhanced antioxidant defence mechanisms. The reduction in oxidative damage was also closely related to elevated CAT and SOD activities. SWE treatment induced the greatest increase in SOD activity (1.00-4.00 fold in leaves and 1.33-4.00 fold in roots) together with substantial enhancement of CAT activity (3.21-11.07 fold in leaves and 1.02-4.07 fold in roots), demonstrating superior ability to activate the ROS scavenging pathway. A similar increase in SOD activity following *Ascophyllum nodosum* extract application has been reported in turf grass [52] and bean plants treated with *Ulva rigida* and *Fucus spiralis* extracts [53]. Likewise, enhanced CAT activity following SWE treatment has been reported in drought and salt stressed plants and Fe deficient tomato roots, suggesting activation of the enzymatic antioxidant defence system [54, 55]. FE and SA also significantly enhanced CAT and SOD activity, although the magnitude of induction was comparatively lower and occasional reductions in CAT activity were observed during individual sampling months in root tissue. In FE treatment, enhanced SOD and CAT activities indicated improved ROS detoxification, thereby enhancing antioxidant activity mediated by beneficial plant microbe interactions. Likewise, Zou et al. [56] demonstrated that inoculation with the endophytic fungus *Falciphora oryzae* significantly upregulated the expression of *CAT*, *APX*, *GR*, *POD*, *GSH1*, and *GSH2* genes, leading to increased antioxidant enzyme activities and glutathione accumulation, thereby enhancing

oxidative stress tolerance in pepper under salt stress. Likewise, SA treatment markedly increased SOD and CAT activities, particularly in leaf tissues, indicating strong activation of the antioxidant defence system in photosynthetically active organs. Yang et al. [57] demonstrated that exogenous SA application enhanced SOD and CAT activities while suppressing the accumulation of H_2O_2 and O_2^-, thereby improving antioxidant capacity through flavonoid and glutathione contents. This response suggested that SA enhances ROS detoxification by stimulating antioxidant enzymes and, thereby, directly or indirectly reducing ROS accumulation, ultimately strengthening cellular redox homeostasis [58]. Although occasional reductions in CAT activity were observed in FE and SA treated roots during specific sampling months, these transient fluctuations may reflect developmental changes in ROS metabolism, temporal adjustments in antioxidant regulation, or compensatory activity of other ROS-scavenging enzymes. The control plants exhibited significantly higher H_2O_2 and MDA contents, accompanied by lower SOD and CAT activities, indicating greater oxidative stress and reduced antioxidant capacity under aeroponic conditions. The limited activities of SOD and CAT impaired ROS detoxification, resulting in H_2O_2 accumulation and enhanced membrane lipid peroxidation. The coordinated induction of SOD and CAT accounts for the reduced H_2O_2 accumulation observed across treatments and highlights the synergistic roles of these enzymes in regulating ROS homeostasis. Overall, the coordinated reduction in MDA and H_2O_2, coupled with increased CAT and SOD activities, demonstrates that elicitor treatments strengthened the antioxidant defence system of *V. jatamansi*.

Elicitor-mediated modulation of essential oil yield and volatile composition

In aeroponic cultivation, elicitor treatments significantly influence the yield and composition of *V. jatamansi* essential oil. Root-drying methods, climatic conditions, plant phenological stage, and cultivation practices further influenced the oil content and chemical profile. Thakur et al. [4] reported essential oil yields ranging from 0.17% to 0.23% (v/w) in *V. jatamansi* roots under different drying conditions. Likewise, Singh et al. [9] observed essential oil yields of 0.60 to 1.66% (v/w) in root and rhizome tissues of *V. jatamansi* collected from diverse agroclimatic regions, indicating that environmental conditions and postharvest processing can substantially influence essential oil accumulation. In our study, the oil content ranged from 1.50 to 2.50% (v/w). The highest oil yield was recorded in the SWE treated plants at 2.50% (v/w). The higher essential oil yield in SWE treated plants is due to the combined effects of aeroponic cultivation and SWE. Aeroponics enhances nutrient uptake and root oxygenation, while SWE supplies phytohormones and bioactive compounds that stimulate photosynthesis

and terpenoid biosynthesis, thereby increasing essential oil accumulation. Nunes et al. [59] claimed that *Ocimum basilicum* plants subjected to the foliar application of SWE from *K. alvarezii* exhibited significantly increased essential oil yield relative to the control. Comparable improvements in essential oil yield were also noted in lemongrass by Yogendra et al. [60] following the administration of SWE from *K. alvarezii* in conjunction with the recommended dose of fertilizer (RFD).

