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The Role Of Selected Growth Regulators And Light Source And Quality In The In Growth, Multiplication, And Enhancement Of Bioactive Compounds In Chrysanthemum Morifolium Plant In Vitro

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Abstract:*The study was carried out in the Central Laboratory for Plant Tissue Culture at the College of Agricultural Engineering Sciences, University of Baghdad, between October 2023 and December 2024. Its aim was to propagate Chrysanthemum morifolium through tissue culture techniques and to explore the influence of specific growth regulators, as well as light source and quality, on both the plant's propagation and its production of secondary metabolites. Four experimental stages were implemented. The first examined the effect of varying sodium hypochlorite (NaOCl) concentrations (0, 5, 10, and 15 mL per 100 mL) on sterilizing plant material. The second investigated how different levels of BA and NAA impacted shoot initiation and multiplication. The third stage assessed the influence of light source and spectral quality on the number and length of shoots, chlorophyll levels, and phenolic compound content. Four levels of BA (0, 0.5, 1 and 1.5 mg L^{-1}) and four concentrations of NAA (0, 0.1, 0.3 and 0.5 mg L^{-1}) were combined with five lighting treatments: white fluorescent, white LED, blue LED, red LED, and a combination of red and blue LED. The best outcome of sterilization treatment was observed by NaOCl treatment at 15 mL for 15 min with single node that produced highest response (100%) on 0.5 mg L^{-1} BA amended media. A significant interaction between BA and NAA concentrations was observed, where 1 mg L^{-1} BA with 0.2 mg L^{-1} NAA yielded the highest average number of shoots (15 per explant), while 0.5 mg L^{-1} BA combined with 0.3 mg L^{-1} NAA produced the longest shoots, averaging 5 cm. White LED light promoted the greatest shoot proliferation (20 shoots per explant), whereas red LED led to the longest shoot growth (3 cm). The red-blue LED mix resulted in the highest chlorophyll content (111.5 mg per 100 g) and supported the greatest accumulation of phenolic compounds, including quercetin, gallic acid, rutin, kaempferol, luteolin, and apigenin, with values of 59, 71.6, 64.5, 50.6, 22.6, and 32.9 µg g^{-1}, respectively.*

Keyword: *Chrysanthemum morifolium, Light LED, NAA, BA, in vitro*

1. INTRODUCTION

Chrysanthemum morifolium is an important global cut flower that is known as the "flower of autumn" since it blooms in a season when there are few other blooming flowers [1], and is an herbaceous perennial from the family Asteraceae. Economically, it is considered the second most important flower worldwide after roses [2]. The plant comprises approximately 40 species, with its native origin traced to East Asia, specifically China, Japan, and Mongolia. Today, it is cultivated worldwide [3].This perennial plant features alternate, lobed, serrated, and aromatic leaves. The flowers are large or small, in isolation or aggregated, varied and bright [4]. Applications of Chrysanthemum morifolium in human medicine include as an antioxidant, anticancer, anti-inflammatory medicine, as well as for the treatment of many diseases. It is also drank as a tea with beneficial health compounds. The plant is rich in secondary metabolites such as phenolic compounds, essential oils, and polysaccharides [5].

Because this taxon is an important ornamental plant and there are many commercial cultivars of it, scientists have been working on micro propagation through plant tissue culture methods. This method offers critical advantages, enabling large-scale plant production independent of growing seasons. Tissue culture allows for the rapid generation of plants genetically identical to the parent, using small explants, within a short time frame and limited space, all year round [6].

