# EFFECT OF LOW-INTENSITY LASER IRRADIATION ON FIELD PERFORMANCE OF MAIZE (*ZEA MAYS* L.) EMERGENCE, PHENOLOGICAL AND SEED QUALITY CHARACTERISTICS

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**Abstract.** Laser application in agriculture has attracted much interest due to the improvement of plant characteristics after laser pre-sowing seed treatment. In this study, *Zea mays* L. seeds were pre-irradiated by a single exposure to blue laser at different intensities (2 and 4 mW/cm<sup>2</sup>) and different irradiation times of 45, 65, 85, and 105s. The field emergence characteristics (i.e., emergence %, mean emergence time, emergence index, vigor index, growth parameters, and seed quality) of laser-induced *Zea mays* L. seeds were determined and compared with those of unirradiated seeds. A randomized complete block design (RCBD) was employed with three replications. The growth characteristics of the seeds exposed to laser for 85s showed significant improvements in terms of the seedling length and leaf number. Additionally, there were observable alterations in the seed emergence percentage (91.66%), emergence index (7.25), and vigor index (520.80) in the seeds irradiated for 85s at a laser intensity of 4 mW/cm<sup>2</sup>. In seeds irradiated for 85 s, both the oil and starch contents were increased by 6.33% and 73.11%, respectively. On the other hand, there was an increase in the protein content of maize seeds (18%) with increased laser intensity. In conclusion, the results of the present study demonstrated that 4 mW/cm<sup>2</sup> of blue laser and the irradiation period of 85s enhanced the emergence process of maize plants.

Keywords: low power, blue laser, exposure time, seed stimulation, physical priming

### Introduction

Laser radiation has been found application in all spheres of engineering due to its basic characteristics, such as coherence, monochromaticity, polarization, and power density. However, these attributes of laser radiation have also made it applicable in biological and agricultural sectors. Upon exposure to laser radiation, certain changes occur in the physiological state of plants and seeds and such changes can either induce or inhibit the development of such plant or seed based on the type of laser radiation used, as well as its intensity and wavelength (Hasan et al., 2020). There are several light-absorbing molecules in nature which mediates the response of organisms to change in the natural light environment. The changes in the light parameter influence various physiological processes (i.e. intra and inter-cellular differentiation, seed germination and seedling growth, photosynthesis, flowering, etc.) depending on the species and developmental

stage or studied organ (He et al., 2017). It has been reported earlier that light is an absolute factor that regulates seed germination in numerous plant species (Jala, 2011).

Several previous studies reported the effects of light on the germination and growth of seeds of plants (Hernandez et al., 2010; Aladjadjiyan, 2012; Qiu et al., 2017; Alsalhi et al., 2018). Some plant seeds require light to grow while the germination of some plant seeds can be inhibited in the presence of light (Hernandez and Michtchenko, 2011; Atif et al., 2020). According to Jala (2011), different levels of exposure time to light can have various influences on the germination parameters of different seeds. Furthermore, when low-power laser light is used on seeds, seedlings, and plants, it produces bio-stimulation (Hernandez et al., 2009, 2010, 2015; Perveen et al., 2010; Hernández and Michtchenko, 2011; Aladjadjiyan, 2012; Jia and Duan, 2013; Hasan et al., 2020).

Without a doubt, the 21<sup>st</sup> century needs to develop technologies to increase the global production of food since one of the main challenges of our time is to feed a growing world population (Foley, 2011; Muhammad-Muaz and Marlia, 2014; Hanafiah et al., 2019) which has been projected to grow by two to three billion of people by the year 2050. This implies that the world population will increase to 9000 million (FAO, 2011), with a simultaneous doubled increase in food cost, the problem of hunger will worsen. At the same time, the need to reduce damage to the environment is considered (Foley, 2011; Aziz et al., 2020a). All these, under the current context and the future climate changes, lead to threatened areas with high temperature (Harun and Hanafiah, 2018; Aziz et al., 2020b) and year forecasts are extremely unfavourable (Field et al., 2012; Fadillah and Marlia, 2016). Therefore, several methods have been introduced to increase crop yield including by laser irradiation.

