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Potential of He-Ne Laser Irradiation and Iron Nanoparticles to Increase Growth and Yield of Pea

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Abstract: The field experiment was carried out during the two growing seasons of 2012/2013 and 2013/2014, at the experimental farm, Faculty of Agriculture, Ain Shams University, Egypt, in order to investigate the effecting of the pre-sowing laser irradiation (He-Ne) combined with different concentrations of iron nanoparticles on growth and yield of pea. Seed laser irradiation treatments were the control (untreated seeds) and 2, 5 and 10 exposure min. Iron oxide nanoparticles experiment by using different concentrations of Fe_3O_4 Nps (0, 100, 300, 500) in addition to 500 ppm chelated iron. Leaf area, dry weight per plant, leaf chlorophyll reading, Fe and Mn, pod protein, pod number and yield per feddan were determined. Results indicated that pre sowing seed He-Ne laser irradiation for 10 min. exposure time combined with 300 INPs increased significantly all tested parametes. In this study, it could be concluded that separately or combined He-Ne laser irradiation at 10 minutes and 300 ppm of iron oxide nanoparticles (Fe_3O_4) gave the best growth parameters and highest yield, compared to the control.

Key words: Pea · Laser irradiation · Iron · Nanoparticles · Growth · Yield

INTRODUCTION

Pea plant (*Pisum sativum* L.) is one main herb belongs to *Fabaceae* family which is characterized by a lot of biochemical compounds. Peas are cultivated for the fresh green seeds, tender green pods, dried seeds and foliage [1]. Green peas are eaten cooked as a vegetable and are marketed fresh, canned, or frozen while ripe dried peas are used whole, split, or made into flour [2]. Leaves are used as a pot herb in Burma and parts of Africa [3]. Peas contain high protein concentration 15.5-39.7% [4].

Helium Neon laser (He-Ne) is a type of gas laser that gain medium consists of a mixture of helium and neon (10:1) inside of a small bore capillary tube, usually excited by a DC electrical discharge. Absten [5] reported that the nature of the interaction between laser light and biological tissue (plant tissue) can be described in terms of reflection, scattering, transmission and absorption. El-Tobgy *et al*. [6] confirmed that the red light laser can induce GA_3 which promotes the complex cycle of GA_3 formation, while the polychromatic light (sunlight) cannot induce this effect. Chinese pine seeds irradiated by He-Ne laser led to an enhancement for seed vigor, germination rapidness, root system expansion and fresh seedling mass [7]. Cucumber seeds irradiated by He-Ne laser stimulated embryonic root growth, photosynthesis rate and peroxidase activity [8]. Also, Osman *et al*. [9] studied effect of He-Ne laser at different exposure periods (5, 10 and 20 min.) with power density of 95 mW/cm^2 on two plants (*Feoniculum vulgare* Mill and *Coriandrum sativum* L.). In most cases, the highest plant heights and number of branches per plant were obtained from the treatment of 20 min exposure. On the other hand, Aguilar *et al.* [10] reported that laser irradiation could cause enhancement of enzyme activities.

The scientific reports have demonstrated that irradiation of seeds of different plants; resulted in an increase of the energy potential of seeds, accelerated maturity makes plants precocious, increased resistance to diseases, influence on alpha-amylase activity and the

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concentration of free radicals in the seeds of several plants which could deactivate seed dormancy, improved germination rate; germination percentage, germinating energy and uniformity of germination, increased seed vigor, impact on the respiration process, photosynthetic activity and chlorophyll and carotenoid contents of seedlings from irradiated seeds [7, 11]. Additionally, El-Kereti [12] found that the wet sweet basil seeds which were exposed to He-Ne laser for 2 min. had the highest yield of herb compared to other treatments. On the other hand, the oil sample obtained from the plants which cultivated from wet seeds exposed to He-Ne laser for 2 min. had a better oil quality than that obtained in other treatments. El–Kereti *et al.* [13] investigated the effect of different laser sources (He-Ne and Ar) on improvement of sweet basil growth and yields separately or combined with nano metal oxides (Fe_3O_4).

