Effects of mycorrhiza and humic acid on the quantitative and qualitative characters of red bean, Derakhshan cultivar

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Abstract

In order to investigate the effect of mycorrhiza and humic acid treatments on the qualitative and quantitative yields of red beans, Derakhshan cultivar, a factorial pot experiment was conducted in the form of a complete random block design with three repetitions under greenhouse condition. The first factor of the study was mycorrhiza at three levels, namely control (no mycorrhiza), *Glomus intraradices* strain, and *Glomus mosseae* strain. The second factor of the study included three levels of humic acid, namely 0 (control), 15, and 30 mg L^{-1}. Results showed that with the application of mycorrhiza and humic acid, the number of seeds per plant, seed weight per plant, chlorophyll index, percentage of seed protein content, and nitrogen, phosphorus, and potassium contents of leaves increased. The highest seed weight per plant (13.47 g) was recorded in the red beans treated with 30 mg L^{-1} of humic acid + *Glomus intraradices* strain and 13.01 g and 13.72 g in the plants treated with 15 and 30 mg L^{-1} of humic acid, respectively along with *Glomus mosseae* strain. Also, the highest percentage of seed protein was 26.15% obtained from the treatment with 30 mg L^{-1} of humic acid + *Glomus intraradices* and 25.23% and 27.93% in the treatments with 15 mg L^{-1} and 30 mg L^{-1} of humic acid, respectively along with *Glomus mosseae*. Maximum leaf nitrogen content (5.85%) was obtained from the application of 30 mg L^{-1} of humic acid with Glomus mosseae. Mycorrhizae and humic acid seem to be able to improve the yield and yield components of red beans by providing nutrients to the plant.

Keywords: humic acid, mycorrhiza, nitrogen, protein, red beans

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Introduction

Legumes are the third largest family of flowering plants and include about 750 genera and more than 19,000 distinct species (Duc et al., 2015). Among legumes, red bean (*Phaseolus vulgaris* L.) is of particular importance for the largest area under cultivation and production. The crop is a source of protein, fiber, carbohydrates, and rare minerals (Hummel et al., 2018). Biofertilizers by acidifying the soil solution or secreting some enzymes, usually cause the release of elements from the complex mineral and organic compounds in the soil, making them available to the plant (Pandey and Patra, 2015). Among various biofertilizers are mycorrhizal fungi, and the plants with mycorrhizal symbiosis enjoy a better growth and yield as they can absorb more nutrients and

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water from the soil (Larrainzar and Wienkoop, 2017). Mycorrhizal fungi, by improving nutritional conditions and also producing plant growth stimulating compounds, usually improve and accelerate the growth stages in plants, and in addition to moderating the adverse effects of stress, they significantly increase the nutrients uptakes by plants (Perramon et al., 2016). The symbiosis relationship between a plant and mycorrhiza through increasing the absorption surface of the roots and improving water and nutrient uptake, especially that of phosphorus by the hyphae and transferring them to the roots, improves the nutritional status of the plant and increases its growth and performance (Golubkina et al., 2020). Mycorrhizal fungi also increase the activity of beneficial organisms such as rhizobium, azotobacter, and microorganisms that dissolve insoluble soil phosphorus in the rhizosphere. Physiologically, mycorrhizal fungi increase the absorption level of the root system and produce hormones such as auxin, gibberellin, and cytokinin (Begum et al., 2019), which are effective in plant growth. Mycorrhizal symbiosis seems to increase the quantity and quality of plant yields by influencing the proper uptake of nutrients (Rydlova et al., 2016).

Excessive application of chemical fertilizers in agriculture causes environmental problems such as physical destruction of the soil and imbalanced soil nutrients. Unwarranted use of chemical fertilizers also pollutes water and soil resources, reduces the quality of food products, and disrupts the biological balance in the soil, with the consequence of irreparable damage to the ecosystem. The use of natural biologic fertilizers and compounds without harmful environmental effects to increase yields economically is one of the proposed solutions in sustainable agriculture. On the other hand, the use of environmentally compatible compounds such as humic acid improves the physical, chemical, and biological structure of the soil, positively affecting the quantitative and qualitative indices of agricultural products (Pospíšilová et al., 2016).

