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# Evaluation Effect of Arbuscular Mycorrhiza Fungi, Biochar and Nitrogen Fertilizer on Important Root Characteristics and Grain Phosphorous Absorption of Wheat cv. Chamran

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## ABSTRACT

**BACKGROUND:** Plant nutrition with nitrogen, biochar, and bacteria leads to the improvement of soil quality, enhanced nutrient supply and facilitated root growth, all of which play a significant role in increasing agricultural productivity and improving crop yields.

**OBJECTIVES:** To investigate the yield reaction of Chamran cultivar wheat and root properties to mycorrhiza and biochar at different levels of nitrogen, an experiment was conducted during two crop years (2017-18) in the southwest of Iran.

**METHODS:** The experiment was designed as a factorial study using a randomized complete block design (RCBD) with four replications. The experimental factors included four levels of nitrogen (applied as urea with 46% nitrogen): 0, 50, 100, and 150kg.N.ha$^{-1}$, two levels of Rhizophagus Irregularis inoculum (without and with application), and three levels of biochar: 0, 4, and 8 ton.ha$^{-1}$.

**RESULT:** The results of the analysis of variance demonstrated significant effects of urea fertilizer, mycorrhiza and biochar on root length, root diameter and root colonization. In this research, the combined use of biochar and urea fertilizer in plants inoculated with mycorrhiza fungi showed better effects on all investigated traits compared to the use of biochar, nitrogen, and mycorrhiza alone. The highest values for root length, root diameter, root dry weight, root volume and root surface density were obtained from the application of 100 and 150 kg.N.ha$^{-1}$ of nitrogen, along with 4 and 8 ton.ha$^{-1}$ of biochar and mycorrhiza. In plants inoculated with mycorrhiza fungi at a biochar level of 4 ton.ha$^{-1}$, there was a significant increase in root length (24%), root colonization rate (9%), root volume (22%) and root surface density (35%) compared to the control plants. This increase was attributed to the improved ability to absorb nutrients and enhance plant growth.

**CONCLUSION:** According to the results of this research, the application of 100 kg.N.ha$^{-1}$ of nitrogen and 4 ton.ha$^{-1}$ of biochar in plants inoculated with mycorrhiza fungi played a vital and effective role in promoting wheat growth and increasing yield by improving the root system.

**KEYWORDS:** *Biofertilizer, Root Colonization, Root length, Root volume, Urea.*

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## 1. BACKGROUND

Wheat is very important as the main food of half of the world's population, for this reason it is considered a strategic plant (Mostajeran *et al*., 2007). The use of nitrogenous chemical fertilizers is one of the ways to solve the nitrogen requirement of agricultural products, which is on one hand the best agricultural input effective in increasing production, and on the other hand, it has a high pollution potential. Excessive use of nitrogen causes surface water pollution. The unbalanced use of nitrogen fertilizers may be caused by their low efficiency, which factors such as losses due to sublimation, leaching, surface runoff, and DE nitrification can be related to this issue. The efficiency of nitrogen consumption shows the increase in the method of absorption, the efficiency of consumption and the method of allocation of yield per increase of each input unit. Today, in order to solve this problem, use biological methods, including the symbiosis of plant roots with microorganisms (Alizadeh Oskouei *et al*., 2014). Biofertilizers are a suitable alternative to chemical fertilizers with the aim of increasing soil fertility and producing sustainable agricultural products. Mycorrhiza symbiosis is one of the most widely known symbiotic relationships between plants and microorganisms and it exists in all ecosystems, so that about 95% of vascular plant species have at least one type of mycorrhiza. Pal and Pandey (2017) investigated the effect of mycorrhiza fungus on the growth and yield of wheat plant and reported that mycorrhiza fungus increased plant height, root length, root biomass, and plant root colonization. A common technology to increase soil fertility is the integrated management of agricultural plants, which includes the use of various animal or organic fertilizers in the soil, but due to the rapid decomposition, the effect of these substances is greatly reduced after a relatively short period, or almost it disappears. Therefore, farmers are required to continuously and annually use these substances in the soil. In addition to creating environmental problems, this issue also leads to an increase in production costs (Sika, 2012). Using biochar as a soil conditioner improves the physical, chemical and biological conditions of the soil. This composition, which turns into biochar at high temperatures, has a fine structure and a special surface. Microspores of biochar can provide a safe environment for the microbes responsible for changing the shape of food elements and reduce the circulation time and increase the bioavailability of food elements. Biochar is a sustainable form of charcoal produced by heating natural materials under high temperatures or without oxygen. This composition has a unique physicochemical structure that leads to an increase in soil fertility and crop yield, especially in degraded soils, which improves soil quality and health, increases crop yield, increases cation exchange capacity, reduces soil acidity, reduces the absorption of toxic substances in the soil and improves the soil structure. It has been shown in many researches that the contamination of grains with mycorrhiza increases the root volume and biomass

(Berta *et al*., 2002). This development is related to the increase of growth hormones (Safapour *et al*., 2012) and proton leakage (Mostajeran *et al*., 2007). In addition to improving the water retention capacity of the soil, biochar is considered as an organic fertilizer and a suitable source of nutrients for plant growth. The results of researches have indicated the reduction of nitrate leaching in soils containing biochar. Anderson and Impiglia (2002) reported that the morphological characteristics of the root change drastically with the change of soil characteristics, climatic conditions and especially the amount of nitrogen in the soil, and therefore these traits can be improved in crops through the optimal use of nitrogen fertilizers.

## 2. OBJECTIVES

Since the global approach and the application of management methods such as the use of biological fertilizers tends to reduce the consumption of chemical fertilizers, this research was conducted in order to know effects of mycorrhiza fungi and biochar on yield and characteristics of wheat plant roots.

## 3. MATERIALS AND METHODS

### *3.1. Field and Treatments Information*

This experiment was carried out during two crop years of 2017-2018 in a field located at south-west of Iran, with a longitude of 49 degrees and 18 minutes east and latitude of 31 degrees and 20 minutes north and a height of 34 meters above sea level. Its climate is dry and tropical, with hot and long summers and mild and short winters. The average temperature in the hot period, which starts from May and lasts until the end of October is about 31.2 degrees Celsius, and its maximum sometimes reaches more than 50 degrees Celsius. During the winter, the average temperature is 14.9 degrees Celsius, and the minimum temperature may rarely fall below zero. The average annual rainfall in this city is about 266 mm and the rainfall period is usually between October and May. Samples were prepared from 0 to 30 cm soil depth and the physical and chemical characters were evaluated. The soil test results are presented in tables (1 and 2).

**Table 1.** Some characteristics of farm soil

| Crop year | Soil Depth (cm) | Soil texture | P (ppm) | K (ppm) | N (%) | OC (%) | EC ($ds.m^{-1}$) | pH |
|---|---|---|---|---|---|---|---|---|
| 2016-2017 | 0-30 | Sandy loam | 9.0 | 120.0 | 0.03 | 0.84 | 4.1 | 6.9 |
| 2017-2018 | 0-30 | Sandy loam | 9.1 | 124.2 | 0.04 | 0.90 | 3.9 | 6.8 |

This experiment was designed as a factorial study using a randomized complete block design (RCBD) with four replications during two crop years (2017-18) in the southwest of Iran. The experimental factors included four levels of nitrogen (applied as urea with 46% nitrogen): 0 ($N_1$), 50 ($N_2$), 100 ($N_3$) and 150 ($N_4$) $kg.N.ha^{-1}$, two levels of Rhizophagus Irregularis inoculum [without ($M_1$) and with application ($M_2$)], and three levels of biochar: 0 ($B_1$), 4 ($B_2$), and 8 ($B_3$) $t.ha^{-1}$.

