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EFFECT OF PLANT GROWTH REGULATOR (SALICYLIC ACID) ON THE PROPAGATION AND VOLATILE OIL PRODUCTION OF ORIGANO (*ORIGANUM VULGARE* L.) *IN VITRO*

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Abstract

The study was carried out in the Laboratory of Plant Tissue Culture/ Department of Horticulture and Landscape Gardening /College of Agricultural Engineering Sciences /University of Baghdad in the period from July 2018 to August 2019. The main aim of the study was the propagating Origanum Vulgare histologically by identifying the combination of appropriate growth regulators for all propagation stages. As well as, study the possibility of stimulating the active compounds (volatile oil) of Origanum Vulgare by adding different concentrations of the growth regulator (salicylic acid) and comparing them with the same active compounds of the mother plant. The seeds were sterilized using a 10% sodium hypochlorite solution for 15 minutes, while the establishment stage, seeds was germinated within five days on Murashige and Skoog (MS) medium free of growth regulators. For the multiplication stage, different concentrations of salicylic acid (1, 2, and 3 $mg.L^{-1}$) were used in combination with 0.5 $mg.L^{-1}$ Benzyl adenine (BA). The addition of salicylic acid gave the highest average shoot length by 11.9, and the concentration of 2 $mg.L^{-1}$ gave the highest average shoot number by 11, while the highest wet weight was 0.316 mg, and the highest dry weight was 0.067. Moreover, the effect of salicylic acid on the dyes has a significant effect in increasing the percentage of dyes, where the concentration of 3 $mg.L^{-1}$ was given the highest Anthocyanin content was 186.25 mg/100g. In addition, the concentration of 2 $mg.L^{-1}$ of salicylic acid gave the highest chlorophyll content was 372.96 mg/100g. As for the effect of salicylic acid on the active compounds of volatile oil, the results showed that the addition of 2 of salicylic acid gave the highest concentrations of the compounds (Al-pinene, Limonene, Carvacrol and Linalool). Finally, the concentration of 1 mg. L^{-1} was characterized by increasing Terpinen and Sapinen concentration, while the compound Thymol has reached 17.37 mg. L^{-1} in the comparison treatment.

Key words *:* Salicylic Acid, Volatile OIL, *Origanum Vulgare* L.

Introduction

Origanum vulgare (Mero, Hibak El-Boy, Atra, wild marjoram) are one of the important aromatic medicinal herbal plants belonging to the Lamiaceae family, Oreganum genus. It is about 43 types, most of them originated in the area around the Mediterranean Sea, which also found in southwestern of Europe and grow on most continents, but with certain environmental conditions. Moreover, the plant contains Flavonoids, Glycoside, water-soluble sugars, Triterpenes, and volatile oils where it's most important components are Carvacrol and Thymol. Oregano used in the treatment of respiratory problems and as an antimicrobial, antiseptic, especially for skin problems, anti-inflammatory, analgesic, anti-depression and reduces the arteriosclerosis, which it produced by many countries such as Turkey and Greece (Sophy Lacoste, 2013). The main components that make up the volatile oils of marjoram are Carvacrol, Thymo, p-cymene, limonene, a-terpinene, y-terpinene, pinene, isoborneol, borneol and a-terpineol (Chun *et al.,* 2004; Martos *et al.,* 2007). The scientific research institutions and the pharmaceutical industry laboratories in the world seeking to use modern techniques, including plant tissue culture in the production of effective compounds and convert them into therapeutic drugs. As the tissue culture has proven to be a useful and effective means of producing many natural materials and medicinal drugs such as compounds of phenols, alkaloids and Glycosides and Saponins of various plants (Phillipson, 1990, Malabagal, Tsay 2004). Studies have shown that growth regulators have a role in stimulating the production of active compounds in addition to their role in the shoot growth, where the growth regulators are one of the axes through

which to increase the concentrations of secondary compounds. Growth regulators work to build proteins, increase cell division, build chlorophyll, activate enzymes and transport nutrients (Hopkins, 1999), resulting in an increase in shoot and thus increases in the plant's ability to construct primary metabolic compounds that convert to secondary metabolic compounds. Its include salicylic acid, which has an effective role in regulating physiological processes within the plant and stimulating the plant to resist biological and non-biological stresses, which is reflected in the increase of the shoot and then produced the secondary compounds (Hayat and Ahmad, 2010). Based on the above, research aims to: propagation of the marjoram plant histologically and study the possibility of stimulating and increasing the production of active compounds (volatile oils) through the addition of different concentrations of growth regulator (Salsilic acid) and compared with the same effective compounds of the mother plant.

