Analysis of Active Compounds and Some Growth Indicators of *Capparis Spinosa* Plant Under In vitro Salt Stress

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Abstract:

Several experiments were conducted to study the effect of benzyl adenine on some growth indices and the effect of salt stress on the production of active compounds of caper plant in vitro, and to compare it with what is produced by in vivo. Different concentrations of sodium chloride were used, and the analysis results showed that the concentration of 90 mM L^{-1} was the highest for the enzyme superoxide dismutase and the enzyme malondialdehyde at a concentration of 60 mM L^{-1}. The concentration of 90 mM L^{-1} also recorded the highest average for the enzyme catalase, while the hydrogen peroxide content reached the highest average at a concentration of 30 mM L^{-1}. The analysis results using the GC/MS device showed that the leaves taken from in vivo achieved som or many different compounds, and the compound that excelled in it was Oleic acid among the compounds and with an area percentage of 13.88%. The compound L-Alanine-4-nitroanilide gave the lowest percentage of area, which was 0.43%. The study also showed that the compound 1-Polypropylene glycol excelled at a concentration of 60 mM $L^{-1}$of sodium chloride over the other compounds, as well as at a concentration of 90 mM L^{-1}, but with a different percentage of area. The area percentage was 18.46% at a concentration of 60 mM L^{-1} and 20.18% at a concentration of 90 mM L^{-1}.

Keywords: Anti-oxidant, Capparis spinosa, GC/MS, in vitro, salt stress

تحليل المركبات الفعالة وبعض مؤشرات النمو لنبات *Capparis spinosa* تحت الإجهاد الملحي خارج الجسم الحي

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الملخص:

نفذت عدة تجارب لدراسة تأثير البنزل ادنين في بعض مؤشرات النمو وكذلك تأثير الإجهاد الملحي في أنتاج المركبات الفعالة لنبات الكبر خارج الجسم الحي ومقارنتها مع ما ينتجه النبات النامي بصورة طبيعية. استخدمت تراكيز مختلفة من كلوريد الصوديوم و بينت نتائج التحليل تفوق التركيز ٩٠ ملي مول لتر$^{-1}$ لأنزيم superoxide dismutase والأنزيم malondialdehyde عند التركيز ٦٠ ملي مول لتر$^{-1}$ ،كما سجل التركيز ٩٠ ملي مول لتر$^{-1}$ اعلى متوسط من أنزيم catalase ، بينما محتوى hydrogen peroxide بلغ أعلى متوسط عند التركيز ٣٠ ملي مول لتر$^{-1}$.فيما بينت نتائج التحليل بجهاز GC/MS ان الاوراق المأخوذة من النباتات النامية طبيعياً قد حققت عدد من المركبات المختلفة وكان المركب الذي تفوق فيها هو مركب Oleic acid من بين المركبات وبنسبة مساحة بلغت ١٣,٨٨ %. فيما أعطى المركب L-Alanine-4-nitroanilide أقل نسب المساحة والذي بلغ ٠,٤٣٪. كذلك بينت الدراسة تفوق المركب 1-Polypropylene glycol عنده المعاملة بالتركيز ٦٠ ملي مول لتر$^{-1}$ من كلوريد الصوديوم على باقي المركبات وكذلك في التركيز ٩٠ ملي مول لتر$^{-1}$ لكن بنسبة مساحة مختلفة حيث بلغت عند التركيز ٦٠ ملي مول لتر$^{-1}$ ١٨,٤٦٪ فيما كانت عند التركيز ٩٠ ملي مول لتر$^{-1}$ ٢٠,١٨٪.

الكلمات المفتاحية: *Capparis spinosa*، الاجهاد الملحي، GC/MS، مضادات الأكسدة، خارج الجسم الحي.

Introduction

The caper plant (*Capparis spinosa* L), belonging to the Capparaceae family, is one of approximately 250 species widely distributed from the Mediterranean to Southeast Asia, Oceania, and Australia (18). Known in Iraq as "Al-Kabar" or "Warad Al-Kabbar," it is a perennial shrub, delete growing wild or cultivated in many regions worldwide. It has been used as a commercial plant and for related products since ancient times, as well as in medicine and cosmetics due to its delete numerous biologically active compounds and beneficial properties (12, 32). Spinosa is one of the important species within the Capparis genus due to its medical, environmental, and economic significance, with capers being nutritionally important as their buds, flowers, and fruits are used as spices (19, 26**).**

The root is used to treat rheumatism, digestive problems, liver and kidney diseases, toothache, headache, and sciatica. The root bark is used as an appetite stimulant, tonic, antidiarrheal, and treatment for spleen diseases. It is also used as an expectorant (24**).** It has also been used to treat asthma, co**u**gh, paralysis, and infectious disorders. It has also been used as an antioxidant and antimicrobial. It also contains the most important active compounds,

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which are alkaloids, phenols, flavonoids, and glycosides. It also contains proteins, vitamins, and fatty acids. These compounds make capers of nutritional and medicinal importance. The phenolic compounds in it are also used as antioxidants (27).

