

**INFLUENCE OF MINERAL FERTILIZATION DEDUCTION WITH  
RHIZOBIUM AND MYCORRHIZA DUAL INOCULATION ON TWO  
BEAN (*PHASEOLUS VULGARIS* L.) CULTIVARS UNDER FIELD  
CONDITIONS**

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**ABSTRACT**

Two field experiments were conducted 2015 and 2016 to study the influence of rhizobium and/or mycorrhiza inoculation under 50% of recommended mineral nitrogen and phosphorus doses compared with 100% recommended doses of nitrogen and phosphorus (control) on growth, pods yield and its components and leaf chemical constituents of snap bean cvs. Paulista and red kidney bean plants. The obtained results, obviously, study revealed that the red kidney bean showed better performance significantly than Paulita for all the studied characters, in both seasons. Fertilization treatment of 100%<sub>NP</sub> and/or 50%<sub>NP</sub> + dual inoculation with rhizobium and mycorrhiza gave the most favorable effect comparing with other fertilization treatments for all studied characters, in both seasons; without significant difference between them; except for 100%<sub>NP</sub> which gave significant higher shoot fresh weight compared with 50%<sub>NP</sub>+RM, in both seasons. The interaction effect between bean cultivars and fertilization treatments showed significant superiority of 100%<sub>NP</sub> or 50%<sub>NP</sub>+ dual inoculation of rhizobium and mycorrhiza with red kidney bean for most cases without significant difference between them, in both seasons. Results of this investigation referred to the ability of reducing nitrogenous and phosphorus mineral fertilization with 50% by using the dual inoculation of mycorrhiza and Rhizobium for achieving the same growth and yield with lowering the consequential environmental hazardous from intensive mineral fertilization use.

**Keywords:** Rhizobium – Okadeen – mycorrhiza – dual inoculation – snap bean – red kidney bean

**INTRODUCTION**

The environmental impact of nitrogen and phosphorus mineral fertilization becomes one of the most important issues in intensive crops production. The excessive use of such two types of fertilizers is causing serious environmental problems (Breeuwsma and Silva, 1992; Ju *et al.*, 2009). Because of the adverse effects of intensive use of mineral fertilization, there is main stream toward using of biofertilizers which considered as renewable source of nutrients of grown plants instead of using mineral fertilization. In addition, biofertilization is low-coast source of nutrients especially for small scale farms (Carvajal-Muñoz and Carmona-Garcia, 2012; Bhattacharjee and Dey, 2014).

Biofertilization is depending on using of plant growth promoting microorganisms (PGPM) which have many beneficial effects on host plants such as enhancing plant growth, increasing the yield and its quality, in addition to increasing the ability of the grown plants against biotic and abiotic stresses (Avis *et al.*, 2008; Nihorimbere *et al.*, 2011; Bhardwaj *et al.*, 2014).

One of the important approaches in sustainable agriculture is the co-inoculation that defined as using of two or more of beneficial microorganisms as biofertilizers. Many research peppers discussed the role of dual inoculation of rhizobium and mycorrhiza as biofertilizers for leguminous crops. Generally, they found that the dual inoculation of rhyzobium and mycorrhiza has many beneficial effects on the leguminous crops such as increasing of the bean plants ability to establish more successful legume-rhizobium symbiosis as well as increasing the mycorrhiza colonization (Badr El-Din and Moawad, 1988; Abd-Alla *et al.*, 2014), decreasing the used quantities of P and/or N chemical fertilizers up to 50% (Ahmed *et al.*, 2006; Talaat and Abdallah, 2008; Dar *et al.*, 2015; Aboutalebian and Malmir, 2017); or may be save more N fertilization under other circumstances (Soares *et al.*, 2016), enhancing the host plants performance in both of vegetative growth or yield and its components traits (Daneils-Hylton and Ahmad, 1994; Geneva *et al.*, 2006; Talaat and Abdallah, 2008; Arumugam *et al.*, 2010; Bhattachaerjee and Sharma, 2012 and Moradi *et al.*, 2013) and also using for controlling the soil porn diseases (Dar *et al.*, 1997 and Aysan and Demir, 2009).

