Heliyon 9 (2023) e20211

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

A new approach on essential oil production of *Origanum onites* L.: Microbial fertilization and microwave extraction

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A R T I C L E I N F O

Keywords:
Origanum onites L
PGPB
Microbial fertilizer (biostimulant)
Yield
Quality
Essential oil

A B S T R A C T

In many studies plant and microbe interaction has been observed due to its stimulating effect on plant oil content. *Bacillus* spp. isolates used as microbial fertilizer/biostimulant have shown a higher potential for enhancing plant production and quality. However, there is still a need for investigating the essential oil content of plants, particularly for the pharmaceutical industry, to increase the targeted essential oil quantity and quality. Thyme is a crucial plant in medicinal aromatic herbal production and export, and its economic value is steadily increasing due to its high essential oil content. This study explores a novel approach to increase essential oil content through microbial fertilization (biostimulation) and microwave extraction methods. The microwave extraction method offers comparable yields to other methods, requiring less time, energy, and only a small amount of water to hydrate the dried plants. Microbial fertilizer formulations were applied to İzmir thyme (*Origanum onites* L.) to assess their effects on yield and quality. Three distinct microbial fertilizer formulations (N, P, NP) containing bacterial isolates of Bacillus subtilis and Bacillus megaterium, as well as a consortium of these two bacteria, were employed in this study. Among the applications on İzmir thyme, the NP formulation (a combination of two bacteria at a dosage of 6 L/ha^{-1}) exhibited the highest efficacy in terms of yield and quality criteria. Moreover, the essential oil ratios (1–2%) obtained from the same fertilizer application, using microwave extraction to enhance quality and accelerate the extraction process, yielded higher than other methods.

This study provides preliminary data and a new approach to regulate and enhance the targeted yield and essential oil content of thyme plants by utilizing microbial fertilizer applications and the microwave extraction method.

1. Introduction

Medicinal aromatic plants exhibit an extensive and highly diverse distribution across Anatolian lands. Anatolia's position as an intercontinental crossroads, characterized by varying climates and ecological profiles, contributes to its remarkable richness in terms of biological diversity. This region serves as a hub for numerous plant families, genera, species, origins, and gene centers. Among these plants, thyme stands out as one of the most prevalent botanical families worldwide [1]. The focus of this study is on İzmir thyme (*Origanum onites* L.), which stands as both the most exported and the most sought-after thyme species. Among the countries importing

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https://doi.org/10.1016/j.heliyon.2023.e20211
Received 29 May 2023; Received in revised form 11 September 2023; Accepted 13 September 2023
Available online 15 September 2023
2405-8440/

thyme from Turkey, the USA holds the top position, accounting for 25% of the imports. Following suit are Germany, Italy, Canada, Poland, the Netherlands, France, Japan, and Australia. The extraction of essential oils from thyme, in particular, finds extensive utilization in various sectors including antiviral, antibacterial, antifungal, insecticidal, medical, and cosmetic industries [2–7]. Thyme extract also finds applications in the food and spice industry [8]. Given these properties, the yield and quality of essential oil production from thyme hold strategic significance for both essential oil producers and commercial consumers.

Fertilization, as widely acknowledged, ranks among the pivotal inputs in agricultural production. Recently, as an eco-friendly substitute for pesticides and chemical fertilizers, organic fertilization has gained prominence, with microbial support emerging as a primary approach. In the cultivation of medicinal aromatic plants like thyme, the employment of fertilizers aligned with organic agriculture or good agricultural practices is strongly recommended. Furthermore, the role of biostimulants in augmenting the yield and quality of secondary metabolites derived from these plants is accentuated [9].

Numerous studies delve into the advantages and implications of thyme essential oil, aiming to amplify its components through various pathways. For example, the antimicrobial constituents of the essential oil can exhibit variability based on the subspecies and varieties of Origanum onites L [10]. The repercussions of Izmir thyme's essential oil are noteworthy; it contributes to prolonging the shelf life of processed foods by impeding the growth of foodborne microorganisms [11]. An examination of antioxidant capacity with respect to vegetation periods within the growth medium showed variations, with the highest carvacrol levels obtained from thyme harvested in July [12]. Additionally, certain investigations indicate the potential for essential oils to serve as a biological control method for weeds [13]. These antecedent studies collectively underscore that environmental factors hold sway in enhancing the potential components of thyme, which, in turn, hold significance across diverse domains.

Microbial inoculants used in agriculture are categorized into two groups: biopesticides and biological fertilizers. Notably, biological fertilizers fall under the umbrella of biostimulants. Biostimulants exert a positive influence on various aspects of plant development, including growth, nutrition, product quality, and yield. These formulations are designed to enhance plant resistance to stress and can encompass microorganisms within their composition [14]. Microorganisms function as activators, contributing to processes such as nitrogen fixation, the augmentation of plant nutrient solubility, the enhancement of iron uptake via siderophore production, and the elevation of volatile organic compound production.