The importance of plant tissue culture technology has. increased in obtaining plants tolerant to various stresses, including saline stress, as plants produce chemical compounds when exposed to different environmental stresses. These stresses affect the biochemical processes responsible for producing these compounds (31). Studies have indicated that stresses have led to variations in the number and quality of compounds identified through GC/MS technology, as stress stimulates the production of many active compounds in most plants by activating other signaling pathways and increasing metabolic and secondary compounds (17), thereby increasing secondary metabolites. Therefore, the presence of external additives to the medium, such as NaCl, may have an impact on the diversity and variation of components and active compounds (36).

Saline stresses alter the morphological, physiological, and chemical responses of plants, as high concentrations of salinity cause osmotic stress by inducing ion toxicity, resulting in the generation of reactive oxygen species that damage DNA, proteins, and lipids (8). Acosta-Motos et al. (1) mentioned that plants have developed several biochemical mechanisms to cope with salt stress, including selective ion accumulation, attempting to exclude or regulate ion uptake, altering photosynthetic pathways, and enhancing the activity of antioxidant enzymes. Studies have indicated that compounds found in the caper plant may inhibit free radicals due to their hydrogen ion concentration (33). Additionally, Baldi et al. (9) observed an increase in the activity of SOD and CAT enzymes at salinity concentrations of 30 and 45 ds m^{-1} in the caper plant. In a study conducted by Alaakl et al. (2), an increase in the content of antioxidant compounds such as proline, MDA, CAT, and SOD was observed in the *Catharanthus roseus* plant when different concentrations of sodium chloride were added in vitro. Therefore, the aim of the study is to investigate the effect of saline stress on the caper plant in vitro to stimulate the production of active compounds and compare them with those in vivo growing in the Anbar region of western Iraq.

Materials and Methods:

The experiments were conducted at the Plant Tissue Culture Laboratory, Department of Horticulture and Landscape Engineering, University of Anbar, from June 2023 to the end of January 2024, to determine the effect of saline stress on the production of active compounds in the caper plant in vitro.

Preparation of the nutrient medium:

The MS (34) nutrient medium was used during both the initiation and multiplication stages. It was prepared by weighing 4.43 grams of MS, and adding 30 grams of sucrose. Benzyl adenine was added at appropriate concentrations for homogenization using a Hot plate stirrer. The components were then mixed thoroughly, and the volume was adjusted to 1 liter with distilled water. The pH was adjusted to 5.7 using a pH meter by adding a few drops of NaOH or HCl. Agar was added to solidify the medium, and after reaching boiling point, it was dispensed into culture tubes. The medium was then heated and sterilized using an Autoclave at a temperature of 121°C and a pressure of 1.04 kg cm^{-2} for 20 minutes.

you can merge the initiation and multiplication stages stage:

After obtaining the plant parts (apical meristem) and washing them, they were sterilized with 3% sodium hypochlorite (3). These parts, with lengths of 0.5 - 1 cm, were then taken and planted on the prepared nutrient medium with different concentrations of benzyl adenine (0.0, 0.5, 1.0, 1.5 mg L^{-1}) at 20 replicates. The planting was conducted inside a growth chamber (hood) and then incubated at a temperature of 25°C for 16 hours of light and 8 hours of darkness for 4 weeks to obtain sterilized plant parts free from pathogenic contaminants, allowing them to grow and develop in subsequent stages (23).

Multiplication stage for apical meristems:

Benzyl adenine growth regulator was added to the MS nutrient medium at concentrations of 0.0, 1.0, 1.5, and 2.0 mg L^-1. A plant explant (apical meristem) with a length of 1 cm was planted in each culture tube, and the cultured tubes were placed in a growth chamber at a temperature of 25°C for 16 hours of light and 8 hours of darkness for 4 weeks (25).

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Experiment for stimulating active compounds:

The plant parts obtained from the multiplication stage were cultured in MS medium prepared with different concentrations of NaCl, namely (0, 30, 60, 90) mmol L^{-1}. The objective was to stimulate and assess antioxidant activity and produce active compounds in vitro.

Extraction of active compounds:

A weight of 150 milligrams was taken from samples of in vivo leaves in Anbar, as well as from leaves exposed to different concentrations of NaCl. 10 milliliters of ethyl alcohol were added, and then placed on a shaker for 24 hours. Subsequently, the extract was filtered using Whatman filter paper. After filtration, it was transferred to

heating for the purpose of alcohol evaporation. Then, analysis was performed using a GC/Mass chromatography device (21).

The experimental design used: The laboratory experiments were conducted following the Completely Randomized Design (C.R.D) with twenty replicates for each treatment during the initiation stage, and ten replicates for each treatment during the multiplication and rooting stages. Data analysis was performed using the Genstat software, and the means were compared using the Least Significant Difference (L.S.D) test at a significance level of 0.05 (6).

Results and Discussion

Initiation of Apical Meristem

The results indicate a significant effect of adding benzyl adenine (BA) on the percentage of dead plant parts after planting the plant parts from the apical meristem (Figure 1). Treatment with 1.5 mg L^{-1} BA resulted in the lowest percentage of dead plant parts, reaching 5.0%, which did not differ significantly from the treatments with 1 mg L^{-1} and 0.5 mg L^{-1} BA. In contrast, the control treatment recorded the highest percentage of dead parts at 50.0%. The results showed that there were no significant differences between the concentrations used in the trait of living plant parts that did not respond to growth. The percentage of living plant parts that responded to growth showed that the 1.5 mg/L treatment was significantly superior, with a percentage of 80%. The 0.5 mg/L treatment had a percentage of 65.0%, which was not significantly different from the 1.5 mg/L treatment. The lowest percentage of responsive plant parts was in the control treatment, at 30.0%.

