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Response of Yield and Yield Components of Three Red Bean (*Phaseolus vulgaris* L.) Genotypes to Co-Inoculation with *Glomus intraradices* and *Rhizobium phaseoli*

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Abstract: The use of biotechnology with the aim of using soil beneficial organisms to produce maximum production, improving soil quality and considering hygiene and environmental safety, has been considered. In order to investigate the effect of biological fertilizers on beans yield and yield components, a field study was conducted during spring and summer 2010 in agricultural experimental station of Arak Islamic Azad University. A factorial experiment was arranged in randomized complete block design with three replication. Three beans varieties Goli (indeterminate), Sayad (Semi determinate) and Derakhshan (determinate) were used. Mycorrhiza fungi including no inoculation and *Glomus intraradices* and three levels of *Rhizobium phaseoli* (no application, strain *Rb*116 and strain *Rb*133) were used. Interaction between Mycorrhiza, *Rhizobium* and cultivars on yield and yield components showed highest seed yield (2664 kg ha^{-1}) and biological yield (6333 kg ha^{-1}) in treatment of inoculation Goli variety with both mycorrhiza (*Glomus intarardices*) and *Rhizobium* strain *Rb*133, respectively. The lowest yield (1172 kg ha^{-1}) was obtained in treatment not using Mycorrhiza fungi and Rhizobium bacteria with variety Derakhshan.

Key words: Co-inoculation · Mycorrhiza · *Rhizobium* · Red beans · Yield · Yield components

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most edible legume representing 50% of grain legume for direct human consumption [1]. In some countries such as Mexico and Brazil, beans are the primary source of protein in human diets [2]. Biological fertilizers contain one or several specific micro-organisms causing more and better development of root systems and components for better absorption [3]. Sufficient amounts of phosphorus for plant nitrogen fixation process requires high energy molecules. Phosphorus uptake from resources, particularly insoluble elements establish a triple symbiosis between plants as hosts, mycorrhizal fungi and Rhizobium, increase nitrogenase enzyme activity and increase legumes yield [4]. Mycorrhizal fungi most important action is crop yield increment, especially in soils with low fertility. Such performance due to absorption increment through the roots, penetrate the soil fungi mycelium and access a greater volume of soil [5]. Mycorrhiza inoculation also increase nutrient uptake. Soil inoculation with mycorrhiza strains significantly increase plant growth and plants phosphorus uptake especially under low supply. Under low phosphorus application, plant roots are strongly infected and consequently increase plant growth, but with high phosphorus level applications, a slight reduction in root occurs [6].

In legumes, Sufficient amount of phosphorus for plant growth and nods formation for nitrogen fixation is essential [7]. Mycorrhizal fungi can improve phosphorus deficiency conditions which has positive effects on nitrogen fixation [7]. Several researches have shown that vesicular arbuscular rnycorrhizal (VAM) fungi can improve immobile nutrients uptake in plants, particularly Phosphorus [8, 9]. Therefore, it is likely that plants infected with VAM fungi can significantly benefit from

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rock phosphate application in these soils. Indeed, VAM-dependent plants species, such as *Stylosanthes* and *Leucaena*, have been known to respond favorably to VAM inoculation at low levels of soil Phosphorus [9, 10].

Mycorrhizal fungi can form symbiosis with field crop under a range of environmental conditions [11, 12]. This symbiosis can enhance nutrient uptake and plant growth under various environmental stress conditions such as salinity, drought and low fertility [13]. The beneficial effect of Mycorrhiza on plant growth was attributed to enhanced phosphorus uptake. The increment in nutrient uptake is proposed to be due to increasing affinity to a particular ion and lowering the threshold concentration for absorption [14].

In symbiosis systems, nitrogen fixation efficiency depends on bacteria strain, plant, host, environmental factors, soil and their interaction. Inoculation with bacteria increases total nitrogen and nitrogen fixation, leghemoglobin, amount of chlorophyll, dry weight, grain yield and forage [15]. Inoculated bean with Rhizobium bacteria, increase performance and reduce the consumption of too much nitrogen fertilizer [16]. One of the major goals of this study is to introduce different applications of biological fertilizers instead of chemical fertilizers and increase beans yield without the usage of artificial chemicals.

