Effect of laser exposure time and rhizobium on germination of clover seeds

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Abstract: This investigation of sub clover germination responses to different combinations of Laser or/and Rhizobium under laboratory conditions. To study effect of irradiation seeds by laser radiation and seeds incubation by Rhizobium combination with laser exposed times 5, 10, and 15 minutes compared in untreated seeds on the germination parameters of clover seeds. Seed germination was monitored every 8 hours for the first 4 days, then inspected every 24 hours for 15 days. Obtained results were showed that using treate seeds by combination laser exposed times and Rhizobium gave higher the germination parameters than seeds which treat by the laser exposed time. By using Laser exsed timesor/and Rhizobium, the results were indicated that the germination parameters of seed cover were decreased for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control). While, the higher values were when using laser exposed time of 5 minutes or/hizobium, but, the lowest values were noticed when using laser exposed time of 15 minutes or/and Rhizobium, because of less than untreated seeds (Control).

Keywords: clover seeds, rhizobium, inoculation, laser, germination parameters

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1 Introduction

Egyptian clover is rightfully considered the first responsible for achieving sustainable fertility of Egyptian lands for more than five thousand years. Clover adds to the soil from 45−90 kg of organic nitrogen per feddan, equivalent to 300−600 kg of fertilizer containing 15% nitrogen.

Alfalfa may be used as a green fertilizer to improve the natural, chemical and biological properties of the soil and increase its production capacity, especially in newly reclaimed lands. Also, the quantities of alfalfa in excess of the needs of animals in the winter and spring seasons can be converted into hay that can be used to feed the animals in the summer season as this period is characterized by a lack of green fodder needed to fill animal needs.

Viliana and Kostov (2015) studied the effect of low and high doses of mineral and organic fertilization on the

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quality of top yield and root mass of alfalfa and effect of mineral and organic fertilization on formation of quality of soil organic matter in dry condition. The doses of 70, 140 and 210 kg ha^{-1} mineral nitrogen (active ingredient) were tested as ammonium nitrate and well-rotted cattle manure. It was found dry mass yield of alfalfa fertilized with manure was to 15.9% higher as compared to dry mass yield obtained from alfalfa fertilized with mineral fertilizer. Sustainable yield index showed the alfalfa crop was more stable under organic fertilization. Alfalfa accumulated to 6027 kg ha^{-1} dry root mass for 4-year period of growing. There was from 614 to 1371 kg ha^{-1} root mass additional with manure application. Manure treated plants showed higher values for nitrogen in dry root mass/nitrogen in dry aboveground mass ratio and plant available nitrogen. Ali et al. (2014) identified the effective characters and their relative importance in improving of BNF, two separate field experiments were conducted under irrigated and rain-fed organic managements of dry. The experiments were laid out in an α-lattice design with two replications and 18 genotypes (eight Iranian ecotypes and ten European cultivars). Plant height was positive and significantly correlated with leaf area index (LAI) and shoot dry matter (DM) under both conditions. Positive correlations were found between biological nitrogen fixation (BNF) and shoot DM (r = 0.61** and 0.87**), irrigated and rain-fed management, respectively). Tounsi-Hammami et al. (2016) explain the effect of nitrogen fertilizer and Rhizobium inoculation on their growth, nodulation and nitrogen content of Hedysarum coronarium L. cultivated on calcareous soil. Rhizobium inoculation enhanced significantly all growth parameters compared to the nitrogen fertilization, especially at the flowering stage. The present investigation attempted to assess the impact of seed pre-treatments on the germination of *Crotalaria persica* (Burm. f.) Merr and *Tephrosia apollinea* (L) Pers. Equally important was our investigation to quantitatively compare different germination parameters used for seed germination studies. The results showed that the final germination was highest for both species under chipping (CHIP), sand paper (SP) and 20 to 90 minutes in concentrated sulfuric acid (SA20 to SA90) (Al-Ansari and Ksiksi, 2016).

Physical dormancy (Baskin and Baskin, 2004) differs between species type, degrees of drought, and the stage of maturation. This type of dormancy can be pre-treated through the use of mechanical resistance or physical impermeability, which depends on the thickness of the seed coat. One widely used chemical to break seed dormancy is the use of sulfuric acid. Effectiveness of the pre-treatment improved as the time of exposure to sulfuric acid increased (Wang et al., 2007). Many procedures for data analysis of seed germination responses are scattered throughout the literature. Many, including Timson germination index, have been reported as a good indicator of seed germination rate (Al-Mudaris, 1998). This index is calculated as the sum of percent germination of seeds at 2 day intervals by the total germination period (Khan and Ungar, 1996).

Alfalfa (*Medicago sativa* L.) has long time ago been recognized and valued as “soil building” legume crop. This crop has the ability to accumulate significantly more nitrogen than other legumes through its deep rooting system and fix atmospheric N2 from 40% to 80% of total plant nitrogen through biological nitrogen fixation (Jarvis, 2005). The goal of such strategies is to maximize the amount of crop output per unit of water, N fertilizer, and pesticide input. Certain farming practiceshave been found to increase efficiency and sustainability of crop production, including integrated pest management, improved drainage control, properly timed water and fertilizer application, and maximizing biological N fixation by incorporating legume crops and enriching soil with N-fixing rhizobia (Rhiz) bacteria (Reid et al., 2005).

Li et al. (2012) observed that the abundance of both microorganisms: ammo-nia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) was negatively correlated with salinity. These conflicting reports indicate the limitations of current understanding about ammonia- oxidizing microorganisms in agricultural soils. Nitrogen fertilization is of great importance to crop yield. Symbiotic N fixation SNF is both initiated and maintained by an active exchange of chemical signals between host plant and Rhiz soil bacteria. Each species of Rhiz interacts only with a particular subset of host plant species. For example, the soil bacterium *Sinorhizobium meliloti* will form a symbiotic partnership with its host plant, alfalfa, but not with other legumes such as soybean or clover (Peck et al., 2006). Many studies have shown that simultaneous infection with rhizobia and some plant growth promoting rhizobacteria (PGPR) can increase growth in respect to Rhizobium inoculation alone in a wide variety of legumes including alfalfa (Hungria et al., 2015; Stajković et al., 2011). Jalali (2000) reported that germination percentage in wheat seed was decreased under drought conditions in which various cultivars 15 species had significant differences in this case. Drought stress in young plants results in metabolic mutations which based on their genotype and perimeter environment the degree of mutation diverse. Germination is the first stage in plant life. Proper environmental conditions for germination of a plant in normal status include enough water,oxygen, light and proper temperature without any external different matter like mineral salts and organic materials which cause germination initiates. By decreasing water potential, absorption of water by the plant is stress which is the most affected. However, to decrease the germination percentage, Drought, water potential must fall below a certain amount (Safarnejad, 2008).

Germination stage of those plants which reproduce through their seeds is very important because of its indirect influence on the plant's concentration. Drought stress usually delay germination process and decreased germination percentage and rate and growth rate (Hamidi, 2000). Alfalfa (*Medicago sativa* L.) was widely planted in arid and semi-arid areas (Safarnejad, 2008), because of its forage, nutritive and high biomass producing values. The use of chemical fertilizers can temporarily relieve the deficiency of fertility, while the long-term use increased not only the cost of agricultural production, but also the deterioration of soil and the selective accumulation of some chemical elements which is harmful to the environment (Mukhtar et al., 2013). They are a part of “green revolution” after which, although not enough, but the plant protein has to be found increasing (Lassaletta et al., 2014). Although signiﬁcant improvement in nitrogen use efficiency has occurred in many countries, an increase of nitrogen fertilization would result in a disproportionately low increase of crop production with further environmental changes (Lassaletta and Grizzetti et al., 2014).

The process is affected by several factors such as temperature variations, oxygen levels, water availability and lack of inhibitory environmental conditions. However, unfavorable conditions in some species could lead to seed dormancy (Yildiztugay and Kucukoduk, 2012). Dormancy was classified into five classes, with physiological dormancy being the most extensive scheme (Baskin and Baskin, 2004). Seed dormancy and germination are complex features relating to higher plants which are influenced by factors that relate to environmental conditions (Koornneef et al., 2002). Seed coat dormancy or physical dormancy play an important role in the success of the germination process. Seed coat dormancy is caused by a hard and impermeable seed coat that prevents imbibitions and some-times gaseous exchange (Yildiztugay and Kucukoduk, 2012).

Moreover, time, rate, homogeneity, and synchronization of germination are also critical issues simply because the dynamics of the germination process are indicative of the ecology of any plant species. These indicators and features are useful for physiologists, seed technologists and ecologists (Ranal and Santana, 2006). Timson’s index is widely used and praised (Ansari et al., 2012; Dwiyanti et al., 2014). They reported with varying examples that different Timson germination indices could still be reached, adding levels of complications and interpretations. Here an attempt is made to assess some of the existing methods relating to germination indices, to compare the information they provide and to correlate them against the most widely used index (*ie.* Timson's). Comparisons are made between Timson's germination index *vs.* six other methods: final germination percentage, coefficient of velocity of germination, germination rate index and the germination index as reported by Al-Mudaris (1998) and Ranal et al. (2009).

The objective of this study was a seed germination analysis of clover (*Trifolium subterraneum* L.) under controlled conditions. Studying the effect of clover germination responses to different combinations of seed irradiation by laser exposure time and seeds incubation by Rhizobium under laboratory conditions, compared with mineral fertilization on the germination parameters of clover seeds, also on shoot, root and plants mass of clover plants were measured.

