PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol x(x): 00–00
https://doi.org/10.14719/pst.1486

HORIZON
e-Publishing Group
HePG

RESEARCH ARTICLE

Influence of mycorrhizal fungi and biochar on nitrogen use efficiency correlated with yield and yield components of wheat

Seyyed Mohammad Amin Sobhani[1], Mojtaba Alavifazel[*1], Mohammad Reza Ardakani[2], Adel Modhej[3], Shahram Lak[1]

[1]Department of Agronomy, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
[2]Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran
[3]Department of Agronomy, Shoushtar Branch, Islamic Azad University, Shoushtar, Iran

*Email: alavifazelm@gmail.com

OPEN ACCESS

ARTICLE HISTORY

Received: 16 September 2021
Accepted: 10 February 2022

Available online
Version 1.0 (Early Access): 14 April 2022

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

Publisher's Note: Horizon e-Publishing Group remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, etc. See https://horizonepublishing.com/journals/index.php/PST/indexing_abstracting

CITE THIS ARTICLE

Sobhani S M A , Alavifazel M, Ardakani M R, Modhej A, Lak S. Influence of mycorrhizal fungi and biochar on nitrogen use efficiency correlated with yield and yield components of wheat. Plant Science Today (Early Access). https://doi.org/10.14719/pst.1486

Abstract

Mycorrhizal symbiosis increases mineral nutrient uptake, especially when nitrogen applies to the fields together with biochar. To evaluate the influence of the biochar application and different levels of nitrogen on nitrogen use efficiency (NUE), yield and yield components of wheat associated with arbuscular mycorrhizal fungi (AMF) A factorial experiment with in randomized complete block design with four replications was implemented for two years (2016-2018). The experimental factors included nitrogen at four levels (urea source with 46% nitrogen) 0, 50, 100 and 150 kg N/ha, *Rhizophagus Irregularis* inoculum at two levels (without and with application), and biochar at three levels 0, 4 and 8 ton/ha. The results demonstrated that plant inoculated with mycorrhiza inoculum combined with 4 ton/ha of biochar showed a significant increase in, root colonization (8%). The application of will be This study proves that application of 100 kg N/ha with an amount of 4 ton/ha of biochar and AMF inoculationhas a useful and effective role in the enhancement of growth and yieldof wheat.

Keywords

Bio-coal; Biological yield; Field-grown wheat; Nitrogen uptake; *Rhizophagus Irregularis*; Root colonization

Introduction

Nutrients, including chemical fertilizers are mainly available to crops through the soil. One of critical nutrients is nitrogen, which is widely used as a fertilizer by most plants and its deficiency limits the quantitative and qualitative yield of crops more than other nutrients (1). It has been reported that nitrogen fertilizers had a significant effect on grain yield and the 1000-grain weight, and this significant increase was observed when up to 150 kg N/ha were used in traits (2). Nitrogen fertilizers have a significant effect on quantitative traits of wheat such as grains/spike, spikelets/spike, 1000-grain weight, grain yield, biological yield and harvest index (3). Plants provide carbon sources needed by the fungus and the fungus supports water and nutrients from the soil to plants. Mycorrhizal hyphae improve a plant's nutritional status, which makes the plant resistant to biotic and abiotic stresses (4, 5). In arid and semi-arid climates such as Iran, soil organic matter content is low due to insufficient vegetation. A common solution to increase soil fertility is integrated crop management which includes the use of various types of manure or organic based amendments in the soil. Biochar is a stable carbon-rich material produced by burning organic matter in oxygen-free or low-oxygen conditions at temperatures below 700°C, a process called pyrolysis (6). The use of biochar as a soil modifier improves soil physical, chemical and biological conditions. The tiny pores of biochar provide a safe environment for plant beneficial microbes, reduce turnaround time and increases the bioavailability of nutrients (7). Biochar leads to an increase in soil fertility crop yield, cation exchange capacity, water holding capacity, decreases soil acidity and soil absorption of toxic substances and improves soil structure (6). In one study, the effect of biochar on wheat yield was investigated and indicated that the application of biochar led to an increase of 56% in grain yield, an improvement in soil properties and an increase in biomass (8). Nitrogen use efficiency and wheat grain yield was affected by different levels of biochar (0, 5, 10, 20, 30, 40 and 50 ton/ha), especially it was observed that at the rate of 5-20 ton/haNUE and grain yield increased significantly (9). The majority of knowledge about mycorrhizal symbiosis combined with biochar is derived from laboratory experiments that use sterilized soil, so these results are not directly relevant to the field (4). The study aimed to test the effect of BC application on wheat biomass and grain yield production, indigenous arbuscular mycorrhizal (AM) fungi propagules and soil microbial community and related to soil quality properties of an Andisol in Southern Chile. Biochars (BCs) were produced from oat hulls (OBC) and pine bark (PBC). Doses of 0, 5, 10 and 20 Mg ha−1 of BCs were applied on soil using wheat as the test crop. The OBC had a significantly higher macronutrients content (N, P, K) than PBC. The highest dose of both BCs significantly improved shoot and root biomass and wheat grain yield. Application of 20 Mg ha−1 of OBC and PBC increased AM spore density and root colonization relative to control treatment. In the same way, the BC application significantly affects the AM mycelium density (10). Since the global approach and the use of management methods such as the use of biological fertilizers tend to reduce the use of chemical fertilizers, this study aims to identify the effects of mycorrhiza and biochar on wheat to increase nitrogen uptake efficiency due to the importance of its production as one It became one of the most important sources of human food.

Table 1. Average biennial meteorological parameters of test site

Month	Rainfall (mm)	Average minimum temperature (°C)	Average maximum temperature (°C)	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

Materials and Methods

Experiment procedure

Experiments were conducted during the 2016-2017 and 2017-2018 seasons in Southwestern Iran, (Longitude, 49° 18, East; Latitude, 31° 20, North; 34 m alt). The average meteorological parameters of the test site are shown in (Table 1). A factorial experiment in the form of a randomized complete block design with four replications implemented. The experimental factors included nitrogen at four levels (urea source with 46% nitrogen);

N1: No fertilizer application

N2: 50 kg per ha

N3: 100 kg per ha

N4: 150 kg per ha

Mycorrhizal symbiosis (Arbuscular Mycorrhizal Fungi- AMF) at two levels; without application (control) and application of (*Rhizophagus irregularis*) with a spore density of 10^5 per gram of carrier at the rate of one kg per ha, which was used at the time of planting and inoculated with seeds.

