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COMPREHENSIVE EVALUATION OF ABIOTIC STRESS RESPONSES AND BIOSTIMULANT MODULATION IN *Thymus* spp.

Doctoral (Ph.D.) Dissertation

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DECLARATION

I declare that the thesis titled “Comprehensive Evaluation of Abiotic Stress Responses and Biostimulant Modulation in *Thymus* spp.” is my own work and that it has not been submitted previously for any degree or examination at any other university.

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Abbreviations

AAE	Ascorbic acid equivalents
ABA	Abscisic acid
Abs.	Absorbance
ABTS	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
AOC	Antioxidant capacity
APX	Ascorbate peroxidase
ANE	Ascophyllum nodosum extract
B_bis	β-bisabolene
B_caryo	β-caryophyllene
C	Control
CAR	Carotenoids
CARV	carvacrol
CAT	Catalase activity
CH_A	Chloropyll a
CH_B	Chloropyll b
CRD	Completely randomised design
D	Drought stress
Dim.	Dimension
DMAPP	Dimethylallyl pyrophosphate
DNA	Deoxyribonucleic Acid
DPPH	2,2-diphenyl-1- picrylhydrazyl
DS	Combined drought and salinity stress
DSW	Dry shoot weight
DW	Dry weight
EC_{50}	Effective concentration
EO	Essential oil
EOC	Essential oil content
eq	Equivalents
FAO	Food and Agriculture Organization
FC	Field capacity
FPP	Farnesyl pyrophosphate
FRAP	Ferric reducing antioxidant power
FSW	Fresh shoot weight
FW	Fresh weight
GAE	Gallic acid equivalents
GC-MS	Gas chromatography–mass spectrometry
GPP	Geranyl pyrophosphate
h	Hour
IPP	Isopentenyl pyrophosphate
IC_{50}	Half-maximal inhibitory concentration
LRI	Linear retention index
MAPs	Medicinal and aromatic plants
meq	Milliequivalents
MFC	Minimum fungicidal concentration
MEP	Methylerythritol phosphate pathway
MIC	Minimum inhibitory concentration
min	Minute
MVA	Mevalonic acid pathway
MUFA	Monounsaturated fatty acids
m/m %	Weight/weight percent
PCA	Principal component analysis
pcs	Pieces
PEG	Polyethylene glycol
POD	Peroxidase activity
PSII	Photosystem II
PUFA	Polyunsaturated fatty acids
p-cym	p-cymene
RDW	Dry root weight
RFW	Fresh root weight
RL	Root length
ROS	Reactive oxygen species
RWC	Relative water content
S	Salinity stress
SS	Soluble sugars
SD	Standard deviation
SH	Shoot length
SOD	Superoxide dismutase
sp./spp.	Species
SSI	Stress susceptibility index
STI	Stress tolerance index
SWC	Soil water capacity
SWt	Saturated weight
PRO	Proline
RT	Retention time
TC1	1st experiment of *T. capitatus* (2024)
TC2	2nd experiment of *T. capitatus* (2025)
Terp	γ-terpinene
Th_Mt_Et	thymol methyl ether
TL1	1st experiment of *T.* × *citriodorus* (2024)
TL2	2nd experiment of *T.* × *citriodorus* (2025)
TPC	Total polyphenol content
TPTZ	2,4,6-tripyridyl-s-triazine
TPSs	Terpene synthases
TP1	1st experiment of *T. pannonicus* (2023)
TP2	2nd experiment of *T. pannonicus* (2024)
TP3	3rd experiment of *T. pannonicus* (2025)
V	Volume
w	Weight

1 Introduction

Within the plant kingdom (Plantae) and under the division Magnoliophyta (commonly known as angiosperms or flowering plants), medicinal and aromatic plants (MAPs) represent a significant and diverse group (Ye et al., 2022). These plants have played a central role in traditional medicine systems across cultures for centuries, largely due to the presence of bioactive secondary metabolites that contribute to their therapeutic potential (Baruah et al., 2024; Islam, 2019). According to the Food and Agriculture Organization (FAO), approximately 52,000 plant species are used globally for medicinal purposes (Schippmann et al., 2002). Most MAPs belong to the class dicotyledons, although some are found among the monocotyledons.

A wide range of plant families is represented among MAPs. The Lamiaceae (Mint family) includes genera such as *Thymus*, *Mentha* and *Lavandula*, all known for their higher essential oil content (EOC) (Alamgir, 2017; Aprotosoaie et al., 2017). The Asteraceae family comprises important medicinal genera like *Matricaria* (chamomile), *Artemisia* and *Echinacea* (Alamgir, 2017; Hanganu & Ahmadi, 2024; Ruzicka et al., 2024). The Apiaceae family includes widely used species such as *Coriandrum sativum*, *Carum carvi* and *Foeniculum vulgare* (Alamgir, 2017; Rafieian et al., 2024; Samojlik et al., 2010). Other notable families include the Fabaceae and Zingiberaceae, which also contribute significantly to the global diversity of medicinal plant species (Alamgir, 2017; Sourabh & Bera, 2024).

Although *Thymus vulgaris* is the most widespread and well-known species of the genus, celebrated for its versatility, evergreen nature, and remarkable therapeutic potential, other *Thymus* species also deserve attention. The richness of *T. vulgaris* in bioactive compounds such as thymol and carvacrol largely explains its extensive use in medicine, cosmetics, and the food industry. Whereas lesser-known species like *Thymus pannonicus*, *Thymus* × *citriodorus*, and *Thymus capitatus* have recently attracted growing scientific interest. These species exhibit distinct chemical compositions and unique aromatic profiles, yet share comparable biological activities, making them promising alternatives for pharmaceutical, nutraceutical, and aromatic applications.

However, plant performance is not determined by genetic background alone; interactions between plants and their environment represent a major source of variability in morphological, physiological, and biochemical traits. In the context of ongoing climate change, drought risk is expected to intensify across many regions during the 21st century. Projections indicate a decline in seasonal rainfall in areas such as southern Europe, North Africa, and parts of southern Africa, further amplifying existing climatic variability and land-use pressures (Knippertz et al., 2003). Rising temperatures and altered precipitation regimes increase evapotranspiration, accelerate soil water loss, and exacerbate soil moisture deficits, ultimately threatening agricultural productivity and water availability (Yu et al., 2024). In parallel, reduced water availability combined with higher evaporation rates can intensify soil salinity, a stressor that severely disrupts plant physiological processes, including water uptake, photosynthesis, and biomass accumulation. Such

effects have already been reported in medicinal plants, including species of the genus *Thymus* (Da Silva et al., 2025; Kramer et al., 2025).

Importantly, under natural and agricultural conditions, plants are rarely exposed to a single stressor. Instead, multiple abiotic stressors, most commonly drought and salinity, often occur simultaneously, creating complex stress scenarios that are more damaging than individual stressors applied in isolation (Mittler, 2006). The combined action of these stressors can overwhelm plant adaptive mechanisms, leading to stronger growth inhibition and metabolic imbalance. As the consequences of climate change are already evident in many agroecosystems, the development of effective and sustainable mitigation strategies has become a necessity rather than an option. In this context, environmentally friendly approaches that enhance plant resilience without increasing chemical inputs are particularly desirable.

Among such approaches, the use of biostimulants, especially seaweed extract-based products, has gained increasing attention as a sustainable tool to support plant performance under abiotic stress. Seaweed biostimulants are known to enhance soil fertility, promote microbial activity, and improve plant physiological status, thereby contributing to more resilient and eco-friendly production systems (Sandhya Rani et al., 2024). Extracts derived from brown algae, such as *Ascophyllum nodosum* extract (ANE) and *Cystoseira barbata*, have been reported to improve nutrient uptake, stress tolerance, and overall plant productivity in a wide range of crops and medicinal plants (Hassan et al., 2021; Staykov et al., 2025). Within this framework, the present study aims to provide a comprehensive evaluation of how *T. pannonicus*, *T.* × *citriodorus*, and *T. capitatus* respond to drought and salinity, applied individually and in combination, and to assess the extent to which seaweed biostimulant application can mitigate stress-induced limitations.

2 Objectives

1. To gain a comprehensive understanding of the effects of imposed abiotic stressors, drought (40% soil water content [SWC]) and salinity (administered progressively at 60, 90, and 120 mM NaCl), applied individually and in combination, on *Thymus pannonicus*, *T.* × *citriodorus*, and *T. capitatus*, by examining responses at three interconnected levels:
   - Morphological and biomass traits, with a focus growth characteristics and associated shoot and root biomass.
   - Physiological traits, including relative water content, photosynthetic pigments, proline accumulation, and soluble sugars.
   - Biochemical and metabolic traits, essential oil (EO) yield and composition, total polyphenol content, antioxidant capacity and hydrogen peroxide level.
2. To assess the potential of a seaweed extract-based biostimulant (*A. nodosum* extract) to mitigate the adverse effects of drought, salinity, and their combination in the three studied *Thymus* species.
3. To compare species-specific physiological and biochemical responses of *Thymus* species under drought and salinity stress, in order to characterise their phenotypic and metabolic diversity and identify inherent adaptive tendencies within the genus.
4. To evaluate the ecological plasticity of the three *Thymus* species by quantifying the magnitude and direction of phenotypic adjustments in response to increasing stress intensity.
5. To quantify the trade-offs between primary growth processes and the accumulation of secondary metabolites under stress conditions, and to determine how these trade-offs vary among species and stress types, as well as the modulatory role of *A. nodosum* extract application in this balance.

3 Literature review

3.1 The genus *Thymus*: pharmacobotanical features and bioactivity

3.1.1 Classification and essential oil diversity of *Thymus* species

The genus *Thymus*, belonging to the Lamiaceae family and the Nepetoideae subfamily, consisting of approximately 250 taxa, encompasses 214 species along with 36 recognised subspecies. It is naturally distributed across the Mediterranean region, especially in the northwest Africa and Iberian Peninsula (Iftikhar et al., 2023; Stahl-Biskup & Venskutonis, 2012). Based on morphological traits and geographical distribution, the *Thymus* genus is divided into eight main sections. The Micantes section comprises North African woody species such as *T. riatarum*, *T. satureioides*, and *T. caespititius*, which are characterised by erect forms, oblong-obovate leaves, and spiciform inflorescences (Belmalha et al., 2017; Morales, 2002). Their essential oils are chemically diverse, with α-terpineol often dominant in *T. caespititius* (Neves et al., 2017; Pinto et al., 2014), while *T. riatarum* populations in Morocco display variability, showing either borneol or γ-terpinene/p-cymene as main constituents (Boubaker et al., 2016; Fadli et al., 2014). The Mastichina section, native to the Iberian Peninsula, includes *T. mastichina* subspecies and *T. albicans*, their essential oils are all rich in 1,8-cineole and linalool (Morales, 2002; Roxo et al., 2020). Similarly, *T. piperella*, the sole member of the Piperella section endemic to Valencia, is distinguished by its verticillaster inflorescence and high levels of p-cymene and carvacrol (Morales, 2002; Ruiz-Navajas et al., 2015) .

Species of the Teucrioides section, originating from the Balkans, exhibit ovate to triangular leaves and verticillaster inflorescences, with p-cymene as a typical compound (Morales, 2002; Pitarokili et al., 2014). The Pseudothymbra section, distributed across the Iberian Peninsula and North Africa, includes nine species with linear, revolute leaves; *T. munbyanus* notably produces carvacrol-rich oils (Benomari et al., 2020; Morales, 2002). The *Thymus* section, predominant in the western Mediterranean, encompasses representative species such as *T. vulgaris*, *T. zygis*, and *T. willdenowii*, with *T. vulgaris* widely known for its thymol-rich essential oil (Morales, 2002; Vouillamoz & Christ, 2020).

The Hyphodromi section, which covers the broader Mediterranean region, includes around 60 species (Morales, 2002); *T. algeriensis*, for instance, exhibits strong chemical polymorphism, producing volatile oils with chief compounds of 1,8-cineole and camphor in Tunisian populations (Zouari et al., 2012), while Algerian accessions may shift toward thymol or terpinyl acetate chemotypes depending on altitude (Hazzit et al., 2009). Finally, the Serpyllum section, comprising about 120 species, is typified by creeping, woody plants such as wild thyme (*T. serpyllum*), whose essential oils are rich in thymol (Galovičová et al., 2021; Morales, 2002).

Within this taxonomic framework, *T. pannonicus*, selected as a model species of the present study, is a well-defined member of section Serpyllum. The hybrid *T.* × *citriodorus*, derived from crosses between *T. pulegioides* and *T. vulgaris*, is generally treated separately in modern classifications,

although it shares several morphological traits with Serpyllum taxa. In contrast, *Thymus capitatus* (currently accepted as *Thymbra capitata*) has historically been assigned to section Micantes, subsection Pseudothymbra, reflecting its distinct morphological and chemical characteristics (Morales, 2002). This taxonomic diversity within the studied taxa provides an appropriate background for the following sections addressing essential oil biosynthesis and the pharmacobotanical characteristics reported for *Thymus* species.

3.1.2 Essential oil biosynthesis in *Thymus* species

Morphologically, essential oil in the Lamiaceae family are produced and stored within specialised glandular trichomes (Figure 1), mainly peltate but also capitate types, which are characteristic features of this plant family (Giuliani & Maleci Bini, 2008). Peltate glandular trichomes represent the primary sites of essential oil biosynthesis and accumulation, acting as natural reservoirs for volatile compounds, particularly monoterpenes (Yamaura et al., 1992). Within each trichome, a group of highly specialised secretory cells actively synthesises essential oils and deposits them in a subcuticular space, which functions as a protective chamber, maintaining the oils in a liquid state and preventing premature evaporation until they are eventually released into the environment (Turner et al., 2000).

The main constituents of essential oil are primarily derived from three major biosynthetic routes. Terpenes are synthesised through the plastidic methylerythritol phosphate (MEP) pathway and the cytosolic mevalonic acid (MVA) pathway, while phenylpropenes originate from the shikimic acid pathway. Both the MEP and MVA pathways generate the fundamental five-carbon isoprenoid building blocks, dimethylallyl pyrophosphate (DMAPP) and isopentenyl pyrophosphate (IPP) (Franz & Novak, 2010; Sapir-Mir et al., 2008).

Within plastids, these units condense to form geranyl pyrophosphate (GPP, C10), the universal precursor for monoterpenes and diterpenes. In contrast, in the cytosol, they combine to produce farnesyl pyrophosphate (FPP, C15), the starting molecule for sesquiterpene biosynthesis (Sun et al., 2022; Tholl, 2015). The diversification of these compounds largely depends on terpene synthases (TPSs), a complex family of enzymes that shape the core skeletons of terpenes and are responsible for the enormous structural diversity observed among mono- and sesquiterpenes (Chen et al., 2011; Stahl-Biskup, 2002; Tholl, 2015).

Among the key bioactive constituents identified in *Thymus* species, thymol, carvacrol, geraniol, linalool, camphor, β-caryophyllene, etc. These major terpenoids, along with diverse secondary metabolites, exhibit remarkable antimicrobial, anti-inflammatory, antioxidant, and anticancer properties (Anwar et al., 2024; Ghasemi Pirbalouti et al., 2015). Such a phytochemical profile underlies the medicinal value of *Thymus* plants, which have shown therapeutic potential in the treatment of cardiovascular disorders, microbial infections, respiratory diseases, and gastrointestinal disturbances (Anwar et al., 2024; Marković et al., 2020; Mazulin et al., 2024).

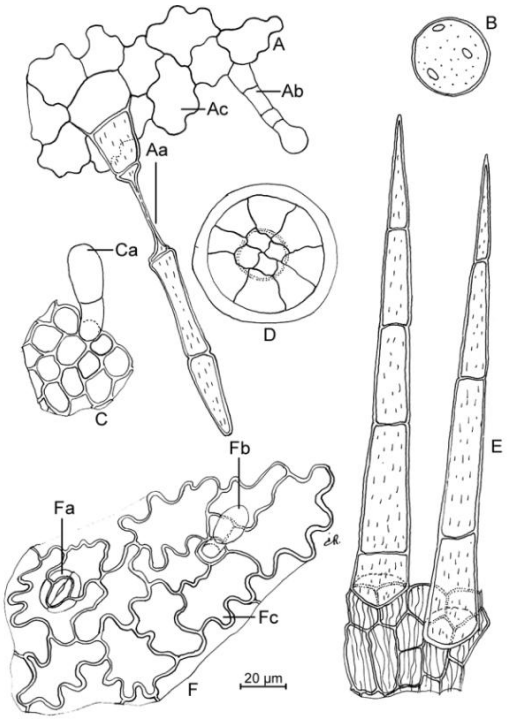

Figure 1. Microscopic characteristics of floral fragments and glandular trichomes in powdered *Thymi herba* (Ph. Eur., 2023, *Thymi herba*, pp. 1752–1754). A: Fragment of the outer epidermis of the corolla (surface view), showing uniseriate, multicellular covering trichomes, often with one collapsed cell (Aa), glandular trichomes with a unicellular head and a multicellular stalk (Ab) and epidermal cells with unthickened walls (Ac); B: Pollen grain, spherical and smooth, with six germinal slit-like pores; C: Epidermal cells from the base of the corolla, isodiametric with slightly thickened walls, bearing a glandular trichome (Ca); D: Peltate glandular trichome (glandular scale) composed of approximately 12 secretory cells, seen in surface view; E: Fragment of the calyx epidermis in surface view, covered by numerous uniseriate covering trichomes with 5–6 cells and a weakly striated cuticle; F: Outer epidermis of the corolla (surface view), showing diacytic stomata (Fa), glandular trichomes (Fb) and epidermal cells with slightly thickened walls (Fc).

3.1.3 Pharmacobotanical features and bioactivity of *Thymus* species applied as model plants of our studies

Thymus vulgaris is the most widely recognised species of the genus, largely because of its richness in thymol and carvacrol and its broad industrial use. Nevertheless, other species such as *T. pannonicus*, *T.* × *citriodorus*, and *T. capitatus* are also important, increasingly attracting scientific attention.

3.1.3.1 *Thymus pannonicus*

T. pannonicus All., commonly known as Pannonian thyme or Hungarian thyme, is a perennial subshrub native to Central and Eastern Europe, particularly associated with the Pannonian Plain and adjacent regions (Chytrý et al., 2019; Jalas, 1972; Simić et al., 2024). It typically inhabits dry grassland slopes, reflecting its strong adaptation to drought conditions, with 38 natural populations recorded in Hungary alone (Borhidi, 1995; Pluhár et al., 2024).

Morphologically, the species is characterised by nearly sessile leaves, featuring faint veins and visible glandular dots, giving the plant a soft, green texture overall. It produces pinkish flowers

arranged in long, unbranched spikes blooming during the summer and has a diploid chromosome number of 2n = 28 (Jalas, 1972; Tutin et al., 1972).

From an ecological perspective, *T. pannonicus* demonstrates a notable adaptability to edaphic conditions. A study by Pluhár et al. (2010) on Hungarian populations revealed that the species predominantly occurs in natural to slightly alkaline soils, with pH values ranging between 6.41 and 7.99. These soils are typically rich in humus and potassium (K_2O), with concentrations often exceeding 200 mg kg^{-1} in several studied populations. Supporting this, Mártonfi et al. (1996) observed its occurrence across a variety of soil types in the Carpathian and Pannonian regions, from low-carbonate substrates to limestone-based soils, often associated with higher nitrogen and oxidisable carbon content.

Essential oil composition and bioactive compounds of *T. pannonicus*

The Pannonian thyme exhibits remarkable variability in both essential oil yield and composition across its natural range. Essential oil yields vary significantly, from as low as 0.1 to as high as 1.9 mL 100 g^{-1} dry weight, depending on ecological conditions within each population (Pluhár et al., 2010, 2024). This chemical diversity has led to the identification of distinct chemotypes; for instance, a study conducted in Serbia revealed three different chemotypes in populations from separate regions: germacrene-D, geranial, and α-pinene (Sostaric et al., 2012). Similarly, several chemotypes have been identified in Hungarian populations, with a notable dominance of thymol-based profiles; these include: thymol (67.50 %); thymol/p-cymene (63.70 % and 11.50 %, respectively); thymol/p-cymene/γ-terpinene (53.58 %, 10.52 %, and 9.63 %, respectively); caryophyllene oxide/α-cubebene/linalool (45.20 %, 15.70 %, and 13.80 %, respectively); β-cadinene/germacrene D (28.82 % and 13.18 %, respectively); and germacrene D/β-caryophyllene/farnesol (29.70 %, 22.00 %, and 10.40 %, respectively), (Pluhár et al., 2010, 2024).

T. pannonicus has emerged as a plant of notable interest in both traditional medicine and modern pharmacological research, largely due to the remarkable richness of its essential oil and phenolic compounds. Its extracts are particularly abundant in polyphenols (150 mg gallic acid (GAE) equivalents g^{-1} extract), flavonoids (34.51 mg rutin eq. g^{-1} extract), rosmarinic acid (80.49 mg g^{-1} extract), salvianolic acid B (41.67 mg g^{-1} extract), luteolin-O-hexuronide (22.01 mg g^{-1} extract), and quercetin-O-hexuronide (3.76 mg g^{-1} extract), as reported by Arsenijević et al. (2016) and Babotă et al. (2023). Boros et al. (2010) also reported about quantitative determination of the polyphenolic patterns of Hungarian wild thyme populations including 8 populations of *T. pannonicus*, where rosmarinic acid was the chief molecule (948.50–1273.60 µg g^{-1} dry weight [DW]) and further phenolic compounds, such as phenolic acids (caffeic acid: 41.40–52.60 µg g^{-1} DW; chlorogenic acid:14.10–207.50 µg g^{-1} DW; ferulic acid: 2.70–11.40 µg g^{-1} DW; p-coumaric acid: 0.60–1.95 µg g^{-1} DW; epicatechin: 0.0–104.0 µg g^{-1} DW) and flavonoids (apigenin: 33.24–120.70 µg g^{-1} DW; rutin: 0.0–67.60 µg g^{-1} DW; naringenin: 4.60–23.60 µg g^{-1} DW; dihydroquercetin: 6.50–48.10 µg g^{-1} DW; quercetin: 1.20–3.40 µg g^{-1} DW; eriodictyol: 1.50–14.50 µg g^{-1} DW) varied in a wide range.

Antioxidant activity of *T. pannonicus*

The rich content of bioactive compounds in *T. pannonicus* plays a key role in its strong antioxidant capacity, as demonstrated by Babotă et al. (2023). The study reported a high 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of 80.71 mg Trolox equivalents g^{-1} extract, a value exceeding that of four other *Thymus* species examined in Romania. Similarly, another study on a *T. pannonicus* chemotype characterised by high levels of thymol (34.90 %) and p-cymene (12.00 %) reported a pronounced DPPH radical inhibition, with a half-maximal inhibitory concentration (IC_{50} = 1009.82 µg mL^{-1}) in samples collected from wild populations in western Romania (Jianu et al., 2015). These findings underscore the exceptional antioxidant strength of *T. pannonicus*, largely attributed to its abundant phenolic and monoterpenoid constituents.

Antimicrobial activity of *T. pannonicus*

T. pannonicus has been reported to show considerable antimicrobial potential against a diverse range of pathogenic microorganisms. According to Arsenijević et al. (2016), its essential oil showed strong inhibitory effects against Gram-positive bacteria such as *Staphylococcus* aureus (MIC: 62.50–500.00 µL mL^{-1}), Gram-negative strains including *Escherichia coli* (MIC: 125.00–500.00 µL mL^{-1}), and opportunistic fungi like *Candida albicans* (MIC: 31.25–62.50 µL mL^{-1}). Additionally, its efficacy extends to other microbes such as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* (Maksimović et al., 2008). *T pannonicus* has also demonstrated targeted activity against *Helicobacter pylori*, a bacterium responsible for chronic gastrointestinal disorders and peptic ulcers, the methanolic extract rich in rosmarinic acid (64 mg g^{-1} of methanolic extract) and the essential oil, dominated by geranial (36.7 %) and neral (27.2 %), exhibited strong antibacterial efficacy against this pathogen (Arsenijević et al., 2024).

Cytotoxic activity of *T. pannonicus*

Beyond its antimicrobial and antioxidant potential, *T. pannonicus* has also proved promising anticancer properties; the deodorised water extract of this species induced up to 90 % cancer cell death in Ehrlich carcinoma cells, underlining its chemopreventive and cytotoxic potential (Ćebović et al., 2018). Furthermore, Babotă et al. (2023) highlighted its inhibitory effects against key enzymes such as α-glucosidase and acetylcholinesterase, making it a valuable candidate for managing metabolic and neurodegenerative diseases.

Antihepatoma activity of *T. pannonicus*

Methanol extracts from *T. pannonicus* in vitro shoot cultures showed notable antihepatoma activity against human HepG2 liver cancer cells. Both extracts, E1 (without hormones) and E2 (with indole-3-acetic acid), inhibited cell growth in a dose and time dependent manner, with E2 exhibiting slightly higher potency (IC_{50} = 77.8 µg mL^{-1}). Chemical analysis identified rosmarinic acid as the main phenolic compound, present in higher amounts in E2. Both extracts and rosmarinic acid caused cell cycle arrest at the G_2/M phase and induced a mild increase in ROS (Reactive

oxygen species) levels, suggesting that the cytotoxic and antiproliferative effects of *T. pannonicus* are largely linked to its high rosmarinic acid content (Gordana et al., 2024).

Biosorption activity of *T. pannonicus*

Interestingly, recent environmental studies have explored the use of *T. pannonicus* beyond medicine. Tekke and Özmal (2022) demonstrated that chemically modified leaves of this species can serve as an efficient and sustainable biosorbent for removing copper from contaminated water, contributing to green remediation technologies.

3.1.3.2 *Thymus × citriodorus*

Commonly known as lemon thyme, *T. × citriodorus* (Pers.) Schreb., is a naturally occurring hybrid resulting probably from the cross between *T. vulgaris* and the geraniol chemotype of *Thymus pulegioides* (Schmidt et al., 2004; Stahl-Biskup & Holthuijzen, 1995). Renowned for its distinct lemon-like aroma, this aromatic plant is native to southern Europe but has since become widely distributed across the Mediterranean region (Toncer et al., 2017). Its appealing scent and hybrid vigour have made it a popular choice in both traditional herbal practices and modern horticulture.

Morphologically, *T. × citriodorus* is a perennial herb that combines a soft herbaceous structure with a woody base. Its upright stems branch directly from the base, forming a compact shape, while the small, oval, and aromatic leaves grow in opposite pairs along the stem. During summer, the plant produces pale pink flowers grouped in tight clusters, the stems are able to root when they come into contact with soil, allowing the plant to spread naturally (Omidbaigi et al., 2009; Stahl-Biskup & Holthuijzen, 1995; Tutin et al., 1972).

T. × citriodorus thrives best under full sun and warm growing conditions, showing a clear preference for well-drained soils with moderate organic enrichment. A study by Vaičiulytė et al. (2022) demonstrated that this hybrid performs particularly well in soils treated with liquid cattle dung, which significantly boosts key nutrients, such as potassium (up to 206 mg kg^{-1}), phosphorus (up to 124 mg kg^{-1}), calcium (up to 808 mg kg^{-1}), total nitrogen (up to 0.83 %), and organic carbon (up to 0.96 %), within a slightly acidic pH range of 6.00–6.40. These nutrients helped the plant grow better and produce more essential oils. In contrast, while humus offered some benefits, its effects were inconsistent and generally less pronounced, suggesting that *T. × citriodorus* prefers a nutrient-rich but balanced soil environment rather than substrates overly saturated with humic content.

Essential oil composition and bioactive compounds of *T. × citriodorus*

The essential oil of *T. × citriodorus* is distinguished by its characteristic lemony aroma and pale-yellow appearance. Essential oil contents vary depending on cultivation conditions and harvest timing; Omidbaigi et al. (2005) reported 1.4 % (w/w) essential oil content based on dry weight, while Bagdat et al. (2011) observed values ranging from 1.308 % to 1.430 %. In Türkiye, Toncer et al. (2017) noted a content of 1.9 % at the flowering stage, which dropped to 1.3 % post-

flowering. The oil of this Turkish chemotype was dominated by terpinolene (71.00 %) and α-terpineol (20.03 %). In contrast, the essential oils of plants grown in Portugal were rich in geraniol (27.50 %) and 1,8-cineole (16.30 %) (Oliveira et al., 2022). Similarly, Chinese-grown samples showed high levels of cis-geraniol (36.01 %) and citral (18.89 %) (Pinpin et al., 2021), while
a Hungarian one contained 39.20 % geraniol and 15.40 % carvacrol (Horváth et al., 2006). These profiles align with findings from Greece, where geraniol reached 47.08 % (Ntalli et al., 2020), emphasising the influence of geographical origin on essential oil composition.

Beyond its distinctive lemon-scented essential oil, *T. × citriodorus* also exhibits notable richness in phenolic compounds. Taghouti et al. (2020) reported high levels of total phenols (165.14 mg caffeic acid eq g^{-1}), total flavonoids (282.93 mg catechin eq g^{-1}), and ortho-diphenols (122.19 mg caffeic acid eq g^{-1}), reflecting the plant's diverse bioactive chemical profile. This complex phytochemical makeup justifies its longstanding use in traditional medicine.

Antimicrobial activity of *T. × citriodorus*

The essential oil of *T. × citriodorus* 'Silver Queen' has shown notable antimicrobial potential; according to recent findings (Steshenko et al., 2021), it exhibited strong antibacterial activity against *Staphylococcus aureus* (inhibition zone: 14.60 ± 1.52 mm) and *Escherichia coli (*19.60 ± 1.85 mm), along with a pronounced fungicidal effect against *Candida albicans* (29.30 ± 2.82 mm). However, no significant activity was observed against *Pseudomonas aeruginosa*. The antimicrobial effects were most strongly correlated with total phenolics and flavonoids, suggesting these compounds play a key role in the plant's bioactivity (Abramovič et al., 2018). These results highlight the promising antibacterial and antifungal properties of this hybrid thyme species for further therapeutic exploration. Additionally, *T.× citriodorus* 'Aureus' essential oil demonstrated significant antifungal activity against *Aspergillus flavus*, a major food contaminant and aflatoxin B1 producer; the oil showed a MIC of 1.0 µL mL^{-1} and a minimum fungicidal concentration (MFC) of 1.0 µL mL^{-1}. This activity is largely attributed to its high geraniol content (60.31 %) and presence of aromatic compounds, making it a promising natural alternative for controlling *A. flavus* growth and aflatoxin contamination (Aprotosoaie et al., 2019). Furthermore, Oliveira et al. (2022) evaluated the anti-acne potential of *T. × citriodorus* essential oil and hydrolate: the essential oil showed strong antimicrobial effects, especially against *Cutibacterium acnes*, *Staphylococcus aureus*, and *S. epidermidis*, with MIC values as low as 0.06 %, it also inhibited biofilm formation and disrupted existing ones. Meanwhile, the hydrolate had milder antimicrobial and anti-biofilm effects but showed higher biocompatibility and anti-inflammatory potential. Overall, the essential oil may be better for short-term treatment of acne flare-ups, while the hydrolate suits daily skin care due to its safety and moderate efficacy.

Antioxidant and antiproliferative activities of *T.* × *citriodorus*

The antioxidant capacity of *T.* × *citriodorus* has been highlighted in several studies using diverse in vitro assays. Rita et al. (2018) reported that infusions of this species exhibited strong DPPH radical-scavenging activity, with an effective concentration 50 % (EC_{50}) value of 0.228 mg mL^{-1}, largely attributed to its high phenolic content, particularly rosmarinic acid. The same study also showed notable effects in β-carotene bleaching and lipid peroxidation inhibition assays, further confirming its antioxidant potential. Complementing these findings, Taghouti et al. (2020) demonstrated that hydroethanolic extracts of *T.* × *citriodorus* showed superior ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical-scavenging activity (1.52 mmol Trolox eq g^{-1}) compared to aqueous decoctions (1.21 mmol Trolox eq g^{-1}), reinforcing the role of extraction method in determining bioactivity. In the same study, the authors demonstrated that extracts from *T.* × *citriodorus* have promising antiproliferative effects, particularly on human colon adenocarcinoma cells (Caco-2). The hydroethanolic extract was the most effective, reducing cell viability with IC_{50} values of 128.2 ± 5.75 µg mL^{-1} after 24 hours and 114.6 ± 4.38 µg mL^{-1} after 48 hours.

Cytoprotective activity of *T.* × *citriodorus*

Beyond its antioxidant and antiproliferative properties, *T.* × *citriodorus* also demonstrates considerable cytoprotective effects under oxidative stress conditions. In the study by Pereira et al. (2013) purified ethanolic extracts of *T.* × *citriodorus* demonstrated cytoprotective effects in human liver cancer cells (HepG2) cells exposed to potassium dichromate-induced oxidative stress. At a concentration of 50 µg mL^{-1}, the extract reduced ROS production by 20 % and increased cell viability by approximately 30 % in the acute exposure model (200 µM for 6 h). A polyphenolic mixture simulating the extract's composition showed similar protection. However, neither the extract nor the mixture was effective in the long-term exposure model (2 µM for 72 h), likely due to the extract's relatively low total phenolic content. The protective effects were mainly attributed to luteolin derivatives.

Nematicidal activity of *T.* × *citriodorus*

Interestingly, the benefits of *T.* × *citriodorus* extend beyond medicinal applications to sustainable agriculture. Its dried powder has demonstrated eco-friendly nematicidal activity, significantly reducing *Meloidogyne incognita* (root-knot nematode) populations by 95 % at 1 g kg^{-1} soil. This treatment also enhanced tomato plant growth, while its unfiltered water extracts improved soil microbial activity. These findings underscore its dual function as both a biopesticide and a biofertiliser, offering a promising green alternative for crop protection and soil health (Ntalli et al., 2020).

3.1.3.3 *Thymus capitatus*

T. capitatus (L.) Hoffmanns. & Link (formerly classified as *Thymbra capitata* (L.) Cav. or *Coridothymus capitatus* (L.) Rchb. f.), is a Mediterranean perennial, woody subshrub commonly known as Zaatar (Bounatirou et al., 2007; Džamić et al., 2015; El Ajjouri et al., 2008; Skoula et al., 2004). It is widely recognised for its aromatic qualities and long-standing use in traditional medicine across the Mediterranean region (Ben El Hadj Ali et al., 2012; Tawaha & Hudaib, 2012).

Morphologically, *T. capitatus* is a compact, woody aromatic shrub with ascending to erect branches that bear small, linear, sessile leaves, each ending in an acute tip. These leaves, which tend to vary slightly with season, are typically around 6–10 mm long and 1.0–1.2 mm wide, and are sparsely ciliate at the base (Morales, 2002; Tutin et al., 1972). Their surfaces show clearly visible oil glands, a characteristic feature of the species. The plant produces dense, cone-shaped flower clusters with oblong-conical bracts. The flowers themselves are small and display delicate shades of purple to pink, consistent with the typical floral structure of the Lamiaceae family, including a bifid upper corolla lip. At the cytological level, *T. capitatus* has a diploid chromosome number of 2n = 30 (Economou-Amilli et al., 1982; Tutin et al., 1972).

In the study of Abdel-Rahman and Migahid (2019), *T. capitatus* was found to thrive in rocky ridge habitats along Egypt's western Mediterranean coast, where it shows uniform distribution and high abundance. These areas feature well-drained, calcareous soils with sand (77–82 %), silt (7–8 %), and clay (10–15 %), and a basic pH (7.63–8.01). Soils are moderately saline (Electrical conductivity: 2.05–3.06 dS m^{-1}) and rich in calcium (up to 10.36 milliequivalents [meq] L^{-1}), sodium (up to 14.28 meq L^{-1}), and potassium (up to 1.46 meq L^{-1}). The presence of *T. capitatus* under these conditions highlights its preference for alkaline, calcareous substrates with moderate salinity, while its growth appears supported by both the rocky terrain and nutrient-rich soil, especially the role of potassium in enhancing development.

Essential oil composition and bioactive compounds of *T. capitatus*

The essential oil yield and composition of *T. capitatus* have been extensively studied across different regions, revealing notable variations. One of its remarkable features is its relatively high essential oil content, which reached up to 2.6 % based on dry plant matter in central Morocco (Tagnaout et al., 2022); this oil was dominated by carvacrol (60.79 %), followed by thymol (12.90 %) and p-cymene (6.21 %). In contrast, samples from northern Morocco showed a slightly lower essential oil content of 2.05 % (Amarti et al., 2008) , while in Tunisia, the value dropped further to around 1.26 %, with carvacrol making up an even higher proportion (71.58 %), alongside 8 % p-cymene and just 0.16 % thymol (Achour et al., 2012). Similar findings came from wild collections in central Tunisia, where the essential oil content was 0.82%, with carvacrol at 65.38 %, accompanied by γ-terpinene (6.75 %) and p-cymene (6.25 %) (Zaïri et al., 2019). This chemical profile closely resembles that of Sicilian *T. capitatus*, which is also characterised by a carvacrol chemotype with 79.90 % carvacrol content (Gonçalves et al., 2017). Interestingly, a different chemotype appears on the Greek island of Lemnos, where Gas chromatography–mass

spectrometry (GC-MS) analysis revealed thymol as the main compound (39.80 %), followed by p-cymene (31.00 %) and a much lower carvacrol content (5.70 %) (Maniki et al., 2023).

In addition to the presence of essential oil, *T. capitatus* is rich in phenolic compounds; (Maniki et al., 2023) reported a total polyphenol content of 231.32 mg GAE g^{-1} EO and a DPPH antioxidant activity of 144.66 µmol Trolox eq g^{-1}, values comparable to those found by Achour et al. (2012), who measured 175.53 mg GAE, with notable amounts of carnosic and rosmarinic acids. The fatty acid profile of the fixed oil of the nutlets has also been analysed (Zaïri et al., 2019), revealing the presence of saturated fatty acids (2.93 g kg^{-1}), monounsaturated fatty acids (MUFA; 0.872 g kg^{-1}) and polyunsaturated fatty acids (PUFA; 0.375 g kg^{-1}). Among these, oleic acid was the predominant MUFA (0.680 g kg^{-1}), while linoleic acid was the predominant PUFA (0.231 g kg^{-1}). The presence of these fatty acids contributes to the oil's antioxidant properties, further enhancing the therapeutic potential of *T. capitatus*.

Antimicrobial activity of *T. capitatus*

T. capitatus essential oil exhibits strong antimicrobial properties against a wide range of pathogens, making it a promising natural agent in both medical and food applications. Its antibacterial effects have been demonstrated against several harmful bacteria, including *Klebsiella oxytoca*, *Enterococcus faecalis*, *Acinetobacter baumannii*, and sensitive strains of *Escherichia coli*, with inhibition zones ranging from 20.7 to 49.9 mm. The MICs were impressively low for sensitive *E. coli* and *Klebsiella pneumoniae* at 2 µL mL^{-1}, although more resistant bacteria like *Staphylococcus aureus* required higher doses (16 µL mL^{-1}). These effects are largely due to the presence of carvacrol, a major component that disrupts bacterial membranes, causing leakage of cell contents and cell death (Tagnaout et al., 2022).

Beyond planktonic bacteria, *T. capitatus* essential oil, rich in oxygenated terpenoids (81.18 %) with thymol as the dominant compound (36 %), has shown notable activity against biofilms of *Staphylococcus epidermidis.* It reduced biofilm bacterial counts by over one log unit and, when combined with chlorhexidine, further enhanced this anti-biofilm effect, an advantage for topical or formulation uses (Alabdullatif et al., 2017). In food preservation, the oil has proven to be effective against common foodborne pathogens like *E. coli*, *Salmonella typhimurium*, *and S. aureus*. When added to minced beef meat, low concentrations (0.01–0.05 %) exhibited bacteriostatic effects, while higher concentrations (1–3 %) showed strong bactericidal action against all tested strains except *Pseudomonas aeruginosa*, which displayed resistance. These results highlight the potential of *T. capitatus* essential oil as a natural preservative to prevent microbial spoilage and improve food safety (Jayari et al., 2018).

The antimicrobial spectrum of *T. capitatus* extends to fungi as well. Its EO strongly inhibits the growth of *Aspergillus niger* and *A. flavus*, reducing mycelial growth by 73.33 % at 10 µL mL^{-1}. This antifungal effect is primarily attributed to the high carvacrol content, supported by minor compounds such as p-cymene and γ-terpinene, which interfere with fungal cell wall enzymes and weaken their structure. The oil may also promote fungal cell aging and death, further reducing

fungal viability (Zaïri et al., 2019). Using microdilution assays, it showed potent activity against *Candida*, *Aspergillus*, and *Rhizopus* species, with MICs ranging from 125 to 500 μg mL^{-1}, confirming its value as a natural antifungal agent (Amakran et al., 2024).

Additionally, Tunisian *T. capitatus* exhibits antiviral activity, notably against herpes simplex virus type 2 (HSV-2). Both its extracts and essential oil inhibited viral infection in vitro, with the ethanolic extract showing the strongest effect (EC_{50} = 2.3 μg mL^{-1}). Three active compounds, β-sitosterol, cinnamaldehyde, and carvacrol, were isolated from this extract, with β-sitosterol demonstrating the highest selectivity index. All three compounds inactivate the virus by directly targeting viral particles, highlighting the therapeutic potential of *T. capitatus* beyond antibacterial and antifungal uses (Toujani et al., 2018).

Antioxidant activity of *T. capitatus*

In various antioxidant assays, the essential oil of *T. capitatus* demonstrated strong free radical scavenging activity and notable antioxidant potential. Using the DPPH method, the oil showed an impressive IC_{50} value of 4.88 ± 0.04 μL mL^{-1}, while the ferric reducing antioxidant power (FRAP) assay revealed an EC_{50} of 0.20 ± 0.02 μL mL^{-1}, indicating effective reduction power. Its total antioxidant capacity measured 11.74 mg ascorbic acid equivalents (AAE) g^{-1} EO, approaching the activity of the synthetic antioxidant butylated hydroxyanisole, which scored 14.33 mg AAE g^{-1} EO (Tagnaout et al., 2022). Supporting these findings, another study reported a similarly strong antioxidant effect with an IC_{50} of 6.4 μg mL^{-1} in the DPPH assay. The antioxidant activity was clearly dose-dependent, with the highest effect at double the IC_{50} concentration (80.90 μg mL^{-1}) and the lowest at half (20.23 μg mL^{-1}). Moreover, the EO demonstrated the ability to inhibit lipid peroxidation, further confirming its promise as a natural source of antioxidants (Bajes et al., 2023).

Cytotoxic activity of *T. capitatus*

Younes et al. (2022) reported that *T. capitatus* collected from Saudi Arabia exhibits notable cytotoxic activity. The ethanolic extract of the leaves showed strong effects against lung cancer cells (A-549), with an IC_{50} of 13.6 μg mL^{-1}, and moderate activity against HepG2, with an IC_{50} of 21.5 μg mL^{-1}. Meanwhile, the stem extract showed moderate cytotoxicity on A-549 cells, with an IC_{50} of 21.38 μg mL^{-1}. Further investigation revealed that the leaves' extract induces cancer cell death by triggering apoptosis, activating key proteins like caspase-3, p53, and Bax, while downregulating the anti-apoptotic protein Bcl-2 and causing cell cycle arrest at the S-phase.

In another study, Kryeziu et al. (2024) explored the cytotoxic effects of *T. capitatus* essential oil against HT-29 colorectal cancer cells. To enhance its stability and prolong its therapeutic action, the oil was encapsulated in liposomes, tiny spherical carriers made from phospholipids. Among different formulations, liposomes made with Phospholipon 90H, a highly purified soy-derived phosphatidylcholine, showed the strongest and most stable cytotoxic activity over six months. This innovative delivery system holds promise for colorectal cancer therapy, although further in vivo research is needed. Supporting these findings, Bajes et al. (2023) found that the EO, rich in thymol (44.34 %) and carvacrol (38.89 %), exerted significant cytotoxic effects against the breast

cancer cell line T47D and the colorectal adenocarcinoma cell line Caco-2. The EO showed higher selectivity for cancer cells compared to normal fibroblasts (MRC5), inducing apoptosis confirmed by deoxyribonucleic acid (DNA) fragmentation, disrupted membrane polarity, and distinctive morphological changes such as cell shrinkage, chromatin condensation, nuclear fragmentation, and cytoplasmic vacuolisation. These results underline the potential of *T. capitatus* as a natural anti-cancer agent.

Hemolytic activity of *T. capitatus*

Moroccan *T. capitatus* essential oil, rich in carvacrol (75.73 %), showed low hemolytic toxicity against human red blood cells at concentrations below 1250 µg mL^{-1}. This low toxicity suggests that it is relatively safe for applications in food preservation and phytopharmaceutical products, supporting its potential as a natural and gentle antimicrobial agent (Amakran et al., 2024).

Pain relief activity of *T. capitatus*

In a study by Gonçalves et al. (2017) , the carvacrol chemotype *T. capitatus*, showed strong pain-relieving effects or antinociceptive. In a test where mice usually lick their paw after feeling pain (called "licking time"), the EO reduced this reaction: at doses of 3, 6, and 12 mg kg^{-1}, the licking time dropped from about 100 seconds (in untreated mice) to 85, 63, and 42 seconds respectively. In nerve tests, a concentration of 500 µg mL^{-1} of the EO reduced the strength of nerve signals by about 80%, likely by blocking sodium channels that help transmit pain signals. These effects were also reversible, meaning the nerves returned to normal afterward.

Pest and parasitic control of *T. capitatus*

T. capitatus essential oil from Morocco contains a high amount of carvacrol (65.96 %), a compound known for its strong bioactive properties. This oil was tested against *Sitophilus oryzae*, a common pest that causes serious damage by infesting and destroying stored grains, leading to significant losses in food supply. The study showed that *T. capitatus* EOs has powerful insecticidal activity against this pest, supporting its traditional use and highlighting its potential as a natural and effective solution for protecting stored grains from infestation (Ainane et al., 2018).

In addition to this, *T. capitatus* EO, composed mainly of carvacrol (65.15 %), along with p-cymene (11.79 %) and γ-terpinene (7.48 %), has demonstrated strong fumigant toxicity against the citrus mealybug, *Planococcus citri*. Laboratory tests revealed nearly complete mortality, with 100 % of larvae and 96 % of adults killed, although larvae were more sensitive. However, it is important to note that the EO also showed considerable toxicity toward *Cryptolaemus montrouzieri*, a beneficial predator, at certain concentrations. This highlights the need to carefully manage its use in pest control to protect helpful insect species (Alloui-Griza et al., 2022).

Beyond its insecticidal properties, *T. capitatus* essential oil also exhibits notable anthelmintic activity. Rich in carvacrol (81.16 %), it effectively inhibited egg hatching *of Haemonchus contortus* in vitro, with an IC_{50} of 1.9 mg mL^{-1}, and reduced adult worm motility by more than 70 % after 8 hours at 1 mg mL^{-1}. In vivo studies in mice infected with *Heligmosomoides polygyrus*

demonstrated significant reductions in parasite loads, 49.5 % in faecal egg counts and 64.5 % in total worm counts, after treatment with the highest dose tested (1600 mg kg^{-1}). These findings suggest that *T. capitatus* EOs holds promise as a natural alternative for controlling gastrointestinal parasites in livestock (Amel et al., 2024).

Preservative and further antimicrobial applications of *T. capitatus*

T. capitatus essential oil has demonstrated strong preservative effects, along with further antimicrobial activity in different practical applications. *T. capitatus* essential oil has proven significant potential as a natural preservative in various contexts. When added at 1 mg L^{-1} to raw and pasteurised milk, it effectively reduced microbial growth, acidity, and fat oxidation, with the strongest preservation observed when combined with pasteurisation. This combination halted bacterial growth by the second day and maintained milk freshness for up to four days, highlighting its promise for extending dairy product shelf life (Ben Jemaa et al., 2018).

Similarly, *T. capitatus* essential oil, containing 73.2 % carvacrol, has been successfully formulated as a pickering emulsion to combat biodeteriogens like cyanobacteria and algae on historic monuments. This eco-friendly application controls microbial growth without leaving residues, maintaining efficacy for up to four months and offering a sustainable solution for cultural heritage preservation (Candela et al., 2019).

3.2 Abiotic stressors and plant adaptive responses

In plant biology, stress is defined as any external factor that negatively affects plant growth, development, or productivity by disturbing cellular homeostasis and normal physiological functioning (Pareek et al., 2010). Abiotic stressors, which include non-living environmental factors, can severely disrupt plant growth and agricultural productivity (Kang et al., 2017). By interfering with physiological processes, altering biochemical pathways, and impeding overall development, these stressors prevent plants from achieving their full genetic potential; the result is often a substantial decline in crop yield, quality, and resilience (Hussaan et al., 2022).

