
Effect of drought stress and helium neon (He-Ne) laser rays on growth, oil yield and fatty acids content in Caster bean (*Ricinus communis L.*)

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Abstract: Pot trial were carried out at greenhouse of National Research Centre, Dokki, Egypt, to evaluate the effect of different irrigation intervals 5,10 and 15 days combined with two exposure time of He-Ne laser rays (0 and 5 min.) on the growth, oil yield and fatty acids constituents of *Ricinus communis*. Plant height, Stem diameter, leaves number leaf area, fresh and dry of leaves weight, as well as relative water content and osmotic potential (atm) were decreased by prolonging irrigation intervals. Carbohydrates % also showed a negative response by extending irrigation intervals. Helium neon (He-Ne) laser rays improved Caster bean growth and decreased osmotic potential followed by increasing relative water content and help plants to complete its life cycle. Palmitic acid is substantial component of the saturated fatty acids, while ricinoleic unsaturated fatty acids is the major one. The highest increment in both ricinoleic and hexacenoic acids was recorded by prolonging irrigation interval up to 10 days.

Keywords: Drought Stress, Water Intervals, *Ricinus communis*, Oil Yield, Fatty Acids and Laser Rays

1. Introduction

Caster bean (*Ricinus communis L.*) Plants used as ornamental plants and are among the oil crops, family Euphorbiaceae cultivated for different medicinal industrial and economic uses of the crop. Castor oil is a vegetable oil obtained by pressing the seeds of the castor plant (*Ricinus communis*) [1]. Castor oil is colorless to very pale yellow liquid with a distinct taste and odor once first ingested. Its boiling point is 313⁰C and its density is 961 Kg/m³ [2]. Castor oil has numerous applications in transportation, cosmetics, pharmaceutical, and manufacturing industries, such as adhesives, brake fluid, caulks dyes electrical liquid dielectric, humectants, inks, machining oils, paints, refrigeration lubricants, rubbers, washing powders and waxes [3]. The United States Food and Drug Administration (FDA) has categorized castor oil as “generally recognized as safe and effective” for over-the-counter use as a laxative with its major site of action the small intestine where it is

digested into Ricinoleic acid [4]. Castor oil is added to Paclitaxel, a mitotic inhibitor used in cancer chemotherapy [5].

Laser radiation is different from all natural forms of light beams in three ways. It is a coherent beam, nearly collimated and monochromic. Helium neon laser (He-Ne) is the most familiar and least expensive gas laser. It emits a fraction of milliwatt to tens of milliwatts (mW) of red light at 632.8 nanometers (nm). As such it has long beam and most common and economical visible laser. The active medium in helium neon laser is a mixture of helium and neon gas at a total pressure of a fraction of atorr to several torrs. The effect of laser on biological tissue may be thermal and non thermal processes. Meanwhile a direct interaction between laser photons and molecules is responsible for photochemical effect [6]. However in this respect [7] used (he-Ne) laser on *Salvia officinalis* dry and wet seeds, and found a higher significant effect on carbohydrate content. In spite of the great economic importance of this oil crop, nothing is known about the effect of laser treatment and water requirement on

growth and oil content on this plant. However, shortage in irrigation water affective negatively on the growth of plants. The objective of this study aimed to evaluate the effect of watering intervals on castor beans seeds with (He-Ne) laser rays.

2. Materials and Methods

This study was carried out during the two growth seasons 2012 and 2013 at the greenhouse of National Research Centre, Dokki, Egypt and aims to investigate the response of castor bean plant (*Ricinus communis L.*) to laser treatment under different irrigation intervals. For cultivation pots 30 cm in diameter and 50 cm in depth were filled with a loamy soil. The experiment in both seasons included six treatments in which combination of three water interval 5, 10 and 15 days and seeds were treated by two Helium neon (He-Ne) laser rays (0 and 5 min. exposure time). The seeds were sown on May in both seasons. Phosphorus fertilizer was added to the soil before sowing, nitrogen and potassium fertilizers were added to the soil according the recommended dose after 30 days from sowing. The irrigation regime were applied after 20 days from sowing, the quantity of water added has been adjusted to bring the soil moisture to field capacity. Treatments were distributed in a complete randomized design with five replicates for each treatment. The irrigation intervals were carried out every 5, 10 and 15 days alone or in combination with laser treatment.

