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Original Article

The Effect of Nano-complex Salicylic Acid-zinc on Vegetative and Phytochemical Characteristics of *Thymus daenensis* Celak.

Amir Reza Tavakoli[1], Abdolhossein Aboutalebi Jahromi[1*], Vahid Rowshan Sarvestani[2] and Abdolrasoul Zakerin[1]

[1] *Jahrom Branch, Islamic Azad University, Jahrom, Iran*
[2] *Fars Agricultural Research Center, Agricultural Research, Education and Extension Organization (AREEO), Shiraz, Iran*

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ABSTRACT

A study was conducted on the effect of nano-complex salicylic acid-zinc on the physiological and biochemical characteristics of *Thymus Daenensis* Celak. The treatments were applied in two stages, 15 days apart, after the establishment, in May as spraying at zero, 0.1, 0.3, and 0.5% (v/v). Based on the results, different levels of nano-complex salicylic acid-zinc led to a change in the vegetative growth indices studied, percentage of essential oil compounds, polyphenolic content, and antioxidant activity. The most important compounds in the essential oil were reported in the control treatment: Carvacrol, Thymol, p-cymene, Borneol, and 1.8-cineole. A 0.5% solution of nano-complex salicylic acid-zinc increased the amount of Carvacrol in essential oil by 80.7% compared to the control. The amount and type of polyphenolic compounds were measured with HPLC. The most important compounds included rosmarinic acid, Thymol, Carvacrol, and Hesperetin. The best treatment for increasing polyphenols was concentration of 0.5% of nano-complex salicylic acid-zinc.

INTRODUCTION

The family Lamiaceae is one of the most diverse and widespread plant families in the study of medicinal plants, and their medicinal importance is due to their essential oil [1]. Thymes are fragrant and medicinal plants from the family Lamiaceae, which have antimicrobial, antifungal, and antioxidant effects. They are widely used in various parts of the world as a drink, food flavoring (spices), and herbal medicine, and are very significant due to their high essential oil yield and valuable phenolic compounds, especially Thymol and Carvacrol. *T. vulgaris* L. and *T. daenensis* Celak are two medicinal species of this family, which have medicinal uses in Iran [2].

Salicylic acid is a new class of plant growth regulators found in all plant dynasties and is effective in many physiological processes in plants at low concentrations. Salicylic acid has been shown to regulate disease resistance and heat production in plants in some cases. Today, salicylic acid is widely known in various forms in plants, and some consider it as one of the plant growth materials [3]. Salicylic acid induces the flowering process, prolongs flower life, delays aging, and speeds up cellular metabolic processes. Maintaining salicylic acid levels may be a prerequisite for the synthesis of auxin or cytokinin. Depending on the plant species, concentration, stages of plant development, and environmental conditions, salicylic acid increases some physiological processes and stops others [4]. The use of salicylic acid in sweet-scented geraniums, peppers, mint, pennyroyal and spikenard has no increase in the amount of essential oil, but it has a positive effect on the quality of the essential oil by reducing the level of toxic compounds in the essential oil [5]. Salicylic acid also has a positive effect on plant vegetative growth. Studies have shown that foliar application of salicylic acid at concentrations of 1, 10 and 100 μM in basil and marjoram increases the number of branches, leaves, and knots of plants, plant height, leaf area, leaf weight, fresh and dry weight of plants, carbohydrates, protein raw materials, amino acids,

*Corresponding author: Jahrom Branch, Islamic Azad University, Jahrom, Iran
Email Address: aa84607@gmail.com

free proline, photosynthetic pigments and levels of micro-elements and reduces the amount of putrescine, spermidine and harmful polyamines [6]. Salicylic acid has also been reported to increase the active ingredient of anthraquinone in tissue culture media in *Oldenlandia diffusa* [7].

The response of medicinal plants to environmental factors including heavy metals, micro- and macro-elements and how their metabolic and physiological changes have always been of interest to researchers. Zinc is an essential micro-element that is also classified as heavy metal and has many structural and functional roles in plant metabolic processes, but both its deficiency and excess amount in soils cause metabolic disorders and inhibit growth in most plant species [8].

