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A comparative studies on the effect of two types of laser on growth, chemical composition and biochemical genetic markerson *Tagetesofficinalis*plant

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Abstract

Background: Tagetes plant *Calendula officinalis,* (Marigold) was grown widely used as annual, perennial and herbaceous ornamentalplant belongs to the family of asteraceae. It is anusually economic plant species utilized in processed forms in modern medicinal industry. Phenolic compounds, carbohydrates, lipids, steroids, tocopherols, terpenoids, vitamin C, carotenoidsare the main constituents of Marigold.Laser rays have attained much attention at different parts of the world for improving growth and quality of plants. In this concern, Laser treatments can modify important components of plant cell and have been reported to affect differentially, the morphology, anatomy, biochemistry and physiology of plant depending on the source and time of laser exposure.

Results: The results indicated that plant height (cm), branches number/plant, stem diameter (mm), root length (cm), flowers number, plant fresh and dry weight, chlorophyll a, chlorophyll b, carotenoids, anthocyanine flowers contents, and electrical conductivity recorded significant increase at 20, 40 min. exposure time of helium-neon and cadmium laser rays. On the contrary, the previously mentioned treatments recorded decrement onanthocyanine stem contents, days from planting to flowering (earlying the flowering), and carbohydrates content. Moreover, the data of SDS PAGE demonstrated, and the effect of laser beam increased proportionally with laser exposure time.

Conclusions: From results it could be concluded that, the high exposure time (40 min.) of laser beam can be used to enhancing growth parameters, chemical constituents and exchange gene expiration which enhances a new protein formulation may provide a better alternative of petals.

Keywords: *Tagets Sp*; Growth; Laser Rays; Chemical Constituents; SDS-PAGE; Gene Expiration

1. Introduction

Tagetes plant *Calendula officinalis,* frequently style as marigold is a rickety of obscurity inconspicuous. Marigold was grown as an ornamental plant widely used as annual, perennial and herbaceous plant belongs to the family of asteraceae. Cultivation of marigold is easy due to its wide adaptability to various soils and climatic conditions. It is anusually economic plant species utilized in raw or in processed forms in modern medicinal industry, worldwide. In addition to the edible uses (i.e. coloring and flavoring agent of food). The main constituents of *Calendula officinalis*are including phenolic compounds, carbohydrates, lipids, steroids, tocopherols, terpenoids, vitamin C, carotenoids, and quinines [1], [2]. Carotenoids extracted from dry petals are used for poultry feeds to improve egg yolk color Broiler's skin [3], [4]. It has medical importance as blood refiner, anti-inflammatory, skin antifungal, blood sugar reduces, and antiviral properties [5], [6].

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It also used as a trap crop for controlling different insects such as tomato fruit borers. Oil extracted from marigold is used in manufacturing perfumes and insect repellents [7].

Laser rays have attained much attention at different parts of the world for improving growth and quality of plants. In this concern, Laser treatments can modify important components of plant cell and have been reported to affect differentially, the morphology, anatomy, biochemistry and physiology of plant depending on the source and time of laser exposure.[8], [9] On *Celosia argenta and Caster bean* reported that significant increasing in plant growth. Also they reported that laser rays could be useful to induce variation in plant improvement. Helium neon (He-Ne) laser rays improved and gains plants resistance to harsh conditions. The laser stimulation of seeds causes the absorption and storage of light energy by plant cells and tissues. A similar process takes place in seeds, which transform the absorbed light energy into chemical energy and then store it. Irradiation with He-Ne laser light increases the energy potential of seeds, increasing the germination capacity and strengthening the plant development against stress conditions [10]. Sodium dodecyl sulphatepolyacrylamide gel electrophoresis (SDS-PAGE) is the widely used biochemical technique for germplasm genetic structure analysis. Protein profiling is important in plant abiotic stress studies, whereas it provides useful information on protein and measuring their stress-dependent change in quantity and activity [11], [12]. Previous studies showed that laser influenced plant growth and metabolism. Whereas, the soluble protein contents in the tagetes seedling were enhanced after using laser irradiated [13], [14]. Similarly, the biochemical characters of the *Isatisindogotica*seedlings, e.g. the concentration of soluble protein and the activities of functional proteins were increased significantly by the laser pretreated [15].

