In Vitro Propagation of *Echinacea purpurea* and Stimulation of the Production of Some Medicinally Active Compounds through the Influence of Selected Growth Regulators and Light Quality.

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Abstract

This study aimed to optimize the in vitro propagation of *Echinacea purpurea* and enhance the production of selected medicinally important bioactive compounds through the evaluation of plant growth regulators and light quality under tissue culture conditions. Shoot tip explants excised from seedlings derived from surface-sterilized seeds were cultured on Murashige and Skoog (MS) medium. The effects of different concentrations of 6-benzyladenine (BA) and α-naphthaleneacetic acid (NAA) were investigated during the multiplication stage, while rooting performance was assessed using various concentrations of indole-3-butyric acid (IBA).

Results showed that the most effective treatment for shoot multiplication was the combination of 1.0 mg L^{-1} BA and 0.3 mg L^{-1} NAA which produced the highest mean number of shoots (17.67 shoots per explant) with marked improvement in other vegetative growth parameters. Medium supplemented with 0.5 mg L^{-1} IBA was better during the stage of rooting; it gave the highest mean number of roots (16.33 roots per plantlet) and a significant increment in root biomass. Light quality was also found to influence the accumulation of bioactive constituents significantly. The leaves under a combined red and blue LED light regime recorded the highest concentration of echinacoside, which was 144.60 µg g^{-1} dry weight compared to other light treatments.

This would prove that the best regulators of growth in plants combined with technologies in lighting based on LED are an effective way to enhance micropropagation efficiency as well as the biosynthesis of valuable pharmacologically compounds in *E. purpurea*. This would be a good way for the sustainable production of high-quality plant material for medicinal and industrial applications.

Keywords: *Echinacea*, BA, NAA, IBA, LED lighting, echinacoside, micropropagation.

1. Introduction

Plants of medical value are the most important repositories of biologically active compounds that are used in the prevention and treatment of various diseases in human beings [1]. Among these, the scientific and commercial interests in Echinacea have been remarkable due to its broad medicinal uses and well-documented pharmacological properties.

The genus *Echinacea* of the family Asteraceae consists of herbaceous perennial species indigenous to North America. Nine species have been recognized within this genus. However, only three of them are commonly used in medical preparations *Echinacea purpurea*, *Echinacea pallida*, and *Echinacea angustifolia* DC. Of these, *E. purpurea* is the most widely cultivated and therapeutically accepted species[1] .

The therapeutic properties of Echinacea are mainly ascribed to its abundant phytochemical

constituents, which include caffeic acid derivatives such as echinacoside, cichoric acid, and caftaric acid, together with flavonoids, polysaccharides, and some lipophilic compounds. These metabolites account for a wide range of biological activities, including immunomodulatory, anti-inflammatory, antioxidant, and antiviral effects.

Other researchers have also previously reported the effectiveness of Echinacea extracts against various viral pathogens like herpes simplex viruses, influenza viruses, and SARS-CoV-2 [2]. This has led to its wide use in pharmaceutical and herbal products for prevention and treatment of respiratory infections and common cold[2 ,3] .

The increased market demand for *Echinacea* products has encouraged the search for more efficient propagation systems that can provide large quantities of uniform, healthy plant material. In this regard, plant tissue culture has provided an ideal biotechnological method for clonal propagation, since it offers rapid clonal propagation with no seasonal constraints regarding year-round production, while ensuring genetic uniformity and high propagation rate[3] .

The success of vitro culture is most closely bound to the success in the application of plant growth regulators which control the division of cells, organogenesis, and general development of the plant. Cytokinin's 6-benzyl adenine (BA) have wide application for the stimulation of shoot induction and multiplication while α-naphthaleneacetic acid (NAA) plays a role in the regulation of morphogenetic responses and vegetative growth. Besides, indole-3-butyric acid (IBA) is accepted as one of the most effective auxins for inducing roots and improving the performance of rooting in plantlets obtained by tissue culture[4] .