Bioeffects due to low laser power in seed pre-planting and in seedlings or plants during their development have been confirmed by numerous studies using seeds of various crops (Osman et al., 2009; Aladjadjiyan, 2012; Hoseini et al., 2013; Kouchebagh et al., 2014a; Śliwka, 2014; Srećković et al., 2014). From the introduction of lasers in the '60s (Nasim and Jamil, 2014), its application began in biological systems (Bessis et al., 1962), dabbling to the agricultural sector as a bio-stimulatory element (BE) of plants and seeds with the ruby laser (Wilczek et al., 2005). He-Ne (Helium-Neon), Ar (Argon), Neodymium- YAG (Nd-YAG), carbon dioxide (CO<sub>2</sub>) and diodes (in different wavelengths -  $\lambda$ ) have been applied to conjecture the use of laser technology for preseding treatment or during some of the phenological stages of the crops (Chen et al., 2005; Hernandez, 2009; Hernandez et al., 2010, 2015; Hernández and Michtchenko, 2011; Aladjadjiyan, 2012; Śliwka, 2014). Laser technology has evolved and has been applied in bio-stimulation processes of seeds and plants. He-Ne laser is the most applied laser as BE of seeds and/or plants since its foray into agriculture until today (Aladjadjiyan, 2012; Hernández et al., 2020).

In this way, it is interesting to study the optimum laser irradiation parameters to produce favourable bioeffects and when applied in the agricultural sector, could, among other effects, increase the performance of products, stems and seeds, reduce the vegetative period, improve the quality of harvest, produce vigorous plants, improve the photosynthetic evolution of plants, break the seed dormancy, stimulate germination, recover the soil, and protect the environment against toxigenic mold and bacteria (Podleśna et al., 2015).

During the bio-stimulation of plants at various developmental phases of the plant, three kinds of photoreceptors (phytochromes, phototropins, and cryptochromes) have been found to absorb light at different wavelengths of 600-750, 320-500 and 500-630 nm,

respectively (Bouly et al., 2007; Levskaya et al., 2009). This implies that the seeds, based on their respective characteristics (physical, chemical, optical, thermal, photothermal, genotypic, phenotypic, etc.) will first take in the light energy and transform it into chemical energy for use in their subsequent growth phases (Jamil et al., 2013). As such, the kinetic equilibrium of seed germination processes can be altered by exposure to laser light, thereby increasing the internal energy of the seeds (Ferdosizadeh et al., 2013) and varying the response according to the seed type and cultivar.

Based on the previous studies, the physiological quality of certain crops has been improved using He-Ne (632.8 nm) and laser diodes (650, 660 nm) bio-stimulation. Among such crops are *Zea mays* L., *Triticum* (Joshi et al., 2012; Jamil et al., 2013), *Carthamus tinctorius* L., *Helianthus annuus* L., *Brassica napus* L. (Ashrafijou et al., 2010; Perveen et al., 2010), *Medicago sativa*, *Vicia faba*, *Lathyrus sativus* L. (Qi et al., 2002; Truchliñski et al., 2002; Wilczek, 2005), *Raphanus sativus* L., *Solanum lycopersicum* L. (Álvarez et al., 2011; Jia and Duan, 2013), *Acacia farnesiana* L., *Ricinus communis* L. (Soliman and Harith, 2009), *Balanites aegyptiaca*, *Celosia argéntea*, *Beta vulgaris* L. (Metwally et al., 2013; Podleśna et al., 2015). Germination is the initial stage of development in the growth of crops. During this stage, the important seed quality parameters that can affect the plant status are the germination rate, seed vigor, and germination uniformity (Mohssen-Nasab et al., 2010). Thus, this study aims to determine the effect of different intensity of blue laser irradiation on maize seeds, in terms of their field emergence parameters, growth, and seed quality.

### **Materials and Methods**

### Study Area

This research was conducted in the experimental field located at the Faculty of Agriculture, University of Baghdad and Laser Laboratory, Institute of Laser for Postgraduate Studies, University of Baghdad (33° 16′ 26″ N, 44° 22′ 39″ E). The experimental site has a hot dry climate in summer season in 2018, the average temperature was in the range of 17.1°C to 31.6°C, with an annual average rainfall of 284.2 mm and average humidity of 45%. Soil samples were randomly collected from the field and transferred to the lab to measure the physical-chemical parameters. The soil was slightly alkaline pH (7.2), salt-free (electrical conductivity 3.8 meq/100 g soil), nitrate and ammonia nitrogen concentrations of 0.0017% and 0.009%, respectively, very high P assimilation rate (43.2 mg/kg), and high K (1.6 mg/L). The soil texture was silt (28.9 g/kg), clay (38.5 g/kg) and sand (32.6 g/kg).

