Effects of Silicon and Salicylic Acid on Growth, Yield, Essential Oil, and Phytochemical Characteristics of Thymus vulgaris L.: A Systematic Review of Experimental Studies Study Design

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Abstract

Background: *Thymus vulgaris L. is one of the important medicinal and aromatic plants, valued for its essential oil and numerous phytochemical constituents.. Exogenous elicitors, including salicylic acid (SA) and silicon (Si) have been studied as sources to enhance plant productivity, stress resistance and phytochemistry.* ***Objective:*** *To compile the experimental evidences systematically in order to evaluate the effect of Si and SA on growth, yield and essential oil production and phytochemical characteristics of T. vulgaris.* ***Methods:*** *A systematic review was carried out based on the PRISMA 2020 guidelines. Experimental studies that met the inclusion and exclusion criteria and tested Si and/or SA on T. vulgaris were retrieved. The seven studies were eligible. There was significant methodological and outcome variability, thus no quantitative meta-analysis.* ***Results:*** *In general, SA was able to improve the production of growth, biomass, essential oils, phenolic solutions and antioxidant related responses depending on the concentration and environmental stress. Potassium silicate enriched plants had higher biomass and essential oil percentage, and plant response to treatment, influenced by co-application and growing conditions.* ***The conclusions****: Si and SA are potential suppliers that are able to increase the productivity and quality of T. vulgaris, but standard factorial experiments and direct combined experiments of both Si and SA are necessary.*

Keywords: *Thymus vulgaris*; salicylic acid; silicon; potassium silicate; essential oil; phenolic compounds; elicitation; systematic review.

1. Introduction

Thymus vulgaris L. Because of its unique phytochemical profile and accompanying biological activities thyme (thyme) is an important medicinal and aromatic plant with a long history of traditional use and a high level of modern interest. Thyme is traditionally applied as a herb in herbal medicines and for health care management while the extracts and essential oil of thyme have been the subject of various research works in the modern period. Pharmacological properties of T. vulgaris are highly related to their secondary metabolites, which show antimicrobial, antioxidant, anti-inflammatory, and other biological activities have been studies. Thyme has thus emerged as an important species in the field of the production of medicinal aromatic plants and production of plant-derived products for various applications such as pharmaceutical, nutraceutical, food and related applications (24; 10; 12).

Essential oil content (EO) is one of the most important quality characteristics of T. vulgaris as it is the quality and content of the essential oil which influences the market value of the seeds and many of their biological properties. Thyme's EO is chemically complex and probably there is a lot of variation between populations, geographical origins and chemotypes. Thymol, carvacrol, p-cymene, and γ-terpinene are the major volatile compounds that are

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usually identified for T. vulgaris, although the concentration of each compound can vary significantly depending on their genetic background and cultivating conditions (30). It is especially significant that the amounts of each constituent chemical can bring about a change in the biological activity and/or therapeutic uses of the EO. Apart from chemical composition, EO yield is one of the significant production parameters and it varies with the genotype, cultivation method, and environment of the thyme production system, which emphasizes the importance of evaluation of the quantity and quality of EO when assessing thyme production systems (32; 11).

Other phytochemical qualities of T. vulgaris also lie outside of its volatile profile, including phenolic and flavonoid compound types, which also impart antioxidant properties to thyme products. The importance of phenolic constituents is of particular interest since they determine the antioxidant activity profile, such as radical-scavenging capacity, which could be modified. But, due to the variability of the phytochemicals found in thyme, there could be differences in its biological properties and medicinal potential (3). Thus, the optimization of the quality of phytochemical by optimizing other agronomic practices would be as critical as increase in biomass or EO production for medicinal aromatic plant production because agronomic practices which increase production but not desirable secondary metabolites are not necessarily improving the quality of the harvested material.(17)

Productivity and phytochemical quality of T. vulgaris is highly sensitive to environmental conditions, water availability being one of the factors affecting the plant performance. A lack of water and drought may limit the growth of plants, slow their rate of biomass accumulation and alter their physiological response to water deficiency. Thus, in thyme, water stress can not only affect primary productivity but also affect metabolic responses which may have implications on accumulation of secondary metabolites and the quality of harvestable crops (28; 29). The effects suggest that, besides biomass production, the availability of water should be taken into account for the biochemical responses, which directly influence the quality of medicinal and aromatic plants.

EO production and composition of T. vulgaris can also vary with environmental conditions. The irrigation regime, and number of water applications, of available water may further influence EO yield and relative abundance of individual volatile constituents, and the harvest timing can cause additional differences in the characteristics of the EO. In this regard, the amount and composition of thyme EO is not a fixed property of the species but dynamic properties depending on the plant growth in connection with environmental conditions (7). Other factors influencing thyme productivity and quality are its soil fertility and cultivation methods. Loss of biomass accumulation and EO characteristics may be affected by nutrient availability, application of organic amendments, growing media, and zinc's application as fertilizer. In particular, manure and nutrient management are found to affect thyme EO quantities and quality; and organic cultivation and growing conditions may affect the production of VOCs (6; 14; 25). The effect of optimization of nitrogen and potassium and other inputs on output of biomass, pigments and essential oil from T. vulgaris has also been studied as a way to enhance the photosynthetic efficiency and biomass and improve output rate of EO (Optimization of nitrogen, potassium and other input parameters on biomass, photosynthetic pigments, and yield of essential oil production of T. vulgaris, 2022). Taken together, the results outlined here suggest that the effects of exogenous biostimulants, on both biomass yield and secondary metabolism, can be highly context-dependent from the environmental and the agronomic point of view.

Silicon (Si), has gained more and more focus in the past few years due to its beneficial effects on altering the responses of plants under environmental stresses. Si is not an essential nutrient to most higher plants, but its accumulation in plant it was hoped that it would contribute to structural support, physiology and bio chemistry of water relations and regulation of

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physiological and biochemical processes related to stress tolerance in plants. There are also links between silicon and antioxidant defense systems and oxidative damage regulation under adverse environmental conditions (17; 5). Physiological effects would form a basis to weigh Si as a plant biostimulant or stress-modulating agent in crop production.