MATERIALS AND METHODS

In order to study the effects of biological fertilizers on yield and yield components of bean, a field study was conducted during spring and summer 2010 in agricultural experimental station of Arak Islamic Azad University. Arak is classified among the temperate climatic regions in Iran with an average annual rainfall of 256 mm. The physical and chemical characteristic of the soil experimental site is presented in Table 1.

A factorial experiment was arranged in randomized complete block design with three replication. Three varieties of bean, Goli (indeterminate) (V1), Sayad (Semi determinate) (V2) and Derakhshan (determinate) (V3) were used in this study. Mycorrhiza fungi including no inoculation (M0) and *Glomus intraradices* (M1) and three levels of *Rhizobium phaseoli*, no application (R0), strain *Rb*116 (R1) and strain *Rb*133 (R2) were used. Seeds inoculation was done in shadow and after drying, inoculated seeds were immediately cultivated. Plant harvest was done manually. For measuring root colonization, gridline method and for roots coloration, Phillip and Hayman [17] methods was used. Statistical analysis was done with SAS (version 9.1.3) statistical software [18]. Duncan method was used for means comparison.

RESULTS AND DISCUSSION

Number of Pods per Plant: Table 2 shows the analysis of variance results. According to this table, regarding simple effects, a significant difference was observed for number of pods per plant among mycorrhiza treatments, different treatments of rhizobium and different varieties ($P< 0.01$, 0.05 and 0.01 respectively) while for interaction effects, only the interaction between varieties and mycorrhiza fungi showed significant difference ($P<0.05$) among treatments. As mentioned in Table 3, the highest number of pods per plant for each simple effect were produced in M1 (18.99), R2 (18.173) and following by R1 (17.407) and finally V1 (21.6). The highest numbers of pods per plant in interaction between mycorrhiza and *rhizobium* treatments were obtained in treatments M1R3 (20.68) and M1R0 (20.18) (Table 4).

For the interaction between macorrhizal fungi and varieties, treatment M1V2 (25.719) had the highest mean of number of pods per plant (Table 5) and for the interaction between rhizobium and varieties, treatments R2V2 (23.17) and R1V2 (22.14) had highest number of pods (Table 6). According to Table 7, the highest number of pods in plant with a total of 28 pods was produced from the treatment of co-inoculation of *Glomus intraradices* and Rb133 with Goli variety (M1R2V1). Lowest number of pods in plant with 11 pods belonged to the treatment of mycorrhizal fungi, non-rhizobium and Derakhshan variety (M1R0V3). Goli variety due to indeterminate growth and producing long stem, have more genetic potential to produce pods. High number of pods per plant in Goli variety was predictable among the varieties as it has the highest yield potential. Sadeghipour *et al.* [19] reported same result. They also showed that indeterminate varieties have more pods per plant. Mahmoud *et al*. [20] reported biological fertilizer (Nitrobin) have significant effect to increase pods in bean.

Table 1: Physical and chemical properties of soil field

Depth (cm)	S. P	EC	pH	TNV (%)	Organic carbon (%)	Total nitrogen (%)	Available P (ppm)	Available K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
0-30	37/5	1/4	7/7	0/18	0/38	0/04	9	200	24	44	32	Clay Loam