Examining plant-fungus interactions, it was observed that leaf phosphorus content increased and shifts occurred in the leaf metabolome of plants within the Plantaginaceae, Fabaceae, and Poaceae families when inoculated with *Rhizophagus irregularis* [15]. In the context of bacteria-plant interaction, the inoculation of basil plants (*Ocimum basilicum*), known for their abundance in secondary metabolites, with *Bacillus subtilis* (GB03) resulted in a substantial elevation in terpene synthesis (from α-terpineol and eugenol) [16]. Similarly, the inoculation of Italian thyme roots with soil bacteria (*P. fluorescens* and *A. brasilense*) has been reported to lead to significant increases in monoterpenes (thymol, carvacrol, sabinen hydrate, and terpinene) [17]. With gene expression regulation at the core of plant-microbe interactions, researchers aim to discern alterations in secondary metabolite-related gene expressions. For instance, moths (*Catharanthus roseus*) inoculated with bacterial strains (*Curvularia* spp., *Choanephora infundibulifera*, *Aspergillus japonicus*, *Pseudomonas* spp.) exhibited heightened expression of genes linked to secondary metabolite production [18].

The mechanisms underlying shifts in plant secondary metabolite levels continue to intrigue researchers, driving ongoing investigations into the exchange of secondary metabolites within plant cells via the plant-microbe relationship. The symbiotic connection between plants and microorganisms significantly contributes to enhancing nutrient uptake in medicinal aromatic plants and fostering secondary metabolite production. The microbial fertilizer formulations employed in this study comprise *Bacillus subtilis* (N) and *B. megaterium* (P) as plant growth-promoting bacteria (PGPB). In this context, a comprehensive understanding of plant-microbe interactions holds pivotal importance for boosting product yield and enhancing quality [19,20].

Within this study, it holds significance to delve into the interplay between thyme plant productivity and essential oil production, taking into careful consideration factors such as soil moisture and nutrient levels. Much akin to other herbal materials, thyme has witnessed significant strides in breeding, cultivation, development, yield enhancement, and overall quality enhancement. The primary focus of this study revolves around pinpointing microbial fertilizer formulations that concurrently stimulate the vegetative growth and yield of İzmir thyme (*Origanum onites* L.), while also scrutinizing the essential oil ratio and its resultant quality. Furthermore, the investigation probes into the influence of varying doses of İzmir thyme (*Origanum onites* L.) on yield and quality parameters.

Tissue culture techniques have been widely employed in numerous studies to enhance essential oil quality [21,22]. The innovation of this study lies in its deviation from traditional tissue culture methods, opting instead for the application of microbes and microbial fertilizers to elevate both yield and essential oil quality. The objective was to enhance the vegetative growth, yield, and essential oil content of İzmir thyme (*O. onites* L.) by implementing diverse doses of microbial fertilizers. These microbial fertilizer compositions encompass *Bacillus subtilis* and *B. megaterium* as plant growth-promoting bacteria (PGPB). Additionally, a novel technique is introduced: Microwave Assisted Extraction (MAE), which offers rapid and efficient extraction of essential oils [23]. Consequently, this research delves into novel avenues for elevating essential oil content via the combined methods of microbial fertilization and microwave extraction.

The central aim of this study is to ascertain the impact of distinct microbial fertilizer formulations and their varying doses on the enhancement of vegetative growth, yield, quality, and essential oil content of İzmir thyme (*Origanum onites* L.). The outcomes of this research are poised to present promising technological breakthroughs in the realms of microbial fertilization (biostimulants) and microwave extraction for thyme essential oil production.

2. Materials and methods

The research was conducted over a span of two years, in the naturally illuminated greenhouse facilities of Konya Regional

Directorate of Forestry Olcay Nursery (coordinates: 37.82266°N, 32.42377°E), spanning the years 2019–2020. The Izmir thyme seeds utilized in the study were sourced from the Aegean Agricultural Research Institute, with the Tayşi-2002 variety serving as the seed origin. The soil texture of the experimental plots was determined using the Bouyoucos Hydrometer method [24]. Soil pH measurements were carried out potentiometrically, employing a "Glass Electrode" pH meter, within a 1:5 soil-water solution [25]. The assessment of lime content was accomplished volumetrically via the Scheibler Klasimeter technique [26]. Determination of soil organic matter content was executed using the Walkey Black Wet Burning method [27], while total nitrogen content was gauged through micro-Kjeldahl methods after wet incineration with a mixture of salicylic acid and salt. For soil phosphorus quantification, extraction with sodium bicarbonate was utilized [28], and potential determination was performed using the low-flame photometric method [29].

2.1. Soil properties

Table 1 presents select physical and chemical analysis outcomes of the soils employed in the experimental plots. Based on the analysis findings, the trial soil was classified as moderately alkaline with a medium humus content.

The research was structured around a random plot experimental design. A total of 7 experimental plots were established, encompassing a control plot without bacterial application to the thyme, and 6 experimental plots where bacteria were applied. In this study, microbial fertilizers containing single and mixed formulations of *Bacillus subtilis* and *Bacillus megaterium* bacteria were administered to İzmir thyme seedlings. Three different doses were employed: 0 L/ha^{-1} (control), 3 L/ha^{-1}, and 6 L/ha^{-1} for each iteration.

The experiment was executed within randomized plots, featuring 4 replications, with each replication accommodating 80 saplings. In total, 560 saplings were distributed across the soil culture pots, adhering to the trial design. Irrigation strategies were tailored to the soil's moisture condition and the microclimate within the greenhouse (coordinates: 37.82266°N, 32.42377°E; steel construction and polycarbonate greenhouse; 215 m^2 growth area; production of 60,000 thyme seedlings in soil culture; misting irrigation system operating at 100 bar pressure; utilization of 4 circulation fans for aeration; temperature regulation via natural gas heating system, maintaining an ideal range of 20–30 °C for thyme production). Prior to commencing the applications, the field capacity of the soil within the pots was determined.

