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Low power continuous wave-laser seed irradiation effect on *Moringa oleifera* germination, seedling growth and biochemical attributes

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ABSTRACT

Recently, laser application in agriculture has gained much attention since plant characteristics were improved significantly in response of pre-sowing seed treatment. Pre-sowing laser seed treatment effects on germination, seedling growth and mineral profile were studied in *Moringa olifera*. *M. olifera* healthy seeds were exposed to 25, 50, 75 mJ low power continuous wave laser light and grown under greenhouse conditions. The seedling growth and biochemical attributes were evaluated from 10-day-old seedlings. The germination parameters (percentage, mean germination time), vigor index, seedling growth (root length, seedling length, shoot fresh weight, root fresh weight, shoot dry weight, root dry weight) enhanced considerably. The laser energy levels used for seed irradiation showed variable effects on germination, seedling growth and mineral profile. The mineral contents were recorded to be higher in seedling raised from laser treated seeds, which were higher in roots versus shoots and leaves. The effect of laser treatment on seedling fat, nitrogen and protein content was insignificant and at higher energy level both nitrogen and protein contents decreased versus control. Results revealed that *M. olifera* germination, seedling growth and mineral contents were enhanced and optimum laser energy level has more acceleratory effect since at three laser energy levels the responses were significantly different. Overall the laser energy levels effect on germination and seedling growth was found in following order; 75 mJ > 50 mJ > 25 mJ, where as in case of fat, protein and nitrogen contents the trend was as; 25 mJ > 50 mJ and 75 mJ. However, this technique could possibly be used to improve the *M. olifera* germination, seedling growth, and minerals contents where germination is low due to unfavorable conditions.

1. Introduction

Recently, laser application in agriculture field gained much attention in controlling plant diseases or monitoring ambient agricultural gases, to control seed transmitted diseases [1–3], environmental sample analysis [4]. A number of studies have been reported regarding laser pre-sowing seed stimulation effect on different crops and vegetable [3,5–14]. Laser seed stimulation results in more seed germination, fast seedling growth, enhanced biochemical and physiological attributes [15,16]. The early plant maturity, changes in energy during germination, radicle and hypocotyl variable responses have also been reported in response of pre-sowing laser treatment [13,14,17]. In He-Ne laser irradiated *Triticum aestivum* higher energy and metabolism rate was observed and resultantly, germination, plant growth, enzyme activity, thermodynamics properties and morphological attributes were affected significantly [7]. Similarly, low power continuous wave He–Ne laser radiation on seed thermodynamic and germination parameters and enzymes activities during germination in sunflower was reported [5]. Moreover, in comparison to physico-chemical pre-sowing seed treatment [13,14], the laser treatment is safe and under the current scenario of environmental pollution [18–40], there is need to adopt green and eco-friendly techniques to enhance the crops productivity.

Moringa oleifera Lam is one of the widely distributed and natural species of a monogeneric family (*Moringaceae*), which is a wild plant and also cultivated since it has applications in various fields of life. *Moringa* is native to the western and sub Himalayan tracts, India, Pakistan, Asia Minor, Africa and Arabia and is also distributed in the Philippines, Cambodia, Central America, North and South America and

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the Caribbean Islands [41]. In Pakistan, *Moringa* is locally known as 'Sohanjna' [42]. The tender pods are used as a vegetable. Also flowers, fruits, leaves, roots are edible, which are also consumed as vegetable. A paste of the leaves is used as an external application for wounds. Moreover, the leaves are the rich source of essential amino acids i.e., methionine, cystine, tryptophan, lysine and is also a valuable source of β-carotene, protein, vitamin C, calcium and potassium with a high content of protein. Decoctions and extracts are also employed in native medicine. This tree is excellent source of natural antioxidants and is used in enhancing the shelf-life of fat containing foods since it has ascorbic acid, flavonoids, phenolics and carotenoids in considerable quantity [42–45]. *M. oleifera* seeds and oil are used in the treatment of arthritis, rheumatism, and hypertension [46]. Oil is also used for frying purposes, lighting as it burns without dense smoke and recently, biodiesel production with high yield is also reported (around 40%) [47–49]. In view of importance of *M. oleifera* tree, there is a need to enhance its productivity. The germination of *M. oleifera* reported to be low due to unfavorable environmental conditions i.e., change in soil chemistry and drought stress etc. [50].

In view of promizing efficiency of laser pre-sowing seed treatment, *Moringa* seed pre-sowing laser treatment has not been reported and in this study laser pre-sowing seed effect on *M. olifera* plant was investigated. The principle objectives were to appraise the *M. olifera* pre-sowing seed laser treatment on germination, growth, biochemical and mineral contents at early growth stages.