The biochar used was prepared from combination of forest trees in northern Iran (Table 3).

*3.2. Farm Management*

The land preparation operation included plowing, mowing, two vertical discs and a trowel at the end of October. In the planting section, two streams of water were built, one for the inflow of water at the beginning of the plots and the other for the exit of excess water at the end of the plots to prevent the mixing of treatment water and the transfer of microorganisms. Each block has 24 plots and each experimental plot includes 7 crop lines with a length of 6 meters and a width of 2.5 meters and the distance between the planting rows was considered to be 20 cm. The grain used was based on the density of 400 plants per square meter and the cultivar used was Chamran cultivar.

**Table 2.** Average biennial meteorological parameters of test site

| Month | Rainfall (mm) | Average Minimum Tem. ($^oC$) | Average Maximum Tem. ($^oC$) | Average minimum relative humidity (%) | Average Maximum relative humidity (%) |
|---|---|---|---|---|---|
| **August** | 0.0 | 32.9 | 48.1 | 14.3 | 49.1 |
| **September** | 0.0 | 26.4 | 42.5 | 13.8 | 43.8 |
| **October** | 0.0 | 21.9 | 38.4 | 11.42 | 35.12 |
| **November** | 12.5 | 17.6 | 32.1 | 10.3 | 28.8 |
| **December** | 25.0 | 8.5 | 24.3 | 8.1 | 26.1 |
| **January** | 29.0 | 8.1 | 21.2 | 7.9 | 25.4 |
| **February** | 28.5 | 12.1 | 22.5 | 8.3 | 26.0 |
| **March** | 14.5 | 14.4 | 30.7 | 13.3 | 31.0 |
| **April** | 10.0 | 19.3 | 37.6 | 14.5 | 28.0 |

To provide the required nutrients, triple superphosphate in the amount of 100 $kg.N.ha^{-1}$ and potassium sulfate in the amount of $150kg.N.ha^{-1}$ according to the soil test were used in all treatments before cultivation. Also, all the biochar in the specified treatments was added to the soil at a depth of 20 cm before planting and then it was mixed with the soil. In order to inoculate wheat grains with mycorrhiza spores, hyphae and roots as a fungal inoculum, the grains were first sterilized by 0.5% sodium hypochlorite solution. These grains were soaked in sterile distilled water for two hours and then they were divided into equal proportions and poured into separate buckets, and then in order to keep the active organs of the fungus on the grains, a thick solution of 20% sugar and Gum Arabic was used. One hour before planting, the grains were completely soaked with inoculation liquid and used along with the control treatment (without spore inoculation), so that first the control treatment and then the other treatments were cultivated in the order of the experimental plan. In order to treat mycorrhiza species (*Rhizophagus irregularis*), mycorrhiza fertilizer with a spore density of 105 per gram of carrier material at the rate of one kilogram per hectare was used at the same time as planting and inoculated with grains. Urea fertilizer (46%) was used to supply the required nitrogen for treatments of 50, 100 and 150 $kg.N.ha^{-1}$. Urea fertilizer according to the soil test (Tables 1 and 2) and based on the determined values for each plot in three distributions: 1/3 before planting, 1/3 at the time of tillering and 1/3 at the time of stem elongation in the form of surface spreading provided to the plant. The first irrigation was done after planting and spreading urea fertilizer in the fields and then regularly according to the needs of the plant every 7 to 10 days. Manual weeding of weeds was done in two stages at the beginning of the stem growth and the stage of the stomach of the stew and without any herbicides for prevent the adverse effect of herbicides on the microorganisms.

3.3. *Measured Traits*

3.3.1. *Root sampling*

Root sampling was done only in first year. Sampling to check root characteristics was done from the depths of 0-30 cm using agar. At the time of handling, two square meters of whole plants were removed from each plot (from the four middle lines with the removal of 0.5 meters from the beginning and the end of each line) with minimal damage and then washed using running water. To separate the soil particles attached to the roots, sodium hexa meta phosphate solution was used and immediately the wet weight of the whole shoot and the wet weight of the root were measured with a precision scale with an accuracy of 0.0001 grams. The volume of the root was calculated through the volume difference created after placing the root in a specific volume of water with an accuracy of 0.1 ml.

**Table 3.** Physical and chemical properties of biochar

| Property | Value |
|---|---|
| **Iodine Value** | 950-1100 $mg.g^{-1}$ |
| **Surface area according to ASTM standard** | 950-1100 $m^2.g^{-1}$ |
| **Methylene blue number** | 150-250 $mg.g^{-1}$ |
| **Amount of moisture** | 8-10% |
| **pH** | 5.8% |
| **Ash** | 4-6% |
| **Carbon** | 84-88% |
| **Gradation** | 0.1mm and <1 |

3.3.2. *Root length, root diameter and root density*

Root length was also calculated using a 1 mm precision ruler and root diameter using a digital caliper, and root density was calculated using the following formula (Ganjali *et al*., 2003).

**Equ. 1.** Root area = 2 × (Length × π × volume) $^{0.5}$

**Equ. 2.** Root surface density = (π × root diameter × root length)

**Equ. 3.** Root density = root dry weight / root volume.

Determining the percentage of root colonization: Also, to determine the percentage of root colonization, after staining, the roots were gridded using the line crossing method (Philips and Hayman, 1970). The sampled roots were washed with water and kept in 5% ethanol solution. Then, in order to remove the color, the roots were placed in a solution of 10% potassium hydroxide for three hours at a temperature of 60 degrees Celsius. In order to neutralize the environment, the roots were placed at room temperature for 4 hours, after which the roots were placed in 0.1 M hydrochloric acid solution.

Then the roots were stained for 12 hours in terpan blue solution (0.01%) according to Giovannetti and Mosse (1989) method. Colored hairy roots about one centimeter long were placed in a petri dish (mesh plate method) which was formed using vertical and horizontal lines of one centimeter squares, spread randomly and then using a microscope, the infected roots and were observed uncontaminated.

3.3.3. *Determining the ratio of dry and wet weight of the root to the volume of the soil*

The wet weight of the root was determined by a digital scale with an accuracy of 0.001. Then the roots were placed inside the oven at 70°C for 48 hours and weighed again with a digital balance scale with an accuracy of 0.001. By dividing the root wet weight and root dry weight by the soil volume, the ratio of root dry and wet weight to the soil volume was calculated (Naseri *et al*., 2019a).

3.3.4. *Measurement of phosphorus*

Grain phosphorus was measured using a spectrophotometer according to the Olsen and Sommers (1982) method. In this way, 0.5 grams of ground plants of each experimental unit were placed in porcelain jars and 5 ml of 0.5 normal magnesium nitrate was added to it. Then it was placed in a bath at a temperature of 100 degrees for 2 hours until 0.5 normal magnesium nitrate was added to it and then it was placed in a bath at a temperature of 100 degrees for 2 hours until the magnesium nitrate completely evaporated. The temperature was 500 degrees Celsius. 5 ml of 2N hydrochloric acid was added to each of the samples. Then they were heated in a Banmari at a temperature of 56 degrees Celsius for 5-10 minutes. After heating, filter paper into 100 ml volumetric flasks. It was transferred to a volume of 100 ml with lukewarm distilled water and after that, 10 ml of this obtained extract was dissolved with 10 ml of 0.5 normal sodium bicarbonate and 2 ml of chlorine and 2 normal tin. Finally, it was added to the volume of 50 ml with distilled water and then read with a spectrophotometer at a wavelength of 660 nm and final amount of plant phosphorus was calculated as a percentage.

