H

EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
BOTANY

ISSN 2090-3812

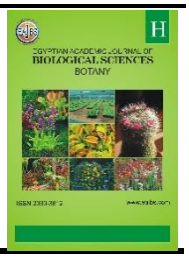
www.eajbs.com

Vol. 13 No.2 (2022)

Citation: Egypt. *Acad. J. Biolog. Sci.* (H. Botany) *Vol.13(2) pp217-233(2022)*
DOI: 10.21608/EAJBSH.2022.281338

Egypt. Acad. Journal Biology. Sci., 13(2):217-233(2022)

Egyptian Academic Journal of Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbsh.journals.ekb.eg.



The Effect of Adding Marine Algae and Jasmonic Acid on Growth Characteristics and Active Substance Yield of Clove Plant *Dianthus Chinensis* L.

Sama Suliman Inad and Rana Hashim Aloush
Saladin Sewage Department, College of Science, University of Tikrit
***E-mail: Sama.s.enad4407@st.tu.edu.iq**

ARTICLE INFO
Article History
Received:15/10/2022
Accepted:14/12/2022
Available:19/12/2022

***Keywords*:**
Dianthus Chinensis L., seaweed extracts, jasmonic acid, flavonoids and tannins.

ABSTRACT

The study was conducted in one of the greenhouses in the Department of Horticulture and Garden Engineering at the College of Agriculture/Tikrit University. For the period between 5/1/2021 - 6/1/2022, *Dianthus Chinensis* L. has been cultivated at the age of four leaves, and then the samples were separated and distributed on containers, and they were classified into three replicates, each replicate containing nine treatments with seaweed extracts with concentrations (0, 5, 10) ml. L-1 and extract of jasmonic acid in concentrations (0, 0.5, 1) ml. L-1, each treatment included four containers in the experimental unit. The study recorded significant differences in the effect of single and overlapping treatments, especially when treated with concentrations of 10 ml. L-1 of seaweed and 1.0 ml. L-1 of jasmonic acid in vegetative growth characteristics (plant height, number of leaves, moist and dry weight of the shoot, length and number of internodes, number of branches, leaf area, percentage of chlorophyll A, B and total), Which are attributed to the nutrients of macro- and trace elements present in the extracts that are important in stimulating growth. Significant differences were recorded with the effect of the treatments on the characteristics of floral growth (the days until buds emergence, length and width of the bud, the days until bud opening, flower diameter and the number of flowers, moist and dry weight of the flower, percentage of carotene), which is considered simple in comparison to the effect on vegetative characteristics, It may due to genetic traits that are slightly affected by stimuli and the environment. Also, there are other significant effects of the extracts on the percentage of volatile oil when treated with A1J0, which was 1.7%, in addition, there was a significant effect of the extracts on the chemical compounds, the concentrations of 10 ml. L-1 and 1 ml. L-1 of seaweed extract and jasmonic acid gave the presence of flavonoids (Quercetin, Apigenin, Rhamntin, Kaempferol), while the concentrations were 5 ml. L-1 and 10 ml. L-1 of seaweed extract with jasmonic acid, respectively, gave the presence of tannins (Gallic acid, Phloroglucino, Tannic acid, Gallotannin, and Pyrogallic acid). The histological study dealt with the variance effectiveness of the aforementioned extracts on the anatomical characteristics of the superficial view of the leaf epidermis (the transverse sections of the leaf blades, the middle vein, the variation in the dimensions of the stomata, the upper, and lower epidermis of the leaves, the variance in the qualitative, quantitative characteristics and the frequency of stomata in the leaves) the stomatal complex type is diacytic which was normal *Dianthus* characteristic.

INTRODUCTION

The world faces wide population growth and development, which has a major role in increasing the need to develop livelihoods, including food sources, the most important of which are crops. Therefore, those concerned resorted to developing chemical and organic

fertilizers that gave immediate results, but their continuous use harmed soil quality and healthy soil microbial communities. And soil fertility and plants are grown in this soil in the long run (Arioli, 2015), and here attention turned to alternatives that provide nutrients and stimulate plant growth with the least harm to plants and the resulting crop. Examples of nutrients are seaweed, algae, and plant hormones (Layek, *et al.,* 2018). Clove Dianthus is a perennial herbaceous plant belonging to the clove family. It is highly branched, reaching a height of more than 60 cm. It has rigid, branching stems with prominent nodes, leaves in opposite pairs, striped in shape, thick in appearance, beautiful terminal flowers in many colors, a five-leaved corolla, and a cylindrical cup to two cups. According to cultivars, flowers vary in size, from small to large (Sreeelekshmi and Siril, 2020). Cloves are severely affected by rain, humidity, and diseases. To obtain flowers of high commercial quality, they must be produced in protected agriculture, as flowers grown in open fields do not comply with international production standards when compared to using a glass or plastic house (Ngan, *et, al.,* 2020). Cloves are severely affected by rain, humidity, and diseases. To obtain flowers of commercial quality, they must be produced in protected cultivation because flowers grown in open fields do not comply with international production standards when compared to using a glass or plastic house (Aalifar, *et al.,* 2019). Cloves are long-day plants, and six pairs of leaves are sufficient for a floral response. Due to the weak sunlight during the day and the high cost of heating in winter, this led to a reduction in the production of cloves in northern Europe and the United States of America (Kumar, *et al.,* 2020), and accordingly moved Clove production to areas that are characterized by lower production costs within unheated plastic houses, easy to ventilate, high productivity, good flowers, as well as protection from extreme climatic conditions to ensure that the growing and continuous demand in the global market for cloves is met (Hemanta, *et, al.,* 2012). Seaweeds are large aquatic algae that belong to the plant kingdom. Seaweeds can thrive in a wide range of harsh environmental conditions and adapt to new ecological surroundings. They produce a variety of healthy primary and secondary metabolites. Therefore, seaweeds are a treasure trove of untapped natural bioactive compounds (Hashem, *et al.,* 2019), as they are effectively used as environmentally friendly biofertilizers in modern agriculture and the improvement of horticultural crops when used in appropriate quantities because they contain high levels of organic matter, which enriches the soil with nutrients. (Hamed, *et al.,* 2018). Biostimulants have increased in recent years, which has greatly helped reduce the use of chemical fertilizers in agricultural production. Among these catalysts is the seaweed extract that contains auxins and cytokinins, in addition to rare elements that positively affect the growth and elongation of cells, which affects the yield plant (Szabo and Hartko, 2009). The treatment with seaweed extract is a good source of active substances. The effect of seaweed extract on the clove plant is because it contains many components, vitamins, and macronutrients, in addition to micronutrients that improve the vegetative growth of the plant, such as increasing the vegetative characteristics such as the number of leaves and the content of chlorophyll, and thus an increase in the efficiency of photosynthesis and representation food (Khan, *et al.,* 2009). This study aimed to investigate the clove plant's response to the addition of seaweed extract and its effect on the vegetative and flowering growth characteristics and the chemical content of some secondary metabolite compounds. And a study of the effect of spraying with the growth regulator jasmonic acid and its effect on the characteristics of vegetative and flowering growth and the plant content of secondary metabolite compounds. Then, the effect of the interaction between the addition of seaweed extract and spraying with jasmonic acid was tested to improve the growth, flowering, and plant content of some secondary metabolites and in some anatomical characteristics of the plant.

MATERIALS AND METHODS

A. Tannins Extraction Procedure:

To isolate Tannin from plant extract powder, 1 gram of the dried and powdered plant material is extracted with 60 ml hexane first. this removes fats, oils, terpenes, waxes, etc. This extract is discarded.

The materials are now subjected to an alcohol extraction, with 50ml of methanol. The extract is evaporated to 2 ml and leaves the crude Tannin mixture.

Many Tannins can be isolated directly from the alcoholic extract by HPLC. This is a separation that works well for Tannin extracted by acidified methanol.

