

IN VITRO EFFECTS OF *CLITORIA TERNATEA* AQUEOUS EXTRACT ON PHYSICOCHEMICAL COMPONENTS AND GROWTH OF *ACHILLEA FRAGRANTISSIMA* L. CULTIVATED UNDER SALINITY STRESS

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Abstract. *Clitoria ternatea* flowers aquatic extract (CTFAE) contains a mixture of essential elements, which promote plant growth and yield. *Achillea fragrantissima* is a medicinal plant native in Saudi Arabia. It has antiviral, anti-neuroinflammatory, anticancer and antispasmodic. Salinity is a primary abiotic environmental challenge, disrupting source-sink relationships, senescence, cellular metabolisms, and ultimately hindering plant growth and development. In this study, a novel strategy for enhancing *A. fragrantissima* propagation under salinity stress by applying a natural plant growth enhancer, CTFAE, to in-vitro plantlets were investigated. The experiment was conducted using a split-plot design with salinity levels as the main factor and CTFAE concentrations as the sub-factor. The CTFAE at concentrations of 0.5 g/L or 1.0 g/L and distinct concentrations of seawater were introduced to the MS media to set salinity level (2000 ppm and 4000 ppm). Our results revealed that CTFAE effectively enhanced *A. fragrantissima* seed germination and plant growth during multiplication and rooting stages, as well as increased the phytochemical compounds in *A. fragrantissima* plants as compared to control treatment. Furthermore, for plants that were exposed to 4000 ppm salinity levels, the application of 0.5 g/L CTFAE was able to alleviate the salinity-induced adverse effects on some of the plant growth parameters.

Keywords: GC-MS, qaysoom, bio-stimulant, abiotic stress, tissue culture

Introduction

Crop productivity and production are negatively impacted by biotic and abiotic stresses resulting in enormous economic losses globally. One of the primary abiotic environmental challenges is salinity, that is brought on by dissolved salt in soil water. Salinity stress disrupts source-sink relationships, speeds up senescence, slows down cellular metabolisms, and ultimately hinders plant growth and development (Sarmoum et al., 2019). Studies have been carried out in recent years to identify the mechanisms underlying salinity tolerance and to create plants that can withstand salt (Murtaza et al., 2024). One of the most crucial stages to lessen the effect of salinity on crops and their production is the search for materials and treatments that boost the resistance and tolerance of salt-intolerant plants. Natural substance and plant extracts that contain

significant and useful substances that enhance other plants' growth and increase their resistance to the negative effects of salinity are among the most important tools used to lessen the effect of salinity on plant production and growth (Alkuwayti et al., 2020; Noreen et al., 2024). The perennial leguminous herb *Clitoria ternatea*, also known as the butterfly pea, has garnered a lot of attention due to its numerous uses in agriculture and medicine. These include use as a crop for cosmetics, traditional medicine, food coloring, and the production of an environmentally friendly insecticide (Jamil et al., 2018). Butterfly pea flowers contain a variety of phytochemical compounds, including flavonoids, anthocyanins, alkaloids, steroids, tannins, and reducing sugars (Escher et al., 2020), which may function as a biostimulant to promote plant development. It is challenging to select crops for salt tolerance based on yield performance in fields because of the significant spatial variation of the salinity level in fields (Pecetti et al., 2024).

Plant biotechnology techniques such as tissue culture have been effectively employed to enhance plant features and efforts to develop stress tolerance to plants (Goda et al., 2017; Khalifa et al., 2023). Because there is greater control over plant growth than in the outside environment and because evaluations are typically favorable to good outcomes in a limited space, *in vitro* cultivation techniques can greatly aid in the study and selection of plant species (Трушкин et al., 2013). Secondary metabolites, which are frequently good sources of bioactive chemicals employed in the pharmaceutical and medical industries, are found in medicinal plants. Asteraceae family: *Achillea fragrantissima* (Forssk.), a fragrant perennial wild herb also known as lavender cotton in English and Qaysoom in Arabic (El-Ashmawy et al., 2016), is considered a fundamental part of Middle Eastern folk medicine. It has been used historically in the Arabian Peninsula to treat a wide range of ailments, including fever, rheumatism, and diabetes (El Fattah et al., 2018; Goda et al., 2023a). *A. fragrantissima* is useful in treating a wide range of ailments, including headaches, exhaustion, smallpox, ophthalmic issues, menstrual difficulties, and lung impacts (Farouk et al., 2019). The essential oil of *A. fragrantissima* has demonstrated efficacy against a variety of drug-resistant bacteria (Zeedan, 2014). According to the literature, no research has been done on the impact of CTFAE as a biostimulant on plant growth. The objective of this investigation is to detailed the specific method of using an aqueous extract of *C. ternatea* flowers (CTFAE) to enhance the growth, yield, and salinity tolerance of *A. fragrantissima* under *in vitro* condition.

Materials and methods

Sources of A. fragrantissima seeds and C. ternatea flower powder

C. ternatea powdered flower was purchased from Earth Circle Organics (USA). Seeds of *A. fragrantissima* were collected at Wadi Harqan, Alqareenah, Riyadh, Saudi Arabia.

Analysis of C. ternatea flower powder compositions

Analyses of macro- and micro-nutrients composition of *C. ternatea* flower powder were performed according to Ryan et al. (2001). Extraction and identification of vitamins were performed as Shindy and Smith (1975); and Qian and Sheng (1998) methods. Fat and total protein were measured according to Chen (2019). The compositions from the above analysis were documented in *Table 2*.

Preparation of aquatic extracts from C. ternatea flower powder

C. ternatea flower powder Aqueous extract was prepared according to Escher et al. (2020) method.

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on seed germination percentage of A. fragrantissima

A. fragrantissima seeds were first soaked in a 70% ethanol solution for 30 seconds, then submerged in a 5% (v/v) sodium hypochlorite solution for 5 minutes, and lastly washed three times with sterile tap water while being exposed to laminar airflow. In 60 ml capacity tubes containing 15 ml of half-strength (1/2X) Murashige and Skoog (MS) basic salts and vitamins (Caisson Labs, Smithfield, UT, USA), supplemented with 30 g/L sucrose (Sigma Aldrich, Saint Louis, MO, USA), and, 7 g/L agar (Caisson Lab, Smithfield, UT, USA), distinct concentrations of seawater (35000 ppm) from the he Arabian Gulf (Uqair, 26.0786 N, 50.0393 E) (*Sup Table 1*) were introduced to the media to produce salinity level (2000 ppm and 4000 ppm) the control treatments containing (1/2X) MS medium only without adding seawater and/or aquatic extract of CTFAE (0.5 g/L and 1.0 g/L). Split-plot design was used for the experiment, with two components, the concentration of the CTFAE (0.0, 0.5 g/L and 1.0 g/L) as a sub-factor and salinity (control, 2000 ppm and 4000 ppm) as the major factor. Disinfested seeds were cultured then, the culture vessels were maintained in a growth chamber (Phillips TLM 40 W/33RS) at 24±2°C with a 16-hour photoperiod and 4000 Lux light intensity. The germination rate was measured after four weeks.

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on multiple shoots induction of A. fragrantissima plants

Shoot tips, measuring between 0.5 and 1.0 cm in length, were obtained from *in vitro* germinated seeds and sub cultured (at the same salinity and CTFAE levels with the germination medium, which the shoot tip was obtained from) on 30 ml of MS medium containing 30 g/L sucrose, 7 g/L agar, and 0.2 mg/L benzyl aminopurine (BAP) (Goda et al., 2023a) in a 200 mL container. Split-plot design was used for the experiment, with two components, the concentration of the CTFAE (0.0, 0.5 g/L and 1.0 g/L) as a sub-factor and salinity (control, 2000 ppm and 4000 ppm) as the major factor. Each treatment consists of ten culture vessels. The longest shoot's height, the explant's fresh weight, the number of shoots, and the callus % were measured after four weeks of culture. To prepare for methanol extraction and phytochemical analysis by GC-MS, a portion of the duplicate explants from each treatment were allowed to air dry at room temperature.

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on rooting induction of A. fragrantissima plants

The shoot tips (0.5–1.0 cm in length) of the 4-week plantlets were separated and cultivated on the same MS medium as previously mentioned, using the same seawater concentrations and CTFAE that were used in the media from which the shoot tips were derived, in addition to 0.4 mg/L 1-naphthaleneacetic (NAA) (Sigma Aldrich) (Goda et al., 2023a) to facilitate roots. Plant fresh weight, root length, number of roots, and plant height were measured after 4 weeks. Some of the rooted plantlets were moved to a plant growth chamber where they were allowed to acclimate in pots filled with a moist mixture

of sand and perlite (1:1). For three weeks, a fine mist was used to irrigate the plants. After four weeks, the proportion of plants that survived was calculated.

Chlorophyll pigment determinations

Using acetone (80%), the chlorophyll a (Chl-a), chlorophyll b (Chl-b), and carotenoids were extracted from the leaves of *A. fragrantissima* plantlets that had developed from the root stage. Following spectrophotometric determination in accordance with A.O.A.C. (1984), the pigments were computed as mg/100 g based on fresh weight.

Mineral composition

Plant samples of *A. fragrantissima* from the rooting stage (4 weeks after culturing on rooting media) were dried at 70°C for 24 h then ground and digested according to Cottenie et al. (1979) method. The modified micro-Kjedahl method was used to determine the amount of nitrogen (N⁺), as explained by Jackson (1967). Phosphorus (P⁺) was calculated using a calorimetric approach using the technique of Murphy and Riley (1962). Using an Atomic Absorption Flame Photometry model Shimadzu-AA7000, the following elements were measured: sodium (Na⁺), potassium (K⁺), magnesium (Mg²⁺), zinc (Zn²⁺), iron (Fe²⁺) and calcium (Ca²⁺) (Mazumdar and Majumder, 2003).

Determination of carbohydrate

The estimate of carbohydrates (ppm) was determined by DuBois et al. (1956) method.

GC-MS Analysis of C. ternatea flower and A. fragrantissima explants

The GC/MS analyses of *C. ternatea* flower and *A. fragrantissima* dry air samples were performed at the Department of chemistry, collage of Science, King Faisal University. *C. ternatea* flower powder and *A. fragrantissima* samples were extracted with methanol, according to El-Ashmawy et al. (2016). The methanol extracts were analysed by gas chromatography coupled with mass spectrometry (GC/MS-QP 2010 Plus), equipped with an auto-sampler AOC-20i, (Shimadzu, Kyoto, Japan). Separation was performed with a 30 m × 0.25 mm × 0.1 µm RTX®-5SilMS capillary column (Restek, Bellefonte, PA, USA). The stationary phase was composed of 5 % diphenyl and 95 % dimethyl-polysiloxane and high purity helium gas (99.9999 %) used as a carrier gas. The computation of composition was according to Lee et al. (2018) with slight modification.

Statistical analysis

The concentration of the *C. ternatea* flower aquatic extract as a sub-factor and salinity as the major factor with 10 repetitions made up the split-plot design of the experiment. ANOVA/MANOVA of Statistica 6 software was used to statistically analyse. The significance of differences among means was performed using the Duncan test at p = 0.05.

Results

Analysis of C. ternatea flower powder compositions

Data in Table 1 showed the GC-MS analysis of the methanol extract of *C. ternatea* flower powder. The analysis identified a wide variety of compounds, including fatty

acids, esters, alcohols, aldehydes, terpenoids, and nitrogen-containing compounds, in the methanol extracts in *C. ternatea* flowers. The most abundant compounds included methyl oleate (22.62%), methyl palmitate (14.33%), palmitic acid (9.67%), and alpha-linoleic acid (10.66%) and ethyl oleate (13.31%) (Table 1). Data in Table 2 showed the phytochemical composition of *C. ternatea* flower includes various macro and micro nutrients and vitamins, such as calcium (340 mg), iron (0.9 mg), vitamin C (200 mg), and small amounts of fats (1.70 g).