The value of Si becomes more significant in abiotic stresses including drought, salinity, heavy metal toxicity etc. and under oxidative stress. The silicon-mediated responses can be the improvement of water status, regulation of reactive oxygen species (ROS), modification of antioxidant defense mechanisms that can help plants to guard their physiology under unfavorable conditions. The impact of these effects is, however, species-dependent, stress dependent, Si availability dependent, and stress experimental environment dependent (2, 4). Si can have effects on stress physiology, as well as interact with pathways that regulate secondary metabolism. The findings in plant systems revealed an effect of Si on antioxidant systems which might interact with changes in phenolic compounds and other secondary metabolites. Stress responses and secondary metabolism are closely associated with the quality of the harvest product, as is particularly applicable to medicinal and aromatic plants (1; 8). However, there are relatively few reports focused specifically on T. vulgaris and how it is affected by potassium-silicate, in conjunction with effects on growth and EO production and phytochemical characteristics.

Another key signaling molecule of interest for both plant stress physiology and secondary metabolism is salicylic acid (SA). The signals of SA is not just a plant growth regulator, but a plant signaling molecule involved in intricate networks that regulate plant growth, development, defense and environmental stimuli responses. It induces various adaptive responses that depend on the interaction with different biochemical and signaling systems, the plant species and developmental stage, mode of application and environmental conditions (9; 27). The above-mentioned properties give rise to growing interest in the use of applied SAs for altering plant performances and tolerance of unfavorable growth conditions.

The antioxidant defense and the oxidative stress responses have been shown to be modulatory in a variety of plant systems for SA. Exogenous SA can affect ROS homeostasis, Antioxidant enzymes, membrane stability etc to effect physiological processes associated with drought and other abiotic stresses. SA will not, however, always respond in a linear manner and too little or too much (and inappropriate!) of a concentration may elicit a response different from optimal concentration(s). Thus, the efficacy of SA will depend on the plant developmental stage, plant density and concentration, stress level, and environmental conditions (16; 18; 19). Experimental studies in T. vulgaris must be interpreted with special caution due to the possibility of variation in the growth, physiological and biochemical responses as a result of variation in experimental conditions.

In addition to stress tolerance, SA is also examined as an elicitor, which has an activating effect on the production of secondary metabolites. Using SA with elicitor can result in changes in phenolic metabolism and accumulation of compounds related to plant defense and biological activity. A similar elicitation method is used on medicinal plants to improve the secondary metabolism giving valuable products, thus suggesting a potential application of SA for the enhancement of phytochemical quality (13; 31). Since EO biosynthesis is also linked to plant secondary metabolism, the metabolic pathways of the plants may also be affected by the action of SA which could impact the yield and chemical composition of EOs. The magnitude and direction of the effects, however, can be expected to be quite diverse, depending on treatment concentration and environment.

The literature reviewed revealed a significant amount of data related to the physiological functions of Si and SA, but it was not very significant with regard to T. vulgaris. The studies show highly variable SA

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concentration, Si source and dose, application method, irrigation regime, different soil conditions, different experimental environments, monitoring results, and co-application of substances. The methodological diversity of the studies renders it difficult to directly compare studies and is one site of apparently inconsistent results related to growth, biomass, EO production and phytochemical responses. Some experiments test Si and/or SA with other nutrient(s) or under certain stress conditions, where an independent effect of the Si/SA treatment cannot be separated from any interactions between the Si/SA treatment and the other stress or nutrient conditions.

Therefore, a review based on focused synthesis of evidence on Si and SA of T. vulgaris is warranted. Even though there are some studies that have investigated the effect of these two elements on plant stress physiology, secondary metabolism, medicinal plants, and aromatic plants in general, there is no statement in the literature that compares and correlates the effect of SA and Si in terms of growth, yield, EO characteristics, and phytochemical traits in T. vulgaris on a systematic basis. Systematic reviews can aid the identification of consistent patterns of treatment response, aid to elucidate the role of concentration and environmental conditions, and highlight key gaps in the current experimental database on T. vulgaris. Specifically, evidence listed will be analyzed to see (1) if responses are different depending on application strategy and stresses applied and (2) if there is enough experimental evidence to warrant the use of combined Si and SA application.

Hence, the purpose of this systematic review was to systematically review the experimental studies that have been conducted to assess the influence of silicon and SA on growth, yield, essential oil yield and phytochemical composition of Thymus vulgaris L. Results from the review of the literature focused on the effects of Si on growth and productivity; effects of SA on growth and yield; changes in phenolic compounds and antioxidant-related traits following Si application; changes in EO content and composition following Si and SA application; effect of foliar application of Si under varying environmental conditions; concentrations, application frequency, and stress level effects on treatment differences; and available data regarding combined application of Si + SA.

2. Materials and Methods

2.1 Study Design

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA 2020) statement guidelines were used to design and report this systematic review. This review aimed to shed light on the effects of silicon (Si) and salicylic acid (SA) on the growth, yield, essential-oil characteristics of Thymus vulgaris L (TM) and its phytochemical properties using an experimental evidence-based approach.

The review question was designed in the Population, Intervention, Comparator, Outcomes, and Study Design format. The experimental material was the plant Thymus vulgaris L. and the treatments were silicon, salicylic acid, silicate or potassium silicate and their combinations with silicon and/or SA. Comparators were treated plants and/or control treatments. Results measured were the plant growth, essential-oil and dry-matter content and yield, essential-oil composition, phenolic substances and flavonoids content, antioxidant activity and other relevant phytochemical characteristics. Experimental studies carried out under field, greenhouse or controlled environmental conditions were eligible to be included.

Severe lack of reported consistency was found in the studies included based on the experimental design, treatment concentration, application methods, environmental conditions, nature of the stress treatments, cultivars/ecotypes, the measured outcome, and the measurement unit; therefore, the evidence was aggregated narratively. A statistical meta-analysis did not take place.

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2.2 Eligibility Criteria

To ensure that eligibility criteria were applied uniformly, they were defined prior to the final stage of studies for selection and then ensured subsequently during title/abstract screening and during full-text screening. Studies qualified for the selection were those that utilized the use of the experimental design approach and that evaluated the role of silicon (Si) and /or salicylic acid (SA) on any pre-defined parameters in relation to plant growth, yield, essential oil, and / or plant phytochemical parameters of the species Thymus vulgaris L.

