MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Effect of cytokinins on Photosynthetic pigments and protein content of micropropagated *Mentha viridis L.* and *Moringa oleifera Lam*under white and violet visible light emitting diodes (LEDs)

Lina; M. Abu-Ziada*[1]; Samia, A. Haroun[1]; Rasha, M. E. Gamel[1]; Mohamed, A. Abbas[1] and Heba Y. El-Banna[2]

[1].*Botany Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt*

[2].*Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University*

***Correspondence to:**Lina M Abu-ziada Email: linamziada@gmail.com*

Received:18/9/2022
Accepted: 24/9/2022

Abstract: The present study was carried out at the tissue culture lab and experimental station of the Vegetable and Floriculture Department, faculty of Agriculture, Mansoura University. Propagation of two medicinal plants grown for 30 days in a protocol wheremature seeds of *Moringa oleifera* was used *invitro* culture, while for *Mentha viridis* the explant material of nodal segment (about 1-1.5 cm) was used. Two types of cytokinins: 6-benzylaminopurin (BAP), and thidiazuron (TDZ) singly at three different concentrations (0, 1, or 2 mgL^{-1}) were used as well as light-emitting diode systems (LEDs) (white as control and violet; the combination of red and blue; 1:1). The results cleared that a general increment in chlorophyll a, chlorophyll b, carotenoids and total pigments by all the used treatments either under white LEDs or violet LEDs illumination in case of the two used plants, as compared to control (free hormone medium). So, under violet LEDs illumination, the maximal value of total pigments (25.717 mg/g dry weight) in *Mentha* was recorded in case of MS medium supplemented with 2 mgL^{-1} TDZ and control medium recorded the least total pigments values (13.587 mg/g, respectively) under white LEDs illumination. In *Moringa* maximum total photosynthetic pigments were 29.967 mg/g, in MS medium with 2 mgL^{-1}BAP under violet LEDs and the minimum value (18.359 mg/g) was in MS medium with 1 mgL^{-1} TDZ under white light conditions; as there wasn't any shoots or leaves at all.The maximal values of total protein content in micropropagated *Mentha* and *Moringa* were in case of MS nutrient medium supplemented with 2 mgL^{-1} TDZ under violet LEDs illumination (46.7007 and 52.164 mg/g fresh weight), respectively. On the other hand, the minimal value of protein content in *Mentha* was in control (free hormone medium) MS medium under white LEDs (21.933 mg/g fresh weight) and in *Moringa* was in MS medium fortified with 1 mgL^{-1} BAP under white LEDs (20.544 mg/g fresh weight).

Keywords*:*Micropropagation,, light emitting diodes (LEDs), photosynthetic pigments, benzyl 6-amino-purine, thidiazuron, Mentha viridis, Moringa oleifera.

1.Introduction

Plant tissue culture is an integral part of plant biotechnology due to the many benefits it offers over traditional methods of propagation. Plant hormones have been shown to improve the success of vegetative propagation from cuttings, but in order to obtain uniform planting materials, tissue culture techniques involving plant materials like nodal segments, indirect organogenesis, multiplication using immature seeds, and regeneration of axillary cotyledons **[1]**.

Plant tissue culture has given new insights into plant biology and has become an important tool for the development of crop species**[2]**. The production of secondary metabolite is also becoming familiar by tissue culture for pharmaceutical use. The integrated approaches of culture systems will provide the basis for the

future development of safe, effective and high-quality products for consumers**[3].**

LightingsystemsinplanttissueculturehavegenerallybeenupdatedinrecentdecadesordesignedbasedonLEDtechnology.Asaresult,variouslightqualityimprovementsarepossible.Light-emittingdiodes(LEDs)asanewlightingtechnologyhaveemergedasanalternativelightsourceforplantsduetotheirwavelengthspecificity,narrowbandwidth,smallsize,solid-stateconstruction,longlifetime,andlowheatgeneration**[4]**.*Moringa oleifera* contains 32 chemical substances with nutritional value, as reported by**[5]**. The leaves and pods of the *Moringa* plant are of great interest due to their high protein content; by eating these parts of the plant, one can fight malnutrition at a low cost. The *Moringa oleifera* plant, specifically its leaves, have been found to contain cytokinin, a plant growth hormone.

Mentha spicata, also known as *Mentha viridis (Lamiaceae)*, is frequently cultivated across the world forits exceptional aroma and economic worth. *M. spicata* is famous for its traditional medical uses, particularly for the treatment of colds, coughs, asthma, fevers, obesity, jaundice, and digestive issues**[6]**. It is also used as a traditional culinary flavouring agent. One of the most widely used species in the genus, *Mentha spicata*, has a long history of use in folk medicine as a digestive and sedative**[6]***Menthaspicata*isaperennialherbofgreatinterestinfolkmedicine**[7]**,especiallyinthetreatmentofgastrointestinaldisorders**[8]**,cosmeticsandmedicines**[5]**.havebiologicalproperties.Essentialoils(Eos)haveshownantibacterial,antifungal,antilarvalandcytotoxiceffects**[9]**.

The natural pigments are essential secondary metabolites, which play multiple roles in thewhole life cycle of plants and are characterized by powerful antioxidant activity. After decades of research and development, multiple benefits of these natural pigments to human health have been explored and recognized and have shown bright application prospects in food, medicine, cosmetics and other industries. Pigments are groups of diverse natural chemical compounds that are synthesized in plants to color our nature and play many important biological functions. Pigments are consumed as essential nutrients and medicinal nutraceuticals in human and animal diets; they are also key determinants of fruit development, appearance and quality, and ultimately, of customer acceptance and market value **[10].** Also, plant-based proteins have been the subject of growing interest from researchers and consumers because of their potential health benefits as well as their positive environmental impact. Plant proteins and their potential for reducing the risk of developing metabolic syndrome, diabetes management, cancer prevention, and weight management were each discussed in many reports.

Thus, the aim of this study was to quantify and compare the content of photosynthetic pigments and protein of *Mentha viridis* and *Moringa oleifera* plants using plant tissue culture protocol assisted by combined use of various factors viz., plant growth regulators (two types of cytokinin: benzylaminopurine and thidiazuron) singly at three different concentrations (0, 1 or 2 mg/L) for each and light emitting diode systems LEDs (white as a control and violet; combination of red and blue; 1:1).

2. Materials and methods

2.1 Plant materials, medium components &chemicals

Mentha viridis(spearmint) pots and Moringa olifera seeds were obtained from the nursery plantation of Mansoura University and selected for apparent uniformity of size and shape. The chemicals used were supplied from Sigma Chemical Company.