Table 1. Phytochemical composition of methanol extracts from *C. ternatea* flower by GC MS

Peak	Compound Name	RT, min	Area %	Molecular Weight (g·mol ⁻¹)	Molecular formula
1	Thymine	9.338	0.02	126.11	C ₅ H ₆ N ₂ O ₂
4	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	11.077	0.05	144.12	C ₆ H ₈ O ₄
5	Hydouracil, 1-methyl-	12.02	0.03	128.13	C ₅ H ₈ N ₂ O ₂
6	Capric acid	14.362	0.02	172.268	C ₁₀ H ₂₀ O ₂
8	DL-Pyroglutamic acid	17.992	0.3	129.11	C ₅ H ₇ NO ₃
9	Methyl dodecanoate	19.485	0.78	214.34	C ₁₃ H ₂₆ O ₂
10	Lauric Acid	20.179	0.92	200.32	C ₁₂ H ₂₄ O ₂
11	Nonanedioic acid, dimethyl ester	20.605	0.2	216.2741	C ₁₁ H ₂₀ O ₄
12	Ethyl dodecanoate	20.774	0.49	228.37	C ₁₄ H ₂₈ O ₂
13	Thiophene, tetrahydro-2-methyl-	21.931	2.87	102.198	C ₅ H ₁₀ S
14	Methyl tetradecanoate	23.894	0.8	242.40	C ₁₅ H ₃₀ O ₂
15	Myristic Acid	25.023	0.49	228.37	C ₁₄ H ₂₈ O ₂
16	Ethyl palmitate	26.282	0.32	284.484	C ₁₈ H ₃₆ O ₂
17	Undecanone, 6,10-dimethyl	28.301	0.07	98.3449	C ₁₃ H ₂₆ O
18	Methyl palmitate	33.205	14.33	270.5	C ₁₇ H ₃₄ O ₂
19	2-Undecanone, 6,10-dimethyl- (Hexahydropseudoionone)	34.667	0.1	149.19	C ₉ H ₁₁ NO
20	Palmitic acid	36.005	9.67	256.42	C ₁₆ H ₃₂ O ₂
21	Palmitic acid, ethyl ester	38.749	3.25	284.4772	C ₁₈ H ₃₆ O ₂
22	N-Nitrosodimethylamine	41.49	0.01	74.083	C ₂ H ₆ N ₂ O
23	Linoleic acid	44.05	10.66	280.4	C ₁₈ H ₃₂ O ₂
24	Oleic acid, methyl ester	44.302	22.62	296.5	C ₁₉ H ₃₆ O ₂
25	6-Octadecenoic acid, methyl ester, (Z)-	44.473	0.6	296.494	C ₁₉ H ₃₆ O ₂
26	Methyl stearolate	45.091	4.3	298.5	C ₁₉ H ₃₈ O ₂
27	Elaidic Acid	45.28	4.62	282.5	C ₁₈ H ₃₄ O ₂
28	Methyl octadec-9-ynoate	45.737	2.57	294.5	C ₁₉ H ₃₄ O ₂
29	Ethyl oleate	45.903	13.31	310.5	C ₂₀ H ₃₈ O ₂
30	9-Tetradecenal	46.047	0.85	210.36	C ₁₄ H ₂₆ O
31	Ethyl stearate	46.495	1.08	312.5	C ₂₀ H ₄₀ O ₂
32	Phytol	47.395	1.62	296.5	C ₂₀ H ₄₀ O
33	Hexadecanal (Palmitaldehyde)	47.6	0.39	240.42	C ₁₆ H ₃₂ O
34	Tridecanol	48.134	0.37	200.36	C ₁₃ H ₂₈ O
35	Eicosanoic acid, methyl ester	49.325	0.46	326.557	C ₂₁ H ₄₂ O ₂
36	Eicosanoic acid, methyl ester (Methyl arachisate)	49.425	0.58	125.13	C ₅ H ₇ N ₃ O
37	(9,12-Octadecadienoic acid (Z,Z)-	49.559	1.03	198.3	C ₁₂ H ₂₂ O ₂
38	4α-Methylandrostan-2,3-diol-1,17-dione	54.37	0.09	280	C ₁₈ H ₃₂ O ₂

Table 2. Chemical composition of *C. ternatea* flower

Chemical	Amount	Unit
Calcium	340	mg
Copper	0.03	mg
Iron	0.9	mg
Magnesium	30	mg
Phosphorus	90	mg
Potassium	155	mg
Zinc	0.2	mg
Protein	0	g
Fat	1.70	g
Carbohydrate	0	g
Vitamin A	7	mg
Vitamin B1	0.04	mg
Vitamin B2	0.1	mg
Vitamin B3	0.45	mg
Vitamin C	200	mg

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on seed germination percentage of *A. fragrantissima*

The data in Figure 1 indicated that an increase in salinity decreased the germination percentage of *A. fragrantissima* seeds, according to single factor analysis of the salinity treatments. The results of the single factor analysis for the CTFAE treatments showed that the seed germination % was positively impacted by both CTFAE concentrations (0.5 g/L and 1 g/L), with the 1 g/L CTFAE performing better than the 0.5 g/L. The enhancing effects of CTFAE treatments enhanced seed germination % under salinity level compared to control salinity when the effects of both the salinity and CTFAE treatments were studied together. Under salinity levels of 2000 and 4000 ppm, the CTFAE at 1 g/L provided the maximum germination percentage.

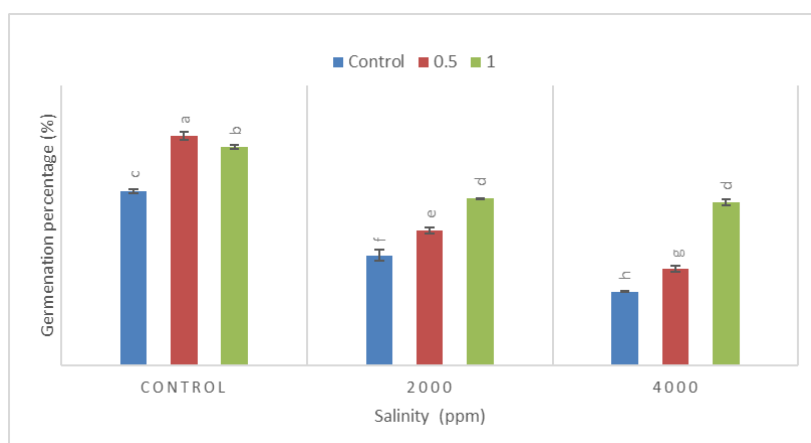


Figure 1. Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on seed germination percentage of *A. fragrantissima*. * Means followed by the same letter are not significantly different at 0.05 level of probability according to Duncan test

Effects of salinity levels, CTFAE (g/L) concentrations, and their interactions on multiple shoots induction of A. fragrantissima

Excision of the shoot tips (1.0 cm) from the in-vitro seedlings was followed by culture on shoots multiplication media supplemented with MS media supplemented with varying salinity levels (control, 2000 and 4000 ppm) and/or CTFAE (0.0, 0.5 and 1.0 g/L). The analyses of the salinity and CTFAE effects on multiplication stage of *A. fragrantissima* are presented in *Table 3* and *Figures 2,3 and 4*. According to the single factor analysis of the salinity treatments, *A. fragrantissima* shoot numbers were shown to decrease in a dose-dependent manner as salinity increased (*Table 3a* and *Figure 2*). In contrast, the height of the longest shoot, the fresh weight of the entire explant, and the callus percentage all significantly increased as the salinity concentration rose (*Table 3a*). Single factor analyses of the effects treatments of CTFAE revealed that a concentration of 0.5 g/L significantly increased the number of *A. fragrantissima* shoots; in contrast, a concentration of 1.0 g/L significantly increased the callus percentage, the fresh weight of the entire explant, and the height of the longest shoot (*Table 3b* and *Figure 3*). Upon combining the analysis of the effects of the salinity and CTFAE concentrations treatments, it was observed that the number of shoots, plant height, and explant fresh weight significantly increased with increasing CTFAE concentrations under salinity control treatment (*Table 3c*). Conversely, the callus % dropped dramatically (*Table 3c* and *Figure 4*). Under the effect of salinity level 2000 ppm, 0.5 g/L CTFAE gave the highest shoot number, and 1.0 g/L gave the length of the longest shoot, fresh weight and callus % (*Table 3c*). With the exception of callus %, which increased with 1.0 g/L of CTFAE, all the above metrics decreased with CTFAE concentrations under salinity levels of 4000 ppm (*Table 3c*).

Table 3. Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on multiple shoots induction of *A. fragrantissima*

Treatments		No. of shoots/ explant (n)	Explant's fresh weight (g)	Length of the longest shoot (cm)	callus %
Salinity (ppm)	CTFAE (g/L)				
(a) Main effect of salinity					
Control		60.25 a*	1.98 b	1.78 b	0.06 c
2000		56.73 b	2.01 a	2.15 a	0.16 a
4000		51.92 c	2.09 a	2.25 a	0.10 b
(b) Main effect of CTFAE					
	0.0	54.25 b	2.01 a	2.13 a	0.15 a
	0.5	61.91 a	1.75 b	1.72 b	0.0 b
	1.0	53.17 b	2.08 a	2.29 a	0.17 a
(c) Interacting effects of salinity CTFAE					
Control	0.0	38 f	1.33 d	1.63 c	0.19 a
	0.5	66.5 c	1.80 c	1.63 c	0.0 b
	1.0	76.25 a	2.83 a	2.0 b	0.0 b
2000	0.0	51.25 d	1.85 c	1.95 b	0.19 a
	0.5	74 b	1.87 c	1.8 c	0.0 b
	1.0	49.25 d	2.28 b	2.63 a	0.25 a
4000	0.0	34.0 c	1.60 c	1.18 c	0.06 b
	0.5	73.5 b	2.85 a	2.75 a	0.0 b
	1.0	48.25 e	1.83 c	2.25 b	0.25 a

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan test



Figure 2. Effects of salinity levels (ppm) on multiple shoots induction of *A. fragrantissima*. (A) is control, (B) is 2000 ppm and (C) is 4000 ppm salinity level respectively. Each square box represents 5 mm x 5mm



Figure 3. Effect of CTFAE (g/L) concentrations on multiple shoots induction of *A. fragrantissima*. (A) is 0.5 g/L CTFAE, (B) is 0.5 g/L CTFAE + 2000 ppm salinity levels and (C) is 0.5 g/L CTFAE + 4000 ppm salinity level respectively. Each square box represents 5 mm x 5 mm



Figure 4. Effect of salinity levels (ppm) and CTFAE (g/l) concentrations on multiple shoots induction of *A. fragrantissima*. (A) is 1 g/L CTFAE, (B) is 1 g/L CTFAE + 2000 ppm salinity levels and (C) is 1 g/L CTFAE 4000 ppm salinity level respectively. Each square box represents 5 mm x 5 mm

Effects of salinity levels, CTFAE (g/L) extract concentrations, and their interactions on rooting induction of *A. fragrantissima*

Table 4a and Figure 5 shows that as the salinity level in the MS media increased, the length of the longest root, root number, and explant fresh weight of *A. fragrantissima* decreased significantly when compared to the control treatments, whereas explant height and callus percentage increased significantly. On the other hand, the CTFAE concentration significantly increased the above parameters of *A. fragrantissima*. with the exception of the callus percentage, which showed the highest significant percentage in the control treatments compared to the CTFAE treatments. The highest values of root

length and number of roots were obtained with 0.5 (ppm) CTFAE, and the highest values of shoot length and explant fresh weight were obtained with 1.0 (ppm) CTFAE (Table 4b). Regarding the examination of the effects of the salinity and CTFAE concentration treatments. Data in Table 4c showed that CTFAE concentrations under control salinity treatment significantly improved the length of the longest root, root number, and explant fresh weight of *A. fragrantissima* as compared to the control treatment. With an increase of 0.5 g/L CTFAE, further increase in CTFAE concentration (1.0 g/L) demonstrated fewer enhancing effects on the above-mentioned growth parameters compared to (0.5 g/L) concentration (Table 4c).