The aim of this investigation is to study the effect of inoculation with rhizobium (R), mycorrhiza (M) and their co-inoculation (RM) in combination with 50% of the recommended doses of N and P mineral fertilization (50%<sub>NP</sub>) on the vegetative growth and yield and its components, and leaves chemical composition of snap bean cvs. Paulista and red kidney plants under field conditions.

## **MATERIALS AND METHODS**

This investigation was conducted in the two successive summer seasons of 2015 and 2016 in Etai El-Barod Research Station, Agriculture Research Center, Egypt during

Two snap bean cultivars were used, Paulista as well as red kidney bean that used in Egypt for local consumption of its green pods. The inoculations were *Rhizobium leguminosarum* biovar. *Phaseoli* (R) that derived from Biofertilizers Unit-The Soil, Water and Environment Research Institute under the commercial name of "Okadeen"; and the mycorrhiza (M) as *Glomus* spp. that was in the form of suspension of spores and root pieces was derived from the of Biofertilization Unit, faculty of Agriculture, Saba Basha, Alex. Univ.

Randomized complete Blocks Design was achieved split plot system; where the snap bean cultivars were randomly allocated in main plots at 22\2

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and 27\2 in the years of 2015 and 2016, respectively. The sub-plots were occupied by fertilization treatments as follows:

- 1- Recommended doses of N and P (100%<sub>NP</sub>) to serve as control.
- 2- Half recommended doses of N and P (50%<sub>NP</sub>).
- 3- Half recommended doses of N and P+inoculation with rhizobium (50%<sub>NP+R</sub>).
- 4- Half recommended doses of N and P+inoculation with Mycorrhiza (50%<sub>NP+ M</sub>).
- 5- Half recommended doses of N and P+dual inoculation with rhizobium and mycorrhiza (50%<sub>NP+RM</sub>).

The recommended doses of nitrogen and phosphorus were 60 kg N fad.<sup>-1</sup> and 30 kg P<sub>2</sub>O<sub>5</sub> fad.<sup>-1</sup>; which used in the forms of ammonium sulfate (20.6%) and mono super phosphate (15%), respectively.

Potassium fertilization was added according to the recommended (48 kg K<sub>2</sub>O fad.<sup>-1</sup>) in the form of potassium sulfate (48%). All other agricultural practices were done as followed in the commercial production of bean crop. Soil samples of the experimental site of the both seasons were collected and physically and chemically analyzed according to the published procedures of Black *et al.* (1965).

**Table 1. The physical and chemical properties of the experimental site in seasons of 2015 and 2016**

Parameter		1 <sup>st</sup> season	2 <sup>nd</sup> season
Physical analysis			
	Clay (%)	60.41	59.61
	Silt (%)	32.50	31.80
	Sand (%)	7.09	8.59
	Texture class	Clay	Clay
Chemical analysis			
	EC (dS/m)	1.93	1.61
	pH	7.70	7.75
	CaCO <sub>3</sub>	3.15	2.45
	O.M%	0.68	0.54
	HCO <sub>3</sub>	0.85	0.70
	Total N (ppm)	41.78	41.09
	Total P (ppm)	2.66	2.34
	Total K (ppm)	74.11	68.34

Rhizobium "Okadeen" inoculum was mixed well with colored sugar solution and added to seeds of the tested bean cultivars which spreading on a clean plastic sheet under shading before sowing. Whereas, the mycorrhiza inoculation was carried out by mixing the maycorrhizal suspension with the

soil, immediately, before the sowing the inoculated bean seeds with rhizobium.