2.2 Time course of the experiment

2.2.1 Surface sterilization of explants

Nodal segments of Mentha viridis were excised using a sharp scalpel then washed for 30 min under running tap water with 4 drops of liquid soap to remove dust particles. The explants were surface sterilized by 25% (v/v) clorox commercial bleach solution (6% sodium hypochlorite) for 6 minutes then finally rinsed three times with distilled sterilized water for 5 min each.

Seeds of Moringa oleifera were washed for 1 hour under running tap water with 4 drops of liquid soap. Next, the seeds were immersed for 30 seconds in 70 % ethanol and transferred to a solution of 25% (v/v) clorox commercial bleach

solution (6% NaOCL) for 20 min, followed by rinsing three times in sterile distilled water. Afterward, the seeds coat was removed inside the laminar flow hood before being cultured.

Culture media and conditions:

Sterile explants were transferred into jars contain 25 ml of MS **[11]**basal medium containing 3% sucrose as a carbon source (Table 1) and supplemented with two types of cytokinin: 6-benzylaminopurine (BAP) and thidiazuron (TDZ) singly at three concentrations (0, 1or 2 mg/L) for each. The medium was solidified with 0.7% plant agar. The pH of the medium was adjusted to 5.75 before adding agar and autoclaved at 121 ^{0}C, 1.1 kg / cm^2 for 25 minutes.

The cultures were incubated in growth chambers at 25 ± 1^{o}C under a 16hrs light/8hrs dark treated with two different light types emitting diodes white LEDs as a control and combination of 1:1 red/blue LEDs (violet LEDs) for 30 days for all treatments.

Table (1): Components of Murashige andSkoog (1962) basal medium.

Constituents	Concentration
Macroelements	
NH_4NO_3	1650 mgL^{-1}
KNO_3	1900 mg L^{-1}
$CaCl_2$. $2H_2O$	440 mg L^{-1}
$MgSO_4$. $7H_2O$	370 mg L^{-1}
$KH2PO_4$	170 mg L^{-1}
Microelements	
H_3BO_3	6.2 mg L^{-1}
$MnSO_4$. $2H_2O$	16.9 mg L^{-1}
$ZnSO_4$. $7H_2O$	8.6 mg L^{-1}
KI	0.83 mg L^{-1}
$NaMoO_4$. $2H_2O$	0.25 mg L^{-1}
$CuSO_4$. $5H_2O$	0.025 mg L^{-1}
$COCl_2$. $6H_2O$	0.025 mg L^{-1}
Na_2 EDTA ($2H_2O$)	37.3 mg L^{-1}
Myo-inositol	80.0 mg L^{-1}
Glycine	2.0 mg L^{-1}
Nicotinic acid (B5)	0.5 mg L^{-1}
Pyridoxine HCL(B6)	0.5 mg L^{-1}
Thiamine – HCL (B1)	0.1 mg L^{-1}

The research involved ten treatments with 20 replicates in a completely randomized design (CRD):

(1) MS medium free hormone (white LEDs as control.

(2) MS medium+1 mg L^{-1}BAP + white LEDs.

(3) MS medium+ 2 mgL^{-1}BAP + white LEDs.

(4) MS medium+ 1 mgL^{-1}TDZ + white LEDs.

(5) MS medium+ 2 mgL-^{1} TDZ + white LEDs.

(6) MS medium free hormone (violet LEDs as control).

(7) MS medium+ 1 mgL^{-1}BAP + violet LEDs.

(8) MS medium+ 2 mgL^{-1}BAP + violet LEDs.

(9) MS medium+ 1 mg L^{-1}TDZ + violet LEDs.

(10) MS medium+ 2 mg L^{-1}TDZ + violet LED.

2.3. Analytical methods

2.3.1. determination of photosynthetic pigments

Using the spectrophotometric approach described by **[12]** for chlorophylls and **[13]** for carotenoids as adopted by **[14]**, the plant photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) were identified in the leaf samples.

3.2.2. Total protein extraction and determination

Extraction and determination according to **[15]**. Protein content was extracted by macerating a known weight of fresh samples in a mortar with 2 ml of extraction buffer (0.2 M tris HCl (pH 6.8), 2% SDS, and 10 % sucrose) then centrifuged at 4000 rpm for 15 minutes. Extraction buffer was prepared by adding 5 g sucrose to 10 ml 1 M tris HCl, 10 ml 10 % SDS and complete to 50 ml with distilled water. The solution was stored at 4°C till used. Protein content in the plant extract was determined spectrophotometrically by Coomassie Brilliant Blue G250 agent.

4.2. Experimental design and statistical analysis

The experiment was factorial with two main factors and 20 replications in a completely randomized design. The first factor was light with two levels: white LEDs and violet LEDs. The second factor was growth regulator with five levels: the control (no growth regulator), 1 mg L^{-1}BAP, 2 mg L^{-1}BAP, 1 mg L^{-1}TDZ, 2 mg L^{-1}TDZ.

Data were subjected to one-way ANOVA, followed by mean separation according to the Duncan's multiple range test

3. Resultsand Discussion

3.1.Photosynthetic pigments of micropropagated *Mentha viridis* (Fig 1)

The results of the current study indicated that, in *Mentha* plantlets in-vitro grown for 30 days showed, compared to control significant increments in chlorophyll a and chlorophyll b content by all the used treatments either under white LEDs or violet LEDs illumination, as compared to control (free hormonal medium). The maximum value of chlorophyll a (11.937 mg/g dry weight) was in MS medium supplemented with 2 mgL^{-1} TDZ under violet LEDs illumination, followed by 11.614 mg/g dry weight in MS medium enriched with 1 mgL^{-1} TDZ under the same light conditions. Chlorophyll b content recorded the maximum value (5.9423 mg/g dry weight) in case of MS medium enriched with 2 mgL^{-1} TDZ, followed by MS medium supplemented with 1 mgL^{-1} TDZ which recorded 5.792 mg/g dry weight, under violet LEDs illumination.

On consequence, the results indicated that, the total chlorophylls were more or less similar to the determined chlorophyll a and chlorophyll b content in the present experiment under white and violet LEDs illumination. Thus, as compared to control, the maximum value was 17.879 mg/g dry weight in caseof MS medium enriched with 2 mgL^{-1} TDZ, followed by 17.406 mg/g dry weight in MS medium supplemented with 1 mg/L TDZ. Whereas, control medium recorded the least values in chlorophyll a (6.356 and 6.562 mg/g, respectively) and in chlorophyll b, (3.2199 and 3.286 mg/g, respectively) and total chlorophylls (9.576 and 9.847 mg/g, respectively), under white and violet LEDs illumination; without significant differences between them.