Furthermore, elicitor treatment significantly influences the volatile compositions of oil. The highest concentration (2.5% (v/w) of essential oil and patchouli alcohol (238.31 mg/L) was detected in the SWE treatment, whereas the marker compound, patchouli alcohol, was found at the highest concentration (43.27%) in the FE treated plants. Similarly, Maaliol (7.76%) was also found in FE treated plants. Interestingly, δ-guaiene (8.62%) decreased from the control (4.97%) in FE treated plants, whereas patchouli alcohol and maaliol increased considerably. The increase in patchouli alcohol and maailol content in FE treatment suggested that beneficial endophytic fungi enhance nutrient uptake, precursor compound availability, and activate defence-related pathways. Endophytes are known to produce phytohormones and other signalling compounds that stimulate secondary metabolism by inducing *terpene synthase* genes and enhancing metabolic flux through the mevalonate (MVA) pathway, thereby promoting the biosynthesis of oxygenated sesquiterpenes such as patchouli alcohol. Supporting our studies, Yuan et al. [61] demonstrated that the endophytic fungus AL12 enhanced sesquiterpenoid biosynthesis by activating the MVA pathway and increasing the levels of carbohydrates, pyruvate, and acetyl-CoA, key precursors for sesquiterpene biosynthesis. Since acetyl-CoA, derived from glycolysis, serves as the primary substrate for the MVA pathway [62], the higher patchouli alcohol content observed in our investigations may be associated with endophyte-induced enhancement of precursor availability and metabolic flux toward sesquiterpene biosynthesis. In contrast, SA treatment reduced patchouli alcohol (35.59%) while increasing β-patchoulene (2.23%), β-gurjunene (2.88%), aromadendrene (6.83%), and α-guajene (4.70%). This enhancement may be attributed to improved nutrient absorption or to alterations in the number of oil glands and in monoterpene production, as documented in prior research on basil and marjoram [63]. Similar enhancements in essential oil accumulation following SA application have been documented in other aromatic plant species, including *Mentha piperita* [64] and lemongrass [65], supporting the positive effect of SA in regulating volatile compound biosynthesis and composition. SWE treatment maintained a relatively high patchouli alcohol content (39.50%) and increased the levels of Seychellene (4.12%), α-santalene (2.10%), β-

selinene (2.29%), and α-selinene (2.65%). Under aeroponic cultivation, efficient nutrient delivery and continuous root oxygenation likely enhanced the biostimulant effect of SWE, resulting in improved essential oil production and maintenance of a desirable volatile profile. The highest essential oil yield (2.50% v/w) observed under SWE treatment further supports its role as an effective biostimulant for enhancing essential oil productivity. Previous studies have also reported that SWE *K. alvarezii* affects essential oil content and composition in basil and lemongrass [60, 66, 59]. Overall, the results demonstrated that elicitors differentially regulate sesquiterpene biosynthesis in *V. jatamansi*. FE inoculation preferentially enhanced pharmaceutically important oxygenated sesquiterpenes, particularly patchouli alcohol and maaliol, whereas SA and SWE mainly altered the abundance of minor volatile constituents. These findings highlighted the potential of combining aeroponic cultivation with elicitor based strategies to improve the yield and quality of *V. jatamansi* essential oil.

