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Physicochemical characterization of essential-oil nanoemulsions and their biostimulant effects on tomato (Solanum lycopersicum L.) seedling growth

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Abstract

Biostimulants are increasingly recognized as sustainable agents for improving plant growth and physiological performance. Among emerging formulations, nanoemulsions have gained considerable attention due to their enhanced stability, bioavailability, and potential to facilitate the delivery of bioactive compounds. In the present study, twelve nanoemulsions prepared from pure essential oil constituents (γ-terpinene, α-pinene, 1,8-cineole, cuminaldehyde, carvacrol, limonene, and cinnamaldehyde) and essential oils of medicinal plants (*Pimpinella anisum*, *Ferula assa-foetida*, *Zataria multiflora*, *Anethum graveolens*, and *Cuminum cyminum*) were investigated for their effects on the seedling growth of tomato (*Solanum lycopersicum* L.). The nanoemulsions were applied as foliar sprays at concentrations of 50, 100, 200, and 400 μL L^{-1} during the vegetative growth stage. The chemical composition of the essential oils was determined using gas chromatography mass spectrometry, while the physicochemical characteristics of the nanoemulsions, including particle size distribution and zeta potential, were analyzed. The results demonstrated that nanoemulsions containing 1,8-cineole exhibited a clear concentration-dependent stimulatory effect, increasing both root and shoot growth by up to 80%. Essential oils of *Pimpinella anisum*, *Ferula assa-foetida*, and *Cuminum cyminum* significantly enhanced root development at concentrations of 400, 100, and 200 μL L^{-1}, respectively. In addition, nanoemulsions derived from *Zataria multiflora*, *Anethum graveolens*, and α-pinene significantly increased shoot biomass at concentrations of 100, 200, and 400 μL L^{-1}, respectively. Overall, 1,8-cineole at the highest tested concentration was identified as the most effective treatment for promoting tomato growth. These findings highlight the potential of essential oil-based nanoemulsions as environmentally friendly biostimulants and support their prospective application in sustainable agricultural systems.

1 Introduction

The use of organic and environmentally friendly inputs as plant growth enhancers has expanded markedly in recent decades, particularly across agricultural systems in the United States and Europe. This trend reflects global concerns related to food security, environmental sustainability, and the health risks associated with the intensive use of synthetic fertilizers. In this context, natural biostimulants including seaweed extracts, beneficial microorganisms, jasmonic acid, salicylic acid, and plant essential oils have gained considerable attention due to their documented ability to enhance plant growth and mitigate various abiotic and biotic stresses [1].

Plant essential oils (EOs) are complex mixtures of volatile, low–molecular weight compounds synthesized through secondary metabolic pathways. They are widely recognized for their antimicrobial, antioxidant, and growth-modulating properties [2]. These bioactivities have encouraged their incorporation into crop production systems as natural agents capable of improving seed germination, seedling vigor, and early plant establishment. However, practical applications of EOs remain limited by their hydrophobicity, chemical instability, and rapid volatilization, which collectively restrict their persistence and bioavailability under greenhouse and field conditions [3].

Nanoformulation technologies particularly nanoemulsions have emerged as promising strategies to overcome these limitations and improve the functional performance of essential oils. Nanoemulsions are kinetically stable dispersions of two immiscible phases, typically oil and water, with droplet diameters generally ranging from 20 to 200 nm. Their nanoscale dimensions enhance solubility, chemical stability, and bioavailability, facilitate controlled release, and reduce volatilization and environmental dissipation [4–8]. Owing to these distinctive physicochemical characteristics, including high surface-area-to-volume ratio, increased reactivity, long-term kinetic stability, and unique optical properties, nanoemulsions have attracted growing interest in agricultural and plant-based applications [9, 10].

Recent studies provide compelling evidence for the dual protective and growth-promoting potential of nanoemulsified essential oils. For example, clove essential-oil nanoemulsions have shown significant efficacy against the fungal pathogen *Neoscytalidium dimidiatum* [11], while other nano-enabled approaches such as copper nanoparticles have improved key growth traits in maize, including plant height, stem diameter, root length, and chlorophyll content, along with reducing Curvularia leaf spot severity [12]. Collectively, these findings underscore the potential of nanoemulsions as versatile agricultural biostimulants.

Tomato (*Solanum lycopersicum* L.) is one of the world's most economically important vegetable crops, with global production exceeding 180 million metric tons annually [13]. In addition to its economic value, tomato fruits are rich in health-promoting bioactive compounds such as lycopene, tocopherols, ascorbic acid, and diverse polyphenols, which contribute antioxidant, anti-inflammatory, and cardioprotective benefits [14, 15]. Therefore, optimizing the growth and biomass accumulation of tomato seedlings through sustainable biostimulant strategies is of significant agricultural and nutritional importance.

Although previous studies have examined the effects of nanoencapsulated essential oils on germination and potential phytotoxicity [6], comprehensive comparative assessments of nanoemulsions derived from different medicinal plant essential oils particularly in relation to their concentration-dependent effects on tomato seedling development remain limited. Addressing this knowledge gap, the present study aimed to evaluate the biostimulant potential of nanoemulsions formulated from selected medicinal plant essential oils on tomato growth, with emphasis on seedling biomass accumulation. Complementary analyses included chemical profiling of the essential oils and detailed physicochemical characterization of the nanoemulsions, focusing on droplet size distribution and zeta potential. We hypothesized that: (i) essential-oil nanoemulsions would produce significant, concentration-dependent improvements in tomato growth; (ii) nanoemulsification would enhance stability and bioavailability while reducing phytotoxicity; and (iii) formulations with smaller droplet sizes and superior physicochemical stability would elicit stronger biostimulant responses compared with less stable formulations.

2 Materials and Methods

2.1 Collection and extraction of essential oils

Medicinal plants were collected from different regions of Iran. Fruits of *Ferula assa-foetida* were harvested from the Khoyeh region, Chaharmahal and Bakhtiari Province (49°49' E; 32°41' N), and *Zataria multiflora* was obtained from the medicinal plant farm of the Faculty of Agriculture, Shiraz University (52°35' E; 29°43' N). The botanical identity of *Ferula assa-foetida* (herbarium no. PM1395) and *Zataria multiflora* (PM1507) was confirmed by taxonomists at the Herbarium of the Pharmacology Department, Shiraz University of Medical Sciences. Fresh fruits of *Anethum graveolens* were obtained from Pakan Seed Company (Isfahan), and fruits of *Cuminum cyminum* and *Pimpinella anisum* were purchased from the Razavi Seed and Seedling Institute (Khorasan Razavi Province). Plant materials were shade-dried, ground into powder, and subjected to hydrodistillation using a Clevenger-type apparatus. Pure analytical-grade compounds including γ-terpinene, α-pinene, 1,8-cineole, cumin aldehyde, carvacrol, limonene, and cinnamaldehyde were purchased from commercial suppliers.