Apart from growth regulators, other environmental factors inside the culture room contribute to the success of in vitro plant development. Of these, light assumes primary importance as it affects photosynthesis, morphogenesis, and secondary metabolite biosynthesis. The recent introduction of Light Emitting Diode (LED) technology has opened possibilities for optimizing tissue culture systems due to low energy requirement, almost no heat, and possibility of specific wavelength delivery matching plant physiological requirements [5]. Different light quality has been reported to differentially regulate plant growth and metabolism. Red light governs phytochrome-mediated responses and shoot elongation; blue light regulates chlorophyll biosynthesis and function of stomata as well as photosynthetic activity. White light offers a broad spectrum that suits the overall growth of plants, whereas combinations of red and blue wavelengths have been reported to increase both biomass production and the accumulation of secondary metabolites in various tissue-cultured plant species[6–8] .

Hence, the present study was conducted to assess the influence of certain plant growth regulators and different LED light regimes on the micropropagation efficiency of Echinacea purpurea along with their possible role in boosting the accumulation of medicinally important bioactive compounds, particularly echinacoside, under in vitro culture conditions.

2.Materials and Method

Plant Material

Experiments were conducted in the Plant Biology Laboratory, Medicinal and Aromatic Plants Research Unit, College of Agricultural Engineering Sciences, University of Baghdad. Seeds of *Echinacea purpurea* were obtained from the Islamic Republic of Iran and used as the source material for the in vitro studies described in this work.

Seeds sterilization

Seeds were surface sterilized with a 1% solution of commercial Clorox (6% sodium hypochlorite) for 20 min and then rinsed four to

five times with sterile distilled water. The whole sterilization process was carried out under aseptic conditions in a laminar airflow cabinet according to the methodology of Samsurizal *et al.* [9].

Seeds germination

Post-surface sterilization, the seeds were plated on hormone-free Murashige and Skoog (MS) medium and then kept in a growth chamber with regulated environmental conditions. The incubation was carried out at 23 ± 2° C with a photoperiod of 16 h light/8 h dark and a light intensity of 1000 lux to facilitate optimum germination as well as subsequent seedling growth.

Branches stock

Aseptic shoot cultures of *Echinacea purpurea* were established from 1-cm shoot tip explants excised from seedlings germinated from surface-sterilized seeds. The explants were cultured on solid Murashige and Skoog (MS) medium without the addition of plant growth regulators to obtain uniform shoot cultures for further in vitro studies.

Shoot Multiplication Stage

After obtaining a sufficient number of shoots, they were transferred to MS multiplication medium supplemented with different concentrations of (BA) (0, 0.5, 1.0, and 1.5 mg L^{-1}) in combination with (NAA) (0, 0.1, 0.3, and 0.5 mg L^{-1}) to evaluate their effects on shoot growth and multiplication. Four weeks after culture initiation, the response of the shoots to the various treatments was assessed by measuring the number and length of shoots, number of leaves, as well as the fresh and dry weights of the resulting vegetative growth [10].

Rooting Stage

For root induction, shoots obtained from the most responsive multiplication treatment were transferred to half-strength Murashige and Skoog (MS) medium containing different levels of indole-3-butyric acid (IBA) (0,0.3,0.5,1.0 mg L^{-1}). Following six weeks of incubation, rooting efficiency was evaluated based on root number, root length, and root biomass, expressed as fresh and dry weights [11].

Effect of Light Quality on the Induction of Secondary Metabolites

Plants obtained from the best treatment during the multiplication stage were used to investigate the effect of light quality on the induction of secondary metabolites. The plantlets were exposed to four different light regimes: white, red, blue, and a combination of red and blue light. After six weeks of light exposure, the concentration of echinacoside in *Echinacea* leaves was determined to evaluate the response of the plants to the different light treatments and their effectiveness in enhancing the accumulation of this biologically active compound [12].