Elicitor-mediated enhancement of the antimicrobial efficacy of essential oil

Elicitor application significantly enhanced the antibacterial activity of *V. jatamansi* essential oil, with the strongest effects observed in the SWE treatment. SWE contains several phytoconstituents that enhance the oil's antimicrobial activity [67]. The antimicrobial efficacy of *V. jatamansi* essential oil exhibited a distinct susceptibility gradient, with greater efficacy against Gram-positive than Gram-negative bacteria. This variation may be linked to broad, distinct cell wall characteristics. The thick, highly porous peptidoglycan matrix of the Gram-positive cell wall lacks a restrictive outer barrier, allowing hydrophobic molecules to readily penetrate the cell and leading to protein denaturation and disruption of energy-producing pathways. Conversely, the complex cell wall of Gram-negative bacteria, consisting of an outer membrane situated outside the thin peptidoglycan layer, serves as a dense lipopolysaccharide shield that restricts the influx of lipophilic molecules [68]. Further, the variations observed within the same Gram's strain indicate that general Gram classification alone cannot completely predict essential oil kinetics. Instead, these intra-gram variations emphasize that species-specific features such as envelope thickness, structural barriers and localized surface charges are important determinants of the vulnerability of an individual microbe to the volatile oil. The higher patchouli alcohol content in SWE may contribute to its enhanced antimicrobial activity. Previous studies have also reported significant antimicrobial activity of *V. jatamansi* essential oil, attributed to its chemical composition [69, 4].

Elicitor-mediated transcriptional regulation of valepotriate biosynthesis

In the present study, elicitor treatment differentially regulated valepotriate accumulation in aeroponically cultivated *V. jatamansi*, with FE consistently producing the highest levels of acevaltrate, didrovaltrate, and valtrate in both leaf and root tissues. FE substantially enhanced acevaltrate accumulation to 0.18% in leaves (4.5 fold) and 0.47% in roots (1.80 fold) compared with the control. Similarly, didrovaltrate increased to 0.23% in leaves (2.55 fold increase) and to 0.53% in roots (1.65 fold increase) under FE treatment. Valtrate, the prominent valepotriate, showed the maximum responses, reaching 1.11% in leaves (2.41 fold) and 3.10% in roots (1.30 fold) under FE. In contrast, SWE produced a moderate improvement, whereas SA exhibited only marginal enhancement for acevaltrate and didrovaltrate and reduced valtrate accumulation. Recently, Thakur et al. [4] reported that valtrate and acevaltrate ranged from 0.45%-1.29% and 0.11%-0.31%, respectively, under different drying methods of *V. jatamansi*. In a similar investigation, Thakur et al. [3] reported that fungal elicitors in *V. jatamansi* significantly enhanced valepotriate production. Espinoza et al. [70] reported that inoculation with *Beauveria bassiana* enhances total alkaloid content in hydroponically cultivated *Allium schoenoprasum* plants. The higher yield of secondary metabolites in fungus treated plants could be due to fungal synthesis of these compounds within plant tissues. Endophytes can also induce host plants to accumulate secondary metabolites [70]. Endophytic fungi are known to activate plant defence responses and contain bioactive compounds such as phytohormones, polysaccharides, and precursor compounds that enhance plant secondary metabolite production [71]. The temporal variation in metabolite accumulation, with a maximum in the 4^{th} month of cultivation, further indicates that valepotriate biosynthesis is developmentally regulated and closely associated with plant maturity. Root tissues consistently accumulated higher concentrations of valepotriates than leaves, with maximum values of 0.47% acevaltrate, 0.53% didrovaltrate, and 3.10% valtrate, suggesting shoot-to-root transportation of valepotriates in *V. jatamansi* [72]. The optimized aeroponic system, characterized by efficient nutrient delivery and continuous root aeration, likely enhanced precursor availability and metabolic activity, thereby amplifying the elicitor response [13]. The synergistic effects of elicitation, aeroponic and temporal optimization present a potential approach for increasing valepotriate yield.