Biochar at three levels;

B1: Not application

B2: 4 tons per ha

B3: 8 tons per ha

Biochar was prepared using a combination of forest tree wastes in northern Iran (Table 2). Mycorrhizal inoculum provided by the Soil and Water Research Institute (SWRI)- Ministry of Agriculture contained a total of 70 active fungal organs per gram of inoculum and has been applied as seed inoculation before sowing the seeds.

Table 2. Physical and chemical properties of biochar

Property	
Iodine Value	950-1100 mg/g
Surface area according to ASTM standard	950-1100 m²/g
Methylene blue number	150-250 mg/g
Amount of moisture	8-10%
pH	5.8%
Ash	4-6%
Carbon	84-88%
Gradation	0.1mm and <1

To inoculate wheat seeds with organs, hyphae and mycorrhizal roots as a fungicide, the seeds were first sterilized with 0.5% sodium hypochlorite solution for 5 min. These seeds were soaked in single sterile distilled water for two hrs and then divided into equal proportions and poured into separate buckets. To keep the active organs of the fungus on the seeds of a concentrated solution of 20% sugar and Arabic gum was used. One hour before sowing, the seeds were completely soaked with inoculum and used with the control treatment (without spore inoculation), so that first the control treatment and then the other treatments were cultured according to the test design. Soil samples from depths of 0-30 cm were taken with an auger. The physical and chemical properties of soil and were determined in the laboratories of SWRI (Table 3).

Table 3. Some characteristics of farm soil

Crop year	Depth of soil (cm)	Texture of soil	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

EC: Electrical Conductivity; OC: Organic carbon; N: Nitrogen

Analytical methods

Each plot consisted of plot consisted of 7 rows having 6 m length and 2.5 m width. Potassium sulfate was used at a rate of 150 kg/ha according to the soil tests in all treatments before planting. Biochar was incorporated into soil to a depth of 20 cm. The AMF inoculum consisted of a mixture of *G. intraradices* spores (10^5 spore/g) and hyphae corn roots were used as a carrier at a rate of 1 kg/ha. The seeds were planted on October 23, 2016 and on November 27, 2017. According to the soil test (Table 1) urea fertilizer was applied to the plant in three splits. One third was provided before planting, one third at tillering time and one third at stem elongation. The first irrigation was conducted after planting and nitrogen application. The irrigation took place regularly every 7 to 10 days. Weed control was done manually without any herbicide to prevent the negative effect of herbicides on mycorrhizal symbiosis. Traits were measured from 2 sqm of each plot were considered for harvesting plants (and were selected from the 4 middle rows after removal of 0.5 m from the beginning and end of each row). Harvest Index (HI) was calculated as a percentage of grain and biological yield. At the physiological maturity stage, 20 plants were randomly selected from the harvested area of each plot to evaluate the yield components. The nitrogen use efficiency was calculated according to Eq. (1):

$$NUE\ (g \cdot g^{-1}) = GY/NS$$

where GY is the grain yield (g) and NS, the N supplied by the fertilizer (g) (11). Measurements of root colonization percentage were taken after cross-staining of the roots using the cross-linking by standard methodology (12). For bleaching, roots were soaked in 10% potassium hydroxide solution for 3 hrs at 60° C. After neutralizing the environment, the roots were placed at room temperature for 4 hrs, after which the roots were immersed in 0.1 M hydrochloric acid solution. The roots were then stained for 12 hrs in Terpan Blue solution (0.01%) following standard methodology (13). Stained hair roots of about one centimeter long were randomly spread in a petri dish (lattice plate method) using vertical and horizontal lines of one centimeter squares. Infection of roots was observed microscopically.

Statistical analysis

Analysis of variance was performed in the form of a factorial experiment with SAS software. The mean comparison was done by LSD test at 5% probability level. Tracing the presence or absence of a linear relationship between the studied variables was calculated as simple correlation coefficients of the traits.

Results and Discussion

Biological yield

The results obtained from the combined analysis of variance indicated that the interaction of the year × nitrogen, year × mycorrhizal symbiosis, year × biochar and also year × mycorrhizal symbiosis × nitrogen × biochar had significant effects on biological yield at the 1% probability level (Table 5). There is a positive and significant correlation between biological yield with harvest index, grain yield, grains/m^2 and spike/m^2 (Table 11).

The reason for the increased biological yield resulting from using biochar and nitrogen can be due to the improvement of soil quality by increasing the availability of nutrients by soil microorganisms and the allocation of elements to shoots and seeds (14). The application of AMF and biochar improve soil physical and chemical properties and thus increase the availability of water and nutrients required by plants. These factors increased the biological yield of the plant (15, 16).

Grain yield

Analysis of variance showed that grain yield significantly increased at the 1% probability level under year and interaction of the year × mycorrhizal symbiosis × nitrogen × biochar (Table 5). The results showed that the correlation between grain yield and grains/m^2 was positive and significant. Among the yield components, grains/m^2 and 1000-grain weight contributed to the increase in grain yield (Table 11).