After seven month from sowing representative plant sample was taken from three replicates randomizly. The growth parameters included plant height, stem diameter, leaves number; leaf area and leaves fresh and dry weight were conducted. Fresh leaves were sampled and the relative water content determined according to [8]. Osmotic pressure was calculated by the methods described by [9]. Another sample of leaves was dried at 70 °c to determine total and soluble carbohydrate % by using) the methods described by [10]. At fruiting stage (250) days from sowing,

seeds were collected to determine oil% by the methods described by [11]. While other treatments can't complete its life cycle.

The GC-MS analysis of the FAMES samples was carried out using gas chromatography – mass spectrometry instrument sands at the Department of Medicinal and Aromatic Plants Research, National Research Centre with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a Tr-5 MS column (30mx0.30mm i.d., 0.25 µm film thickness).Analyses were carried out using helium as carriages at a flow ate of 1.0mL/min. and a split ratio of 1:10 using the following temperature program: 60 C° for 1 min; rising at 3.0 c/min. to 300 C° and held for 1 min. The injector and detector were held at 300 C°. Diluted samples (1:10 hexane/v) of 0.2 µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral rang of m/z 40-450.

The recorded data (means of the two growing seasons) were statistically analyzed according to the procedure of Snedecor and Cochran [12], where the means of the studied treatments were compared by using L.S.D. test at 0.05 of probability.

3. Results

3.1. Effect of Irrigation Intervals

Data presented in table (1) show that, the least growth parameters expressed in plant height, stem diameter, leaves number, leaf area, fresh and dry weight of leaves were recorded when plants irrigated every 15 days. While 5 and 10 days intervals provided with adequate supply of water which resulted in increasing the previous mentioned parameters with in significant differences between treatments.

Table (1). Effect of irrigation Intervals on growth and physiological parameters of Caster bean plant (Mean of two seasons of 2012& 2013).

Irrigation Intervals treatments (days)	Growth parameters				Physiological parameters				
	Plant height (cm)	Stem diameter (mm)	Leaves number	Leaf area cm ²	Leaves fresh weight (g)/plant	Leaves dry weight (g)/plant	Carbohydrate %	Osmotic pressure (atm)	RWC Relative Water content%
5	1.33	1.28	32.5	114.51	282.09	73.86	3.65	6.01	57.25
10	0.945	1.02	24.66	73.41	168.24	44.56	3.25	4.95	52.70
15	0.828	0.85	19.83	49.83	90.19	22.97	3.06	3.73	45.61
LSD at 0.05%	0.203	0.13	1.46	17.85	16.75	3.69	0.247	1.20	17.33

Significantly relative water content% (RWC %) as well as osmotic potential (atm) were decreased by prolonging irrigation intervals up to 15 days. The highest relative water content was recorded when plants irrigated every 5 and 10 days without significant difference. Expanding irrigation intervals from 5 up to 15 days plants tended to decrease

significantly carbohydrate percentage. The difference between carbohydrate % under irrigation every 10 or 15 days was not enough to reach the level of significant response

3.2. Effect of (He-Ne) Laser Rays

Table (2). Effect of laser rays treatments on growth and physiological parameters of Caster bean plant (Mean of two seasons of 2012& 2013).

He-Ne laser Min.	Growth parameters				Physiological parameters					
	Plant height (cm)	Stem diameter (mm)	Leaves number	Leaf area cm ²	Leaves fresh weight (g)/plant	Leaves dry weight (g)/plant	Carbohydrate %	Osmotic pressure (atm)	RWC Relative Water content%	
0 min.	0.89	0.91	25.22	68.18	157.99	40.81	3.27	5.59	50.92	
5 min.	1.17	1.20	26.11	90.32	202.36	53.45	3.36	4.20	52.78	
LSD at 0.05%	0.203	0.159	1.58	12.07	8.13	3.15	0.232	1.18	10.50	

As shown in table (2) Laser treatment (He-Ne laser) rays had no significant increase on plant height and leaves number of Caster bean plants. On the contrary the highest increment was in leaf area which reached 32.47% followed by stem diameter 31.86% then fresh and leaves matter which reached 28.08% and 30.97% respectively, over the control.

Data in the same table also show that, carbohydrate percentage as well as relative water content was increased but insignificant in response to (He-Ne laser) rays treatment. However osmotic potential (atm) of the cell sap was significantly lower than the control plants by about 24.86 % less than the control.