Materials and Methods

The study was carried out in the Laboratory of Plant Tissue Culture/ Department of Horticulture and Landscape Gardening /College of Agricultural Engineering Sciences / University of Baghdad in the period from July 2018 to August 2019. Where the experimental program included, Initiation stage**,** where the Source of explants was Seeds

Preparation of medium

The available medium MS (1962 Skoog, Murashige) produced by the Holland Company Duchefa that weights 4.9 $g.L^{-1}$ in all propagation stages. As the Sucrose was added by 30 $g.L^{-1}$, and the plant growth regulators were added after basic solutions were prepared according to the type of experiment and then the pH (Potenz Hydrogen) was adjusted to 5.7 ± 1 Using hydrochloric acid HCL or Sodium hydroxide NaOH by (1N). Subsequently, the volume completed to a liter and Agar type (Agar-Agar) by 7 g. L^{-1} was added to the medium in order to homogeneity of ingredients and dissolve the Agar, then the medium was heated using a hot plate magnetic stirrer until homogenization and then distributed in a culture tube with 10 ml and covered with appropriate cover.

Sterilization

One of the important processes on which the success of its tissue culture depends is sterilization and includes: - Sterilization of used tools, where all the tools used such as blades, tweezers and petri dish were sterilized using Autoclave at 121°C and at a pressure of 1.04 kg. cm^{-2} for 30 minutes. In addition, 99% concentration of ethyl alcohol is used to sterilize tweezers and blades and burn them with a Bunsen burner after each planting process into the Laminar air flow cabinet, and the planting cabinet and hands were sterilized with ethyl alcohol at a concentration of 70%. Secondly, the Medium sterilization, where the Tubes contained medium was sterilized by Autoclave at 121°C under 1.04 kg pressure.cm^{-2} for 15 minutes, and then the medium left to cool and hardens at room temperature until it was ready for planting. Finally, seeds sterilizing and planting, the seeds were washed with running water, then with dishwashing liquid and then with running water. Subsequently, sterilized by the commercial minor at a concentration of 10% with the addition of a drop of tween-20, and then planted on a medium MS free of growth regulators. The cultures were incubated in the growth room light 1000 Lux for 16 hours/ day at a temperature of 25 ± 2 °C, and the light conditions were 16 hours lighting and 8 hours darkness.

Multiplication stage

The vegetative growth resulting from the Initiation stage was planted on MS medium supplementing with salicylic acid concentrations (0, 1, 2, and 3) mg. L^{-1} with 0.5 $mg.L^{-1}$ from BA and the following measurements were then made:- measuring the number and length of the shoot, the wet and dry weight. Furthermore, the measuring the amount of chlorophyll dye ($mg.100^{-1}g$) According to (Goodwin 1976), the total chlorophyll content was calculated according to the following equation: -

Total chlorophyll = 8.02 (663) +20.2 (645). As well as, the Measure the amount of Anthocyanin dye ($mg.100^{-1}g$) was achieved As reported in (Ranganna, 1977) according to the equation:- Anthocyanin dye (AD) ($mg.100^{-1}g$)

$$AD = \frac{\text{dilution volumex total volumex device reading}}{\text{sample volumex sample taken for device reading}} \over 98.2$$

Finally, the Concentrations of active substances were measured, where the test was conducted in the laboratory of the Ministry of Science and Technology/ Environment and Water Department, by using (Gas chromatography) GC device model Shimadzh 2010. The sample area was based on when estimating the concentrations of these substances after comparing them with the area of the standard active substances according to the following equation: - Sample concentration (SC)

$$SC = \frac{\text{standard substance concentration} \times \text{sample area}}{\text{standard substance area}} \times \frac{\text{dilution times number}}{\text{volume}}$$

Results and Discussion

Effect of salicylic acid on shoot average number and length of marjoram plant

The results of table 1 showed that the comparison treatment was superior by giving the highest average number of shoot was 13.00 shoot/explant, and did not differ significantly from the concentration of 2 mg.L^{-1} which gave 11.00 shoot/explant, while it was significantly different from the rest of the treatments. Moreover, for the effect of salicylic acid in the shoot length, the concentration of 1 mg.L^{-1} was superior by giving the highest average shoot length 11.90 cm, which differed from the comparison treatment, that gave the lowest average amounted 3.50 cm, while did not differ significantly from the concentration of 2 mg.L^{-1}, which reached 10.30 cm.