Separation Condition:

1. mobile phase70:30methanol: waterv/v
2. separation of Tannin mixture reversed phase C-18 (50× 2.6 mm ID) column, 5µm particle size ID.
3. flow rate 2 ml/min
4. temperature experiment 33 c.
5. uv detector 249 nm.
6. standard 50 µm /ml.

Description Equipment:

1.shimadzu HPLC-10, double pump model HPLC-10A Shimadzu, japan.
2. reodyne 7125 injectors equipped with 20 µl injection loop.
3. uv detector set at 249 nm.

Concentration = area in sample/area in standard * standard Concentration. *dilution
Percentage %= concentration partial / concentration total
Dilution for all specimens = 2

B. Flavonoids Extraction Procedure:

To isolate Flavonoids from leaf plant extract powder, 1 gram of the dried and powdered leaf plant material is extracted with 80 ml isohexane first. This removes fats, oils, terpenes, waxes, etc. This extract is discarded.

The materials are now subjected to an alcohol extraction, with 30ml of ethanol. The extract is evaporated to 2 ml and leaves the crude flavonoid mixture.

Many Flavonoids can be isolated directly from the alcoholic extract by HPLC. This is a separation that works well for flavonoids extracted by acidified ethanol.

Separation Condition:

1. mobile phase70:30ethanol: waterv/v
2. separation of flavonoid mixture on reversed phase C-18 (250× 6.4 mm ID) column, 5µm particle size ID.
3. flow rate 2 ml/min
4. temperature experiment 25 c.
5.Uv detector 259 nm.
6. Standard 50 µm /ml.

Description equipment:

1.shimadzu HPLC-10, double pump model HPLC-10A Shimadzu, japan.
2. reodyne 7125 injectors equipped with 20 µl injection loop.
3. UV detector set at 259 nm.

Concentration = area in sample/area in standard * standard Concentration. *Dilution
Percentage %= concentration partial / concentration total
Dilution for all specimens = 1

C. Anatomical Study:

1. Epidermis Preparation:

The epidermis was prepared from soft samples, a part was taken from the middle of the fully developed leaf so that it included the middle vein, part of the blade, and the edge, and the stripping-off method was used to obtain the upper and lower epidermis by using forceps with two fine ends. The epidermis was spread on a glass slide, and glycerine was placed on it. Then, it was covered with a slide cover and ready for examination and study. Using a graduated ocular lens scale, the models were examined under a compound microscope, Olympus type. (5-10) epidermal cells were measured, the dimensions of the radial wall and the tangential wall were studied, the shape of the normal epidermal cells, the dimensions of the guard cells, and the shape of the stomata complexes were studied, and the frequency was calculated. Stomata according to the equation:

Stomata frequency = number of stomata per mm2. According to Stace (1965), the terms given in Dilcher (1974) were used.

2- Preparation of Transverse Sections:

The vertical sections of the species under study were studied based on the soft samples that facilitated they are obtaining from the field by immersing them in sodium hydroxide (NaOH) solution at a concentration of 1% for (24-48) hours, then rinsed with distilled water to remove the basic solution from them, and then fixed in FAA solution) for a period of (18-24 hours, and when used, they are rinsed with 70% alcohol, and the rest of the steps are passed, or the dry samples are boiled with water for (5) minutes, then transferred to 70% alcohol until used. The sections were prepared according to the following steps:

1) Killing and Fixation: Soft parts of the leaf and flower heads were taken and cut into small pieces ranging in length from (2-5) mm, then transferred to small vials and placed in them approximately 20 milliliters of the formaldehyde acetic alcohol solution that was prepared. Prepare it according to the method of Johnson (Johnson, 1940) for a period ranging between (18-24) hours:

Ethyl alcohol 50ml, Glacial acetic acid 5ml, Formaldehyde (37%-40%) 10ml, and Distal water 35ml

2) Washing and Dehydration: The samples were washed twice with ethyl alcohol at a concentration of 70% to ensure that traces of the fixative were removed and kept in 70% alcohol.) two hours in each concentration, then placed in absolute ethyl alcohol for two hours to eliminate the remaining water in the preserved samples.

3) Clearing and Infiltration: After the process of washing and priming, the liquefaction and impregnation process was carried out by passing the samples through a series of a mixture of absolute ethyl alcohol and xylene in volume ratios (1:3, 1:1, 3:1) and then with pure xylene for two hours each according to the SAS method. (Sass, 1958), then half of the xylene containing the samples was poured. An amount of liquid paraffin was added to the oven at a temperature of (55-60) C for one hour for the paraffin to replace the evaporated xylene. The paraffin was poured, and pure liquid paraffin was added instead. Inside the oven, samples were left in the oven for a period ranging between (4-5) days to remove traces of xylene. Paraffin was poured, and pure liquid paraffin was placed instead. The samples were left in the oven for two hours (I repeated this process 5-6 times) for the last time. I left for a whole night, Al-Mashhadani (Al-Mashhadani, 1992).

4) Embedding and Mounting: Custom molds of appropriate sizes were prepared, and a quantity of hot molten wax was poured into them at a degree of (60-55) Om. The samples were transferred to square-shaped molds and placed in the desired direction. The samples were labeled and left cold for a full day to ensure their hardening. It was sufficient, then the wax molds were removed while preserving their identities, and thus they became ready for cutting. Then the wax molds were fixed on wooden pieces designated as carriers after they were trimmed using a blade for that purpose until the molds became parallel-rectangular to

be ready for cutting with a Rotary Microtome of Bright type and a thickness of (10). -15) Micrometer. After cutting, the strips containing the samples were placed in a water bath with a temperature ranging between (40-45) Om to flatten the strips. Then the strips were carried on clean glass slides previously coated with a thin smear of glycerin-albumin adhesive, and the slides were placed on a plate. It is heated at (40-45) o C for a period of (4-12) hours to fix the tapes on the slats and remove wrinkles.

5) Dewaxing and Staining: According to Sass (1958), glass slides containing plant sections were passed through the following solutions:

A- Xylene from (2-4) hours at 50 Om twice.b- Xylene to absolute alcohol 1:1 for five minutes.

C- Descending series of ethyl alcohol 30%, 50%, 70%, 80%, 96% for 5 minutes each.

D - Safranin dye 0.2% dissolved in ethyl alcohol at a concentration of 50% for a period of (2-24) hours.

C- An ascending series of ethyl alcohol 90%, 80%, 70%, 50%, and 30% for 5 minutes for each concentration.

h- Fast green dye at a concentration of 1% in absolute ethyl alcohol for (3-5) seconds.

g- Absolute ethyl alcohol for (5) minutes.

d- Xylene and Clove oil in a 1:1 volume ratio for (5) minutes.D- Xylene for (3) minutes twice.

After that, the slides were loaded with permanent mounting by placing a drop of adhesive (Canada balsam) on the sections, and the cover slide was placed gently, and the slides were transferred to a hot plate at a temperature of (40-45) C for a full day to be dried Jonson, (1940). The sections were then examined under an Olympus-type compound microscope and photographed with a camera installed on the compound microscope.

RESULTS AND DISCUSSION

1. Effect of Jasmonic Acid and Seaweed on The Percentage of Oil Content:

The different treatments significantly affected the percentage of oil content, as the addition of seaweed was recorded at a concentration of 5 ml. L-1 had the highest percentage of 1.7, while this percentage decreased to 0.8 in the comparison treatment (Table 1). This may be attributed to the fact that marine algae are a rich source of protein and antioxidants, which reduces stress, increases plant efficiency in metabolic processes, and, thus, increases secondary metabolite compounds. And its ratio to the vegetable part (zielewiz, 2020).

While the remaining treatments gave different percentages, adding seaweed was recorded at a concentration of 10 ml. L-1 and jasmonic acid at a concentration of 0.5- and 1.0-ml. L-1, a ratio of 1.2, and the use of seaweed at a concentration of 0.5 and jasmonic acid at a concentration of 10 ml. The liter-1 scored a ratio of 1.4. This may indicate that the studied trait was affected by jasmonic acid, which participates in the physiological and chemical processes associated with plant growth and development and helps the plant to adapt to various stresses (Raza, *et al.*, 2020), also; jasmonic acid has a role in increasing the manufacture of carbohydrates and secondary compounds in the flowering parts. Thus, the percentage of oil increased (Al-Shofili, 2013). It is worth noting that the rest of the values ranged between the two extremes (0.9 and 1.5).