The content of carotenoids of *Mentha* plantlets determined in-vitro grown for 30 days, cleared that comparing to the control medium, this pigment increased generally by the used treatments. Thus, MS medium fortified with 1 & 2 mgL^{-1}thidiazuron as a growth regulator recorded the highest carotenoid values (7.516&7.838 mg/g dry weights, respectively), under violet LEDs illumination; without significant differences between them. The calculated content of total pigments of *Mentha* plantlets *invitro* grown for 30 days, cleared that comparing to the control medium, total pigments increased significantly by the used treatments. So, under violet LEDs illumination, the maximum value of total pigments (25.717 mg/g dry weight) was recorded in case of MS medium supplemented with 2 mgL^{-1} TDZ, followed by 24.922 mg/g dry weight in MS medium enriched with 1 mg L^{-1} TDZ; also, without significant differences between them. In consequence, control medium recorded the least carotenoid values (4.0113and 4.918 mg/g, respectively) and total pigments values (13.587 and 14.765 mg/g, respectively) under white and violet LEDs illumination; without significant differences between them.

Inthisregard,**[16]**showedthatEucalyptus,Musa andSpathiphyllumexhibitedhighergrowthininvitrogrownplantsunderacombinationofredandbluelightcomparedtowhitelight(W).(R/B,4/1).**[17]**reportedthatCunninghamialanceolataplantsinvitroundermonochromaticredlighthadthehighestchlorophylla,chlorophyllb,totalchlorophyllcontents,andphotosystemII,comparedwithmonochromaticblueandgreenlight.Wenotedthatthephotochemicalefficiencyof(PSII)wasdemonstrated.Furthermore,**[18]**suggeststhatbluelightisbeneficialforchlorophyllaccumulationandincreasingthechlorophylla/bratio.**[19]**stated that regarding the application of BAP, the lowest mean value of chlorophyll a of in vitro propagated *Aloe vera* (2.15 mg/L) was recorded at 0 mg/L BAP and the greatest mean value of chlorophyll a (3.11 mg/L) was recorded at level of 3 mg/L BAP. He also recorded that the concentration of 3mg/L BAP gave the highest mean value (1.42 mg/L) of chlorophyll b and the maximum mean values of carotene (1.53 to 1.57 mg/L). Moreover,**[20]**stated that chlorophyll level increased by BAP treatments on pot grown population of a local variety of cowpea (*Vigna unguiculata*) Kanannado, with greater increase in the 200 ppm BAP treated plants.

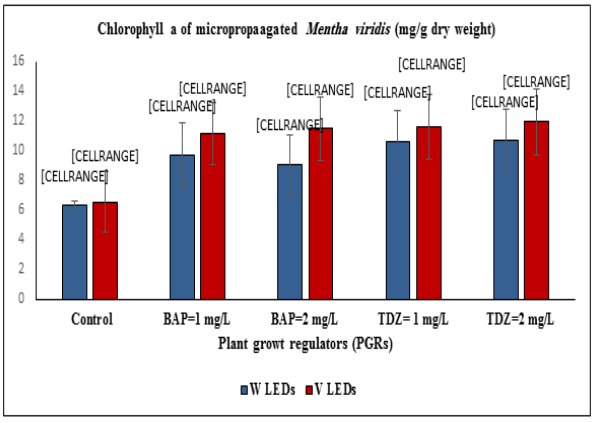
As the control medium recorded the least values under white and violet LEDs illumination in this investigation, according to **[21],** the amount of chlorophyll currently obtained in tissue cultures is significantly lower than that found in the mesophyll cells of whole plants of the same species, and the rate of chlorophyll formation on exposure of cultured cells to the light is significantly slower than the response of etiolated organized tissues.

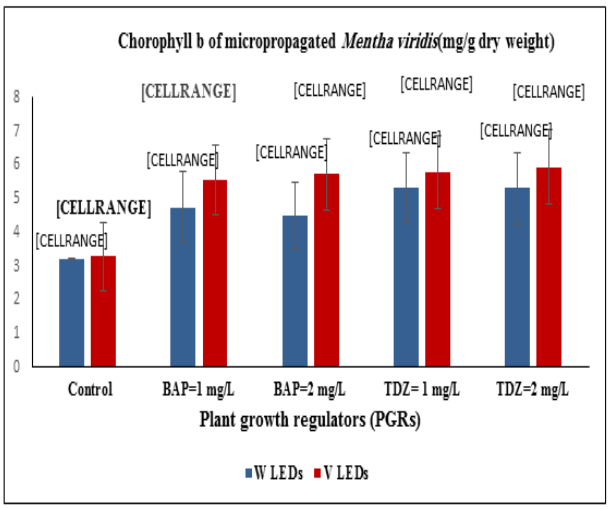
In support of **[22]**, which claimed that LEDs did not improve the rooting of shoots but increased the photosynthetic pigments content under B/R, it was noted in the current study the superiority of violet LED illumination on pigments production of *Mentha* plantlets i*nvitro* grown for 30 days. This facilitated the acclimatization process of *invitroMusa acuminata* plantlets. According to the results of 30 days of *invitro* growth of *Mentha* plantlets **[23]**. In photosynthesis, light quality is crucial because it affects how light is absorbed by chlorophyll. **[24]** found that plants lighted with a blue light treatment for more than 6 days had significantly lower total chlorophyll contents than controls. They also found that the effect of red LED light treatment on carotenoid content was significantly reduced by 57%.Studies on *Mentha* plantlets show that blue light plays a key role in the synthesis of chlorophyll and increases the chlorophyll content of plantlets when grown *invitro* for 30 days **[25]**. A study by *Phalaenopsis***[26]** has verified this. The synthesis of chlorophyll a and b and carotenoids was enhanced by red: blue light, but not by blue or red light, according to recent studies on *Verbena offcinalis* callus cultures **[27]**. Additionally, **[28]** noted that plantlets of *Pyrus communis L*. cultivated under red LEDs have much less chlorophyll and carotenoids in their photosynthetic pigment.

Carotenoids are a crucial component of the reaction centre and antenna of the photosynthetic process **[29, 30]**. They serve a variety of purposes in plants, including defence mechanisms against oxidative stress in addition to their direct function in photosynthesis **[31].**Reactive oxygen species can be directly scavenged by carotenoids, protecting cells from membrane deterioration and reactive oxygen species-mediated chlorophyll breakdown. According to **[32]**, cucumber plants produced more carotenoids when blue light was elevated to 50% of the total light spectrum. It has been demonstrated that higher amounts of carotenoids act as a cumulative defensive mechanism that is correlated with high light intensity or high levels of blue. This agrees with the findings of the study.