3.4. *Statistical Analysis*

The results of the data variance uniformity test (Bartlett's test) were not significant in the two years of the research implementation, so composite analysis was performed in the form of factorial test with SAS software (Ver.8). Comparison of means was done by LSD test at 5% probability level.

## 4. RESULT AND DISCUSSION

4.1. *Root length*

The effect of amounts of nitrogen, mycorrhiza and biochar and the interaction effect of urea fertilizer, mycorrhiza and biochar in terms of root length were significant at the 1% probability level (Table 4). The maximum root length with an average of 16.83 cm was obtained from the treatment of applying 50 $kg.N.ha^{-1}$, which showed an increase of 8% compared to the treatment of 100 $kg.N.ha^{-1}$ (Table 5).

**Table 4.** Results of combined analysis of variance of the effects of treatments on studied traits

| S.O.V | df | Root length | Root diameter | Root Colonization | Root dry weight | Root volume |
|---|---|---|---|---|---|---|
| Replication(R) | 3 | 3.01 | 0.05 | 0.2 | 0.49 | 0.1 |
| Mycorrhiza (M) | 1 | 15.84** | 1.04n.s | 2.34* | 2.32** | 0.86n.s |
| Biochar (B) | 2 | 111.54** | 0.94n.s | 7.88** | 18.16** | 13.54** |
| Nitrogen (N) | 3 | 7.45** | 0.55n.s | 39.62** | 1.55** | 25.74** |
| M*B | 2 | 9.5** | 1.32** | 68.46** | 1.63** | 4.67** |
| M*N | 3 | 67.28** | 0.48n.s | 47.51** | 10.65** | 0.8n.s |
| B*N | 6 | 66.31** | 0.62n.s | 10.99** | 11.6** | 4.9** |
| B*N*M | 6 | 145.27** | 0.72* | 6.88** | 23.34** | 7.27** |
| Error | 69 | 1.25 | 0.21 | 0.42 | 0.22 | 0.37 |
| CV (%) | | 6.9 | 12.5 | 1.81 | 12.09 | 9.81 |

ns, * and **: no significant, significant at the 5% and 1% probability levels, respectively.

**Continue table 4.**

| S.O.V | df | Root surface density | Root density | Ratio of root weight to soil volume | Ratio of root dry weight to soil volume | Phosphorus percentage of grains |
|---|---|---|---|---|---|---|
| Replication (R) | 3 | 4.34 | 0.01 | 11197143 | 446108937 | 0.001 |
| Mycorrhiza (M) | 1 | 86.26* | 0.11* | 1665483543n.s | 15105067n.s | 2.07** |
| Biochar (B) | 2 | 40902** | 0.69** | 483031123n.s | 2150324682n.s | 0.071** |
| Nitrogen (N) | 3 | 1426** | 0.5** | 75314497n.s | 3241395052* | 0.508** |
| M*B | 2 | 4052** | 0.3** | 743030509n.s | 4686341506** | 0.158** |
| M*N | 3 | 16767** | 0.241** | 816748313n.s | 597770908n.s | 0.554** |
| B*N | 6 | 12940** | 0.38** | 965155106n.s | 1863058474n.s | 0.430** |
| B*N*M | 6 | 30555** | 0.72** | 1000222012n.s | 2699645473* | 0.406** |
| Error | 69 | 10.89 | 0.007 | 1003501662 | 1066020903 | 0.001 |
| CV (%) | | 1.73 | 12.97 | 21.3 | 69.1 | 4.62 |

ns, * and **: no significant, significant at the 5% and 1% probability levels, respectively.

Morphological characteristics of roots such as root length change drastically with changes in soil characteristics, climatic conditions, and especially the amount of nitrogen in the soil, and therefore these characteristics can be managed in crops through the optimal use of nitrogen fertilizers (Anderson and Impiglia, 2002). Also, although the positive effects of nitrogen in increasing the number, weight and volume of roots have been fully proven, there are many reports about the effect of high consumption ratios of this element in preventing the increase in the number, volume and length of wheat roots which was consistent with the results of this research. In this research, the use of mycorrhiza and 4 ton.ha$^{-1}$ of biochar had the highest root length with an average of 19.12 cm, and the absence of mycorrhiza and biochar had the lowest root length with an average of 14.56 cm (Table 6). The highest and lowest root length belonged to the treatment of 100 kg.N.ha$^{-1}$ and the use of mycorrhiza (18.91 cm) and 100 kg.N.ha$^{-1}$ and the absence of mycorrhiza (13.25 cm), respectively (Table 7). The increase in nitrogen causes the roots to expand and become larger and absorb more nutrients and moisture from the soil and the presence of mycorrhiza causes changes in the root morphology in such a way that the spread of mycorrhiza mycelium associated with the internal tissues of the root increases the length of the root (Jiriaie *et al*., 2014). Davis *et al*. (2002) also showed that the root length in the plant infected with mycorrhiza was greater than the root length of the plant without mycorrhiza, which was consistent with the results of this research. In the investigation of the interaction of nitrogen and biochar, the range of changes in the average root length varied from 19.37 cm in the treatment of 150 kg.N.ha$^{-1}$ and 4 ton.ha$^{-1}$ of biochar to 12.12 cm in the treatment of no mycorrhiza ton biochar (Table 8). The highest root length was related to the fertilizer treatment of 150 kg.N.ha$^{-1}$ and the use of mycorrhiza and 4 ton.ha$^{-1}$ of biochar, and the lowest root length was related to the treatment of 50 kg.N.ha$^{-1}$, no use of mycorrhiza and biochar (Fig. 1). It can be stated that urea and biochar fertilizers, after being added to the soil, increase the rate of nitrogen transformation in the short term and as a result increase the usable nitrogen of soil through the mineralization of resistant soil organic matter and the immobilization of nitrogen in the form of organic compounds. On the other hand, the presence of mycorrhiza causes changes in the morphology of the root, and the release of mycorrhiza mycelium associated with the internal tissues of the root increases the length of the root, as a result, nutrients are absorbed more (Vamerial *et al*., 2003). These results were consistent with the reports of Riad *et al*. (2018) and Seyed Sharifi *et al*. (2003).

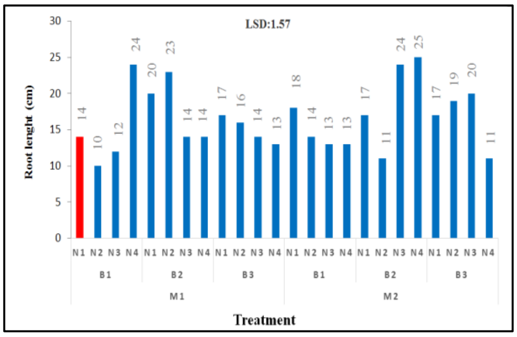


**Fig. 1.** Comparison of the mean trilateral Root lenght. N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$: 100, $N_4$: 150 kg.N.ha$^{-1}$, B (biochar) $B_1$ no application, $B_2$: 4, $B_3$: 8 t.ha$^{-1}$, M (mycorrhiza), $M_1$: no application, $M_2$: application.

4.2. *Root diameter*

The interaction effects of biochar and mycorrhiza and the triple interaction effects of nitrogen, mycorrhiza and biochar on root diameter were significant at the 1% probability level (Table 4). The interaction effect of mycorrhiza and biochar on root diameter was significant (Table 4). The highest and lowest root diameters with averages of 4 and 3.31 cm were related to the treatments of mycorrhiza application and 4 $t.ha^{-1}$ of biochar and mycorrhiza application and 8 $t.ha^{-1}$ of biochar respectively (Table 6). Also, the triple interaction effects of nitrogen, mycorrhiza and biochar on root diameter were significant (Table 4). The highest and lowest root diameter belonged to treatments of applying 100 $kg.N.ha^{-1}$, mycorrhiza and 4 $ton.ha^{-1}$ of biochar, and the treatment of applying 150 $kg.N.ha^{-1}$, using mycorrhiza and 8 $ton.ha^{-1}$ of biochar (Fig. 2).