2.2 GC–MS analysis of essential oils

Chemical composition of essential oils was determined using GC–MS (Agilent 7890A/5975C) equipped with a DB-5 silica capillary column (30 m × 0.25 mm, 0.25 μm). The oven temperature program included 60, 220, 260, and 280°C. Helium served as the carrier gas at 30.6 cm s^{-1} with a split ratio of 1:100. Ionization energy was 70 eV, scan time 1 s, and mass range 40–300 m/z. Constituents were identified by comparison with standard mass spectral libraries.

2.3 Preparation of nanoemulsions

Nanoemulsions were prepared by mixing essential oils with Tween 80 and incubating the mixture at 37°C for 12 h. The mixture was then added to 10 mL of double-distilled water under continuous stirring at 900 rpm. After 12 h incubation at 25°C, the volume was adjusted to 50 mL. The final concentration of each nanoemulsion stock was 2000 μL L^{-1} and was diluted to obtain the required treatment concentrations. Fresh solutions were prepared before each foliar application.

2.4 Characterization of nanoemulsions

Particle size (hydrodynamic diameter), polydispersity index (PDI), and zeta potential were measured using a nanoparticle analyzer (SZ-100, Horiba, Japan) equipped with a 532 nm, 10 mW laser. Measurements were performed at room temperature.

2.5 Experimental conditions

The study was conducted in 2023 in the laboratory, growth chamber, and research greenhouse of the Department of Plant Production and Genetics, Faculty of Agriculture, Shiraz University, Bajgah, Iran (52°58' E; 29°72' N; 1810 m a.s.l.). Environmental parameters were controlled throughout the study.

2.6 Plant material and experimental design

Tomato seeds (*Solanum lycopersicum* L.) were obtained from Felat 111 Company and sown in 1-kg pots containing a cocopeat–loam mixture. Three seeds were sown in each pot. After emergence, seedlings were thinned to retain one uniform plant per pot. Plants were grown in a controlled growth chamber at

25 ± 1°C with a 16 h light / 8 h dark photoperiod and regulated relative humidity. The experiment was arranged in a completely randomized design (CRD) with four replications.

2.7 Foliar application of nanoemulsions

Twelve nanoemulsions were prepared using the following essential oils and pure compounds: γ-terpinene, α-pinene, 1,8-cineole, cumin aldehyde, carvacrol, *Pimpinella anisum*, *Ferula assa-foetida, Zataria multiflora, Anethum graveolens, limonene, Cuminum cyminum*, and cinnamaldehyde. Nanoemulsions were applied as foliar sprays at concentrations of 50, 100, 200, and 400 μL L^{-1}. Controls included distilled water and distilled water containing Tween 80. Foliar application was performed at the two-leaf stage by spraying 1 mL of solution on each seedling. Spraying was carried out until the leaf surfaces were uniformly wetted without reaching the runoff point. Plants were harvested two weeks later at the 5–6 leaf stage.

2.8 Measurement of growth parameters

Shoots and roots were separated and dried at 70°C for 72 h until reaching constant weight. Dry weights of shoots, roots, and total biomass were recorded using an analytical balance with a sensitivity of 0.0001 g.

2.9 Statistical analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA). Prior to analysis, data were checked for normality and homogeneity of variance. Analysis of variance (ANOVA) was conducted, and mean comparisons were performed using the least significant difference (LSD) test at the 1% and 5% probability levels. Pearson correlation analysis was used to evaluate relationships among measured traits. Multivariate relationships among variables were further explored using principal component analysis (PCA). Scatter plot matrices, polar heat maps of Pearson correlations, and PCA biplots were generated to visualize relationships among the studied variables. Graphs were prepared using Microsoft Excel 2016.

3 Results

3.1 Chemical composition of essential oils used for nanoemulsion preparation

GC–MS analysis revealed that the main constituents of *Zataria multiflora* essential oil were α-pinene (1.1%), β-myrcene (0.8%), α-terpinene (0.97%), p-cymene (4.5%), γ-terpinene (3.5%), linalool (3.6%), carvacrol methyl ether (1.2%), thymol (27.9%), and carvacrol (50.7%). Minor constituents included β-caryophyllene (2.2%), aromadendrene (0.6%), spathulenol (0.6%), and caryophyllene oxide (0.7%) (Table 1). In *Anethum graveolens* essential oil, carvone (59.2%) and limonene (14.2%) were identified as the dominant compounds. The essential oil of *Pimpinella anisum* was mainly composed of trans-anethole (81.8%). In *Ferula assa-foetida* fruit essential oil, the most abundant constituents were the Z-

and E-isomers of 1-propenyl-sec-butyl disulfide. In addition, the major components of *Cuminum cyminum* fruit essential oil were cumin aldehyde (31.13%), p-cymene (21.71%), and β-pinene (19.00%) (Table 1).

Table 1
Chemical composition Zataria multiflora, Anethum graveolens, Pimpinella anisum, Cuminum Cyminum (CC), Ferula assa-foetida and Cuminum cyminumfruit essential oil.

Compounds name	**RT (min)[a]**	***Zataria multiflora***	***Anethum graveolens***	***Pimpinella anisum***	***Ferula assa-foetida***	***Cuminum cyminum***
α-Pinene	5.704	1.18	1.14			1.3
β-Pinene	6.852				7.93	19
β-Myrcene	6.951	0.85				
Myrcene	6.951		0.85			
α-Terpinene	7.761	0.97	0.85			
p-Cymene	8.047	4.59				21.71
Limonene	8.059		14.22	5.56		
γ-Terpinene	8.99	3.50	2.10			9.79
Linalool	10.14	3.68	1.04			
Terpinolene	12.06					0.55
Z-1-Propenyl-sec-butyl disulfide	12.30				37.15	
E-1-Propenyl-sec-butyl disulfide	12.51				24.42	
Terpinen-4-ol	12.92					0.44
Trans-dihydro carvone	13.25			0.94		
Bis (1-methylpropyl) disulfide	13.32				1.45	
Myrtenol	13.74					0.83
Carvacrol methyl ether	13.99	1.21				
Carvone	15.48		59.20	6.94		
Cumin aldehyde	14.65					31.13
α-Terpinen-7-al	15.68					3.66
γ-Terpinen-7-al	15.86					11.21
CIS-Dihydro carvone	15.89		6.30			

Compounds name	RT (min)[a]	*Zataria multiflora*	*Anethum graveolens*	*Pimpinella anisum*	*Ferula assa-foetida*	*Cuminum cyminum*
Thymol	15.99	27.99				
trans-Dihydro carvone	15.99		7.45			
Trans-Anethole	16.03			81.82		
Carvacrol	16.44	50.79				
β-Caryophyllene	18.92	2.251	1.03			
Aromadendrene	19.38	0.62				
β-Selinene	19.54				0.84	
γ-Himachalene	20.47			1.98		
α-Zingibirene	20.68			0.76		
β-Dihydo agarofuran	21.18				5.23	
Spathulenol	22.99	0.67				
Caryophyllene oxide	23.07	0.75				
Dill apiole	23.09		4.00			
α-Eudesmol	23.69				1.23	
δ-Selinene	24.10				6.86	
Valencene	24.36				1.87	
β-Cadinene	24.63				1.67	
Guaiol	24.79				1.52	

Table 2
Mean droplet size, polydispersity index, and zeta potential of nanoemulsions prepared from Zataria multiflora, Anethum graveolens, Pimpinella anisum, Cuminum Cyminum (CC), Ferula assa-foetida and Cuminum cyminumfruit essential oils.