Studies were considered if they were original experimental research articles where T. vulgaris L. was involved and treatment and a control or comparison condition was clearly defined. Eligible interventions included Si, silicate, potassium silicate or Si/SA (Si + salicylic acid). Studies had to include at least one outcome related to the study of plant height, number of branches, fresh or dry biomass, herbage yield, dry matter yield, essential oil content, essential oil yield, essential oil composition, total phenol concentration, total flavonoid concentration, individual phenols, antioxidants activity, or the main essential oil components (thymol, carvacrol, p-cymene, γ-terpinene). Only peer reviewed publications that provided enough methodological and outcome information to be considered for eligibility assessment and for data extraction were included.

Review articles, systematic reviews, book chapters, editorials, studies with insufficient experimental and data reports, duplicate publications and studies on species other than T. vulgaris were excluded. Researches that did not look at silicon, salicylic acid or essential-oil or any pre-planned growth or yield or essential oil or phytochemical results were also discarded. Publications which lacked certain information or experimental data were not included because it was not possible to establish eligibility and/or obtain the relevant experimental data.

If there were more than one Thymus species in an eligible publication only data that could be specifically attributed to T. vulgaris were considered. Likewise, other additional treatments, environmental stresses and other co-interventions that met all the inclusion criteria were included in the study, but these were noted in the data extraction process because of their importance to ensure that neither the use of SI alone nor the use of SAL by itself is being attributed to the whole study.

2.3 Information Sources

The literature search was done through five bibliographic electronic databases: Scopus, Web of Science Core Collection, PubMed, Science Direct and Google Scholar. The search strategy used to achieve highest retrieval of relevant experimental studies was to use the terms related to the target species and the terms to silicon, silicates, essential oil and phytochemical features of these.

The reference lists of potentially eligible publications were also hand screened to look for relevant publications that were not found in the electronic database searches. The last search dated up to September 2026.

2.4 Search Strategy

Combinations of species-specific, intervention-specific and outcome-related terms were used to develop the search strategy. Thym and thymus vulgaris" were the two principle species specific terminology. Intervention-related terms that were included were: “salicylic acid”, “salicylate”, “silicon”, “silicate” and “potassium silicate”. Some outcome-related terms included were “essential oil”, “volatile oil”, “phenolic”, “flavonoid”, “antioxidant” and “phytochemical”.

Search terms used included: (“Thymus vulgaris” OR thyme) AND (“salicylic acid”); (“Thymus vulgaris” OR thyme) AND (“phenolic”).OR (“flavonoid”).OR

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(“essential oil” OR silicon).OR (“potassium silicate”); (“Thymus vulgaris” OR thyme) AND (antioxidant*).OR (“phenolic”).OR (“flavonoid”).OR (“potassium silicate”); (“Thymus vulgaris” OR thyme) AND (“potassium silicate”).OR (“silicon”).OR “Thyme”.OR thyme.OR “potassium silicate”.OR “silicon”.OR “silicon dioxide”.OR “potassium dioxide”.OR Silicon dioxide.OR Potassium dioxide.

Depending on the required of each database, search syntax, boolean operators, truncation symbols and field restriction were adapted. Available in the Supplementary Material is the literature search strategy or how the literature was identified on the complete database search.

2.5 Study Selection Process

There were four stages in the study selection process: identification, screening and eligibility assessment, and final inclusion. Records that were retained and excluded at every stage were documented, and used to create the corresponding PRISMA 2020 flow diagram.

Total of 86 record was identified through literature search. After collection consolidation, 18 duplicate records were eliminated accounting for 20.93% of the records identified initially. This left 68 different records for title and abstract screening, or 79.07% of the records identified initially.

A total of 68 due to distinct records were excluded from the title and abstract screening process based on the pre-established inclusion/exclusion criteria. Of the 343 screened records, 72.06% were not selected for full-text screening and 19 (27.94%) records were advanced to full-text screening.

The criteria for full inclusion/exclusion were predefined and the full text of the 19 potentially eligible articles was later examined. Full-text selection 12 articles were excluded (63.16% of articles assessed). In total, seven experimental studies met all inclusion criteria were included in the definitive qualitative synthesis which accounted for 36.84% of the complete text articles evaluated.

In the end, it was discovered that there were 86 records identified, 18 were eliminated as duplicates, in total there were 68 records that were screened for the title and abstract, 49 were outside the scope of inclusion, 19 of the original 68 were full-text articles screened for eligibility with 12 of those being excluded, leaving 7 that were included in the qualitative systematic synthesis of the studies. The final inclusion rate was 8.14% of the total of records initially identified. The actual inclusion rate was 10.29% compared to the number of unique records that were screened.

Reasons for exclusion at the full-text stage cited were testing non-T. vulgaris species, lack of a relevant growth or yield, essential-oil or phytochemical outcome, and insufficient experimental information to extract reliable data. A count of studies which met each of the individual exclusion criteria was documented for presentation in the final PRISMA flow diagram and in additional screening documentation.

All seven of the included studies received qualitative, narrative synthesis. There was significant methodological and outcome variability among studies included in the review to not permit meta-analysis. Therefore, seven studies were used for qualitative synthesis and none of the studies were used for a quantitative meta-analysis.

The screening process: 86 records were identified, 18 of these were duplicates, 68 of the records screened were unique records, 49 full text articles excluded during title and abstract screening, 19 full text articles assessed for eligibility, 12 full text articles excluded, and the seven studies were included in the final systematic review. The study selection process, including identification, screening, eligibility, and final inclusion, is summarized in the PRISMA 2020 flow diagram (Figure 1).