# Laser Irradiation Experiment

A blue laser source of 100 mW power (410 nm) was used in this study. A 100 cm distance was maintained between the laser and the seed samples. The seeds were positioned in a steady manner on the side facing the embryo and hanged in the air (supported on one side by a heat dissipater). To ensure that the laser diode operates in a normal mode for a prolonged period, it was placed in a special bed in the modules' ribbed part to provide good cooling. Optics were used in the lasers to ensure the provision of the optimum laser power. Additionally, the irradiation power intensity of the laser was set at 2 and 4 mW/cm<sup>2</sup>, the intensity was measured using a power meter. The blue laser was used to irradiate the maize seeds for different exposure times of 45, 65, 85 and 105 s. The

optimum period for each treatment was monitored using a time controller device. The parts of the device and the positioning of the treated seeds are depicted in *Table 1*.

Excremental condition	Details				
Laser type	Diode laser				
Wavelength	410 nm				
Power intensity	2, and 4 mW/cm <sup>2</sup>				
Laser exposition time	45, 65, 85, and 105 s				
Wave emission	Continuous (CW)				
Beam size	2 mm				
Distance from sample	100 cm				
Cultivar treated	Baghdad 3				

Table 1. Experimental conditions used for seed pre-treatment of maize

# **Experimental Design**

In summer 2018, a  $4x^2$  factorial experimental design was conducted for blue laser exposure times ( $T_1$ = 45,  $T_2$ = 65,  $T_3$ = 85 and  $T_4$ = 105 s) at 410 nm, two intensities ( $P_1$ = 4 and  $P_2 = 2 \text{ mW/cm}^2$ ), and a control (without exposure to laser) in a design of complete blocks with three replications. The land was fallow, harrowed and furrowed with machinery. Planting was carried out manually in dry soil at a plant density of 6667 plants/ha, and inter-plant distance of 25 cm. In this study, 600 seeds were pretreated before sowing in the field. The size of the experimental plot was 2 meters wide by 1.5 meters long. Total irradiated seeds were 1600. This study used maize seed variety (Baghdad 3 cultivar) provided by the Office of Agriculture Research, Baghdad. The seed variety was grown during the planting season (spring-summer) in July 2018 in the experimental field. The seeds lot was first standardized with respect to size and colour, followed by seeds dipped into a sodium hypochlorite solution (1% v/v) for sterilization and then rinsed in tap water for several time periods and left to dry by room temperature prior to irradiation. From each treatment group, six seeds were selected and measured for thicknesses using a Vernier instrument. The observed average thickness of the seeds was 3.9 mm.

The fertility of the fields was improved with 520 kg/ha of compost (46:18 of N:  $P_2O_5$ ) and prior to sowing, the soil was fertilized with 436 kg/ha of urea (1/2 before sowing and the rest at the flowering stage). During the growing period, weeding was done manually while insects were controlled chemically using diazinon at the rate of 6 kg/ha which was applied at 20 and 35 days after sowing. The crops were harvested manually after 110 days from the date of sowing. The drying of the ears was natural in a ventilated place and in the shade. It was manually de-grained when the moisture content of the grain was around 15% and the grain was stored at a temperature of 25°C.

# Parameters Measurement

A daily count of seedling emergence was conducted while the other seedling parameters (emergence %, emergence index, vigor index) were recorded after the viable seeds have emerged (no new seed emergence observed). The mean emergence time (MET) was calculated using Eq. 1 as follows:

$$MET = \Sigma Dn / \Sigma n$$
 (Eq.1)

where D = number of days that elapsed from the first day of sowing, and n = the number of emerged seeds on day D.

Emergence index (EI) was calculated according to The Association of Official Seed Analysis (1978) formula in *Eq.* 2:

$$EI = No. of emerged seeds / Day of first count + ...+ No. of emerged seeds/Day of final count (Eq.2)$$

Emergence speed was calculated by *Eq. 3*:

$$Es = \sum Ni / Di$$
 (Eq.3)

where, Ni = number of seeds emerged per day. Di =Number of days (daily germination). Seedling vigor index was calculated as below (*Eq. 4*):

Vigor Index (VI) = emergence (%) x Seedling length (cm) 
$$(Eq.4)$$

Flowering dates: Dates when 100% of the plants in a plot attained anthesis and incipient silk extrusion were recorded and expressed as days after planting, as well as stem diameter, and seedling leaves number at 21<sup>th</sup> days after planting were measured.

Seedling heights at 21<sup>th</sup> day after planting: This is the measure of a distance from the surface of the soil to the flag leaf-bearing nodes. Fresh weight was determined and dry weight was calculated by drying seedling (at 21<sup>th</sup> day after planting) in an oven at 75°C until the weight remained constant. The top ear was determined from 5 randomly selected plants in a plot and the mean of the measurements was determined and presented as the plant height. The proximate composition of the grain, including protein, starch, and oil were analysed after harvesting based on the method prescribed by AACC (2000).