Nanotechnology opens a large scope of novel application in the fields of biotechnology and agricultural industries, because nanoparticles (NPs) have unique physicochemical properties, i.e., high surface area, high reactivity, tunable pore size and particle morphology [14]. It permits broad advances in agricultural research, such as reproductive science and technology, conversion of agricultural and food wastes to energy and other useful byproducts through enzymatic nano bioprocessing, disease prevention and treatment in plants using various nanocides [15]. Nanotechnology helps to agricultural sciences and reduces environmental pollution by production of pesticides and chemical fertilizers by using the nano particles and nano capsules with the ability to control or delay delivery, absorption and more effective, environmentally friendly and production of nano-crystals to increase the efficiency of pesticides for application of pesticides with lower dose. Nano nutrients have small particle size (3 – 50 nano meters), with large surface area when sprayed on to leaves it delivers all nutrients to chlorophyll directly, it enhances efficiency of photosynthesis hence plants grow well and give high yields, good keeping qualities, higher resistance to pests and diseases due to presence of silica in the formulation. Researchers from their findings suggested both positive and negative effects on plant growth and development and the impact of engineered nanoparticles (ENPs) on plants depends on the composition, concentration, size and physical and chemical properties of ENPs as well as plant species [14].

Metal nanoparticles are considered to modify physiological and biochemical processes in plants thereby affecting their germination and growth favorably or otherwise. It can induce the efficiency of chemical energy production in photosynthetic systems The chlorophyll in photosynthetic reaction center binds to the NPs and nano crystals there by forming a novel hybrid system that may produce ten times more excited electrons due to Plasmon resonance and fast electron–hole separation the enhancement mechanisms may help in the design of artificial light- harvesting systems [16]. The main advantage of using magnetic nanoparticles is that they allow a very specific localization of the particles to release their load, which is of great interest in the study of nanoparticulate delivery for plants [15]. Moghadam *et al.* [17] tested the effect of iron nanofertilizer on spinach and reported that nano fertilizer causes 58 and 47% increase in wet weight and maximum leaf surface index, respectively comparing to use of no fertilizer. Iron nano fertilizer led to maximum value of aerial organs dry weight for sugar beet type and minimum value was obtained for treating both types of spinach without using fertilizer. Studying leaf area index trend and growth rate diagrams indicate that nano fertilizer has a positive effect on all plant growth steps. Bakhtiari *et al.* [18] also studied the effect of different concentrations of iron nanoparticles (0, 0.01%, 0.02%, 0.03% and 0.04%) on wheat, they reported that the highest values of spike weight, 1000 grain, biologic yield, grain yield and protein content were achieved in 0.04% Fe concentration and the lowest values were achieved in the control.

The aims of this study is to study the effect of iron oxides nanoparticles on the yield and growth of *Pisum sativum* at different concentrations (0ppm, 100 ppm and 300 ppm and 500 ppm and 500 ppm chelated iron, in addition to study the effect of irradiation by (He-Ne) at different exposure periods (0, 2, 5, 10 min) combined with different concentrations of nano particles on the vegetative growth, yield, biochemical and mineral elements contents.

MATERIALS AND METHODS

The field experiment was carried out during the two growing seasons of 2012/2013 and 2013/2014, at the experimental farm, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt, in order to investigate the effecting of the pre-sowing laser irradiation (He-Ne) combined with different concentrations of iron nanoparticles on growth and yield of pea (*Pisum sativum* L.). Seeds of pea “Master-B” cultivar were sown on the 1st of September 2012/2013 and 2013/2014 seasons. The area of the experimental plot was

9 m^2 consisted of three rows, each row was 4.3 m length and 0.7 m width. The plant distance was 15 cm apart on one side. Cultural management, disease and pest control programs were followed according to the recommendations of the Egyptian Ministry of Agriculture.

The Experimental Treatments

He-Ne Laser Irradiation: Seed He-Ne laser irradiation (wavelength 632.8 nm, power density 75.0 mW, beam diameter 1.0 mm model Griat, U.S.A) treatments, i. e. control, periods 2, 5 and 10 min exposure. Laser applications have been carried out at the National Institute of Laser Enhanced Sciences (NILES), Laser Technology Centre, Cairo Univ., Giza, Egypt. Laser light emitted from the standard device is generally characterized in terms of power (in units of watts and milliwatts). The power levels vary from laser type to another [19]. The energy (in joules) is defined as the power × time interval during which it is emitted, i.e. energy (Joules) = power (W) × time (sec). The laser beam has other parameters which are especially important [20, 21]. For most visible laser types, the laser beam power may be characterized by a Gaussian curve in two dimensions, (a Gaussian spot). For this type of beam, the spot size (radius = r) is defined as the distance from the center of the beam to the point on the edge where the power density of $1/e^2$ of total power. The beam diameter is then twice the spot size. The power density or irradiance is then defined as the power applied per unit area of target tissue. Fluence is defined as the energy applied to an area of target tissue, i.e. Fluence = power density (W/cm^2) x time (sec). Choosing the proper power, spot size, exposure time, mode and wavelength enables the worker to perform the task appropriately [19]. He-Ne laser of power 3mW was used for red light irradiation of pea seeds. The spot diameter of He-Ne laser was 2 mm, so we did not need to use a divergence lens to have the desired power density (75 mW/cm^2). The 2 mm spot diameter was enough for one seed exposure in each time.