**Table 5.** Mean comparison effect of treatment on studied traits

| Treatment | Root length (Cm) | Root diameter (Cm) | Root Coloni-zation (%) | Root dry weight (g) | Root Volume ($cm^3$) |
|---|---|---|---|---|---|
| **Mycorrhiza (M)** | | | | | |
| **Non- application** | 15.83* | 3.81 | 36.33 | 3.78 | 6.17 |
| **application** | 16.64 | 3.6 | 36.02 | 4.10 | 6.35 |
| **LSD 5%** | 0.45 | 0.18 | 0.26 | 0.19 | 0.25 |
| **Biochar (B)** | | | | | |
| **Non- application** | 14.78 | 3.59 | 35.9 | 3.28 | 5.82 |
| **4 $ton.ha^{-1}$** | 18.34 | 3.9 | 35.87 | 4.76 | 5.96 |
| **8 $ton.ha^{-1}$** | 15.59 | 3.62 | 36.75 | 3.78 | 7.01 |
| **LSD 5%** | 0.55 | 0.23 | 0.32 | 0.23 | 0.30 |
| **Nitrogen (N)** | | | | | |
| **Non- application** | 15.54 | 3.87 | 35.33 | 3.68 | 6.36 |
| **50 $kg.N.ha^{-1}$** | 16.83 | 3.62 | 35.5 | 3.83 | 7.31 |
| **100 $kg.N.ha^{-1}$** | 16.08 | 3.79 | 35.79 | 4.28 | 6.54 |
| **150 $kg.N.ha^{-1}$** | 16.5 | 3.54 | 38.08 | 3.98 | 4.84 |
| **LSD 5%** | 0.64 | 0.26 | 0.37 | 0.27 | 0.35 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

**Continue table 5.**

| Treatment | Root surface density ($km^2.cm^{-3}$) | Root density ($kg.m^{-3}$) | Ratio of root weight to soil volume | Ratio of root dry weight to soil volume |
|---|---|---|---|---|
| **Mycorrhiza (M)** | | | | |
| **Non- application** | 189.52* | 0.64 | 144514 | 47644 |
| **application** | 191.41 | 0.71 | 152845 | 46850 |
| **LSD (5%)** | 1.34 | 0.03 | 12900 | 13296 |
| **Biochar (B)** | | | | |
| **Non- application** | 163.93 | 0.65 | 144861 | 39426 |
| **4 $ton.ha^{-1}$** | 231.12 | 0.83 | 148548 | 46540 |
| **8 $ton.ha^{-1}$** | 176.34 | 0.54 | 152628 | 55775 |
| **LSD (5%)** | 1.64 | 0.04 | 15799 | 16284 |
| **Nitrogen (N)** | | | | |
| **Non- application** | 198.25 | 0.71 | 147557 | 64596 |
| **50 $kg.N.ha^{-1}$** | 179.25 | 0.53 | 147949 | 40392 |
| **100 $kg.N.ha^{-1}$** | 193.16 | 0.60 | 147888 | 40963 |
| **150 $kg.N.ha^{-1}$** | 190.5 | 0.68 | 151324 | 43037 |
| **LSD (5%)** | 1.9 | 0.05 | 18243 | 18803 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

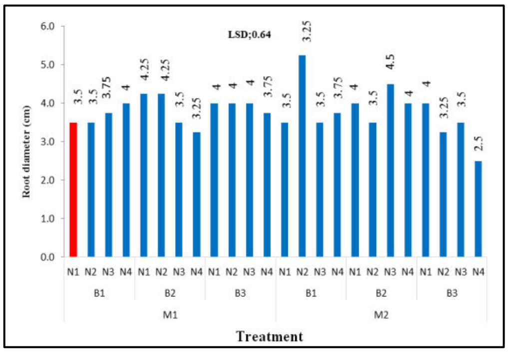


**Fig. 2.** Comparison of the mean trilateral effects on Rootdiameter. N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$:100, $N_4$: 150 $kg.N.ha^{-1}$, B (biochar) $B_1$ no application, $B_2$: 4, $B_3$: 8 $ton.ha^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

In this research, the application of nitrogen and biochar and mycorrhiza symbiosis through proper nutrition increased the root diameter, in such a way that plant infected by mycorrhiza fungus absorbs the unavailable phosphorus (which is located at a far distance from its roots) through its mycelium, as a result, they cause more nutrients to be absorbed by the roots. In this regard, Chaudhry *et al*. (2016) reported that the use of nitrogen fertilizer and biochar along with proper irrigation has provided water and nutrients and better root development. In this study, the use of 4 $t.ha^{-1}$ of biochar had a larger root diameter than 8 $t.ha^{-1}$ of biochar. Therefore, it seems that the application of a moderate amount of biochar is better than its high amount due to the creation of optimal conditions for plant growth (Jemal and Abebe, 2016). Other researchers have also pointed out the better effect of a balanced amount of biochar compared to its low and large amounts. Adjusting the pH and nutrients of the soil in a balanced level and as a result better plant growth in the treatments of moderate amounts of biochar is one of the reasons why this treatment is better than using large amounts of biochar (Jemal and Abebe, 2016). In many researches, it has been shown that the contamination of grains with mycorrhiza causes an increase in root volume and diameter (Berta *et al*., 2002). This development is related to the increase of growth hormones (Safapour *et al*., 2012) and also proton leakage (Mostajeran *et al*., 2007). Proton leakage improves water and solute absorption by mycorrhiza plants (Alizadeh and Alizadeh, 2008). In alkaline conditions, there is not only the limitation of root growth and the insolubility of many essential elements and the reduction of water absorption, but the abundance of hydroxyl ions in the soil reduces the effect of proton leakage by this system, which can lead to root development and many other changes (Mostajeran *et al*., 2007). These results were consistent with reports of Abbaspour *et al*. (2018) who pointed out positive role of nitrogen, biochar and biochar in increasing root diameter.

4.3. *Root colonization*

The effects of mycorrhiza treatments, urea and biochar and their mutual effects on root colonization were significant (Table 4). Based on the average comparison results, the highest root colonization with an average of 38.08% was obtained from the treatment of 150 $kg.N.ha^{-1}$ of urea fertilizer (Table 5). The interaction of mycorrhiza and biochar on root colonization showed that the highest root colonization was assigned to the treatment of no mycorrhiza and 8 ton.ha$^{-1}$ of biochar at the rate of 37.68%, which was not statistically significantly different from the treatment of mycorrhiza and no biochar use, and the lowest colonization 34.37% of the roots were assigned to the treatment of non-use of mycorrhiza and biochar (Table 6). The lowest percentage of root colonization (36.75%) was attributed to the treatment of 50 kg.N.ha$^{-1}$ and no mycorrhiza use, and the highest percentage of the root colonization (38.25%) was assigned to the treatment of 150 kg.N.ha$^{-1}$ and the use of mycorrhiza (Table 7). The triple interactions of nitrogen, mycorrhiza and biochar on root colonization were significant (Table 4). The highest and lowest percentage of root colonization were related to the treatments of applying 100 kg.N.ha$^{-1}$, using mycorrhiza and not using biochar, and the treatment of not using nitrogen, not using mycorrhiza and biochar (Fig. 3). In this research, the use of urea fertilizer, biochar and mycorrhiza fungi had positive and synergistic effects on wheat plants, which the researchers attributed to the effect of biochar and nitrogen in increasing the growth of hairy roots (the presence of abundant hairy roots is a suitable environment for the penetration of fungi into the root cells) and increasing the longitudinal growth of the mushroom mycelium and their penetration into the lower layers of the soil, which increases the plant's access to nutrients. The synergistic effects of biological fertilizer (*Mycorrhiza fungi*) increased the percentage of root colonization because fungal spores can grow in biochar pores and cause an increase in root colonization by fungi. Also, the use of balanced chemical fertilizers stimulates mycorrhiza colonization in the plant, while the use of chemical fertilizers containing unusually high or low amounts of nitrogen causes a decrease in mycorrhiza fungus colonization (Gholami and Koocheki, 2001). Biochar through different mechanisms such as improving the physical and chemical properties of the soil, changing the signaling compounds between the fungus and the plant and reducing the toxicity of toxic compounds causes an increase in the colonization of plant roots with mycorrhiza fungi and as a result increases availability of water and nutrients required by plants (Fathi Gerdelidani and Mir Seyed Hosseini, 2015). Results of this research were consistent by reports of Pal and Pandey (2017).