EO	Size (nm)	Polydispersity Index (PDI)	Zeta Potential (mV)
Zataria multiflora	158.575 ± 24.16	0.58 ± 0.15	-50.15 ± 1.05
Cuminum cyminum	369.05 ± 13.71	0.46 ± 0.06	-51.00 ± 0.08
Pimpinella anisum	205.4 ± 18.06	0.46 ± 0.11	-52.13 ± 1.68
Anethum graveolens	229.475 ± 13.61	0.48 ± 0.06	-41.00 ± 1.28
Ferula assa-foetida	267.4 ± 0.51	0.51 ± 0.09	-48.7 ± 1.37
Cinnamaldehyde	290.125 ± 101.45	0.42 ± 0.91	-50.40 ± 1.87
Cumin	226.5 ± 20.69	0.45 ± 0.10	-32.10 ± 1.12
α-Pinene	334.8 ± 194.31	0.31 ± 0.58	-37.68 ± 2.95
1,8-Cineole	370.1 ± 25.68	0.46 ± 0.08	-45.08 ± 1.53
Carvacrol	248.775 ± 46.05	0.68 ± 0.15	-42.28 ± 1.27
γ-Terpinene	151.875 ± 8.62	0.55 ± 0.08	-48.90 ± 2.14
Limonene	110.825 ± 17.75	1.33 ± 0.56	-23.75 ± 1.97

3.2 Physicochemical properties of nanoemulsions

The structural characteristics of the prepared nanoemulsions are presented in Table 2. The mean droplet size ranged from 110.82 to 370.10 nm. The largest droplet size was observed in the 1,8-cineole nanoemulsion, whereas the smallest droplet size was recorded for the limonene nanoemulsion. The polydispersity index (PDI) varied between 0.31 and 1.33. The highest PDI value was associated with limonene nanoemulsion, while the lowest value was observed for α-pinene nanoemulsion. Zeta potential values ranged from − 52.1 to − 23.7 mV. The highest negative zeta potential was recorded for the limonene nanoemulsion, whereas the lowest value was observed for the *Pimpinella anisum* nanoemulsion (Table 2).

3.3 Effects of essential-oil nanoemulsions on tomato growth parameters

Application of essential-oil nanoemulsions significantly affected tomato growth parameters, including shoot dry weight, root dry weight, total dry weight, shoot-to-root ratio, and growth index (Table 3).

Table 3
Results of analysis of variance (ANOVA) for the effects of essential oils on growth parameters of tomato.

Treatment	df	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Total dry weight (g plant^{-1})	Shoot/Root	Growth index
	49	**0.0012****	**0.0006****	**0.0024****	**0.7166****	**0.2163****
Error	150	0.0003	0.0001	0.0006	0.1684	0.0579
CV (%)		19.31697	18.21175	16.02482	21.69779	27.69447

* and **: significant at $p \leq 0.05$ and $p \leq 0.01$, respectively.

Shoot dry weight showed different responses depending on the type and concentration of nanoemulsion. The nanoemulsion of 1,8-cineole caused a concentration-dependent increase in shoot dry weight, with approximately 82% enhancement at 400 µL L^{-1} compared with the control. The nanoemulsion of *Anethum graveolens* also increased shoot dry weight at concentrations of 50 and 200 µL L^{-1}. Similarly, cinnamaldehyde nanoemulsion significantly increased shoot dry weight at 100 and 400 µL L^{-1}. In contrast, limonene and γ-terpinene nanoemulsions showed a concentration-dependent inhibitory effect on shoot dry weight. Among the components of *Zataria multiflora* essential oil, thymol application resulted in a reduction in shoot dry weight with increasing concentration (Fig. 1).

The analysis of variance showed that nanoemulsion treatments significantly influenced root dry weight. The highest root dry weight values were observed in treatments containing *Pimpinella anisum, Cuminum cyminum*, and *Ferula assa-foetida* essential oils, with values of 0.0938, 0.0934, and 0.0869 g plant^{-1} at concentrations of 400, 200, and 100 µL L^{-1}, respectively. Graphical analysis indicated that root dry weight increased by approximately 40% with increasing concentration of the 1,8-cineole nanoemulsion. Moreover, treatments with *Ferula assa-foetida* (100 µL L^{-1}), *Pimpinella anisum* (200 µL L^{-1}), and *Cuminum cyminum* (400 µL L^{-1}) produced significantly higher root dry weight compared with the control (Fig. 2). In most essential oils, except cumin aldehyde, *Pimpinella anisum*, and 1,8-cineole, increasing the concentration from 50 to 400 µL L^{-1} resulted in a decrease in root dry weight.

Total plant dry weight was also significantly affected by nanoemulsion treatments. The highest biomass accumulation was observed in plants treated with *Ferula assa-foetida* nanoemulsion at 100 µL L^{-1} (0.207 g) and 1,8-cineole nanoemulsion at 400 µL L^{-1} (0.206 g). Conversely, the application of 400 µL L^{-1} nanoemulsions of *Zataria multiflora*, *Anethum graveolens*, *Cuminum cyminum*, γ-terpinene, and limonene resulted in the lowest total dry weights. However, nanoemulsions of cinnamaldehyde, *Pimpinella anisum*, 1,8-cineole, α-pinene, and carvacrol at 400 µL L^{-1} increased total dry weight by 68.2%, 66.3%, 92.5%, 70.1%, and 60.7%, respectively, compared with the water control (Fig. 3).

The shoot-to-root ratio was significantly affected by nanoemulsion concentration at the 5% probability level ($p \leq 0.05$). The highest value (2.79) was observed in the *Ferula assa-foetida* nanoemulsion at 50 µL L^{-1}. In most treatments, increasing concentration from 50 to 400 µL L^{-1} resulted in a reduction in the shoot-to-root ratio. In contrast, in *Cuminum cyminum*, 1,8-cineole, α-pinene, and carvacrol nanoemulsions, increasing the concentration from 50 to 400 µL L^{-1} increased this ratio by 19.73%, 28.8%, 48%, and 14.6%, respectively (Fig. 4).