Iron Nanoparticles: Iron oxide nanoparticles experiment performed by using different concentrations of Fe_3O_4 Nps (0, 100, 300 and 500 ppm) and bulk chelated iron (500 ppm).

Preparation of Iron Nanoparticles (INPs) Oxides: The Fe_3O_4 magnetic nanoparticles were prepared based on the co-precipitation of Fe^{3+} and Fe^{2+} with a molar ratio of 3:2 under the aqueous ammonia (0.3 $mol.L^{-1}$) as precipitating agent [22]. Briefly, mixing ferrous chloride tetrahydrate and ferric chloride in 100 ml deionized water was done under vigorous stirring and with the protection of nitrogen to prepare total concentration of 0.015 $mol.L^{-1}$ ferrite solution. Aqueous ammonia (0.3 $mol.L^{-1}$) was dropped slowly into the mixture solution until the pH value was titrated to 11.0 [23]. It can be observed that the solution became black due to the formation of Fe_3O_4 particles. The sample was kept reacting in water bath at 50°C for 30 min under vigorous stirring and dispersed in ultra-sonicator for another 30 min. The black mixture was then aged at 80°C for 1 h. After that, the prepared product was transferred to a large beaker and dissociated from liquor with the ferromagnet. Finally, the Fe_3O_4 magnetic nanoparticles were washed with distilled water until the pH value descended to 7.0 and were collected in the vacuum drying chamber after being dried for 4 h at 60°C.

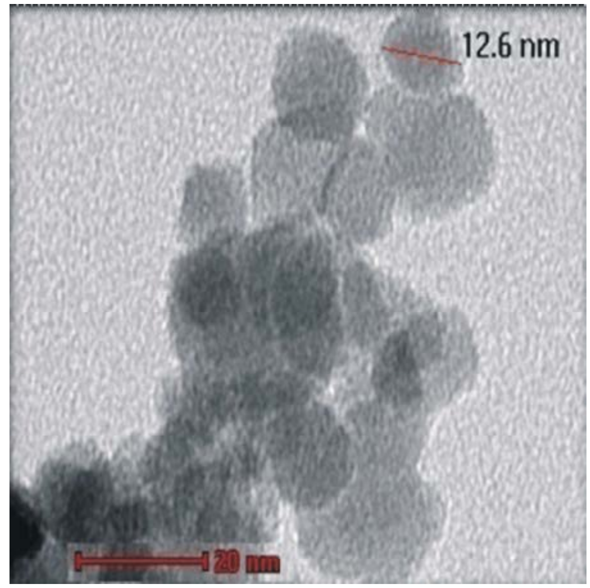

Fig. 1: TEM imaging of the prepared Fe_3O_4 NPs revealed a spherical shape of the particles, with an average size of around 12.6 nm

Characterization of Fe_3O_4 (Magnetic Nanoparticles): The size and shape of Fe_3O_4 magnetic nanoparticles were observed directly by TEM (Transmission Electron Microscope). The electron acceleration voltage was 60 kV. The sample was prepared as follows: a few prepared Fe_3O_4 magnetic nanoparticles were put in absolute alcohol and decentralized in an ultrasonicator for 20 min. It was dropped to carbon-coated copper grid and dried at room temperature. Figure (1) show the synthesize magnetite nano particles revealed a spherical shape and an average particle size of 12.6 nm.

The Experimental Design: The experiment was laid out in a split plot design with three replicates. Seed laser irradiation (He-Ne) treatments were assigned in the main plots but foliar applications of iron nanoparticles oxides were distributed in the sub-plots.

Sampling and Collecting Data

Vegetative Characteristics: Three plants were chosen at random from each plot at 75 days from sowing to study the following parameters; leaf area and dry weight per plant [24]. Leaf chlorophyll reading (SPAD) was determined using the recently full expanded mature upper leaf of 5 plants in the middle row per plot. A digital chlorophyll meter, Minolta SPAD-502, (Minolta Company, Japan) was used.

Each ashed sample was dissolved in 0.1 N HCl and the volumes were completed to the mark (100 ml). This solution was used for the quantitative determination of Fe and Mn by atomic absorption spectrophotometer (Thermo Jarrell Ash Model AA SCAN1). All the determinations were carried out with an air-acetylene gas mixture at a rate of 5 L/min according to the method described by Reuter [25].

Yield and its component: pod number and yield per feddan were calculated. Pod total protein percentage (dry weight) was measured colorimetrically according to A.O.A.C. [24].