Table (1) Effect of jasmonic acid and seaweed on the percentage of oil content

Transactions	%	A1J1	1.0 de
A0J0	0.8 e	A1J2	1.4 bc
A0J1	1.3 bc	A2J0	0.9 e
A0J2	1.5 ab	A2J1	1.2 cd
A1J0	1.7 a	A2J2	1.2 cd

Table 2: Effect of jasmonic acid and seaweed on flavonoids of chemical compounds in clove leaves

Transactions	Flavonoids			
	Quercetin	Apigenin	Rhamntin	Kaempferol
A0J0	16.9257 f			22.0616 c
A2J0	35.3743 a	19.6974 b		17.7558 d
A2J2	30.6109 c	23.7886 a	42.4682 a	17.2468 d
A1J1	14.0184 g	22.9273 a		8.7685 f
A1J2	18.0333 d	8.7598 c	18.0333 d	14.2637 e
A2J1	11.8239 h	7.21833 c	19.3421 d	30.9776 a
A0J1	22.0993 e		25.5705 c	
A0J2	15.5259 fg		29.1507 b	9.0206 f
A1J0	33.7386 b	22.9812 a	43.5071 a	27.6519 b

*Similar letters in one column mean that there are no significant differences between them at the level of 0.05

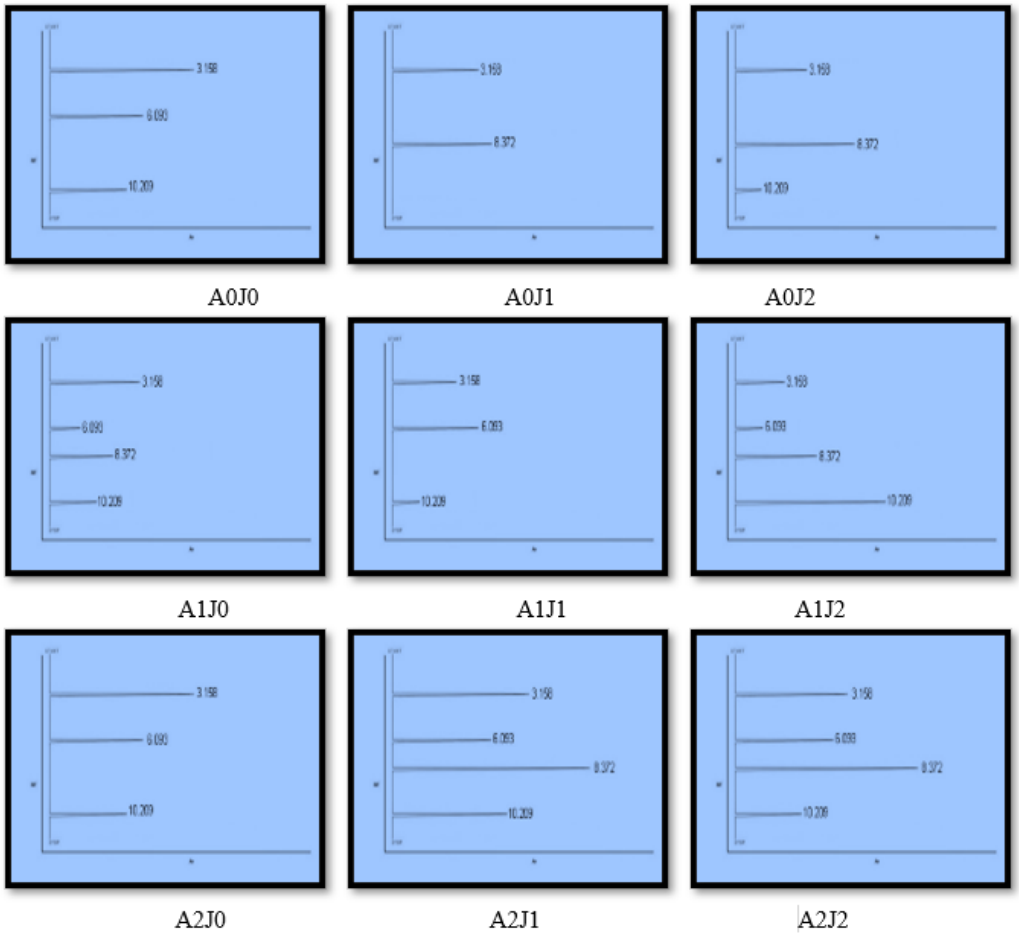


Fig. 1: The chromatography curve of the active substances.

1.1. Results of the Effect of Treatment with Jasmonic Acid and Seaweed on Theanines:

Table 3 shows a chapter on 5 tannin compounds (Gallic acid, PHIoroglucinol, Tannic acid, GaIIotannin, and pyrogallic acid). The comparison treatment was recorded with jasmonic acid at a concentration of 0.5 ml. L-1 gave the highest rate of gallic acid, amounting to 49.4281, while the lowest rate of gallic acid was recorded using marine algae at a concentration of 10 ml. L-1 and the concentration are 0.5 ml. L-1 of jasmonic acid was 3.6099, while the values ranged between the two concentrations mentioned above.

The percentage of PHoroglucinol increased in the comparison treatment as it gave 75.8375, while the percentage decreased when the concentration was added 10 ml. L-1 of marine algae nested with 0.5 mL concentrate. L-1 of jasmonic acid reached the lowest percentage of 12.3858, and the rest of the percentages ranged between the values mentioned above.

The use of seaweed at a concentration of 10 ml. L-1 and jasmonic acid at a concentration of 1.0 ml. L-1 increased tannic acid and gall tannin, where the ratios reached 46.0635 and 22.2159, respectively. In contrast, these ratios decreased to record the lowest level of tannic acid when using a concentration of 10 ml. L-1 of marine algae reached 19.9500, and the lowest level of Gallootannin was recorded using marine algae at a concentration of 10 ml. L-1 and 0.5 ml. L-1 Jasmonic acid. PyrogaIIic acid's highest percentage was when the concentration was 1.0 ml. L-1 with the comparison treatment 0 amounted to 46.6410, while this percentage decreased to 22.9290 when treated with seaweed at 10 ml. L-1 and a concentration of 0.5 mL. L-1 and the remaining values ranged between the limits mentioned above.

From the previous results, we note that the percentage of separated materials decreased to the lowest levels when using the concentration of 10 ml. L-1 of seaweed concentrate 0.5 mL. L-1 of jasmonic acid, except for Tannic acid, recorded a low level of 22. 2797.The comparison treatment recorded the absence of GaIIotannin &PyrogaIIic acid compounds, while the comparison treatment recorded jasmonic acid at a concentration of 0.5 ml. L-1 The absence of three compounds: Tannic acid, Gaotannin, and Pyrogallic acid, and the use of marine algae at a concentration of 5 ml was recorded. L-1 with a concentration of 0.5 mL. L-1 absence of gallic acid, and tannic acid, while the treatment with seaweed was recorded at a concentration of 10 ml. L-1 and concentration 0.5, 1.0 ml. L-1 of jasmonic acid and the presence of all identified compounds. Marine algae on theanines, as the highest rate of the gallic acid compound, was 49.4281 in treatment A0J1, while the lowest rate for the same substance was 3.6099 in treatment 1A2J. As for the effect of the treatments on the compound Phloroglucinol, the highest rate was 75.8375 in treatment 2A2J, while the lowest rate was when Treatment A2J1, which amounted to 12.3858, and the table showed the effect of the transactions on the rate of the tannic acid compound, whose highest value was 46.0635 in treatment A2J2, and the lowest rate of the same compound was 19.9500 in treatment A2J0, while the highest rate of the compound was 22.2159 in treatment A2J2, while the lowest rate was in treatment A1J2 and reached 3.0950. Finally, regarding the effect of the treatments on the compound pyrogallic acid, the highest rate was 46.6410 in treatment A0J2, while the lowest rate for the same compound was 22.9290 in treatment A2J1.

