



Modulating secondary metabolism in *Pimpinella anisum* and *Foeniculum vulgare* under climate change-induced abiotic stress: A review on quality optimization

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ABSTRACT

Climate change scenarios have imposed serious abiotic stresses mainly drought soil salinity and become one of most important factors that influence sustainable agricultural production on earth. Both Anise (*Pimpinella anisum* L.) and Fennel (*Foeniculum vulgare* Mill.) are valuable medicinal and aromatic plants (MAPs) which are cultivated all over world due to their oil yields and biological activity of their bioactive compounds. Abiotic stresses limit vegetative growth, biometric characters and raw seed yield; however, at the same time these factors induce plants' defense system promoting secondary metabolites production. This review aimed to provide a detailed overview about molecular mechanisms and regulations in Anise and Fennel plant responding to drought and salinity stresses. An overview is presented about accumulation of secondary metabolites like trans-anethole, fenchone and polyphenol compounds induced by these stresses that act as survival mechanism to overcome oxidative stress. In addition, recent advancements in overcoming cellular damages and improvement of medicinal compounds quality through external application of signaling compounds like salicylic acid, jasmonates and chitosan are also discussed.

KEYWORDS: *Pimpinella anisum*, *Foeniculum vulgare*, secondary Metabolism, Climate Change, drought.

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مراجعة لتحسين جودة وتنظيم الايض الثانوي في نباتي الينسون والشمر تحت ظروف الاجهاد البيئي الناجم من التغيرات المناخية

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المخلص

تفرض التغيرات المناخية ضغوطاً بيئية شديدة، مثل الجفاف وملوحة التربة، مما يهدد استدامة الإنتاج الزراعي العالمي. ويركز هذا المقال العلمي على نباتي الينسون والشمر، وهما من النباتات الطبية والعطرية ذات الأهمية الاقتصادية الكبيرة بفضل زيوتها الطيارة ومركباتها الحيوية. تسبب الجفاف والملوحة في تقليل النمو الخضري للمحصول وتقليص حجم وجودة إنتاج البذور الخام. وتحفز هذه الضغوط نظام الدفاع الداخلي للنباتات، مما يدفعها لزيادة إنتاج "المستقلبات الثانوية" (مثل مركبات الترانس-أنيثول، والفينثون، والبوليفينول) كآلية للبقاء ومقاومة الإجهاد التأكسدي. كما أن دراستنا تستعرض الآليات والتنظيمات الجزيئية التي تتبعها النباتات للاستجابة لهذه الظروف القاسية. وتمت مناقشة طرق تقليل الأضرار الخلوية وتحسين جودة المركبات الطبية عبر الرش الخارجي للمركبات، مثل حمض الساليسيليك، والياسمونات، والكيروزان.

كلمات مفتاحية: نبات الشمر، نبات الينسون، الايض الثانوي، التغير المناخي، الجفاف.

INTRODUCTION

1. Economic importance :

Human-induced climate change and agricultural resilience is among most impactful scientific inquiries to date (Chele et al.,2021). As levels of greenhouse gas emissions continue to expand into atmosphere, global climates are becoming more drastically altered than ever before producing extreme events such as droughts, unusual rainfall patterns and soil salinity especially within dry climates (M, H. and M., F., 2024). These new developments threaten to impair growth of medicinal and aromatic plants (MAPs), which are far more sensitive to weather changes than cereal grains. Two highly important MAPs, *Pimpinella anisum* L. (anise) and *Foeniculum vulgare* Mill. (fennel) are

members of Apiaceae family (Ghassemi-Golezani et al., 2026). Both crops have been widely used since ancient times throughout regions of the Mediterranean, Southern Europe, and Asia (Nouripour-Sisakht et al., 2025). Traditionally they have played important roles in medical practices such as unani, ayurvedic and traditional Arabic medicine.