## Statistical Analysis

The acquired data in this study were analyzed using Genstat® Statistical software version 19. The software was used to analyze the analysis of variance (ANOVA) at a significance level of p < 0.01; the Fisher's test was used to define the observed levels of significant differences between the means of the datasets. All the measurements were performed in triplicates.

#### Results

*Table 2* shows the seed emergence percentage and other parameters. Evidently, each laser exposure time and intensity significantly influenced the field emergence percentage. The percentage of emergence was found to be increased over the control, reaching a maximum seed emergence percentage of 91.6% by seeds irradiated with 4 mW/cm<sup>2</sup> laser intensity for 85 s. A decreased percentage was observed at 65 s as the lowest seed emergence percentage of 58.6 and 62.5% was recorded from the seeds irradiated at 65 s and the un-irradiated control seeds, respectively. The increased percentage of emergence was significantly different (p < 0.01) when compared to the control seeds.

Treatments	emergence %	emergence index	emergence speed (hour)	mean emergence time (hour)	vigor index	plant height (cm/plant <sup>-</sup> )	leaves number/ plant	fresh weight (g)	dry weight (g)
$T_1P_1$	87.50 <sup>b</sup>	6.41 <sup>ab</sup>	43.33°	51.00 <sup>de</sup>	282.80 <sup>e</sup>	20.20 <sup>c</sup>	8.20 <sup>ab</sup>	1.89 <sup>a</sup>	0.70 <sup>a</sup>
$T_1P_2$	83.33°	6.33 <sup>abc</sup>	48.80 <sup>d</sup>	53.12 <sup>e</sup>	345.60°	21.60 <sup>bc</sup>	7.80 <sup>bc</sup>	1.84 <sup>a</sup>	0.64 <sup>a</sup>
$T_2P_1$	58.66 <sup>f</sup>	4.16 <sup>d</sup>	63.18 <sup>e</sup>	64.19 <sup>f</sup>	$148.80^{i}$	18.60 <sup>c</sup>	$8.40^{ab}$	1.84 <sup>a</sup>	0.56ª
$T_2P_2$	83.33°	6.00 <sup>bc</sup>	48.95 <sup>d</sup>	46.67 <sup>bc</sup>	240.00 <sup>g</sup>	20.00 <sup>c</sup>	7.00 <sup>cd</sup>	2.28 <sup>a</sup>	0.41ª
<b>T</b> <sub>3</sub> <b>P</b> <sub>1</sub>	91.66ª	7.25 <sup>a</sup>	25.71ª	33.00 <sup>a</sup>	520.80 <sup>a</sup>	25.13 <sup>a</sup>	8.20 <sup>ab</sup>	3.66 <sup>a</sup>	0.70 <sup>a</sup>
T <sub>3</sub> P <sub>2</sub>	87.50 <sup>b</sup>	6.58 <sup>ab</sup>	37.60 <sup>b</sup>	44.57 <sup>b</sup>	377.60 <sup>b</sup>	23.60 <sup>ab</sup>	8.80 <sup>a</sup>	$4.00^{\mathrm{a}}$	0.71ª
$T_4P_1$	83.33°	6.16 <sup>abc</sup>	43.20 <sup>c</sup>	49.71 <sup>cd</sup>	$266.00^{\mathrm{f}}$	19.00 <sup>c</sup>	7.20 <sup>cd</sup>	2.70 <sup>a</sup>	0.50 <sup>a</sup>
T <sub>4</sub> P <sub>2</sub>	66.66 <sup>d</sup>	5.16 <sup>cd</sup>	42.50 <sup>c</sup>	54.25 <sup>e</sup>	288.40 <sup>d</sup>	20.60 <sup>bc</sup>	7.80 <sup>bc</sup>	2.32 <sup>a</sup>	0.49 <sup>a</sup>
Control	62.50 <sup>e</sup>	4.58 <sup>d</sup>	38.60 <sup>b</sup>	45.33 <sup>b</sup>	188.00 <sup>h</sup>	18.80 <sup>c</sup>	6.80 <sup>d</sup>	2.34 <sup>a</sup>	0.46 <sup>a</sup>
Т	*	*	*	*	*	*	Ns	Ns	Ns
Р	Ns	Ns	Ns	Ns	*	Ns	Ns	Ns	Ns
T*P	*	Ns	*	*	*	*	*	Ns	Ns
LSD	2.47	1.23	2.14	3.14	2.64	3.48	0.91	2.59	0.84