Statistical Analysis: Data of the two seasons were arranged and statistically analyzed using Mstatic (M.S.). The comparison among means of the different treatments was determined, as illustrated by Snedecor and Cochran [26].

RESULTS AND DESCUSSION

Leaf Area: Data presented in Table (1) indicated that there were significant differences in leaf area among the tested He-Ne laser irradiation. In this respect, seed pea plants treated with pre-sowing He-Ne laser irradiation for 10 min. exposure time showed the highest leaf area value compared with the other He-Ne laser irradiation treatments during the two seasons of study. This result may be attributed that seed pre-treated with laser have to absorb more energy from the surroundings than that of the control in the course of the individual development because laser broke the kinetic equilibrium of germination seeds and enhanced the internal energy of seeds. It was reported that as an open system, the living organism must exchange energy with the surrounding system to keep its high order state of the system when this order is broken [27]. Consequently, the biochemistry and physiology metabolisms of the plants pre-treated with laser were accelerated and finally plant growth was enhanced and leaf area and biomass were augmented notably [28]. Koper *et al*. [29] on tomato reported that laser light significantly increased leaf area. The stimulating effect of laser radiation was also observed on leaf area of spring wheat plants [30]. In addition, The increase in leaf area on caster bean 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 [31].

Concerning the foliar application of Fe_3O_4 NPs, 300 ppm concentration resulted in the highest significant leaf area value, while the concentration of 0 ppm gave the lowest value which did not differ significantly from the bulk source ferric cholorid at 500 ppm in the two seasons. This may be attributed to that iron has higher penetration efficiency, higher tissue mobility and it is an essential for the synthesis of chlorophyll and photosynthesis. In this respect, El Kereti *et al.* [12] found that leaf area of *Ocimum basilicum* had a significant increase for nano iron oxides concentrations compared to the control plants.

Respecting the interaction, combined treatment of He-Ne laser irradiation for 10 min. exposure time with 300 and 500 ppm INPs increased leaf area. The analysis of flag and penultimate leaves indicates an ability of laser light to increase the blade area. This effect has an undoubted related to the biostimulating influence of short exposure to laser light, observed also in barley [32].

Plant Dry Weight: The seed pea plants exposure with pre-sowing He-Ne laser irradiation for 10 min gave the highest values for plant dry weight as compared with other exposure periods (0, 2, 5 min.) (Table 2), these results were true in both seasons. The seed stimulation through He–Ne laser radiation not only affects the thermodynamic parameters of germinating seeds but also the physiological metabolism and development of seedlings. Therefore, the role of laser pre-treatment was a long-term effect. The underlying mechanism might be the phytochrome was probably induced by the He–Ne laser radiation of 632.8 nm because its wavelength is close to the absorbing wavelength of Pr (phytochrome of red light) subsequently, the activities of related enzymes, which were modulated by phytochrome, could be enhanced and phytochrome-mediated responses, e.g. the decomposition rate of lower entropy macromolecule, are accelerated.

Table 1: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on leaf area (cm^2) of pea plants in the two seasons (2012/2013 and 2013/2014)

He-Ne laser period (minute)	Iron nanoparticles (ppm) 0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	30.9 gh	39.1 d-g	44.6 bc	35.8 e-h	28.5 h	35.8 D
2	34.0 f-h	40.3 c-f	46.1 bd	40.2 c-f	40.2 c-f	40.1 C
5	38.0 d-g	43.2 b-e	47.9 bc	41.2 c-f	47.9 bc	43.7 B
10	50.8 b	60.3 a	60.5 a	62.9 a	48.1 bc	56.5 A
Mean	38.4 C	45.7 B	49.8 A	45.0 B	41.2 C	
	2nd season					
0	28.6 i	30.9 hi	48.2 bc	41.3 d-f	39.2 e-g	37.6 D
2	34.0 gh	35.9 f-h	60.6 a	48.0 bc	40.2 e-g	43.7 C
5	43.3 cd	39.2 e-g	60.2 a	47.2 bc	40.3 e-g	46.1 B
10	44.7 ce	46.1 bd	62.9 a	50.7 b	40.3 e-g	48.9 A
Mean	37.6 C	38.0 C	58.0 A	46.8 B	40.0 C	

Means followed by different letters are significantly different at P $\leq$ 0.5 level; Duncan's multiple range test

Table 2: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on plant dry weight (g) per plant of pea in the two seasons (2012/2013 and 2013/2014)