Abiotic stressors are generally classified into two broad categories: physical and chemical. Physical stressors include drought or water scarcity, the opposite extreme of waterlogging or flooding, as well as temperature extremes, rising temperatures leading to heat stress, and falling temperatures causing cold stress or, in more severe cases, frost damage. Other examples are irradiation stress from excessive light or UV exposure, mechanical stress caused by wind, hail, or physical injury, and soil-related constraints such as compaction or poor aeration (Álvarez & Acosta-Motos, 2022; Mondal et al., 2020). Chemical stressors, on the other hand, encompass factors like salinity, often resulting from elevated NaCl levels in the soil, heavy metal contamination, and nutrient imbalances or deficiencies that limit plant growth. Importantly, salinity can also induce water deficit, as salts decrease the osmotic potential of the soil, reducing water availability for plants unless they adjust their root osmotic potential (Pareek et al., 2010). Each of these stress types can act alone or in combination, often compounding their negative

effects on plant health and productivity (Álvarez & Acosta-Motos, 2022; Hussaan et al., 2022). In the following sections, particular emphasis will be placed on drought and salinity stressors, given their prevalence and severe impact on global agriculture.

3.2.1 Drought stress

Drought stress arises when a plant's water demand exceeds the supply available through root uptake, often as a result of limited soil moisture. This imbalance is strongly influenced by soil characteristics, such as texture, structure, and organic matter content, which determine water retention and availability (Meunier et al., 2022). As one of the most pressing environmental challenges in agriculture, drought stress has profound implications for global food security. It is primarily driven by prolonged periods of low or irregular rainfall, coupled with rising temperatures and greater climatic variability. These conditions accelerate soil moisture loss, lower plant water potential, and disrupt essential physiological processes (Ranjan et al., 2023). The severity of drought's impact depends on its frequency, timing, intensity, and duration, with climate change further amplifying plant vulnerability. Ultimately, drought stress not only compromises plant growth and productivity but also causes substantial economic losses for farming communities worldwide (Rai & Rai, 2020).

The effect of drought stress on *Thymus* spp. is multifaceted, impacting growth, physiological processes, biochemical and hormonal responses.

3.2.1.1 Morphological and production biological responses to drought stress

Drought stress markedly constrains the growth and morphology of *Thymus* species, with reductions across fresh and dry biomasses, and anatomical traits. In *T. eriocalyx*, water limitation reduced plant fresh weight from 10.3 g plant^{-1} at 80 % of field capacity (FC) to 6.3 g plant^{-1} at 40 % FC, while shoot dry weight declined from 1.20 to 0.85 g plant^{-1}. Similarly, the crop growth rate dropped from 0.15 to 0.09, and the relative growth rate from 0.15 to 0.12 (Amiri et al., 2018). Comparable declines have been reported in *T. vulgaris*, where drought reduced shoot fresh weight by 50 % and root fresh weight by 66 %, thereby lowering the root/shoot ratio from 0.68 to 0.47 compared with the control (70–80 % soil water content). Water limitation also induced pronounced anatomical modifications: the stem cross-sectional area decreased from 0.652 to 0.455 mm², xylem area from 0.217 to 0.157 mm², and leaf cross-sectional area from 0.653 to 0.158 mm². Moreover, the mean xylem vessel diameter declined from 4.29 to 1.18 µm, while cuticle thickness nearly doubled, rising from 0.79 to 1.70 µm (Farag et al., 2019). In another study, severe drought (⅓ FC) significantly diminished multiple growth traits in *T. vulgaris*: root length was reduced, root dry weight fell by 49 %, shoot dry weight by 37 %, leaf dry weight by 36 %, branch number by 84 %, stem length by 33 %, and leaf area by 59 % compared with well-watered plants (Pazoki et al., 2012).

3.2.1.2 Physiological responses to drought stress

In addition to these morphological adjustments, drought stress strongly influences the physiological performance of *Thymus* species, with marked differences among cultivars and species. In *T. vulgaris*, reducing soil water content to 30–40 % led to pronounced declines in photosynthetic pigments, with chlorophyll a, chlorophyll b, and carotenoids decreasing from 3.46 to 2.36, 1.98 to 1.60, and 1.75 to 1.47 mg g^{-1} fresh weight (FW), respectively, compared with plants grown under optimal SWC (70–80 %). In parallel, osmolyte accumulation was triggered, as reflected by higher levels of soluble sugars (7.19 to 10.79 mg g^{-1} FW), proline (3.72 to 5.26 µmol g^{-1} FW), and free amino acids (0.25 to 0.40 mg g^{-1} FW) (Farag et al., 2019). Similarly, *T. eriocalyx* displayed pigment adjustments, where chlorophyll increased slightly (0.013 to 0.016 mg g^{-1} FW) under 40 % FC, while chlorophyll b decreased (0.010 to 0.006 mg g^{-1} FW). Under the same conditions, proline content rose by 67 % and soluble carbohydrates by 6.45 %, whereas protein content declined by 26.50 % relative to the control (Amiri et al., 2018). Beyond these biochemical shifts, differences in drought tolerance between cultivars were evident in photosynthetic performance. In *T. vulgaris* cv. Varico3, net photosynthesis remained stable at 20 µmol m^{-2} s^{-1} until the 8th day of stress, while the sensitive cv. Wagner experienced a 50 % reduction by the 4th day. Stomatal conductance mirrored this divergence, decreasing gradually by 0.2 mmol m^{-2} s^{-1} after 12 days in Varico3, but dropping more sharply (0.4 mmol m^{-2} s^{-1}) within only 4 days in 'Wagner'. Leaf water potential also declined, reaching –3 MPa in the tolerant and –5 MPa in the sensitive cultivar (Mahdavi et al., 2020). Consistent with these patterns, *T. capitatus* under 70 % SWC exhibited reduced leaf water content (53.64 % compared to 66.34 % at full irrigation) and a marked decline in phosphorus concentration from 1.41 to 0.96 mg g^{-1} DW (García-Caparrós et al., 2019).

3.2.1.3 Biochemical responses to drought stress

Beyond morphological and physiological adaptations, *Thymus* species respond to drought stress through significant changes in biochemical composition and volatile compounds, which help them cope with water deficit. Drought stress can markedly influence essential oil composition and secondary metabolites in *Thymus* species. In *T. eriocalyx*, water deficit at 40 % FC enhanced certain active compounds, with thymol increasing by 21.14 %, while other major volatiles, such as p-cymene and γ-terpinene, decreased by 9.07 % and 2.50 %, respectively, compared with 80 % FC (Amiri et al., 2018). Similarly, in *T. vulgaris*, plants under 30–40 % SWC exhibited elevated ROS (from 1.76 to 2.80 µmol g^{-1} FW), malondialdehyde (from 1.43 to 2.07 µmol g^{-1} FW), total soluble phenols (from 1.67 to 2.16 mg g^{-1} FW), and enhanced enzymatic activity, including polyphenol oxidase (from 1109 to 1308 Unit min^{-1} mg^{-1} protein), compared with well-watered controls (Farag et al., 2019). Drought-induced alterations in volatile profiles were cultivar-dependent: the tolerant *T. vulgaris*. 'Varico3' displayed peaks in thymol, ocimene, α-thujene, γ-terpinene, β-caryophyllene, o-cymene, germacrene-D, and β-myrcene on the 12th day of stress, followed by slight decreases by the 14th day; in contrast, the sensitive 'Wagner' variety showed

early peaks in β-myrcene, β-pinene, α-thujene, o-cymene, ocimene, γ-terpinene, α-phellandrene, thymol, β-caryophyllene, and germacrene-D by the 4th day, with sharp reductions by the 8th day (Mahdavi et al., 2020). In *T.* × *citriodorus* , drought simulated by 4 % polyethylene glycol (PEG-6000) reduced chlorophyll content and essential oil yield (from 9 to 6 µL g^{-1} DW) but increased certain volatiles such as geraniol (from 4.2 to 21.9 %) and carvacrol (from 0 to 31 %), while enhancing root absorption capacity and raising the root-to-shoot ratio to optimise water uptake (Tátrai et al., 2016). Conversely, in *T. capitatus*, essential oil content declined only slightly from 46.36 to 44.65 mL kg^{-1} DW under 70 % SWC compared with full irrigation (García-Caparrós et al., 2019). In *T. lotocephalus* subjected to 7 % PEG, drought stress reduced total salvianolic acids (0.89 to 0.33 g kg^{-1} extract), caffeic acid (46.00 to 23.00 mg kg^{-1}), rosmarinic acid (53.00 to 23.90 g kg^{-1}), sagerinic acid (2.88 to 0.97 g kg^{-1}), and total flavonoids (8.21 to 7.80 g kg^{-1}), with dihydromorelloflavone and luteolin-7-glucuronide as the major detected compounds. Antioxidant capacity, assessed via FRAP, DPPH, and ABTS, decreased significantly under PEG treatment (Mansinhos et al., 2022). Environmental conditions also modulate volatiles in wild populations: in Valencia, Spain, seasonal changes associated with summer conditions, including reduced water availability, were accompanied by an increase in 1,8-cineole content *in T. vulgaris* (1,8-cineole chemotype) from 21.8–43.2 % in late spring to 42.6–68.5 % in summer (Llorens-Molina & Vacas, 2017).

3.2.1.4 Hormonal responses to drought stress

Hormonal regulation in response to drought stress plays a crucial role in *Thymus* species, modulating growth and adaptive mechanisms. In *T. serpyllum*, water deficit triggers increase in salicylic acid, gibberellic acid, and auxins, which are vital for stress signalling and maintaining growth under adverse conditions. Similarly, in *T. vulgaris*, drought elevates levels of salicylic acid and jasmonic acid, key regulators of defence and tolerance pathways. These hormonal adjustments illustrate the intricate strategies thyme employs to coordinate growth, activate protective mechanisms, and enhance survival under water-limited conditions (Moradi, 2018).

3.2.2 Salinity stress

Salinity refers to the accumulation of soluble salts, primarily sodium chloride (NaCl), in soil or water to levels that adversely affect plant growth and development (Childs & Hanks, 1975). Soils affected by salinity often display visible white salt crusts on the surface, localised salt spots, and prolonged waterlogging after rainfall. Plants respond differently to these conditions depending on their evolutionary adaptations: halophytes are naturally tolerant to salinity, whereas glycophytes are more sensitive and exhibit reduced growth. The degree of tolerance and the rate at which growth declines vary among species (Ondrasek et al., 2009). Soil salinity represents a widespread threat to agriculture worldwide, compromising crop productivity and challenging sustainable farming practices. According to the FAO (2021) assessment, over 6 % of the global subsoil is affected by salinity. In *Thymus* species, salinity stress not only diminishes growth and essential oil production but also disrupts cellular integrity, causing increased oxidative damage to cell membranes (Zrig et al., 2019).

3.2.2.1 Morphological and production biological responses to salinity stress

Experimental studies on different *Thymus* species consistently show that salinity stress limits vegetative growth, although the degree of sensitivity varies among species and stress levels. In vitro germination of *Thymus cilicicus* revealed that increasing salinity stress markedly inhibited growth, reducing both development and shoot formation. The species displayed tolerance up to 50 mM NaCl, while exposure to 100 mM caused pronounced growth suppression, accompanied by visible stress symptoms such as leaf curling and necrosis (Agar et al., 2022). Similar trends were reported in *T. serpyllum*, where NaCl concentrations of 100 and 200 mM reduced stem length by approximately 15 % and 35 %, respectively, compared to non-saline controls, while root elongation remained relatively unaffected (Koźmińska et al., 2021). In *T. vulgaris*, both shoot and root dry weight declined progressively with increasing salinity: shoot biomass dropped from 5.8 g at 0 mM to 4.9 g at 100 mM, and root biomass decreased from 1.1 g to 0.8 g across the same gradient (Najafian et al., 2009). Interestingly, another study on *T. serpyllum* reported that aboveground biomass remained stable between 0 and 100 mM NaCl (around 6.1–6.4 g DW per pot) and only showed significant reduction at 200 mM (4.86 g DW). Root biomass was more resilient, with no notable decline until 400 mM NaCl, where it dropped from 2.8 g to 1.86 g DW per pot (Kim & Eom, 2009). These findings suggest that moderate salinity can be tolerated by some *Thymus* species without drastic morphological or biomass impairment, but higher salt concentrations eventually inhibit shoot development.

3.2.2.2 Physiological responses to salinity stress

Salinity stress has been shown also to trigger diverse physiological responses across *Thymus* species, with the severity of the effects depending on both the species and salt concentration. In *T. serpyllum*, exposure to 100 and 200 mM NaCl caused a marked decline in photosynthetic pigments: total chlorophyll dropped by 33 % and 51 %, respectively. At the same time, osmoprotective compounds accumulated, with total soluble proteins rising by 14 % and 25 % respectively, and free amino acids increasing by 47 % and 105 % respectively. Ion analysis revealed that Na^+ and Cl^- concentrations nearly doubled under 200 mM NaCl compared to the control, while K^+ declined from around 40 to 25 g kg^{-1} DW, indicating ionic imbalance. Despite these metabolic shifts, shoot and root water content showed only a slight, non-significant decline at the highest salinity level (Koźmińska et al., 2021).

In *T. vulgaris*, similar trends have been reported, though responses may vary with developmental stage or experimental design. Under 120 mM NaCl, soluble sugars increased substantially to 250 mg g^{-1} FW compared with 150 mg g^{-1} FW in the control, suggesting active osmotic adjustment. However, protein content declined to 2.2 mg g^{-1} FW at the same salinity level. Photosynthetic pigments were severely affected: chlorophyll a, chlorophyll b, and carotenoids decreased by 50 %, 36 %, and 77 %, respectively (Razavizadeh & Mohagheghiyan, 2015). Gas-exchange parameters corroborate this sensitivity; as salt levels increased from 0 to 50 and 100 mM, photosynthetic rates dropped from 10.2 to 7.0 and 3.0 μmol m^{-2} s^{-1} respectively, while transpiration fell from 0.17 to

0.09 and 0.05 mol m^{-2} s^{-1} respectively. Interestingly, water-use efficiency peaked at 50 mM (2.3) before declining at 100 mM (1.4), compared with 2.1 in the control. Electrolyte leakage followed a similar intermediate pattern, rising to 43 % at 50 mM but remaining lower at both 0 and 100 mM (32 % and 35 %, respectively), suggesting membrane damage is not strictly linear with salinity (Najafian et al., 2009).

Seed germination studies further highlight interspecific variability. In *T. vulgaris*, salinity reduced germination and seedling vigour depending on both salt type and osmotic potential. Seeds tolerated NaCl up to –0.3 MPa and KCl up to –0.2 MPa, whereas $CaCl_2$ and $MgCl_2$ caused significant inhibition even at –0.2 and –0.1 MPa, respectively (Stefanello et al., 2018). Similarly, *T. daenensis* and *T. kotschyanus* exhibited progressive reductions in germination, root length, and shoot development under NaCl stress treatments of 0, –3, –6, and –9 bar. Germination remained relatively stable at mild levels but dropped sharply beyond –6 bar, indicating moderate tolerance during early developmental stages (Khoshsokhan et al., 2011). A broader comparison involving *T. fedtschenkoi*, *T. migricus*, and *T. daenensis* under salinity up to –12 bar showed that germination declined significantly at –8 bar but did not reach zero even at the most extreme level. Among the three, *T. migricus* was the most sensitive, whereas *T. daenensis* and *T. fedtschenkoi* showed better tolerance and maintained superior root branching. Notably, *T. daenensis* preserved the highest number of secondary roots under stress conditions (Eisvand & Kalibar, 2013).

Additional evidence from *T. serpyllum* confirms the decline in photosynthetic pigments under prolonged salinity. In mature leaves, chlorophyll a content dropped from full control levels to 89.9 % and 81.7 % at 100 and 200 mM NaCl, respectively. Chlorophyll b showed a comparable trend, decreasing to 90.7 % and 82.5 % relative to the control. These reductions, while gradual, reflect the cumulative stress imposed on the photosynthetic apparatus (Kim & Eom, 2009).

Collectively, these findings reveal that *Thymus* species employ a combination of osmotic adjustment, ion regulation, and stress-induced metabolic shifts to cope with salinity. However, the threshold of tolerance varies widely by species and growth stage, with pigment stability, germination capacity, and ion homeostasis emerging as key indicators of resilience.

3.2.2.3 Biochemical responses to salinity stress

Salinity triggers distinct biochemical adjustments in *Thymus* species, particularly in antioxidant activity, membrane stability, and secondary metabolite production. In *T. serpyllum*, both peroxidase (POD) and catalase (CAT) activities rise noticeably under moderate salinity stress. At 100 mM NaCl, POD activity increases by about 30 %, while CAT shows an even stronger response, rising by approximately 83 %. This enzymatic upregulation reflects an active defence against oxidative stress. Consistent with this, malondialdehyde, a marker of lipid peroxidation, rises from 5 nmol g^{-1} DW in the control to 13 and 23 nmol g^{-1} DW at 100 and 200 mM NaCl, respectively, suggesting increasing membrane damage as stress intensifies. At the same time, total

polyphenol decline significantly, dropping from 270 to 220 µg GAE g^{-1} DW at 200 mM NaCl, indicating a possible shift in metabolic allocation or phenolic oxidation (Koźmińska et al., 2021).

In *T. vulgaris*, salinity affects both essential oil composition and phenolic accumulation. At 120 mM NaCl compared to the control, the proportion of the major component thymol decreases from roughly 26.1 % to 18.1 %. Other constituents also shift: p-cymene rises slightly (19.2 % to 21.9 %) and γ-terpinene increases (15.4 % to 22.4 %), while compounds such as α-pinene (3.6 % to 1.4 %) and linalool (2.3 % to 0.9 %) decline. Minor constituents like borneol, camphene, and α-terpinolene follow similar trends. Interestingly, while essential oil composition changes, total phenolic content increases with rising salinity. From 0.9 mg g^{-1} FW at 0 mM NaCl, phenolics climb gradually to 1.1, 1.3, 1.7, and 1.7 mg g^{-1} FW at 80, 100, 120, and 150 mM NaCl, respectively, suggesting a compensatory antioxidant response (Razavizadeh & Mohagheghiyan, 2015).

3.2.2.4 Hormonal responses to salinity stress

Hormonal regulation appears to play a central role in salinity tolerance in *T. serpyllum*. Abscisic acid (ABA) levels increase sharply as salt concentration rises, but responses differ between mature and young leaves. In mature leaves, ABA levels rise from approximately 1000 to 2500 and 2900 pmol g^{-1} at 0, 100 and 400 mM NaCl respectively. Young leaves show even stronger hormonal activation, with levels reaching about 4200, 4500, and 6000 pmol g^{-1} at the same respective treatments. Notably, these spikes are most pronounced 24 hours after stress initiation, followed by a decline and return to baseline around 72 hours. This rapid but transient increase in ABA suggests an early signalling role for stomatal regulation, osmotic adjustment, and stress signalling rather than a sustained accumulation (Kim & Eom, 2009).

3.2.3 Combined stresses

While the effects of individual abiotic stressors on *Thymus* growth and metabolism have been widely documented, studies investigating the combined impact of multiple stressors remain very limited. This research gap is particularly evident within the *Thymus* genus; however, valuable insights can be drawn from related aromatic species within the Lamiaceae family. These comparative studies suggest that the simultaneous application of stressors often causes non-additive and sometimes synergistic effects, leading to more severe physiological and biochemical disturbances than single stress alone.

For instance, Stefanakis et al. (2024) examined the combined influence of drought (50 % irrigation) and varying salinity levels (0–150 mM NaCl) on several Lamiaceae species, including *Mentha spicata*, *Origanum onites*, and *Origanum dictamnus*. In *M. spicata*, total chlorophyll content declined progressively from 3.60 mg g^{-1} FW under control conditions to 2.25 mg g^{-1} FW at 150 mM NaCl and 50 % of irrigation level, indicating severe detriment of the photosynthetic apparatus under combined stress. A similar trend was observed in *O. onites*. Meanwhile, osmoprotective compounds such as proline accumulated markedly in *O. dictamnus*, increasing from 0.08 to 0.40 mg g^{-1} FW, while oxidative stress markers such as H_2O_2 rose sharply in

M. spicata from 0.03 to 0.79 µmol g^{-1} FW. These findings show that drought amplifies the oxidative impact of salinity, compelling plants to activate intricate defence mechanisms. This highlights the strong interconnection between osmotic and oxidative responses under combined stress.

Similarly, Gholamnia et al. (2021) reported that exposing *Mentha piperita* to a combined treatment of 120 mM NaCl and 35 °C as a heat stress for 72 h led to profound metabolic shifts. Compared to the control (0 mM NaCl, 25 °C), phenolic content more than doubled (1.3 to 3.3 mg g^{-1} FW), and proline increased from 2.2 to 4.0 mg g^{-1} FW, reflecting an intensified antioxidant defence. However, total chlorophyll (from 1.70 to 0.40 mg g^{-1} FW), carotenoids (from 4.5 to 2.4 mg g^{-1} FW), soluble sugars (from 110 to 40 mg g^{-1} FW), and the ionic balance: K^+/Na^+ ratio (from 8.8 to 0.8) all dropped drastically. Concerning biomass values, shoot fresh weight declined from 4.3 to 2.5 g, whereas root fresh weight unexpectedly increased from 14 to 26 g, likely as an adaptive strategy to enhance water and ion uptake under stress conditions. These contrasting changes reflect a trade-off between growth and survival, where metabolic energy is redirected toward protection rather than biomass production.

In another example, *Artemisia sieberi* alba (syn. *A. herba alba*), a desert-adapted species, showed remarkable but limited resilience when subjected to combined drought (50 % irrigation) and heat stress (37 °C). Compared with the control, plant height, shoot, and root fresh weights declined by 24 %, 20 %, and 31 %, respectively, while stomatal conductance dropped from 0.13 to 0.04 mmol H_2O m^{-2} s^{-1} and relative water content from 58 % to 47 %. Water-use efficiency decreased sharply from 7.5 to 2.2 kg m^{-3}, and photosynthetic rate fell from 11 to 2 µmol CO_2 m^{-2} s^{-1}. In contrast, stress indicators such as H_2O_2 and proline increased by 140 % and 128 %, respectively. These results reveal that even xerophytic plants can reach a point where their protective mechanisms are no longer enough to counter the damaging effects of combined stress (Alhaithloul, 2019).

In *Mentha spicata*, the simultaneous exposure to 150 mM NaCl and 60 µM copper (as a heavy-metal stress) caused severe morphological inhibition and significant biochemical alterations. The essential oil's main constituent, carvone, declined from 75.6 % in the control to 73.2 % under combined stress. Likewise, total phenolic content decreased to 8 µmol GAE g^{-1} FW compared to 11 µmol GAE g^{-1} FW in the control, and to the higher values recorded under single stress treatments (14 µmol GAE g^{-1} FW under NaCl alone and 13 µmol GAE g^{-1} FW under copper alone). Antioxidant capacity, measured by FRAP, also dropped to 7 mg Trolox g^{-1} FW under combined stress, compared to 24 mg Trolox g^{-1} FW in the control and 26 mg Trolox g^{-1} FW under NaCl alone. Such results suggest antagonistic interactions between the two stressors, where simultaneous ion toxicity and osmotic imbalance may limit the plant's ability to sustain secondary metabolite synthesis (Chrysargyris et al., 2019).

Generally, together on *Mentha*, *Origanum*, and *Artemisia* species show that combined stress usually increase oxidative and osmotic problems and change how plants manage their metabolism.

This evidence offers useful guidance for future research on *Thymus* species, where such combined stress studies are still very limited.

3.3 Biostimulants as emerging tools for enhancing plant performance and stress resilience: a focus on seaweed extract-based products

Biostimulants are natural substances or microorganisms that enhance seed germination, stimulate plant growth, improve nutrient uptake, and boost tolerance to environmental stress. They are generally classified into two main groups: microbial and non-microbial biostimulants, both offering sustainable alternatives to synthetic agricultural inputs (Deepika et al., 2025). The following subsection outlines the principal types within each category.

3.3.1 Classification of biostimulants : Microbial and non-microbial

Microbial biostimulants consist primarily of beneficial bacteria, such as plant growth-promoting rhizobacteria, and fungi like arbuscular mycorrhizal fungi. These microorganisms contribute to plant performance through multiple biochemical mechanisms. They improve nutrient availability via solubilisation processes (Ali et al., 2024), stimulate the production of phytohormones such as auxins and gibberellins that promote root development and plant vigour (Albasri et al., 2024), and activate antioxidant defence systems, enabling plants to better withstand drought, salinity, and other stressors (Albasri et al., 2024; Ali et al., 2024).

Non-microbial biostimulants, derived from natural organic or inorganic sources, include a wide range of formulations. Protein hydrolysates, obtained from plant or animal proteins through chemical or enzymatic hydrolysis, are known for enhancing crop yield and quality (Ciriello et al., 2024; Malécange et al., 2023). Humic substances, such as humic and fulvic acids, improve soil structure and nutrient retention, leading to stronger plant development (Kaviya et al., 2025). Among inorganic biostimulants, compounds like zinc oxide and silicon play crucial roles in improving nutrient efficiency, modulating hormonal pathways, and activating antioxidant mechanisms that enhance plant resilience (Asif et al., 2023). Chitosan, a polysaccharide derived from shellfish exoskeletons, strengthens plant defence responses and stimulates the synthesis of secondary metabolites, offering protection against both biotic and abiotic stress (Sun et al., 2023). Finally, seaweed extracts, rich in bioactive compounds, are widely recognised for improving nutrient uptake and significantly enhancing plant tolerance to adverse conditions (Rai et al., 2021). Given their increasing relevance, the following subsections provide a more detailed focus on seaweed-based biostimulants.

3.3.2 Seaweed-derived biostimulants: types and their application

Seaweeds, also known as macroalgae, are multicellular photosynthetic organisms broadly classified into three main groups: brown algae (Ochrophyta), green algae (Chlorophyta), and red algae (Rhodophyta), (Yousef et al., 2024). Thriving in marine ecosystems, they are among the most productive primary biomass sources on Earth, contributing significantly to global carbon sequestration. Rich in carbohydrates, proteins, lipids, polysaccharides, vitamins, and minerals, seaweeds support complex symbiotic relationships with marine organisms while also serving as

valuable raw materials for the food, cosmetic, and pharmaceutical industries (Abdel-Kareem & ElSaied, 2022; Yousef et al., 2024).

Beyond their ecological and industrial relevance, seaweeds have gained considerable attention in agriculture for their potential as natural biostimulants. Extracts derived particularly from brown species such as *Ascophyllum nodosum* extract, found in cold water of the northern Atlantic Ocean, have been shown to stimulate root development, enhance nutrient uptake efficiency, and improve plant tolerance to abiotic stress (Cardozo et al., 2007; Hussain et al., 2021; Mukherjee & Patel, 2020). Depending on the formulation, seaweed extract-based products can be applied as seed primers, foliar sprays, or soil amendments, offering a sustainable alternative to synthetic fertilisers. Their multifunctional nature, combining nutritional support with stress mitigation, makes them one of the most promising tools for modern climate-resilient agriculture (Gautam et al., 2024).

3.3.3 Chemical composition of seaweed extracts: focus on *Ascophyllum nodosum*

Seaweed extracts derived from *A. nodosum* are widely recognised for their rich and diverse biochemical composition, which underpins their strong bioactive potential. Polysaccharides represent one of the most abundant components, typically ranging from 347 to 528 mg g^{-1} DW (Baardseth, 1970). Fucoidans, sulphated polysaccharides accounting for approximately 10 % of the biomass, are primarily located in the intercellular spaces of the medullary tissue, which lies between the cortex and central axis of the thallus. Alongside fucoidan, alginate constitutes 20–26 % of the dry matter and is almost entirely composed of mannuronic acid. Laminarin contributes a smaller fraction (2–5 %), while mannitol represents around 5–8 % (Bruno et al., 2023). Other minor carbohydrates such as fucose, arabinose, galactose, glucose, xylose, and mannose have also been identified (Gómez-Mascaraque et al., 2021). The extraction method plays a critical role in determining both yield and molecular characteristics; for instance, accelerated solvent extraction at 160 °C for 18 minutes can recover exceptionally high alginate and fucoidan fractions (~ 38.3 % and 39.4 %, respectively), whereas harsher conditions (200 °C) lead to their degradation (Bruno et al., 2023).

Phenolic compounds, particularly phlorotannins such as phloroglucinol derivatives like 7-phloroeckol, form another key class of metabolites, contributing to the extract's antioxidant and enzyme-inhibitory properties. Depending on the extraction protocol, *A. nodosum* can yield around 19.1 mg GAE g^{-1} DW (ultrasound-assisted extraction), 115 mg GAE g^{-1} DW (acid extraction), or as low as 15.06 mg GAE g^{-1} DW (alkaline extraction) (Bruno et al., 2023). Free amino acids account for roughly 5–10 % of the dry weight, with glutamic and aspartic acids being dominant (Baardseth, 1970), while lipids contribute a modest amount of 2–4 % (Baardseth, 1970; Kumari et al., 2023). Pigments such as chlorophyll a (600–1000 mg kg^{-1} DW), violaxanthin (60–130 mg kg^{-1} DW), and fucoxanthin (170–270 mg kg^{-1} DW) are also present, with carotenoids peaking between 40–100 mg kg^{-1} DW, particularly in May during gamete ripening. Furthermore, ANE contain essential vitamins (e.g., B12 and K) and are mineral-rich, supplying notable amounts of phosphorus (0.61 g kg^{-1} DW), calcium (8 g kg^{-1} DW), potassium (11.4 g kg^{-1} DW), magnesium

(6 g kg^{-1} DW), nitrogen (17 g kg^{-1} DW), as well as iron (372.8 mg kg^{-1} DW), manganese (48.2 mg kg^{-1} DW), zinc (100.2 mg kg^{-1} DW), and copper (10 mg kg^{-1} DW) (Bruno et al., 2023).

3.3.4 Physiological and biochemical effects of *A. nodosum* extracts

In the study of Rezaei et al. (2024), the application of ANE to *T. vulgaris* resulted in a pronounced improvement in both growth and metabolic activity. Essential oil content increased markedly from 0.45 % in the control to 0.59 %, 0.62 %, and 1.00 % with seaweed extract concentrations of 2.5, 5.0 and 10.0 mL L^{-1}, respectively. A similar trend was observed in secondary metabolite accumulation: total flavonoid levels rose from 1.00 mg quercetin g^{-1} DW in untreated plants to 1.25 and 1.50 mg quercetin g^{-1} DW at 5.0 and 10.0 mL L^{-1} applications. Overall physiological development followed the same dose-dependent pattern, with notable increases in plant height, stem diameter, and both fresh and dry biomass of aerial parts. Photosynthetic pigments were also positively affected; total chlorophyll content increased progressively from 0.846 mg g^{-1} FW in the control to 0.885, 0.907, and 0.934 mg g^{-1} FW under 2.5, 5, and 10 mL L^{-1} treatments respectively, alongside a parallel enhancement in carotenoid concentration. These results clearly demonstrate that ANE not only stimulates vegetative growth but also reprograms secondary metabolism toward higher bioactive compound accumulation, reinforcing its potential as a natural growth promoter in MAPs.

Both foliar and soil applications of ANE clearly acted as powerful growth enhancers, but each route seemed to stimulate *Satureja hortensis* L. in slightly different ways. Soil drenching provided a more sustained supply of nutrients and bioactive molecules to the root zone, which translated into stronger biomass accumulation and higher essential oil percentages. Foliar spraying, on the other hand, appeared to trigger a faster physiological response, boosting plant height and branching by delivering hormones like auxins and cytokinins directly through the leaves. Interestingly, when both methods were combined (foliar and soil application), the effects were not simply additive but synergistic, resulting in the highest dry matter and essential oil yields. This suggests that ANE do not act as simple fertilisers, but rather as metabolic activators that regulate growth and secondary metabolism through multiple pathways, depending on how they are delivered (Nasiri et al., 2025).

In a related experiment of Pacheco et al. (2019) examining the influence of ANE on *Achillea millefolium* L., the response of photosynthetic pigments and secondary metabolites showed a dose-dependent pattern. The chlorophyll a content initially decreased significantly at 6 mL L^{-1} of ANE but rose again at the highest concentration of 9 mL L^{-1}, suggesting a possible threshold effect where excessive bioactive compounds may temporarily disrupt chlorophyll synthesis before adaptation occurs. In contrast, chlorophyll b, total chlorophyll, anthocyanin, and carotenoid levels remained largely unaffected across all tested concentrations (3,6 and 9 mL L^{-1}), indicating that pigment biosynthesis in *A. millefolium* may be relatively stable under ANE-derived stimulation. On the other hand, total polyphenol content increased progressively with higher concentrations of *A. nodosum* extract, reaching significantly elevated levels at 6 and 9 mL L^{-1} compared to the untreated control. Interestingly, antioxidant activity remained unchanged at 3 and 6 mL L^{-1} but

declined significantly at 9 mL L^{-1}, implying that very high doses may trigger metabolic shifts away from antioxidant accumulation. Morphological and biomass traits, improved consistently with increasing concentrations of the ANE, highlighting its overall growth-promoting potential despite the variable effects on biochemical parameters.

In a recent study Farruggia et al. (2024) conducted on *Salvia officinalis* L., foliar application of four different biostimulants led to remarkable improvements in plant growth and productivity compared to the untreated control. Plants treated with biostimulants exhibited significant increases in plant height, chlorophyll content, relative water content, as well as total fresh and dry biomass yields. The essential oil yield was also notably enhanced, rising from 9.7 to 10.9 kg ha^{-1} during the first experimental year and from 15.4 to 18.6 kg ha^{-1} in the second. Moreover, the qualitative composition of the essential oil was influenced by the biostimulant treatments, with several compounds displaying distinct variations in their relative abundance, reflecting a differential metabolic response to each applied biostimulant.

In an experiment conducted on *Mentha* × *piperita* and *Ocimum basilicum*, the application of ANE at 7 mL L^{-1}, either as a soil drench or foliar spray, markedly enhanced the essential oil composition. In *M.* × *piperita*, the concentrations of L-menthone and L-menthol increased to 32.4 % and 32.6 % under soil drench, and to 31.1 % and 31.2 % under foliar spray, compared with 29.2 % and 29.1 % in the control, respectively. Similarly, in *O. basilicum*, the levels of linalool and methyl chavicol were significantly higher under both application methods, reflecting a stimulation of terpenoid biosynthesis. Moreover, the essential oils obtained from ANE-treated plants exhibited stronger antibacterial activity than those from untreated controls. The oils from *M.*× *piperita* and *O. basilicum* were particularly effective against *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus flavus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Among treatments, the soil drench application showed the highest antibacterial potential, outperforming foliar sprays. These results suggest that ANE not only improves the biochemical quality of essential oil but also enhances their antimicrobial properties, especially when applied through the soil (Elansary et al., 2016).

3.3.5 Role of seaweed extracts in enhancing abiotic stress tolerance

Recent studies have highlighted the remarkable potential of seaweed extract-based biostimulants, particularly *A. nodosum* extracts, in alleviating abiotic stress across aromatic and medicinal plants belonging to the Lamiaceae and Apiaceae families. In *T. vulgaris*, for instance, the application of seaweed extract under drought stress conditions significantly enhanced the relative water content, chlorophyll a and b, total polyphenol, and total flavonoids by 38 %, 32 %, 36 %, 17 %, and 45 %, respectively, compared with the drought-stressed control. Biochemical stress markers were also favourably modulated, proline content decreased from 3.8 to 2.7 µg g^{-1}, and soluble sugars dropped by 60 %, suggesting improved osmotic balance. Likewise, antioxidant enzyme activities, including CAT, SOD, and ascorbate peroxidase (APX), showed a similar tendency, indicating an enhanced oxidative defence system. Importantly, seaweed extract application also

improved the EO percentage under drought stress conditions, elevating it from 2.2 % in untreated plants to 3.3 % under ANE-treated plants, demonstrating its effectiveness in sustaining both physiological and biochemical performance under water deficit (Rahimi et al., 2022).

In an Iranian study investigating the influence of seaweed extract on the seeds of *T. daenensis* under saline conditions, Najafi et al. (2024) found that the application of seaweed extract significantly improved germination performance at specific concentrations. At 2 %, the extract notably accelerated germination time in plants exposed to 100 and 150 mM NaCl stress, whereas no significant effect was observed under 50 mM or control conditions. The germination rate was also enhanced at 0.5 % seaweed extract concentration, particularly under 100 mM salinity. Morphological traits such as plant height and biomass showed the greatest improvement at 0.5 % seaweed extract, especially in plants subjected to 100 and 150 mM NaCl, suggesting that the biostimulant effect was more pronounced under moderate to severe stress. Moreover, proline accumulation, a common indicator of osmotic stress, increased progressively with salinity in untreated plants. However, seaweed extract application effectively reduced proline levels across all treatments, with the 2 % concentration demonstrating the strongest mitigation effect, indicating a reduction in stress severity and an improvement in physiological balance.

Similar positive outcomes were reported in *Cuminum cyminum* (Apiaceae) subjected to different field capacities (70 %, 30 %, and 10 %). Across all moisture levels, seaweed extract-treated plants exhibited higher EO yields compared with untreated controls. The most pronounced effect was observed at 70 % field capacity, where EOs yield reached 9.6 L ha^{-1} in treated plants versus 7.4 L ha^{-1} in the control. Even under severe drought (10 % FC), seaweed extract-treated plants maintained higher EO production (8.0 L ha^{-1}) compared with 6.3 L ha^{-1} in the control. In addition, chlorophyll a and b contents, as well as antioxidant enzymes such as CAT and POD, increased significantly under seaweed extract treatments, reflecting improved physiological resilience and stress tolerance (Rahgoshahi et al., 2023).

3.3.6 Mechanism of action of seaweed extract

When *A. nodosum* extracts are applied to plants, their bioactive components, including polysaccharides (laminaran, fucoidan, alginate), betaines, phenolics, polyamines, micronutrients, and trace amounts of phytohormones, interact first at the leaf or root surface, where they can be absorbed through cuticular pores or root epidermal cells (Fernández et al., 2021). These compounds act as elicitors, triggering early molecular signaling pathways that resemble plant responses to beneficial stress (Jayaraj et al., 2008).

After absorption, ANE constituents stimulate nutrient uptake systems, notably nitrate and sulfate transporters, by upregulating the corresponding genes, thereby enhancing nitrogen and sulfur assimilation and improving photosynthetic and metabolic efficiency (De Saeger et al., 2019). This stimulation increases the expression of high-affinity transporters (such as NRT2 and SULTR families), which not only enhance ion uptake but also improve root hair density and length, allowing for a greater absorption surface. The increased nutrient influx supports amino acid

biosynthesis, chlorophyll formation, and enzyme activation necessary for photosynthetic metabolism (Nair et al., 2012; Rayirath et al., 2009).

At the hormonal level, ANE does not simply deliver hormones but modulates endogenous hormone homeostasis. It downregulates auxin-responsive genes, reducing excessive elongation while promoting balanced growth, and simultaneously upregulates cytokinin-related pathways by repressing cytokinin-oxidase genes, leading to enhanced cell division and shoot development (Ali et al., 2022). Minor adjustments also occur in abscisic acid metabolism, fine-tuning stomatal regulation and water conservation under stress (Kumari et al., 2023).

Physiologically, these changes translate into enhanced photosynthetic activity, evidenced by higher chlorophyll content, improved photochemical efficiency of photosystem II (PSII), and upregulation of photosystem-stabilising genes such as photosystem II subunit S and violaxanthin de-epoxidase, which protect chloroplasts from photoinhibition (Santaniello et al., 2017). The stabilisation of thylakoid membranes by ANE-derived antioxidants and osmolytes also improves electron transport rates and reduces the accumulation of ROS in chloroplasts (Shukla et al., 2018). Moreover, the enhancement of Rubisco activity and carbonic anhydrase under ANE treatment promotes CO_2 fixation and carbohydrate production, increasing overall energy availability for growth and stress recovery (Shukla et al., 2018). ANE also boosts carbon and nitrogen metabolism, maintaining a higher carbohydrate reserve and balanced C:N ratio, which supports continuous growth even under adverse conditions (Nair et al., 2012). This metabolic balance contributes to the synthesis of compatible solutes and amino acids, such as proline and glycine betaine, that stabilise proteins and cellular structures during dehydration or salinity stress (Nair et al., 2012; Rayirath et al., 2009).

Under abiotic stressors like drought or freezing, ANE functions as a priming agent. It prepares plants to respond faster by enhancing antioxidant defence systems, increasing the activities and gene expression of enzymes like SOD and APX (Nikoogoftar-Sedghi et al., 2023). In cold stress, treated plants accumulate more osmoprotectants such as proline and soluble sugars, maintain membrane integrity through improved unsaturated fatty acid composition, and activate cold-responsive transcription factors like DREB1A/CBF3, COR15A, and COR78, which strengthen tolerance (Rayirath et al., 2009).

Ultimately, ANE acts not as a nutrient supplement but as a biostimulators regulator, it reprograms plant metabolism and gene expression to enhance nutrient assimilation, optimise hormonal signalling, increase photosynthetic capacity, and activate protective stress mechanisms. This holistic modulation integrates metabolic, hormonal, and transcriptomic networks, allowing plants to maintain homeostasis even under fluctuating environmental pressures. The continuous crosstalk between these pathways results in faster recovery after stress removal and sustained growth vigour. The result is a physiologically robust plant with improved growth, yield potential, and resilience to environmental stressors (Nair et al., 2012; Rayirath et al., 2009; Santaniello et al., 2017).

4 Materials and methods

4.1 Experimental design and procedures

4.1.1 Plant material and growth conditions

All experiments were conducted under greenhouse conditions at the Buda Campus of the Hungarian University of Agriculture and Life Sciences (Budapest). The greenhouse primarily served to protect the plants from rainfall, and environmental factors such as temperature and humidity were not actively controlled. However, all treatments were conducted simultaneously under the same conditions, ensuring that differences observed between treatments reflect the experimental effects rather than environmental variability (Figure 2).

Figure 2. Experimental *Thymus* plants grown under greenhouse conditions (Budapest, 2025)

As summarised in Table 1, a total of seven experiments were carried out over a period of three years (2023, 2024 and 2025). Among them, three involved *Thymus pannonicus*, which was propagated using the root division method from mother plants growing in the open-field collection of *Thymus* taxa, belonging to the Medicinal Plant Unit, Experimental and Research Station, Hungarian University of Agriculture and Life Sciences (Budapest-Soroksár). This propagation was performed approximately two months before each experiment. The young plants were first grown in 0.4 L pots under greenhouse conditions until sufficient development, after which they were transferred to the experimental greenhouse at the Buda Campus.

Thymus × *citriodorus* plants were purchased from Etnoflóra Company (Budapest) and used for Experiment No. 3 (TL1). After the shoots were harvested, the remaining plants were carefully maintained under controlled conditions to encourage natural regeneration of new shoots. Once these shoots had fully developed, they were propagated by cuttings to produce new plants for Experiment No. 6 (TL2). The propagated plants were then kept under phytotron conditions in 0.4 L pots until the start of the next experimental phase.

Table 1. Summary of experimental design, species, treatments, and stress conditions applied (Budapest, 2023–2025).

Experiment	Species	Labels	Date	Total number of plants	Drought level	Salinity level	*A. nodosum* concentration	Treatments
1	*T. pannonicus*	TP1	24/05/2023–30/06/2023 (36 days)	100	**70 % SWC**: Control, salinity stress, control with ANE, salinity stress with ANE. **40 % SWC**: Drought stress, combined stress, drought stress with ANE, combined stress with ANE.	60 mM NaCl	Not applied	4 treatments: 1/Control 2/Drought stress 3/Salinity stress 4/Combined stress
2	*T. pannonicus*	TP2	27/05/2024–08/07/2024 (41 days)	80		90 mM NaCl		
3	*T. × citriodorus*	TL1	23/04/2024–03/06/2024 (41 days)	60		120 mM NaCl		
4	*T. capitatus*	TC1	10/06/2024–21/07/2024 (41 days)	80		90 mM NaCl		
5	*T. pannonicus*	TP3	30/05/2025–23/07/2025 (53 days)	80		120 mM NaCl	8 ml ANE L^{-1} water	8 treatments: 1/Control 2/Drought stress 3/Salinity 4/Combined stress 5/Control + ANE 6/Drought stress + ANE 7/Salinity stress + ANE 8/Combined stress + ANE
6	*T. × citriodorus*	TL2	23/05/2025–16/07/2025 (53 days)	80				
7	*T. capitatus*	TC2	07/06/2025–30/07/2025 (53 days)	80				

SWC: Soil water capacity; ANE: *Ascophyllum nodosum* extract.

Thymus capitatus plants were developed from seeds collected from wild populations growing in the Aousja Hills, located in northern Tunisia within the Bizerte Governorate (37°09′35.6″ N, 10°06′00.9″ E). The seeds were soaked in gibberellic acid (100 ppm) for 24 hours in February 2024, then placed in Petri dishes for approximately two weeks to achieve full germination under phytotron conditions (15 °C, 16-hour photoperiod). The resulting seedlings were transplanted into 0.4 L pots and maintained for three months before being transferred to the greenhouse to start Experiment No. 4 (TC1). After harvesting the shoots, the original plants were kept under favourable conditions to promote natural regeneration of new shoots. Once fully developed, these regenerated shoots were propagated by cuttings to obtain new plants for Experiment No. 7 (TC2), following the same procedure applied to TL2.

Before starting each experiment, all plants underwent an acclimatisation period after being transferred to the greenhouse to minimise transplant stress. Uniform 2 L pots were used for all experimental setups.

As summarised in Table 1, the experimental design for the first four experiments included a control group maintained at 70 % SWC, while drought-stressed plants received only 40 % SWC at each irrigation (Figure 3). Salinity stress treatments were applied at 70 % SWC, and combined stress involved the simultaneous application of both drought and salinity treatments. In the final three experiments, the same four treatment groups were included, each duplicated with the addition of *A. nodosum* extract, to evaluate the combined effect of biostimulant application under stress conditions.

Figure 3. Measuring plant weight to determine water requirements for SWC per irrigation (Budapest, 2025).

4.1.2 Growth substrate and soil water capacity

The substrate used in all experiments consisted of a homogeneous mixture of 50 % peat moss (Kekkilä Professional, Kekkila Oy, Ratatie 11 A, FI-01300 Vantaa, Finland; produced in Estonia), 30 % potting soil (Mr. Garden, AGRO CS Slovakia s.r.o., 98401 Lučenec), 15 % perlite (Magyar Perlit Ltd. Lepsény, Hungary) with a particle size of 0–6 mm and a pH of 6.8–7.0, and 5 % sand. The physicochemical characteristics of the peat moss, potting soil, and the final substrate mixture are presented in Table 2.

Table 2. Physicochemical properties of the substrate components and final mixture.

Properties	**Peat moss**	**Potting soil**	**Final substrate mixture**
pH	5.90 ± 0.50	5.50 ± 0.50	5.99*
N (m/m %)	0.30	0.10	0.18*
P_2O_5 (m/m %)	0.10	0.01	0.053*
K_2O (m/m %)	0.10	0.03	0.059*
Dry matter (m/m %)	nd	30.00	9.00*
Total water-soluble salts (m/m %)	2.0	nd	1.00*
Organic matter content (m/m %)	70.00	75.00	57.50*

nd: no data.

*: Values calculated as weighted averages of all components, assuming perlite and sand contribute negligible nutrients.

Before initiating each experiment, the SWC of the substrate was determined using the Reynolds (1970) gravimetric method. Briefly, a portion of the substrate mixture was oven-dried at 105 °C for 24 hours to determine its dry weight (DW). Another portion (same weight as the first portion) was saturated with water to obtain the saturated weight (SWt). The SWC was then calculated using the following formula:

$$SWC\ (\%) = [(SWt - DW)/DW] \times 100$$

This procedure ensured accurate determination of the substrate's moisture-holding capacity prior to plant cultivation, providing uniform growing conditions across all experiments.

4.1.3 Water supply, salinity treatments, and *A. nodosum* extract applications

For irrigation, tap water was used as the baseline, containing a NaCl concentration of 1.037 mM. Additional NaCl (food-grade table salt) was added as required to achieve the desired salinity levels in each experiment. The physicochemical characteristics of the tap water are presented in Table 3. Irrigation was performed twice weekly, and in salinity treatments, 50 mL of saline solution was applied per plant at each irrigation event. The remaining water required to reach the predefined soil water content was supplied using tap water, thereby ensuring uniform moisture conditions across treatments.

Salinity stress was imposed progressively in order to avoid osmotic shock. In TP3, TL2, and TC2, NaCl concentration was increased stepwise: 30 mM at the first irrigation, 60 mM at the second, 90 mM at the third, and 120 mM from the fourth to the fifteenth irrigation. Thus, plants received

twelve applications of 50 mL at 120 mM during the experimental period. In TP2 and TC1, salinity was applied at 30 mM and 60 mM during the first two irrigations, followed by 90 mM from the third to the twelfth irrigation, corresponding to ten applications at 90 mM. In TL2, concentrations increased from 30 mM to 60 mM and 90 mM during the first three irrigations and were then maintained at 120 mM from the fourth to the twelfth irrigation, resulting in nine applications at the final concentration. In TP1, lower salinity levels were used, beginning with 15 mM, 30 mM, and 45 mM during the first three irrigations, and maintained at 60 mM from the fourth to the eleventh irrigation, corresponding to eight applications at 60 mM. Plant measurements were conducted after repeated saline irrigations, thus reflecting cumulative salt exposure rather than the effect of a single application.