Table 2. Field emergence and growth components after seed pre-treatment with blue laser

The exposure time:  $T_1=45$  s,  $T_2=65$  s,  $T_3=85$  s, and  $T_4=105$  s. Power density:  $P_1=4$  mW/cm<sup>2</sup>, and  $P_2=2$  mW/cm<sup>2</sup>. Means of treatments were compared using the least significant difference (LSD) at  $p \le 0.01$ . Mean values with the same letters in the same column are statistically equal (Fisher's, a = 0.01)

A similar trend was also observed in the mean emergence time as shown in *Table 3*. The seeds irradiated with 4 mW/cm<sup>2</sup> laser intensity for 65 s needed 64.19 h as the average emergence time, whereas those irradiated for 85 s required only 33 h, showing a significant difference in the respective emergence times. The emergence speed was observed to be 25.7 h for seeds exposed to 4 mW/cm<sup>2</sup> laser intensity for 85 s, showing a considerable increase compared to seeds irradiated for 85s at a laser intensity of 2 mW/cm<sup>2</sup> (37.6 h) and un-irradiated treatment group (38.6 h). This reduction in emergence speed for the seeds exposed to laser treatment (T<sub>3</sub>P<sub>2</sub> and T<sub>3</sub>P<sub>1</sub>) was significant at (p < 0.01) when compared to the control seeds. There was also a linear improvement in the seedling vigor index by 520 in seeds treated with a laser power intensity of 4 mW/cm<sup>2</sup> for 85 s of 4 mW/cm<sup>2</sup> (P < 0.01), which was statistically significant in comparison to the control (4.58).

The maximum plant height, fresh and dry weights of seedling were observed in seeds treated with laser for 85 s. The plant height and the number of leaves were increased by a single exposure to laser for 85 s (p < 0.01) compared to the control set. Laser irradiation for 85 s at 4 mW/cm<sup>2</sup> significantly increased the plant height (25.13 cm) for the seedlings from the control seeds (18.8 cm). The laser treatments also significantly affected the number of leaves per maize plant as shown in *Table 2*. Increases in the number of leaves per plant for both power densities were observed. The best results (8.8 leaves/plant) were exhibited by seeds irradiated with 2 mW/cm<sup>2</sup> laser intensity for 85 s, followed by those irradiated with 4 mW/cm<sup>2</sup> laser intensity for 85 s (8.4 leaves/plant). The minimum number of leaves per plant (6.8) was obtained in the un-irradiated control. There was no statistically significant difference between the observed effects of treatments of the fresh and dry weights of the maize seeds (*Table 2*).

Traatmonte	Protein content	Oil content	Starch content	Stem diameter	Days to Anthesis	Days to Silking
Treatments	(%)	(%)	(%)	(cm)	(day)	(day)
$T_1P_1$	16.30 <sup>ab</sup>	3.08 <sup>c</sup>	68.78 <sup>cd</sup>	2.30 <sup>bc</sup>	60.40 <sup>b</sup>	61.50 <sup>a</sup>
$T_1P_2$	14.60 <sup>bc</sup>	6.22 <sup>a</sup>	71.25 <sup>b</sup>	1.90 <sup>c</sup>	59.33 <sup>b</sup>	63.90 <sup>a</sup>
$T_2P_1$	16.67 <sup>ab</sup>	3.95 <sup>bc</sup>	66.30 <sup>cd</sup>	2.20 <sup>bc</sup>	58.92 <sup>ab</sup>	63.30 <sup>a</sup>
$T_2P_2$	16.70 <sup>ab</sup>	6.18 <sup>a</sup>	69.40 <sup>c</sup>	1.90 <sup>c</sup>	59.13 <sup>ab</sup>	62.70 <sup>a</sup>
<b>T</b> <sub>3</sub> <b>P</b> <sub>1</sub>	18.00 <sup>a</sup>	6.33 <sup>a</sup>	76.83ª	3.50 <sup>a</sup>	56.20ª	62.25ª
$T_3P_2$	18.20 <sup>a</sup>	6.22 <sup>a</sup>	73.11ª	2.80 <sup>abc</sup>	58.72 <sup>ab</sup>	64.00 <sup>a</sup>
$T_4P_1$	13.70 <sup>c</sup>	3.81 <sup>bc</sup>	71.76 <sup>b</sup>	2.30 <sup>bc</sup>	61.40 <sup>b</sup>	64.10 <sup>a</sup>
$T_4P_2$	14.80 <sup>bc</sup>	4.27 <sup>bc</sup>	63.82 <sup>e</sup>	2.60 <sup>abc</sup>	60.83 <sup>b</sup>	65.40 <sup>a</sup>
Control	16.80 <sup>ab</sup>	4.37 <sup>b</sup>	70.12 <sup>bc</sup>	2.90 <sup>ab</sup>	61.40 <sup>b</sup>	63.00 <sup>a</sup>
Т	*	*	*	Ns	*	Ns
Р	Ns	*	Ns	Ns	Ns	Ns
T*P	Ns	*	*	*	*	Ns
LSD	2.47	1.23	1.76	0.91	2.97	3.60