Table 4. Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on rooting induction of *A. fragrantissima*

Treatments		Length of the longest Root (cm)	Shoot length (cm)	No. of roots/ explant (n)	Explant's fresh weight (g)	Callus %
Salinity (ppm)	CTFAE (g/L)					
(a) Main effect of salinity						
Control		2.06 a*	1.54 b	3.67 a	1.5 a	0.22 c
2000		1.55 c	1.65 a	2.3 b	1.11b	0.30 b
4000		1.87 b	1.61 a	1.33 c	0.92 c	0.42 a
(b) Main effect of CTFAE						
	0.0	0.75 b	1.44 b	1.4 b	1.02 a	0.52 a
	0.5	3.33 a	1.56 b	4.22 a	1.2 a	0.14 c
	1.0	1.5 b	1.83 a	1.78 b	1.32 a	0.25 b
(c) Interacting effects of salinity and CTFAE						
Control	0.0	1.4 c	1.63 abc	1.0 d	0.93 bc	0.57 b
	0.5	4.3 a	1.33 c	8.67 a	1.87 a	0.08 e
	1.0	1.83 b	1.66 abc	2.33 c	1.70 a	0.0 f
2000	0.0	1.3 c	1.83 ab	0.9 d	0.80 bc	0.25 d
	0.5	1.88 b	1.38 c	3.5 bc	1.48 ab	0.57 b
	1.0	2.67 b	1.84 ab	3.0 bc	0.8 3bc	0.0 f
4000	0.0	0.0 c	1.33 c	0.0 e	0.37 c	0.42 c
	0.5	5.67 a	1.5 bc	4.0 b	0.93 bc	0.08 e
	1.0	0.0 c	2.0 a	0.0 e	1.47 ab	0.75 a

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan test

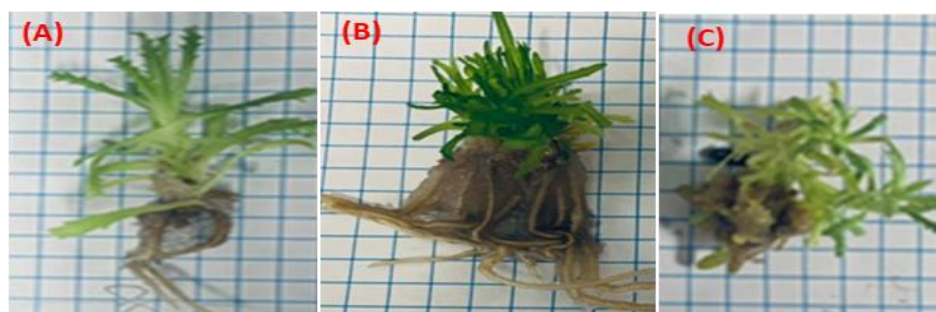


Figure 5. Effect of salinity (ppm) and CTFAE (g/L) on root induction of *A. fragrantissima*. (A) is control treatment, (B) is 0.5 g/L CTFAE and (C) is 4000 ppm salinity level + 1.0 g/L CTFAE, respectively. Each square box represents 5 mm x 5 mm

Under the influence of a salinity level of 2000 ppm, 0.5 g/L produced the highest fresh weight of explant and callus percentage, while 0.5 and 1.0 g/L CTFAE produced significantly longer and higher number of roots as compared to that without CTFAE (Table 4c). At salinity levels of 4000 ppm, no root was induced at the (0.0 and 1.0 g/L) CTFAE; however, the maximum plant height, plant fresh weight, and callus percentage were formed at 1.0 g/L CTFAE (Table 4c). The acclimatization techniques used in this study proved to be effective. When regenerated plantlets were placed in soil, they survived 80% of the time (data not shown).

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on the chlorophyll a, b and carotenoid contents of A. fragrantissima

Salinity and CTFAE increased photosynthetic pigment (Chl a, Chl b, and carotenoids) contents in *A. fragrantissima* leaves compared to control treatments (Table 5a and b), based on single factor analyses. Analysis of the interaction effects of salinity and CTFAE revealed that, at control salinity, the presence of CTFAE significantly raised the Chl a, Chl b, and carotenoid content (Table 5c). Under 2000 ppm salinity, the application of CTFAE boosted Chl a, Chl b, and carotenoid levels compared to 2000 (ppm) salinity level (without CTFAE) (Table 5c). While adding CTFAE to explants grown at salinity (4000 ppm) reduced the concentrations of Chl a, b, and carotenoid, the variations in carotenoid were not statistically significant when compared to the 4000 ppm salinity treatment (without CTFAE) (Table 5c).

Table 5. Effects of salinity levels (ppm), *C. ternatea* flower Aquatic extract (g/L) concentrations, and their interactions on the compositions (mg/100 g fresh weight of leaves) of chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoids in *A. fragrantissima* in-vitro plantlets rooting stage

Treatments		Chl a	Chl b	Carotenoids
Salinity (ppm)	CTFAE (g/L)	(mg/100 g F.W.)	(mg/100 g F.W.)	(mg/100 g F.W.)
(a) Main effect of salinity				
Control		171.894 b*	239.909 c	181.151 b
2000		195.866 a	265.541 a	208.685 a
4000		173.544b	249.054b	181.676 b
(b) Main effect of CTFAE				
	0.0	165.287b	158.124 c	187.419c
	0.5	188.430 a	270.549 a	192.819 a
	1.0	187.586 a	225.831 b	191.274 b
(c) Interacting effects of salinity and CTFAE				
Control	0.0	128.99 j	211.401h	154.887f
	0.5	186.249 c	228.657f	190.158 c
	1.0	200.433 b	279.669 c	198.407 c
2000	0.0	182.645e	192.11 i	201.175 b
	0.5	199.438 b	323.821 a	205.598 b
	1.0	205.513 a	280.689b	219.285 a
4000	0.0	184.218 d	273.978d	188.087 d
	0.5	179.603 f	259.171 e	186.702 d
	1.0	156.811 i	214.012g	170.239 e

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan test

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on the mineral contents in A. fragrantissima leaves

Tables 6a and 7a reveal that a rise in salinity significantly decreased the quantity of N and Fe, but significantly increased the K, Mg, and Ca content in *A. fragrantissima* leaves compared to the salinity control treatment. Salinity levels of 4000 ppm greatly reduced the amount of P and Zn, while salinity levels of 2000 ppm significantly increased these elements (Tables 6a and 7a). The amount of Na decreased significantly at a salinity level of 2000 ppm in the explant leaves (Tables 6a and 7a). The application of CTFAE resulted in increases in N, K, and Zn content, as well as decreases in P, Mg, Ca, and Na content, and these effects were significant when compared to the control treatment (without CTFAE) (Tables 6b and 7b). Fe content increased substantially at 0.5 g/L and decreased significantly at 1.0 g/L CTFAE as compared to the control without CTFAE (Tables 6b and 7b), respectively. The combined effects of CTFAE and salinity results showed that, compared to the control salinity treatment, plants treated with 0.5 and 1.0 g/L CTFAE contained the highest N, P, K, Mg, Ca, and Fe but the lowest Na and Fe contents (Tables 6c and 7c), respectively. Under salinity (2000 and 4000 ppm), the plants treated with CTFAE also contained the highest N, but lowest Ca and Na compared with the control (without CTFAE), respectively (Tables 6c and 7c). Under 2000 ppm salinity, the P content increased significantly in the salinity control (without CTFAE), whereas Ca and Mg elements increased significantly in the salinity control (without CTFAE) treatments at 4000 ppm salinity levels. Under 2000 ppm salinity, plants treated with 0.5 g/L CTFAE contained the least amount of K and Mg, whereas plants treated with 1.0 g/L contained the highest amounts of the same components. A similar impact was demonstrated under 4000 ppm salinity levels for K and Zn elements, respectively (Tables 6c and 7c).

Table 6. Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on Nitrogen (N), Phosphorus (P), potassium (K), magnesium (Mg) and Calcium (Ca) content (ppm) in *A. fragrantissima* in-vitro plantlets rooting stage

Treatments		N	P	K	Mg	Ca
Salinity (ppm)	CTFAE (g/L)					
(a) Main effect of salinity						
Control		1992.17 a*	5.09 b	231.59 c	2.42 c	15.55 c
2000		1627.80 c	6.47 a	248.03 b	2.51 b	17.79 a
4000		1881.70 b	4.86 c	248.48 a	2.52 a	15.84 b
(b) Main effect of CTFAE						
	0.0	1607.13 c	6.18 a	191.80 c	2.49a	17.98 a
	0.5	1823.88 b	4.80 c	210.66 b	2.47 c	14.74 c
	1.0	2090.65 a	5.44 b	325.64 a	2.49 b	16.47 b
(c) Interacting effects of salinity and CTFAE						
Control	0.0	1583.30 g	5.03 e	101.94 i	2.42 i	13.56 h
	0.5	1961.35 c	5.21 d	241.69 e	2.42 h	15.45 f
	1.0	2431.85 a	5.03 e	351.16 a	2.43 f	17.71 c
2000	0.0	1577.75 i	9.51 a	201.75 f	2.52 c	20.46 a
	0.5	1677.30 f	4.51 g	196.19 g	2.49 f	17.45 d
	1.0	1688.35 e	5.38 c	347.15 b	2.53 b	15.45 f
4000	0.0	1660.35 g	3.99 h	271.72 d	2.54 a	19.92 b
	0.5	1833.00 d	4.69 f	194.09 h	2.50 e	11.36 i
	1.0	2151.75 b	5.90 b	279.62 c	2.51 e	16.25 e

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan test

Table 7. Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on sodium (Na), zinc (Zn), and iron (Fe) content (ppm) in *A. fragrantissima* in-vitro plantlets rooting stage

Treatments		Na	Zn	Fe
Salinity (ppm)	CTFAE (g/L)			
(a) Main effect of salinity				
Control		3437.456 b*	0.038 b	5.497 a
2000		2642.76 c	0.0656 a	4.9775 b
4000		4749.995 a	0.0231 c	4.143 c
(b) Main effect of CTFAE				
	0.0	4868.41 a	0.02585 c	5.068 b
	0.5	2913.59 b	0.06468 a	5.169 a
	1.0	3048.17 b	0.0362 b	4.381 c
(c) Interacting effects of salinity and CTFAE				
Control	0.0	4334.955 b	0.02065 h	6.63395 a
	0.5	2136.985 i	0.04985 b	5.356 c
	1.0	3840.425 d	0.04355 c	4.5014 f
2000	0.0	2880.595 f	0.03055 e	5.02885 d
	0.5	2726.855 g	0.12865 a	5.5792 b
	1.0	2320.835 h	0.03755 d	4.325 g
4000	0.0	7389.665 a	0.02635 g	3.5416 i
	0.5	3876.935 c	0.01555 i	4.57025 e
	1.0	2983.235 e	0.0275 f	4.317 h

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan test

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on the carbohydrate contents in *A. fragrantissima*

Table 8 shows the effect of a single factor analysis of salinity treatment, CTFAE, and their interaction on carbohydrate content in *A. fragrantissima* leaves during the rooting stage. The data showed that salinity levels as well as CTFAE significantly increased carbohydrate contents, with the greatest effect of 1.0 g/L CTFAE under salinity control treatments as well as 2000 ppm and 4000 ppm compared to salinity and CTFAE control treatments.

Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on the compositions of essential oil prepared from *A. fragrantissima* in-vitro plantlets multiplication stage

Different salinity levels (control, 2000, and 4000 ppm) have an impact on the phytochemical composition of methanol leaf extracts from *A. fragrantissima*, as Table 9 illustrates. Methyl alpha-D-glucopyranoside (monosaccharide) had the highest area% in the 4000-ppm group (33.13%) in comparison to the control (29.68%), and it decreased as salinity increased (18.77% at 2000 ppm). Methyl beta-D-glucopyranoside, a monosaccharide, rose with salinity in comparison to the control (3.37%; 6.8% at 2000 ppm and 6.64% at 4000 ppm). As salinity rose, the concentration of melatitose (a disaccharide) significantly decreased; the control group had the greatest area (6.12%), while the 4000 ppm group had the lowest (2.57%).