2.2. Bacterial strains, biofertilizer properties and applications

In this study, microbial fertilizers (N, P, NP) comprising two distinct strains were employed. Specifically, N stands for *Bacillus subtilis*, P represents *B. megaterium*, and NP combines both *Bacillus subtilis* and *B. megaterium* in a consortium-based formulation. *Bacillus* spp. exhibit rapid multiplication, enveloping the roots and leaves of plants to produce metabolites that enhance nutrient absorption. These fertilizers enable atmospheric nitrogen fixation and contribute to the reduction of chemical fertilizer use. Beyond elevating yield and quality, they confer resistance against environmental stressors and partially function as biocontrols, restraining plant pathogens.

Bacillus subtilis (N), retaining its vitality and metabolic activity on leaves, fosters leaf development by promoting the synthesis of natural organic acids, amino acids, auxins, and cytokinins. It facilitates robust root development, enhancing root penetration and growth. During initial plant stages, from seed germination and root formation to the emergence of hairy roots, and onward to leaf and stem maturation, *Bacillus subtilis* enhances productivity.

Similarly, *Bacillus megaterium* (P) promotes seed germination and root establishment in early plant phases and supports leaf and stem growth in subsequent stages. It facilitates the uptake of crucial nutrients, including NH_4 and NO_3, particularly phosphorus (P), and essential elements such as Ca, Mg, Fe, Mn, Zn, and Cu that might be less accessible in the soil.

In the research, microbial fertilizers were administered to İzmir thyme seedlings across different dosages (0 L/ha^{-1} (control), 3 L/ha^{-1}, and 6 L/ha^{-1}) and various formulations (control isolates of N and P, and NP formulations). These applications were mixed with 100 L. of water and designed ratios before being applied. Initial application took place after thyme germination, with additional seedling root dipping during the diversion period. The first planting of seedlings commenced on March 15, 2019, and harvest occurred in the second week of June 2020. Throughout the first year, applications were conducted bi-monthly, while in the second year, applications were aligned with the designated dosages until harvest.

2.3. Irrigation applications

Among the abiotic factors impacting plant development, water deficiency emerges as one of the most pivotal. Water stress

Table 1
Some physical and chemical properties of the soils used in the experiment.

Soil Properties	Values	Soil Properties	Values
Sand (%)	36.60	Electrical conductivity (mS/cm)	0.20
Clay (%)	34.60	Organic matter (%)	3.04
Dust (%)	28.83	N (%)	0.16
Lime (%)	35.22	P (ppm)	45
pH (1:2,5)	8.05	K (ppm)	275

significantly influences plants' morphological and physiological attributes, as well as their yield and overall quality. To address this crucial aspect, both soil moisture and greenhouse microclimate conditions were meticulously considered in the context of irrigation applications.

Before commencing the applications, the field capacity was determined. This entailed saturating tubes with water and allowing them to undergo free flow, with the tubes covered to curb evaporation. Upon cessation of drainage, the tubes were weighed, and the field capacity was calculated based on the weight.

In the realm of irrigation water application, all pots received irrigation at 80% of the field capacity rate. Adhering to the field capacity, water equivalent to the average decrease every three days was replenished in the tubes. Post-planting, the tube positions were periodically shifted in a predetermined sequence, altering once every seven days. This strategic alteration ensured that the thyme seedlings experienced uniform exposure to growth conditions between successive applications.

2.4. Maintenance and harvest operations

Thyme seedlings underwent the trial without the application of any chemical pesticides. For fresh thyme harvesting, the 2 nd week of June 2020 was chosen, coinciding with the onset of flowering. Harvesting of the herb was executed by carefully cutting with scissors, maintaining a height of 4–6 cm above the soil surface. This approach ensured that the roots and the immediate surroundings of the seedlings remained unharmed.

2.5. Growth and yield parameters

Plant height was ascertained by measuring the section of the main shoot from the soil level to the shoot's apex using a ruler. To determine fresh herb yield, plants were gently cut at a height of 4–6 cm above the soil using garden shears, followed by weighing.

Dry herb yield (g/plant) was calculated by drying the thyme in the shade and then measuring the weight. For the calculation of drug leaf yield (g/plant), the dried thyme was blended with a stick and sifted through a 4 mm mesh sieve, followed by sorting.

The drug petiole ratio (PR) was determined using the subsequent equation:

PR = (drug leaf/(drug leaf + stem)) * 100 [30]

2.6. Essential oil extraction and path analysis

For the extraction of essential oil, the microwave-assisted water distillation method was employed [23]. In this technique, 150 mL of distilled water was added to 50 g of the sample at 35 °C, followed by a 30-min duration. Subsequently, distillation was carried out by applying 550 W after the 30-min waiting period. The essential oil quantity was determined upon collection from the graduated region of the Clevenger apparatus. To ensure the absence of water in the collected essential oils, anhydrous sodium sulfate was introduced into the bottles. These bottles were stored at 4 °C for subsequent analysis.