Effect of salicylic acid on the total dry and wet weight of marjoram (g)

(Table 2) shows the effect of different concentrations of salicylic acid on the wet and dry weight of marjoram plant, where the concentration of 2 mg.L^{-1} was significantly superior by giving the highest average wet weight that amounted to 0.682 g compared with the comparison treatment which gave the lowest wet weight of 0.316 g. As for dry weight, the concentration of 2 mg.L^{-1} was superior by giving the highest average dry weight of 0.067 g compared with the comparison treatment which gave the lowest dry weight of 0.028 g.

Table 1: Effect of salicylic acid on the shoot average number (shoot/explant) and shoot average length (cm) of marjoram planted on MS medium containing 0.5 mg.L^{-1} BA after 6 weeks of cultivation.

mg .L^{-1} SA	Number of shoot /explant	Shoot length cm
0	13.00	3.50
1	9.00	11.90
2	11.00	10.30
3	5.00	8.20
L.S.D	2.06	2.12

Effect of salicylic acid on the average chlorophyll dye (mg/100gm) of marjoram planted on MS medium containing 0.5 mg.L^{-1} BA after 6 weeks of cultivation

(Table 3) data showed that there were a significant difference between the treatments, and the concentration of 2 mg.L^{-1} was significantly superior by giving the highest chlorophyll dye ratio of 372.96 mg/100gm, which differed significantly from the rest of the treatments.

Effect of salicylic acid on the average Anthocyanin dye (mg.100 $^{-1}$g) wet weight) of marjoram planted on MS medium containing 0.5 mg.L^{-1} BA after 6 weeks of cultivation

Table 2: Effect of salicylic acid on wet and dry weight (gm) of marjoram planted on MS medium containing 0.5 mg.L^{-1} BA after 6 weeks of cultivation.

SA	Wet weight	Dry weight
0	0.316	0.028
1	0.494	0.052
2	0.682	0.067
3	0.342	0.041
L.S.D	0.134	0.015

Table 3: Effect of salicylic acid on average chlorophyll dye (mg/100gm) of marjoram planted on MS medium containing 0.5 mg.L^{-1} BA after 6 weeks of cultivation.

SA	Chlorophyll dye (mg/100gm)
0	122.52
1	262.28
2	372.96
3	264.81
L.S.D	4.81

weeks of cultivation

The results of table 4 showed that the plant content of the Anthocyanin dye was significantly affected by salicylic acid treatments, where the concentration of 3 mg.L^{-1} was given the highest rate was 186.25 (mg.100 $^{-1}$g) wet weight compared to the comparison treatment that gave 14.07(mg.100 $^{-1}$g) wet weight.

Effect of salicylic acid on concentration of volatile oil compounds of marjoram plant

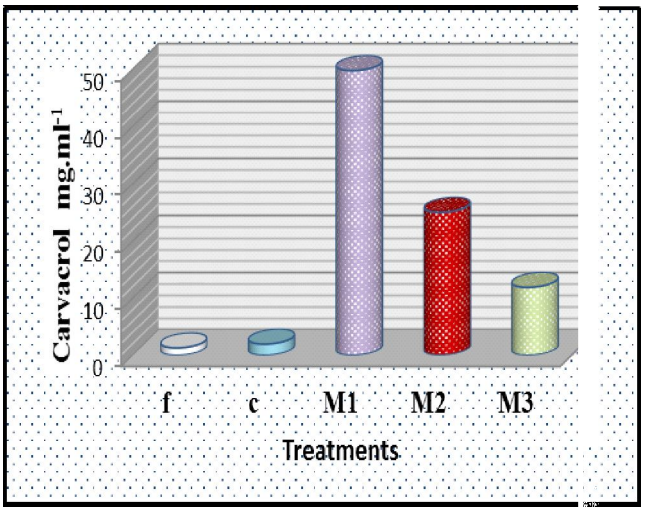
The results of table 5 shows the effect of different concentrations of salicylic acid on the concentrations of medical compounds. It was observed that there was a clear effect of the salicylic acid addition in increasing the concentration of active compounds, especially the concentration of 2 mg.L^{-1}, which gave the highest concentration of (Al-pinene, Limonene, Carvacrol, Linalool compounds) compared with the comparison treatment. While the concentration of 1 mg.L^{-1} in was characterized by increasing the concentration of Sabinen and Terpinen, while the Thymol compound was 17.37 mg.L^{-1} in