He-Ne laser period (minute)	Iron nanoparticles (ppm) 0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	6.02 j	7.20 fg	9.81 b	6.67 hi	6.45 i	7.23 D
2	7.08 gh	7.50 ef	9.99 b	7.65 e	7.01 gh	7.83 C
5	8.5 d	8.85 cd	9.95 b	8.48 d	8.49 d	8.85 B
10	10.00 b	9.00 c	10.55 a	9.77 b	8.89 cd	9.64 A
Mean	7.88 C	8.14 B	10.08 A	8.14 B	7.71 C	
	2nd season					
0	5.80 m	6.85 l	11.65 d	7.01 l	5.70 m	7.410D
2	6.7 l	7.66 k	12.67 c	8.49 j	8.99 i	8.90 C
5	9.26 i	9.20 i	13.75 b	9.65 h	9.81 gh	10.34B
10	11.15 e	10.06 g	14.40 a	13.43 b	10.55 f	11.93A
Mean	8.23 D	8.44 C	13.14 A	9.65 B	8.76 C	

Means followed by different letters are significantly different at P $\leq$ 0.5 level; Duncan's multiple range test

Accordingly, the entropy and internal energy of seeds were enhanced during seed germination. So the basis of the stimulation mechanism in plant physiological stage is the synergism between the polarized monochromatic laser beam and the photoreceptors [33]. Also, Metwally *et al.* [31] found that He-Ne laser rays improved Caster bean growth and help plants to complete its life cycle.

As for the effect of Fe_3O_4 NPs, the same data in Table (2) indicate that foliar application of 300 ppm Fe_3O_4 NPs concentration produced the highest values of plant dry weight compared with other tested foliar applications during the two seasons of growth. These results also were demonstrated by Sheykhbaglou *et al.* [34] who reported that the iron oxide NPs have been found to increase soybean pod and leaf dry weight. It was added that iron deficiency inhibited leaf growth, cell number, size and cell division, as well as chlorophyll, protein, starch and sugar content. Thus, the fresh and dry weight of herb could be decreased. Iron is necessary for the biosynthesis of chlorophyll and cytochrome, besides the function of iron in the metabolism of chloroplast RNA, leading to increase in the biosynthesis materials (produced and accumulated), consequently, the growth was enhanced [35].

Respecting the effect of the interaction, the same data indicate that He-Ne laser irradiation for 10 min. exposure time combined with 300 ppm INPs foliar spray led to the highest plant dry weight. These results show that the interaction between the studies of two factors has a positive effect on the characteristics of general growth.

Chlorophyll Reading: Data in Table (3) showed that the irradiation of He-Ne laser increased the chlorophyll reading whenever the exposure time increased along the two seasons. The He-Ne laser irradiation could raise the activities of SOD, CAT and APX enzymes in plants which

Table 3: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on chlorophyll reading (SPAD) of pea plants in the two seasons (2012/2013 and 2013/2014)

	Iron nanoparticles (ppm)					
He-Ne laser period (minute)	0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	35.6 j	43.3 h	43.4 h	44.4 f-h	42.9 h	41.9 D
2	38.3 i	43.8 gh	46.3 ce	44.2 gh	43.9 gh	43.3 C
5	38.7 i	46.0 ce	49.8 b	45.5 d-f	45.1 e-g	46.7 B
10	39.3 i	46.5 cd	53.5 a	47.1 c	47.0 c	44.2 A
Mean	38.0 C	44.9 B	48.3 A	45.3 B	44.7 B	
	2nd season					
0	35.6 l	39.3 j	46.1 d	38.3 k	38.7 jk	39.6 D
2	43.3 hi	45.1 ef	47.1 c	42.9 i	43.8 g-i	44.4 C
5	43.4 g-i	46.0 d	47.0 c	45.6 de	43.9 gh	45.2 B
10	44.4 fg	46.3 cd	53.5 a	49.8 b	44.2 f-h	47.6 A
Mean	41.7 C	44.2 B	48.4 A	44.2 B	42.7 BC	

Means followed by different letters are significantly different at $P \leq 0.5$ level; Duncan's multiple range test

resulted in accelerated the plant physiological metabolism and increased plant growth [36]. Additionally, suitable doses of laser irradiation improved the concentration of chlorophyll of Basil [37]. Also, Sebanek *et al.* [38] found an increase of chlorophyll content in the leaves of pea plants which were grown from laser light irradiated seeds.