2 Materials and methods

2.1 Seed germination and treatments

Seeds of clover (Trifolium Alexandrinum L.) variety which will use in this study, were collected from from the Field Crops Research Institute, Agricultural Research Center (ARC), Giza, Egypt. Seeds of clover were screened to remove empty or broken seeds from each population. The experiment was carried out at the Water, Soils and Evironment Research Institute, Agricultural Research Center, Egypt., during the 2020 growing season.

2.2 Clover seeds treated in Rhizbium:

1- Rhizbium rate: A two-node bag (200 g) is sufficient for seeds of one acre for old lands.

2- Gummy solution: Prepare a sugar solution (Four tablespoons of sugar dissolved in 2 cups of water).

3- Mixed seeds by solution and Rhizobium: A large plastic mattress is placed in a shady place or in the early morning, then the seeds to be inoculated are placed on it, then all of them are mixed with the sugar solution, where all the seeds are moistened and then the two nodules bag is opened after confirming the date of validity on the package and added to the seeds and mixing well to ensure distribute all the contents of the bag to the seed.

2.3 Laser and Rahzobium treatments

Seeds of clover were established by direct seeding of 20 seeds for each the Petry dish, one layer of filter paper and covered with a second layer. Each pre-treatment was replicated four times. The seeds were supplied daily with distilled water during the germination trial, which lasted fifteen days. In order to determine the germination parameters of clover seeds (germination percentage, root and shoot lengths and vigority index of seeds), under various laser times and Rhizobium by using factorial experiment in complete randomize design with four replications. The seeds were divided two groups, each group is including three treatments. One group of them, seeds were exposed to laser radiations (632.8 nm) for three different laser times, which were 5, 10, 15 min, and another group was incubated by Rhizobium and exposed to same laser times, by adding two seed samples, first as control and second as incubating by Rhizobium, and fresh water was used to irrigate.

2.4 Laser setup

Seeds clover exposure by laser beam for seeds irradiation were carried out in the Laboratory of Laser Applications in Agricultural Engineering at the National Institute of Laser Enhanced Science (NILES), Cairo University, for evaluation of clover plants during germination period by seeds treatments by He-Ne Laser 632.8 nm irradiation and Rhizobia incubation treatments.

2.5 Description of the apparatus

The opto- electronic apparatus was manufactured and developed to provide irradiation clover seeds samples with different doses. The Opto – electronic apparatus is shown in Figure 1, consists of the following main parts:

Stand holder, it was designed and fabricated of iron as a shape square with length 45 mm and total length 900 mm , it contains seven holes to adjust the height and direction of laser and beam expander to insure a good aliment of the laser beam on measurement area. The stand holder was fixed as a vertical on an optical bench by two screw bolts.

2.6 Beam expander

The reverse Galilean telescope design provides a certain angular magnification, called the Expander Power. The beam diameter is first increased in size by its power. The expander was used in the optical unit in front of Helium-Neon laser. Each laser beam size 1 mm expanded in order to cover area 20X30 mm2. The specifications of beam expander were as follows:

Dimensions of the expander with length of 280 mm , maximum and minimum diameters of 110 and 50 mm.

Source of manufacture: Egypt, made in hard Aluminum, electrostatic black paint.

Beam expansion power : 50 X

Lenses: (1 st Diverging lens : F = 20 mm; Dia.= 15 mm); (2 nd Diverging lens: F = 2.2 mm; Dia.= 10 mm); and (Collimating lens : F = 240 mm; Dia. = 100 mm, (F is the focal lens)).

2.7 Optical bench

The optical bench was fabricated from stainless steel. The dimensions of this bench were 880, 580 and 45 mm in length, width and height, respectively, with screw holes 6 mm diameter and the distance between them was 50 mm. Bench was manufactured in USA sitting on the second frame.

Table 1 Setup of specification of the experiment

Frist group with Nile water			
Control with N2	5 minutes	10 minutes	15 minutes
R1	R1	R1	R1
R2	R2	R2	R2
R3	R3	R3	R3
R4	R4	R4	R4
Second group Nile water			
Control with Rhizobia	5 minute Laser and Rhizobia	10 minute Laser and Rhizobia	15 minute Laser and Rhizobia
R1	R1	R1	R1
R2	R2	R2	R2
R3	R3	R3	R3
R4	R4	R4	R4

Control with N2 5 minutes 10 minutes 15 minutes

(a) Sampling of clover seed germination by laser irradiation in Lab

Control with Rhizobia 5 minute Laser and Rhizobia 10 minute Laser and Rhizobia 15 minute Laser and Rhizobia

(b) Clover seed germination by laser irradiation and Rhizobium incubation in Lab

Figure 2 Clover seed germination by laser irradiation or/and Rhizobium incubation in Lab

2.8 Measurements

Seeds were considered germinated when a visible radicle protrusion (≥ 2 mm) occurred, at which point they were removed (Soltani et al., 2002). Results are analysed in graphical form.

Seed germination was monitored every 8 hours for the first 4 days, then inspected every 24 hours for 14 days.

Radicle and plumule lengths: They were measured by vicul capilor .

The vigour index (VI) of the seed was estimated as suggested by Abdul-Baki and Anderson (1973) as follow:

Vigour index = [germination percentage × mean (radicle length + plumule lenght] / 100

Germination percentage (GP) was defined as the final accumulated seed germination (%) at the end of each experiment.

Germination rate (GR) of individual seeds or percentiles in a seed population is defined as the inverse of time taken by that seed or percentile to complete germination. Since there were 50 seeds per replicate, each germinated seed counted as 2%. The 50th seed percentile (25th seed in the replicate) to germinate was considered the median percentile, as this would be the median seed to germinate under optimum conditions.

Specific root length (SRL) as cm g^{-1} = root length (cm)/root weight (g).

Specific root length (SRL) is probably the most frequently measured morphological parameter of the roots. It is believed that it characterize the changes of roots depending on size and type of the fertilization. According to Ostonen et al. (2007) specific root length decreased significantly under fertilization.

Percentage of survival seedlings in the recovery test was calculated as:

Survival seedlings/germinated seeds × 100

Timson's germination index was calculated using the formula reported by Ajmal and Ungar (1998).

Timson germination index (TGI) = ΣG/T, where G is the percentage of seed germinated per day, and T is the germination period.

2.9 Data analysis

Data of germination parameters were subjected to variance analysis of using SAS 8.0 (Statistical Analysis System), and using graphs to show the interacting effect of laser or/ and Rhizobium on germination behaviour.

2.10 Setup of germination experiments

Lab Germination experiments were divided into two groups, the experiments were used Laser or/and Rhizobium treatments as shown in Table 1 and Figure 2.

3 Results and discussion

3.1 Effect of laser or/and Rhizobium on germination percentage (GP)

GP was affected by laser exposure time (LET) or/and Raizobium (Rhiz). Responses of GP to LET indicates that, under 5, 10, and 15 min., the average GP was high (98% and 99%) over a untreated sample (control) 88%. GP was still above 88% at 5 min or and Rhizobium. The laser exposed time (5 min) or/and Rhizobium for this experiment (Figures 3 and 4) were the optimal treatments for GP when laser exposed time or/and laser 5 min, and GP reduced when laser exposed time was 10 min. But, at laser exposed time or/and Rhizobium 15 min., was didn't acceptable germination percentage (82% and 85%), because of less than untreated samples (88%).

3.2 Effect of Laser radiation or / and Rhizobium incubation on germination seed percentages

By using Laser radiation, the results were indicated that the germination percentages of clover seeds were decreased by 98%, 92%, and 82% for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 88%. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the germination percentages of clover seeds were decreased by 99%, 97%, and 85% for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 90%. (Figures 3 and 4)

There is a positive correlation able to between germination percentage and shoot length. Also, there is a positive correlation between the ratio of root to shoot length with shoot length. There is a positive correlation between the germination percentage with root length and vigor index. The results indicated that they possess a higher germination percentage, shoot length and vigour index, have been affected by laser exposed time or/and Rhizobium during the germination stage than the untreated seeds (Control). Norman et al. (1998) state that the ecological significance of therm coinhibition for clovers is that when temperatures are >30 ℃ after false breaks, even soft-seeded clovers will not germinate.