Tissue culture technology has provided multiple opportunities for the continuous production of these compounds without being restricted to specific seasons, thanks to the ability to control environmental conditions and the components of the nutrient medium required by the plant. To enhance the ability of plants to produce such compounds, elicitors are commonly used, as they represent effective tools that

contribute to increasing the yield of active substances. Light and salicylic acid are among the non-living elicitors [7]. As is well-established, light is an important environmental factor for plant growth and development, it not only controls the growth and morphogenesis of plant, but also is a major factor for both the primary and secondary metabolism. Plants sense light through a diversity of photoreceptors that absorb photons in red as well as blue regions of the light spectrum. It is, thus, necessary to supply an appropriate light source in plant tissue culture laboratories. A very frequent light source that is utilized in the work at such labs are fluorescent lamps providing light with wavelengths between 400 and 700 nm. However, this range is not fully utilized by plant cultures, and it emits a constant intensity that may have other negative effects on plants. Recently, LED lamps have been proposed as a versatile light source for various applications in plant tissue culture due to their numerous advantages [8].

Based on the above, the objective of the study was to: Investigate the effect of the cytokinin (BA) and auxin (NAA) on plant growth and shoot multiplication, as well as to determine the influence of light source and quality on shoot growth, chlorophyll content, and phenolic compound accumulation in Chrysanthemum morifolium under in vitro conditions

2. MATERIALS AND METHODS

The experiment was conducted in the Plant Tissue Culture Laboratory of the Department of Horticulture and Landscape Gardening, College of Agricultural Engineering Sciences, University of Baghdad. Chrysanthemum morifolium seedlings were obtained from a private nursery, produced by the Dutch company Decorum.

The ready-made MS medium (Murashige and Skoog, 1962) produced by the Dutch company HIMedia was used at a concentration of 4.9 g L^{-1}. Sucrose was added at 30 g L^{-1}, and plant growth regulators were incorporated after being prepared as stock solutions according to the experiment type. The pH was adjusted to 5.7 ± 0.1 using 1N hydrochloric acid (HCl) or sodium hydroxide (NaOH), then the volume was completed to one liter. Agar-Agar was added at 7 g L^{-1}. To homogenize the components and dissolve the agar, the culture medium was heated using a hot plate magnetic stirrer until complete homogenization. The medium was then distributed into culture tubes (10 ml per tube) and covered with appropriate caps. All tools used in the experiment (scalpel holders, forceps, and Petri dishes) were sterilized in an oven at 160°C for two hours. Additionally, 99% ethanol was used for sterilizing forceps and scalpels, followed by flame sterilization using a Bunsen burner after each transfer operation inside the laminar air flow cabinet.

The work cabinet and hands were sterilized with 70% ethanol. Distilled water used in the culture process was sterilized using an autoclave at 121°C under 1.04 kg cm^{-2} pressure for 30 minutes, then left to cool before use. Culture tubes containing the medium were sterilized in the autoclave at 121°C under 1.04 kg cm^{-2} pressure for 15 minutes, then left to cool and solidify at room temperature until ready for culturing.

2.1 Initiation Phase

2.1.1. Sterilization Procedure

Shoots were collected from the mother plant, with flowers and leaves removed, then cut into appropriate sizes. The explants were placed under running tap water for 30 minutes, followed by washing with liquid soap and distilled water. They were then transferred to a laminar airflow cabinet for surface sterilization using commercial bleach (NaOCl) at different concentrations (0, 5, 10, 15 mL per 100 mL distilled water) for 15 minutes, with the addition of a few drops of Tween-20. Subsequently, the explants were rinsed three times with sterile distilled water (5 minutes per wash). After ten days, contamination rates were recorded as follows:

$$\textbf{Contamination percentage} = \frac{\text{Number of contaminated explants}}{\text{Total number of explants}} \times 100$$

2.1.2 Effect of BA, NAA, and Their Interaction on Explant Response Rate

Plant segments (shoot tips and single nodes) obtained from the optimal sterilization treatment were cultured on MS medium supplemented with varying concentrations of BA (0, 0.5, 1, 1.5 mg L^{-1}) in combination with NAA (0, 0.1, 0.3, 0.5 mg L^{-1}). After six weeks, the response percentage was calculated.