The mycorrhizal symbiosis increases root uptake and transport of nutrients to the roots by spreading hyphae and altered plant root morphology and increases thus also uptake of nitrogen provided by the fertilizer. Mycorrhizal symbiosis with roots through the uptake of water and

Table 5. Results of combined analysis of variance concerning the effects of experimental treatments on studied traits

S.O.V	df	Biological Yield	Grain Yield	Harvest Index (HI)	Spikes/m²	Spikelets/ spike	Grains/ spikelet	Grains/m²	1000-grain Weight	Nitrogen use efficiency
Year(Y)	1	2336165*	2383169*	19936.12^{ns}	75373^{ns}	131.937**	0.871**	451049393^{ns}	10440.05^{ns}	689.3^{ns}
Replication (Year)	6	12135	3250	59.61	292	0.005	0.167	2897546	7.695	2.90
Mycorrhiza (M)	1	179557^{ns}	63201^{ns}	56.68^{ns}	9259^{ns}	0.11^{ns}	0.206^{ns}	31381006^{ns}	112.55^{ns}	15.30^{ns}
YM	1	93689**	66466^{ns}	208.04^{ns}	3334**	0.273^{ns}	0.002^{ns}	2894038^{ns}	303.51^{ns}	13.98**
Biochar (B)	2	95470^{ns}	82746^{ns}	695.63^{ns}	666^{ns}	48.768^{ns}	0.287^{ns}	92014800^{ns}	30.6^{ns}	19.94^{ns}
YB	2	22938**	31125^{ns}	363.95^{ns}	1416^{ns}	3.492^{ns}	0.609^{ns}	29246278^{ns}	13.27^{ns}	8.71^{ns}
Nitrogen (N)	3	79321^{ns}	131292^{ns}	1371.78*	57835*	28.166**	0.528^{ns}	163020119*	37.66^{ns}	3732.75*
YN	3	44309**	23010^{ns}	78.74^{ns}	5465^{ns}	0.645^{ns}	0.146^{ns}	13546404^{ns}	103.06^{ns}	297.30**
MB	2	14256^{ns}	6779^{ns}	96.72^{ns}	19054^{ns}	1.696^{ns}	0.175^{ns}	20226598^{ns}	66.151^{ns}	54.25^{ns}
YMB	2	1642^{ns}	7338^{ns}	172.00^{ns}	2143^{ns}	1.023^{ns}	0.062^{ns}	2975917^{ns}	150.39**	10.19^{ns}
MN	3	8343^{ns}	3749^{ns}	40.97^{ns}	19161^{ns}	4.4088^{ns}	0.097^{ns}	23736583*	61.66^{ns}	10.59
YMN	3	2228^{ns}	2735^{ns}	53.35^{ns}	3627^{ns}	7.7862^{ns}	0.086^{ns}	2156290^{ns}	21.22^{ns}	2.54^{ns}
BN	6	23191^{ns}	16494^{ns}	255.65^{ns}	6185^{ns}	7.282^{ns}	0.666^{ns}	21213971^{ns}	14.05^{ns}	54.65*
YBN	6	20552^{ns}	20366^{ns}	321.22*	7089^{ns}	4.389^{ns}	0.804*	26230927*	35.92^{ns}	10.68^{ns}
MBN	6	44103^{ns}	26459^{ns}	268.29*	43564*	4.477^{ns}	0.207^{ns}	26307466*	47.86*	283.25**
YMBN	6	23827**	8573**	51.03^{ns}	7985**	2.174**	0.127^{ns}	4654281*	8.89^{ns}	18.83**
Error	138	4816	1962	42.78	470	0.007	0.124	1980828	4.78	3.24
C.V. (%)		9.6	17.3	19.2	5.6	0.9	13	14	8.7	10

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

Table 11. Correlation between measured traits

Traits	Biological yield	Grain yield	Spike/m²	Harvest index	Spikelets/ spike	Grains/ spikelets	1000-grain weight	Grains/m²
Grain yield	0.79**							
Spike/m²	0.55*	0.60*						
Harvest index	0.55*	0.93**	0.53^{ns}					
Spikelets/spike	0.54^{ns}	0.65*	0.03^{ns}	0.62*				
Grains/spikelets	0.44^{ns}	0.62*	0.26^{ns}	0.64*	0.46^{ns}			
1000-grain weight	0.28^{ns}	0.47*	0.06^{ns}	0.47^{ns}	0.08^{ns}	0.09^{ns}		
Grains/m²	0.78**	0.92**	0.67**	0.85**	0.70**	0.68**	0.12^{ns}	
Nitrogen use efficiency	0.036^{ns}	-0.072^{ns}	-0.332^{ns}	-0.201^{ns}	-0.244^{ns}	-0.153^{ns}	-0.072^{ns}	-0.359^{ns}

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

nutrients increases photosynthesis, leading to more productivity and improved grain growth and yield (17). Biochar and mycorrhizal fungi could have a positive effect on grain yield by increasing nutrient uptake, improved plant moisture conditions and increased photosynthesis and concentration of plant hormones (17). The application of biochar with nitrogen fertilizer also facilitates nitrogen mineralization by decreasing the C/N ratio, which also resulting in improved total nitrogen of the soil (18, 19). The results obtained from the step-by-step analysis of multivariate linear regression (Table 4), show that grain yield (Y) is a function of Harvest index (X_1) and Biological Yield (X_2). Based on the step-by-step method, this model justified about 98.47% of the changes due to variable factors. Harvest Index and the Biological Yield were very influential on grain yield.

Table 4. Step-by-step regression steps for grain yield as a function variable and other traits as an independent variable

Variable added to the model	Step-by-step regression step	
	1	2
Constant number	-65.64	-250.13
Harvest index	9.42**	7.24**
Biological yield		0.362**
Explanatory coefficient	87.32	98.47

The step-by-step regression coefficients in the last step are significant at the probability levels of 1% (step 1) and 5% (step 2)

$$Y = -250.13 + 7.24(X_1) + 0.362(X_2)$$

$$R^2 = 98.47$$

Y= Grain yield, X_1= Harvest index weight, X_2= Biological yield

Harvest index (HI)

The results obtained from the combined analysis of variance indicated that the simple effect of nitrogen fertilizer, interaction of year × nitrogen × biochar and triple interaction of the mycorrhizal symbiosis × nitrogen × biochar on harvest index had significant at the 5% probability level (Table 5). The highest harvest index was obtained from the treatments of application of 150 kg N/ha, application of AMF and 8 ton/ha of biochar, while the lowest harvest index was obtained from the treatment of application of 50 kg N/ha, non-application of AMF and application of 8 ton/ha biochar (Fig.1). With increasing nitrogen fertilizer levels, the harvest index was increased. The reason for the increase in harvest index can be attributed to better absorption of nutrients. Plants absorb nutrients and increased leaf area index which provide better absorption of solar radiation and produced more assimilates directed to the grain formation (3). Increasing harvest index of maize due to the application of biochar in soil is the result of its effects on improving soil physical, chemical, and biological properties that have led to increasing grain yield (20). Due to the porous structure of biochar and its high surface area, nutrient leaching is prevented and, thus, the efficiency of nitrogen fertilizer has been increased. The results of this study are similar with the investigations conducted earlier (21).

