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The effect of abiotic stress (drought, salinity, heat) on gene expression and secondary metabolite production in endemic Iraqi medicinal and aromatic plants and the potential use of growth regulators to enhance their tolerance

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Abstract
Abiotic stresses like drought, salinity, and heat pose significant threats to medicinal plant productivity and phytochemical quality, this study investigated the morphological, physiological, biochemical, and gene expression responses of a medicinal plant to varying intensities of drought, salinity ([NaCl]), and heat stress, and evaluated the ameliorative potential of salicylic acid (SA) and gibberellic acid (GA_3), differences between two geographical origins (Shuwan and Huwayjah) were also assessed. All stress factors significantly ($p<0.001$) reduced growth parameters (stem height, dry weight, leaf area) and photosynthetic efficiency, while increasing oxidative stress markers (MDA, H_2O_2), plants responded by upregulating antioxidant enzymes (SOD, CAT, POD) and accumulating secondary metabolites, including essential oil components (e.g., linalool, camphor), total phenolics, flavonoids (e.g., rosmarinic acid), coriandrin, and ursolic acid. Gene expression analysis revealed upregulation of key genes in the MEP (DXR, HDR) and phenylpropanoid (PAL, CHS) pathways, sA application (15-20% mitigation) effectively alleviated stress impacts, reducing oxidative damage by up to 40% and enhancing antioxidant enzyme activity, notably, SA combined with moderate salinity increased essential oil yield by 20% and provided the highest economic return ($915/ha). GA_3 promoted stem elongation but reduced root mass, the Shuwan origin exhibited significantly higher stress tolerance (e.g., 25% higher salt tolerance) compared to Huwayjah, correlating with more favorable native soil and climatic conditions, extracts from SA-treated, drought-stressed plants showed enhanced antimicrobial and antioxidant activities, these findings highlight SA's potential for improving stress resilience and phytochemical value in medicinal plants, and underscore the importance of ecotype selection for cultivation in stress-prone environments.

Keywords: Abiotic stress, salicylic acid, gibberellic acid, medicinal plants, oxidative stress

Introduction
Within the grand theatre of terrestrial existence, where life's intricate ballet unfolds against a backdrop of perpetual environmental flux, plants stand as sentinels of resilience and biochemical ingenuity, their silent, sessile nature has, over evolutionary eons, necessitated the development of an extraordinarily sophisticated chemical arsenal - a panoply of secondary metabolites (SMs) - that serve not merely as passive byproducts of primary metabolism, but as dynamic agents mediating their intricate dialogue with a biotic and abiotic world fraught with challenges [1, 2], these compounds, a dazzling array of terpenoids, phenolics, alkaloids, and myriad other structural classes, are the very crucible of plant defense, communication, and adaptation, enabling survival against herbivory, pathogen onslaught, and the relentless pressures of environmental extremes [3, 4], for humanity, this phytochemical legacy has been a cornerstone of existence, providing not only sustenance but also a pharmacopoeia of unparalleled richness, from which remedies for ailments and enhancers of well-being have been drawn since the dawn of civilization [5], the profound medicinal and aromatic virtues ascribed to countless plant species are, in essence, a testament to the biological activities inherent in these SMs, their molecular structures often exquisitely tailored to interact with specific physiological targets in other organisms, including humans [6].

However, this delicate and vital interplay between plant biochemistry and environmental stability is now facing an existential crisis of unprecedented magnitude, as the spectre of global climate change casts an ever-lengthening shadow, manifesting as a dramatic intensification of abiotic stressors such as drought, soil salinization, and extreme temperature fluctuations [7, 8], these environmental perturbations are not mere inconveniences to plant life; they are profound physiological assaults that recalibrate the entirety of plant metabolism, forcing a diversion of precious energy and resources from growth towards survival and defense [9], the biosynthesis of SMs, being energetically demanding and often tightly regulated by environmental cues, is exquisitely sensitive to such stresses, plants under duress frequently exhibit significant alterations in their SM profiles, sometimes leading to an accumulation of specific protective compounds, while in other instances, a detrimental decline in valuable phytochemicals can occur, thereby impacting their medicinal efficacy, aromatic quality, and overall ecological fitness [10, 11], understanding the nuanced molecular choreography that dictates these stress-induced phytochemical shifts - from the initial perception of the stress signal at the cellular membrane, through the labyrinthine signal transduction cascades involving phytohormones and reactive oxygen species (ROS), to the ultimate reprogramming of gene expression governing SM biosynthetic pathways - is a scientific imperative of the highest order [12, 13].

It is within this critical nexus of plant stress physiology, molecular biology, and phytochemistry that the quest for strategies to bolster plant resilience and optimize the production of beneficial SMs gains profound urgency, the judicious application of plant growth regulators (PGRs), such as salicylic acid (SA) and gibberellins (GAs), offers a tantalizing prospect for modulating plant responses to abiotic stress, potentially enhancing tolerance mechanisms and fine-tuning the metabolic flux towards the synthesis of desired compounds [14, 15], this endeavor is particularly pertinent for endemic medicinal and aromatic plants (MAPs) that are not only integral to regional biodiversity and traditional healthcare systems but are also increasingly vulnerable to environmental degradation, in Iraq, a cradle of ancient civilizations with a rich heritage of phytomedicine, endemic MAPs face acute threats from escalating aridity, soil degradation, and thermal extremes, jeopardizing both their ecological persistence and their socio-economic value [16].

This investigation, therefore, embarks on a deeply focused exploration into the intricate responses of *Coriandrum sativum* L. (coriander), a plant of immense economic, medicinal, and culinary significance in Iraq, particularly in the semi-arid environs of Kirkuk, including the agriculturally vital regions of Hawija and Shuan, by subjecting coriander to meticulously controlled drought, salinity, and heat stresses, this study aims to unravel the consequent modulations in the expression of key genes within pivotal SM biosynthetic pathways, notably the methylerythritol phosphate (MEP) pathway crucial for terpenoid synthesis, and to correlate these genetic shifts with quantitative changes in a spectrum of pharmacologically active SMs, the specific phytochemical targets include the major constituents of its essential oil - linalool, geranyl acetate, and camphor, recognized for their antimicrobial and neuropharmacological attributes; alkaloids such as coriandrine and diazine derivatives, implicated in sedative and antispasmodic activities; phenolic compounds like rosmarinic acid and quercetin, lauded for their potent anti-inflammatory and antioxidant capacities; flavonoids including apigenin and luteolin, vital for cellular protection against oxidative damage; and triterpenes like ursolic acid, which plays a role in plant immunity and exhibits diverse human health benefits [17, 18], furthermore, the potential of SA and GA3 to ameliorate stress-induced damage and enhance the production of these bioactive molecules will be rigorously assessed, linking these interventions to changes in plant antioxidant enzyme machinery, the ultimate ambition is to forge a comprehensive understanding, from gene to metabolite to functional outcome, that can inform strategies for the sustainable cultivation and enhanced medicinal valorization of this vital Iraqi plant resource against the backdrop of an increasingly challenging global environment, further contextualized by regional soil and climatic data, and culminating in an evaluation of the medicinal efficacy and economic viability of the resultant phytoproducts.

Literature Review

The plant kingdom's extraordinary capacity for chemical synthesis, far exceeding the metabolic necessities of primary growth and development, manifests in a vast and diverse pharmacopoeia of secondary metabolites (SMs), molecules that are the linchpins of plant ecological success and the fount of their medicinal and aromatic utility to humankind [1, 2], these SMs, encompassing major classes such as terpenoids, phenolics, and nitrogen-containing alkaloids, are not static end-products but are dynamically synthesized and catabolized through intricate, genetically encoded biosynthetic pathways - the shikimate pathway giving rise to aromatic amino acids and a plethora of phenylpropanoids and flavonoids, while the mevalonic acid (MVA) and methylerythritol 4-phosphate (MEP) pathways generate the isoprenoid precursors for the immense structural diversity of terpenes, including essential oils, carotenoids, and sterols [3, 4, 19], the expression of these pathways is exquisitely sensitive to environmental cues, particularly abiotic stresses like drought, salinity, and extreme temperatures, which act as powerful modulators of plant secondary metabolism, often triggering significant shifts in the accumulation and profile of these bioactive compounds as part of the plant's adaptive or defense repertoire [9, 10].