Following the determination of the best result obtained from the shoot initiation experiment, the plant parts were cultured according to the following experiments:

2.2Multiplication Phase

2.2.1 Effect of Different Concentrations of BA and NAA and their Interaction on the Multiplication of Plant Parts: Shoots were cultured on MS medium supplemented with different concentrations of BA (0, 0.5, 1, 2 mg L^{-1}) in combination with NAA at concentrations of (0, 0.1, 0.2, 0.3 mg L^{-1}). After 6 weeks of culture, measurements were taken, including the number and length of shoots.

Effect of Light Source and Quality and their Interactions on Shoot Multiplication and the Stimulation of Phenolic Compound Production: Shoots resulting from the best treatment obtained in the first experiment were exposed to the following light treatments: a. Fluorescent lamps (FL white) b. White LED c. Blue LED d. Red LED e. Interaction between Red and Blue LED. After 6 weeks, measurements were taken, including the number and length of shoots, chlorophyll pigment content, and the quantification of active compounds (phenolic compounds).

Chlorophyll pigment concentration was estimated according to the method described by Dere et al., (9).

2.2.3. Extraction of Phenolic Compounds

1.5 grams of ground leaves were taken, and 40 mL of chloroform was added. The mixture was continuously stirred for 24 hours at ambient temperature. The extract was then placed in an ultrasonic disruptor for 15 minutes, after which 100 mL of butanol was added. The mixture was transferred to a separating funnel, and the polar organic layer (butanol) was collected and transferred to a rotary evaporator to obtain a dry extract. This process was repeated 3 times to obtain a sufficient quantity of extract before analysis.

2.3. Analysis Conditions

The examination was conducted in the laboratories of the Ministry of Science and Technology – Department of Environment and Water, [1] according to the method provided by Mradu et al., (10), using a High-Performance Liquid Chromatography (HPLC) instrument, model (Sykam), of German origin. The mobile phase is methanol: distilled water: formic acid (5:25:70) and the separation column as used a C18-ODS column (25 cm × 4.6 mm) to separate phenolics. UV detector detection wavelength was 280 nm, and the mobile phase flow rate was 1 mL/min.

2.4. Statistical Analysis

All experiments were analyzed using a Completely Randomized Design (CRD) with one or two factors and 10 replicates per treatment. The results were analyzed using the GenStat statistical software to study the effects of different factors and their interactions on the studied traits. Significant differences between means were compared using the Least Significant Difference (LSD) test (11).

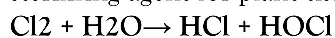
3. RESULTS AND DISCUSSION

3.1. Initiation Stage

3.1.1. Effect of Commercial Sodium Hypochlorite on Contamination Percentage of Plant Explants (Shoot Tip, Single Node) of Chrysanthemum morifolium In Vitro

The results presented in Table 1 show that different concentrations of commercial sodium hypochlorite significantly affected the reduction of contamination. The 15 mL concentration was extremely pure and was significantly different from other treatments. Control treatment, however, showed the highest contamination percentage 100%, and was not significantly different from 5 mL that had 85% contamination. But this was in stark contrast to the 10 mL treatment wherein we achieved 60% contamination rate.

The results also shows no significant differences between the types of plant explants (shoot tip or single node). Regarding the interaction between sodium hypochlorite concentration and explant type, the results indicated that the treatment with 15 mL applied to both shoot tips and nodal segments performed significantly better than the other treatments, resulting in zero contamination (0%). It can be observed that contamination decreases as the concentration of sodium hypochlorite increases, reaching complete elimination at 15%. This is attributed to the effectiveness of hypochlorous acid (HOCl), a strong oxidizing agent formed when chlorine reacts with water, as shown in the following chemical equation (12). These findings are consistent with those of Ramawat (13), who reported that NaOCl is an effective sterilizing agent for plant tissues, capable of eliminating pathogenic microorganisms.



These results align with those obtained by Zafarullah et al., (14) in their study on the surface sterilization of shoot tips of Chrysanthemum morifolium cultured in vitro. They are also in agreement with Kazeroonian et al., (15), who successfully obtained contamination-free cultures from chrysanthemum nodes using a 2% NaOCl solution for 10 minutes.