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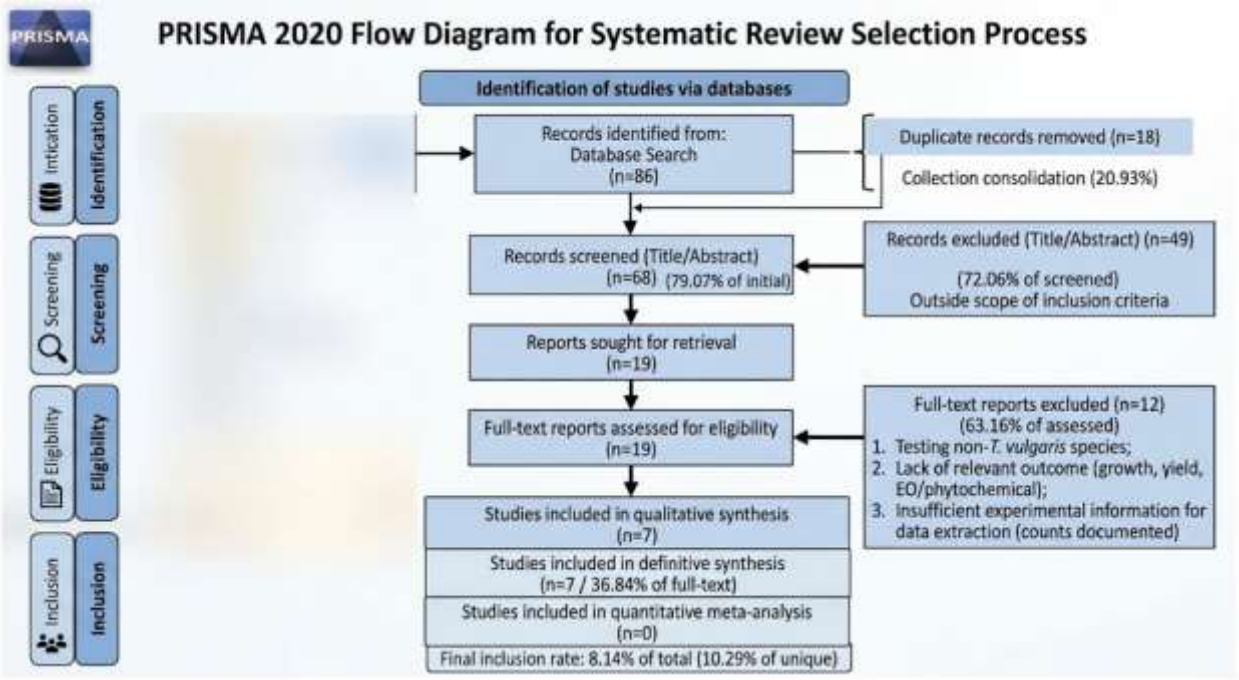


Figure 1. PRISMA 2020 flow diagram of the study selection process

2.6 Data Extraction

A structured framework for data extraction was used to systematically extract data from each eligible study. Data that were extracted included bibliographic information, experimental location and conditions, plant material, cultivar/ecotype if specified, experimental design, number of replicates, type of treatment, treatment concentration, method of application, frequency of application, length of treatment, irrigation or environmental conditions and additional stressors or co-interventions.

All the outcome data were obtained for plant growth, biomass, yield, essential-oil characteristics and phytochemical response. Growth and yield variables were plant height, number of branches, fresh biomass, dry biomass, herbage yield, and dry matter yield. The essential-oil content, essential-oil yield, and relative composition of the major volatile constituents were essential-oil variables. The phytochemical variables included the total phenolic content, total flavonoid content, antioxidant activity, individual phenolics, pigments, and biochemical characteristics mentioned in the original studies.

For numerical data, statistical formatting when presented in one publication has been reproduced as it appears in that publication and the units are reported as reported. If there was a mean or scale used for measures of variability, the value was not transformed for reporting. The outcome was not reported or could not be reliably identified in the available publication, so as not to mislead the reader, the outcome was recorded as not reported (NR); this also applied in the case of non-sound bites. In the cases of non-sound bite and where it did not appear to be reported or was unable to be verified from the available publication, the outcome was recorded as not reported (NR). Blanks were not filled with values from other studies and there were no unreported treatment means estimated.

If multiple experimental factors were used (water stress, heavy-metal stress, biochar, seaweed extract/chitosan, or combined foliar nutrition), the

data used for two or more treatments were separated and documented so that they could be interpreted along with the specific experimental factor.

2.7 Quality Assessment

This methodological appraisal of the studies included focussed on predefined domains of relevance for experimental studies on plants. The criteria included were whether the experimental design was appropriate, whether the experiments were replicated sufficiently, whether an untreated or suitable control treatment was included, the clarity and completeness of reporting on outcomes, and whether the appropriate statistical analysis of treatment effects used was appropriate.

Specific attention was made to whether the experimental design was sufficient to meet the research questions, whether the number of biological replicates was mentioned and reported, treatment and control groups were suitably identified and whether appropriate statistical analyses were provided for the results reported. Transparency in reporting of the concentrations of the treatment, application methods, experimental conditions and statistical procedures were also taken into account.

Due to differing experimental designs and reporting practices across these included studies, a quality assessment was not conducted using a universal numerical quality score, rather just in a qualitative manner. Individual studies were not given a numerical rating or composite score. Rather, the methodology strengths and weaknesses were recorded for each study and taken into account in assessing the consistency and reliability of the reported results.

The methodological quality was assessed by focusing on five key domains: experimental design, experimental replication, control treatment, reporting of outcomes and statistical analysis. Experimental design was considered appropriate if the study had the key elements of the experimental design, such as factorial or randomized design (when applicable) and the design was clear. Replication was evaluated based upon reporting of the number of biological replicates and whether replication was sufficient or described. The appropriateness and availability of an untreated group or corresponding control group were evaluated as a measure of control. The completeness and clearness of the reported measurements for growth, yield, essential-oil, and phytochemical were applied to conduct the evaluation of the outcome reporting. Evaluation of the appropriateness of the statistical approach and transparency of the use of statistical methods to identify treatment effects and statistical significance was evaluated.

Each included experimental study was appraised for the characteristics related to reporting and methodology that we believed may have influenced the risk of bias. Potential sources of bias from an inadequate experimental design/replication, inappropriate or insufficient control treatments, unintentionally missing out on outcome reporting, inadequate or unclear statistical analysis were assessed. Risk of bias was applied mostly to support the interpretation of the evidence, not exclusion of studies because of methodological shortcomings. Studies were included if they met all of the pre-defined eligibility criteria with methodology that was considered "weak" for participating in the synthesis of the findings, and methodology was taken into account when synthesizing and interpreting findings.