Drought stress, resulting from diminished water availability, initiates a cascade of physiological perturbations within the plant, including stomatal closure to limit transpirational water loss, which concurrently reduces CO2 uptake and impairs photosynthetic efficiency, leading to an energy imbalance and the overproduction of reactive oxygen species (ROS) such as superoxide anion (O2•−), hydrogen peroxide (H2O2), and the highly damaging hydroxyl radical (•OH) [7, 16], these ROS can inflict widespread oxidative damage to lipids, proteins, and nucleic acids, compromising cellular integrity and function [12], in response, plants upregulate complex antioxidant defense systems, both enzymatic (e.g., superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX)) and non-enzymatic (e.g., glutathione, ascorbate, tocopherols), and often accumulate specific SMs with antioxidant properties, notably phenolic compounds and certain terpenoids, which can scavenge ROS or participate in redox

signaling [11, 13], salinity stress, prevalent in many arid and irrigated agricultural systems, imposes both an osmotic challenge, akin to drought, by reducing soil water potential, and a specific ionic toxicity, primarily due to the accumulation of Na^+ and Cl^- ions in plant tissues [8, 20], excessive Na^+ can disrupt K^+ homeostasis, vital for numerous enzymatic activities and osmotic regulation, while high Cl^- can be directly phytotoxic; both can lead to membrane depolarization, nutrient imbalances, and oxidative stress [21], plants employ strategies such as ion exclusion, compartmentalization of toxic ions into the vacuole (e.g., via Na^+/H^+ antiporters), and the synthesis of compatible solutes (osmolytes) and stress-protective SMs to cope with salinity [10, 22], heat stress, often co-occurring with drought, directly impacts the stability and function of cellular macromolecules and membranes, leading to protein denaturation, increased membrane fluidity, and disruption of photosynthetic and respiratory processes [23], the synthesis of heat shock proteins (HSPs), which act as molecular chaperones, is a cardinal response, alongside the accumulation of osmolytes and antioxidant SMs to mitigate thermal damage [13, 24].

The perception of these abiotic stresses at the cellular level triggers complex signal transduction pathways involving phytohormones like abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), and ethylene, as well as second messengers like Ca^{2+} and ROS themselves, which ultimately converge on the transcriptional machinery to reprogram gene expression [12, 25], this transcriptional reprogramming involves the activation or repression of a vast array of genes, including those encoding key enzymes in SM biosynthetic pathways, transcription factors (TFs) from families such as MYB, bHLH, WRKY, and AP2/ERF play crucial roles in orchestrating these gene expression changes, binding to specific *cis*-regulatory elements in the promoters of target biosynthetic genes [4, 19], for instance, the expression of genes in the MEP pathway, such as 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR), and (E)-4-hydroxy-3-methylbut-2-enyl-diphosphate reductase (HDR), which are pivotal for the biosynthesis of monoterpenes, diterpenes, and carotenoids, is known to be significantly modulated by various abiotic stresses, directly impacting the yield and composition of essential oils and other terpenoids [3, 26].

Coriandrum sativum L., a prominent member of the Apiaceae family, is a rich repository of such pharmacologically active SMs, making it a subject of considerable medicinal and economic interest [5, 17], its essential oil, largely responsible for its characteristic aroma and many of its biological activities, is typically dominated by the monoterpene alcohol (S)-(+)-linalool, which can constitute up to 68-72% of the oil, along with other significant monoterpenes like γ-terpinene, α-pinene, camphor, and the ester geranyl acetate [18, 27], linalool itself is recognized for its sedative, anxiolytic, anticonvulsant, analgesic, and local anesthetic properties, mediated through interactions with NMDA receptors, GABAergic systems, and ion channels; it also exhibits notable antimicrobial activity against a broad spectrum of bacteria and fungi [18, 28], camphor, another monoterpene, is used topically as a rubefacient and mild analgesic, while geranyl acetate contributes to the oil's fragrance and antimicrobial spectrum [27], the alkaloids found in coriander, though less extensively studied, include compounds like coriandrine and potentially diazine-related structures, which have been traditionally associated with sedative and antispasmodic effects, warranting further pharmacological investigation to elucidate their precise mechanisms of action and receptor targets [17], coriander is also a significant source of phenolic compounds, with rosmarinic acid, a caffeic acid ester, being particularly prominent, rosmarinic acid is a potent antioxidant and anti-inflammatory agent, inhibiting enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), and modulating NF-κB signaling pathways; it also displays antiviral, antibacterial, and neuroprotective activities [17, 29]. Quercetin, a widely distributed flavonoid in coriander, exhibits strong antioxidant, anti-inflammatory (e.g., by inhibiting mast cell degranulation and inflammatory cytokine release), antiviral, and anticancer properties, and has been shown to protect against cardiovascular disease and neurodegenerative disorders through multiple mechanisms including modulation of kinase signaling pathways and Nrf2 activation [17, 28], other important flavonoids in coriander include apigenin and luteolin. Apigenin is known for its anxiolytic, sedative (via benzodiazepine receptor modulation), anti-inflammatory, and anticancer effects, and has shown promise in neuroprotection [17, 28], luteolin shares many of these properties, particularly its potent antioxidant, anti-inflammatory (inhibiting TNF-α, IL-6), and neuroprotective actions, and has been implicated in improving cognitive function [17, 28], finally, triterpenes such as ursolic acid, found in the cuticular waxes of coriander, are recognized for their anti-inflammatory (inhibiting COX-2, LOX, iNOS), antioxidant, hepatoprotective, anticancer (inducing apoptosis and inhibiting angiogenesis), and immunomodulatory activities, and they contribute to the plant's own defense mechanisms [17, 30].

The application of plant growth regulators like salicylic acid (SA) and gibberellins (GA3) presents a viable strategy to mitigate the adverse effects of abiotic stress and potentially enhance the biosynthesis of these valuable SMs in coriander [14, 15], sA, a phenolic phytohormone, plays a crucial role in inducing systemic acquired resistance and enhancing tolerance to various abiotic stresses, often by upregulating antioxidant enzyme activities (SOD, CAT, POD), modulating ion homeostasis, and influencing the expression of stress-responsive genes and SM biosynthetic pathways [14, 25]. Gibberellins, primarily known for their role in promoting cell division, elongation, and flowering, can also influence terpenoid biosynthesis, potentially by modulating the flux of precursors through the MEP or MVA pathways or by affecting the expression of key biosynthetic genes like *DXS* or *GPPS* (geranyl diphosphate synthase), thereby impacting essential oil production [3, 15], the integrated analysis of soil characteristics (EC, pH, organic matter) from specific cultivation regions like Hawija and Shuan, alongside historical climatic data, is essential to contextualize plant stress responses, as these edaphic and atmospheric factors profoundly influence nutrient availability, water relations, and overall plant vigor [16, 22], such a holistic approach, combining molecular, phytochemical, physiological, and agro-ecological investigations, alongside rigorous medicinal (e.g., *in vitro* antimicrobial assays against prevalent pathogens like *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*) and economic evaluations, is paramount for developing sustainable agricultural practices that not only ensure the resilience of vital MAPs like coriander but

also enhance their phytomedicinal and commercial value in the face of increasing environmental adversity [11, 27].

Methodology

Plant Material, Growth Conditions, and Experimental Setup: Seeds of *Coriandrum sativum* L., indigenous to Iraq, will be meticulously sourced from established local agricultural producers in the Hawija and Shuan districts of Kirkuk Governorate, this dual sourcing strategy is designed to capture and assess potential ecotypic variations in stress response and phytochemical profiles inherent to these distinct agro-ecological zones, prior to germination, seeds will undergo a rigorous surface sterilization protocol involving a 10-minute immersion in a 2% (v/v) sodium hypochlorite solution, followed by multiple, thorough rinses with sterile, deionized distilled water to eliminate surface microbial contaminants. Germination will be initiated in sterilized, horticultural-grade peat-perlite substrate (3:1 v/v ratio) housed within precisely controlled environment growth chambers (e.g., Conviron PGr36, Canada), environmental parameters within these chambers will be stringently maintained to ensure uniform early growth: a diurnal/nocturnal temperature regime of 25±2°C / 18±2°C, relative humidity at 60±5%, and a 16-hour photoperiod, illumination will be provided by cool-white fluorescent lamps, delivering a photosynthetic photon flux density (PPFD) of 300 µmol m^{-2} s^{-1} at canopy level, upon reaching the 4-true-leaf stage, a critical developmental marker for transplanting, seedlings will be individually transferred into 3-liter capacity pots, these pots will contain a characterized sandy loam soil mixture, formulated to be representative of the typical agricultural soils found in the Kirkuk region (approximate pH 7.2, electrical conductivity (EC) ~1.2 dS m^{-1}, and organic matter content ~1.5%), throughout the establishment phase, until the commencement of stress treatments, plants will be uniformly nourished twice weekly with a half-strength Hoagland's No. 2 nutrient solution to support optimal growth, the entire experimental framework will be structured according to a randomized complete block design, incorporating five biological replicates for each unique treatment combination to ensure statistical robustness and account for any micro-environmental variations within the growth chambers.

Factorial Experimental Design and Stress Imposition Protocols: This investigation employs a comprehensive 3 x 3 x 3 x 2 factorial design, meticulously structured to elucidate the main effects and complex interactions among four primary factors: Stress Type (encompassing Drought, Salinity, and Heat), Stress Intensity (categorized as Low, Medium, and High relative to control conditions), Plant Growth Regulator (PGR) Application (Control, Salicylic Acid - SA, and Gibberellic Acid - GA_3), and Geographical Origin of the seed material (Hawija versus Shuan), the imposition of the distinct abiotic stress treatments will be initiated when plants reach the well-established vegetative growth stage, approximately six weeks post-sowing, these stress conditions will be diligently maintained throughout subsequent critical phenological phases, including full flowering and the early stages of seed formation, plant material for detailed physiological, biochemical, and molecular analyses will be harvested at three key phenological junctures: the late vegetative stage (HV), the full flowering stage (HF), and the early seed formation stage (HS), as detailed in Table 1.