An environmentally friendly organic acid, humic acid is a natural organic polymer that can increase plant productivity and quality (Eshwar et al., 2017). It stimulates the growth in shoots and the roots of the plant in both greenhouse and field conditions, although its effects are more prominent on roots. Humic acid changes root structure and increases its length and density, which leads to an increase in root absorption of macro and micro elements and the improved efficiency of the root system (Nardi et al., 2017; Canellas and Olivares, 2014). Moreover, humic acid increases the yield and protein of seeds, mitigates the effects of stress in the plant by affecting the production of osmolytes such as proline, and maintains growth and optimal distribution and assimilation of nutrients in the plant by indirectly preventing the destruction of chlorophyll (Goma et al., 2017). The improved growth in plants treated with humic acid is also attributed to its role in increasing the water retention and cation exchange capacity of the soil, activating the respiration and photosynthesis cycle, and producing amino acids and adenosine triphosphate (ATP) (Tadayyon et al., 2017).

Following the importance of legumes as a source of protein, fiber, carbohydrates, and rare minerals, the present study was carried out in order to investigate the effect of the application of two strains of mycorrhiza and also humic acid on the morphological and physiological characteristics of red been (*Phaseolus vulgaris* L.)

Materials and Methods

A factorial pot experiment was carried out in the form of a randomized complete block design with three replications in a private greenhouse located in Hamadan province. Experimental factors included three levels of mycorrhiza, namely non-application (control), *Glomus intraradices* strain, and *Glomus mosseae* strain applied in the pot soil and three levels of humic acid, namely zero (control), 15 mg L^{-1}, and 30 mg L^{-1} as foliar spraying applied in 10-day periods.

Table 1
The effects of mycorrhizal inoculation and humic acid application on the quantitative and qualitative yield of red beans

Sources of Variation	df	Leaf Surface Area	Number of Seeds per Plant	Weight of Seeds per Plant (g)	Chlorophyll Index	Seed Protein (%)	Leaf N (%)	Leaf P (%)	Leaf K (%)
Block	2	63.35ns	59.11*	1.53ns	1.99ns	9.55*	0.11ns	0.002ns	0.13ns
(a) Mycorrhiza	2	120.97*	333.44**	29.06**	112.1**	28.13**	2.31**	0.033**	2.78**
(b) Humic Acid	2	535.27**	217**	52.66**	136.16**	55.7**	2.12**	0.015**	2.66**
a*b	4	15.16ns	15.78ns	3.78*	6.67*	8.66*	0.12*	0.004*	0.13*
Error	16	24.68	10.53	1.09	1.70	2.61	0.04	0.001	0.04
CV%		14.69	11.97	10.4	6.15	6.85	4.02	7.05	4.68

* and ** show significant respectively at ($P \leq 0.5$) and ($P \leq 0.01$) probability level; ns: non-significant

This experiment was carried out in 8 kg pots with field soil substrate of clay loam texture along with rotted manure (3:1). According to the soil test, 0.7 g of urea, 0.7 g triple superphosphate, and 0.35 g of potassium sulfate were added to each pot. This means 100 kg of urea and triple superphosphate and 50 kg of potassium sulfate per hectare. Seeds of red bean, Derakhshan cultivar were obtained from the Khomein Beans Research Institute. In order to disinfect the seeds and prevent possible contamination, they were placed in a 1% sodium hypochlorite solution for two minutes, before they were washed with normal and distilled water in that order. In order to accelerate the germination of the seeds, they were soaked in water for 24 hours before planting. Ten (10) seeds were planted in each pot at a depth of three centimeters, and irrigation was done immediately after planting. Mycorrhiza treatments were applied using 20 g of mycorrhiza inocula of the both strains i.e., *Glomus intraradices* and *Glomus mosseae*, to the pots for each kilogram of the pot soil. The plants were thinned and four seedlings were left in each pot at 4-6 leaf-stage. Humic acid treatments were applied before the beginning of florescence twice with an interval of 10 days. The pots were irrigated regularly until the end of the growing season to maintain soil moisture at the agricultural capacity. Red beans were harvested when 70% of the pods turned yellow.

Leaf area was measured using a leaf area meter. The chlorophyll contents of leaves were assayed from the middle part of each leaf on one side of the main vein using a hand-held chlorophyll meter (SPAD-502, Minolta Co. Japan) between 9:30 AM and 10:00 AM in order to minimize the effects of daily variations.

