

The Effect of Different Light Spectrum Ratios and Photosynthetic Photon Flux Density (PPFD) on Some Agronomic and Physiological Traits in *Artemisia annua* L.

Mahtab Namdaran Gooran, Saeid Jalali Honarmand* and Danial Kahrizi

Department of Plant Production and Genetics, Faculty of Science and Agricultural Engineering, Razi University, Kermanshah, Iran

Article History

Received: 05 April 2021
Accepted: 10 June 2021
© 2012 Iranian Society of Medicinal Plants.
All rights reserved.

Keywords

Artemisia
Full-spectrum
Light quality
Ultraviolet
Wavelength

ABSTRACT

In order to investigate the effect of the ratio of different light spectra on the growth and physiological traits of the medicinal plant *Artemisia annua* L., an experiment was conducted based on a randomized complete block design with three replications. Experimental treatments were six levels of different light wavelengths including control (base light: full spectrum), base light + ultraviolet spectrum, base light+blue light spectrum, base light + green light spectrum, base light + red light spectrum and base light + far-red light spectrum. The results showed that different light ratios influenced all the measured traits. Accordingly, the application of all light treatments significantly increased the dry weight of the *Artemisia* plant. Also, the light treatments had significant ($P \leq 0.01$) effects on plants height, contents of chlorophyll a and b, total chlorophyll, carotenoids and anthocyanin. The percentage and yield of plant essential oils were impressed significantly ($P \leq 0.01$) under the application of different light wavelengths. Although UV increased the content of the essential oil, it reduced the yield of the essential oil due to the reduction in the dry weight of plants. According to the results of this study, it can be concluded that a combination of base light with blue light can increase the biomass yield as well as percentage and yield of essential oils of *Artemisia*, compared to control.

INTRODUCTION

Artemisia annua L. is an important medicinal plant, belongs to the family Asteraceae and native to China. *Artemisia* is widely interested in its artemisinin content, a secondary metabolite that is considered to be an effective drug in the treatment of malaria, even against drug-resistant strains such as *Plasmodium falciparum*. Moreover, the anti-tumor effects of artemisinin, as well as its role in the treatment of infectious diseases such as schistosomiasis, leishmaniasis, and hepatitis B, have been reported in several studies [1]. Artemisinin has the potential to treat a wide range of cancers, including breast cancer, leukemia, colon cancer, and lung carcinomas [2]. The low concentration of artemisinin (0.01% to 1% of the plant dry weight) [3] makes it relatively expensive and it is difficult to respond to the high demand for it (more than 100 million artemisinin-based

combination therapies courses per year) [4]. Various approaches including the chemical synthesis [5] and genetic engineering of the pathway genes involved in artemisinin biosynthesis in *A. annua* [6,7] have been attempted to increase artemisinin production but none have been successful because of high cost and complexity. So, extraction of artemisinin from plant tissues is currently the most economical strategy.

Among various environmental factors, light is one of the most important variables that affecting the photomorphogenesis and photosynthesis of plants [8]. Light is also an essential energy source for plant photosynthesis and an important signal for plant growth and development. Changes in light quality have a profound effect on plant growth, especially in photosynthesis [9]. Diets containing fresh or processed herbs have a positive relationship with preventing cardiovascular diseases, chronic diseases,

*Corresponding author: Department of Plant Production and Genetics, Faculty of Science and Agricultural Engineering, Razi University, Kermanshah, Iran
Email Address: sjhonarmand@yahoo.com

and certain types of cancer, due to high concentrations of phytonutrients such as essential oils, phenolic compounds, flavonoids, and carotenoids [10,11]. The secondary metabolites are organic compounds that are not directly involved in the primary metabolic processes of the growth, development, or reproduction of plants. They play an important role in plant defense against insects and pathogens, act as attractants to pollinators and seed dispersers in reproductive processes, and some may create a competitive advantage as poisons for rival species [12]. Additionally, these metabolites have been used in various industries such as medicines, flavorings, dyes, fibers, glues, oils, waxes, and perfumes [13].

Chlorophyll is the material base of plant photosynthesis. Its concentration and composition directly influence the photosynthetic rate of the leaves. Chlorophyll is synthesized via a complex biosynthetic pathway: glutamate is converted to 5-aminolevulinic acid (ALA), protoporphyrin IX (Proto IX) is biosynthesized, magnesium is inserted into Proto IX to form Mg-proto IX, proto chlorophyll (Pchl_{id}) is turned into Pchl_{id} a, and chlorophyll_{id} a is esterified into chlorophyll a [14]. Chlorophyll biosynthesis requires light [15]. The formation of photosynthetic pigment is controlled by different light qualities. At the same time, different photosynthetic pigments absorb different light spectrum. Blue light is generally considered to be beneficial for the formation of Chl a. Higher Chl a/b ratios are observed under blue light and lower Chl a/b ratios under red light [16]. Blue light improves gene expression of MgCH, GluTR and FeCH which regulates the synthesis of chlorophyll [17] and promotes chlorophyll synthesis [18,19]. Red light is not conducive to the formation of chlorophyll, because of the reduction in tetrapyrrole precursor 5-aminolevulinic acid [20]. Therefore, this study aimed to investigate the effect of different ratios of light spectra on the agronomic, morphological, and physiological characteristics of the Artemisia plant.