Table 2: Analysis of variance of experimental traits

Treatment	df	Number of pods per plant	Number of seeds per plant	Number of seeds per pod	100 seeds weight	Seed yield	Biological yield	Root colonization
R	2	40.90*	951.94**	0.60*	22.19*	1213780.99**	1861957.50^{ns}	*14/67
M	1	259.60**	3439.22**	0.02^{ns}	0.03^{ns}	3249093.42**	3224691.86^{ns}	229/40**
Rh	2	55.83*	602.35*	0.01^{ns}	1.34^{ns}	1033797.90**	7244601.97**	^{ns}3/07
Rh × M	2	11.30^{ns}	276.61^{ns}	0.17^{ns}	1.62^{ns}	147747.23^{ns}	164898.08^{ns}	1/11^{ns}
V	2	389.55**	3060.98**	1.44**	462.02**	1352430.77**	20117746.82**	26/45**
M ×V	2	54.50*	590.50*	0.09^{ns}	19.70^{ns}	172967.95*	547511.18^{ns}	13/75*
Rh ×V	4	0.84^{ns}	49.76^{ns}	0.15^{ns}	4.36^{ns}	105587.88^{ns}	892899.36^{ns}	^{ns}3/37
M × Rh × V	4	2.39^{ns}	77.20^{ns}	0.18^{ns}	3.41^{ns}	55050.69^{ns}	617430.71^{ns}	^{ns}0/94
Error	34	12.22	142.61	0.16	6.48	64750.32	809046.66	2/93
CV (%)		16.81	18.39	12.76	9.14	13.70	15.72	5/51

ns, * and **: Non significant and significant $P<0.05$ and $P< 0.01$ respectively. M: Mycorrhiza; R: Replication; Rh: Rhizobium; V: Variety.

Table 3: Mean comparison of mycorrhiza, rhizobium and varieties simple effects on yield and yield components

	Mean			squares			
Treatments	Number of pods per plant	Number of seeds per plant	Number of seeds per pod	100 seed weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Root colonization(%)
M0	14.60 b	45.35 b	3.08 a	27.87 a	1611.32 b	4314.7 a	28.99b
M1	18.99 a	61.31 a	3.12 a	27.82 a	2101.90 a	4803.4 a	33.11a
R0	14.813 b	46.99 b	3.09 a	27.61a	1580.13 b	3826.6 b	30.63 a
R1	17.407 a	54.66 a	3.08 a	28.14 a	1984.82 a	4917.0 a	31.45 a
R2	18.173 a	58.33 a	3.13 a	27.77 a	2004.89 a	4933.6 a	31.08 a
V1	21.60 a	61.81 a	2.88 b	23.99 c	2157.53 a	5642.50 a	32.23 a
V2	16.52 b	59.87 a	3.42 a	25.95 b	1791.12 b	4504.30 b	31.11 a
V3	12.30 c	38.31 b	2.99 b	33.58 a	1621.18 c	3530.30 c	29.81 b

Means with the same letter in each column have no significant difference ($P<0.05$). M0, M1: Control (no use of mycorrhiza fungi) and fungi application (*Glomus intraradices*): R0, R1, R2: Control (no use of rhizobium), rhizobium *Rb*116 application and rhizobium *Rb*133 application; V1, V2, V3: Goli, Sayad and Derakhshan.

Table 4: Mean comparison of interaction between various treatments of mycorrhizal fungi and rhizobium on yield and yield componenets

	Mean			squares			
Treatments	Number of pods per plant	Number of seeds per plant	Number of seeds per pod	100 seed weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Root colonization(%)
M0R0	13.52 b	43.00 b	3.146 a	27.92 a	1428.0 c	3474.1 b	28.69 b
M1R0	20.18 a	62.78 a	3.064 a	28.08 a	2235.4 a	5127.4 a	32.56 a
M1R2	16.10 b	50.99 b	3.04 a	27.31 a	1732.3 b	4179.0 ab	33.35 a
M1R3	20.68 a	70.16 a	3.26 a	28.06 a	2338.0 a	5103.9 a	33.42 a
M0R1	14.63 b	46.54 b	3.10 a	28.21 a	1734.2 b	4706.6 a	29.55 b
M0R3	15.66 b	46.50 b	3.00 a	27.48 a	1671.8 b	4763.4 a	28.73 b

Means with the same letter in each column have no significant difference ($P<0.05$). M0, M1: Control (no use of mycorrhiza fungi) and fungi application (*Glomus intraradices*): R0, R1, R2: Control (no use of rhizobium), rhizobium *Rb*116 application and rhizobium *Rb*133 application.