Table 4: Effect of salicylic acid on average Anthocyanin dye (mg.100 $^{-1}$g) wet weight) of marjoram planted on MS medium containing 0.5 mg.L^{-1} BA after 6 weeks of cultivation.

mg .L^{-1} SA	Anthocyanin dye (mg/100g)
0	14.07
1	41.37
2	119.63
3	186.25
L.S.D	13.74

Table 5: Effect of salicylic acid in the concentration of volatile oil compounds in the shoot resulting from multiplication stage of the marjoram plant after 6 weeks of cultivation on MS medium.

Treatments	Carvacrol	Thymol	Sabinen	Terpinen	comphor	Linalool	Limonene	AL-pinene	Mayrcene
0	1.93	17.37	19.40	1.40	1.33	1.33	0.37	2.97	2.83
1	2.23	6.33	28.54	2.30	1.41	5.23	0.38	2.97	4.43
2	14.93	11.54	27.77	1.90	1.80	9.33	0.75 a	4.04	4.43
3	5.07	9.23	19.83	1.60	1.40	2.85	0.38 b	3.24	3.98
$LSD_{0.5}$	1.59	1.36	6.56	0.96	0.94	1.49	0.28	0.93	1.81



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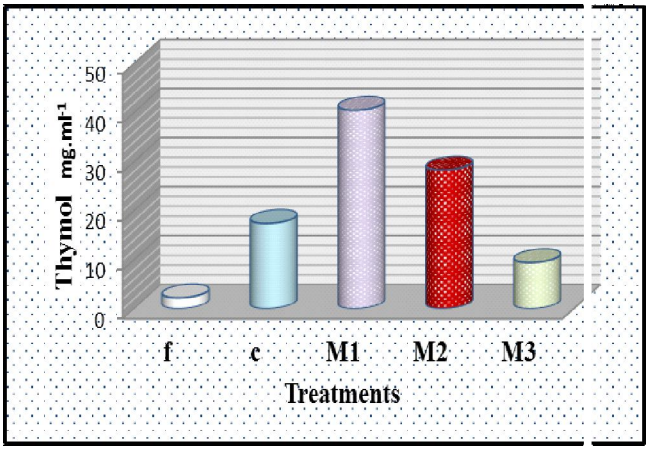

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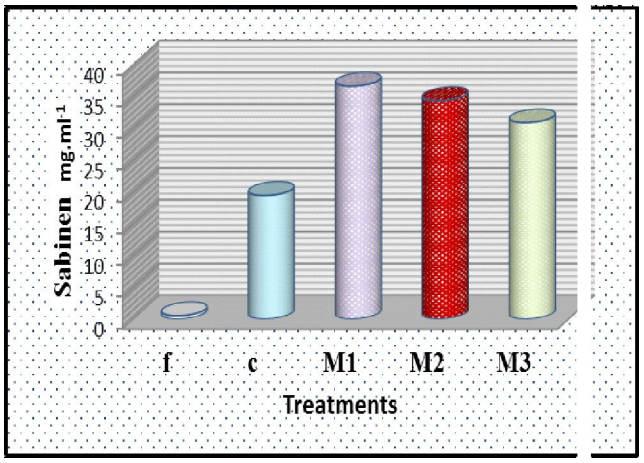