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Table (1): Effect of commercial bleach concentrations and plant part type on contamination percentage (%)

Plant Part	Commercial Bleach Concentrations				Mean
	0	**5**	**10**	**15**	
Shoot Tip	100	80	50	0	57.5
Node	100	90	70	0	65
LSD (for Plant Part)	**28**				**N. S**
Mean	100	85	60	0	
LSD (for Bleach Concentration)	**19.8**				

3.1.2. Effect of BA and NAA concentrations and their interaction on the response percentage (%) of plant parts (shoot apex and single node) of Chrysanthemum morifolium In vitro

The results indicated that a BA concentration of 0.5 mg L^{-1} significantly affect explant response rates, yielding the highest response percentage of 45%. This concentration showed statistically significant differences compared to other BA concentrations, except for 1 mg L^{-1} BA. Regarding explant type, single nodes significantly outperformed shoot tips, achieving a 45% response rate compared to 13.1% for shoot tips.

For NAA concentrations, the control treatment (0 mg L^{-1}) showed superior performance with a 43.7% response rate, differing significantly from other NAA concentrations. The table further revealed significant interactions between BA concentrations and explant types, where the combination of 0.5 mg L^{-1} BA with single nodes produced the highest response rate (72.5%), significantly differing from other treatments.

The two-way interaction between BA and NAA concentrations was found to be significant as well. The highest rate of response was induced by 0.5 mgl^{-1}BA and 0 mgl^{-1}NAA (65% response) with statistical difference as compared to the other treatments, except for1 mgl^{-1}BA and 0 mgl^{-1}NAA (50% response). As well, the explant type × NAA concentration interaction was also highly variable, with single node explants combined with 0 mg L^{-1} NAA producing the best response (70%).

The three-way interaction between BA concentration, explant type, and NAA concentration showed particularly notable results. The combination of single nodes with 0.5 mg L^{-1} BA and 0 mg L^{-1} NAA produced a remarkable 100% response rate, significantly outperforming most other treatments. Comparable results were observed with: Single nodes + 0.5 mg L^{-1} BA + 0.1 mg L^{-1} NAA (90%); Single nodes + 1 mg L^{-1} BA + 0 mg L^{-1} NAA (80%); Single nodes + 1.5 mg L^{-1} BA + 0 mg L^{-1} NAA (70%)

The effectiveness of BA concentrations can be attributed to cytokinin's role in stimulating cell division and differentiation in cultured shoots, leading to vegetative shoot formation (21). The rate of bud induction and growth depends on both the concentration and type of cytokinins and auxins in the culture medium. Higher cytokinin/auxin ratios typically promote lateral bud formation by directing nutrients toward shoot development, whereas higher auxin/cytokinin ratios tend to stimulate root formation (16). Cytokinins facilitate cell division by enhancing tRNA synthesis, which subsequently promotes protein and enzyme production necessary for plant growth (17). The differential response among explants may reflect variations in physiological age and cellular maturity (18).

These findings align with previous studies (15, 19, 20).

Table 2. Effect of BA and NAA concentrations and their interaction on the response percentage (%) of cultured plant parts (shoot apex, single node) of Chrysanthemum morifolium in vitro

BA conc. (mg L^{-1})	Plant part	NAA concentration (mg L^{-1}) 0	0.1	0.3	0.5	Mean
0	**apex**	10	0	0	0	2.5
	node	30	0	10	0	10
0.5	**apex**	30	10	20	10	17.5
	node	100	90	60	40	72.5
1	**apex**	20	20	30	20	22.5
	node	80	60	40	30	52.5
1.5	**apex**	10	10	20	0	10
	node	70	50	40	20	45