2.8 Data Synthesis

Data extracted were synthesized by utilizing a structured narrative approach. The synthesis was evaluated under the prism of the principal effects which occurred under the three areas of growth and yield, essential-oil characteristics and phytochemical and biochemical responses. In each domain, findings were compared with the type of intervention used, the concentration of the intervention, how it was applied, conditions of the experiment, and whether any other factors of the environment and/or abiotic were present. The difference between the effect of normal growing conditions and under stress conditions was particularly emphasised, especially water deficit. With respect to the salicylic acid treatments, the

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synthesis took into consideration the influence that treatment concentration might have on the observed responses and the treatment timing and method of application and environmental conditions that influenced the response. The synthesis classified the silicon sources used, especially potassium silicate; and between single applications of silicon and simultaneous applications with other foliar nutrients and/or biostimulatory treatments. For this reason the substantial methodological and experimental variation of studies included, the narrative synthesis was chosen. Various differences were noted in experimental design, plant material, treatment concentrations, application techniques, environmental and irrigation conditions, experimental stressors, outcome definition, statistical means, measurement unit, and in the measuring techniques. But these differences hindered the ability toPool the study results quantitatively. In doing so, the findings were synthesized based on identifying patterns of response within and across studies instead of by computing pooled effect estimates. When numerical data on treatment response were reported, the response both from the direction of the numerical changes and from the statistical significance reported by the original authors was taken into account. Great attention was given to results that were common to the independent studies, and to responses that seemed to be related to concentration of treatment, environmental conditions or other experimental factors. If co-interventions or combination of more than one experimental factor, e.g., water stress, heavy metal exposure, biochar, seaweed extract, chitosan or combined foliar nutrition, were used, these were taken into account as well in the interpretation. They were considered in deciding if the response observed was likely due to salicylic acid or silicon alone, or if the finding was due to the combined effects of the intervention and other experimental conditions. A quantitative meta-analysis was not conducted due to the wide-ranging diversity of study design, interventions, outcome measures and the way these were reported. The results are therefore given in the form of a narrative synthesis of the experimental evidence, in systematic fashion.

3. Results

3.1 Study Selection

Eighty-six records were found during the predetermined literature search. 18 duplicates (20.93%) were removed leaving 68 unique records (79.07%) for title and abstract screening. From these, 49 records (72.06%) were excluded and 19 articles (27.94%) were finally assessed for full-text. After a full text evaluation 12 articles (63.16%) were excluded and 7 studies (36.84%) fulfilled all eligibility criteria and were used in the qualitative synthesis. A quantitative meta-analysis was not performed.

Table 1. Summary of the study-selection process

%	n	PRISMA stage
100.00	86	Records identified
20.93	18	Duplicates removed
79.07	68	Records after duplicate removal
100.00	68	Records screened
72.06	49	Records excluded during screening
27.94	19	Full-text articles assessed

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63.16	12	Full-text articles excluded
36.84	7	Studies included in qualitative synthesis
0.00	0	Studies included in meta-analysis
8.14	7/86	Overall inclusion from initial records

3.2 Characteristics of Included Studies

Seven experimental studies were included in the eligibility criteria over the last seven years (2015-2022). It is noted that six studies concerned themselves with the action of salicylic acid (SA; 85.71%) and one with potassium silicate (14.29%). The studies were conducted in Egypt (n = 3; 42.86%), Iran (n = 3; 42.86%), and the Czech Republic (n = 1; 14.29%).

Table 2. General characteristics of the included studies

Principal outcomes	Main experimental condition	Concentration/rate	Intervention	Year	Country	Study
Growth, biomass, pigments, MDA, EO	Field	SA: 0.5, 1 mM	SA + citric acid	2015	Egypt	Miri et al.
Phenolics, flavonoids, antioxidant activity, EO	100, 75, 50, 25% field capacity	0, 1, 2, 3 mM	SA	2018	Egypt	Khalil et al.
EO content, yield and composition	Field	Treatment-specific	SA + foliar NPK	2018	Czech Republic	Pavela et al.
Growth, physiology, EO composition	Well-watered and water stress	0, 100, 150 ppm	SA	2019	Iran	Mohammadi et al.
Dry matter, phenolics, EO	Normal and withholding irrigation	0, 100, 150, 200 mg L^{-1}	SA	2020	Iran	Mohammadi et al.
Growth, biomass, pigments, EO	Sandy-soil field; two seasons	6, 9, 12 mL L^{-1}	Potassium silicate	2020	Egypt	Waly et al.
Growth, biomass, biochemical	Greenhouse;	SA: 0, 150, 300 µM	SA + Pb +	2022	Iran	Rezaei et al.

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responses	Pb/biochar stress	biochar

In total, 6/7 (85.71%) studies evaluated the effects of SA and 1/7 (14.29%) the effects of silicon. There was no eligible factorial study that investigated the effect of Si + SA on T. vulgaris.

3.3 Effects of Salicylic Acid on Growth and Yield

In general, the growth and biomass-related responses to SA were positive, but varied with concentration and environmental conditions.

Table 3. Selected quantitative growth and yield responses to SA

Significance	Response	SA treatment	Control	Outcome	Study
P = 0.041	+17.24%	1.02 (0.5 mM)	0.87	Fresh weight (kg m^{-2})	Miri et al. (2015)
P = 0.041	+24.14%	1.08 (1 mM)	0.87	Fresh weight (kg m^{-2})	Miri et al. (2015)
P = 0.013	+30.30%	0.43 (0.5 mM)	0.33	Dry weight (kg m^{-2})	Miri et al. (2015)
P = 0.013	+21.21%	0.40 (1 mM)	0.33	Dry weight (kg m^{-2})	Miri et al. (2015)
Significant	NR	1958.83 (200 mg L^{-1})	NR	Total dry matter (kg ha^{-1})	Mohammadi et al. (2020)
Significant	NR	Improved under SA	NR	Growth/biomass	Khalil et al. (2018)

According to (20), the best conditions for fresh weight was found to be 1 mM SA while they obtained the best condition for dry weight was at 0.5 mM SA. The answer was, therefore, non-uniform concentration dependent over biomass variables.

3.4 Effects of Salicylic Acid on Phenolic and Antioxidant Characteristics

Water deficit condition showed the highest response to phytochemicals. The results of this study were consistent with the results of (15) who reported significant increase in the content of total phenolics and flavonoids after the application of SA under severe water stress.