The concentration of echinacoside, one of the major medicinally active compounds in *Echinacea* leaves, was determined using High-Performance Liquid Chromatography (HPLC) (SYKAM, Germany) equipped with a Diode Array Detector (DAD). The chromatographic separation was performed under isocratic conditions using a mobile phase consisting of 0.1% formic acid and acetonitrile at a ratio of 80:20 (v/v). The mobile phase was delivered at a flow rate of 1.5 mL min^{-1}, and an injection volume of 100 µL of *Echinacea* extract was employed. Detection and quantification of echinacoside were carried out at a wavelength of 330 nm using the PDA detector [13]:

$$\textit{Sample concentration} = \frac{\textit{Standard concentration x sapmle area x Dilutiontimes}}{\textit{Standard area}}$$

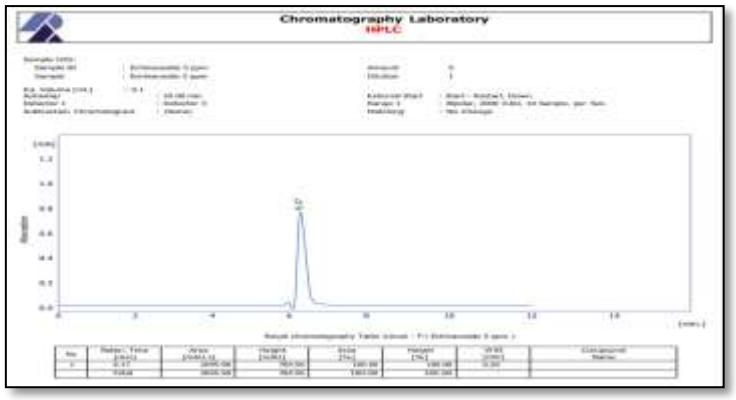

Fig 1. Standard echinacoside of leaves) mg.g-1).

Statistical design

The study utilized a factorial experimental design incorporating 10 repetitions within a completely randomized design (CRD) [14].

3.Results and Discussion

3.1. Effect of BA, NAA, and Their Interaction on Shoot Length of *Echinacea purpurea*

Table (1) presents the results of the study. Shoot length was significantly affected by the concentrations of benzyl adenine (BA) and naphthalene acetic acid (NAA). Among the treatments of BA, the control treatment showed the best shoot elongation with a mean shoot length of 4.667 cm, while that with BA added showed relatively lower values. The same trend was observed for NAA, where the hormone-free medium gave the best mean shoot length compared to the other NAA levels tested. A significant interaction was also observed between the levels of BA and NAA. The combination of 0.5 mg L^{-1} BA and 0.0 mg L^{-1} NAA proved to be the most effective as it gave the maximum mean shoot length of 6.667 cm, which was significantly higher than that obtained from the other treatment combinations.

Table 1. Effect of BA and NAA and Their Interaction on the Mean Shoot Length (cm) of Echinacea Plants.

BA mg L^{-1}	NAA mg L^{-1}				Mean BA
	0.0	**0.1**	**0.3**	**0.5**	
0.0	3.000	1.833	2.667	1.000	4.667
0.5	6.667	5.000	4.333	2.667	4.625
1	4.000	4.667	2.667	2.333	3.417
1.5	4.000	3.333	3.000	4.667	3.750
L.S.D (0.05)	4.417	3.708	3.167	5.167	**Mean NAA**
0.512	0.512				1.025

3.2. Effect of BA, NAA, and Their Interaction on the Mean Number of Shoots in *Echinacea purpurea*

The data presented in Table (2) demonstrated that shoot multiplication was significantly influenced by both BA and NAA concentrations, as well as by their interaction. Among the BA treatments, 1.0 mg L^{-1} BA proved to be the most effective concentration, producing the highest mean number of shoots (13.42 shoots $plant^{-1}$), whereas the control treatment recorded the lowest value (3.08

shoots plant^{-1}). Likewise, NAA significantly affected shoot proliferation, with 0.3 mg L^{-1} NAA yielding the greatest mean number of shoots (9.33 shoots plant^{-1}), surpassing all other treatments.