The measured characters were vegetative growth characters (plant height, cm; branches number plant<sup>-1</sup>; leaf area plant<sup>-1</sup>, cm<sup>2</sup>; leaves number plant<sup>-1</sup>; shoot fresh and dry weights plant<sup>-1</sup>, g) and yield and its components (pods number plant<sup>-1</sup>; average pod weight, g; pods yield plant<sup>-1</sup>, ton fad.<sup>-1</sup>). For leaves chemical composition; nitrogen (%) was determined using microkjeldahal method (Chapman and Pratt, 1961), Phosphorus and potassium (%) were estimated using flam photometer according to Temminghoff and Houba (2004). The photosynthetic pigments (mg/g f.w.) have been measured spectrophotometrically as described by Gunes *et al.* (2007) using acetone 90% at wave lengths of 663,644 and 470 nm.

Data were statistically analyzed using CoStat program (Version 6.4, CoHort, USA, 1998–2008). Least Significant Difference test (LSD) was used at 0.05 probability level to verify the significance between treatments.

## **RESULTS AND DISCUSSION**

The results shown in Table (2) clearly showed that the two tested cultivars were significantly differing for all vegetative growth characters. The red kidney cultivar exhibits higher mean values than Paulista, in both seasons. Regarding the effect of fertilization treatments, the results revealed significant effect on all the vegetative characters, in both years. Fertilization treatment 100%<sub>NP</sub> or 50%<sub>NP+RM</sub> was found to give the highest mean values of plant height, leaves area plant<sup>-1</sup> and shoot dry weight plant<sup>-1</sup> compared to other fertilization treatments without significant differences between them. In the contrary, the fertilization treatment 100%<sub>NP</sub> gave significant heavier shoot fresh weight plant<sup>-1</sup> comparing to the other treatments, in both seasons. Such finding refers to that the dual inoculation+50%<sub>NP</sub> may result in producing more dry matter than 100%<sub>NP</sub>.

The interaction between bean cultivars and fertilization treatments was found to be significant for all characters listed in Table (2). The best interaction was found between red kidney cultivar and each of 100%<sub>NP</sub> or 50%<sub>NP+RM</sub>, in both seasons.

There is equality in effect of 100% of recommended N and P mineral fertilization with dual inoculation with adding 50% of the recommended doses of N and P on the most studied vegetative growth characters of bean cultivars. Such effect suggested synergistic effect between mycorrhiza and rhizobium that increase the ability to supply bean plants with more nutrients as provided by 100%<sub>NP</sub> as well as enhancing plant growth which interpreted in higher mean values of vegetative growth traits if compared with 50%<sub>NP+R</sub> or 50%<sub>NP+M</sub>.

Many previous researches, also, emphasized on the synergistic effect between rhizobium and mycorrhiza and their positive effect on vegetative

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growth (Ibijbijen *et al.*, 1996 on bean; Geneva *et al.*, 2006 on pea; Abd-Alla *et al.*, 2014 on faba bean). The growth increment of the host plants, also, could be due to that mycorrhiza produce at least two gibberellin-like substances and four substances with the properties of cytokinins (Barea and Azcón-aguilar, 1982) in addition to that *Rhizobium phaseoli* was found to produce GAs and IAA (Atzorn *et al.*, 1988) and increased nutrients uptake, which, consequently, enhancing the overall performance of bean plants

Aryal *et al.* working on bean plants (2003) reported that, nitrogen fixation under the inoculation of rhizobium bacteria needs high levels of phosphorus in the host plant tissues that provided by inoculation with mycorrhiza. Also, they found that mycorrhiza was present more colonization if inoculated with rhizobium, compared to singly inoculation with each one. Moreover, the enhancement of the whole performance of the dual inoculated plants could be attributed to mycorrhiza facilitating the acquisition and mobilization of certain elements such as P, Fe, K and other minerals that involve in synthesis of nitrogenase enzyme and leghemoglobin (Abd-Alla *et al.*, on faba bean, 2014; Püschel *et al.* on *Medicago* spp., 2017)