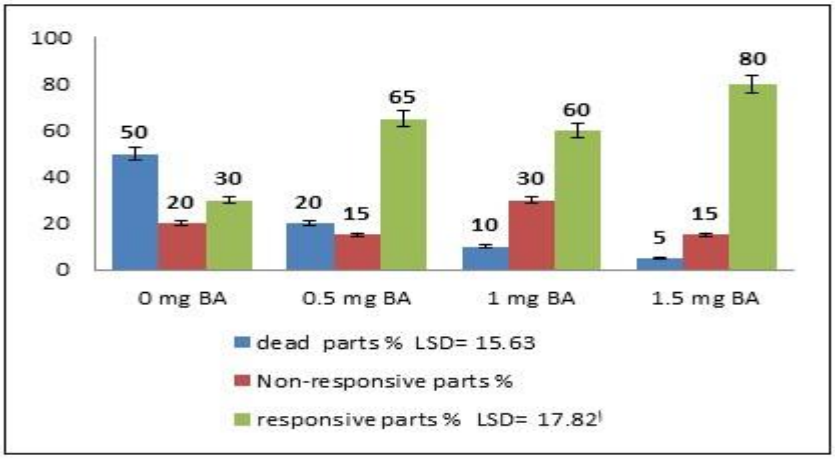

Figure 1. Effect of benzyl adenine on the initiation stage of the apical meristem of caper plant

The plant parts (apical meristems) exhibited varied responses depending on the different concentrations of benzyl adenine added to the MS medium. The use of benzyl adenine during the initiation stage without others may be attributed to its high efficiency, which depends on the presence of double bonds in the adenine ring, as it possesses three double bonds compared to kinetin and 2ip, and other cytokinins. Therefore, its efficiency in stimulating cell division is higher (14). The reason for the higher response of live parts to the growth of the apical meristem in the medium supplied with a concentration of 1.5 mg L^{-1} of BA could be attributed to the optimal ratio of cytokinins and to the internal content of other regulators in the apical meristem of Caper plants, as the presence of this mixture of regulators causes cell division and increased growth. Cytokinins play an effective role in stimulating cell division and growth rate, thereby increasing the growth rate, which is consistent with the findings of Elmaghrabi (15). Additionally, the lack of growth response by the apical meristem despite its viability may be due to differences in hormonal content and cell maturity (28). Its growth response may be attributed to the significant role of cytokinins in breaking apical dominance and stimulating lateral bud growth due to its effective role in RNA and protein synthesis, as well as enzyme formation in plant cells, which aligns with the findings of Al-Badrani (4). Furthermore, Daffalla (13) found an increase in the number of buds in Capparidaceae with increasing levels of BA.

Multiplication of apical meristem

The results of the statistical analysis in Figure 2 showed that the treatment with 1.5 mg L^{-1} BA significantly outperformed all other treatments with the highest number of leaves at 7.30 leaves $plantlet^{-1}$, while the treatment with no growth regulators recorded the lowest number of leaves at 3.50 leaves per $plantlet^{-1}$. The results also indicated significant differences in branch length, with the treatment of 1.5 mg L^{-1} BA significantly outperforming all other treatments by providing an average length of 3.78 cm, while the control treatment recorded the shortest branch length of 1.95 cm. Regarding the number of branches, the results showed that the treatment with 1.5 mg L^{-1} BA significantly outperformed all other treatments with the highest average number of branches, which was 3.50 branches $plantlet^{-1}$, while there were no significant differences among the other three treatments, and the treatment with 0 mg L-1 recorded the lowest number of branches, which was 1.70 branches $plantlet^{-1}$.

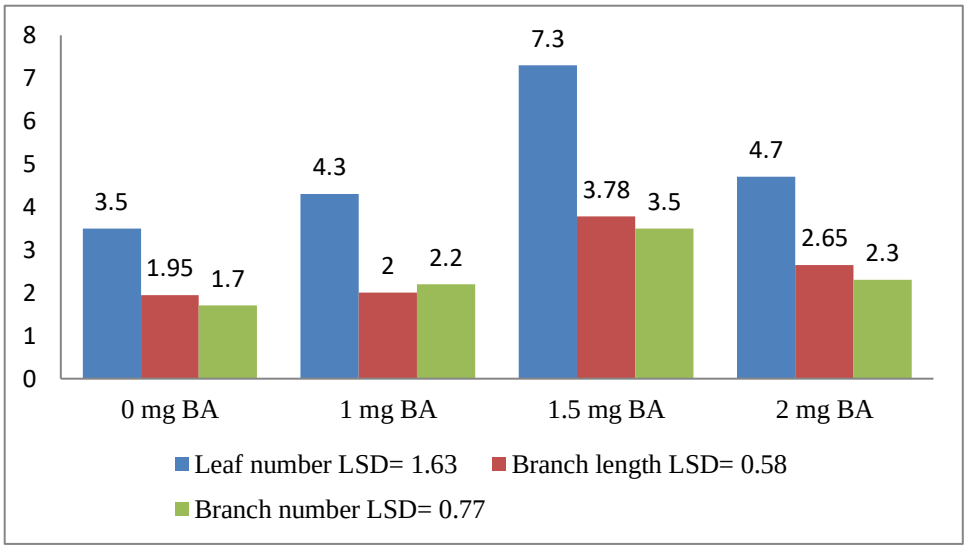

Figure 2: The effect of benzyl adenine (BA) on the multiplication of Caper apical meristems