Table 3. Flowering and seed quality components after seed pre-treatment with blue laser

The exposure time:  $T_1=45$  s,  $T_2=65$  s,  $T_3=85$  s, and  $T_4=105$  s. Power density:  $P_1=4$  mW/cm<sup>2</sup>, and  $P_2=2$  mW/cm<sup>2</sup>. Means of treatments were compared using the least significant difference (LSD) at  $p \le 0.01$ . Mean values with the same letters in the same column are statistically equal (Fisher's, a = 0.01)

Table 3 also presents the effect of laser exposure on the oil content of the seeds. The laser-treated seeds and the control seeds presented a comparable level of oil content. As shown in Table 3, there were significant differences in the oil content of the seed. Generally, the oil contents of the seeds were increased by exposure to laser for different exposure times, where seeds exposed for 85 s to 4 mW/cm<sup>2</sup> laser intensity showed higher oil content (6.33%), followed by those exposed to  $2 \text{ mW/cm}^2$  laser intensity (6.22%). The control seeds showed the lowest oil content (4.37%). Highest levels of total protein content and starch content were recorded in seeds exposed to laser for 85 s compared to the control (p < 0.01). The exposure of maize seeds to laser at different exposure times had a significant influence on the protein content of the seeds (Table 3). An increase in the exposure time to laser was generally found to increase the protein content of the seeds, with the highest protein content of 18.2% being observed in seeds exposed to  $2 \text{ mW/cm}^2$ laser power for 85 s, followed by 18% for seeds exposed to 4 mw/cm<sup>2</sup> laser power for 85 s. The control seeds had a protein content of 16.8%. The protein content increased gradually, and the maximum activities were noted in seedlings derived from 85 s laser irradiated seeds, whereas the starch content showed maximum activity at 85 s which was found to be significant (p < 0.01) compared to the control group. The different energy densities of laser irradiation also increased the stem diameter during the harvest stage. Pre-sowing exposure to 4 mW/cm<sup>2</sup> laser power for 85 s was observed to significantly increase the stem diameter of the plants (3.5 cm) compared to the stems of the control seeds (2.9 cm).

Regarding the days to silking, *Table 3* shows a marked difference in the days to silking of the maize plants exposed to laser for different exposure times. However, there was no statistically significant difference between the observed effects. The shortest period (61.5 day) was observed in plant seeds exposed to 4 mW/cm<sup>2</sup> blue laser for 45 s compared to 65.4 day of the control group. There were also significant differences in the Days to Anthesis as presented in *Table 3*. In general, using different exposure times led to an

increase in the Days to Anthesis. For the blue laser, 85 s of exposure at  $4 \text{ mW/cm}^2$  has a shorter period to Anthesis (56.2 day), followed by seeds irradiated with a power density of 2 mW/cm<sup>2</sup> (58.72 day). The control group had the longest period to Anthesis (61.4 day).

## Discussion

Seed germination is a crucial stage in plant development and can be considered as a determinant for plant productivity. Physiological and biochemical changes followed by morphological changes during germination are strongly related to seedling survival rate and vegetative growth which consequently affect yield and quality. Seed stimulates the embryo to produce phytohormones mainly gibberellic acid (GA) which can diffuse to aleurone layer and initiate a signaling cascade resulting in the synthesis of a-amylases and other hydrolytic enzymes.

Phytochromes are involved in the sensing of the light environment by seeds, and the control of germination by red and far-red light was one of earliest phytochrome-mediated responses described Phytochromes can affect the growth capacity of the embryo and/or the constraint imposed by seed tissues around it. Much evidence exists for the role of phytochromes in promoting the synthesis of gibberellins (GAs), which are important stimulants for germination (García-Martinez and Gil, 2001; Hilhorst et al., 2018; Ribalta et al., 2019). Phytochromes also play a role in regulating the sensitivity to GAs (Mishra and Khurana, 2017). Recently, phytochromes have also been shown to be involved in the degradation of abscisic acid, the major plant hormone that maintains dormancy (Miransari and Smith, 2014).