MATERIAL AND METHODS

This research was conducted in 2017 based on a randomized complete block design with three replications on the Artemisia plant in the research laboratory of Agricultural and Natural Resources

Campus, Razi University. Initially, an optical shelf in 3 floors and 2 rows including 6 compartments was made and in all floors, the full spectrum of light was installed in the same way using a fluorescent lamp of the Narva brand and a Power LED lamp.

Experimental treatments in each of the classes included: 1- Control (base light or a full spectrum of light with a wavelength of 400-840 nm and 107 $\mu\text{mol}/\text{m}^2/\text{s}$ photosynthetic photon flux density (PPFD) and irradiance 213 mW/m^2 at a maximum wavelength of 657 nm and combining 16% blue light, 9% green light, 63% red light and 12% far-red), 2- Combining base light and ultraviolet light spectrum with a maximum wavelength of 365 nm and 51 mW/m^2 energy, 3- Combination of base light and blue light spectrum with a maximum wavelength of 447 nm and 103 $\mu\text{mol}/\text{m}^2/\text{s}$ PPFD, 4- Combination of base light and green light spectrum with a maximum wavelength of 520 nm and 25 $\mu\text{mol}/\text{m}^2/\text{s}$ PPFD, 5- Combination of base light and red light spectrum with a maximum wavelength of 659 nm and 120 $\mu\text{mol}/\text{m}^2/\text{s}$ PPFD and 6- Base light and far-red light spectrum with a maximum wavelength of 739 nm and 106 $\mu\text{mol}/\text{m}^2/\text{s}$ PPFD (Figure 1). Spectra and the whole data mentioned above were measured using a UPRtek PG100N spectrometer.

Artemisia seeds were prepared from the National Center for Genetic and Biological Resources of Iran and planted in pots with 10 cm in diameter and 12 cm in height for each light treatment in three replications. The pots were filled with a soil mixture of sand, field soil, and manure (1:1:1). To obtain the desired density (5 plants per pot), at first 7 seeds were planted in each pot and after the emergence, additional plants were weeded. The duration of the experiment was 3 months and under photoperiod conditions, 16 hours of light and 8 hours of darkness and air temperature (day/night) were as 25/20 for all treatments. Irrigation was also done twice a week. The plants were harvested before flowering and when they were in the vegetative phase. The studied agronomic and morphological traits were including plant height, number of lateral branches, fresh and dry weight of the aerial part of the plant. In order to measure dry weight, the plants were cut from the soil surface and dried in the shade three months after growth. Then their weight was expressed per unit area of the pot.