3.2. Photosynthetic pigments content of micropropagated *Moringa oleifera* (Figure 2)

In this study, comparing to the control values, the highest chlorophyll a content in *Moringa invitro* was 14.191 in MS medium with 2 mg/L BAP under white light followed by 12.904in the same MS medium but under violet LEDs illumination. Moreover, the highest chlorophyll b content was 9.9043in MS medium with 2 mg/L BAP followed by 8.1037 in MS medium with 1 mg/L BAP also but under violet LEDs illumination. Maximum total chlorophylls and total photosynthetic pigments were 22.8087 and 29.967 mg/g respectively, in MS medium with 2 mg/L BAP under violet LEDs. The minimum values of chlorophyll a or b or total chlorophylls or total pigments were in MS medium with 1 and 2 mg/L TDZ under white or violet light conditions as there wasn't any shoots or leaves at all. Whereas this treatment (2 mg/L TDZ under violet light conditions) recorded the highest carotenoids value (8.6497), comparing to the control value.For clarification the reason that TDZ treatments recorded the minimum pigments values, it is worthy to mention that by these treatments no differentiation shown in the *M. oleifera* propagated explants**[33]**.





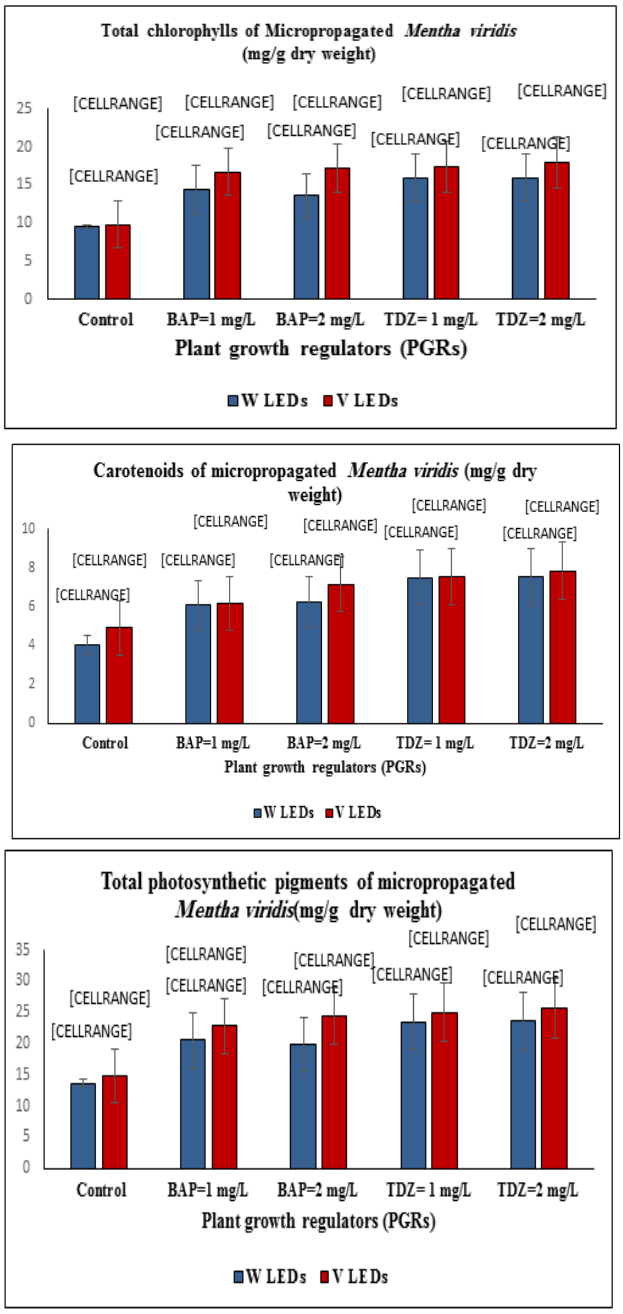


Figure (1): Photosynthetic pigments of micropropagated *Mentha viridis*(mg/g dry weight) after 30 days from transplanting under two different visible light emitting diodes (White &Violet) LEDs**.**

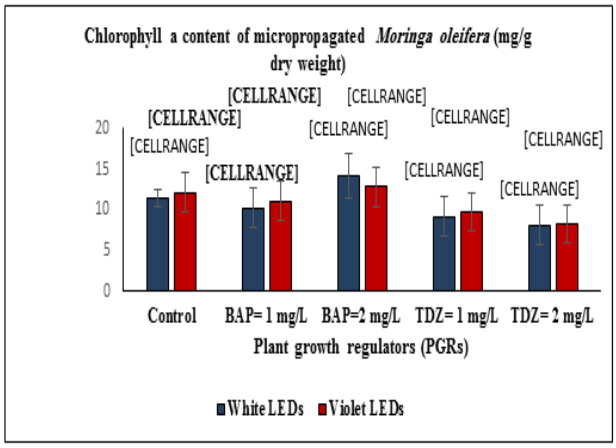
Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

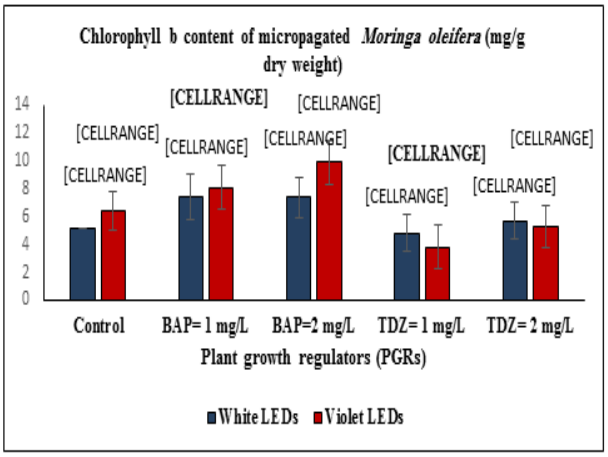
In support, **[34]**reported that the highest content of chlorophyll a, b and carotenoids was reported in pear plants *invitro* cultivated with BAP in the nutrient medium under white LED light, and the lowest in plants grown with metatopolin (mT) (type of cytokinins) under mixed (red &blue) LED light.**[35]**also reported similar results and **[34]**stated that white LEDs increased chlorophylls and carotenoids contents of *Lippia filifolia*.