Table 3: The effect of jasmonic acid and seaweed on the theanines of the chemical compounds in the leaves of the clove plant.

Transactions	Teanines				
	Gallic acid	PHloroglucinol	Tannic acid	Gallotannin	pyrogallic acid
A0J0	40.2801 d	75.8375 a	23.5316 e		
A2J0	37.6254 e	24.6838 e	19.9500 f		45.7058ab
A2J2	17.0232 f	35.3601 c	46.0635 a	22.2159 a	23.2752 d
A1J1		24.8995 e		13.0093 b	44.8589bc
A1J2	17.4617 f	26.0301 d	42.6827 b	3.0950 d	43.1121 c
A2J1	3.6099 g	12.3858 h	22.2797 e	8.1987 c	22.9290 d
A0J1	49.4281 a	42.7799 b			
A0J2	43.9706 c	22.4488 f	36.8568 c		46.6410 a
A1J0	45.8218 b	20.2274 g	34.2285 d		44.4420bc

*Similar letters in one column mean that there are no significant differences between them at the level of 0.05

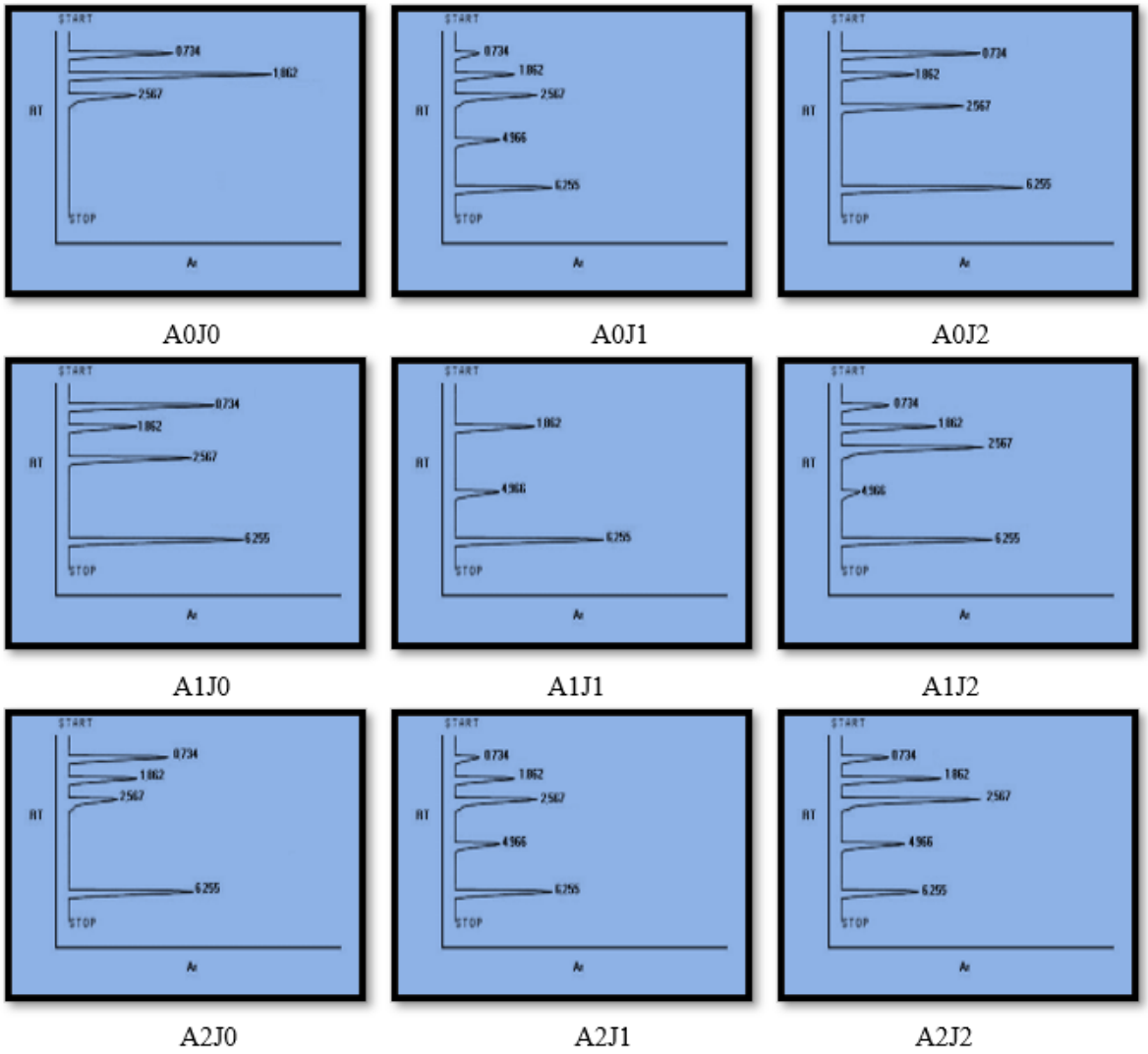


Fig. 2: The chromatogram of the active compounds.

1.2. Discussing the Effect of Treatment with Jasmonic Acid and Seaweed on The Chemical Compounds of The Chinese Clove Plant:

From the above results, it was found that there is a significant effect when using jasmonic acid and seaweed on the rate of active chemical compounds within the clove plant, that jasmonic acid plays a key role in regulating the indoor plant and that its concentration is sufficient to perform the physiological role of the plant. It acts as an essential signal in the plant hormone network and interacts with auxins, gibberellins, salicylate, and brassinosteroids to regulate plant growth and disease resistance. Sharma *et al.* (2019) showed that seaweed extracts are biologically active substances used in agricultural and horticultural crops and have many beneficial effects in terms of improving the quantity and quality of production. The use of seaweed extracts as natural organic stimulants in sustainable agriculture has increased to avoid excessive fertilizer use and improve mineral uptake (Rouphael *et al.*, 2018), as biostimulants can act directly on the plant and its physiological vitality or by improving soil conditions, i. Metabolic efficiency to induce increased yield, enhance crop quality, increase plant tolerance, facilitate nutrient uptake, enhance product quality traits including sugar content, color, fruit seed, etc., make water use more efficient and enhance soil fertility, especially by promoting the development of microorganisms In supplementary soils (Ayyat, & Abdel-Mola, 2020), and from those above, we conclude that

this may affect metabolites in general and secondary metabolites in particular, which affected the formation and increase of flavonoids and tannins.

2. Anatomical Study:

2.1. Surface View Of the Leaf Epidermis:

The results shown in Table (4) indicated a variation in anatomical characteristics about the vertical sections of the leaf blades and the middle vein. The upper surface reached 5µm compared to the comparison treatment, which recorded 2µm, which did not differ significantly from the treatment A1J0 A2J1. In comparison, the treatments at the level of the lower surface gave the highest rate of 4µm recorded by the treatment A1J2, superior to the treatment of A0J0, which gave a rate of 1µm, which did not differ in the treatments A1J0 A2J1, while recorded Transaction A0J1 µm3.

It can be seen that the treatment A1J2 gave the highest rate on the upper and lower surface levels and that the treatments A1J0 and A2J1 did not record a significant difference in comparison treatments.

The rate of epidermal thickness is important, as the results showed a significant difference between the treatments, as treatment A0J2 recorded the highest rate of the thickness of the upper epidermis, which amounted to 29µm compared to the comparison treatment, which recorded 24µm. In comparison, treatment A1J1 recorded an average of 18µm, much lower than the comparison treatment. About the lower epidermis, A1J1 recorded the lowest rate of 12 µm, which is similar to that recorded by the A2J0 treatment, which was lower than the comparison treatment, which recorded 16 µm for the thickness of the lower epidermis.