Although micro-elements are needed by plants in small amounts, they play a prominent role in plant growth and development [9]. The results of several studies indicate the effect of micro-elements and heavy metals on the quantitative and qualitative performance of medicinal plants [10,11]. The use of fertilizers to improve soil fertility and plant production, regardless of the type of cultivation or environmental conditions, is inevitable. It has been reported that about 50 percent of the increase in crop production has been due to the use of fertilizers in agriculture. In the last four decades, despite many efforts, the efficiency of the use of nutrients in agricultural products, which is the ratio of kilograms of plant dry matter per kilogram of nutrients consumed, has remained constant [12]. Furthermore, in order to maintain the current level of agricultural production, the application of various conventional fertilizers at a high level and for a long time in the agricultural sector has created serious environmental problems at the global level [13]. In the field of sustainable agriculture (production of agricultural products with appropriate quality and quantity without damaging the environment forever), the application of nanotechnology as one of the promising ways to significantly increase the production of food needed by the population is rapidly growing in the world [14]. The diameter of the pores of plant cell walls is in the range of 5 to 20 nm; therefore, nanometer-sized structures are important in many aspects of plant biology [15]. Nano-fertilizers are carriers of nutrients in dimensions of 3 to 40 nanometers and have the ability to properly transport the ions of nutrients at a dimension of 9 to 40 nanometers due to their high specific surface area [16]. The use of nanotechnology in the production of fertilizers may lead to optimal release and increase the efficiency of nutrient uptake in fertilizers, resulting in significant economic and environmental benefits [13].

Since the plant used in this research is an important agricultural product that is important in terms of essential oils and medicinal compounds, and on the other hand, for improving the cultivation of this plant and determining its nutritional issues, the effect of nano-complex salicylic acid-zinc on the vegetative properties and the level of compounds of *T. daenensis* Celak. were studied.

MATERIAL AND METHODS

The experiment was carried out using a randomized complete block design with three replications on a farm at Agricultural and Natural Resources Research and Education Center in Shiraz City, Fars Province, Iran, at an average height of about 1486 meters above sea level, an average rainfall of 337 mm and an average temperature of 18 Celsius degrees. The seeds were prepared from the mentioned center and planted in a greenhouse in an equal mixture of soil, sand, and rotted manure in early February of 2018. In the 6- to 8-leaf stage, 5 plants for each plot, in late March, they were transferred to the field in plots of 1.5 × 3 meters at 30cm spacing on rows, 50cm apart, and they remained in the field until the time of their exploitation and the necessary care was taken.

Table 1 Results of physical and chemical analysis of experimental farm soil before the experiment

Parameter	Unite	value	Parameter	Unite	value
Clay	%	28.3	Potassium (K)	mg/kg	499
Silt	%	45.1	Phosphorous (P)	mg/kg	15.4
Sand	%	31.6	Nitrogen (N)	%	0.07
Cooper (Cu)	mg/kg	1.52	Organic carbon (OC)	%	0.68
Manganese (Mn)	mg/kg	19.8	pH	-	7.52
Zinc (Zn)	mg/kg	1.55	Electrical Conductivity (EC)	Ds/m	7.6
Iron (Fe)	mg/kg	7.6	-	-	-

The soil texture was a loamy clay soil and its pH was 7.3. Table 1 shows some of the physical and chemical properties of the soil at the test site. The foliar application was started in late June in two stages, 15 days apart, after the establishment and sufficient vegetative growth of plants.

The levels of the fertilizer used were zero, 0.1, 0.3, and 0.5% (v/v) of nano-complex salicylic acid-zinc, both before flowering and at the full-flower stage in three replications. To prevent sunburn and its destructive effect on the nano-complex, spraying was done at sunset. The plants were harvested at the flowering stage. Nano-complex salicylic acid-zinc (n [$Zn(SA)_2$] were purchased from "Zist Nano Fanavaran Atie Pajooh" company in Fars Science and Technology Park, Iran.

Essential Oil Extraction and Measurement of Essential Oil Content

Immediately after harvesting, the plants of each replication were dried at room temperature, the weighing and their dry weight were calculated until they reached a constant weight [17]. The essential oil was extracted by hydro-distillation using an all-glass Clevenger type apparatus. Extraction was performed in three replications for three hours. Resulting essential oil was kept in a dark-colored container at 4 °C after dewatering. Quantitative analysis was performed with GC by integrating the area under the curve by normalizing the surface and by device software. Qualitative analysis and identification of compounds in GC was performed by calculating the quartz index and matching the results with the results of the analysis with the GC /MS. The standard is not used in the identification of compounds. In the analysis of essential oil compositions with GC and GC/MS and in the absence of standard compounds, there is no need to draw a calibration curve and calculate the relevant competency figures. The extracts obtained by soaking in solvent were injected into the HPLC machine. The analysis was performed by external standard method and with the standards of polyphenol compounds in HPLC analysis.