LSD		**33.8**				**16.9**
	BA	**0**	**0.1**	**0.3**	**0.5**	**Mean BA**
BA	**0**	20	0	5	0	6.2
*	**0.5**	65	50	40	25	45
NAA	**1**	50	40	35	25	37.5
	1.5	40	30	30	10	27.5
LSD		**23.9**				**11.9**
Plant part* NAA	Plant	**0**	**0.1**	**0.3**	**0.5**	**Mean part**
	apex	17.5	10	17.5	7.5	13.1
	node	70	50	37.5	22.5	45
LSD		**16.9**				**8.4**
NAA		**0**	**0.1**	**0.3**	**0.5**	
	Mean	43.7	30	27.5	15	
LSD		**11.9**				

Figure 1: Initiation of cultures on MS medium in vitro

3.2. Multiplication Stage

3.2.1. Effect of BA and NAA and Their Interaction on the Average Number of Shoots in Chrysanthemum morifolium In Vitro

The results presented in Table (3) indicate that BA concentrations had a significant effect on increasing the number of shoots. The concentration of 1 mg L^{-1} BA proved most effective, producing the highest average of 8.5 shoots per explant which gave the lowest average of 1 shoot per explant which was significantly higher than the control treatment that yielded the lowest average of only 1 shoot per explant. This result was not significantly different from that of the 2 mg L^{-1} BA concentration, which produced an average of 8.25 shoots per explant.

As for the effect of NAA, the table shows that the concentration of 0.2 mg L^{-1} led to the best performance, with the highest average number of shoots at 9.5 shoots per explant, while the control treatment recorded the lowest value at 1.5 shoots per explant.

The same table also reveals a significant interaction between BA and NAA concentrations. The combination of 1 mg L^{-1} BA with 0.2 mg L^{-1} NAA resulted in the highest number of shoots, with an average of 15 shoots per explant. This value was significantly higher than those obtained from all individual NAA treatments and the combination of 0.5 mg L^{-1} BA with 0 mg L^{-1} NAA, which resulted in only 1 shoot per explant.

Figure (2): Shoot multiplication of Chrysanthemum morifolium cultured on MS medium supplemented with different concentrations of BA and NAA after 6 weeks

Table (3): Effect of BA and NAA and their interaction on the average number of shoots of Chrysanthemum morifolium (shoots per explant) after 6 weeks of in vitro culture on MS medium.

BA conc. (mg L⁻¹)	NAA concentration (mg L⁻¹)				Mean
	0	0.1	0.2	0.3	
0	1	1	1	1	1
0.5	1	7	10	4	5.5
1	2	12	15	5	8.5
2	2	10	12	9	8.25
LSD	1.34				0.67
Mean	1.50	7.50	9.50	4.75	
LSD	0.67				

3.2.2. Effect of BA and NAA and Their Interaction on the Average Shoot Length of Chrysanthemum morifolium In Vitro

The results presented in the table (4) suggest that different concentrations of BA and NAA significantly affected the shoots length. The 0.5 mg L^{-1} concentration of BA produced, much higher shooting length (3.05 cm) than 2 mg L^{-1} concentrations, which resulting in the lowest average of 1.9 cm. The 2 mg L^{-} treatment was not quite different from other concentrations.

For the NAA, the length of the highest shoot was seen in the concentration of 0.3 mg L, which reached 3.5 cm, was much higher than the results of other treatments except for 0.2 mg L^{-1} concentration giving 2.42 cm on average.

For interaction between BA and NAA, the results indicate that a combination of 0.5 mg L^{-1} with 0.3 mg L^{-1} NAA significantly outperformed most other treatments, except for a 1 mg L^{-1} BA with 0.3 mg L^{-1} NAA, , which produced the highest shoot length of 5 cm.

Table (4): Effect of BA and NAA and their interaction on the average shoot length of Chrysanthemum morifolium (cm) after 6 weeks of in vitro culture on MS medium.