The aim of this study to investigate the effect of two types of laser on growth, chemical composition, genetic on *Tagetes officinalis* plant.

2. Methods

The study was carried out at the greenhouse of National Research Centre, Dokki, and Cairo, Egypt during seasons 2019-2020, to investigate the response of vegetative growth and chemical parameters of Tagetes plants under irradiation conditions of helium cadmium (He-Cd and He-Cd) laser. For cultivation, pots 30 cm in diameter and 30 in depth were filled with a loamy sandy soil (2:1 by volume) the physical and chemical characteristics of the soil are show in Table (1).Nitrogen and potassium fertilizers were added to the soil according to the recommended dose of Ministry of Agriculture after three months from planting.The experiment consisted of four for each kind of laser treatments included the control. Helium cadmium laser were used for exposing seedlings (10 cm length) at the wave length of blue laser (460) and red laser (650 nm) and output power60 and103 Mw/cm^2.Seedlings plantation was in two seasons on February 2019 and 2020 after treated with helium cadmium and helium neon laser, whereas the exposure times were (0, 20, 30 and 40 min.) for two types of laser. After four months from planting, representative plant sample was taken from three replicates randomly. The growth and flower parameters included plant height (cm), leaves number/plant, plant length (cm), root length (m), fresh and dry weight of leaves, number of days to flowering and other parameters were recorded. Leaves samples were dried at 70 c^o and another samples of fresh leaves were collected for determine some chemical constituents. Determination of pigments content (mg/g F.W.) (Chlorophylla, b and Carotenoids) was carried out according to the method described by [16] anthocyanin analysis according to [17] carbohydrate (mg/g D.W.)Was carried out according to the method described by [18] protein (mg/ml) was carried outaccording to [19], Morphological data were subjected to a statistical analysis according to [20] and the means were compared. The design of the expirments was a Complete Randomized Design. The following experiment was designed to obtain details about the response of Tagetes plants two laser types under different exposure time.

Table 1 Soil chemical analysis

Analyses type		**Soluble Kations /ppm**				**Soluble Inions /ppm**			
PH	EC	Ca	Mg	Na	K	CO_3	HCO_3	Cl	SO_4
7.64	0.93	3.5	1.5	3.6	0.8	-	0.9	4.8	3.7

2.1. SDS-Protein electrophoresis in leaves

Fractionation electrophoresis was performed under identical conditions on sodium dedocylsulphate polyacrylamide gel (SDS-PAGE) (12%W/V) vertical slab using BIORAD Techware 1.5 mm according to the method of [21]as modified by [22]**.** The molecular weights of proteins were estimated relative to marker, a wide range molecular weight protein

(Fermentas com.).Gels were photographed scanned, analyzed using Gel Doc VilberLourmat system to capture the image and to calculate band intensities. The design of the experiments was a Complete Randomized Design.

Figure 1 Tagates seedlings and blue and red laser rays

3. Results

3.1. Effect of laser types

Table 2 The effect of color laser on vegetative growth and flowering of Tagetes plant. (Means of two seasons)

Treatments	Characteristics							
	Plant height	Branch number	Stem diameter (cm)	Root length (cm)	Fresh weight leaves (g)	Dry weight of leaves (g)	Flower No.	Number of days to flower
Blue laser	22.5	20.83	0.91	9.54	21.07	4.48	12.08	50.75
Red laser	19.4	10.41	0.88	7.75	14.63	3.11	11.33	50.85
LSDat0.5%	0.851	0.776	0.065	0.72	0.78	0.166	0.91	0.74

Table 3 The effect of color laser on chemical constituents ofTagetes plant. (Means of two seasons)