To measure the amount of essential oil, first, the essential oil collection bottles were weighed up to two decimal places by a digital scale and then weighed again after collecting the essential oil in the bottles. Their weight difference showed the amount of essential oil by weight, with which a simple proportion, the amount of essential oil was measured as weight percent.

Features of the Devices used

Gas Chromatography Device

Gas chromatography device (Agilent Technologies A7890), HP-5 column at a length of 30m and a diameter of 0.32mm, stationary phase layer thickness of 0.25µm, column temperature programming from 60 to 210 °C with an increase temperature by 3 °C per minute, detector type: FID at 290 °C, carrier gas: nitrogen at a rate of one milliliter per minute, injection temperature of 280°C

Gas Chromatography-mass Spectrometry

Gas chromatography-mass spectrometry (Agilent Technologies A5975), HP-5MS column at a length of 30 meters and a diameter of 0.25mm, stationary phase layer thickness of 0.25µm, column temperature programming from 60 to 210 °C with an increase in temperature by 3 °C per minute, and 210 to 240 with an increase in temperature by 20 °C per minute, injection chamber temperature: 280 °C, ionization energy: 70 electron volts, carrier gas: helium.

Identifying the Constituent Compounds of Essential Oils

The percentage of the constituent compounds of each essential oil after separation was calculated along with the retention index. Identification of VCs was based on the calculation of their retention indices under temperature-programmed conditions for n-alkanes (C8-C25) and the oil on a HP-5 column under the same chromatographic conditions. Further identification was made by matching their recorded mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature [18].

Essential oil Extraction and Measurement of Phenolic Compounds

The procedure for phenolic compounds determination was carried out according to the modified method established by Justesen *et al* [19]. HPLC analysis was carried out on an Agilent 1200 series, equipped with a Zorbax Eclipse XDB-C18 column (4.6 × 5 µm i.d.; × 150 mm film thickness, RP), and a photodiode array detector (PDA). Elution was monitored at 280 and 320nm. The gradient elution program was selected at an elution speed of 1ml per minute and an injection volume of 20 µl. The gradient mobile phase included 1% methanol and 1% formic acid.

Method of Making a Nano-complex of Salicylic Acid and Zinc

Salicylic acid (2 mM) was dissolved in water (5ml) at 25 °C Zn $(OAc)_2$ (1 mM) in 2ml of distilled water was added to this solution. This mixture was prepared using an ultrasonic device with a special probe. The total chemical reaction was performed under a constant range of 90% for 30mins. Ultrasonic irradiation was performed without any cooler, so that during the reaction the temperature reached about 40 °C. The mixture was cooled to room temperature to allow the solvent to evaporate. After placing the mixture in the vicinity of the air, the resulting bright yellow powder was dried and then kept in a vacuum for 18 hours.

Statistical analysis

Data analysis was performed using SAS and Excel software. The means obtained after analysis of variance were compared by LSD test at $P < 0.05$.

RESULTS

Investigating vegetative traits

Table 2 shows the results of analysis of the variance of the effect of foliar application of nano-complex salicylic acid-zinc on the vegetative traits of *T. daenensis* Celak. Based on the results, the effect of foliar application of nano-complex salicylic acid-zinc on plant height, fresh weight, dry weight, and the ratio of fresh weight to dry weight was significant at $P < 0.05$, and on inflorescence height, leaf length and leaf width was significant at $P < 0.01$ and it had no significant effect on the number of branches per the plant.

All vegetative traits measured increased to 0.3% fertilizer level and then decreased. The foliar application of nano-complex salicylic acid-zinc at 0.3% level increased plant height, fresh weight, dry weight, inflorescence height, leaf length, leaf width and the ratio of fresh weight to dry weight, compared to the control without foliar application (32.5, 26.7, 19.2, 38.3, 22.2, and 27.08%, respectively) (Table 3).