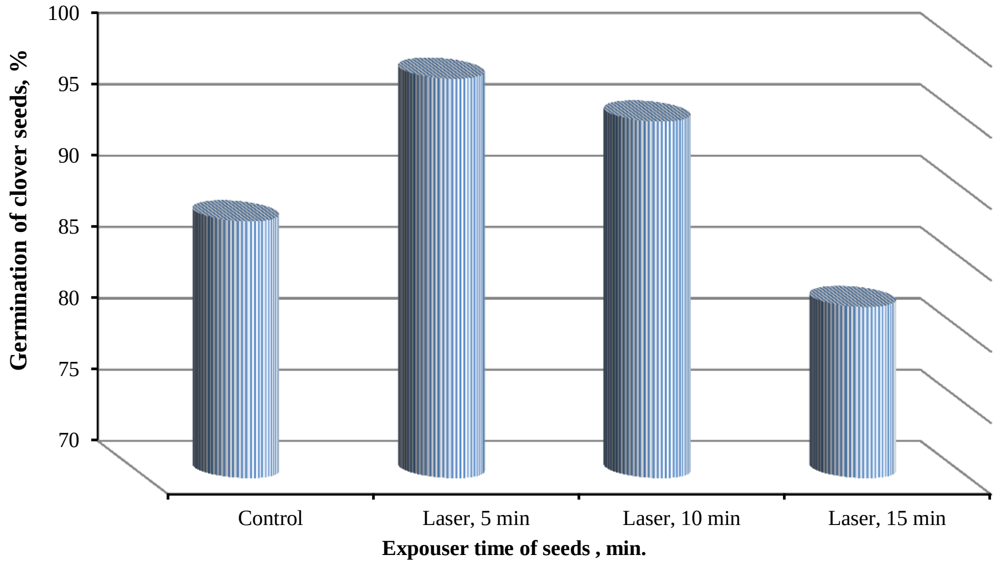

Figure 3 Effect of laser exposure time on germination percentage of clover seeds

3.3 Effect of Laser radiation or/and Rhizobium incubation on germination seed percentages

By using Laser radiation, the results were indicated that the percentage of surviving seedlings of clover seeds were decreased by 98%, 94%, 95.79%, and 93.90% for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 94.32%. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the germination percentages of clover seeds were decreased by 97.98%, 96.88%, and 92.94% for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 95.67%. (Figures 5 and 6). Increasing and spreading the role of biofertilizers such as *Rhizobium* would decrease the necessity for chemical composts and decline adverse environmental effects. Consequently, in the improvement and application of ecological agricultural techniques, biofertilization is of great importance in relieving environmental pollution and the corrosion of nature (Elkoca et al., 2008).

Clover seedlings were found obviously promoted after being inoculated with laser exposed time or/and Rhizobium. Survival rate of seedlings, shoot height, root length, root volume, leaf area, individual number of leaves per plant, biomass and P uptake percentage of the *M. sativa* varieties were found remarkably increased than *control group*. German et al. (2000) observed that associative nitrogen fixing bacteria and phosphate solubilizing bacteria play a positive role in promoting the increase of root length, plant size and root surface area, as well as the assimilation of water and nutrient, and help to strengthen the respiration in root .

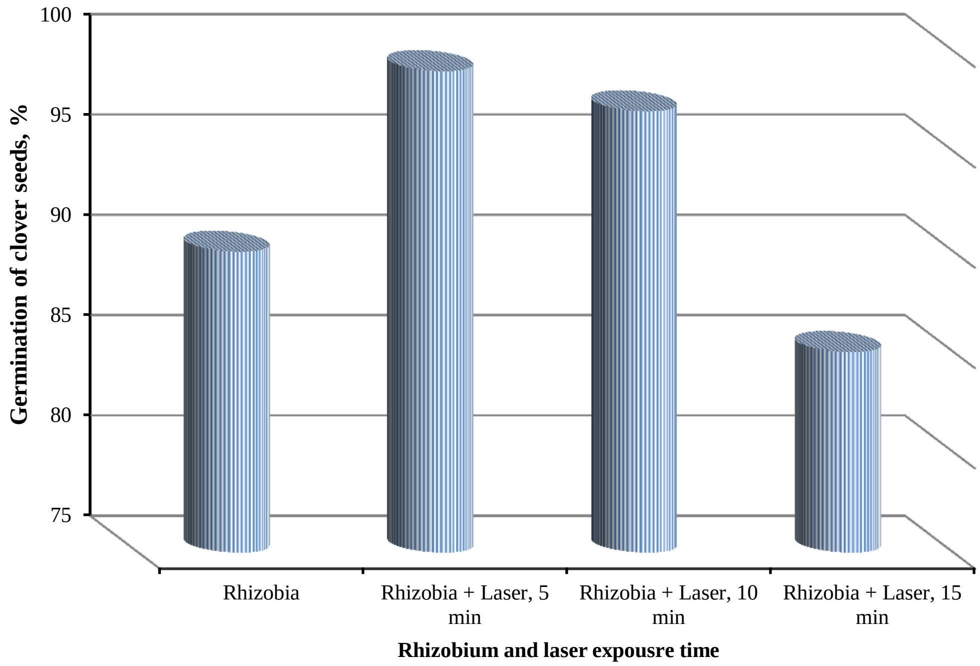

Figure 4 Effect of Rhizobium on germination percentage of clover seeds

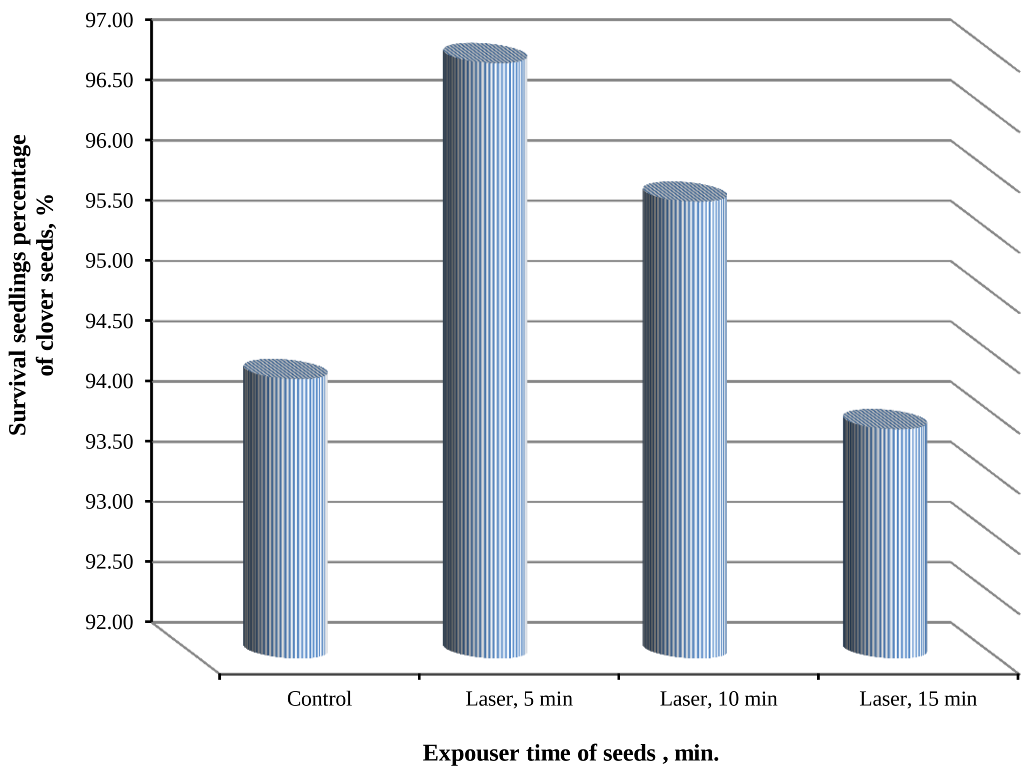

Figure 5 Effect of laser exposure time on survival seedlings percentage of clover seeds

3.4 Effect of Laser radiation or/and Rhizobium incubation on root, shoot and plant lengths of clover

By using Laser radiation, the results were indicated that the root lengths of the clover plant were decreased by (2.9, 2.4, and 1.8 cm for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 3.4 cm. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the root lengths of the clover plant were decreased by 3.6%, 2.4%, and 2.1% for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 3.4 cm.

By using Laser radiation, the results were indicated that the shoot lengths of the clover plant were decreased by 16, 15, and 12 cm for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 13 cm. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the shoot lengths of the clover plant were decreased by 19, 17, and 13 cm for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 15 cm.

By using Laser radiation, the results were indicated that the lengths of the clover plant were decreased by 18.9, 17.4, and 13.8 cm for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 16.4 cm. While, By using a combination of Laser radiation and Rhizobium, the results were indicated that the lengths of the clover plant were decreased by 22.4, 19.4, and 15.1 cm for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 18.6 cm.

In this study, some clover cultivar in terms of germination percentage and rate, plumule length, the ratio of radicle to plumule and vigority index of seed indicated that there is a significant difference ($p< 0.01$). There is a discrepancy among cultivars in terms of germination rate reduction. Notable the reduction in germination percentage among cultivars begins from potential -9 bar, so that the amount of germination reduction in this density is less in treated seeds by nitrogen fertilizer than laser and/or Rhizobium seeds in comparison with other cultivars. Obtained results indicated that by increasing laser exposed time, the root length was decreased,which the more decrease in length continues up to the increased laser exposed time (15 min). and after that the length of the root was decreased. Other studies, Abdul-baki and Anderson (1973), Busso et al. (1998) reported that the radicle length was reduced under water stress. These differences might be due to tolerance genes in chromosome structure and high moisture absorption by resistant cultivars (Hamidi, 2000).

Results pointed that expose clover seeds to laser exposed times from 5 to 15 min., germination percentage reached to 82%, root and shoot lengths were decreased (16 and 3.4 cm) and their appearance was effected. (Control) which were (13 and 2.9 cm) , but at laser exposed times of 15 min., which were the lowest lengths of (12 and 1.8 cm). The author pointed that expose soybean cultivars to drought stress from 0-16 bar reduced the osmotic potential, germination percentage reached to 81%, radicle and plumule lengths were decreased and their appearance was effected. By decreasing water potential the length of plumule in studying cultivars was reduced. Under drought conditions, plumule length was more affected than the radical length (Hamidi, 2000).