Treatments	Characteristics							
	Chl. a (Mg/g.f.w.)	Chl b (Mg/g.f.w.)	Carotenoids (Mg/g.f.w.)	Flower Anthocyanin content (Mg/g.f.w.)	Leaves Anthocyanin Content (Mg/g.f.w.)	Carbohydrate Content (Mg/g.D.W.)	Electric. conductivity	Protein (mg/ml)
Blue laser	1.06	0.375	1.85	0.24	0.170	22.15	83.007	0.660
Red laser	1.19	0.425	1.97	0.71	0.197	22.96	8.960	0.860
LSDat0.5%	0.006	0.006	0.003	0.009	0.004	0.07	0.074	0.007

Datain Table (2 and 3) showed that, blue laser recorded increased significant most growth parameters (plant height, branch number, stem diameter, root length, flower number and fresh and dry weight of leaves), compared with red color laser. Whereas both of two colors achieved the same value on the day from planting to flower. On the contrary red color laser recorded significant increment in all of chemical constituents of Tagetes plant compared to blue light laser treatments (Table 3).

Table 4 The effect of times exposure of laser on vegetative growth and flowering ofTagetes plant. (Means of two seasons

Treatments	Characteristics								
	Plant Height	Branch Number	Stem diameter (cm)	Root length (cm)	Fresh weight of leaves (g)	Dry weight of leaves (g)	Flower No.	Number of days to flower (days)	Protein (mg/ml)
0 min.	16.33	10, 66	0.685	5.50	15.40	3.25	7.83	59.83	0.543
20min.	22.66	14.83	1.010	9.16	22.53	4.80	14.66	42.66	0.688
30min.	22.16	18.50	0.948	9.91	23.00	4.90	9.83	49.33	0.777
40min.	22.66	18.50	0.975	10.00	23.36	4.98	14.50	50.83	1.052
LSDat0.5%	1.20	1.09	0.097	1.03	1.56	0.328	1.29	1.05	0.001

3.2. Effect of time exposure

We can conclude from Table (4 and 5) in general indicated that, all exposure times of laser treatments (20, 30 and 40 min.) recorded increment in vegetative growth parameters compared with zero min (control). Laser exposure time. Treated plants with 40 min. exposure time recorded the highest values on vegetative growth parameters, followed by 30 min. laser exposure time in most cases compared with 20 min. laser and o min. laser exposure time (control).

Table 5 The effect of times exposure of laser on chemical constituents of Tagetes plant. (Means of two seasons)

Treatments	Characteristics							
	Chl.a (Mg/g.f.w.)	Chl b (Mg/g.f.w.)	Carotenoids (Mg/g.f.w.)	Flower Anthocyanin content (Mg/g.f.w.)	Leaves Anthocyanin Content (Mg/g.f.w.)	Carbohydrate Content (Mg/g.D.W.)	Electric conductivity	Protein (mg/ml)
0 min.	0.80	0.272	1.31	0.181	0.236	41.04 1	7.83	0.543
20min.	1.53	0.538	2.62	0.665	0.190	15.65	114.29	0.688
30min.	0.86	0.297	1.52	0.488	0.163	18.42	104.60	0.777
40min.	1.32	0.493	2.19	0.545	0.146	15.11	97.22	1.052
LSDat0.5%	0.009	0.009	0.004	0.014	0.006	0.103	0.105	0.001

The previous mentioned exposure times treatments achieved increased in chemical constituents of Tagets plants in comparison with 0 min. exposure time. Data in table (5) also showed the response of chemical constituents to laser application, treated plants with 20 min. recorded the highest values on (chlorophyll a, chlorophyll b, carotenoids, flowers anthocyanine content and electrical conductivity) of Tagetes plants and followed by 40 min. exposure time compared with 30 and 0 min. exposure time. On the other hand the previous mentioned treatments recorded decrements in carbohydrates and anthocyanine content of leaves compared with 0 min. exposure time (control).