Essential Oil Compounds

Table 4 shows the results of the effect of different levels of nano-complex salicylic acid-zinc on the level and type of essential oil compounds in *T. daenensis* Celak. The study of compounds showed a maximum of 42 compounds of essential oils in *T. daenensis* under different treatments of a nano-complex of salicylic acid and zinc, but in the essential oil of the control plant, 38 different compounds accounted for 92.8% of all known essential oil compounds (Table 4). In all treatments, the most important essential oil compounds included Carvacrol, Thymol, p-cymene, Borneol, and 1,8-Cineole. The control treatment also included Carvacrol (28.5%), Thymol (27.15%), p-cymene (13.6%), Borneol (4.46%), and 1.8-cineole (4.26%).

A comparison of the means showed that with increasing concentration of nano-complex salicylic acid-zinc from zero to 0.5%, a Carvacrol content increased by 19.65, 43.79, and 80.7%, respectively. The highest and lowest percentages of Thymol were related to the control treatment (27.15%) and 0.5% of nano-complex salicylic acid-zinc (8.46%), respectively. The use of nano-complex salicylic acid-zinc at 0.1% to 0.3% levels had no significant effect on the percentage of Thymol compared to the control treatment and then significantly reduced the percentage of Thymol at 0.5% level. This trend of change was similar for p-cymene and Borneol compounds. 1.8-Cineole decreased in 0.1% and 0.3% of nano-complex salicylic acid-zinc compared to the control treatment but increased by 5.6% compared to the control at the level of 0.5% of nano-complex salicylic acid-zinc. The highest increase and decrease among the compounds of essential oils due to the use of nano-complex salicylic acid-zinc at the rate of 0.5% were related to (E)-*b*-ocimene (6.37-fold increase) and Thymol methyl ether (77.9%), respectively, compared to the control. Nano-complex salicylic acid-zinc seems to have an effect on the number of compounds as well. The compounds decreased with increasing concentration of nano-complex salicylic acid-zinc compared to the control, but this reduction is limited to the highest concentration of nano-complex salicylic acid-zinc. However, compounds above 0.5% are preserved at different levels of nano-complex salicylic acid-zinc.

Polyphenol Contents

With the standard injection of Sigma Corporation and plant extracts, 17 polyphenols were evaluated. Seven (7) out of 17 polyphenols were not observed in any of the treatments (Table 6). The analysis of variance of the levels of caffeic acid, quercetin, p-coumaric acid, Carvacrol, vanilin, trans-ferulic acid, Hesperetin, rosmarinic acid, Thymol, and their total contents under different amounts of a nano-complex of salicylic acid and zinc showed a significant difference at 1% level (Table 5). Furthermore, the results of the analysis of variance of extract samples showed that polyphenols of caffeic acid, quercetin, p-coumaric acid, Carvacrol, Vanilin, trans-ferulic acid, Eeugenol, Hesperetin, rosmarinic acid, and Thymol with different amounts in the extract of *T. daenensis* were under the influence of different amounts of compounds of nano-complex salicylic acid-zinc (Table 6).

Table 6 shows the results of the study of 17 polyphenolic compounds by calculating the area under HPLC curves and comparing them with standard curves for *T. daenensis* at diverse levels of foliar application of nano-complex salicylic acid-zinc. Investigating the total phenolic compounds in plant extract showed that the highest level of phenolic compounds at 516.43 mg/l was related to foliar application of nano-complex salicylic acid-zinc at 0.5% level (Fig. 1). An examination of the phenolic compounds in plant extract showed that the highest phenolic compounds of was related to rosmarinic acid at 207.2mg/l in the control treatment (235.3 mg/l in 0.5% fertilizer foliar application). Spraying with nano-complex salicylic acid-zinc at the rates of 0.1, 0.3, and 0.5% caused an increase in this substance at 6.9, 4.2, and 13.5%, respectively. Thymol content also decreased due to fertilizer foliar application. The amount of Thymol in the control treatment was 161 mg/l, which decreased by 32.49, 28.4, and 31.08%, respectively, because of fertilizer foliar application at the rates of 0.1, 0.3, and 0.5%. A Hesperetin content decreased compared to the control treatment. The amount of this substance in control treatments and fertilizer foliar application at concentrations of 0.1, 0.3, and 0.5 was 16, 12.6, 14.3, and 13.5 mg/l, respectively.