3.5 Effect of Laser radiation or/and Rhizobium incubation on specific shoot length, Specific root length and root/shoot ratios plants:

By using Laser radiation, the results were indicated that the specific shoot length (SSHL) of the twenty clover plants was decreased by 147.47, 142.18, and 124.40 cm g^{-1} for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, therefore, the treated seeds with laser exposed times 5 and 10 minutes were higher than untreated seeds (Control) which was 140.35 cm g^{-1}. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the specific shoot length (SSHL) of the clover plants was deccreased by 163.52, 157.41, and 142.18 cm g^{-1} for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 154.47 cm g^{-1} (Figures 11 and 12).

By using Laser radiation, the results were indicated that the Specific root length (SRL)of the twenty clover plants was decreased by 104.62, 80.00, and 69.05 cm g^{-1} for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 77.42 cm g^{-1}. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the Specific root length (SRL) of the twenty clover plants was decreased by 124.14, 100.00, and 80.00 cm g^{-1} for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 59.26 cm g^{-1} (Figures 13 and 14).

By using Laser radiation, the results were indicated that the root/shoot ratios of the twenty clover plants were decreased by 0.18, 0.16, and 0.15 for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 0.26. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the weights of the root/shoot ratios of the clover plants were decreased by 0.18, 0.14, and 0.16 for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 0.24

Sadr (1989) in study on alfalfa plant argues that in this plant dedication of carbon hydrates to root and leave is in precedence than stem. Also leavs and roots have priority in absorbing water and photosynthesis process for plant and that they are more protected than stem, therefore the increase in ratio of radicle length to plumule length was decreasd in growth of aerial organs (Safarnejad, 2008).

Hamidi (2000) indicated that the ratio of radicle to plumule length was increased by increasing drought stress. The ratio of radicle length to plumule length is a flexibility trait which it was increased as the result of drought stress (Safarnejad, 2008).