Today the industries predominantly driving supply and demand of both crops are pharmacological and chemical based on the unique composition of volatile and non-volatile phytochemicals produced by each species (Jaiswal and Agrawal, 2021). Essential oil (EO), found in the dried schizocarps of both plants, is implemented widely in carminative, expectorant, antimicrobial, and anti-inflammatory medicines (IB et al., 2015). Both crops aroma compounds, directed by these EO's are also key players in the culinary spice market for both foods as well as beverages and cosmetics (SEMİZ et al., 2023). While historical cultivation strategies emphasized maximizing above ground biomass and crude seed yield (kg/ha) (Nattan and Koussao, 2026), Phyto medical industries face strict legislative constraints that require chemical standardization and higher levels of individual markers such as trans-Anatole and fenehone (Wang et al., 2026).

Unfortunately, these conditions that promote vegetative growth will produce a diluted yield of desired secondary metabolites (Belkhadi and Hediji, 2024). Environmental stress below pestilent levels can act as a natural signal to shunt carbon flux away from primary metabolism and toward secondary metabolism (Sorkhi et al., 2021). Therefore, a new field of medicinal agriculture can be summarized by manipulation of this stress; or “environmental elicitation” with the purpose of improving phytochemical characteristics at time of harvest. Thus, it is critical to understand how anise and fennel shift their metabolic responses to environmental stress if we are to transition from conventional cultivation to high-throughput quality-focused medicinal production (Mansour, 2009).

2 .Morpho-physiological changes due to drought and salinity stress plants

Pimpinella anisum and *Foeniculum vulgare* encounter when exposed to drought and salinity develop numerous morphological and physiological changes in an attempt to sustain homeostasis throughout their cells (Soussi et al., 2023). Hyper-accumulation of sodium Na^+ and chloride Cl^- in soil matrix is what causes salinity stress; therefore, presenting plants with two forms of stress. An initial osmotic stress that limits the uptake of water via roots and a later deferred ionic stress that poisons cells by inhibiting key enzymatic functions (Sharangi et al., 2021). This same thermodynamic principal applies to drought stress in which less energy is available in soil water for plants to reverse the severe matric suction and sustain homeostatic turgor pressure within their cells (Alburqan et al., 2026). Morphologically, both stresses cause similar significant decreases in plant height, number of umbels/plants, number of umbels lets, and drastic reductions in the weight of a thousand seeds (Duman and Ödemiş, 2026). Physiologically, the immediate response to water deficit is the stomata

closure of leaves which is triggered by rapid de novo synthesis and root-to-shoot translocation of ABA to foliar guard cells (Atherton et al.,2023). Although loss of water via transpiration is decreased, this compensation is met with limitations to diffusion of internal CO₂ into the mesophyll resulting in large decreases in leaf relative water content (RWC) and net photosynthetic rate (Osama et al.,2019). This limitation of the Calvin cycle over-reduces the photosynthetic electron transport chain as more photons are being absorbed by chloroplasts than can be used in carbon fixation reactions. This excess of electrons is directly donated to molecular oxygen creating highly reactive Oxygen Species (ROS) including superoxide radical (O₂⁻), hydrogen peroxide (H₂O₂), and hydroxyl radical (. OH) (Şişman et al.,2025).

To avoid uncontrollable lipid, protein, and nucleic acid oxidation anise and fennel increase production of enzymatic and non-enzymatic antioxidants (Ahmad et al.,2021). These plants perform osmotic adjustment by de novo synthesizing and accumulating intracellular concentrations of low molecular-weight, non-toxic compatible solutes also known as osmoprotectants. Mainly proline, glycine betaine and total soluble carbohydrates which aid in stabilizing protein tertiary structure, osmotic cell turgor, and thylakoid membrane integrity. While this is occurring anise and fennel also up-regulate their intrinsic antioxidant enzyme systems. Superoxide dismutase (SOD) is the first antioxidant enzyme to combat ROS and functions by simply dismutation (O₂⁻) into (H₂O₂). H₂O₂ is then enzymatically broken down into water and oxygen through a very systematic pathway consisting of catalase (CAT), guaiacol peroxidase (GPX), and the enzymes involved in the ascorbate-glutathione cycle. These enzymes include acrobat peroxidase (APX) and glutathione reeducate (GR) (Eldeeb and EM, 2024).

