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EFFECT OF Low Level Laser (LLL) ON GROWTH, YIELD AND ESSENTIAL OIL QUALITY AND QUANTITY OF *ARTEMISIA ANNUA* PLANT

Hany H.Abdelhamid [1], Gamal Mahmoud Ghazal[2], Tarek Abd-Allah El-Tayeb[3]

1. National Organization for Drug Control and Research (NODCAR)- Giza - Egypt
2. Applied research center of medicinal plants , National Organization for Drug ontrol and esearch (NODCAR)- Giza – Egypt
3. National Institute of Laser Enhanced Sciences (NILES), Photobiology and photochemistry -Cairo University- Egypt

Abstract
The high demand global for food and drugs is the goal for which studies and research conducted to get safe food and medicine, so the laser light is considered a clean technology because it produces eco-friendly plants and significantly reduces using fertilizers and chemicals. *Artemisia annua* is one of the most important medicinal crops. The current study set out to investigate the effect of using the low-level laser (LLL) on growth, yield and essential oil, nutrients and primary metabolites of Artemisia *Annua* as carbohydrates and photosynthesis pigments. Pre-sowing laser irradiation of seeds results in a significant increase in vegetative parameters and active constituents, particularly with the He-Ne laser at a 10-minute exposure time, where plant height increased to 51.94 % and branch number increased to 54%. Exposure with the Blue diode laser led to an increase in total carbohydrates to 50.37% over untreated seeds, indicating nutrients were significantly improved by He-Ne laser, including calcium, which increased up to 76.35%. Physiological and biochemical changes occurred in the plant due to photochemical reactions between the laser beam and photoreceptors in the seeds of *Artemisia annua.*

Key words; *Artemisia annua*, Laser Irradiation, helium-neon laser, diode laser, growth, yield, Plant height, N.P.K, . Calcium, Carbohydrate, Essential oil yield, photochemical reaction.

1. INTRODUCTION

Artemisia annua is a well-known member of the Asteraceae family. The Asteraceae family is one of the largest flowering plant families, with nearly 23,000 species distributed across 1,500 genera [1]. Artemisia *Annua* is a Chinese annual herb that grows naturally as part of the steppe vegetation in the northern parts of Chatar and Suiyan provinces at elevations of 1,000–1,500 meters above sea level. Artemisia *Annua,* also known as "Qinghai," has a long history in Chinese traditional medicine, then in Africa and Asia for treating fever and chills [2], making it one of the most medicinal plants. Consequently, numerous researches and studies have been conducted to collect medicinal properties that can treat various diseases [3].

Artemisia *Annua* is a plant that is rich in active ingredients and nutrients such as carbohydrates and minerals such as potassium, phosphorus, and calcium [4]. Besides, due to the essential oil found in the plant and also the herbs, it is used to treat gastrointestinal helminthiasis, diarrhoea, and skin rashes and has a good therapeutic ability [5-7]. The whole plant has traditionally been used as an antimalarial, a food additive, and an anti-inflammatory [8]. Artemisia annua has attracted the attention of scientists and researchers due to the significant role and activity of artemisinin and essential oil in antimalarial and antiplasmodial efficacy while also having low toxicity [9]. Monoterpenoids, sesquiterpenoids, triterpenoids, flavonoids, alkaloids, steroids, and coumarins are found in plant tissues, particularly leaves, flowers, and fruits [10].

Calcium plays a critical role as an ion channel that facilitates signal transduction in plant cells via hormones. Several studies have revealed a strong relationship between photoreceptors such as phytochrome in the plasma membrane and growth hormones [11].

Numerous researchers focused their efforts on isolating and studying the components of Artemisia oil, revealing that the oil contains significant amounts of α-pinene, camphor, 1,8-cineol, and Artemisia ketone [12]. Essential oils can be described as either products or blends of aromatic substances, which were characterized as pure chemical compounds, and it is becoming volatile if found in natural conditions. [13].

Compared to chemical enhancement, using lasers in agriculture is a low-cost and environmentally friendly physical tool for increasing and enhancing agricultural production [14, 15]. Laser treatment of plant seed has been shown to have several benefits for agricultural production and the enhancement of plant active constituents [16-20].

The diode laser and the helium-neon laser are regarded as the most important types of lasers for the biostimulation of plants [21-23].

*Corresponding author e-mail: hanyh8945@gmail.com (Hany H Abdelhamid Badr)
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Osman et al. [24] investigated the effect of laser helium-neon (He-Ne) light at a wavelength of 632.8 nm on dry seeds of anise and cumin plants before sowing at various exposure times. Their findings indicated that laser light improved and increased production while also increasing the volatile oil content from 3.87 % to 9.1% after 20 minutes of exposure compared to untreated seeds. Using lasers on plant seeds improved all properties at all exposure periods. Abo Rekab et al**.** [25] investigated that the effect of diode laser treatments on palm. Metwally et al. [17] studied the positive impact of three types of visible light laser Helium-neon (He-Ne), cadmium neon (Cd-Ne) and Argon (Ar) on photosynthetic pigments of Balanites *aegyptiaca* plantlets compared to untreated plants. Aftab and Younus [16] demonstrated that red laser treatment significantly affected the biochemical processes in wheat seeds and the plant's dry and fresh weight.