Table 8. Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on carbohydrate content (ppm) of *A. fragrantissima* in-vitro plantlets rooting stage

Treatments		Carbohydrate (ppm)
Salinity (ppm)	CTFAE (g/L)	
(a) Main effect of salinity		
Control		10104.5 a*
2000		6541.833 c
4000		6850.166 b
(b) Main effect of CTFAE		
	0.0	6220.5 b
	0.5	6120.166 c
	1.0	11155.8 a
(c) Interacting effects of salinity and CTFAE		
Control	0.0	5747.5 h
	0.5	9873.5 c
	1.0	14692.5 a
2000	0.0	5962.5 g
	0.5	6741.5 f
	1.0	6921.5 e
4000	0.0	6951.5 d
	0.5	1745.5 i
	1.0	11853.5 b

* Means followed by the same letter within a column are not significantly different at 0.05 level of probability according to Duncan test

The concentration of alpha-lactose, a disaccharide, increased with salinity; it peaked at 4000 ppm (1.15%) compared to lower levels at 2000 ppm (0.65%) and control (0.36%). Ester compounds, (R)-dimethyl 2-hydroxysuccinate, peak at 2000 ppm (1.51%) and slightly increase with salinity. The 5-acetyl valeric acid, methyl ester is only present in the control (1.36%), and it is missing at greater salinities. While methyl palmitate declined as salinity rose, methyl tetradecanoate stayed constant throughout treatments (fatty acid esters). With increasing salinity, fatty acids and their derivatives, such as pentadecylic acid, decreased (from 7.36% in the control to 4.94% at 4000 ppm). While methyl 9-octadecenoate significantly rose at 4000 ppm (2.6%), methyl oleate decreased as salinity increased. Phenolic substances, the level of 2,4-Di-tert-butylphenol rose to 2000 ppm (1.01%) and then somewhat down to 4000 ppm (0.97%). A rise of 4000 ppm (1.64%) was seen in 3,5-Di-tert-butyl-4-hydroxybenzaldehyde. Terpenoids and other organic compounds, Megastigmatrienone and (Z)-9-Hexadecenoic acid, methyl ester, both showed decreased levels with increased salinity. N-nitrosohexamethyleneimine (organic hydrazide), present only at 4000 ppm (3.18%). Acetylacetophenone and propofol, emerged only at 4000 ppm. Beta-Sitosterol (Sterol), Only present in the control (0.83%). n-nitrosodiethylamine and 2(1H)-quinolinone, 4-methyl, present only at higher salinity levels.

The results presented in Table 10 and Sup. Table 2 demonstrated the impact of varying concentrations of CTFAE on the phytochemical composition of methanol leaf extracts obtained from *A. fragrantissima* in vitro plantlets throughout the multiplication stage.

Table 9. Effect of different concentration Salinity levels (ppm) on the phytochemical composition of methanol leaf extracts from *A. fragrantissima* in-vitro plantlets multiplication stage

Compound Name	Area%		
	Salinity levels (ppm)		
	Control	2000	4000
Melezitose	6.12 a*	5.6 b	2.57 c
pyrrolidin-2-ylmethanol	3.12c	5.88 a	3.99 b
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-4,4,6-Trimethyl-1,3-oxazinane-2-thione	10.19 b	13.11b	9.42c
(R)-Dimethyl 2-hydroxysuccinate	0.31a	0 b	0 b
5-Acetyl Valeric acid, methyl ester	1.31 b	1.51 a	1.11 b
DL-Proline, 5-oxo-, methyl ester	1.36 a	0 b	0 b
Anisole	0.37b	0.36 b	0.39 a
Acetophenone	1.02 a	0.24 b	0.31 b
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	0.29 a	0 b	0 b
2,4-Di-tert-butylphenol	4.52 a	0 b	0 b
Lauric acid methyl ester	0.88 c	1.01 a	0.97 b
o-Ethoxybenzylamine	0.28 a	0.26 b	0.25 b
Methyl alpha-D-glucopyranoside	0.76 a	0 b	0 b
Methyl tetradecanoate	29.68 b	18.77 c	33.13 a
Methyl beta-D-glucopyranoside	0.33 a	0.30 a	0.30 a
Myristic Acid	3.37 b	6.8 a	6.64 a
alpha-Lactose	0.48 a	0.31 b	0 c
Beta-Sitosterol	0.36 c	0.65 b	1.15 a
(Z)-9-Hexadecenoic acid, methyl ester	0.83 a	0 b	0 b
Methyl palmitate	0.84 a	0.42 b	0.5 b
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	2.17 a	1.82 b	1.64 c
(S)-10-camphorsulfonic acid	0.41 b	0 c	1.64 a
2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl	3.93 a	1.15 b	0.79 c
Pentadecylic Acid	4.97 a	0 b	0 b
Megastigmatrienone	7.36 a	6.81 b	4.94 c
9,19-Cyclolanostan-3-ol, acetate, (3beta)	0.82 a	0.52 b	0.53 b
1-Nonadecene	0.28 a	0 b	0 b
Methyl oleate	0.74 b	0.76 b	0.89 a
Methyl 9-octadecenoate	3.39 a	2.91 b	0.49 c
Methyl stearate	0.49 b	0.44 b	2.6 a
9,12-Octadecadienoic acid	0.51 a	0.51 a	0 b
5-Hydroxymethylfurfural	1 b	1.14 a	1.15 a
octadecanoic acid	1.78 a	1.51 b	1.34 b
cis-9-Tricosene	1.26 a	1.11 b	1.03 b
1-Hexacosanol	0.12 c	0.18 b	0.23 a
Undecane	0.92 a	0.82 a	0.5 b
	0 b	0.03 a	0 b

Compound Name	Area%		
	Salinity levels (ppm)		
	Control	2000	4000
(S)-2,3,4,5-tetrahydropyridine-2-carboxylic acid	0 b	0.17 a	0 b
N-nitrosodiethylamine	0 b	0.08 a	0 b
1,4-Anhydro-d-mannitol	0 b	0.15 a	0 b
2(1H)-Quinolinone, 4-methyl-	0 c	2.32 a	2.2 a
Ribitol	0 c	8.36 a	5.38 b
2-Pentanone	0 b	1.65 a	0 b
Guanosine	0 c	2.99 b	3.08 b
Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	0 b	1.13 a	0 b
Cardenolide	0 b	1.3 a	0 b
2-Deoxy-D-ribose	0 b	0.13 a	0 b
(Z,E)-7,11-Hexadecadien-1-yl acetate	0 b	1.17 a	0 b
DL-1,2-Hexanediol	0 b	0.33 a	0 b
N-Nitrosohexamethyleneimine	0 b	0 b	3.18 a
Oxamic hydrazide	0 b	0 b	0.79 a
Durenol	0 b	0 b	0.22 a
Acetylacetophenone	0 b	0 b	0.24 a
Propofol	0 b	0 b	0.19 a
(-)-(Z)-Verbenol	0 b	0 b	0.22 a
Octyl beta-D-glucopyranoside	0 b	0 b	1.32 a
1,6-Anhydro-.beta.-D-glucopyranose	0 b	0 b	0.26 a
2,2-Dimethylglutaric acid	0 b	0 b	0.59 a
Methyl stearolate	0 b	0 b	0.27 a
(Z)-9-Tricosene	0 b	0 b	0.08 a

* Means followed by the same letter within a row are not significantly different at 0.05 level of probability according to Duncan test

The data revealed that alpha-lactose and methyl alpha-d-glucopyranoside was only present in the control treatments, whereas melezitose and Methyl beta-D-glucopyranoside was highest in the control and significantly reduced with CTFAE concentration. (R)-dimethyl 2-hydroxysuccinate and 5-acetyl valeric acid, methyl ester exclusive to the control group (1.31% and 1.36%, respectively). Methyl palmitate displayed erratic reactions, peaking at 1 g/L CTFAE extract (2.3%). Methyl tetradecanoate was detected on control and 1 g/L CTFAE. When CTFAE was used at 0.5 g/L, 2,4-di-tert-butylphenol levels increased to 1.12% from the control's 0.88%. Only the control group (0.41%) contains 3,5-di-tert-butyl-4-hydroxybenzaldehyde. (S)-10-camphorsulfonic acid and 2-cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl compounds significantly increased with CTFAE 1g/L concentration compared to control treatment. Methyl oleate and methyl 9-octadecenoate were elevated at 1g/L of CTFAE, whereas pentadecylic acid and methyl stearate were only detected in the control (7.36% and 0.51%, respectively).

Table 10. Effect of different concentration CTFAE (g/L) on the phytochemical composition of methanol leaf extracts from *A. fragrantissima* in-vitro plantlets multiplication stage

Compound Name	Area%		
	CTFAE (g/L)		
	Control	0.5	1
Melezitose	6.12 a*	3.29 c	4.32 b
pyrrolidin-2-ylmethanol	3.12 c	4.65 a	3.64 b
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-4,4,6-Trimethyl-1,3-oxazinane-2-thione	10.19 b	11.53 a	11.26 a
(R)-Dimethyl 2-hydroxysuccinate	0.31 a	0 b	0 b
5-Acetyl Valeric acid, methyl ester	1.31 a	0 b	0 b
DL-Proline, 5-oxo-, methyl ester	1.36 a	0 b	0 b
Anisole	0.37 b	0.37 b	0.56 a
Acetophenone	1.02 a	0 b	0 b
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	0.29 a	0 b	0 b
2,4-Di-tert-butylphenol	4.52 a	0 c	2.26 b
Lauric acid methyl ester	0.88 b	1.12 a	0.73 b
o-Ethoxybenzylamine	0.28 b	0.36 a	0.3 ab
Methyl alpha-D-glucopyranoside	0.76 c	22.14 a	12.98 b
Methyl tetradecanoate	29.68 a	0 b	0 b
Methyl beta-D-glucopyranoside	0.33 a	0 b	0.34 a
Myristic Acid	3.37 a	3.29 a	2.04 b
alpha-Lactose	0.48 a	0 b	0 b
Beta-Sitosterol	0.36 a	0 b	0 b
(Z)-9-Hexadecenoic acid, methyl ester	0.83 a	0 b	0 b
Methyl palmitate	0.84 a	0.6 b	0.68 b
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	2.17 a	1.67 b	2.3 a
(S)-10-camphorsulfonic acid	0.41 a	0 b	0 b
2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl	3.93 b	3.79 b	7.22 a
Pentadecylic Acid	4.97 c	5.12 b	10.39 a
Megastigmatrienone	7.36 a	0 b	0 b
9,19-Cyclolanostan-3-ol, acetate, (3beta)	0.82 b	0.89 b	1.17 a
1-Nonadecene	0.28 a	0 b	0 b
Methyl oleate	0.74 b	0c	1.02 a
Methyl 9-octadecenoate	3.39 b	2.62 c	3.91 a
Methyl stearate	0.49 a	0 b	0 b
9,12-Octadecadienoic acid	0.51 a	0 b	0 b
5-Hydroxymethylfurfural	1 b	0c	2.51 a
octadecanoic acid	1.78 a	0 b	0 b
cis-9-Tricosene	1.26 c	1.76 b	2.41 a
1-Hexacosanol	0.12 a	0 b	0 b
2(1H)-Quinolinone, 4-methyl-	0.92 a	0.49 b	0c
	0c	1.04 b	1.9 a

Compound Name	Area%		
	CTFAE (g/L)		
	Control	0.5	1
N-Nitrosohexamethyleneimine	0 b	2.98 a	0 b
N-Allylmorpholine	0 b	0.42 a	0 b
4-Methoxy-2,5-dimethylbenzaldehyde	0 b	1.11 a	0 b
Guanosine	0 b	1.97 a	0 b
Decane, 1-iodo-	0 b	0.35 a	0 b
1-Butanamine, N,N-dimethyl-	0 b	0.38 a	0 b
Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	0 b	0.83 a	0 b
Beta-Sitosterol	0 b	0.85 a	0 b
2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl-	0 c	5.12 b	10.39 a
Palmitic acid	0 b	7.39 a	7.29 a
1,2-Hexanediol	0 b	0.49 a	0b
1-Octadecanol	0 c	1.03 a	0.34 b
Phytol	0 c	0.61 b	0.9 a
9,12,15-Octadecatrienoic acid, methyl ester	0 b	2.76 a	2.72 a
Tetraethoxypropane	0 b	0 b	1.31 a
2-Methyl-2-propyl-1,3-propanediol	0 b	0 b	2.01 a
4-Chloroanisole	0 b	0 b	0.42 a
Camphor oxime	0 b	0 b	0.32 a

* Means followed by the same letter within a row are not significantly different at 0.05 level of probability according to Duncan test

The levels of terpenoids such as phytol, megastigmatrienone, and 9,12,15-octadecatrienoic acid methyl ester were found to be higher at 1 g/L (1.17%, 0.9%, and 2.76%) when CTFAE was present. Whereas the presence of n-nitrosohexamethyleneimine is limited to 0.5 g/L (2.98%). Beta-sitosterol, present only on CTFAE at 0.5 g/L (0.83%). 1-Octadecanol was increased with CTFAE (1.03% at 0.5 g/L). Tetraethoxypropane and 2-methyl-2-propyl-1,3-propanediol were only present at 1 g/L (2.01% and 1.31%, respectively).