2.1 Effects of abiotic stress on production & composition of volatile oil:

In Apiaceae plants, chemical composition of essential oils varies tremendously according to microclimate of the growing plant (Shinde et al.,2024). Volatile compounds of anise and fennel are produced and accumulate in internal glands called vittae, also known as oil ducts. These ducts are located inside the seed pericarp, running parallel to the length of seed (Ascensao, 2019). Absolute essential oil production (L/ha) decreases with stress (reflecting the decrease in total seed weight), but percent essential oil composition of seeds (% dry weight basis) often increases significantly (*) with moderate drought or salt stress. (Younesi et al.,2013)

Stress → Phenylpropanoid Pathway: Phenylalanine —> PAL enzyme INDUCED ---> trans-Anethole
↑ (Pharmacological Marker)

Trans-anethole is major chemical contributor to the medicinal properties of both *P.anisum* and *F.vulgare*. As a phenylpropanoid, trans-anethole is produced through shikimate metabolic pathway. The percent (%) trans-anethole of volatile oil has been reported to increase with moderate drought stress. (A, M. and A., S, 2023) Such increase is associated with up regulation of phenylalanine

ammonia-lyase (PAL) and other enzymes responsible for trans-anethole production. PAL is the gateway enzyme that channels primary metabolites like phenylalanine towards phenylpropanoid pathway. (Elmas et al.,2024) Therefore, drought stress inhibits primary growth, causing excess carbon skeletons and NADPH to be redirected towards production of reduced metabolites such as terpenoids and phenylpropanoids. (D, Z. and M., S., 2025)

F. vulgare responds to different levels of EC with genotypic variation in ratio of major monoterpenes fenchone, estragole (aka methyl chavicol), and limonene. (Khammassi et al.,2022) Moderate salt stress can change chemo type of fennel plant by decreasing the percentage of the potential carcinogen estragole, while boosting levels of beneficial compounds such as fenchone and trans-Anatole. This is useful information to pharmaceutical companies who demand food grade essential oils with low straggle content to meet stringent international export standards. Notice that only moderate stress was described in above studies. If drought or salt stress is severe or prolonged, then the oil ducts shrivel up and volatile oils will quickly evaporate or become oxidized (Gayathri et al.,2024) .

3 .Production of phenolic and antioxidant capacity

In addition to essential oils, secondary metabolite production of both *P. anisum* and *F. vulgare* is comprised of several non-volatile compounds including phenolic acids and flavonoids (Dwivedi et al.,2018). Polyphenols are stored in the vacuole of plant cells and serve as a potent backup defense system against acute oxidative stress. When antioxidant enzyme activity (SOD & CAT) can no longer keep up with stress production from prolonged drought or saline exposure, plants will immediately trigger increased transcription of genes responsible for CHS and HCT (Naghdyzadegan et al.,2022). Chalcone synthase, hydroxycinnamoyl- CoA shikimate/quintet hydroxycinnamoyl transferees. CHS and HCT are enzymes responsible for the biosynthesis of phenolic and flavonoids in plants. This increased transcription results in significant increases in TPC and TFC production throughout the plant, especially in seeds.

ROS and DROS scavenging phenolic in stressed fennel/anise include Rosmarinus acid, chromogenic acid, caffeic acid, rutin and derivatives of quercetin (Athanasiadis et al.,2023). Structurally these compounds all contain multiple hydroxyl groups on aromatic rings, allowing them to serve as effective hydrogen or electron donors (Rosler et al.,1997). Instead of important cellular components like lipid membranes, they will readily react with and neutralize ROS such as singlet oxygen and hydroxyl radicals.

As a result, multiple studies using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS assays have shown extracts taken from saline or water stressed anise and fennel contain significantly higher antioxidant capacity than irrigated, stress free controls (Raze, 2023). This equates to a more useful end product for production of functional food ingredients and nutraceuticals as demand for natural

antioxidants from plant sources continues to rise to replace synthetic additives (Karahuseyin, 2023) .

4 .Agronomic interventions for quality optimization and stress mitigation

Fine tuning agricultural management is a promising option to conserve biomass production and enhance accumulation of secondary metabolites in anise and fennel (Kumar et al.,2017). When plants are exposed to high levels of stress, depending exclusively on the activation of the plant's own defense responses leads to economic losses in terms of overall seed production. Thus, modern approaches aim to treat plants with environmentally compatible chemical elicitors/signaling molecules to induce changes in plant metabolism. One such signaling molecule is salicylic acid (SA), an endogenous phenolic compound which modulates multiple physiological responses involved in systemic acquired resistance (SAR) (Gupta et al.,2026).