To ensure precise control of water availability, pots were weighed individually after each 50 mL saline application using a precision balance. Based on the recorded weight, tap water was added until the predefined soil water content (SWC) was reached. For control and salinity treatments, SWC was maintained at 70 % of the substrate's maximum water-holding capacity, whereas drought and combined stress treatments were maintained at 40 % SWC. The maximum water-holding capacity of the substrate was determined gravimetrically at the beginning of the experiment as described previously. For example, in TC1, the soil mixture had a dry weight of 296.35 g and a maximum water-holding capacity of 869.5 g (293.4 %). Taking into account the weight of the pot and plant, the final target weight was approximately 1030 g at 70 % SWC and 770 g at 40 % SWC. These target weights were maintained throughout the experimental period at each irrigation event.

Table 3. Physicochemical properties of tap water used for irrigation.

pH	Electric conductivity	Sodium ions (Na^+)	Chloride ions (Cl^-)	Sodium Chloride (NaCl)
7.33	494 µS cm^{-1}	20.31 mg L^{-1}	40.23 mg L^{-1}	60.54 mg L^{-1} ~ 1.037 mM

The *A. nodosum* extract used in the experiments was purchased from Terra Aquatica Company (Fleurance, France), and its base composition is presented in Table 4. The extract was applied at a concentration of 8 mL L^{-1} and delivered to each plant via irrigation (100 mL per plant) once every two weeks, starting from the first week of experiments TP3, TL2, and TC2. In total, four seaweed extract applications were performed in each of these experiments.

In salinity-stressed plants receiving seaweed treatment, the extract solution was applied first, followed by the scheduled 50 mL saline solution. Afterwards, tap water was added as needed to reach the predefined soil water content, as described above. This procedure ensured consistent salt exposure while maintaining uniform moisture levels across treatments.

Table 4. Composition of Terra Aquatica *A. nodosum* seaweed extract base cream (diluted 25 % in water).

Parameters	Quantity
pH	4.20 to 4.50
Specific gravity	1.07 % w/w
Organic matter	78 % w/w
Mineral content	22 % w/w
Alginate	26.60 % w/w
Laminarin	11.20 % w/w
Fucoidan	7.10 % w/w
Mannitol	10.10 % w/w
Polyphenols	6.90 %
Protein/amino acids	7.00 %
Nitrogen	1.00 %
Betaines	150 ppm
All the above are based on dry matter	
Plant growth hormone equivalents	
Cytokinin	45 ppm
Auxin	182 ppm
Gibberellin	30 ppm
Inorganic/mineral content	
Potassium	2.20 %
Phosphorus	0.16 %
Chlorine	4.80 %
Sulphur	1.70 %
Calcium	1.20 %
Magnesium	1.80 %
Iodine	0.12 %

ppm: parts per million; w/w: mass percentage.

4.2 Determination of morphological parameters and masses

4.2.1 Shoot growth parameters and masses

4.2.1.1 Shoot length

One day before harvest, the shoot length of each plant was measured from the soil surface at the base of the stem to the apical tip of the longest shoot.

4.2.1.2 Number of shoots per plant

The number of shoots was determined one day prior to harvest by counting all the shoots emerging from the basal part of each plant at the soil surface.

4.2.1.3 Shoot fresh and dry weights

At the end of each experiment, all plants were harvested, and their fresh weights were immediately recorded individually. The samples were then left to air-dry in a shaded, well-ventilated area until a constant weight was achieved, after which their dry weights were determined.

4.2.2 Root growth parameters and masses

4.2.2.1 Root length, fresh and dry weights

For root-related measurements, five plants per treatment were randomly selected on the harvest day. Roots were carefully separated from the substrate, washed gently with tap water to remove adhering soil particles, and lightly blotted to remove excess moisture. Root length and root fresh weight were recorded immediately. Afterwards, the roots were air-dried naturally until reaching a constant weight to determine their dry mass.

4.3 Determination of physiological parameters

4.3.1 Photosynthetic pigments contents

The contents of chlorophyll a, chlorophyll b, and carotenoids were determined following the methods of Lichtenthaler and Wellburn (1983) and Mackinney (1941). For each treatment, fresh leaf samples (w = 0.1 g) were collected from five randomly selected plants, serving as independent biological replicates. Each sample was ground in a pre-cooled mortar with 3 mL of 80 % acetone, a small pinch of Na_2CO_3, and a few quartz grains until a homogeneous mixture was obtained as illustrated in Figure 4. A further 6 mL of 80 % acetone was then added to enhance pigment extraction. The extract was transferred into a centrifuge tube, adjusted to a final volume of V = 10 mL, and centrifuged at 1000 × g for 3 min by a Heraeus Biofuge Primo centrifuge (Thermo Fisher Scientific Inc, Waltham, USA).

The absorbance (abs.) of the supernatant was measured at 480, 644, and 663 nm using a Thermo Fisher Scientific Evolution 201 spectrophotometer (Thermo Fisher Scientific Inc, Waltham, USA) against 80 % acetone as a blank. The pigment concentrations were calculated using the following equations:

Chlorophyll a: (12.7 × Abs. 663 – 2.69 × Abs. 644) × (V/w)
Chlorophyll b: (22.9 × Abs. 644 – 4.68 × Abs. 663) × (V/w)
Total chlorophyll: (20.2 × Abs. 644 + 8.02 × Abs. 663) × (V/w)
Carotenoids (Total): (5.01 × Abs. 480) × (V/w)

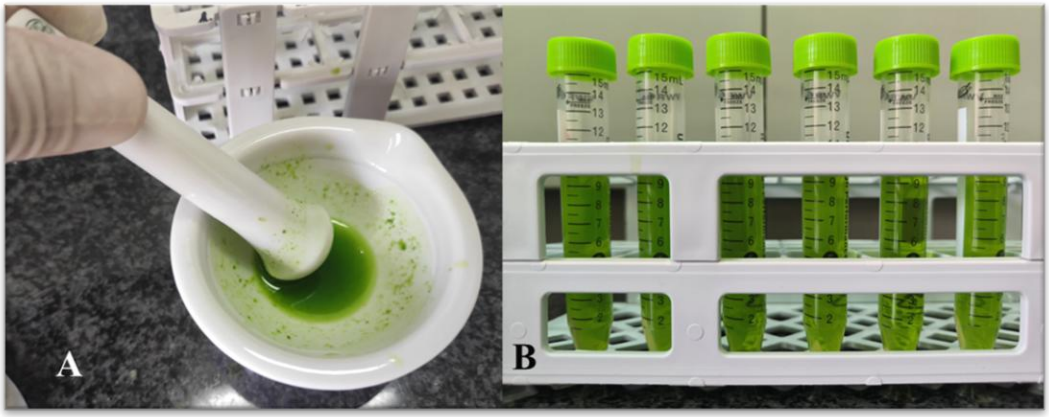

Figure 4. Leaf pigment extract (A) and the supernatant obtained after centrifugation (B).

4.3.2 Relative water content (RWC)

To assess the water status of the leaves, RWC was measured following the method of Orsini et al. (2010). For each treatment, five plants were randomly selected, and 20 fresh leaves were collected per plant, serving as independent biological replicates. The fresh weight (FW) was recorded, then leaves were immersed in distilled water at 4 °C for 24 hours (Figure 5). After gently blotting off excess water the saturated weight (SWt) was determined. Finally, the samples were oven-dried at 105 °C for 48 hours to determine the dry weight (DW). The RWC was calculated using the following equation:

$$RWC\ (\%) = [(FW - DW) / (SWt - DW)] \times 100$$

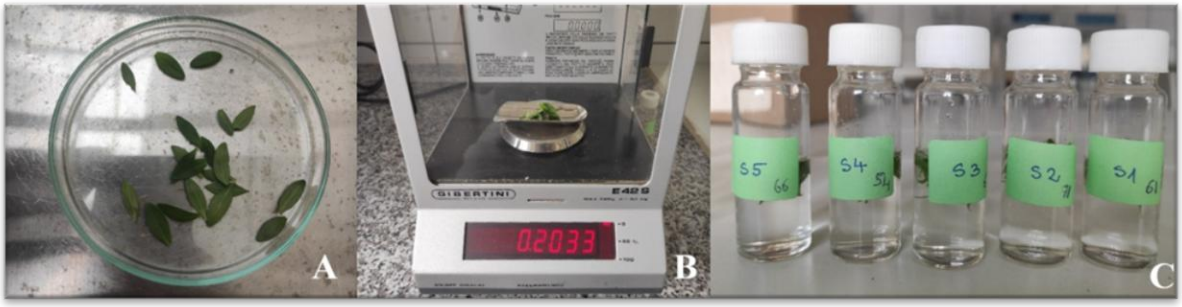

Figure 5. Steps of the RWC assay showing leaves (A), weighing (B), and soaking in distilled water (C).

4.3.3 Proline content

Proline content was quantified following the method described by Bates et al. (1973), as the proline assay illustrated in Figure 6-A. During the final week of the experiment, four plants were randomly selected from each treatment, and 0.5 g of fresh tissue was homogenised in 3 % sulfosalicylic acid using a mortar and pestle. Proline content was determined using these four independent biological replicates per treatment. The homogenate was filtered through Whatman filter paper, and 2 mL of the filtrate was mixed in a test tube with 2 mL of freshly prepared ninhydrin reagent (1.25 g ninhydrin dissolved in 30 mL glacial acetic acid and 20 mL of 6 M phosphoric acid) and 2 mL of glacial acetic acid.

The mixture was vortexed and incubated in a boiling water bath for 1 h, then immediately cooled in an ice bath to stop the reaction. Afterward, 4 mL of toluene was added, and the solution was vigorously vortexed to allow phase separation (Figure 6-B). The absorbance of the upper layer was measured at 520 nm using a spectrophotometer (Thermo Fisher Scientific, Evolution 201) with toluene as the blank. Proline concentration (µmol proline g^{-1} FW) was determined using a standard calibration curve prepared with known concentrations of L-proline (2, 4, 6, 8 and 10 µg ml^{-1}).

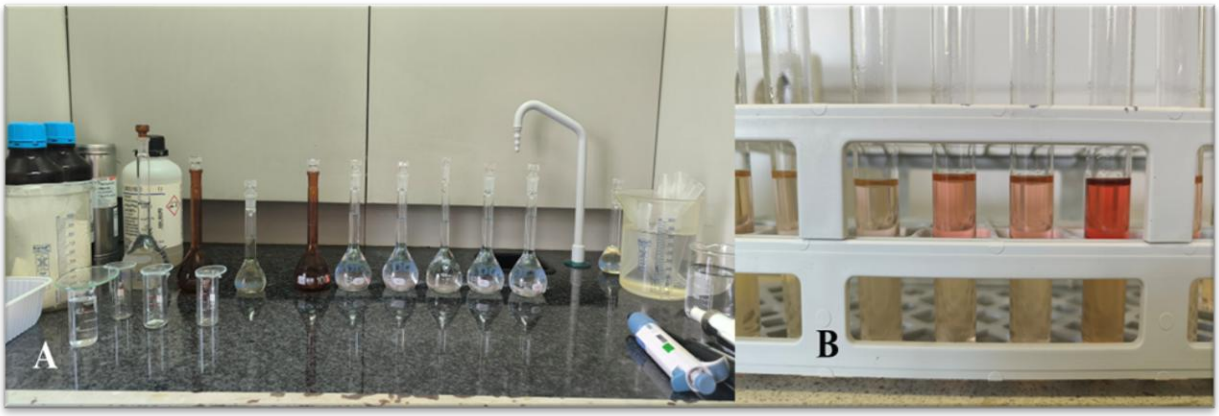

Figure 6. Proline assay showing laboratory setup (A) and reaction mixture after toluene addition (B).

4.3.4 Soluble sugar content

Soluble sugars were quantified using the Trevelyan and Harrison (1952) method. Fresh plant material (0.4 g) was collected from four randomly selected plants per treatment (four independent biological replicates) and homogenised in 10 mL of 95 % ethanol. The mixture was incubated in a water bath at 85 °C for 20 minutes to extract soluble sugars, then centrifuged by Heraeus Biofuge Primo centrifuge (Thermo Fisher Scientific Inc, Waltham, USA) at 10 000 × g for 10 minutes (Heraeus Biofuge Primo centrifuge). This extraction step was repeated twice, and all supernatants were combined for analysis.

For colour development, 5 mL of ice-chilled anthrone reagent (prepared by adding 500 mL of concentrated sulfuric acid to 200 mL of distilled water) was placed in a test tube, followed by the addition of 1 mL of the sugar extract. After cooling for 5 minutes, the mixture was heated in a boiling water bath for 10 minutes, then rapidly cooled in an ice bath for 2 minutes to stop the reaction and stabilise the colour.

The absorbance of the resulting green solution was measured at 620 nm using a Thermo Fisher Scientific Evolution 201 spectrophotometer (Thermo Fisher Scientific Inc, Waltham, USA), with a 2:1 mixture of sulfuric acid and water serving as the blank. Soluble sugar concentration (mg glucose g^{-1} FW) was calculated using a standard calibration curve prepared with known glucose concentrations (20, 40, 60, 80 and 100 µg ml^{-1}).

4.4 Determination of biochemical parameters

4.4.1 Essential oil content

For each treatment, four bulk samples of dried plant material were prepared. Each bulk consisted of four individual plants mixed together and represented one biological replicate, resulting in four biological replicates per treatment. Within each bulk, plants were randomly selected, homogenised, and finely chopped prior to extraction. Between 10 and 15 g of the plant material (depending on the experiment) were placed into a round-bottom flask with distilled water and subjected to hydrodistillation for 2 hours using a Clevenger-type apparatus (Figure 7),

following the procedure outlined in the Hungarian Pharmacopoeia (Ph. Hg., 2004, *Thymi herba*, pp. 2359–2360). Distillation time was counted from the moment heating was initiated. The essential oil volume was read directly from the calibrated arm of the Clevenger apparatus at the end of the distillation period.

Simultaneously, a 2 g portion of each sample was oven-dried at 105 °C for 3 hours to determine its dry weight. The essential oil yield was then calculated on a dry weight basis and expressed as milliliters per 100 grams of dry weight (mL 100 g^{-1} DW), after applying moisture correction to determine absolute dry weight. The essential oil obtained from each bulk sample was subsequently stored at 4 °C in sealed tubes until GC and GC–MS analyses. The results were compared with the quality standard established for *Serpylli herba*, which specifies a minimum essential oil content of 0.3 mL 100 g^{-1} DW (Ph. Eur., 2023, *Serpylli herba*, pp. 1777–1778).

Figure 7. Clevenger-type apparatus used for the hydrodistillation.

4.4.2 Essential oil composition

The composition of the essential oils was analysed using gas chromatography (GC) coupled with mass spectrometry (MS). The GC–MS analysis was performed on an Agilent 6890N GC system (Agilent Technologies, USA) connected to an Agilent 5975 Inert mass selective detector. The GC chromatographic system was fitted with an HP-5 capillary column (30 m × 0.35 mm internal diameter, 0.25 µm film thickness). The temperature began at 60 °C, increasing at 3 °C min^{-1} up to 240 °C, and held for 5 min at the final temperature. Helium served as the carrier gas at a flow rate of 1 mL min^{-1}. A 0.2 µL aliquot of a 1% (V/V) solution of each essential oil in n-hexane was injected in split mode (30:1). Injector and detector temperatures were maintained at 250 °C. The mass spectrometer operated in electron ionisation mode at 70 eV, scanning a mass range of m/z 50–550 in full-scan acquisition.

The relative percentage composition of individual constituents was determined based on peak area normalisation, and compounds were identified by comparing the obtained mass spectra and linear retention indices (LRIs) with those reported in the literature. The LRIs were calculated according to the Van Den Dool and Kratz (1963) equation, using a homologous series of n-alkanes, and compound identities were further confirmed by comparison with reference libraries (NIST MS Search 2.0, Wiley 275) and an in-house database. The relative abundance of each compound was expressed as a percentage of the total chromatogram area, following the order of elution.

4.4.3 Total polyphenol content (TPC)

The remaining portion of the bulk plant samples used for essential oil distillation was ground into fine powder, sieved, and 0.5 g was weighed in triplicate for each bulk sample. To each portion, 50 mL of boiling distilled water was added, and the mixture was left to stand at room temperature for 24 hours to ensure complete extraction of the bioactive compounds. The following day, the extracts were filtered (Figure 8), and aliquots were transferred into two Eppendorf tubes per sample, serving as technical replicates, and stored at −20 °C for further analysis. Additionally, 20 mL of each extract was evaporated to dryness to determine the dry weight of the extract.

The total polyphenol content was determined following the Folin–Ciocalteu method Singleton and Rossi (1965). For each extract sample, three independent replicates were prepared. In a set of six test tubes, 2.5 mL of Folin–Ciocalteu reagent was mixed with 490 µL of distilled water. Then, 10 µL of each extract replicate was added at 10-second intervals, followed by the addition of 2 mL of sodium carbonate (Na_2CO_3) solution at the same timing. The tubes were incubated in a warm water bath until a stable colour developed. The absorbance of the reaction mixture was measured at 760 nm using a Thermo Fisher Scientific Evolution 201 spectrophotometer (Thermo Fisher Scientific Inc, Waltham, USA). The TPC was expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW), calculated from a standard calibration curve prepared with known concentrations of gallic acid.

Figure 8. Preparation and aqueous extraction of powdered plant material for biochemical analysis.

4.4.4 Antioxidant capacity (AOC)

The FRAP assay was used to evaluate the antioxidant capacity of the plant extracts, following the method described by Benzie and Strain (1996). For each extract sample, three independent replicates were prepared. In a series of nine test tubes, 1.5 mL of freshly prepared FRAP reagent was added to each tube. The reagent consisted of a mixture of 50 mL of acetate buffer, 5 mL of TPTZ (2,4,6-tripyridyl-s-triazine) solution, and 5 mL of ferric chloride ($FeCl_3$) solution. Subsequently, 40 µL of distilled water was added to each tube. The reaction was initiated by adding 10 µL of the plant extract sample, in triplicate, at 30-second intervals. After thorough mixing, the absorbance of each reaction mixture was measured at 593 nm using a Thermo Fisher Scientific Evolution 201 spectrophotometer (Thermo Fisher Scientific Inc, Waltham, USA). The antioxidant capacity was expressed as milligrams of ascorbic acid equivalents per gram of dry weight (mg AAE g^{-1} DW), calculated from a standard calibration curve prepared with known concentrations of ascorbic acid.

4.4.5 Hydrogen peroxide (H_2O_2) content

Hydrogen peroxide content was determined according to the method of Patterson et al. (1984). Four plants were randomly selected from each treatment (four independent biological replicates), and 1 g of fresh tissue was homogenised in 2 mL of cold acetone using a pre-chilled mortar and pestle. The homogenate was centrifuged at 10 000 × g for 10 minutes (Heraeus Biofuge Primo centrifuge, Thermo Fisher Scientific Inc, Waltham, USA), and 1 mL of the resulting supernatant was transferred into a clean test tube. To this, 0.1 mL of titanium reagent (20 % $TiCl_2$ in concentrated HCl) was added and mixed thoroughly, followed by 0.2 mL of 17 M ammonia to facilitate the formation of a titanium-peroxide precipitate. The mixture was centrifuged again at 10 000 × g for 3 minutes.

The precipitate was washed five times with cold acetone, each time vortexed and centrifuged at 10 000 × g for 3 minutes to remove impurities. The final pellet was dissolved in 3 mL of 2 N sulfuric acid until complete dissolution. The absorbance of the solution was then measured at 410 nm using a spectrophotometer (Thermo Fisher Scientific, Evolution 201), with 2 N sulfuric acid as the blank. Hydrogen peroxide concentration (µmol H_2O_2 g^{-1} FW) was calculated using a standard calibration curve prepared with known concentrations of H_2O_2 (0.1, 0.2, 0.4, 0.6, and 0.8 µM).

4.5 Determination of stress indexes

4.5.1 Stress susceptibility index (SSI)

The stress susceptibility index measures how strongly plant performance is affected by stress compared with its growth under optimal conditions, taking into account the overall mean yield reduction across all treatments. This index serves as an indicator of the plant's sensitivity to the applied stress factors. The SSI was calculated according to the formula proposed by Fischer and Maurer, (1978):

$$SSI = \frac{\left(1 - \frac{Ys}{Yp}\right)}{SI}$$

where:
Ys: plant weight under stress conditions,
Yp: average weight under control (non-stress) conditions,
SI: represents the stress intensity, determined as:

$$SI = 1 - \left(\frac{\overline{Ys}}{Yp}\right)$$

Where: $\overline{Ys}$ is the average weight of plants of all treatments under stress.

4.5.2 Stress tolerance index (STI)

The stress tolerance index is used to identify treatments that maintain relatively high productivity under stressful environments compared to their performance in optimal conditions. This index reflects the ability of plants to combine tolerance with good yield potential. It was calculated following the method of Fernandez (1993) using the equation:

$$STI = \frac{(Ys \times Yp)}{(Yp)^2}$$

where:
Ys: plant weight under stress conditions,
Yp: average weight under optimal (control) conditions.

4.6 Statistical analysis

All experiments were conducted following a completely randomised design (CRD). A one-way analysis of variance (ANOVA) was applied to the first four experiments (TP1, TP2, TL1, and TC1) to evaluate the effects of the applied stress treatments. For the last three experiments (TP3, TL2, and TC2), a two-way ANOVA was employed to assess both the individual and interactive effects of stress and *A. nodosum* extract treatments. Post-hoc comparisons were performed using Tukey's HSD test or the Games–Howell test, depending on the homogeneity of variances. The level of statistical significance was set at $p \leq 0.05$. Prior to analysis, data were tested for normality using the Shapiro–Wilk test and for variance homogeneity through Skewness and Kurtosis statistics.

The raw data were organised and summarised in Microsoft® Excel® (Microsoft 365 MSO, Version 2510 Build 16.0.19328.20244), where clustered column charts and pie diagrams were also created. All statistical analyses were carried out using IBM SPSS Statistics (version 27), while RStudio (version 2024.12.0 Build 467) was used to generate the clustered heatmaps and principal component analyses (PCA).

5 Results

5.1 Evaluation of *T. pannonicus* experiments

5.1.1 Effects of applied stressors and *A. nodosum* extract on the morphology and biomass of *T. pannonicus*

5.1.1.1 Effect of applied stressors and *A. nodosum* extract on the shoot length

The effects of the different stress treatments applied in TP1 and TP2 on the shoot parameters of *T. pannonicus* are summarised in Table 5, while Table 6 presents the combined influence of stress and *A. nodosum* (ANE) in TP3. Regarding shoot length, in TP1, control (C, 70 % SWC) plants and those exposed to 60 mM salinity (S) showed similar shoot length, whereas drought (D) and combined stress (DS) treatments resulted in significantly shorter shoots. No significant difference was observed between D and DS, suggesting that at 60 mM NaCl, the addition of salinity to drought did not further reduce the shoot length beyond the effect of water deficit alone. In TP2, the same trend persisted despite the increased salinity level (90 mM); both D and DS significantly decreased shoot length (38.72 cm and 35.10 cm, respectively) compared with C and S (46.55 cm and 43.57 cm, respectively), and they were statistically separated into distinct groups, indicating that the combination of drought and salinity imposed stronger growth inhibition than drought alone. In TP3, with the highest salinity level (120 mM), S began to align with D and DS, all showing lower shoot length compared with the control, reflecting the increasing sensitivity of *T. pannonicus* to higher salinity. However, across all stress treatments, ANE application did not significantly affect shoot length ($p \leq 0.05$), indicating that its influence on shoot elongation was limited under the applied conditions.

5.1.1.2 Effects of applied stressors and *A. nodosum* extract on the number of shoots

The number of shoots per plant in *T. pannonicus* was clearly affected by both stress treatments and ANE application across the three experiments, as shown in Tables 5 and 6. In TP1, control plants produced the highest number of shoots (29.72 pcs plant^{-1}), which was not significantly different from drought and salinity (60 mM), but was significantly higher than the combined treatment (26.76 pcs plant^{-1}), highlighting the additive negative effect of simultaneous drought and salinity. In TP2, all treatment groups differed significantly ($p \leq 0.05$), with C again showing the highest number of shoots, followed by S (90 mM), D (40 % SWC), and DS, indicating that both drought and higher salinity progressively limited shoot initiation. TP3 exhibited a similar trend, with shoot number declining under stress conditions. The application of ANE had a more pronounced effect in this trait, promoting shoot initiation in the control, alleviating the negative impact of drought stress by restoring number of shoots closer to control values, and mitigating the severe reduction caused by the combined stress, increasing shoot number by approximately 22 %. In contrast, ANE had no significant effect on number of shoots under salinity stress.

5.1.1.3 Effects of applied stressors and *A. nodosum* extract on the fresh shoot weight

Regarding fresh shoot weight, in TP1, drought stress significantly ($p \leq 0.05$) reduced fresh shoot weight (12.98 g plant^{-1}) compared with the control and salinity treatments (21.87 g plant^{-1} and 21.21 g plant^{-1} respectively). At 60 mM, salinity alone did not markedly affect fresh shoot weight relative to the control, but its combination with drought (DS) led to the most pronounced decline in fresh shoot weight. In TP2, where the experiment was repeated using a higher salinity level (90 mM), salinity by itself, together with the D (40 % SWC) also induced a clear reduction in fresh shoot weight, and their combination resulted in a negative impact similar to that observed in TP1. In TP3, at an even higher salinity level (120 mM), fresh shoot weight followed the same general trend as in TP1 and TP2, with DS showing the lowest values. The application of ANE significantly improved fresh shoot weight under DS (DS+ANE, + 77 %), whereas under the other treatments: control with ANE, drought with ANE and salinity and ANE, the differences in fresh shoot weight were attenuated and became statistically indistinguishable as shown on Figure 9.

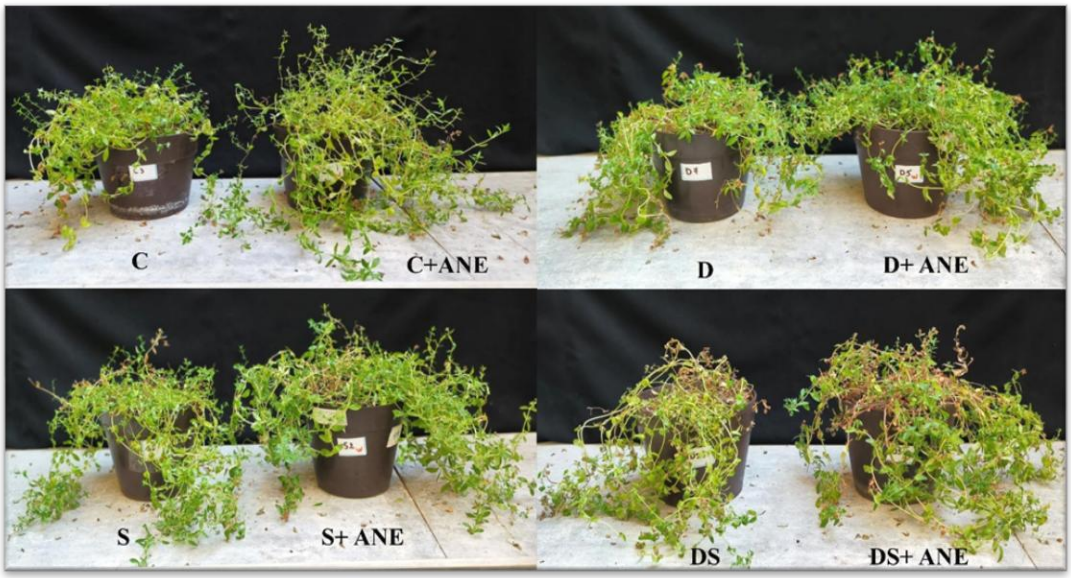

Figure 9. *T. pannonicus* plants in TP3 under different treatments applied. C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.1.1.4 Effects of applied stressors and *A. nodosum* extract on the shoot dry weight

As shown in Table 5, dry shoot weight in TP1 was significantly reduced by drought and combined stress, whereas the control and salinity at 60 mM maintained higher and statistically similar values (6.05 g plant^{-1} and 5.34 g plant^{-1}, respectively). Increasing salinity to 90 mM in TP2 was still not sufficient to significantly reduce dry shoot weight compared with the control, although a slight decline was evident relative to the previous year. In this second experiment, D and DS again emerged as the most harmful treatments, resulting in the lowest significant dry shoot weight values.

Table 5. Shoot parameters of *T. pannonicus* in TP1 (2023, 60 mM salinity) and TP2 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		Shoot length (cm)		**Number of shoots** (pcs plant^{-1})		**Fresh shoot weight** (g plant^{-1})		**Dry shoot weight** (g plant^{-1})	
Experiment		TP1 (60 mM) **	TP2 (90 mM) **	TP1 (60 mM) *	TP2 (90 mM) **	TP1 (60 mM) **	TP2 (90 mM) **	TP1 (60 mM) **	TP2 (90 mM) **
Treatments	**C**	37.94 ± 4.65^{a}	46.55 ± 3.59^{a}	29.72 ± 4.06^{a}	46.10 ± 2.14^{a}	21.87 ± 3.45^{a}	26.77 ± 5.40^{a}	5.84 ± 1.13^{a}	5.89 ± 1.48^{a}
	D	33.33 ± 5.32^{b}	38.72 ± 3.04^{b}	29.60 ± 4.99^{ab}	28.30 ± 3.54^{c}	12.98 ± 3.81^{b}	15.42 ± 4.32^{c}	3.93 ± 1.16^{b}	3.58 ± 0.97^{b}
	S	37.92 ± 3.62^{a}	43.57 ± 4.40^{a}	29.32 ± 3.63^{ab}	42.15 ± 2.75^{b}	21.21 ± 4.53^{a}	22.19 ± 4.76^{b}	6.05 ± 1.32^{a}	5.10 ± 1.11^{a}
	DS	33.68 ± 4.89^{b}	35.10 ± 3.56^{c}	26.76 ± 2.76^{b}	22.20 ± 2.48^{d}	9.45 ± 9.96^{c}	12.13 ± 3.00^{c}	3.20 ± 0.78^{b}	3.24 ± 0.79^{b}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. TP1: 1st experiment of *T. pannonicus*; TP2: 2nd experiment of *T. pannonicus*; pcs: pieces; C: control; D: drought stress; S: salinity stress; DS: combined stress; *: $p \leq 0.05$; **: $p < 0.01$.

Table 6. Shoot parameters of *T. pannonicus* in TP3 (2025, 120 mM salinity) under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		Shoot length (cm)	**Number of shoots** (pcs plant^{-1})	**Fresh shoot weight** (g plant^{-1})	**Dry shoot weight** (g plant^{-1})
Treatments	**C**	51.00 ± 6.75^{Ac}	36.20 ± 3.88^{Ac}	21.50 ± 4.69^{Ab}	5.35 ± 2.10^{Aa}
	C + ANE	53.10 ± 7.17^{Ac}	41.30 ± 4.74^{Bb}	21.92 ± 1.57^{Aa}	5.64 ± 1.01^{Aa}
	D	44.00 ± 5.77^{Aab}	29.10 ± 3.51^{Ab}	19.77 ± 3.57^{Ab}	5.20 ± 1.19^{Aa}
	D + ANE	45.80 ± 4.52^{Aab}	33.40 ± 2.55^{Ba}	22.97 ± 3.50^{Aa}	6.22 ± 1.09^{Aa}
	S	47.30 ± 6.04^{Aab}	29.50 ± 3.84^{Ab}	16.40 ± 4.33^{Aab}	4.42 ± 1.70^{Aa}
	S + ANE	50.90 ± 6.74^{Aab}	29.00 ± 3.26^{Aa}	18.86 ± 5.98^{Aa}	5.56 ± 1.37^{Aa}
	DS	43.80 ± 4.70^{Aa}	22.70 ± 1.89^{Aa}	10.29 ± 4.77^{Aa}	3.53 ± 1.17^{Aa}
	DS + ANE	47.70 ± 2.98^{Aa}	27.70 ± 4.35^{Ba}	18.23 ± 5.19^{Ba}	5.32 ± 1.77^{Ba}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). pcs: pieces; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

In TP3, as indicated in Table 6, despite the higher salinity level (120 mM), no significant differences were detected among the stress treatments in the absence of ANE, although DS continued to show the numerically lowest dry shoot weight. Notably, the application of ANE improved dry shoot weight only under DS, raising it from 3.53 g plant^{-1} to 5.32 g plant^{-1}. Under ANE-treated conditions, all treatments became statistically similar, with dry shoot weight values clustering within a narrow range and no distinct differences detectable among them.

5.1.1.5 Effects of applied stressors and *A. nodosum* extract on the root length

Root length is a key indicator of the ability of *T. pannonicus* to explore the soil for water and nutrients, and in our experiments, it was clearly affected by both stress treatments and ANE application (Tables 7 and 8). In TP1, control and salinity plants had similar root length, drought caused a slight but non-significant reduction, whereas combined stress resulted in the shortest roots, with values significantly lower than control and salinity. This indicates that the simultaneous action of drought and salinity more strongly restricted root elongation than either factor alone. In TP2, a similar pattern was observed; however, at the higher salinity level (90 mM), S also showed a significant reduction in root length compared with the C, suggesting that from this level onward, salinity alone started to limit root growth. In TP3, all four treatments without ANE displayed relatively similar root length, but ANE application markedly changed the response profile: it significantly increased root length under D (by about 23 %) and S (by about 14 %), and alleviated the negative impact of the combined stress in DS (by about 18 %), while not adversely affecting the control. This highlights the capacity of ANE to stimulate root elongation particularly under stress conditions.

5.1.1.6 Effects of applied stressors and *A. nodosum* extract on the fresh weight of roots

Concerning root fresh weight, *T. pannonicus* showed clear changes in response to the different stress treatments and ANE application, as presented in Tables 7 and 8. In TP1, under 60 mM salinity, root fresh weight of control and salinity plants remained similar, with no significant reduction under salinity alone. Both drought and combined stress tended to reduce root fresh weight compared with control and salinity, but only combined stress was clearly separated statistically, indicating the strongest inhibition under combined stress. In TP2, C and S (90 mM) again showed similarly and significantly higher root fresh weight (7.09 g plant^{-1} and 6.63 g plant^{-1}, respectively) than D and DS, with no significant difference between the latter two. This pattern reinforces the idea that water deficit is the main driver of root fresh weight reduction, while salinity up to 90 mM has a limited direct effect on root biomass when water supply is adequate. In TP3, combined stress was clearly and significantly lower than the other treatments in the absence of ANE. However, ANE application significantly compensated the negative impact of D, S, and DS on root fresh weight: S+ANE reached the highest root fresh weight, while D+ANE and DS+ANE tended to approach the values of C+ANE, as shown in Figure 10, the impact of the applied factors on TP3 roots development is illustrated.

5.1.1.7 Effects of applied stressors and *A. nodosum* extract on the dry weight of roots

For root dry weight, *T. pannonicus* also showed consistent and marked responses to stress and ANE application across the three years, as presented in Tables 7 and 8. In TP1, control plants had the highest root dry weight, but this was only significantly ($p \leq 0.05$) higher than combined stress, which showed the lowest value, while drought and salinity displayed intermediate root dry weight that did not differ significantly from either control or combined stress. In TP2, despite the higher salinity level, a similar pattern was observed: the control still maintained the highest root dry weight, and only D became significantly lower than the control, whereas S and DS remained statistically intermediate. In TP3, at 120 mM, salinity (1.00 g plant^{-1}) separated from the control group and, together with combined stress (0.67 g plant^{-1}), formed a significantly lower group, while drought (1.40 g plant^{-1}) and control (1.45 plant^{-1}) remained statistically similar. In this third year, ANE did not markedly affect root dry weight in C and D, but it significantly improved root dry weight under S (by about 63 %) and DS (by about 116 %) compared with the non-treated plants, raising their values to levels comparable with the control treated with ANE.

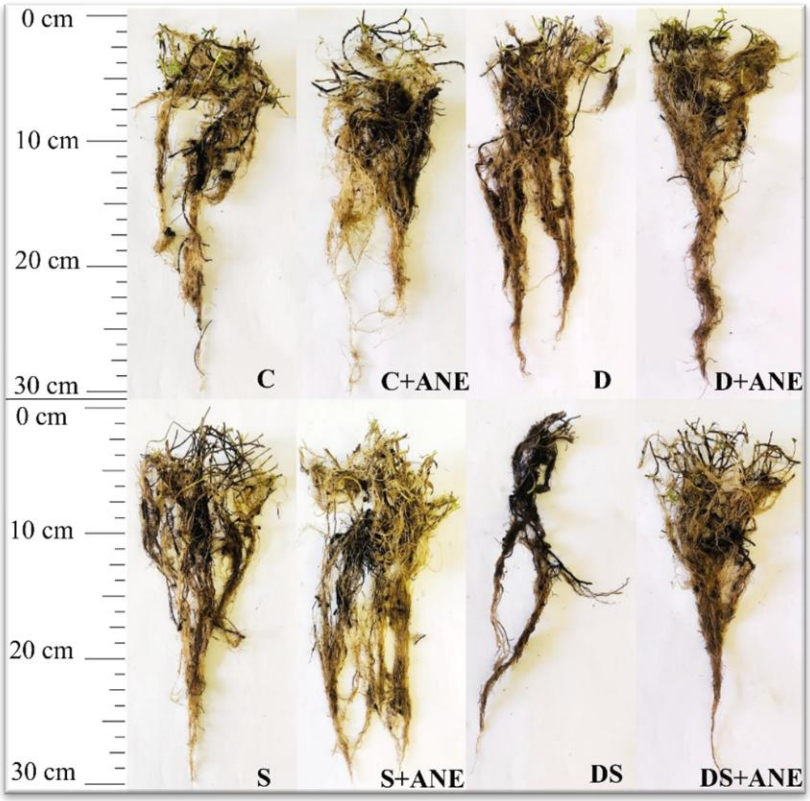

Figure 10. Effect of the applied treatments on root development in *T. pannonicus* in TP3. C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

Table 7. Root parameters of *T. pannonicus* in TP1 (2023, 60 mM salinity) and TP2 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA**.**

		Root length (cm)		**Fresh root weight** (g plant^{-1})		**Dry root weight** (g plant^{-1})	
Experiment		TP1 (60 mM) *	TP2 (90 mM) **	TP1 (60 mM) *	TP2 (90 mM) **	TP1 (60 mM) *	TP2 (90 mM) **
Treatments	**C**	29.32 ± 5.41^{a}	32.25 ± 1.25^{a}	5.37 ± 3.24^{ab}	7.09 ± 0.34^{a}	1.56 ± 0.69^{a}	1.86 ± 0.71^{a}
	D	24.60 ± 5.38^{ab}	24.05 ± 2.16^{c}	3.00 ± 1.62^{ab}	3.47 ± 0.87^{b}	0.78 ± 0.37^{ab}	0.62 ± 0.41^{b}
	S	30.50 ± 6.25^{a}	28.05 ± 1.51^{b}	5.76 ± 1.50^{a}	6.63 ± 0.47^{a}	1.20 ± 0.28^{ab}	1.36 ± 0.38^{ab}
	DS	19.90 ± 3.25^{b}	21.50 ± 2.38^{c}	2.12 ± 1.11^{b}	3.13 ± 0.24^{b}	0.53 ± 0.30^{b}	0.52 ± 0.36^{b}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. TP1: 1st experiment of *T. pannonicus*; TP2: 2nd experiment of *T. pannonicus*; C: control; D: drought stress; S: salinity stress; DS: combined stress; *: $p \leq 0.05$; **: $p < 0.01$.

Table 8. Root parameters of *T. pannonicus* in TP3 (2025, 120 mM salinity) under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		Root length (cm)	**Fresh root weight** (g plant^{-1})	**Dry root weight** (g plant^{-1})
Treatments	**C**	22.33 ± 0.58^{Aab}	5.56 ± 0.23^{Ab}	1.45 ± 0.22^{Ab}
	C + ANE	24.00 ± 1.00^{Aab}	7.54 ± 0.16^{Ba}	1.61 ± 0.20^{Aa}
	D	23.33 ± 2.52^{Ab}	5.61 ± 0.26^{Ab}	1.40 ± 0.08^{Ab}
	D + ANE	28.67 ± 1.15^{Bc}	6.76 ± 0.65^{Ba}	1.55 ± 0.07^{Aa}
	S	19.33 ± 0.58^{Aa}	4.82 ± 1.19^{Ab}	1.00 ± 0.28^{Aa}
	S + ANE	22.00 ± 1.00^{Ba}	9.54 ± 0.27^{Bb}	1.63 ± 0.07^{Ba}
	DS	20.83 ± 1.04^{Aab}	2.66 ± 0.36^{Aa}	0.67 ± 0.13^{Aa}
	DS + ANE	24.67 ± 0.58^{Bb}	7.22 ± 0.91^{Ba}	1.45 ± 0.10^{Ba}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.1.2 Effects of applied stressors and *A. nodosum* extract on the physiology of *T. pannonicus*

5.1.2.1 Effects of applied stressors and *A. nodosum* extract on the photosynthetic pigments

Tables 9 and 10 illustrate the responses of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids to stress treatments and ANE application across the three experiments. In TP1, none of the chlorophyll parameters differed significantly among treatments; chlorophyll a, chlorophyll b, and total chlorophyll remained broadly similar across the four treatments. In TP2, however, one-way ANOVA revealed significant differences: control had the highest chlorophyll a (2338.38 µg g^{-1} FW), chlorophyll b (822.45 µg g^{-1} FW), and total chlorophyll (3160.85 µg g^{-1} FW), followed by S, which showed slightly lower but statistically similar values. Drought and combined stress exhibited clear reductions in all chlorophyll pigments, with DS showing the lowest levels, highlighting the stronger negative impact of combined drought and salinity.

In TP3, with salinity increased to 120 mM, S alone caused a significant decline in chlorophyll a, chlorophyll b, and total chlorophyll compared with C, whereas DS continued to have the lowest pigment concentrations. *A. nodosum* extract application had a pronounced effect under DS, significantly restoring chlorophyll a (from 1232.30 to 1875.42 µg g^{-1} FW), chlorophyll b (from 687.34 to 970.31 µg g^{-1} FW), and total chlorophyll (from 1919.11 to 2844.97 µg g^{-1} FW), while its impact under single stress treatments was smaller or inconsistent. Overall, chlorophyll content remained stable at 60 mM, declined under drought and combined stress at 90 mM, and showed the clearest recovery under DS when ANE was applied at 120 mM.

Carotenoid content followed a similar pattern. In TP1, no significant differences were observed among treatments, reflecting stability under moderate salinity stress. In TP2 (90 mM), salinity remained similar to control, drought was slightly reduced but not significantly, while combined stress showed the lowest carotenoid content and differed significantly from control and salinity. In TP3 (120 mM), all stressed plants without ANE had lower carotenoids than C, with DS being most affected. *A. nodosum* extract significantly restored carotenoid levels under D (from 607.5 µg g^{-1} FW to near control values) and markedly under DS (from 443.39 to 630.59 µg g^{-1} FW), whereas C+ANE and S+ANE showed only minor, non-significant increases.

5.1.2.2 Effects of applied stressors and *A. nodosum* extract on the relative water content

Relative water content (RWC) reflects the internal water status of the plant and is a key indicator of drought and salinity tolerance in *T. pannonicus*. The evolution of this parameter across the three experiments is shown in Tables 11 and 12. In TP1, control and salinity maintained similarly high RWC values, whereas drought and combined stress significantly reduced RWC compared with both C and S. This pattern indicates that in TP1 (at 60 mM), water deficit is the main factor driving the decline in leaf hydration, while salinity alone still allows leaves to preserve a relatively stable water status.

Table 9. Photosynthetic pigment parameters of *T. pannonicus* in TP1 (2023, 60 mM salinity) and TP2 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		Chlorophyll a (µg g^{-1} FW)		**Chlorophyll b** (µg g^{-1} FW)		**Total chlorophyll** (µg g^{-1} FW)		**Carotenoids** (µg g^{-1} FW)	
Experiment		TP1 (60 mM) ^{ns}	TP2 (90 mM) **	TP1 (60 mM) ^{ns}	TP2 (90 mM) **	TP1 (60 mM) ^{ns}	TP2 (90 mM) **	TP1 (60 mM) ^{ns}	TP2 (90 mM) **
Treatments	**C**	1776.43 ± 251.11	2338.98 ± 101.16^{a}	699.34 ± 96.94	822.45 ± 100.26^{a}	2475.15 ± 328.23	3160.85 ± 157.08^{a}	549.68 ± 72.39	583.49 ± 43.66^{a}
	D	1983.13 ± 116.12	1913.74 ± 53.90^{bc}	754.98 ± 47.16	635.15 ± 13.75^{b}	2737.44 ± 126.90	2548.89 ± 50.00^{b}	581.91 ± 29.57	538.49 ± 25.26^{ab}
	S	1905.95 ± 274.28	2060.32 ± 243.82^{ab}	769.3 5± 114.89	789.10 ± 41.55^{a}	2684.68 ± 388.14	2849.42 ± 225.15^{ab}	580.66 ± 79.31	570.52 ± 35.19^{a}
	DS	1910.75 ± 173.83	1606.06 ± 179.87^{c}	786.77 ± 94.22	509.64 ± 27.10^{c}	2696.84 ± 254.21	2115.71 ± 205.20^{c}	609.88 ± 33.40	481.46 ± 12.32^{b}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. FW: fresh weight; TP1: 1st experiment of *T. pannonicus*; TP2: 2nd experiment of *T. pannonicus*; C: control; D: drought stress; S: salinity stress; DS: combined stress; ^{ns}: non-significant; **: $p < 0.01$.

Table 10. Photosynthetic pigment parameters of *T. pannonicus* in TP3 (2025, 120 mM salinity) under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		Chlorophyll a (µg g^{-1} FW)	**Chlorophyll b** (µg g^{-1} FW)	**Total chlorophyll** (µg g^{-1} FW)	**Carotenoids** (µg g^{-1} FW)
Treatments	**C**	2001.99 ± 55.40^{Ac}	1047.82 ± 59.67^{Ac}	3048.99 ± 112.96^{Ac}	692.05 ± 20.07^{Ac}
	C + ANE	2030.90 ± 23.28^{Ab}	970.71 ± 97.08^{Aa}	3000.82 ± 91.52^{Aa}	682.53 ± 31.31^{Ab}
	D	1854.81 ± 140.26^{Abc}	945.77 ± 109.85^{Abc}	2799.84 ± 249.25^{Abc}	607.05 ± 35.07^{Ab}
	D + ANE	1861.10 ± 145.44^{Aab}	964.63 ± 123.56^{Aa}	2824.98 ± 168.89^{Aa}	673.85 ± 21.06^{Bab}
	S	1525.48 ± 236.49^{Aab}	742.88 ± 67.58^{Aab}	2267.77 ± 199.00^{Aab}	516.36 ± 41.44^{Aa}
	S + ANE	1599.48 ± 210.68^{Aa}	912.79 ± 130.32^{Aa}	2511.59 ± 219.92^{Aa}	591.31 ± 53.58^{Aa}
	DS	1232.30 ± 119.89^{Aa}	687.34 ± 67.51^{Aa}	1919.11 ± 139.95^{Aa}	443.39 ± 27.77^{Aa}
	DS + ANE	1875.42 ± 37.95^{Bab}	970.31 ± 17.27^{Ba}	2844.97 ± 55.06^{Ba}	630.59 ± 23.91^{Bab}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). FW: fresh weight; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

In TP2, the general pattern remained similar; however, at 90 mM, S shifted closer to D in terms of RWC and no longer clearly separated from it, suggesting that at this higher level, salinity alone also begins to impair leaf water status. In TP3, stress again induced a reduction in RWC, with C displaying the highest value (83.43 %) and DS the lowest (69.82 %), while D and S occupied intermediate positions that were not significantly different from either extreme.

Interestingly, the two-way ANOVA in TP3 did not reveal a significant interaction between stress and ANE, indicating that *A. nodosum* application did not markedly restore leaf water content, but only induced small numerical increases under stress.

5.1.2.3 Effect of applied stressors and *A. nodosum* extract on the proline content

Proline accumulation was used as a biochemical marker of stress response in *T. pannonicus* under drought, salinity, and their combination, as shown in Tables 11 and 12. In TP2, proline content was lowest in control and significantly higher in all stressed plants. Drought and salinity accumulated comparable amounts of proline (2.66 and 2.80 µmol proline g^{-1} FW respectively), both significantly lower than combined stress (3.80 µmol proline g^{-1} FW), indicating that proline levels rise with increasing stress intensity and complexity. In TP3, proline again increased with stress, and absolute values were higher than in TP2 at 120 mM, particularly in S and even more in DS, confirming a stronger stress signal under the more severe salinity regime.

Under *A. nodosum* treatments in TP3, ANE significantly reduced proline accumulation at high salinity: from 3.78 to 3.00 µmol proline g^{-1} FW in S, and from 4.71 to 3.73 µmol proline g^{-1} FW in DS, while having no marked effect in control or under drought. This pattern suggests that ANE mitigated stress perception or intensity under salinity and combined stress, lowering the need for proline overaccumulation without altering basal proline levels in non-stressed plants.