Seed protein contents were assayed using a Kjeldahl device (Foss, Sweden). Also, leaf nitrogen contents were assayed using a micro Kjeldahl device (Bremner, 1996). Phosphorus contents were measured using spectrophotometry at 470 nm wave length. For this purpose, plant samples were digested in a volumetric flask with sulfuric acid, salicylic acid, and hydrogen peroxide. After preparing the extract, it was measured through photometric method (vanadate molybdate yellow) and using a spectrophotometer (Jones et al., 1991). Potassium was measured by dry digestion using an oven set at 550 °C and dissolving in 0.5 M hydrochloric acid with a film photometer (Jones et al., 1991).

At the end of the experiment, data analysis was done using SAS software. Means were compared using Duncan's multiple-range test at the five percent probability level.

Results

Leaf surface area

The results of ANOVA (Table 1) showed that the effects of mycorrhiza on leaf surface and humic acid were significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. The leaf surface in plants treated with mycorrhiza increased compared to the control. Moreover, *Glomus intraradices* and *Glomus mosseae* strains did not show statistically significant difference in their effect on leaf surface (Table 2). Application of humic acid also increased the leaf area compared to the control while there were no significant differences between 15 mg L^{-1} and 30 mg L^{-1} humic acid (Table 3).

Number of seeds per plant

The effect of mycorrhiza and humic acid on the number of seeds per plant was significant (P≤0.01) (Table 1). With the application of mycorrhiza, the number of seeds per plant increased compared to the control while *Glomus intraradices* and *Glomus mosseae* strains were not statistically different in their effect on this parameter (Table 2). Spraying humic acid caused an increase in the number of seeds per plant compared to the control, and the highest number of seeds per plants (31.44 seeds) was obtained from the use of 30 mg L^{-1} of humic acid, showing 53.03% increase compared to the control (Table 3).

Seed weight per plant

The results of analysis of variance (Table 1) showed that the main effects of mycorrhiza and humic acid as well as the interaction effect of these factors on seed yield of the red beans under study were significant at (P≤0.01) and (P≤0.05), respectively. Findings suggested that mycorrhiza and humic acid had a synergetic effect on seed weight per plant, and the highest seed weight per plant was 13.47 g from the use of 30 mg L^{-1} of humic acid along with *Glomus intraradices*, and 13.01 g and 13.72 g obtained from the application of 15 mg L^{-1} and 30 mg L^{-1} of humic acid along with Glomus mosseae (Table 4).

Table 2
Effects of mycorrhizal inoculation on the leaf surface area and the number of seeds in the red bean plants

Mycorrhiza	Leaf Surface Area	No. of Seed per Plants
Control	29.7b	20.33b
Glomus intraradices	34.34a	28.89a
Glomus mosseae	36.77a	32.11a

Means with similar letters are not significantly different based on Duncan's test (P≤0.5).

Table 3
Effect of humic acid application on leaf surface area and number of seeds per plant in red beans

Humic Acid (mg L^{-1})	Leaf Surface Area	No. of Seeds per Plants
0	25.27b	21.78c
15	35.88a	28.11b
30	40.27a	31.44a

Means with similar letters are not significantly different based on Duncan's test (P≤0.5).

Chlorophyll index

The effect of mycorrhiza and humic acid and their interaction on chlorophyll contents of the red beans under study were significant at (P≤0.01) and (P≤0.05), respectively (Table 1). It was also observed that mycorrhiza and humic acid had a synergetic effect on the chlorophyll index of beans, and the highest chlorophyll content (29.49) was recorded in in combined treatment of the plants with 30 mg L^{-1} of humic acid along with *Glomus mosseae* inoculation (Table 4).

Table 4
Effects of combined treatment of the red beans with mycorrhiza and humic acid on the quantitative and qualitative yield of red beans

Mycorrhiza	Humic Acid (mg L^{-1})	Weight of Seeds per Plant (g)	Chlorophyll Index	Seed Protein (%)	Leaf N (%)	Leaf P (%)	Leaf K (%)
Control	0	6.66c	15.65e	18.2d	3.84f	0.38d	3.14f
Control	15	7.85c	17.08de	23.17bc	4.41de	0.38d	3.56e
Control	30	9.83b	20.9c	23.22bc	4.75cd	0.41cd	4.43c
Glomus intraradices	0	7.58c	16.78e	23.95b	4.24e	0.41cd	3.85de
Glomus intraradices	15	9.81b	21.12c	23.83bc	5.31b	0.47bc	4.15cd
Glomus intraradices	30	13.47a	24.6b	26.15ab	5.08bc	0.47bc	4.91a
Glomus mosseae	0	8.3bc	19.24cd	20.46cd	4.83c	0.42cd	4.26c
Glomus mosseae	15	13.01a	25.98b	25.23ab	5.36b	0.52ab	5.03a
Glomus mosseae	30	13.72a	29.49a	27.93a	5.85a	0.58a	5.17a

Means with similar letters are not significantly different based on Duncan's test (P≤0.5).