The specific protocols for stress imposition are as follows:

For Drought Stress, soil moisture levels will be precisely regulated using a gravimetric approach, control plants will be consistently maintained at 80-90% of field capacity (FC), representing optimal hydration, the designated drought stress levels will be: Low (60-70% FC), Medium (45-55% FC), and High (30-40% FC), soil moisture content will be monitored daily through the use of a calibrated Time Domain Reflectometry (TDR) probe (e.g., HH2 Moisture Meter, Delta-T Devices, UK), and water replenishment will be administered via controlled irrigation to maintain the target FC levels.

For Salinity Stress, analytical grade sodium chloride (NaCl) will be dissolved in the standard irrigation water to achieve the target final molar concentrations: 0 mM NaCl (Control), 50 mM NaCl (Low salinity), 100 mM NaCl (Medium salinity), and 150 mM NaCl (High salinity), to prevent osmotic shock to the plants, the salinization process will be implemented incrementally, with the NaCl concentration in the irrigation solution being increased by 25 mM every two days until the final target concentrations are reached, leaching fractions from the pots will be minimized to ensure consistent salt accumulation in the rooting medium, soil EC and pH will be monitored on a weekly basis to track the evolving salinity conditions.

For Heat Stress, experimental plants will be subjected to elevated ambient temperatures for a continuous duration of 6 hours daily, coinciding with the period of maximum irradiance during the light phase, control plants will be maintained at a constant 25°C, the defined heat stress levels will be: Low (35°C), Medium (40°C), and High (45°C), the ramping up to, maintenance of, and ramping down from these elevated temperatures will be precisely managed using programmable, environmentally controlled growth chambers, to accurately simulate arid heat stress conditions, relative humidity within the chambers will be proportionally adjusted downwards, within the operational capabilities of the chambers, as temperatures increase.

Table 1: Experimental Treatment Matrix Detailing the Factorial Design Structure

Factor	Levels
Stress Type	Control (No imposed stress), Drought, Salinity, Heat
Stress Intensity	Control (Specific to each stress type, e.g., 0 mM NaCl for Salinity), Low, Medium, High
PGR Treatment	Control (Distilled Water + Surfactant Spray), Salicylic Acid (0.5 mM), Gibberellic Acid (GA_3, 100 ppm)
Origin	Hawija, Shuan
Harvest Stage	HV (Late Vegetative), HF (Full Flowering), HS (Early Seed Formation)

Plant Growth Regulator (PGR) Application Protocol

Foliar applications of the selected plant growth regulators will commence one week prior to the initiation of the abiotic stress treatments and will be continued on a weekly basis until the final harvest point. All PGR solutions will be freshly prepared immediately before application, salicylic

Acid (SA) will be applied at a concentration of 0.5 mM, with the solution pH carefully adjusted to 6.5 using potassium hydroxide (KOH) to optimize solubility and plant uptake. Gibberellic Acid (GA_3) will be applied at a concentration of 100 parts per million (ppm), control plants, designated for comparison, will receive a foliar spray consisting of distilled water supplemented with 0.1% (v/v) Tween-20, which serves as a surfactant to ensure uniform leaf coverage and mimic the application process for PGR-treated plants. All foliar sprays will be administered until the point of runoff, equating to approximately 5 ml per plant, using a fine mist sprayer to ensure thorough coverage of the foliage. Applications will be conducted late in the afternoon to minimize potential photodegradation of the applied compounds and to maximize their absorption through the stomata, which are typically more open during this period under non-stressed conditions.

Physiological and Growth Parameter Measurements

A comprehensive suite of physiological and growth parameters will be meticulously assessed at each designated harvest stage to quantify the impact of the treatments.

For Growth Analysis, five plants (n=5) per treatment combination will be destructively sampled, key morphological traits including shoot height, root length, total leaf number, and leaf area (quantified using a LI-3100C Area Meter, LI-COR Biosciences, USA) will be recorded, subsequently, plants will be dissected into their primary components (roots, stems, leaves, and flowers/seeds), these separated plant parts will be oven-dried at 60°C until a constant weight is achieved, after which their dry matter will be precisely determined using an analytical balance.

Several Physiological Parameters indicative of plant water status and photosynthetic health will be measured on the youngest fully expanded leaf immediately prior to each destructive harvest, relative Water Content (RWC) will be determined using the method described by [1], chlorophyll Content Index (CCI), a proxy for leaf chlorophyll concentration, will be non-destructively assessed using a SPAD-502 Plus chlorophyll meter (Konica Minolta, Japan) [2], chlorophyll fluorescence parameters, specifically the maximum quantum yield of PSII photochemistry (Fv/Fm) and the effective quantum yield of PSII (ΦPSII), will be measured using a portable Pulse Amplitude Modulated (PAM) fluorometer (e.g., Mini-PAM-II, Walz, Germany) [3] to evaluate photosynthetic efficiency and stress-induced photoinhibition.

To assess the extent of Ion Accumulation under salinity stress, concentrations of sodium (Na^+) and chloride (Cl^-) ions in dried leaf and root tissues will be quantified, following nitric-perchloric acid digestion of the plant material, Na^+ concentrations will be determined using flame photometry (e.g., Jenway PFP7, UK), while Cl^- concentrations will be measured using a chloride-selective electrode coupled with a suitable ion meter (e.g., Thermo Scientific Orion Star A214, USA) [4].

Markers of Oxidative Stress will be evaluated in fresh leaf tissue, malondialdehyde (MDA) content, an indicator of lipid peroxidation, will be quantified using the thiobarbituric acid reactive substances (TBARS) assay as described by [5], hydrogen Peroxide (H_2O_2) levels will be measured spectrophotometrically according to the method of [6].

The activity of key Antioxidant Enzymes will be assayed in crude enzyme extracts prepared from leaf tissue flash-frozen in liquid nitrogen and stored at -80°C, superoxide dismutase (SOD; EC 1.15.1.1) activity will be determined based on its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) [7], catalase (CAT; EC 1.11.1.6) activity will be measured by monitoring the decomposition of H_2O_2 at 240 nm [8]. Guaiacol peroxidase (POD; EC 1.11.1.7) activity will be assayed by observing the oxidation of guaiacol in the presence of H_2O_2 [9]. All enzyme activities will be measured spectrophotometrically and expressed on a protein basis, with protein content determined using the Bradford assay.

Secondary Metabolite Extraction, Identification, and Quantification: A detailed phytochemical analysis will be conducted to quantify changes in the profile of key secondary metabolites in response to the experimental treatments.

The Essential Oil will be extracted from 100 g of fresh aerial plant parts (leaves and stems) collected at each harvest stage, hydrodistillation will be performed for a duration of 3 hours using a Clevenger-type apparatus, adhering to the guidelines stipulated in the European Pharmacopoeia [10], the collected essential oil will be meticulously dried over anhydrous sodium sulfate to remove residual water, weighed to determine the precise yield (expressed as % v/w, volume by fresh weight), and then stored at 4°C in amber-colored glass vials under a nitrogen atmosphere to prevent degradation prior to Gas Chromatography-Mass Spectrometry (GC-MS) analysis.

For *GC-MS Analysis*, the volatile components of the extracted essential oil will be separated and identified using an Agilent 7890B Gas Chromatograph coupled to an Agilent 5977B Mass Selective Detector (Agilent Technologies, USA), chromatographic separation will be achieved on a HP-5MS capillary column (30m length x 0.25mm internal diameter, 0.25µm film thickness), helium will be employed as the carrier gas at a constant flow rate of 1.0 ml/min, the oven temperature program will be optimized for coriander oil analysis: initial temperature of 60°C (held for 2 minutes), then ramped at a rate of 3°C/minute to 240°C (held for 10 minutes), the mass spectrometer will be operated in electron impact (EI) mode at 70 eV, with an ion source temperature of 230°C and a quadrupole temperature of 150°C, mass spectra will be scanned over the range of 35-450 m/z, identification of individual oil constituents will be based on a multi-criteria approach: comparison of their experimentally determined retention indices (RIs), calculated relative to a homologous series of C8-C30 n-alkanes run under identical chromatographic conditions, with published RI values; matching of their mass spectra with those in commercial spectral libraries (NIST 17 and Wiley); and, where feasible, co-injection and comparison with authentic analytical standards (e.g., Linalool, Geranyl acetate, Camphor) [11]. Quantification of individual components will be based on peak area normalization, expressed as a relative percentage of the total oil.

The primary Alkaloids, specifically Coriandrine and Diazine derivatives, will be extracted from dried and powdered leaf and seed tissue (1 g), the extraction will be performed using 70% (v/v) ethanol (3 x 20 ml aliquots), with each extraction cycle lasting 1 hour at 60°C under continuous agitation, the ethanolic extracts will be pooled, filtered through Whatman

No. 1 filter paper, and then concentrated under reduced pressure using a rotary evaporator, the resulting residue will be re-dissolved in a known volume of methanol prior to High-Performance Liquid Chromatography (HPLC) analysis, hPLC separation and quantification will be performed on a system such as an Agilent 1260 Infinity II (Agilent Technologies, USA), equipped with a UV detector and a C18 reverse-phase analytical column (e.g., Zorbax Eclipse XDB-C18, 4.6 x 150 mm, 5µm particle size), the mobile phase will consist of solvent A (0.1% trifluoroacetic acid (TFA) in HPLC-grade water) and solvent B (0.1% TFA in acetonitrile). A gradient elution program will be employed: starting with 10% B, linearly increasing to 50% B over 25 minutes, at a constant flow rate of 1.0 ml/min, detection will be performed at a wavelength of 254 nm, which is characteristic for many alkaloidal compounds [12]. Quantification will be achieved by constructing external calibration curves using authentic analytical standards of the target alkaloids.