Talaat and Abdallah (2008) revealed that dual inoculation of rhizobium and mycorrhiza with 50% of N and P mineral fertilization was more effective in enhancing all the studied growth characters of faba bean plants such as plant height, leaf area, shoot dry weight when compared with 100% of mineral N and P. On cowpea, Arumugam *et al.* (2010) reported that the dual inoculation with rhizobium and mycorrhiza was found to be efficient more than individual inoculation for growth criteria. Wang *et al.* (2011) found that the co-inoculation with rhizobium and mycorrhiza of soybean plants was more effective under low levels of N and P. They also stated that co-inoculation enhancing each of rhizobium nodulation and mycorrhiza colonization. Bhattarai *et al.* (2011) mentioned that the Rhizobium and mycorrhiza were found to be effective and well symbiotic to the Red Kidney bean which reflects on growth parameters. Also, Gardezi *et al.* (2012) reported that the mycorrhizal inoculation and nitrogen fixation provided higher mean values of plant height, leaf area and aerial dry weight of common bean plants. On garden pea, Bai *et al.* (2016) reported that the dual inoculation with rhizobium and mycorrhiza were resulted in significant increases of the studied vegetative growth traits and contributed to decreasing N and P mineral fertilization up to 25%.

### **Yield and its components**

The mean values in Table (3) showed the main effects of bean cultivars and fertilization treatments and their interaction on yield and its components. The two tested bean cultivars exhibit significantly differ performance towards pods number plant<sup>-1</sup>, average pod weight and total pods yield. The red kidney bean cultivar gave higher mean values of such characters, in both seasons.

Fertilization treatments; 100%<sub>NP</sub> and 50%<sub>NP+RM</sub> resulted in, significantly, higher values of pods number plant<sup>-1</sup>, average pod weight and total pods yield fad<sup>-1</sup> than other treatments. The difference between applied 100%<sub>NP</sub> and 50%<sub>NP+RM</sub> on pods yield and its components was not significant.

The effect of interaction between bean cultivars and fertilization treatments seemed to be significant for yield and its components characters, in both seasons. The fertilization treatments of 100%<sub>NP</sub> or 50%<sub>NP+RM</sub> could be considered as a good treatment for achieving the best yield and its components traits for bean plants.

When N and P mineral fertilization was deducted to 50%, the dual inoculation was found to be more effective in enhancing yield and its components characters compared to solo inoculation treatment. It could be a result of enhancing the vegetative growth of the host plants which positively reflected on the yield and its components characters.

The results of Aryal *et al.* (2003) were in harmony with the results of this investigation. They stated that dual inoculation of bean plants significantly increased bean pod yield compared to singly inoculated plants with rhizobium or mycorrhiza. Likewise, Talaat and Abdallah (2008) stated the positive significant effect of dual inoculation with rhizobium and mycorrhiza in addition to 50% of the recommended doses of N and P on yield and its components characters of faba bean when compared to fertilization with 100% of recommended doses of mineral N and P. This positive effect may be attributed to the increase of nutrients uptake, phytohormones and modify the microbial balance.

**Table 2. Effect of bean cultivars and fertilization treatments and their interaction on vegetative growth characters during seasons of 2015 and 2016.**