5.1.2.4 Effects of applied stressors and *A. nodosum* extract on the soluble sugars content

Soluble sugars were quantified as another key osmolyte contributing to osmotic adjustment and stress tolerance in *T. pannonicus*, as presented in Tables 11 and 12. In TP2, soluble sugars content was lowest in control and significantly ($p \leq 0.05$) higher in all stressed plants. Drought and salinity accumulated similar, intermediate sugar levels, whereas combined stress showed the highest soluble sugars, indicating that soluble sugars increase as stress intensity and complexity rise. In TP3, one-way ANOVA again revealed a clear upward trend in soluble sugars from C to D to S, although these three treatments were not statistically different from each other; only DS displayed a statistically distinct and marked increase, confirming that combined drought and salinity elicit the strongest sugar accumulation.

A. nodosum extract application in TP3 significantly ($p \leq 0.05$) reduced soluble sugars in all stressed plants, by about 17 % in D+ANE, 13 % in S+ANE, and 12 % in DS+ANE, yet their values remained higher than those of C+ANE. This indicates that while the stress-induced gradient in soluble sugars is preserved, it is attenuated under ANE treatment, suggesting a partial alleviation of stress intensity rather than a complete normalisation of sugar levels.

Table 11. Relative water content, proline and soluble sugars parameters of *T. pannonicus* in TP1 (2023, 60 mM salinity) and TP2 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		RWC (%)		**Proline** (µmol proline g^{-1} FW)		**Soluble sugars** (mg glucose g^{-1} FW)	
Experiment		TP1 (60 mM) **	TP2 (90 mM) **	TP1 (60 mM)	TP2 (90 mM) **	TP1 (60 mM)	TP2 (90 mM) **
Treatments	**C**	78.10 ± 2.00^{a}	87.40 ± 1.35^{a}	nd	1.62 ± 0.48^{a}	nd	22.83 ± 1.75^{a}
	D	65.08 ± 6.00^{b}	82.29 ± 1.16^{bc}	nd	2.66 ± 0.67^{b}	nd	29.99 ± 1.30^{b}
	S	76.11 ± 3.00^{a}	84.40 ± 2.05^{ab}	nd	2.80 ± 0.17^{b}	nd	32.65 ± 0.83^{b}
	DS	67.23 ± 6.00^{b}	79.70 ± 1.70^{c}	nd	3.80 ± 0.18^{c}	nd	39.00 ± 1.29^{c}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. RWC: relative water content; FW: fresh weight; TP1: 1st experiment of *T. pannonicus*; TP2: 2nd experiment of *T. pannonicus*; nd: not determined; C: control; D: drought stress; S: salinity stress; DS: combined stress; **: $p < 0.01$.

Table 12. Relative water content, proline and soluble sugars parameters of *T. pannonicus* in TP3 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		RWC (%)	**Proline** (µmol proline g^{-1} FW)	**Soluble sugars** (mg glucose g^{-1} FW)
Treatments	**C**	83.43 ± 3.61^{Ab}	1.57 ± 0.09^{Aa}	25.24 ± 1.36^{Aa}
	C + ANE	85.23 ± 4.91^{Aa}	1.46 ± 0.12^{Aa}	22.41 ± 1.22^{Aa}
	D	74.67 ± 2.53^{Aab}	2.39 ± 0.27^{Ab}	27.36 ± 1.21^{Ba}
	D + ANE	77.14 ± 9.50^{Aa}	2.16 ± 0.25^{Ab}	22.83 ± 1.69^{Aa}
	S	74.26 ± 3.94^{Aab}	3.78 ± 0.35^{Bc}	27.25 ± 0.72^{Ba}
	S + ANE	78.62 ± 3.91^{Aa}	3.00 ± 0.25^{Ac}	23.67 ± 1.63^{Aab}
	DS	69.82 ± 4.73^{Aa}	4.71 ± 0.32^{Bd}	31.26 ± 0.68^{Bb}
	DS + ANE	75.18 ± 2.33^{Aa}	3.73 ± 0.16^{Ad}	27.44 ± 1.72^{Ab}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). RWC: relative water content; FW: fresh weight; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.1.3 Effects of applied stressors and *A. nodosum* extract on the biochemical traits of *T. pannonicus*

5.1.3.1 Effects of applied stressors and *A. nodosum* extract on the essential oil content

Essential oil content (EOC) is a critical quality trait in *T. pannonicus*, reflecting both genetic potential and environmental influences, including stress conditions. Quantitative measurements of EOC across the three experiments are presented in Tables 13 and 14. In TP1, control plants exhibited the highest EOC (0.91 ml 100 g^{-1} DW), significantly higher than those in drought and combined stress, which showed the lowest values (0.48 and 0.52 ml 100 g^{-1} DW, respectively). Salinity had an intermediate effect, not significantly different from either C or D. In TP2, EOC in salinity was significantly lower than in the control (which maintained the highest EOC) and its values were statistically similar to those of drought and combined, all showing notable reductions. TP3 without ANE showed a similar pattern to TP2, with control again having the highest EOC.

In contrast, when *A. nodosum* was applied, all stressed treatments experienced significant enhancements in EOC: D+ANE increased from 0.19 to 0.38 ml 100 g^{-1} DW, S+ANE from 0.19 to 0.29 ml 100 g^{-1} DW, and DS+ANE from 0.13 to 0.20 ml 100 g^{-1} DW, while no significant changes were observed in C. This demonstrates that ANE alleviates the negative impact of stress on essential oil production, restoring EOC closer to control levels (0.31 ml 100 g^{-1} DW).

5.1.3.2 Effects of applied stressors and *A. nodosum* extract on the total polyphenol

Total polyphenol content (TPC) was quantified as a key secondary metabolite, and the results are presented in Tables 13 and 14. In TP1, drought alone significantly ($p \leq 0.05$) reduced TPC compared with control, whereas salinity had a milder effect. The combined stress caused the largest decrease, indicating that simultaneous exposure to drought and salinity strongly suppresses polyphenol accumulation. In TP2, under higher salinity (90 mM), control maintained the highest TPC (137.88 mg GAE g^{-1} DW), significantly higher than S (122.78 mg GAE g^{-1} DW) and DS (118.23 mg GAE g^{-1} DW), while D (133.85 mg GAE g^{-1} DW) was statistically similar to the control. Drought and salinity were also statistically comparable, showing that moderate drought or salinity alone had less impact than their combination.

In TP3, with salinity increased to 120 mM, TPC declined further under stress, with C and D showing statistically similar, higher values than S and DS. Two-way ANOVA revealed a significant interaction between ANE and the combined application of 120 mM NaCl and 40 % SWC, where ANE application increased TPC by approximately 45 % in DS. This suggests that *A. nodosum* exerts a protective effect on secondary metabolism, helping to maintain polyphenol accumulation under severe abiotic stress.

5.1.3.3 Effects of applied stressors and *A. nodosum* extract on the antioxidant capacity

Antioxidant capacity (AOC) reflects the ability of *T. pannonicus* to neutralise reactive oxygen species, making it a key indicator of stress tolerance. Measurements of AOC across the experiments are presented in Tables 13 and 14. In TP1, drought and salinity alone did not significantly reduce AOC (148.42 and 152.48 mg AAE g^{-1} DW, respectively) compared with control (162.18 mg AAE g^{-1} DW). However, the combined stress caused a significant decline, indicating that simultaneous exposure to multiple stressors has a stronger negative effect on antioxidant capacity. In TP2, despite the higher salinity level (90 mM), no statistically significant differences were detected, although DS showed the numerically lowest AOC. In TP3, D maintained AOC levels similar to C, suggesting that drought alone has a minor effect, whereas S at 120 mM significantly reduced AOC. The combination of D (40 % SWC) and S (120 mM of NaCl) produced the most pronounced decrease in AOC across all experiments.

As observed for TPC, ANE application significantly enhanced AOC under combined stress. In DS+ANE, AOC increased from 110.14 to 153.86 mg AAE g^{-1} DW, effectively restoring antioxidant capacity close to control levels. D+ANE and S+ANE showed slight to moderate improvements, but these changes were not statistically significant. These results highlight that *A. nodosum* preferentially mitigates oxidative stress under the most severe stress conditions.

5.1.3.4 Effects of applied stressors and *A. nodosum* extract on the hydrogen peroxide content

Hydrogen peroxide (H_2O_2) is a key reactive oxygen species, and its accumulation reflects the level of oxidative stress in *T. pannonicus*, making it an important parameter for evaluating plant stress responses. Quantitative data for H_2O_2 are summarised in Tables 13 and 14. In TP2, all treatments were statistically separated from one another: Drought caused a moderate but significant increase in H_2O_2 (2.26 µmol H_2O_2 g^{-1} FW) compared with the control (0.51 µmol H_2O_2 g^{-1} FW), salinity led to a stronger accumulation (3.11 µmol H_2O_2 g^{-1} FW), and combined stress induced the highest levels (4.03 µmol H_2O_2 g^{-1} FW). This pattern clearly indicates that salinity and drought exert additive effects on oxidative stress.

In TP3, focusing on stress treatments without ANE, a similar trend to TP2 was observed. H_2O_2 content increased from C to D, and further under S and DS, with S and DS becoming statistically similar, although DS still showed the highest numerical value. This confirms that H_2O_2 accumulation intensifies with stress severity and complexity. With *A. nodosum* application, no statistically significant changes in H_2O_2 were detected across treatments, even though stressed plants treated with ANE often showed slightly lower H_2O_2 values than their non-treated groups. Thus, while there is a numerical tendency toward reduced ROS in ANE-treated plants, these TP3 data do not allow us to conclude a significant mitigation of H_2O_2 at the statistical level.

Table 13. Biochemical parameters of *T. pannonicus* in TP1 (2023, 60 mM salinity) and TP2 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		EOC (ml 100 g^{-1} DW)		**TPC** (mg GAE g^{-1} DW)		**AOC** (mg AAE g^{-1} DW)		**H_2O_2** (µmol H_2O_2 g^{-1} FW)	
Experiment		TP1 (60 mM) **	TP2 (90 mM) **	TP1 (60 mM) **	TP2 (90 mM) **	TP1 (60 mM) **	TP2 (90 mM) ns	TP1 (60 mM) ns	TP2 (90 mM) **
Treatments	**C**	0.91 ± 0.09^{a}	0.64 ± 0.06^{a}	150.02 ± 16.35^{a}	137.88 ± 9.85^{a}	162.18 ± 20.91^{a}	138.87 ± 30.70	nd	0.51 ± 0.07^{a}
	D	0.48 ± 0.18^{b}	0.45 ± 0.05^{b}	127.21 ± 7.72^{b}	133.85 ± 8.88^{ab}	148.42 ± 20.52^{a}	136.27 ± 29.96	nd	2.26 ± 0.34^{b}
	S	0.69 ± 0.12^{ab}	0.44 ± 0.05^{b}	141.77 ± 18.92^{a}	122.78 ± 19.87^{bc}	152.48 ± 34.35^{a}	134.81 ± 18.80	nd	3.11 ± 0.18^{c}
	DS	0.52 ± 0.11^{b}	0.41 ± 0.08^{b}	101.49 ± 20.45^{c}	118.23 ± 10.21^{c}	106.57 ± 30.28^{b}	116.76 ± 27.69	nd	4.03 ± 0.29^{d}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. EOC: essential oil content; TPC: total polyphenol content; GAE: gallic acid equivalents; AOC: antioxidant capacity; AAE: ascorbic acid equivalents; H_2O_2: hydrogen peroxide; FW: fresh weight; DW: dry weight; TP1: 1st experiment of *T. pannonicus*; TP2: 2nd experiment of *T. pannonicus*; nd: not determined; C: control; D: drought stress; S: salinity stress; DS: combined stress; ^{ns}: non-significant; **: $p < 0.01$.

Table 14. Biochemical parameters of *T. pannonicus* in TP3 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		EOC (ml 100g^{-1} DW)	**TPC** (mg GAE g^{-1} DW)	**AOC** (mg AAE g^{-1} DW)	**H_2O_2** (µmol H_2O_2 g^{-1} FW)
Treatments	**C**	0.30 ± 0.10^{Ab}	201.01 ± 24.67^{Ab}	186.87 ± 18.89^{Ac}	0.44 ± 0.03^{Aa}
	C + ANE	0.31 ± 0.00^{Ab}	194.40 ± 18.52^{Ab}	179.33 ± 11.35^{Ab}	0.35 ± 0.14^{Aa}
	D	0.19 ± 0.08^{Aa}	162.08 ± 16.47^{Ab}	180.59 ± 12.81^{Abc}	2.03 ± 0.10^{Ab}
	D + ANE	0.38 ± 0.08^{Bb}	169.21 ± 18.43^{Aab}	185.67 ± 6.55^{Ab}	1.90 ± 0.12^{Ab}
	S	0.19 ± 0.05^{Aa}	117.93 ± 25.93^{Aa}	156.68 ± 30.02^{Ab}	2.87 ± 0.15^{Ac}
	S + ANE	0.29 ± 0.11^{Bb}	139.98 ± 24.73^{Aa}	175.36 ± 15.31^{Ab}	2.37 ± 0.16^{Abc}
	DS	0.13 ± 0.03^{Aa}	108.59 ± 21.68^{Aa}	110.14 ± 18.01^{Aa}	3.12 ± 0.18^{Ac}
	DS + ANE	0.20 ± 0.11^{Ba}	157.46 ± 24.05^{Ba}	153.86 ± 14.75^{Ba}	2.87 ± 0.15^{Ac}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). EOC: essential oil content; TPC: total polyphenol content; GAE: gallic acid equivalents; AOC: antioxidant capacity; AAE: ascorbic acid equivalents; H_2O_2: hydrogen peroxide; FW: fresh weight; DW: dry weight; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.1.3.5 Effects of applied stressors and *A. nodosum* extract on the essential oil composition

The GC–MS results for the three experiments on *T. pannonicus* are presented in Tables 29, 30, and 31 in the Appendices. In TP1, 14 compounds were identified and grouped into three main classes: monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes. Monoterpene hydrocarbons (camphene, α-terpinene, p-cymene, γ-terpinene) accounted for 25.30 % in the control and decreased under stress, reaching 21.46 % in the combined stress. Oxygenated monoterpenes (1,8-cineole, camphor, borneol, thymol methyl ether, carvacrol methyl ether, thymol, carvacrol) formed the dominant group (56.88 % in C) and showed slight reductions under individual stress conditions (55.79 % in D and 52.98 % in S). Sesquiterpenes (β-caryophyllene, β-bisabolene) showed the opposite tendency, being lowest in C (6.44 %) and highest in DS (9.30 %).

In TP2, 21 compounds were identified. New constituents such as 1-octen-3-ol (aliphatic compound), bicyclogermacrene, spathulenol, and thymoquinone appeared in trace amounts (0.33–1.86 %). Despite this slightly richer profile, the relative proportions of the main groups and their trends under stress were broadly similar to TP1. In TP3, again 21 compounds were identified. In the control, monoterpene hydrocarbons represented 26.25 % and oxygenated monoterpenes 58.77 %, both decreasing progressively with increasing stress severity, whereas sesquiterpenes started at 11.49% in the control and increased under stress. Across stress treatments in TP3, ANE application clearly attenuated these shifts, tending to return the proportions of the main groups toward values closer to the control.

Among all detected compounds, four major components were consistently abundant and were therefore selected for one-way and two-way ANOVA (Tables 15 and 16): thymol, p-cymene, β-bisabolene, and thymol methyl ether. Thymol, the principal constituent of *T. pannonicus* EO, reached its highest proportion in C in TP1 (34.89 %), and was significantly reduced in D and DS (26.52 % and 24.08 %, respectively). In TP2, a similar pattern was observed, but D showed only a moderate, non-significant reduction, closer to S and not clearly different from C. In TP3, despite the higher salinity, neither D nor S alone significantly reduced thymol compared with C, while DS caused a clear and significant decrease. Under combined stress, ANE increased thymol from 23.18 % to 26.17 %, and in the other stressed treatments ANE produced partial numerical increases, suggesting a tendency to restore thymol toward control-like levels.

The proportion of p-cymene, a monoterpene closely related to thymol, showed a more nuanced response. In TP1, p-cymene increased significantly under S compared with C and DS, while D was intermediate. This indicates that at 60 mM NaCl, salinity alone tends to enhance p-cymene, but this effect disappears when salinity is combined with drought (DS). In TP2, no significant differences were detected among treatments, although a slight numerical decline was observed from C (15.30 %) to DS (12.52 %). In TP3, in the absence of *A. nodosum*, p-cymene was highest in C (18.96 %) and significantly higher than in all stress treatments: D (9.35 %), S (7.83 %), and DS (5.08 %), with no differences among the latter three.

Table 15. Relative percentages (%) of the main volatile compounds of *T. pannonicus* in TP1 (2023, 60 mM salinity) and TP2 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		thymol (%)		***p-cymene*** (%)		**β-bisabolene** (%)		**thymol methyl ether** (%)	
Experiment		TP1 (60 mM) **	TP2 (90 mM) **	TP1 (60 mM) *	TP2 (90 mM) ns	TP1 (60 mM) **	TP2 (90 mM) ns	TP1 (60 mM) **	TP2 (90 mM) ns
Treatments	**C**	34.89 ± 3.65^{a}	35.75 ± 1.18^{a}	16.25 ± 1.97^{a}	15.30 ± 0.96	4.79 ± 0.68^{a}	3.27 ± 0.13	9.33 ± 1.58^{a}	9.10 ± 2.51
	D	26.52 ± 3.85^{b}	30.72 ± 3.33^{ab}	16.78 ± 3.50^{ab}	14.32 ± 5.89	6.37 ± 1.67^{b}	5.11 ± 1.27	13.86 ± 1.87^{b}	13.62 ± 2.49
	S	29.20 ± 2.09^{ab}	31.08 ± 1.44^{ab}	21.29 ± 2.11^{b}	13.18 ± 0.16	4.76 ± 0.83^{a}	4.55 ± 0.91	9.30 ± 1.02^{a}	13.48 ± 1.07
	DS	24.08 ± 3.42^{b}	25.60 ± 2.22^{b}	16.12 ± 2.89^{a}	12.52 ± 4.22	6.79 ± 1.13^{b}	7.59 ± 3.61	15.89 ± 3.24^{b}	14.52 ± 2.44

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. TP1: 1st experiment of *T. pannonicus*; TP2: 2nd experiment of *T. pannonicus*; nd: not determined; C: control; D: drought stress; S: salinity stress; DS: combined stress; ns: non-significant; *: $p \leq 0.05$; **: $p < 0.01$.

Table 16. Relative percentages (%) of the main volatile compounds of *T. pannonicus* in TP3 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		thymol (%)	**p-cymene** (%)	**β-bisabolene** (%)	**thymol methyl ether** (%)
Treatments	**C**	28.15 ± 3.99^{Aa}	18.96 ± 3.00^{Ab}	8.33 ± 2.15^{Aa}	14.59 ± 2.56^{Aa}
	C + ANE	29.82 ± 1.49^{Aa}	17.61 ± 0.45^{Aa}	7.74 ± 0.56^{Aa}	15.80 ± 2.78^{Aa}
	D	28.31 ± 3.24^{Aa}	9.35 ± 3.24^{Aa}	10.26 ± 3.71^{Aa}	22.45 ± 3.01^{Bb}
	D + ANE	28.26 ± 3.60^{Aa}	15.42 ± 3.82^{Aa}	9.16 ± 2.94^{Aa}	15.04 ± 3.21^{Aa}
	S	26.31 ± 3.28^{Aa}	7.83 ± 2.67^{Aa}	22.58 ± 3.58^{Bb}	20.49 ± 1.78^{Bb}
	S + ANE	28.71 ± 1.00^{Aa}	11.94 ± 2.04^{Aa}	10.80 ± 3.45^{Aa}	18.26 ± 2.54^{Aa}
	DS	23.18 ± 3.62^{Ab}	5.08 ± 2.77^{Aa}	25.15 ± 2.67^{Bb}	20.59 ± 0.92^{Bb}
	DS + ANE	26.17 ± 4.79^{Ba}	12.41 ± 2.68^{Aa}	10.13 ± 2.23^{Aa}	16.12 ± 13.00^{Aa}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

Two-way ANOVA did not detect a significant interaction between stress and ANE, even though p-cymene tended to increase numerically in stressed plants treated with ANE toward values close to C+ANE (17.61 %), as exemplified by the rise from 9.35 % (D) to 15.42 % (D+ANE).

β-bisabolene, an important sesquiterpene in *T. pannonicus* EO, generally displayed an inverse pattern to thymol and p-cymene. In TP1, its proportion increased significantly ($p \leq 0.05$) under drought and combined stress compared with control and salinity. In TP2, despite the higher salinity, β-bisabolene did not show statistically significant variation among treatments, suggesting a weaker or more unstable response at this level. In TP3 without ANE, S (120 mM) and DS (120 mM + 40% SWC) markedly increased β-bisabolene (22.58 % and 25.15 %, respectively), whereas C and D remained much lower (8.33 % and 10.26 %). Under ANE application, β-bisabolene was strongly and significantly reduced under S and DS (from 22.58 % to 10.80% and from 25.15 % to 10.13 %, respectively), bringing these treatments back into the same statistical group as C+ANE (7.74 %) and D+ANE (9.16 %).

Thymol methyl ether, another oxygenated monoterpene related to thymol, showed a broadly similar behavior to β-bisabolene. In TP1, D and particularly DS tended to enhance thymol methyl ether compared with C and S, whereas in TP2 no clear, significant response to stress was detected. In TP3, in the absence of ANE, all stress treatments (D, S, DS) substantially increased thymol methyl ether relative to C, reproducing the pattern observed in TP1 but now with S (at 120 mM) joining D and DS. Under ANE, this stress-induced rise was systematically reduced in all three stress conditions, with thymol methyl ether in D+ANE, S+ANE, and DS+ANE shifting back toward the control range.

5.1.4 Evaluation of stress indices in *T. pannonicus*

The stress susceptibility (SSI) and tolerance (STI) indices across the three experiments are illustrated in Figures 11 and 12, respectively. Across years, SSI reveals a dynamic pattern of sensitivity in *T. pannonicus*. In TP1, drought (SSI = 1.02) and combined stress (SSI = 1.20) were already classified as susceptible ($SSI \geq 1$), whereas salinity alone was only moderately limiting (SSI = 0.83). In TP2, despite the higher salinity level (90 mM), D and DS remained in the susceptible class (SSI = 1.10 and 1.45, respectively), while S stayed in the moderate range (SSI = 0.64). By TP3, D appeared less constraining: it shifted into the moderate class without ANE (SSI = 0.59) and even became favourable under ANE application (SSI = −0.31), indicating that D+ANE plants outperformed the non-stressed reference. In contrast, salinity and especially combined stress persisted as the main limiting factors in TP3. Salinity alone remained susceptible (SSI = 1.34), though it improved to a moderate level with ANE (SSI = 0.72). Combined drought–salinity represented the harshest condition, being highly susceptible without ANE (SSI = 2.88), but shifting to the moderate class when ANE was applied (SSI = 0.87).

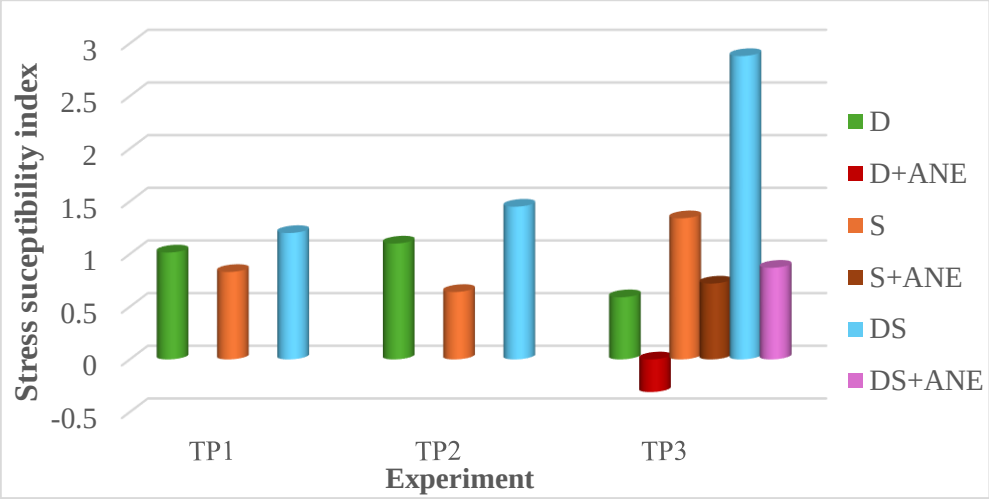

Figure 11. Stress susceptibility index of *T. pannonicus* across all the experiments (TP1, TP2, TP3). D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

Across the three experiments, stress tolerance index values confirmed that *T. pannonicus* performed better under salinity alone (up to 90 mM) than under combined drought-salinity. In TP1, STI was lowest under combined stress (0.08) and slightly higher under drougth (0.11), while salinity displayed the highest index (0.18), indicating that biomass was more strongly preserved under salinity than under the combined stress. In TP2, all STI values increased, with S again reaching the highest value (0.82), followed by D (0.57) and DS (0.44), reinforcing the idea that salinity alone was less detrimental than drought or especially their combination.

By TP3, at the highest salinity level (120 mM), drought clearly showed a high STI (0.91), which increased further with ANE (1.05), revealing a strong improvement in drought performance. In contrast, S and S+ANE displayed intermediate STI values (0.75 and 0.86), while DS remained the most limiting condition with the lowest STI (0.47). However, DS+ANE increased sharply to 0.84, indicating a substantial recovery of productivity under combined stress when ANE was applied.

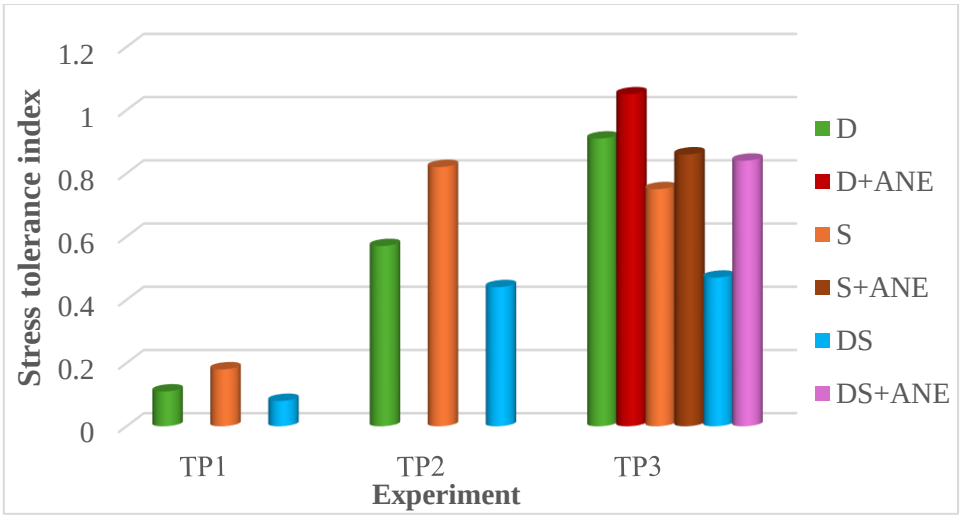

Figure 12. Stress tolerance index of *T. pannonicus* across all the experiments (TP1, TP2, TP3). D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.1.5 Correlation and principal component analysis of *T. pannonicus* parameters

The correlations among the morphological, biomass, physiological, and biochemical parameters of *T. pannonicus* in TP3 are illustrated in the clustered heatmap in Figure 13. The heatmap clearly organises the variables into two main correlation blocks: a performance (growth) block and a stress response block. The performance block (red region) includes thymol, shoot length, fresh and dry shoot weights, root length, root fresh weight, chlorophyll a, chlorophyll b, carotenoids, EOC, AOC, TPC, RWC and p-cymene, while the stress-response block (blue region) groups H_2O_2, proline, soluble sugars, and the stress-inducible volatiles β-bisabolene and thymol methyl ether.

Within the performance block, strong positive correlations are evident. chlorophyll a, chlorophyll b and carotenoids cluster closely with RWC and root length, indicating that better leaf water status and longer roots are associated with higher pigment content. AOC and TPC occupy the same high-correlation area as the photosynthetic pigments, suggesting that antioxidant capacity and polyphenols tend to increase in conditions where pigments are maintained, pointing to a coordinated protective and photosynthetic response. Morphological traits and yields such as fresh shoot weight, dry shoot weight, shoot length, root fresh weight and root length also show moderate positive correlations with EOC and thymol ($r < 0.5$), implying that plants with greater biomass generally tend to produce more essential oil and thymol.

Conversely, H_2O_2, proline, soluble sugars, β-bisabolene and thymol methyl ether form a compact sub-cluster that is negatively correlated with pigments (chlorophyll a, chlorophyll b, carotenoids), RWC, TPC, AOC and, to a lesser extent, with growth parameters, EOC and thymol. This pattern indicates that when oxidative and osmotic stress markers are high, water status, pigment content, antioxidant and polyphenol levels, biomass, EOC and thymol tend to be reduced.

Although the heatmap is based on correlations among variables rather than on treatments, its structure reflects clear groupings of stress-related and growth-related traits. Higher levels of H_2O_2, proline, soluble sugars, β-bisabolene, and thymol methyl ether cluster together and are associated with reduced growth parameters, pigment content, and thymol. In contrast, higher shoot length, fresh and dry shoot weights, root length, root fresh weight, pigment levels, RWC, AOC, TPC, EOC, and thymol form a separate cluster linked with improved water status and biomass-related traits.

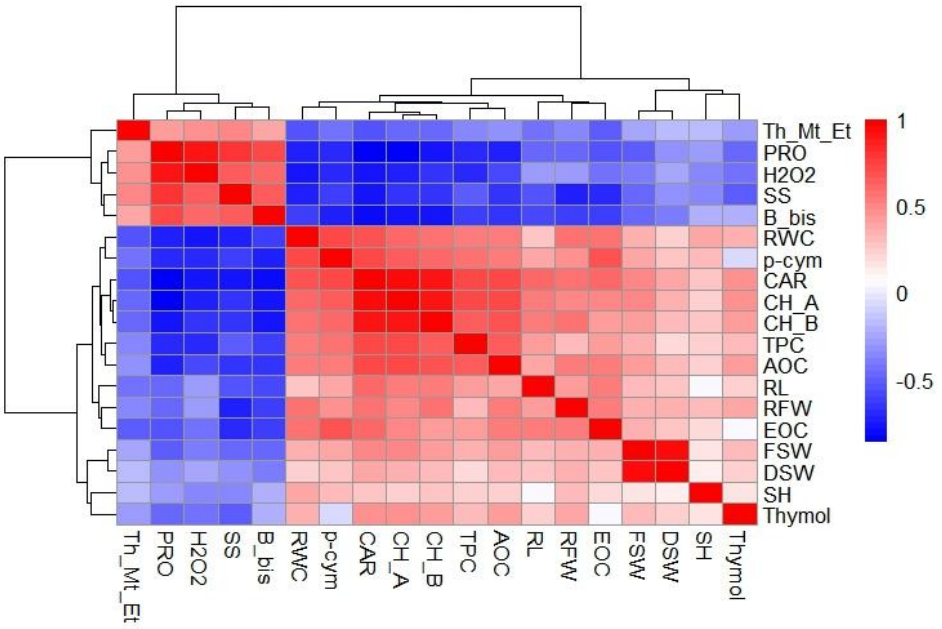

Figure 13. Hierarchical clustering heatmap of morphological, biomass, physiological and biochemical parameters of *T. pannonicus* in TP3. SH: shoot length; DSW: dry shoot weight; FSW: fresh shoot weight; EOC: essential oil content; RFW: fresh root weight; RL: root length; AOC: antioxidant capacity; TPC: total polyphenol content; CH_B: chlorophyll b; CH_A: chlorophyll a; CAR: carotenoids; p-cym: p-cymene; RWC: relative water content; B_bis: β-bisabolene; SS: soluble sugars; H_2O_2: hydrogen peroxide; PRO: proline; Th_Mt_Et: thymol methyl ether.

The biplot of the principal component analysis for *T. pannonicus* in TP3 is shown in Figure 14. Dim1 (54.4 %) represents the main gradient and clearly separates the severe stress treatments on the left (DS, S) from the control and ANE associated treatments on the right (C, C+ANE, D+ANE). Dim2 (8.3 %) represents a secondary gradient that slightly distinguishes drought from salinity contexts.

In terms of treatment positions, DS (−6.2, −0.4) lies at the extreme left of Dim1, showing the strongest association with stress related traits. S (−3.2, 0.0) also occupies the left side, indicating a clear stress signal but less extreme than DS. Closer to the origin, DS+ANE (−0.2, 0.5) shifts toward a more neutral zone, suggesting a partial shift in the overall response profile under ANE. Nearby, D (−0.2, 0.5) occupies a slightly higher position on Dim2, indicating a moderate drought effect that is still closer to recovery than S. S+ANE (0.3, 0.5) moves further toward the favourable side of Dim1 compared with S, suggesting some improvement under ANE. On the right-hand side, D+ANE (2.4, 0.6) is clearly shifted toward the vigour biomass vectors, indicating that ANE effectively mitigated drought. Finally, C (3.2, −0.8) and C+ANE (3.6, −0.5) lie far to the right and slightly lower on Dim2, in the region associated with the highest growth, pigment and essential oil related loadings.

The orientation of the variable vectors confirms this structure. Stress and defence markers (proline, H_2O_2, soluble sugars, β-bisabolene and thymol methyl ether) point toward the left side of the biplot, where DS and S are located, indicating positive associations with these severe stress treatments

and strong oxidative and osmotic stress, as well as a shift in essential oil composition toward stress-related volatiles, and negative associations with C, C+ANE and D+ANE. In contrast, growth, water status and photosynthetic traits, fresh shoot weight, dry shoot weight, root fresh weight, RWC, chlorophylls, carotenoids, AOC, TPC, EOC, thymol and p-cymene, are oriented toward the right, aligning with C, C+ANE and D+ANE. This shows that well-watered with lower salinity stress and ANE-treated plants maintain higher biomass, better water status, richer pigment pools and a more favourable, thymol rich EO profile. Intermediate traits such as shoot length and root length tend to associate with D, DS+ANE and S+ANE, reflecting partial stress effects or partially corrected stress responses.

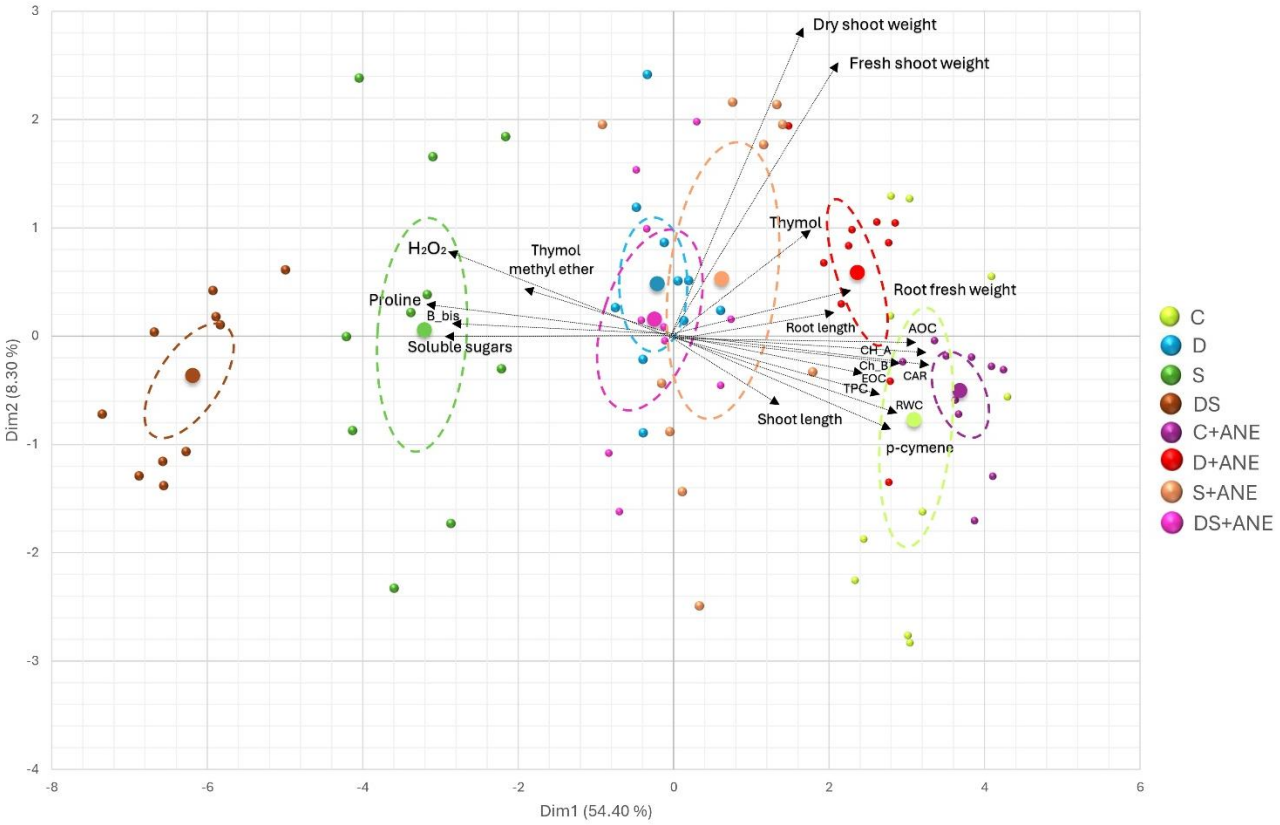

Figure 14. Biplot of principal component analysis illustrating the relationships among the studied parameters of *T. pannonicus* when subjected to various stress treatments and *A. nodosum* extract conditions in TP3. Dim: dimension; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract; EOC: essential oil content; RWC: relative water content; AOC: antioxidant capacity; TPC: total polyphenol content; CH_B: chlorophyll b; CH_A: chlorophyll a; CAR: carotenoids; B_bis: β-bisabolene; H_2O_2: hydrogen peroxide.

5.2 Evaluation of *T.* × *citriodorus* experiments

5.2.1 Effects of applied stressors and *A. nodosum* extract on the morphology and biomass of *T.* × *citriodorus*

5.2.1.1 Effects of applied stressors and *A. nodosum* extract on the shoot length

The results of the one-way ANOVA for TL1 and the two-way ANOVA for TL2 for all studied morphological and biomass traits are summarised in Tables 17 and 18 respectively. Concerning shoot lengths, in TL1, the tallest plants were observed in the control (C, 37.06 cm), with values significantly higher than all other treatments. Drought (D) alone (32.93 cm) led to a moderate reduction in length compared with the control, whereas the shortest plants were recorded under combined stress (DS, 29.46 cm), which was significantly lower than both C and D, highlighting the strong negative impact of the simultaneous action of 120 mM NaCl and 40 % SWC. The salinity treatment (S, 32.53 cm) showed an intermediate shoot length, not significantly different from either D or DS, suggesting a gradual decline in length as stress intensity increased.

In TL2, the two-way ANOVA did not detect significant differences in shoot length among the stress treatments within the same *A. nodosum* extract (ANE) level, indicating a more attenuated stress effect on this trait compared with TL1. However, when the interaction between stress and ANE was considered, a significant improvement was evident under drought: *A. nodosum* extract application increased shoot length from 40.95 to 45.05 cm in D+ANE.

5.2.1.2 Effect of applied stressors and *A. nodosum* extract on the number of shoots

The number of shoots of *T.* × *citriodorus* under the different treatments is reported in Tables 17 and 18. In TL1, the control produced the highest number of shoots (130 pcs plant^{-1}), significantly exceeding all stress treatments. Drought alone (113 pcs plant^{-1}) resulted in a clear reduction compared with the control yet still maintained significantly higher values than both salinity and combined stress. Under salinity (84.25 pcs plant^{-1}) and combined drought–salinity (77.50 pcs plant^{-1}), the number of shoots dropped markedly and to a similar extent, with both treatments being significantly lower than C and D but not different from each other, confirming the strong negative effect of salt and its combination with drought on branching.

In TL2, without ANE application, a slightly different pattern from TL1 emerged. Drought and salinity shifted towards an intermediate position, becoming statistically similar to each other. Drought also became statistically comparable to the C, while S remained significantly higher than DS, which continued to show the lowest number of shoots. When *A. nodosum* was applied, a pronounced improvement in number of shoots was observed under all stress conditions: number of shoots increased by approximately 6 %, 13 %, and 8 % in D+ANE, S+ANE, and DS+ANE, respectively, relative to their corresponding non-ANE treatments. The relative ranking among treatments with ANE mirrored that of the unstressed and stressed groups without *A. nodosum* extract: C+ANE plants still had the highest number of shoots, DS+ANE remained the lowest, and D+ANE and S+ANE occupied intermediate positions.

5.2.1.3 Effects of applied stressors and *A. nodosum* extract on the fresh shoot weight

In TL1, where plants were exposed to 120 mM salinity, fresh shoot weight was the highest in the control (42.12 g plant^{-1}), and this value was significantly higher than in the salinity (30.38 g plant^{-1}) and in the combined stress (23.31 g plant^{-1}). Although DS showed the lowest mean, it was statistically grouped together with S. Drought alone caused a slight reduction in the fresh shoot weight, but this decrease was not statistically different from other treatments in TL1.

In TL2, a similar pattern was observed as in the previous year without *A. nodosum* extract application: S and DS significantly reduced fresh shoot weight compared with control and drought when no ANE was used. When *A. nodosum* extract was applied, a clear improvement appeared under combined stress, with DS+ANE increasing from 44.91 g plant^{-1} to 47.40 g plant^{-1}. Differences among *A. nodosum* treatments themselves were not statistically significant but applying ANE to stressed plants tended to bring their fresh shoot weights closer to that of C+ANE, a pattern evidenced in Figure 15.

5.2.1.4 Effects of applied stressors and *A. nodosum* extract on the shoot dry weight

As shown in Table 17, the one-way ANOVA for shoot dry weight in TL1 revealed no significant differences among all treatments. Statistically, all stress treatments and the control maintained similar dry shoot weight, despite minor numerical decreases under stress. In TL2, when no ANE was applied, the highest dry shoot weight values were observed in C and D (13.85 g plant^{-1} and 12.99 g plant^{-1}, respectively), which were significantly higher than the lowest dry shoot weight recorded under DS (10.94 g plant^{-1}). The S treatment (12.05 g plant^{-1}) occupied an intermediate position, not significantly different from any other group.

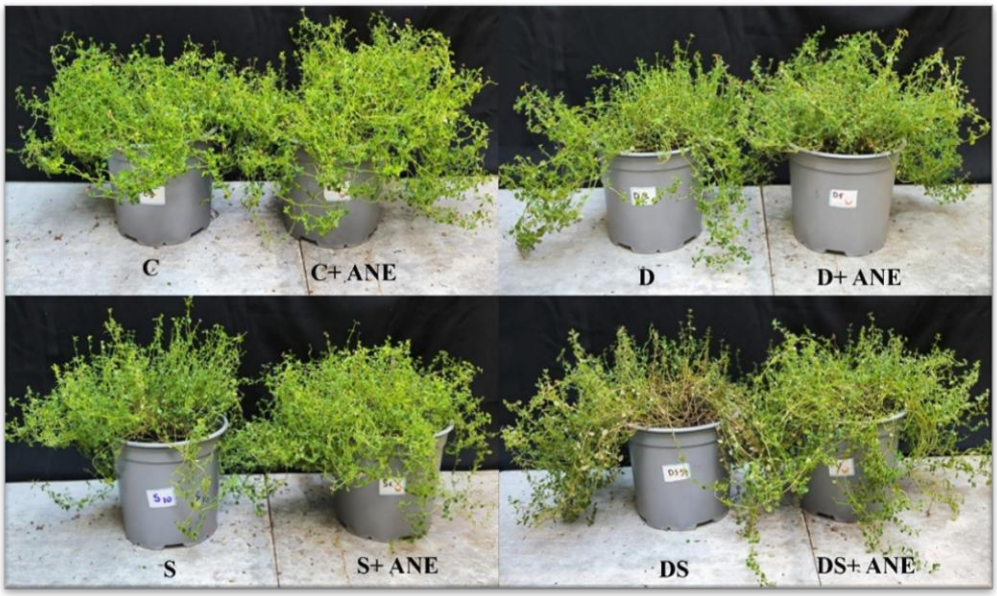

Figure 15. *T.* × *citriodorus* plants in TL2 under different treatments applied.
C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *A. nodosum* extract.

With ANE application, a significant increase in dry shoot weight was detected only under combined stress (DS+ANE), rising from 10.94 to 12.75 g plant^{-1}, while no statistically relevant effects of ANE were observed under individual stress treatments or in the control, and differences among ANE-treated groups were not significant, either.

5.2.1.5 Effects of applied stressors and *A. nodosum* extract on the root length

Regarding root length, the response of *T.* × *citriodorus* to drought, salinity, and their combination showed consistent tendencies in both experiments, as reported in Tables 17 and 18. In TL1, with 120 mM NaCl, the one-way ANOVA indicated that control (28.08 cm) and drought (26.18 cm) shared the same statistical group, meaning that drought alone did not significantly shorten roots compared with the control. By contrast, salinity (22.83 cm) significantly reduced root length relative to both C and D, and the combination of 120 mM NaCl with 40 % SWC led to the most pronounced and significant reduction (18.50 cm), highlighting the strong impact of combined stress on root elongation.

In TL2, root length appeared more stable when comparing stress treatments within each *A. nodosum* application. In the absence of ANE, no significant differences in root length were detected among the stress treatments, and the same absence of significance was observed within the ANE-treated groups. Moreover, no significant interaction between stress and ANE was found, indicating that ANE did not measurably modify root length under any stress treatment in TL2.

5.2.1.6 Effects of applied stressors and *A. nodosum* extract on the fresh root weight

Regarding fresh root weight, the response of *T.* × *citriodorus* to drought, salinity, and their combination, with and without ANE, showed clear treatment-related differences in both experiments, as presented in Tables 17 and 18. In TL1, the control recorded the highest root fresh weight (34.60 g plant^{-1}), significantly higher than all stress treatments. Drought alone (25.30 g plant^{-1}) led to a marked reduction compared with the control, while the combined drought–salinity treatment (20.76 g plant^{-1}) produced the lowest root fresh weight, significantly lower than both C and D. The salinity treatment (22.00 g plant^{-1}) occupied a statistical intermediate position between D and DS, indicating a progressive decline in root biomass with increasing stress severity.

In TL2, despite using the same salinity level (120 mM), the response pattern was slightly modified. Drought and salinity became statistically distinct, with D and C showing similar and higher root fresh weight values compared with S and DS. Salinity and combined stress, in turn, were statistically similar to each other and remained at the lower end of the scale, confirming the particularly detrimental impact of salt, especially when combined with drought (Figure 16). When *A. nodosum* extract was applied, the two-way ANOVA revealed that ANE significantly increased root fresh weight (by 46 %) only under combined stress, while differences among the ANE-treated groups themselves remained non-significant.

5.2.1.7 Effects of applied stressors and *A. nodosum* extract on the dry root weight

The dry root weight in TL1, as shown in Table 17, followed a pattern very similar to that observed for root fresh weight, with a gradual decline as stress intensity increased. Root dry weight reached its highest value in the control (8.58 g plant^{-1}) and dropped to its lowest under combined stress (5.60 g plant^{-1}), highlighting the pronounced negative impact of the combined action of drought and salinity on belowground biomass.

In TL2, when no ANE was applied, C (3.61 g plant^{-1}) remained significantly higher than DS (2.36 g plant^{-1}), while D (3.02 g plant^{-1}) and S (2.58 g plant^{-1}) occupied intermediate positions, without clear statistical separation from either control or combined stress. Under *A. nodosum* extract application, the two-way ANOVA showed no strong statistical differences ($p \leq 0.05$) in dry root weight among C+ANE, D+ANE, S+ANE, and DS+ANE, despite a slight enhancement were observed in DS+ANE, indicating a general convergence of values under ANE treatment.