Table 4. Quantitative phenolic and antioxidant responses

Calculated response	SA + stress treatment	Control	Outcome	Study
+705.88%	68.5 ± 1.2	8.5 ± 0.3	Total phenolics (mg GAE g^{-1} DW)	Khalil et al. (2018)
+426.23%	32.1 ± 0.1	6.1 ± 0.3	Total flavonoids (mg rutin eq. g^{-1} DW)	Khalil et al. (2018)
−50.81%	36.6 ± 0.9	74.4 ± 0.4	DPPH IC_{50} (μg mL^{-1})	Khalil et al. (2018)

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NR; reported ≈58% increase	18.431	NR	Total phenolics (mg GAE g^{-1} DW)	Mohammadi et al. (2020)
−25.25%	0.367 (0.5 mM)	0.491	MDA (nM g^{-1})	Miri et al. (2015)
−4.48%	0.469 (1 mM)	0.491	MDA (nM g^{-1})	Miri et al. (2015)

The treatment effect for MDA was significant in Miri et al. ($P = 0.002$). The decrease in DPPH IC_{50} values observed by (15)., suggest enhanced radical scavenging capacity by the lower IC_{50} values being indicative of higher antioxidant activity in the DPPH assay.

3.5 Effects of Salicylic Acid on Essential-Oil Characteristics

SA affected the content and yield of essential oils. The magnitude and direction of the response varied with concentration and water availability, however.

Table 5. Essential-oil responses to SA

Response	SA treatment	Control	Outcome	Study
+31.35%	58.07 (0.5 mM)	44.21	EO production (mL m^{-2})	Miri et al. (2015)
+24.54%	55.06 (1 mM)	44.21	EO production (mL m^{-2})	Miri et al. (2015)
NR	1.00 ± 0.06 (2 mM + 25% FC)	NR	EO content (% v/w)	Khalil et al. (2018)
NR	2.66 (100 mg L^{-1} + withholding irrigation)	NR	EO content (%)	Mohammadi et al. (2020)
+18.76 to +42.47%	SA + NPK	Control	EO yield	Pavela et al. (2018)
Treatment-associated increase	62.1–64.1	53.1–62.7	Thymol (%)	Pavela et al. (2018)

Results indicated that Miri et al. had statistically significant effect on EO production ($P = 0.025$). The results of (23)., as just stated, are tentative as the application of both foliar NPK and SA cannot be separated.

3.6 Effects of Silicon Application

Only one study (14.29%) investigated silicon directly. (33) evaluated potassium silicate at 6, 9, and 12 mL L^{-1} under field conditions.

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Table 6. Quantitative responses to potassium silicate

12 mL L⁻ ¹	9 mL L⁻ ¹	6 mL L⁻ ¹	Variable	Cut	Season
15.2	13.3	12.0	Plant height (cm)	1st	1
18.5	14.3	13.6	Plant height (cm)	2nd	1
85.3	53.3	40.6	Branches plant⁻ ¹	1st	1
80.6	61.0	47.0	Branches plant⁻ ¹	2nd	1
32.99	30.39	18.87	Fresh weight (g plant⁻ ¹)	1st	1
13.13	10.16	6.86	Dry weight (g plant⁻ ¹)	1st	1
34.54	28.98	23.50	Fresh weight (g plant⁻ ¹)	2nd	1
14.23	13.13	7.63	Dry weight (g plant⁻ ¹)	2nd	1
22.3	21.0	19.0	Plant height (cm)	1st	2
24.0	22.3	20.3	Plant height (cm)	2nd	2
55.43	38.29	36.36	Fresh weight (g plant⁻ ¹)	1st	2
21.96	19.13	14.33	Dry weight (g plant⁻ ¹)	1st	2
63.71	53.37	44.46	Fresh weight (g plant⁻ ¹)	2nd	2
31.66	20.33	19.66	Dry weight (g plant⁻ ¹)	2nd	2

At the first cut in the first season, increasing potassium silicate from 6 to 12 mL L⁻ ¹ increased fresh weight from 18.87 to 32.99 g plant⁻ ¹ (+74.83%) and dry weight from 6.86 to 13.13 g plant⁻ ¹ (+91.40%). At the second cut, the corresponding increases were 46.98% and 86.50%, respectively.

Table 7. Essential-oil percentage under potassium silicate

12 mL L⁻ ¹	9 mL L⁻ ¹	6 mL L⁻ ¹	Cut	Season
0.34%	0.32%	0.29%	1st	1
0.35%	0.33%	0.30%	2nd	1
0.36%	0.35%	0.32%	1st	2
0.39%	0.37%	0.34%	2nd	2

Relative to 6 mL L⁻ ¹, the 12 mL L⁻ ¹ treatment increased EO percentage by 17.24%, 16.67%, 12.50%, and 14.71% across the four season × cut combinations, respectively. These values describe the change between the reported potassium-silicate

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concentrations and should not be interpreted as pooled treatment effects.

3.7 Influence of Environmental Conditions

The main environmental factor modifying the SA response was water availability. Khalil et al. compared 100%, 75%, 50%, and 25% field capacity while (21). applied normal irrigation and withheld irrigation.

Table 8. Environmental conditions and observed responses

Main observed response	Evidence	Environmental condition
Maximum dry matter: 1958.83 kg ha^{-1} with 200 mg L^{-1} SA	Mohammadi et al. (2020)	Normal irrigation
Increased phenolics, flavonoids and antioxidant activity	Khalil et al. (2018)	Water deficit
Highest reported phenolic and flavonoid responses with 3 mM SA	Khalil et al. (2018)	25% field capacity
Maximum EO content: 2.66% with 100 mg L^{-1} SA	Mohammadi et al. (2020)	Withholding irrigation
Improved physiological and biochemical traits with SA	Mohammadi et al. (2019)	Water stress
SA interacted with Pb and biochar for growth and biochemical responses	Rezaei et al. (2022)	Pb stress
Increasing potassium silicate generally increased growth and EO content	Waly et al. (2020)	Sandy-soil field conditions

Therefore, it is suggested that environmental changes affected SA responses with the phytochemical changes being especially significant under water deficit. The best treatment concentration was found to vary, however, depending on the outcome measured.

3.8 Influence of Application Method and Concentration

The majority of studies included used a foliar method for drug delivery. Three applications of Miri and Waly were reported at about 15-day intervals. Concentration-response relationships were not identical for different outcomes.