The most significant percentage of changes due to foliar application of nano-complex salicylic acid-zinc was related to the polyphenolic compound of quercetin, whose concentration in the treatment of 0.5% nano-complex salicylic acid-zinc increased from 0.004mg/l in the control treatment to 0.34mg/l (Table 6).

The concentration of Thymol was significantly reduced because of fertilizer foliar application. The amount of Thymol in the control treatment was 161mg/l, which decreased by 32.49, 28.4, and 31.08%, respectively, because of fertilizer foliar application at the rates of 0.1, 0.3, and 0.5%. A Hesperetin content decreased compared to the control treatment. The amount of this substance in control treatments and fertilizer foliar application at concentrations of 0.1, 0.3, and 0.5 was 16, 12.6, 14.3, and 13.5mg/l, respectively.

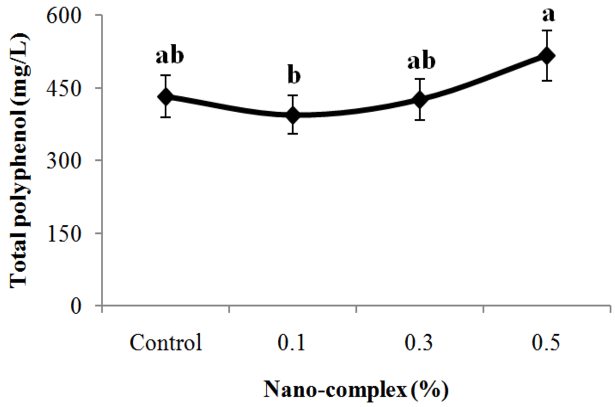

Fig. 1 Changes in the total polyphenols measured due to different amounts of nano-complex salicylic acid-zinc

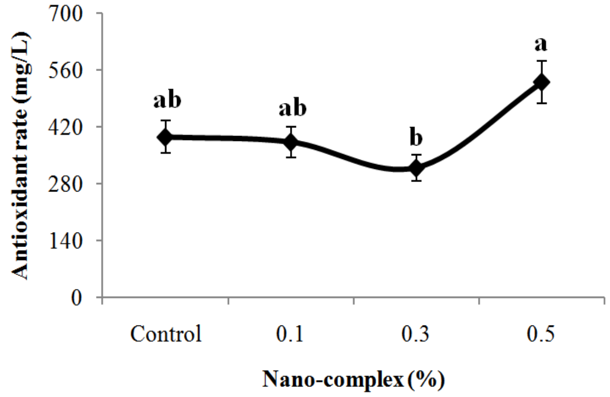

Fig. 2 Changes in the antioxidants rate measured due to different amounts of nano-complex salicylic acid-zinc

By comparing the mean caffeic acid levels in control treatments and levels of 0.1% and 0.5% of nano-complex salicylic acid-zinc, they were in a statistical group without significant differences. The most elevated concentration of this compound was related to the 0.3% level of nano-complex salicylic acid-zinc. There was an increasing trend due to the increase in p-coumaric acid levels. The lowest and highest amounts of this compound were observed for the control treatment and 0.5% of fertilizer, respectively. Carvacrol levels had a 2.2-fold increase compared to the control treatment due to the use of nano-complex salicylic acid-zinc at 0.5% (Table 6).

Changes in Antioxidants Due to Foliar Application of a Nano-complex of Salicylic Acid and Zinc

The most elevated antioxidant concentration in the plant was obtained by foliar application of nano-complex salicylic acid-zinc at 0.5%. The antioxidant concentration of the plant in this treatment increased by 25.4% compared to the control (without foliar application). The concentration of antioxidants in the plant was significantly affected by different amounts of nano-complex salicylic acid-zinc. The most significant antioxidant concentration in the plant was obtained by foliar application of nano-complex salicylic acid-zinc at 0.5%. The antioxidant concentration of the plant in this treatment increased by 25.4% compared to the control (without foliar application) (Fig. 2).