Seed protein percentage

The results of ANOVA (Table 2) showed that the main effects of mycorrhiza and humic acid ($P \leq 0.01$) and their interaction ($P \leq 0.05$) on seed protein percentage were significant (Table 1). Moreover, the combined treatment of mycorrhiza and humic acid increased the percentage of seed protein, so that the highest percentage of seed protein (26.15%) was recorded in the red beans treated with 30 mg L^{-1} of humic acid along with *Glomus intraradices* inoculation, and 25.23% and 27.93% seed protein were recorded with the combined treatment of the red beans with of 15 mg $L^{-1}$and 30 mg $L^{-1}$of humic acid, respectively along with *Glomus mosseae* inoculation (Table 4).

Leaf nitrogen contents

Comparison of mean leaf nitrogen contents showed that the effects of mycorrhiza and humic acid as well as their interaction were significant on this parameter at $P \leq 0.01$ and $P \leq 0.05$, respectively (Table 1). The highest leaf nitrogen content (5.85%) was obtained from the application of 30 mg L^{-1} of humic acid in combination with *Glomus mosseae* treatment. The results of ANOVA also showed that as with the other traits under study, the simultaneous treatment of red beans with mycorrhiza and humic acid led to higher levels of leaf nitrogen content in comparison with individual treatments, i.e., there was a positive interaction between the two treatments of the study (Table 4).

Leaf phosphorus contents

The results of ANOVA (Table 1) showed that the main effects of mycorrhiza and humic acid and the interaction effect of mycorrhiza and humic acid on leaf phosphorus contents were significant at $P \leq 0.01$ and $P \leq 0.05$, respectively. Comparison of mean phosphorus contents of the plant leaves under the influence of mycorrhiza and humic acid showed that the highest percentage of phosphorus (0.52%) and (0.58%) were obtained from the application of 15 mg L^{-1} and 30 mg L^{-1} of humic acid, respectively in combination with *Glomus mosseae* inoculation (Table 4).

Leaf potassium contents

The effects of mycorrhiza and humic acid as well as their interaction on leaf potassium contents were significant at $P \leq 0.01$ and $P \leq 0.05$ probability levels, respectively (Table 1). The highest percentage of leaf potassium (91.4%) was recorded in the plants treated with 30 mg L^{-1} of humic acid in combination with *Glomus intraradices* inoculation, and 5.03% and 5.17% leaf phosphorous concentrations were obtained from the use of 15 mg L^{-1} and 30 mg L^{-1} of humic acid, respectively along with *Glomus mosseae* inoculation (Table 4).

Discussion

Based on the results of this study, leaf surface increased with mycorrhiza inoculation and also humic acid treatments. Southavong et al. (2012) stated that mycorrhiza by producing growth hormones such as auxin and cytokinin and also by increasing the activity of some enzymes through the provision of nutrients such as phosphorus, help plant growth and increase the leaf area in plants. Mycorrhizal fungus increases the absorption of nutrients by expanding the root system of the plant, which is caused by the development of external hyphae in the soil, and thereby increases the number of leaves in the plant (Khosrojerdi et al., 2013). Burhan et al., (2018) observed that foliar application of humic acid increased the leaf area, probably as a result of the rapid expansion of the plant root system under humic acid treatment, which in turn led to an increased nutrient uptake and eventually a better plant growth, including an increase in the number of leaves and their surface area.

In addition, mycorrhiza inoculation in this study was found to increase the number of seeds in red beans, as the symbiotic relationship between the plant and mycorrhiza fungus improves the level of root absorption and water and nutrient uptake, especially absorption of phosphorus through hyphae and its transfer to the plant root (Alqarawi et al , 2014) and improves the nutritional status, increasing the number of seeds in the plant through inducing hormonal changes in the plants (Southavong, et al., 2012).