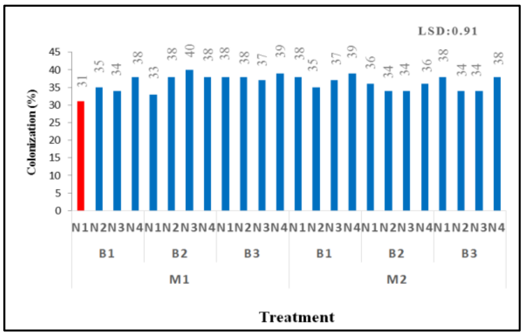


**Fig. 3.** Comparison of the mean trilateral effects on root colonization. N(Nitrogen) $N_1$: no application $N_2$: 50, $N_3$:100, $N_4$: 150 kg.N.ha$^{-1}$, B (biochar) $B_1$ no application, $B_2$: 4, $B_3$: 8 t.ha$^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

**Table 6.** Mean comparison of mycorrhiza × biochar effects on studied traits

| Mycorrhiza (M) | Biochar | Root length (Cm) | Root diameter (Cm) | Root Colonization (%) | Root dry weight (g) |
|---|---|---|---|---|---|
| Non- application | Non-application | 15* | 3.68 | 34.37 | 3.18 |
| | 4 ($t.ha^{-1}$) | 17.56 | 3.81 | 36.93 | 4.43 |
| | 8 ($t.ha^{-1}$) | 14.93 | 3.93 | 37.68 | 3.54 |
| application | Non-application | 14.56 | 3.50 | 37.43 | 3.38 |
| | 4 ($t.ha^{-1}$) | 19.12 | 4 | 34.81 | 5.09 |
| | 8 ($t.ha^{-1}$) | 16.25 | 3.31 | 35.81 | 4.01 |
| LSD (5%) | | 0.78 | 0.32 | 0.45 | 0.33 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

**Continue table 6.**

| Mycorrhiza (M) | Biochar | Root volume ($cm^3$) | Root surface density ($km^2.cm^{-3}$) | Root density ($kg.m^{-3}$) | Ratio of root dry weight to soil volume |
|---|---|---|---|---|---|
| Non- application | Non-application | 5.31 | 169.8 | 0.51 | 38540 |
| | 4 ($t.ha^{-1}$) | 6.2 | 217.2 | 0.70 | 35530 |
| | 8 ($t.ha^{-1}$) | 6.99 | 181.6 | 0.71 | 68860 |
| application | Non-application | 6.33 | 158.1 | 0.60 | 40310 |
| | 4 ($t.ha^{-1}$) | 5.71 | 245.1 | 0.96 | 57550 |
| | 8 ($t.ha^{-1}$) | 7.03 | 171.1 | 0.56 | 42690 |
| LSD (5%) | | 0.42 | 2.32 | 0.05 | 2291 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

4.4. *Root dry weight*

The results obtained from the analysis of variance table confirm that nitrogen, mycorrhiza and biochar treatments and their mutual effects on root dry weight were significant (Table 4). The highest dry weight of the roots was related to the treatment of 100 $kg.N.ha^{-1}$ (4.28g), which showed a 14% increase compared to the treatment of no nitrogen application (Table 5). In this regard, Feiziasl *et al*. (2014) reported that nitrogen consumption could significantly increase root wet weight, root dry weight, total root volume, and the ratio of root volume to soil volume, and this increase was linear. It continued until the last level of nitrogen consumption. Also, the interaction of mycorrhiza and biochar on root dry weight showed that the highest root dry weight was attributed to the treatment of mycorrhiza application and 4 $ton.ha^{-1}$ of biochar (5.09g) and the lowest root dry weight was assigned to the treatment of no mycorrhiza and biochar application (3.18g). In this study, the use of mycorrhizae and biochar contributed more than 38% in increasing the dry weight of the root compared to not using them (Table 6). The comparison of the average interaction of urea fertilizer and mycorrhiza on root dry weight showed that the application of mycorrhiza and 100 $kg.N.ha^{-1}$ had the highest root dry weight, which showed a 44% increase compared to the treatment of 100 $kg.N.ha^{-1}$ and no mycorrhiza (Table 7). The results of comparing the average effect of urea fertilizer and biochar showed (Table 8), that the highest root dry weight was obtained from the treatment of 4 $ton.ha^{-1}$ of biochar and 150 $kg.N.ha^{-1}$, which was different from the treatment of 4 $ton.ha^{-1}$ of biochar and 150 $kg.N.ha^{-1}$ was not statistically significant, and the lowest root dry weight was assigned to the treatment of no nitrogen and no biochar application, which showed a 57% decrease compared to the application of biochar. The triple interactions of nitrogen, mycorrhiza and biochar on root dry weight were significant (Table 4). The highest and the lowest root dry weight were respectively applied to the treatments of 150 $kg.N.ha^{-1}$, mycorrhiza consumption and 4 $ton.ha^{-1}$ of biochar (which was not statistically significantly different from the treatment of 100 $kg.N.ha^{-1}$ and the use of mycorrhiza and 4 $ton.ha^{-1}$ of biochar) and the treatment of application of 50 $kg.N.ha^{-1}$, lack of application of mycorrhiza and biochar was related (Fig. 4). Plant roots provide a suitable habitat for the activity of many soil microorganisms. In this regard, the increase in the weight of root dry matter in urea, biochar and mycorrhiza fertilizer treatments can be due to the increase in the absorption of water and nutrients and the better transfer of these substances. With the increase of root colonization, the root system of the host plant is developed and causes an increase in the absorption level of the roots due to the penetration of the fungal hyphae threads in the soil, and as a result, the root has access to a larger volume of the soil and the efficiency of water absorption and nutrient elements and dry matter production increase (Vamerial *et al*., 2003).

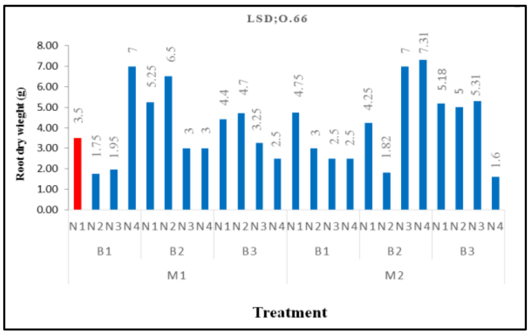


**Fig. 4.** Comparison of the mean trilateral effects on the Root dry wieght. N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$: 100, $N_4$: 150 $kg.N.ha^{-1}$, B (biochar) $B_1$ no application, $B_2$: 4, $B_3$: 8 $ton.ha^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

It seems that the increase in root dry matter weight in mycorrhiza treatments compared to non-mycorrhiza treatments is due to increased absorption of water and nutrients and better transport of these substances, which is confirmed by other researchers (Pal and Pandey, 2017). Also, Ali Sial *et al*. (2019) stated that combination of biochar and chemical fertilizers in the soil improved plant growth, grain yield and soil fertility.