Also, the concentration of Fe_3O_4 NPs at 300 ppm resulted in a highest significant increase of chlorophyll reading, while 0 ppm concentration gave the lowest value in the two seasons. These results are in harmony with the findings of Jia *et al.* [39] who reported that the foliar treatment of basil with varying concentrations of iron oxides NPs significantly increased the chlorophyll content. Also, El kereti *et al.* [13] found that the highest concentration of Fe_3O_4 NPs (30 mg/L) gave the maximum chlorophyll content because of whenever iron concentration increased will increase the chlorophyll production consequently, enhance the growth rate. This increase is as a result of the function of iron as a stimulator of the activity of chlorophyll synthesis enzymes. In addition previous studies indicated that iron function in the synthesis of a specific kind of RNA that in turn regulates chlorophyll synthesis. Iron, a cofactor for approximately 140 enzymes [40] that plays an important role in the photosynthetic reactions and it is one of the essential elements for growth and development of plants, including chlorophyll synthesis, thylakoid synthesis and chloroplast development [41]. In addition, Ghafari and Razmjoo [42] found that nano-iron oxide application produced the highest chl a, chl b and total chlorophyll contents on wheat. Iron application increased chl a, b and total chlorophyll contents as compared with the control, but there was no marked differences between iron rates on these traits.

Regarding the effect of the interaction between different doses of He-Ne laser irradiation and foliar application of Fe_3O_4 NPs, data show that pre sowing He-Ne laser irradiation for 10 min combined with 300 ppm Fe_3O_4 NPs foliar applied gave the hieghst values of chlorophyll reading in the two seasons. The combined treatment of different doses of He-Ne laser irradiation without foliar application of Fe_3O_4 NPs often reported the lowest values and had the same effect of the combined application of He-Ne laser and the 500 ppm of the bulk. In this concern, cucumber seeds irradiated by He-Ne Laser increased the quantity of the photosynthetic products and the content of plastid pigments in leaves [8].

Iron and Manganese Contents: Data in Tables (4 and 5) showed that laser irradiation resulted in a significant increase of Fe and Mn concentrations and they increased with all lasers used treatments. Our results were in close agreement with the findings of Truchlinski *et al*. [43] who reported that the Triticale seeds irradiated by laser caused the increase of mineral element contents, notably that of Mn and Fe. In the case of laser treatment the stimulation was due once again to the increasing energy supply of seeds. In that case the photon energy of laser radiation was absorbed by chlorophyll and directly affected the photosynthetic intensity. The effect of enhancement of preliminary seeds water soaking could be attributed to water inhibition by cells. That should have made the cell membrane thinner [44]. In this respect, Aly and El-Faramawy [45] found that chemical composition on *Vicia faba* leaves significant increased by He-Ne irradiation treatment.

Table 4: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on iron (ppm) of pea leaves in the two seasons (2012/2013 and 2013/2014)

	Iron nanoparticles (ppm)					
He-Ne laser period (minute)	0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	200 m	214 l	256 h	267 g	212 l	230 D
2	227 k	247 i	352 e	352 e	239 j	284 C
5	242 ij	285 f	389 c	362 d	354 e	326 B
10	256 h	287 f	451 a	366 d	428 b	358 A
Mean	232 E	258 D	362 A	337 B	308 C	
	2nd season					
0	164 j	237 fg	200 h	204 h	187 i	198 D
2	227 g	257 de	242 f	229 g	245 ef	240 C
5	249 ef	327 b	327 b	282 c	278 c	293 B
10	263 d	336 b	351 a	289 c	279 c	304 A
Mean	226 B	289 A	280 A	251 B	247 B	

Means followed by different letters are significantly different at $P \leq 0.5$ level; Duncan's multiple range test

Table 5: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on manganese (ppm) of pea leaves in the two seasons (2012/2013 and 2013/2014)

	Iron nanoparticles (ppm)					
He-Ne laser period (minute)	0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	49 kl	52 ij	68 d	56 g	48 l	55 D
2	50 k	59 f	73 c	67 d	56 gh	61 C
5	51 jk	59 f	79 a	74 bc	65 e	66 B
10	54 hi	66 de	81 a	81 a	75 b	71 A
Mean	51 D	59 C	75 A	70 B	61 C	
	2nd season					
0	46 k	44 kl	41 l	40 l	60 j	46 D
2	60 j	110 fg	111 f	100 h	61 j	89 C
5	94 i	161 a	135 d	112 f	100 h	121 B
10	108 g	162 a	142 c	147 b	130 e	138 A
Mean	77 E	119 A	107 B	100 C	88 D	

Means followed by different letters are significantly different at $P \leq 0.5$ level; Duncan's multiple range test

Table 6: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on protein percentage (dry weight basis) of pea pods in the two seasons (2012/2013 and 2013/2014)