Table 5: Mean comparison of interaction between mycorrhizal fungi and varieties on yield and yield components

	Mean			squares			
Treatments	Number of pods per plant	Number of seeds per plant	Number of seeds per pod	100 seed weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Root colonization(%)
M0V1	17.46 b	47.22 cd	2.927 c	22.86 c	1801.3 bc	5320.3 ab	29.17 c
M0V2	14.81 bc	54.94 bc	3.48 a	26.27 b	1581.7 cd	4138.1 cd	29.42 c
M0V3	11.54 c	33.89 e	2.927 c	34.48 a	1450.9 d	3485.7 d	28.37 d
M1V1	18.193 b	64.80 b	3.36 ab	25.64 b	2000.5 b	4870.6 bc	35.29 a
M1V2	25.719 a	76.39 a	2.93 c	25.13 bc	2513.7 a	5964.8 a	32.80 b
M1V3	13.059 c	42.74 de	3.07 bc	32.68 a	1791.5 bc	3574.9 d	31.25 b

Means with the same letter in each column have no significant difference ($P<0.05$). M0, M1: Control (no use of mycorrhiza fungi) and fungi application (*Glomus intraradices*); V1, V2, V3: Goli, Sayad and Derakhshan.

Table 6: Mean comparison of the interaction effect of rhizobium and varieties on yield and yield components

	Mean			squares			
Treatments	Number of pods per plant	Number of seeds per plant	Number of seeds per pod	100 seed weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Root colonization(%)
R0V1	19.45 abc	55.92 ab	2.87 bc	24.25 bc	2018.9 abc	4694.8 cd	31.18 bc
R1V1	22.14 ab	61.93 a	2.93 bc	23.35 c	2283.8 a	6326.4 a	33.44 a
R2V1	23.17 a	67.57a	2.85 bc	24.37 bc	2169.9 ab	6326.4 ab	32.09 ab
R0V2	14.79 de	56.42 ab	3.58 a	25.95 bc	1511.7 d	3962.6 cde	30.54 bc
R1V2	16.75 cde	59.80 a	3.31 abc	26.75 b	1904.2 bc	4943.8 bc	31.40 abc
R2V2	17.96 bcd	63.39 a	3.37 ab	25.17 bc	1957.4 bc	4606.6 cd	31.39 abc
R0V3	10.19 f	28.64 c	2.83 c	32.64 a	1209.8 e	2822.3 e	30.16 bc
R1V3	13.33 ef	42.25 b	3.00 bc	34.34 a	1766.5 cd	3480.7 de	29.52 c
R2V3	13.38 ef	44.04 b	3.16 abc	33.76 a	1887.3 bc	4287.8 cd	29.75 c

Means with the same letter in each column have no significant difference (P<0.05). R0, R1, R2: Control (no use of rhizobium), rhizobium *Rb*116 application and rhizobium *Rb*133 application; V1, V2, V3: Goli, Sayad and Derakhshan.

Table 7: Mean comparison of the interaction effect of mycorrhizal fungi, rhizobium and varieties on yield and yield components

