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Tagetes officinalis Oil Production under Photobiology Treatments

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ABSTRACT

Tagetes plant was grown widely as an herbaceous ornamental plant belonging to the family of Asteraceae. It is an economic plant species utilized in processed forms in modern medicinal processes. The main constituents of Marigolds are Phenolic compounds, carbohydrates, lipids, steroids, tocopherols, terpenoids, vitamin C, and carotenoids. From the results, it could be concluded that with the high exposure time (40 minutes two types of laser recorded the highest values in oil content compared with control and other laser treatments. The results of phenolic and flavonoid contents for essential oil from Tagetes treated with blue and red laser revealed a significant ($P \leq 0.05$) increase after laser treatment especially in red laser treatment which recorded (17.08 mg GAE/g) after 15 mins compared to control (13.25 mg GAE/g) and blue laser (16.49 mg GAE/g) at the same time. A similar observation was noticed in total flavonoid content, which increases with increasing exposure time to both types of laser. Twenty-nine volatile compounds were identified which comprise about 99.6%, 99.13%, 99.66%, 99.58%, and 99.27% in control and blue as well as red laser after 10 and 15 mins. respectively. The data revealed that the main volatile constituents were terpenes either mono or sesquiterpenes and oxygenated sesquiterpenes. Dihydro-Tagetone was considered the major volatile compound in all samples under investigation with a concentration of control (26.31%) and exhibited a pronounced increase in treated samples with concentrations of 28.56% and 27.91% after 15 mins. treatment with red and blue laser respectively.

INTRODUCTION

Tagetes plant (*Calendula officinalis*), (Marigold) is grown widely as an herbaceous ornamental plant belonging to the family of Asteraceae. It is an economic plant species utilized in processed forms in modern medicinal processes. The main constituents of Marigold are Phenolic compounds, carbohydrates, lipids, steroids, tocopherols, terpenoids, vitamin C, and carotenoids. In addition to the edible uses (i.e. coloring and flavoring agents of food). The main constituents of *Calendula officinalisare* include phenolic compounds, carbohydrates, lipids, steroids, tocopherols, terpenoids, vitamin C, carotenoids, and quinines Shahrbabakiet al (2017). Carotenoids extracted from dry petals are used for poultry feeds to improve the egg yolk color of the boiler's skin. Singh (2014). It has medical importance as a blood refiner, anti-inflammatory, skin antifungal, blood sugar reduction, and antiviral properties. Baranidharan et al (2020). Laser rays have attained much attention in different parts of the world for improving the growth and quality of plants. In this concern, Laser treatments can modify important components of

plant cells and have been reported to affect differentially, the morphology, anatomy, biochemistry, and physiology of plants depending on the source and time of laser exposure. Sami et al. (2013) and *Celosia argenta* and Sami et al. (2014) Caster bean reported that significant increase in plant growth. Also, they reported that laser rays could be useful to induce variation in plant improvement. Previous studies showed that laser influenced plant growth and metabolism. Whereas, oil contents in the tagetes seedling flowers were enhanced after using laser irradiation (Govilet al. 1991; Cai et al. 2000).

The extracted oil from tagetes has several applications in food products with antimicrobial activity and is used as flavoring and fragrance in perfumes. Also, the oil had medicinal properties such as anticancer, hypotensive, and ant inflammatory effects (Rajesh et al. 2012; Oliveira et al.2015). The essential oil of tagetes has been shown to be an effective free radical scavenger, and the ethanol extract is reportedly effective against parakeratosis (Khan and Evans, 1996; Gutierrez et al. 2006). Nowadays, there is increased attention to environmentally safe potential strategies for aromatic and medicinal plants to improve the morphological, physicochemical, and genetic agronomical traits. Among the physical elicitors UV as well as gamma irradiation and laser treatments have been applied in various studies to enhance seed germination, and improve the growth parameters and metabolite production (Thoratet al.2021; Saadet al. 2021). The current investigation has been undertaken to isolate the essential oil of tagetes after treatment with two types of laser (red and blue)at different times and evaluate its effect on phytochemicals, antioxidant as well and volatile oil composition. Therefore, the aim of this study was to investigate the effect of two types of laser on the chemical composition of oil contents in the Tagetes officinalis plant.