Through the multiplication stage, the addition of cytokinins during the vegetative multiplication stage of different plant species is of great importance due to their effectiveness in stimulating axillary buds without the need to remove apical meristem due to their resistance to the action of auxins (40). Additionally, the significance of adding cytokinins lies in their ability to stimulate cell division and lateral branch formation, as mentioned earlier. This explains the possibility of benzyl adenine at a concentration of 1.5 mg L^{-1}, which provided the highest leaf count for the apical meristem; these results were consistent with the findings of Musallam *et al.*, (35). Furthermore, the increase in branch number, especially with the 1.5 mg L^{-1} benzyl adenine treatment, may be attributed to its positive effects on leaf and branch multiplication, reducing apical dominance. Additionally, it plays a role in attracting and accumulating metabolic substances at the sites of lateral bud initiation, as well as directly stimulating RNA, protein, and chlorophyll synthesis (22). Moreover, the presence of BA leads to hormonal imbalance in regions abundant in auxins, resulting in the breaking of apical dominance and stimulation of lateral bud growth (5). Numerous studies have highlighted the role of cytokinins when used at optimal concentrations in tissue culture, forming attraction zones in lateral buds, thereby enhancing nutrient uptake and promoting bud growth (37).

The effect of adding sodium chloride on catalase enzyme (CAT) : The statistical analysis results presented in Figure 3 demonstrate the significant effect of adding sodium chloride to the nutrient medium. It showed a statistically superior performance for the treatment with 90 mmol L^{-1}, with the highest average catalase (CAT) activity in plants at 243.3 $AUmin^{-1}$ g^{-1}, while the control treatment yielded the lowest average at 91.5 $AUmin^{-1}$ g^{-1}.

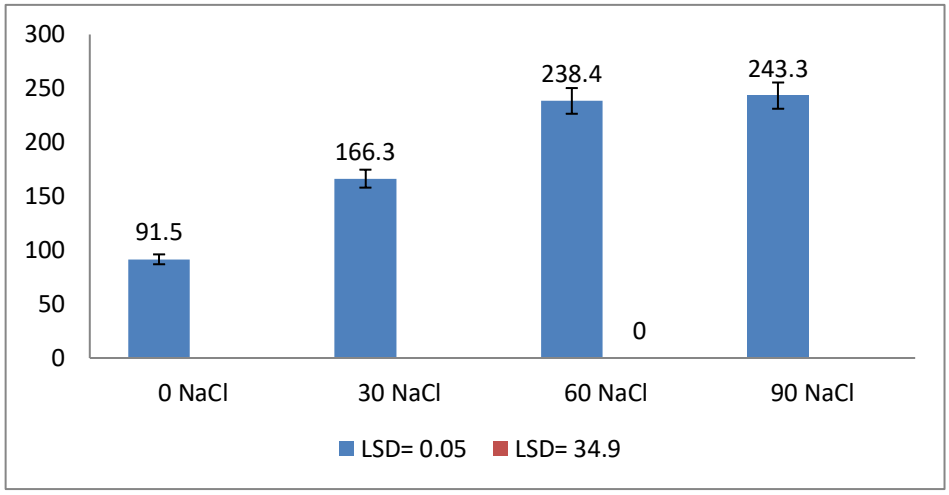

Figure 3: The effect of salt concentrations on catalase enzyme in Caper

The effect of sodium chloride addition on hydrogen peroxide (H_2O_2) concentration:

The statistical analysis results indicate that the treatment with a concentration of 30 mmol L^{-1} exhibited significant superiority over the other treatments (Figure 4). This treatment yielded the highest average hydrogen peroxide (H_2O_2) content at 1.89 µmol $100mg^{-1}$ D.W. In contrast, the control treatment showed the lowest H_2O_2 content at 0.30 µmol $100mg^{-1}$ D.W.

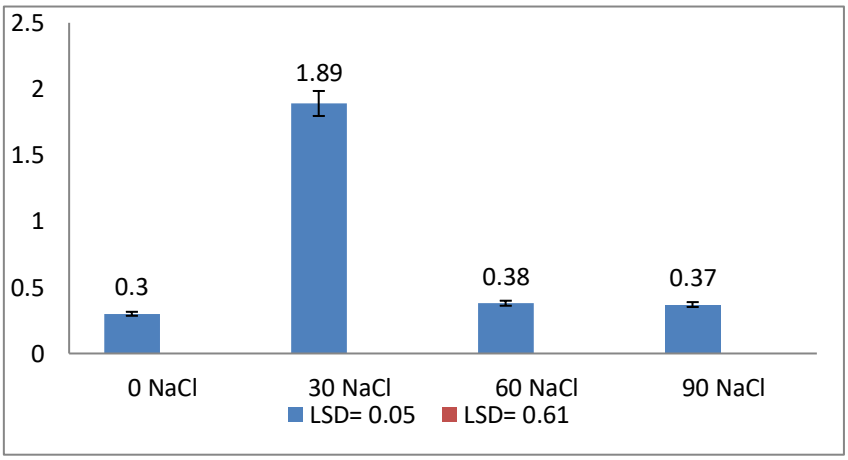

Figure (4): The effect of salt concentrations on the hydrogen peroxide content in Caper.