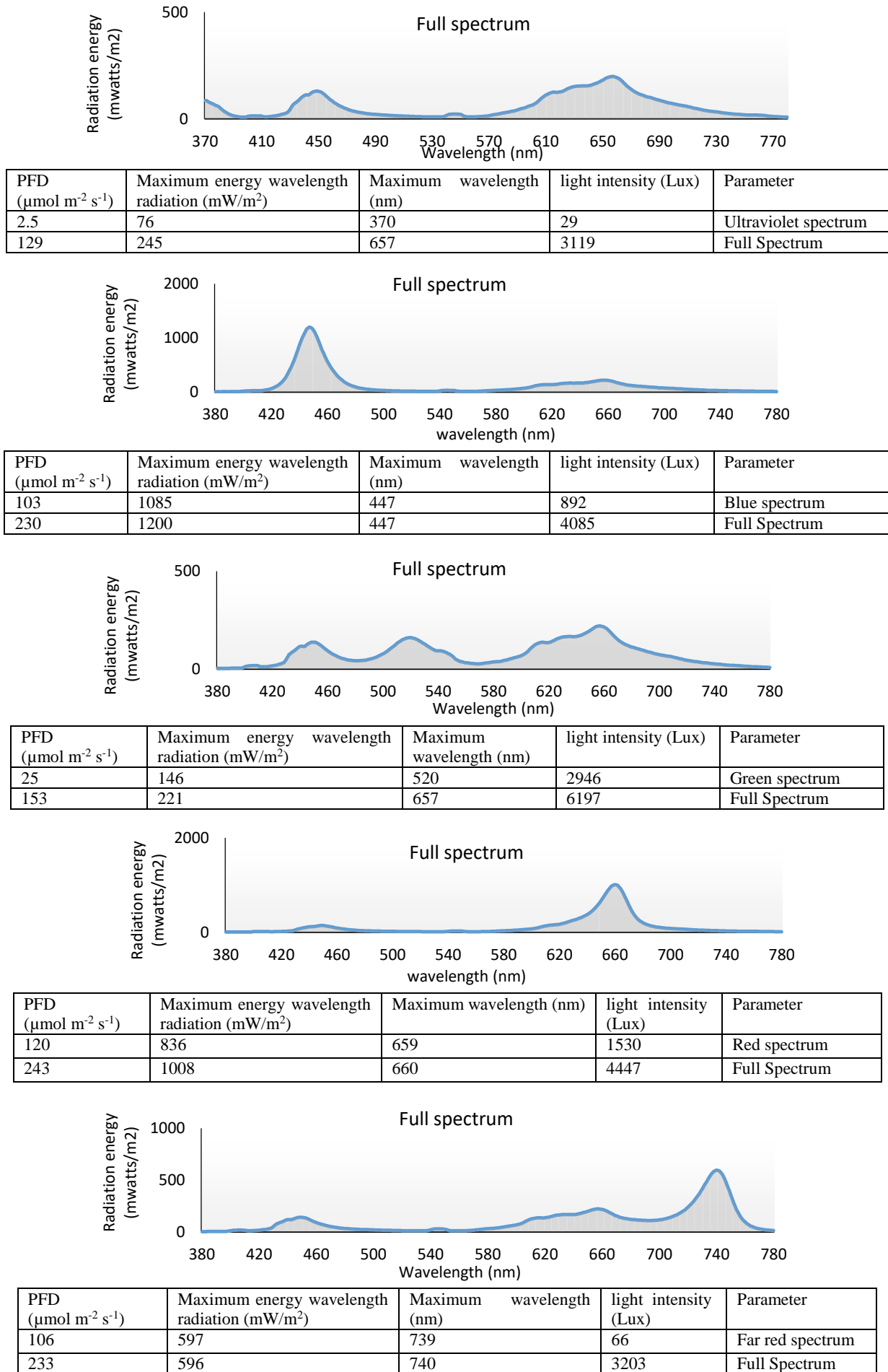


Fig. 1 Optical spectrum of experimental treatments.

Physiological traits including the content of chlorophyll and carotenoids were measured according to Arnon (1967) at wavelengths of 663 nm for chlorophyll a, 645 nm for chlorophyll b and 470 nm for carotenoids by ELISA (BioTek, Powerwave, XS2) and then quantified. Anthocyanin content was determined according to Nadernejad *et al.* (2013) [21]. The essential oil was extracted by hydrodistillation. First, 50 g of the shadow dried shoot with 500 ml of distilled water has been placed in a Clevenger balloon and after the mixture reached the boiling point, the essential oil was extracted for three hours. Then the percentage of essential oil was recorded and finally, the yield of essential oil was calculated based on the yield of biomass.

The data were analyzed after checking for normality with SAS software version 4.9. The main effects were compared using Fisher's least significant difference (LSD) test.

RESULTS

The results of the analysis of variance of different traits showed that in most traits there is a significant difference compared with control treatment (Table 1). The effect of light on the height of the *Artemisia* plant had a significant difference ($P \leq 0.01$) compared with the control treatment. The highest height of the *Artemisia* plant (33.77 cm) was related to far-red light treatment (Fig. 2). The light treatments had a significant ($P \leq 0.01$) effect on plant dry weight (Table 1). Blue light treatment (8.926 g) without significant difference with far-red treatment had the highest value and control treatment (6.47 g) without significant difference with green, red and ultraviolet treatments had the lowest value (Fig. 3). In this study, far-red light increased the stem height and subsequently increased plant dry weight compared to the control treatment. The number of lateral branches significantly ($P \leq 0.01$) affected under light treatments (Table 1). The highest number of lateral branches was obtained from red light treatment and the lowest amount of mentioned trait was obtained from far-red light (Fig. 4). According to the results of the analysis of variance, the content of chlorophyll a, chlorophyll b and total chlorophyll were significantly ($P \leq 0.01$) affected by light treatments. The highest amount of chlorophyll a was related to blue light

treatment (6.323 mg/g fresh leaf weight) and the lowest amount of this trait was related to green light treatment (2.543 mg/g fresh leaf weight) (Fig. 5). Also, the highest amount of chlorophyll b was related to blue light treatment (4.753 mg/g fresh leaf weight) and the lowest amount of this trait was related to green light treatment (2.174 mg/g fresh leaf weight) (Figure 6). The highest and lowest total chlorophyll content were related to blue light treatment (11.067 mg/g fresh leaf weight) and green light treatment (4.717 mg/g fresh leaf weight), respectively (Fig. 7). There were significant ($P \leq 0.01$) differences between light treatments in the content of anthocyanins and carotenoids. So the highest amounts of these traits were related to the blue light (12.451 and 0.561 mg/g, respectively) and the lowest contents were related to green light treatment (8.623 and 0.385 mg/g fresh leaf weight) (Fig. 8 and 9, respectively). The percentage and yield of essential oils significantly ($P \leq 0.01$) affected under the application of light treatments (Table 2). The highest percentage of essential oil (0.28%) was related to blue light without significant difference with UV and green light and the lowest percentage of essential oil (0.20%) was related to control treatment (Fig. 10). Also, the yield of *Artemisia* essential oil yield was significantly ($P \leq 0.01$) affected by light treatments (Table 2). The highest essential oil yield (1.146 ml/m²) was related to blue light and the lowest yield of essential oil (0.64 ml / m²) was related to the control (Fig. 11).