Furthermore, we examined the expression of iridoid pathway genes to better understand the enhanced valepotriate content. The results showed a relationship between valepotriate accumulation and gene expression level. Gene expression analysis revealed tissue, elicitor, and time dependent regulation of iridoid biosynthesis and gene expression patterns. In leaf tissue, SA showed comparatively broad transcriptional activation with maximum expression of *GES*

(2.57 fold, 3rd month) and *SLS* (2.61 fold, 3rd month). SWE preferentially enhanced *7DLS* (2.51 fold, 3rd month), *IS* (2.91 fold, 6th month), and *7DLGT* (2.65 fold, 6th month), whereas FE showed maximum induction of *G10H* (2.66 fold, 3rd month), correlating with higher levels of acevaltrate, didrovaltrate and valtrate content. Root tissue exhibited stronger induction of several genes, particularly under FE treatment. The highest expression was observed for *SLS* (4.63 fold, FE, 5th month), *7DLS* (3.55 fold, SA, 6th month), *IS* (3.21 fold, FE, 1st month), *GES* (3.35 fold, FE, 1st month) and *7DLGT* (3.16 fold, SWE, 6th month), which correlate with elevated content of acevaltrate, didrovaltrate and valtrate in root tissue. The temporal expression pattern further suggested the sequential activation of pathway components. Early induction of *GES* and *IS* may support precursor formation, whereas enhanced expression of *SLS*, *7DLS*, and *7DLGT* during later stages may contribute to downstream terpenoid metabolism. The accumulation of maximum transcript levels at different cultivation periods, together with maximum valepotriate accumulation around the 4th month, indicates that transcriptional activation precedes or accompanies metabolite accumulation and may contribute to subsequent valepotriate production. *G10H*, encoding a cytochrome P450 monooxygenase involved in terpenoid biosynthesis, was strongly upregulated by FE and SWE in leaf and root, potentially contributing to boosted valepotriate production [73]. Similarly, *GES*, which catalyses geraniol formation, showed increased expression in response to elicitor treatments in both tissues, suggesting coordinated activation of terpenoid/iridoid biosynthetic pathways [74]. Comparable results have been documented in earlier research indicating that different environmental and elicitor stimuli can activate overlapping specialized metabolic pathways in *V. jatamansi*. Spectral LED treatments enhanced the expression of *GES, G10H,* and *SLS* in *in vitro* cultures [1], while drought stress and FE elicitation also upregulate several genes in *V. jatamansi* [2, 3]. The overlap between these previously reported responses and the present expression profiles supports the involvement of a common stress-responsive transcriptional network underlying specialized metabolite biosynthesis. Furthermore, elevated expression of biosynthetic genes may be associated with the ability of fungal-derived elicitors to activate host defence signalling. Recognition of fungal molecular signals can stimulate ROS and hormone-mediated pathways, particularly those involving JA, SA, and ethylene, which subsequently regulate transcription factors controlling specialized metabolism [75]. Endophytes may also modify precursor availability and host metabolic status, thereby supporting increased flux through specialized metabolic pathways [71]. Overall, the present results demonstrated that SA, FE, and SWE differentially regulate biosynthetic gene expression, with FE exerting particularly strong effects in roots, SA producing broader

activation of pathway genes, and SWE promoting specific late-stage iridoid-associated responses. The agreement with previous LED, drought, and fungal elicitation studies further suggested that both biotic and abiotic stimuli can influence regulatory mechanisms controlling iridoid biosynthesis in *V. jatamansi*. These findings provide molecular support for the use of targeted elicitation as a strategy for manipulating specialized metabolite production under controlled aeroponic cultivation.

Conclusions

The findings demonstrated that the combined use of aeroponics and elicitor treatment is a robust approach for the sustainable production of superior-quality biomass and metabolites in *V. jatamansi*. Elicitor-specific responses enable targeted enhancement of key traits, with SA improving morphological growth, FE contributing to biomass and valepotriate accumulation, and SWE boosting essential oil yield and patchouli alcohol accumulation. Enhanced photosynthetic performance, antioxidant defence, expression of iridoid biosynthetic genes and antimicrobial potential further demonstrate the physiological and molecular benefits of elicitation. Future research should focus on optimizing elicitor dosage, application timing, and combinations to improve secondary metabolite and essential oil biosynthesis in aeroponically cultivated crops. The integration of transcriptomic and metabolomic investigations may clarify the molecular networks responsible for elicitor-induced metabolite accumulation. Additionally, scaling up optimized elicitation protocols and conducting targeted profiling of essential oil constituents could support the standardized commercially viable production of high-value phytochemicals.