	Mean			squares			
Treatments	Number of pods per plant	Number of seeds per plant	Number of seeds per pod	100 seed weight (g)	Seed yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Root colonization(%)
M0R0V1	cde 15.65	efgh 42.28	bc 2.79	cd 22.65	de 1729.9	bcde 4024.1	27/84 g
M0R0V2	cde 14.62	56.29 bcde	a 3.71	bc 27.16	fg 1306.1	cde 3691.7	28/91 fg
M0R0V3	10.30 e	h 30.43	abc 2.93	a 23.95	g 1247.9	e 2706.6	29/34 fg
M0R1V1	cde 18.00	51.00 cdefg	abc 2.95	d 22.12	cde 1903.3	a 6319.9	30/81 defg
M0R1V2	cde 13.76	51.34 bcdefg	abc 3.26	bc 27.17	de 1736.4	abcd 4642.6	29/90 efg
M0R1V3	de 12.14	efgh 37.29	abc 3.08	a 35.33	efg 1562.9	de 3157.2	27/95 g
M0R2V1	cde 18.73	cdefg 48.37	c 2.75	cd 23.80	de 1770.8	ab 5616.9	28/88 fg
M0R2V2	16.05 cde	bcde 57.19	abc 3.47	cd 24.48	def 1702.7	bcde 4079.9	29/47 fg
M0R2V3	de 12.19	fgh 37.28	bc 2.78	a 34.15	efg 1541.9	abcd 4593.2	27/83 g
M1R0V1	ab 23.25	abc 69.56	abc 2.91	cd 25.86	abc 2307.8	ab 5365.5	34/52 abc
M1R0V2	14.96 cde	55.56 bcde	abc 3.45	cd 24.78	de 1717.3	bcde 4233.6	32/17 bcdef
M1R0V3	10.09e	26.86 h	2.73 c	31.34 ab	1171.7 g	2937.9 de	30.99 defg
M1R1V1	26.29a	72.86 ab	2.91 bc	24.58 cd	2664.3 a	6332.9 a	36/06 a
M1R1V2	19.74 bc	68.26 abcd	3.35 abc	26.32 cd	2072.0 c	5245.0 ab	32/91 bcde
M1R1V3	14.51 cde	48.37 defgh	2.93 abc	33.34 a	1970.0 cde	3804.3 bcde	31/10 defg
M1R2V1	27.61 a	86.76 a	2.95 abc	24.94 cd	2569.1 ab	6195.9 a	35/30 ab
M1R2V2	19.88 bc	69.59 abc	3.27 abc	25.87 cd	2212.2 bc	5133.3 ab	33.31 abcd
M1R2V3	14.57 cde	54.14 bcdef	3.55 ab	33.37 a	2307.8 bc	3982.5 bcde	31/67 cdef

Means with the same letter in each column have no significant difference (P<0.05). M0, M1: Control (no use of mycorrhiza fungi) and fungi application (*Glomus intraradices*): R0, R1, R2: Control (no use of rhizobium), rhizobium *Rb*116 application and rhizobium *Rb*133 application; V1, V2, V3: Goli, Sayad and Derakhshan.

Number of Seeds per Plant: Regarding simple effects of mycorrhiza, rhizobium and varieties, significant differences (P< 0.01, P< 0.05 and P< 0.01, respectively) was observed for number of seeds per plant. Among the interactions, only a significant difference (P<0.05) was observed between mycorrhiza and varieties. According to Table 3, the highest number of seeds per plant for each simple effect were produced in M1 (61.31), R2 (58.33) and following by R1 (54.66) and finally V1 (61.81) following by V2 (59.87). No significant difference was observed regarding the interaction between various levels of mycorrhiza and rhizobium treatments but according to Table 4, the highest number of seeds per plant was produced in treatment M1R3 (70.16). According to Table 5, the highest and lowest mean number of seeds per plant for the interaction between mycorrhiza and varieties were produced in treatments M1V2 (76.39) and M0V3 (33.89) respectively. Considering the interaction between various treatments of rhizobium and varieties, treatment R2V1 with a mean of 67.57 produced the highest number of seeds per plant (Table 6). Among all treatments, the highest and lowest number of seeds per plant were produced in treatments M1R2V1 (86.76) and R0V3 (28.64) respectively (Tables 7 and 6). Goli variety with an indeterminate growth habit has more potential for pod production than other varieties. Thus in conditions that both mycorrhiza and rhizobium exist in the soil, plants produce the highest number of pods. Derakhshan with determinate growth habit, have fewer pod numbers per plant than Goli and Sayad varieties. Ortas [6] reported that legumes P and N uptake and nodulation increases when inoculated with mycorrhiza. Low mycorrhiza efficiency leads to lower number of nodulation will result a deficiency of P and N nutrition in soybean. Thus it is recommended to use the natural sources such as rhizobium and mycorrhiza fungi inoculation for growth plant and consequently reducing N and P fertilizer application [21].