DISCUSSION

The results showed that with increasing the level of nano-complex salicylic acid-zinc up to 0.3% level, plant height, fresh weight, dry weight, inflorescence height, leaf length, leaf width, and the ratio of fresh weight to dry weight significantly increased and then decreased at 0.5% level. These results are consistent with the results of the study done by Gharib [20]. Gharib's study showed that the number of shoots, plant height, and leaf area increased with the foliar application of salicylic acid in basil. In most cases, salicylic acid is an organic compound that affects physiological activity in plants [21]. Excessive use of nano-complexs also reduced growth index rates. In general, nano-complex salicylic acid-zinc appears to be able to improve the absorption of nutrients up to 0.3%, which in turn increases appearance traits, including plant height, and length and number of internodes. The results showed that 0.5% of nano-complex salicylic acid-zinc had the highest increase in the essential oil levels of Carvacrol, 1.8-cineole, and linalool (10.7%). At the level of 0.3% of nano-complex salicylic acid-zinc, the essential oils of Thymol, thymoquinone, 3-octanone, terpinyl acetate, sabinene (17.8%), at the level of 0.1, α-pinene, caryophyllene oxide, terpineol, camphor spathuenlenol, terpinen-4-ol, and Thymol methyl ether (21.4%), and the rest of the essential oils (53.77%) in the treatment without the use of nano-complex salicylic acid-zinc were the highest. Increasing the amount of essential oil because of the foliar application of plants with salicylic acid may be due to increased vegetative growth, higher nutrient uptake by roots resulting from increased plant photosynthetic activity as well as changes in the population of essential oil-producing glands in leaves and flowers [20].

Table 2 The results of the analysis of variance of the traits measured of *T. daenensis* Celak. under the influence of different concentrations of nano-complex salicylic acid-zinc

Dependent variables	Independent variables (Mean squares)		C.V%
	Fertilizer treatment	Error	
Plant height	40.1*	4.3	10.7
No. of branches	2.3 ^{ns}	17.8	21.6
Fresh weight (FW)	149.2 *	124.8	20.1
Dry weight (DW)	7.9 *	7.6	15.1
Inflorescence height	5.7 **	0.5	12.4
Leaf length	10.97 **	1.96	9.9
Leaf width	1.4 **	0.6	19.8
FW/DW ratio	0.09 *	0.03	6.1

^{ns} Not significant, * and ** Significant at $P< 0.05$ and $P< 0.01$, respectively.

Table 3 Effect of foliar application of different concentrations of nano-complex salicylic acid-zinc on the vegetative characteristics of *T. daenensis* Celak.

Treatment \ Attributes	Plant height (cm)	Fresh weight (g/plant)	Dry weight (g/plant)	Inflorescence height (cm)	Leaf length (mm)	Leaf width (mm)	FW/DW ratio
Control	16.2 c	45.7 b	16.4 b	4.47 c	12.6 b	3.5 bc	2.8 b
nano-complex 0.1%	16.5 c	56.7 a	17.7 b	4.50 c	12.3 b	3.3 c	3.2 a
nano-complex 0.3%	24.0 a	62.4 a	20.3 a	7.33 a	16.2 a	4.8 a	3.1 a
nano-complex 0.5%	20.2 b	57.4 a	18.3 b	6.00 b	15.3 a	4.0 b	3.1 a

Means in each column with same letters are not significant difference according to LSD ($P< 0.05$).

Table 4 MS/GC analysis of the compounds in the essential oil of *T. daenensis* Celak. under the treatment of nano-complex salicylic acid-zinc (values in percent)