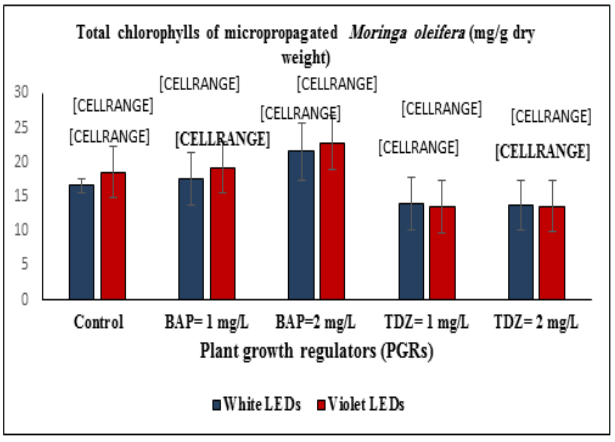
However, when *Gerbera jamesonii* was grown in a test tube under red/blue LED light as opposed to separate blue and red LED lights, larger quantities of chlorophyll a and b were found **[36]**. Shoots of *Stevia rebaudiana* subjected to red/blue LED light contained more carotenoids than those exposed to blue LED light **[37]**. Additionally, while looking at the total chlorophyll content in different *Lippia alba* chemotypes, **[38]** found that leaves grown under LED (blue/red) illumination had higher levels of photosynthetic pigment.

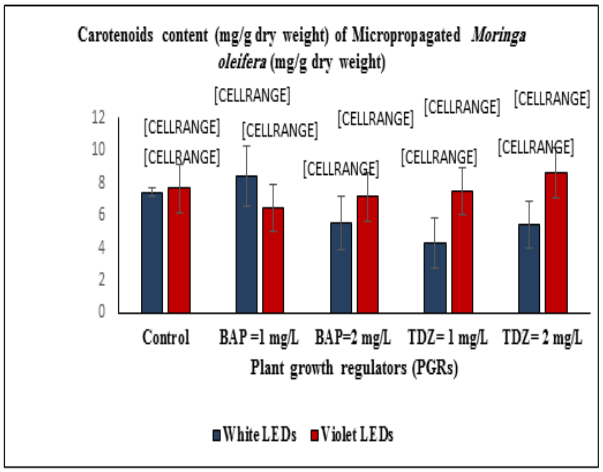
The quality and intensity of the light being emitted has an impact on the production of chlorophylls a, b, and carotenoids, with the combined blue and red wavelengths having the greatest photosynthetic efficiency **[39]**.As it affects how light is absorbed by pigments involved in photosynthesis, light quality is crucial to photosynthesis **[23]**. According to **[40]**, blue:red (B:R)=1:1 LED lights aided in the growth of rice seedlings and produced the highest amounts of carotenoid and chlorophyll as well as the highest rates of photosynthetic activity. Under the blue: red = 1:1 LED, rice seedlings produced abundant photosynthetic products more efficiently. In most research, one of the important criteria for assessing the impact of LEDs has been the amount of chlorophyll in cultured cells and/or tissues. Chlorophyll concentrations are a trustworthy sign of a healthy plant. High levels of chlorophyll indicate healthy photosynthetic activity as well as the nutritional state of plants **[41]**.Examination of the data cleared the excellence of violet LED light, in harmony with these results, cultures of *Euphorbia***[42]**,*T.japonicum***[43]**, *Doritaenopsis***[44]**, grapes **[18]**, cotton**[45]**,*Oncidium***[46]**, sugarcane**[47]**, *L. alba***[35]**, *Stevia***[37]**and blueberry**[48,49]** showed that cultures exposed to blue LEDs or mixes of red and blue LEDs had higher chlorophyll contents than cultures exposed to monochromatic red LED treatments. Moreover, red LED-induced increases in chlorophyll content were seen in banana shoot cultures**[50]** and *R. glutinosa***[51]**.White LEDs were also found to

improve chlorophyll productionin*vanilla***[52]**and sugarcane**[53]**.









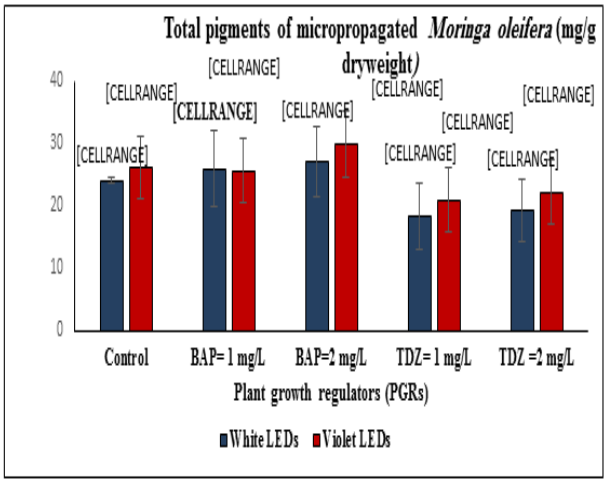


Figure (2): Photosynthetic pigments of micropropagated *Moringa oleifera* (mg/g dry weight) after 30 days under two different visible light emitting diodes (White &Violet) LEDs**.**

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

3.3. Total protein content of micropropagated *Mentha viridis* (Figure 3a)

Regarding total protein content in the current study, it significantly increments by the used treatments under both white or violet LEDs illumination, comparing to control medium. Hence, control medium recorded the least total protein values (21.933 and 30.1536mg/g fresh weights, respectively) under white and violet LEDs illumination; with a significant difference between them. Whereas, under violet LEDs illumination, the maximum value of total protein (46.7007 mg/g fresh weights) was recorded in case of MS medium supplemented with 2 mg/L TDZ, followed by 44.947 mg/g fresh weights in MS medium enriched with 1 mg/L TDZ; also, with a significant difference between them. These are in harmony with some authors such as **[54]**in the balloon flower (*Platycodon grandiflorum*), who observed higher levels of water-soluble proteins and endogenous PGRs in all LED light treatments.While, **[37]**reported that a higher total protein content of *invitroVanilla planifolia* plantlets was observed under fluorescent light.

As the protein content recorded the least values in control medium under white and violet LEDs illumination. According to **[55]**, while the total protein content of *Brassica*

oleracea L calluses was the lowest (1.37), it greatly rose in regenerated plantlets (8.57) in conditions containing TDZ. The degree of genetic stability at the level of gene expression, according to **[56]**, is also reflected in the changes in the protein profiles of the regenerated plants. The level of free amino acids will be higher during stressful situations to prevent cellular oxidative damage **[57]**. These results confirmed the obtained results of this study as well as further demonstrate that nutritional quality of plants could be varied by selecting special light sources under controlled growth environments.