3.3. Effect of Interaction between Watering Intervals and Laser Rays Treatment

Table (3). Effect of Water intervals and He-Ne laser rays and their interaction on growth and physiological parameters of Caster bean plant (Mean of two seasons of 2012& 2013).

Irrigation Intervals days	He-Ne laser Min.	Growth parameters				Physiological parameters				
		Plant height (cm)	Stem diameter (mm)	Leaves number	Leaf area cm ²	Leaves fresh weight (g)/plant	Leaves dry weight (g)/plant	Carbohydrate %	Osmotic pressure (atm)	RWC Relative Water content%
5	0	1.20	1.10	33.00	106.30	271.59	69.96	3.13	4.49	56.61
	5	1.46	1.46	32.00	122.26	292.60	77.76	4.16	2.97	57.90
10	0	0.78	0.85	23.33	66.26	128.33	33.13	3.40	4.67	45.23
	5	1.11	1.20	26.00	80.56	208.15	56.00	3.10	4.36	45.98
15	0	0.69	0.78	19.33	32.00	74.04	19.34	3.30	4.61	50.51
	5	0.96	0.93	20.33	67.66	106.34	26.60	2.83	5.19	54.89
L.S.D. at 0.05 %		0.608	0.479	1.013	5.28	2.63	2.01	0.412	3.61	4.28

It is clear from the data in Table (3) that the tallest plant (cm) stem diameter (mm), leaf area (cm²), fresh and dry weight (g). Also, carbohydrate % and relative water content were obtained by exposing caster bean seeds to (He-Ne) laser rays and irrigated every 5 days. The lowest values for the previously mentioned parameters were recorded when plants irrigated every 15 days with zero (He-Ne laser) rays.

3.4. Effect of Irrigation Intervals, Laser Rays and its Combination on Costar Oil Yield

Oil yield in caster seeds was not affected by laser treatments whereas apparent decrease was induced by prolonging irrigation intervals from 5 days to 10 days. The decrease compared with the control value reached 20.02 %. However, laser treatments encourage caster bean plants to complete life cycle until fruiting.

Table (4). Effect of Water intervals and He-Ne laser rays (5 min.) and their interaction on oil yield of Caster bean plant (Mean of two seasons of 2012& 2013).

Irrigation Intervals days	0	5		10		15	
He-Ne laser Min.	0	0	5	0	5	0	5
Oil %	35.15	-	35.58	-	28.11	-	-

*Some treatments can't complete its life cycle

3.5. Effect of Irrigation Intervals, Laser Rays and its Combination on Costar Oil Fatty Acids

Table (5). Effect of Water intervals and He-Ne laser rays (5 Min.) and their interaction on the percentage of fatty acids composition of Caster bean plant (Main of two seasons of 2012& 2013).

Irrigation Intervals days	He-Ne laser Min.	Saturated fatty acids			Unsaturated fatty acids					
		Palmitic	Stearic	Total	Linoleic	Oleic	Ricinoleic	Hexaecenoic	Octadecenoic	Total
control	0	8.69	5.89	14.58	16.20	14.85	50.27	0.0	4.10	85.42
5	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	7.20	5.89	13.09	16.20	14.61	49.02	1.77	3.46	85.06
10	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	7.63	5.36	13.26	15.74	13.32	51.75	2.33	3.60	86.74
15	0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*Some treatments cannot complete its life cycle

It is event from table (5). The saturated fatty acids palmitic, which constituent a substantial component of the saturated fatty acid in costar oil were decreased slightly by prolonging irrigation intervals under (He-Ne) rays treatments. The reduction reached 17.14 % and 12.19 % when plants irrigated every 5 and 10 days respectively.

As for the unsaturated fatty acids, ricinoleic is the major one and laser had no marked effect on this respect. Prolonging irrigation intervals up to 10 days combined with laser treatment a decrease was observed in linoleic and oleic acid compared with the control plants. The highest increments in both ricinoleic and Hexaecenoic acids were obtained by prolonging irrigation intervals up to 10 days interacted with laser. Generally, control plant has the highest palmitic acid and the lowest Hexaecenoic acid.

4. Discussion

It is clearly apparent from previous work that prolonging irrigation intervals for caster bean plant decreased plant height, leaf area as well as fresh and leaves dry weight. The decrease in plant length under water stress condition was obtained by [13] on *Japane ment*, [14] on *Amaranthus* species; [15] on cotton (*Cossypium hirsutum*), where they indicated that such decrease in cell elongation resulting from water shortage which led to a decrease in each of cell turgor, cell volume and eventually cell growth and/or due to blocking up of xylem and phloem vessels, thus hindering any translocation.