The treatments A1J1 and A0J1 did not achieve a significant difference from the comparison treatment, while the treatment A0J2 recorded 20 µm, outperforming all the studied treatments. The thickness of the mesophyll tissue of the leaf was greatly affected by the treatments applied in the study, where the treatment A0J2 gave the highest rate of thickness in the columnar and spongy layer of 44-81 µm. It was superior to the comparison treatment, which recorded 35-61 µm, respectively, for the columnar and spongy tissues. In comparison, the average thickness of the mesophyll tissue decreased as it reached 29 µm and 50 µm for the columnar and spongy tissues, and the rates ranged between these values for the treatments.

The stimulatory effect of the stimulants in the current study was in line with previous studies on tomatoes treated with seaweed extracts, which indicated that the stimulatory effect of different concentrations among the tested plants resulted in a differential, crop-specific response in terms of plant morphological features (for example, leaf permeability and epidermal thickness) (Rouphael, & Colla, 2018). Also, a recent study by Di Mola et al. (2019) stated that plant biostimulants could be considered a sustainable production tool to increase the yield of leafy vegetables in low-fertility soils.

This may be explained by the fact that marine algae extracts contain biologically active molecules such as amino acids (tryptophan, glutamic, and aspartic acids), LDPH-soluble peptides, polysaccharides, phenolic compounds, and phytohormones (abscisic acid, auxins, brasylinostrotin) (Colla *et al.,* 2017) These former molecules present in seaweeds and biostimulants may have triggered the signal transduction pathway by eliciting the synthesis of endogenous plant hormones, thereby increasing cell growth and improving epidermis in the leaf (Bhattacharyya, *et, al.* 2015).

In a recent study involving the treatment of tomato and soybean plants with jasmonic acid extract, the result was that it caused significant changes in the formation and thickness of the epidermis and the density of stomata. It increased the relative content of phenolic compounds and cutin in the leaf epidermis of the plant cuticle (Li *et al.,* 2018).

Some previous reports indicated that the dynamic responses of cuticular wax synthesis to unfavorable environmental conditions might also be mediated by other

phytohormones increasing total wax content along with structural changes in surface wax and potential effects on cuticular properties (Yeats & Rose, 2013), Hormonal treatments also led to changes in epidermal permeability. They increased aldehydes and ketones, in addition to transformations between wax components of varying polarities (Yuan, 2020). The study examined collenchyma tissue in the anterior mediastinum, and the plates showed that angular collenchyma tissue was found in treatments A0J0, A0J2, A1J1, and A2J1. At the same time, its presence was not observed in the rest of the treatments. This may be attributed to the effect of the treatments in protecting the plant from external stress and pathogens, thus increasing metabolic activities and photosynthesis, which led to the growth of cells and angular collenchyma tissue that gives flexibility to the places where it is present (Hu, *et al.*,2018).Regarding the number of vascular bundles in the cross-sectional section, it recorded the highest rate of 14 vascular bundles, while this rate decreased to 9 in treatments A1J2 and A2J0. In contrast, treatment A0J2 recorded a rate close to the comparison treatment A0J1, and A1J0 gave an average of 10 bundles. The reason for the variation in the significant effect of the extracts on the study samples may be due to the role of cytokinins in improving growth in general and encouraging the growth of lateral buds and vascular tissues (Wu & Lin, 2000).

As for the size of the vascular cylinder, it was found that the seaweed extract increased the size of the vascular cylinder as a result of an increase in the number and diameter of the vascular units, and this is attributed to the action of cytokinins in the extract that promote the division of parenchymal tissue cells within the conducting tissues. Also, the contents of the seaweed extract may stimulate the cambium. The formation of new vascular bundles increases stem diameter (Marhoon, & Abbas, 2015).

Table (4): Quantitative characteristics of the cross-sections of leaf blades and midribs in some treatments belonging to the genus Dianthus, measured in micrometers.

Qualities transactions	Cuticle thickness rate		Leaf cuticle thickness		The thickness of the mesophyll tissue		Dimensions of mesophyll cells		Presence of collenchyma tissue in the middle vein	Type of thickening of the collenchyma tissue	Average number of vascular bundles per cross section
	Lower	upper	Lower	upper	squishy	Al Emadi	Width	Length			
A0J0	1	2	16	24	35	61	11	20	+	angular	14
A0J1	3	5	16	26	29	50	10	21	-	-	10
A0J2	2	3	20	29	44	81	12	18	+	angular	13
A1J0	1	2	16	28	30	78	10	20	-	-	10
A1J1	2	5	12	18	29	64	9	17	+	angular	11
A1J2	4	5	15	20	41	53	14	21	-	-	9
A2J0	2	3	12	23	30	71	13	24	-	-	13
A2J1	1	2	18	20	36	59	9	16	+	angular	9
A2J2	2	5	15	20	33	60	12	18	-	-	12

Average of five replications for each type studied (-) absence of the adjective, (+) presence of the adjective.

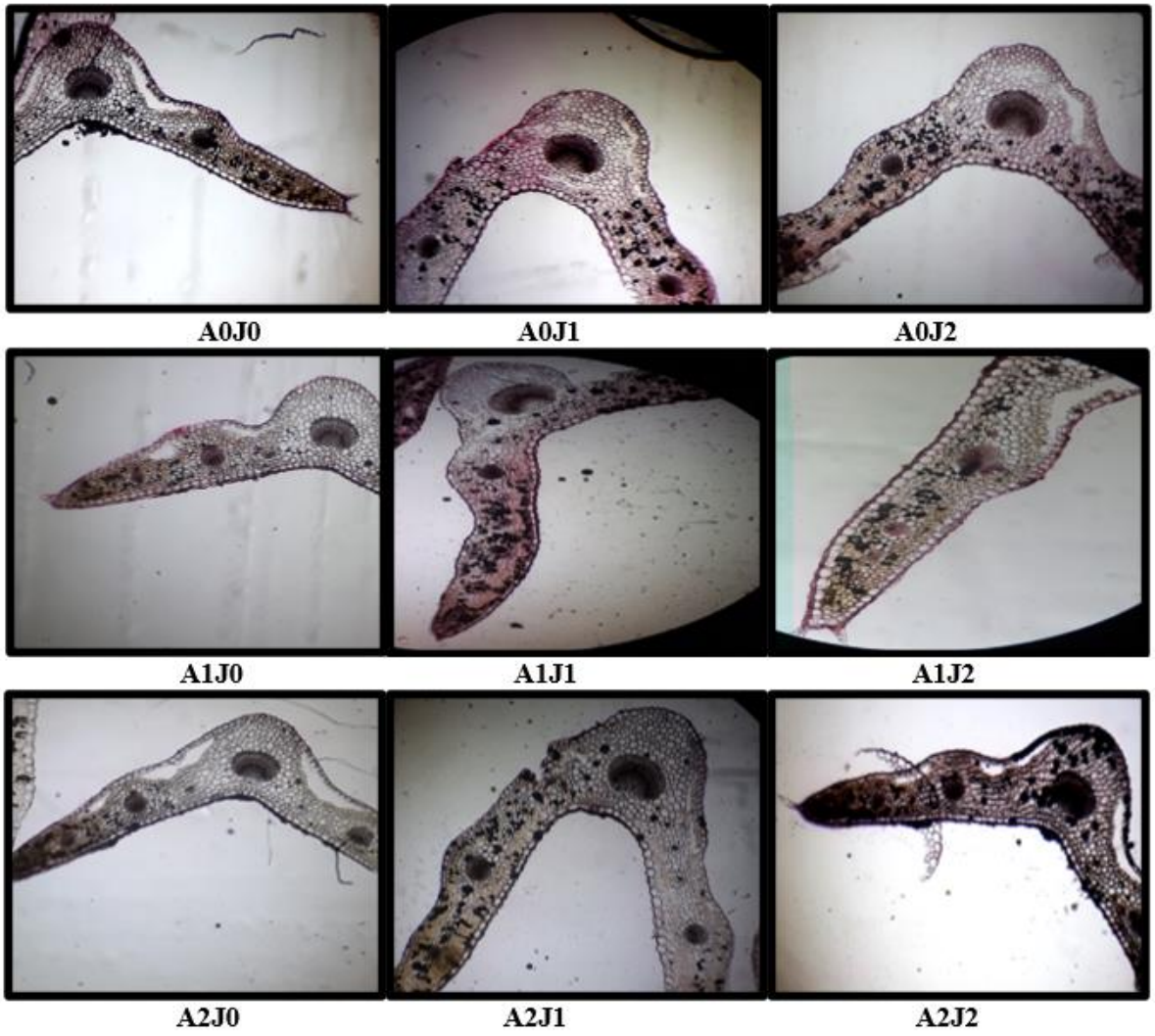


Fig. 3: Cross-sectional section of leaves of some species belonging to the genus Dianthus studied 10X.