Fertilization treatments (B)	Cultivars (A)					
	2015		Mean	2016		Mean
	Paulista	Red kidney bean		Paulista	Red kidney bean	
<b>Plant height (cm)</b>						
100% <sub>NP</sub>	30.70	36.97	<b>33.83</b>	32.60	35.07	<b>33.83</b>
50% <sub>NP</sub>	25.63	26.77	<b>26.2</b>	27.47	28.60	<b>28.03</b>
50% <sub>NP</sub> +R	27.6	31.03	<b>29.32</b>	29.63	31.70	<b>30.67</b>
50% <sub>NP</sub> +M	29.23	34.2	<b>31.72</b>	31.50	33.30	<b>32.40</b>
50% <sub>NP</sub> +RM	31.03	38.93	<b>34.98</b>	32.57	34.97	<b>33.77</b>
<b>Mean</b>	<b>28.84</b>	<b>33.58</b>		<b>30.75</b>	<b>32.73</b>	
L.S.D.	A=2.76	B=2.29	AB=3.24	A= 1.45	B= 1.35	AB= 1.91
<b>Leaf area plant<sup>-1</sup> (cm<sup>2</sup>)</b>						
100% <sub>NP</sub>	425.24	433.33	<b>429.29</b>	433.83	464.81	<b>449.32</b>
50% <sub>NP</sub>	374.99	406.88	<b>390.94</b>	356.46	363.59	<b>360.02</b>
50% <sub>NP</sub> +R	396.05	416.44	<b>406.25</b>	383.37	418.09	<b>400.73</b>
50% <sub>NP</sub> +M	410.56	422.36	<b>416.46</b>	416.93	441.80	<b>429.36</b>
50% <sub>NP</sub> +RM	424.90	433.31	<b>429.11</b>	431.52	462.76	<b>447.14</b>
<b>Mean</b>	<b>406.35</b>	<b>422.47</b>		<b>404.42</b>	<b>430.21</b>	
L.S.D.	A= 5.51	B=9.54	AB=13.49	A= 11.88	B=20.32	AB=28.73
<b>Shoot fresh weight<sup>-1</sup> (g)</b>						
100% <sub>NP</sub>	80.86	86.33	<b>83.60</b>	75.61	83.70	<b>79.65</b>
50% <sub>NP</sub>	57.73	63.87	<b>60.80</b>	54.84	63.50	<b>59.17</b>
50% <sub>NP</sub> +R	66.71	71.60	<b>69.15</b>	59.69	69.10	<b>64.39</b>
50% <sub>NP</sub> +M	72.72	77.10	<b>74.91</b>	66.85	73.31	<b>70.08</b>
50% <sub>NP</sub> +RM	78.12	82.34	<b>80.23</b>	70.54	78.52	<b>74.53</b>
<b>Mean</b>	<b>71.23</b>	<b>76.25</b>		<b>65.51</b>	<b>73.63</b>	
L.S.D.	A=1.62	B= 3.15	AB= 4.46	A= 1.90	B= 4.22	AB= 5.97
<b>Shoot dry weight<sup>-1</sup> (g)</b>						
100% <sub>NP</sub>	14.93	18.12	<b>16.52</b>	16.56	17.19	<b>16.87</b>
50% <sub>NP</sub>	12.85	15.19	<b>13.89</b>	12.62	15.43	<b>14.02</b>
50% <sub>NP</sub> +R	14.11	15.77	<b>14.94</b>	14.48	16.17	<b>15.33</b>
50% <sub>NP</sub> +M	14.62	17.03	<b>15.83</b>	15.43	17.10	<b>16.27</b>
50% <sub>NP</sub> +RM	15.37	18.62	<b>17.00</b>	16.19	17.15	<b>16.67</b>
<b>Mean</b>	<b>14.32</b>	<b>16.95</b>		<b>15.06</b>	<b>16.61</b>	
L.S.D.	A= 0.68	B= 0.64	AB=0.90	A=1.07	B= 0.54	AB= 0.77

Daniels-Hylton and Ahmed (1994) and Safapour *et al.* (2011) stated that the using of dual inoculation with red kidney bean can be achieved better yield compared to single inoculation with rhizobium or mycorrhiza. Also, Bhattarai *et al.* (2011) reported that the rhizobium-mycorrhiza inoculation is the most favorable treatment for enhancing the red kidney bean yield and its components. Similar findings were reported by Aboutalebian and Malmir (2017) on soybean where found to give the maximum mean values of yield and yield components when inoculated with rhizobium and mycorrhiza if the chemical nitrogen fertilization was reduced from 60 to 30 kg N ha<sup>-1</sup>.

**Table 3. Effect of bean cultivars and fertilization treatments and their interaction on yield and its components characters during seasons of 2015 and 2016.**