A significant interaction was also observed between BA and NAA concentrations. The combination of 1.0 mg L^{-1} BA and 0.3 mg L^{-1} NAA produced the highest shoot multiplication rate, reaching 17.67 shoots plant^{-1}, and significantly outperformed all other treatment combinations. These findings indicate that the appropriate balance between cytokinin and auxin concentrations plays a critical role in enhancing shoot proliferation efficiency in *Echinacea purpurea* cultures.

Table 2. Effect of BA and NAA and Their Interaction on the Mean Number of Shoots (shoot plant^{-1}) of Echinacea Plants.

BA mg L^{-1}	NAA mg L^{-1}				Mean BA
	0.0	0.1	0.3	0.5	
0.0	1.33	2.33	3.33	5.33	3.08
0.5	6.33	7.00	7.67	9.67	7.67
1	11.33	11.00	17.67	13.67	13.42
1.5	11.00	11.01	8.67	5.67	9.08
L.S.D (0.05)	7.50	7.83	9.33	8.58	Mean NAA
0.997	0.997				1.994

3.3. Effect of BA and NAA and Their Interaction on the Mean Number of Leaves (leaf plant^{-1}) in Echinacea Plants

The results presented in Table (3) demonstrated a significant effect of benzyl adenine (BA) concentrations on the number of leaves produced. The treatment supplemented with 1.0 mg L^{-1} BA recorded the highest mean number of leaves (6.50 leaves plant^{-1}), showing a significant superiority over all other treatments, whereas the control treatment produced the lowest mean value (3.17 leaves plant^{-1}). Regarding the effect of naphthalene acetic acid (NAA), the treatment containing 0.3 mg L^{-1} NAA achieved the highest mean number of leaves (5.50 leaves plant^{-1}), significantly exceeding the other concentrations as well as the control treatment, which recorded the lowest values. Furthermore, the interaction between BA and NAA concentrations significantly influenced leaf production. The combination of 1.0 mg L^{-1} BA and 0.3 mg L^{-1} NAA resulted in the highest mean number of leaves, reaching 9.67 leaves plant^{-1}, and differed significantly from all other interaction treatments.

Table 3. Effect of BA and NAA and Their Interaction on the Mean Number of leaves (leaf plant^{-1}) of Echinacea Plants.

BA mg L^{-1}	NAA mg L^{-1}				Mean BA
	0.0	0.1	0.3	0.5	
0.0	4.33	2.00	3.00	3.33	3.17
0.5	5.33	3.67	5.33	4.67	4.75
1	4.67	5.67	9.67	6.00	6.50
1.5	5.67	4.67	5.00	4.33	4.92
L.S.D (0.05)	4.00	4.83	5.50	5.00	Mean NAA
0.588	0.588				1.176

3.4. Effect of BA and NAA and Their Interaction on the Mean Fresh Weight (g) of Echinacea Plants

The results presented in Table (4) demonstrated a significant effect of benzyl adenine (BA) concentrations on the fresh weight of Echinacea plants. The treatment supplemented with 1.0 mg L^{-1} BA recorded the highest mean fresh weight (31.74 g plant^{-1}), showing a significant superiority over all other treatments, whereas the control treatment exhibited the lowest mean fresh weight (23.92 g plant^{-1}). With respect to the effect of naphthalene acetic acid (NAA), the treatment containing 0.3 mg L^{-1} NAA significantly outperformed the other concentrations, producing the highest mean fresh weight of 30.01 g plant^{-1}, while the control treatment recorded the lowest values. Furthermore, the interaction between BA and NAA concentrations resulted in significant differences among treatments. The combination of 1.0 mg L^{-1} BA and 0.3 mg L^{-1} NAA produced the highest mean fresh weight, reaching 33.65 g plant^{-1}, and

was significantly superior to all other interaction treatments.