These results were consistent with the results of other researchers such as Riad *et al*. (2018).

4.5. *Root volume*

The root volume under the simple effect of biochar and nitrogen was significant at the 1% of probability level. The interaction effects of mycorrhiza and biochar, nitrogen and biochar and the triple interaction of nitrogen, mycorrhiza and biochar were also significant at the 1% probability level on root volume (Table 4). The use of mycorrhiza and 8 $ton.ha^{-1}$ of biochar had the highest root volume ($7cm^3$) and the treatment without mycorrhizae and biochar had the lowest root volume ($5.31cm^3$) (Table 6). The levels of 4 and 8 $t.ha^{-1}$ of biochar at the level of 50 and 100 $kg.N.ha^{-1}$ had the highest root volume (Table 8). The triple interaction effects of nitrogen, mycorrhiza and biochar on root volume were significant (Table 4). The highest and lowest root volume belonged to the treatments of applying 100 $kg.N.ha^{-1}$ and using 8 $ton.ha^{-1}$ of biochar and mycorrhiza, and no application of nitrogen, using mycorrhiza and 4 $ton.ha^{-1}$ of biochar (Fig. 5). Root volume also increased with increasing levels of urea fertilizer, biochar and mycorrhiza consumption. An increase in nitrogen causes the roots to expand and absorb more moisture from the soil. The increase in root volume indicates the further development of the root, which makes it possible to increase the ability to absorb more water and nutrients in a larger volume of soil (Seyed Sharifi *et al*., 2018). Mycorrhiza fungi, by increasing the absorption level of the roots as well as releasing acids and acidifying the rhizosphere environment, dissolve immobile elements and make them usable for the host plant. It has also been proven that the correct balance between nitrogen and phosphorus increases root growth and volume. Therefore, it can be concluded that fungal treatments along with nitrogen and biochar, by providing the correct balance between nitrogen and other less mobile elements, such as phosphorus and microelements, have caused a positive and significant effect on the volume of plant roots (Egamberdiyeva, 2007). In this regard, Dianat maharluei *et al.* (2018) reported that biochar and mycorrhiza probably increased the root volume due to their increasing effect on the availability of nutrients and water needed by the plant, as well as the production of plant growth stimulants, including growth hormones, and that was consistent with the results of this research. On the other hand, Vamerial *et al*. (20003) stated that plant roots provide a suitable habitat for the activity of many soil microorganisms. In this regard, the increase in the volume and weight of the root dry matter in mycorrhiza treatments can be due to the increase in the absorption of water and nutrients and the better transfer of these substances. Also, Fathi Gerdelidani anf Mir Seyed Hosseini (2015) stated that biochar can affect soil organisms directly and indirectly. Fungal spores can grow in the pores of biochar and cause an increase in volume and root colonization by fungi.

**Table 7.** Mean comparison of mycorrhiza × nitrogen effects on studied traits

| Mycorrhiza (M) | Nitrogen | Root length (Cm) | Root Colonization (%) | Root dry weight (g) |
|---|---|---|---|---|
| Non-application | Non-application | 16.66* | 33.5 | 4.16 |
| | 50 ($kg.N.ha^{-1}$) | 16.41 | 36.75 | 4.09 |
| | 100 ($kg.N.ha^{-1}$) | 13.25 | 36.83 | 2.73 |
| | 150 ($kg.N.ha^{-1}$) | 17 | 37.91 | 4.16 |
| Application | Non-application | 17 | 37.16 | 4.39 |
| | 50 ($kg.N.ha^{-1}$) | 14.66 | 34.25 | 3.27 |
| | 100 ($kg.N.ha^{-1}$) | 18.91 | 34.75 | 4.93 |
| | 150 ($kg.N.ha^{-1}$) | 16 | 38.25 | 3.79 |
| LSD (5%) | | 0.91 | 0.52 | 0.38 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

**Continue table 7.**

| Mycorrhiza (M) | Nitrogen | Root surface density ($km^2.cm^{-3}$) | Root density ($kgm^{-3}$) |
|---|---|---|---|
| Non- application | Non- application | 201.5 | 0.6 |
| | 50 ($kg.N.ha^{-1}$) | 204.3 | 0.54 |
| | 100 ($kg.N.ha^{-1}$) | 155.08 | 0.48 |
| | 150 ($kg.N.ha^{-1}$) | 197.16 | 0.95 |
| Application | Non- application | 195 | 0.83 |
| | 50 ($kg.N.ha^{-1}$) | 155.58 | 0.52 |
| | 100 ($kg.N.ha^{-1}$) | 231.25 | 0.71 |
| | 150 ($kg.N.ha^{-1}$) | 183.83 | 0.77 |
| LSD (5%) | | 2.68 | 0.18 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

These results were consistent by reports of Pal and Pandey (2003) and Ali Sial *et al*. (2019) who pointed out positive role of nitrogen, biochar and increase root volume.

4.6. *Root surface density*

The effect of nitrogen, mycorrhiza and biochar treatments and their mutual effects on root surface density were significant (Table 4).

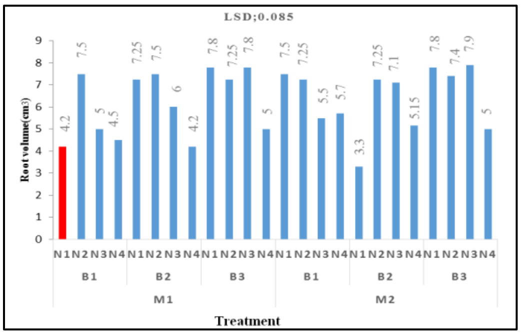


**Fig. 5.** Comparison of the mean trilateral effects on the Root volume. N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$:100, $N_4$: 150 kg.N.ha$^{-1}$, B (biochar) $B_1$ no application, $B_2$: 4, $B_3$: 8 t.ha$^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

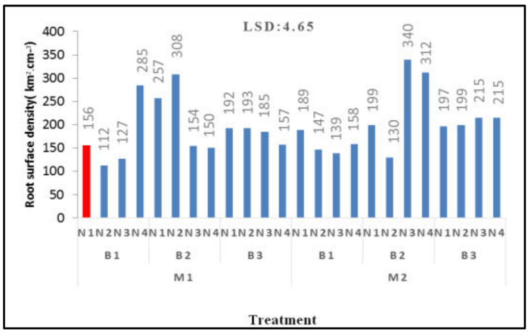


**Fig. 6.** Mean comparison trilateral effects on Root surface density. N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$: 100, $N_4$: 150 kg.N.ha$^{-1}$, B (biochar) $B_1$: no application, $B_2$: 4, $B_3$: 8 t.ha$^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

The highest root surface density (245.1km$^2$.cm$^{-3}$) was assigned to the treatment of mycorrhiza application and 4 ton.ha$^{-1}$ of biochar, and the lowest root surface density (158.1km$^2$.cm$^{-3}$) was assigned to the treatment of mycorrhiza application and no biochar application. It was 35% less (Table 6). The application of mycorrhiza and 100 kg.N.ha$^{-1}$ resulted in the highest root surface density (231.25km$^2$.cm$^{-3}$) (Table 7). The interaction effect of urea fertilizer and biochar showed (table 8), that the highest density of the root surface (247.25 km$^2$.cm$^{-3}$) from the treatment of application of 4 ton.ha$^{-1}$ of biochar and 100 kg.N.ha$^{-1}$ and the lowest density of the root surface (119.8 km$^2$.cm$^{-3}$) was assigned to the treatment of 8 ton.ha$^{-1}$ of biochar and 150 kg.N.ha$^{-1}$ (Table 4). The highest and lowest density of the root surface was related to the treatments of application of 100kg.N.ha$^{-1}$, application of mycorrhiza and 4 ton.ha$^{-1}$ biochar, and the treatment of application of 50kg.N.ha$^{-1}$, no application of mycorrhiza and biochar (Fig. 6).