The effect of sodium chloride addition on malondialdehyde (MDA).:

The statistical analysis results presented in Figure 5 demonstrate the significant effect of sodium chloride on the content of the enzyme MDA in plants. Specifically, the treatment with a concentration of 60 mmol L^{-1} recorded the highest MDA content at 2.50 AU µmol g-1 FW. In contrast, the control treatment achieved the lowest MDA concentration at 0.35 µmol g-1 FW.

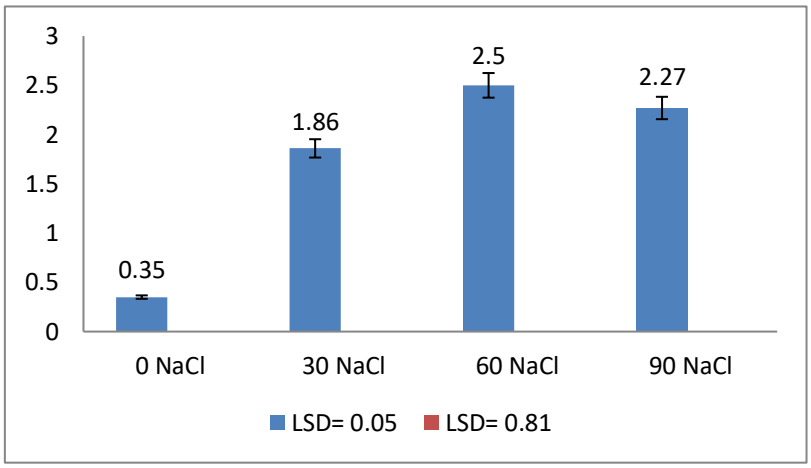

Figure 6: The effect of salt concentrations on the Superoxide dismutase enzyme in Caper.

Antioxidant enzymes play an important and effective role as a protective system for plants and increase their tolerance to salt stress and other stresses (7). The increase in antioxidant activity reduces oxidative stress, increases osmotic pressure, and selective uptake, i.e., the absorption of beneficial ions, and works to prevent the accumulation of ions with excessive toxicity, which helps the plant to tolerate salt stress (38). The plant maintains optimal levels of ROS species through a number of complex defense mechanisms that consist of the enzymatic system such as Superoxide dismutase, Catalase, and Peroxidase enzymes, as well as non-enzymatic defense systems such as vitamins and phenols. An increase in RSO compound concentrations above a certain limit due to salt stress exceeds the plant's ability to scavenge these compounds. This is where the plant will be exposed to a state of stress called oxidative stress, which is a state of imbalance between antioxidants and pro-oxidants (39).

We can conclude here that the increase in sodium chloride concentrations led to an increase in SOD enzyme activity. The reason for this may be that this enzyme is the first line of defense for the cell against free radical damage, as it removes ROS and converts highly toxic ROS to hydrogen peroxide. The increase in NaCl concentration led to an increase in MDA, which is attributed to a decrease in antioxidants and an increase in free radical production that led to lipid peroxidation of membranes (16). The results are consistent with the results of Kusvuran (30) on melon plants and the results of Yasar (42) on eggplant tissue cultures. These results also do not agree with those of Vieira Santos (41), who showed that the activity of antioxidant enzymes, especially the CAT enzyme, decreased in sunflower plants when treated with salt concentrations in vitro.

The effect of different concentrations of sodium chloride and the estimation of active compounds using GC/MS.

Based on the GC/MS analysis results presented in Table 1, the active compounds found in the leaves of the Caper wild plant in the Anbar region of western Iraq were identified. The analysis revealed that Oleic acid compound showed superiority among the compounds with an area percentage of 13.88% and a retention time of 23.85. This was followed by the Polypropylene glycol compound with an area percentage of 11.98% and a retention time of 6.14. While, the compound L-Alanine-4-nitroanilide exhibited the lowest area percentage at 0.43% and retention time of 28.64.

Table 1: Retention time, area percentage, and types of secondary metabolites in the leaves of Caper plant in vivo found in Anbar