Table 4. Effect of BA and NAA and Their Interaction on Mean Fresh Weight (g) of Echinacea Plants.

BA mg L⁻ ¹	NAA mg L⁻ ¹				Mean BA
	0.0	0.1	0.3	0.5	
0.0	21.10	24.68	23.23	26.66	23.92
0.5	26.48	28.28	29.58	28.57	28.23
1	30.69	29.41	33.65	33.23	31.74
1.5	31.46	30.99	32.44	31.60	31.62
L.S.D (0.05)	27.43	28.34	30.01	29.73	Mean NAA
0.683	0.683				1.367

3.5. Effect of BA and NAA and Their Interaction on the Mean dry Weight (g) of Echinacea Plants

The results of Table 5 show that benzyl adenine (BA) concentration had a significant effect on Echinacea plant dry weight. The treatment with 1.0 mg L⁻ ¹ BA supplement had the highest mean dry weight of 0.445 g plant⁻ ¹, showing significant superiority over all other treatments, while the control treatment had the lowest mean dry weight, at 0.290 g plant⁻ ¹. For the effect of naphthalene acetic acid (NAA), the treatment with 0.3 mg L⁻ ¹ NAA significantly outperformed the other concentrations, giving the highest mean dry weight of 0.387 g plant⁻ ¹; the control treatment gave the lowest values. It also showed that there are significant differences between the interaction treatments due to the concentrations of BA and NAA. The combination of 1.0 mg L⁻ ¹ BA and 0.3 mg L⁻ ¹ NAA produced the highest mean dry weight, at 0.530 g plant⁻ ¹, and was significantly superior to all other interaction treatments.

Table 5. Effect of BA and NAA and Their Interaction on Mean dry Weight (g) of Echinacea Plants.

BA mg L⁻ ¹	NAA mg L⁻ ¹				Mean BA
	0.0	0.1	0.3	0.5	
0.0	0.335	0.386	0.391	0.435	0.290
0.5	0.312	0.259	0.288	0.301	0.386
1	0.367	0.398	0.530	0.488	0.445
1.5	0.373	0.348	0.339	0.346	0.351
L.S.D (0.05)	0.346	0.347	0.387	0.392	Mean NAA
0.012	0.012				0.025

3.6. Effect of Light Quality on echinacoside in *Echinacea* leaves

The results presented in Table (6) indicated a significant effect of light quality on the echinacoside content in the leaves of Echinacea plants. The combined red and blue light treatment showed a significant superiority, recording the highest echinacoside concentration of 144.60 µg, whereas the white light treatment produced the lowest concentration of 86.90 µg. These findings suggest that light quality plays a crucial role in stimulating the accumulation of biologically active compounds in vitro-grown Echinacea plants. Fig 2.

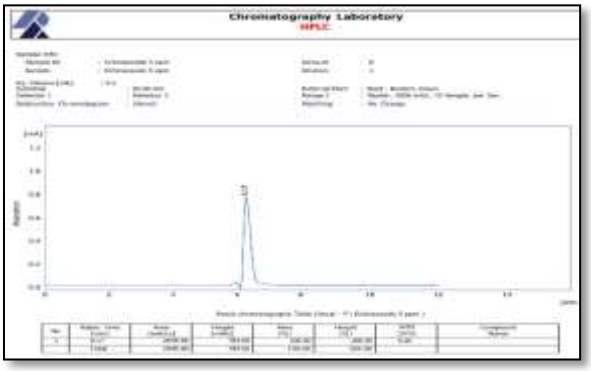

fig2. Best treatment red + blue light. of echinacoside

Table 6. Effect of Light Quality on echinacoside in *Echinacea* leaves

Light Quality	Echinacoside
White	86.90
Red	124.60
Blue	97.80
Red+ Blue	144.60
LSD 0.05	3.823

3.7.Effect of IBA Concentrations on Root Length and Number of Roots in Echinacea Plants.