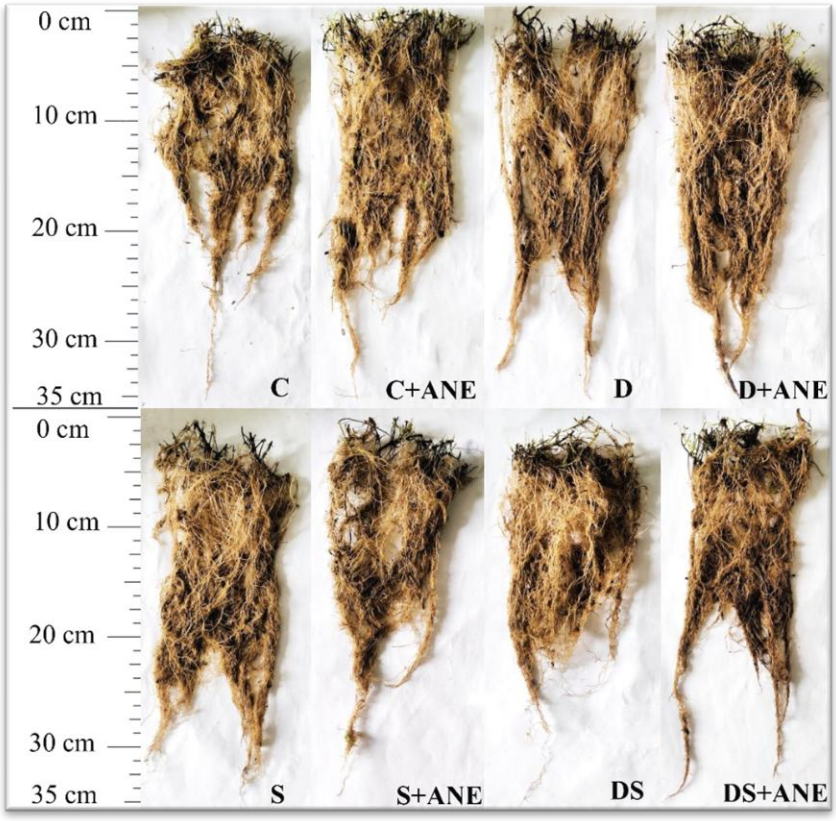

Figure 16. Effect of the applied treatments on root development in *T. × citriodorus in* TL2. C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

Table 17. Morphological and biomass parameters and biomass of *T.* × *citriodorus* in TL1 (2024, 120 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		Shoot length (cm)	**Number of shoots** (pcs plant^{-1})	**Fresh shoot weight** (g plant^{-1})	**Dry shoot weight** (g plant^{-1})	**Root length** (cm)	**Fresh root weight** (g plant^{-1})	**Dry root weight** (g plant^{-1})
Experiment		TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) ^{ns}	TL1 (120 mM) *	TL1 (120 mM) *	TL1 (120 mM) *
Treatments	**C**	37.06 ± 4.07^{a}	130 ± 7.70^{a}	42.12 ± 9.04^{a}	9.71 ± 2.98	28.08±1.66^{a}	34.60 ± 1.67^{a}	8.58 ± 0.44^{a}
	D	32.93 ± 2.31^{b}	113 ± 8.83^{b}	32.97 ± 7.75^{ab}	8.98 ± 1.98	26.18±1.54^{a}	25.30 ± 0.92^{b}	7.08 ± 0.53^{b}
	S	32.53 ± 3.46^{bc}	84.25 ± 3.30^{c}	30.38 ± 13.52^{b}	8.54 ± 2.26	22.83±1.26^{b}	22.00 ± 3.27^{bc}	6.07 ± 0.92^{bc}
	DS	29.46 ± 2.87^{c}	77.50 ± 6.13^{c}	23.31 ± 9.43^{b}	8.38 ± 2.24	18.50±1.24^{c}	20.76 ± 1.54^{c}	5.60 ± 0.29^{c}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. TL1:1st experiment of *T.* × *citriodorus*; pcs: pieces; C: control; D: drought stress; S: salinity stress; DS: combined stress; ^{ns}: non-significant; *: $p \leq 0.05$; **: $p < 0.01$.

Table 18. Morphological and biomass parameters and biomass of *T.* × *citriodorus* in TL2 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		Shoot length (cm)	**Number of shoots** (pcs plant^{-1})	**Fresh shoot weight** (g plant^{-1})	**Dry shoot weight** (g plant^{-1})	**Root length** (cm)	**Fresh root weight** (g plant^{-1})	**Dry root weight** (g plant^{-1})
Treatments	**C**	43.90 ± 2.64^{Aa}	69.90 ± 4.38^{Ac}	48.30 ± 5.20^{Ab}	13.85 ± 1.31^{Ab}	33.25 ± 2.75^{Aa}	19.52 ± 2.64^{Ab}	3.61 ± 0.56^{Ab}
	C + ANE	43.75 ± 2.28^{Aa}	71.70 ± 4.17^{Ac}	49.99 ± 7.38^{Aa}	14.33 ± 1.87^{Aa}	33.50 ± 1.29^{Aa}	21.43 ± 3.75^{Aa}	3.48 ± 0.50^{Aa}
	D	40.95 ± 2.52^{Aa}	66.00 ± 3.59^{Abc}	46.63 ± 6.79^{Ab}	12.99 ± 1.72^{Ab}	30.50 ± 4.80^{Aa}	18.44 ± 4.12^{Ab}	3.02 ± 0.45^{Aab}
	D + ANE	45.05 ± 3.88^{Ba}	70.00 ± 1.70^{Bb}	48.36 ± 4.72^{Aa}	13.26 ± 1.54^{Aa}	31.25 ± 2.22^{Aa}	17.86 ± 4.77^{Aa}	3.28 ± 0.74^{Aa}
	S	41.80 ± 4.66^{Aa}	62.50 ± 3.84^{Ab}	45.36 ± 7.05^{Aa}	12.05 ± 1.62^{Aab}	29.00 ± 4.24^{Aa}	15.12 ± 1.40^{Aa}	2.58 ± 0.35^{Aab}
	S + ANE	45.15 ± 4.01^{Aa}	70.80 ± 2.53^{Bb}	47.65 ± 10.80^{Aa}	13.01 ± 1.43^{Aa}	30.00 ± 6.48^{Aa}	17.32 ± 4.13^{Aa}	2.80 ± 0.73^{Aa}
	DS	41.30 ± 4.37^{Aa}	56.90 ± 3.93^{Aa}	44.91 ± 5.69^{Aa}	10.94 ± 1.35^{Aa}	28.75 ± 3.10^{Aa}	14.71 ± 5.07^{Aa}	2.36 ± 0.81^{Aa}
	DS + ANE	41.80 ± 2.20^{Aa}	61.50 ± 4.14^{Ba}	47.40 ± 3.92^{Ba}	12.75 ± 1.51^{Ba}	32.38 ± 4.03^{Aa}	21.41 ± 3.39^{Ba}	3.14 ± 0.80^{Aa}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). pcs,: pieces; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.2.2 Effects of applied stressors and *A. nodosum* extract on the physiology of *T.* × *citriodorus*

5.2.2.1 Effects of applied stressors and *A. nodosum* extract on the photosynthetic pigments

The one-way ANOVA for TL1 and the two-way ANOVA for TL2, summarising all physiological parameters of *T.* × *citriodorus*, are presented in Tables 19 and 20. Regarding chlorophyll pigments, TL1 showed a consistent pattern across treatments. Drought alone had minimal effect, with total chlorophyll reaching 2901.20 µg g^{-1} FW, statistically similar to the control, indicating that water deficit at the applied level did not strongly impair chlorophyll a and b content. Salinity, however, caused a significant reduction in total chlorophyll (1931.70 µg g^{-1} FW), while the combination of drought and salinity produced the lowest pigment levels (1813.50 µg g^{-1} FW), statistically comparable to salinity alone, demonstrating the pronounced negative impact of salt and combined stress on chlorophyll accumulation.

In TL2, a slightly different trend was observed. Without *A. nodosum* extract, chlorophyll a was significantly reduced under S and DS, with DS reaching the lowest value (1288.60 µg g^{-1} FW), whereas drought alone had an intermediate non-significant effect (1615.30 µg g^{-1} FW), slightly similar to the control. *A. nodosum* extract application notably increased chlorophyll a under salinity (1588.29 µg g^{-1} FW compared with 1321.97 µg g^{-1} FW for S alone), highlighting a protective effect, while variation among stress treatments within the same ANE level remained limited. For chlorophyll b, all stress treatments, individually or combined, significantly lowered pigment content relative to the control, and ANE application did not result in significant improvement. Total chlorophyll was also significantly reduced under S (2073.74 µg g^{-1} FW) and DS (2112.34 µg g^{-1} FW), with drought alone showing intermediate values (2458 µg g^{-1} FW) not significantly different from S or DS. *A. nodosum* extract application significantly enhanced total chlorophyll only under salinity (2436.27 µg g^{-1} FW compared with 2073.74 µg g^{-1} FW for S alone), while within each ANE level, differences among stress treatments were generally minor, suggesting a stabilising effect of the ANE.

Carotenoid content were also evaluated in both trials. In TL1, carotenoids appeared more sensitive to stress than chlorophyll pigments: all stressed treatments, whether applied individually or in combination, showed a significant decline in carotenoid levels and were statistically different from each other. The lowest value was recorded under DS (431.73 µg g^{-1} FW), indicating a progressive reduction in carotenoids as stress intensity increased, with combined drought–salinity exerting the strongest negative effect.

In TL2, in the absence of *A. nodosum* extract, the overall pattern was similar to TL1. Control plants showed the highest carotenoid content (697.77 µg g^{-1} FW), while DS again displayed the lowest (498.50 µg g^{-1} FW). Drought and salinity occupied intermediate positions (554.36 and 525.42 µg g^{-1} FW, respectively) and were not clearly separated from either the control or DS. With *A. nodosum* extract application, no significant interaction between stress and ANE was detected. Numerically, ANE tended to slightly increase carotenoid content under all stress conditions, but

these differences were not statistically significant, and all ANE-treated groups remained within the same statistical range, with stressed plants still showing somewhat lower values than the control.

5.2.2.2 Effects of applied stressors and *A. nodosum* extract on the relative water content

The responses of *T.* × *citriodorus* relative water content (RWC) to drought, salinity, and their combination, with and without ANE application, revealed clear patterns in how stress and the biostimulant influence plant water status, as shown in Tables 19 and 21. In TL1, the one-way ANOVA indicated a significant separation among all treatments: control plants had the highest RWC (88.44 %), reflecting well-hydrated leaves, and RWC declined progressively with increasing stress severity. Drought showed the next highest value (82.97 %), followed by salinity (77.79 %), while combined stress recorded the lowest RWC (71.13 %), corresponding to a reduction of 17.31 % compared with the control, and highlighting the strong impact of combined drought–salinity on leaf hydration.

In TL2, the same overall pattern was maintained: the control again exhibited the highest RWC and DS the lowest, while D and S occupied intermediate positions, partially overlapping with both C and DS. This suggests that, even in TL2, combined stress clearly depresses RWC, whereas drought and salinity alone induce more moderate decreases. *A. nodosum* extract application significantly increased RWC only under combined stress, raising DS values by about 13.54 % and bringing them close to the control range, while no significant differences were detected among C+ANE, D+ANE, S+ANE, and DS+ANE.

5.2.2.3 Effects of applied stressors and *A. nodosum* extract on the proline content

To better understand the osmotic adjustment strategy of *T.* × *citriodorus* under drought, salinity, and their combination, with and without ANE application, changes in proline accumulation were evaluated, as shown in Tables 19 and 20. In TL1, a basal proline level was observed under non-stress conditions (2.40 µmol g^{-1} FW), significantly lower than in all stressed treatments. Both drought and salinity markedly increased proline content (6.01 and 7.26 µmol g^{-1} FW, respectively), indicating a strong stress-induced accumulation. The highest proline level was recorded under DS (9.51 µmol g^{-1} FW), significantly exceeding all other treatments and highlighting the particularly strong osmotic and ionic constraints imposed by combined drought–salinity.

In TL2, comparing only the stressed plants without ANE, the same pattern as in the first year was observed, with proline reaching its maximum under DS and intermediate values under D and S. The two-way ANOVA further revealed that ANE application significantly reduced proline accumulation in several treatments: by about 14 % in C+ANE, 7 % in D+ANE, and 19 % in DS+ANE, while no significant change was detected in S+ANE. Importantly, the qualitative ranking among treatments remained the same in the presence of ANE, with control plants still showing the lowest proline levels, single stress treatments intermediate, and combined stress treatment the highest.

Table 19. Physiological parameters of *T.* × *citriodorus* in TL1 (2024, 120 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		Chlorophyll a (µg g^{-1} FW)	**Chlorophyll b** (µg g^{-1} FW)	**Total chlorophyll** (µg g^{-1} FW)	**Carotenoids** (µg g^{-1} FW)	**RWC** (%)	**Proline** (µmol proline g^{-1} FW)	**Soluble sugars** (mg glucose g^{-1} FW)
Experiment		TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) *	TL1 (120 mM) **	TL1 (120 mM) **
Treatments	**C**	2117.36 ± 29.78^{a}	784.57 ± 54.79^{a}	2901.20 ± 77.07^{a}	584.54 ± 30.18^{a}	88.44 ± 2.07^{a}	2.40 ± 0.20^{a}	21.80 ± 1.45^{a}
	D	1989.20 ± 127.56^{a}	732.67 ± 23.18^{a}	2721.20 ± 150.74^{a}	543.83 ± 14.60^{b}	82.97 ± 0.15^{b}	6.01 ± 0.80^{b}	33.32 ± 4.14^{b}
	S	1442.08 ± 298.45^{b}	490.08 ± 131.70^{b}	1931.70 ± 429.41^{b}	440.62 ± 86.60^{c}	77.79 ± 1.76^{c}	7.26 ± 0.81^{b}	43.67 ± 4.43^{c}
	DS	1358.00 ± 48.75^{b}	455.89 ± 31.11^{b}	1813.50 ± 65.50^{b}	431.73 ± 9.31^{d}	71.13 ± 3.83^{d}	9.51 ± 1.77^{c}	48.11 ± 2.31^{c}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. RWC: relative water content; FW: fresh weight; TL1:1st experiment of *T.* × *citriodorus*; C: control; D: drought stress; S: salinity stress; DS: combined stress; *: $p \leq 0.05$; **: $p < 0.01$.

Table 20. Physiological parameters of *T.* × *citriodorus* in TL2 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		Chlorophyll a (µg g^{-1} FW)	**Chlorophyll b** (µg g^{-1} FW)	**Total chlorophyll** (µg g^{-1} FW)	**Carotenoids** (µg g^{-1} FW)	**RWC** (%)	**Proline** (µmol proline g^{-1} FW)	**Soluble sugars** (mg glucose g^{-1} FW)
Treatments	**C**	1806.89 ± 133.82^{Ab}	1022.77 ± 155.07^{Ab}	2828.89 ± 237.48^{Ac}	697.77 ± 147.54^{Ab}	85.15 ± 4.46^{Ab}	1.92 ± 0.19^{Aa}	14.43 ± 2.39^{Aa}
	C + ANE	1792.05 ± 95.13^{Aa}	1238.01 ± 80.03^{Ab}	3029.20 ± 109.63^{Ab}	805.11 ± 16.56^{Ab}	85.46 ± 4.41^{Aa}	1.66 ± 0.09^{Ba}	12.66 ± 3.56^{Aa}
	D	1615.30 ± 90.45^{Ab}	843.61 ± 59.31^{Aa}	2458.26 ± 128.96^{Ab}	554.36 ± 59.26^{Aab}	76.14 ± 5.16^{Aab}	5.56 ± 0.20^{Ab}	21.24 ± 1.32^{Ab}
	D + ANE	1621.38 ± 189.51^{Aa}	890.12 ± 135.19^{Aa}	2510.82 ± 314.42^{Aa}	624.62 ± 81.13^{Aa}	82.55 ± 3.13^{Aa}	5.18 ± 0.10^{Bb}	14.85 ± 2.63^{Bab}
	S	1321.97 ± 142.80^{Aa}	752.34 ± 103.70^{Aa}	2073.74 ± 44.92^{Aa}	525.42 ± 52.43^{Aab}	79.34 ± 4.02^{Aab}	5.87 ± 0.40^{Ab}	26.19 ± 2.08^{Abc}
	S + ANE	1588.29 ± 108.02^{Ba}	848.64 ± 128.08^{Aa}	2436.27 ± 210.30^{Ba}	549.85 ± 119.44^{Aa}	82.86 ± 2.41^{Aa}	5.38 ± 0.27^{Ab}	13.79 ± 3.33^{Ba}
	DS	1288.60 ± 61.14^{Aa}	824.33 ± 197.31^{Aa}	2112.34 ± 186.80^{Aab}	498.50 ± 39.51^{Aa}	67.54 ± 3.46^{Aa}	8.97 ± 0.38^{Ac}	30.46 ± 3.13^{Ac}
	DS + ANE	1496.22 ± 287.72^{Aa}	872.16 ± 216.63^{Aa}	2367.73 ± 181.62^{Aa}	542.46 ± 28.59^{Aa}	81.08 ± 3.94^{Ba}	7.31 ± 0.30^{Bc}	21.88 ± 0.54^{Bb}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). RWC: relative water content; FW: fresh weight; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.2.2.4 Effects of applied stressors and *A. nodosum* extract on the soluble sugars content

To further characterise the osmotic adjustment of *T.* × *citriodorus* under drought, salinity, and their combination, we also examined the accumulation of soluble sugars. In TL1, control exhibited the lowest soluble sugar content (21.80 mg glucose g^{-1} FW). Drought induced a significant increase to 33.32 mg glucose g^{-1} FW, while salinity and combined stress promoted even stronger accumulations, reaching 43.67 and 48.11 mg glucose g^{-1} FW, respectively. This reveals a clear, progressive rise in soluble sugars from control to drought, salinity, and finally combined stress, with the highest values recorded in the salinity-containing treatments.

In TL2, the same general pattern was observed in plants without *A. nodosum*, with a stepwise increase in sugar content from C to D, S, and DS, mirroring the gradient of stress severity. When ANE was applied, however, sugar levels under D, S, and DS were significantly reduced compared with their respective non-ANE treatments (by about 30 %, 47 %, and 28 %, respectively), indicating a marked attenuation of stress-induced sugar accumulation.

5.2.3 Effects of applied stressors and *A. nodosum* extract on the biochemical traits of *T.* × *citriodorus*

5.2.3.1 Effects of applied stressors and *A. nodosum* extract on the essential oil content

All biochemical parameters for *T.* × *citriodorus* are summarised in Tables 21 and 22. In TL1, with 120 mM NaCl as the salinity stress, control plants recorded the highest essential oil content (EOC) at 0.449 ml 100 g^{-1} DW, significantly higher ($p \leq 0.05$) than all other treatments, indicating optimal conditions for EO accumulation. Drought significantly reduced EOC to 0.303 ml 100 g^{-1} DW. Salinity caused a slightly stronger decline than drought, though not significantly different from D, while the combination of drought and salinity resulted in the lowest EOC (0.186 ml 100 g^{-1} DW), significantly lower than C and D but statistically similar to S, highlighting the pronounced negative effect of combined stress on EO production.

In TL2, the pattern differed somewhat. Only DS caused a clear and significant reduction in EO content (−31 %), while the other treatments maintained statistically similar levels. *A. nodosum* extract application significantly improved EOC under drought (+18 %) and combined stress (+21 %), and although EO levels under S+ANE also increased, the difference was not statistically significant. Comparisons among all ANE-treated groups revealed no significant differences, suggesting that in the presence of ANE, EO content remains relatively stable even under stress conditions.

5.2.3.2 Effects of applied stressors and *A. nodosum* extract on the total polyphenol

Total polyphenol content (TPC) was quantified in both trials in *T.* × *citriodorus*, as presented in Tables 21 and 22. In TL1, TPC reached its highest value in the control (242.79 mg GAE g^{-1} DW), which was statistically similar to drought (238.60 mg GAE g^{-1} DW), indicating that drought alone had little impact on phenolic accumulation. In contrast, salinity significantly reduced TPC to

192.95 mg GAE g^{-1} DW, and combined stress further lowered it to 160.74 mg GAE g^{-1} DW, in comparison to C and D.

In TL2, despite the same salinity level (120 mM), only combined stress treatment caused a significant decline in TPC, decreasing from 235.08 mg GAE g^{-1} DW in C to 207.00 mg GAE g^{-1} DW in DS, while other individual stress treatments did not differ significantly from the control. When *A. nodosum* extract was applied, its positive effect on phenolic metabolism became evident: TPC increased in all stressed treatments, by about 23 % in D+ANE, 26 % in S+ANE, and 8.42 % in DS+ANE, highlighting the capacity of *A. nodosum* extract to boost phenolic content under adverse conditions. Interestingly, when comparing *A. nodosum* extract treatments among themselves, C+ANE and DS+ANE showed significantly lower TPC than D+ANE and S+ANE, suggesting that phenolic accumulation is particularly stimulated in plants subjected to single stress treatments in the presence of ANE.

5.2.3.3 Effects of applied stressors and *A. nodosum* extract on the antioxidant capacity

For deeper insight into the redox balance of *T. × citriodorus* under stress and *A. nodosum* extract application, total antioxidant capacity was evaluated, as shown in Tables 21 and 22. In TL1, control displayed the highest AOC (150.60 mg AAE g^{-1} DW), statistically similar to drought (147.70 mg AAE g^{-1} DW), indicating that drought alone did not markedly compromise antioxidant capacity. In contrast, salinity significantly ($p \leq 0.05$) reduced AOC to 93.28 mg AAE g^{-1} DW, while combined stress caused an even stronger decline to 62.28 mg AAE g^{-1} DW, severely impairing the antioxidant system compared with the other treatments.

In TL2, AOC followed the same general tendency observed for many other traits in *T. × citriodorus*: values decreased numerically with increasing stress severity, but only DS showed a statistically significant reduction (about −27 % compared with C), while D and S remained statistically similar to the control. In this trial, *A. nodosum* application produced one of the most remarkable responses across the experiments: AOC increased significantly in all four treatments, C+ANE, D+ANE, S+ANE, and DS+ANE, by approximately 43 %, 54 %, 83 %, and 92 %, respectively. This pattern indicates that ANE consistently enhances AOC, with particularly strong stimulation under salinity and combined drought–salinity. Among the *A. nodosum* extract-treated groups, S+ANE exhibited the highest AOC, significantly surpassing the other treatments.

5.2.3.4 Effects of applied stressors and *A. nodosum* extract on the hydrogen peroxide

The results of hydrogen peroxide analysis in TL1 and TL2 are summarised in Tables 21 and 22. In TL1, under non-stress conditions, control showed a basal H_2O_2 level of 0.67 μmol H_2O_2 g^{-1} FW, significantly lower than all stressed treatments. Drought already induced a marked and significant increase in H_2O_2 (2.50 μmol H_2O_2 g^{-1} FW) compared with the control, indicating the onset of oxidative stress. The highest H_2O_2 levels were recorded under salinity and combined stress (3.41 and 3.74 μmol H_2O_2 g^{-1} FW, respectively), which were statistically similar to each other, pointing to a strong oxidative burden in plants exposed to salinity, either alone or combined with drought.

In TL2, control again maintained the lowest H_2O_2 concentration, while values increased stepwise in D and S, which displayed similar levels in this trial, and reached their maximum under DS, significantly higher than in all other treatments. *A. nodosum* extract application significantly reduced H_2O_2 accumulation under salinity and combined stress, lowering values from 2.52 to 1.84 µmol H_2O_2 g^{-1} FW in S+ANE and from 3.75 to 2.84 µmol H_2O_2 g^{-1} FW in DS+ANE. By contrast, only minor changes were observed in C+ANE and D+ANE, indicating a limited effect under non-stress or mild stress conditions.

5.2.3.5 Effects of applied stressors and *A. nodosum* extract on the essential oil composition

Tables 32 and 33 in the Appendices detail the volatile oil components of *T. × citriodorus* essential oils in TL1 and TL2 as determined by GC-MS. In TL1, a total of 17 compounds were identified, which can be classified into three groups: oxygenated monoterpenes (linalool, borneol, nerol, thymol methyl ether, neral, geraniol, geranial, thymol, geranyl acetate, geranyl butanoate, geranyl 2-methyl butanoate), sesquiterpenes (β-caryophyllene, β-bisabolene, caryophyllene oxide), and aliphatic compounds (1-octen-3-ol, 3-octanone, 3-octanol). Oxygenated monoterpenes dominated the profile, reaching 84.99 % under combined stress and peaking at 88.96 % in control. Aliphatic compounds followed a similar decreasing trend with stress, showing the lowest proportion in DS (1.49 %) compared with 2.38 % in control. Conversely, sesquiterpenes exhibited the opposite pattern, increasing under stress: C (6.27 %), D (9.12 %), S (10.50 %), DS (11.11 %).

In TL2, GC-MS identified 18 compounds, which were grouped similarly to TL1. The response of these compound groups to stress mirrored TL1. Regarding *A. nodosum* extract, it tended to stabilise compound levels close to the control. For instance, oxygenated monoterpenes in C were 92.14 %, decreased to 87.73 % under DS, but with ANE, C+ANE retained 91.22 % and DS+ANE increased to 89.48 %. Similar stabilising effects were observed in sesquiterpenes and aliphatic compounds, particularly in DS, while D and S showed variable responses when ANE was applied.

Among the identified compounds, the four most abundant, geraniol, geranial, neral, and β-bisabolene, were selected as representative of *T. × citriodorus as* shown in tables 21 and 22. Geraniol, the most dominant, was highly sensitive to stress in TL1, decreasing from 60.40 % in C to 52.80 % in D, 55.41 % in S, and 50.09 % in DS, with no significant differences among the three stressed groups. TL2 confirmed this trend, with geraniol highest in C and progressively decreasing from D to S to DS, where all stressed groups were significantly different from each other. Two-way ANOVA showed that ANE increased geraniol only in DS (+8.49 %), with no effect on other groups. Comparisons among ANE treatments indicated that geraniol proportions remained statistically stable across treatments.

Geranial, the second most abundant compound, displayed distinct patterns across treatments. In TL1, its lowest proportion was in the control (12.29 %), not significantly different from drought and salinity. Drought tended to slightly increase geranial more than S, but the difference remained statistically insignificant, whereas DS clearly raised geranial to 15.29 %. In TL2, a slightly different pattern was observed: the highest geranial level was in S (17.18 %), significantly different

Table 21. Biochemical parameters of *T.* × *citriodorus* in TL1 (2024, 120 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		EOC (ml 100 g^{-1} DW)	**TPC** (mg GAE g^{-1} DW)	**AOC** (mg AAE g^{-1} DW)	**H_2O_2** (µmol H_2O_2 g^{-1} FW)	**geraniol** (%)	**geranial** (%)	**neral** (%)	**β-bisabolene** (%)
Experiment		TL1 (120 mM) *	TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) **	TL1 (120 mM) *	TL1 (120 mM) **	TL1 (120 mM) *
Treatments	**C**	0.449 ± 0.08^{a}	242.79 ± 10.27^{a}	150.60 ± 19.38^{a}	0.67 ± 0.12^{a}	60.40 ± 0.58^{a}	12.29 ± 0.85^{a}	8.26 ± 0.07^{a}	2.55 ± 0.34^{a}
	D	0.303 ± 0.03^{b}	238.60 ± 34.67^{a}	147.70 ± 20.54^{a}	2.50 ± 0.07^{b}	52.80 ± 3.94^{b}	14.36 ± 0.89^{ab}	9.57 ± 0.6^{bc}	3.90 ± 0.65^{ab}
	S	0.200 ± 0.02^{bc}	192.95 ± 30.48^{b}	93.28 ± 18.31^{b}	3.41 ± 0.55^{c}	55.41 ± 1.24^{b}	12.48 ± 0.24^{a}	8.38 ± 0.57^{ab}	4.14 ± 0.85^{ab}
	DS	0.186 ± 0.01^{c}	160.74 ± 18.47^{b}	62.28 ± 8.69^{c}	3.74 ± 0.14^{c}	50.09 ± 2.18^{b}	15.29 ± 1.44^{b}	10.39 ± 0.53^{c}	4.25 ± 0.46^{b}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. EOC: essential oil content; TPC: total polyphenol content; GAE: gallic acid equivalents; AOC: antioxidant capacity; AAE: ascorbic acid equivalents; H_2O_2: hydrogen peroxide; FW: fresh weight; DW: dry weight; TL1:1st experiment of *T.* × *citriodorus*; C: control; D: drought stress; S: salinity stress; DS: combined stressors; *: $p \leq 0.05$; **: $p < 0.01$.

Table 22. Biochemical parameters of *T.* × *citriodorus* in TL2 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		EOC (ml 100 g^{-1} DW)	**TPC** (mg GAE g^{-1} DW)	**AOC** (mg AAE g^{-1} DW)	**H_2O_2** (µmol H_2O_2 g^{-1} FW)	**geraniol** (%)	**geranial** (%)	**neral** (%)	**β-bisabolene** (%)
Treatments	**C**	0.55 ± 0.04^{Aa}	235.08 ± 31.91^{Aa}	106.65 ± 24.62^{Ab}	1.33 ± 0.26^{Aa}	62.60 ± 0.22^{Ad}	13.52 ± 0.36^{Aa}	10.61 ± 0.27^{Aa}	1.33 ± 0.10^{Aa}
	C + ANE	0.56 ± 0.07^{Aa}	248.73 ± 27.10^{Aa}	152.78 ± 38.55^{Ba}	1.23 ± 0.08^{Aa}	59.80 ± 2.56^{Aa}	15.27 ± 0.91^{Ab}	10.57 ± 0.63^{Ab}	1.40 ± 0.25^{Aa}
	D	0.49 ± 0.05^{Aa}	233.56 ± 42.33^{Aa}	100.23 ± 29.62^{Ab}	2.45 ± 0.26^{Ab}	58.82 ± 1.29^{Ac}	15.44 ± 2.04^{Aab}	10.12 ± 0.46^{Ba}	2.43 ± 0.34^{Ab}
	D + ANE	0.58 ± 0.10^{Ba}	287.19 ± 29.28^{Bb}	154.40 ± 39.50^{Ba}	2.01 ± 0.16^{Ab}	60.62 ± 1.59^{Aa}	13.05 ± 0.35^{Aa}	9.06 ± 0.21^{Aa}	1.92 ± 0.39^{Aa}
	S	0.48 ± 0.07^{Aa}	226.08 ± 25.58^{Aa}	104.31 ± 18.18^{Ab}	2.52 ± 0.17^{Bb}	55.45 ± 0.59^{Ab}	17.18 ± 0.97^{Ab}	13.27 ± 0.57^{Bb}	2.28 ± 0.07^{Bb}
	S + ANE	0.54 ± 0.06^{Aa}	284.60 ± 44.14^{Bb}	190.61 ± 40.69^{Bb}	1.84 ± 0.21^{Ab}	57.53 ± 1.88^{Aa}	15.42 ± 0.57^{Bb}	11.04 ± 0.56^{Ab}	1.44 ± 0.10^{Aa}
	DS	0.38 ± 0.11^{Ab}	207.00 ± 31.75^{Ab}	77.70 ± 23.00^{Aa}	3.75 ± 0.36^{Bc}	50.49 ± 1.08^{Aa}	16.09 ± 1.22^{Aab}	13.87 ± 1.17^{Bb}	3.56 ± 0.15^{Bc}
	DS + ANE	0.46 ± 0.10^{Ba}	244.45 ± 27.63^{Ba}	148.16 ± 32.27^{Ba}	2.84 ± 0.21^{Ac}	58.98 ± 0.82^{Ba}	14.91 ± 0.28^{Bb}	10.30 ± 0.06^{Ab}	1.36 ± 0.16^{Aa}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). EOC: essential oil content; TPC: total polyphenol content; GAE: gallic acid equivalents; AOC: antioxidant capacity; AAE: ascorbic acid equivalents; H_2O_2: hydrogen peroxide; FW: fresh weight; DW: dry weight; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

from C (13.52 %). *A. nodosum* extract application significantly decreased geranial in S (from 17.18 to 15.42 %) and DS (from 16.09 to 14.91 %), while D and C remained stable. Comparisons among ANE treatments showed that geranial proportions were maintained across treatments.

Neral exhibited a similar tendency to geranial. In TL1, control recorded the lowest level (8.26 %), significantly lower than drought and combined stress. Drought alone or combined with salinity increased neral, while S had only a mild effect. In TL2, only S and DS significantly increased neral compared with C and D, with D remaining stable. *A. nodosum* extract application significantly reduced neral in all stressed groups: D+ANE (−1.06 %), S+ANE (−2.23 %), and DS+ANE (−3.57 %), producing more uniform levels across treatments.

β-bisabolene, a minor sesquiterpene (1–4 %), tended to increase under stress. In TL1 and TL2, combined stress showed the highest levels (4.25 % and 3.56 %, respectively), while drought and salinity were intermediate. *A. nodosum* extract slightly reduced β-bisabolene under salinity and combined stress, suggesting a modest suppressive effect.

5.2.4 Evaluation of stress indices in *T.* × *citriodorus*

The stress susceptibility indices (SSI) for TL1 and TL2 together outline a clear gradient in the sensitivity of *T.* × *citriodorus* to drought, salinity, and their combination, while also emphasising the mitigating effect of *A. nodosum* extract (Figure 17). In TL1, *T.* × *citriodorus* behaves as drought-tolerant (SSI = 0.12), moderately sensitive to salinity (SSI = 0.87), and highly susceptible to combined drought–salinity (SSI = 2.00), showing that the interaction between water deficit and salinity is far more damaging than either stress alone. In TL2, where *A. nodosum* extract was included, the pattern becomes more nuanced: in the absence of ANE, all three stress treatments (D, S, DS) fall in the susceptible range (SSI ≥ 1), with DS remaining the harshest condition. However, when *A. nodosum* extract is applied, drought clearly shifts into the tolerant category (D+ANE, 0.32), salinity moves to a moderate susceptibility level (S+ANE, 0.61), and combined stress also improves from high susceptibility to a moderate level (DS+ANE, 0.71).

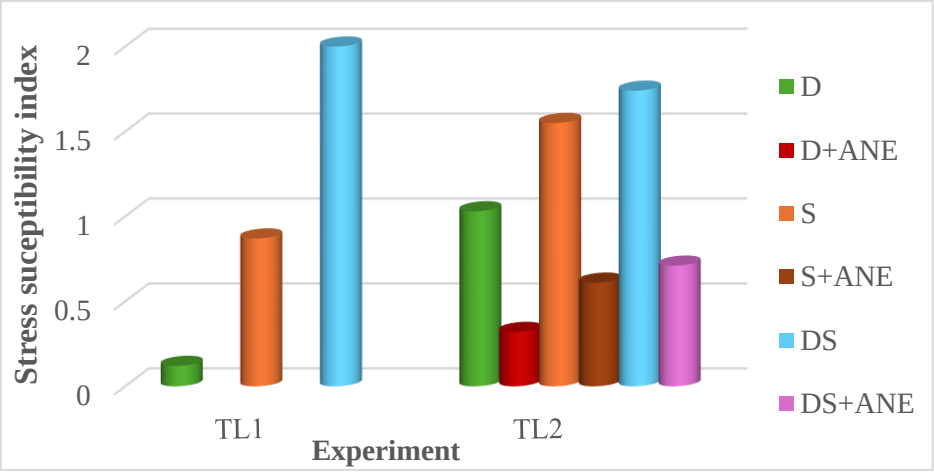

Figure 17. Stress susceptibility index of *T.* × *citriodorus* across all the experiments (TL1, TL2). D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

The stress tolerance index (STI) values confirm the SSI-based interpretation of *T.* × *citriodorus* performance under drought, salinity, and their combination, as shown in Figure 18. In TL1, drought displays a relatively high STI (0.95), indicating that plants are able to maintain performance quite well under water deficit. Salinity leads to a moderate reduction (STI = 0.68), whereas combined drought–salinity shows the lowest STI (0.26), reflecting a drastic loss of productivity under DS and supporting its high susceptibility.

In TL2, STI values are overall higher than in TL1 for comparable stress treatments, but the same hierarchy is preserved: D and D+ANE (0.94–0.98) show the best performance, salinity treatments (S and S+ANE: 0.79 and 0.92) occupy an intermediate position, and DS (0.57) remains the weakest, although DS+ANE (0.90) displays a marked recovery. The comparison between +ANE and –ANE treatments in TL2 reveals this *A. nodosum* extract has almost no effect on drought STI (both values are already high), slightly improves STI under salinity (from 0.79 to 0.92), and very strongly enhances performance under combined stress (from 0.57 to 0.90).

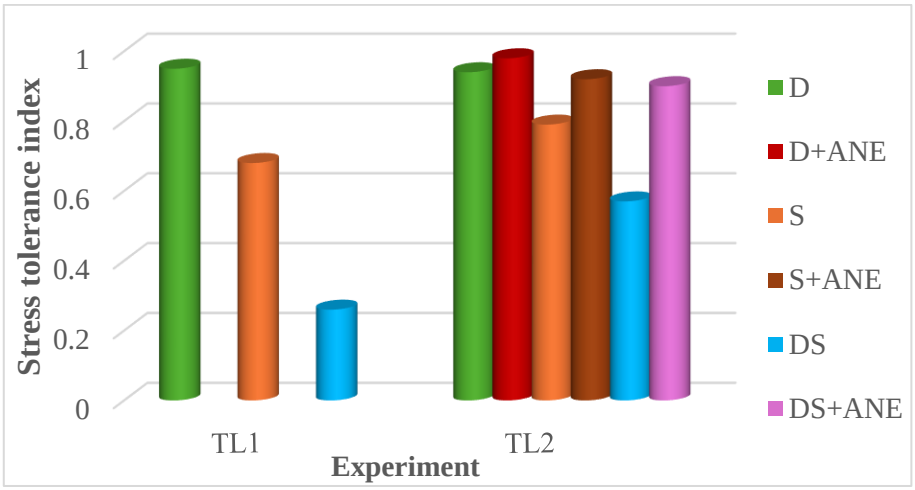

Figure 18. Stress tolerance index of *T.* × *citriodorus* across all the experiments (TL1, TL2). D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.2.5 Correlation and principal component analysis of *T.* × *citriodorus* parameters

The correlations among the morphological, biomass, physiological, and biochemical parameters of *T.* × *citriodorus* in TL2 are shown in the clustered heatmap in Figure 19. The heatmap reveals two clearly separated main clusters, each grouping parameters that are strongly interrelated according to Pearson's correlation.

The first cluster corresponds to stress markers and stress-related volatiles. Proline, H_2O_2, soluble sugars, β-bisabolene, geranial, and neral form a compact group with very strong positive correlations among themselves, represented by yellow to light-green colours. These variables are, in turn, negatively correlated (dark blue) with almost all growth-related, pigment, and water-status traits. This configuration highlights a clear trade-off: as stress markers rise, growth performance, hydration status, and photosynthetic capacity tend to decline.

The second cluster brings together the growth and performance related parameters. Within this block, several meaningful sub-structures can be distinguished. Geraniol, the key volatile in *T.* × *citriodorus*, shows positive correlations with pigments (chlorophyll a and carotenoids), RWC, EOC, and TPC, indicating that higher geraniol levels are associated with better physiological status and improved essential oil quality rather than with stress conditions. The photosynthetic pigment sub-block (chlorophyll a, chlorophyll b, carotenoids) is strongly correlated ($r > 0.5$) with RWC, geraniol, EOC, and TPC, suggesting that good water relations and preserved pigment integrity support higher phenolic accumulation and essential-oil production.

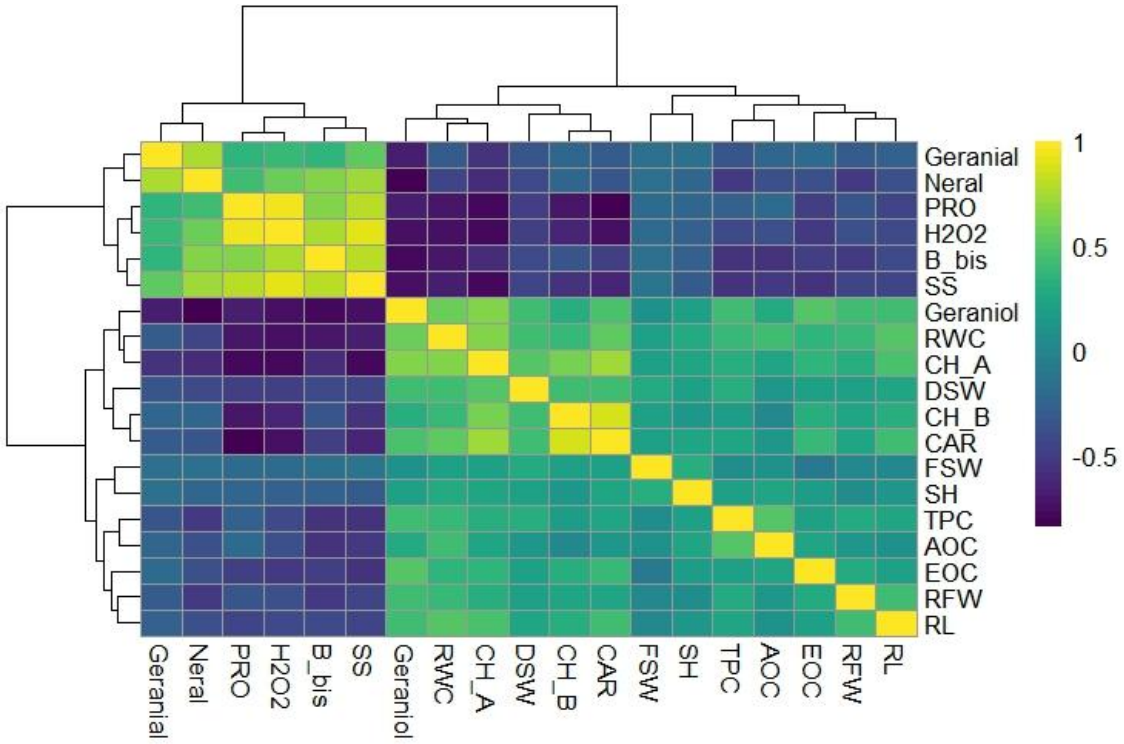

Figure 19. Hierarchical clustering heatmap of morphological, biomass, physiological and biochemical parameters of *T.* × *citriodorus* in TL2. SH: shoot length; DSW: dry shoot weight; FSW: fresh shoot weight; EOC: essential oil content; RFW: fresh root weight; RL: root length; AOC: antioxidant capacity; TPC: total polyphenol content; RWC: relative water content; CH_B: chlorophyll b; CH_A: chlorophyll a; CAR: carotenoids; B_bis: β-bisabolene; SS: soluble sugars; H_2O_2: hydrogen peroxide; PRO: proline.

Another sub-block includes root traits and some biomass related parameters (root length, root fresh weight, dry shoot weight, shoot length) and, to a lesser extent, fresh shoot weight, along with RWC, EOC, geraniol, TPC, AOC, and chlorophyll a. These correlations are generally positive but moderate ($0 < r < 0.5$), indicating that plants with greater shoot and root biomass tend to show somewhat higher antioxidant capacity, phenolic content, and EO yield. This pattern points to a plausible mechanistic chain: longer and more massive roots enhance water uptake, which improves RWC; better hydration helps maintain chlorophyll and carotenoid levels, sustaining photosynthesis and allowing more carbon to be channelled into secondary metabolites (phenolics, essential oil). Since these relationships are moderate rather than very strong, root and shoot biomass contribute to, but do not fully determine, essential oil quality.

The biplot of the principal component analysis for *T.* × *citriodorus* in TL2 is shown in Figure 20. The first principal component, Dim1 (47 %), represents the main gradient from severe stress on the left to vigorous, better performing or only slightly stressed plants on the right. Dim2 (10 %) mainly separates biostimulated (*A. nodosum* extract-treated) from non-biostimulated treatments, with the exception of the control.

Regarding treatment positions, the PCA clearly shows a distinct separation of DS, located furthest to the left at (−5.6; 0.9), representing a highly stressed profile clearly isolated from all other groups. Also on the left side, but closer to the centre, S (−2.8; 0.6) indicates that salinity alone is still strongly stressful, though less extreme than combined drought–salinity. Near the origin, D (−0.4; 0) reflects a moderate drought effect, with some deviation from the control but not as drastic as S or DS. Just below on Dim2, DS+ANE (−0.3; −1.5) forms a specific response pattern: *A. nodosum* extract under combined stress shifts plants away from the "pure stress" DS cluster towards the central zone, but with a distinct biochemical profile. Slightly to the right of the origin and below it, S+ANE (0.3; −1.2) suggests a clear improvement compared with S, moving towards the performance zone, although still not reaching the full control area. D+ANE (2.0; −1.6) lies clearly on the positive side of Dim1, indicating a strong recovery under drought when *A. nodosum* extract is applied. Control (3.4; 0.8) is positioned on the right, reflecting healthy, vigorous plants under optimal conditions, and close to it, furthest to the right and highest on Dim2, located C+ANE (3.6; 2.1), the best-performing group, indicating that ANE further enhances growth, pigments, and/or water status even in the absence of stress.

The orientation of the variable vectors reinforces this structure. On the left side, proline, soluble sugars, H_2O_2, β-bisabolene, geranial, and neral, all defence or stress related markers, are oriented towards DS and S. Their association with these treatments indicates that plants exposed to strong combined drought–salinity or salinity alone accumulate osmolytes, reactive oxygen species, and stress-related volatiles. On the right side, most growth and performance related parameters, RWC, chlorophyll a and b, carotenoids, fresh shoot weight, dry shoot weight, shoot length, root fresh weight, root length, and EOC, point towards C and C+ANE, indicating better water balance, a stronger photosynthetic apparatus, and higher biomass under control and *A. nodosum* extract-treated control conditions. TPC and AOC are positioned closer to D+ANE and, to some extent, DS+ANE and S+ANE, suggesting that *A. nodosum* extract under drought, and to a lesser degree under combined and salt stress, promotes an antioxidant- and phenolic-rich profile, which may contribute to improved tolerance.

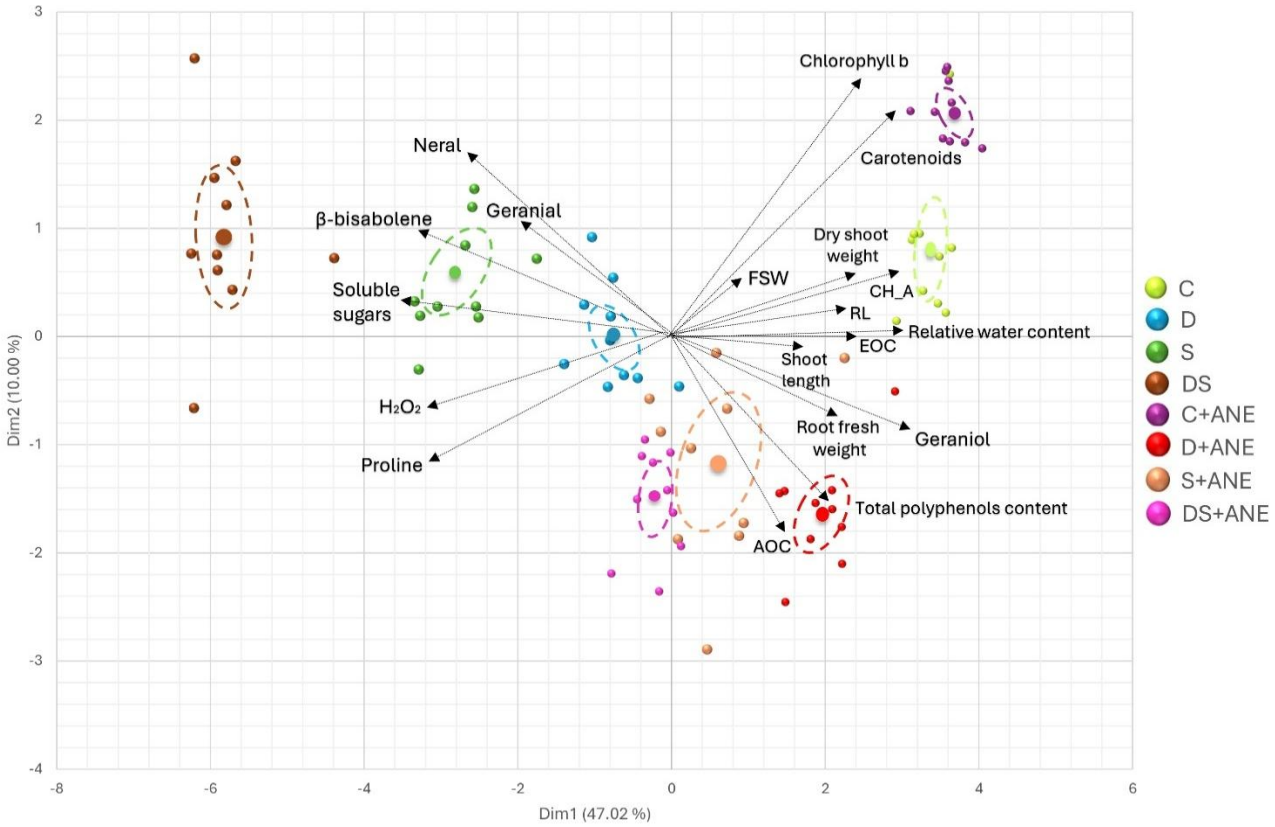

Figure 20. Biplot of principal component analysis illustrating the relationships among the studied parameters of *T.* × *citriodorus* when subjected to various stress and *A. nodosum* extract conditions in TL2. Dim: dimension; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract; FSW: fresh shoot weight; EOC: essential oil content; RL: root length; AOC: antioxidant capacity; CH_A: chlorophyll a; H_2O_2: hydrogen peroxide.

5.3 Evaluation of *T. capitatus* experiments

5.3.1 Effects of applied stressors and *A. nodosum* extract on the morphology and biomass of *T. capitatus*

5.3.1.1 Effects of applied stressors and *A. nodosum* extract on the shoot length

The morphological and biomass analysis of shoot and root parameters for *T. capitatus* in TC1 and TC2 is presented in Tables 23 and 24, respectively. Regarding shoot length in TC1, plants subjected to combined stress (DS) were the smallest, with a significant reduction in shoot elongation to 20.70 cm compared to control (C) and salinity (S) treatments, highlighting the strong inhibitory effect of simultaneous drought (D, 40 % SWC) and salinity (90 mM NaCl). Control plants were the tallest at 27.50 cm, significantly exceeding DS and D. Drought alone caused a moderate decrease in shoot length to 22.25 cm, statistically lower than the control, while salinity plants reached 25.50 cm, an intermediate value not significantly different from control or drought. Control plants were the tallest at 27.50 cm, significantly exceeding DS and D. In TC2, where a higher salinity level (120 mM) was applied, only the combined stress group exhibited a significant decrease in shoot length, about 15 % shorter than the control. Regarding *A. nodosum* extract (ANE)-treated plants, no significant enhancement of shoot length was observed across any treatment, although numerically ANE extract appeared to buffer stress effects, resulting in more

uniform shoot elongation. Comparisons among ANE extract treatments revealed no differences between them.