Fertilization treatments (B)	Cultivars (A)					
	2015		Mean	2016		Mean
	Paulista	Red kidney bean		Paulista	Red kidney bean	
	<b>Pods No. plant<sup>-1</sup></b>					
100% <sub>NP</sub>	21.90	29.39	<b>25.65</b>	25.76	26.73	<b>26.25</b>
50% <sub>NP</sub>	14.16	18.82	<b>16.49</b>	17.26	19.43	<b>18.35</b>
50% <sub>NP</sub> +R	17.38	22.16	<b>19.77</b>	19.89	22.71	<b>21.30</b>
50% <sub>NP</sub> +M	19.16	25.04	<b>22.10</b>	22.78	23.93	<b>23.35</b>
50% <sub>NP</sub> +RM	21.38	29.16	<b>25.27</b>	25.56	27.05	<b>26.31</b>
<b>Mean</b>	<b>18.79</b>	<b>24.91</b>		<b>22.25</b>	<b>23.97</b>	
L.S.D.	A= 1.97	B= 1.48	AB= 2.10	A=0.48	B= 1.53	AB= 2.16
	<b>Average pod weight (g)</b>					
100% <sub>NP</sub>	3.58	3.98	<b>3.78</b>	3.69	4.45	<b>4.07</b>
50% <sub>NP</sub>	2.72	2.80	<b>2.76</b>	2.65	3.03	<b>2.84</b>
50% <sub>NP</sub> +R	3.19	3.39	<b>3.29</b>	3.24	3.53	<b>3.39</b>
50% <sub>NP</sub> +M	3.39	3.64	<b>3.51</b>	3.55	4.06	<b>3.80</b>
50% <sub>NP</sub> +RM	3.52	3.93	<b>3.72</b>	3.73	4.41	<b>4.07</b>
<b>Mean</b>	<b>3.28</b>	<b>3.55</b>		<b>3.37</b>	<b>3.90</b>	
L.S.D.	A= 0.03	B= 0.18	AB= 0.26	A= 0.04	B= 0.18	AB= 0.25
	<b>Total pods yield (Ton fad.<sup>-1</sup>)</b>					
100% <sub>NP</sub>	2.87	3.77	<b>3.32</b>	2.59	3.30	
50% <sub>NP</sub>	1.45	1.99	<b>1.72</b>	1.79	2.34	<b>2.07</b>
50% <sub>NP</sub> +R	2.05	2.82	<b>2.43</b>	2.25	2.62	<b>2.43</b>
50% <sub>NP</sub> +M	2.36	3.22	<b>2.79</b>	2.57	2.91	<b>2.74</b>
50% <sub>NP</sub> +RM	2.79	3.75	<b>3.27</b>	2.65	3.33	<b>2.89</b>
<b>Mean</b>	<b>2.16</b>	<b>2.95</b>		<b>2.37</b>	<b>2.90</b>	
L.S.D.	A= 0.51	B= 0.19	AB= 0.27	A= 0.15	B= 0.18	AB= 0.25

**Leaves chemical composition**

Tables (4) and (5) showed the main effects of bean cultivars, fertilization treatments and their interactions on leaves content of N, P, K and photosynthetic pigments during the seasons of 2015 and 2016.

Data in Table (4) showed that, generally, red kidney cultivar had, significantly, higher mean values of N, P and K contents than Paulista cultivar, in both seasons with few exceptions. Leaves P and K contents of Paulista and red kidney cultivars were too small to be significant in 2015 and 2016, respectively.

The fertilization treatments, in both seasons of study, revealed significant effect on leaves N, P and K contents of bean plants (Table, 4). Positive significant effect of N and K contents were found in plants which received 100%<sub>NP</sub> or 50%<sub>NP</sub>+RM fertilization compared to other treatments, in both years. Maximum significant effect of leaves P was found in plants supplied with 50%<sub>NP</sub>+RM in comparison with other fertilization treatments, in both years.



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As shown in Table (4), the interaction effect between two studied factors was found to be significant for N, P and K leaf contents, in both seasons of study. Application of 100%<sub>NP</sub> or 50%<sub>NP+RM</sub> fertilization treatment in combination with red kidney bean gave the highest mean values for the leaves N, P and K contents, in both seasons.