Abbreviations

FE	Fungal Endophyte
SWE	Seaweed Extract
SA	Salicylic Acid
EC	Electrical Conductivity
IHBT	Institute of Himalayan Bioresource Technology
SCADA	Supervisory Control and Data Acquisition
PPFD	Photosynthetic Photon Flux Density
PDA	Potato Dextrose Agar
ITS	Internal Transcribed Spacer

CTAB	Cetyltrimethylammonium Bromide
PDB	Potato Dextrose Broth
QYmax	Maximum Quantum Efficiency of PSII
ϕPSII	Effective Quantum Yield of PSII
NPQ	Non-Photochemical Quenching
qP	Photochemical Quenching Coefficients Based on the Puddle Models
qL	Photochemical Quenching Coefficients Based on the Lake Models
qN	Non-Photochemical Quenching Coefficient
Rfd	Relative Fluorescence Decrease Ratio
MDA	Malondialdehyde
TBA	Thiobarbituric Acid
TCA	Trichloroacetic Acid
H_2O_2	Hydrogen Peroxide
SOD	Superoxide dismutase
NBT	Nitroblue Tetrazolium Chloride
CAT	Catalase
MIC	Minimum Inhibitory Concentration
MBC	Minimum Bactericidal Concentration
GES	*Geraniol Synthase*
G10H	*Geraniol 10-Hydroxylase*
IS	*Iridoid Synthase*
7DLS	*7-Deoxyloganetic Acid Synthase*
7DLGT	*7-Deoxyloganetic Acid Glucosyl Transferase*
SLS	*Secologanin Synthase*
FW	Fresh Weight
DW	Dry Weight
PSII	Photosystem II
RFD	Recommended Dose of Fertilizer
MVA	Mevalonate

Author contribution

Amit Kumar, Ashish R. Warghat: Conceived the concept and framed the experimental design, execution of all experiments, data analysis and investigation, original manuscript writing and editing, supervision, funding acquisition. **Ankita Thakur, Shiv Shanker Pandey**: Endophyte isolation and characterization, manuscript writing and editing. **Shashi Rani, Seema Devi:** Data analysis and investigation. **Priyanka Parmar:** Enzymatic analysis. **Arushi Katoch, Dharam Singh**: Antimicrobial analysis, manuscript writing and editing. **Anil Kumar, Dinesh Kumar:** UPLC analysis, manuscript writing and editing. **Vivesh Sood, Shiv Kumar:** Essential oil extraction, GC-MS analysis. **Mohit Sharma:** Essential oil extraction, manuscript writing and editing. **Ramjeelal Meena:** Nursery raising and providing planting material for experimental work

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Supplementary information

The data are available in the article and its online supplementary material.

Declarations

Ethics approval and consent to participate: Not applicable.

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Availability of data and materials

All data generated or analyzed during this study are included in this article and its supplementary information files. The data used to support the findings of this study are available from the corresponding author on reasonable request.

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Legends to figures and tables

Fig. 1 Schematic representation of the aeroponic cultivation process of *Valeriana jatamansi*

Fig. 2 Effect of SA, FE, and SWE on morphological traits of aeroponically cultivated *Valeriana jatamansi*. a) shoot length, b) leaf length, c) leaf width, and d) root length. Data are presented as mean ± standard error (SE) (n = 15). Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, $P \leq 0.05$). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract

Fig. 3 Effect of SA, FE, and SWE on biomass yield of aeroponically cultivated *Valeriana jatamansi*. a) Leaf FW, b) Root FW, c) Leaf DW, and d) Root DW. Data are presented as mean ± standard error (SE) (n = 3). Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, $P \leq 0.05$). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; FW, fresh weight; DW, dry weight