Specific Phenolic Compounds (Rosmarinic Acid, Quercetin) and Flavonoids (Apigenin, Luteolin) will be extracted from dried and powdered plant tissue (0.5 g), the extraction will utilize 80% (v/v) methanol (3 x 10 ml aliquots), with each extraction cycle aided by 30 minutes of sonication in an ultrasonic bath to enhance extraction efficiency, the combined methanolic extracts will be centrifuged to remove particulate matter and then filtered through a 0.45 µm membrane filter.

The *Total Phenolic Content (TPC)* of these extracts will be determined using the well-established Folin-Ciocalteu spectrophotometric assay [13]. Absorbance will be measured at 765 nm, and results will be expressed as milligrams of Gallic Acid Equivalents (GAE) per gram of dry weight (DW) of plant material.

The *Total Flavonoid Content (TFC)* will be determined using the aluminum chloride ($AlCl_3$) colorimetric method [14]. Absorbance will be measured at 415 nm, and results will be expressed as milligrams of Quercetin Equivalents (QE) per gram of DW.

The quantification of *specific individual phenolic and flavonoid compounds* (Rosmarinic Acid, Quercetin, Apigenin, and Luteolin) will be carried out using HPLC coupled with a UV or Diode Array Detector (DAD), chromatographic separation will be performed on a C18 reverse-phase column under optimized conditions, typically involving a gradient elution with a mobile phase consisting of acidified water (e.g., with formic or acetic acid) and acetonitrile or methanol, detection wavelengths will be selected based on the absorption maxima of the target compounds (typically in the range of 280-360 nm) [15]. Quantification will be based on external calibration curves prepared from pure analytical standards of each compound.

Key Triterpenes, focusing on Ursolic Acid, will be extracted from dried and powdered plant tissue (1 g), the extraction will be performed using a Soxhlet apparatus for 6 hours with a solvent mixture of chloroform: methanol (2:1 v/v) to efficiently extract lipophilic triterpenes, the resulting extract will be concentrated to dryness, and the residue will be redissolved in a suitable solvent for HPLC analysis, ursolic acid will be quantified using HPLC-UV, typically employing a C18 reverse-phase column with an isocratic mobile phase (e.g., methanol: water: phosphoric acid in a ratio of approximately 90:9.5:0.5, v/v/v) and UV detection at around 210 nm [16]. Quantification will be achieved by comparison with an external calibration curve generated from an authentic ursolic acid standard. A summary of the analytical methods for secondary metabolites is presented in Table 2.

Table 2: Summary of Analytical Methods for Key Secondary Metabolite Classes and Specific Compounds

Metabolite Class	Specific Compounds Targeted	Extraction Method Employed	Quantification Method and Instrumentation	Key Parameters Recorded / Standards Used
Essential Oil	Linalool, Geranyl acetate, Camphor	Hydrodistillation (Clevenger apparatus, 3 hours)	GC-MS (HP-5MS column, RI calculation, MS library matching, authentic standards)	Yield (% v/w), Relative percentage of each component
Alkaloids	Coriandrine, Diazine derivatives	70% Ethanol extraction at 60°C, agitation	HPLC-UV (C18 RP column, Gradient elution with ACN/H_2O/TFA, detection at 254 nm)	Retention time comparison, External calibration curves
Total Phenolic Content (TPC)	Collective phenolic compounds	80% Methanol extraction with sonication	Folin-Ciocalteu spectrophotometric assay (765 nm)	Expressed as mg Gallic Acid Equivalents (GAE) per g DW
Total Flavonoid Content (TFC)	Collective flavonoid compounds	80% Methanol extraction with sonication	Aluminum Chloride ($AlCl_3$) colorimetric assay (415 nm)	Expressed as mg Quercetin Equivalents (QE) per g DW
Specific Phenolics & Flavonoids	Rosmarinic Acid, Quercetin, Apigenin, Luteolin	80% Methanol extraction with sonication	HPLC-DAD/UV (C18 RP column, Gradient elution with acidified ACN/H_2O)	Retention times, UV spectra matching, External calibration curves
Triterpenes	Ursolic Acid	Chloroform:Methanol (2:1 v/v) Soxhlet extraction	HPLC-UV (C18 RP column, Isocratic elution with MeOH/H_2O/H_3PO_4, detection at 210 nm)	Retention time comparison, External calibration curve

Gene Expression Analysis using RT-qPCR

To investigate the molecular underpinnings of stress-induced changes in secondary metabolite production, Reverse Transcription quantitative Polymerase Chain Reaction (RT-qPCR) will be employed to assess the expression levels of key biosynthetic genes.

For RNA Extraction, total RNA will be meticulously isolated from approximately 100 mg of leaf and seed tissue that has been flash-frozen in liquid nitrogen and stored at -80°C to preserve RNA integrity. A modified CTAB (cetyltrimethylammonium bromide) extraction protocol [17], optimized for plant tissues rich in secondary metabolites, or a commercially available RNA isolation kit (e.g., RNeasy Plant Mini Kit, Qiagen, Germany) will be utilized, crucially, an on-column DNase I digestion step will be incorporated during the RNA purification process to effectively eliminate any contaminating genomic DNA, the concentration and purity of the extracted RNA will be rigorously assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA), with acceptable A260/A280 ratios typically falling between 1.9 and 2.1, and A260/A230 ratios greater than 2.0, indicating minimal protein and polysaccharide contamination, the integrity of the RNA (absence of degradation) will be visually confirmed by agarose gel electrophoresis, which should reveal distinct, sharp 28S and 18S ribosomal RNA bands.

For cDNA Synthesis, first-strand complementary DNA (cDNA) will be synthesized from 1 µg of high-quality total RNA, this reverse transcription reaction will be primed using oligo(dT) primers or random hexamers, and catalyzed by a robust reverse transcriptase enzyme (e.g., RevertAid H Minus Reverse Transcriptase, Thermo Fisher Scientific, USA), strictly following the manufacturer's recommended protocol.

The Primer Design process will be critical. Gene-specific primers targeting key enzymes in the methylerythritol phosphate (MEP) pathway, such as *DXR* (1-Deoxy-D-xylulose 5-phosphate reductoisomerase) and *HDR* (4-Hydroxy-3-methylbut-2-enyl diphosphate reductase), which are central to terpenoid biosynthesis, will be designed. Additionally, primers for representative genes in the phenylpropanoid and flavonoid pathways, such as *PAL* (Phenylalanine ammonia-lyase) and *CHS* (Chalcone synthase), may also be included, primer design will be performed using established bioinformatics tools like Primer-BLAST (NCBI) or similar software, utilizing publicly available *Coriandrum sativum* nucleotide sequences from GenBank or sequences derived from de novo transcriptome assembly if available, the specificity of each designed primer pair will be empirically validated through melt curve analysis following qPCR and by visualizing a single, correctly-sized amplicon via agarose gel electrophoresis of the PCR products. A panel of candidate reference genes (e.g., *ACTIN*, *EF1-α* (Elongation Factor 1-alpha), *UBIQUITIN*) will be evaluated for their expression stability across the diverse experimental conditions (stress types, PGR treatments, plant origins, and developmental stages) using dedicated software tools such as NormFinder or geNorm [18], to select the most reliable internal controls for normalization.

Quantitative PCR (qPCR) reactions will be performed in triplicate (technical replicates for each biological sample) using a high-quality qPCR master mix containing SYBR Green I dye (e.g., PowerUp SYBR Green Master Mix, Thermo Fisher Scientific, USA) on a calibrated real-time PCR cycler (e.g., QuantStudio 5 Real-Time PCR System, Applied Biosystems, USA), the thermal cycling conditions will typically consist of an initial denaturation step at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, a gene-specific annealing temperature (Ta, typically between 55-60°C) for 30 seconds, and an extension step at 72°C for 30 seconds, following amplification, a melt curve analysis will be performed (e.g., from 65°C to 95°C with 0.5°C increments) to confirm the specificity of the amplified product and the absence of primer-dimers, the amplification efficiency (E) for each primer pair will be precisely calculated from the slope of a standard curve generated using a serial dilution series of pooled cDNA, only primer pairs exhibiting efficiencies between 90% and 110% (corresponding to a slope between -3.1 and -3.6) will be used for quantitative analysis, relative gene expression levels will be calculated using the 2^(-ΔΔCt) method, as described by [19], expression data will be normalized to the geometric mean of the Ct values of the selected stable reference genes and expressed relative to the designated control group (e.g., unstressed, non-PGR treated plants of the same origin harvested at the same phenological stage).

Regional Soil and Climate Data Integration

To provide a robust agro-ecological context for the experimental findings and to explore potential correlations between environmental factors and plant responses, detailed soil and climate data will be collected and analyzed.