Row	Compounds	Retention index (R.I.)	Percentage of the compound in each level of nano-complex			
			Nano-complex level (%)			
			Control (0)	0.1	0.3	0.5
1	Tricyclene	922	0.06[a]	0.00[a]	0.00[a]	0.00[a]
2	α-Thujene	927	0.97 a	0.69 b	0.36 c	0.74 b
3	α-Pinene	934	1.69 a	1.80 a	0.52 c	0.95 b
4	Camphene	950	1.39 a	1.37 a	0.89 b	1.11 c
5	Sabinene	974	0.26 b	0.14 b	0.60 a	0.25 b
6	β-Pinene	978	0.57 a	0.45 a	0.47 a	0.50 a
7	3-Octanone	985	0.40 ab	0.33 ab	0.46 a	0.22 b
8	Myrcene	989	0.90 a	0.58 b	0.57 b	0.88 a
9	α-Phellandrene	1001	0.09 a	0.00 a	0.00 a	0.00 a
10	p-Mentha-1(7),8-diene	1004	0.07 a	0.00 a	0.00 a	0.00 a
11	α-Terpinene	1016	0.33 a	0.00 b	0.00 b	0.00 b
12	p-Cymene	1024	13.60 a	11.04 ab	8.88 ab	7.11 b
13	Limonene	1028	0.72 a	0.71 a	0.37 b	0.49 b
14	1,8-Cineole	1031	4.26 a	3.42 ab	2.66 b	4.50 a
15	(E)-β-Ocimene	1050	0.08 c	0.35 ab	0.30 b	0.51 a
16	γ-Terpinene	1057	1.76 a	0.92 c	0.92 c	1.35 b
17	cis-Sabinene hydrate	1068	0.78 a	0.54 b	0.59 b	0.42 b
18	Terpinolene	1088	0.30 a	0.20 b	0.00 c	0.26 ab
19	Linalool	1099	1.93 b	1.23 b	1.49 b	5.06 a
20	trans-Pinocarveol	1137	0.20 b	0.33 a	0.00 c	0.15 b
21	Camphor	1145	0.57 b	0.81 a	0.58 b	0.51 b
22	Borneol	1166	4.46 ab	4.79 a	4.84 a	4.07 b
23	Terpinen-4-ol	1178	0.46 a	0.49 a	0.36 a	0.36 a
24	α-Terpineol	1190	0.35 b	0.63 a	0.39 b	0.51 ab
25	Thymol methyl ether	1233	0.59 a	0.63 a	0.30 b	0.13 b
26	Carvacrol methyl ether	1243	0.14 b	0.00 c	0.00 c	1.87 a
27	Thymoquinone	1251	0.79 d	1.11 c	2.17 a	1.60 b
28	Thymol	1290	27.15 a	24.03 a	26.29 a	8.46 b
29	Carvacrol	1298	28.50 b	34.31 b	40.98 ab	51.51 a
30	δ-Elemene	1336	0.00 b	2.10 a	0.00 b	0.00 b
31	α-Terpinyl acetate	1348	0.13 b	0.22 b	0.42 a	0.29 ab
32	β-Elemene	1391	0.00 b	0.00 b	0.00 b	0.14 a
33	(E)-Caryophyllene	1421	1.91 a	1.74 b	1.12 c	0.83 d
34	(E)-Caryophyllen	1423	0.00 b	0.00 b	0.00 b	0.83 a
35	α-Humulene	1451	0.09 a	0.00 a	0.00 a	0.00 a
36	Germacrene D	1481	0.17 a	0.13 a	0.00 b	0.00 b
37	Bicyclogermacrene	1497	0.30 ab	0.23 b	0.28 ab	0.47 a
38	β-Bisabolene	1506	0.75 a	0.66 ab	0.68 ab	0.51 b
39	δ-Cadinene	1512	0.00 c	0.00 c	0.47 b	0.68 a
40	(E)-γ-Bisabolene	1532	0.34 b	0.35 b	0.30 b	0.51 a
41	Thymohydro quinone	1555	0.00 b	0.00 b	0.00 b	0.13 a
42	Spathulenol	1576	0.53 a	0.61 a	0.56 a	0.32 b
43	Caryophyllene oxide	1582	1.64 b	1.90 a	0.92 c	0.63 d
44	α-Cadinol	1652	0.00 b	0.00 b	0.00 b	0.82 a

Means in each row with same letters are not significant difference according to LSD ($P< 0.05$).

Table 5 Summary of results of the analysis of variance of different amounts of phenolic compounds in plant extract

Dependent variables	Independent variables (Mean squares)		C.V%
	Fertilizer treatment	Error	
Caffeic acid	5.0 **	0.1	19.2
Quercetin	0.090 **	0.001	18.7
p-Coumaric acid	3.5 **	0.1	23.4
Carvacrol	4981.2 *	204.9	19.1
Vanilin	5.9 **	0.2	24.0
Trans-ferulic acid	101.1 *	2.0	18.1
Eugenol	227.4 *	8.8	23.2
Hesperetin	6.2 **	8.0	20.1
Rosmarinic acid	416.5 **	1940.4	20.1
Thymol	1850.8 **	633.7	20.2

* and ** Significant at $P< 0.05$ and $P< 0.01$, respectively.