The results presented in Table (7) revealed a significant effect of different indole-3-butyric acid (IBA) concentrations on root number and root length in Echinacea plants. The treatment with 0.5 mg L^{-1} IBA was significantly superior in root number, registering the highest mean value of 7.33 roots $plant^{-1}$ over other treatments. Control treatment gave the highest mean value of root length, which was 7.00 cm; on the other hand, the lowest mean value of 2.67 cm was recorded for 0.5 mg L^{-1} IBA treatment. These results reveal that root number and root length responded differently to IBA concentrations in the rooting medium.

Table 7. Effect of IBA Concentrations on Root Length and Number of Roots in Echinacea Plants.

IBA (mg. l^{-1})	Root length (cm)	Number of roots
0	7.00	7.33
0.3	6.00	9.67
0.5	2.67	16.33
1.0	4.67	14.67
LSD 0.05	1.537	1.719

3.8. Effect of IBA Concentrations on the Fresh and Dry Weights of Echinacea Roots

Results in Table (8) show a significant effect of indole-3-butyric acid (IBA) concentrations on fresh and dry weights of Echinacea roots. The treatment with 0.5 mg L^{-1} IBA showed a significant superiority; it had the highest mean fresh weight of 0.426 g compared to the control treatment, which had the lowest mean fresh weight of 0.311 g. It also had the highest mean dry weight of 0.231 g compared to the control treatment, which had the lowest mean dry weight of 0.102 g. These results indicate that the addition of 0.5 mg L^{-1} IBA enhanced root growth and promoted the accumulation of root biomass compared with the other treatments (fig. 3).

Table 8. Effect of IBA Concentrations on the Fresh and Dry Weights of Echinacea Roots

IBA (mg. l^{-1})	Dry weight (g)	Fresh weight (g)
0	0.231	0.102
0.3	0.261	0.154
0.5	0.426	0.311
1.0	0.356	0.212
LSD 0.05	0.052	0.004

Fig3. Best treatment IBA (0. 5 mg. L^{-1}) in Echinacea Roots.

4.Discussion

The significant increase in shoot number observed in Table (2) with the addition of benzyl adenine (BA) will be attributed to the fact that it is an effective cytokinin in plant tissue culture. BA will contribute to the release of apical dominance and also stimulate axillary bud development by promoting nutrient sink formation and directing assimilates toward meristematic tissues that are actively growing. This will then enhance shoot initiation and proliferation since cell division will be promoted. BA is one of the most commonly used cytokinin's in tissue culture techniques due to its stability and resistance to degradation compared with other plant growth regulators, which brings about an improvement in the efficiency of shoot induction and multiplication [15].

Superiority noticed in the proliferation of shoots can also be due to the proper balance of cytokinins and auxins in the medium of culture. This balance

of hormones reduces the effect of inhibition of apical dominance, stimulates the outgrowth of axillary buds, and differentiates vascular tissue, thus permitting translocation of water and nutrients to tissues that are actively growing. This is seen as a significant increase in the number of shoots (Table 2), number of leaves (Table 3), fresh weight (Table 4), and dry weight (Table 5) which will finally enhance growth and development of the plant as a whole [16].

The results obtained with naphthalene acetic acid (NAA) may be due to its character as a relatively stable synthetic auxin. In many applications of tissue culture, NAA is more effective than the natural auxin indole-3-acetic acid (IAA) because it is not rapidly degraded and is less prone to photodecomposition or to conjugation with organic compounds in the culture medium-sugars, alcohols, and amino acids, for example. These properties allow NAA to keep its physiological activity for longer periods within plant tissues [17, 18, 19].