ID	Retention time	Area	Active compounds	Group
1	4.303	1.83	2,4-Dihydroxypropiophenone,	Phenol
2	4.762	1.39	Octamethylcyclotetrasiloxane	Siloxane
3	5.134	0.72	2-Acrylamidoglycolic acid	Amide
4	5.385	2.77	Cinmethylin	Ether
5	5.679	1.36	Eucalyptol	Alcohol
6	5.809	7.87	1,1-Oxydi-2-Propanol	Alcohol
7	6.147	11.98	Polypropylene glycol	Alcohol
8	6.424	8.08	1-(2-Methoxy-1-methylethoxy) propan-2-ol	Ether
9	6.779	3.90	3-(3-Hydroxyphenyl)-3-hydroxypropanoic acid	Phenol
10	7.064	0.70	Sarcosine, N-(3-cyclopentylpropionyl)-, tetradecyl ester	Alkaloid
11	7.384	1.92	Sarcosine, N-(3-cyclopentylpropionyl)-, pentadecyl ester	Alkaloid
12	7.687	1.14	3,4-methylenedioxyamphetamine	Benzodioxoles
13	8.492	2.65	l-Menthol	Alcohol
14	8.899	1.10	Cyclotetrasiloxane, octamethyl	Siloxane
15	9.211	0.50	Pentasiloxane, dodecamethyl	Siloxane
16	9.436	0.96	5,'6-Cyclo-O-thymidine	Alkaloid
17	9.643	4.94	Dehydrogeraniol	Aldehyde
18	10.206	4.73	trans-Citral	Aldehyde
19	10.924	0.48	2-Amino-2,4-dimethylpentanenitrile	Nitrile
20	11.297	0.99	Margaric acid, TBDMS	Fatty acid ester
21	11.669	2.35	3-'Hydroxypentobarbital	Alkaloid
22	11.980	0.49	Sarcosine, N-(3-cyclopentylpropionyl)-, tetradecyl ester	Alkaloid
23	14.646	0.84	3-Methylnordazepam	Alkaloid
24	14.915	1.20	2,6-bis(3-chlorophenyl) pyrrolo[3,4-f] isoindole-1,3,5,7-tetrone	Alkaloid
25	17.399	0.56	Hydroxynorephedrine	Alkaloid
26	17.676	0.71	Tetradecamethylhexasiloxane	Siloxane
27	21.112	2.11	Methyl palmitate	Fatty acid ester
28	21.666	7.66	Myristic acid	Fatty acid
29	23.232	0.76	Methyl octadeca-9,12-dienoate	Fatty acid ester
30	23.310	2.26	11-Octadecenoic acid, methyl ester	Fatty acid ester
31	23.630	0.47	Methyl stearate	Fatty ester
32	23.856	13.88	Oleic Acid	Fatty acid
33	24.132	2.05	Oleic acid	Fatty acid
34	24.409	0.59	Arginine	Amino acid
35	52.561	0.65	Cyanoacetylurea	Amide
36	26.366	0.46	Phenylephrine	Amino alcohol
37	26.963	0.72	Trimethylsilyl l-alanylalaninate#	TMS derivative
38	27.469	1.04	GLYCERYL RICINOLEATE	Fatty acid ester
39	28.648	0.43	L-Alanine-4-nitroanilide	Amine
40	30.321	0.74	2,3,4-Trimethoxyphenylacetonitrile	Nitrile

The results of the GC/MS analysis in Table 2 indicated differences in the quality of the active compounds extracted from the entire plant parts of the Caper grown in an MS nutrient medium devoid of any salt concentrations. The superiority was observed in the compound Polypropylene glycol with an area percentage of 24.02% and a retention time of 6.11. This was followed by the compound 2-Propanol, 1, 1-oxybis with an area percentage of 15.18% and a retention time of 5.76. The compound Cyclotrisiloxane, hexamethyl achieved the lowest among them, with an area of 0.60% and a retention time of 29.78.

Table 2: Effect of concentration (0.0) on the production of some secondary metabolites in caper plantlets tissues in vitro.

ID	Retention time	Area	Active compounds	Group
1	4.294	1.97	1,1,3,3,5,5-Hexamethyltrisiloxane	Siloxane
2	4.701	1.30	3-Hydroxy-butyraldehyde	Aldehyde
3	5.368	3.19	Cinmethylin	Ether
4	5.653	1.20	Eucalyptol	Alcohol
5	5.766	15.18	1,1-'Oxydi-2-propanol	Ether
6	6.112	24.02	Polypropylene glycol	Alcohol
7	6.398	7.87	Dihydromyrcenol	Alcohol
8	6.675	1.21	3-(3-hydroxybutan-2-yloxy) butan-2-ol	Ether
9	7.376	2.76	2-Norbornanol	Alcohol
10	7.679	1.53	3,4-methylenedioxyamphetamine	benzodioxoles
11	8.484	3.47	l-Menthol	Alcohol
12	8.899	1.86	2,3-Dimethoxyamphetamine	benzodioxoles
13	9.401	0.79	Oxazepam, 2TMS derivative	Alkaloid
14	9.643	6.51	N-{7-[(3-fluorophenyl) carbonyl]-2,3-dihydro-1,4-benzodioxin-6-yl} cyclopropane carboxamide	Alkaloid
15	10.206	6.18	trans-Citral	Aldehyde
16	11.314	0.65	Imidazole, 2-amino-5-[(2-carboxy) vinyl]	Alkaloid
17	11.712	0.70	(R)-alpha-Terpinyl acetate	Oxide
18	21.112	2.32	Methyl palmitate	Fatty acid ester
19	21.683	4.10	Pentadecanoic acid	Fatty acid
20	23.224	0.69	methyl trans-12-octadecenoate	Fatty acid ester
21	23.310	2.26	Methyl Elaidate	Fatty acid ester
22	23.847	7.87	Oleic Acid	Fatty acid
23	24.124	1.01	Oleic Acid	Fatty acid
24	27.430	0.77	Hydroxynorephedrine	Alkaloid
25	29.784	0.60	Hexamethylcyclotrisiloxane	Siloxane

The GC/MS analysis results presented in Table 3 indicate that the growth of plant tissues in MS medium supplemented with a concentration of 30 mM NaCl did not differ from those in the salt-free medium. The dominance was observed again in the compound Polypropylene glycol, but with a lower area percentage of 23.73% and a retention time unchanged from in vivo leaves at 6.14. Compound 2-Propanol, 1,1'-oxybis showed variation compared to the control treatment, with an area percentage of 16.58% and a retention time of 5.80. While, the compound Methyl stearate exhibited the lowest area percentage at 0.51% and retention time of 23.63.