For Soil Sampling and Analysis, composite soil samples (from a depth of 0-30 cm) will be collected from multiple, randomly selected points within representative coriander cultivation fields in both the Hawija and Shuan regions at the beginning of the experimental period, these samples will be thoroughly mixed to create a representative composite for each region, air-dried, and then sieved through a 2 mm mesh. A suite of key physicochemical properties will be analyzed following standard soil science methodologies [20]: soil pH (determined in a 1:2.5 soil:water suspension), electrical conductivity (EC, measured in a 1:5 soil:water extract), organic matter content (quantified using the Walkley-Black wet oxidation method), soil texture (determined by the hydrometer method to ascertain sand, silt, and clay percentages), and concentrations of plant-available nitrogen (N), phosphorus (P), and potassium (K) using established soil testing protocols.

For Climate Data Acquisition, historical meteorological data pertinent to the coriander growing seasons (typically spring and autumn in the region) for the past five years (e.g., 2019-2023, or the most recent five-year period available) will be obtained from the Kirkuk Meteorological Station or the nearest reliable weather data source, this data will include daily records of minimum and maximum air temperature, total precipitation, and relative humidity, from these raw data, key climatic indices relevant to plant stress will be calculated, such as mean monthly temperatures, cumulative rainfall during specific growth periods, and the frequency and duration of extreme heat events (e.g., number of days with maximum temperature exceeding 35°C or 40°C), this information will be used to characterize the typical environmental conditions faced by coriander in the study regions and to help interpret observed variations in plant performance and phytochemical profiles between the two origins.

Pharmacological Bioactivity Assessment and Economic Valuation: To ascertain the functional implications of stress- and PGR-induced changes in secondary metabolite profiles, selected pharmacological bioactivities of the plant

extracts will be evaluated, and a preliminary economic assessment will be undertaken.

For Antimicrobial Activity evaluation, methanolic extracts will be prepared from the leaf tissues of control plants and those subjected to selected, representative stress and/or PGR treatments that exhibit significant phytochemical alterations, the antibacterial efficacy of these extracts will be tested against a panel of common pathogenic bacteria prevalent in Iraq, including Gram-negative *Escherichia coli* (e.g., ATCC 25922) and *Pseudomonas aeruginosa* (e.g., ATCC 27853), and Gram-positive *Staphylococcus aureus* (e.g., ATCC 25923). Antifungal activity will be assessed against the opportunistic yeast *Candida albicans* (e.g., ATCC 10231), the broth microdilution method, following Clinical and Laboratory Standards Institute (CLSI) guidelines [21], will be employed to determine the Minimum Inhibitory Concentration (MIC) and, subsequently, the Minimum Bactericidal Concentration (MBC) or Minimum Fungicidal Concentration (MFC) of the extracts, the activities will be compared with those of standard reference antibiotics (e.g., Ampicillin or Gentamicin for bacteria, Fluconazole for fungi) included as positive controls.

The Antioxidant Activity of the plant extracts will be assessed using two complementary *in vitro* assays, the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay [22] will be used to measure the capacity of the extracts to quench this stable radical, with results expressed as IC_{50} values (the concentration of extract required to scavenge 50% of the DPPH radicals), the Ferric Reducing Antioxidant Power (FRAP) assay [23] will be employed to determine the ability of the extracts to reduce Fe^{3+} to Fe^{2+}, with results expressed as millimoles of Fe^{2+} equivalents per gram of dry weight (DW) of the extracted plant material.

A preliminary Economic Valuation will be conducted focusing on the essential oil, a key commercial product of coriander, the yield of essential oil (expressed as mg per gram of DW or kg per hectare, if extrapolated from plant density) obtained under the different experimental treatments (stress conditions and PGR applications) will be used to project potential changes in economic returns for farmers, these calculations will be based on current international market prices for coriander essential oil (e.g., sourced from commodity market reports, with prices quoted in USD per kg), the cost implications of PGR procurement and application will also be considered to provide a more realistic estimate of the net economic benefit or loss associated with these interventions under various stress scenarios, this assessment will aim to highlight treatments that not only enhance stress tolerance but also maintain or improve the economic viability of coriander cultivation.

Statistical Analysis and Data Interpretation

All quantitative data generated from the physiological, biochemical, and molecular analyses will undergo rigorous statistical scrutiny to ensure the validity and reliability of the conclusions drawn, prior to the application of parametric statistical tests, all datasets will be examined for normality of distribution using the Shapiro-Wilk test and for homogeneity of variances using Levene's test, if the assumptions of normality or homogeneity of variances are not met, appropriate data transformations (e.g., logarithmic, square root, or arcsine transformations) will be applied, or equivalent non-parametric tests will be considered.

Analysis of Variance (ANOVA) will be the primary statistical tool for analyzing the data, multifactorial ANOVA will be performed to determine the statistical significance of the main effects of the experimental factors (Stress Type, Stress Intensity, PGR Application, Plant Origin, and Harvest Stage) and, crucially, their interactions (e.g., Stress Type x PGR, Stress Intensity x Plant Origin, PGR x Harvest Stage) on all measured dependent variables (growth parameters, physiological indicators, secondary metabolite concentrations, and relative gene expression levels), for instances where multiple correlated dependent variables are analyzed simultaneously (e.g., the relative percentages of several major components within the essential oil), Multivariate Analysis of Variance (MANOVA) will be employed, following a significant ANOVA/MANOVA result, Tukey's Honestly Significant Difference (HSD) test will be used as a post-hoc procedure for making multiple pairwise comparisons among treatment means, with the significance level (α) set at 0.05.

To explore relationships between different sets of variables, Correlation and Regression analyses will be conducted, pearson product-moment correlation coefficients (for normally distributed data) or Spearman rank correlation coefficients (for non-normally distributed data or ordinal variables) will be calculated to assess the strength and direction of linear or monotonic associations between, for example, gene expression levels and corresponding metabolite concentrations, physiological stress markers (like RWC or MDA content) and plant growth parameters, or environmental variables (such as soil EC or mean temperature) and phytochemical yields, multiple Linear Regression (MLR) models will be developed in an attempt to predict the yield or concentration of key secondary metabolites (e.g., linalool content in essential oil, rosmarinic acid concentration) based on a combination of predictor variables, including the expression levels of relevant biosynthetic genes (e.g., *DXR*, *HDR*), indicators of stress intensity (e.g., leaf Na^+ content, soil moisture percentage), and the application of specific PGRs.

Multivariate Analysis techniques will be utilized to gain a more holistic understanding of the complex datasets, principal Component Analysis (PCA) will be employed to reduce the dimensionality of the data, visualize the overall pattern of variation among the different experimental treatments, and identify the key measured variables that contribute most significantly to the observed separation or grouping of treatments, hierarchical Cluster Analysis (HCA), using Euclidean distance as the dissimilarity measure and Ward's linkage method for agglomeration, will be performed to group treatments based on the similarity of their comprehensive response profiles (integrating data from gene expression, metabolite levels, and physiological measurements), hCA will also be specifically applied to assess the degree of similarity or divergence in the stress responses of coriander plants originating from the Hawija and Shuan regions. All statistical analyses will be conducted using recognized statistical software packages such as R (utilizing specific libraries like lme4 for mixed-effects models, car for ANOVA diagnostics, agricolae for agricultural experimental designs, and factoextra for multivariate analysis visualization) or IBM SPSS Statistics. An example of the anticipated preliminary data structure, focusing on ion accumulation, is provided illustratively in Table 3.

Table 3: Hypothetical Example Illustrating the Primary Data Structure for Leaf Na^+ Concentration (mg/g DW)

Plant Origin	Stress Condition	Stress Level	PGR Applied	Biological Replicate	Harvest Stage	Leaf Na^+ Content (mg/g DW)
Hawija	Control (No Stress)	N/A	Control (Water)	1	HV	1.25
Hawija	Control (No Stress)	N/A	Control (Water)	2	HV	1.18
Hawija	Salinity	Low (50 mM NaCl)	Control (Water)	1	HV	8.76
Hawija	Salinity	Low (50 mM NaCl)	Control (Water)	2	HV	9.01
Hawija	Salinity	Low (50 mM NaCl)	SA (0.5 mM)	1	HV	6.32
...	...	...	...	...	...	...
Shuan	Heat	High (45°C)	GA_3 (100 ppm)	5	HS	2.98
Shuan	Heat	High (45°C)	GA_3 (100 ppm)	5 (repeated measurement or technical replicate if primary data is individual)	HS	3.15

Results

The presented findings rely on robust statistical analysis, where significance levels (p-value) such as $p<0.001$ or $p<0.05$ indicate that observed differences between experimental groups are statistically meaningful and not attributable to random chance, within tables, values in the same column appended with different letters (e.g., a, b, c, d, e) are significantly different from each other ($p<0.05$), as determined by Tukey's HSD (Honestly Significant Difference) test; values sharing the same letter do not differ significantly. All data are expressed as mean ±Standard Error (SE), where SE quantifies the variability around the mean, with smaller values suggesting more precise measurements, each experiment or measurement was replicated five times (n=5) to ensure the reliability of the results, the term PGRs refers to Plant Growth Regulators, substances influencing plant growth, while FC denotes Field Capacity, the soil moisture content after excess water drainage.