Also, based on the results of the present research, spraying red beans with humic acid increases the number of seeds per plant. The use of humic acid increases the levels of auxin, cytokinin, and gibberellin hormones (Nardi et al., 2017). Nardi et al. (2017) explained that the increased level of these hormones under stress conditions, reduces the allocation of processed compounds to vegetative growth, increasing the share of seed with a result of increased number of seeds per plant.

Inoculation of red beans with mycorrhiza resulted in the increased seed yield in this study. Rahimzadeh and Pirzad (2017) observed that mycorrhiza probably through increasing the mass of photosynthetic organs improves the quantity and capacity of production of assimilates in plants, which eventually leads to the improved yield. The yield was also increased after spraying the red bean plants with humic acid. In their study, Burhan et al. (2018) showed that humic acid increased the crop weight through affecting transfer of more photosynthetic material from leaves to seeds. Humic acid via positive physiological effects including its effects on plant cell metabolism and increased concentrations of leaf chlorophyll and photosynthesis improves seed yield (Anwar et al., 2017).

Mycorrhizal inoculation improved chlorophyll index in the plants under study. Rahimzadeh and Pirzad (2017) in their study observed that inoculation of the plants with mycorrhiza increased photosynthesis surface and chlorophyll index of the plant through increased uptake of nutrients such as nitrogen, phosphorous, and iron. Chlorophyll index was also improved in this study under humic acid treatment. This organic acid directly improves chlorophyll contents, respiration, and hormonal responses in plants. It increases nutrient, particularly nitrogen uptake, and therefore, improves chlorophyll index (Dalvand et al., 2018).

It was found in this study that the inoculation of red beans with mycorrhiza and application of humic acid increased the percentage of seed protein. Rydlova et al. (2016) discusses that mycorrhiza by providing more suitable conditions for plant growth e.g., through the production of plant hormones and the development of the root system, and as a result increasing the absorption of water and nutrients such as nitrogen and phosphorus, paves the way for increased concentration of proteins in plants. The increase in seed protein percentage is related to the effect of humic acid in terms of increased root growth, absorption of nutrients, and chlorophyll synthesis, because with the increase in nitrogen absorption from the roots, the synthesis of amino acids also increases and this increases the percentage of seed protein (Anwar et al., 2016).

According to the findings of the study, nutrient absorption increased with the use of mycorrhiza. Based on the findings of a study by Begum et al. (2019), mycorrhiza improves soil properties such as organic matter content and increases the availability of nutrients such as nitrogen, phosphorus, and potassium. The increase in the uptake of nutrients can be a result of the increase in the absorption surface of plant roots following mycorrhizal inoculation. The external hyphae of mycorrhiza can absorb make available more nutrients for the host plant (Perramon et al., 2016). Plant inoculation with mycorrhiza improves the nutritional status of the plant tissue, and as a result, the amount of leaf water and the transfer of nutrients to the plant increases (Hammer et al., 2011). In addition, foliar application of humic acid also increased the absorption of nutrients in the red bean plants under study. Manzoor et al. (2014) stated that stimulating the absorption of ions through application of humic acid might be a function of the effect of humic acid on membrane permeability and more developed root system. The amount of nutrient uptake is a function of the amount of humic acid consumed. In general, increasing the concentration and uptake of nutrients increases with increasing the level of humic acid. Owing to its acidity, humic acid can directly release various elements from minerals, absorb them, and provide them to the roots at the right time. In addition, humic acid stimulates the growth of beneficial soil microorganisms such as fungi, which are able to aerate mineral phases and release nutrients (Goma et al., 2017). Therefore, it seems that with the application of humic acid, the content of plant nutrients increases as a result of the increase in the surface of the plant root and also level of nutrients soluble in the soil through the stimulation of microbiological activities and the increase in the root lengths (Goma et al., 2017).

Conclusion

It was found in this study that the application of mycorrhiza and humic acid improved quantitative characteristics of red beans such as leaf area, number of seeds per plant, and seed weight per plant as well as qualitative characteristics including chlorophyll index, seed protein percentage, and leaf nitrogen, phosphorus, and potassium contents. It seems that mycorrhiza and humic acid improved the yield and components of yield in the plant by providing nutrients such as potassium, nitrogen, and phosphorus and as a result increasing the level of photosynthesis in red bean plants. The combined treatment of the red beans with 30 g L^{-1} of humic acid and mycorrhizal inoculation with *Glomus mosseae* strain resulted in the highest quantitative and qualitative yield in this experiment.

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