2. MATERIALS AND METHODS

Location and Duration:

This work was conducted over two consecutive seasons: 2016–2017 and 2017–2018, at the Medicinal Plants, Applied Research Center Farm (ARCMP), National Organization for Drug Control And Research (NODCAR), Giza, Egypt. *Artemisia annua* L. Seeds were obtained from the experimental Faculty of Pharmacy farm, Cairo University, Giza, Egypt.

2.1. Laser Irradiation:

For field cultivation, seeds were classified into four major groups, and each contained 19.5 g seeds. The four groups were subdivided in the same manner. The first group served as a control, while the other three were exposed to three different types of lasers (e.g., He-Ne (632.8 nm, 23 mW), Diode Blue (405 nm, 78 mW), and Diode Green (517 nm, 32 mW)). Each laser-irradiated group was subdivided into four additional subgroups based on the laser exposure duration (5, 10, 15 and 20 minutes). Each sub-group contained 1.5 g seeds for each laser exposure time. The power densities for all used lasers were adjusted nearly to be 92 mW/ Cm^2 by changing the laser spot diameter using different diverging lenses. [26].

The first seeds were sown on foam trays (1m*1m for the area) on 15th February 2016 and 2017 (respectively) inside a greenhouse in a bed of sand loamy soil and Patmos (2:1), where they germinated after 10-15 days. They were two months after sowing the seeds (on 15th April in both seasons, 2016 and 2017), at the height of 15-20 cm for the seedlings.

Data Recorded:

2.2. Vegetative Parameters:

Three replicates were randomly taken to determine vegetative growth parameters; the following data were recorded in each season:

Plant height (cm)., dry herb yield/feddan (ton). The herb was dried at 88 °C until it reached a constant weight [27]. The dry weights of herb feddan were calculated by multiplying the fresh and dry weights/plant by the number of plants/feddan.

Chemical analysis of internal components

2.2.1. Total chlorophyll content: Chlorophyll contents were all determined calorimetrically in fresh leaf samples (mg/g fresh matter), according to Saric et al. [28].

2.2.2. Determination of N, P and K elements:

Nitrogen content (N) was determined as (N%) by the modified micro-Kjeldahl method, as previously described by FAO [29]. Regarding phosphorus as (P%), it was estimated using the Chapman and Parker technique [30]. Potassium (K) was determined as (K%) using a flam-photometer (Jeneway SN: 20158051101 Japan), according to Brown and Lilleland's procedure [31].

2.2.4. Determination of Calcium:

Calcium concentration was determined as (Ca%) in herb dry weight using an atomic absorption spectrophotometer; Perkin Elmer Analyst 400 (SN: 20158051101), USA.

2.2.5. Extraction of Essential Oil.

The percentage of essential oil in the plant was determined by hydrodistilling dry herb for 3 hours using a Clevenger-type apparatus (Boro G - India), according to the Egyptian Pharmacopoeia method [32]. The obtained essential oil was dried over anhydrous sodium sulfate to determine the oil percentage and then filtered.

The essential oil of Artemisia *Annua* was analyzed utilizing gas-liquid chromatography (GLC) using an Agilent Technologies GC Model: 6890 Chromatograph equipped with a flame ionization detection (FID) detector and an HP5column, Model G1530A -USA, according to Rohloff's guidelines [33]. The constituents of the essential oil of Artemisia *Annua* were identified by comparing their relative and absolute retention times with those of authentic standards. The essential oil composition was reported as a relative percentage of the total peak area.

2.3. Experimental Design and Statistical Analysis.

Treatments were arranged in a randomized complete block design (RCBD) with three replicates of each treatment. Each block date was subjected to standard analysis of variance (ANOVA) to determine the values of the least significant difference (L.S.D.). According to Sendecor and Cochran [34]**,** data were statistically analyzed using Statistical Package for the Social Sciences (MINITAB 16) software. The method of Duncan's multiple range tests was applied to the comparison between means, according to Waller and Duncan [35].

3.RESULTS

3.1.Vegetative Parameters:

3.1.1.-Plant height

According to the results in **Table 1**, irradiation of He-Ne, Diode Blue, and Diode Green lasers significantly affected plant height during the two seasons of the experiment ($p \leq 0.05$). He-Ne laser treatment resulted in the tallest plants among the different treatments, measuring 198.133 and 211 cm in the first and second seasons, respectively. All treatments increased plant height significantly compared to the control, in which the plant heights measured 146.83 and 157 cm in the first and second seasons, respectively. In terms of the effect of laser exposure time, the tallest plants were obtained at 5 minutes for all treatments, measuring 201.6 and 215.25 cm in the first and second seasons, respectively. Concerning the interaction between type of laser (Blue, Green, and Red) and laser exposure time, the He-Ne laser exposure at 630 nm for 10 minutes resulted in maximum values of 282.67 and 303 cm for plant heights in the first and second seasons, respectively, compared to the other combinations. On the other hand, the shortest plants were found in laser Green (530 nm) at 20 minutes, measuring

141.33 and 145.67 cm in the first and second seasons, respectively.