Number of Seeds per Pod: Analysis of variance of number of seeds per pod (Table 2) revealed only a significant difference ($P<0.01$) among varieties. As shown in Table 3, Sayad variety (V2) had the highest number of seeds per pod (3.42). Fungi and bacterial treatments separately and in combination did not affect number of seeds per pod. The highest number of seeds per pod in this study was obtained in treatment M0R0V2 (Table 7) with a mean 0f 3.71 seeds per pod which indicate that Sayad variety (V2) had the highest genetic potential of producing seeds in pods. The results indicated that as number of seeds per pod is a genetic trait, it is not affected by environmental effects.

Sharifi and Haghnia, [22] reported that biological fertilizer Nitroxin affected wheat yield and yield components but the mean number of grains per spike was not affected by treatment. Radwan and Awad also reported that *Azotobacter* and *Azospirillum* do not affect number of seeds in pod in Peanuts.

100 Seed weight: As shown in Table 2, only a significant difference ($P<0.01$) was observed among varieties for 100 seed weight. Mean comparison for 100 seed weight (Table 3) showed that the highest and lowest 100 seed weight belongs to V3 (Derakhshan) and V1 (Goli) varieties (33.58 and 23.99 g respectively). As mentioned in Table 2, mycorrhiza, rhizobium, combination of both fungi and bacteria and the interaction of all three factors did not show any significant difference among treatments.

Although the highest and lowest 100 seed weight were obtained in treatments M0R1V3 (35.33 g) and M0R1V1 (22.12 g) respectively (Table 7). This result comparing to the simple effect of varieties confirms that varieties have different genetical potential in producing seeds. Westermann and Crother [23] reported that beans erect varieties have higher 100 seed weight compared to prostrate varieties. Sharif *et al.* [24] reported that *Azospirillum* increasesed 100 seed weight in rice. Adholeya and Prakash [25] also reported the same results. Their study on effects of compost and bio-fertilizer showed that bio-fertilizer increased 100 seed weight in beans.

Seed Yield: According to Table 2, a significant difference ($P<0.01$) was observed for seed yield by considering only simple effects of mycorrhiza fungi, rhizobium and varieties treatments. As shown in Table 3 treatment M1 produced higher seed yield (2101.90 kg ha^{-1}). For bacteria treatments R2 (2004.89 kg ha^{-1}) followed by R1 (1984.82 kg ha^{-1}) produced higher seed yield compared to control while among varieties, V1 (2157.53 kg ha^{-1}) had the highest seed yield.

Regarding interactions between factors, only interaction between mycorrhiza and varieties showed a significant difference ($P<0.05$). According to Table 5 the highest and lowest seed yield were produced in treatments M1V2 (2513.7 kg ha^{-1}) and M0V3 (1450.9 kg ha^{-1}) respectively. Other interactions did not show significant deference among treatments but the highest seed yield productions in each category were produced in M1R3 (2338.0 kg ha^{-1}), R1V1 (2283.8 kg ha^{-1}) and M1R1V1 (2664.3 kg ha^{-1}) as mentioned in Tables 4, 6 and 7, respectively.

Our results are similar to Sadeghipour *et al.* [19]. Isik *et al.* [26] reported that varieties with unlimited growth have the ability to produce higher seed yield compared to varieties with limited growth habit. Neveen *et al.* [27] reported dual inoculation increase plant productivity. Ali *et al.* [28] reported inoculated *Pisum sativum* L. had significantly higher nodule number, nodule weight, root weight, shoot weight, seed yield and foliage yield compared to non-inoculated plants.

Goli varieties (V1) due to unlimited growth and more pods per plant considered to have higher performance than Sayad and Derakhshan although having lower 100 seed weight.

Biological Yield: Due to analysis of variance (Table 2), considering simple effects, a significant difference ($P<0.01$) was observed among varieties and rhizobium treatments. As shown in Table 3, Goli variety (V1) had the highest biological yield (5642.50 kg ha^{-1}). Regarding simple effect of rhizobium on biological yield, treatments R2 (4933.6 kg ha^{-1}) followed by R1 (4917.0 kg ha^{-1}) produced the higher biological yield. Although there was no significant difference between mycorrhiza treatments but M1 had higher biological yield (4803.4 kg ha^{-1}).