Fig. 4 Effect of SA, FE, and SWE on Chlorophyll fluorescence parameters of aeroponically cultivated *Valeriana jatamansi*. a) QY_max, b) ϕPSII, c) NPQ, and d) qN. Data are presented as mean ± standard error (SE) (n = 3). Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, $P \leq 0.05$). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; QY_max, maximum photosynthetic efficiency of PSII; ϕPSII, yield of PSII; NPQ, non-photochemical quenching; qN, non-photochemical quenching coefficient

Fig. 5 Effect of SA, FE, and SWE on Chlorophyll fluorescence parameters of aeroponically cultivated *Valeriana jatamansi.* a) qP, b) qL, and c) Rfd. Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, $P \leq 0.05$). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; qP, photochemical quenching based on the puddle model; qL, photochemical quenching based on the lake model; Rfd, relative fluorescence decrease ratio

Fig. 6 Effect of SA, FE, and SWE on antioxidant activity of aeroponically cultivated *Valeriana jatamansi.* a) Leaf MDA, b) Root MDA, c) Leaf H_2O_2, and d) Root H_2O_2. Different letters indicate statistically significant differences between treatments (Duncan's multiple range test,

P ≤ 0.05). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; MDA, malondialdehyde; H_2O_2, hydrogen peroxide

Fig. 7 Effect of SA, FE, and SWE on antioxidant activity of aeroponically cultivated *Valeriana jatamansi.* a) Leaf SOD, b) Root SOD, c) Leaf Catalase, and d) Root Catalase. Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, P ≤ 0.05). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; SOD, superoxide dismutase

Fig. 8 Effect of SA, FE, and SWE on valepotriate content of aeroponically cultivated *Valeriana jatamansi.* a) Leaf acevaltrate, b) Root acevaltrate, c) Leaf didrovaltrate, d) Root didrovaltrate, e) Leaf valtrate, and f) Root valtrate. Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, P ≤ 0.05). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract

Fig. 9 Effect of SA, FE, and SWE on iridoid biosynthesis pathway gene expression in leaf tissue of aeroponically cultivated *Valeriana jatamansi.* a) *7DLS*, b) *GES*, c) *SLS*, d*) IS*, e) *G10H*, and f) *7DLGT.* Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, P ≤ 0.05). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; *7DLS, 7-deoxyloganetic acid synthase; GES, geraniol synthase; SLS, secologanin synthase; IS, iridoid synthase; G10H, geraniol 10-hydroxylase; 7DLGT, 7-deoxyloganetic acid glucosyl transferase*

Fig. 10 Effect of SA, FE, and SWE on iridoid biosynthesis pathway gene expression in root tissue of aeroponically cultivated *Valeriana jatamansi.* a) *7DLS*, b) *GES*, c) *SLS*, d*) IS*, e) *G10H*, and f) *7DLGT.* Different letters indicate statistically significant differences between treatments (Duncan's multiple range test, P ≤ 0.05). SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; *7DLS, 7-deoxyloganetic acid synthase; GES, geraniol synthase; SLS, secologanin synthase; IS, iridoid synthase; G10H, geraniol 10-hydroxylase; 7DLGT, 7-deoxyloganetic acid glucosyl transferase*

Table 1 Chemical composition (%) of essential oil extracted from the roots of aeroponically cultivated *V. jatamansi*. SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; RT, retention time; RI cal, retention index calculated; RI lit. retention index from the literature

Table 1 Chemical composition (%) of essential oil extracted from the roots of aeroponically cultivated *V. jatamansi*