5.3.1.2 Effects of applied stressors and *A. nodosum* extract on the number shoots

As shown in Tables 23 and 24, the number of shoots of *T. capitatus* was also influenced by the applied stressors. In TC1, control plants produced the highest number of shoots, with an average of 29.25 pcs plant^{-1}, significantly ($p \leq 0.05$) higher than all stressed treatments. The lowest number of shoots was recorded under combined stress (14.25 pcs plant^{-1}), which was significantly lower than C and S but statistically similar to drought (17.00 pcs plant^{-1}). Drought showed an intermediate mean, overlapping statistically with both DS and salinity (S, 19.25 pcs plant^{-1}), confirming that all stress conditions reduced branching, with the strongest inhibition under DS.

In TC2, a somewhat different pattern emerged. Drought maintained an intermediate position and was not statistically different from other treatments, while salinity and combined stress clearly and significantly reduced shoot number compared with the control. The application of *A. nodosum* slightly improved number of shoots under salinity, increasing it from 34.80 pcs plant^{-1} in S to 38.50 pcs plant^{-1} in S+ANE. Under drought and combined stress, the effect of *A. nodosum* extract was more limited but tended to narrow the gap between control and stressed plants.

5.3.1.3 Effects of applied stressors and *A. nodosum* extract on the fresh shoot weight

Regarding fresh shoot weight, in TC1, where 90 mM NaCl was applied as salinity stress, one-way ANOVA showed that control plants had a significantly higher fresh shoot weight (12.69 g plant^{-1}) than all stressed treatments. Drought, salinity, and combined stress displayed statistically similar fresh shoot weight values (7.94, 9.18, and 6.53 g plant^{-1}, respectively), confirming a clear reduction in shoot biomass compared with the control. In TC2, where a higher salinity level (120 mM) was used, the control again maintained significantly higher fresh shoot weight than stressed treatments, indicating a similar response pattern to TC1. Two-way ANOVA further revealed a significant interaction between stress and *A. nodosum* extract application: ANE markedly improved fresh shoot weight in drought and salinity by approximately 32 % and 20 %, respectively, whereas no noticeable improvement was observed in DS+ANE. Overall, drought and salinity strongly reduced the fresh shoot weight of *T. capitatus*. In TC1, all stress treatments (D, S, and DS) significantly decreased shoot biomass relative to the control, with no marked differences among them, highlighting the high sensitivity of shoot growth to both water and salt limitation. In TC2, the application of *A. nodosum* extract partially mitigated these negative effects, as reflected by the higher fresh shoot weight and the reduced gap between control and stressed plants when *A. nodosum* extract was applied, as displayed in Figure 21.

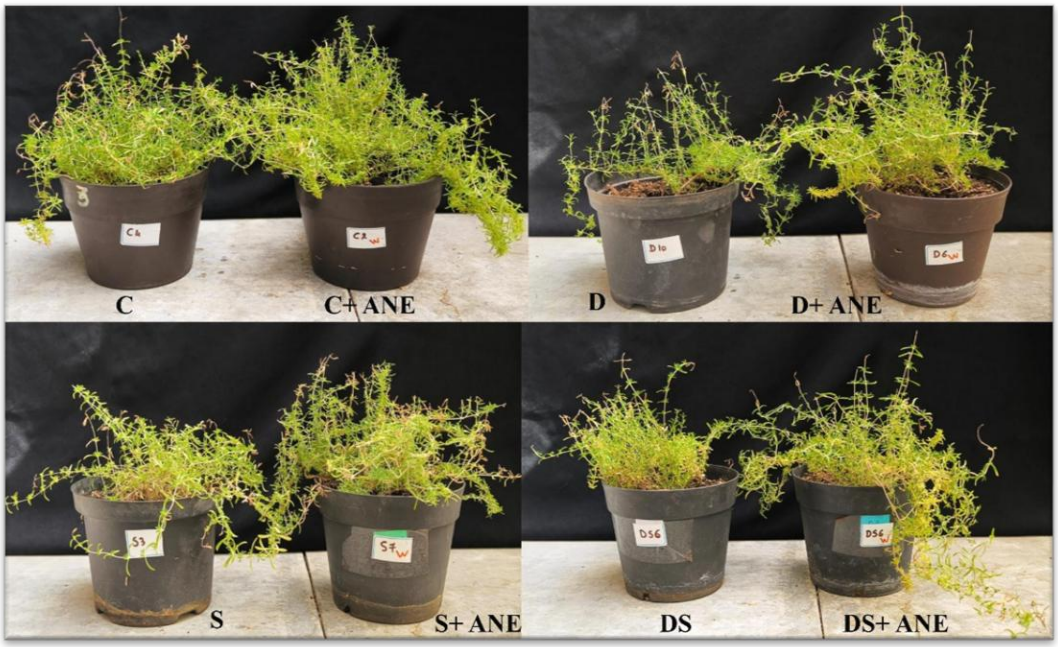

Figure 21. *T. capitatus* plants in TC2 under different treatments applied. C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.3.1.4 Effects of applied stressors and *A. nodosum* extract on the dry shoot weight

As shown in Tables 23 and 24, the dry shoot weight of *T. capitatus* in TC1 was highest in control plants (2.61 g plant^{-1}), significantly exceeding the values observed under drought and combined stress, which were the lowest at 1.52 and 1.44 g plant^{-1}, respectively. This highlights the pronounced negative impact of water deficit and combined stress on dry biomass accumulation. Salinity alone had an intermediate effect (2.09 g plant^{-1}), not significantly different from either the control or the other stress treatments. In TC2, surprisingly, two-way ANOVA did not detect significant differences among stressed treatments, nor any significant effect of *A. nodosum* extract application, despite a slight numerical increase in dry shoot weight across all treatments with *A. nodosum* extract. This indicates that, while drought and combined stress moderately reduced dry shoot biomass in TC1, salinity alone had a comparatively smaller effect. Unlike fresh shoot weight, dry biomass was minimally influenced by *A. nodosum* extract in TC2, suggesting that ANE primarily helps to maintain water content and turgor (reflected in fresh weight) rather than directly promoting the accumulation of structural dry matter.

5.3.1.5 Effects of applied stressors and *A. nodosum* extract on the root length

Root length of *T. capitatus* was also affected by drought, salinity, combined stress, and *A. nodosum* extract application, as shown in Tables 23 and 24. In TC1, control plants exhibited the longest roots (39.53 cm), significantly exceeding all stressed treatments, while D, S, and DS showed much shorter roots (23.98, 26.75, and 23.00 cm, respectively) with no significant differences among them. In TC2, a similar trend was observed, with all stress treatments reducing root length relative to the control. Two-way ANOVA revealed a significant effect of ANE, which increased root length under drought by 78 % and under salinity by 21.5 %, while no significant enhancement was

recorded under combined stress. Notably, D+ANE showed the highest root length in the trial (31.50 cm), even exceeding control values, and was significantly higher than DS+ANE.

5.3.1.6 Effects of applied stressors and *A. nodosum* extract on the fresh weight of roots

Fresh root weight was also evaluated, and the ANOVA results for TC1 and TC2 are presented in Tables 23 and 24. In TC1, all stress treatments, whether applied individually or in combination, significantly reduced root fresh weight compared with the control, while no differences were detected among them. This indicates that, for this trait, combined stress treatment does not statistically exacerbate the reduction in root biomass beyond the effect of single stress treatment. In TC2, when considering treatments without ANE, control again showed the highest root fresh weight (4.90 g plant^{-1}), although it was not statistically different from S, which remained in the same statistical group as C and D despite its numerical reduction (2.63 g plant^{-1}). Drought and combined stress exhibited the lowest root fresh weight values (2.20 and 2.50 g plant^{-1}, respectively), pointing out a marked decrease in root biomass under water deficit and combined stress (Figure 22).

Under ANE treatment, root fresh weight was markedly improved under D and S, increasing by approximately 145 % and 100 %, respectively, and more than doubling the values of the corresponding untreated plants. No significant differences were detected among treatments within the ANE level, suggesting a homogenising effect on root biomass.

5.3.1.7 Effects of applied stressors and *A. nodosum* extract on the dry weight of roots

The dry root weight of *T. capitatus* was strongly influenced by drought, salinity, combined stress, and, in TC2, by *A. nodosum* extract application, as shown in Tables 23 and 24. In TC1, a pattern similar to that observed for root fresh weight was found: all stress treatments (D, S, and DS) significantly reduced root dry weight (0.23, 0.29, and 0.18 g plant^{-1}, respectively) compared with the control (0.74 g plant^{-1}), with no significant differences among the stressed groups. In TC2, under the higher salinity level (120 mM), the same trend persisted, with all stressed treatments reducing root dry weight relative to the control. *A. nodosum* extract application significantly increased root dry weight only in S+ANE (1.09 g plant^{-1} compared with 0.59 g plant^{-1} in S), while other treatments, notably D+ANE, showed numerical improvements that were not statistically significant, indicating a partial compensatory effect of *A. nodosum* extract on stress-induced reductions.

Table 23. Morphological and biomass parameters and biomass of *T. capitatus* in TC1 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		Shoot length (cm)	**Number of shoots** (pcs plant^{-1})	**Fresh shoot weight** (g plant^{-1})	**Dry shoot weight** (g plant^{-1})	**Root length** (cm)	**Fresh root weight** (g plant^{-1})	**Dry root weight** (g plant^{-1})
Experiment		TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **
Treatments	**C**	27.50 ± 5.52^{a}	29.25 ± 2.49^{a}	12.69 ± 6.09^{a}	2.61 ± 1.16^{a}	39.53 ± 1.25^{a}	4.36 ± 0.45^{a}	0.74 ± 0.10^{a}
	D	22.25 ± 4.17^{bc}	17.00 ± 3.85^{bc}	7.94 ± 3.31^{b}	1.52 ± 0.67^{b}	23.98 ± 1.84^{b}	1.65 ± 0.40^{b}	0.23 ± 0.05^{b}
	S	25.50 ± 3.71^{ab}	19.50 ± 4.37^{b}	9.18 ± 3.05^{b}	2.09 ± 0.68^{ab}	26.75 ± 4.34^{b}	2.03 ± 0.75^{b}	0.29 ± 0.10^{b}
	DS	20.70 ± 3.84^{c}	14.25 ± 3.53^{c}	6.53 ± 2.86^{b}	1.44 ± 0.52^{b}	23.00 ± 2.94^{b}	1.33 ± 0.25^{b}	0.18 ± 0.08^{b}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. TC1: 1st experiment of *T. capitatus*; pcs: pieces; C: control; D: drought stress; S: salinity stress; DS: combined stress; ^{ns}: non-significant; **: $p < 0.01$.

Table 24. Morphological and biomass parameters and biomass of *T. capitatus* in TC2 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		Shoot length (cm)	**Number of shoots** (pcs plant^{-1})	**Fresh shoot weight** (g plant^{-1})	**Dry shoot weight** (g plant^{-1})	**Root length** (cm)	**Fresh root weight** (g plant^{-1})	**Dry root weight** (g plant^{-1})
Treatments	**C**	32.20 ± 3.33^{Ab}	41.80 ± 6.18^{Ab}	18.63 ± 5.48^{Ab}	4.25 ± 1.83^{Aa}	21.25 ± 4.19^{Ab}	4.90 ± 1.49^{Ab}	0.88 ± 0.25^{Ab}
	C + ANE	32.90 ± 5.33^{Aa}	44.70 ± 4.01^{Ab}	19.38 ± 5.55^{Aa}	4.46 ± 2.44^{Aa}	22.75 ± 3.77^{Aab}	5.44 ± 1.80^{Aa}	1.19 ± 0.37^{Aa}
	D	30.40 ± 4.40^{Ab}	36.30 ± 5.31^{Aab}	16.76 ± 4.70^{Aa}	4.06 ± 1.50^{Aa}	17.75 ± 1.71^{Aa}	2.20 ± 0.74^{Aa}	0.50 ± 0.17^{Aa}
	D + ANE	31.50 ± 5.68^{Aa}	39.00 ± 3.86^{Aab}	22.07 ± 7.87^{Ba}	5.57 ± 2.18^{Aa}	31.50 ± 3.06^{Bb}	5.40 ± 2.72^{Ba}	0.97 ± 0.44^{Aa}
	S	27.60 ± 4.77^{Ab}	34.80 ± 3.55^{Aa}	15.84 ± 5.73^{Aa}	3.45 ± 1.78^{Aa}	18.00 ± 1.73^{Aa}	2.63 ± 1.18^{Aab}	0.59 ± 0.17^{Aa}
	S + ANE	30.90 ± 4.79^{Aa}	38.50 ± 4.35^{Bab}	19.05 ± 5.54^{Ba}	4.73 ± 1.94^{Aa}	22.13 ± 2.95^{Bab}	5.26 ± 1.18^{Ba}	1.09 ± 0.24^{Ba}
	DS	27.40 ± 6.02^{Aa}	35.10 ± 4.53^{Aa}	12.26 ± 3.84^{Aa}	3.41 ± 1.10^{Aa}	18.00 ± 1.08^{Aa}	2.50 ± 1.01^{Aa}	0.54 ± 0.19^{Aa}
	DS + ANE	27.50 ± 3.57^{Aa}	34.90 ± 3.18^{Aa}	13.98 ± 2.20^{Aa}	4.39 ± 1.89^{Aa}	18.75 ± 2.22^{Aa}	3.50 ± 1.06^{Aa}	0.76 ± 0.24^{Aa}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). pcs, pieces; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

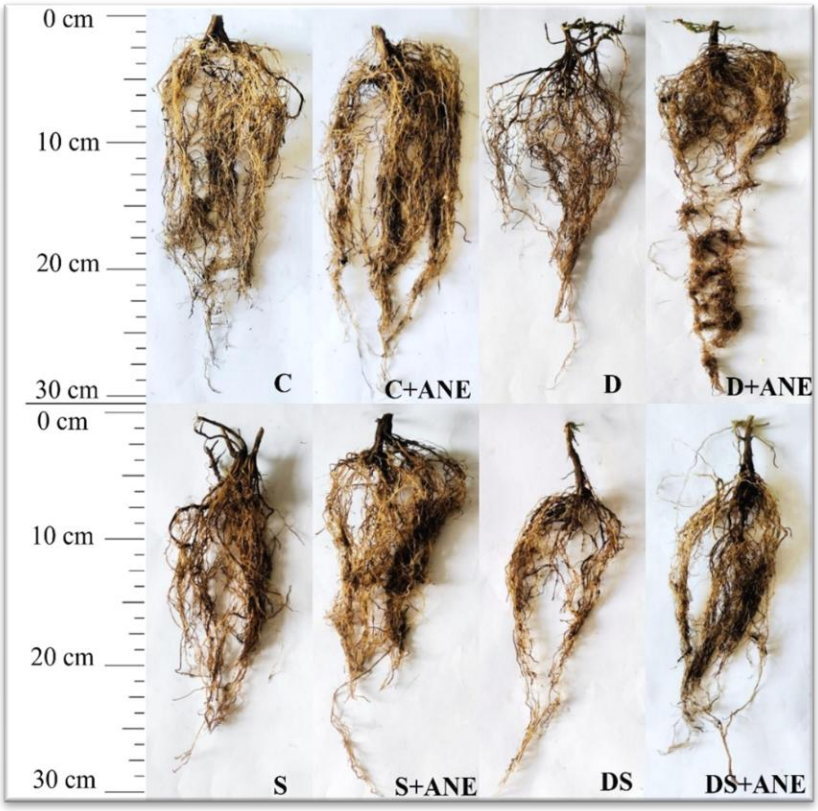

Figure 22. Effect of the applied treatments on root development in *T. capitatus* in TC2. C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.3.2 Effects of applied stressors and *A. nodosum* extract on the physiology of *T. capitatus*

5.3.2.1 Effects of applied stressors and *A. nodosum* extract on the photosynthetic pigments

The impact of the applied stressors on the physiological traits of *T. capitatus* in TC1, as revealed by one-way ANOVA, is summarised in Table 25, while the two-way analysis for TC2 is presented in Table 26. In TC1, with 90 mM NaCl and 40 % SWC as salinity and drought levels, respectively, chlorophyll a, chlorophyll b and total chlorophyll reached their highest values in the control (897.98, 286.37 and 1184.07 µg g^{-1} FW, respectively), all significantly higher than in the stressed treatments. Drought, salinity and combined stress significantly reduced all chlorophyll fractions compared with C, with the lowest total chlorophyll recorded under DS (488.79 µg g^{-1} FW), highlighting a strong stress-induced impairment of the photosynthetic pigment pool.

In TC2, for treatments without *A. nodosum* extract, chlorophyll a and chlorophyll b did not differ significantly among treatments, placing control at a similar statistical level to the stressed groups. However, total chlorophyll again followed a clearer pattern, with C showing the highest value (1335.51 µg g^{-1} FW) and DS the lowest (645.19 µg g^{-1} FW), these two being significantly different, while D and S occupied intermediate positions and overlapped statistically with both C and DS. The two-way ANOVA further revealed a significant interaction between stress and ANE. *A. nodosum* extract application increased chlorophyll a, chlorophyll b and total chlorophyll in the control by approximately 7 %, 17 % and 11 %, respectively, and induced an even stronger

enhancement in DS plants (60 %, 67 % and 87 %, respectively), whereas under drought and salinity its effect was modest and did not result in significant recovery.

Carotenoids were quantified in parallel with chlorophyll pigments. In TC1, carotenoid content followed a pattern similar to chlorophyll a and chlorophyll b: all three stressed treatments showed significantly reduced levels, statistically indistinguishable from one another, while C displayed the highest content (260.52 μg g^{-1} FW). In TC2, C again had the highest carotenoid levels, whereas DS showed the lowest values; D and S caused a moderate reduction and occupied intermediate positions, sharing similar statistical levels with both C and DS. *A. nodosum* extract application significantly increased carotenoids in C (+15 %) and, even more markedly, in DS (+81 %), strongly improving the carotenoid pool compared with their respective untreated counterparts. Among ANE-treated groups, carotenoid levels became statistically similar across stress conditions, with DS+ANE being notably raised towards C+ANE.

5.3.2.2 Effects of applied stressors and *A. nodosum* extract on the relative water content

Leaf relative water content (RWC) in *T. capitatus* was influenced by both the type of stress applied and, in TC2, by the presence of *A. nodosum* extract, as shown in Tables 25 and 26. In TC1, control plants exhibited the highest RWC (90.84 %), significantly exceeding all stressed treatments. Drought caused a notable reduction to 86.83 %, while salinity had an intermediate effect (88.58 %), not significantly different from C or D. The combined stress treatment resulted in the lowest RWC (79.09 %), significantly lower than control and the other treatments, indicating the strongest disruption of leaf hydration when both stressors act together.

In TC2, under the higher salinity level (120 mM), S significantly reduced RWC compared with C, which maintained the highest leaf water content. Drought occupied an intermediate position, statistically similar to both C and S, while DS again showed the lowest RWC, significantly below all other treatments. *A. nodosum* extract application significantly improved RWC only under drought, raising it from 84.29 % to 88.67 %, restoring leaf hydration to values comparable to or slightly above C+ANE. In contrast, RWC under salinity and combined stress remained lower, and ANE had no significant effect in these conditions.

5.3.2.3 Effects of applied stressors and *A. nodosum* extract on the proline content

The proline content of *T. capitatus* responded strongly to drought, salinity, combined stress, and under severe stress, to ANE application, as shown in Tables 25 and 26. In TC1, proline reached its highest level under combined stress (7.76 μmol g^{-1} FW), significantly exceeding all other treatments. Drought and salinity individually also induced significant proline accumulation (6.13 and 5.28 μmol g^{-1} FW, respectively), reflecting osmotic stress, while the control maintained the lowest level (2.80 μmol g^{-1} FW). In TC2, a similar pattern was observed in the absence of ANE, except that drought became statistically similar to the control. *A. nodosum* extract application reduced proline significantly only under combined stress (from 3.77 to 2.94 μmol g^{-1} FW), indicating a clear stress-alleviating effect. Slight decreases were also noted under D+ANE and S+ANE, approaching C+ANE levels, but these changes were not statistically significant.

Table 25. Physiological parameters of *T. capitatus* in TC1 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		Chlorophyll a (µg g^{-1} FW)	**Chlorophyll b** (µg g^{-1} FW)	**Total chlorophyll** (µg g^{-1} FW)	**Carotenoids** (µg g^{-1} FW)	**RWC** (%)	**Proline** (µmol proline g^{-1} FW)	**Soluble sugars** (mg glucose g^{-1} FW)
Experiment		TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **
Treatments	**C**	897.98 ± 86.05^{a}	286.37 ± 33.66^{a}	1184.07 ± 117.74^{a}	260.52 ± 14.47^{a}	90.84 ± 1.24^{a}	2.80 ± 0.32^{a}	14.43 ± 0.70^{a}
	D	437.78 ± 117.44^{b}	151.97 ± 36.33^{b}	589.61 ± 148.93^{b}	151.93 ± 32.34^{b}	86.83 ± 1.11^{b}	6.13 ± 0.70^{b}	29.27 ± 5.69^{b}
	S	452.18 ± 35.13^{b}	158.72 ± 30.96^{b}	610.75 ± 63.05^{b}	152.43 ± 24.01^{b}	88.58 ± 1.90^{ab}	5.28 ± 0.23^{b}	27.59 ± 5.57^{b}
	DS	363.09 ± 45.06^{b}	125.82 ± 19.39^{b}	488.79 ± 52.86^{b}	113.35 ± 18.52^{b}	79.09 ± 2.06^{c}	7.76 ± 0.79^{c}	36.74 ± 6.03^{b}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. RWC: relative water content; FW: fresh weight; TC1: 1st experiment of *T. capitatus*; C: control; D: drought stress; S: salinity stress; DS: combined stress; **: $p < 0.01$.

Table 26. Physiological parameters of *T. capitatus* in TC2 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		Chlorophyll a (µg g^{-1} FW)	**Chlorophyll b** (µg g^{-1} FW)	**Total chlorophyll** (µg g^{-1} FW)	**Carotenoids** (µg g^{-1} FW)	**RWC** (%)	**Proline** (µmol proline g^{-1} FW)	**Soluble sugars** (mg glucose g^{-1} FW)
Treatments	**C**	898.81 ± 3.63^{Aa}	437.05 ± 42.45^{Aa}	1335.51 ± 39.39^{Ab}	319.97 ± 14.17^{Ab}	87.68 ± 2.49^{Ac}	0.95 ± 0.08^{Aa}	9.93 ± 1.05^{Aa}
	C + ANE	964.84 ± 38.06^{Ba}	512.76 ± 13.75^{Ba}	1477.21 ± 47.89^{Ba}	367.90 ± 15.97^{Ba}	88.43 ± 1.92^{Ab}	0.91 ± 0.17^{Aa}	9.11 ± 0.44^{Aa}
	D	824.32 ± 103.46^{Aa}	412.65 ± 52.52^{Aa}	1236.64 ± 197.21^{Aab}	285.90 ± 56.59^{Aab}	84.29 ± 1.23^{Abc}	1.40 ± 0.22^{Aab}	13.70 ± 1.70^{Aa}
	D + ANE	920.79 ± 171.36^{Aa}	446.47 ± 62.23^{Aa}	1366.91 ± 311.63^{Aa}	358.72 ± 91.83^{Aa}	88.67 ± 1.53^{Bb}	1.18 ± 0.12^{Aab}	12.15 ± 2.55^{Aa}
	S	633.18 ± 148.70^{Aa}	477.97 ± 75.28^{Aa}	1110.83 ± 144.93^{Aab}	251.00 ± 90.88^{Aab}	82.42 ± 0.83^{Ab}	1.77 ± 0.06^{Ab}	21.84 ± 4.53^{Ab}
	S + ANE	715.86 ± 96.52^{Aa}	415.37 ± 59.85^{Aa}	1130.93 ± 127.68^{Aa}	274.55 ± 27.68^{Aa}	84.24 ± 2.50^{Aab}	1.63 ± 0.09^{Ab}	18.97 ± 1.34^{Ab}
	DS	501.52 ± 25.92^{Aa}	243.82 ± 10.87^{Aa}	645.19 ± 29.13^{Aa}	168.67 ± 16.10^{Aa}	76.34 ± 2.34^{Aa}	3.77 ± 0.28^{Bc}	25.68 ± 2.71^{Ab}
	DS + ANE	800.80 ± 36.21^{Ba}	405.98 ± 14.58^{Ba}	1206.46 ± 68.64^{Ba}	304.94 ± 26.15^{Ba}	80.24 ± 2.39^{Aa}	2.94 ± 0.36^{Ac}	23.17 ± 3.72^{Ab}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). RWC: relative water content; FW: fresh weight; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

5.3.2.4 Effects of applied stressors and *A. nodosum* extract on the soluble sugars content

The soluble sugar content of *T. capitatus* was strongly affected by drought, salinity, combined stress, and, in TC2, by *A. nodosum* extract application under stress conditions, as shown in Tables 25 and 26. In TC1, control plants exhibited the lowest soluble sugar content (14.43 mg glucose g^{-1} FW), while all stressed treatments (D, S, DS) showed a significant increase compared with the control. The highest accumulation occurred under combined stress (36.74 mg glucose g^{-1} FW), indicating that sugars are mobilised as osmoprotectants and energy reserves under adverse conditions.

In TC2, soluble sugar content increased progressively with stress severity, with S and DS showing the highest values under the higher salinity level, whereas D remained statistically similar to the control. *A. nodosum* extract application slightly reduced sugar accumulation across all treatments, but two-way ANOVA did not detect a significant interaction between stress and ANE, suggesting that osmotic adjustment through sugars is predominantly stress driven rather than influenced by biostimulant application in *T. capitatus*.

5.3.3 Effects of applied stressors and *A. nodosum* extract on the biochemical traits of *T. capitatus*

5.3.3.1 Effects of applied stressors and *A. nodosum* extract on the essential oil content

The influence of the applied factors on the biochemical traits of *T. capitatus* in both experiments is shown in Tables 27 and 28. Essential oil content (EOC) displayed a clear response to stress in TC1: all treatments differed significantly, with the control showing the highest EOC (3.22 ml 100 g^{-1} DW). Salinity resulted in a moderate reduction (2.57 ml 100 g^{-1} DW), followed by drought (2.26 ml 100 g^{-1} DW), while the lowest value appeared under combined stress (2.13 ml 100 g^{-1} DW). In TC2, however, the two-way ANOVA did not detect significant differences among the stress treatments, indicating statistical similarity despite a numerical trend toward higher EOC in D, S, and DS compared with the control. *A. nodosum* extract application significantly increased EOC in non-stressed plants (from 1.54 to 2.11 ml 100 g^{-1} DW), while its effect under stress remained modest; D+ANE, S+ANE, and DS+ANE reached values comparable to C+ANE and were consistently higher than their respective untreated counterparts, although the interaction was not statistically significant.

The contrasting behaviour of EOC between the two trials appears closely linked to plant age, and structural allocation. In TC1, plants originated from seed and were only four months old when the stress treatments began; their biomass was dominated by leaves (~ 70 %) as presented in Figure 23, making essential oil production particularly vulnerable to water deficit and combined stress. By contrast, TC2 plants were seven months old, propagated from cuttings, and exhibited a woodier structure with a higher stem proportion (~ 42 %) and a reduced leaf share (~ 58 %). This shift in leaf–stem balance likely contributed to the greater stability of EOC under stress in TC2. While the

small but consistent increase in EOC following *A. nodosum* extract application in this second trial also suggests that biostimulants can subtly enhance secondary metabolite production.

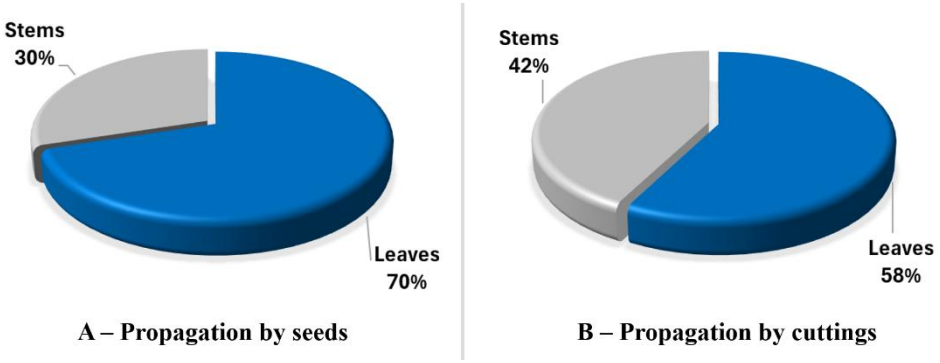

Figure 23. Proportions of stems and leaves in plants propagated by seeds in TC1 (A) and by cuttings in TC2 (B).

5.3.3.2 Effects of applied stressors and *A. nodosum* extract on the total polyphenol content

The total polyphenol content (TPC) of *T. capitatus* in both experiments is summarised in Tables 27 and 28. In TC1, the one-way ANOVA identified combined stress as the treatment producing the highest TPC (123.29 mg GAE g^{-1} DW), significantly exceeding all other groups. The control (111.26 mg GAE g^{-1} DW), drought (107.41 mg GAE g^{-1} DW), and salinity (108.94 mg GAE g^{-1} DW) remained statistically similar, although the control consistently showed slightly higher values than the individual stress treatments. This pattern indicates that only severe simultaneous constraints were capable of stimulating a marked increase in phenolic accumulation.

In TC2, the response shifted: the control grouped statistically with DS, forming a high TPC cluster, while drought and salinity alone resulted in lower polyphenol levels. Salinity, in particular, produced the lowest TPC and differed significantly from all other treatments. *A. nodosum* extract application profoundly altered this pattern, fully restoring phenolic content under individual stress treatments, rising from 175.43 to 210.29 mg GAE g^{-1} DW in D+ANE and from 153.67 to 210.43 mg GAE g^{-1} DW in S+ANE, bringing these values close to those observed in C+ANE and DS+ANE.

5.3.3.3 Effect of applied stressors and *A. nodosum* extract on the antioxidant capacity

The antioxidant capacity (AOC) of *T. capitatus* in both experiments is summarised in Tables 27 and 28. In TC1, the one-way ANOVA revealed that combined stress did not stimulate antioxidant activity; on the contrary, it produced the lowest AOC value (72.80 mg AAE g^{-1} DW), closely followed by salinity (78.18 mg AAE g^{-1} DW). Both treatments were significantly lower than the control, which showed the highest antioxidant capacity (102.75 mg AAE g^{-1} DW). Drought occupied an intermediate position, remaining statistically indistinguishable from all other groups. A comparable pattern emerged in TC2, with drought joining salinity and combined stress to form the lowest AOC cluster, again contrasting with the higher values observed in control plants.

A. nodosum extract application markedly altered this outcome. In TC2, it significantly enhanced AOC under salinity (from 141.98 to 159.02 mg AAE g^{-1} DW) and produced an even stronger improvement under combined stress, where AOC increased from 134.99 to 177.79 mg AAE g^{-1} DW, surpassing not only the untreated stressed plants but also all other *A. nodosum* extract-treated groups. After *A. nodosum* extract addition, C+ANE, D+ANE, and S+ANE showed largely comparable antioxidant levels, indicating that the *A. nodosum* extract effectively narrowed the physiological gaps between treatments.

5.3.3.4 Effects of applied stressors and *A. nodosum* extract on the hydrogen peroxide

Hydrogen peroxide content was evaluated as a stress marker in *T. capitatus*, with results presented in Tables 27 and 28. In TC1, control plants exhibited the lowest baseline H_2O_2 level (1.82 µmol g^{-1} FW), significantly lower than all stressed treatments, confirming minimal oxidative stress. Both drought and salinity induced moderate increases (6.13 and 5.28 µmol g^{-1} FW, respectively), while combined stress triggered the strongest oxidative burst (7.76 µmol g^{-1} FW), significantly exceeding all other treatments.

In TC2, where a higher salinity level (120 mM) was applied, a similar pattern was observed; however, salinity alone became statistically comparable to drought and combined stress, indicating that the added salinity intensified oxidative stress. *A. nodosum* extract application slightly reduced H_2O_2 levels across all treatments, with a significant 11 % decrease observed only under combined stress.

5.3.3.5 Effects of applied stressors and *A. nodosum* extract on the essential oil composition

The GC–MS profile of *T. capitatus* in TC1 (Table 34, Appendices) revealed ten volatile compounds grouped into monoterpene hydrocarbons (α-pinene, β-myrcene, α-terpinene, p-cymene, γ-terpinene), oxygenated monoterpenes (linalool, borneol, thymol, carvacrol), and the sesquiterpene β-caryophyllene. Oxygenated monoterpenes dominated the essential oil, reaching their highest proportion under DS (84.74 %), followed by D (83.06 %) and S (82.09 %), with the lowest values in C (80.47 %). Sesquiterpenes showed a similar trend, peaking in DS (3.10 %). By contrast, monoterpene hydrocarbons displayed the opposite pattern, with the greatest proportion in C (14.77 %) and progressive reductions under stress. TC2 (Table 35, Appendices) exhibited the same compound classes and a comparable stress response pattern. In ANE-treated plants, monoterpene hydrocarbons and sesquiterpenes were generally enhanced under stress, with values approaching those of C+ANE.

Among all volatiles, four major compounds were selected for ANOVA (Tables 27 and 28). Carvacrol, the principal constituent of *T. capitatus* oil, increased markedly under stress. In TC1, its highest values appeared in DS (82.58 %) and S (79.59 %), both exceeding D (80.73 %) and C (78.86 %); D remained statistically similar to the other groups. In TC2, DS again showed the highest, significant carvacrol levels, while C, D, and S formed a uniform lower group. *A. nodosum* extract application did not significantly affect carvacrol in any treatment, though a slight, no significant decline was noted in DS+ANE.

Table 27. Biochemical parameters of *T. capitatus* in TC1 (2024, 90 mM salinity) under the different stress treatments, evaluated by one-way ANOVA.

		EOC (ml 100 g^{-1} DW)	**TPC** (mg GAE g^{-1} DW)	**AOC** (mg AAE g^{-1} DW)	**H_2O_2** (µmol H_2O_2 g^{-1} FW)	**carvacrol** (%)	**p-cymene** (%)	**γ -terpinene** (%)	**β-caryophyllene** (%)
Experiment		TC1 (90 mM) *	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **	TC1 (90 mM) **
Treatments	**C**	3.22 ± 0.18^{a}	111.26 ± 7.01^{a}	102.75 ± 8.54^{a}	1.82 ± 0.39^{a}	78.86 ± 0.65^{a}	5.25 ± 0.11^{a}	5.86 ± 0.35^{a}	2.10 ± 0.04^{a}
	D	2.26 ± 0.12^{b}	107.41 ± 8.32^{a}	85.88 ± 15.29^{ab}	6.13 ± 0.70^{b}	80.73 ± 0.58^{ab}	4.94 ± 0.15^{a}	4.22 ± 0.04^{bc}	2.56 ± 0.12^{ab}
	S	2.57 ± 0.04^{c}	108.94 ± 5.14^{a}	78.18 ± 9.64^{b}	5.28 ± 0.23^{b}	79.59 ± 0.48^{b}	4.93 ± 0.11^{a}	4.61 ± 0.11^{b}	2.43 ± 0.33^{a}
	DS	2.13 ± 0.01^{b}	123.29 ± 10.14^{b}	72.80 ± 16.37^{b}	7.76 ± 0.79^{c}	82.58 ± 1.43^{b}	3.89 ± 0.64^{b}	3.34 ± 0.69^{c}	3.10 ± 0.32^{b}

Means ± SD are displayed for the data. Distinct letters within the same column denote significant differences between the treatment means. EOC: essential oil content; TPC: total polyphenol content; GAE: gallic acid equivalents; AOC: antioxidant capacity; AAE: ascorbic acid equivalents; H_2O_2: hydrogen peroxide; FW: fresh weight; DW: dry weight; TC1: 1st experiment of *T. capitatus*; C: control; D: drought stress; S: salinity stress; DS: combined stress; *: $p \leq 0.05$; **: $p < 0.01$.

Table 28. Biochemical parameters of *T. capitatus* in TC2 (2025, 120 mM salinity), under the different stress and *A. nodosum* extract treatments, evaluated by two-way ANOVA.

		EOC (ml 100 g^{-1} DW)	**TPC** (mg GAE g^{-1} DW)	**AOC** (mg AAE g^{-1} DW)	**H_2O_2** (µmol H_2O_2 g^{-1} FW)	**carvacrol** (%)	**p-cymene** (%)	**γ -terpinene** (%)	**β-caryophyllene** (%)
Treatments	**C**	1.54 ± 0.17^{Aa}	201.64 ± 13.14^{Ac}	172.23 ± 10.40^{Ab}	0.90 ± 0.09^{Aa}	75.45 ± 1.87^{Ab}	6.58 ± 0.26^{Ac}	1.81 ± 0.28^{Aa}	2.78 ± 0.45^{Aa}
	C + ANE	2.11 ± 0.19^{Ba}	208.53 ± 10.13^{Aa}	166.59 ± 2.96^{Aab}	0.86 ± 0.08^{Aa}	76.73 ± 2.96^{Aa}	6.23 ± 0.45^{Aa}	2.00 ± 0.75^{Aa}	2.37 ± 0.26^{Aa}
	D	1.88 ± 0.15^{Aa}	175.43 ± 16.01^{Bb}	144.22 ± 16.35^{Aa}	3.61 ± 0.46^{Ab}	77.99 ± 2.59^{Ab}	6.15 ± 0.90^{Abc}	1.46 ± 0.30^{Aa}	3.56 ± 0.28^{Ba}
	D + ANE	2.14 ± 0.16^{Aa}	210.29 ± 14.22^{Aa}	155.07 ± 12.69^{Aa}	3.16 ± 0.36^{Ab}	78.07 ± 2.19^{Aa}	5.85 ± 1.67^{Aa}	1.62 ± 0.49^{Aa}	2.81 ± 0.35^{Aab}
	S	1.95 ± 0.11^{Aa}	153.67 ± 12.72^{Ba}	141.98 ± 13.35^{Ba}	4.07 ± 0.17^{Abc}	77.85 ± 1.22^{Ab}	5.05 ± 0.45^{Ab}	1.04 ± 0.32^{Aa}	3.52 ± 0.66^{Aa}
	S + ANE	2.02 ± 0.14^{Aa}	210.43 ± 21.97^{Aa}	159.02 ± 10.14^{Aab}	3.78 ± 0.30^{Abc}	76.22 ± 0.51^{Aa}	6.19 ± 0.39^{Ba}	1.64 ± 0.22^{Ba}	3.40 ± 0.25^{Ab}
	DS	2.13 ± 0.10^{Aa}	207.29 ± 13.83^{Ac}	134.99 ± 11.67^{Ba}	4.51 ± 0.10^{Bc}	80.32 ± 1.86^{Aa}	2.76 ± 0.20^{Aa}	1.22 ± 0.51^{Aa}	4.24 ± 0.79^{Ab}
	DS + ANE	2.20 ± 0.13^{Aa}	212.88 ± 32.78^{Aa}	177.79 ± 29.99^{Ab}	4.01 ± 0.28^{Ac}	76.59 ± 2.03^{Aa}	3.78 ± 1.56^{Aa}	1.62 ± 0.56^{Aa}	3.29 ± 0.27^{Ab}

Means ± SD are displayed for the data. Different lowercase letters within a column indicate significant differences among treatment categories (C, D, S, DS) for the same *A. nodosum* extract application (without or with ANE), whereas different uppercase letters indicate significant differences between *A. nodosum* extract applications within the same treatment category ($p \leq 0.05$). EOC: essential oil content; TPC: total polyphenol content; GAE: gallic acid equivalents; AOC: antioxidant capacity; AAE: ascorbic acid equivalents; H_2O_2: hydrogen peroxide; FW: fresh weight; DW: dry weight; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract.

p-cymene, the second most abundant compound, reached its highest proportion in the control during TC1 (5.25 %), statistically similar to drought (4.94 %) and salinity (4.93 %), but significantly higher than DS (3.89 %). In TC2, DS again showed the lowest p-cymene content, while the increased salinity level (120 mM) caused a significant reduction even in C. Drought occupied an intermediate position between C and S. *A. nodosum* extract enhanced p-cymene only under salinity, raising S+ANE from 5.05 % to 6.19 %; changes in the remaining groups were minor and nonsignificant.

γ-Terpinene followed a pattern nearly identical to p-cymene. In TC1, the highest level appeared in the control (5.86 %), followed by the salinity (4.61 %), while combined stress showed the lowest value (3.34 %). Drought fell between S and DS and did not differ statistically from them. In TC2, no significant differences emerged despite a similar ranking of treatments. *A. nodosum* extract significantly increased γ-terpinene only under salinity, elevating S+ANE from 1.04 % to 1.62 %.

β-Caryophyllene, a minor sesquiterpene, increased under combined stress. In TC1, combined stress reached 3.10 %, exceeding control and drought, while salinity was intermediate. A similar trend was observed in TC2. Application of *A. nodosum* extract slightly reduced β-caryophyllene under drought but had little effect in other treatments.

5.3.4 Evaluation of stress indices in *T. capitatus*

Across the two experiments, stress susceptibility index (SSI) values revealed distinct shifts in how *T. capitatus* responded to drought, salinity, and combined stress, along with a clear enhancement effect of *A. nodosum* extract in TC2 as indicated in Figure 24. In TC1, drought (0.98) and salinity (0.73) fell within the moderate range, indicating that single stress treatment caused noticeable but not severe reductions, while combined stress already reached a susceptible level (1.28). Under the harsher TC2 conditions, all plants without ANE application became susceptible (SSI ≥ 1), with combined stress showing extreme sensitivity (3.28). *A. nodosum* extract application markedly altered this pattern: drought shifted from susceptibility to strong tolerance (SSI = –1.47), salinity improved from susceptible (1.54) to clearly tolerant (0.16), and although combined stress remained in the susceptible category, its severity was reduced (from 3.28 to 2.44).

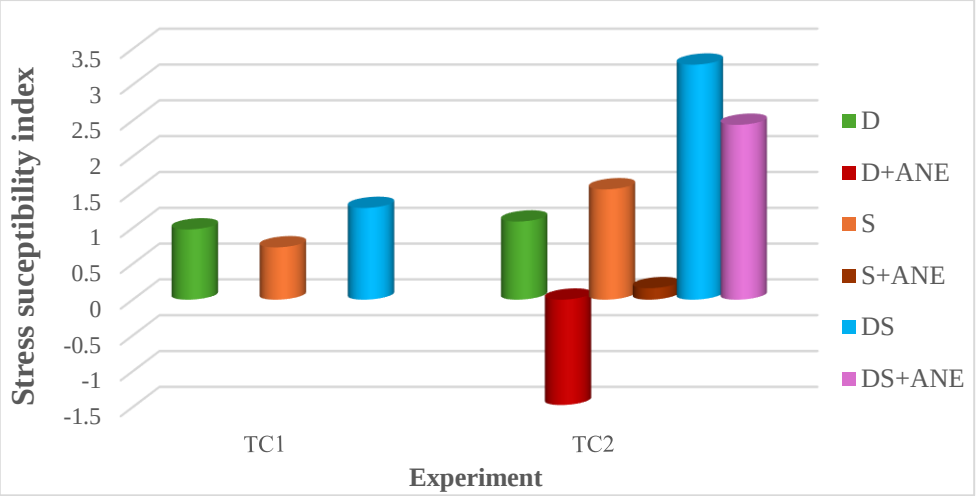

Figure 24. Stress susceptibility index of *T. capitatus* across all the experiments (TC1, TC2). D: drought stress; S: salinity stress; DS: combined stress; ANE: *A. nodosum* extract.

Stress tolerance index (STI) values indicate that *T. capitatus* maintained moderate performance under drought and salinity, with a clearer enhancement appearing in TC2, particularly when *A. nodosum* extract was applied as seen in Figure 25. In TC1, drought (0.62) and salinity (0.72) showed intermediate stability, whereas combined stress produced the lowest STI (0.51), reflecting a stronger yield decline under DS. In TC2, drought performance increased markedly and became the most resilient stress condition (0.88), with ANE elevating it further to 1.16, demonstrating a pronounced biostimulant effect. Salinity remained within a moderate range (0.83) but nearly matched non-stressed performance when paired with ANE (0.98). Combined stress again resulted in the weakest values (0.64), though ANE offered partial mitigation (0.73).

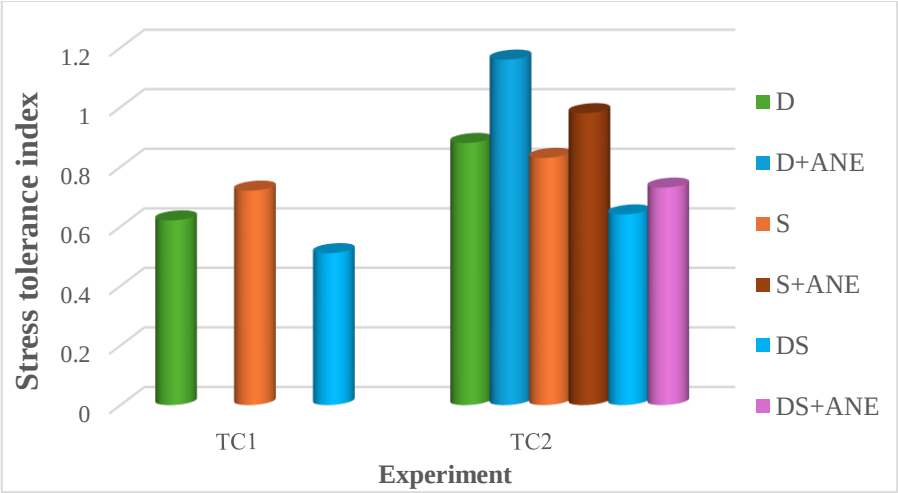

Figure 25. Stress tolerance index of *T. capitatus* across all the experiments (TC1, TC2). D: drought stress; S: salinity stress; DS: combined stress; ANE: *A. nodosum* extract.

5.3.5 Correlation and principal component analysis of *T. capitatus* parameters

The correlations among the morphological, biomass, physiological, and biochemical traits of *T. capitatus* in TC2 are presented in the clustered heatmap in Figure 26. Two coherent modules can be seen. The first is a stress block comprising proline, H_2O_2, soluble sugars, β-caryophyllene, EOC, and carvacrol, all showing strong positive correlations with one another. Opposite to this, a performance block groups pigments (chlorophyll a, chlorophyll b, carotenoids), RWC, biomass traits, TPC, AOC, p-cymene, and γ-terpinene, which are positively interrelated and negatively associated with the stress block.

Within the stress cluster, carvacrol displays only moderate internal association ($r < 0.5$) and shows clear negative correlations with pigments, RWC, and AOC, indicating that higher carvacrol levels coincide with lower chlorophylls, reduced hydration, and diminished antioxidant capacity. EOC follows a similar pattern, though its associations with carvacrol and β-caryophyllene are weaker. At the upper left of the heatmap, proline, soluble sugars, H_2O_2, and β-caryophyllene form a compact blue sub-cluster ($r > 0.5$), strongly inversely related to pigments, RWC, and AOC ($r < –0.5$), reflecting the typical trade-off where oxidative and osmotic stress intensifies as water status and pigment integrity decline.