Essential oil ratios were gauged volumetrically (mL/g), with oil ratios expressed as % essential oil yields (mL/100 g drug herb). Analytical evaluation was performed at the Anadolu University Herbal, Pharmaceutical, and Scientific Research Application-Research Center using both GC and GC/MS methods. In the GC method, the Agilent 7890B GC System was employed with an Agilent HP-Innowax column (60 m × 0.25 mm inner diameter x 0.25 μm film thickness). The injection temperature and detector temperature were set at 250 °C. The temperature program consisted of 60 °C for 10 min, followed by a ramp of 4 °C/min to 220 °C for 10 min, and then a gradual increase of 1 °C/min to 240 °C, maintained for 80 min. The carrier gas used was helium at a flow rate of 0.7 mL/min.

For GC/MS analysis, the conditions encompassed an Agilent 7890B GC 5977B Mass Selective Detector System, with the same Agilent HP-Innowax column. Injection temperature was set at 250 °C, while ion source temperature was 230 °C. Electron impact (EI) ionization mode was employed with an electron energy of 70 eV. The mass range analyzed spanned from 35 to 450 *m/z*. The temperature program for GC/MS mirrored that of GC. The carrier gas remained helium, with a flow rate of 0.7 mL/min. Furthermore, the Wiley 9-Nist 11 Mass Spectral Database was utilized for comprehensive evaluation.

2.7. Data analysis

All data acquired from the thyme experimental plots underwent comprehensive statistical analysis within the SPSS software, specifically utilizing IBM SPSS Statistics 20. This analysis encompassed various statistical procedures, including variance analysis, correlation testing, and multiple comparison tests. The primary objective was to uncover significant differences between the diverse applications employed in the study.

The normal distribution of variables was assessed using the Shapiro-Wilk test. Variables that did not adhere to the normal distribution were presented along with their median, minimum, and maximum values. Given the non-normal distribution of certain variables, the "Kruskal Wallis H″ nonparametric test was selected for the comparison between thyme groups.

In addition to comparisons, the associations between variables were explored using the "Spearman Correlation Coefficient." Throughout the analysis, the significance level for tests was established at 0.05.

3. Results and discussion

This study employed three distinct formulations of microbial fertilizers/biostimulants, namely N, P, and NP, during the cultivation of İzmir thyme (*Origanum onites* L.). The primary aim was to enhance both yield and quality outcomes. Following the applications implemented on İzmir thyme, it was deduced that the most impactful fertilizer formulation, demonstrating substantial efficacy concerning yield and quality criteria, was NP (a combination of two bacteria with a dosage of 6 L/ha^{-1}). This formulation showcased considerable potential in comparison to other alternatives.

The combined action of these strains exhibited a synergistic effect, enabling the achievement of nutrient sufficiency through two mechanisms: fixation of atmospheric free nitrogen and the conversion of immobilized phosphate compounds in the soil into plant-accessible forms (as illustrated in Fig. 1).

Findings from the İzmir thyme fertilizer application studies revealed remarkable results. The application of NP at a dosage of 6 L/ha^{-1} demonstrated a substantial increase in fresh herb productivity (43%), drug herb yield (44%), and drug leaf yield (44%) when compared to the control group. Within the same dosage range, individual fertilizer applications yielded distinct outcomes: N(6 L/ha^{-1}) application exhibited a yield increase of 14–15%, while P(6 L/ha^{-1}) application led to a reduction of 33–38% in yield parameters.

Emerging research underscores the pivotal role of plant-microbe interactions in influencing secondary metabolite production and overall productivity. The emphasis on natural and organic drug herb production, which often entails fewer side effects, further elevates the significance of medicinal plants in contemporary contexts [31].

Avcı's research documented varying outcomes in different *Origanum onites* L. clones collected from Western Anatolian flora, cultivated using conventional methods. Fresh herb yields ranged from 1779.8 to 4854.6 kg/ha^{-1}, drug herb yields spanned 612.3–1602.4 kg/ha^{-1}, while drug leaf yields varied between 408.4 and 953.4 kg/ha^{-1}. Essential oil ratios fluctuated between 2.77% and 5.56%. These productivity values were influenced by diverse environmental factors, including topography and climate.

Kutlu's exploration delved into the application of the most effective nitrogen-fixing and phosphate-dissolving PGPR isolates to *Origanum onites* L. In İzmir thyme, PGPR inoculations exhibited unexpected potency, promoting development, yield, and quality criteria. Reports indicated that through bacterial interventions, nutrient intake, leaf macro and microelement quantities, drug herb yield, drug leaf yield, essential oil ratio, and fundamental components within the essential oil could be enhanced [32,33]. Moreover, bacterial inoculations as biostimulants/biofertilizers in the production of other medicinal herbs have showcased their effectiveness on productivity. For instance, Abdul-Jaleel documented increased biomass yield and alkaloid content in the medicinal plant Vinca (*Catharanthus roseus*) due to the inoculation of *Pseudomonas fluorescens* bacteria [34]. Similarly, Abdelaziz's work highlighted how PGPR bacteria applications in rosemary cultivation resulted in heightened yield and quality when compared to conventional (NPK) fertilization [35].