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Table 9. Concentration-related responses

Highest treatment	Outcome showing highest response	Concentrations	Intervention	Study
1 mM	Fresh weight	0.5, 1 mM	SA	Miri et al. (2015)
0.5 mM	Dry weight	0.5, 1 mM	SA	Miri et al. (2015)
0.5 mM	EO production	0.5, 1 mM	SA	Miri et al. (2015)
3 mM	Phenolics/flavonoids	1–3 mM	SA	Khalil et al. (2018)
2 mM	EO content	1–3 mM	SA	Khalil et al. (2018)
200 mg L^{-1}	Dry matter	100–200 mg L^{-1}	SA	Mohammadi et al. (2020)
100 mg L^{-1}	EO content under withholding irrigation	100–200 mg L^{-1}	SA	Mohammadi et al. (2020)
12 mL L^{-1}	Growth/biomass/EO	6–12 mL L^{-1}	KSi	Waly et al. (2020)

The results show that there is no single optimal concentration for all the growth, yield,essential-oil and phytochemical results.

3.9 Evidence Gap: Combined Silicon and Salicylic Acid Application

There was no included study evaluating a factorial study of treatment Si + SA in T. vulgaris. Thus, there was no study that demonstrated evidence of combined application, while 6/7 studies (85.71%) had evidence of application of SA alone or in combination with other applications, and 1/7 studies (14.29%) had evidence for application of potassium silicate.

Table 10. Evidence distribution and major gaps

Percentage	Studies (n)	Evidence category
85.71%	6	Salicylic acid investigated
14.29%	1	Silicon investigated
0.00%	0	Direct Si + SA combination
42.86%	3*	Studies involving water stress
57.14%	4*	Studies involving additional co-interventions
0.00%	0	Quantitative meta-analysis

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Classification is based on the nature of co-interventions/stress conditions as a study level characteristic, and is not mutually exclusive among the categories. This evidence gap is the absence of direct Si + SA experiments. It is recommended that future studies use factorial designs comprising of control, SA only, Si only and Si + SA treatments and outcome should be expressed as standardized growth, yield, essential oil, phenols, flavonoids and antioxidants. This would enable a more thorough evaluation of potential additive and interactive effects and would make it possible to have theoretically meaningful quantitative synthesis in future.

4. Discussion

In the present systematic review seven experimental studies were found that assessed salicylic acid (SA) and/or silicon (Si) on the plant of Thymus vulgaris L. All available evidence suggests that SA can affect plant growth, biomass production, phytochemical accumulation, antioxidant related characteristics and essential oil production while evidence about silica is still rather limited. The effectiveness of these treatments did not hold constant across both treatments and environment; that is, the responses to them are highly sensitive to the experimental conditions. Importantly, there were no studies included in which the combined use of silicon and SA was directly evaluated, and it is therefore unknown whether these may have an additive, synergistic or antagonistic effect.

Salicylic acid had measurable activity on plant growth and biomass under some experimental treatments and conditions, but the extent and direction of the response were based on treatment concentration and experimental conditions, indicating that it is an environmentally-responsive plant compound. (20) found an increase in fresh and dry biomass under foliar SA application and the response varied between 0.5 mM and 1 mM. The results indicate that the increase in plants growth with the increase in SA concentration is not always linear, and that sometimes the concentration at which the best response occurs can vary between different growth dimension. When used as a biostimulant, SA can exhibit such variation, especially when evaluating its effects in relation to other agronomic traits: An increase in one trait may not be associated with an optimal response in one or more other traits.

Water availability, especially in goads, was the major factor influencing the response of T. vulgaris to SA. In water-stressed plants, the accumulation of phenolics and flavonoids and antioxidant activity were significantly altered in thyme plants exposed to SA, which suggests that application of SA might influence the biochemical response of thyme plants to water stress (15). Concerns with regard to effectiveness of SA cannot be considered in isolation from irrigation regimes, as (21) showed that traits and essential oil constituents varied between well-watered and water-stressed plants. The results suggest that the intensity of stress could be considered to be one of the main modifiers of SAs and not merely an extra experimental variable.

The effects of SA on yield and phytochemical characteristics also were associated with treatment conditions. With foliar SA treatment, under water stress conditions, (22) observed increased yields and phytochemical compounds, with the optimum application rate depending on the phytochemical compound. This result confirms that there is not an optimum level of SA for T. vulgaris. Rather, the concentration required may be dependent upon the main production goal, which can be biomass growth, essential oil production, phenolic enrichment, or another characteristic with respect to quality. Moreover, (26) showed that the physiological response of SA exposed to lead was also impacted by interaction effects between biochar, which further suggested that the context of chemical and environmental conditions may also influence the physiological response of SA with lead stress.

The evidence records too indicate that SA can affect essential oil production and composition. (20) also

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noted that essential oil production showed variations after the application of SA and that they had various range of response in essential oil yield. (15) also showed that under water stress levels, application of SA resulted in changes in essential oil composition and quality. The results are significant because essential oil percentage, essential oil yield and individual concentrations of constituents will not necessarily be equivalent indicators of product quality. The responses observed also suggest that an increase in biomass does not always lead to a proportionally more elevated concentration of essential oils.

The results of (23) also supports the notion that foliar nutrition with essential oil technology could be one way to alter essential oil production and composition; the experiment in their study involved foliar NPK nutrition combined with the essential oil technology of SA. Therefore any changes that were observed in this study must be considered as evidence of combined foliar nutritional intervention and should not be attributed solely to the operational of SA. However, it is relevant when synthesising evidence that co-application with other nutrients or biostimulants may lead to plant responses being altered and to the effects being more challenging to attribute to the specific nutrient or biostimulant treatment.

Evidence on silicon was much more limited than the evidence on SA. Among the studies included only (33) investigated potassium silicate in T. vulgaris. The reported parameters of growth, biomass, essential oils production, essential oils content, and essential oils production per unit volume have been increased with increasing potassium silicate, starting at 6ml/l and 12ml/l. The largest tested concentration consistently resulted in the largest reported concentrations. While these results suggest a positive effect on potassium silicate, it is advisable to interpret results from this study with caution, as it was based on a single setting and also comprised extra treatments with additional treatments with seaweed extract and chitosan. So far, no general optimum concentration of silicate has been established, and whether the observed effects were due to silicate alone is not clear.