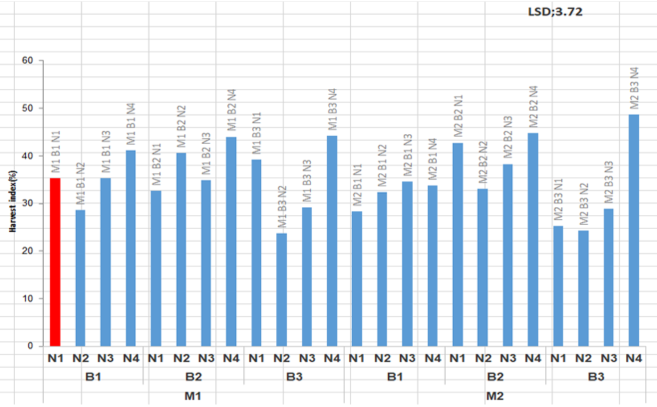

Fig. 1. Comparison of the mean trilateral effects on the Harvest index. N (Nitrogen) N1: no application, N2: 50, N3:100, N4: 150 kg/ha, B (biochar) B1: no application, B2: 4, B3: 8 tons/ha, M (mycorrhiza) M1: no application, M2: application

Spikes/m2

The main effect of nitrogen fertilizer on the spikes/m^2 was significant at the 5% probability level (Table 5). Also interaction between year × mycorrhizal symbiosis, nitrogen × biochar × mycorrhizal symbiosis and year × mycorrhizal symbiosis × nitrogen × biochar on the spikes/m^2 were significant (Table 5).

There is a positive and significant correlation between spikes/m^2 and grains/spike, which increases with the number of spikes. A significant correlation was observed between the number of spikes per unit area and the grains/m^2 and the grain yield in wheat (Table 11). The highest spikes/m^2 was related to plants treated with 100 kg N/ha, and without AMF and biochar, while the lowest spikes/m^2 was obtained from the treatment of application of 50 kg N/ha, application of AMF and application of 4 ton/ha biochar (Fig. 2). With increased nitrogen fertilizers, the growth of vegetative buds is stimulated, and as a result, the number of spikes per unit area increased. The presence of sufficient nutrients in the soil improves the nutritional status of the plant, increases the efficiency of photosynthetic conversion to dry matter and thus increases the number of spikes (16). The application of nitrogen fertilizer caused an increase in the number of tillers and spikes per unit area in wheat plants (22).

Spikelets/spike

Combined analysis of data variance showed that the simple effect of the year and nitrogen fertilizer and also interaction between year × mycorrhizal symbiosis × nitrogen × biochar on the spikelets/spike were significant at the 1% probability level (Table 5).

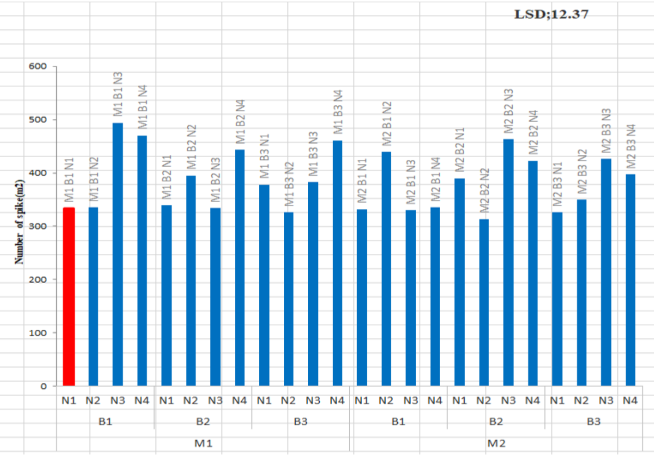

Fig. 2. Comparison of the mean trilateral effects on the Number of spike/m^2. N (Nitrogen) N1: no application, N2: 50, N3:100, N4: 150 kg/ha, B (biochar) B1: no application, B2: 4, B3: 8 tons/ha, M (mycorrhiza) M1: no application, M2: application

Proper nitrogen availability leads to high photosynthetic activity. Accordingly, assimilates are produced to strengthen spikelet developments. Thus, a more incredible spikelets/spike was formed (23). The nitrogen fertilizer application was increasing in the share of reproductive organs, including the number of spikelets per spike (24). The addition of biochar combined with nitrogen and biofertilizer enhanced the crop growth of wheat. In this study, improved spike growth and yield response to co-application of nitrogen fertilizer, biochar and biofertilizer agree with the results of (25) who reported yield improvements had been observed in response to biochar application that was combined with biochemical fertilizer. Mycorrhizal fungi can significantly improve small grain crop growth, nitrogen uptake and yield (26). Biochar and plant-growth-promoting biofertilizers may be used as a strategy to solubilize and increase nutrient availability and improve plant yield (27).

Grains/spikelet

The combined analysis of data variance showed that simple effect of the year and interaction between year × nitrogen × biochar had a significant effect on the grains/spikelet at 1 and 5% probability level respectively (Table 5). Nitrogen increases wheat yield through increasing number of spikes, grains/spike and 1000-grains weight. It seems that increasing the nitrogen leads to proper nutrition of the plant and the photosynthetic level of the plant is increased, and the plant with more synthesis of assimilates strengthens the productive buds of spikelets, and thus, a greater number of grains is formed in the plant (28).