This study showed the superiority of violet LED light for protein production. According to **[58]** and **[59]**, shorter wavelengths of the same spectrum were beneficial for protein accumulation, and blue LEDs were effective for protein accumulation in potato plantlets *invitro*. In agreement with our results, blue light at 445 nm was found to be more beneficial for protein accumulation in potato plantlets in vitro than blue light at 465 nm.It was observed in this study the excellence of violet LEDs illumination in protein synthesis. In harmony, blue LEDs was good for protein accumulation in potato plantlets *invitro*, according to **[59]** and shorter wavelengths of the same spectrum were favorable for protein accumulation. According to **[58]** blue light at 445 nm was found to be more advantageous for protein accumulation in potatoplantlets in vitro than blue light at 465 nm, according to**[60]**,which was consistent with our findings**.**

[61]reported that red-blue light can also increase the content of protein in *Vigna radiate*.**[62]**declared that monochromatic LEDs with emission peaks 465, 630 and 660 nm induced a ≈ 20% decrease of protein levels as compared to LEDs peaking at 405 nm and cool- and warm white LEDs. **[63]**reported that for monochromatic light, the blue light recorded the most accumulation of amino acids and proteins compared to other lights. Also, plantlets exposed to red and blue light had the maximum levels of pigments.**[40]**declared that the protein content in the rice seedlings was the highest under the red LED light, although significant differences were not observed.

3.4. Total protein content of *Moringa oleifera* (Figure 3b)

Nearly all the used treatments recorded protein content higher than those recorded in case of control. So, the maximum content of protein was 52.1637 mg/g fresh weight in *Moringainvitro* grown in MS medium with 2 mg/L TDZ under violet light, followed by 46.847 mg/g fresh weight in MS medium contained 2 mg/L BAP under the same light. While, the minimum content of protein was 20.544mg/g fresh weight in *Moringainvitro* grown in MS medium with 1 mg/L BAP under white light. Already *Moringa* organs contain high amount of protein, *Moringa*'s fruits, flowers & leaves contain 5 to 10% protein in average**[64],** its pods reported to contain 2.5 g protein/100g.

Recently, the leaves of the *Moringa* plant are a good source of flavonoids, phenolics, and carotenoids as well as β-carotene, protein, vitamin C, calcium, and potassium **[65, 66, 67]**.However, it has been discovered that a number of horseradish (*Moringa oleifera*) tree parts, including the leaves, roots, bark, seeds, flowers, and immature pods, have therapeutic characteristics. When the *Moringa* crop was harvested at a 30 day cutting interval with broad spacing, the nitrogen content was 6.11 percent, the potassium content was 9.14 percent, and the ascorbate content was 89.73 µg g-1 **[68]**. The phosphorous content was 3.40 percent.

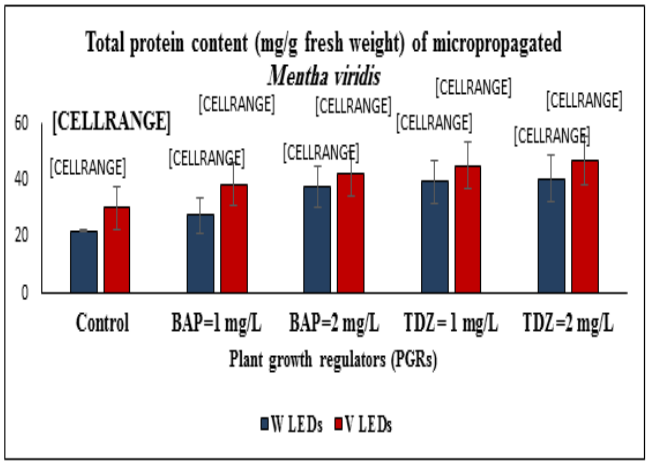


Figure (3a): Total protein content of micropropagated *Mentha viridis* (mg/g fresh weight) after 30 days from transplanting under

two different visible light emitting diodes (White &Violet) LEDs.

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

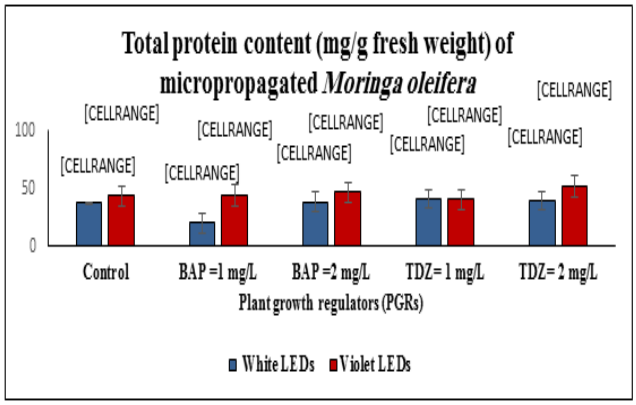


Figure (3b): Total protein content of micropropagated *Moringa oleifera* (mg/g fresh weight) after 30 days under two different visible light emitting diodes (white &violet) LEDs.

Values listed represent the mean ± standard error (SE). Different superscript letters refer to significant variation; with the least significant difference (LSD) at $p \leq 0.05$.