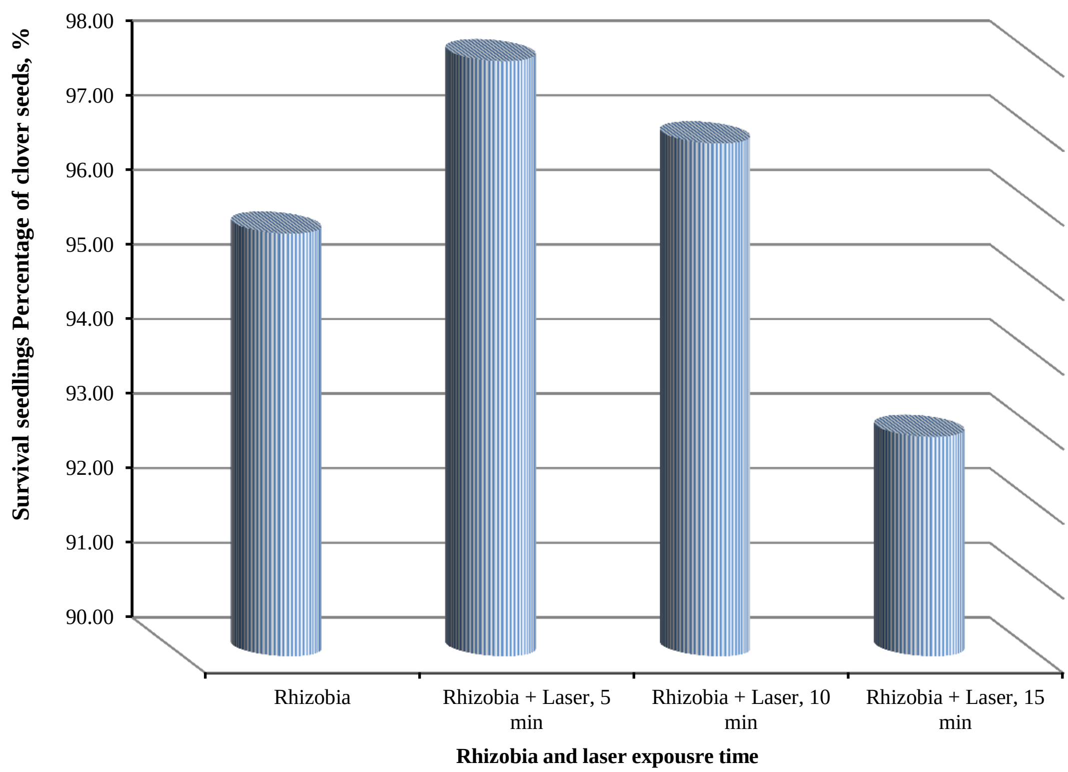

Figure 6 Effect of Rhizobiz on survival seedlings percentage of clover seeds

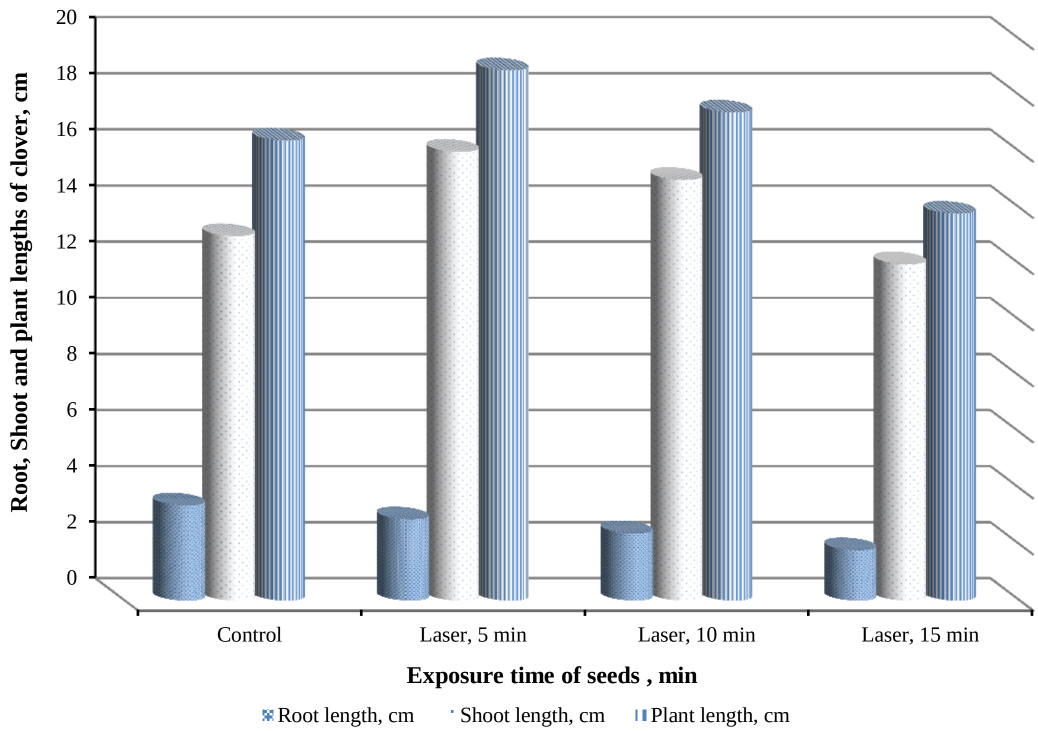

Figure 7 Effect of laser exposure time on plant lengths of clover

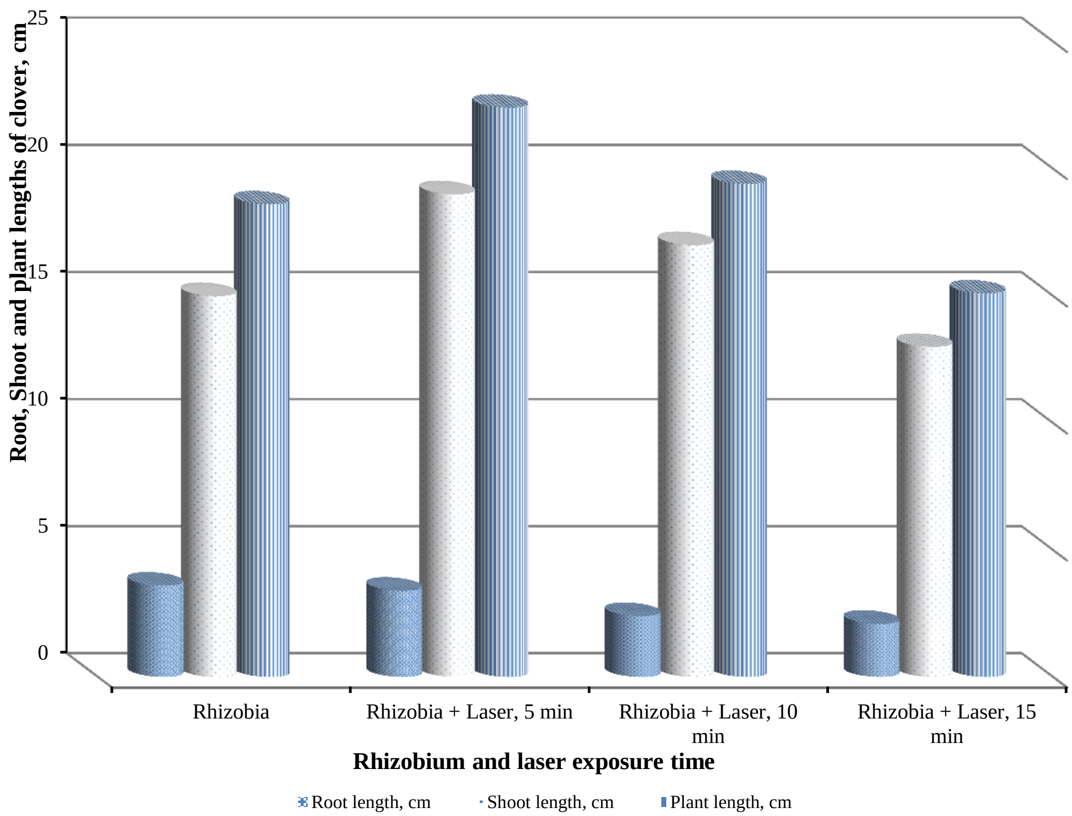

Figure 8 Effect of Rhizobium and laser exposure time on plant lengths of clover

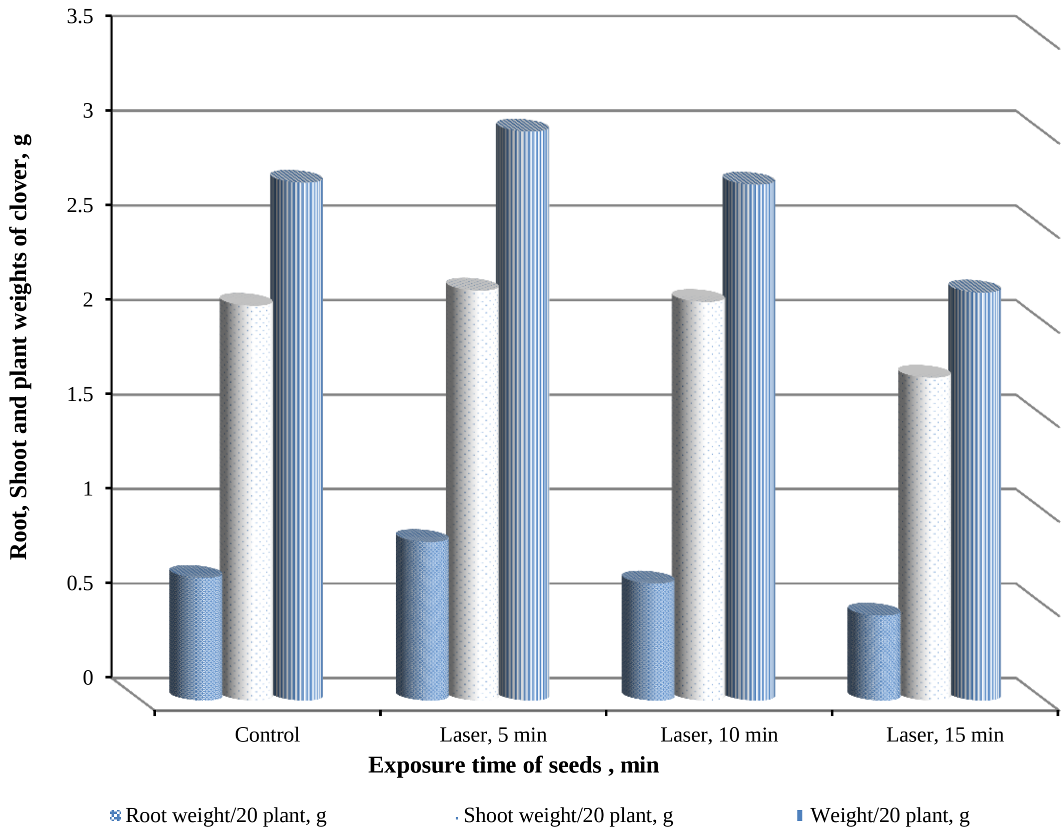

Figure 9 Effect of laser exposure time on plant weights of clover

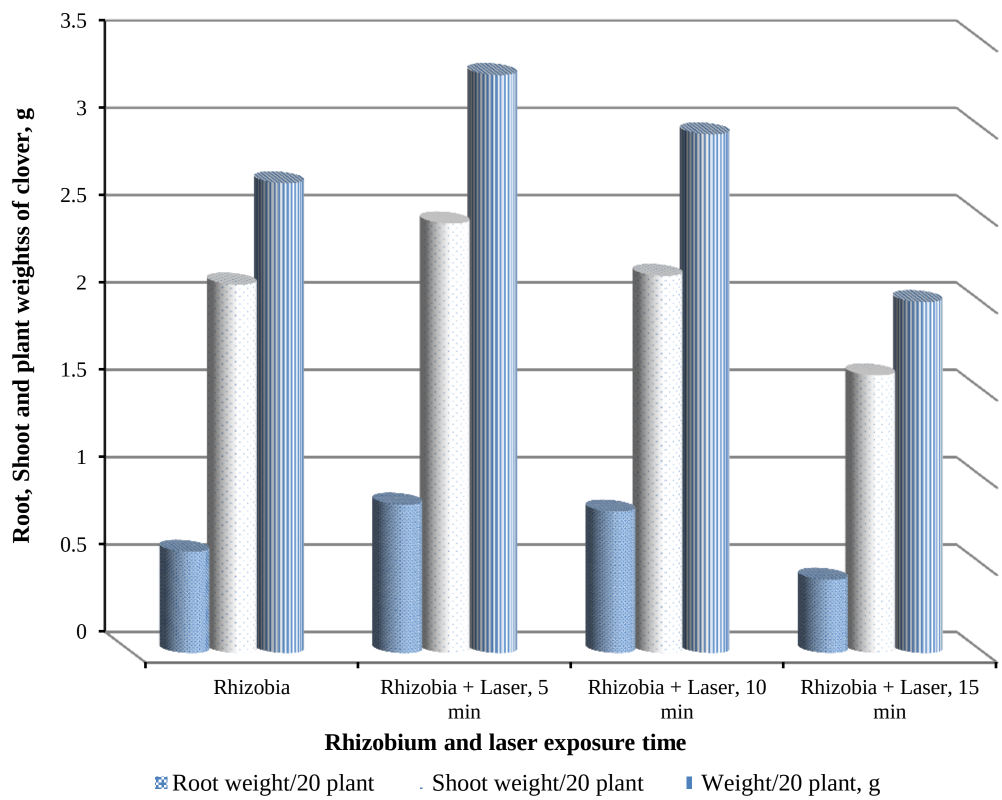

Figure 10 Effect of Rhizobium and laser exposure time on plant weights of clover

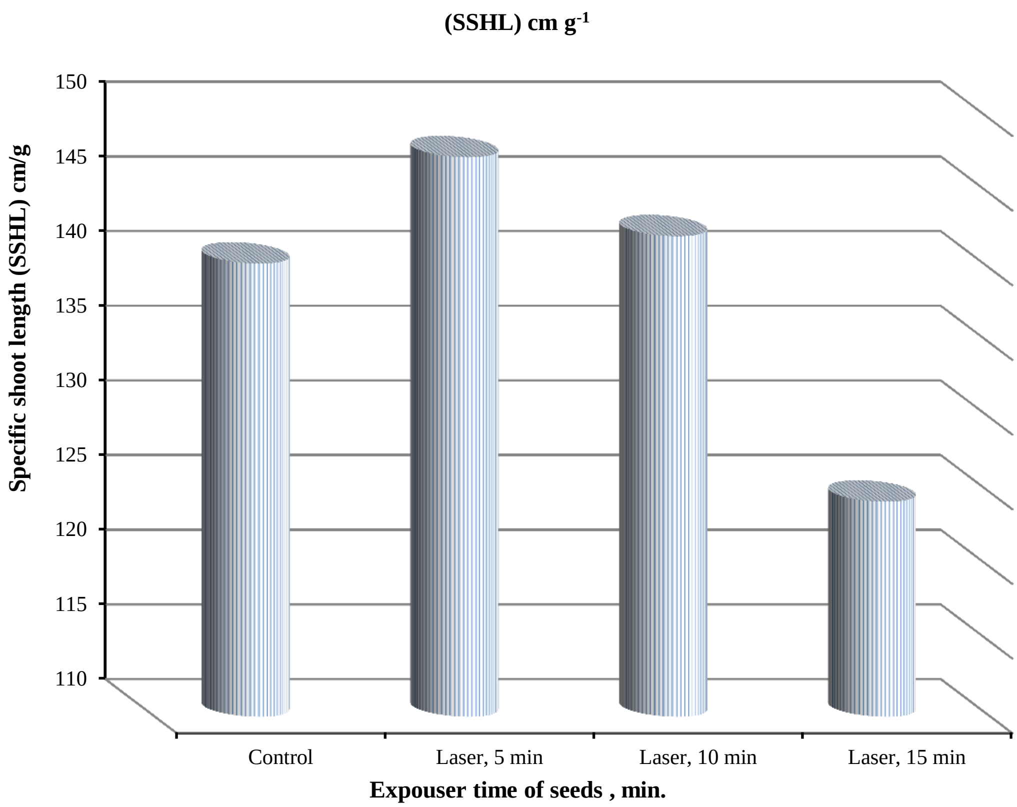

Figure 11 Effect of laser exposure time on SSHL of clover plant germination

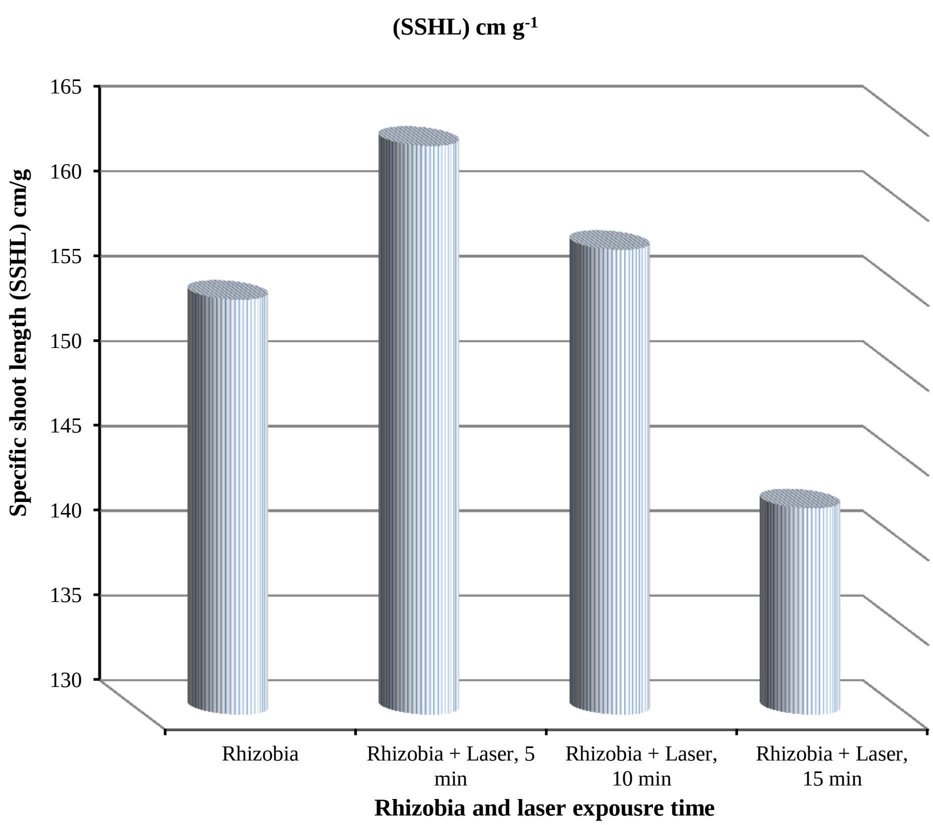

Figure 12 Effect of Rhizobium and laser exposure time on SSHL of clover plant germination

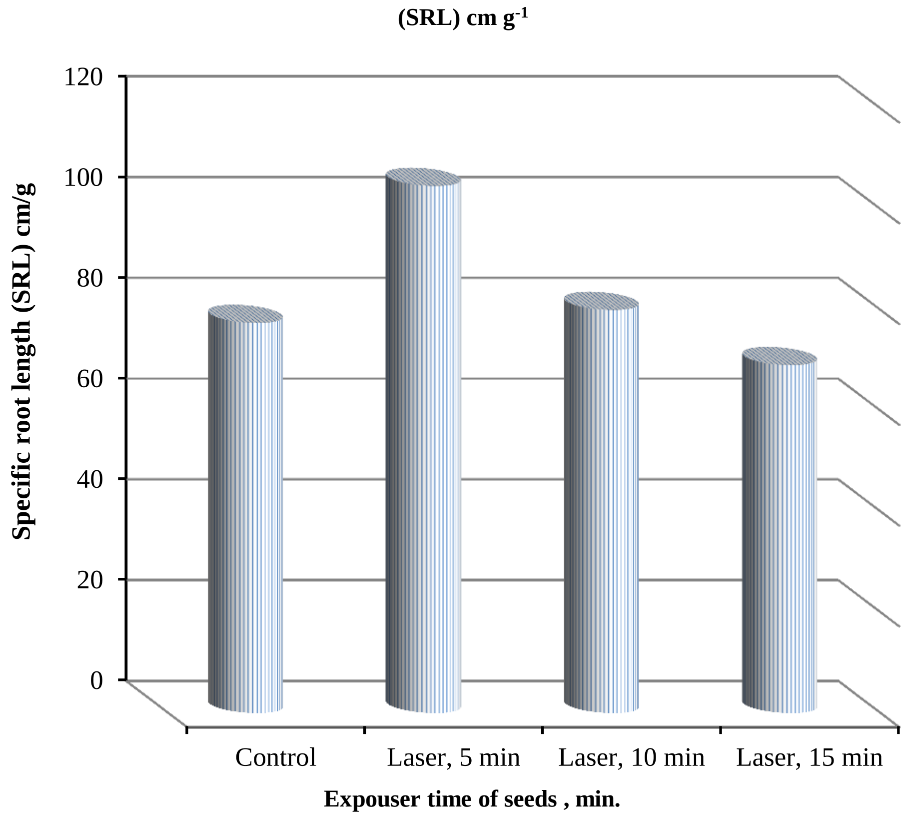

Figure 13 Effect of Rhizobium and laser exposure time on SRL of clover plant germination

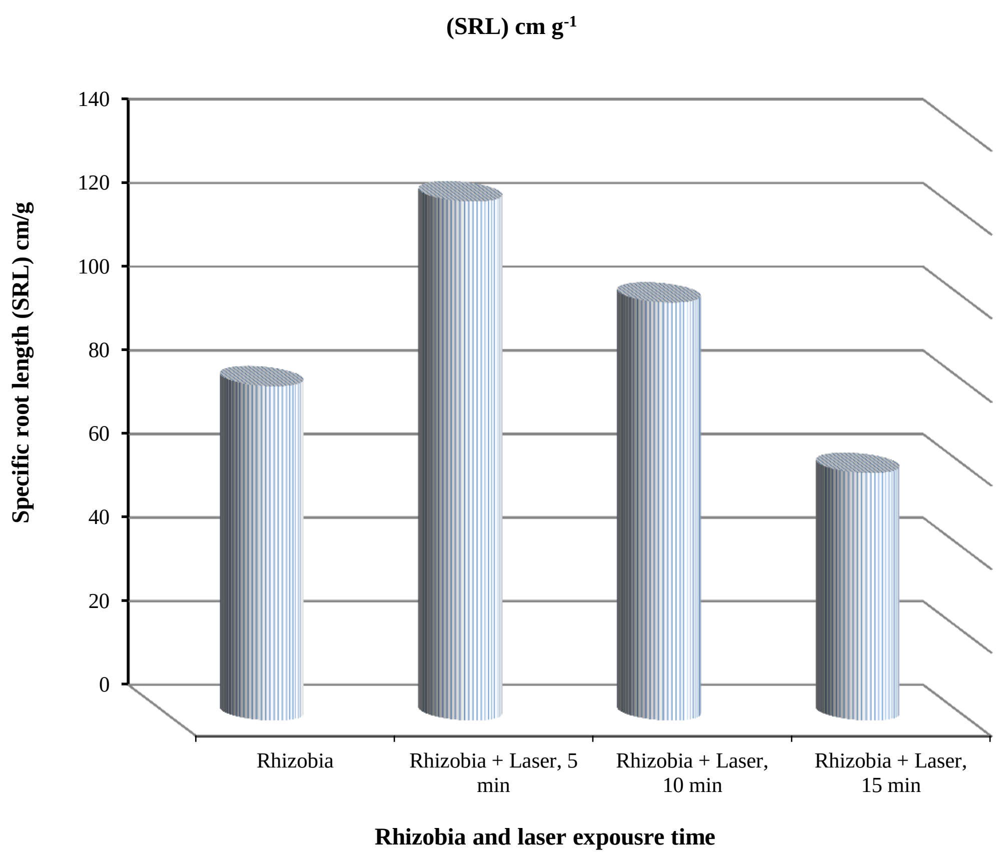

Figure 14 Effect of Rhizobium and laser exposure time on SRL of clover plant germination

3.6 Effect of Laser radiation or/and Rhizobium incubation on root, shoot and plant weights

By using Laser radiation, the results were indicated that the root weights of the twenty clover plants were decreased by 0.84, 0.62, and 0.45 g for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 0.65 g. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the root weights of the twenty clover plants were decreased by 0.85, 0.81, and 0.42 g for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 0.58 g.

By using Laser radiation, the results were indicated that the shoot weights of the twenty clover plants were decreased by 2.17, 2.11, and 1.71 g for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 2.09 g. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the shoot weights of the twenty clover plants were decreased by 2.46, 2.16, and 1.59 g for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 2.11 g. (Figure 15).