	Iron nanoparticles (ppm)					
He-Ne laser period (minute)	0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	28.4 cd	30.3 b-d	35.6 a-c	30.3 b-d	30.6 b-d	31.1 C
2	30.6 b-d	31.8 a-d	36.9 a-c	33.4 a-c	31.3 a-d	32.8 C
5	35.3 a-c	32.5 a-d	37.5 a-c	36.9 a-c	34.1 a-c	35.3 B
10	36.3 a-c	36.6 a-c	39.1 ab	40.3 a	35.9 d	37.6 A
Mean	32.7 C	32.81 C	37.3 A	35.2 B	33.0 C	
	2nd season					
0	26.1 d-f	20.3 f	31.3 b-e	25.3 ef	27.2 d-f	26.0 D
2	29.7 c-e	31.6 b-e	33.4 a-d	30.0 b-e	31.6 b-e	31.3 C
5	30.9 b-e	35.6 a-c	33.4 a-d	39.1 a	35.0 a-c	34.8 B
10	31.9 f	36.8 a-c	37.5 ab	39.4 a	36.9 a-c	36.5 A
Mean	29.7 A	31.1 A	33.91 A	33.5 A	32.7 A	

Means followed by different letters are significantly different at $P \leq 0.5$ level; Duncan's multiple range test

The foliar application of 300 ppm INPs gave the highest iron and manganese content in pea leaves compared with other foliar application treatments in the first season. Application of 100 or 300 ppm INPs foliar application gave the highest iron content in the second season while, foliar application of 100 ppm INPs gave the highest manganese content in pea leaves in the second season. In this respect, Iron oxides NPs have a high reactivity because of its high surface area. These features simplify the absorption of fertilizers that produced in nano scale [46]. In this respect, Vattani *et al.* [47] found that application of nano-chelate levels increased accumulation of iron spinach leaves.

With regard to the effect of the interaction between He-Ne laser irradiation and INPs foliar application, He-Ne laser irradiation for 10 min. exposure time combined with 300 ppm INPs foliar application gave the highest iron content compared with other interaction treatments in the two seasons. The He-Ne laser irradiation for 5 or 10 min. exposure time combined with 300 or 500 ppm INPs foliar application gave the highest manganese content in the first season while, He-Ne laser irradiation for 5 or 10 min. exposure time combined with 100 ppm INPs foliar application gave the highest manganese content in the second season.

Protein Percentage of Pods: Increasing protein percent was generally observed, due to pre sowing He-Ne laser irradiation treatments comparing with control plants (Table 6). Application of pre sowing He-Ne laser irradiation for 10 min gave the maximum increase in protein content in the pods in both seasons. This result may be attributed that, many enzymes control the plant growth and development so the basis of this mechanism might be the existence of phytochrome which are sensory photoreceptors that regulate growth and development of plants in the response to light stimuli [48].

The data of our work revealed that protein content increases through the application of 300 ppm INPs in the first season while, insignificantly in the second season. Iron and manganese are two important elements in enzyme structure involved in amino acid biosynthesis and thus amino acids are the base of protein synthesis [49].

The interactions among the studied factors, generally, indicated that He-Ne laser irradiation plus INPs foliar application treatments at all used concentration gave the highest values of protein content of pod compared with control plants in both seasons.

Pod Number and Yield: Data in Tables (7 and 8) showed that He-Ne laser irradiation resulted in significant increases of number of pods and yield and they were reduced as long as the exposure period was reduced leading to the lowest results at 0 min exposure.

Our results indicated that foliar application with iron NPs at 300 ppm increased the number of pods in the two seasons. Respecting pod yield same trend was observed, illustrated that foliar application of Fe_3O_4 NPs with 300 ppm concentration had a significant positive effect on pod weight, while other treatments didn't differ significantly from each other as was obvious in the second season. This is due to in foliar spray through iron coupled with direct uptake of ferrous iron by leaves resulting in higher production of chlorophyll, dry matter and higher ferrous iron content in leaves [50]. This obvious enhancement in morphological characterization may be due to the role of iron in plant metabolism, such as activating catalase enzymes associated with superoxide dismutase, as well as in photorespiration, nitrogen fixation and the glycolate pathway [35]. Furthermore, Sheykhbaglou *et al.* [34] reported that the iron oxide NPs have been found to increase soybean pod and leaf dry weight. Also, Fe_3O_4 NPs have been reported as facilitators for iron and photosynthesis transfer to the leaves of peanut [51]. Finally the interaction of plant cell with the nanoparticles resulted in modification of plant gene expression and associated biological pathways which ultimately affect plant growth and development [15]. Iron is the most important rare elements which is required for plants. It is a key element in cell metabolism and it is involved in photosynthesis, respiration, enzymes activity, etc. Iron is essential for plants growth and under Fe deficiency conditions, chlorophyll production disturbs and plants show interveinal chlorosis, which appears first in younger tissues because iron is not easily translocated inside plant body [18].