From chemical, physical, and biological standpoints, the endophytic and rhizospheric environments exhibit pronounced disparities [36]. Plant exudates rich in organic matter enhance the nutritional quality of the rhizosphere environment in contrast to bulk soil. However, rhizospheric organisms must adeptly navigate soil competition and environmental variables to ensure their survival. In contrast, the endophytic environment stands as more stable and uniform, evincing reduced susceptibility to environmental fluctuations. Mechanisms driving plant growth promotion by endophytes and rhizosphere bacteria align closely. These mechanisms encompass nitrogen fixation, nutrient solubilization, the production of growth hormones and enzymes vital for plant growth regulation, as well as biocontrol functions [37].

The study also subjected the active bacteria to statistical evaluation regarding their potential utility as biostimulants and/or biological fertilizers for İzmir thyme production. The Kruskal Wallis H Test analysis ($p < 0.05$) conducted during the experiment unveiled a positive and significant relationship between fresh/wet herb weights ($r = 0.28$), drug herb weights ($r = 0.37^{**}$), and drug

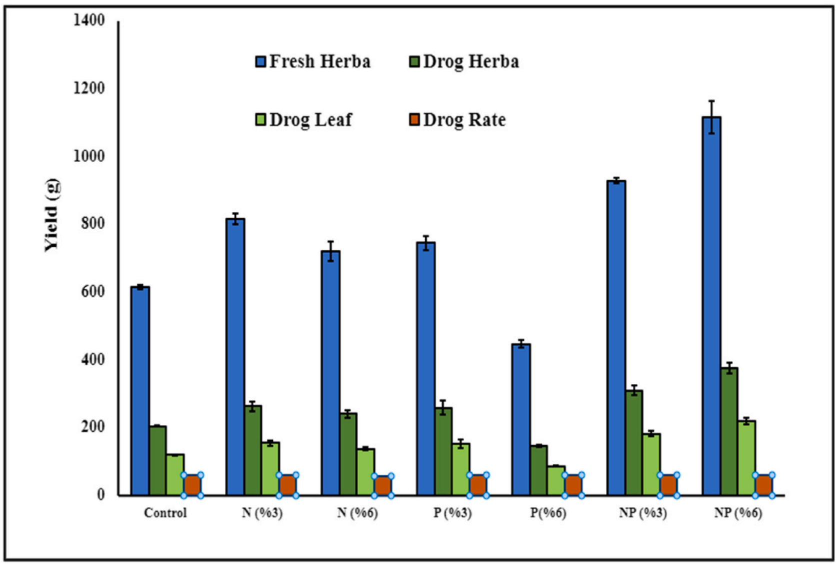

Fig. 1. The application effects on fresh herb, drog herb and drog leaf yield(g) of İzmir Thyme.

leaf weights (r = 0.39*) (refer to Tables 2–4). In light of these results, the hierarchy of biofertilizer formulations applied in İzmir thyme production, based on their effectiveness across tested parameters, was delineated as "Control < N(3%) < NP(3%) < NP(6%)". Among these formulations, the application of NP at a dosage of 6 L/ha^{-1} emerged as the most impactful compared to the control group.

The thyme groups examined within the research exhibit a statistically significant difference concerning drug rates ($p < 0.001$). The homogeneity of thyme groups with respect to drug rates is presented in Table 5.

A positive correlation is evident among the fresh herb weights, drug herb weights, drug leaf weights, and drug rates of thyme encompassed in the study.

As the fresh herb weights of thyme escalate, there is a concomitant increase observed in drug herb weights, drug leaf weights, and drug rates ($p < 0.001$, $r = 0.981$; $p < 0.001$, $r = 0.970$; $p = 0.002$, $r = 0.261$, respectively).

Similarly, an elevation in drug herb weights correlates with amplified drug leaf weights and drug rates ($p < 0.001$, $r = 0.989$; $p = 0.001$, $r = 0.271$, respectively).

The interrelationship among fresh herb weights, drug herb weights, drog leaf weights, and drog ratios of thyme underwent analysis via Spearman Correlation Analysis (**$p < 0.01$) (refer to Table 6). Notably, a noteworthy association is observed between the drug ratio of thyme and the increase in drug leaf weights ($p < 0.001$, $r = 0.301$).

The evaluation analyses conducted in the experiment unveiled a noteworthy positive relationship between the coexistence of the two different bacteria and the attainment of high-dose fresh herb, drug herb yield, drug leaf yield, and drug rate. The activity of bacteria displayed variations contingent upon bacterial strains. The increase in drug ratio resulting from N, P, and NP formulation applications was determined to be 57.3%, 58.6%–59%, respectively. It can be asserted that bacterial activity is a multifaceted process relying on more than one mechanism, rendering it challenging to expound the impact of bacterial activity on vegetative growth with a solitary parameter.

Numerous studies have underscored the efficacy of nitrogen and phosphorus fertilizers, as well as harvest timing, on the drug-to-plant ratio in medicinal aromatic plants. Nevertheless, the augmentation of vegetative components, prompted by escalated nitrogen doses, can lead to increased plant water content and potentially lower drug herb yield. Similar scenarios have been observed in the context of phosphorus dose applications [38].

In this experiment, it can be inferred that the synergistic effect of these two bacteria, addressing nitrogen and phosphorus requirements, plays a pivotal role. Plants engage in diverse interactions across various ecoregions (competitive, exploitative, waste, compensatory, and dense) with microbes inhabiting the rhizosphere, phyllosphere, and endosphere. While there is a wealth of research on plant-microbe interactions, much of the focus has been directed toward mitigating pathogenic impacts, such as infections, or investigating responses to abiotic stress [39–42]. However, the favorable aspects of these interactions have long garnered attention alongside their protective ecological role. Directing specific bacteria as biostimulants in plant microbiome interactions has been documented to exert significant effects on the production of primary and secondary metabolites [43].