Grains/m2

Combined analysis of data variance showed that the simple effect of nitrogen fertilizer, triple interaction of experimental factors, interaction of year × nitrogen × biochar, mycorrhizal symbiosis × nitrogen, and also year × mycorrhizal symbiosis × nitrogen × biochar had significant on grains/m^2 at the 5% probability level (Table 5). The highest grains/m^2 was allocated to the treatment of the application of 150 kg N/ha and non-application of biochar and AMF, while the lowest grains/m^2 was obtained from the treatment of application of 50 kg N/ha, non-application of AMF and application of 8 ton/ha biochar (Fig. 3). Nitrogen increases wheat yield through increasing number of spikes, grains/spike and 1000-grains weight. In general, yield

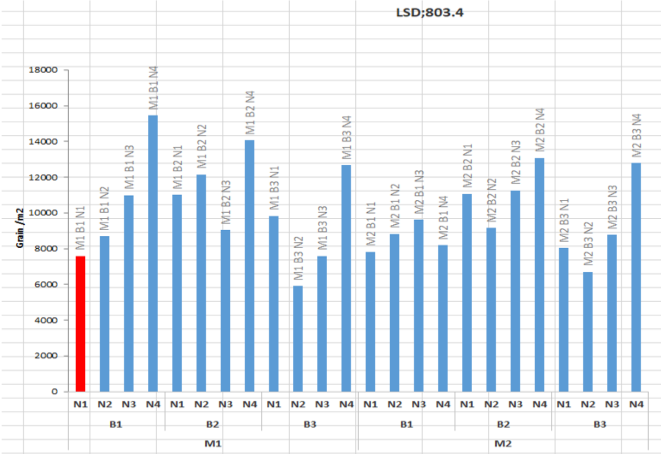

Fig. 3. Comparison of the mean trilateral effects on Grain /m^2. N(Nitrogen) N1: no application N2: 50, N3:100, N4: 150 kg/ha, B (biochar) B1 no application, B2: 4, B3: 8 tons/ha, M (mycorrhiza) M1: no application, M2: application

components in wheat are under the direct influence of nitrogen (29). It seems that increasing the nitrogen leads to proper nutrition of the plant, and the photosynthetic level of the plant is increased, and the plant with more synthesis of assimilates strengthens the productive buds of spikelets, and thus, a greater number of grains is formed in the plant (28).

1000-grains weight

Results showed that the triple interaction of year × mycorrhizal symbiosis × biochar and also mycorrhizal symbiosis × nitrogen × biochar on 1000-grains weight were significant at 1 and 5% probability level respectively (Table 5). The correlation coefficients showed a positive and significant correlation between 1000-grain weight with grain yield, which indicates that 1000-grain weight increases with increasing grain yield (Table 11). The lowest and highest 1000-grains weight were related to the treatment of non-application of nitrogen, application of AMF with 8 ton/ha of biochar, and the treatment of non-application of nitrogen and AMF with 8 ton/ha of biochar, respectively (Fig. 4).

In this study, it seems that at low nitrogen levels due to the allocation of more materials to a smaller number of grains, their weight has also increased, which was consistent with the results of (30). Accordingly, less N fertilizer is required if provided together with biochar (31). The use of mycorrhiza fungi, due to the positive role of these microorganisms in the absorption of water and nutrients, especially phosphorus and its transfer to host plant cells, improved plant growth, increased photosynthesis and production of assimilating. As a result, in the grain filling stage, sufficiently produced assimilates were transferred to the kernels and large grains were

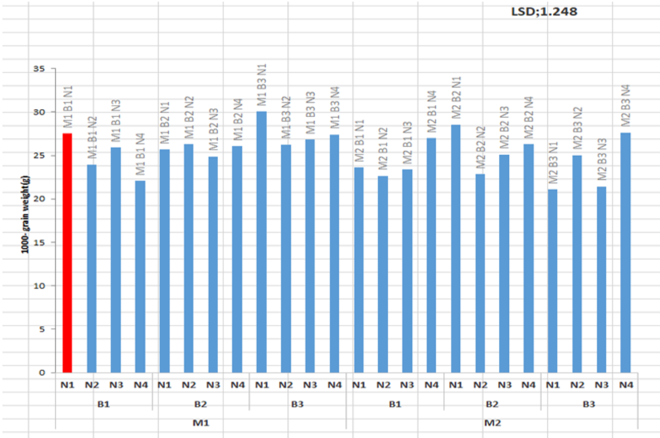

Fig. 4. Comparison of the mean trilateral effects on 1000-grain weight. N (Nitrogen) N1: no application N2: 50, N3:100, N4: 150 kg/ha, B (biochar) B1 no application, B2: 4, B3: 8 tons/ha, M (mycorrhiza) M1: no application, M2:

produced. As a result, in the grain filling stage, sufficiently grown sap was transferred to the seeds and large grains with acceptable weight were produced.

Nitrogen use efficiency (NUE)

The main effect of nitrogen fertilizer and interaction between biochar × nitrogen fertilizer on NUE was positive and significant at 5% probability level (Table 5). Also interaction of the year × nitrogen, year × mycorrhizal symbiosis, triple interaction of experimental factors and intertaction of year × nitrogen × biochar × mycorrhizal symbiosis were significant on NUE at 1% probability level (Table 5).

The highest of NUE were allocated to the treatment 100 kg N/ha, 8 ton/ha of biochar and non-application of AMF (Fig. 5) and this treatment had a high efficiency of nitrogen consumption, which shows the effect of biochar application in increasing cation exchange capacity and

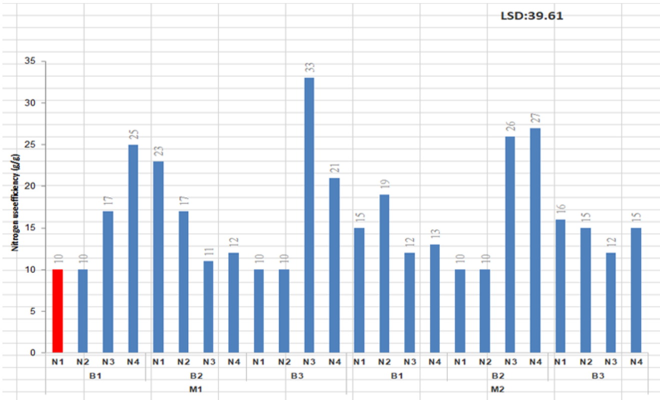

Fig. 5. Comparison of the mean trilateral effects on the nitrogen use efficiency. N (Nitrogen) N1: no application, N2: 50, N3:100, N4: 150 kg/ha, B (biochar) B1: no application, B2: 4, B3: 8 tons/ha, M (mycorrhiza) M1: no application,

better nitrogen retention, preventing its leaching and thus better plant access to nitrogen and increasing yield and reducing nitrogen fertilizer consumption (1, 9, 28).