Table 6 The interaction between color laser and laser times exposure on vegetative growth and flowering of Tagetes plant. (Means of two seasons)

Treatments	Characteristics							
	Plant Height	Branch Number	Stem diameter	Root length	Fresh weight	Dry weight of leaves	Flower No	Number of days

			(cm)	(cm)	of leaves (g)	(g)		to flower (days)
He-Cd 0 min.	17.00	15.66	0.716	6.00	15.40	7.66	3.25	57.66
He-Cd20min.	23.33	21.66	0.99	9.33	22.53	12.33	4.80	43.33
He-Cd30min.	25.66	25.00	0.79	12.83	23.00	11.33	4.90	51.66
He-Cd40min.	24.00	21.00	1.00	10.00	23.36	17.00	4.98	49.66
He-Ne0min.	15.66	5.66	0.65	5.00	10.75	8.00	2.29	62.00
He-Ne 20min.	22.00	8.00	1.02	9.00	16.03	17.00	3.42	42.00
He-Ne 30min.	18.66	12.00	0.92	7.00	11.46	8.33	2.43	47.00
He-Ne 40min	21.33	16.00	0.95	10.00	20.26	12.00	4.32	52.00
LSDat0.5%	1.70	1.55	0.555	1.45	1.57	1.83	1.24	1.49

3.3. Effect of interaction between types of laser and time exposure

Figure 2 different laser treatments compared with control

Data in Table (6) cleared that, the interaction between laser type (blue and red) and exposure times (20, 30 and 40 min.) recorded significant increase in almost cases of vegetative growth parameters compared with two color of laser interacted with 0 min (control).The interaction between (blue laser + 30 min. exposure) recorded the highest values on plant height (25.65 cm), branches number (25) root length (12.83 cm) while, the interaction between blue laser + 40 min. achieved increased in previous mentionedparameters and recorded the highest values in stem diameter (1, 00 mm), flower number (17) and fresh and dry weight of leaves (23.36g and 4, 98 g) respectively, compared with the interaction between the two color of laser and zero exposure time (control). However, earlier flowers were induced by red and blue laser and 20 min.It passed the control (the two color of laser + 0 min. exposure) about (14 and 15 days) earlier in flowering period.

Data in Table (7) showed that, the interaction between laser type and time exposure recordedinsignificanteffect in almost cases on chemical constituents.

The lowest values in carbohydrate and leaves anthocyanin content obtained from the interaction between (blue laser and 40 min. exposure time) recorded (0.092 and 0.160) and (red laser and 20 min.) recorded (3.82 and 15.04), respectively compared with all other treatments.

Table 7 The effect of the interaction between color laser and laser times exposure on chemical constituents of Tagetes plant. (Means of two seasons)

Treatments	**Characteristics**							
	Chl.a (Mg/g.f.w.)	**Chl b (Mg/g.f.w.)**	**Carotenoids (Mg/g.f.w.)**	**Flower Anthocyanin content (Mg/g.f.w.)**	**Leaves Anthocyanin content (Mg/g.f.w.)**	**Carbohydrate Content (Mg/g.D.W.)**	**Electrical conductivity**	**Protein (mg/ml)**
He-Cd 0 min.	0.801	0.265	1.310	0.183	0.223	38.48	17.80	0.541
He-Cd20min.	1.180	0.397	2.160	0.301	0.221	16.27	100.78	0.543
He-Cd30min.	1.020	0.374	1.770	0.156	0.146	20.04	114.80	0.638
He-Cd40min.	1.270	0.467	2.170	0.335	0.092	13.82	98.65	0.921
He-Ne0min.	0.801	0.280	1.320	0.180	0.250	43.60	17.86	0.546
He-Ne 20min.	1.88	0.680	3.08	1.030	0.160	15.04	127.80	0.833
He-Ne 30min.	0.707	0.220	1.270	0.820	0.180	16.80	94.40	0.916
He-Ne 40min	1.380	0.520	2.220	0.860	0.200	16.40	95.80	1.183
LSDat0.5%	4.29	4.20	1.95	2.30	2.870	0.146	0.146	1.442