The effects of salinity and CTFAE on the phytochemical content of methanol leaf extracts from *A. fragrantissima* in-vitro plantlets multiplication stage were displayed in Table 11 Sup. Table 2. Melezitose was found to be most concentrated in the (2000 ppm + 1 g/L CTFAE) group (6.45%), but the lowest amount was seen when salinity was combined with 1 g/L of CTFAE (2.34% at 4000 ppm + 1 g/L CTFAE). The maximum concentrations of methyl alpha-D-glucopyranoside and beta-D-glucopyranoside were 4000 ppm + 1 g/L CTFAE 4000 ppm + 1 g/L CTFAE (33.1% and 11.06%), respectively. (R)-Dimethyl 2-hydroxysuccinate present at its highest level with the control (1.36%), and decreased with salinity and CTFAE treatments while, 5-Acetyl Valeric acid, methyl ester present only in the control (1.36%). The methyl laurate, 1-methylpiperidine, 1,4-diacetylbenzene, 1,4-anhydro-d-mannitol octyl beta-d-glucopyranoside, 2,2-dimethylglutaric acid, beta-Lactose and 1-tridecene found only in the plant which treated with 4000 ppm + 1 g/L CTFAE (Table 11).

Table 11. Effect of Interacting effects of salinity (ppm) and CTFAE (g/L) on the phytochemical composition of methanol leaf extracts from *A. fragrantissima* in-vitro plantlets multiplication stage

Compound Name	Area%				
	Effects of salinity (ppm) and CTFAE (g/L)				
	Control	2000+0.5	2000+1	4000+0.5	4000+1
Melezitose	6.12 b*	2.44 d	6.45 a	4.32 c	2.34 d
pyrrolidin-2-ylmethanol	3.12 e	5.97 c	4.2 d	8.88 a	6.27 b
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	10.19 c	8.57 d	12.47 a	10.78 b	7 e
4,4,6-Trimethyl-1,3-oxazinane-2-thione	0.31 b	0 c	0 c	0 c	0.8 a
(R)-Dimethyl 2-hydroxysuccinate	1.31 a	0.85 b	0 c	0 c	0.82 b
5-Acetyl Valeric acid, methyl ester	1.36 a	0 b	0 b	0 b	0 b
DL-Proline, 5-oxo-, methyl ester	0.37 b	0.44 b	0.86 a	0.92 a	0.31 c
Anisole	1.02 a	0 b	0 b	0 b	0 b
Acetophenone	0.29 a	0 b	0 b	0 b	0 b
1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	4.52 a	3.92 b	4.11 a	3.25 c	0 d
2,4-Di-tert-butylphenol	0.88 b	0.51 c	1.03 a	0 d	0 d
Lauric acid methyl ester	0.28 a	0 b	0 b	0 b	0 b
o-Ethoxybenzylamine	0.76 a	0 b	0 b	0 b	0 b
Methyl alpha-D-glucopyranoside	29.68 b	26.45c	13.42 e	19.61 d	33.1 a
Methyl tetradecanoate	0.33 c	0.49 b	0.64 a	0.63 a	0.34 c
Methyl beta-D-glucopyranoside	3.37 d	4.92 c	1.55 e	5.04 b	11.06 a
Myristic Acid	0.48 a	0 b	0 b	0 b	0 b
alpha-Lactose	0.36 a	0 b	0 b	0 b	0 b
Beta-Sitosterol	0.83 a	0 b	0 b	0 b	0 b
(Z)-9-Hexadecenoic acid, methyl ester	0.84 b	0.46 c	0.8 b	1.02 a	0.53 c
Methyl palmitate	2.17 b	1.47 c	4.06 a	3.78 a	2.31 b
3,5-Di-tert-butyl-4-hydroxybenzaldehyde	0.41 a	0 c	0 c	0.3 b	0 c
(S)-10-camphorsulfonic acid	3.93 b	3.65 b	6.38 a	3.38 b	0 c
2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl	4.97 b	0 c	8.45 a	0 c	0 c
Pentadecylic Acid	7.36 a	0 b	0 b	0 b	0 b
Megastigmatrienone	0.82 b	1.15 a	0.75 b	0.78 b	0.52 c
9,19-Cyclolanostan-3-ol, acetate, (3beta)	0.28	0 b	0 b	0 b	0 b
1-Nonadecene	0.74 c	0.82 b	0.68 c	1.0 a	0.98 a
Methyl oleate	3.39 c	2.62 d	6.97 a	6.18 b	3.37 c
Methyl 9-octadecenoate	0.49 c	0 d	0.77 b	0.91 a	0.55 c
Methyl stearate	0.51 a	0 b	0 b	0 b	0 b

Compound Name	Area%				
	Effects of salinity (ppm) and CTF AE (g/L)				
	Control	2000+0.5	2000+1	4000+0.5	4000+1
9,12-Octadecadienoic acid	1.0 a	0 c	0 c	0 c	0.36 b
5-Hydroxymethylfurfural	1.78 a	0 c	0 c	0 c	0.84 b
octadecanoic acid	1.26 d	1.58 c	2.07 a	1.71 b	0.96 e
cis-9-Tricosene	0.12 a	0 b	0 b	0 b	0 b
1-Hexacosanol	0.92 b	0.96 b	1.46 a	0 d	0.56 c
Palmitic acid	0 e	6.56 a	4.31 c	5.66 b	3.43 d
1-Octadecanol	0 c	0.56 b	1.01 a	0 c	0 c
Camphor oxime	0b	0.4 a	0 b	0 b	0 b
Furaneol	0b	9.575 a	0 b	0 b	0 b
1-nitrosoazepan-3-ol	0 b	1.85 a	0 b	0 b	0 b
Mannose	0 c	1.34 a	0 c	0.8 b	1.4 a
Beta-Sitosterol	0b	1.4 a	0 b	0 b	0 b
D-erythro-Pentose, 2-deoxy	0b	0.36 a	0 b	0 b	0 b
beta-Caryophyllene	0c	0.44 a	0 c	0.5 b	0 c
Methyl linoleate	0b	1.77 a	0 b	0 b	0 b
9,12-Tetradecadien-1-ol, acetate, (9Z,12E)-	0c	2.11 a	1.22 b	0 c	0 c
N-nitrosodiethylamine	0 b	0 b	0.51 a	0 b	0 b
2,3-Dihydro-2,5-dihydroxy-6-methyl-4-H-pyran-4-one	0 b	0 b	2.77 a	2.99 a	0 b
1,1,3,3-Tetramethoxypropane	0 b	0 b	1.52 a	0 b	0 b
4-Chloroanisole	0 d	0.29 c	0.33 b	0.31 b	0.57 a
1-Pentadecanol	0 b	0 b	0.1a	0 b	0 b
2-Methyl-1,4-bis(trimethylsiloxy)butane	0 b	0 b	0.89 a	0 b	0 b
Hydroquinone	0 b	0 b	0.98 a	0 b	0 b
1-FLUORONONANE	0 b	0 b	0 b	0.55 a	0 b
4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a α ,4 β ,4a β ,7 α ,7a β ,7b α)]-	0 b	0 b	0 b	0.44 a	0 b
Citronellyl propionate	0 b	0 b	0 b	0.08 a	0 b
Anhydro-d-mannosan	0 b	0 b	0 b	0.13 a	0 b
Carvone Epoxide	0 b	0 b	0 b	0.25 a	0 b
8,11-Octadecadienoic acid, methyl ester	0 c	0 c	0c	0.59 a	0.49 b
stearic acid	0 c	0 c	0c	1.04 a	0.83 b
Erucamide	0 b	0 b	0 b	0.36 a	0 b
1,1,3,3-Tetraethoxypropane	0 b	0 b	0 b	1.16 a	0 b
Carcinogen	0 b	0 b	0 b	1.32 a	0 b
2,6-Diethylcyclohexanone	0 b	0 b	0 b	0.54 a	0 b
Quinuclidine	0 b	0 b	0 b	0.3 a	0 b
Methyl laurate	0 b	0 b	0 b	0.56 a	0.29 a

Compound Name	Area%				
	Effects of salinity (ppm) and CTFAE (g/L)				
	Control	2000+0.5	2000+1	4000+0.5	4000+1
1-methylpiperidine	0 b	0 b	0 b	0 b	0.2 a
1,4-Diacetylbenzene	0 b	0 b	0 b	0 b	0.45 a
1,4-Anhydro-d-mannitol	0 b	0 b	0 b	0 b	2.56 a
Octyl beta-D-glucopyranoside	0 b	0 b	0 b	0 b	1.85 a
2,2-Dimethylglutaric acid	0 b	0 b	0 b	0 b	0.21 a
beta-Lactose	0 b	0 b	0 b	0 b	0.3 a
1-Tridecene	0 b	0 b	0 b	0 b	0.07 a

* Means followed by the same letter within a row are not significantly different at 0.05 level of probability according to Duncan test

Discussion

Plant products are thought to be restricted by salt stress. Thus, a variety of substances have been used to reduce the negative consequences of salt. Chemical analysis of *C. ternatea* flower powder reveals the presence of several compounds, including macro- and micro-nutrients, vitamins, fat, as well as fatty acids, esters, alcohols, aldehydes, terpenoids, and nitrogen-containing compounds which important for plant growth and its health, and particularly important in light of climate change as they can contribute to beneficial roles in plant growth and development (Niu et al., 2022). *C. ternatea* flower powder's GCMS analysis reveals that it contains 4 α -Methylandrostane-2,3-diol-1,17-dione and stigmasterol is a steroid, which play a vital role in plant growth regulation by affecting cell elongation, division, and differentiation (Wei and Li, 2020). They also improve plant resistance to abiotic stimuli such as salinity, dehydration, and heat (Wei and Li, 2020; Chaudhuri et al., 2022). Terpene phytol is a precursor of vitamin E and chlorophyll that aids in plant development and metabolism (Byju et al., 2013; Niu et al., 2022). A variety fatty acid has been found in the *C. ternatea* flower powder including linoleic, oleic and palmitic. Linoleic acid, an essential part of membrane phospholipids, has an impact on the membrane's fluidity and integrity (Prochowska et al., 2024). It is the precursor of jasmonic acid, a plant hormone implicated in defense and stress responses (Zhang et al., 2019). It strengthens a plant's defenses against abiotic stresses such as drought and salinity (Ahmad et al., 2024). Oleic acid influences lipid metabolism, which in turn enhances seed germination and seedling vigor (Dhaliwal et al., 2024). Numerous hormonal signaling mechanisms that control growth and development are influenced by oleic acid (Yang et al., 2024). It has been demonstrated to enhance plants' reactions to stress (Gogna et al., 2020). Saturated fatty acids like palmitic and stearic acid are involved in membrane construction and energy storage (Ma et al., 2021).