DISCUSSION

According to the results of this study, the increase in height due to the treatment of far-red spectrum and subsequently the increase in dry weight of the stem increased the dry weight of the medicinal plant *Artemisia*. The effect of blue light on vegetative growth, especially in forage crops and medicinal plants, has been proven, so by changing the ratio of different wavelengths of light in controlled conditions, greenhouse and vertical agriculture, and especially by increasing the ratio of blue light and ultraviolet light in UVA range can affect the quantity and quality of plant essential oils. In a study on two cultivars of basil (*Ocimum basilicum* L.), light treatments with a high red/far-red ratio with a low growth rate led to a decrease in stem height.

Table 1 Analysis of variance of the effect of light treatments on morphophysiological traits of *A. annua* L.

SOV	df	MS							
		Height	Number of side branches	Dry weight	Clorophyll a	Clorophyll b	Total Clorophyll	Anthocyanins	Cartonoid
Replication	2	6.13	0.38	1.60	0.09	0.26	0.08	0.49	0.001
Treatment	5	179.62**	2.05**	2.96**	4.99**	2.47**	13.58**	6.25**	0.01*
Error	10	3.78	0.65	0.50	0.11	0.20	0.45	0.25	0.004
CV	-	12.83	12.01	19.32	7.22	14.60	8.55	8.97	14.27

Table 2 Analysis of variance of the effect of light treatments on the percentage and yield of *A. annua* L. essential oil

SOV	df	MS	
		Percentage of essential oils	Essential oil function
Replication	2	0.0008	0.059
Treatment	5	0.004**	0.16**
Error	10	0.005	0.018
CV	-	10.12	15.39

* and **: significant at 5% and 1% probability levels, respectively.

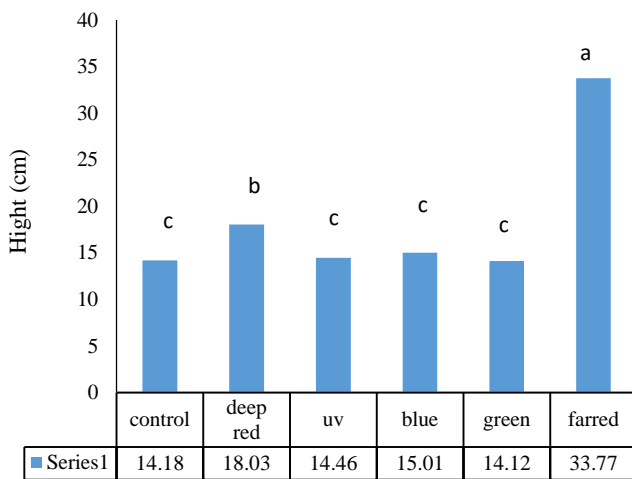


Fig. 2 The effect of light treatments on *A. annua* L. plant height.

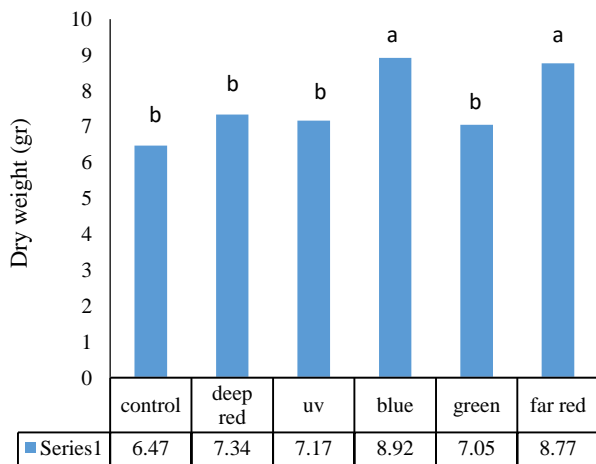


Fig. 3 The effect of light treatments on *A. annua* L. plant dry weight

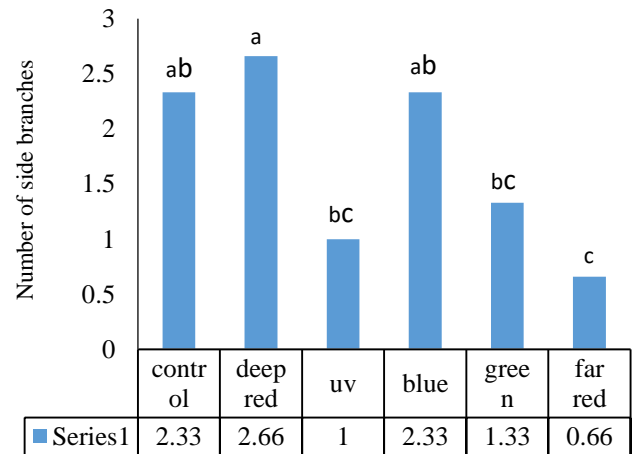


Fig. 4 The effect of light treatments on *A. annua* L. plant number of side branches.