By using Laser radiation, the results were indicated that the weights of the twenty clover plants were decreased by 3.01, 2.73, and 2.16 g for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 2.74 g. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the weights of the twenty clover plants were decreased by 3.31, 2.97, and 2.01 g for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 2.69 g (Figure 16).

Figures show the effect of laser exposed time or/and *Rhizobium meliloti* application on shoot length (A), root length (B), and dry mass (D) of alfalfa seedling. It was noticed that the shoot, root lengths, and biomass of seedlings were *promoted more* effectively by laser exposed time or/and Rhizobium at 5 and 10 min *than untreated seeds (Control).*

Biomass of clover seedlings inoculated with laser exposed time or and Rhizobium were increased at 5 minutes over others treatment (laser exposed time or/and Rhizobium (10 and 15 minutes) but the untreated seeds was the higher than treated seeds at 15 minutes. So, the lowest biomass samples become the treated seeds by 15 minutes.

Justes et al. (2001) considered that Alfalfa plants without nitrogen fertilization had a significantly lower root dry mass than plants with nitrogen fertilization.

3.7 Effect of Laser radiation or / and Rhizobium incubation on vigor index and TGI plants

By using Laser radiation, the results were indicated that the vigor index (VI) of the twenty clover plants decreased by 18.52, 16.53, and 11.32 for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 14.43. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the vigor index (VI) of the clover plants was decreased by 22.18, 18.82, and 12.84 for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 16.74 (Figures 17 and 18).