2. Materials and Methods

2.1. Moringa oleifera Seed Collection

M. oleifera seeds were kindly supplied by Ayyub Agriculture Research Institute, Faisalabad. The seeds free from any deformity were handpicked from seed lot and tested for purity (Eq. (1)) and moisture contents (Eq. (2)).

$$Purity = \left\{\frac{Wieght\ of\ pure\ seed}{Weight\ of\ impure\ seed}\right\} \times 100 \quad (1)$$

$$Moisture\ contents\ (\%) = \left\{\frac{Seed\ wieght\ before\ drying\ -\ wieght\ of\ dry\ seed}{Seed\ wieght\ before\ drying}\right\} \times 100 \quad (2)$$

2.2. Laser Treatment of Seeds

The laser treatments were performed in triplicate and experiments were conducted under Randomized Complete Block Design (RCBD). Before laser irradiation, seeds were soaked in distilled water for 3 h, air-dried and subjected to He–Ne CW-laser (632.8 nm, density 1 mW mm^{-1}, beam diameter 1 mm) irradiation at 25, 50 and 75 mJ from the embryonic side of the seed and laser set up is reported elsewhere [7]. The irradiated seeds were sown and grown under greenhouse conditions. Un-irradiated seeds were considered as control.

2.3. Sowing and Growing Conditions

The sand was thoroughly washed with deionized water, dried for in sun light and dried sand was leveled in pots. Before sowing, seeds were sterilized with 0.05% $HgCl_2$ solution for 10 min and washed with distilled water. The seeds were planted in pots (30 × 25 cm) under complete randomized design in triplicate (Table 1). Nine healthy seeds were sown in each pot at a depth of ~2.5 cm. Before sowing, the sand in the pots was saturated with Hoagland's nutrient solution (half-strength) and then sand was moistened with water and kept moist (80%) by spraying water daily. The pots with seeds were kept in artificial glass greenhouse at 25.8 °C and under visible light of approximately 900 mmol m^{-2} s^{-1} for 8 h/day. The number of emerged seeds was recorded daily according to the seedling evaluation in the Handbook of the Association of Official Seed Analysis (1990) until germination ceased and germination percentage, mean germination time were calculated. After 10 days, the plants were harvested and vigor indices, growth and biochemical parameters were estimated.

Table 1
Experimental design for the irradiation of seeds (T = laser energy and R = replicate).

Treatment → Replication ↓	$T_{0\ (0\ mJ)}$	$T_{1\ (25\ mJ)}$	$T_{2\ (50\ mJ)}$	$T_{3\ (75\ mJ)}$
R_1	T_0R_1	T_1R_1	T_2R_1	T_3R_1
R_2	T_0R_2	T_1R_2	T_2R_2	T_3R_2
R_3	T_0R_3	T_1R_3	T_2R_3	T_3R_3

Germination percentage was computed at the end of 7th day of germination as already reported elsewhere [51] (Eq. (3)).

$$Germination\ (\%) = \frac{Seed\ germinated}{Total\ number\ of\ seeds} \times 100 \quad (3)$$

Mean germination time (MGT) was calculated according to the relation shown in Eq. (4), where *n* is the number of seeds germinated on day *D*, and *D* is the number of days counted from the beginning of the germination [51].

$$MGT = \frac{\sum D_n}{\sum n} \quad (4)$$

Vigor index I (VI I) and vigor index II (VI II) were calculated as shown in Eqs. (5) and (6), respectively.

$$VI\ I = Germination\ (\%) \times seedling\ length\ (root + shoot) \quad (5)$$

$$VI\ II = Germination\ (\%) \times seedling\ dry\ weight\ (root + shoot) \quad (6)$$

The lengths (root and shoot) (cm) and weight (fresh and dry) (g) of 10-day-old plants were calculated with the help of measuring scale and electric balance form three randomly selected plant, respectively and data was averaged [51]. The number of branches and number leaves/plant were counted from three randomly selected plants, and data was averaged.

Nitrogen was estimated by micro-Kjeldhal's method [52]. The mineral contents (Ca, Cu, Fe, Mg and Zn) were analyzed using Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan) following AOAC methods [53]. Briefly, digestion mixture was prepared by dissolving 0.42 g of Se and 14 g of $LiSO_4{\cdot}2H_2O$ in 350 mL of H_2O_2, mixed well and 420 mL H_2SO_4 were added slowly while keeping the mixture an ice bath. Dried plant sample (0.1 g) was taken and 1 mL of digestion mixture was added and placed on hot plate. The temperature was increased gradually from 50 °C to 200 °C. On blackening of flask contents, 0.5 mL of $HClO_4$ was added, heated again till the contents turned colorless. After cooling, the solution was diluted up to 50 mL, filtered and used for the minerals estimation. The mineral qualitative analysis was also performed using LIBS [54]. Briefly, Pulsed Q-switched Nd:YAG (Quantel Brilliant) was used for mineral analysis. The laser set up for this experimentation is already reported in detail [51]. The sample was subjected to Nd:YAG laser at 200 mJ at 1064 nm wavelength and emission spectrum was recorded with the help of spectrometer coupled with LIBS detection system and Q-switch of the Nd:YAG laser.

3. Results and Discussion

Different techniques have been used to enhance the germination of *M. oleifera* since germination is low due to un-suitable soil and environmental conditions [55]. Present research was conducted to investigate the laser pre-sowing effect on various characteristics of *M. oleifera*. *M. oleifera* germination, growth, biochemical and mineral