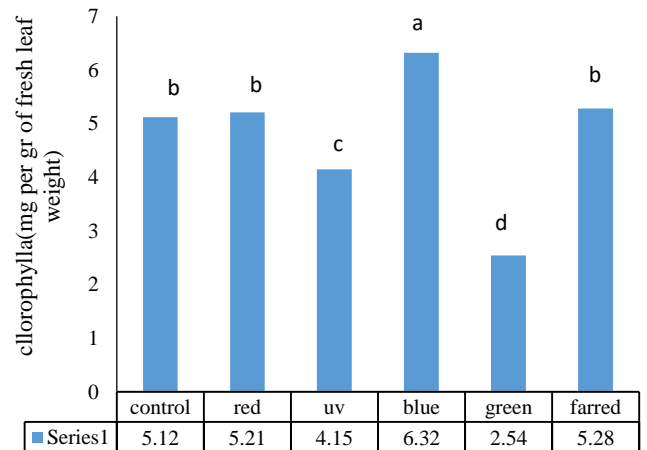


Fig. 5 The effect of light treatments on *A. annua* L. plant chlorophyll.

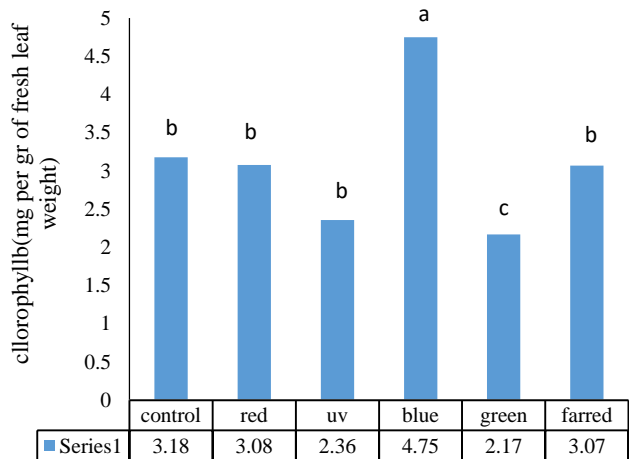


Fig. 6 The effect of light treatments on *A. annua* L. plant chlorophyll

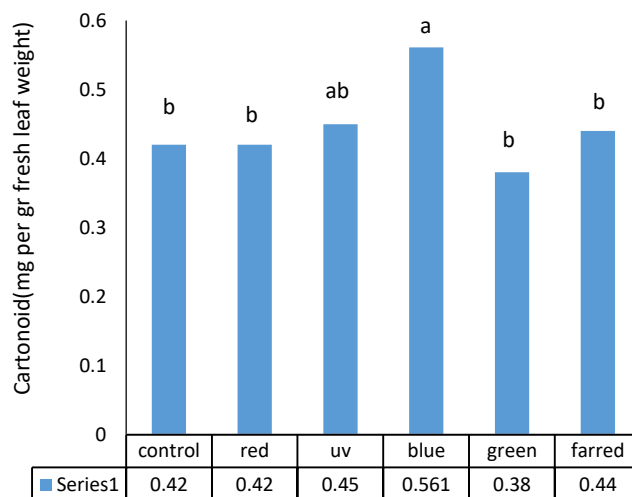


Fig. 9 The effect of light treatments on *A. annua* L. plant carotenoid.

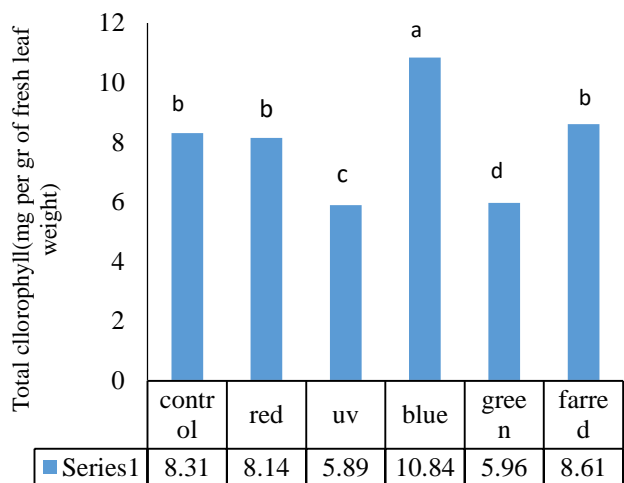


Fig. 7 The effect of light treatments on *A. annua* L. plant total chlorophyll.