Biochar can change the dynamics of nutrients by affecting the nitrogen cycle and providing options to minimize the loss of nitrogen through ion exchange, absorption and non-dynamization and increasing food supply (32). The use of biochar alone has its limitations and has little effect. The simultaneous use of biochar with nitrogen fertilizers can achieve valuable benefits by improving nitrogen mineralization conditions. In other words, there is better coordination between nitrogen mineralization and plant demand (uptake). Therefore, it can be concluded that biochar, especially at low levels of nitrogen fertilizer application, has a significant effect on the efficiency of nitrogen application, which is consistent with the findings of the earlier study (33). It has been shown in several studies that increasing nitrogen consumption significantly reduced nitrogen consumption efficiency in wheat plants (34). Various experiments have shown that increasing nitrogen application reduces nitrogen use efficiency (35).

Root colonization

Analysis of variance showed that the simple effects of nitrogen fertilizer, biochar and all interactions on this trait were significant at the 1% probability level. (Table 6). The rate of colonization enhanced with increasing consumption of mycorrhiza, nitrogen fertilizer and biochar (Table 7). According to the results in Table 8, the highest rate of colonization was related to the consumption of 8 tons of biochar and 150 kg of nitrogen fertilizer per ha. The highest rate of colonization was obtained in the simultaneous application of mycorrhizal inoculum and nitrogen fertilizer at a rate of 150 kg/ha (Table 9). The lowest rate of colonization was obtained in the non-application of mycorrhiza and biochar (Table 10).

The highest and lowest percentages of root colonization were related to the treatments with 100 kg N/ha, 4 ton/ha of biochar and non-application of AMF and control treatment, respectively (Fig. 6).

Table 6. Results of combined analysis of variance on root colonization

S.O.V	df	colonization
Replication	3	0.205
Mycorrhiza (M)	1	2.344 *
Biochar (B)	2	7.88**
Nitrogen (N)	3	39.62**
MB	2	68.46**
MN	3	47.510**
BN	6	10.99**
MBN	6	6.88**
Error	69	0.42
C.V. (%)		1.8

** and *: Mean squares of treatments were significant at 1% and 5% probability levels respectively

Table 7. Comparison of the mean of simple effects on colonization

	Colonization
Mycorrhiza (M)	
Non- application	33.36
Application	36.02
LSD 5%	26.0
Biochar (B)	
Non- application	35.9
4 ton/ha	35.87
8 ton/ha	37.75
LSD 5%	0.32
Nitrogen (N)	
Non- application	33.35
50 kg/ha	35.5
100 kg/ha	35.79
150 kg/ha	38.08
LSD 5%	0.37

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

Table 8. Comparison of the mean of biochar × nitrogen effects on root colonization

Biochar	Nitrogen	colonization
Non- application	Non- application	34.37
	50 kg ha	35.00
	100 kg ha	35.62
	150 kg ha	38.62
4ton/ha	Non- application	34.00
	50 kg ha	35.75
	100 kg ha	36.50
	150 kg ha	37.25
8ton/ha	Non- application	37.62
	50 kg ha	35.75
	100 kg ha	35.25
	150 kg ha	38.67
LSD 5%		0.64

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

Table 9. Comparison of the mean of mycorrhiza × nitrogen effects on root colonization

Mycorrhiza	Nitrogen	colonization
Non- application	Non- application	33.50
	50 kg ha	36.75
	100 kg ha	36.83
	150 kg ha	37.91
Mycorrhiza	Non- application	37.16
	50 kg ha	34.25
	100 kg ha	34.75
	150 kg ha	38.25
LSD 5%		0.52

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

Table 10. Comparison of the mean of mycorrhiza × biochar effects on root colonization

Mycorrhiza	Biochar	colonization
Non- application	Non- application	34.37
	4ton/ha	36.39
	8ton/ha	37.68
Mycorrhiza	Non- application	37.43
	4ton/ha	34.81
	8ton/ha	35.81
LSD 5%		0.45

The mean of treatments that differ from LSD is significantly different at the 5% level

The results showed that the synergistic effects of biological fertilizer (mycorrhizal fungus) increased the percentage of root colonization because it seems that fungal spores can grow in biochar pores and increase root colonization by the fungus (37). Application of nitrogen fertilizer, biochar and mycorrhizal fungi provides root cells. It increases the longitudinal growth of fungal mycelium and their penetration into the subsoil, which increases the plant's access to nutrients. Also, the use of balanced

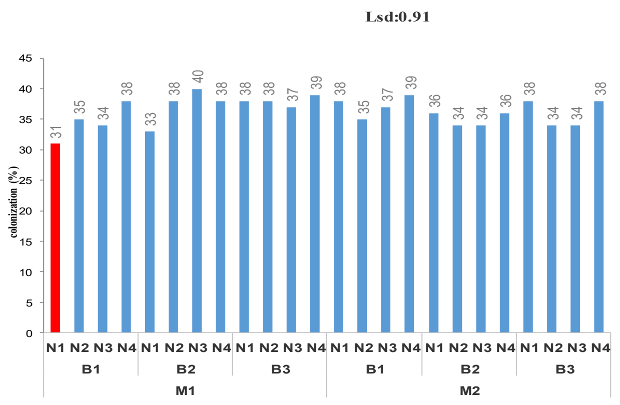

Fig. 6. Comparison of the mean trilateral effects on root colonization. N (Nitrogen) N1: no application N2: 50, N3:100, N4: 150 kg/ha, B (biochar) B1 no application, B2: 4, B3: 8 tons/ha, M (mycorrhiza) M1: no application, M2:

chemical fertilizers stimulates mycorrhizal colonization in the plant. In contrast the use of chemical fertilizers containing unusually high or low levels of nitrogen reduces the colonization of mycorrhizal fungi (36). Biochar increases the colonization of plant roots with mycorrhizal fungi through various mechanisms such as improved physical and chemical properties of soil, changed the signaling compounds between fungi and plants and reduced the toxicity of toxic compounds and thus increasing the availability of water and nutrients (38-40).