For the germination index (GI), chipping and all SA exposures (except 90 minutes) revealed high means for both *C. persica* and *T. apollinea*, while SP also resulted in high germination index for *C. persica*. The mean rate of seed germination was highest under sand rubbing and distilled water (0.9 and 0.8, respectively) for *C. persica* and under SA20 (1.3), for *T. apollinea*. (Al-Ansari and Ksiksi, 2016).

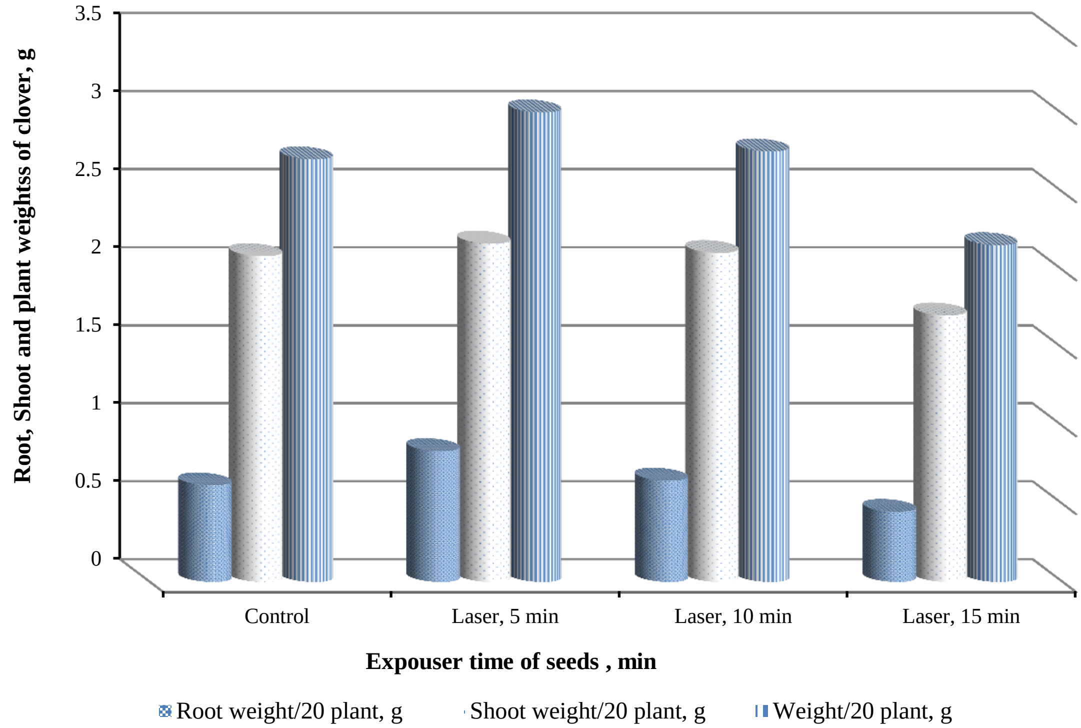

Figure 15 Effect of laser exposure time on plant weight of clover

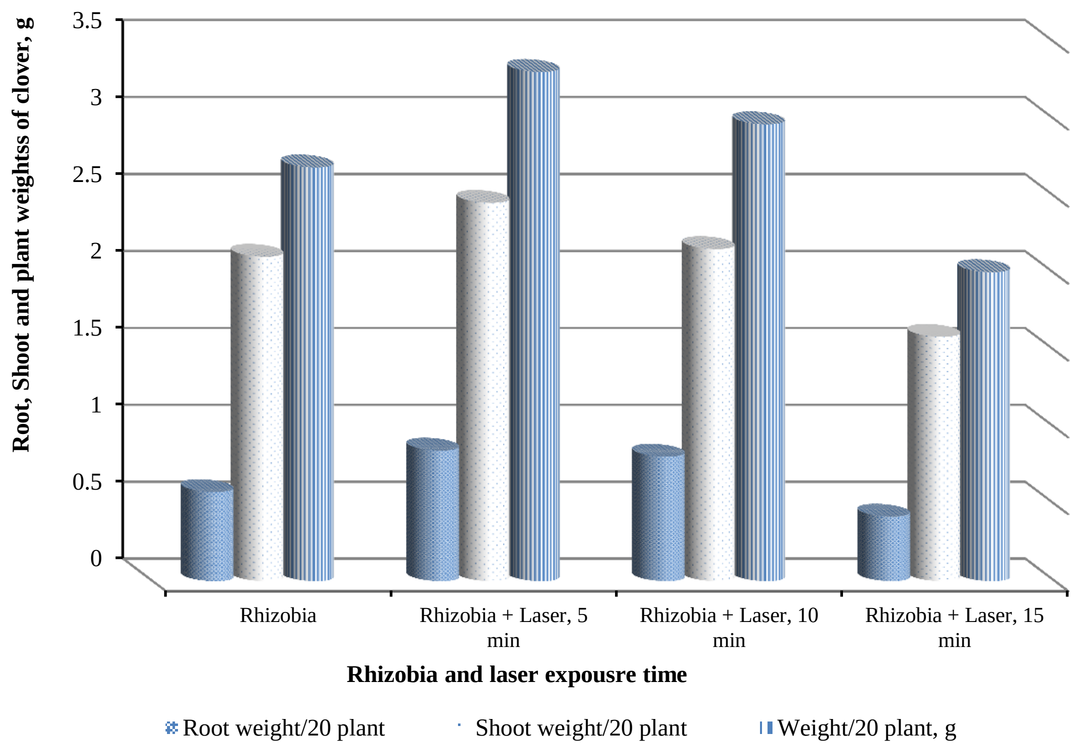

Figure 16 Effect of Laser or and Rhizobiz on plant weight of clover

By using Laser radiation, the results were indicated that the GP/Period (TGI) of the twenty clover plants decreased by 6.53, 6.33, and 5.47 for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control) which was 5.87. While, By using combination of Laser radiation and Rhizobium, the results were indicated that the GP/Period (TGI) of the twenty clover plants was decreased by 6.60, 6.47, and 5.67 for combination Rhizobium and seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples as Rhizobium only (Control) which was 6.00 g. (Figures 19 and 20)

Timson Germination Index (TGI) showed high values for most pre-treatments. The highest TGI recorded was 45.6% for SA30. As for the correlation between the germination parameters, it is recommended that final germination percent coupled with germination index are used as germination parameters. They do not require complex calculations, while they complement each other in giving a representative evaluation of seed germination (Al-Ansari and Ksiksi, 2016).

Data also showed that the vigor index of clover seeds was decreased by increasing at Laser or/and Rhizobium. This reduction in laser with 15 minutes or / and Rhizobium (12.84 and 11.33). While, were less reduction in in laser with 5 minutes or / and Rhizobium (22.04 and 8.52), but they were intermittent reduction in laser with 10 minutes or / and Rhizobium (18.82 and 16.53). (Figures 11 and 12). Germination rate index and the germination index provided reliable information. The germination rate index gave the highest correlation between seed characteristics and seedling germination (Ahmad, 2001).

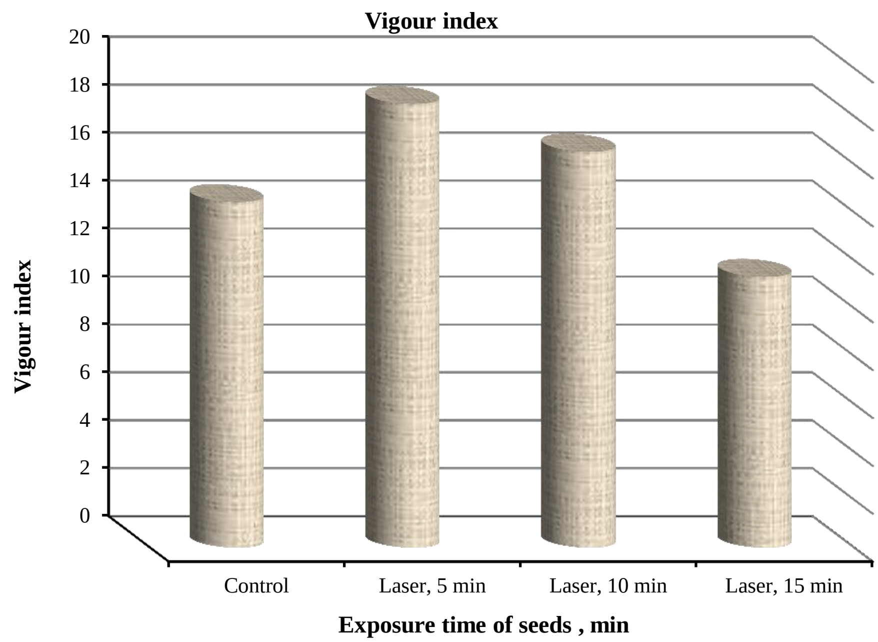

Figure 17 Effect of laser exposure time on vigour index of clover plant germination

An attempt of TGR was made to assess the correlations between Timson germination index (TGI), a widely used, germination index, to break the dormancy for seed clover.

The highest germination was observed in treating seeds by Rhizobia and exposure with Laser for 5 min (99%). Timson Germination Index (TGI) also showed high values for all treatments; except of treated seeds by exposure laser time for 15 min. High laser exposure time may have been the most important factor leading to this lack of germination.