3.1.2. Dry herb yield/feddan:

As detailed in Table (3), The results clearly confirmed the extent of the significant differences. It was found that the highest values of the average herb dry weight yield obtained by He-Ne laser treatment were 8.95 and 9.15 Ton/fed for the 1st and 2nd season, respectively when compared these results to the others types of laser and D. Green laser treatment was the lowest (6.72, 6.92 Ton/fed) respectively, It was noticeable that 5 minute was the best time exposure led to an increase in herb dry weight yield for both seasons were recorded (9.34 and 9.6 Ton/fed) respectively. At the same time, in contrast, increasing in time to 20 minutes caused to dropped of herb dry weight yield to 4.85 and 5.02(Ton/fed) for the first and second season. Regarding the interaction between the effects of varying laser and exposure periods of time In both seasons, the highest yield of dry herb weights by ton (with values of 14.98 and 15.26 Ton/fed for the first and second seasons, respectively) were those of plants exposed to He-Ne laser and 10 minute of time exposure, whereas the lowest dry herb weights (with values of 433.33 and 451.67 g/plant in first and second seasons, respectively). While the lowest values of yield (herb dry weights) were obtained with laser D. Green treatment for 20 minutes (3.73 & 3.95 Ton / Fadan) for both seasons.

3.2. Total Chlorophyll Content

The obtained results in a table (4) shows the effect of laser types and varying time exposure had a significant effect on total chlorophyll content (mg/g fresh matter) for the 1st and 2nd seasons. As observed, the highest values of the mean total chlorophyll (mg/g fresh matter), for both seasons, were obtained by He-Ne laser, were (4.02 and 4.2) mg/g fresh matter, respectively. In the case of ten minutes, gave the highest value of total chlorophyll (mg/g fresh matter) in the 1st and 2nd season cultivation seasons respectively, (4.111 and 4.27 mg/g fresh matter), followed by 5minute was achieved the second-highest (3.65 and 3.84 mg/g fresh matter) in the same respect order. The results were tabulated showed that the best result for total chlorophyll (mg/g fresh matter) of *Artemisia annua* was achieved by exposing plants to He-Ne laser for 10 minutes, was recorded (6.55 and 7 mg/g fresh matter) for both seasons flowed by D .Blue laser at 5minute (4.76 and 7mg/g fresh matter), One the other side laser D. Green has recorded the lowest value as well as gave a negative result when compared to all other treatments.

Table (1) Effect of different type of Laser and different exposure time during two seasons on plant height (cm.) for *Artemisia annua* L plant

Plant Height (cm.)										
First season						Second season				
Laser type						Laser type				
Exposure Time	Laser Control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630nm)	Mean	Laser Control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630nm)	Mean
0 min	146.83 h	146.83 h	146.83 h	146.83 h	146.83d	157 i	157 i	157 i	157 i	157d
5 min	146.83 h	238.33 b	181.33 d	231.67b	201.6a	157 i	252.33 b	206.33d	245.33c	215.25a
10 min	146.83 h	186.1 c	165.66ef	282.67a	195.34b	157 i	194 e	188.33ef	303a	210.58b
15 min	146.83 h	163.66 f	153.67 g	170.33e	158.62c	157 i	180.33 g	164. h	182.67fg	171.0c
20 min	146.83h	150 gh	141.33 i	154.67g	148.21d	157 i	162 hi	145.67 j	167 h	157.91d
Mean	146.83 d	177.1 b	157.76 c	198.13a		157 d	189.13b	172.26 c	211 a	
LSD Time					2.375	LSD Time				3.09
LSD Laser					2.13	LSD Laser				2.75
LSD Time *laser					4.75	LSD Time *laser				6.16

Mean that do not share a letter are significantly different- Data with different letters have significant difference (> LSD value)

Table (2) : The herb Dry weight Yield by (Ton/fed) *of Artemisia Annua* L plant with different type of Laser and different exposure time during two seasons.

The herb Dry weight Yield by (Ton/fed)										
First season						Second season				
Laser type						Laser type				
Exposure time	Laser control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630nm)	Mean	Laser control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630nm)	Mean
0 min	6.48h	6.48h	6.48h	6.48h	6.48c	6.61h	6.61h	6.61h	6.61h	6.61c
5 min	6.48h	9.96c	9.2d	11.71b	9.34a	6.61h	10.32c	9.49d	11.97b	9.6a
10 min	6.48h	7.27f	8.42e	14.98a	9.29b	6.61h	7.53f	8.62e	15.26a	9.51b
15 min	6.48h	5.93i	5.79j	6.83g	6.26d	6.61h	6.10i	5.92j	6.99g	6.41d
20 min	6.48h	4.41l	3.73m	4.77k	4.85e	6.61h	4.58l	3.95m	4.94k	5.02e
Mean	6.48d	6.81b	6.72c	8.95a		6.61d	7.03b	6.92c	9.15a	
LSD Time					0.02660.	LSD Time				0.0170
LSD Laser					0.0238	LSD Laser				0.0152
LSD Time *laser					0.0532	LSD Time *laser				0.0341

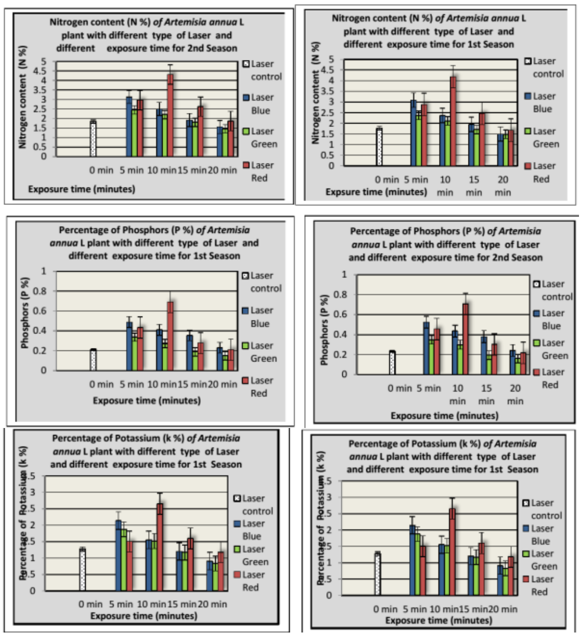
Mean that do not share a letter are significantly different- Data with different letters have significant difference (> LSD value)

Table (3): Total Chlorophyll (mg/g fresh matter) of *Artemisia Annua* L plant with different type of Laser and different exposure time during two seasons.