Table 3: Effect of 30 mM NaCl concentration on the production of some secondary metabolites in Caper plantlets tissues in vitro.

ID	Retention time	Area	Active compounds	Group
1	4.294	1.61	2-nitrobenzaldehyde	Aldehyde
2	5.134	0.55	3-Hydroxybutanal	Aldehyde
3	5.316	1.52	1,4-Cineole	Ether
4	5.653	1.48	Eucalyptol	Alcohol
5	5.809	16.58	1,1-'Oxydi-2-propanol	Ether
6	6.147	23.73	Polypropylene glycol	Ether
7	6.398	7.58	Dihydromyrcenol	Alcohol
8	6.787	5.25	2,5-Dihydroxybenzaldehyde	Aldehyde
9	7.376	2.54	β-Fenchol	Cyclic terpeneoid
10	7.679	1.40	3,7-Diacetamido-7H-s-triazolo[5,1-c]-s-triazole	Amide
11	8.215	0.93	Isoborneol	Cyclic terpenoid
12	8.492	3.57	l-Menthol	Alcohol
13	8.890	1.66	Octamethylcyclotetrasiloxane	Siloxane
14	9.392	0.68	NordazepamTMS	Alkaloid
15	9.643	6.38	8-Mercaptomenthone	Ketone
16	10.206	5.95	trans-Citral	Aldehyde
17	11.703	0.77	Terpinyl acetate	Terpenoid
18	21.103	2.07	Methyl palmitate	Fatty acid ester
19	21.683	3.49	palmitic acid	Fatty acid
20	23.224	0.79	linoleic acid	Fatty acid
21	23.310	2.24	Methyl vaccinate	Fatty acid ester
22	23.630	0.51	Methyl stearate	Fatty acid ester
23	23.855	7.13	Elaidic acid	Fatty acid
24	24.124	0.87	Oleic Acid	Fatty acid
25	27.447	0.74	Propionylurea	Amide

The results of the analysis in Table 4 demonstrate that the compound Polypropylene glycol exhibited superiority over the other compounds with an area percentage of 18.46% and a retention time of 6.19 when treated with a concentration of 60 mM NaCl. This was lower than the area percentage achieved by the same compound at the 30 mM NaCl concentration, with no difference in retention time. Following Polypropylene glycol, the compound 2-Propanol, 1,1-oxybis achieved an area percentage of 5.86, followed by the compound 9-Octadecenoic acid, (E) with an area percentage of 9.58 and a retention time of 23.84. The compound Phthalic acid, cyclohexyl neopentyl ester recorded the lowest area percentage at 0.63% and a retention time of 8.44 when treated with a concentration of 60 mM NaCl.

Table 4: Effect of 60 mM NaCl concentration on the production of some secondary metabolites in Caper plantlets tissues in vitro.

ID	Retention	Area	Active compounds	Group
1	5.307	1.81	1,4-Cineole	Ether
2	5.671	2.96	Hexamethylcyclotrisiloxane	Siloxane
3	5.861	14.39	1,1-'oxydi-2-propanol	Ether
4	6.190	18.46	Polypropylene glycol	Ether
5	6.372	6.10	Dihydromyrcenol	Fatty alcohol
6	6.528	1.01	Tris(trimethylsiloxy)arsine	Semiochemicals
7	6.796	4.43	3,3,6,6,7,7-hexamethyl-1,2,4,5,8,9-	Terpenoid
8	7.367	2.99	Fenchol	Alcohol
9	7.670	1.87	1,3-Adamantanediacetamide	Alkaloid
10	8.224	1.09	2-(4-cyclohexylphenoxy)-N-(6-methylpyridin-2-yl) acetamide	Alkaloid
11	8.449	2.80	3-(2-methylpropyl) cyclohexene	Terpenoid
12	8.614	0.63	Phthalic acid, cyclohexyl neopenty l ester	Glycoside
13	8.839	0.91	- (-)α-Terpineol	Terpenol
14	9.393	0.69	Nordazepam TMS	Alkaloid
15	9.635	8.15	trans-Citral	Aldehyde
16	10.198	7.95	cis-Citral	Aldehyde
17	11.297	0.83	Cyclostyle (diethyl)borane	Terpenoid
18	11.704	0.91	α-Terpinyl acetate	Terpenoid
19	21.103	2.34	methyl palmitate	Fatty acid ester
20	21.675	4.53	palmitic acid	Fatty acid
21	23.224	0.80	Methyl linoleate	Fatty acid ester
22	23.310	2.53	Methyl elaidate	Fatty acid ester
23	23.847	9.58	Elaidic acid	Fatty acid
24	24.124	1.19	cis-13-Octadecenoic acid	Fatty acid
25	27.430	1.04	3-Bornanone, semicarbazone	Terpenoid