Recent studies underscore the significant impact of plant-microbe interactions on the production of secondary metabolites and overall productivity. As a result, the utilization and investigation of biofertilizers and biostimulants have gained widespread attention, driven by the imperative for natural and/or organic production in the cultivation of medicinal aromatic plants [31]. The concentration and composition of essential oils (EOs) in plants play crucial ecological roles. For instance, the heightened synthesis of EOs in plants serves as a defensive mechanism against microorganisms [5]. In the context of this study, the inoculation of *B. subtilis* and *B. megaterium* through microbial fertilizer formulations led to an increase in the EOs yield of Izmir thyme. It is plausible to suggest that this escalation is not solely attributed to increased biomass, but rather to the enhanced biosynthesis of terpenes originating from the metabolites of biofertilizer bacteria, acting as potent biostimulants. The comprehensive fat content (as shown in Table 7) and the constituent compounds identified with high concentrations are documented in Table 8 and illustrated in Fig. 2. The composition of essential oils determined in this study for Izmir thyme aligns with findings from studies conducted by Kutlu and Güngör [33,44].

Essential oil was extracted through Microwave-Assisted Extraction (MAE) from plants harvested subsequent to the application of biofertilizers. The comprehensive yields of volatile compounds derived from *O. onites* L. are presented in Table 7. The outcomes revealed that MAE of O. onites L. exhibited a significantly higher oil yield (2.0% ± 0.01) with N (6%) fertilizer application compared to the control, signifying statistical significance ($p < 0.05$).

Golmakani and Rezaei's research also reported similar outcomes for *Thymus vulgaris* essential oil, specifically a yield of 2.52% with microwave-assisted extraction, albeit with an extended extraction duration (2 h) and higher power wattage (990 W) [45]. Another study explored supercritical water extraction for Thymbra spicata essential oil, revealing that higher extraction temperatures corresponded to increased oil yield. They achieved a comparable extraction efficiency of 2% at 100 °C [46]. Uysal documented similar

Table 2
Examination of Thyme Groups in the Scope of the Study in terms of Fresh Herb Weights(g).

	n	Median	Min	Max	Chi-square	p	Paired comparisons
Control	20	604	540	728	124,91	<0,001	Control < N(3%),NP(3%),NP(6%)
N(3%)	20	822.5	725	925			
N(6%)	20	710	610	840			N(3%) < NP(6%)
P(3%)	20	745.5	659	820			N(6%) < NP(3%),NP(6%)
P(6%)	20	455.5	358	515			P(3%) < NP(3%),NP(6%)
NP(3%)	20	909.×5	791	1100			P(6%) < N(6%),P(3%),N(3%),NP(3%), NP(6%)
NP(6%)	20	1067	987	1350			

Kruskal Wallis H Test, $p < 0.05$.

Table 3
Examination of Thyme Groups in the Scope of the Research in terms of Drog Herba Weights(g).

	n	Median	Min	Max	Chi-square	p	Paired comparisons
Control	20	201	180	242	118.8	<0.001	Control < N(3%),NP(3%),NP(6%)
N(3%)	20	269.5	190	300			
N(6%)	20	236	203	280			N(3%) < NP(6%)
P(3%)	20	248.5	225	359			N(6%) < NP(3%),NP(6%)
P(6%)	20	145.5	119	167			P(3%) < NP(6%)
NP(3%)	20	303	263	366			P(6%) < N(6%),P(3%),N(3%),NP(3%), NP(6%)
NP(6%)	20	355.5	329	459			

Kruskal Wallis H Test, $p < 0.05$.

Table 4
Examination of Thyme Groups in the Scope of the Study in terms of Drog Leaf Weights(g).

	n	Median	Min	Max	Chi-square	p	Paired comparisons
Control	20	117.5	105	142	11.885	<0.001	Control < N(3%),NP(3%),NP(6%)
N(3%)	20	158	111	176			
N(6%)	20	137.5	110	161			N(3%) < NP(6%)
P(3%)	20	145.5	132	211			N(6%) < NP(3%),NP(%6)
P(6%)	20	85.5	70	98			P(3%) < NP(6%)
NP(3%)	20	178	154	215			P(6%) < N(6%),P(3%),N(%3),NP(3%), NP(6%)
NP(6%)	20	208.5	193	270			

Kruskal Wallis H Test, $p < 0,05$.

Table 5
Examination of Thyme Groups in the Scope of the Research in terms of Drog Ratios.

	n	Median	Min	Max	Chi-square	p value
Control	20	58.6	58.3	58.7	10.967	0.089
N(3%)	20	58.7	58.4	58.8		
N(6%)	20	58.6	39.3	58.8		
P(3%)	20	58.6	58.4	58.8		
P(6%)	20	58.6	58.2	58.8		
NP(3%)	20	58.7	58.5	58.8		
NP(6%)	20	58.7	58.6	58.8		

Kruskal Wallis H Testi, $p < 0,05$.

Table 6
The relationship between fresh herb weights, drog herba weights, leaf herbal weights and drog ratios of thyme within the scope of the research.