BA conc. (mg L⁻¹)	NAA concentration (mg L⁻¹)				Mean
	0	0.1	0.2	0.3	
0	2	3	3.5	3.3	2.95
0.5	2.6	2.4	2.2	5	3.05
1	2.5	2	2	4.2	2.67
2	2.3	2	2	1.5	1.9
LSD	1.07				0.54
Mean	2.35	2.35	2.42	3.5	
LSD	0.54				

The increase in shoot number may be attributed to the effect of cytokinins, particularly when used at appropriate concentrations in tissue culture. These compounds play a role in breaking apical dominance and in creating zones of attraction within lateral buds, which in turn encourages the movement and accumulation of nutrients and essential elements needed for the growth of vegetative buds. As a result, bud growth is stimulated, leading to an increase in shoot number (21). Of the cytokinins, BA is one of the most commonly used in tissue culture because of its stability and resistance to rapid degradation. It is thought to promote the differentiation of vascular tissues and xylem in buds and stems to increase nutrient transfer, thus stimulating growth of lateral shoots (22).

A suitable balance between auxins and cytokinins plays a key role in overcoming apical dominance by encouraging the development of connecting vascular tissues between lateral buds and the main vascular system of the explant. This helps supply necessary nutrients to the growing buds, ultimately promoting shoot growth and increasing branching (17). Regarding shoot length, several scientific studies have highlighted the important role of auxins—particularly NAA—at appropriate concentrations in tissue

culture. In comparison to conditions lacking auxins and with only cytokinins present, auxins are known to enhance apical dominance and suppress the development of lateral shoots. This suppression leads to an elongation of the main shoots (23). These findings are consistent with what was reported by Mehedi et al., (24) and Chowdhuryet al., (25).

Figure (3): Shoot length of Chrysanthemum morifolium cultured on MS medium supplemented with different concentrations of BA and NAA after 6 weeks.

3.3 Effect of Light Source and Light Quality on the Average Number and Length of Shoots of Chrysanthemum morifolium In Vitro

The results shown in Table (5) demonstrate the effect of both the light source and light quality on the average number and length of shoots. In terms of light source, LED lighting significantly outperformed fluorescent lighting (FL), producing an average of 18 shoots per explant compared to 15 shoots per explant under fluorescent light.
As for light quality, it had a significant effect on shoot number. The mix of red/blue LED light provided the most shoots (20 per explant), followed closely by white LED light (18 per explant).
For shoot length, there were no significant differences between the 2 light sources. Nevertheless, in light quality, the longest shoots (3 cm) were achieved by red LED light and the shortest (1.2 cm) under white LED light.

Table (5): Effect of light source and light quality on the average number of shoots (shoots per explant) and shoot length (cm) of Chrysanthemum morifolium cultured on MS medium containing 1 mg L^{-1} BA and 0.2 mg L^{-1} NAA after 6 weeks of in vitro culture.

Light source	Shoot number (shoot plant-1)	Shoot length (cm)
White FL	15	1.5
White LED	18	1.2
Red LED	13	3
Blue LED	10	2
Red/Blue LED	20	1.3
LSD	**2.08**	**0.63**

3.3.1 Effect of light source and quality on chlorophyll pigment concentration

As can be seen from Table 6, the influence of light source and its spectral quality on chlorophyll concentration in Chrysanthemum Callus cultures is very important. LED lighting was a more effective light source with ozone production of 73.6 mg per 100 g of fresh weight, approximately 1.5 times (1.5×) higher than under FL (48.9 mg per 100 g). This 50.7% enhancement in chlorophyll content under LEDs likely stems from their spectral precision and higher light energy efficiency. Among different light spectra, the combined red-blue LED treatment generated the highest chlorophyll accumulation (111.5

mg per 100 g), while white LED produced the lowest concentration (73.6 mg per 100 g) among LED variants.

Table (6): Effect of light source and light quality on the average chlorophyll pigment concentration (mg 100 g^{-1}) in Chrysanthemum morifolium cultured on MS medium containing 1 mg L^{-1} BA and 0.2 mg L^{-1} NAA after 6 weeks of cultivation.