MATERIALS AND METHODS

The study was carried out at the greenhouse of the National Research Centre, Dokki, and Cairo, Egypt during seasons 2019-2020, to investigate the response chemical parameters of oil contents on 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 loamy sandy soil (2:1 by volume) the physical and chemical characteristics of the soil are shown in Table (1). Nitrogen and potassium fertilizers were added to the soil according to the recommended dose of the Ministry of Agriculture after three months from planting. The experiment consisted of four for each kind of laser treatment including the control. Helium cadmium laser was used for exposing seedlings (10 cm length) at the wavelength of a blue laser (460) and red laser (650 nm) and output power 60 and 103 Mw/cm2. Seedlings plantation was in two seasons in February 2020 and 2021 after being 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, a representative plant sample was taken from three replicates randomly. Flowers samples were collected in the two seasons and weighted to extract the essential, oil (100 gm) fresh weight from flowers were weighted and hydro-distilled for 3 hours using Cleveger-type apparatus methods Cleveger (1928).

Table 1: Physical and chemical parameters of soil samples

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

Preparation of Essential oil

The volatile oil was obtained by passing over anhydrous Na2SO4 to strip it of any water, while the oils were kept in sealed glass bottles covered with aluminium foil at 20°C until required.

Determination of total phenolic content

The total phenolic content of essential oil methanolic extract was estimated by the Folin–Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi (1965), using gallic acid as a standard phenolic compound. Briefly, 50 ul (three replicates) of the filtered extracts were mixed

with 450 ul of distilled water and 2.5 ml of 0.2 N Folin–Ciocalteu reagent. After 5 min, 2 ml of saturated sodium carbonate (75 g/l) was added. The absorbance of the resulting blue-colored solution was measured at 765 nm after incubation at 30 oC for 1.5 h with intermittent shaking. Quantitative measurements were performed, based on a standard calibration curve of six points: 20, 100, 200, 300, 400, and 500 mg/L of gallic acid in 80% methanol. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram of oil.

Total flavonoids

The total flavonoid content of the prepared extracts was determined by the method of Davis et al. (1980). The extract (100 µL) was placed in a test tube before adding 1 mL of diethylene glycol reagent and 100 µL of 1 N NaOH. The mixture was shaken vigorously and incubated at 37 ^{o}C for 1 hr before measuring the absorbance at 420 nm. A standard curve was prepared using rutin. The total flavonoid content was expressed as rutin equivalents (RE) in milligrams per gram of oil.

Determination of total antioxidant activity
ABST assay

The antioxidant capacity assay of essential oil methanolic extract was carried out using the improved ABTS+ method, as described by Re et al. (1999). Briefly, ABTS+ radical cation is generated by reacting 7 mM ABTS+ and 2.45 mM potassium persulfate via incubation at room temperature (23 oC) in the dark for 12–16 h. The ABTS+ solution was diluted with 80% HPLC-grade ethanol to an absorbance of 0.700 ± 0.040 at 734 nm and equilibrated at 30 ^{o}C. Plant extracts were diluted with distilled water or 80% methanol, such that after the introduction of a 30 µL aliquot of each dilution into the assay, it produced from 20% to 80% inhibition of the blank absorbance. To 3.0 ml of diluted ABTS+, 30ul of each plant extract solution was added and mixed thoroughly. The reactive mixture was allowed to stand at room temperature for 6.0 min and the absorbance was recorded immediately at 734 nm.BHT and ascorbic acid were used as a positive control. The results are expressed as IC50 values (ug/ml), the concentration required to cause a 50% ABST+ inhibition (Re et al. 1999).