The results in Table 5 demonstrate the effect of adding a salt concentration of 90 mM NaCl to the nutrient medium. The GC/MS analysis revealed the presence of several active compounds with varying area percentages compared to other concentrations. The compound Polypropylene glycol exhibited dominance over other compounds at the same concentration, with an area percentage of 20.68%, which is higher than the area percentage at 60 mM NaCl concentration (18.46%) but lower than that at 30 mM NaCl concentration (23.73%). The retention time for Polypropylene glycol at 90 mM concentration was 6.09. Following Polypropylene glycol, the compound 2-Propanol, 1,1-oxybis achieved a respective area percentage of 17.03 with a retention time of 5.74. This was followed by Oleic Acid with an area percentage of 9.16 and a retention time of 23.85. The compounds Methyl stearate and Cyclohexene, 3,3,5-trimethyl recorded area percentages of 0.54% and 0.57%, respectively, with retention times of 23.62 and 11.30.

Table 5: Effect of 90 mM NaCl concentration on the production of some secondary metabolites in Caper plantlets tissues in vitro.

ID	Retention time	Area	Active compounds	Group
1	4.295	1.35	2-Methoxy-2'-methyl-stilbene	Stilbene
2	5.117	0.63	3-Hydroxybutanal	Aldehyde
3	5.325	1.89	1,4-Cineole	Ether
4	5.740	17.03	1,1'-Oxydi-2-propanol	Ether
5	6.095	20.68	Polypropylene glycol	Ether
6	6.398	8.28	Dihydromyrcenol	Fatty alcohol
7	6.649	1.28	3,3'-oxybis-2-butanol	Ether
8	7.376	2.60	β-Fenchol	Terpenoid
9	7.670	1.72	1-Terpinenol	Terpenol
10	8.466	3.59	dl-Menthol	Alcohol
11	8.839	0.76	L-Terpineol	Terpenol
12	9.393	0.62	Octadecane-12-on-1-ol, TMS	Siloxane
13	9.635	7.87	trans-Citral	Aldehyde
14	10.198	7.84	Cis-Citral	Aldehyde
15	11.305	0.57	ε-Cyclogeraniolene	Terpenoid
16	11.704	0.84	α-Terpinyl acetate	Terpene
17	21.103	2.30	methyl palmitate	Fatty acid ester
18	21.674	4.00	palmitic acid	Fatty acid
19	23.224	0.97	Methyl linoleate	Fatty acid ester
20	23.310	2.65	Methyl elaidate	Fatty acid ester
21	23.622	0.54	Methyl stearate	Fatty acid ester
22	23.856	9.16	Oleic Acid	Fatty acid
23	24.124	1.17	Oleic Acid	Fatty acid
24	27.430	1.04	Methoxamine	Amine
25	30.312	0.68	4-(4-methoxyphenoxy)-1,2,5-oxadiazol-3-amine	Amine

Based on the GC/MS analysis results, differences in the types of compounds between in vivo in their native environment and plants exposed to various concentrations of sodium chloride are observed in vitro. The compound Oleic acid showed superiority in the leaves of in vivo (Table 1), while the results indicated the dominance of Polypropylene glycol when plants were exposed to sodium chloride concentrations (Tables 2, 3, 4, 5). The results also suggested that the addition of different concentrations of sodium chloride stimulated the formation of some other secondary compounds, in addition to the variation in area percentage and retention time of the resulting compounds at different NaCl concentrations.

Studies have indicated antioxidant activity in extracts from various parts of the Caper plant, all showing significant antioxidant activity. This may be attributed to the wide distribution of these antioxidants throughout the plant, such as tocopherols and carotenoids, which protect its cells from oxidation (20). Caper is rich in many chemical compounds possessing free radical scavenging properties and capable of capturing various reactive oxygen species (ROS) (29).

Plant chemical analyses of different parts of the Caper plant have revealed rich sources of compounds, as indicated by research on their enhanced health effects and active components (11). Tissue culture technology holds high value for research into plant biological processes to aid in the production of secondary metabolites by exposing plant tissues to stress in vitro. This is the reason for the increase in secondary metabolites in Caper plants at saline concentrations compared to in vivo occurring compounds produced by the plant. Therefore, the presence of NaCl in the medium and its components may have an impact on the diversity and variation of active components and compounds (36). Salinity reduces plant growth and development and affects a wide range of physiological and metabolic processes in plants (10). Plants have evolved complex mechanisms to adapt to osmotic, ionic, and oxidative stress caused by salinity. Hence, the presence of various compounds at each tested concentration may be attributed to the plants' ability to tolerate salt stress and produce these active compounds (43). Moreover, this difference could be due to the various growth inputs in vitro (44).

a) Initiation stage, (b) and (c) Multiplication stage, (d) and (e) Salinity stress, (f) Plant extract.

Conclusion

In this study, it could be outcome that the highest concentrations of benzyl adenine significantly increased the responsive parts in caper to be initiated with decreased the dead parts. In contrast, the antioxidative system was improve under the highest concentrations of salts up of 60 mmol per liter. Another point is that the profiling of phytochemicals was occurred under salt stress. However, the secondary metabolites naturally biosynthesized in caper were best.

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