The highest germination was observed in Tamarindus indica seeds treated with 50% Sulfuric acid exposure (Muhammad and Amusa, 2004). Timson Germination

Index (TGI) also showed high values for all treatments; except for SA90. High acid exposures may have been the most important factor leading to this lack of germination.

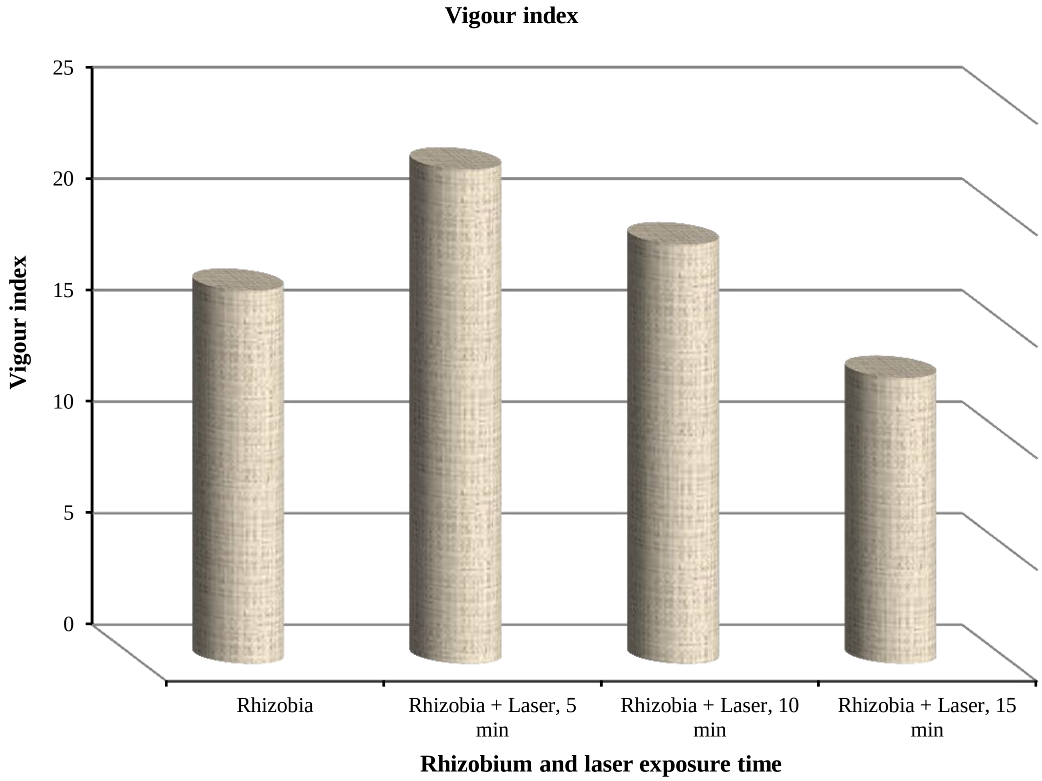

Figure 18 Effect of Rhizobium and laser exposure time on vigour index of clover plant germination

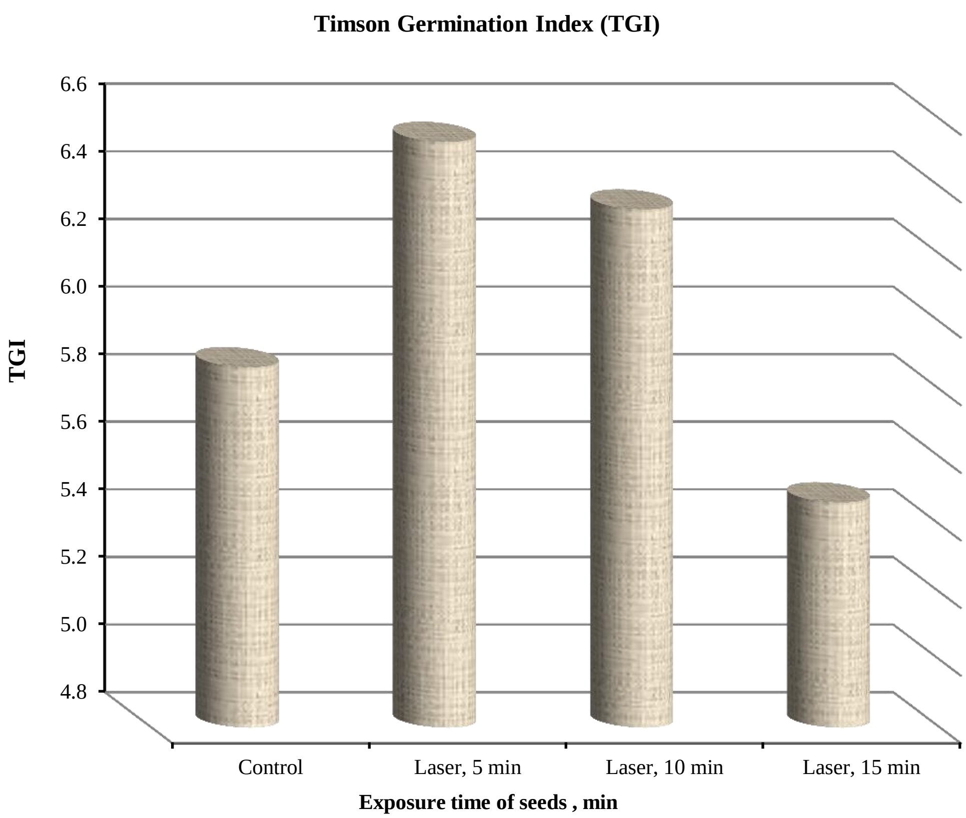

Figure 19 Effect of laser exposure time on TGI of clover plant germination

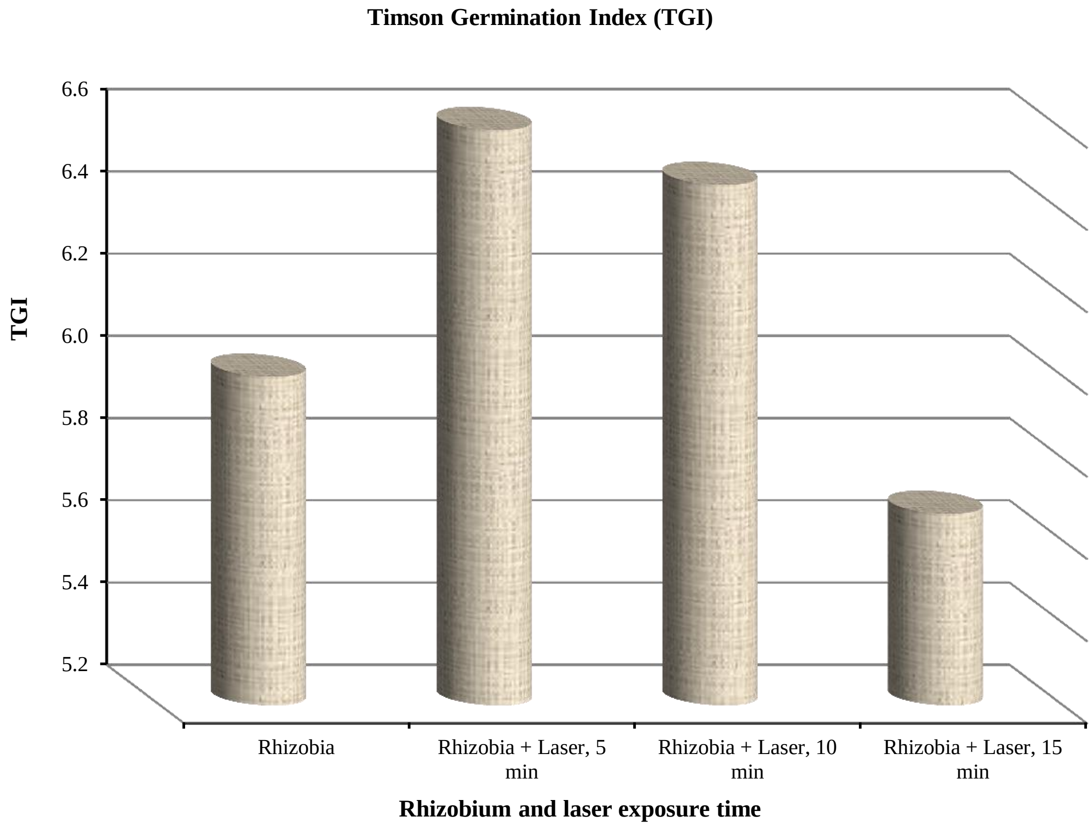

Figure 20 Effect of Rhizobium and laser exposure time on TGI of clover plant germination

4 Conclusion

To study effect of irradiation seeds by laser radiation and seeds incubation by Rhizobium combination with laser exposed times 5, 10, and 15 minutes compared in untreated seeds on the germination parameters of clover seeds. Obtained results were oncluded s following: using treate seeds by combination laser exposed times and Rhizobium gave higher the germination parameters than seeds which treat by the laser exposed time. By using Laser exsed timesor/and Rhizobium, the results were indicated that the germination parameters of seed cover were decreased for seed irradiation with Laser radiation of 5, 10 and 15 minutes, respectively, compared with untreated samples (Control). While, the higher values were when using laser exposed time of 5 minutes or/hizobium, but, the lowest values were noticed when using laser exposed time of 15 minutes or/and Rhizobium, because of less than untreated seeds (Control).

Conflict of Interest

The authors confirm that this article content has no conflict of interest, the authors declare that there are no conflicts of interests regarding the publication of this manuscript.

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