2.2. Variation in the dimensions of the stomata in the upper and lower epidermis of leaves in some treatments:

The results are shown in table 5 a significant difference in the dimensions of the stomata at the level of the upper surface, where the treatment A1J0 recorded (22 x 30), which was the highest compared to the comparison treatment, which gave an average of (21 x 25), and the dimensions decreased in the treatment A1J1 to record the lowest values It reached (21 x 16) and were similar about the average height in treatment A2J0, which achieved an average of (21 x 17). At the same time, it ranged between the rates mentioned in the treatments.

The changes can be explained by the fact that the plant hormones present in the used treatments can mediate in regulating stomata and controlling the dimensions of the stomata pores and their activity, the most important of which is the uptake of carbon dioxide for photosynthesis in mesophyll cells (Khokon, 2010). Guard cells respond to numerous environmental and endogenous signals and regulate ion channels and transporters of solutes in their membranes (Assmann, &Jegla, 2016). The resulting changes in guard cell size and puffiness lead to the opening or closing of stomata, depending on light conditions, intracellular CO2 concentration, and humidity. Pathogen-associated molecular patterns also trigger air, soil water availability, and stomata closure to prevent the invasion of pathogenic microorganisms in plants (Zamora *et al.*, 2021).

Table 5: Variation in the dimensions of the stomata and the upper and lower epidermis of the leaves in some treatments belonging to the genus Dianthus, measured in micrometers.

Characters transactions	Stomata in Lower Surface		Stomata in Upper Surface		The average length × average width of normal cells	
	Width	Length	Width	Length	Lower Surface	Upper Surface
A0J0	19	28	21	25	24×62	21×50
A0J1	14	25	17	25	26×65	18×81
A0J2	16	30	15	27	23×85	14×66
A1J0	20	30	22	30	25×50	26×52
A1J1	15	25	16	21	21×71	28×80
A1J2	18	30	22	26	20×59	22×41
A2J0	18	25	17	21	20×83	16×73
A2J1	21	27	15	27	21×47	12×35
A2J2	20	24	14	25	19×42	15×30

An average of five replications for each type of study (-) absence of the adjective, (+) presence of the adjective

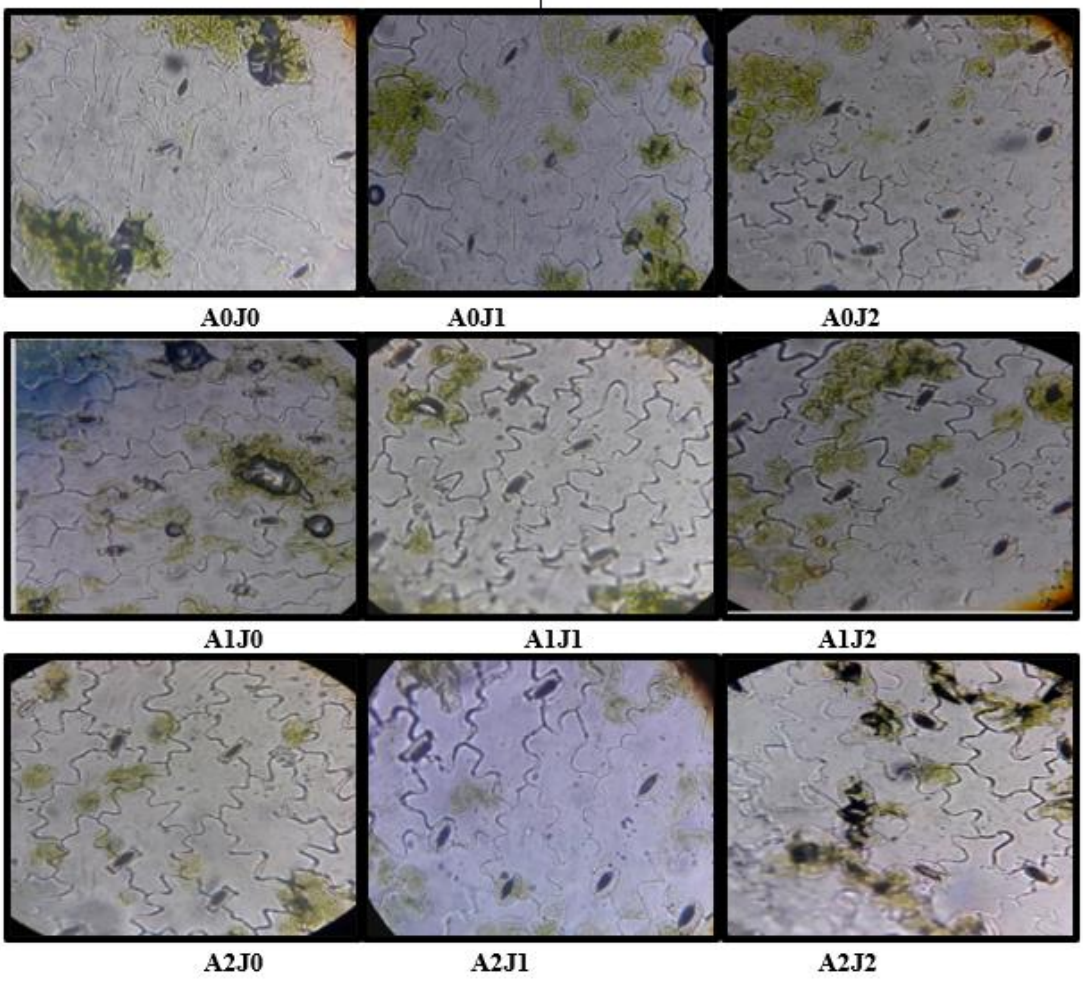


Fig. 4: Stomata and normal epidermal cells (lower surface) of some species of the genus Dianthus studied 10X**.**

2.3. Variation In the Qualitative and Quantitative Characteristics and The Frequency of Stomata in Leaves of Some Treatments:

The study coefficients affected the number of stomata, as the treatment A0J2 gave the highest average stomata frequency, reaching 58 on the upper surface and 86 on the lower surface, superior to the comparison coefficient A0J0, which gave an average of 38 stomata frequency in the upper surface and 40 in the lower surface.

While the treatment A1J2 gave the lowest rate of stomata frequency in the leaf, which reached 29 on the upper surface and 36 on the lower surface, where it recorded the

lowest rate compared to the unit of comparison of these values. As for the ratio between the upper and lower surface, the comparison treatment recorded the highest ratio of 0.95, while the treatment A0J2 recorded a ratio of 0.67.

A recent study using foliar spraying of seaweed extract found that the plants significantly reduced the effects of drought stress on basil plants. Furthermore, reduce electrolyte leakage (Esmaielpour&Fatemi, 2020).

A recent study confirmed plant hormones' involvement in regulating plant growth and stomata movement. They described jasmonic acid (JA) as a natural growth regulator known for modifying plant morphological, physiological, and biochemical processes in response to drought, cold, and salt stress (Li *et al*., 2020).