Total Chlorophyll											
First season						Second season					
Laser type						Laser type					
Exposure time	Laser control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630nm)	Mean	Laser control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630n m)	Mean	
0 min	2.81g	2.81g	2.81g	2.81g	2.81 d	2.71h	2.71h	2.71h	2.71h	2.71e	
5 min	2.81g	4.76 b	3.53 d	3.50 de	3.65b	2.71h	5 b	4.07 c	3.61 e	3.84b	
10 min	2.81g	3.48 de	3.60 d	6.55 a	4.111a	2.71h	3.6 e	3.77 d	7 a	4.27a	
15 min	2.81g	3.10 f	2.67h	3.87 c	3.114c	2.71h	3.2 f	2.81gh	4.2 c	3.23c	
20 min	2.81g	2.47 gh	2.35 i	3.39 e	2.82d	2.71h	2.9g	2.51 i	3.5 e	2.9d	
Mean	2.18 d	3.381 b	2.994 c	4.02 a		2.71d	3.48b	3.17 c	4.2 a		
LSD Time					0.06250	LSD Time				0.0695	
LSD Laser					. 0.0559	LSD Laser				0.0616	
LSD Time *laser					0.125	LSD Time *laser				0.0137	

Mean that do not share a letter are significantly different- Data with different letters have significant difference (> LSD value)

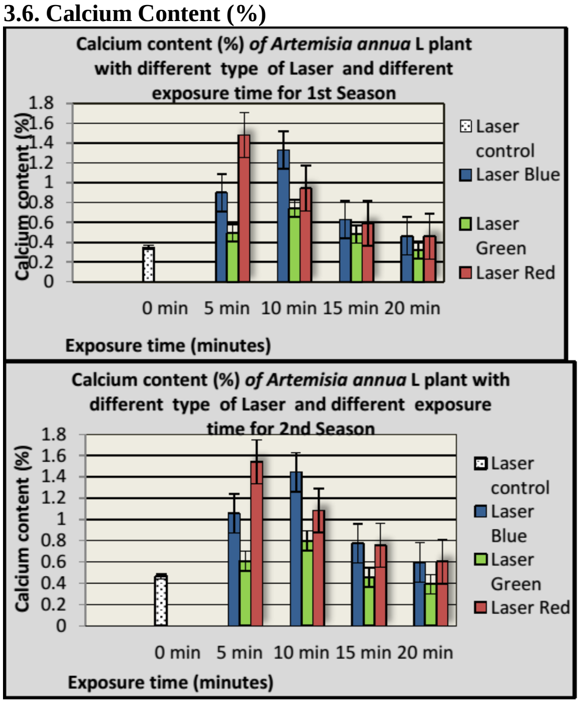
3.4.Nitrogen, Phosphorus and Potassium (NPK) content



Fig(1) NPK Nitrogene, Potassium and Phosphorus content (%) under the effect of different laser types, radiation and different time exposure during two seasons, Error bars represent the standard Error Values are mean of 3 experiments with ± SE. Statistical data determined by ANOVA followed by Duncan's multiple-range.

Data presented in **Fig (1,2 and 3).**The data reveal that laser radiation had a significant effect on N, P and K content (%) in all laser types when compared to the no-radiation control in both seasons studied. The He-Ne laser gave higher N, P and K content (%) when compared to the other of types. All exposure time treatments showed significant differences in NPK content percentage when compared to non-treated control. With respect to the interaction, the NPK content when exposed to He-Ne laser at 10 minutes gave the highest values: (4.186 and 4.31%) for N% and (0.692 and 0.707%) for the P% and the K% were (2.655 and 2.81 %) compared to the other combinations in 1st and 2nd season, respectively. Whereas, the lowest content of N,P and K percentage were found in the laser green at 20 minutes, the N% recoded (1.49 and 1.46%), P% (0.150 and 0.162%) and (0.83 and 0.9 %) for K% compared to control (1.77 and 1.84%), (0.213 and 0.233%) and (1.275 and 1.22%) for N,P and (0.234 and 0.253%) for K%, respectively in respect order by season..

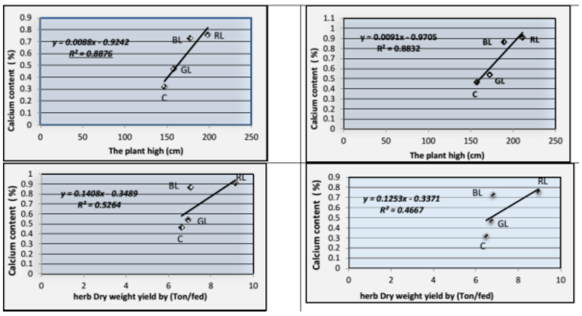
3.6. Calcium Content (%)



Fig(2): Calcium Content content (%) under effect of different laser types radiation and different time exposure during two seasons, Error bars represent the standard Error Values are mean of 3 experiments with ± SE. Statistical data determined by ANOVA followed by Duncan's multiple-range.