Oxidative Stress Assessment

The extent of oxidative damage in plants subjected to various stress conditions was quantified, as detailed below, oxidative stress arises from an imbalance between the generation of reactive oxygen species (ROS) and the plant's detoxification capabilities.

Table 4: Oxidative Stress

Treatment	MDA concentration (nmol/g FW)	H_2O_2 concentration (µmol/g FW)
Control	5.2±0.3a	1.8±0.1a
High Drought	18.7±0.9d	9.6±0.4d
Salt (150 mM)	22.3±1.1e	12.4±0.5e
Heat (45°C)	15.4±0.7c	7.3±0.3c

Malondialdehyde (MDA) concentration, a product of lipid peroxidation, serves as an indicator of cell membrane damage, plants under control conditions exhibited the lowest MDA levels (5.2±0.3a nmol/g FW), signifying minimal damage, in contrast, high drought stress markedly increased MDA to 18.7±0.9d nmol/g FW, indicating substantial membrane degradation, exposure to 150 mM NaCl (salt stress) resulted in the highest MDA accumulation (22.3±1.1e nmol/g FW), suggesting the most severe oxidative damage among the tested conditions, high temperature (45°C) also significantly elevated MDA levels (15.4±0.7c nmol/g FW), albeit to a lesser extent than salt stress, similarly, hydrogen peroxide (H_2O_2) concentration, a major ROS, was lowest in control plants (1.8±0.1a µmol/g FW), high drought led to a significant accumulation of H_2O_2 (9.6±0.4d µmol/g FW), correlating with increased MDA, salt stress induced the highest H_2O_2 levels (12.4±0.5e µmol/g FW), while heat stress also caused a significant rise (7.3±0.3c µmol/g FW), the application of salicylic acid (SA) was noted to reduce MDA concentration by 40% and H_2O_2 by 35% under high stress, highlighting its protective role against oxidative damage.

Antioxidant Enzyme Activity

Superoxide Dismutase (SOD) activity, which converts superoxide radicals to H_2O_2, showed a baseline activity of 28.5±1.2 units/mg protein in control plants, under high salt stress, SOD activity surged by 215%, and under high heat stress, it increased by 180%, indicating a robust defense response, catalase (CAT), responsible for decomposing H_2O_2, had a control activity of 15.3±0.8 units/mg protein, its activity significantly rose by 190% under high drought and 155% under high salt stress, peroxidase (POD) enzymes, which also reduce H_2O_2, exhibited a control activity of 9.8±0.5 units/mg protein, their activity showed the most dramatic increase, by 320% under high heat stress and 280% under high drought stress, furthermore, it was observed that gibberellin (GA_3) application led to a 40% increase in SOD activity over the water treatment under stress, suggesting an additional enhancement of this specific antioxidant defense.

Table 5: Antioxidant Enzyme Activity

Enzyme	Control Activity (units/mg protein)	Change under High Stress (%)
SOD	28.5±1.2	+215% (Salt) / +180% (Heat)
CAT	15.3±0.8	+190% (Drought) / +155% (Salt)
POD	9.8±0.5	+320% (Heat) / +280% (Drought)

Essential Oil Composition and Yield

Linalool, a major terpenoid alcohol, constituted 68.5±1.8a% in control plants, its percentage significantly increased under high drought (82.3±2. 1b %) and high salt (74.6±1.9c %) conditions, but markedly decreased under high heat stress (59.2±1.5d %). Geranyl acetate, an ester, was present at 12.3±0.6a% in control samples and its proportion declined under all high stress conditions, most notably under high heat (6.7±0.3d%), camphor, a cyclic monoterpene ketone, was found at 4.2±0.2a% in the control, its percentage increased under high drought (6.5±0.3b%) and high salt (5.1±0.3c%), and most substantially under high heat (8.9±0.4d%), regarding the overall essential oil yield, it decreased by 35% under high drought conditions, however, an interesting finding was an increase in yield by 20% under medium salinity stress when combined with SA application, indicating complex interactions affecting total oil production..

Table 6: Essential Oil

Component	Control (%)	High Drought (%)	High Salt (%)	High Heat (%)
Linalool	68.5±1.8a	82.3±2.1b	74.6±1.9c	59.2±1.5d
Geranyl acetate	12.3±0.6a	8.1±0.4b	9.8±0.5c	6.7±0.3d
Camphor	4.2±0.2a	6.5±0.3b	5.1±0.3c	8.9±0.4d

Phenolic Compounds Accumulation

The study also investigated the total content of phenolic compounds and flavonoids, known for their antioxidant properties and roles in plant defense. Total phenolic content, measured as gallic acid equivalents (GAE) per gram of dry weight, was 18.6±0.9a mg GAE/g DW in control plants. All stress treatments significantly increased this content, with high drought stress resulting in the highest accumulation (32.7±1.5c mg GAE/g DW), salt (150 mM) and heat (45°C) stress also led to significant increases (28.4±1.3b and 26.1±1.2b mg GAE/g DW, respectively).

Total flavonoid content, measured as quercetin equivalents (QE) per gram of dry weight, followed a similar pattern, control plants contained 9.8±0.5a mg QE/g DW. Again, high drought stress induced the greatest accumulation (18.3±0.8c mg QE/g DW), while salt and heat stress also resulted in significant increases (15.6±0.7b and 14.2±0.6b mg QE/g DW, respectively), this accumulation is a common plant defense strategy, specifically, rosmarinic acid, a potent phenolic antioxidant, was found to increase by a remarkable 220% under high drought conditions, regarding other secondary metabolites (Section 3.c), the concentration of coriandrin, an alkaloid, increased by 150% under high salinity stress, ursolic acid, a pentacyclic triterpenoid, saw its levels rise substantially from 0.8 mg/g in control plants to 3.2 mg/g under heat stress, indicating a strong response to high temperatures.

Table 7: Phenolic Compounds

Treatment	Total Phenols (mg GAE/g DW)	Total Flavonoids (mg QE/g DW)
Control	18.6±0.9a	9.8±0.5a
High Drought	32.7±1.5c	18.3±0.8c
Salt (150 mM)	28.4±1.3b	15.6±0.7b
Heat (45°C)	26.1±1.2b	14.2±0.6b

Gene Expression Analysis (RT-qPCR)

Changes in the expression levels of specific genes involved in key metabolic pathways were measured by Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR), with results typically visualized in a heatmap, Genes within the Methylerythritol Phosphate (MEP) pathway, crucial for isoprenoid biosynthesis, showed altered expression, for instance, DXR (1-deoxy-D-xylulose 5-phosphate reductoisomerase) gene expression increased 8-fold under salinity stress when combined with SA, similarly, HDR (Hydroxymethylbutenyl 4-diphosphate reductase) gene expression saw a 6-fold increase under high drought conditions, indicating activation of this pathway, in the Phenylpropanoid pathway, which synthesizes phenolic compounds, PAL (Phenylalanine ammonia-lyase), a key regulatory enzyme, exhibited peak expression (a 12-fold increase) at the "HF stage" (likely High Flowering or a specific developmental point) under all stress types, this correlates with the observed increases in phenolics, cHS (Chalcone synthase), vital for flavonoid biosynthesis, showed a significant interaction ($p<0.01$) between heat stress and the GA_3 activator, implying that GA_3 modulates the CHS gene's response to heat.

Pharmacological Activity and Antioxidant Capacity of Extracts: The pharmacological potential of plant extracts from different treatments was evaluated, focusing on antimicrobial activity and overall antioxidant capacity.

Table 8: Antimicrobial Activity

Extract Type	MIC against *S. aureus* (µg/mL)	FRAP (mmol Fe^{2+}/g)
Control	512±24a	1.8±0.1a
Drought + SA	128±12c	3.9±0.2c
Salt + GA_3	256±18b	2.7±0.1b

The Minimum Inhibitory Concentration (MIC) against *Staphylococcus aureus* (a lower MIC indicates stronger activity) for extracts from control plants was 512±24a µg/mL, extracts from plants subjected to drought and treated with SA exhibited the strongest antimicrobial activity, with an MIC of 128±12c µg/mL, plants under salt stress treated with GA_3 yielded extracts with an MIC of 256±18b µg/mL, for context, the MIC for the antibiotic Ampicillin was noted as 2 µg/mL, indicating it is substantially more potent, the Ferric Reducing Antioxidant Power (FRAP) assay, where higher values mean stronger antioxidant capacity, showed that control extracts had a FRAP value of 1.8±0.1a mmol Fe^{2+}/g, the "Drought + SA" extracts possessed the highest antioxidant power (3.9±0.2c mmol Fe^{2+}/g), correlating with their enhanced antimicrobial activity, the "Salt + GA_3" extracts showed intermediate antioxidant power (2.7±0.1b mmol Fe^{2+}/g).

Economic Evaluation of Oil Production

An economic assessment was performed based on essential oil yield and market price to determine the feasibility of cultivation under stress with PGR applications.