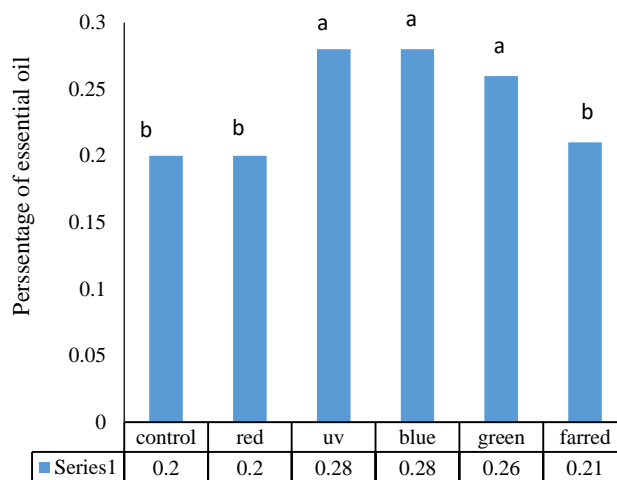


Fig. 10 The effect of light treatments on *A. annua* L. plant percentage of essential oil.

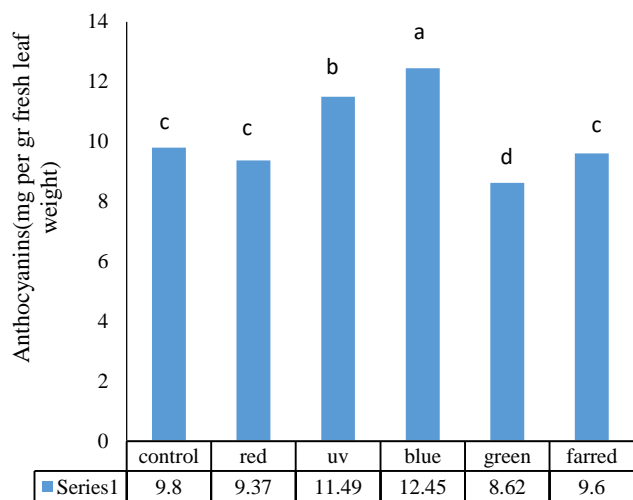


Fig. 8 The effect of light treatments on *A. annua* L. plant anthocyanins.

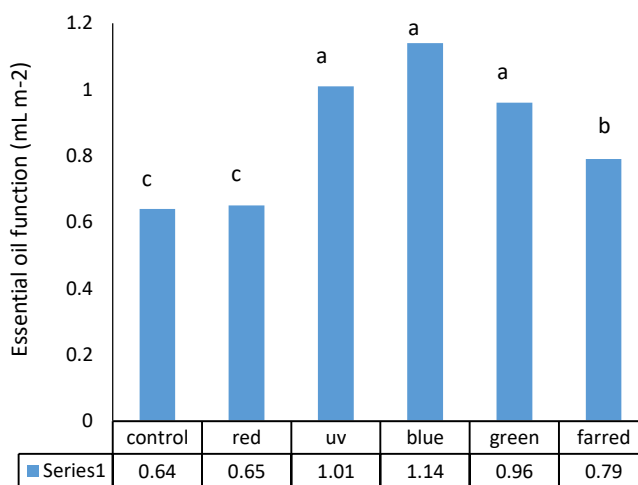


Fig. 11 The effect of light treatments on *A. annua* L. plant essential oil function

While in light treatment with a low red/far-red ratio, low circumference for both basil cultivars led to an increase in stem height [22]. In another experiment, the effect of far-red and blue light on the development, length of stem and root of basil, was observed that the maximum stem length obtained in far-red light treatment. The increase in stem length was due to increased internal gibberellin on the plant due to light treatment. Gibberellin affects the division of mitosis in the terminal meristem, and also increased the length and number of cells [23]. In this experiment, similar results were obtained in relation to an increase in plant height under the application of red light treatment. Also, blue light as a signal controls the action of opening and closing the stomata and increasing the aperture conductivity by increasing the density and size of the stomata [24,25]. In an experiment, the effects of white, green, blue, yellow light spectra and dark on the anatomy and chemical composition of Amla (Indian grape) were investigated. According to the results, the best morphophysiological reactions including root weight, stem height, number of leaves, number and height of branches were observed in blue light treatment [26]. In the present study, the highest content of chlorophyll, carotenoids and anthocyanins was obtained under the application of the blue light treatment, compared to other wavelengths in the PAR range, which is consistent with previous research [25]. In another experiment, blue light increased the chlorophyll a/b ratio and increased the activity of the enzymes rubisco and phosphoenolpyruvate carboxylase, in addition to the effect on the opening of stomata. The stomata modify the photosynthesis of each unit of leaf area and increase the photosynthetic efficiency [27]. Chlorophyll content increases under blue light [28]. Light stimulates the expression of genes involved in the chlorophyll biosynthesis pathway or inhibits genes that encode chlorophyll-degrading enzymes, and causes the accumulation of chlorophyll in leaves. Blue light increases the concentration of anthocyanins, carotenoids and chlorophyll, in comparison with the control (fluorescent white light) [29]. Light can affect the synthesis of carotenoids by affecting the genes encoding the biosynthesis pathway of geranyl pyrophosphate. Carotenoids are involved in the defense systems that gradually replace the anthocyanin defense system of young leaves as the leaf matures [30]. One of the reasons for the increase