4. References

1. Bukar, S.M. and Abba, H.M. (2022).,Macro- and micropropagation of Moringa oleifera Lam (Moringaceae): A mini review. Asian *journal of plant biology*, **4(1)**: 20-25.
2 Khan, T., et al., (2021) Plant in vitro culture technologies; a promise into factories of secondary metabolites against COVID-19. Frontiers in Plant Science,. **12**:356.
3 Lemma, D.T., et al., (2020). Micropropagation of medicinal plants. International *Journal of Plant Breeding and Crop Science*, **7**:796-802.
4. Barceló-Muñoz, A., et al. (2021), Effect of LED lighting on physical environment and microenvironment on in vitro plant growth and morphogenesis: The need to standardize lighting conditions and their description. plants,.**11**(60): 1-35.
5. Price, M. (2007) ,The Moringa Tree. ECHO Tech..
6. Mahendran, G., et al (2021).,The traditional uses, phytochemistry and pharmacology of spearmint (Mentha spicata L.). *Journal of Ethnopharmacology*,. 278.
7. Zengin, G.,et al. (2022),Novel perceptions on chemical profile and biopharmaceutical properties of Mentha spicata extracts: adding missing pieces tothe scientific puzzle. Plants, **11**: 233.
8. Mahboubi, M.,Mentha spicata L. essential oil, (2021) phytochemistry and its effectiveness in flatulence. *J. Tradit. Complement, Med.*,.**11**: 75–81.
9. Piras, A., et al., (2021) Antifungal activity of essential oil from Mentha spicata L. and Mentha pulegium L. growing wild in Sardinia Island (Italy). Nat. Prod. Res.,. **35**: 993–9
10. Takei, K., et al.,. (2015). Galactomyces fermentation filtrate prevents T helper 2-mediated reduction of filaggrin in an aryl hydrocarbon receptor-dependent manner. Clin Exp Dermatol, **40**, 786-793
11. Murashige T, Skoog F (1962), A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plantarum,. **15**:473–497.
12. Arnon, D.I., (1949)Copper enzymes in isolated chloroplasts, polyphenoxidase in beta vulgaris. Plant Physiol.,. **24**: 1- 15.
13. Horváth, G., et al(1972).,Effect of light intensity on the formation of carotenoids in normal and mutant maize leaves. Phytochem.,. **11**: 183–187.
14. Kissimon, J., (1999) Analysis of the photosynthetic pigment composition. Proceedings of the International Workshop and Training Course on Microalgal Biol. and Biotech. Mosonmagyar, Hungary.,. 13- 26.
15. Bradford, M.M., A rapid and (1976).sensitive method for the quantification of microgram quantities of protein utilizing the principal of protein-dye-binding. Anal. Biochem., **72**: 248-251.
16. Nhut, D.T. and Nam, N.B.**,** (2010) Light-emitting diodes (LEDs): an artificial lighting source for biological studies. The third international conference on the development of biomedical engineering in Vietnam. Berlin, Heidelberg: Springer,. P. 134–9.

17. Zhou, J.Y., et al. (2016.)**,** Effect of light quality on the growth of tissue culture chlorophyll and chlorophyll fluorescence in Cunninghamia lanceolata. Chinese JTrop Crops., **37**(9):1759–65 (in Chinese).
18. Poudel, P.R., et al(2008).,Effect of red- and blue-light-emitting diodes on growth andmorphogenesis of grapes. Plant Cell Tiss Organ Cult.,. **92**:147–153.
19. Attaya, A.S., (2021). LED Light technology as a source of illumination and a promising method for Stevia rebaudiana elite propagation. *Egypt J. Agron*., 43: 123–132.
20. Mukhtar, F.B., et al., (2009) Effect of benzyl amino purine (BAP), coconut milk (CM) and manure applications on leaf senescence and yield in photoperiod sensitive cowpea variety (Kanannado). Afric. *J. Plant Sci.*,**.3(7):**142-146.
21. George, E.F., (2008)The Background. In: George, E.F.; Hall, M. A.& De Klerk, G. J., editors. Plant Tissue Culture Procedure – Background. Netherlands: Springer,**.** pp. 1–28.
22. Trivedi, A. and Sengar, R.S., (2017)**.** Effect of various light-emittimg diodes on growth and photosynthetic pigments of banana (Musa acuminata) CV. grande naine in vitro plantlets. International *Journal of Chemical Studies*, **5(5)**: 1819-1821.
23. Topchiy, N. M., et al., (2005). The effect of additional red irradiation on the photosynthetic apparatus of Pisum sativum. Photosynthetica, **43**(3):451–456.
24. Ma, Y.L., et al., (2021).Solvent effect on phenolics and antioxidant activity ofHuangshan Gongju (Dendranthema morifolium (Ramat) Tzvel. cv. Gongju) extract. Food Chem. Toxicol., **147**: 111875.
25. Li, H.M., et al. (2013)., Effects of different light resources on growth of upland cotton (Gossypium hirsutum L.) seedlings. Crop Sci Accept.
26. Ren, G.P., et al., (2016) Effect of LED in different light qualities on growth of Phalaenopsis plantlets. Chin. Bull. Bot.,. **51**: 81–88.
27. Kubica , P., et al., (2020) Verbena officinalis (Common Vervain) - A Review on the Investigations of This Medicinally Important Plant Species. Planta Med,. **86**(17):1241-1257.
28. Nacheva, L., et al., (2021) Effect of LED lighting on the growth of micropropagated pear plantlets (Pyrus communis L.). International Symposium on Light in Horticulture. Proceedings of the 5th Balkan Scientific Conference on Biology.,**.** pp: 1-8.
29. Ustin, S.L., et al., (2009) Retrieval of foliar information about plant pigment systems from high resolution spectroscopy. Remote Sensing of Environment,. **113**: S67-S77.
30. Yaroshevich, I.A., et al., (2015) Functional interpretation of the role of cyclic carotenoids in photosynthetic antennas via quantum chemical calculations. Computational and Theoretical Chemistry,. **1070**: 27-32.
31. Gill, S.S. and Tuteja, N., (2010). Reactive Oxygen Species and Antioxidant Machinery in Abiotic Stress Tolerance in Crop Plants. Plant Physiology and Biochemistry, **48**: 909-930.
32. Hogewoning, S.W., et al., (2010) Blue light dose-.responses of leaf photosynthesis, morphology, and chemical composition of Cucumis sativus grown under different combinations of red and blue *light. J. Exp.* Bot., **61**: 3107–3117.
33 Abouziada, L.M., et al. (2022), Secondary metabolites, hormonal homeostasis, and antioxidant enzymes of Moringa oleifera in response to white or violet in response to white or violet combined with cytokinins under tissue culture conditions.Notulae Botanicae Horti Agrobotanici Cluj-Napoca,.**50**(3):12879.
34 . Dimitrova, N., et al (2021)., Influence of biostimulators regoplant and charkor on growth and development of micropropagated pear plants at acclimatization stage. Proceedings of the 5th Balkan Scientific Conference on Biology,. pp: 17-24.
35. Chaves, M., et al (2020)., Influence of colorful light-emitting diodes on growth, biochemistry, and production of volatile

organic compounds in vitro of Lippia filifolia (Verbenaceae). *Journal of Photochemistry and Photobiology Biology*,. **212**: 112040.