The higher density of the root surface in nitrogen, mycorrhiza and biochar application treatments can be attributed to the increase in the availability of water and nutrients as a result of mycorrhiza symbiosis and the production of more photosynthetic substances during the growth period (Naseri *et al*., 2019a). In this regard, Kapoor *et al*. (2011) stated that the mycorrhiza hyphae threads are divided into two categories, some of them enter the plant root system and cause a decrease in the concentration of abscisic acid and increase the amount of cytokinin. This action increases water absorption and expands the root system of the plant. The second group of hyphae threads are outside the root system, these hyphae threads secrete phosphorus-soluble organic acids such as malic acid, which increase the absorption of phosphorus by the plant, and finally, the sum of these factors increases the growth and development of the plant, in the conditions mycorrhiza fungi are used.

In the investigation of wheat root surface density, the results showed that grain inoculation with mycorrhiza fungus could increase the root surface density by 20% (Jiriaie *et al*., 2014). Also, Naseri *et al*. (2019b) stated that mycorrhiza fungus can increase the dry weight of roots and aerial parts through proper fertilization, so that plants infected with mycorrhiza fungus can absorb phosphorus that is not available to plants that are far away from their roots are supposed to absorb through their mycelium's and as a result cause more nutrients to be absorbed by roots.

4.7. *Root density*

The effects of urea fertilizer and biochar treatments and the mutual effects of urea fertilizer, biochar and mycorrhiza on root density were significant at the 1% probability level (Table 4). Mycorrhiza and biochar interaction was also significant on root density (Table 4). The highest and lowest root density with averages of 0.96 and 0.51kg.m$^{-3}$ belonged to the treatments of using mycorrhiza and 4 t.ha$^{-1}$ of biochar and no application of mycorrhiza and biochar, respectively (Table 6). The lowest root density (0.48kg.m$^{-3}$) was obtained from the treatment of 100 kg.N.ha$^{-1}$ and the absence of mycorrhiza, which was 62% lower than the treatment of 150 kg.N.ha$^{-1}$ and the absence of mycorrhiza (Table 7). The average comparison of the mutual effect of urea fertilizer and biochar showed (Table 8), that the highest root density (1.5 kg.m$^{-3}$) was obtained from the treatment of 150 kg.N.ha$^{-1}$ and the absence of biochar. The highest and lowest root density were respectively related to the treatments of application of 150 kg.N.ha$^{-1}$ and absence of biochar and mycorrhiza, and application of 100 kg.N.ha$^{-1}$, absence of application of mycorrhiza and biochar (Fig. 7). In this research, the application of nitrogen significantly increased the root density, which indicates a further increase in root growth due to the application of nitrogen. In this regard, Rostami and Ghobadi (2018) reported that the effect of nitrogen treatment on root volume, root dry weight, root length, root surface and root density was significant, which was consistent with the results of this research. In this study, biochar and mycorrhiza had a negative effect on root density. Some studies, including Naseri *et al*. (2019b), have pointed out the effect of mycorrhizae on root density. They stated that the hydraulic conductivity of the root system of mycorrhiza plants is higher than that of non-mycorrhiza plants, which is due to the increase of the root surface or length of the mycorrhiza roots, which was contrary to the results of this research.

4.8. *Ratio of root wet weight to soil volume (RDM)*

The results obtained from the analysis of variance table confirm that nitrogen, mycorrhiza and biochar treatments and their interaction effects on the ratio of root weight to soil volume were not significant (Table 4). The results confirm that different amounts of urea fertilizer, mycorrhiza and biochar had the same effect on the ratio of root wet weight to soil volume.

**Table 8.** Comparison of the mean of biochar × nitrogen effects on studied traits

| Biochar | Nitrogen | Root length (Cm) | Root Colonization (%) | Root dry weight (g) |
|---|---|---|---|---|
| **Non- application** | **Non-application** | 12.2* | 34.37 | 2.03 |
| | **50 ($kg.N.ha^{-1}$)** | 12.12 | 35 | 2.3 |
| | **100 ($kg.N.ha^{-1}$)** | 12.5 | 35.62 | 2.22 |
| | **150 ($kg.N.ha^{-1}$)** | 18.50 | 38.62 | 4.75 |
| **4 $ton.ha^{-1}$** | **Non-application** | 18 | 34 | 4.75 |
| | **50 ($kg.N.ha^{-1}$)** | 17 | 35.75 | 4.164 |
| | **100 ($kg.N.ha^{-1}$)** | 19 | 36.50 | 5 |
| | **150 ($kg.N.ha^{-1}$)** | 19.37 | 37.25 | 5.15 |
| **8 $ton.ha^{-1}$** | **Non- application** | 16.50 | 37.62 | 4.21 |
| | **50 ($kg.N.ha^{-1}$)** | 17.50 | 35.75 | 4.58 |
| | **100 ($kg.N.ha^{-1}$)** | 16.75 | 35.25 | 4.28 |
| | **150 ($kg.N.ha^{-1}$)** | 16 | 38.37 | 3.87 |
| **LSD (5%)** | | 1.11 | 0.64 | 0.46 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

**Continue table 8.**

| Biochar | Nitrogen | Root volume ($cm^3$) | Root surface density ($km^2.cm^{-3}$) | Root density ($kg.m^{-3}$) |
|---|---|---|---|---|
| **Non- application** | **Non-application** | 5.87 | 172.5 | 0.61 |
| | **50 ($kg.N.ha^{-1}$)** | 7.37 | 129.37 | 0.41 |
| | **100 ($kg.N.ha^{-1}$)** | 5.25 | 132.75 | 0.5 |
| | **150 ($kg.N.ha^{-1}$)** | 4.78 | 221.1 | 1.11 |
| **4 $ton.ha^{-1}$** | **Non-application** | 5.31 | 228.1 | 1.01 |
| | **50 ($kg.N.ha^{-1}$)** | 7.25 | 218.5 | 0.55 |
| | **100 ($kg.N.ha^{-1}$)** | 6.55 | 247.25 | 0.73 |
| | **150 ($kg.N.ha^{-1}$)** | 4.73 | 230.6 | 1.03 |
| **8 $ton.ha^{-1}$** | **Non-application** | 7.9 | 194.1 | 0.52 |
| | **50 ($kg.N.ha^{-1}$)** | 7.32 | 192 | 0.63 |
| | **100 ($kg.N.ha^{-1}$)** | 7.82 | 199.5 | 0.56 |
| | **150 ($kg.N.ha^{-1}$)** | 5 | 119.8 | 0.43 |
| **LSD (5%)** | | 0.60 | 3.29 | 0.08 |

*Mean of treatments that differ from LSD is significantly different at the 5% level.