Nanoemulsion treatments also significantly influenced the growth index ($p \leq 0.05$). The highest value was observed for *Ferula assa-foetida* nanoemulsion at 100 µL L^{-1}. The treatment with 1,8-cineole at 400 µL L^{-1} ranked next and was not significantly different from the highest treatment. These treatments increased the growth index by approximately 2.3- and 2.2-fold compared with the water and Tween controls (Fig. 5).

3.4 Pearson correlation and principal component analysis

Scatter plot matrix and polar heat map analyses revealed strong positive correlations between growth index and shoot dry weight, root dry weight, and total dry weight. In contrast, the growth index showed a negative correlation with the shoot-to-root ratio (Fig. 6a,b).

Principal component analysis showed that the first two principal components explained 99.20% of the total variance, with PC1 and PC2 accounting for 60.98% and 38.22%, respectively. In the PCA biplot, nanoemulsions of α-pinene, 1,8-cineole, and *Ferula assa-foetida* were located in the upper-right quadrant, where total dry weight and growth-related indices had the highest loadings, indicating a strong association with enhanced plant growth. Cinnamaldehyde and *Anethum graveolens* were positioned in the upper-left quadrant and were more closely associated with shoot dry weight and shoot-to-root ratio. In contrast, γ-terpinene and *Zataria multiflora* were distributed in the lower-right quadrant, near root dry weight, indicating a stronger relationship with root biomass development (Fig. 6c).

4 Discussion

The present study demonstrates that nanoemulsions derived from medicinal plant essential oils exert differential and concentration-dependent effects on tomato growth, biomass allocation, and overall plant performance. These varied responses are closely linked to both the chemical composition of the essential oils and the physicochemical properties of the corresponding nanoemulsions, such as droplet size, polydispersity index (PDI), and zeta potential.

The differential effects observed on tomato growth can be attributed to the distinct chemical profiles of the tested essential oils, as revealed by GC–MS analysis. Phenolic monoterpenes such as thymol and carvacrol dominate in *Zataria multiflora*, carvone in *Anethum graveolens*, trans-anethole in *Pimpinella anisum*, sulfur-containing disulfides in *Ferula assa-foetida*, and cuminaldehyde and p-cymene in *Cuminum cyminum*. These bioactive constituents possess well-documented antimicrobial and allelopathic

properties. Such properties may explain the contrasting stimulatory and inhibitory effects observed on plant growth parameters [16–18].

Nanoemulsions containing 1,8-cineole and *Ferula assa-foetida* consistently enhanced biomass accumulation and growth index values, indicating a biostimulatory effect at optimal concentrations. Moderate increases in shoot and root biomass might result from improved nutrient uptake, enhanced membrane permeability, or modulation of phytohormonal balance induced by low concentrations of terpenoid compounds. Conversely, nanoemulsions rich in limonene and γ-terpinene exhibited concentration-dependent growth inhibition. Such inhibition may be due to membrane disruption, oxidative stress induction, or interference with cellular metabolism at higher doses. Similarly, increasing thymol concentration led to reduced shoot dry weight, supporting previous findings that phenolic monoterpenes can exert phytotoxic effects at elevated levels [9, 11].

The droplet size range (110–370 nm) and zeta potential values (− 23.7 to − 52.1 mV) indicate that the prepared nanoemulsions were relatively stable. Although smaller droplet sizes, as observed in the limonene nanoemulsion, theoretically enhance surface area and bioavailability, this may also intensify phytotoxic effects at higher concentrations. On the other hand, higher absolute zeta potential values (e.g., in *Cuminum cyminum*) suggest improved colloidal stability, potentially facilitating sustained interaction with leaf surfaces and controlled release of active compounds. Therefore, plant growth responses likely reflect a delicate balance between delivery efficiency and possible phytotoxicity.

The significant effects observed on the shoot-to-root ratio indicate that nanoemulsions influenced biomass partitioning. In most treatments, increasing the concentration reduced the shoot-to-root ratio, suggesting preferential root development under moderate stress conditions. This adaptive response may help plants allocate more resources to roots, optimizing water and nutrient uptake [17, 18]. The positive correlation between growth index and root dry weight further supports this interpretation. However, some nanoemulsions (e.g., *Cuminum cyminum*, 1,8-cineole, α-pinene, and carvacrol) demonstrated an increased shoot-to-root ratio at higher concentrations, indicating differential regulation of biomass allocation. Such variability implies that individual essential oil components may differently interact with hormonal pathways governing root and shoot development [19, 20, 21].

Strong positive correlations between the growth index and total, shoot, and root dry weights confirm that biomass accumulation is the principal factor driving improved plant performance. The negative association between growth index and shoot-to-root ratio suggests that treatments enhancing overall growth are accompanied by proportionally greater root biomass allocation, possibly improving resource acquisition efficiency.

Principal component analysis further illustrated treatment-specific growth patterns. Nanoemulsions of α-pinene, 1,8-cineole, and *Ferula assa-foetida* were closely associated with total dry weight and growth-related indices, underscoring their potential as effective biostimulants. Conversely, cinnamaldehyde and *Anethum graveolens* were more related to shoot biomass characteristics, while γ-terpinene and *Zataria multiflora* clustered with root development. These distinct patterns indicate that essential oil

nanoemulsions modulate specific growth components depending on their chemical makeup and concentration.

Collectively, these findings suggest that nanoemulsions from medicinal plant essential oils can act either as growth promoters or inhibitors depending on their composition and applied concentration. Low to moderate concentrations of certain nanoemulsions, particularly those from *Ferula assa-foetida* and 1,8-cineole, enhanced tomato growth and biomass accumulation, highlighting their potential as plant biostimulants. Conversely, higher concentrations of monoterpene-rich nanoemulsions such as limonene and γ-terpinene may induce phytotoxic effects, limiting their practical agricultural application.

5 Conclusion

The present study demonstrates that nanoemulsions derived from medicinal plant essential oils significantly affect tomato growth, biomass production, and biomass allocation patterns in a concentration-dependent manner. The results clearly show that both the chemical composition of essential oils and the physicochemical characteristics of their nanoemulsions determine the magnitude and direction of plant responses. Among the tested treatments, nanoemulsions based on *Ferula assa-foetida* and 1,8-cineole exhibited the most pronounced positive effects on total dry weight and growth index, highlighting their potential as effective plant growth-promoting formulations. In contrast, nanoemulsions rich in limonene and γ-terpinene caused inhibitory effects at higher concentrations, emphasizing the critical need for dose optimization. Strong positive correlations between growth index and biomass parameters, along with principal component analysis results, confirmed that enhanced plant performance was primarily associated with increased total and root dry weight. Furthermore, changes in the shoot-to-root ratio suggest that certain nanoemulsions may influence biomass partitioning, potentially improving resource acquisition efficiency. Overall, these findings indicate that essential oil nanoemulsions could serve as promising biostimulant candidates under controlled conditions when properly formulated and applied at optimal concentrations. However, application at inappropriate doses may result in phytotoxic effects. Therefore, further research into their physiological and molecular mechanisms, as well as validation at field scale, is recommended to support their sustainable use in agricultural systems.