One of the most frequent abiotic stressors that lowers crop plant yield is salt stress. In our research, saline levels reduced *A. fragrantissima* seed germination percentage and the growth of the explant at the multiplication and root stages over the control treatment with the exception of the multiplication stage, where we discovered that moderate salinity (2000 ppm) levels improved the explant's growth parameters compared to control treatment. These findings are consistent with past research on the responses of many plant species to salt stress during germination (Gholami et al., 2012; Molnar et al., 2024a,b; Arafa et al., 2024; Elkhodary et al., 2024).

Elevated salinity reduced seed water intake exacerbates the impacts of stress by reducing imbibition and seed turgescence (Tarchoun et al., 2022). Raising the saline concentration inhibits root development, and germination percentage via lowering the osmotic potential. Salinity also causes ion toxicity and oxidative stress which reduced plant growth (Atta et al., 2023). At low concentrations, salinity has a significant positive impact on shoot growth *in vitro* due to its ability to increase osmolarity (Gholami et al., 2012). The concentration of photosynthetic pigments and carbohydrate in *A. fragrantissima* leaves increased with increased salt levels, these results are consistent with findings from other plants (Trifunović-Momčilov et al., 2021; Zhang et al., 2024). Increased photosynthetic pigment levels in *A. fragrantissima* plants cultivated in salinity conditions—2000 ppm and 4000 ppm—play a crucial antioxidant role in mitigating the negative effects produced by the formation of reactive oxygen species (ROS) (Aslam et al., 2016). Salinity causes osmotic stress and reduces the amount of water that plants can absorb. Through controlling osmotic adjustment and carbon storage in plants, soluble carbohydrates serve critical functions as osmolytes in salt stress (Ahmad et al., 2017). The nutritional composition of *A. fragrantissima* leaves was strongly impacted by salinity, showing significant rises in potassium (K), magnesium (Mg), calcium (Ca) and sodium (Na) and significant reductions in nitrogen (N), iron (Fe), phosphorus (P) and zinc (Zn). Toxic ions including Na build up in high level of salinity, causing ion toxicity and reducing nutrient intake, which worsens damage to plant tissues and cells, may be the cause of the decrease in N and Fe (Isayenkov and Maathuis, 2019). Reduced zinc levels from high salinity affect photosynthesis and sugar transport in plants, which alters the control of carbohydrate metabolism and slows down plant growth (Fan et al., 2021). On the other hand, the elevated K, Mg, and Ca levels could be the result of the plant trying to preserve ionic homeostasis by counteracting the osmotic stress (Osakabe et al., 2013). The phytochemical profile of *A. fragrantissima* methanol leaf extracts is considerably impacted by salinity, with changes observed in sugars, esters, fatty acids, phenolics, and other organic compounds. The specific rise or fall in certain sugars, esters, fatty acids, phenolic compounds, terpenoids, and other organic compounds points to the distribution of resources in response to the stress of salt. Accumulation of sugar acts as an osmolyte to mitigate the deleterious consequences of salt stress (Almodares et al., 2008). Fatty acids may be involved in the plant's defense system against salt stress during development (Aziz et al., 2015). Phenolic molecules, such as 2,4-Di-tert-butylphenol and 3,5-Di-tert-butyl-4-hydroxybenzaldehyde, increase in response to salinity stress. phenolic compounds that scavenge reactive oxygen species (ROS) generated during stress. This helps shield biological tissues from oxidative damage (Rodrigues et al., 2024).

In our study, we found that CTFAE increased the percentage of *A. fragrantissima* seeds that germinated as well as the explant's growth during the multiplication and root stages as well as certain phytochemical component in plant tissue when compared to the control treatment. This increase was also associated with higher levels of photosynthetic pigments, carbohydrates, nutrients like N, P, K, Mg, Ca, and Zn in plant tissue. Suggests that the aquatic extract of CTFAE is a very promising bio stimulant, plant extracts with active ingredients that promote plant development also produced the similar outcomes (El Sherif et al., 2020; Peron et al., 2024; Kovács et al., 2024; Kaniyassery et al., 2024; Sohrabi et al., 2025). The current study's observations of the CTFAE enhancing effects on seed germination %, explant growth during multiplication, and rooting stages in *A. fragrantissima* plants cultivated under high saline (4000 ppm) were consistent with other results on a variety of plant species maintained under salinity (Mutlu-Durak et al.,

2023; Melito et al., 2024; Kovács et al., 2024; Salvage et al., 2024). In addition to the previously mentioned characteristics, CTFAE increased the amount of carbohydrates and decreased the buildup of Na ions while boosting N, P, and K ions in plant cells exposed to 4000 ppm salt. Plants under salt stress have been shown to have higher osmo-tolerance in response to increases in K (Van Zelm et al., 2020). The plant under high salinity level also exhibited higher concentrations of certain effect compounds, including melezitose, methyl alpha-D-glucopyranoside, and beta-D-glucopyranoside, which are involved in defense and stress reactions (Al-Khayri et al., 2023).

Conclusions

CTFAE, used alone or in conjunction with varied salinity levels, boosted the growth and phytochemical compound of the *A. fragrantissima* plant, making it an especially effective biostimulant. Our findings showed that applying 1 g/L CTFAE resulted in the largest growth rate in the multiplication stage, whereas applying 0.5 g/l resulted in the highest growth parameter in the rooting stage. *A. fragrantissima* grows less in high salinity than the control and thrives in intermediate salinity (2000 ppm). At 4000 ppm of salinity, 0.5 g/LCTFAE might reduce the harmful effects of salt stress.

Supplementary materials. *Table S1:* title; Effects of salinity levels, CTFAE concentrations, and their interactions on the phytochemical composition of methanolic leaf extracts from *A. fragrantissima in-vitro* plantlets.

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Conflicts of Interest. The authors declare no conflict of interest.

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APPENDIX

Sup. Table 1. Chemical properties and compositions of the seawater

Salinity Level (ppm)	Cations (mg/L)			Anions (m/L)				Sodium Adsorption Ratio (SAR)
	Ca ²⁺	Mg ²⁺	Na ⁺	CO ₃ ²⁻	HCO ₃ ⁻	SO ₄ ²⁻	Cl ⁻	
35000	520	1500	13.044	24	171	3100	23000	65

Sup. Table 2. Effects of salinity levels (ppm), CTFAE (g/L) concentrations, and their interactions on the phytochemical composition of methanolic leaf extracts from *A. fragrantissima* in-vitro plantlets

Treatments		Peak	Essential Oil Compounds	RT, min	Area, %	Molecular Weight (g/mol)	Molecular Formula
Salinity level (ppm)	CTFAE (g/L)						
Control	0						
		1	Melezitose	8.587	6.12	504.44	C18H32O16
		2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.868	10.19	144.12	C6H8O4
		3	pyrrolidin-2-ylmethanol	10.681	3.12	101.15	C5H11NO
		5	4,4,6-Trimethyl-1,3-oxazinane-2-thione	11.351	0.31	159.25	C7H13NOS
		6	(R)-Dimethyl 2-hydroxysuccinate	11.696	1.31	162.14	C6H10O5
		7	5-Acetyl Valeric acid, methyl ester	12.385	1.36	240.32	C12H16O3S
		9	DL-Proline, 5-oxo-, methyl ester	13.861	0.37	157.17	C6H9NO3
		10	Anisole	14.136	1.02	108.14	C7H8O
		11	Acetophenone	14.6	0.29	120.15	C6H5COCH3
		12	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	15.045	4.52	151.12	C4H9NO5
		14	2,4-Di-tert-butylphenol	15.627	0.88	206.32	C14H22O
		15	Lauric acid methyl ester	15.837	0.28	214.34	C13H26O2
		16	o-Ethoxybenzylamine	16.732	0.76	151.21	C9H13NO
		17	Methyl alpha-D-glucopyranoside	17.321	29.68	194.18	C7H14O6
		18	Methyl tetradecanoate	18.312	0.33	242.40	C15H30O2
		19	Methyl beta-D-glucopyranoside	18.568	3.37	194.18	C7H14O6
		20	Myristic Acid	18.707	0.48	228.37	C14H28O2
		21	alpha-Lactose	18.767	0.36	342.30	C12H22O11
		22	Beta-Sitosterol	18.889	0.83	414.70	C29H50O
		23	(Z)-9-Hexadecenoic acid, methyl ester	20.317	0.84	254.41	C16H30O2
		24	Methyl palmitate	20.543	2.17	270.50	C17H34O2
		25	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	20.6	0.41	234.33	C15H22O2

		26	(S)-10-camphorsulfonic acid	20.668	3.93	231.29	C10H15O4S-
		27	2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl	20.79	4.97	164.24	C11H16O
		28	Pentadecylic Acid	20.906	7.36	242.40	C15H30O2
		29	Megastigmatrienone	20.998	0.82	190.28	C13H18O
		30	9,19-Cyclolanostan-3-ol, acetate, (3beta)	21.973	0.28	470.80	C32H54O2
		31	1-Nonadecene	22.173	0.74	266.50	C19H38
		32	Methyl oleate	22.317	3.39	296.50	C19H36O2
		33	Methyl 9-octadecenoate	22.375	0.49	296.50	C19H36O2
		34	Methyl stearate	22.567	0.51	298.5 0	C19H38O2
		35	9,12-Octadecadienoic acid	22.608	1	280.40	C18H32O2
		36	5-Hydroxymethylfurfural	22.666	1.78	504.44	C ₆ H ₆ O ₃
		38	octadecanoic acid	22.896	1.26	284.50	C18H36O2
		39	cis-9-Tricosene	24.09	0.12	322.6	C23H46
		40	1-Hexacosanol	25.855	0.92	382.70	C26H54O
2000	0						
		1	Melezitose	8.575	5.6	504.44	C18H32O16
		2	Undecane	8.976	0.03	156.31	C11H24
		3	pyrrolidin-2-ylmethanol	9.121	0.17	101.15	C5H11NO
		4	N-nitrosodiethylamine	9.705	0.08	102.14	C4H10N2O
		5	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.875	13.11	144.12	C6H8O4
		6	1,4-Anhydro-d-mannitol	10.336	0.15	164.16	C6H12O5
		7	pyrrolidin-2-ylmethanol	10.718	5.88	101.15	C5H11NO
		8	2(1H)-Quinolinone, 4-methyl-	10.972	2.32	159.18	C10H9NO
		10	Ribitol	11.486	8.36	152.15	C5H12O5
		11	(R)-Dimethyl 2-hydroxysuccinate	11.687	1.51	162.14	C6H10O5
		12	2-Pentanone	12.413	1.65	86.13	C5H10O
		13	DL-Proline, 5-oxo-, methyl ester	13.845	0.36	157.17	C6H9NO3
		14	Anisole	14.119	0.24	108.14	C7H8O