The treatment efficacy seems to be influenced greatly by environmental stress. In the studies included, the effect of the interaction of SA with water stress and other stress conditions was variable on T. vulgaris. In one case, under severe water deficit, the changes in phenolic and flavonoid accumulation were more pronounced in SA treatments than in the other case where the biggest change in biomass was seen under another irrigation + concentration combination (15; 22). As stated earlier, the effect of treatment also might be dependent on the type of stress condition, and other soil amendments might also affect the interaction between SA and Pb, as demonstrated by (26). Together, these results argue for a treatment concentration dependent interpretation of the efficacy of SA use as opposed to assuming that one concentration will be consistently effective in different environments.

In a practical sense the available evidence indicate that foliar SA may be useful in modifying the growth, biomass, essential oil production, and phytochemical properties of T. vulgaris. However, these findings do not validate the use of a set universal concentration for a specific SA for both production purposes and environmental settings. The treatment selection should rather be based on the trait to be treated, irrigation system used, degree of stress, concentration, treatment application, and interaction with other treatment inputs. Silicon is also promising for the improvement of growth and essential oil-related parameters, but findings are significantly limited and more information is needed to make recommendations to growers for the application of silicon.

This literature review revealed that there is a significant gap in the field of research on silicon and SA in combination for T. vulgaris. In none of the studies included in this review carried out a comparison between a control no-treated and a control SA (silicon + SA group). At this time, there is no published evidence from the articles that are

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included here to assess any additive, synergistic, antagonistic, or independent effect of these treatments when they are combined. In further studies factorial design should be used to separate the effect of the two treatments and both agronomic and biochemical responses should be examined.

The standardisation of experimental protocols would be additional useful to increase the comparability of studies. Future studies should include the concentration of SA in standardized units, and indicate the silicon source, volume per application, frequency of application, developmental stage of plants being treated, irrigation regime, stage of harvest, essential oil extraction method and analytical conditions for the GC–MS analysis. Essential oil yield, percentage, and concentration of phytochemicals per kg of biomass and per kg of essential oil should be reported per unit and consistent denominators. More reliable comparison of these and possible eventual quantitative synthesis if there are enough homogeneous studies would be made easier by such standardization.

Validation over the long- and short-term is also needed. Much of the available evidence comes from single experiments performed under particular environment/stress conditions, making the results less applicable to commercial production units since they are hard to generalize. There should therefore be multi-season field evaluation of the stability of the treatments as determined under the following conditions: soil types, irrigation regimes, and across years. Along with the biological response, studies in the future should explore the reproducibility and cost-efficiency of any observed increase in biomass, essential oil yield and quality of thyme to evaluate how such improvements reflect the merit of cultivating thyme.

The present review has many merits such as specificity to T. vulgaris, experimental evidence and agronomic, essential oil and phytochemical outcome. Environmental conditions and application characteristics as well as co-treatments have been considered in the review and are important for interpretation of the differences in the experiments. The conclusions are however limited by the small number of eligible studies, due to the predominance of SA studies in comparison to silicon studies, co-interventions and the variations in treatment concentration and units used, as well as substantial between-study differences in methods. These restrictions made it impossible to carry out a meaningful quantitative pooling and therewith it was decided to use a narrative synthesis. Results clearly suggest that the both species possess potential to alter various production and quality traits of T. vulgaris, but the magnitude and direction of the effects vary with target trait, environment, concentration of the species and experiment design.

5. Conclusions

The findings obtained through the systematic review analysis suggest that salicylic acid (SA) has significant potential to modify the growth, yield, essential oil (EO) production physiology, phenolic accumulation, antioxidant responses, and physiological performance of Thymus vulgaris L. The application of SA was mostly correlated with increased biomass production, EO-related traits, phenolic accumulation, antioxidant responses, and physiological performance under water-deficit or other stress conditions across the included experimental study. However, none of these effects was uniform and seemed to be a function of application level, irrigation type, stress intensity, genotype or ecotype, and other treatments. Thus, any statements about the universal positive or concentration independent effects of SA on thyme should be interpreted with caution. Optimization of the application conditions is necessary.

Evidence for silicon (Si), especially potassium silicate, was less than for SA. The results presented indicated that the application of potassium silicate has the potential to enhance vegetative growth, biomass production and EO percentage of T. vulgaris. However, the contributing influence of Si is difficult to interpret due to the few studies and because Si has been used in conjunction with other inputs. The

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available evidence, therefore, supports the possible application of Si as a stress-modulating and productivity-enhancing treatment, but is not enough to define the dose-response relationships and general guidance for the cultivation of T. vulgaris.

The results also show that both exogenous treatments and environmental factors are responsive in the production of EO and phytochemical quality. The interaction between treatment concentration and growing condition was often significant for changes in the EO content and composition, phenolic compounds, antioxidant-related traits and for biomass. Therefore, the effectiveness of treatment should be evaluated based on both the yield and composition of the EO and the biomass and phytochemical characteristics, and not just one of these production factors.

There were a number of evidence gaps identified. There were very few studies available, and they generally measured different outcomes, tests were performed at different concentrations, different application techniques, different environmental conditions, and different methods of treatment. The choice of whether to draw conclusions about additive, synergistic or antagonistic effects between Si and SA in T. vulgaris itself was restricted due to the lack of direct evaluation between their combined application in T. vulgaris. Furthermore, the existing evidence lacks data relating to long-term field trial performance and how effectively treatments reproduce the effects each treatment delivers across varieties and environments, as well as information on the correlation between treatment-induced changes in EO quantity and stability of the chemical composition.

Well-designed factorial experiments involving Si, SA, and Si x SA interaction as well as proper untreated controls in both non-stress and defined-stress environments should be conducted in future studies. Standardized application rates and dates, growth rate assessment, biomass measurement, EO yield, determination of EO composition, phenolic and flavonoid content, antioxidant activity, and indispensable physiological parameters evaluation should be tested in studies. Adaptability of treatments to different soil and climatic conditions requires multi-season field trials. It would enhance our evidence base for optimizing strategies of application of Si and SA and also for developing scientifically sound strategies to increase the productivity and/or phytochemical value of T. vulgaris.

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