Conclusion

In this study, the combined use of *Rhizophagus Irregularis*, biochar and nitrogen fertilizer did not lead to any homogenizing (antagonistic) effect on wheat growth. Interaction of the use of 4 tons per ha of biochar had a more significant effect on most of the measured traits than 8 tons per ha of biochar. Therefore, it seems that the use of a moderate amount of biochar is better than its high amounts due to the creation of optimal conditions for plant growth. In this study soil nutrients in a balanced way and as a result of better plant growth in the treatments of a medium amount of biochar is one of the reasons why this treatment is better than the high application of biochar. In this research, biochar production has caused the optimal use of agricultural and industrial waste and can be used to protect the environment and reduce pollutants and convert waste into useful compounds. Production of this substance and its consumption in Iranian soils where organic matter deficiency is common and its high stability to maintain the level of soil organic matter and improve their physical and chemical properties can be considered as one of the best and easiest methods. According to the obtained results, the use of 100 kg of nitrogen per ha along with the consumption of 4 tons per ha of biochar can be recommended due to their positive role on growth of wheat.

Acknowledgements

The authors would like to acknowledge all technicians who actively supported the field and lab works during the two years of this experiment.

Authors contributions

SMAS Collected the data (field and lab works), data analysis, writing the article. MA and MRA performed the research concept and design, field works, writing the article, critical revision of the article and final approval of the article. AM contributed to research conception and design, statistical analysis, writing the article. SL contributed to research conception, design and lab work.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no competing interests.

Ethical issues: None

References

1. Belete F, Dechassa N, Molla A, Tana T. Effect of nitrogen fertilizer rates on grain yield and nitrogen uptake and use efficiency of bread wheat (*Triticum aestivum* L.) varieties on the Vertisols of central highlands of Ethiopia. Agriculture and Food Security. 2018;7:1-12. https://doi.org/10.1186/s40066-018-0231-z.
2. Litke K, Gaile Z, Ruza A. Effect of nitrogen fertilization on winter wheat yield and yield quality. Agronomy Research. 2018;16:500-09. https://doi.org/10.15159/ar.18.064.
3. Mosanaei H, Ajamnorozi H, Dadashi MR, Faraji A, Pessarakli M. Improvement effect of nitrogen fertilizer and plant density on wheat (*Triticum aestivum* L.) seed deterioration and yield. Emirates Journal of Food and Agriculture. 2017;29:899-910. https://doi.org/10.9755/ejfa.2017.v29.i11.1500.
4. Pellegrino E, Opik M, Bonari E, Ercoli L. Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. Soil Biology and Biochemistry. 2015;84:210-17. https://doi.org/10.1016/j.soilbio.2015.02.020.
5. Shabani G, Ardakani MR, Chaichi MR, Friedel JK, Khavazi K. Effect of different fertilizing treatments on nutrient uptake in annual medic (*Medicago scutellata* cv. robinson) under irrigated and dry farming systems. Journal of Agricultural Science and Technology. 2015;17:299-310. Available at: http://journals.modares.ac.ir/article-23-10126-en.html.
6. Fiaz K, Malik SA, Younis U, Danish S, Raza Shah MH, Niaz S. Drought impact on Pb/Cd toxicity remediated by biochar in *Brassica campestris*. Journal of Soil Science and Plant Nutrition. 2014;14:845-54. https://doi.org/10.4067/S0718-95162014005000067.
7. Gaskin JW, Speir RA, Harris K, Das K, Lee RD, Morris LA, Fisher DS. Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status and yield. Agronomy Journal. 2010;102:623-33. https://doi.org /10.2134/agronj2009.0083.
8. Mahmoud E, Ibrahim M, Abd El-Rahman L, Khader A. Effects of biochar and phosphorus fertilizers on phosphorus fractions, wheat yield and microbial biomass carbon in *Vertic torrifluvents*. Communications in Soil Science and Plant Analysis. 2019;50:362-72. https://doi.org/10.1080/00103624.2018.1563103
9. Sun HJ, Zhang HC, Shi WM, Zhou MY, Ma XF. Effect of biochar on nitrogen use efficiency, grain yield and amino acid content of wheat cultivated on saline soil. Plant, Soil and Environment. 2019;65:83-89. https://doi.org/10.17221/525/2018-PSE.
10. Curaqueo G, Roldan A, MutisA, Panichini M, Perez-San Martín A, Meier S, Mella R. 2021. Effects of biochar amendment on wheat production, mycorrhizal status, soil microbial community, and properties of an Andisol in Southern Chile. Field Crops Research. 273(1): 1-12. https://doi.org/10.1016/j.fcr.2021.108306.

11. Foulkes MJ, Hawkesford MJ, Barraclough PB, Holdsworth MJ, Kerr S, Kightley S, Shewry PR. Identifying traits to improve the nitrogen economy of wheat: recent advances and future prospects. Field Crops Research. 2009;114:329-42. https://doi.org/10.1016/j.fcr.2009.09.005.

12. Philips J, Hayman D. Improved procedures for cleaning roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Journal of Transactions of the British Mycological Society. 1970;55:158-61. https://doi.org/10.1016/S0007-1536(70)80110-3

13. Giovannetti M, Mosse B. An evaluation of techniques to measure vesicular arbuscular infection in roots. New Phytology. 1980;84:489-500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x

14. Ali Sial T, Lan Zh, Wang L, Zhao Y, Zhang J, Kumbhar F, et al. Effects of different biochars on wheat growth parameters, yield and soil fertility status in a silty clay loam soil. Molecules. 2019;24:2-19. https://doi.org/10.3390/24091798.

15. Zhang M, Gao B, Varnoosfaderani S, Hebard A, Yao Y, Inyang M. Preparation and characterization of a novel magnetic biochar for arsenic removal. Bioresource Technology. 2013;130:457-62. https://doi.org/10.1016/j.biortech.2012.11.132

16. Zee TE, Nelson NO, Newdigger G. Biochar and nitrogen effects on winter Wheat growth, kansas agricultural experiment station research reports. Agronomy Crop Sciences Commons. 2017;3:1–8. https://doi.org /10.4148/2378-5977.

17. Cardoso M, Kuyper TW. Mycorrhizal and tropical soil fertility. Agricuitra, Ecosystems and Environment. 2006;116:72-84. https://doi.org /10.1016/j.agee.2006.03.011.