Application of salicylic acid at optimal concentrations (0.5 to 1.5 mM) can alleviate osmotic and oxidative stress (Akula and Ravishankar, 2011). After being taken up by the leaf tissue, SA improves cellular metabolism by maintaining photosynthetic efficiency and cellular membrane stability as well as reducing lipid peroxidation. This occurs because SA triggers an increase in expression of genes related to important antioxidant enzymes, including SOD and APX (Kumari et al.,2017). Increases in these enzymes ensures the quick conversion of H₂O₂ before it can accumulate and harm proteins found in chloroplasts (Yang et al.,2023). As a result, SA-treated anise and fennel exhibit greater RWC and photosynthesis under drought conditions (Conrath, 2025).

Application of elicitors, such as chitosan - beta-(1-4)-linked polysaccharide polymer which resembles the fungal cell wall mimicry molecules (Pooja et al.,2023) can also be beneficial in horticultural crops. Elicitation through chitosan takes place when it attaches to receptor sites present in plasma membrane activating intracellular signaling for accumulation of secondary metabolites meant for plant defense (S., et al.,2020). Spraying on leaves has been proven to activate the phenylpropanoid pathway in Anise and fennel by inducing the PAL enzyme activity (El Allaoui et al.,2024). Increase in PAL activity has shown to directly correlate with elevated % of volatile fraction; trans-Anatole, present in extracted essential oil (Zhang and Zhang, 2022). Moreover, chitosan creates a nan film over stomata pores reducing luxury water loss and not interfering with diffusion of CO₂ molecules physically (Rahmatullah et al.,2018). Along with natural elicitors, holistic plant nutrition techniques through nanoparticles have been gaining popularity recently (Esim and Güneş, 2021). Nanoparticles in seed or leaf application act as Nano-fertilizers which apart from supplementing nutritional deficits aid in eliciting positive responses to abiotic stresses. For instance, Zinc oxide nanoparticles/ZnO NPs/seaweed extracts in hyper saline environments help maintain K⁺/Na⁺ ratio within cytoplasm by preventing excessive entry of Na⁺ which can otherwise hamper enzymatic activity within plant cells (Shan et al.,2015). Halting Na⁺ toxicity keeps premature senescence of basal leaves at bay and allows

continual photo-assimilates flux to flower heads, securing marketable yield of seeds (Moghimipour et al.,2017).

The use of soil amendments like PGPR and AMF can help stabilize soil content in the long run (Gill and Tuteja, 2010). PGPR and AMF colonize rhizosphere of Anise and fennel increasing root surface area accessible for absorption of water from subterranean layers through hypha networks (Di et al.,2019). *Bacillus/Pseudomonas* species of PGPR produce IAA and ACC-deaminase which help reduce ethylene concentration produced by plants under stress. This reduction helps keep the inhibition of root growth at bay allowing continuous uptake of P and N required for synthesis of oxygenated volatile terpenoids (Sukasri et al.,2023) .

5.Evaluation of analytical methods

A sensitive and standardized analysis is required to evaluate variations in these secondary metabolites caused by stress conditions. (Sahad et al.,2014) Traditional approaches for extracting essential oils from Apiaceae species were based on hydro-distillation performed with the aid of a Clevenger-type device. (Reverchon, 1997) Although this approach is still employed on an industrial scale to evaluate essential oil yields, some drawbacks have been recognized including the loss of heat-labile monoterpene hydrocarbons and lengthy extraction procedures that may lead to artifacts production. (Reverchon and De, 2006) Many recent laboratories are beginning to employ technologies including Supercritical Fluid Extraction (SFE) CO₂ and Microwave-Enhanced Hydro distillation (MAHD) to bypass these limitations. (Huzar et al.,2018)