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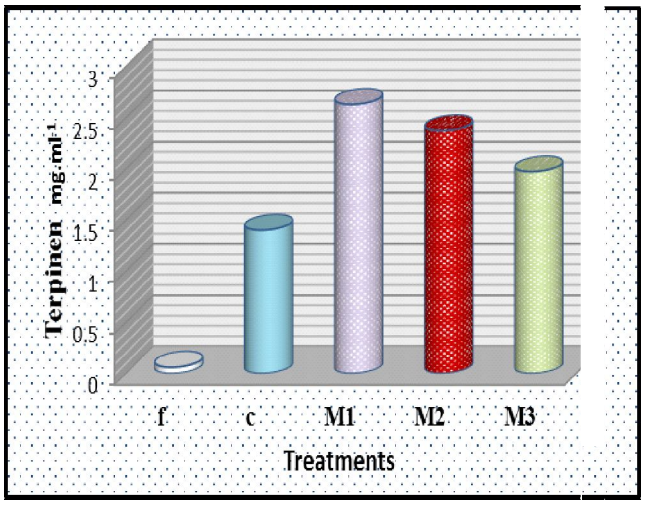

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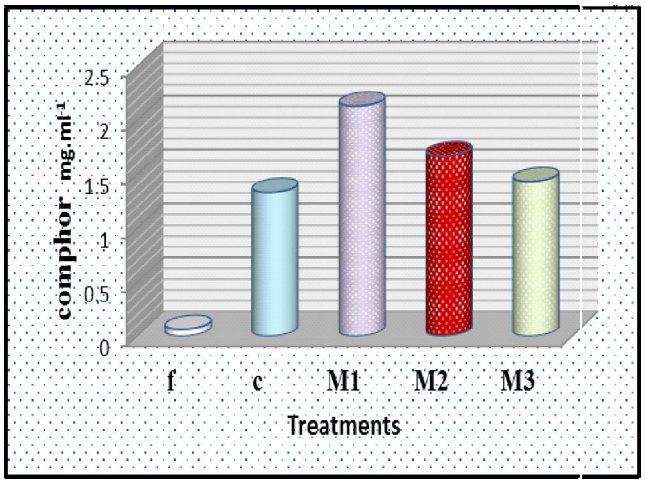
comparison treatment.

Discussion

The results showed that salicylic acid has the effective role in improving vegetative traits in tissue culture, as it was observed that the effect of SA in increasing the shoot length may be due to its role in stimulating the internal hormones such as ouxin IAA and gibberlin GA3 (Talaat *et al.,* 2014, EL-bassiony *et al.,* 2014). The increase in wet and dry weight may be attributed to the fact that the salicylic acid is characterized by its stimulating effect that result from its regulation of plant physiological and biochemical processes such as cell division, differentiation and elongation. As well as, it stimulates the absorption of elements and control of their transition, increase the permeability of cellular membranes of ions, accelerate the process of carbon and protein synthesis and also increases the activity of enzymatic processes and photosynthesis. Which leads to increased growth and the accumulation of dry matter in the plant (Hayat *et al.,* 2007; Raskin, 2003; Blokhina, 2003; EL-Tayeb, 2005). As for the increase of plant content of dyes was due to the role of salicylic acid in speeding up the formation of dyes such as chlorophyll and carotene and increase the



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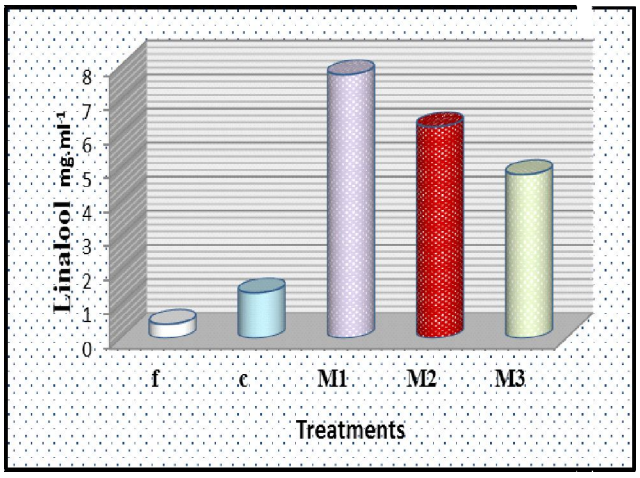