Declarations

Author contributions

Mohammad Hassani: conceptualization, data curation, formal analysis and methodology. Seyed Abdolreza Kazemeini: project administration, conceptualization, data curation, formal analysis and methodology, visualization, writing original draft. Mozhgan Alinia: literature search, data curation, formal analysis and methodology, writing original draft, writing-review and editing. Mohammad Hamidian: conceptualization. Behnam Asgari Lajayer: writing-review and editing. All authors have read and agreed to the published version of the manuscript.

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Figures

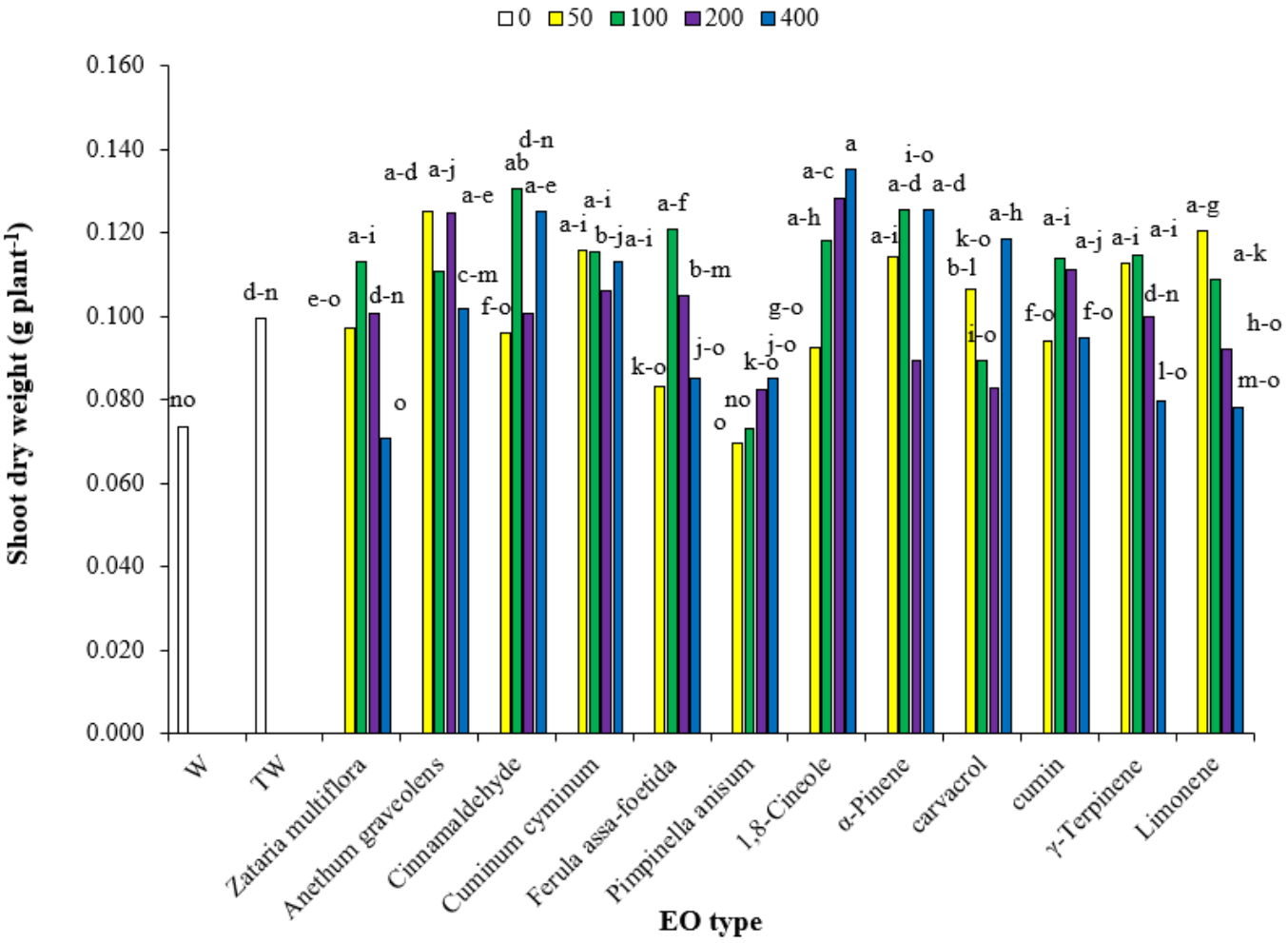

Figure 1

The effect of different concentrations (50, 100, 200 and 400 µL L^{-1}) of twelve nanoemulsions prepared from pure essential oil compounds (γ-Terpinene, α-Pinene, 1,8-Cineole, Cumin, Carvacrol, Limonene and Cinnamaldehyde) and Medicinal Plants (*Pimpinella anisum*, *Ferula assa-foetida*, *Zataria multiflora*, *Anethum graveolens* and *Cuminum cyminum*) on shoot dry weight of tomato. Data are presented as the means of four replicates. Different letters in the columns show significant differences ($P < 0.05$) according to LSD test.