		Fresh Herb	Drug Herb	Drug Leaf	Drug Ratio
Fresh Herb	r		0.981**	0.970**	0.261**
	p		<0.001	<0.001	0.002
Drug Herb	r			0.989**	0.271**
	p			<0.001	0.001
Drug Leaf	r				0.301**
	p				<0.001
Drug Ratio	r				
	p				

*Spearman Correlation Analysis **$p < 0.01$.*

findings for the essential oil of Thymbra spicata using solvent-free microwave extraction [47]. Considering our findings and those of other studies, Microwave extraction demonstrates comparable yields to alternative methods, while requiring less time, energy, and a minimal amount of water to rehydrate the dried plants [23].

The oil quality of the thyme groups examined in the study displayed a range of variation from 89.6 to 92.7. Notably, the highest oil quality was observed in the NP(6%) fertilizer application group, achieving a value of 92.7. Following this, the P(3%) group exhibited an oil quality value of 91.6, the control group showed a value of 90.9, the P(6%) group recorded a value of 90.9, and the N(3%) group demonstrated a value of 90.9. Conversely, the lowest oil quality was found in the N(6%) fertilizer application group.

For the individual oil components, the α-Terpinene component showcased variation between 1.9 and 4.1. Interestingly, the highest value was identified in the control group, while the lowest was observed in the NP(6%) fertilizer application group.

Similarly, the oil quality of the linalool component exhibited variation ranging from 2.2 to 7.1. The N(6%) fertilizer application

Table 7
Values of essential oil ratios detected in thyme groups within the scope of the study.

Groups	Essential Oil Ratios(%)
Control	1 mL
N(3%)	1.9 mL
N(6%)	2 mL
P(3%)	1.8 mL
P(6%)	1.6 mL
NP(3%)	1.6 mL
NP(6%)	1.6 mL

Table 8
Relative percentages of essential oil compositions detected in thyme groups within the scope of the study.

	ɤ-Terpinene	Linalool	*p*-Cymene	β- Caryophyllene	Thymol	Carvacrol	Total
Control	4.1	4.9	1.0	1.9	4.2	70.5	91.6
N(3%)	3.7	6	1.3	1.7	6.2	71.7	90.6
N(6%)	3.7	7.1	1.6	1.5	6.6	69.1	89.6
P(3%)	2.7	3.9	1.2	1.4	6.3	76.4	91.9
P(6%)	3.5	5.1	1.4	1.5	7.6	71.8	90.9
NP(3%)	3.9	2.4	0.6	1.7	8.1	76.0	92.7
NP(6%)	1.9	2.2	0.7	1.5	4.7	82.1	92.4