Although according to Table 2 no significant difference were observed for interactions between the factors but the highest biological yield for each interaction were M1R0 with 5127.4 kg ha^{-1}, M1V2 with 5964.8 kg ha^{-1}, R1V1 with 6326.4 kg ha^{-1} and M1R1V1 with 6332.9 kg ha^{-1} (Tables 4, 5, 6 and 7 respectively). Kathleen *et al.* [29] reported biological yield (dry weight) increment of many plants inoculated with mycorrhizal fungi. Geneva *et al.* [30] reported co-inoculation of pea plants significantly increased plant biomass, photosynthetic rate, nodulation and nitrogen fixation activity in comparison to single inoculation with *Rhizobium leguminosarum*. Ardakani *et al.* [31] reported alfalfa co-inoculation with mycorrhizal fungi and r*hizobium* increased shoot dry weight as well as BNF at the first and second harvest. Stancheva *et al.* [32] also reported that co-inoculation significantly increased total P content in pea plant tissues and seed yield.

Root Colonization: Regarding simple effects, a significant difference ($P< 0.01$) was observed among mycorrhiza and varieties treatments for root colonization percentage. Among the interactions, only a significant difference ($P<0.05$) was observed between mycorrhiza and varieties. According to Table 3, the highest root colonization percentage among mycorrhiza treatments was produced in M1 (33.11%) treatment. Among varieties, V1 (32.23) followed by V2 (31.11) had higher root colonization percentage (Table 3). No significant difference was observed regarding the interaction between various levels of mycorrhiza and rhizobium treatments but according to Table 4, the highest root colonization percentage was produced in treatment M1R3 (33.42%). According to Table 5 the highest root colonization percentage for the interaction between mycorrhiza and varieties were produced in treatments M1V1 (35.29%). Amirabadi *et al.* [33] studied the effects of *Azotobacter*, different amounts of chemical phosphorus and mycorrhizal fungi on corn and reported that the combination treatment of mycorrhizal fungi and *Azotobacter* had the highest percentage of root colonization. Stancheva *et al.* [32] reported co-inoculation significantly increased total percentage of root colonization in pea.

It seems that regarding mycorrhiza symbiosis ability with host roots, root colonization increase by increment of plant root amount. Also nitrogen fixation by rhizobium creates an environment without food shortage which results in increment of root establishment which leads to a higher level of symbiosis between fungi and plant roots.

CONCLUSION

Mycorrhizal fungi and rhizobium treatments increased grain yield compared to controls. Considering Goli variety, the yield difference between the two highest yielded treatments M1R1V1 (combination of mycorrhizal fungi, rhizobium strain 116 and Goli variety) and M1R2V1 (combination of mycorrhizal fungi, rhizobium strain 133 and Goli variety; treatments with highest yield) with control treatment (M0R0V1) is 934.4 and 839.2 kg/ha^{-1} respectively and with the lowest treatment (M1R0V3) is 1416.4 and 1321.2 kg/ha^{-1} respectively. Considering Sayad variety, the yield difference between the two highest yielded treatments M1R2V2 (combination of mycorrhizal fungi, rhizobium strain 133 and Sayad variety) and M1R1V2 (combination of mycorrhizal fungi, rhizobium strain 116 and Sayad variety) with control treatment (M0R0V2) is 906.1 and 765.9 kg/ha^{-1} respectively and with the lowest treatment (M1R0V3) is 1040.5 and 900.3 kg/ha^{-1},respectively. Considering Derakhshan variety, the yield difference between the two highest yielded treatments M1R2V3 (combination of mycorrhizal fungi, Rhizobium strain 133 and Derakhshan variety) and M1R1V3 (combination of mycorrhizal fungi, rhizobium strain 116 and Derakhshan variety; treatments with highest yield) with control treatment (M0R0V3) is 1059.9 and 722.1 kg/ha^{-1} respectively and with the lowest treatment (M1R0V3) is 1136.1 and 798.3 kg/ha^{-1} respectively. Thus regarding our results in this study, the use of biological fertilizers for sustainable agricultural purposes and healthy food production is recommended.

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