Table 6 Type and level of polyphenols in *T. daenensis* Celak. [Celak] because of different amounts of nano-complex salicylic acid-zinc

Row	Polyphenols (mg lit/l)	Nano-complex level (%)			
		Control (0)	0.1	0.3	0.5
1	Sinapic acid	ND	ND	ND	ND
2	Gallic acid	ND	ND	ND	ND
3	Catechin	ND	ND	ND	ND
4	Caffeic acid	0.001 b	0.001 b	2.580 a	0.001 b
5	Chloregenic acid	ND	ND	ND	ND
6	Quercetin	0.0040 b	0.0001 c	0.0001 c	0.3490 a
7	p-Coumaric acid	0.188 c	1.521 b	1.610 b	2.846 a
8	Coumarin	ND	ND	ND	ND
9	Carvacrol	37.9 b	31.9 b	58.3 b	121.0 a
10	Vanilin	0.000 c	1.477 b	3.124 b	2.705 a
11	Trans-ferulic acid	9.929 a	0.000 b	0.000 b	10.177 a
12	Hesperedin	ND	ND	ND	ND
13	Ellagic acid	ND	ND	ND	ND
14	Eugenol	0.000 b	16.420 a	14.952 a	19.571 a
15	Hesperetin	16.004 a	12.610 a	14.334 a	13.524 a
16	Rosmarinic acid	207.195 a	221.684 a	215.912 a	235.264 a
17	Thymol	161.049 a	108.720 b	115.308 ab	110.997 b

Means in each row with same letters are not significant difference according to LSD ($P< 0.05$).
ND: no determine.

In a study, the foliar application of salicylic acid increased growth and uptake of elements and changes in the number of secretory glands and biosynthesis of monoterpenes, as well as increasing the amount of essential oil [20, 22].

In the present study, it was discovered that the number and type of essential oil compounds in the treatments were different. Although in compounds above 0.5% at different levels of a nano-complex of salicylic acid and zinc, there was no significant difference, in terms of the amount of each compound, there was a difference. This was in line with the results of the studies done on basil and marjoram plants. Percentage change and retention of key compounds are consistent with previous research [20,23].

The most elevated level of the compound obtained from thyme in the study was Carvacrol, and the other major compounds included Thymol, p-cymene, Borneol, and 1.8-cineole, respectively. Medicinal plants are abundant sources of secondary metabolites, i.e., the sources of the active ingredients of many drugs. However, with changes in plant metabolism, salicylic acid appears to alter secondary plant material, including essential oil compounds, a process similar to the role of salicylic acid in creating stress resistance.

Phenolic compounds in thyme plant are among the most significant antioxidant products, used in the pharmaceutical industry. Many studies have shown that acid by binding to membrane receptors, produces active oxygen, NO, protein kinases, and salicylic acid [24]. By directly affecting the transcription of genes and enzymes involved in the production of secondary metabolites, these changes increase the production of these compounds. Salicylic acid, by affecting the phenylalanine enzyme and increases the level of ammonia-lyase lead to activate the phenylpropanoid pathway and increase the production of phenolic compounds [25, 26]. Salicylic acid induces the accumulation of total phenolic compounds by increasing the activity of the PAL enzyme. Therefore, salicylic acid carries out a significant role in the process of messenger transmission, which induces the biosynthesis of total phenolic compounds and the expression of defense genes in plants. The use of salicylic acid in rice plants has led to the induction of the expression of genes for proteins involved in phenylpropanoid metabolism and the synthesis of phenolic compounds [27]. There was, in addition, an increase in phenolic compounds under the influence of salicylic acid in the sage plant [28].

CONCLUSION

Based on the results, different levels of salicylic acid-zinc led to a change in the vegetative growth indices studied, percentage of essential oil compounds, polyphenolic content, and antioxidant activity. The most important compounds in the essential oil were reported in the control treatment: Carvacrol, Thymol, p-cymene, Borneol and 1.8-cineole, respectively. A 0.5% solution (v/v) of nano-complex salicylic acid-zinc increased the amount of Carvacrol in the essential oil compared to the control. The most important compounds including Rosmarinic acid, Thymol, Carvacrol, and Hesperetin were the types of polyphenolic compounds. In this study, the best treatment for increasing polyphenols was a concentration of 0.5% (v/v) of nano-complex of salicylic acid and zinc. Based on the results, in general, it can be said that applying a concentration of 0.3% of nano-complex salicylic acid-zinc improves crop traits and a concentration of 0.5% increases phenolic and antioxidant compounds.

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