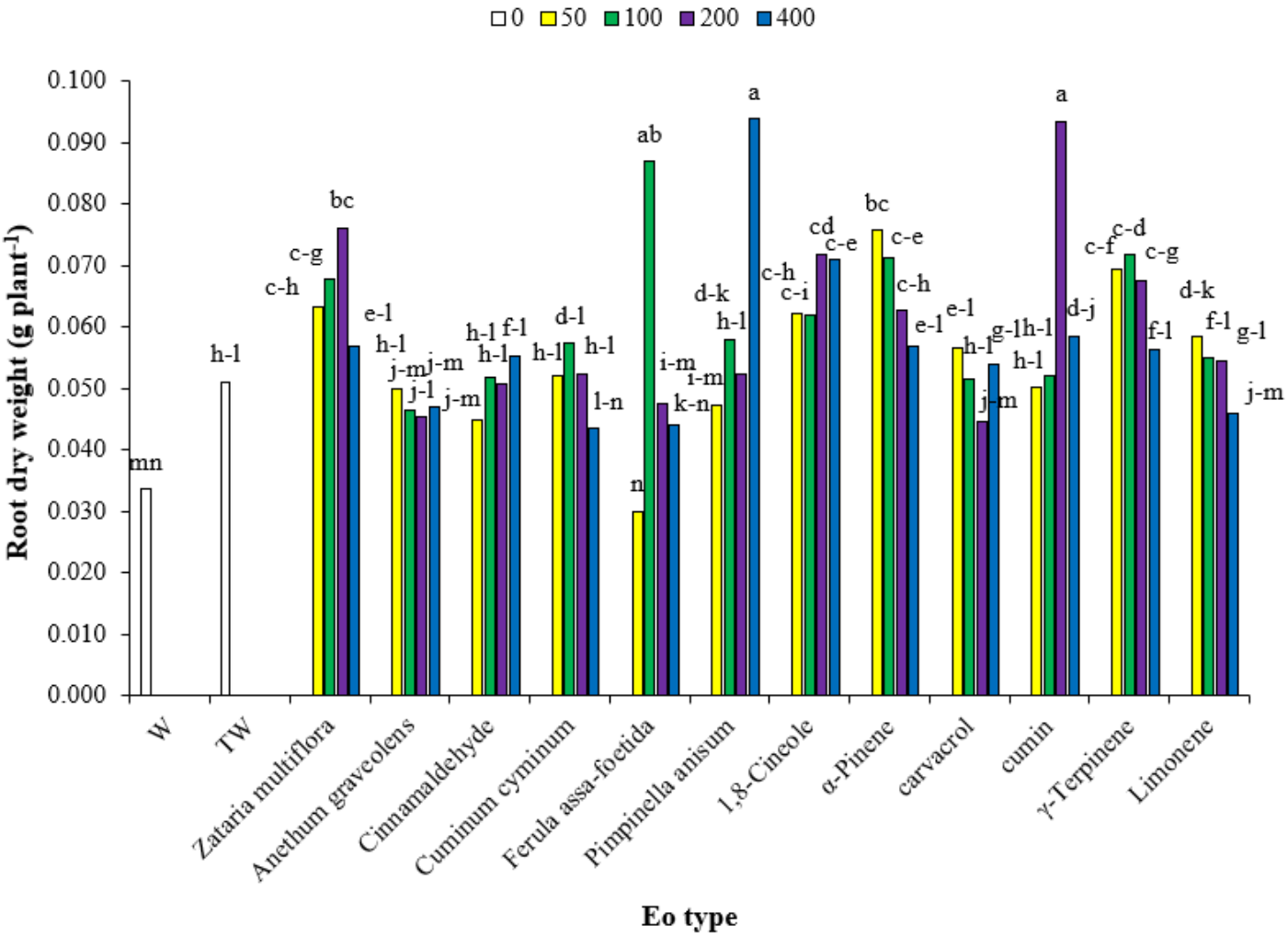

Figure 2

The effect of different concentrations (50, 100, 200 and 400 µL L^{-1}) of twelve nanoemulsions prepared from pure essential oil compounds (γ-Terpinene, α-Pinene, 1,8-Cineole, Cumin, Carvacrol, Limonene and Cinnamaldehyde) and Medicinal Plants (*Pimpinella anisum*, *Ferula assa-foetida*, *Zataria multiflora*, *Anethum graveolens* and *Cuminum cyminum*) on root dry weight of tomato. Data are presented as the means of four replicates. Different letters in the columns show significant differences ($P < 0.05$) according to LSD test.