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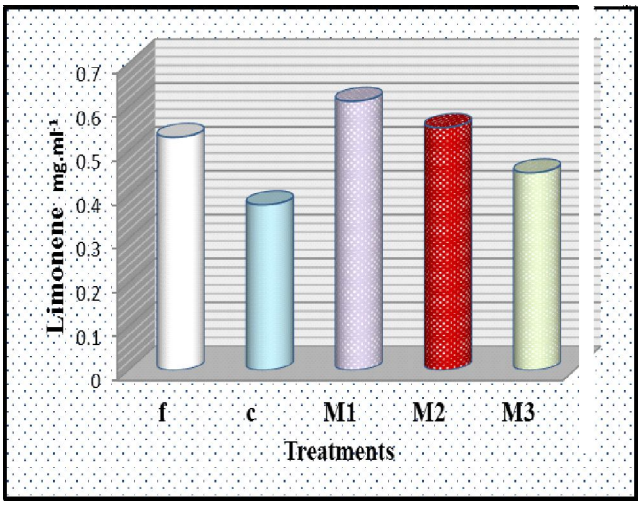

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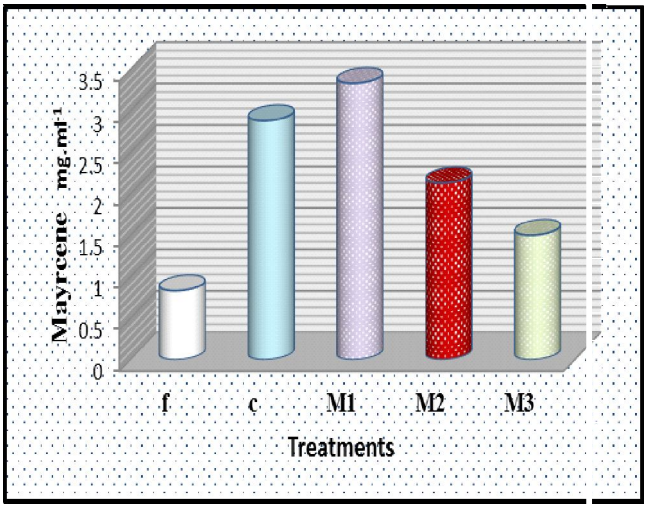

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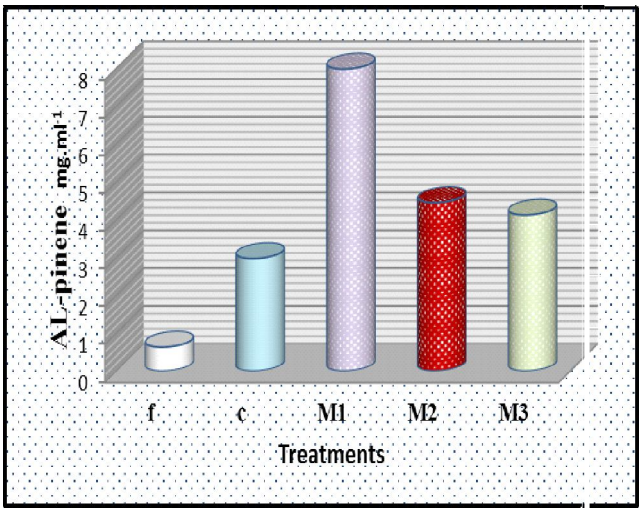

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accumulation of processed food that have a role in the construction the molecule of chlorophyll. This is what (Mohammed and Younis 1991 and Ahmed, Hayat 2007) pointed out. As for the increase in concentrations of active substances may be attributed to the reason that the salicylic acid has the effective role in regulating physiological processes and reduce oxidation occurs in the cell membranes and thus improve the permeability of nutrients.

Conclusions

1. The addition of salicylic acid to the medium gave the highest rate of shoot length and the highest rate of wet and dry weight.
2. The addition of salicylic acid gave the highest concentration of chlorophyll and Anthocyanin dyes.
3. The addition of salicylic acid at a concentration of 2mg.L^{-1} has led to increased concentrations of volatile oil compounds (Al-pinene, Limonene, Carvacrol, Linalool).

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