**Table 4. Effect of bean cultivars and fertilization treatments and their interaction on leaves chemical composition during seasons of 2015 and 2016.**

Fertilization treatments (B)	Cultivars (A)					
	2015		Mean	2015		Mean
	Paulista	Red kidney bean		Paulista	Red kidney bean	
<b>Nitrogen (%)</b>						
100% <sub>NP</sub>	3.54	3.73	<b>3.63</b>	3.21	3.35	<b>3.28</b>
50% <sub>NP</sub>	2.46	2.66	<b>2.56</b>	2.56	2.62	<b>2.59</b>
50% <sub>NP+R</sub>	3.05	3.47	<b>3.26</b>	2.88	3.03	<b>2.96</b>
50% <sub>NP+M</sub>	3.29	3.39	<b>3.34</b>	3.04	3.09	<b>3.06</b>
50% <sub>NP+RM</sub>	3.51	3.69	<b>3.60</b>	3.18	3.47	<b>3.33</b>
<b>Mean</b>	<b>3.17</b>	<b>3.39</b>		<b>2.97</b>	<b>3.11</b>	
L.S.D.	A= 0.20	B=0.14	AB=0.20	A=0.11	B= 0.10	AB= 0.14
<b>Phosphorus (%)</b>						
100% <sub>NP</sub>	0.33	0.38	<b>0.35</b>	0.33	0.35	<b>0.34</b>
50% <sub>NP</sub>	0.20	0.22	<b>0.21</b>	0.22	0.24	<b>0.23</b>
50% <sub>NP+R</sub>	0.24	0.26	<b>0.25</b>	0.24	0.27	<b>0.26</b>
50% <sub>NP+M</sub>	0.32	0.35	<b>0.34</b>	0.29	0.34	<b>0.32</b>
50% <sub>NP+RM</sub>	0.36	0.39	<b>0.37</b>	0.35	0.37	<b>0.36</b>
<b>Mean</b>	<b>0.29</b>	<b>0.31</b>		<b>0.29</b>	<b>0.31</b>	
L.S.D.	A= 0.02	B=0.02	AB=0.03	A= 0.03	B= 0.02	AB= 0.04
<b>Potassium (%)</b>						
100% <sub>NP</sub>	2.45	2.68	<b>2.56</b>	2.63	2.65	<b>2.64</b>
50% <sub>NP</sub>	1.92	1.93	<b>1.93</b>	1.75	2.06	<b>1.90</b>
50% <sub>NP+R</sub>	2.38	2.41	<b>2.40</b>	2.31	2.33	<b>2.32</b>
50% <sub>NP+M</sub>	2.40	2.44	<b>2.42</b>	2.42	2.46	<b>2.44</b>
50% <sub>NP+RM</sub>	2.47	2.65	<b>2.56</b>	2.54	2.78	<b>2.66</b>
<b>Mean</b>	<b>2.32</b>	<b>2.42</b>		<b>2.33</b>	<b>2.46</b>	
L.S.D.	A=0.25	B=0.10	AB=0.14	A= 0.10	B= 0.20	AB= 0.28

Table (5) shows the mean values of photosynthetic pigments as affected by the two studied main factors (bean cultivars and fertilization treatments) and their interactions. The main effect of bean cultivars showed significant effect of chlorophyll a, chlorophyll b, total chlorophyll and carotene with superiority of red kidney bean compared with Paulista, in both seasons.

In most cases, the data in Table (5) revealed that 100%<sub>NP</sub> and 50%<sub>NP+RM</sub> fertilization treatments were significantly differed from other fertilization treatments which they gave the highest mean values of the photosynthetic pigments, in both seasons. Whereas, the treatment of 50%<sub>NP</sub> was give the significantly lowest mean values of such traits, in both seasons.

The results of photosynthetic pigments traits that listed in Table (5) were, also, exhibit a significant interaction between bean cultivars and fertilization treatments, in both seasons.

As mentioned before, the co-inoculation between rhizobium and mycorrhiza was found to have synergistic relationship which provided more mycorrhizal colonization or more nitrogen fixation activity compared to singly inoculation (Kucey and Bonetti, 1988; Wang *et al.*, 2011; Bulgarelli *et al.*, 2017).