Data showed in **Fig (5)** reveal that a significant difference in a calcium (%) of *Artemisia annua* .It was found that the highest values of content calcium (%) obtained by He-Ne laser treatment were 0.759 and 0.912 (%) for the 1st and 2nd season, respectively, when compared these results to the others types of laser, it found that the diode green laser was the lowest values which recorded 0.472, 0.542 (%) respectively. It was noticed that 10 minutes was the best time exposure led to an increase in the calcium (%) for both seasons were recorded (0.835 and 0.958 %) respectively. On the other side, 20 minutes of exposure time showed decrease in calcium content (%) to 0.391 and 0.515(%) for the first season and second season respectively, so, twenty minutes was less pronounced comparably with control (0.32, 0.466 %) during two seasons. The highest values of calcium content (%) were 1.48 and 1.54 % in the first and second seasons, respectively obtained by exposing to He-Ne laser and10minute of time exposure while using diode green laser at 20 minutes gave the lowest values (with values of 0.323 and 0.392 %) in first and second seasons, respectively.

3.7. Relationship between Calcium content and the plant height



Fig(3): Calcium Content content (%) under effect of different laser types, radiation and different time exposure during two seasons

Fig (3) shows the relationship between Calcium (%) and plant high(cm) with laser light treatment during first and second season, which is explained by the Figure (6).The data indicated that the presence of a strong relationship expressed by the R- squared value on the chart, where (R2 =0.8876) and (R2 = 0.8832) for the first and second season respectively. The results obtained in the case the He-Ne laser effect confirmed that the plants which have the highest values of Calcium content by percentage (0.759 and 0.912%) have the tallest plants (198.133 and 211cm) during two successive seasons with respect to the effect of diode green laser, plants which have the lowest values of plant high (cm) where recorded 172.26 and 157.76 cm for both season Respectively contains the lowest values of calcium content as a percentage (0.472 and 0.542%) and vice versa for two axis in these relationship for both of two seasons respectively. Regression analysis between Calcium content (%) and plant high(cm) showed almost best appropriate. The correlation coefficient represents the harmonization extent between them. As presented, **the Fig (C and D)** shows the relationship between Percentage of Calcium (%) and herb Dry weight yield by (Ton/fed) with effect of three types of laser light during two successive seasons. It clearly shows that there is a correlation, but it is not strong, (R2 = 0.4667) for the first season and (R2 = 0.5264) As it presented that the highest value recorded for calcium content (0.759 and 0.912%) for first and second seasons, respectively led to the largest dry weight of herb (8.95 and 9.15 tons / fad) and vice versa during the first and second seasons respectively, and this correlation was recorded under the influence of Red laser. While in the presence of the green laser, the results showed that the low calcium content as percentage (0.472 and 0.542%) gave the lowest dry weight for the plant (6.72 and 6.92 tons / fad) and vice versa for the first and second seasons, respectively.

Table (4)Effect of different type of Laser and different exposure time during two seasons on essential oil Yield by Liter / fed of Artemisia *Annua L*

Essential oil yield										
First season						Second season				
Laser type						Laser type				
Exposure time	Laser control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630nm)	Mean	Laser control	Laser Blue (405 nm)	Laser Green (530nm)	Laser Red (630nm)	Mean
0 min	37.66i	37.66i	37.66i	37.66i	37.66e	41.14k	41.14k	41.14k	41.14k	41.14e
5 min	37.66i	63.27d	56.8f	79.56b	59.15b	41.14k	70.86e	61.50f	85.65b	64.79b
10 min	37.66i	52.54g	57.51e	119.5 a	66.80a	41.14k	56.56g	63.11f	124.92a	71.43a
15 min	37.66i	44.86h	44.85h	63.56d	47.73c	41.14k	48.06 i	50.06 h	78.59 c	54.46c
20 min	37.66i	37.43 i	21.27 j	65.45c	40.45d	41.14k	45.58 j	26.5 l	76.77d	47.53d
Mean	37.66d	47.15 b	43.48 c	73.15 a		41.14d	52.44b	48.46 c	81.41a	
LSD Time					0.707	LSD Time				0.8095
LSD Laser					0.632	LSD Laser				00.75
LSD Time *laser					1.414	LSD Time *laser				1.619

Mean that do not share a letter are significantly different- Data with different letters have significant difference (> LSD value)

• 3.9. Essential Oil Yield

Data tabulated in **Table (4)** reveal that a significant difference in essential oil yield by Liter /Fadan of *Artemisia Annua L* plant between various types of laser irradiations and different time exposure during the two seasons of experiment ($p \leq 0.05$). The highest values of essential oil Yield by (L / Feddan) obtained by He-Ne laser treatment were (73.15 and 81.41 L / Feddan) for the 1st and 2nd season, respectively, when compared to the others types of laser, while the diode green laser recorded the lowest values (43.48, 48.46 L/ Feddan) in the same respect order, respectively. It was noticed that 10 minutes was the best time exposure led to the maximum values of essential oil yield for both seasons (66.80 and 71.43 L/ Feddan respectively. On the other hand, 20 minutes of exposure time showed decreased to the lowest values in essential oil Yield (40.45 and 47 L/ Feddan) for both seasons. Regarding the multifactor effect resulted from laser types and exposure time reflected the significant effects during the two seasons. The highest values of the essential oil were assessed at 10 minutes for He-Ne laser (119.5 and 124.90) respectively in first and second seasons), while the lowest value were achieved at 20 minutes for laser D- Green obtained (21.27 and 26.5) in the same respect order.