	15	Guanosine	14.962	2.99	283.24	C10H13N5O5
	16	2,4-Di-tert-butylphenol	15.612	1.01	206.32	C14H22O
	17	Lauric acid methyl ester	15.822	0.26	214.34	C13H26O2
	18	Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	16.716	1.13	180.20	C10H12O3
	19	Methyl alpha-D-glucopyranoside	17.281	18.77	194.18	C7H14O6
	20	Methyl tetradecanoate	18.296	0.3	242.40	C15H30O2
	21	Methyl beta-D-glucopyranoside	18.56	6.8	194.18	C7H14O6
	22	Myristic Acid	18.692	0.31	228.37	C14H28O2
	23	alpha-Lactose	18.738	0.65	342.30	C12H22O11
	24	Cardenolide	18.865	1.3	342.50	C23H34O2
	25	2-Deoxy-D-ribose	19.939	0.13	134.13	C5H10O4
	26	(Z)-9-Hexadecenoic acid, methyl ester	20.299	0.42	254.41	C16H30O2
	27	Methyl palmitate	20.525	1.82	270.50	C17H34O2
	28	(S)-10-camphorsulfonic acid	20.65	1.15	231.29	C10H15O4S-
	29	(Z,E)-7,11-Hexadecadien-1-yl acetate	20.771	1.17	280.40	C18H32O2
	30	Pentadecylic Acid	20.891	6.81	242.40	C15H30O2
	31	Megastigmatrienone	20.98	0.52	190.28	C13H18O
	32	DL-1,2-Hexanediol	21.163	0.33	118.17	C6H14O2
	33	1-Nonadecene	22.154	0.76	266.50	C19H38
	34	Methyl oleate	22.299	2.91	296.50	C19H36O2
	35	Methyl 9-octadecenoate	22.358	0.44	296.50	C19H36O2
	36	Methyl stearate	22.55	0.51	298.50	C19H38O2
	37	9,12-Octadecadienoic acid	22.591	1.14	280.40	C18H32O2
	38	5-Hydroxymethylfurfural	22.647	1.51	504.44	C ₆ H ₆ O ₃
	40	octadecanoic acid	22.877	1.11	284.50	C18H36O2
	41	cis-9-Tricosene	24.706	0.18	322.60	C23H46
	42	1-Hexacosanol	25.836	0.82	382.70	C26H54O
4000	0					

	1	Melezitose	8.526	2.57	504.44	C18H32O16
	2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.864	9.42	144.12	C6H8O4
	3	pyrrolidin-2-ylmethanol	10.691	3.99	101.15	C5H11NO
	4	2(1H)-Quinolinone, 4-methyl-	10.97	2.2	159.18	C10H9NO
	6	N-Nitrosohexamethyleneimine	11.342	3.18	128.17	C6H12N2O
	7	Ribitol	11.551	5.38	152.15	C5H12O5
	8	Oxamic hydrazide	11.675	0.79	103.08	C2H5N3O2
	10	Durenol	12.8	0.22	150.22	C10H14O
	11	DL-Proline, 5-oxo-, methyl ester	13.841	0.39	157.17	C6H9NO3
	12	Anisole	14.12	0.31	108.14	C7H8O
	13	p-Acetylacetophenone	14.587	0.24	162.18	C10H10O2
	14	Guanosine	14.954	3.08	283.24	C10H13N5O5
	15	Propofol	15.201	0.19	178.27	C12H18O
	16	2,4-Di-tert-butylphenol	15.615	0.97	206.32	C14H22O
	17	Lauric acid methyl ester	15.825	0.25	214.34	C13H26O2
	18	(-)-(Z)-Verbenol	16.719	0.22	152.23	C10H16O
	19	Methyl alpha-D-glucopyranoside	17.374	33.13	194.18	C7H14O6
	20	Methyl tetradecanoate	18.298	0.3	242.40	C15H30O2
	21	Methyl beta-D-glucopyranoside	18.558	6.64	194.18	C7H14O6
	22	alpha-Lactose	18.734	1.15	342.30	C12H22O11
	23	Octyl beta-D-glucopyranoside	18.839	1.32	292.37	C14H28O6
	24	1,6-Anhydro-.beta.-D-glucopyranose	19.939	0.26	162.14	C6H10O5
	25	(Z)-9-Hexadecenoic acid, methyl ester	20.301	0.5	254.41	C16H30O2
	26	Methyl palmitate	20.526	1.64	270.50	C17H34O2
	27	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	20.583	0.24	234.33	C15H22O2
	28	(S)-10-camphorsulfonic acid	20.652	0.79	231.29	C10H15O4S-
	30	Pentadecylic Acid	20.89	4.94	242.40	C15H30O2
	31	Megastigmatrienone	20.983	0.53	190.28	C13H18O

		32	2,2-Dimethylglutaric acid	21.179	0.59	160.17	C7H12O4
		33	1-Nonadecene	22.156	0.89	266.50	C19H38
		34	Methyl stearolate	22.233	0.27	294.50	C19H34O2
		35	Methyl 9-octadecenoate	22.301	2.6	296.50	C19H36O2
		36	Methyl oleate	22.367	0.49	296.50	C19H36O2
		37	9,12-Octadecadienoic acid	22.592	1.15	280.40	C18H32O2
		38	5-Hydroxymethylfurfural	22.649	1.34	504.44	C ₆ H ₆ O ₃
		40	octadecanoic acid	22.877	1.03	284.50	C18H36O2
		41	cis-9-Tricosene	24.708	0.23	322.60	C23H46
		42	1-Hexacosanol	25.838	0.5	382.70	C26H54O
		43	(Z)-9-Tricosene	27.706	0.08	322.60	C23H46
Control	0.5						
		1	Melezitose	8.561	3.29	504.44	C18H32O16
		2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.886	11.53	144.12	C6H8O4
		3	pyrrolidin-2-ylmethanol	10.716	4.65	101.15	C5H11NO
		4	2(1H)-Quinolinone, 4-methyl-	10.981	1.04	159.18	C10H9NO
		6	N-Nitrosohexamethyleneimine	11.343	2.98	128.17	C6H12N2O
		7	(R)-Dimethyl 2-hydroxysuccinate	11.694	1.11	162.14	C6H10O5
		10	DL-Proline, 5-oxo-, methyl ester	13.844	0.37	157.17	C6H9NO3
		11	N-Allylmorpholine	14.708	0.42	127.18	C7H13NO
		12	4-Methoxy-2,5-dimethylbenzaldehyde	14.825	1.11	164.20	C10H12O2
		13	Guanosine	14.955	1.97	283.24	C10H13N5O5
		14	Decane, 1-iodo-	15.358	0.35	268.19	C10H21I
		15	2,4-Di-tert-butylphenol	15.616	1.12	206.32	C14H22O
		16	1-Butanamine, N,N-dimethyl-	15.701	0.38	101.19	C6H15N
		17	Lauric acid methyl ester	15.826	0.36	214.34	C13H26O2
		18	Benzaldehyde, 6-hydroxy-4-methoxy-2,3-dimethyl-	16.72	0.83	180.20	C10H12O3
		19	Methyl alpha-D-glucopyranoside	17.328	22.14	194.18	C7H14O6

	20	Methyl beta-D-glucopyranoside	18.56	3.29	194.18	C7H14O6
	21	alpha-Lactose	18.743	0.94	342.30	C12H22O11
	22	Beta-Sitosterol	18.876	0.85	414.70	C29H50O
	23	(Z)-9-Hexadecenoic acid, methyl ester	20.303	0.6	254.41	C16H30O2
	24	Methyl palmitate	20.529	1.67	270.50	C17H34O2
	25	(S)-10-camphorsulfonic acid	20.656	3.79	231.29	C10H15O4S-
	26	2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl-	20.779	5.12	164.24	C11H16O
	27	Palmitic acid	20.9	7.39	256.42	C16H32O2
	28	Megastigmatrienone	20.983	0.89	190.28	C13H18O
	29	1,2-Hexanediol	21.175	0.49	118.17	C6H14O2
	30	1-Octadecanol	22.159	1.03	270.49	C18H38O
	31	Methyl oleate	22.303	2.62	296.50	C19H36O2
	32	Phytol	22.4	0.61	296.50	C20H40O
	33	9,12-Octadecadienoic acid	22.602	2.96	280.40	C18H32O2
	34	9,12,15-Octadecatrienoic acid, methyl ester	22.658	2.76	292.50	C19H32O2
	36	octadecanoic acid	22.886	1.76	284.50	C18H36O2
	37	cis-9-Tricosene	24.711	0.49	322.60	C23H46
	40	1-Hexacosanol	25.841	2	382.70	C26H54O
	42	(Z)-9-Tricosene	27.71	0.71	322.60	C23H46
Control	1					
	1	Melezitose	8.562	4.32	504.44	C18H32O16
	2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.865	11.26	144.12	C6H8O4
	3	pyrrolidin-2-ylmethanol	10.669	3.64	101.15	C5H11NO
	4	2(1H)-Quinolinone, 4-methyl-	10.975	1.9	159.18	C10H9NO
	6	Tetraethoxypropane	11.691	1.31	220.31	C11H24O4
	7	2-Methyl-2-propyl-1,3-propanediol	12.364	2.01	132.20	C7H16O2
	8	DL-Proline, 5-oxo-, methyl ester	13.841	0.56	157.17	C6H9NO3
	10	4-Chloroanisole	14.129	0.42	142.58	C7H7ClO

		11	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	14.863	2.26	151.12	C4H9NO5
		12	2,4-Di-tert-butylphenol	15.621	0.73	206.32	C14H22O
		13	Lauric acid methyl ester	15.831	0.3	214.34	C13H26O2
		14	Oxotremorine	16.726	0.28	206.28	C12H18N2O
		15	Methyl alpha-D-glucopyranoside	17.182	12.98	194.18	C7H14O6
		16	Methyl tetradecanoate	18.306	0.34	242.40	C15H30O2
		17	Methyl beta-D-glucopyranoside	18.545	2.04	194.18	C7H14O6
		18	Nonanoic acid	18.692	0.84	158.24	C9H18O2
		19	beta-Caryophyllene	18.882	0.59	220.35	C15H24O
		20	(Z)-9-Hexadecenoic acid, methyl ester	20.308	0.68	254.41	C16H30O2
		21	Methyl palmitate	20.535	2.3	270.50	C17H34O2
		22	(S)-10-camphorsulfonic acid	20.662	7.22	231.29	C10H15O4S-
		23	2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl-	20.786	10.39	164.24	C11H16O
		24	Palmitic acid	20.9	7.29	256.42	C16H32O2
		25	Megastigmatrienone	20.991	1.17	190.28	C13H18O
		27	1-Nonadecene	22.165	1.02	266.51	C19H38
		28	Methyl linoleate	22.241	0.42	294.50	C19H34O2
		29	Methyl oleate	22.309	3.91	296.50	C19H36O2
		30	Phytol	22.404	0.9	296.50	C20H40O
		31	9,12-Octadecadienoic acid	22.605	2.51	280.40	C18H32O2
		32	9,12,15-Octadecatrienoic acid, methyl ester	22.661	2.72	292.50	C19H32O2
		34	octadecanoic acid	22.895	2.41	284.50	C18H36O2
		35	1-Octadecanol	24.082	0.34	270.49	C18H38O
		36	Camphor oxime	24.502	0.32	167.25	C10H17NO
		40	(Z)-9-Tricosene	25.847	3.43	322.60	C23H46
		42	1-Hexacosanol	27.718	1.31	382.70	C26H54O
2000	0.5						
		1	Melezitose	8.526	2.44	504.44	C18H32O16