-Carotene bleaching assay

The carotene bleaching method is based on the loss of the yellow colour of -carotene due to its reaction with radicals formed by linoleic acid oxidation in an emulsion. The rate of -carotene bleaching can be slowed down in the presence of antioxidants (Kulisicet al.2004). -Carotene (2.0 mg) was dissolved in 20 ml chloroform and to 4.0 ml of this solution, linoleic acid (40 mg) and Tween 40 (400 mg) were added. Chloroform was evaporated under a vacuum at 40 oC and 100 ml of oxygenated ultra-pure water was added, then the emulsion was vigorously shaken. Reference compounds (BHT and ascorbic acid) and essential oils were prepared in methanol. The emulsion (3 ml) was added to a tube containing 0.2 ml of different concentrations of essential oils (1, 3, 5 and 7 mg/ml) and extract (1, 10, 100 and 200 ug/ml). The absorbance was immediately measured at 470 nm and the test emulsion was incubated in a water bath at 50 oC for 120 min, when the absorbance was measured again. BHT and ascorbic acid were used as positive control. In the negative control, the essential oil extracts were substituted with an equal volume of methanol. The antioxidant activity (%) of the essential oils was evaluated in terms of the bleaching of the -carotene using the following formula:

% Inhibition = [(At -Ct)/C0-Ct)] X100

where At and Ct are the absorbance values measured for the test sample and control, respectively, after incubation for 120 min, and C0 is the absorbance values for the control measured at zero time during the incubation. The results are expressed as IC50 values (ug/ml), the concentration required to cause a 50% -carotene bleaching inhibition. Tests were carried out in triplicate.

Gas chromatography–flame ionization detector (GC–FID) analysis

The essential oil analyses were carried out using Agilent 7890 GC equipped with a flame ionization detector, an electronic pressure control injector and a capillary column (HP-5 Innowax: 30 m X 0.25 mm; 0.25 um film thickness); carrier gas, He at 1.0 ml/min; split ratio, 1:20. The oven temperature was programmed from 60 to 250ºC at the rate of 5ºC/min. and finally, the temperature of 250ºC was kept constant for 10 minutes. Subsequent GC working conditions were as follows: carrier gas was He with a constant flow rate of 1.0 mL/min.

Ionization voltage was kept at 70 eV. MS working conditions were as follows: the temperature of the ion source and the interface were 200 and 250ºC, respectively, and the mass range was scanned from 43 to 456 m/z. The injector and detector temperatures were 250 and 300ºC, respectively.

Gas chromatography-mass spectrometry (GC–MS) analysis

GC/MS analysis was performed on Agilent 7890 GC coupled to a 5977 MS detector with electron impact ionization (70 eV). An HP-5-MS capillary column (30 m X 0.25 mm coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25 um film thickness) was used. Oven temperature was programmed to rise from 60 to 250 oC at a rate of 5 oC/min; the transfer line temperature was 250 °C. The carrier gas was He with a flow rate of 1.0 ml/min and a split ratio of 60:1. Scan time and mass range were 1 s and 40–300 m/z, respectively.

Identification of the volatile constituents

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of n-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST (NIST, 2011) and the homemade MS library built up from pure substances and components of known essential oils, as well as by comparison of their retention indices with literature (Adams, 2007).

RESULTS AND DISCUSSION

We can conclude from Table (2) in general, all exposure times of laser treatments (0, 20, 30, and 40 min.) recorded increments in oil content compared with zero min (control). Laser exposure time. Treated plants with 40 min. exposure time for the two laser types recorded the highest values, followed by 30 min. the laser exposure time of two laser types. The oil content of tagetes flowers was increased by laser treatment and surpassed the control plants. This may be due to the formation of GA and the main biological activity of growth hormones and enzyme activity enhancing under laser effect, Sami et al (2014) on *Ricinus communis* plant.