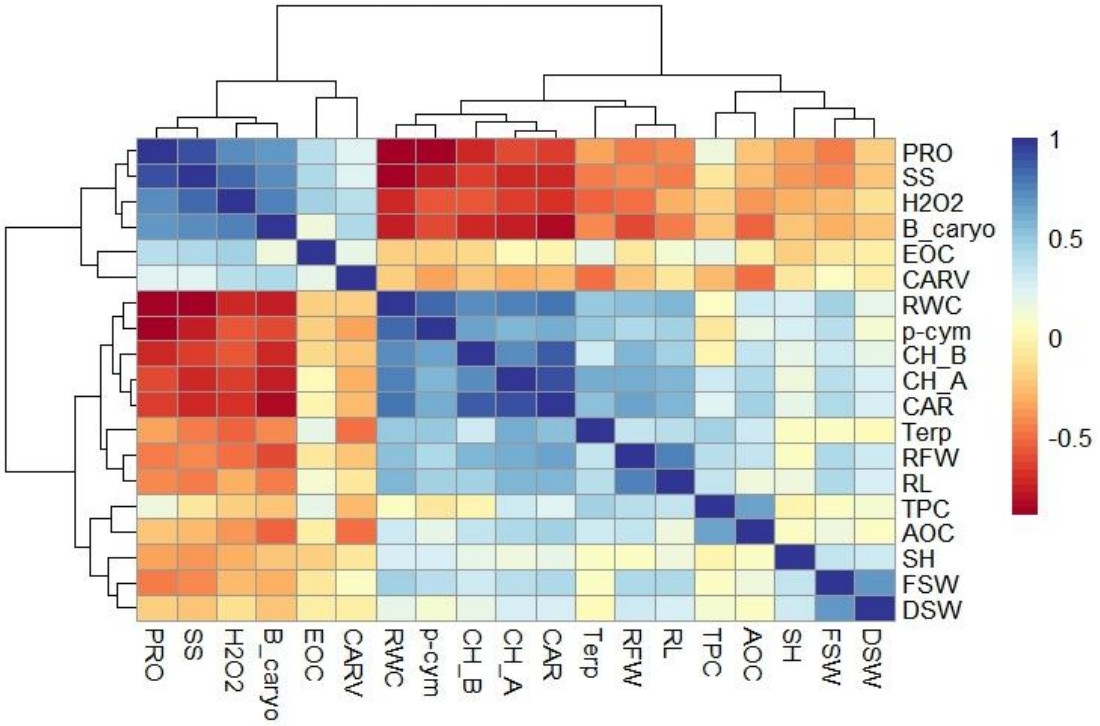

Figure 26. Hierarchical clustering heatmap of morphological, biomass, physiological and biochemical parameters of *T. capitatus* in TC2. SH: shoot length; DSW: dry shoot weight; FSW: fresh shoot weight; EOC: essential oil content; RFW: fresh root weight; RL: root length; AOC: antioxidant capacity; TPC: total polyphenol content; RWC: relative water content; CH_B: chlorophyll b; CH_A: chlorophyll a; CAR: carotenoids; p-cym: p-cymene; B-caryo: β-caryophyllene; CARV: carvacrol; SS: soluble sugars; H_2O_2: hydrogen peroxide; PRO: proline; Terp: γ-terpinene.

In the performance module, a distinct blue block links pigments, relative water content, and p-cymene ($r > 0.5$), highlighting how well-hydrated plants sustain higher chlorophyll and carotenoid levels that, in turn, support p-cymene production. TPC and AOC, however, show only weak positive correlations with RWC and shoot biomass, suggesting that they do not simply scale with plant vigour. The biomass group (fresh shoot weight, dry shoot weight, shoot length, root fresh weight, root length) also maintains weak positive associations with several quality traits, but not as tightly as the pigment, RWC and AOC cluster, indicating that growth performance contributes to essential oil quality without being its primary driver.

The biplot of the principal component analysis for *T. capitatus* in TC2 is shown in Figure 27. Dim1 (46.10 %) represents the primary gradient and clearly separates the strongest stress responses on the left (DS, S) from the control and well-performing treatments on the right (C, C+ANE, D+ANE). Dim2 (11.80 %) represents a secondary gradient that differentiates ANE-treated plants from those without ANE.

Regarding treatment positions, the PCA distinctly separates the DS group at the farthest left (−5.7; 0.7), reflecting the highest total stress load. Slightly less negative on Dim1, S is located at (−2.4; −2.1), indicating salinity stress of moderate intensity. Closer to the origin, D is positioned at (−0.6; −1.4), showing a moderate drought response while still being clearly stress-affected. Moving from DS toward the centre, DS+ANE (−1.6; 2) indicates a partial physiological relief under *A. nodosum* extract treatment. Slightly right of centre, S+ANE (0.4; 0.5) occupies an intermediate position between stressed and fully healthy groups, reflecting partial recovery from salinity. Further to the right along positive Dim1, D+ANE (2.8; 0.3) demonstrates strong drought recovery, indicating maximal improvement of drought resilience through *A. nodosum* extract application. Normal C plants are positioned nearby (2.8; −0.7), showing high performance under non-stress conditions, while C+ANE is located at the farthest right (3.8; 0.4), suggesting that ANE enhances both vigour and metabolic activity in unstressed plants.

The orientation of variable vectors further strengthens this structure. Proline, H_2O_2, soluble sugars, β-caryophyllene, and carvacrol point sharply toward S and DS, indicating increased osmolyte production, oxidative activity, and stress-induced volatiles under harsh conditions. In contrast, traits associated with physiological integrity, RWC, chlorophyll a, chlorophyll b, carotenoids, fresh shoot weight, dry shoot weight, shoot length, root length, root fresh weight, and p-cymene, align with C and C+ANE, reflecting strong water balance, pigment stability, and biomass accumulation. AOC, TPC, EOC, and to a lesser extent γ-terpinene cluster nearer to DS+ANE, S+ANE, and D+ANE, marking a metabolic reinforcement pathway where *A. nodosum* extract partially restores physiological status while strongly enhancing biochemical defences. These patterns suggest that *A. nodosum* extract *A. nodosum* extract application shifts stressed *T. capitatus* away from the “damage zone” and toward a more resilient, metabolically strengthened phenotype.

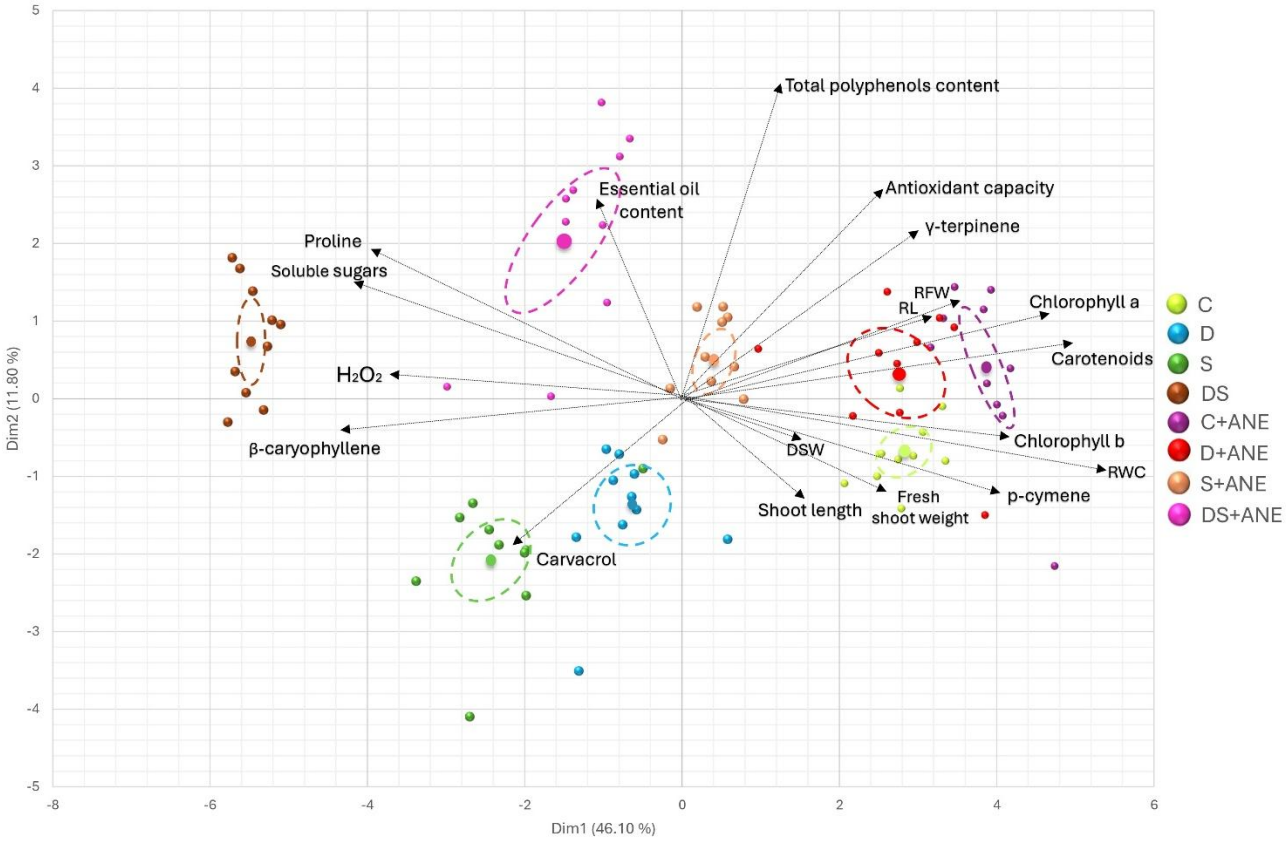

Figure 27. Biplot of principal component analysis illustrating the relationships among the studied parameters of *T. capitatus* when subjected to various stress and *A. nodosum* extract conditions in TC2. Dim: dimension; C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract; DSW: dry shoot weight; RFW: fresh root weight; RL: root length; RWC: relative water content; H_2O_2: hydrogen peroxide.

6 Discussion

6.1 Morphological and production biological stress responses of *Thymus* spp.

In *T. pannonicus*, **shoot traits** showed a clear sensitivity to stress, with drought causing reductions of up to 20 % in shoot number and 14% in shoot length in TP3, emphasising its dominant inhibitory effect on aerial growth. Moderate salinity (60–90 mM) produced little change, indicating a certain tolerance at these concentrations, whereas 120 mM salinity reduced shoot traits, like fresh shoot weight by up to 24 %, and even more strongly under combined stress (up to 51 %), revealing an additive negative effect between osmotic and ionic pressures. Root responses followed a similar pattern: drought markedly reduced root biomass and length, while moderate salinity remained mostly tolerable. However, at 120 mM, root length declined by up to 31 %, and the effect intensified under combined stress, confirming that water deficit (40 % SWC) together with high salinity are the primary factors limiting both aerial and belowground growth in this species.

In *T.* × *citriodorus*, shoot traits were particularly sensitive when drought and salinity acted together, with reductions reaching 21 % in dry shoot weight and 19 % in shoot number in TL2. Single drought caused only minor changes in both trials, but high salinity (120 mM) became markedly more damaging when coupled with water deficit, pointing to a synergistic interaction between osmotic and ionic stress. Root biomass showed a similar tendency, declining by up to 35 % in dry root weight under combined stress, despite the stability seen of root length in TL2.

In *T. capitatus*, shoot growth declined under both drought and salinity, with combined stress causing reductions of up to 34 % in fresh weight and 20 % in dry weight in TC2, indicating a pronounced sensitivity to concurrent water and salt stress. Even single drought (40 % SWC) or high salinity (90–120 mM) was sufficient to reduce shoot performance, although dry weight and shoot length remained relatively more stable, suggesting a degree of structural adjustment under stress. Root traits showed a similar pattern, with biomass consistently decreasing and the strongest suppression observed under combined treatment, reflecting the limited capacity of this species to maintain growth when exposed to simultaneous osmotic and ionic constraints.

Previous studies show comparable trends across thyme species, confirming drought as a major limiting factor for biomass production. In *T. vulgaris*, both fresh and dry root weights drop under water scarcity (Abd Elbar et al., 2019), and Arpanahi et al. (2020) reported significant reductions in shoot and root dry weights in *T. vulgaris* and *T. daenensis*. Short drought periods can already impair growth; Mahdavi et al. (2020) observed a 20 % decrease in shoot dry weight in *T. vulgaris* ‘Varico3’ after only 14 days. Severe drought further reduced root dry mass by 28 % in *T. daenensis* (Arpanahi et al., 2020). *T. capitatus* can tolerate salinity up to 100 mM NaCl (Kim & Eom, 2009), yet still exhibits a clear decline in fresh biomass under drought (García-Caparrós et al., 2019). These studies reinforce the consistent sensitivity of thyme species to water deficit and salinity, aligning closely with our results.

The reductions in shoot and root growth under drought and salinity arise from a series of interconnected physiological constraints. The strong positive correlations observed between shoot and root traits and pigment levels further support these explanations. Salinity leads to the accumulation of Na^+ and Cl^- in the cytoplasm, disrupting water uptake and metabolic processes, reducing photosynthetic efficiency, and altering hormonal balance (Arif et al., 2020; Wang et al., 2007, 2008). Drought, in turn, restricts water availability, limiting cell expansion and division (Brunner et al., 2015; Farooq et al., 2009; Litvin et al., 2016), while also decreasing chlorophyll content and impairing gas exchange (Siddique et al., 2016). Both stress treatments ultimately reduce nutrient uptake (Ashraf et al., 2013) and pigment levels, weakening the photosynthetic machinery. When acting together, these osmotic and ionic pressures create a highly restrictive physiological environment that severely limits biomass accumulation above- and belowground.

6.2 Physiological stress responses of *Thymus* spp.

Across the experiments, *T. pannonicus* maintained relatively stable **chlorophyll** and **carotenoid** levels under moderate salinity (60 mM), but pigment concentrations declined sharply in TP2, under drought and higher salinity (90 mM), particularly under combined drought–salinity stress. At 120 mM NaCl, chlorophyll a, chlorophyll b, and carotenoids dropped significantly under salinity alone (by 24 %, 34 % and 36 % respectively), with the lowest values consistently recorded under the combined treatment.

In *T.* × *citriodorus*, drought had little effect on pigments, whereas high salinity (120 mM) and combined stress caused marked reductions in chlorophylls and carotenoids (up to 19–29 %), indicating progressive impairment of the photosynthetic system under severe stress. These changes suggest reduced light-harvesting and photoprotective capacity under increasing osmotic and ionic pressure.

In *T. capitatus*, drought, 90 mM salinity, and especially combined stress caused strong reductions in chlorophylls and carotenoids (up to 44–52 %), indicating high sensitivity of the photosynthetic apparatus to osmotic and ionic stress. At higher salinity (120 mM), chlorophylls remained relatively stable, whereas carotenoids decreased under combined stress, suggesting general inhibition of pigment biosynthesis and reduced photoprotective capacity under severe stress conditions.

These findings are consistent with previous studies reporting declines in leaf pigments under drought in *T. vulgaris* L. (Farag et al., 2019) and *T. daenensis* Celak (Bistgani et al., 2017). Similarly, Bistgani et al. (2019) observed in *T. vulgaris* that total chlorophyll decreased from 3.3 mg g^{-1} FW at 0 mM salinity to 2.4 mg g^{-1} FW at 90 mM, aligning with our results, where total chlorophyll decreased from 2.9 mg g^{-1} FW (0 mM) to 1.9 mg g^{-1} FW (120 mM). Alijani et al. (2024) also confirmed that salinity stress reduces photosynthetic pigments in *Achillea millefolium* L., supporting the negative impact of salinity stress on pigment stability. Interestingly, other abiotic stressors can have opposite effects, as UV-B exposure increased chlorophyll content in

T. vulgaris (Azadi et al., 2021), and *T. sibthorpii* leaves collected in winter chilling showed higher chlorophyll levels than summer leaves (Lianopoulou et al., 2013).

In fact, abiotic stressors contribute to the excessive generation of ROS, including superoxide radicals and H_2O_2, as observed in our study. This oxidative stress arises from an imbalance between ROS production and the plant's antioxidant defences. Excess ROS can degrade chlorophyll, impair photosynthesis, and ultimately restrict plant growth (Hussain et al., 2019). In addition, reduced cellular RWC leads to chloroplast shrinkage, while ROS attack lipids in thylakoid membranes, where chlorophylls and carotenoids reside. This triggers lipid peroxidation, compromising membrane integrity, disrupting ATP and NADPH production, and reducing carbon assimilation in the Calvin cycle. Consequently, PSII function is impaired, leading to diminished photosynthetic efficiency (Killi et al., 2020; Pospíšil & Yamamoto, 2017; Santakumari & Berkowitz, 1991; Yalcinkaya et al., 2019).

Across the three experiments, leaf **relative water content** in *T. pannonicus* was mainly reduced by drought and combined stress, while moderate salinity (up to 90 mM) allowed leaves to maintain hydration levels close to the control. At 120 mM, RWC declined under combined stress (by 13.61 %), indicating that severe water deficit coupled with high salinity strongly disrupts leaf water balance, although the species shows partial tolerance to moderate salinity.

Across both experiments, *T.* × *citriodorus* exhibited a consistent decline in RWC from control to drought (40 % SWC), salinity (120 mM NaCl), and combined drought–salinity. TL1 shows a clear stepwise reduction, whereas TL2 confirms the same trend with slightly narrower differences between drought and salinity, suggesting that osmotic restriction, reduced water uptake, and potential root and membrane impairment contribute to the progressive decline in hydration, with combined stress exerting the strongest limitation in *T.* × *citriodorus* (by 17.61 %).

The results also show that *T. capitatus* can partially maintain RWC under individual drought or salinity treatments up to 90 mM, but combined drought–salinity causes a marked decline (by 11.34 % in TC2). The gradual reduction in RWC with increasing stress intensity indicates that osmotic and ionic pressures reinforce each other under combined conditions, substantially limiting water acquisition and retention.

Limited water availability is a direct consequence of the decrease in RWC, disrupting transpiration and photosynthesis and ultimately reducing plant growth and productivity, as previously documented in *T. vulgaris* and *T. serpyllum* (Mohasseli & Sadeghi, 2019). Supporting this, Ashrafi et al. (2022) reported that under 40% FC, RWC dropped to 45 % in *T. vulgaris* and 70 % in *T. kotschyanus* compared to optimally irrigated plants (90 % FC). These values are even lower than those recorded in our conditions (74.67 % in TP3, 76.14 % in TL2 and 84.29 % in TC2), suggesting that the overall impact in our setup was comparatively moderate. Similarly, salinity at 120 mM NaCl has been shown to reduce RWC and simultaneously suppress leaf area, shoot length, and other growth parameters (Meguekam et al., 2021).

Under drought stress, reduced soil moisture directly restricts water availability, decreasing water content within plant tissues and causing a decline in RWC (Shivakrishna et al., 2018; Trifilò et al., 2023). The resulting drop in turgor pressure limits cell expansion, thereby constraining growth and development in *T.* × *citriodorus* (Tátrai et al., 2016). Under salinity, osmotic stress arises from the accumulation of Na^+ and Cl^-, which disrupt nutrient balance, especially by restricting access to essential ions like K^+ and Ca^{2+}. This ionic imbalance compromises water absorption at both the root level and within tissues, further exacerbating the decline in RWC (Guo et al., 2015; Nassar et al., 2020).

Stress indicators in *T. pannonicus*, including **proline**, **soluble sugars**, and **hydrogen peroxide**, showed a clear and coordinated increase with rising stress intensity, reflecting activation of protective metabolic and signalling responses. Proline and soluble sugars reached their highest levels under 120 mM salinity (increasing by 200 % and 24 %, respectively), supporting their role in osmotic adjustment under severe osmotic and ionic stress. Hydrogen peroxide also peaked under combined stress, indicating enhanced oxidative signalling and stress pressure as environmental constraints intensified, showing that *T. pannonicus* actively engages both osmoprotective and stress-response pathways to cope with extreme conditions.

In *T.* × *citriodorus*, proline, soluble sugars, and H_2O_2 similarly increased progressively with stress intensity, with the highest accumulations under combined drought–salinity stress (up to 367 %, 111 %, and 182 %, respectively, in TL2), indicating strong activation of osmotic and metabolic protective mechanisms. H_2O_2 mirrored this pattern, signalling intensified oxidative stress under combined constraints, which likely contributes to impaired leaf hydration, growth, and photosynthetic efficiency, demonstrating a tightly coordinated activation of protective mechanisms that buffer the plant against escalating water and ionic challenges.

In *T. capitatus*, proline, soluble sugars, and H_2O_2 accumulated markedly, reaching their highest levels under combined drought–salinity stress (up to 297 %, 159 %, and 401 %, respectively, in TC2), indicating strong activation of osmotic and stress-defence mechanisms. The simultaneous elevation of these osmolytes and H_2O_2 reflects intensified oxidative and ionic pressure under severe stress, suggesting that *T. capitatus* enhances protective biochemical pathways to maintain cellular homeostasis.

These patterns are consistent with previous findings in thyme species. Farag et al. (2019) and Shahroudi et al. (2023) reported increased proline and total soluble sugars in *T. vulgaris* L. and *T. daenensis* under drought, while Arpanahi et al. (2020) observed that proline in *T. daenensis* rose from 3 μmol g^{-1} FW under control conditions to 15 μmol g^{-1} FW under severe drought (40–45 % FC). In our study, all *Thymus* spp. displayed a similar trend, with proline increasing from 2.4 μmol g^{-1} FW at optimal irrigation to 6 μmol g^{-1} FW under drought in case of TL1, suggesting a conserved stress-response mechanism across *Thymus* species.

Physiologically, stress-induced stomatal closure limits CO_2 uptake, disturbing chloroplast function and causing an imbalance in ATP/NADPH utilisation, which in turn disrupts the photosynthetic

electron transport chain. Excess electrons are transferred to oxygen, generating superoxide radicals (O_2^-) via the Mehler reaction, subsequently converted into H_2O_2. Mitochondrial electron leakage contributes similarly to H_2O_2 formation (Gill & Tuteja, 2010; Hippeli & Elstner, 1996; Wise & Naylor, 1987). H_2O_2 functions not only as a signal molecule but also enhances proline accumulation by upregulating pyrroline-5-carboxylate synthase (P5CS) and downregulating proline dehydrogenase (ProDH), while modulating sugar metabolism to increase soluble sugar content (Couée et al., 2006; Liu et al., 2020). These osmolytes perform multiple protective roles: they adjust cellular osmotic balance, stabilise proteins and membranes, and scavenge ROS, collectively mitigating oxidative damage and strengthening the plant's resilience to drought and salinity (Li et al., 2022; Mansour & Salama, 2020; Shafi et al., 2019; Suprasanna et al., 2016; Tiwari, 2024).

6.3 Biochemical stress responses and stress indices of *Thymus* spp.

Across the three experiments, *T. pannonicus* showed a marked decline in **essential oil content** under drought, salinity (from 90 mM), and especially under combined stress (up to 57 % in TP3), indicating high sensitivity of secondary metabolism to severe abiotic constraints. In addition to reducing total EO yield, stress induced compound-specific changes: thymol remained relatively stable under single stress but decreased under combined stress (by 5 % in TP3); p-cymene increased under moderate salinity but declined as stress intensified, whereas β-bisabolene and thymol methyl ether were enhanced under high salinity and combined stress. These shifts suggest that stress does not uniformly suppress secondary metabolism but rather redirects metabolic pathways toward specific compounds under adverse conditions.

In *T.* × *citriodorus*, drought, salinity, and especially their combination reduced essential oil content, with the strongest decline under combined stress (up to 31 % in TL2), indicating limited maintenance of secondary metabolism under severe stress conditions. Stress also altered oil composition: geraniol decreased (by 12 % under combined stress in TL2), while geranial, neral, and β-bisabolene increased, shifting the oil profile toward citral-type aldehydes and sesquiterpenes, which may contribute to stress adaptation and cellular protection.

In *T. capitatus*, responses differed between experiments, partly reflecting differences in plant age and biomass structure. Older plants, characterised by a higher proportion of woody tissues relative to leaves, showed more stable essential oil levels (TC2) compared with younger plants with leaf-dominated biomass (TC1), which may help explain the different response patterns observed between experiments, although other minor factors may also contribute. In TC1, drought, 90 mM salinity, and combined stress caused strong reductions in essential oil content (up to 34 % under combined stress). In contrast, TC2 showed greater stability in oil yield under stress. Stress also modified oil composition in a compound-specific manner: carvacrol increased (by 6.45 % under combined stress in TC2), whereas p-cymene and γ-terpinene generally declined, while β-caryophyllene accumulated under dual stress. These shifts suggest a stress-responsive

reprogramming of secondary metabolism that favours compounds associated with stress defence and cellular protection.

Overall, our findings emphasise the negative impact of drought and salinity, especially in combination, on essential oil production in *Thymus* spp. These results align with reports by Mohasseli and Sadeghi (2019), who documented reduced EOC and thymol levels in *T. daenensi*s and *T. vulgaris* under water stress; with Askary et al. (2018), who found that EO production declined at 40 % FC; and with Alavi et al. (2013) who reported similar reductions in *T. daenensis* although some compounds (carvacrol, γ-terpinene) increased. PEG-induced drought in *T. vulgaris* likewise decreased thymol and carvacrol (Razavizadeh et al., 2019), consistent with our *T. pannonicus* findings. The dominance of carvacrol in our *T. capitatus* oils supports earlier Tunisian reports on the same species too (Bounatirou et al., 2007). Conversely, our results diverge from those of Amiri et al. (2018), stating an increased EOC, such as in *T.* × *citriodorus* (by 33.6 % and 24.5 % increases at 60 % and 40 % FC, respectively in comparison to well-watered plants) and in *T. eriocalyx*. In the same study, stress-driven increases in selected compounds have also been observed in *T. eriocalyx*, where thymol, carvacrol, and trans-caryophyllene rose under drought stress. Our results also reflect the study of Németh-Zámbori et al. (2016), who found increases in terpinene type components at the expense of thymol and carvacrol in *T. vulgaris* under drought stress.

Indeed, under stress conditions, an alteration in metabolic processes can take place to mitigate the damaging effects of ROS by modifying the biosynthesis of specific terpenoids based on their functional roles (Wang et al., 2018). In our *T.* × *citriodorus* experiments, for example, geraniol, a monoterpenoid alcohol, can be oxidised into geranial and neral (citral), a pathway supported by earlier biochemical studies (Hagvall et al., 2020; Silva et al., 2022). This oxidation not only contributes to ROS detoxification but also explains the observed decrease in geraniol and the corresponding rise in geranial and neral under stress conditions. While this mechanism remains inferential without gene- or enzyme-level validation, it strongly suggests a stress-induced rerouting within the geraniol pathway. A similar principle may underlie the changes observed across the other species: in *T. pannonicus*, β-bisabolene and thymol methyl ether increased at the expense of thymol, while in *T. capitatus*, β-caryophyllene rose as carvacrol, p-cymene, and γ-terpinene declined. This pattern may represent a species-specific adaptive mechanism that preserves the functional and commercial quality of the essential oil (including therapeutic attributes such as anticancer activity) under adverse environmental conditions (Paoli et al., 2023; Silva et al., 2022). Nevertheless, further targeted research is needed to confirm these biochemical routes and to clarify their significance for essential oil composition and function.

Across the three experiments, **total polyphenol content** in *T. pannonicus* was mainly reduced by salinity (from 90 mM) and combined drought–salinity, while drought alone had little effect. In TP3, drought did not significantly change polyphenol levels, indicating limited phenolic metabolic response to water deficit alone. In contrast, salinity and combined stress reduced polyphenols by

up to 41–46 %, suggesting that ionic and osmotic stress together generate a higher physiological burden, limiting the plant's capacity to sustain phenolic biosynthesis under severe conditions.

Across TL1 and TL2, *T.* × *citriodorus* showed moderate sensitivity of total phenolic content to stress. In TL1, salinity (120 mM) and especially combined drought–salinity reduced TPC (by 21 % and 34 %, respectively), while drought alone had little effect, suggesting that ionic stress limits phenolic metabolism more than water deficit. In TL2, TPC declined mainly under combined stress, indicating that phenolic responses depend on stress severity and are more pronounced under multiple simultaneous stressors.

In *T. capitatus*, total phenolic content was strongly influenced by stress complexity. Phenolic accumulation was mainly stimulated under combined drought–salinity stress, suggesting that simultaneous osmotic and ionic stress generates stronger oxidative signalling that activates phenolic biosynthesis. In contrast, single stress treatments had weaker effects and sometimes reduced TPC, particularly in TC2. The consistently higher phenolic levels under combined stress (by 11 % and 3 %), indicate that *T. capitatus* prioritises phenolic synthesis as a protective response under multiple simultaneous stress conditions.

Similar responses in *T. capitatus* have been reported by Razavizadeh and Mohagheghiyan (2015), who observed increased polyphenol accumulation under stress conditions in *T. vulgaris*, supporting the idea that stress can activate secondary metabolism as a defensive mechanism. Likewise, (Zrig et al., 2019) documented a 15 % increase in TPC in *T. vulgaris* following two weeks of exposure to 150 mM salinity. Such responses are closely linked to the activation of the phenylpropanoid pathway, particularly through the upregulation of phenylalanine ammonia-lyase (PAL), which catalyses the conversion of phenylalanine into cinnamic acid, a key precursor of polyphenols (Amjad et al., 2024; Astaneh et al., 2018). In stress-tolerant Mediterranean species such as *T. capitatus*, it appears that single stress treatment do not exceed the activation threshold required to stimulate phenolic synthesis, whereas combined stress does. Similar patterns have been reported in *Eucalyptus globulus*, where individual drought or heat stress resulted in lower trans-cinnamic acid levels than their combination, which induced higher phenolic accumulation (Correia et al., 2018). This supports the concept that under moderate stress, carbon is preferentially allocated to maintenance and photosynthesis, while under severe stress, defensive secondary metabolism becomes a priority (Chaves et al., 2009; Selmar & Kleinwächter, 2013).

On the other hand, the role of polyphenols as ROS scavengers and protectors against oxidative damage has been well documented (Lang et al., 2024; Wang et al., 2016), which helps explain the contrasting responses observed in *T.* × *citriodorus* and *T. pannonicus*. In these species, the rate of phenolic utilisation may exceed biosynthesis under stress, resulting in a net decrease in TPC under both individual and combined treatments. Supporting this interpretation, studies on *Ligularia fischeri* showed that drought-induced reductions in polyphenols were associated with the downregulation of key biosynthetic genes such as LfHCT and LfHQT4, leading to lower production of caffeoylquinic acids and flavonoids (Park et al., 2023). Similarly, Lugo-Cruz et al.

(2018) reported decreased polyphenol content in *Zea mays* bran under drought stress, attributing this decline to the active consumption of hydroxycinnamic acids for radical scavenging and photoprotection against stress-induced ROS.

In *T. pannonicus*, **antioxidant capacity** decreased sharply under salinity (from 120 mM) and combined drought–salinity stress, whereas drought alone had little effect (by 16 %, 41 % and 3 % respectively), indicating that high ionic and combined stress impose a strong oxidative burden, which the plant's own defence system cannot fully handle, leaving it vulnerable to reactive oxygen species.

In *T.* × *citriodorus*, antioxidant activity was also strongly affected by salinity and even more by combined drought–salinity, while drought alone caused only minor changes. TL1 showed a clear drop in AOC under salinity and combined stress (by 58 %), suggesting that oxidative pressure under these conditions exceeds the plant's protective capacity and may damage membranes, proteins, and the photosynthetic apparatus. TL2 confirmed that combined stress is the most limiting condition, highlighting how cumulative stress factors reduce the plant's ability to maintain redox balance.

In *T. capitatus*, AOC declined under both moderate and severe stress, with the strongest reductions observed under salinity (90 mM) and combined drought–salinity (24 % and 29 % respectively). Younger, seed-propagated plants (TC1) were particularly sensitive, showing that these stress treatments exceeded their antioxidant capacity. Older, cutting-propagated plants (TC2) started with higher baseline AOC but still experienced reductions under drought (40 % SWC) and salinity (120 mM), indicating that even more mature plants have a limited ability to cope with oxidative pressure when stress is strong or combined.

These results differ from reports by Szabó et al. (2022), who observed increased antioxidant capacity under drought, and from Razavizadeh and Mohagheghiyan (2015) and Zrig et al. (2019) ,who found that salinity enhanced enzymatic antioxidant activity in thyme. This variability highlights the complexity of plant stress responses, which depend not only on the type, intensity, and duration of stress, but also on the species and its developmental stage. Although enzymatic antioxidant activities were not directly measured in the present study, our findings agree with those of Khosh-Khui et al. (2012), who reported that prolonged drought reduced antioxidant capacity and total phenolic content in *T. vulgaris*. Similarly, Németh-Zámbori et al. (2016) showed that drought imposed at 25 % SWC significantly decreased AOC (by 21 %) and TPC (by 18 %) in the roots of *T. vulgaris* compared with control conditions (70 % SWC), while it did not significantly affect AOC or TPC in the shoots, emphasising that antioxidant responses in thyme are highly context-dependent and can vary substantially with experimental conditions.

Even though TPC and compounds such as carvacrol increased under combined stress in *T. capitatus*, their antioxidant potential was still insufficient to neutralise excessive ROS, while other species showed a net decrease in TPC under stress. This suggests that enzymatic defences like SOD, CAT, and APX may be overwhelmed or downregulated, limiting the plant's capacity to

cope with oxidative stress (Thakur & Garg, 2022) and highlighting the need for both biochemical and enzymatic mechanisms to maintain cellular protection under harsh conditions.

In *T. pannonicus*, **SSI** and **STI** values indicated improved drought tolerance across experiments, while high salinity (120 mM) and especially combined drought–salinity caused the greatest yield losses. Drought stress became more manageable over time, particularly in TP3, whereas salinity and combined stress remained within the susceptible range. This pattern highlights the stronger physiological burden imposed by ionic stress and its interaction with water deficit. The high SSI under combined stress in TP3 confirms that simultaneous osmotic and ionic constraints severely limit productivity in this species.

In *T. × citriodorus*, SSI and STI values showed a clear hierarchy of stress tolerance. The species maintained relatively stable performance under drought alone, while salinity imposed moderate constraints. However, combined drought–salinity caused the strongest reductions in growth, water status, and physiological performance. Low STI and high SSI under combined stress confirm the high physiological cost of simultaneous osmotic and ionic stress. Differences between TL1 and TL2, with slightly lower tolerance in TL2, suggest that plant developmental stage or prior stress exposure may influence sensitivity, while confirming that combined stress is the most limiting condition for this species.

In *T. capitatus*, SSI and STI responses varied with stress type and experimental conditions. Plants showed moderate tolerance to individual drought or salinity, while their combination imposed the strongest limitations, reflecting the higher metabolic and physiological cost of dual stress exposure. Sensitivity was higher in TC2, where drought, salinity, and especially combined stress reduced performance, suggesting that plant age or physiological status may influence stress vulnerability. Low STI values under combined stress in both trials confirm that simultaneous osmotic and ionic stress severely restricts growth and biomass production, even in this relatively tolerant species.

The combined stress condition emerges as the most detrimental, as evidenced by its distinct separation in the principal component analysis and the strong clustering of stress markers in the heatmap, highlighting the enormity of the physiological and biochemical impact. These responses are regulated by different signalling pathways: drought primarily activates ABA dependent mechanisms for water conservation, while salinity triggers ion-specific pathways to manage ion balance (Wang et al., 2018). Both stress treatment induce ROS signalling and engage calcium and mitogen-activated protein kinase (MAPKs) cascades that control stress responsive genes and antioxidant defences (Choudhury et al., 2013; Kovtun et al., 2000). Though our study didn't directly examine these pathways, they likely underlie the physiological changes observed and warrant further investigation.

6.4 Biostimulant-mediated stress mitigation of *A. nodosum* extract

Morphological and biomass responses

In *T. pannonicus*, *A. nodosum* extract mainly improved plant performance under severe combined drought–salinity stress, where it partially restored **shoot** and **root growth**. Shoot biomass responded mainly under strong stress, while shoot length was relatively stable, indicating limited effect on vertical growth but better support of overall biomass production. Shoot number was moderately improved across treatments. Root traits showed the strongest response, with ANE enhancing root biomass and elongation under stress, helping maintain a more functional root system under adverse conditions. Overall, *A. nodosum* extract acted as a stress-mitigation treatment, particularly under severe environmental constraints.

A. nodosum extract in *T. × citriodorus*, mainly acted as a stress buffer under combined drought–salinity conditions. Its effects on shoot length were limited, but it helped reduce losses in shoot biomass and maintained shoot number, contributing to better canopy stability under stress. Root responses were weaker, with ANE mainly supporting root fresh biomass under severe stress, while root length and dry weight showed only minor recovery, suggesting that ANE primarily supports aboveground stability rather than root development in this species.

In *T. capitatus*, *A. nodosum* extract supported both shoot and root performance under stress. It moderated shoot biomass losses under drought and salinity and helped stabilise shoot elongation, and partially preserved shoot number, although it could not fully compensate under severe combined stress. Root traits responded more strongly, with ANE improving root biomass and elongation under single stress treatments, helping maintain water and nutrient uptake and enhancing overall plant stability, with greater benefits observed under moderate stress conditions.

Our results are in accordance with previous findings in *T. vulgaris*, where *A. nodosum extract* proved effective in enhancing several morphological and biomass traits under deficit irrigation conditions. Plant length increased by 26 %, shoot number by 39 %, and total dry weight by 52 % (Rahimi et al., 2022). Similar trends were reported in *Salvia officinalis* L. subjected to drought stress at 18 % and 7 % FC and treated with *Ulva rigida* seaweed extract; in this study, shoot length was significantly enhanced at 18 % FC when a higher extract concentration (75 %) was applied, whereas leaf number increased under both drought levels at lower extract concentration (25 %). Notably, even control plants treated with seaweed extract showed higher leaf numbers, while leaf area was consistently improved across all extract concentrations (25 %, 50 %, and 75 %) at both FC levels (Mansori et al., 2015). Comparable responses were also observed in *Solanum lycopersicum* L., where ANE application under drought stress led to a general improvement in morphological and biomass traits, including shoot and root fresh weight as well as shoot and root dry matter content, at both flowering and fruit-set stages (Campobenedetto et al., 2021).

Physiological responses

The application of *A. nodosum* extract in *T. pannonicus* mainly improved **photosynthetic pigment** stability under severe stress, particularly under combined drought–salinity at 120 mM, where chlorophylls and carotenoids were partially restored. This suggests enhanced photoprotection and photosynthetic function under high stress pressure. In contrast, ANE had only minor effects on the **relative water content**, indicating that its protective action is mainly related to metabolic and physiological stabilisation rather than improved leaf hydration.

In *T. × citriodorus*, *A. nodosum* extract partially mitigated pigment loss, mainly by preserving chlorophyll a and total chlorophyll under salinity stress, suggesting better protection of the photosynthetic apparatus and reduced oxidative damage. ANE had little effect on RWC under single stress, but under combined drought–salinity it improved plant water status, indicating that its protective effects are most pronounced under severe, multiple stress conditions.

The application of *A. nodosum* extract in *T. capitatus* partially restored chlorophyll and carotenoid levels, particularly under severe combined drought–salinity stress, helping maintain photosynthetic stability, while its effect under single stress was weaker. ANE also improved RWC under drought, suggesting enhanced leaf hydration, but this benefit decreased under salinity and combined stress. Overall, ANE mainly supported photosynthetic protection under severe stress, whereas improvements in water status were more evident under moderate stress conditions.

A. nodosum extract consistently moderated stress responses in *T. pannonicus*. Under salinity and combined stress, **proline** accumulation was reduced and the increase in **soluble sugars** was attenuated, suggesting a lower physiological stress burden and reduced reliance on osmotic adjustment. In contrast, **H_2O_2** levels were only slightly affected, indicating limited reduction of oxidative pressure. These results indicate that ANE alleviated stress mainly through improved physiological regulation rather than strong suppression of stress signalling.

A similar but more pronounced physiological modulation was observed in *T. × citriodorus*. *A. nodosum* extract consistently reduced proline and soluble sugar accumulation under drought, salinity, and combined stress, suggesting reduced osmotic stress perception in treated plants. H_2O_2 levels were also lowered under severe stress, indicating improved antioxidant protection and cellular stability, confirming that ANE enhances physiological resilience mainly by improving internal stress regulation and protecting cellular functions under adverse conditions.

In *T. capitatus*, the physiological response to *A. nodosum* extract was more selective. The strongest effects occurred under combined drought–salinity stress, where proline accumulation was reduced and H_2O_2 levels were partially lowered, while soluble sugars remained largely unchanged. This suggests that stress mitigation was mainly achieved through reduction of oxidative pressure rather than strong osmotic adjustment. Benefits under single stress were limited, but these responses confirm the role of ANE in reducing physiological strain under severe, multiple stress conditions.

The capacity of seaweed extract to mitigate abiotic stress through physiological regulation has already been documented in several plant species, supporting the patterns observed in the present study. For example, a crude extract from the brown macroalga *Fucus spiralis* effectively alleviated drought stress in *Raphanus sativus* L.; under 40 % FC, seaweed extract application increased relative water content by 17.07 % compared with untreated stressed plants (Er-rqaibi et al., 2025). This response closely mirrors the improvements in water status observed in our TL2 and TC2 experiments. In the same study, stress markers such as proline, soluble sugars, and malondialdehyde were substantially reduced by seaweed extract treatment (23.45 %, 44 %, and 27.36 %, respectively), in line with our findings. However, unlike our results, chlorophyll and carotenoid levels were not fully restored, highlighting that pigment responses to seaweed extract application may vary depending on species, stress intensity, and extract composition.

Consistent with our observations under salinity stress conditions, similar physiological benefits were reported in *Oryza sativa* L. exposed to 200 mM NaCl, where the application of ANE significantly enhanced chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content under both weekly and biweekly treatments (Shahzad et al., 2023). These improvements were accompanied by higher net photosynthetic rate, transpiration rate, stomatal conductance, and water use efficiency, indicating a coordinated reinforcement of photosynthetic performance and gas exchange. At the same time, seaweed extract application markedly reduced key stress indicators, including hydrogen peroxide, superoxide anion, lipid peroxidation (malondialdehyde), and lipoxygenase activity, further supporting its role in limiting oxidative damage under saline conditions.

Biochemical responses

In *T. pannonicus*, *A. nodosum* extract markedly improved **essential oil content** under stress, with the strongest effect under combined drought–salinity. ANE also helped stabilise **essential oil composition** by maintaining thymol levels and limiting the stress-induced increase of β-bisabolene and thymol methyl ether, while having little effect on p-cymene. **Total polyphenol content** and **antioxidant capacity** were also partially restored under severe stress, suggesting that ANE supports secondary metabolism and redox balance, helping preserve both oil quantity and quality mainly under high stress conditions rather than under optimal growth conditions.

A similar protective effect was observed in *T.* × *citriodorus*, where *A. nodosum* extract mitigated stress-induced reductions in essential oil content, particularly under drought and combined stress, while maintaining stable EO levels under salinity. ANE also helped preserve oil composition by maintaining higher geraniol levels and limiting excessive increases in geranial, neral, and β-bisabolene. Total polyphenol content and antioxidant capacity were enhanced, especially under salinity and combined stress, indicating improved metabolic and oxidative protection rather than complete suppression of stress effects. These responses suggest that ANE supports secondary metabolism and plant defence systems, helping maintain EO yield, quality, and cellular stability under severe stress conditions.

In *T. capitatus*, *A. nodosum* extract had a moderate but positive effect on essential oil metabolism. EOC was slightly enhanced mainly under control conditions, while under stress ANE primarily helped stabilise oil composition rather than strongly increase yield. ANE partially restored early monoterpenes such as p-cymene and γ-terpinene under salinity without promoting excessive carvacrol accumulation. Total polyphenol content and antioxidant capacity were also improved, especially under salinity and combined stress, indicating better metabolic and redox regulation rather than strong stimulation of secondary metabolite production. These responses suggest that ANE helps maintain a balanced essential oil profile and supports antioxidant defence mechanisms under stress conditions.

Our findings are consistent with previous reports highlighting the stress-mitigating potential of seaweed extracts. In *Lavandula angustifolia*, application of ANE had no effect on non-stressed plants, yet under drought, EO yields increased from 0.55 to 0.75 % DW, and under salinity stress (100 mM, twice weekly), yields rose from 0.58 to 0.70 % DW (Spagnuolo et al., 2025). Similarly, in radish exposed to drought (40 % FC), TPC increased, but treatment with *Fucus sativus* L. extract restored TPC to near-normal levels, reducing it by 13 % (Er-rqaibi et al., 2025). Aligning with these observations, cultivated *T. vulgaris* treated with ANE under drought conditions exhibited a marked increase EO percentage, from 2.2 % to 3.3 %, alongside enhancements in total flavonoids and polyphenol by 45 % and 36 %, respectively. Antioxidant enzyme activities, including CAT, SOD, and APX, were also significantly elevated by 58 %, 34 %, and 45 %, reflecting a strengthened oxidative defence system (Rahimi et al., 2022).

Across the three *Thymus* species, *A. nodosum* extract application consistently reinforced stress resilience, as reflected in both **SSI** and **STI**, with the most pronounced benefits observed under drought and salinity. In *T. pannonicus*, treatment shifted drought susceptibility toward moderate tolerance and partially alleviated the negative effects of salinity and combined stress. Similarly, in *T.* × *citriodorus*, ANE reduced vulnerability under salinity and combined stress, stabilised yield, and enhanced STI, likely through improvements in RWC, osmotic adjustment, antioxidant capacity, and EO preservation. In *T. capitatus*, ANE not only mitigated drought effects but also induced overcompensation, improved salinity tolerance, and partially offset combined stress impacts. Together, these findings highlight that ANE strengthens physiological regulation, water relations, and antioxidant defences, ultimately supporting growth and biomass maintenance under challenging conditions, and underscore its practical potential as a biostimulant for enhancing *Thymus* performance under multi-factorial stress.

The stress-mitigating effects of *A. nodosum* extracts are multifaceted, encompassing morphological, biomass, physiological, and biochemical responses that enhance plant resilience to both abiotic and biotic challenges. Our results indicate that ANE exerts its strongest influence under combined drought–salinity stress, with moderate effects under individual stress treatments and only minor impacts in unstressed plants. As shown in Table 4, ANE contains a rich mixture of bioactive compounds, including polysaccharides, phytohormones, and minerals, that collectively promote plant health and productivity under adverse conditions. Cytokinins, auxin-

like compounds, ABA-modulating factors, and other signalling molecules act as early stress sensors, initiating cascades involving calcium fluxes, ROS, and MAPK pathways, which ultimately reprogram stress and growth-related gene expression (Dubiella et al., 2013; Jayaraj et al., 2008; Mercier et al., 2001).

These early signalling events enhance photosynthetic performance, maintain plant morphology, and stimulate secondary metabolism. ANE-treated plants show increased chlorophyll content due to enhanced biosynthesis, reduced degradation, and improved nitrogen assimilation, which together preserve photosystem II under drought and salinity (Fan et al., 2013). Morphologically, ANE modulates the auxin–cytokinin balance, promoting root growth and branching while delaying shoot senescence, thereby improving water and nutrient uptake and sustaining aboveground biomass under stress conditions (Santaniello et al., 2017). By maintaining carbon assimilation and limiting oxidative damage, ANE also supports secondary metabolism, including the production of essential oils and polyphenols (Fan et al., 2013; Jannin et al., 2013).

These findings reinforce the priming role of ANE rather than its function as a direct nutrient source, explaining why its effects are limited under control conditions where physiological homeostasis is already maintained. Under combined drought–salinity stress, overlapping osmotic and oxidative pressures create a wider margin for biostimulant-induced improvement. Seaweed extracts enhance antioxidant defences, stabilise photosynthesis, and promote ABA-mediated signalling, with their protective effects becoming more pronounced as stress severity increases (Calvo et al., 2014; Van Oosten et al., 2017). This coordinated activation of multiple defence pathways explains why ANE often confers greater benefits under combined stress than under individual stress treatments or in non-stressed plants (Rouphael & Colla, 2020; Shukla et al., 2019).

6.5 The phenotypic and metabolic diversity among *Thymus* spp. under various stress conditions

In the present study, the three *Thymus* species were evaluated to assess their responses to drought and salinity stress. As discussed above, these species showed contrasting morphological, biomass, physiological, and biochemical responses when exposed to stress factors, highlighting their phenotypic and metabolic diversity under adverse conditions and allowing comparison of their species-specific adaptive characteristics. However, since the last-year experiments (TP3, TL2, TC2) were conducted simultaneously, with only a few days difference between measurements, and since plants were at a comparable developmental stage and grown under the same environmental conditions, cross-species comparisons in this subsection were mainly focused on physiological and biochemical traits. This approach reduces potential confounding effects related to growth stage or environmental variation, while allowing comparison of species-level adaptive tendencies, potentially associated with their biological background, rather than attributing differences strictly to genetic factors.

Photosynthetic pigments, including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, showed marked species-specific sensitivity to salinity stress (120 mM) and to its

combination with drought (40 % SWC). In *T. pannonicus* (TP3) and *T.* × *citriodorus* (TL2), pigment contents declined significantly under these stress conditions, indicating a higher vulnerability of the photosynthetic apparatus. In contrast, *T. capitatus* (TC2) maintained comparatively stable pigment levels, even under severe stress. This stability highlights a greater phenotypic resilience of *T. capitatus* in preserving the integrity and functionality of its photosynthetic system under harsh environmental conditions, suggesting an intrinsic, species-specific protective strategy rather than a transient stress response.

Stress indicators further supported this interspecific differentiation. Proline accumulation increased under stress in nearly all treatments across the 2025 experiments, with the notable exception of drought-stressed *T. capitatus*, where proline levels remained close to control values. When the relative increase in proline content from control to the most severe stress treatment was considered, clear differences emerged among species, reaching approximately 97 %, 200 %, and 367 % in TC2, TP3, and TL2, respectively. The markedly lower proline accumulation in *T. capitatus*, despite exposure to comparable stress intensity, indicates a reduced reliance on osmotic adjustment through proline synthesis. This pattern suggests that *T. capitatus* employs alternative, intrinsic tolerance mechanisms that limit cellular damage and reduce the need for strong metabolic reprogramming. Other stress-related metabolites, such as soluble sugars and hydrogen peroxide, showed more variable trends among species and treatments, with differences often being less pronounced, thereby limiting their usefulness for defining clear interspecific strategies. Nevertheless, the overall stress-marker profile reinforces the existence of distinct intrinsic response strategies within the *Thymus* genus.