S.N.	Compound name	RT value	RI Cal.	RI Lit.	Control	SA	FE	SWE	% Range
1	α-Fenchene	8.0539	-	-	1.27	1.99	-	1.61	1.27-1.99
2	β-Patchoulene	22.309	1389	1379	1.21	2.23	0.54	1.24	0.54-2.23
3	β-Elemene	22.5062	1395	1389	1.47	1.1	1.07	1.24	1.07-1.47
4	α-Santalene	23.3843	1424	1416	1.81	-	1.32	2.1	1.32-2.1
5	β-Gurjunene	23.8994	1442	1431	2.75	2.88	2.11	2.86	2.11-2.88
6	α-Guaiaene	24.0585	1447	-	5.33	-	-	5.21	5.21-5.33
7	α-Guajene	24.0712	1447	-	2.7	4.7	-	-	2.7-4.7
8	Aromadendrene	24.2621	1454	1439	4.71	6.83	-	6.0	4.71-6.83
9	Seychellene	24.3257	1456	1444	4.06	3.34	3.47	4.12	3.34-4.12
10	α-Guaiene	24.0395	1446	1437	4.79	-	2.94	4.13	2.94-4.79
11	α-Patchoulene	24.7392	1469	1454	3.57	3.55	2.31	3.71	2.31-3.71
12	α-Gurjunene	24.7839	1471	1409	0.66	0.29	-	0.79	0.29-0.79
13	Italicene	25.1464	1482	1405	1.07	1.17	-	1.23	1.07-1.23
14	β-Selinene	25.4901	1493	1489	0.52	-	0.3	2.29	0.3-2.29
15	α-Selinene	25.7699	1502	1498	2.38	2.36	1.49	2.65	1.49-2.65
16	α-Humulene	25.8717	1505	1452	-	2.39	2.58	-	2.39-2.58
17	δ-Guaiene	26.1325	1515	1437	8.62	7.45	4.97	7.95	4.97-8.62
18	α-Maaliene	26.4888	1528	-	2.11	1.89	-	2.05	1.89-2.11
19	Kessane	26.7433	1537	1529	0.69	0.62	0.64	0.62	0.62-0.69
20	Maaliol	27.9457	1578	1566	4.99	4.66	7.76	4.39	4.66-7.76
21	Viridflorene	28.5882	1599	-	1.01	1.28	1.29	1.0	1.0-1.29
22	Patchouli alcohol	30.8404	1682	1656	40.32	35.59	43.27	39.5	35.59-43.27
23	Longifolenaldehyde	31.1394	1693	-	1.78	1.64	0.94	1.83	0.94-1.83

SA, salicylic acid; FE, fungal endophytes; SWE, seaweed extract; RT, retention time; RI cal, retention index calculated; RI lit. retention index from the literature



V. jatamansi plants cultivated in the nursery



Sterilized plants with 0.05% formalin and 1% Bavistin



Sterilized net pots and clay pebbles



Plants acclimatized in liquid nutrient solution

Plant layers analyzed for:

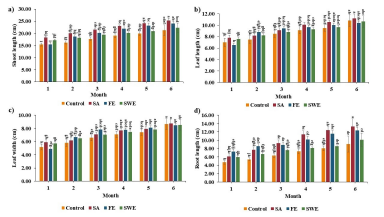
- Morphological traits
- Physiological performance
- Antioxidant enzyme capacity
- Valepotriates accumulation
- Essential oil production
- Gene expression analysis

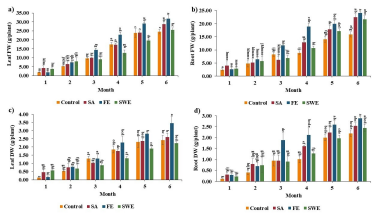


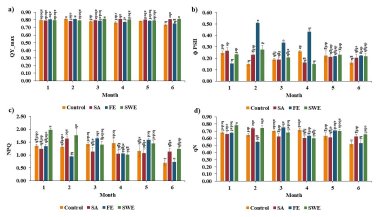
Treated plants showed improved growth

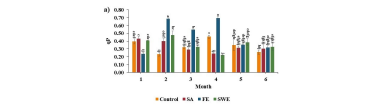


Foliar application of biostimulants and elicitors

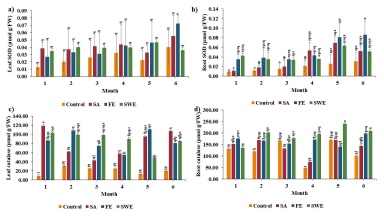

a)
Plant height (cm)
Month
b)
Leaf length (cm)
Month
c)
Leaf width (cm)
Month
d)
Spike length (cm)
Month
Control
SA
FE
SWE