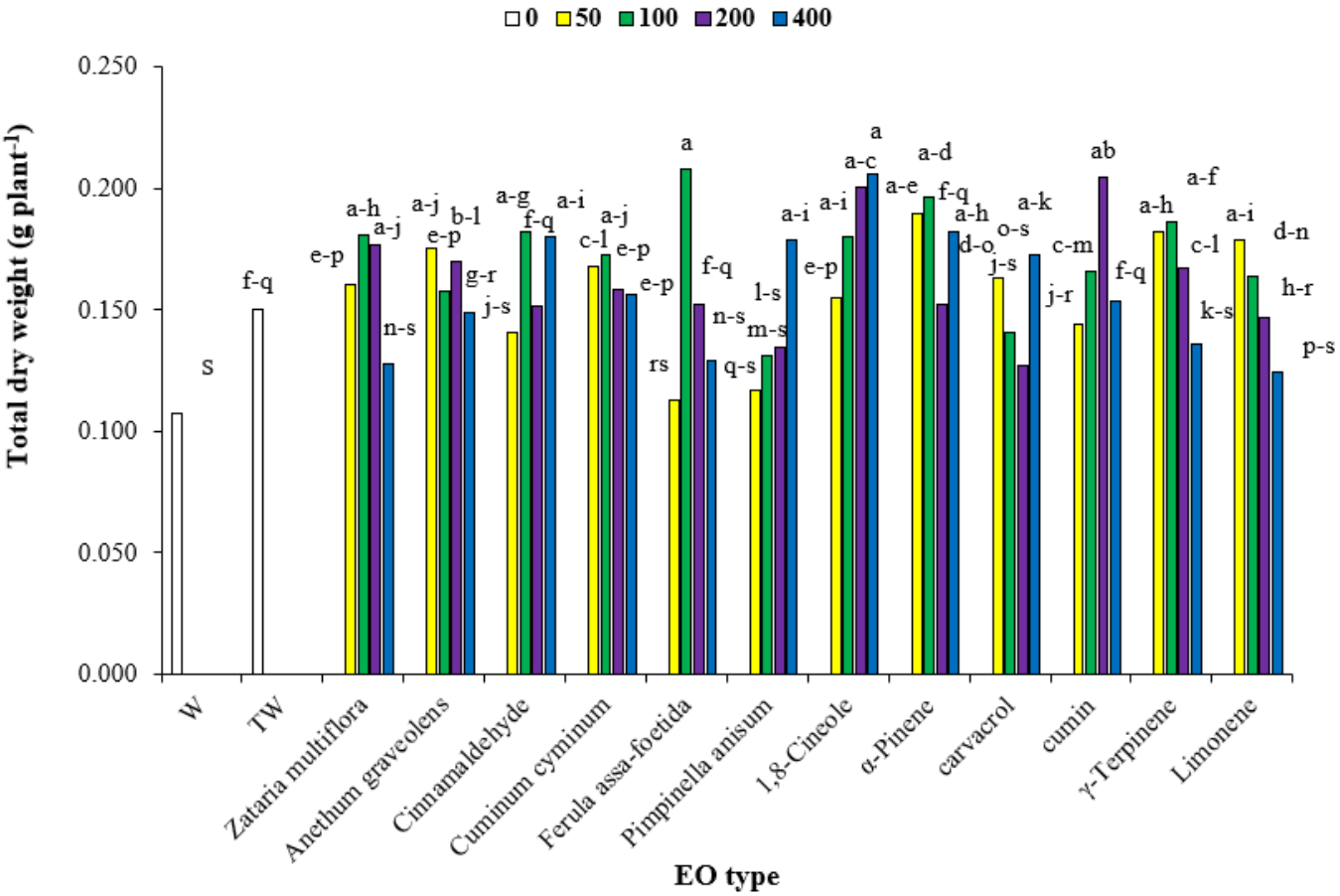

Figure 3

The effect of different concentrations (50, 100, 200 and 400 µL L^{-1}) of twelve nanoemulsions prepared from pure essential oil compounds (γ-Terpinene, α-Pinene, 1,8-Cineole, Cumin, Carvacrol, Limonene and Cinnamaldehyde) and Medicinal Plants (*Pimpinella anisum*, *Ferula assa-foetida*, *Zataria multiflora*, *Anethum graveolens* and *Cuminum cyminum*) on total dry weight of tomato. Data are presented as the means of four replicates. Different letters in the columns show significant differences ($P < 0.05$) according to LSD test.

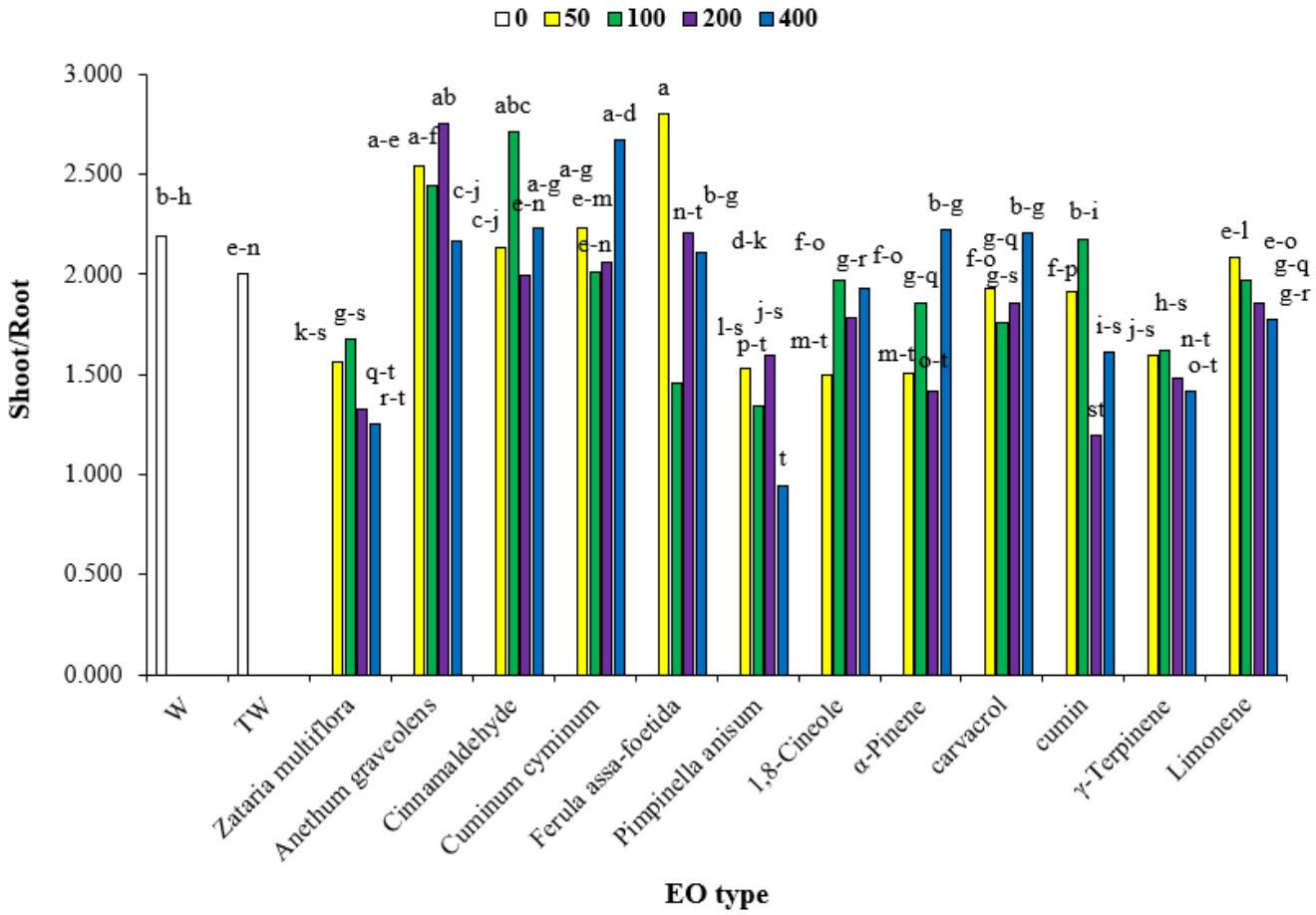

Figure 4

The effect of different concentrations (50, 100, 200 and 400 µL L^{-1}) of twelve nanoemulsions prepared from pure essential oil compounds (γ-Terpinene, 1,8-Cineole, α-Pinene, Carvacrol, Cumin, Limonene and Cinnamaldehyde) and Medicinal Plants (*Pimpinella anisum*, *Ferula assa-foetida*, *Zataria multiflora*, *Anethum graveolens* and *Cuminum cyminum*) on shoot/root ratio of tomato. Data are presented as the means of four replicates. Different letters in the columns show significant differences ($P < 0.05$) according to LSD test.