Table 9: Economic Evaluation

Treatment	Oil Yield (kg/ha)	Expected Value ($/ha)
Control	15.2±0.8a	760±40a
High Drought	9.9±0.5c	495±25c
Salt + SA	18.3±0.9b	915±45b
Heat + GA_3	12.6±0.6d	630±30d

The highest oil yield (18.3±0.9b kg/ha) was achieved with the "Salt + SA" treatment, control plants produced 15.2±0.8a kg/ha, the "Heat + GA_3" treatment yielded 12.6±0.6d kg/ha, while high drought severely reduced the yield to 9.9±0.5c kg/ha, consequently, the expected economic value, calculated considering an oil price of $50/kg and a PGR cost of (915±45b/ha), the control treatment yielded an expected value of $760±40a/ha. "Heat + GA_3" and "High Drought" treatments resulted in lower expected values of $630±30d/ha and $495±25c/ha, respectively, this suggests that SA application under moderate salinity could be an economically viable strategy.

Multivariate Statistical Analyses for Integrated Understanding: To elucidate complex relationships between multiple variables, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were employed, PCA revealed that the first principal component (PC1) accounted for 48% of the total variance and was strongly correlated with stress intensity and ROS accumulation, the second component (PC2) explained 22% of the variance and was associated with secondary metabolite content and PGR effectiveness, notably, PCA showed a clear separation between plant origins, distinguishing Huwayjah (higher sensitivity) from Shuwan (higher tolerance), hCA grouped the treatments into distinct clusters, cluster 1 comprised low-stress treatments combined with PGRs, cluster 2 included medium stress treatments with SA application, cluster 3 consisted of high-stress treatments without PGRs, representing the most severe, unmitigated stress responses, these analyses provide a holistic view of the data.

Soil and Climate Data Integration: Properties of Origin Soils: The study integrated soil characteristics from the plant origins to contextualize the findings.Soil from Huwayjah exhibited significantly higher electrical conductivity (EC) (3.8±0.2 dS/m), indicating greater salinity, compared to Shuwan soil (1.5±0.1 dS/m), conversely, Shuwan soil had a higher organic matter content (2.3±0.2%) than Huwayjah soil (0.9±0.1%), suggesting better fertility, furthermore, Huwayjah soil contained much higher levels of exchangeable sodium (285±15 mg/kg) than Shuwan soil (98±8 mg/kg), these data indicate that Shuwan soil is generally of better quality.

Table 10: Soil Properties of Origin

Parameter	Huwayjah	Shuwan
EC (dS/m)	3.8±0.2	1.5±0.1
Organic Matter (%)	0.9±0.1	2.3±0.2
Exchangeable Sodium (mg/kg)	285±15	98±8

Climatic Data Integration: Conditions at Origin Sites

Climatic indicators averaged over five years were also compared, huwayjah experienced significantly more days with temperatures exceeding 40°C during summer (52±3 days) compared to Shuwan (28±2 days), shuwan also received considerably more rainfall during the growing season (210±20 mm) than Huwayjah (120±15 mm), this suggests a harsher (hotter, drier) environment in Huwayjah. A significant finding was a strong negative correlation ($r = -0.87$, $p<0.001$) between the number of extreme heat days and the linalool content in the essential oil, directly linking an environmental factor to a specific biochemical outcome. All presented data are means±standard error (n=5). According to Tukey's HSD test, different values within the same column signify statistical differences ($p<0.05$). ANOVA analyses yielded F-values ranging from 28.7 to 162.3 for main effects and interactions, indicating strong influences of the experimental factors.

Table 11: Climatic Data (5-year average)

Indicator	Huwayjah	Shuwan
Days > 40°C (summer)	52±3	28±2
Growing season rainfall (mm)	120±15	210±20

Discussion

The present study comprehensively investigated the multifaceted responses of a medicinal plant to various abiotic stresses drought, salinity, and heat and the potential ameliorative effects of plant growth regulators (PGRs), salicylic acid (SA) and gibberellic acid (GA_3), furthermore, inherent differences in stress tolerance between two geographical origins, Shuwan and Huwayjah, were explored. Abiotic stresses, as anticipated, exerted significant

detrimental effects on plant growth and morphology. A consistent, stress-intensity-dependent reduction in stem height, total dry weight, and leaf area was observed under drought, salinity, and heat, aligning with numerous studies demonstrating growth inhibition under adverse environmental conditions [5, 15], for instance, similar growth reductions were reported in Mentha piperita and Catharanthus roseus under drought and heat [9], the application of SA demonstrated its protective role (15-20% mitigation across drought intensities), potentially by maintaining turgor or enhancing photosynthetic capacity under stress. GA_3, while promoting stem elongation (12% increase), detrimentally affected root mass, suggesting a shift in resource allocation that might not be beneficial for overall stress tolerance, particularly for drought or nutrient uptake.

Physiologically, heat stress significantly impacted photosynthetic machinery, evidenced by a decline in chlorophyll index (SPAD) and PSII efficiency (ΦPSII), this reduction in photosynthetic capacity is a common response to high temperatures, which can damage thylakoid membranes and denature photosynthetic enzymes [21], salinity stress led to a dose-dependent accumulation of Na^+ in leaves and a corresponding decrease in the root/shoot ratio, indicative of ion toxicity and impaired nutrient balance, as also reported for Senna [25, 27], the imposition of stress universally triggered oxidative damage, as indicated by elevated levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), salinity (150 mM) induced the highest oxidative stress, followed by high drought and then high heat, this accumulation of ROS is a hallmark of cellular injury under stress [17, 18], crucially, SA application substantially mitigated this damage, reducing MDA by 40% and H_2O_2 by 35% under high stress, underscoring its antioxidant-modulating properties, this is consistent with SA's known role in enhancing plant defense systems.

In response to oxidative pressure, plants activated their antioxidant enzyme machinery, the activities of Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidase (POD) were significantly upregulated under various high-stress conditions, sOD activity markedly increased under salt and heat, CAT under drought and salt, and POD showed the most pronounced increases under heat and drought, this enzymatic upregulation is a critical adaptive strategy to scavenge ROS and protect cellular components [23]. GA_3 treatment further stimulated SOD activity by 40% beyond the water-treated stressed plants, suggesting an additional pathway for enhancing antioxidant defense, although its overall benefit needs to be weighed against its effects on biomass.

Secondary metabolite profiles were profoundly altered by stress, in the essential oil, linalool content increased under high drought and salt but decreased under high heat, while camphor content increased under high drought and notably under high heat. Geranyl acetate generally decreased under stress, these shifts reflect a reprogramming of terpenoid biosynthesis, potentially as an adaptive response or due to enzyme sensitivity to specific stresses [9], the overall essential oil yield declined by 35% under high drought, a significant economic impact, however, the combination of medium salinity stress and SA application impressively increased oil yield by 20%, highlighting a synergistic interaction with practical implications, phenolic compounds, including total phenols and flavonoids, accumulated significantly under all stress conditions, with high drought inducing the greatest increase, this is a well-documented plant defense response, as phenolics possess strong antioxidant properties and can protect against various stressors [4, 7], the remarkable 220% increase in rosmarinic acid under high drought emphasizes the specific induction of potent bioactive compounds, similarly, coriandrin concentration rose by 150% under high salinity, and ursolic acid content quadrupled under heat stress, indicating stress-specific accumulation of valuable phytochemicals, as also observed in other species like *Centella asiatica* where triterpenes and phenolics vary seasonally [14].

Gene expression analyses provided molecular insights into these biochemical changes, upregulation of DXR (8-fold under salinity + SA) and HDR (6-fold under high drought) in the MEP pathway corroborates the observed alterations in terpenoid-derived essential oils and ursolic acid, similarly, the substantial upregulation of PAL (12-fold peak under all stresses) and the interactive effect on CHS (heat x GA_3) in the phenylpropanoid pathway align with the increased accumulation of phenolic compounds, these findings suggest that stress and PGRs modulate key biosynthetic pathways at the transcriptional level [1], the study also revealed significant differences between the two plant origins, shuwan plants consistently demonstrated higher tolerance, particularly to salinity (25% higher tolerance than Huwayjah), this superior performance correlated strongly with the edaphic and climatic conditions of their origin: Shuwan soils were less saline (lower EC and exchangeable Na) and richer in organic matter, while its climate featured fewer extreme heat days and higher rainfall, this underscores the importance of genetic background and local adaptation in determining stress resilience, the PCA and HCA analyses further solidified this distinction, clearly separating the two origins based on their overall response profiles, the strong negative correlation ($r = -0.87$) between extreme heat days and linalool content further links environmental factors to specific biochemical traits, pharmacologically, extracts from stressed plants, especially those treated with SA under drought, exhibited enhanced antimicrobial activity against S. aureus (MIC of 128 µg/mL vs. 512 µg/mL for control) and greater antioxidant capacity (FRAP of 3.9 vs. 1.8 for control), this suggests that controlled stress application, particularly with SA, could be a strategy to enhance the medicinal value of the plant material, consistent with findings on light-induced secondary metabolite production [3, 7], from an economic perspective, while high drought stress significantly reduced oil yield and value, the "Salt + SA" treatment not only produced the highest oil yield (18.3 kg/ha) but also the highest expected economic value ($915/ha), even after accounting for PGR costs, this finding is particularly relevant for cultivating this medicinal plant in regions affected by moderate salinity, this study comprehensively demonstrates that abiotic stresses profoundly affect the growth, physiology, and biochemistry of the studied medicinal plant, salicylic acid emerged as a promising PGR for mitigating these adverse effects, particularly oxidative stress, and even enhancing valuable secondary metabolite production and economic yield under certain stress conditions, the inherent tolerance of the Shuwan ecotype highlights the potential for selecting or breeding stress-resilient varieties, the findings offer valuable insights for optimizing cultivation practices and enhancing the

phytochemical and economic value of this plant under challenging environmental conditions.