36. Pawłowska, B., et al. (2018**),**Impact of LED light sources on morphogenesis and levels of photosynthetic pigments in Gerbera jamesonii grown in vitro. Hortic. Environ. Biotechnol.,**.59**: 115–123.
37. Ramı´rez-Mosqueda, M.A., et al. (2017)., The effect of light quality on growth and development of in vitro plantlet of Stevia rebaudiana Bertoni. Sugat Tech., **19**(3): 331-336.
38. Batista, D.S., et al., (2016) Light quality affects in vitro growth and essential oil profile in Lippia alba (Verbenaceae). In Vitro Cell Dev Biol—Plant,. **52**:276–282.
39 Dutta Gupta, S. and Karmakar, A., (2017) Machine vision based evaluation of impact of light emitting diodes (LEDs) on shoot regeneration and the effect of spectral quality on phenolic content and antioxidant capacity in Swertia chirata, J*ournal of photochemistry and photobiology B,*.**174**: 162-172.
40. YU, L., et al., (2020) Effects of light-emitting diodes on tissue culture plantlets and seedlings of rice (Oryza sativa L.). *Journal of Integrative Agriculture.*, **19**(7): 1743–1754.
41. Liu, Y., et al., (2006) Leaf chlorophyll readings as an indicator for spinach yield and nutritional quality with different nitrogen fertilizer applications. *J Plant Nutr*.,. **29**: 1207–1217.
42. Dewir, Y.H., et al.,The(2006). effects of paclobutrazol, light emitting diodes (LEDs) and sucrose on flowering of Euphorbia millii plantlets invitro. *Eur J Hortic Sci*., **71**(6):240–244.
43. Moon, H.K., et al.**,** (2006). Growth of Tsuru-rindo (Tripterospermum japonicum) cultured in vitro under various sources of light-emitting diode (LED) irradiation. *J. Plant Biol.*, **49**: 174–179.
44. Shin, K.S., et al., (2008)The effect of light quality on the growth and development of in vitro plants Act breaks and rearrangements Doritaenopsis. Plant Physiology,**.30**: 339–343.
45. Li, H., et al., (2010)Effect of light-emitting diodes on growth and morphogenesis of upland cotton (Gossypium hirsutum L.) plantlets in vitro. Plant Cell Tiss Organ Cult.,. **103**:155–163.
46. Mengxi, L., et al. (2011),Effects of different spectral lights on Oncidium PLBs induction, proliferation, and plant regeneration. Plant Cell Tiss Organ Cult.,. **106**:1–10.
47. Maluta, F.A., et al. (2013),In vitro culture of sugarcane exposed to different lightsources. Pesqui Agropecu Bras.,. **48**(9):1303–1307.
48. Hung, C.D., et al. (2016),In vitro proliferation and ex vitro rooting of microshoots of commercially important rabbiteye blueberry (Vaccinium ashei Reade) using spectral lights. Sci Hortic., a. **211**:248–254.
49. Hung, C.D., et al. (2016)b,LED light for in vitro and ex vitro efficient growth of economically important highbush blueberry (Vaccinium corymbosum L.). Acta Physiol Plant.,. **38**:152.
50. Vieira, L.D.N., et al., (2015) Light-emitting diodes (LED) increase the stomata formation and chlorophyll content in Musa acuminata (AAA) 'Nanicão Corupá' in vitro plantlets. Theor Exp Plant Physiol.,. **27**(2):91–98.
51 . Manivannan, A., et al., (2015).Blue LED light enhances growth, phytochemical contents, and antioxidant enzyme activities of Rehmannia glutinosa cultured in vitro. Hortic Environ Biotechnol., **56**(1):105–113.
52. Bello-Bello, J.J., et al (2016).,Effect of LED light quality on in vitro shoot proliferation and growth of vanilla (Vanilla planifolia Andrews). *Afr J Biotechnol.*,. **15**(8):272–277.
53. Silva, M.M.A., et al., (2016)The effect of spectral light quality on in vitro culture of sugarcane. Acta Sci Biol Sci.,. **38** (2):157–161.
54 . Liu, M., et al. (2014**),**Evaluation of leaf morphology, structure and biochemical substance of balloon flower (Platycodon grandiflorum (Jacq.) A. DC.) plantlets in

vitro under different light spectra. Scientia Horticulturae,**. 174**(1):112–118.
55. Srivastava, S., et al. (2017),TDZ-induced plant regeneration in Brassica oleracea L. var. botrytis: effect of antioxidative enzyme activity and genetic stability in regenerated plantlets. In Vitro Cell.Dev.Biol.—Plant,. **53**:598–605.
56. Mamdouh, D., et al. (2021),Genetic stability, phenolic, flavonoid, ferulic acid contents, and Antioxidant Activity of Micropropagated Lycium schweinfurthii Plants. Plants,. **10**: 2089.
57 .Moyer, R.A., et al. (2002)., Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. *J. Agr. Food Chem.*, **50**: 519–525.
58 . Chang, H., et al. (2009).,Effects of light quality on microtuber induction of Solanum tuberosum. *Chinese J. Appl. Ecol.*, **20**(**8**):1891–1895.
59. Johkan, M., et al. (2010), Blue light-emitting diode light irradiation of seedlings improves seedling quality and growth after transplanting in red leaf lettuce. Hort science,. **45**(12):1809-1814.
60. Li, Y., et al., (2018).Variation in leaf chlorophyll concentration from tropical to cold-temperate forests: association with gross primary productivity. Ecol. Indic., **85**: 383–389.
61. Amitrano C., et al. (2018),Light fertilization affects growth and photosynthesis in mung bean (Vigna radiata) *plants. J. Environ. Account. Manage*.,). **6**: 295-304.
62 .Schulze, P.S.C., et al., (2016). Effect of light quality supplied by light emitting diodes (LEDs) on growth and biochemical profiles of Nannochloropsis oculata and Tetraselmis chuii. *Algal research journal*, **16**: 387-398.
63. Elsabaa, N.A., et al., (2022) Response of potato growth in vitro to LED spectral exchange. *J. of Plant Production, Mansoura Univ.*,. **13** (7):281 – 286.
64. (F/FRDP) (1992) Forestry/Fuelwood Research and development Project, Growing Multipurpose Trees on Snlall Farms. Bangkok, Thailand. Winrock. International.
65. Mehwish, H.M., et al. (2021).,Therapeutic potential of Moringa oleifera seed polysaccharide embedded silver nanoparticles in wound healing. *International journal of biological macromolecules*, **184**: 144-158.
66. Xiong, Y., et al**.,** (2021) Virucidal activity of Moringa A from Moringa oleifera seeds against Inuenza A Viruses by regulating TFEB. International Immunopharmacology,. **95**: 107561.
67. Daghaghele, S., et al., (2021)Intensication of Extraction of Antioxidant Compounds from Moringa Oleifera Leaves Using Ultrasound-Assisted Approach: BBD-RSMDesign. *International Journal of Fruit Science*,.**21**(1): 693-705.
68. Basra, S.M.A., et al., (2015) Biomass production and nutritional composition of Moringa oleifera under different cutting frequencies and planting spacings. *Int. J. Agric. Biol*.,. 17.