in anthocyanin content is that the biosynthesis of anthocyanins requires enzymes whose expression is regulated by the quality of light so that blue light affects the synthesis of anthocyanins by stimulating the Phenylpropanoide pathway [31]. In an experiment on *Brassica campestris* L., blue light treatment with a wavelength of 440-470 nm and an intensity of 80 $\mu\text{mol}/\text{m}^2/\text{s}$ was the highest, which led to an increase in anthocyanins content [27]. It was reported that UV radiation had a variety of effects on the production of secondary metabolites, including increases in the concentration of phenolic compounds, isoprenes and terpenoids [32]. In aromatic plants, the biosynthesis of terpenoids is significantly affected both quantitatively and qualitatively by growth regulators [24]. The biosynthesis of terpenoids is based on primary metabolisms such as photosynthesis and the oxidative pathway for carbon stabilization [33]. Therefore, the production of these compounds may increase as plant growth conditions improve and initial metabolism increases. However, this possibility contradicts hypotheses of growth-differentiation balance and carbon-nutrient balance regarding the accumulation of secondary metabolites in the plant [34]. In this case, several studies have reported improved plant development along with increased production of secondary compounds, especially essential oils, under light treatments. Therefore, increasing biomass production as a result of increasing primary metabolism can also increase essential oil yield. It can be seen that the yield of essential oil in *Artemisia* increased under the influence of biomass and the percentage of essential oil. Finally, according to the obtained results, it can be stated that blue and UV light with a significant difference compared to other treatments and especially compared to the control, has increased the essential oil yield. As a result, the production of *Artemisia* can be increased by using the mentioned treatments under controlled conditions.

REFERENCES

1. Weathers P.J., Elkholy S., Wobbe K.K. The biosynthetic pathway and its regulation in *Artemisia annua*, a terpenoid rich species, *In Vitro Cell. Dev. Biol.: Plant.* 2006;42:309-317.
2. Mannan A., Ahmed I., Arsad W., Asim M., FQureshi R.A., Hussain I., Mirza B. Survey of Artemisinin production by diverse *Artemisia* species in northern Pakistan, *Malar.* 2010;3099:310.