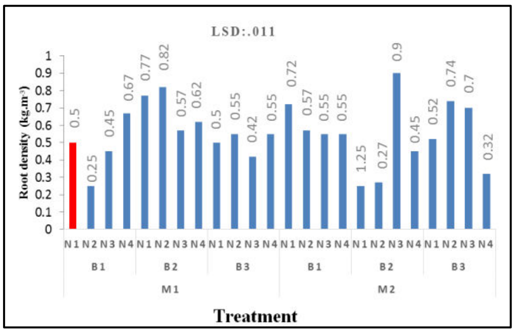


**Fig. 7.** Mean comparison of trilateral effects on the Root density. N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$:100, $N_4$: 150 kg.N.ha$^{-1}$, B (biochar) $B_1$: no application, $B_2$: 4, $B_3$: 8 ton.ha$^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

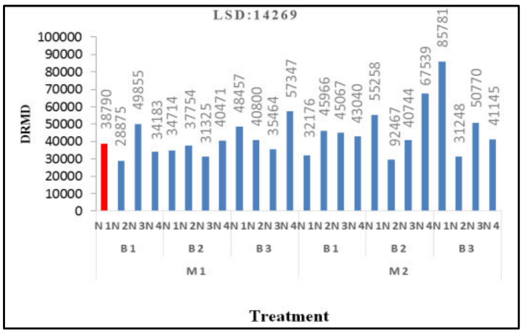


**Fig. 8.** Comparison of the mean trilateral effects on the DRMD. N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$:100, $N_4$: 150 kg.N.ha$^{-1}$, B (biochar) $B_1$: no application, $B_2$: 4, $B_3$: 8 ton.ha$^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

4.9. *Ratio of root dry weight to soil volume*

The simple effects of urea fertilizer treatment and the interaction effects of biochar and mycorrhiza at the 1% of probability level and the triple interaction effects of nitrogen, mycorrhiza and biochar on this component were significant at the 5% of probability level (Table 4). The highest and lowest ratio of root dry weight to soil volume was related to the treatments of application of 50 kg.N.ha$^{-1}$ and 4 t.ha$^{-1}$ biochar and the use of mycorrhiza and application of 50 kg.N.ha$^{-1}$, no application of mycorrhiza and biochar (Fig. 8). In the soils of dry areas, due to the lack of organic matter, it is necessary to use nitrogenous fertilizers and biochar, and the wheat plant responds positively to the use of nitrogenous chemical fertilizers. On the other hand, the secretion of substances that regulate plant growth and the production of growth-stimulating hormones by fungi can stimulate the development and expansion of the root, and as a result, have a positive effect on the root dry weight and the volume of the soil.

In this regard, Svoboda *et al*. (2006) studied the effect of nitrogen consumption on the distribution and expansion of winter wheat roots and concluded that the effect of nitrogen on the expansion of root length in the whole soil volume was positive and significant. Also, Faiziasal *et al*. (2014) also showed that compared to the control treatment (without nitrogen consumption), nitrogen consumption significantly increased root fresh weight, root dry weight, total root volume, and the ratio of root volume to soil volume. Sen *et al*. (2003) also reported a similar result regarding the increase between the amount of nitrogen consumed with root volume and its dry weight. Abdelaziz *et al*. (2007) have reported an increase in the absorption level of roots per unit of soil volume with nitrogen consumption. The research results of Vamerial *et al*. (2003) indicate that plant roots provide a suitable habitat for the activity of many soil microorganisms. In this regard, the increase in the weight of the root dry matter in mycorrhiza treatments can be due to the increase in the absorption of water and nutrients and the better transfer of these substances. These results were consistent with the findings of Naseri *et al*. (2019a) and Garg and Chandel (2010).

4.10. *Grain phosphorus content*

The effect of nitrogen, mycorrhiza and biochar treatments and their mutual effects on the phosphorus percentage of grains was significant (Table 4). The highest percentage of phosphorus belonged to the fertilizer treatment of 100kg.N.ha$^{-1}$ and the use of mycorrhiza and 4 t.ha$^{-1}$ of biochar, and the lowest amount of this attribute belonged to the treatment of the use of 50 kg.N.ha$^{-1}$, without the use of mycorrhiza and biochar (Fig. 9). Increasing soil nitrogen increases the absorption of phosphorus by the plant through increasing the growth of aerial organs and roots, as well as changing the metabolism of the plant and increasing the usability and solubility of phosphorus. In each of the biochar levels, with mycorrhiza and nitrogen consumption, the amount of grain phosphorus increased, and this significant increase in the amount of grain phosphorus indicates the important and positive role of mycorrhiza symbiosis in helping plants absorb nutrients, especially phosphorus from the soil. It seems that the presence of mycorrhiza causes changes in the morphology of the root and the release of mycorrhiza mycelium associated with the internal tissues of the root increases the length of the root, which increases the absorption of nutrients such as phosphorus (Vamerial *et al*., 2003). In this regard, Alqarawi *et al*., (2014) reported that plant symbiosis with mycorrhiza by increasing the level of root absorption, absorption of water and nutrients, especially phosphorus by hyphae threads and its transfer to the plant root, improves the nutritional status and increases plant yield. On the other hand, Aktinson *et al*. (2010) observed that biochar affects soil phosphorus availability and phosphorus absorption by plants indirectly by changing the environment of microorganisms. Also, mycorrhiza fungi increase the elements in the soil, especially the less mobile elements such as phosphorus, which are less absorbable by the plant in the normal state, and by expanding their hyphae threads network in the soil, they increase the level and speed of plant root absorption.

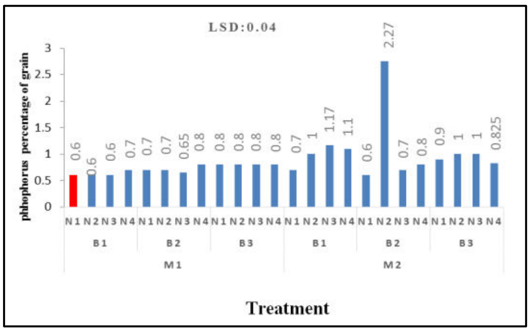


**Fig. 9.** Mean comparison of trilateral effects on the phosphorus percentage of grains N (Nitrogen) $N_1$: no application, $N_2$: 50, $N_3$:100, $N_4$: 150 kg.N.ha$^{-1}$, B (biochar) $B_1$: no application, $B_2$: 4, $B_3$: 8 ton.ha$^{-1}$, M (mycorrhiza) $M_1$: no application, $M_2$: application.

## 5. CONCLUSION

The amount of urea fertilizer has a significant effect on root characteristics and can play a crucial role in root development, ultimately leading to increased plant yield and growth. Moreover, the combined use of biochar and mycorrhiza fungi enhances water and mineral absorption, resulting in higher crop yields. In plants inoculated with mycorrhiza fungus and treated with 4 tons per hectare of biochar, root length increased by 24%, root colonization rate by 9%, root volume by 22%, and root surface density by 35% compared to control plants. Furthermore, the combination of biochar and nitrogen chemical fertilizer demonstrated better effects on fiber properties compared to using biochar or chemical fertilizer alone. This improvement is attributed to the presence of more pores and a specific surface area in biochar, which reduces fertilizer wastage and leaching, allowing plants to better absorb nutrients. Based on the obtained results and recognizing the crucial role of the root system in facilitating the transport of water and nutrients to aerial parts of the plant, it is strongly recommended to adopt the following approach: utilize 100 $kg.N.ha^{-1}$ along with the application of 4 $t.ha^{-1}$ of biochar and mycorrhiza fungi. This combination significantly improves the root structure, ultimately leading to a substantial increase in crop yield.

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## FOOTNOTES

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