3.4. Biochemical genetic markers

As for the electrophoretic banding pattern of proteins extracted from leaves of tagetes plants was used to study three dosage effects of two laser. As shown in Fig. 2 and their denstrometric analysis are illustrated in table (7) the presence and absence of band were assessed with (1) and (0), respectively. One novel band with molecular weight 43 KDa appeared in (red laser 40 min) exposure of laser treatment. However, this band becomes invisible clearly in blue laser 40 min. exposure treatment. Moreover, two novel bands were detected with molecular weight 46 KDa in (treatments blue laser 30 and red laser 30 min.) exposure time. On the contrary, the band with molecular weight 33 KDa was absent in control while showed in all exposure of two laser treatments. On the other hand, the band with molecular weight 38 KDa was observed in control and (treatments blue laser 30, red laser 30 and 0 laser min.) while absent in all exposure of two laser treatments.

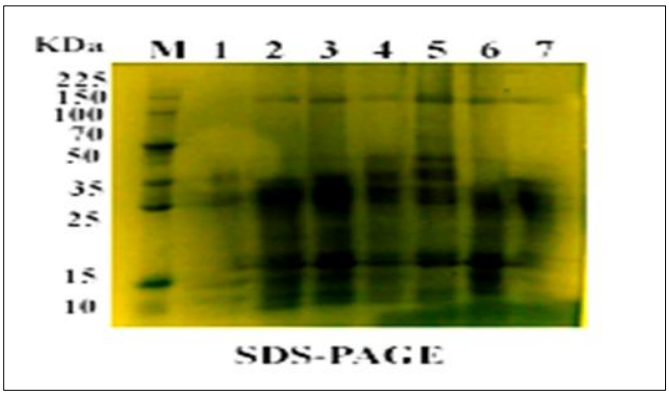

Figure 3 SDS-PAGE of tagetes leaves protein (total protein) of the three different dosage of two laser radiation which was detected in treatment samples compared to control plants

4. Discussion

The presented work aimed to study the effect of laser rays on growth, flowering, and some chemical constituents and gene expiration of Tagetes Sp. plant. The assessment of the response of Targets plants to these treatments was undertaken by measuring growth parameters and some chemical constituents. From the results obtained in this investigation it is clear that, laser rays led to increase in the plant height, branches number, root length, and both of fresh and dry weights in compared with the untreated plantsthis results hold true with both types of laser. The data also revealed that the increase in plant height was followed by increase in the branches number which induced high fresh and dry weights. This maybe for rolling biological effect of GA in cell elongation through the weaken cell wall by enzymes induction, which promoted by laser treatments [23], [24].

The laser treatments increased growth regulators and protein, which may explain the increase of electrical conductivity under the higher doses of laser rays. In this respect, [25] reported that auxins induced net synthesis of RNA and protein; So it means that laser enhanced GA_3 formation and encourage the release of IAA which had promotive effect on root growth, nutrient and water uptake and this reflected in plant growth [26] revealed that irradiated *Adansonia digitata* seeds with He-Ne laser induced high Plant growth parameters compared with control.[27] they mentioned that exposing *Celosia argentea* seeds to (He-Ne) laser for 2min recoded significantly increase in number of root and length of root per shootlet compared to control.

[28] Studied the effect of the pre-sowing laser biostimulation of Jacaranda" (*Jacaranda mimosifolia*) and "mezquite" (*Prosopislaevigata*).They mentioned that, the 30 second treatment produced a considerable effect on root parameters. These results hold true with [29] on wheat seeds.

Leave is considering sensitive argan to light period and light type. The flower hormone (Florigen) is configured in the leaves and transmitted to apex regions through the phloem to enhance flowering. Laser radiation was found to have an effect on the flowering time (delay, earlier and preventing flowers) of plan.The laser effect varies depending on the laser color, the power output used, the exposure time and the plant kind exposed to the laser.