3.10. Chemical Composition of Artemisia *Annua* essential oil:

Table (5).chemical constitunts by percentage (%) of Artemisia *Annua*,s essential oil at different laser tybes and diiferent time exposure .

Essential oil components	No.	control	Laser He-Ne (632nm Red)				Laser Diode (512nm Green)				Laser Diode (405nm Blue)			
			5 min	10 min	15 min	20 min	5 min	10 min	15 min	20 min	5 min	10 min	15 min	20 min
α-thujene	1	0.42	0.88	1.31	1.86	0.75	0.41	0.56	0.40	0.54	2.11	2.51	0.64	0.61
α -Pinene	2	2.00	3.70	3.20	3.26	3.93	1.54	1.64	0.82	0.40	3.71	4.20	1.43	1.42
Camphene	3	4.47	3.21	4.06	4.73	4.12	2.51	2.50	3.31	2.42	1.84	3.06	2.84	3.73
1,8 cineole	4	10.29	8.95	7.43	8.29	8.65	5.94	4.75	3.64	5.53	7.24	6.14	7.45	7.56
Artemisia ketone	5	8.98	15.45	18.36	15.34	12.53	12.46	11.85	7.41	8.30	9.40	8.75	9.54	7.96
Cis-Sabinene hydrate	6	3.20	3.67	4.33	4.30	3.73	4.56	4.35	7.31	6.34	6.49	6.00	5.50	6.63
Terpinolene	7	1.41	0.76	1.06	2.21	1.92	1.62	1.08	0.84	0.00	0.80	1.40	1.53	2.40
Trans-Sabinene hydrate	8	1.75	2.42	2.65	2.54	2.10	2.25	1.95	0.71	0.80	0.97	1.08	2.32	1.52
T rans β-ocimene	9	4.35	2.85	2.76	2.52	2.22	3.44	5.32	7.41	7.01	6.31	3.91	5.63	4.56
Champhor	10	24.43	19.85	19.97	18.24	19.19	23.52	25.20	20.42	21.44	30.42	32.39	27.34	27.02
Borneol	11	6.52	5.40	4.67	4.67	4.36	5.89	6.32	4.54	6.52	5.40	4.40	6.40	4.01
Myrtenal	12	5.21	5.92	5.97	5.55	7.40	5.40	5.43	5.42	7.20	6.31	5.27	4.95	5.80
Myrtenol	13	1.46	0.00	0.00	0.00	0.00	0.44	1.49	0.73	0.53	1.02	1.73	1.54	1.50
Trans-carveol	14	1.70	2.01	1.82	2.12	2.95	0.62	0.84	2.41	1.61	1.73	1.51	1.42	1.69
Cis-carveol	15	3.82	4.37	4.81	5.51	4.40	3.46	4.62	6.22	3.69	3.34	3.84	3.49	2.62
Eugenol	16	3.41	2.94	2.69	1.87	3.86	2.34	2.40	2.72	2.51	1.63	3.76	3.42	4.47
Benzyle 2-methyl butyrate	17	2.72	4.60	3.79	4.43	6.02	7.33	7.75	4.41	4.57	1.79	1.30	2.21	1.53
Tranc- Caryophyllene	18	8.46	8.64	6.27	7.70	5.73	7.72	6.23	8.65	7.84	6.22	5.44	5.72	7.43
β- farnesene	19	4.00	2.94	3.40	3.50	5.22	6.93	3.40	7.41	9.51	1.30	1.64	5.10	1.83
β- Selinene	20	0.75	0.67	0.70	0.78	0.71	1.73	2.03	3.62	2.72	1.27	1.75	1.75	5.79
Ledenoxid	21	0.66	0.96	0.80	0.64	0.61	0.87	0.79	2.27	1.17	0.95	0.42	0.00	0.00
		100	100	100	100	100	100	100	100	100	100	100	100	100

Result data tabulated in Table **(5)** reveal that The main constituent of the essential oil champhor was the predominant component recording (24.43%), followed by 1, 8 cineole which recorded (10.29%), then Artemisia ketone (8.98%) accompanied by trans- caryophyllene which amounted (8.46%).

Concerning the effect of laser type or the exposure time on the chemical constituents of essential oil, large fluctuations in essential oil components were existed due to the aforementioned treatments as *Artimisia* ketone recorded the highest value in Laser He-Ne (Red) at 10 minutes exposure time amounting (18.36%) compared to control (8.98%). While, Camphor showed its highest value in Laser Diode (512nm Green) at 10 minutes exposure time recording (32.39%) compared to control (24.43%).

4.Discussion

The role of laser light applications with low intensity as bio-stimulation if used on seeds has been recently discussed [38-41]. Unlike lasers, which are considered eco-friendly stimulators, other stimuli, such as chemical fertilizers, are harmful to both plants and humans due to the way they are transferred from plants and soil to humans [42, 43].

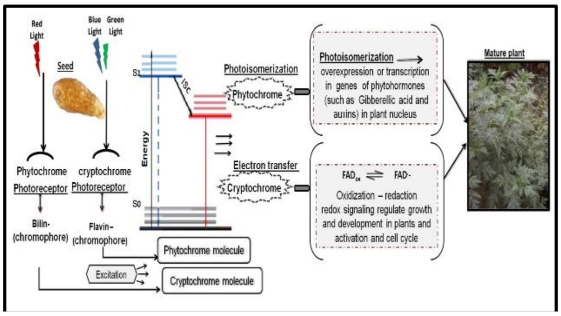
Numerous studies corroborated our current findings by demonstrating beneficial effects on growth parameters, branch number, total biomass, and crop yield.