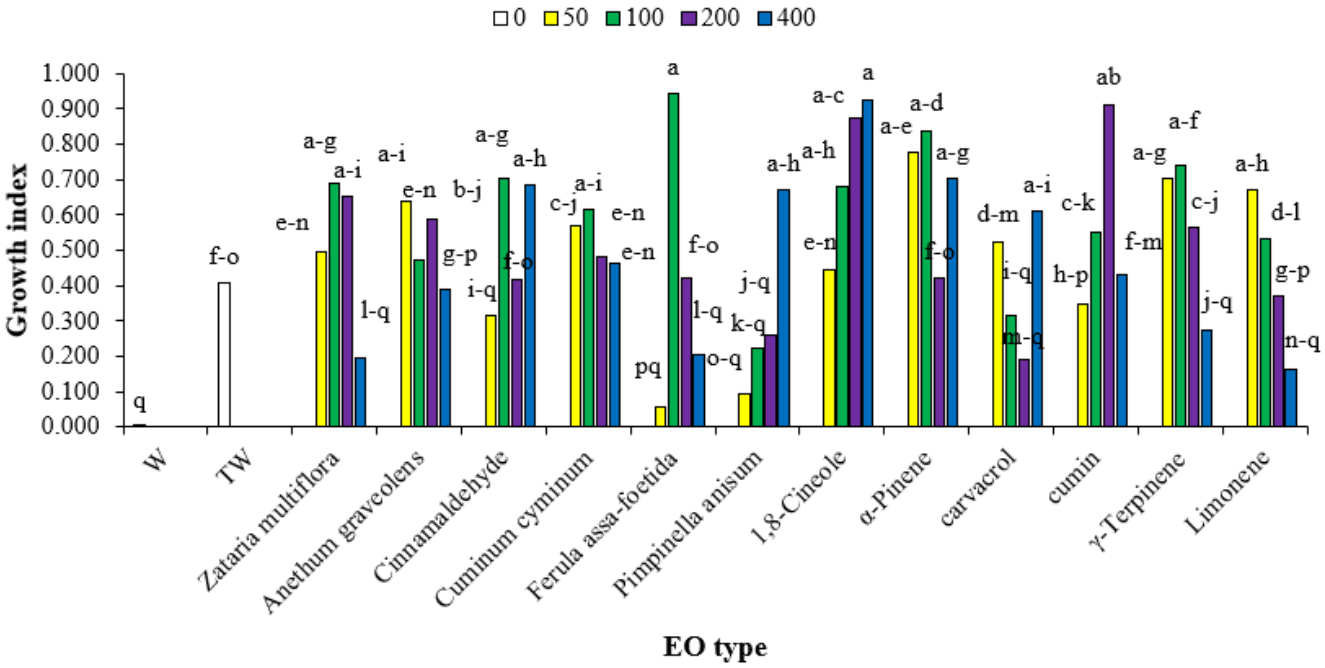

Figure 5

The effect of different concentrations (50, 100, 200 and 400 µL L^{-1}) of twelve nanoemulsions prepared from pure essential oil compounds (γ-Terpinene, α-Pinene, 1,8-Cineole, Cumin, Carvacrol, Limonene and Cinnamaldehyde) and Medicinal Plants (*Pimpinella anisum*, *Ferula assa-foetida*, *Zataria multiflora*, *Anethum graveolens* and *Cuminum cyminum*) on growth index of tomato. Data are presented as the means of four replicates. Different letters in the columns show significant differences ($P < 0.05$) according to LSD test.

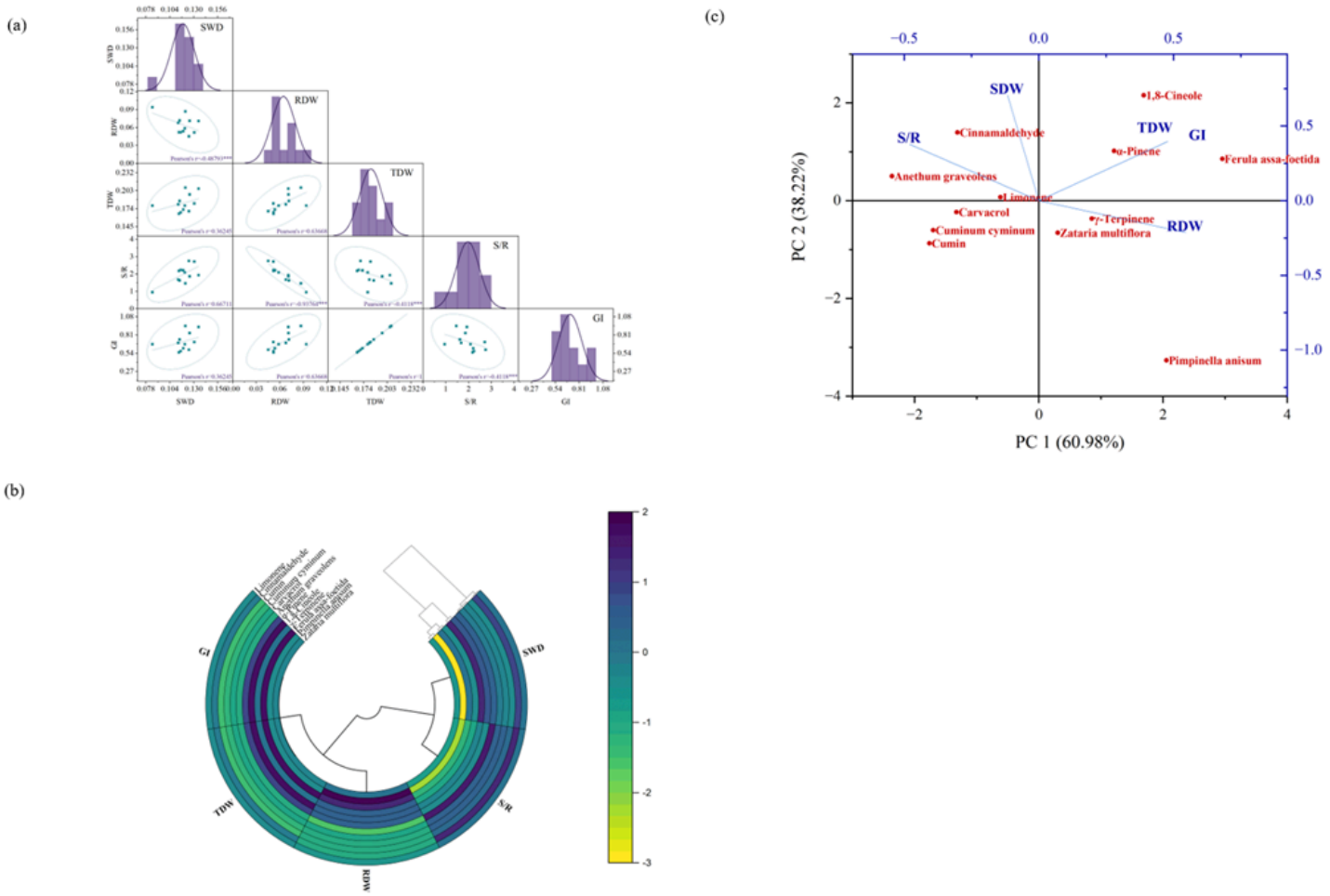

Figure 6

A scatter plot matrix (a), polar heat map of the Pearson correlations (b) and an analysis PCA-Biplot (c) of the principal component analysis for effect of twelve nanoemulsions prepared from pure essential oil compounds (γ-Terpinene, α-Pinene, 1,8-Cineole, Cuminaldehyde, Carvacrol, Limonene and Cinnamaldehyde) and Medicinal Plants (*Pimpinella anisum*, *Ferula assa-foetida*, *Zataria multiflora*, *Anethum graveolens* and *Cuminum cyminum*) on growth parameters of tomato. SDW: Shoot dry weight, RDW: Root dry weight, TDW: Total dry weight, GI: Growth index, S/R: Shoot/root ratio.