These effects of laser application may be due to synthetic compounds such as (IBA, cyocel, NAA, ethereal and others) or may be natural hormones. In this respect, on gebera plants, [23] found that, GA, cytokinen, ABA and IBA increased and accompanied by increments in growth and flowering in most cases.

The application of laser treatments He-Ne and He-Cd enhanced the flowering, these results agreed with [30] on *Viciafaba* Moreover, the flowering enhancement by laser rays on *Eustomagrandiflorum* plants was reported by [31]. Another study was done by [23] recorded that laser rays delayed flowering period of gerbera plants, whereas[8] mentioned that treating the plants with helium neon laser (He-Ne) significantly increased delaying flowering as compared with untreated plants.

The increased in photosynthetic pigments in leaves and flowers anthocyanine content as a result of treated plants with both type of laser this maybe for laser rays effect which promote GA formation which increases photosynthetic pigments contents and sugar concentration this results hold true with [32] on *Pisumsativum* and [33] on *Jojopa Sp.*, they studied the effect of He-Ne laser. They showed that the seeds were treated with He-Ne laser rays significantly increased

chlorophyll a and b, totalchlorophyll as well as carotenoidscompared to untreated plants.[34] on vinca plants found that, cadmium and helium neon laser rays able to enhance anthocyanin content. Anthocynine was increased with laser rays, these results confirmed by [35].

In additional to study the effect of two laser radiation on vegetative growth we studied its effect on gene expression which appeared in total protein banding profiles. In this study, tagetes seedling exposed to two laser rays in different times (0, 20, 30 and 40 min.), have been studied for the analysis of protein profiles to examine their effects by SDS-PAGE technique. Several variations of electrophoresis were observed for the separation of proteins. These variations included increasing in bands intensity and their appearance. The laser irradiation could produce its effects through its light and electromagnetic effects [36] or through temperature effects [37]. Whereas, our results showed bands whose synthesized or changed in their intensities were directly induced by the perception of laser. These results agree with [38] who showed changes in the protein functional activities. Similarly, the physiological characters of the seedlings, and the biochemical characters of the seedlings, e.g. soluble protein and the activities of functional protein were increased significantly by the laser pretreated [39], [40].These biochemical results could reflex the same effect of dosage exposing time on vegetative. The biochemical changes, vegetative growth parameters increased by increasing the dosage time (blue laser 20 and red laser 20 min. exposure time), then these parameters decreased at blue laser 40 min. exposure time. The exposition time to laser radiation is very important to produce stimulation effects and these agree with [41]. Laser ray induced changes in protein banding patterns and their intensities. Exposing plants to laser ray could be useful method to make variations and plant improvement [8].

Table 8 Densitometric profile for total protein profiles of the non-treated (control) and three laser dosages effects on tagetesplants

Band No	**M.W Kda**							
		1	**2**	**3**	**4**	**5**	**6**	**7**
1	165	1	1	1	1	1	1	1
2	48	1	1	1	1	1	1	1
3	46	0	0	0	1	1	0	0
4	43	0	0	0	0	1	0	0
5	38	1	0	0	1	1	0	0
6	33	0	1	1	1	1	1	1
7	28	1	1	1	1	1	1	1
8	27	1	1	1	1	1	1	1
9	25	1	1	1	1	1	1	1
10	18	1	1	1	1	1	1	1
11	17	1	1	1	1	1	1	1
12	14	1	1	1	1	1	1	1
13	13	1	1	1	1	1	1	1
14	11	1	1	1	1	1	1	1
Total		11	11	11	13	14	11	11

5. Conclusion

Thecurrentresearchconcludedthatthe laser irradiation can affect plant morphology, flowering, chemicalconstituents, andgenemutagenesis. The high exposure time (40 min.) of laser beam can be used to enhancing growth parameters, chemical constituents and exchange gene expiration which enhances a new protein formulation may provide a better alternative of petals.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

Statement of ethical approval

The manuscript does not contain studies involving human participants, human or animal data, and animal or human tissue.

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Authors' contributions

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