Besides, it has been reported that He-Ne laser treatment before sowing can increase the interior energy of seeds, improve cotyledon enzymes, boost cell metabolism, and enhance cell division (mitosis), resulting in an increase in the length of the plant during early growth, the content of lipid peroxidation products, and cell metabolic changes [19, 20, 39].

In the present study, the modes of photochemical reaction are an important tool in understanding the role of light in the plant. There are several photoreceptors inside seed cells, and the phytochrome and cryptochrome are critical photosensory modules in the plant. Phytochromes are a type of photoreceptor that reacts to red and far-red light via a chromophore (bilin tetrapyrrole protein). On the molecular level, photoexcitation of phytochrome results in photoisomerization of the molecules, which results in overexpression or transcription of growth hormone genes in the plant nucleus (such as Gibberellic acid and auxins) [44, 45]. Consequently, significant advancements in large-scale transcriptional reprogramming in plant growth and development have been observed [46].

Cryptochromes are photoreceptors for blue light-absorbing flavoproteins such as FAD (flavin adenine dinucleotide), which act as cofactors for enzymes and as chromophores for biologically active signalling that is stimulated by blue light and reversed by green light [47, 48]. Photochemical reactions are characterized by photoconversion, which is driven by electron transfer between the reduction and oxidation cycles FAD_{ox}<-------> FAD^- [49, 50].

There are interactions between cellular redox signalling and phytohormone signalling, where cellular oxidation enhances plant growth regulation. Furthermore, the cycles of oxidation and reduction play a critical role in activating the cell cycle and triggering genetic and epigenetic control, which enhances plant growth and physiological development [51].



Fig(4) mechanism of photochemical reaction of light with photoreceptor in plant seeds and transformation of chemical energy action into the all parts of the plant

Regarding the effect of the three types of laser (Red, Blue and green) on parameters of photosynthetic pigments in the Sequoia sempervirens plant, it has been demonstrated that the effect of red laser light was more pronounced at 5 min and caused an increment in total chlorophyll to 407.8% compared to control, while green laser gave the highest value of carotenoids (232.46%) compared to control. These results match those obtained in the previous study [17].

According to the findings, an increase in the carbohydrates content of dry leaves of *Artemisia annua* was observed. Study reported that supplementary exposure to blue and red light resulted in the maximal accumulation of carbohydrates compounds [51]. It can be attributed to that the spectral energy allocation of red and blue light is nearly identical to the absorption spectrum of chlorophyll and xanthophyll, respectively.

In contrast to the mother plant or seedling, which contain chloroplasts, dry seeds contain etioplasts that converted to mature chloroplasts during germination to initiate photosynthesis [53].

In angiosperms, light is required to differentiate the etioplast into the chloroplast. Blue light enhances the expression of MgCH, GluTR, and FeCH genes, which are involved in chlorophyll synthesis [45]. A similarity between the effect of laser exposure on vegetative measurements and the content of nutrients such as nitrogen, phosphorus, and potassium was observed in the current study. Any increase in the concentration of these elements results in an increase in the number of nucleic acids and phospholipids in cell membranes and potassium in the cellular sap of the vacuole, which exerts dominance over the cells' osmotic pressures and electrical balance [54].

There is a good relationship between calcium signalling and auxin signalling. Thus, it has an influential role in regulating morphogenesis plant tissue formation [54].

The results have shown that red light has the most significant effect on calcium in plant tissues, which support previous ones [58].

The relationship between phytochrome and calcium content and the activation of calcium function in plants has been investigated, where phytochrome serves as a photoreceptor for the red light, and phytochrome acts as a carrier and facilitates an increase in calcium ions when exposed to red light [59, 60].

The relationship between calcium and vegetative characteristics in plants is due to the ability of calcium ions to transmit various cell signals, the most important of which are hormones and light signals [55]. Our findings indicate that 46.67% and 52.64% of dry herb weight (ton/fad) in the first and second seasons, respectively, are attributed to the calcium content. In contrast, the remainder of the ratio is attributed to other factors. According to the data in the relationship, calcium contributed 88.76 % of plant height (cm) in the first season and 88.32 % in the second season.

Calcium is required for growth and differentiation at all stages of plant development, from embryogenesis to reproduction (60-62). As a result, calcium and Ca^{2+} have been dubbed "the missing link in auxin action" [57]. The results indicated that the laser light improved the relationship between calcium content (Ca%) and plant height (cm).

The relationship between calcium content (Ca%) and dry herb weight is stronger and larger than the relationship between calcium content (Ca%) and herb dry weight expressed by the R-squared value on the chart, where R2 = 0.8876 and R2 = 0.8832 in the first and second seasons, respectively.

Calcium may act as a signalling molecule for various hormones, but auxins work at a higher rate than the other hormones, at 88.76% and 88.32% in the first and second seasons, respectively. Auxins are critical for cell elongation in plants [59]. Our findings may aid in the understanding of plant height increment.

According to Silva et al. [60], the significant increase in essential oil yield caused by light radiation was due to increased herb biomass production.