18. Ali K, Arif M, Jan T. Integrated use of biochar: A tool for improving soil and wheat quality of degraded soil under soil wheat-maiza cropping pattern. Pakistan Journal of Botany. 2015;47:233-40.

19. Akom M, Boateng N, Otoo1 E, Dawoe E. Effect of biochar and inorganic fertilizer in Yam (*Dioscorea rotundata* Poir) production in a forest agroecological zone. J Agric Sci. 2015;7:211-22. https://doi.org/ 10.5539/JAS.V7N3P211.

20. Major J, Rondon M, Molina D, Riha SJ, Lehmann J. Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant and Soil. 2010;33:117-28. https://doi.org /10.1007/s11104-010-0327-0.

21. Gebremedhin GH, Haileselassie B, Berhe D, Belay T. Effect of biochar on yield and yield Components of wheat and post-harvest soil properties in Tigray, Ethiopia. Fertilizers and Pesticides. 2015;6:158-62. https://doi.org/10.4172/jbfbp.1000158.

22. Mousavi SK, Feyzian M, Ahmadi AR. Effects of nitrogen application on wheat growth in the Lorestan Province. Iranian Journal of Soil Research. 2010;2:135-42.

23. Bavar M, Heidari Sharif Abad H, Noormohamadi Gh. The effects of different levels of nitrogen on yield and yield components of rainfed wheat in two regions of North Khorasan. Open Journal of Ecology. 2016;6:443-51. http://dx.doi.org/10.4236/oje.2016.67042

24. Khan Z, Rahman MH, Haider Gh, Amir R, Ikram RM, Ahmad Sh et al. Chemical and biological enhancement effects of biochar on wheat growth and yield under arid field conditions. Sustainability. 2021;13:5890. https://doi.org/10.3390/su13115890

25. Akhtar SS, Li G, Andersen MN, Liu, F. Biochar enhances yield and quality of tomato under reduced irrigation. Agricultural Water Management. 2014;138:37-44. https://doi.org/10.1016/j.agwat.2014.02.016

26. Dal Cortivo C, Ferrari M, Visioli G, Lauro M, Fornasier F, Barion G et al. Effects of seed-applied biofertilizers on rhizosphere biodiversity and growth of common wheat (*Triticum aestivum* L.) in the field. Frontiers in Plant Science. 2020;11:72. https://doi.org/10.3389/fpls.2020.00072

27. Sarfraz R, Hussain A, Sabir A, Fekih IB, Ditta A, Xing S. Role of biochar and plant growth promoting rhizobacteria to enhance soil carbon sequestration. A review. Environmental Monitoring and Assessment. 2019;191:1-3. https://doi.org/10.1007/s10661-019-7400-9

28. Mardukhi B, Rejali F, Daei G, Ardakani MR, Malakouti MJ, Miransari M. Mineral uptake of mycorrhizal wheat (*Triticum aestivum* L.) under salinity stress. Communications in Soil Science and Plant Analysis. 2015;46:343-57. http://doi.org/10.1016/j.jplph.2008.09.013

29. Hatfield JL, Prueger JH. Nitrogen over-use, under-use and efficiency. In: Proceedings of the 4th International Crop Science Congress. 2004; Brisbane, Australia.

30. Saber Z, Pirdashti H, Esmaeili MA, Abbasian A. The effect of plant growth promoting rhizobacteria, nitrogen and phosphorus on relative agronomic efficiency of fertilizers, growth parameters and yield of wheat (*Triticum aestivum* L.). Agroecology. 2013;5 (1):39-49. (In Persian).

31. Arif M, Asad A, Umair M, Munsif F, Kawsar A, Inamullah M, Saleem M, Ayub G. Effect of biochar, FYM and mineral nitrogen alone and in combination on yield components of maize. Sarhad Journal of Agriculture. 2012;28:191-95.

32. Clough TJ, Condron LM, Kammann C, Müller C. A review of biochar and soil nitrogen dynamics. Agronomy. 2013;3:275-93. https://doi.org /10.3390/agronomy3020275.

33. Liu Z, He T, Cao T, Yang T, Meng J, Chen W. Effects of biochar application on nitrogen leaching, ammonia volatilization and nitrogen use efficiency in two distinct soils. Journal of Soil Science and Plant Nutrition. 2017;17(2):515-28. http://dx.doi.org/10.4067/S0718-95162017005000037

34. Asplund L, Bergkvist G, Weih M. Proof of concept: nitrogen use efficiency of contrasting spring wheat varieties grown in greenhouse and field. Plant and Soil. 2014;374:829-42. https://doi.org/10.1007/s11104-013-1895-6.

35. Mandic V , Krnjaja V, Tomic Z, Zorica Bijelic Z, Simic A, Ruzic Muslic D et al. Nitrogen fertilizer influence on wheat yield and use efficiency under different environmental conditions. Chilean Journal of Agricultural Research. 2015;75(1):92-97. http://doi.org/10.4067/S0718-58392015000100013

36. Gholami A, Kochaki A. Mycorrhiza in sustainable agriculture (Translation). Shahroud University Press. 2001; 212 p.

37. Fathi Gardelidani A, Mirsaid Hosseini. Various aspects of biochar effects in soil quality improvement. 2015 June; International Conference on Applied Research in Agriculture.

38. Pal A, Pandey S. Role of arbuscular mycorrhizal fungi on plant growth and reclamation of barren soil with wheat (*Triticum aestivum* L.) crop. International Journal of Soil Science. 2017;12:25-31. https://doi.org/10.3923/ijss.2017.25.31

39. Habibi S, Meskarbashee M, Farzaneh M. Effect of mycorrhizal fungus (*Glomus* spp) on wheat (*Triticum aestivum* L.) yield and yield components with regard to irrigation water quality. Iranian Journal of Field Crops Research. 2015;13(3):471-84. https://doi.org/10.22067/gsc.v13i3.51155

40. Ilkaee MN, Paknejad F, Zavareh M, Ardakani MR, Kashani A. Prediction model of leaf area in soybean (*Glycine max* L.). American Journal of Agricultural and Biological Sciences. 2011; 6:110-13. https://doi.org/10.3844/ajabssp.2011.110.113

§§§