Regarding essential oil productivity, *T. capitatus* again demonstrated a high degree of resilience, as EOC remained remarkably stable across all stress treatments in TC2. In contrast, EOC in *T.* × *citriodorus* was negatively affected only under combined drought and salinity stress, whereas in *T. pannonicus*, both individual stress treatments and combined stress led to a clear reduction in oil yield. These contrasting patterns indicate that EO biosynthesis in *T. capitatus* appears to be more tightly regulated and less susceptible to environmental perturbation, whereas in the other species it seems more closely associated with growth performance and stress intensity. This distinction further supports the presence of species-specific metabolic strategies within the genus.

Metabolic diversity among the studied species became even more evident when focusing on EO composition and its modulation under stress. GC–MS analysis performed in the 2025 experiments revealed 21 identified compounds in *T. pannonicus*, dominated by thymol (26.17–29.82 %) and p-cymene (7.83–18.96 %), defining a clear thymol chemotype. In this species, monoterpene hydrocarbons and oxygenated monoterpenes generally declined with increasing stress intensity, whereas sesquiterpenes increased. In *T.* × *citriodorus*, 18 compounds were identified, with geraniol (55.45–62.60 %), geranial (13.05–17.18 %), and neral (9.06–13.87 %) as dominant constituents. Notably, monoterpene hydrocarbons were absent in this species, while the remaining chemical groups followed trends broadly similar to those observed in *T. pannonicus* under stress. In *T. capitatus*, only 10 compounds were detected, indicating a chemically simpler but highly

specialised profile. As in *T. pannonicus*, compounds were grouped into monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes; however, their stress-related dynamics differed markedly; while monoterpene hydrocarbons and sesquiterpenes followed trends comparable to those observed in *T. pannonicus*, oxygenated monoterpenes increased with stress severity, in contrast to the reductions observed in the other species. *T. capitatus* was clearly characterised as a carvacrol chemotype (75.45–80.32 %), with p-cymene and γ-terpinene as key precursors. Focusing on the dominant compounds, thymol in *T. pannonicus* declined only under combined stress, whereas geraniol in *T.* × *citriodorus* decreased under both individual and combined stress treatments. In contrast, carvacrol in *T. capitatus* was preserved under individual stress treatments and even increased under combined stress, underscoring the exceptional stability and intrinsic regulation of its chemotype. The three PCA analyses further confirmed the central role of these dominant compounds in structuring species-specific chemical responses under stress conditions.

These findings are consistent with previous reports highlighting extensive chemodiversity within the *Thymus* genus. Although linalool is the dominant compound in species such as *T. moesiacus*, *T. jankae*, *T. vandasii*, *T. longicaulis*, and *T. sibthorpii*, substantial variation exists in accompanying constituents such as geranyl acetate, geraniol, and linalyl acetate (Trendafilova et al., 2023). Similarly, *T. atticus*, *T. leucotrichus*, and *T. striatus* are characterised by high sesquiterpenoid contents, particularly β-caryophyllene, while *T. thracicus* exhibits a mixed mono-/sesquiterpene chemotype dominated by β-myrcene, cis-sabinene hydrate, τ-cadinol, and elemol (Trendafilova et al., 2021, 2023). Collectively, these observations indicate that species-specific intrinsic factors play a major role in shaping both phenotypic and metabolic diversity within the genus *Thymus*. The distinct physiological stability, metabolic regulation patterns, and essential oil profiles observed among *T. capitatus*, *T. pannonicus*, and *T.* × *citriodorus* underline how inherent species characteristics influence stress responses and secondary metabolism. Such diversity represents an important adaptive asset within the genus, enabling different species to persist and maintain functional and phytochemical integrity under contrasting and often limiting environmental conditions.

6.6 Ecological and phenotypic plasticity of *Thymus* spp. under stress conditions

Phenotypic plasticity describes the capacity of plants to modify functional traits in response to environmental fluctuations, whereas ecological plasticity reflects the breadth of conditions under which a species can maintain performance and persist across habitats (Bakhtiari et al., 2019; Sultan, 2000). This dual concept is particularly relevant for perennial aromatic taxa such as *Thymus*, which naturally occupy stress-prone environments and rely on flexible physiological and metabolic strategies to cope with climatic variability.

The three PCA analyses performed for the 2025 experiments (TP3, TL2, and TC2) provide an integrated view of trait coordination under drought, salinity, and combined stress, allowing plasticity to be assessed through multivariate shifts rather than isolated parameters. In this

framework, phenotypic plasticity was interpreted as the degree of movement along Dim1 between the control and stress treatments (see Figures 14, 20 and 27). Drought stress (40 % SWC) consistently resulted in the smallest shift from the control in all species, indicating that moderate water limitation remained within the adaptive range of the three *Thymus* taxa. Nevertheless, *T.* × *citriodorus* exhibited a larger displacement than *T. pannonicus* and *T. capitatus*, suggesting a higher sensitivity to drought-induced changes in its physiological and biochemical profile. Salinity stress (120 mM) produced a more pronounced shift away from the control, with *T.* × *citriodorus* and *T. pannonicus* showing greater displacement than *T. capitatus*, pointing to a stronger salinity-driven reorganisation of traits in these species. Combined drought and salinity stress generated the largest separation from the control, pushing all species to the extreme negative side of Dim1; however, the magnitude of this shift was still lower in *T. capitatus* compared to other species, indicating a comparatively higher capacity to maintain functional coherence under compounded stress.

These PCA patterns reveal contrasting plasticity strategies within the genus. *T. capitatus* displayed limited phenotypic displacement but higher trait stability, reflecting a conservative strategy characterised by reduced responsiveness yet strong resistance to environmental perturbation. In contrast, *T. pannonicus* and *T.* × *citriodorus* exhibited larger multivariate shifts, indicative of greater phenotypic plasticity but also higher sensitivity, as stress triggered broader physiological and metabolic reconfiguration. In this context, plasticity should not be interpreted as superior performance, but rather as the degree of adjustment required to cope with stress; thus, the lower plasticity of *T. capitatus* reflects inherent robustness rather than limited adaptability.

Across species, the PCA further highlights the role of stress intensity in defining the limits of plasticity. Drought alone produced moderate trait reorganisation and remained within the plastic range of all three species, whereas salinity, particularly when combined with drought, pushed plants beyond their optimal adaptive window. This pattern supports the concept that phenotypic plasticity can buffer moderate stress, but extreme or combined stress expose genetic and physiological constraints, leading to marked divergence from control conditions.

The contrasting plasticity patterns observed among the three *Thymus* species can be explained by their distinct ecological origins. *T. capitatus* is a Mediterranean species naturally exposed to prolonged summer drought, high solar radiation, and rocky soils, with flowering and essential oil production typically occurring under limited water availability (Abdel-Rahman & Migahid, 2019). The Tunisian provenance of the population used in this study therefore aligns well with the PCA results, which revealed a limited displacement from the control but high overall stability under stress. This behaviour indicates a conservative adaptive strategy, where core physiological and metabolic functions are maintained rather than extensively adjusted when stress intensifies. In contrast, *T. pannonicus*, originating from cooler temperate regions characterised by lower summer temperatures and higher precipitation (Simić et al., 2024), as reflected by the population used in this study originating from Hungary and *T.* × *citriodorus*, a hybrid adapted to moderately warm and sunny environments (Vaičiulytė et al., 2022), both exhibited larger multivariate shifts

under salinity and combined stress. Despite their different ecological backgrounds, these two species showed comparable intermediate plasticity, characterised by stronger trait adjustments but reduced physiological stability compared with *T. capitatus*. Across all species, the addition of ionic stress to water deficit emerged as the key factor driving plants beyond their optimal adaptive range, clearly exposing differences in ecological plasticity within the *Thymus* genus.

6.7 Trade-offs among growth, stress tolerance, and secondary metabolite production in *Thymus* spp.

In the current study, we aimed to investigate whether *Thymus* spp. reallocates resources from growth to the synthesis of bioactive compounds when exposed to drought (40% SWC), salinity (120 mM NaCl), or their combination, and to examine the role of *A. nodosum* extract in modulating this trade-off.

Across the three *Thymus* species, the heatmap and PCA consistently reveal a clear physiological trade-off that defines the baseline response to abiotic stress in the absence of *A. nodosum* extract supplementation. Stress-only treatments cluster with osmotic and oxidative markers: proline, H_2O_2 and soluble sugars, while opposing traits linked to plant performance, including biomass accumulation, photosynthetic pigments and RWC. This antagonistic pattern indicates that as stress intensity increases, resources are preferentially diverted toward defence and osmotic adjustment, at the expense of growth, pigment stability and tissue hydration. In all species, plants positioned along the stress-dominated axis exhibit reduced biomass and impaired physiological functioning, whereas those associated with higher RWC, pigment concentrations and biomass show lower stress marker accumulation. These multivariate relationships establish a robust baseline trade-off: activation of defence mechanisms under drought and salinity inherently constrains growth and physiological performance, reflecting the cost of stress tolerance before any biostimulant-mediated mitigation is considered.

The multivariate patterns further indicate that abiotic stress drives a marked reallocation of metabolic priorities from growth-related processes toward secondary metabolism. Across the three *Thymus* species, essential oil traits and stress-associated compounds, particularly β-bisabolene, geranial, neral, carvacrol, β-caryophyllene, tend to align with stress-dominated treatments, whereas photosynthetic pigments, RWC and biomass traits cluster with control or high-performance conditions. This opposition suggests that stress does not merely suppress growth but actively redirects carbon and energy away from primary metabolism, including photosynthesis and biomass formation, toward the synthesis of defensive secondary metabolites. As stress intensity increases, pigment integrity and photosynthetic capacity decline, while the accumulation of essential oil constituents linked to chemical defence becomes more pronounced. Collectively, these relationships support the view that drought and salinity promote a defence-oriented metabolic shift in *Thymus*, enhancing secondary metabolite production at the expense of growth and photosynthetic efficiency.

A. nodosum extract application reshapes the stress-induced trade-off without eliminating it, creating an attenuated balance among growth and defence in stressed *Thymus* plants. In D+ANE, S+ANE, and DS+ANE treatments, PCA positions shift toward intermediate space among the extreme stress and high-performance clusters, reflecting a partial rebalancing of physiological and metabolic states. *A. nodosum* extract-treated plants exhibit enhanced AOC, increased TPC, improved EOC, and notable gains in shoot and root growth as well as pigment stability, indicating a coordinated reinforcement of both metabolism and physiology under stress. These patterns highlight that ANE buffers the growth–defence balance: it supports defensive metabolism while simultaneously stabilising key physiological traits. Rather than removing the stress-imposed trade-off, ANE attenuates it, providing a nuanced modulation that preserves secondary metabolism and mitigates the severity of stress impacts.

Under drought and salinity, trade-offs among growth and defence are a natural part of *Thymus* biology. *A. nodosum* extract does not remove these stress constraints, but it helps plants cope more efficiently by acting as a metabolic buffer. In stressed plants, ANE redirects resources toward antioxidant and secondary metabolite production while also supporting pigments, shoot and root growth, and essential oil traits. This balanced reinforcement reduces the cost of activating defence mechanisms, easing the impact of stress without completely restoring all growth traits. Basically, ANE reshapes the growth-defence trade-off, helping plants tolerate adverse conditions while maintaining both metabolic and physiological performance.

7 Conclusion and recommendation

This study evaluated the stress responses of three *Thymus* species, *T. pannonicus*, *T.* × *citriodorus*, and *T. capitatus*, through a comprehensive, multi-year experimental framework. In total, 560 plants were examined across seven experiments conducted over three consecutive years, of which 240 plants were specifically used to assess the mitigating potential of a *A. nodosum* extract during the final experimental year. A wide range of morphological, biomass, physiological, and biochemical traits were integrated to capture both growth performance and metabolic adjustments under drought (40 % soil water capacity), salinity (60, 90 and 120 mM) and their combination.

For *T. pannonicus*, the results clearly demonstrated a progressive stress response that depended on salinity intensity. At 60 mM NaCl (first experiment of *T. pannonicus*), salinity alone induced only minor deviations from the control, confirming a relatively high tolerance at this level, whereas drought exerted a stronger negative effect. The combined stress consistently caused the most pronounced reductions, indicating a clear synergistic interaction. When salinity was increased to 90 mM in the second experiment, *T. pannonicus* expressed moderate sensitivity, particularly under combined stress, while drought responses remained comparable to those observed in the previous year. At 120 mM NaCl (third experiment of *T. pannonicus*), salinity effects approached those of combined stress, reflecting a threshold beyond which tolerance mechanisms were insufficient. Notably, *A. nodosum* extract application under these severe conditions partially restored plant performance, positioning stressed plants between single drought and control levels, highlighting its role as a stress-mitigating rather than growth-promoting input.

In *T.* × *citriodorus*, both experimental years revealed a consistent response pattern. In the first experiment of *T.* × *citriodorus*, drought negatively affected plant performance, but salinity at 120 mM imposed a stronger limitation, with the combined treatment being the most detrimental. This pattern was largely reproduced in the second experiment, suggesting a stable species response rather than year-specific variability. Importantly, *T.* × *citriodorus* did not behave as an intermediate or highly sensitive species; instead, its response profile closely resembled that of *T. pannonicus*, indicating a comparable level of stress tolerance. *A. nodosum* extract application markedly reshaped plant responses under stress, bringing both single and combined stress treatments closer to the control and control with *A. nodosum* extract groups, reinforcing its protective effect under adverse conditions.

For *T. capitatus*, stress-induced reductions were observed across all measured traits; however, the magnitude of differences among treatments remained relatively moderate compared to the other species. In first experiment of *T. capitatus*, drought and salinity at 90 mM caused comparable declines, while their combination exerted a stronger but still less extreme effect. In the second experiment, increasing salinity to 120 mM led to a more pronounced deterioration, yet single salinity and drought treatments maintained intermediate performance between control and combined stress. *A. nodosum* extract application proved particularly effective in this species,

alleviating the negative effects of salinity and combined stress and restoring drought-treated plants to levels comparable with the unstressed control, indicating a strong biostimulant–species interaction.

Overall, this study confirms the markedly deleterious impact of combined drought and salinity, especially at 120 mM NaCl, on the morphological, biomass, physiological, and biochemical performance of all three *Thymus* species. At the same time, it clearly demonstrates that *A. nodosum* extract can alleviate stress-induced damage, particularly under combined stress conditions, by restoring plant performance toward control levels. Importantly, the biostimulant showed limited effects under non-stress conditions, suggesting that its application is most justified as a targeted stress-management strategy rather than as a routine agronomic input.

To further elucidate the mechanisms underlying these responses, future research should focus on enzymatic antioxidant systems (e.g., Superoxide dismutase, catalase, peroxidase) and molecular-level investigations to confirm the physiological and metabolic pathways involved in stress mitigation and *A. nodosum* extract-induced tolerance. From a practical perspective, the present findings support the recommendation of *A. nodosum* extract application for farmers cultivating *T. pannonicus* and *T.* × *citriodorus*, particularly under the increasing risks of drought and salinity. This is especially relevant for Hungarian production systems, where reduced summer precipitation and prolonged dry periods have become more frequent and represent a critical constraint during key developmental stages of *Thymus* species. In addition, the use of *A. nodosum* extract-based biostimulants may also be recommended for *T. capitatus*, a species widely distributed and traditionally harvested in Tunisia, where cultivation under rainfed and marginal conditions exposes plants to recurrent water and salinity stress. In this context, biostimulant application could represent a sustainable strategy to enhance stress resilience and support the domestication and cultivation of this economically and ecologically valuable species.

8 NEW SCIENTIFIC RESULTS

1. A concentration-dependent salinity response pattern was demonstrated in *Thymus pannonicus*: low to moderate salinity (60–90 mM) induced only minor changes, whereas high salinity (120 mM) clearly triggered growth reduction and stronger physiological-biochemical adjustments, including increased photosynthetic pigments amounts, relative water content, antioxidant capacity and β-bisabolene percentage. This response pattern confirmed a species-specific salinity tolerance threshold.
2. Metabolic rerouting in *Thymus pannonicus* was proved: stress reduced thymol while increased sesquiterpene and other oxygenated monoterpene levels in the essential oil. These opposite trends indicate compound-specific regulation rather than uniform suppression of essential oil biosynthesis. *Ascophyllum nodosum* extract modulated these pathways differently by enhancing selected biosynthetic branches, indicating regulation at the level of metabolic processes.
3. Stress-related modulation of citral components in *Thymus* × *citriodorus* was verified: Drought and salinity altered the biochemical profile of this species, revealing that stress induced a decline in the geraniol ratio, with a concomitant increase in the geranial and neral percentages, reflecting stress-related metabolic adjustments.
4. Enhanced sensitivity to combined stress in *Thymus capitatus* was validated: while drought or salinity applied individually caused moderate reductions in growth, water status, and pigment levels, their combination exerted a cumulative and more pronounced negative effect across all traits, revealing a higher sensitivity to simultaneous stress than to single stress.
5. Drought tolerance enhancement by *Ascophyllum nodosum* extract in *Thymus capitatus*: it was demonstrated that under drought conditions, the extract markedly improved relative water content, pigment levels, and growth, while oxidative stress remained lower than in untreated plants. Stress tolerance indices shifted from susceptible to strongly tolerant, highlighting a clear protective effect under water deficit.
6. Stress-dependent action of *Ascophyllum nodosum* extract was confirmed: the extract caused only minor or non-significant effects in unstressed *Thymus pannonicus*, *T.* × *citriodorus* and *T. capitatus*, whereas its impact became markedly stronger under drought and salinity. Physiological and biochemical traits responded more prominently under stress conditions, indicating a consistently stress-dependent effect.

9 Summary

Although *Thymus vulgaris* is the most widely known and extensively studied species of the genus, several other *Thymus* species remain comparatively underexplored despite their valuable aromatic and biological properties. This thesis therefore focuses on *Thymus pannonicus*, *T.* × *citriodorus*, and *T. capitatus* as alternative genetic resources with potential agronomic and industrial relevance. Their responses to drought and salinity were investigated, as these abiotic stressors are becoming increasingly frequent and severe under ongoing climate change. Because plants are rarely exposed to a single stress factor in natural environments, the combined application of drought and salinity was included to better reflect realistic field conditions. Given the central role of secondary metabolism in *Thymus*, particularly essential oil production, this work aimed to elucidate how stress affects plant growth, physiological status, oil yield and composition and other biochemical traits. In parallel, a biostimulant derived from the brown seaweed extract *A. nodosum* was tested as a natural, eco-friendly alternative to chemical inputs, with the hypothesis that it could alleviate stress effects while supporting essential oil production under adverse conditions. In addition to evaluating stress and *A. nodosum* extract responses, this study examined phenotypic and metabolic diversity, ecological plasticity, and growth-metabolite trade-offs, while also assessing whether essential oil yield and quality could be maintained under stress.

A total of seven experiments were conducted over three years: three on *T. pannonicus* (TP1, TP2, TP3), two on *T.* × *citriodorus* (TL1, TL2), and two on *T. capitatus* (TC1, TC2). Plants were grown in 2 L pots and the irrigation was applied twice weekly, with drought treatments maintained at 40 % soil water capacity, while control and salinity treatments were maintained at 70 % soil water capacity. Salinity levels were progressively applied at 60 and 90 mM in the first experiments and increased to 120 mM in the final year. Combined stress consisted of the simultaneous application of drought and salinity. *Ascophyllum nodosum* extract was applied every two weeks at a volume of 100 mL per plant, with a concentration of 8 mL L^{-1} in the final-year experiments (TP3, TL2, TC2). Morphological and biomass traits included shoot length, shoot number, fresh and dry shoot biomass, root length, and fresh and dry root weights. Physiological parameters comprised chlorophyll a, chlorophyll b, carotenoids, relative water content, proline, and soluble sugars. Biochemical analyses included essential oil content and composition, total phenolic content, antioxidant capacity, hydrogen peroxide, as well as stress susceptibility and stress tolerance indices.

T. pannonicus displayed stress responses that were strongly dependent on stress intensity, allowing clear thresholds of tolerance to be identified. Drought, high salinity (120 mM), and especially their combination resulted in marked reductions in shoot and root biomass, shoot number, shoot length, and photosynthetic pigments. These coordinated declines indicate that under extreme stress, both carbon assimilation and structural growth are constrained. In contrast, intermediate salinity levels (60–90 mM) applied alone caused only minor changes in morphological, biomass traits and pigment content (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids),

demonstrating that *T. pannonicus* is able to tolerate moderate salt exposure without major physiological disruption. The progressive accumulation of proline, soluble sugars, and H_2O_2 with increasing stress severity reflects active osmotic adjustment and oxidative signalling rather than immediate cellular failure. Relative water content declined mainly under drought and combined stress at 120 mM, indicating that water retention mechanisms were engaged but insufficient under the most severe conditions. *A. nodosum* extract application moderated these stress effects, particularly by enhancing root growth across all stress treatments and improving shoot development under combined stress, thereby supporting water uptake and plant stability. This mitigation was mirrored in the biochemical profile: antioxidant capacity and total polyphenol content, which declined under severe stress, were restored by *A. nodosum* extract application, suggesting reinforcement of antioxidant and phenolic pathways. Essential oil content, dominated by thymol, p-cymene, β-bisabolene, and thymol methyl ether, declined under drought and salinity from 90 mM onward, with the strongest reduction under combined stress. However, *A. nodosum* extract stabilised essential oil yield and moderated compositional shifts, notably by preserving thymol dominance and limiting stress-induced increases in β-bisabolene and thymol methyl ether. Principal component analysis in TP3, supported these patterns by placing the control on the positive side of Dim1, drought-treated plants in an intermediate position, salinity further to the left, and combined stress at the extreme negative side, reflecting increasing stress severity; notably, *A. nodosum* extract shifted all stressed treatments closer to the control, indicating partial recovery of growth- and pigment-related traits. These results indicate that *T. pannonicus* combines tolerance to intermediate salinity with a clear reallocation toward osmotic, oxidative, and secondary metabolic defences under severe stress, a response that is effectively buffered by *A. nodosum* extract application.

T. × *citriodorus* responded to drought and salinity with a stress pattern broadly comparable to *T. pannonicus*, though with distinct physiological and metabolic nuances. Exposure to 120 mM salinity and its combination with drought caused moderate reductions in shoot length, shoot number, and shoot and root biomass, while drought alone had a more limited impact on growth. Photosynthetic pigments declined mainly under salinity and combined stress, indicating that ionic stress rather than water limitation was the primary driver of photosynthetic disruption in this species. Stress marker accumulation: proline, soluble sugars, and H_2O_2, increased markedly with stress severity, pointing to an active osmotic and oxidative adjustment strategy rather than stress avoidance. The partial decline in relative water content further indicates controlled dehydration rather than loss of water balance. Antioxidant capacity and total polyphenol content decreased mainly under salinity and combined stress, suggesting that antioxidant and phenolic pools were challenged under severe conditions. *A. nodosum* extract application substantially improved shoot and root biomass, shoot number, and relative water content under combined stress, while also enhancing antioxidant capacity and total polyphenol content across all stress treatments. This coordinated improvement highlights the capacity of *A. nodosum* extract to reinforce both physiological stability and secondary metabolism. Essential oil content, dominated by geraniol, geranial, neral, and β-bisabolene, declined with increasing stress severity, particularly under

combined stress. Nevertheless, *A. nodosum* extract application preserved essential oil yield and moderated compositional shifts by maintaining geraniol levels and limiting excessive accumulation of geranial and neral. The principal component analysis similarly showed the strongest displacement under combined stress, followed by salinity and then drought, while *A. nodosum* extract application consistently moved stressed plants toward the control cluster, highlighting its buffering effect on physiological and metabolic responses, indicating that *T.* × *citriodorus* is not intrinsically stress-sensitive but rather responds to severe stress through metabolic reprogramming that can be effectively mitigated by biostimulant support.

T. capitatus exhibited the most stable overall response to drought and salinity, although stress effects were still evident and followed trends comparable to the other species. Under drought, 90 mM salinity in TC1,120 mM salinity in TC2 and combined stress, reductions in shoot and root biomass, shoot number, root length, and photosynthetic pigments were observed, confirming that stress constrained growth and photosynthesis. Stress markers: proline, soluble sugars, and H_2O_2, increased with stress severity, demonstrating active stress perception and response, while relative water content remained relatively stable, suggesting more effective water regulation. Antioxidant capacity generally declined under stress while total polyphenol content decreased in single stress treatment but increased in combined stress, though their responses differed between TC1 and TC2, pointing to context-dependent metabolic regulation. *A. nodosum* extract application reinforced this stability by improving growth mainly under single salinity, maintaining pigment levels under combined stress, enhancing relative water content under drought, and strengthening antioxidant defences. Essential oil content, dominated by carvacrol, p-cymene, γ-terpinene, and thymol methyl ether, showed contrasting responses between experiments: in TC1 (90 mM), essential oil yield and dominant compounds slightly declined under stress, whereas in TC2 (120 mM), essential oil production increased with stress intensity. This divergence likely reflects differences in plant age, with older, cutting-derived plants in TC2 showing a greater capacity to sustain or enhance secondary metabolism under stress. In TC2, *A. nodosum* extract contributed to stabilising essential oil yield and preserving carvacrol dominance. Regarding the principal component analysis, it revealed smaller stress-induced shifts overall, with combined stress still causing the greatest displacement; across treatments, *A. nodosum* extract application brought stressed plants closer to the control, reflecting enhanced trait stability. Overall, *T. capitatus* combines inherent physiological stability with flexible metabolic regulation, enabling it to maintain growth, water relations, antioxidant defences, and essential oil composition under increasing stress intensity.

The three *Thymus* species showed clear species-specific differences in their physiological and biochemical responses. *T. capitatus* maintained relatively stable pigment levels (chlorophyll a, chlorophyll b, total chlorophyll, carotenoids) and showed lower accumulation of stress markers (proline, soluble sugars, H_2O_2), indicating greater intrinsic resilience to abiotic stress. In contrast, *T. pannonicus* and *T.* × *citriodorus* exhibited greater fluctuations, reflecting higher sensitivity to stress and stronger reliance on osmotic adjustment mechanisms. Essential oil composition further highlighted species-specific metabolic patterns: thymol dominated in *T. pannonicus*, geraniol,

geranial, and neral in *T.* × *citriodorus*, and carvacrol, p-cymene, and γ-terpinene in *T. capitatus*, with stress differentially affecting minor and dominant compounds across species. These patterns suggest substantial species-specific diversity in phenotypic traits and secondary metabolism, contributing to distinct adaptive responses under abiotic stress conditions.

Phenotypic plasticity, assessed via principal component analysis, varied among species. *T. capitatus* displayed limited trait displacement under drought and salinity, reflecting a conservative, robust strategy, whereas *T. pannonicus* and *T.* × *citriodorus* showed larger shifts, revealing higher plasticity but also greater sensitivity. Moderate drought remained within the adaptive range for all species, while combined stress pushed them toward physiological limits. These patterns underscore that plasticity reflects the degree of adjustment needed rather than superior performance, with ecological origin influencing the balance between stability and flexibility.

Under drought, salinity, and combined stress, *Thymus* spp. consistently exhibited a trade-off between growth and defence. As stress markers: proline, soluble sugars, and H_2O_2, accumulated, growth, shoot and root biomass, pigments, and relative water content declined, reflecting the metabolic cost of stress tolerance. In parallel, in most cases, stress reduced total polyphenol content, antioxidant capacity, and essential oil content, indicating a general constraint on secondary metabolism; however, essential oil composition was selectively modulated, with some volatile compounds maintained or enhanced while others declined, depending on their defensive role. An exception was observed in *T. capitatus*, where total polyphenol content increased under combined stress and essential oil content increased in TC2, reflecting a species-specific metabolic strategy under severe stress. *A. nodosum* extract application attenuated this trade-off by supporting both growth and physiological stability while sustaining secondary metabolism, partially preserving essential oil yield and stabilising the proportions of dominant compounds, thereby reducing the negative impact of stress without fully eliminating it.

10 Appendices

10.1. Bibliography

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10.2. Further appendices

Table 29. Essential oil composition (%) of *T. pannonicus* (TP1 – 2023) under various treatments.

Components	RT	LRI	**C**	**D**	**S**	**DS**
camphene	5.95	952	0.58	0.46	0.50	0.37
α-terpinene	7.79	1018	1.38	0.89	1.11	0.69
p-cymene	8.09	1026	16.25	16.78	21.29	16.12
1,8-cineol	8.38	1034	0.96	1.24	1.13	1.26
γ-terpinene	9.20	1056	7.09	6.55	5.68	4.28
unknown*	9.45	1065	3.18	3.90	3.71	4.28
camphor	12.68	1144	2.95	3.13	2.79	3.42
borneol	13.43	1162	2.23	3.13	3.37	4.02
thymol methyl ether	16.20	1228	9.33	13.86	9.30	15.89
carvacrol methyl ether	16.61	1238	6.35	7.81	7.04	7.50
thymol	18.81	1290	34.89	26.52	29.20	24.08
carvacrol	19.20	1300	0.17	0.10	0.15	0.11
β-caryophyllene	23.68	1420	1.65	2.13	1.90	2.51
β-bisabolene	27.23	1508	4.79	6.37	4.76	6.79
Total identified (%)	–	–	91.79	92.86	91.93	92.09
Monoterpene hydrocarbons	–	–	25.30	24.68	28.58	21.46
Oxygenated monoterpenes	–	–	56.88	55.79	52.98	56.28
Sesquiterpenes	–	–	6.44	8.50	6.66	9.30

C: control; D: drought stress; S: salinity stress; DS: combined stress; RT: retention time; LRI: linear retention index.

Table 30. Essential oil composition (%) of *T. pannonicus* (TP2 – 2024) under various treatments.

Components	RT	LRI	**C**	**D**	**S**	**DS**
1-octen-3-ol	6.48	974	1.14	0.72	0.74	0.55
β-myrcene	6.99	995	0.97	0.76	0.47	0.47
α-terpinene	7.79	1018	1.45	0.99	0.48	0.62
p-cymene	8.09	1026	15.31	14.33	13.2	12.52
1,8-cineol	8.38	1034	0.72	0.56	0.77	0.86
γ-terpinene	9.20	1056	9.21	5.75	6.23	5.22
cis-sabinene hydrate	9.73	1070	2.07	1.793	3.19	2.53
linalool	10.76	1097	1.56	1.36	1.62	1.27
camphor	12.68	1144	0.08	0.46	1.79	1.09
borneol	13.43	1162	2.43	2.19	3.98	3.28
thymol methyl ether	16.20	1228	9.10	13.62	13.5	14.52
carvacrol methyl ether	16.61	1238	8.23	9.14	9.40	9.70
thymoquinone	17.16	1251	0.33	0.71	0.98	0.81
thymol	18.81	1290	35.75	30.73	31.1	25.60
carvacrol	19.20	1300	0.17	0.16	0.15	0.18
β-caryophyllene	23.68	1420	0.61	1.07	0.97	1.31
bicyclogermacrene	26.81	1497	0.62	1.10	0.40	1.07
β-bisabolene	27.23	1508	3.28	5.11	4.55	7.59
spathulenol	29.98	1584	0.99	1.86	0.95	1.47
caryophyllene oxide	30.20	1590	0.45	1.01	0.66	0.90
Total identified (%)	–	–	94.48	93.46	95.10	91.61
Monoterpene hydrocarbons	–	–	26.94	21.83	20.38	18.83
Oxygenated monoterpenes	–	–	60.44	60.72	66.38	59.84
Sesquiterpenes	–	–	5.95	10.15	7.53	12.34
Aliphatic compounds	–	–	1.14	0.72	0.74	0.55

C: control; D: drought stress; S: salinity stress; DS: combined stress; RT: retention time; LRI: linear retention index.

Table 31. Essential oil composition (%) of *T. pannonicus* (TP3 – 2025) under various treatments.

Components	RT	LRI	C	D	S	DS	C+ANE	D+ANE	S+ANE	DS+ANE
1-octen-3-ol	6.48	974	0.31	0.25	0.08	0.00	0.33	0.35	0.35	0.33
β-myrcene	6.99	995	0.28	0.13	0.06	0.00	0.21	0.40	0.21	0.22
α-terpinene	7.79	1018	0.40	0.10	0.03	0.00	0.18	0.56	0.15	0.22
p-cymene	8.09	1026	18.96	9.35	7.83	5.08	17.61	15.42	10.94	12.41
1,8-cineol	8.38	1034	0.77	0.71	0.36	0.07	0.78	0.96	0.91	0.87
γ -terpinene	9.20	1056	6.61	1.81	1.28	0.56	4.21	3.75	1.63	3.32
cis-sabinene hydrate	9.73	1070	2.56	2.57	1.96	0.19	2.70	3.43	2.34	2.35
linalool	10.76	1097	0.69	0.89	0.48	0.06	0.92	1.06	1.10	0.91
camphor	12.68	1144	1.95	3.44	2.27	0.65	2.82	3.16	4.00	3.01
borneol	13.43	1162	1.80	2.55	1.08	0.46	2.24	2.50	2.70	3.35
thymol methyl ether	16.20	1228	14.59	22.45	20.49	20.59	15.80	15.04	18.26	16.12
carvacrol methyl ether	16.61	1238	8.08	10.17	10.15	6.16	8.77	8.81	9.36	8.95
thymoquinone	17.16	1251	0.07	0.55	0.26	0.23	0.13	0.22	0.61	0.25
thymol	18.81	1290	28.15	28.31	26.31	23.18	29.82	28.26	28.71	26.17
carvacrol	19.20	1300	0.11	0.08	0.03	0.08	0.13	0.12	0.30	0.21
β-caryophyllene	23.68	1420	0.74	1.67	1.49	2.87	1.06	1.29	2.17	2.02
Germacrene D	26.18	1482	0.60	0.55	0.57	2.42	1.24	0.61	0.90	1.08
bicyclogermacrene	26.81	1497	0.56	0.44	0.27	3.03	0.98	0.38	0.83	1.41
β-bisabolene	27.23	1508	8.33	10.26	22.58	25.15	7.74	9.16	10.80	10.13
spathulenol	29.98	1584	0.61	0.47	0.43	5.15	0.66	0.51	0.78	1.82
caryophyllene oxide	30.20	1590	0.65	1.54	1.05	2.59	0.86	1.00	1.41	1.90
Total identified (%)	–	–	96.82	98.30	99.07	98.53	99.18	97.00	99.46	97.05
Monoterpene hydrocarbons	–	–	26.25	11.39	9.20	5.64	22.21	20.13	13.93	16.17
Oxygenated monoterpenes	–	–	58.77	71.72	63.39	51.67	64.11	63.56	68.29	62.19
Sesquiterpenes	–	–	11.49	14.93	26.39	41.21	12.54	12.95	16.89	18.36
Aliphatic compounds	–	–	0.31	0.25	0.08	0.00	0.33	0.35	0.35	0.33

C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract; RT: retention time; LRI: linear retention index.

Table 32. Essential oil composition (%) of *T.* × *citriodorus* (TL1 – 2024) under various treatments.

Components	RT	LRI	**C**	**D**	**S**	**DS**
1-octen-3-ol	6.48	974	0.37	0.26	0.27	0.23
3-octanone	6.71	984	0.63	0.30	0.39	0.41
3-octanol	6.96	994	1.38	1.00	0.96	0.85
linalool	10.76	1097	0.17	0.38	0.31	0.30
borneol	13.43	1162	1.39	1.76	1.60	1.68
nerol	16.15	1227	1.03	1.44	1.29	1.43
thymol methyl ether	16.20	1228	2.04	2.56	2.06	2.18
neral	16.58	1238	8.26	9.57	8.38	10.39
geraniol	17.20	1252	60.40	52.80	55.41	50.09
geranial	17.86	1268	12.29	14.36	12.48	15.29
thymol	18.81	1290	1.50	1.13	0.66	0.45
geranyl acetate	22.43	1388	0.28	0.44	0.46	0.36
β-caryophyllene	23.68	1420	2.05	2.53	2.38	3.00
β-bisabolene	27.23	1508	2.55	3.90	4.14	4.25
geranyl butanoate	29.33	1566	1.21	1.87	2.03	2.16
caryophyllene oxide	30.20	1590	1.67	2.69	3.98	3.86
geranyl 2-methyl butanoate	30.44	1597	0.39	0.62	0.73	0.66
Total identified (%)	–	–	97.61	97.62	97.53	97.59
Oxygenated monoterpenes	–	–	88.96	86.93	85.41	84.99
Sesquiterpenes	–	–	6.27	9.12	10.50	11.11
Aliphatic compounds	–	–	2.38	1.56	1.62	1.49

C: control; D: drought stress; S: salinity stress; DS: combined stress; RT: retention time; LRI: linear retention index.

Table 33. . Essential oil composition (%) of *T.* × *citriodorus* (TL2 – 2025) under various treatments.

Components	RT	LRI	**C**	**D**	**S**	**DS**	**C+ANE**	**D+ANE**	**S+ANE**	**DS+ANE**
1-octen-3-ol	6.48	974	0.78	0.67	0.76	0.66	0.80	0.78	0.92	0.72
3-octanone	6.71	984	1.64	1.11	1.31	1.02	1.20	1.34	1.43	1.32
3-octanol	6.96	994	1.24	1.12	1.12	1.11	1.00	1.11	1.18	1.16
linalool	10.8	1097	0.24	0.23	0.23	0.35	0.26	0.33	0.30	0.30
borneol	13.4	1162	0.61	0.85	0.73	1.30	0.74	1.02	1.00	1.15
nerol	16.2	1227	1.99	2.08	1.97	2.24	1.95	2.04	3.19	2.04
thymol methyl ether	16.2	1228	0.75	0.69	0.66	0.89	0.68	0.82	0.98	0.91
neral	16.6	1238	10.61	10.12	13.27	13.87	10.57	9.06	11.04	10.30
geraniol	17.2	1252	62.60	58.82	55.45	50.49	59.80	60.62	57.53	58.98
geranial	17.9	1268	13.52	15.44	17.18	16.09	15.27	13.05	15.42	14.91
thymol	18.8	1290	0.45	0.38	0.32	0.31	0.43	0.39	0.41	0.28
carvacrol	19.2	1300	0.33	0.66	0.59	0.81	0.42	0.30	0.25	0.31
geranyl acetate	22.4	1388	0.16	0.20	0.21	0.21	0.18	0.26	0.28	0.24
β-caryophyllene	23.7	1420	1.83	1.88	1.74	1.85	1.79	2.25	1.84	1.59
β-bisabolene	27.2	1508	1.33	2.43	2.28	3.56	1.40	1.92	1.44	1.36
geranyl butanoate	29.3	1566	0.64	0.67	0.59	0.86	0.67	0.91	0.71	0.79
caryophyllene oxide	30.2	1590	0.82	1.06	0.89	1.62	0.91	1.32	1.29	1.47
geranyl 2-methyl butanoate	30.4	1597	0.24	0.24	0.23	0.31	0.25	0.35	0.29	0.27
Total identified (%)	–	–	99.75	98.63	99.54	97.55	98.33	97.83	99.49	98.08
Oxygenated monoterpenes	–	–	92.14	90.38	91.43	87.73	91.22	88.15	90.40	89.48
Sesquiterpenes	–	–	3.98	5.37	4.91	7.03	4.10	5.49	4.57	4.42
Aliphatic compounds	–	–	3.66	2.90	3.19	2.79	3.00	3.23	3.53	3.20

C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract; RT: retention time; LRI: linear retention index.

Table 34. Essential oil composition (%) of *T. capitatus* (TC1 – 2024) under various treatments.

Components	RT	LRI	**C**	**D**	**S**	**DS**
α-pinene	5.56	938	0.49	0.29	0.26	0.11
β-myrcene	6.99	995	1.52	1.20	1.20	0.77
α-terpinene	7.79	1018	1.65	1.24	1.34	0.90
p-cymene	8.09	1026	5.25	4.94	4.93	3.89
γ-terpinene	9.20	1056	5.86	4.22	4.61	3.34
linalool	10.76	1097	1.07	1.62	1.70	1.50
borneol	13.43	1162	0.38	0.55	0.61	0.48
thymol	18.81	1290	0.16	0.16	0.19	0.18
carvacrol	19.20	1300	78.86	80.73	79.59	82.58
β-caryophyllene	23.68	1420	2.10	2.56	2.43	3.10
Total identified (%)	–	–	97.33	97.51	96.85	96.85
Monoterpene hydrocarbons	–	–	14.77	11.89	12.34	9.01
Oxygenated monoterpenes	–	–	80.47	83.06	82.09	84.74
Sesquiterpenes	–	–	2.10	2.56	2.43	3.10

C: control; D: drought stress; S: salinity stress; DS: combined stress; RT: retention time; LRI: linear retention index.

Table 35. Essential oil composition (%) of *T. capitatus* (TC2 – 2025) under various treatments.

Components	RT	LRI	C	D	S	DS	C+ANE	D+ANE	S+ANE	DS+ANE
α-pinene	5.56	938	0.16	0.19	0.18	0.15	0.16	0.19	0.16	0.13
β-myrcene	6.99	995	0.95	0.86	0.90	0.98	1.04	0.90	0.91	0.84
α-terpinene	7.79	1018	1.81	1.46	1.04	1.22	2.00	1.62	1.64	1.62
p-cymene	8.09	1026	6.58	6.15	5.05	2.76	6.23	5.85	6.19	3.78
γ -terpinene	9.20	1056	6.31	4.68	5.00	6.04	6.32	5.49	5.47	6.24
linalool	10.76	1097	1.93	1.59	1.87	1.74	1.64	1.41	1.90	1.47
borneol	13.43	1162	0.58	0.81	1.08	0.28	0.21	0.31	0.55	0.35
thymol	18.81	1290	0.18	0.13	0.23	0.11	0.10	0.13	0.13	0.15
carvacrol	19.20	1300	75.45	77.99	77.85	80.32	76.73	78.07	76.22	76.59
β-caryophyllene	23.68	1420	2.78	3.56	3.52	4.24	2.37	2.81	3.40	3.29
Total identified (%)	–	–	96.72	97.42	96.72	97.85	96.79	96.78	96.57	94.45
Monoterpene hydrocarbons	–	–	15.81	13.34	12.17	11.15	15.75	14.05	14.37	12.61
Oxygenated monoterpenes	–	–	78.14	80.52	81.03	82.45	78.68	79.92	78.80	78.56
Sesquiterpenes	–	–	2.78	3.56	3.52	4.24	2.81	3.40	3.29	3.29

C: control; D: drought stress; S: salinity stress; DS: combined stress; ANE: *Ascophyllum nodosum* extract; RT: retention time; LRI: linear retention index.

Table 36. F and p values from two-way ANOVA for stress, *A. nodosum* extract, and their interaction for *T. pannonicus* (TP3 – 2025).

Trait	**F** (stress)	**p** (stress)	**F** (ANE)	**p** (ANE)	**F** (stress × ANE)	**p** (stress × ANE)
FSW	7.297	0.000	7.261	0.223	1.494	0.000
DSW	2.508	0.066	8.581	0.005	0.714	0.047
Shoot length	6.528	0.001	4.929	0.030	0.168	0.917
Shoot number	35.307	0.000	13.216	0.001	1.956	0.128
RFW	14.157	<0.001	153.260	<0.001	13.095	<0.001
RDW	10.006	0.001	40.588	<0.001	5.972	0.006
Root length	19.676	<0.001	46.532	<0.001	2.541	0.093
Chlorophyll a	15.572	0.000	10.575	0.005	6.937	0.003
Chlorophyll b	6.030	0.006	7.002	0.018	4.574	0.017
Total chlorophyll	13.256	0.000	10.662	0.005	6.389	0.005
Carotenoids	27.185	0.000	34.061	0.000	8.771	0.001
RWC	6.240	0.005	3.050	0.100	0.170	0.915
Proline	140.831	0.000	27.062	0.000	4.381	0.020
Soluble sugars	19.170	0.000	45.841	0.000	0.415	0.745
EOC	1.780	0.019	3.950	0.046	0.710	0.038
TPC	22.88	0.000	7.080	0.010	3.130	0.032
AOC	30.90	0.000	10.830	0.002	6.540	0.001
H_2O_2	175.687	0.000	7.825	0.013	1.114	0.373
thymol	0.750	0.038	0.640	0.034	0.090	0.045
p-cymene	2.670	0.053	2.410	0.140	0.540	0.061
B-bisabolene	4.270	0.021	9.210	0.000	2.460	0.000
Th_Mt_Et	0.780	0.000	2.340	0.000	0.750	0.041

F: F-statistic; p: probability value; ANE: *Ascophyllum nodosum* extract; FSW: fresh shoot weight; DSW: dry shoot weight; RFW: fresh root weight; RDW: dry root weight; RWC: relative water content; EOC: essential oil content; TPC: total polyphenol content; AOC: antioxidant capacity; H_2O_2: hydrogen peroxide; Th_Mt_Et: thymol methyl ether.

Table 37. F and p values from two-way ANOVA for stress, *A. nodosum* extract, and their interaction for *T.* × *citriodorus* (TL2 – 2025).

Trait	**F** (stress)	**p** (stress)	**F** (ANE)	**p** (ANE)	**F** (stress × ANE)	**p** (stress × ANE)
FSW	0.790	0,049	1.850	0.178	0.020	0.009
DSW	7.500	0.000	6.360	0.014	0.970	0.414
Shoot length	1.680	0.179	6.383	0.014	1.832	0.149
Shoot number	46.942	0.000	43.561	0.000	2.167	0.099
RFW	0.000	0.202	3.583	0.070	1.255	0,031
RDW	3.116	0.045	1.560	0.224	0.689	0.568
Root length	1.400	0.267	1.029	0.320	0.297	0.827
Chlorophyll a	11.213	0.000	4.565	0.043	1.693	0.195
Chlorophyll b	8.550	0.000	3.987	0.057	0.609	0.616
Total chlorophyll	22.178	0.000	10.177	0.004	0.894	0.458
Carotenoids	13.769	0.000	4.697	0.040	0.400	0.755
RWC	2.867	0.058	4.889	0.037	1.103	0.367
Proline	573.420	0.000	40.950	0.000	8.870	0.001
Soluble sugars	25.000	0.000	48.370	0.000	4.490	0.018
EOC	2.702	0.048	2.615	0.019	0.232	0.051
TPC	7.370	0.000	48.240	0.000	2.870	0.039
AOC	8.150	0.000	181.300	0.000	3.570	0.015
H_2O_2	77.320	0.000	32.370	0.000	3.360	0.045
geraniol	30.760	0.000	20.630	0.000	18.720	0.000
geranial	6.730	0.002	5.690	0.027	5.970	0.004
neral	39.220	0.000	72.820	0.000	13.760	0.000
β-bisabolene	27.800	0.000	98.890	0.000	29.500	0.000

F: F-statistic; p: probability value; ANE: *Ascophyllum nodosum* extract; FSW: fresh shoot weight; DSW: dry shoot weight; RFW: fresh root weight; RDW: dry root weight; RWC: relative water content; EOC: essential oil content; TPC: total polyphenol content; AOC: antioxidant capacity; H_2O_2: hydrogen peroxide.

Table 38. F and p values from two-way ANOVA for stress, *A. nodosum* extract, and their interaction for *T. capitatus* (TC2 – 2025).

Trait	**F** (stress)	**p** (stress)	**F** (ANE)	**p** (ANE)	**F** (stress × ANE)	**p** (stress × ANE)
FSW	3.490	0.021	3.450	0.068	0.480	0.045
DSW	0.903	0.444	5.666	0.020	0.455	0.715
Shoot length	3.680	0.016	0.130	0.720	0.480	0.698
Shoot number	10.640	0.000	4.299	0.042	0.604	0.614
RFW	2.823	0.060	11.875	0.002	1.414	0.263
RDW	2.907	0.055	14.628	0.001	0.457	0.021
Root length	3.925	0.021	13.780	0.001	4.883	0.009
Chlorophyll a	5.442	0.009	5.099	0.038	0.825	0.499
Chlorophyll b	3.591	0.037	2.665	0.122	2.069	0.354
Total chlorophyll	5.955	0.006	6.074	0.025	1.895	0.171
Carotenoids	5.450	0.009	10.704	0.005	1.277	0.316
RWC	27.978	0.000	11.093	0.004	1.113	0.373
Proline	173.698	0.000	14.126	0.002	4.820	0.014
Soluble sugars	41.019	0.000	3.322	0.087	0.193	0.900
EOC	1.246	0.046	3.933	0.065	0.888	0.008
TPC	8.643	0.000	36.893	0.000	8.200	0.000
AOC	6.434	0.001	20.454	0.000	7.820	0.000
H_2O_2	200.469	0.000	8.889	0.009	0.882	0.471
carvacrol	0.689	0.472	0.615	0.444	0.727	0.551
p-cymene	14.165	0.000	1.036	0.324	1.178	0.349
γ -terpinene	1.751	0.197	3.284	0.089	0.297	0.827
β-caryophyllene	7.415	0.002	9.115	0.008	0.976	0.428

F: F-statistic; p: probability value; ANE: *Ascophyllum nodosum* extract; FSW: fresh shoot weight; DSW: dry shoot weight; RFW: fresh root weight; RDW: dry root weight; RWC: relative water content; EOC: essential oil content; TPC: total polyphenol content; AOC: antioxidant capacity; H_2O_2: hydrogen peroxide.

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