In this respect [16] also, reported that drought has adverse effect on plant height of *Catharanthus roseus* plants. Also, [17] added that there is an inverse relationship between increasing the severity of drought and the number of leaves formed on stressed plants. Such reduction in number of leaves due to water stress attributed to its direct effect on cell division which arose from reduction on nucleic acid synthesis and/or enhancement of its break down [18]. The reduction in leaves number in response to stress can also be attributed to enhancement of leaf abscission due to hormonal imbalance which arose from increased ABA and decreased IAA levels in treated plants [19] if compared to untreated plants.

The drought also controls the fresh weight, the decline in fresh weight due to the decrease in water contents of stressed plant cell and tissue, which lose their turbot and thus shrinks. The decrease in both fresh and dry weight of stressed shoots reveled the influence of water on stimulatory and regulating the photosynthetic enzymes which influence both the fresh and dry weight production [20]. In this respect, [21] reported that, the water stress caused major reduction in plant height, leaves number, leaf area index, fresh and dry weight of cotton (*Gossypium hirsutum*) plants and some Cucurbitacea members. They added that the growth of plants is controlled by roles of cell division and enlargement, as well as by the supply of organic and in inorganic compounds required for the synthesis of new protoplasm and cell wall and water stress not only affect morphological appearance but also changes biomass ratio.

Our results are in harmony with those obtained by [22] they revealed that caster bean plants that were irrigated every 5 and 10 days, caused increase in plant growth. However high soil water potential throughout the growing season is necessary to maintain crop growth and high economic yield. Moreover [23] on *Hibiscus sabdariffa L.* cited that addition of adequate water decreased absisic acid and increased cytokinins, gibberellins and indole acetic acid hormone which reflecting good plant growth. Under water stress conditions 15 days irrigation intervals both relative water content and osmotic potential decreased. These results were in harmony with [24] on Soybean leaves however; this may be due to the reduction of photosynthetic pigments under water stress conditions compared with well watered plants. Thus water is essential in the maintenance of turgor which is essential for cell enlargement and growth.

The present study show that the exposure to water stress decreased total carbohydrate %, this results hold true with the finding of [25] on faba bean leaves. The decline of poly saccharides in response to prolonging irrigation intervals were similar to those results obtained by [26] and [27]. They attributed this decline in carbohydrate to soil water deficiency which helps certain chemical stimulus absisic acid (ABA) through zylem vessels to leaves of stressed plants which led to stomatal closure and this leading to decline in growth. Also [28] on *Gossypium hirsutum*)

recorded that the leaves sucrose levels were increased, but starch level was decreased. Our results of the previous work showed the same trend of [29], they published that a (He-Ne) ray was efficient on growth of *Ricinus communis* plants. Statistical analyses showed increase in plant length, stem diameter and number of leaves, this may be reflected the effect of these rays on cell division which continues to all parts of plant at vegetative stage or may be the main biological active gibberellic acid formation is promoted by red light (He-Ne laser) treatment.

The data also show that the increase of leaves number induced high fresh and dry weight of leaves, thus may be due to the role of gibberellic acid in cell elongation, where GA may cause elongation by induction of enzymes that weaken the cell wall [30]. In this respect [31] concluded that He-Ne laser treatment exhibited remarkable increase in leaf area and cytokinin content in gerbera leaves.

Total carbohydrate percentages of *Ricinus communis* leaves were increased by He-Ne laser treatment and surpassed the control plants. This may be due to the formation of GA [31] which promoted by red light that caused increase in soluble and non soluble carbohydrates [32]. The present study also shows that, He-Ne laser treatment decreased osmotic potential in plants followed by an increase in relative water content. This means that may be (He-Ne) treatment can withstand stress by increasing photosynthetic pigments, growth parameters IAA, GA, Cytokinin as mentioned by [31]. The high relative water content was attained by exposure the seeds to He-Ne rays followed by insignificance difference by those grown under drought condition (irrigated every 15 days). However, the tolerance of most plants to stress was due to their ability to build up high negative water and osmotic potential, so that it maintained the uptake of water from soil.

The present results of fatty acids constituents are in harmony with those obtained by [1], who cited that castor oil fatty acids are ricinoleic, oleic, linoleic, linolenic, stearic and palmitic acids, who added that ricinoleic acid is the major one.

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