Table 6: Variation in the qualitative and quantitative characteristics and the frequency of stomata in the leaves in some treatments belonging to the genus Dianthus

Characters transactions	Frequency of leaf stomata			Qualitative traits	
	Upper Surface	Lower Surface	Ratio U/L	The type of stoma model	The nature of the walls of epidermal cells
A0J0	38	40	0.95	orthogonal	very wavy
A0J1	49	52	0.94	orthogonal	Medium waviness
A0J2	58	86	0.67	divergent and orthogonal	very wavy
A1J0	48	61	0.78	divergent and orthogonal	Medium waviness
A1J1	37	45	0.82	orthogonal	Low wavy
A1J2	29	36	0.80	orthogonal	Low wavy
A2J0	35	44	0.79	orthogonal	Medium waviness
A2J1	30	38	0.78	orthogonal	Medium waviness
A2J2	32	42	0.76	orthogonal	Low wavy

* The stomatal frequency was calculated on the basis of (mm2) and not on the basis of the microscope field only.

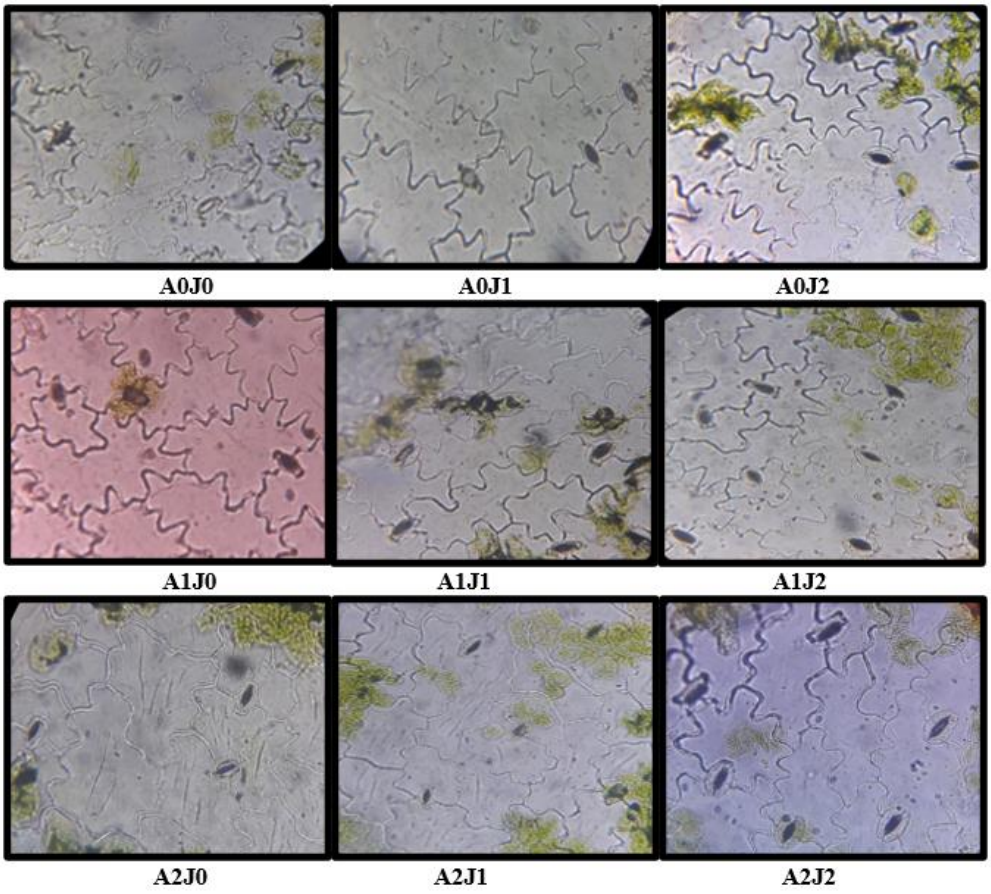


Fig.5: Stomata and normal epidermal cells (upper surface) of some species of the genus Dianthus studied 40X.

2.4. Floral Buds:

Table (7) shows that the treatment A2J0 gave the highest rate of flower calyx thickness, which amounted to 76µm, superior to the rate of comparison treatment, which amounted to 35µm, which led to an increase in leaf thickness; As for the lowest rate, it was recorded by the A2J2 treatment, which reached 30 µm, which may indicate that increasing the number of treatments led to a decrease in the growth rate and cell expansion (Cokrowati, et al., 2022). As for the average thickness of the petal leaf, treatment A2J0 gave the highest rate of 79µm, and it succeeded in the average thickness of the calyx in the petal leaf compared to the control treatment.

The lowest rate was in treatment A2J2 and reached 45µm; the rest of the treatments ranged between these two values. From the current results, we note that the mean of the thickness of the flower cup and the mean of the thickness of the petal leaf in treatment A0J2, which gave 85 and 55 µm, respectively, and in treatment A1J1, which had a rate of 66, 61 µm.The average diameter of the ovary recorded a rise, as it reached the highest average diameter in the A1J0 treatments and reached 140µm, outperforming the comparison treatment that gave 123µm in A0J1. In contrast, the lowest average was recorded at 101. The values ranged between the mentioned rates. (Kularathne, *et al*., 2021) conducted a study on ornamental plants by adding extracts that confirmed that they have positive effects, as they improve flower size, shape, length, chlorophyll, and fresh flower weight because they contain cytokines and auxins.

Table 7: Quantitative characteristics of the cross-sections of unopened floral buds in some treatments belonging to the genus Dianthus measured**.**

Adjectives transactions	Flower bud clip shape	The thickness of the floral calyx	Thickness of the petal leaf	Egg clip shape	The eggs diameter	Number of ovarian lobes
A0J0	circular	35	49	Oval - round	123	3-4
A0J1	oval	44	57	Oval - round	101	3
A0J2	oval	55	58	oval	134	3
A1J0	oval	49	51	Oval - round	140	4
A1J1	circular	61	62	oval	125	3
A1J2	Elongated oval	52	70	oval	105	3
A2J0	oval	76	79	Oval - round	119	4
A2J1	circular	48	65	oval	112	3-4
A2J2	circular	30	45	oval	125	4

Average of five replications for each type studied. Unripe, unopened flower buds were used.

Conclusions:

There is a significant positive effect when using seaweed extracts and jasmonic acid on the growth of the Chinese carnation Dianthus chinensis. The effect was significant on vegetative growth and some flowering traits as the concentration exceeded 10 ml. L-1 of seaweed extract significantly affected the traits: number of leaves, fresh and dry weight, number and length of internodes, and chlorophyll. At the same time, the addition of jasmonic concentration was 5 ml. L-1 in the description of plant height and number of branches. The overlap of the two concentrations was 1.0- and 10 ml. L-1 of jasmonic acid and seaweed had a significant effect on most of the vegetative growth traits. The treatment was recorded as 1.0 ml. L-1 of jasmonic acid significantly affected flower growth characteristics, flower bud length and width, number of flowers, and fresh and dry weight of the flower. The treatment with a concentration of 5 ml.L-1 seaweed recorded the highest percentage of oil. The treatment was also recorded with two concentrations, 1.0- and 10 ml. L-1 of seaweed and jasmonic showed the presence of all chemical compounds, which differed significantly from the control treatment, which did not record the presence of most chemical compounds. The

treatments also recorded an effect on the anatomical characteristics, as the dimensions of the stomata differed in the upper epidermis from the lower leaves, in addition to the variation in the qualitative and quantitative characteristics and the frequency of stomata.

REFERENCES

Aalifar, M., Arab, M., Aliniaeifard, S., Dianati, S., ZareMehrjerdi, M., Limpens, E., &Serek, M. (2019). Embryogenesis efficiency and genetic stability of Dianthus caryophyllus embryos in response to different light spectra and plant growth regulators. *Plant Cell, Tissue and Organ Culture (PCTOC)*, 139(3), 479-492.