Hernandez et al. (2010, 2011) presented a detailed review of the use of laser treatment for plant stimulation. The review suggested that laser light can be used in agriculture for seeds bio-stimulation based on the additive interaction between the laser beam (polarized and monochromatic) and the photoreceptors that absorb it. This interaction activates several biological processes in the exposed seeds. However, the level of effect of laser treatment is a function of the laser wavelength, the output power, and the period of exposure.

According to several reports, various laser output powers have been investigated (Govil et al., 1991; Jia and Duan, 2013) to 5 mW (Hernandez et al., 2010). Different periods of exposure have also been studied, ranging from 30 s (Hernández and Michtchenko, 2011) to 120 min (Khalifa and El Ghandoor, 2011). In the present study, the highest emergence rate was presented by seeds exposed to 4 mW/cm<sup>2</sup> laser intensity for 85 s which is comparable to earlier reports by Cwintal et al. (2010). According to several authors, laser treatment can also cause some levels of damage to plant cells and tissues (Jia and Duan, 2013). As per Salvaev et al. (2007) and Hernandez et al. (2010), there are two specific responses which can be induced upon exposure of cells to laser light: (i) a rapid stress effect which increases the level of lipid peroxidation products generation, and (ii) series of secondary reactions due to the adaptive metabolic changes which can elicit some morphogenetic processes. The present results agreed with previous findings by Taie et al. (2014) who reported the maximum germination percentage of some Merremia sp. upon exposure to laser light. In a study on Stevia seeds, Goettemoeller and Ching (1999) reported that the two weeks-delay and the low seed germination percentage observed in the control set could be attributed to the importance of light to the germination of Stevia seeds (light-requiring seeds) as the exposed seeds showed better germination percentage compared to the control. All light-requiring seeds have also been earlier reported to show dormancy (Taiz and Zeiger, 2011), while the importance of light to seed germination and plant growth has been reported (Hernandez et al., 2009).

However, upon completion of germination, Jala (2011) recorded the highest germination percentage in seeds treated with laser light. The present results on germination show deviation from Jala (2011) but in line with Colbach et al. (2002) on Alopecurus myosuroides and Ambika (2007) on Chromolaena odorata seeds. Horizontal and vertical expansion of shoot, particularly leaves, is a genetically-controlled developmental process (Tsukaya, 1998) and irradiation with blue light seems to cause imbalance in the expression of the concerned genes, leading to inhibition of leaf expansion. Furthermore, the improved rate of seed germination could be attributed to the matter and energy transfer processes involved in the germination and growth of seeds (Abu-Elsaoud et al., 2013). For instance, different intensities of laser treatment have been reported to induce the germination of wheat and maize seeds, as well as some vegetables (Asghar et al., 2016). The spectral influence of laser treatment on seed germination has also been investigated by Chen et al. (2005) in which laser light was reported to induce changes in the normal plant functions, and elicited rapid cell division, resulting in a rapid rate of initial growth and development. Therefore, the observed positive effects of laser light treatment on the seed germination parameters in the present study could be attributed to an improved rate of laser-induced cell division. Some reports have also reported that laser treatment is a physical process involves the absorption and storage of radiant energy by the cells and tissues of plants. This is also applicable to seeds as they first absorb the radiation energy before its subsequent transformation into chemical energy for subsequent use (El-Naggar et al., 2012; Hedimbi and Singh, 2012; AlSalhi et al., 2018).

According to Hernandez et al. (2010), the pre-sowing exposure of various seeds (like rice, maize, tomato, radish, peas, cucumber, lettuce, onion, etc.) to laser has a significant influence on their germination parameters. The seeds of vegetables have been reported as the most sensitive seeds to laser stimulation compared to cereals (Gładyszewska, 2006; Hernandez et al., 2015). Laser radiation of different exposure times (45, 65, 85, and 105 s) as used in the present study showed different effects on maize plants. Statistical analysis of this work reveals that plant height, number of leaves and stem diameter increased by laser treatments and this may reflect the effect of laser on cell division of shoot taps of the exposed shoots. This effect continued in the cell division of all parts of the plant at both vegetative and flowering stages. Our results reveal that the exposure to blue laser rays for 85 s and 4 mW/cm<sup>2</sup> laser intensity had the most pronounced effect in increasing the growth criteria for maize plant and decreased emergence time. A similar effect was also noticed by Podleśna et al. (2015). These results agree with Chen et al. (2005) who showed changes in the protein functional activities. There were also increases in the physiological and biochemical characteristics of the seedlings after exposure to laser (Chen et al., 2005; Qiu et al., 2013). The exposure time to laser radiation is very important to produce stimulation effects and these agree with the report of Rimal et al. (2014).