During SFE-CO₂, reduced temperatures and higher pressure are used, maintaining the configuration of phenylpropanoids and minimizing oxidation of sensitive components such as fennel and straggles. (Chan et al.,2021) Once extracted, gas chromatography mass spectrometry (GC-MS) and flame ionization detection (GC-FID) are usually used to analyze and quantify the composition of the volatile fraction obtained. (Gandhimathi et al.,2023) GC-MS analysis is used to positively identify trace volatiles by computer matching mass spectral fragmentations to libraries (i.e., NIST and Wiley). (Guizelini et al.,2024) Non-volatile compounds, such as polyphenols, are typically evaluated by HPLC-diode array detection (DAD) or HPLC-electrospray ionization mass spectrometry (ESI-MS). (Shi et al.,2025) This equipment allows researchers to isolate, identify, and quantify overlapping flavonoids and phenolic acids (e.g., rosmarinic acid, chromogenic acid, caffeic acid). Absolute changes in the mg/g of individual antioxidants after specific agronomic stress treatments can then be determined based on known chromatographic profiles. This precise accounting is essential for producers because slight variations in ratios could affect the biological activity of the product (Al Murad et al.,2020).

6 .Constraints, unknowns, and practicality considerations :

While the aforementioned practice of capitalizing on a mitigated abiotic stressor to improve the phytochemical output of *A. foeniculum* and *P. anisum* sounds encouraging on paper, many logistical constraints and unknowns must be overcome before practices can be realistically applied to crops on a commercial level (Adjewor, 2025). For example, controlling for extraneous variables is difficult in large open-field productions since multiple abiotic stressors are often presented at once in nature (e.g., a drought year with high temperatures and strong UV light). While linear metabolic responses can be easily measured and analyzed within greenhouse experiments testing one stressor at a time, field crops undergoing multiple dynamic stresses that have yet to be cross mapped will often display unpredictable and noisy chemical profiles (Dai, 2024).

Determining an ideal threshold between biomass loss and secondary metabolite build up also proves to be challenging at times (Weiner et al., 2021). Should the stress exceed a certain period of time or intensity, the economic burden of producing a smaller seed mass will drastically overshadow any benefits gained from an increased percentage of oil (Pouyani et al., 2025). Ideally, mathematic models and agronomic blueprints would need to be created to identify the finite "window of stress" that each crop variant and soil type can sustain (Lassoued et al., 2023).

Even from a financial standpoint, many of the aforementioned mitigation tactics require growers to initially invest in capital-heavy technology such as computerized deficit irrigation schedules or consistent, large-volume purchases of synthetic signaling molecules such as chitosan and nanoparticles (Purwanto et al., 2025). Without price premiums from pharmaceutical companies requiring verified, standardized inputs, these increased production costs may limit the techniques usage in developing countries where both anise and fennel are currently being cultivated as low input crops (Vishwakarma et al., 2026). Moreover, little research has been done on the potential ecotoxicology of bio accumulated nanoparticles in farmed soils or if they could make their way into the human body through medical extracts.

CONCLUSION

Chemo preventive secondary metabolites production in sweet fennel and anise represents a promising approach for obtaining functional food/feed from these medicinal crops. While severe water deficit and soil salinization predictably decreases biomass accumulation and overall seed production of *F. vulgare* and *P. anisum*, appropriate mild levels of such abiotic stresses constitute a cheap and sustainable elicitation strategy to positively manipulate OE quality by enhancing trans- Anatoles and fenchone levels as well as augmenting polyphenol phytoalexin biosynthesis. Coupling deficit irrigation regimes with elicitors (salicylic acid, jasmonates), chitosan, nanoparticles could represent

the future strategy of farmers for increasing important bioactive compounds production on field basis. On the other hand, further studies are needed to understand how the expression of entry point genes for phenylpropanoid and torpedoed metabolic pathways are modulated under combined stresses. Also, isolation of stress-induced promoters from those genes through transcriptomics and metabolomics studies can be helpful to enhance production of desirable bioactive metabolites under stress conditions in future. Finally, implementation of future knowledge should be focused on multi-location multi-year field experiments to develop protocols for standardization of botanical raw materials using stress agriculture according to climate/weather conditions of each area to supply pharmaceutical industries worldwide.

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