Arioli, T.; Mattner, S.W.; Winberg, P.C(2015). Applications of seaweed extracts in Australian agriculture: Past, present and future. *Journal* of *Applied Phycology*, 27, 2007–2015

Assmann, S.M. & Jegla, T. (2016) Guard cell sensory systems: recent insights on stomatal responses to light, abscisic acid, and CO2. *Current Opinion in Plant Biology*, 33, 157–167

Ayyat, A. M., & Abdel-Mola, M. A. M. (2020). Response of Tagetespatula plants to foliar application of potassium silicate and seaweed extract under various irrigation intervals. *Scientific Journal of flowers and Ornamental Plants*, 7(4), 513-526.

Battacharyya, D.; Babgohari, M.Z.; Rathor, P.; Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae*,196, 39–48.

Colla, G.; Hoagland, L.; Ruzzi, M.; Cardarelli, M.; Bonini, P.; Canaguier, R.; Rouphael, Y. Biostimulant (2017). action of protein hydrolysates: Unraveling their effects on plant physiology and microbiome. *Frontiers in* Plant Science, 8, 2202

Di Mola, I., Cozzolino, E., Ottaiano, L., Giordano, M., Rouphael, Y., Colla, G., & Mori, M. (2019). Effect of vegetal-and seaweed extract-based biostimulants on agronomical and leaf quality traits of plastic tunnel-grown baby lettuce under four regimes of nitrogen fertilization. *Agronomy*, 9(10), 571.

Dilcher, D.L. (1974). Approaches to The Identification of Angiosperm Leaf Remains. *Botanical Review*, Vol.40,1-157.

Esmaielpour, B., &Fatemi, H. (2020). Effects of seaweed extract on physiological and biochemical characteristics of basil (Ocimumbasilicum L.) under water-deficit stress conditions. *Journal of Science and Technology of Greenhouse Culture*, 11(1).

Hamed, S.M.; Abd El-Rhman, A.A.; Abdel-Raouf, N.; Ibrahim, I.B.M. (2018). Role of marine macroalgae in plant protection & improvement for sustainable agriculture technology. Beni-Suef Univ. *Journal of Basic & Applied Sciences*, 7, 104–110

Hashem, H. A., Mansour, H. A., El-Khawas, S. A., &Hassanein, R. A. (2019). The potentiality of marine macro-algae as bio-fertilizers to improve the productivity and salt stress tolerance of canola (Brassica napus L.) plants. *Agronomy*, 9(3), 146.

Hemanta L., K. V. Jayaprasad, D. P. Kumar, R. K. Manohar and G. Gopinath. (2012). Effect of levels of fertigation on yield and flower quality of different varieties of carnation (Dianthus caryophyllus L.) under naturally ventilated polyhouse. *Mysore Journal of Agricultural* Sciences, 46 (2): 245-250.

Hu, Q., Min, L., Yang, X., Jin, S., Zhang, L., Li, Y., ... & Zhang, X. (2018). Laccase GhLac1 modulates broad-spectrum biotic stress tolerance via manipulating phenylpropanoid pathway and jasmonic acid synthesis. *Plant physiology*, 176(2), 1808-1823.

Jonson, a.c.(1940). microscopic preparation in plant tissues. botanical journal of American union.press. USA. P:30-38.

Khan, W., Rayirath, U. P., Subramanian, S., Jithesh, M. N., Rayorath, P., Hodges, D. M., et al. (2009). Seaweed extracts as biostimulants of plant growth and development. *Journal of Plant Growth Regulator,* 28, 386–399. doi: 10.1007/s00344-009-9103-x.

Khokon, M.A.R., Okuma, E., Hossain, M.A., Munemasa, S., Uraji, M., Nakamura, Y. et al. (2010) Involvement of extracellular oxidative burst in salicylic acid-induced stomatal closure in Arabidopsis. *Plant, Cell & Environment,* 34, 434–443

Kularathne, M. N., Srikrishnah, S., &Sutharsan, S. (2021). Effect of Seaweed Extracts on Ornamental Plants: Article Review. *Current Agriculture Research Journal,* 9(3).

Kumar P, Partap M, Ashrita, Rana D, Kumar P, Ashish RW (2020) Metabolite and expression profiling of steroidal alkaloids in wild tissues compared to bulb derived in vitro cultures of Fritillaria roylei – high value critically endangered Himalayan medicinal herb. *Industrial Crops and Products,* 145:111945

Layek, J.; Das, A.; Idapuganti, R.G.; Sarka, D.; Ghosh, A.; Zodape, S.T.; Lal, R.; Yadav, G.S.; Panwar, A.S.; Ngachan, S.; *et al*. (2018). Seaweed extract as organic bio-stimulant improves productivity and quality of rice in eastern Himalayas. *Journal* of *Applied Phycology*,30, 547–558.

Li, S., Hamani, A. K. M., Si, Z., Liang, Y., Gao, Y., &Duan, A. (2020). Leaf gas exchange of tomato depends on abscisic acid and jasmonic acid in response to neighboring plants under different soil nitrogen regimes. *Plants,* 9(12), 1674

Marhoon, I. A., & Abbas, M. K. (2015). Effect of foliar application of seaweed extract and amino acids on some vegetative and anatomical characters of two sweet pepper (Capsicum Annuum L.) cultivars. *International Journal of Research Studies in Agricultural Sciences,* 1(1), 35-44.

Ngan, H. T. M., Tung, H. T., Van Le, B., &Nhut, D. T. (2020). Evaluation of root growth, antioxidant enzyme activity and mineral absorbability of carnation (Dianthus caryophyllus “Express golem”) plantlets cultured in two culture systems supplemented with iron nanoparticles. *Scientia Horticulturae,* 272, 109612.

Raza, A., Charagh, S., Zahid, Z., Mubarik, M. S., Javed, R., Siddiqui, M. H., &Hasanuzzaman, M. (2021). Jasmonic acid: a key frontier in conferring abiotic stress tolerance in plants. *Plant Cell Reports,* 40(8), 1513-1541.

Rouphael, Y., Giordano, M., Cardarelli, M., Cozzolino, E., Mori, M., Kyriacou, M. C., ... &Colla, G. (2018). Plant-and seaweed-based extracts increase yield but differentially modulate nutritional quality of greenhouse spinach through biostimulant action. *Agronomy,* 8(7), 126.

Rouphael, Y.; Colla, G. (2018). Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. *Frontiers in Plant Scienc*, 9. https://doi.org/10.3389/fpls.2018.01655

Sharma, P., A. Abrol, A. Qureshi and S. Sharma (2019). "Role of biostimulants with special reference to Panchgavya and Jeevamrit in floriculture-a review." *Agric international,* 6(1): 23-32.

Sreelekshmi, R., &Siril, E. A. (2020). Influence of polyamines on hyperhydricity reversion and its associated mechanism during micropropagation of China pink (Dianthus chinensis L.). *Physiology and Molecular Biology of Plants,* 26(10), 2035-2045.

Stace, C. A. (1965). Cuticular Studies as an aid to Plant Taxonomy. *Bulletin of the British Museum (Natural History). Botany,*4(1): 3-78.

Szabo, V. & K. Hrotko. (2009). Preliminary Results of biostimulator treatments on Crataegus and Prunus stockplants. *Bulletin UASVM Horticulture,* 66(1): 223-228.

Wu, Y. T and C.H. Lin, (2000). Analysis of cytokinin activity in commercial aqueous seaweed extract. *Gartenbauwissenschaft,* 65:170–173

Yuan, Z., Jiang, Y., Liu, Y., Xu, Y., Li, S., Guo, Y., & Ni, Y. (2020). Exogenous hormones influence Brassica napus leaf cuticular wax deposition and cuticle function. *PeerJ* 8:e9264 https://doi.org/10.7717/peerj.9264

Zamora, O., Schulze, S., Azoulay-Shemer, T., Parik, H., Unt, J., Brosche, M., ... &Kollist, H. (2021). Jasmonic acid and salicylic acid play minor roles in stomatal regulation by CO2, abscisic acid, darkness, vapor pressure deficit and ozone. *The Plant Journal*, 108(1), 134-150