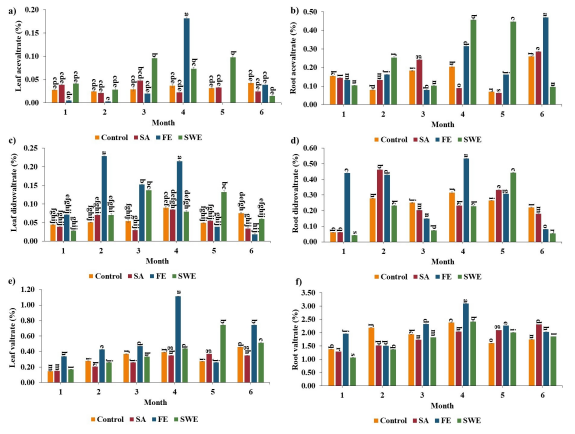

a)
Leaf FW (g/plant)
Month
Control
SA
FE
SWE
b)
Root FW (g/plant)
Month
Control
SA
FE
SWE
c)
Leaf DW (g/plant)
Month
Control
SA
FE
SWE
d)
Root DW (g/plant)
Month
Control
SA
FE
SWE


a)
QY max
Month
Control
SA
FE
SWE
b)
Φ PSII
Month
Control
SA
FE
SWE
c)
NPQ
Month
Control
SA
FE
SWE
d)
qN
Month
Control
SA
FE
SWE

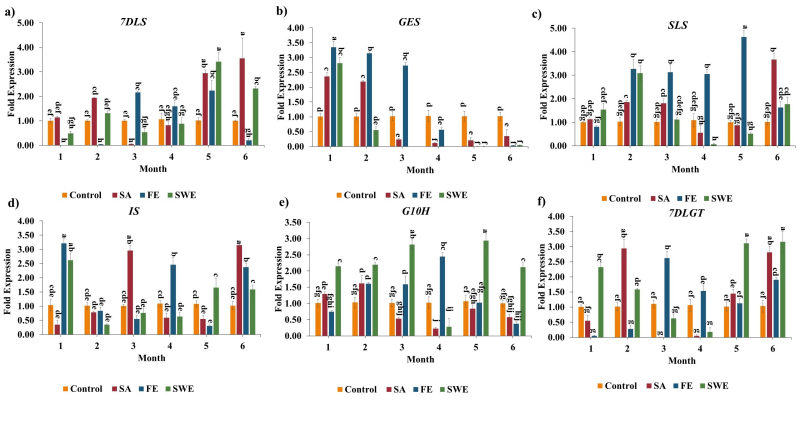

a)
gs
Month
Control
SA
FE
SWE
b)
gs
Month
Control
SA
FE
SWE
c)
PH
Month
Control
SA
FE
SWE

a)
Leaf NDA (cmol g-1DW)
Month
Control
SA
TE
SWE
b)
Root NDA (cmol g-1DW)
Month
Control
SA
TE
SWE
c)
Leaf DEA (nmol g-1DW)
Month
Control
SA
TE
SWE
d)
Root DEA (nmol g-1DW)
Month
Control
SA
TE
SWE


a)
Leaf NOD (mmol g-1FW)
Month
b)
Root NOD (mmol g-1FW)
Month
c)
Leaf nitrate (mmol g-1FW)
Month
d)
Shoot nitrate (mmol g-1FW)
Month
Control
SA
FE
SWE


a)
Leaf neoxalitrate (%)
Month
b)
Root neoxalitrate (%)
Month
Control
SA
FE
SWE
c)
Leaf dihtrosilicate (%)
Month
d)
Root dihtrosilicate (%)
Month
e)
Leaf vultrate (%)
Month
f)
Root vultrate (%)
Month

a)
7DLS
b)
GES
c)
SLS
d)
IS
e)
G10H
f)
7DLGT
Fold Expression
Month
1
2
3
4
5
6
Control
SA
FE
SWE


a)
7DLS
b)
GES
c)
SLS
d)
IS
e)
G10H
f)
7DLGT
Fold Expression
Month
Control
SA
FE
SWE

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