Table 2: Effect of laser type and exposure time on oil flower content of Tagetes plant (Means of two seasons)

		Helium neon laser (red)			Cadmium neon laser (blue)		
Treatments	**0 min.**	**20 min.**	**30 min.**	**40 min.**	**20 min.**	**30 min.**	**40 min.**
Oil content (ml)	**1.7**	**1.9**	**2.5**	**3**	**2**	**3**	**3.5**

The phenoglic and flavonoid contents of essential oil from Tagetes treated with blue and red laser are given in(Table 3).The obtained results revealed that there is a significant (P≤0.05) increase in the determined phytochemicals after laser treatment especially in red laser treatment which recorded (17.08 mg GAE/g) after 40 mins compared to control (13.25 mg GAE/g) and blue laser (16.49 mg GAE/g) at the same time (Table 3). A similar observation was noticed in total flavonoid content which increases with increasing exposure time to both types of laser. The maximum value was found in the red laser after 40 mins (5.91 mg RE/g) compared to control and blue laser at the same time which had (4.96 and 5.31 mg RE/g) respectively (Table X).The obtained results are in good agreement with Katarzyna et al. (2020)who mentioned that the increase of phytochemicals such as phenolic and carotenoid content of T. wittm depend on the time of irradiation for seeds.Also, our results were confirmed byMahmood et al. (2021)who mentioned that laser light could improve the yield of plant metabolic pathways and enhance the production of biomass in a species as well as the dose-specific manner in sunflowers.The studied phytochemicals either phenolics or flavonoids are considered the most abundant antioxidants responsible for the plant defense mechanisms.In the literature, the effect of laser on phytochemicals and metabolites depends on the source of light and time of treatment (Etxeberriaet al. 2016; Cui and Lei, 2019).

The data in (Table 3) shows the effect of Tagetes essential oil antioxidant activity after red and blue laser treatments on assays of ABST+ and -carotene. The assay results expressed as IC50 in comparison with BHT and ascorbic acid. The data in (Table 3) showed that as the time of laser treatment

increased an increase in antioxidant activity occurred at all studied times. Generally, the red laser treatments were stronger than the blue laser. In comparing ions with standard antioxidants the investigated samples were lower than BHT, but higher than ascorbic acid. A similar observation was reported by Chen and Han (2014) who found an increase in antioxidant activity after laser light treatment of wheat. Based on the results reported by Ohnishi et al. (1994) the antioxidant activity of tagetes may be correlated with the phenolic compounds such as chlorogenic and caffeicacids which possess peroxyl radical antioxidant activity higher than ascorbic acid and tocopherols. All the studied doses and types of laser antioxidant activity could be therefore attributed to the proton donor ability and direct scavenging of the bioactive constituents (Brand-Williams et al. 1995). Therefore, it is important to extend our study to shade the light of the phenolic composition of the selected doses and type of laser using HPLC-MS analysis.

Table (3) Effect of laser type and exposure time on phytochemicals and antioxidant activity of Tagetes

Treatment	Control	Blue laser			Red laser		
		20	30	40	20	30	40
Total phenolics(GAE mg/g)	13.25±0.08	15.37±0.02[a]	16.43±0.13[b]	16.49±0.05	15.98±0.11[a]	16.92±0.21[b]	17.08±0.17[b]
Total flavonoids (RE mg/g)	4.96±0.12	5.09±0.14	5.28±0.15[a]	5.31±0.07[a]	5.76±0.09[b]	5.83±0.16[b]	5.91±0.12[b]
Antioxidant activity(IC_{50})							
ABST	25.34±0.17	19.35±0.08	17.36±0.04	15.73±0.05	14.32±0.06	12.85±0.07	9.67±0.08
-Carotene	46.82±0.14	31.94±0.02	28.47±0.12	21.76±0.09	24.37±0.16	19.45±0.09	15.24±0.03
BHT	8.13±.02						
Ascorbic acid	54.16±0.03						

Values are given as mean ± SD (n= 3); Values with the same letters within the same column are not significant

Volatile compounds analysis

The variations in tagetes essential oil composition after treatment with red and blue laser for different times were subjected to analysis by GC and GC-MS and the identified constituents with their relative concentrations are given in Table 4. A total of twenty-nine were identified which comprise about 99.6%, 99.13%, 99.66%, 99.58% and 99.27% in control and red as well as blue laser after 30 and 40 mins respectively. The data revealed that the main volatile constituents were terpenes either mono or sesquiterpenes and oxygenated sesquiterpenes. Dihydro-Tagetone was considered the major volatile compound in all samples under investigation with the concentration of control (26.31%) and exhibited a pronounced increase in the treated sample with concentrations of 28.56% and 27.91% after 40 mins. treatment with red and blue laser respectively (Table 4).Recently, studies referred to the improvement of essential oil yield and its precursors in anise after laser treatment as mentioned by Okla et al. (2021) which supported our data.