The increase in shoot length can be attributed to the role of auxins, particularly NAA, in promoting tissue growth and cell elongation when applied at optimum concentrations, especially in the presence of balanced cytokinin levels. The synergistic interaction between auxins and cytokinins promotes both cell division and cell elongation, resulting in better shoot growth with longer shoots. The findings of the present study are in agreement with Mehedi et al. [20] and Chowdhury et al [21] who reported that cytokinin–auxin interaction improved vegetative growth and increased micropropagation efficiency under in vitro culture condition.

The better performances of cultures developed under LED light with those developed under fluorescent light (FL) may be ascribed to the special features of LED technology wherein it can emit wavelengths in perfect control that are needed physiologically by in -vitro-grown plants. The use of LED lamps is also more energy-efficient with lower heat emissions compared to conventional fluorescent lamps which create a better light environment for plant growth as well as secondary metabolite production [22].

The results in Table (6) show that the treatment with combined red and blue LEDs significantly increased the content of echinacoside in the leaves of Echinacea plants. This could be the direct effect of the light quality on the regulation of metabolic processes participating in the biosynthesis of secondary metabolites. Both red light (630-665 nm) and blue light (440-480 nm) fall within the effective absorption spectra of photosynthetic pigments and photoreceptors. As a result, these wavelengths improve the efficiency of photosynthesis and also stimulate the production of primary carbon metabolites, which are necessary precursors for the biosynthesis of phenolic compounds and biologically active glycosides, including echinacoside [23].

Also, blue light will switch on the genes and enzymes that have to do with secondary metabolic pathways; on the other hand, red light will improve photosynthetic performance and increase photosynthetic assimilation accumulation. The two wavelengths in combination have a synergistic effect in promoting the synthesis and accumulation of secondary metabolites in the plant tissues. Perhaps this synergism can explain the significantly higher content of echinacoside observed in the combined red and blue light treatment as compared to the other light treatments.

The results obtained are in line with some previous studies that emphasized the pivotal role of light quality in the regulation of secondary metabolite production by medicinal plants in vitro. According to Kim et al. [24] and Moon et al. [25], red and blue lights either individually or in combination increased physiological activity and hence stimulated the accumulation of bioactive compounds in tissues. Similarly, Silva et al. [26] and Bajwa et al. [27] reported that secondary metabolites of medicinal plants were more strongly induced when specific LED wavelengths were applied to in vitro cultures of these plants (fig. 2).

The significant increase in root number as well as fresh and dry root weights (Tables 7 and 8) (fig 3.) may be attributed to the pivotal role of auxins in promoting root induction under in vitro culture conditions. A high auxin-to-cytokinin ratio in the

culture medium is considered a key factor in stimulating the initiation and development of adventitious roots [28]. Indole-3-butyric acid (IBA) is among the most widely used auxins for rooting due to its high efficiency in inducing root formation and increasing root numbers through the promotion of cell division and differentiation at root initiation sites [29].

Furthermore, IBA enhances the differentiation of meristematic cells toward the root developmental pathway and stimulates physiological processes associated with root growth, resulting in increased root biomass and higher fresh and dry root weights. These findings are consistent with those reported by Ioio et al. [30] and Moubayidin et al. [31], who demonstrated the central role of auxins in regulating root organogenesis through the control of cell division and differentiation. The present results also agree with those of Sushma Rani [32] and Li et al. [33], who reported that appropriate concentrations of IBA significantly improved rooting response and promoted root system development in in vitro-cultured plants, including medicinal species such as Echinacea.

5.Conclusion

The results of the present study demonstrate that the optimized use of plant growth regulators and light quality significantly improved the micropropagation efficiency of *Echinacea purpurea* and enhanced the accumulation of echinacoside, a major medicinally important bioactive compound. These findings further indicate that the integration of plant tissue culture techniques with LED-based lighting systems represents an effective biotechnological strategy for the large-scale production of high-quality medicinal plants with enhanced phytochemical content under in vitro.

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