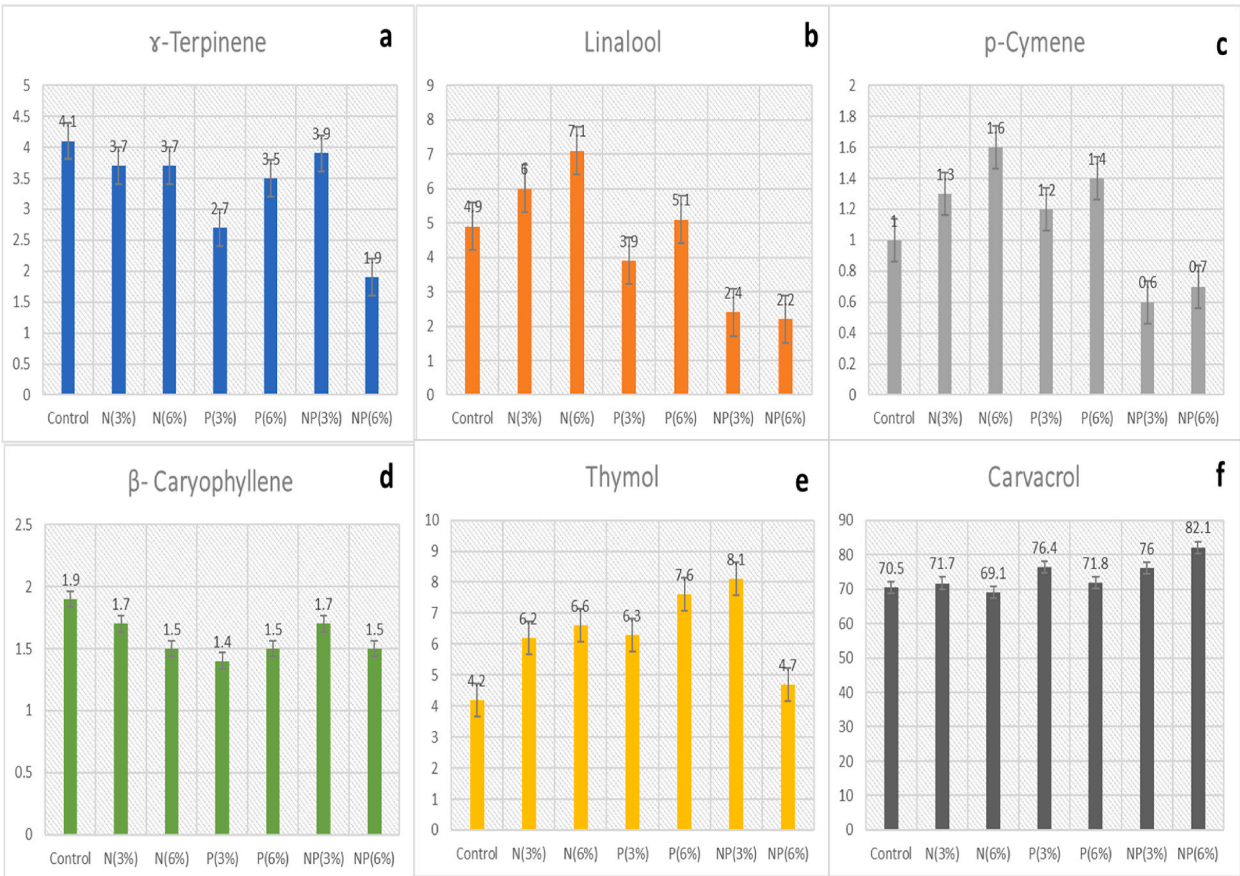

Fig. 2. The Thyme Essential oils: (a) ɤ-Terpinene (b) Linalool (c) *p*-Cymene (d) β- Caryophyllene (e) Thymol (f) Carvacrol changing amounts with the microbial fertilizer applications.

group displayed the highest value, while the lowest was seen in the NP(6%) fertilizer application group.

The *p*-Cymene component's oil quality spanned from 0.6 to 1.6. The N(6%) fertilizer application group displayed the highest value, and the lowest was observed in the NP(3%) fertilizer application group.

The β-Caryophyllene component's oil quality fluctuated between 1.4 and 1.9. The control group showcased the highest value, whereas the lowest value was observed in the P(3%) fertilizer application group for thyme.

The thymol component's oil quality ranged from 4.7 to 9.2. The control group exhibited the highest value, and the lowest value was associated with the NP(6%) fertilizer application.

Lastly, the carvacrol component's oil quality varied from 69.1 to 82.1. Notably, the NP(6%) applied thyme group demonstrated the highest value, while the lowest value was found in the N(6%) thyme applied fertilizer group, as illustrated in Table 8 and Fig. 2.

In our study, we employed the innovative and environmentally friendly microwave extraction method for oil extraction, avoiding the use of chemical-heavy methods like hexane distillation that might leave residues in oil contents. This approach offers a safe and pure extraction method for obtaining fixed and essential oils, applicable in various sectors such as medicine, food, cosmetics, human and animal health, and plant protection. It serves as an innovative and secure approach for such oil production.

Throughout our research, variations in essential oil ratios were observed across different formulations and doses. Essential oil qualities can indeed fluctuate under distinct growth and stress conditions. Our findings revealed that, when evaluating the essential oil's main components in İzmir thyme (Origanum onites L.), the highest ratios of carvacrol, linalool, and *p*-cymene were achieved with N(6 L./ha^{-1}) fertilizer application, facilitated by microbial fertilizer/biostimulant application.

Carvacrol, being a prominent essential oil in thyme, holds significance due to its widespread applications. NP(6 L./ha^{-1}) application demonstrated efficacy in enhancing both fresh drug herb yield and carvacrol essential oil efficiency. Furthermore, the N(6 L./ha^{-1}) dose showed promise for enhancing other active ingredients such as linalool and *p*-cymene. By leveraging specific strain efficiencies, it's conceivable that further exploration and optimization could enhance the efficiency of essential oils like linalool and *p*-cymene, which require targeted efforts.

4. Conclusions

Based on the comprehensive findings and evaluations presented, the most effective fertilizer application for achieving optimal yield and quality criteria in İzmir thyme cultivation is the NP formulation at a rate of 6 L./ha-1. This formulation, consisting of a consortium of bacteria with nitrogen-fixing and phosphate-solubilizing properties, serves as a promising microbial fertilizer/biostimulant for medicinal aromatic plant cultivation.

Highlighting the advantages of microbial fertilizers, particularly in terms of enhancing plant nutrition, is crucial. These formulations not only promote plant growth but also contribute to increasing the essential components of the plant, thereby enhancing their bioavailability. The application of such microbial fertilizers to medicinal aromatic plants, as demonstrated by the significant yield, quality, and essential oil quantity improvements in İzmir thyme, can play a pivotal role in boosting production and enhancing competitiveness in international markets.

Utilizing microwave extraction offers comparable yields to traditional methods while requiring less time, energy, and a smaller amount of water for hydrating dry plants. This study highlights the advantages of this technique over conventional industrial distillation methods to achieve maximum essential oil yield and quality from İzmir thyme.

For future research, it will be essential to further optimize combinations like the ones explored in our study to enhance the growth of medicinal and aromatic plants targeted for essential oil production. This optimization can significantly contribute to the industry's goal of increasing essential oil production for various applications.

Author contribution statement

Nurgül KITIR ŞEN, Ph.D.: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ahmet Duran: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Data availability statement

Data included in article/supp. material/referenced in article.

Note

During the preparation of this work the author(s) used [ChatGPT] in order to [GRAMMAR EDITING]. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Nurgul Kitir Sen reports financial support and equipment, drugs, or supplies were provided by Konya Food and Agriculture

University.

Acknowledgment

This study received support from Konya Food and Agriculture University and Konya Forest Nursery Directorate. The preparation of this study was aided by utilizing data from the 'Effects Of Some Microbial Fertilizer Formulations On Yield And Quality Of Izmir Oreganum (Origanum Onites L.)' graduate thesis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20211.

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