The obtained results are in good agreement with Craveiro et al. (1988) who mentioned that the Brazilian T. minuta rich in dihydrotagetone. Piperitenone and piperitone were the most dominant monoterpenoids with concentrations of 16.34% and 13.28% in control sample and exhibited pronounced increase after laser treatment especially red laser after 40 mins which recorded concentration of 19.25% and 16.72% of piperitenone and piperitone respectively (Table 4). Our results confirmed by Laosinwattana et al. (2018) who found that monoterpenes are the major volatile compounds in the aerial parts of T. erecta which represent about 46.3%-97.3%.In the present study,sesquiterpenoids such as caryophyllene was found with low concentration varied from 0.89% after treatment with blue laser for 30 mins and 0.24% after 40 mins compared to control sample which showed 0.59% (Table 4)and completely disappeared after blue laser treatment. The obtained data are in contrast to those reported by Resmi et al. (2018) who found that sesquiter

penoids like caryophyllene were considered the second major volatile compounds in tagetes. The variation in the volatile composition may be due to the species, aerial parts, and method of isolation as well as environmental conditions.

Table 4: Effect of laser type and exposure time on volatile oil composition of Tagetes

Volatile compounds	LRI [a]	Control	Red		Blue	
			30	40	30	40
α-Pinene	938	0.52	1.02	1.08	1.95	1.98
Sabinene	971	0.68	0.78	0.85	0.62	0.81
Myrcene	992	0.28	0.34	0.18	0.03	1.24
α-Phellandrene	1005	0.05	n.d	0.02	0.17	0.65
O-Cymene	1026	n,.d	n.d	0.16	0.92	n.d
Limonene	1027	6.13	7.16	8.17	7.56	7.85
Sylvestrene	1029	3.19	2.08	1.06	1.82	0.46
(Z)-β-Ocimene	1031	0.82	0.12	n.d	n.d	0.09
(E)-β-Ocimene	1045	0.65	0.37	0.13	0.28	0.12
Dihydro-Tagetone	1048	26.31	27.18	28.56	27.84	27.91
Linalool	1092	1.04	0.62	0.34	1.62	0.29
(E)-Tagetone	1138	1.93	0.35	0.18	0.58	0.16
(E)-Myroxide	1142	4.12	1.29	0.72	0.67	0.54
(Z)-Tagetone	1152	6.52	8.16	9.13	7.28	8.63
Borneol	1167	0.31	0.18	0.01	0.16	0.05
Terpinen-4-ol	1176	0.95	0.52	n.d	1.41	0.73
(E)-Isocitral	1178	0.42	0.49	0.38	n.d	1.04
p-Cymen-8-ol	1183	7.85	9.14	9.57	9.54	9.83
α-Terpineol	1186	2.39	1.34	0.59	1.78	0.25
Verbenone	1195	0.54	0.27	0.13	0.05	1.14
(E)-Ocimenone	1227	1.85	0.34	0.54	0.91	0.59
Piperitone	1248	13.28	15.26	16.72	15.67	16.02
Piperitenone	1341	16.34	18.36	19.25	18.52	18.74
Geranyl acetate	1380	0.25	0.17	0.38	n.d	0.07
β-Elemene	1388	n.d	0.05	0.61	0.01	0.05
(Z)-Jasmone	1393	2.17	1.63	n.d	0.03	n.d
β-caryophyllene	1416	0.59	0.89	0.24	n.d	n.d
α-Humulene	1457	0.28	0.64	0.19	0.02	0.01
Germacrene D	1482	0.14	0.38	0.47	0.14	0.02
Total		**99.6**	**99.13**	**99.66**	**99.58**	**99.27**

a: LRI: Linear retention indices; b: Values are expressed as relative area percentage; n.d: not detected

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