	2	Furaneol	9.575	0.74	128.13	C ₆ H ₈ O ₃
	3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.864	8.57	144.12	C ₆ H ₈ O ₄
	4	pyrrolidin-2-ylmethanol	10.73	5.97	101.15	C ₅ H ₁₁ NO
	5	2(1H)-Quinolinone, 4-methyl-	10.983	1.09	159.18	C ₁₀ H ₉ NO
	7	1-nitrosoazepan-3-ol	11.337	1.85	144.17	C ₆ H ₁₂ N ₂ O ₂
	8	R)-Dimethyl 2-hydroxysuccinate	11.692	0.85	162.14	C ₆ H ₁₀ O ₅
	10	DL-Proline, 5-oxo-, methyl ester	13.842	0.44	157.17	C ₆ H ₉ NO ₃
	11	4-Chloroanisole	14.126	0.29	142.58	C ₇ H ₇ ClO
	13	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	14.999	3.92	151.12	C ₄ H ₉ NO ₅
	14	2,4-Di-tert-butylphenol	15.618	0.51	206.32	C ₁₄ H ₂₂ O
	16	Methyl alpha-D-glucopyranoside	17.356	26.45	194.18	C ₇ H ₁₄ O ₆
	17	Methyl tetradecanoate	18.302	0.49	242.40	C ₁₅ H ₃₀ O ₂
	18	Methyl beta-D-glucopyranoside	18.567	4.92	194.18	C ₇ H ₁₄ O ₆
	19	Mannose	18.74	1.34	180.16	C ₆ H ₁₂ O ₆
	20	Beta-Sitosterol	18.877	1.4	414.70	C ₂₉ H ₅₀ O
	21	D-erythro-Pentose, 2-deoxy	19.947	0.36	134.13	C ₅ H ₁₀ O ₄
	22	beta-Caryophyllene	20.1	0.44	220.35	C ₁₅ H ₂₄ O
	23	(Z)-9-Hexadecenoic acid, methyl ester	20.305	0.46	254.41	C ₁₆ H ₃₀ O ₂
	24	Methyl palmitate	20.532	1.47	270.50	C ₁₇ H ₃₄ O ₂
	25	(S)-10-camphorsulfonic acid	20.658	3.65	231.29	C ₁₀ H ₁₅ O ₄ S-
	27	Palmitic acid	20.899	6.56	256.42	C ₁₆ H ₃₂ O ₂
	28	Megastigmatrienone	20.988	1.15	190.28	C ₁₃ H ₁₈ O
	29	1-Nonadecene	22.16	0.82	266.51	C ₁₉ H ₃₈
	30	Methyl oleate	22.305	2.62	296.50	C ₁₉ H ₃₆ O ₂
	31	Phytol	22.399	0.56	296.50	C ₂₀ H ₄₀ O
	32	Methyl linoleate	22.6	1.77	294.50	C ₁₉ H ₃₄ O ₂
	33	9,12-Tetradecadien-1-ol, acetate, (9Z,12E)-	22.657	2.11	252.39	C ₁₆ H ₂₈ O ₂
	35	octadecanoic acid	22.886	1.58	284.50	C ₁₈ H ₃₆ O ₂

		36	Camphor oxime	24.492	0.4	167.25	C10H17NO
		37	Methyl oleate	24.712	0.4	296.50	C19H36O2
		38	1-Octadecanol	24.77	0.45	270.49	C18H38O
		39	1-Hexacosanol	25.842	0.96	382.70	C26H54O
		41	(Z)-9-Tricosene	27.712	0.15	322.60	C23H46
2000	1						
		1	Melezitose	8.537	6.45	504.44	C18H32O16
		2	N-nitrosodiethylamine	9.65	0.51	102.14	C4H10N2O
		3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.853	12.47	144.12	C6H8O4
		4	pyrrolidin-2-ylmethanol	10.639	4.2	101.15	C5H11NO
		5	2,3-Dihydro-2,5-dihydroxy-6-methyl-4-H-pyran-4-one	10.971	2.77	144.12	C6H8O4
		7	1,1,3,3-Tetramethoxypropane	11.689	1.52	164.20	C7H16O4
		10	DL-Proline, 5-oxo-, methyl ester	13.844	0.86	157.17	C6H9NO3
		11	4-Chloroanisole	14.126	0.33	142.58	C7H7ClO
		12	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	14.758	4.11	151.12	C4H9NO5
		13	1-Pentadecanol	15.517	0.1	228.41	C15H32O
		14	Methyl dodecanoate	15.831	0.61	214.34	C13H26O2
		15	Methyl alpha-D-glucopyranoside	17.1	13.42	194.18	C7H14O6
		16	2-Methyl-1,4-bis(trimethylsiloxy)butane	17.46	0.89	248.51	C11H28O2Si2
		17	Methyl tetradecanoate	18.307	0.64	242.40	C15H30O2
		18	Methyl beta-D-glucopyranoside	18.524	1.55	194.18	C7H14O6
		19	Hydroquinone	18.569	0.98	110.11	C6H6O2
		20	1-FLUORONONANE	18.717	0.55	146.25	C9H19F
		22	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1a α ,4 β ,4a β ,7 α ,7a β ,7b α)]-	18.879	0.44	222.37	C15H26O
		23	Citronellyl propionate	19.584	0.08	212.33	C13H24O2
		24	Anhydro-d-mannosan	19.936	0.13	162.14	C6H10O5
		25	Carvone Epoxide	20.102	0.25	166.22	C10H14O2

		26	(Z)-9-Hexadecenoic acid, methyl ester	20.308	0.8	254.41	C16H30O2
		27	Methyl palmitate	20.534	4.06	270.50	C17H34O2
		28	(S)-10-camphorsulfonic acid	20.658	6.38	231.29	C10H15O4S-
		29	2-Cyclopenten-1-one, 3-methyl-2-(2Z)-2-pentenyl	20.78	8.45	164.24	C11H16O
		30	Palmitic acid	20.887	4.31	256.42	C16H32O2
		31	Megastigmatrienone	20.989	0.75	190.28	C13H18O
		32	1-Nonadecene	22.164	0.68	266.51	C19H38
		33	8,11-Octadecadienoic acid, methyl ester	22.241	0.59	294.50	C19H34O2
		34	Methyl oleate	22.308	6.97	296.50	C19H36O2
		35	Methyl 9-octadecenoate	22.367	0.77	296.50	C19H36O2
		36	Phytol	22.402	1.01	296.50	C20H40O
		37	octadecanoic acid	22.563	2.07	284.50	C18H36O2
		38	cis-9-Hexadecenal	22.654	1.92	238.41	C16H30O
		39	9,12-Tetradecadien-1-ol, acetate, (9Z,12E)-	22.784	1.22	252.39	C16H28O2
		41	Spiro[4.5]decane	23.151	0.1	138.25	C10H18
		42	1-Nonadecene	24.08	0.17	266.51	C19H38
		44	1-Hexacosanol	25.844	1.46	382.70	C26H54O
		46	(Z)-9-Tricosene	27.716	0.18	322.60	C23H46
4000	0.5						
		1	Melezitose	8.524	4.32	504.44	C18H32O16
		2	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.85	10.78	144.12	C6H8O4
		3	pyrrolidin-2-ylmethanol	10.686	8.88	101.15	C5H11NO
		4	2,3-Dihydro-2,5-dihydroxy-6-methyl-4-H-pyran-4-one	10.971	2.99	144.12	C6H8O4
		5	Palmitic acid, trimethylsilyl ester	11.186	1.37	328.60	C19H40O2Si
		6	1,1,3,3-Tetraethoxypropane	11.69	1.16	220.31	C11H24O4
		7	Carcinogen	12.332	1.32	144.13	C4H8N4O2
		8	2,6-Diethylcyclohexanone	12.442	0.54	154.25	C10H18O
		9	DL-Proline, 5-oxo-, methyl ester	13.836	0.92	157.17	C6H9NO3

		10	4-Chloroanisole	14.124	0.31	142.58	C7H7ClO
		11	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-	14.833	3.25	151.12	C4H9NO5
		12	Quinuclidine	15.502	0.3	111.18	C7H13N
		13	2,4-Di-tert-butylphenol	15.618	1.03	206.32	C14H22O
		14	Methyl laurate	15.828	0.56	214.34	C13H26O2
		16	Methyl alpha-D-glucopyranoside	17.156	19.61	194.18	C7H14O6
		17	N-Methylindole	17.461	0.45	131.17	C9H9N
		18	Methyl tetradecanoate	18.304	0.63	242.40	C15H30O2
		19	Methyl beta-D-glucopyranoside	18.534	5.04	194.18	C7H14O6
		20	Mannose	18.733	0.8	180.16	C6H12O6
		21	Isopropyl hexanoate	18.821	0.45	158.24	C9H18O2
		22	beta-Caryophyllene	18.882	0.5	220.35	C15H24O
		23	(Z)-9-Hexadecenoic acid, methyl ester	20.306	1.02	254.41	C16H30O2
		24	Methyl palmitate	20.532	3.78	270.50	C17H34O2
		25	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	20.592	0.3	234.33	C15H22O2
		26	(S)-10-camphorsulfonic acid	20.656	3.38	231.29	C10H15O4S-
		28	Palmitic acid	20.889	5.66	256.42	C16H32O2
		29	Megastigmatrienone	20.987	0.78	190.28	C13H18O
		30	1-Nonadecene	22.161	1	266.51	C19H38
		31	8,11-Octadecadienoic acid, methyl ester	22.239	0.49	294.50	C19H34O2
		32	Methyl oleate	22.307	6.18	296.50	C19H36O2
		33	Methyl 9-octadecenoate	22.367	0.91	296.50	C19H36O2
		34	octadecanoic acid	22.562	1.71	284.50	C18H36O2
		35	cis-9-Hexadecenal	22.652	1.39	238.41	C16H30O
		37	stearic acid	22.885	1.04	284.50	C18H36O2
		38	Erucamide	24.711	0.36	337.60	C22H43NO
		39	(Z)-9-Tricosene	25.843	0.52	322.60	C23H46
4000	1						

	1	Melezitose	8.525	2.34	504.44	C18H32O16
	2	(S)-2,3,4,5-tetrahydropyridine-2-carboxylic acid	9.1	0.23	127.14	C6H9NO2
	3	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	9.857	7	144.12	C6H8O4
	4	pyrrolidin-2-ylmethanol	10.706	6.27	101.15	C5H11NO
	7	4,4,6-Trimethyl-1,3-oxazinane-2-thione	11.342	0.8	159.25	C7H13NOS
	8	(R)-Dimethyl 2-hydroxysuccinate	11.69	0.82	162.14	C6H10O5
	10	1-methylpiperidine	12.86	0.2	99.17	C6H13N
	11	4-Chloroanisole	13.845	0.57	142.58	C7H7ClO
	12	DL-Proline, 5-oxo-, methyl ester	14.128	0.31	157.17	C6H9NO3
	13	1,4-Diacetylbenzene	14.594	0.45	162.18	C10H10O2
	15	1,4-Anhydro-d-mannitol	14.837	2.56	164.16	C6H12O5
	17	Methyl laurate	15.833	0.29	214.34	C13H26O2
	18	Methyl alpha-D-glucopyranoside	17.314	33.1	194.18	C7H14O6
	19	Methyl tetradecanoate	18.307	0.34	242.40	C15H30O2
	20	Methyl beta-D-glucopyranoside	18.564	11.06	194.18	C7H14O6
	21	Mannose	18.74	1.4	180.16	C6H12O6
	22	Octyl beta-D-glucopyranoside	18.842	1.85	292.37	C14H28O6
	23	(Z)-9-Hexadecenoic acid, methyl ester	20.309	0.53	254.41	C16H30O2
	24	Methyl palmitate	20.537	2.31	270.50	C17H34O2
	27	Palmitic acid	20.893	3.43	256.42	C16H32O2
	28	Megastigmatrienone	20.991	0.52	190.28	C13H18O
	29	2,2-Dimethylglutaric acid	21.177	0.21	160.17	C7H12O4
	30	beta-Lactose	21.709	0.3	342.30	C12H22O11
	32	1-Nonadecene	22.166	0.98	266.51	C19H38
	33	9,12-Octadecadienoic acid	22.243	0.36	280.40	C18H32O2
	34	Methyl oleate	22.31	3.37	296.50	C19H36O2
	35	Methyl 9-octadecenoate	22.375	0.55	296.50	C19H36O2
	36	octadecanoic acid	22.566	0.96	284.50	C18H36O2

		37	5-Hydroxymethylfurfural	22.658	0.84	504.44	C ₆ H ₆ O ₃
		38	stearic acid	22.886	0.83	284.50	C ₁₈ H ₃₆ O ₂
		42	1-Hexacosanol	25.847	0.56	382.70	C ₂₆ H ₅₄ O
		46	1-Tridecene	28.094	0.07	182.35	C ₁₃ H ₂₆