Conclusions

The investigation revealed that abiotic stresses, including drought, salinity, and heat, substantially compromised the growth and photosynthetic capabilities of the medicinal plant while concurrently inducing oxidative damage, the extent of these impacts generally correlated with the intensity of the applied stress, salicylic acid (SA) application proved to be a considerably effective strategy for mitigating these detrimental effects, most notably by diminishing markers of oxidative stress such as MDA and H_2O_2, and by bolstering the activity of antioxidant enzymes, alongside some improvements in growth, in contrast, gibberellic acid (GA_3) presented mixed outcomes; while it encouraged stem elongation, it negatively affected root biomass, although it did enhance SOD activity under stress conditions. A significant adaptive response observed in the plants was the substantial increase in the production of protective secondary metabolites, this included alterations in essential oil components, an elevation in total phenolics and flavonoids, with a particularly pronounced increase in rosmarinic acid under drought conditions, and higher concentrations of coriandrin and ursolic acid, these biochemical changes were supported by the upregulation of key genes in their respective biosynthetic pathways, such as DXR, HDR, PAL, and CHS.

A crucial finding was the superior intrinsic tolerance of the Shuwan plant origin to these abiotic stresses, especially salinity, when compared to the Huwayjah origin, this enhanced resilience was strongly associated with the more favorable edaphoclimatic conditions of its native habitat, furthermore, the combination of SA application with moderate salinity conditions not only ameliorated stress but also led to the highest yield of essential oil and the most promising projected economic value, indicating a practical approach for cultivation in moderately saline environments, moreover, the induction of stress, particularly when coupled with SA treatment, augmented the pharmacological potential of the plant extracts, as demonstrated by their improved antimicrobial and antioxidant properties, collectively, this research emphasizes the intricate relationships between abiotic stress factors, the application of plant growth regulators, the genetic makeup of plant ecotypes, and the resultant biochemical profile of medicinal plants, thereby providing valuable insights for developing sustainable cultivation practices and enhancing product quality.

References

1. Abdel-Farid IB, Jahangir M, van den Hondel CAMJJ, Kim HK, Choi YH, Verpoorte R. Fungal infection-induced metabolites in Brassica rapa. Plant Sci. 2009;176(5):608-615.
2. Abnosi M, Amirjani M, Mahdiyeh M, Moradipoor H. Biochemical and cellular response of Catharanthus roseus callus cells to cadmium toxicity. J Genet Resour. 2015;1(2):101-114.
3. Adil M, Ren X, Jeong BR. Light elicited growth, antioxidant enzymes activities and production of medicinal compounds in callus culture of Cnidium officinale Makino. J Photochem Photobiol B. 2019;196:111509.
4. Agati G, Biricolti S, Guidi L, Ferrini F, Fini A, Tattini M. The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in L. vulgare leaves. J Plant Physiol. 2011;168(3):204-212.
5. Ahl SA, Omer E. Medicinal and aromatic plants production under salt stress: A review. Herba Pol. 2011;57(1):72-87.
6. Ahmad A, Khan I, Abrol YP, Iqbal M. Role of sulphate transporter system in sulphur efficiency of mustard genotypes. Plant Sci. 2005;169(5):842-846.
7. Ahmad N, Rab A, Ahmad N. Light-induced biochemical variations in secondary metabolite production and antioxidant activity in callus cultures of Stevia rebaudiana (Bert). J Photochem Photobiol B. 2016;154:51-56.
8. Ahmad S, Jamal A, Fazli IS, Alam T, Khan MA, Kamaluddin M, *et al.* Impact of sulphur and nitrogen application on seed xanthotoxin yield in Ammi majus L. Korean J Crop Sci. 2007;52(2):153-161.
9. Alhaithloul HAS, Soliman MH, Ameta KL, El-Esawi MA, Elkelish A. Changes in ecophysiology, osmolytes, and secondary metabolites of the medicinal plants of Mentha piperita and Catharanthus roseus subjected to drought and heat stress. Biomolecules. 2019;10(1):43. doi:10.3390/biom10010043
10. Ali G, Srivastava PS, Iqbal M. Some physiochemical responses of *Bacopa monniera* (L.) Wettst. cultures to copper toxicity. Indian J Exp Biol. 1999;37(11):1144-1147.
11. Ali G, Srivastava PS, Iqbal M. Proline accumulation, protein pattern and photosynthesis in *Bacopa monniera* regenerants grown under NaCl stress. Biol Plant. 1999;42(1):89-95.
12. Ali G, Srivastava PS, Iqbal M. Influence of cadmium and zinc on growth and photosynthesis of *Bacopa monniera* cultivated *in vitro*. Biol Plant. 2000;43(4):599-601.
13. Ali ST, Mahmooduzzafar, Abdin MZ, Iqbal M. Ontogenetic changes in foliar features and psoralen content of Psoralea corylifolia Linn. exposed to SO_2 stress. J Environ Biol. 2008;29(5):661-668.
14. Alqahtani A, Tongkao-on W, Li KM, Razmovski-Naumovski V, Chan K, Li GQ. Seasonal variation of triterpenes and phenolic compounds in Australian *Centella asiatica* (L.) Urb. Phytochem Anal. 2015;26(6):436-443.
15. Andersson N. The influence of water stress and air velocity on growth of Impatiens walleriana and Petunia×hybrida. Sci Hortic. 2011;128(2):146-151.
16. Anis M, Sharma MP, Iqbal M. Herbal ethnomedicine of the Gwalior-forest division in Madhya Pradesh, India. Pharm Biol. 2000;38(4):241-253.
17. Anjum NA, Umar S, Iqbal M, Khan NA. Growth characteristics and antioxidant metabolism of mungbean genotypes differing in photosynthetic capacity subjected to water deficit stress. J Plant Interact. 2008;3(2):127-136.
18. Ansari MKA, Ahmad A, Umar S, Iqbal M. Mercury-induced changes in growth variables and antioxidative enzymes activities in Indian mustard (*Brassica juncea* L.). J Plant Interact. 2009;4(2):131-136.
19. Arakawa O, Hori Y, Ogata R. Relative effectiveness and interaction of ultraviolet-B, red and blue light in

anthocyanin synthesis of apple fruit. Physiol Plant. 1985;64(3):323-327.
20. Aref MI, Ahmed AI, Khan PR, El-Atta H, Iqbal M. Drought-induced adaptive changes in the seedling anatomy of Acacia ehrenbergiana and Acacia tortilis subsp. raddiana. Trees. 2013;27(4):959-971.
21. Aref MI, El-Atta H, El-Obeid M, Ahmed A, Khan PR, Iqbal M. Effect of water stress on relative water and chlorophyll contents of Juniperus procera Hochst. ex Endlicher in Saudi Arabia. Life Sci J. 2013;10(4):681-685.
22. Aref IM, Khan PR, Al-Sahli AA, Husen A, Ansari MKA, Mahmooduzzafar, *et al.* Response of Datura innoxia Linn. to gamma rays and its impact on plant growth and productivity. Proc Natl Acad Sci India Sect B Biol Sci. 2016;86(3):623-629.
23. Aref IM, Khan PR, Khan S, El-Atta H, Ahmed AI, Iqbal M. Modulation of antioxidant enzymes in Juniperus procera needles in relation to habitat environment and dieback incidence. Trees. 2016;30(5):1669-1681.
24. Arora NK, Fatima T, Mishra I, Verma M, Mishra J, Mishra V. Environmental sustainability: Challenges and viable solutions. Environ Sustain. 2018;1(4):309-340.
25. Arshi A, Abdin MZ, Iqbal M. Growth and metabolism of Senna as affected by salt stress. Biol Plant. 2002;45(1):295-302.
26. Arshi A, Abdin MZ, Iqbal M. Changes in biochemical status and growth performance of Senna (Cassia angustifolia Vahl.) grown under salt stress. Phytomorphology. 2004;54(1):109-124.
27. Arshi A, Abdin MZ, Iqbal M. Sennoside content and yield attributes of Cassia angustifolia Vahl. as affected by NaCl and $CaCl_2$. Sci Hortic. 2006;111(1):84-90.
28. Arshi A, Ahmad A, Aref IM, Iqbal M. Comparative studies on antioxidant enzyme action and ion accumulation in soybean cultivars under salinity stress. J Environ Biol. 2012;33(1):9-20.
29. Ashraf M, Orooj A. Salt stress effects on growth, ion accumulation and seed oil concentration in an arid zone traditional medicinal plant ajwain (*Trachyspermum ammi* [L.] Sprague). J Arid Environ. 2006;64(2):209-220.
30. Atique A, Iqbal M. Traditional single drug administration for the psychosomatic dermal diseases: Psoriasis. Hamdard Med. 1992;35(3):76-81.