In terms of the effect of light quality (certain wavelengths) on essential oil compounds, our findings are consistent with those of a previous study, which reported that exposure to blue light increased the amount of sesquiterpene group compounds while red light enhanced the amount of monoterpene group compounds [60]. Low-level visible-spectrum laser light demonstrated a significant effect on the quality of essential oils. Blue light activated the metabolic pathway of the sesquiterpenes biosynthesis in plants.

The results obtained in this research study are analogous to those reported by Metwally et al., [17] on the similar three types of laser (Red, Blue and green) influence on parameters of photosynthetic pigments in Sequoia sempervirens plant, confirmed that the effect of red laser light was more pronounced at 5min and caused increment in total chlorophyll to 407.8% compared to control, while green laser gave the highest value of carotenoids (232.46% compared to control).

Our results in the present study showed that increment carbohydrates content in dry leaves of *Artemisia annua* plant, Study [2] reported that the supplementary exposure to blue light and red light drove to a maximal accumulation of carbohydrates compounds, this due to the spectral energy allocation of red and blue light almost corresponds with the absorption spectrum of chlorophyll and photosynthesis. As contrary to the mother plant or seedling which contains chloroplasts, dry seeds contain etioplasts which changed into mature chloroplasts to start photosynthesis once germination began [53].

Light is necessary for differentiation of etioplast to chloroplast in angiosperms**.** Blue light improves gene expression of MgCH, GluTR and FeCH which regulates the synthesis of chlorophyll[44.]

We notice from our results a similarity in the effect produced from laser exposure on the vegetative measurements and the content of nutrients such as nitrogen, phosphorus and potassium. Any increase in the content of these elements leads to an increase in nucleic acids and phospholipids in cell membranes and potassiumrole in the cellular sap of vacuole which dominance cells osmotic pressures and electrical balance. [53].

Muller et *al* ., [60] indicated that the significant increase in the yield of essential oil under effect of light radiation was due to increase herb biomass production.

Regarding our data on the effect of light quality (certain wavelengths) on essential oil compounds, Our results are in accordance with those obtained by Noguchi and Amaki [59], reported that exposure to blue light exhibited increment in amounts of sesquiterpene group compounds, and in the same time red light showed a raise amounts of monoterpene group compounds. Low-level laser light with visible spectrum showed an effect with significant differences in the quality of essential oil. Blue light activated the metabolic pathway of the sesquiterpenes biosynthesis in plants.

5-CONCLUSION

Our current study has shown the Artemisia annua plant, which is one of the most important medicinal plants, whose leaves and herbs contain many active ingredients, especially essential oil.

Laser technology could provide tremendous development in agriculture to improve morphological characteristics and improve productivity. Improvements in the quantity of products from the herb or the quantity and quality of the extracted volatile oil, when exposing plant seeds to laser radiation, our study's best results were by using He-Ne laser upon exposure for 10 minutes were in many vegetative characteristics, the plant's content of elements and nutrients. While the use of green diode laser improved carotenoids, and blue diode laser improved total carbohydrates also at 5 minutes. Moreover, 10 minutes, but sometimes a 20-minute exposure period, led to decreased results.

The study suggested that the explanation of the action of laser beams on the plant is due to the conversion and utilization of light energy that took place through the interaction between the light and the receptors in the plant. Low-Level Laser (LLL) can activate chemical, biological, and genetic pathways in the plant and transferring to the most of its tissues in all stages of its life.

6-REFERENCES

1. Bremer K, Anderberg A. Asteraceae: cladistics & classification (No. Sirsi) i9780881922752. 1994.
2. Feng X, Cao S, Qiu F, Zhang B. Traditional application and modern pharmacological research of Artemisia annua L. *Pharmacol. Ther*. 2020;216:107650.
3. Alesaeidi S, Miraj S. A systematic review of anti-malarial properties, immunosuppressive properties, anti-inflammatory properties, and anti-cancer properties of Artemisia annua. *Electron Physician*. 2016;8(10):3150.
4. Brisibe EA, Umoren UE, Brisibe F, Magalhäes PM, Ferreira JF, Luthria D, et al. Nutritional characterisation and antioxidant capacity of different tissues of Artemisia annua L. *Food Chem*. 2009;115(4):1240-6.
5. VERDIAN RM, SADAT EE, HAJI AA, Fazeli M, PIRALI HM. Chemical composition and antimicrobial activity of Artemisia annua L. essential oil from Iran. 2008.
6. Arab H, Mardjanmehr S, Shahbazfar A, Rassouli A, Abdollahi M, Nekouie O. Toxicopathologic effects of artemisinin in broiler chickens following a single oral dose: an LD50 study. *Int J Poultry Sci*. 2009;8(8):808-12.
7. Ferreira JF, Luthria DL, Sasaki T, Heyerick A. Flavonoids from Artemisia annua L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. *Molecules*. 2010;15(5):3135-70.
8. Graziose R, Ann Lila M, Raskin I. Merging traditional Chinese medicine with modern drug discovery technologies to find novel drugs and functional foods. *Curr Drug Discov Technol*. 2010;7(1):2-12.
9. Liu NQ, Schuehly W, von Freyhold M, van der Kooy F. A novel purification method of artemisinin from Artemisia annua. *Ind Crops Prod*. 2011;34(1):1084-8.
10. Snedecor GW, Cochran WG. Statistical methods. IOWA. Iowa State University Press Starkstein, SE, & Robinson, RG (1989) Affective disorders and cerebral vascular disease. *BJPsych*. 1980;154:170-82.

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