3. Liu C., Zhao Y., Wang Y. Artemisinin: current state and perspectives for Biotechnological production of an antimalarial drug, *Appl. Microbiol. Biotechnol.* 2006;72:11-20.
4. Mutabingwa T.K. Artemisinin-based combination therapies (ACTs): best hope for malaria treatment but inaccessible to the needy, *Acta Trop.* 2005;95:305-315.
5. Avery M.A., Chong W.K., Jennings M., White C. Stereoselective total synthesis of (+)-artemisinin, the antimalarial constituent of *Artemisia annua* L. *J Am. Chem. Soc.* 1992;114:974-979.
6. Chen D.H., Ye H.C., Li G.F. Expression of a chimeric farnesyl diphosphate synthase gene in *Artemisia annua* L. transgenic plants via *Agrobacterium tumefaciens*-mediated transformation, *Plant Sci.* 2000;155:179-185.
7. Ro D.K., Paradise E.M., Ouellet M., Fisher K.J., Newman K.L., Ndungu J.M., Ho K.A., Eachus R.A., Ham T.S., Kirby J., Chang M.C.Y., Withers S.T., Shiba Y., Sarpong R., Keasling J.D. Production of the antimalarial drug precursor artemisinic acid in engineered yeast, *Nature.* 2006;440:940-943.
8. Avercheva O.V., Berkovich Y.A., Erokhin A.N., Zhigalova T.V., Pogosyan S.I., Smolyanina S.O. Growth and photosynthesis of Chinese cabbage plants grown under light-emitting diode-based light source. *Russ J Plant Physiol.* 2009;56:14-21.
9. Fukuda N., Fujitan M., Ohta Y., Sase S., Nishimura S., Ezura H. Directional blue light irradiation triggers epidermal cell elongation of abaxial side resulting in inhibition of leaf epinasty in geranium under red light condition. *Sci Hortic.* 2008;115:176-182.
10. Nishioka N., Nishimura T., Ohya K., Sumino M., Malayeri S., Goto E., Inagaki N., Morota T. Light Quality Affected Growth and Contents of Essential Oil Components of Japanese Mint Plants; International Workshop on Greenhouse Environmental Control and Crop Production in Semi-Arid Regions: Tucson, AZ, USA; 2008; pp. 431-436.
11. Asami D.K., Hong Y.J., Barrett D.M., Mitchell A.E. Comparison of the total phenolic and ascorbic acid content of freeze-dried and air-dried marionberry, strawberry, and corn grown using conventional, organic, and sustainable agricultural practices. *J Agric Food Chem.* 2003;51:1237-1241.
12. Croteau R., Kutchan T.M., Lewis N.G. Natural products (secondary metabolites). *Biochem Mol Biol Plants.* 2000;24:1250-1319.
13. Croteau R., Kutchan T.M., Lewis N.G. Natural products (secondary metabolites). *Biochem. Mol Biol. Plants.* 2000;24:1250-1319.
14. Tanaka R., Tanaka A. Tetrapyrrole biosynthesis in higher plants. *Plant Biol.* 2007;58:321-346.
15. Hooper J.K., Eggink L.L. Assembly of light-harvesting complex II and biogenesis of thylakoid membranes in chloroplasts. *Photosynth Res.* 1999;61:197-215.
16. Marsac N.T., Houmard J. Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. *FEMS Microbiol Rev.* 1993;1:119-189.
17. Wang H., Gu M., Cui J.X., Shi K. Effects of light quality on CO₂ assimilation, chlorophyll-fluorescence quenching, expression of Calvin cycle genes and carbohydrate accumulation in *Cucumis sativus*. *J Photochem Photobiol B.* 2009;96:30-37.
18. Poudel P.R., Kataoka I., Mochioka R. Effect of red-and blue light- emitting diodes on growth and morphogenesis of grapes. *Plant Cell Tiss Org.* 2008;2:147-153.
19. Kurilcik A., Canova M.R., Dapkuniene S., Zilinskaite S., Kurilcik G. In vitro culture of *Chrysanthemum* plantlets using lightemitting diodes. *Cent Eur J Biol.* 2008;2:161-167.
20. Sood S., Gupta V., Tripathy B.C. Photoregulation of the greening process of wheat seedlings grown in red light. *Plant Mol Biol.* 2005;59:269-287.
21. Nadernejad N.A., Ahmadimoghdam J., Hossyinfard S Poorseyedi S. Study of the rootstock and cultivar effect in PAL activity, production of phenolic and flavonoid compounds on flower, leaf and fruit in Pistachio (*Pistacia vera* L.). *Iranian Journal of Plant Biology.* 2013;5:95-109.
22. Whitelam G., Halliday K. Light and Plant Development. Blackwell Publishing, Oxford, p.313. with UV-B, *J. Plant Physiol.* 2007;147:589-592.
23. Abinaya M., Prabhakaran S., Nur H., Chung H., Byoung R. Blue LED Light Enhances Growth, Phytochemical Contents, and Antioxidant Enzyme Activities of *Rehmannia glutinosa* Cultured in Vitro. *Environ. Biotechnol.* 2015;56:105-113.
24. Sakalauskien E.S., Samuolien G., Brazaityt A. Supplementary UV-B irradiation effects on basil (*Ocimum basilicum* L.) growth and phytochemical properties. *J. Food Agric. Environ.* 2012;10:342-346.
25. Boccalandro H.E., Giordano C.V., Ploschuk E.L., Piccoli P.N., Bottini R., Casal J.J. Phototropins but not cryptochromes mediate the blue light-specific promotion of stomatal conductance, while both enhance photosynthesis and transpiration under full sunlight. *Physio Plant.* 2012;158:1475-84.
26. Cristiane P.V., Marcos V., Leal-costa E., Schwart T., Tavares R., Machado K., Celso L. Light spectra affect the morphoanatomical and chemical features of clonal *Phyllanthus tenellus* Roxb grown in vitro. *Soc.* 2015;114:69-119.
27. Li H., Tang M., Xu M., Liu Z.G., Han X.Y. Effects of different light sources on the growth of non-heading Chinese cabbage (*Brassica campestris* L.). *J Agric Sci.* 2012;4:262-273.
28. Lillo C., Appenroth K.J. 2001. Light regulation of nitrite reductase in higher plants: which photoreceptors are involved? *Plant Biology.* 2012; 3: pp. 455-465.
29. Terfa M.T., Poudel M.S., Roro A.G., Gislerod H.R., Olsen J.E., Torre S. Light emitting diodes with a high proportion of blue light affects external and internal

- quality parameters of pot roses differently than the traditional high pressure sodium lamp. *Acta Hort.* 2012a;956:635-642.
30. Candan N., Tarhan L. Changes in chlorophyll-carotenoid contents, cytokinins. *Plant growth regulation.* 2003;32:359-567.
31. Kopsell D.A., Kopsell D.E., Lefsrud M.G., Curran-Celentano J., Dukach L.E. Variation in lutein, carotene, and chlorophyll concentrations among Brassica oleraceae cultivars and seasons. *HortScience.* 2004;39:361-364.
32. Mackerness S.A.H. Plant responses to ultraviolet-B (UV-B: 280-320 nm) stress: What are the key regulators?. *Plant Growth Regul.* 2000;32:27-39.
33. Singh N., Luthra R., Sangwan R. Oxidative pathways and essential oil tissue-specific expression of an anionic peroxidase in zucchini. *Plant Physiology.* 1990b;26:2129-2136.
34. Coley P.D. Costs and benefits of defense by tannins in a neotropical tree complex patterns of terpenoid gene expression. *Biotechnol Bioeng.* 1986;83:653-667.