From the in-vitro growth data, it was observed that different exposure times to blue laser increased the germination and shoot multiplication rates of maize plants. This was supported by the reports of Hwida et al. (2012) on *Balanites aegyptiaca* and Cotoneaster horizontalis and Dănăilă et al. (2011) on Petunia hybrid and Dianthus caryophyllus plants. According to earlier reports on *Balanites aegyptiaca*, the maximum number of shootlets per explant increases with laser treatments (Lobna et al., 2014; Rania et al., 2015). The maize seeds exposed to blue laser for 85 s had the longest plants compared to the control.

These results showed the same trend with the reports of Ali et al. (2014), Lobna et al. (2014), and Kouchebagh et al. (2014b). The laser-induced cell elongation has been reported to increase the level of gibberellic acid which results in increased cell vacuoles (Mahmoud and Ibrahem, 2000). Regarding the number of leaves, the seeds exposed to blue laser for 85 s has plants with the highest number of leaves per plant compared to the control. This observation agreed with the reports of earlier studies (MacLeod and Millar, 1962; Kamiya and Martinez, 1999; Aguilar et al., 2015).

Osman et al. (2009) and Aguilar et al. (2015) suggested that laser radiation could induce faster rates of enzymatic activities within the cells of the exposed seeds. This could also be due to the endogenous GA content and its role in cell growth. It is believed that GA can induce cell growth by inducing enzymes that reduce the integrity of the cell wall (MacLeod and Millar, 1962). Seeds exposed to blue laser for 85 s produced plants with the longest stem diameter and dry and fresh weights compared to the control. Plant growth is generally controlled by several factors, such as enzymes and hormones like gibberellic acid (GA<sub>3</sub>) and cytokinin. Kamiya and Martinez (1999) observed that exposure to laser plays a significant role in GA<sub>3</sub> formation and endogenous level of GA<sub>1</sub>. The resulting increase in GA<sub>3</sub> response manifests in better cell growth, reduced cell wall integrity, proteolytic enzymes production, increased auxin content, and increased sugar concentration; can also increase the osmotic pressure of the cell sap. The physical manifestations of the increased cell elongation due to laser radiation are increased plant height and number of branches, as well as increased number of flowers (Ali et al., 2014; Rania et al., 2015). Exposure to laser can also increase the nitrogen content and result in increased protein content. This is necessary for the development of plant organs (such as the branches and umbels) (Osman et al., 2009). The study by Mahmoud and Ibrahem (2000) showed that laser irradiation can increase cell number, nucleic acids, and phospholipids membranes. It can also increase the potassium and phosphorus contents, thereby leading to the elongation of laser-irradiated cells.

However, further details are needed on the effects of different laser irradiation as they remain inexplicable (Samuilov and Garifullina, 2007). Coherent laser light beams excite electrons and promote the biophoton and entropy emission, thereby triggering an increase in the internal energy of the exposed material. The transient action of laser irradiation has also been implicated in the stimulation of various functional activities and higher plants' resistance to biotic disease (Rassam, 2010; Podleśny et al., 2012; Srećković et al., 2014; Tang et al., 2019). The role of laser technology in the agricultural sector is being evaluated from its bio-stimulatory role. Despite the reported positive influences of laser irradiation (visible to near IR), its light-regulated mechanism and role in plants are yet to be understood (Aguilar et al., 2015). Laser irradiation is emerging as a novel agricultural practice owing to its positive role in seed germination, seeds biochemical composition, enzyme activities, plant growth, seed quality, stress resistance, fruit size, and yield characteristics. Corn seeds stained with methylene blue were treated by Hernández et al. (2011) using a laser diode, a wavelength of 655 nm with 27.4 mW of power and 5 min of exposure. In these seeds, the number of seeds infected by Fusarium spp. was reduced, suggesting the possibility of using laser irradiation as a means of disease control in maize seeds, thereby improving the quality of seeds and the final product derived from plants emerging from such seeds.

# Conclusion

In the present study, the pre-sowing exposure of maize seeds to blue laser light at 4 mW/cm<sup>2</sup> laser intensity and 85 s exposure time significantly improved the seedling emergence rate and improved the seed emergence uniformity. Significant modification was also observed in the stages of plant growth. Such use of the laser is technically feasible and could be one of the solutions to increase seed emergence, seedling growth and establishment of more efficient maize seeds. However, future study is needed to understand the mechanism underlying phytohormones by the optimum blue laser pretreatment.

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