Concerning the interaction, all combination of iron had a good effect with increment the exposure time of He-Ne laser irradiation on pod number and yield. These works support opinion about the relation of the He-Ne laser and GA_3 formation. This may be due to coherent light (red light laser) the only possible way to promote GA3â hydroxyls gene S3h1 expression. The positive relationship between irradiation and flowering may be due to GA which has been seen to promote seed germination [52], while a gibberellin derivative was found to promote floral induction in *Chionochloamacra* [53].

Table 7: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on number of pods per plant of pea in the two seasons (2012/2013 and 2013/2014)

	Iron nanoparticles (ppm)					
He-Ne laser period (minute)	0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	8.2 i	9.7 j	9.4 k	9.6 j	10.5 i	9.5 D
2	10.2 i	11.2 g	12.7 e	10.9 h	11.1 g	11.2 C
5	10.3 i	12.7 e	14.7 c	11.5 f	12.1 f	12.3 B
10	12.7 e	15.2 b	17.7 a	15.4 b	13.5 d	14.9 A
Mean	10.4 C	12.2 B	13.6 A	11.9 B	11.8 B	
	2nd season					
0	8.1 i	9.2 hi	12.6 cd	10.6 e-g	9.9 gh	10.1 D
2	9.6 gh	10.2 f-h	13.4 bc	11.1 ef	10.0 f-h	10.9 C
5	10.9 ef	10.8 ef	14.0 ab	11.6 de	10.8 ef	11.6 B
10	11.4 ef	12.7 cd	14.8 a	14.7 a	12.8 cd	13.3 A
Mean	10.0 D	10.7 C	13.7 A	12.0 B	10.9 C	

Means followed by different letters are significantly different at P ≤ 0.5 level; Duncan's multiple range test

Table 8: Effect of He-Ne laser irradiation and iron nanoparticles (INps) on yield (ton/Fed.) of pea plants in the two seasons (2012/2013 and 2013/2014)

	Iron nanoparticles (ppm)					
He-Ne laser period (minute)	0 INps	100 INps	300 INps	500 INps	500 chelated iron	Mean
	1st season					
0	1.52 j	1.99 i	2.54 e-h	1.97 i	2.27 g-i	2.06 D
2	1.76 i	2.54 e-h	3.37 ab	2.64 d-g	2.36 f-i	2.54 C
5	2.13 hi	2.70 d-g	3.45 ab	2.84 d-f	2.61 d-g	2.75 B
10	2.91 c-e	3.02 b-d	3.79 a	3.21 bc	3.44 ab	3.27 A
Mean	2.08 C	2.56 B	3.29 A	2.66 B	2.67 B	
	2nd season					
0	1.58 j	1.78 hi	2.13 d	1.67 ij	1.87 gh	1.81 D
2	1.69 ij	1.98 ef	2.51 b	2.03 d-f	1.91 e-g	2.03 C
5	2.03 d-f	2.35 c	2.51 b	2.06 de	1.93 ef	2.18 B
10	2.34 c	2.54 b	3.43 a	2.42 c	2.45 c	2.64 A
Mean	1.91 B	2.16 B	2.65 A	2.05 B	2.04 B	

Means followed by different letters are significantly different at P ≤ 0.5 level; Duncan's multiple range test

Recently, gibberellin derivatives have been shown to have significant effects on budbreak, vegetative shoot growth and flowering in *Metrosideroscollina* [54]. This conclusion is consistent with those of previous studies [55, 56] which reported that the dealing with laser induces to produce a large number of morphological and physiological changes in higher plants. In those studies, exposure to laser beam had shown a linear increase in growth, including dry mass gain, leaf size consequently the yield of many plants [57]. In this respect, Mohammed [58] reported the effect of He-Ne on sage plant (*Savia officinalis*) at different exposure time (0.2.5.10 min), found in laser at 5 min have higher yield of herb compared with the other types of laser, on other side in wet case which exposed to He-Ne laser for 10 min provided salvia plant of higher amounts of oil after cultivation.

The satisfactory effect of He-Ne laser irradiation and Fe_3O_4 NPs on pod number and yield might be due to increasing leaf area (Table 1), plant dry weight (Table 2), chlorophyll (Table 3), iron and manganese concentration (Table 4 and 5), as previously mentioned.

CONCLUSIONS

From our results we can conclude that combined treatment of He-Ne laser irradiation for 10 min. with 300 ppm of Fe_3O_4 NPs foliar application had a clear and a significant increase on growth and yield of pea along the two seasons.

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