Light source		Chlorophyll pigment concentration (mg 100 g^{-1})
	White FL	48.9
	White LED	73.6
	Red LED	80.1
	Blue LED	95.7
	Red/Blue LED	111.5
LSD		**5.55**

3.3.2. Effect of Light Quality on the Concentration of Phenolic Compounds in Chrysanthemum morifolium In Vitro

Table 7 also indicates that light quality is involved in enhancing phenolic compound biosynthesis. The highest accumulation levels, under both red and blue LED, were detected for quercetin, gallic acid, rutin, kaempferol, luteolin, and apigenin of 59, 71.6, 64.5, 50.6, 22.6, and 32.9 µg g^{-1}, respectively. These values were significantly greater than those obtained under all other light conditions, except red LED, which exceeded the red-blue LED treatment in luteolin content, with a production of 18.9 µg g^{-1}. but it did not exceed it in any of the other compounds

In contrast, the white fluorescent (FL) treatment produced the lowest levels of phenolic compounds: 20.6, 33.6, 30.6, 25.9, 9, and 18.7 µg g^{-1} for quercetin, gallic acid, rutin, kaempferol, luteolin, and apigenin, respectively. Notably, the kaempferol and apigenin concentrations under fluorescent light were equal to those recorded under blue LED light.

Table (7): Effect of light quality on the concentration of phenolic compounds (µg g^{-1}) in Chrysanthemum morifolium cultured on MS medium containing 1 mg L^{-1} BA and 0.2 mg L^{-1} NAA after 6 weeks of in vitro cultivation.

Phenolic compounds	Light type FL White	LED White	LED Red	LED Blue	LED Red	$LSD_{0.05}$
Qurcetine	20.6	30.6	57.5	33.6	59.0	4.88**
Gallic acid	33.6	38.9	69.2	38.8	71.6	5.07**
Rutin	30.6	36.9	61.5	37.9	64.5	3.95**
Kaempferol	25.9	32.4	47.9	25.9	50.6	4.38**
Luteolin	9.0	14.8	18.9	10.5	22.6	3.42**
Apigenin	18.7	23.6	29.8	18.7	32.9	3.35**

Several unique features of LED technology, (including the ability to tailor emitted wavelength to the growth stage) can account for the better performance of cultures grown under LED vs FL (Table 26), and the lower heat output compared to fluorescent lamps (26). Moreover, the improved performance of cultures compacted under red and blue LED lights on the tested parameters could be attributed to the specific bands of red light (630-665 nm) and blue light (440-480 nm). This spectral range is conveniently close to the peak absorption of light by chlorophyll and the other pigments, and hence is the best for stimulating all classes of photoreceptors. In response, photosynthetic activity is stimulated to support additional biosynthesis of growth promoting nutrients and secondary metabolites (27).These results were similar to those of Bello-Bello et al.,(28).

Plant response to the effect of light quality varies depending on the plant species. For instance, the best shoot length for in vitro orchid cultures was observed under a mixture of red and blue LED light, while Anthurium cultures exhibited the best shoot length under blue LED light. These results also align with the findings of Kim et al.,(29) and Moon et al.,(30) in their study on the effect of light quality on in vitro Chrysanthemum, where cultures grown under a mixture of blue and red light showed the highest concentration of chlorophyll pigment. Additionally, these results are consistent with the findings of Bello-Bello et al.,(28).

Figure 4. Shoots of Chrysanthemum morifolium cultured on MS medium under different types of lighting

4. CONCLUSION

Sterile plant explants were successfully obtained using 15 mL/100 mL^{-1} of commercial sodium hypochlorite (NaOCl) for 15 minutes. the highest response rate of 100% was achieved when single nodes were cultured on MS medium containing 0.5 mg L^{-1} BA without adding NAA. The addition of 1 mg L^{-1} BA with 0.2 mg L^{-1} NAA outperformed other treatments in producing the highest average number of shoots. Regarding the light source, LED lighting significantly surpassed fluorescent lighting by yielding the highest average number of shoots, chlorophyll content, and secondary metabolites accumulation. Regarding light quality, the interaction between red and blue LED light resulted in the highest values for shoot number, chlorophyll concentration, and phenolic compound production.

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