This synergistic relation was found to affect, positively, the chemical composition of bean plants. Firstly, rhizobium provides the host plants with significant amounts of N and some phytohormones-like substances (Atzorn *et al.*, 1988). Secondly, it has been well stated that the mycorrhiza fungal mycelium provides extra extinction of the host plant root system which secure more P to the host plants in addition to considerable amounts of N, K and other nutrients and its capability to produce some phytohormones-like substances (Barea and Azcón-aguilar, 1982; Clark and Zeto, 2000; Govindarajulu *et al.*, 2005; Abd-Alla *et al.*, 2014; Püschel *et al.*, 2017).

Thus, the synergistic effect between rhizobium and mycorrhiza resulted in enhancing the concentrations of N, P and K as well as the photosynthetic pigments, which reflects on increasing the growth and yield, of the host plants as emphasized by many researchers (Hazarika *et al.* on green gram, 2000; Aryal *et al.* on bean, 2003; Rajasekaran *et al.* on groundnut, 2006; Arumugam *et al.* on cowpea, 2010 and Tajini *et al.* on common bean, 2012 and Bhattacharjee and Sharma on pigeon pea, 2012 and 2015). The efficiency of the tripartite symbiosis among bean, rhizobium and mycorrhiza was found to be more pronounced under low phosphorus conditions (Mortimer *et al.*, 2008).

It could be concluded that the using of the dual inoculation of mycorrhiza and rhizobium as "Okadeen" for bean plants was found to be the best application for better bean growth, yield and chemical composition, in addition to deducting about 50% of the nitrogenous and phosphorus mineral fertilization which consequently decrease the production costs and lowering the environmental hazards that derived from intensive use of mineral fertilization.

**Table 5. Effect of bean cultivars and fertilization treatments and their interaction on leaves photosynthetic pigments during seasons of 2015 and 2016.**

Fertilization treatments (B)	Cultivars (A)					
	2015			2016		
	Paulista	Red kidney bean	Mean	Paulista	Red kidney bean	Mean
<b>Chlorophyll a (mg/g)</b>						
100% <sub>NP</sub>	1.25	1.45	<b>1.35</b>	1.35	1.43	<b>1.39</b>
50% <sub>NP</sub>	0.78	1.02	<b>0.9</b>	0.75	0.98	<b>0.86</b>
50% <sub>NP</sub> +R	1.04	1.29	<b>1.16</b>	0.94	1.24	<b>1.09</b>
50% <sub>NP</sub> +M	1.13	1.41	<b>1.27</b>	1.19	1.48	<b>1.33</b>
50% <sub>NP</sub> +RM	1.23	1.44	<b>1.34</b>	1.34	1.45	<b>1.39</b>
<b>Mean</b>	<b>1.08</b>	<b>1.32</b>		<b>1.11</b>	<b>1.31</b>	
L.S.D.	A= 0.16	B=0.07	AB=0.09	A= 0.08	B= 0.10	AB= 0.14
<b>Chlorophyll b (mg/g)</b>						
100% <sub>NP</sub>	0.96	1.26	<b>1.11</b>	1.05	1.16	<b>1.10</b>
50% <sub>NP</sub>	0.63	0.82	<b>0.73</b>	0.53	0.76	<b>0.65</b>
50% <sub>NP</sub> +R	0.86	0.99	<b>0.92</b>	0.81	1.01	<b>0.91</b>
50% <sub>NP</sub> +M	0.96	1.09	<b>1.03</b>	0.96	1.11	<b>1.04</b>
50% <sub>NP</sub> +RM	1.01	1.24	<b>1.13</b>	1.04	1.21	<b>1.12</b>
<b>Mean</b>	<b>0.88</b>	<b>1.08</b>		<b>0.88</b>	<b>1.05</b>	
L.S.D.	A= 0.12	B=0.11	AB=0.13	A= 0.15	B= 0.10	AB= 0.14
<b>Total chlorophyll (mg/g)</b>						
100% <sub>NP</sub>	2.20	2.71	<b>2.46</b>	2.40	2.59	<b>2.50</b>
50% <sub>NP</sub>	1.41	1.84	<b>1.63</b>	1.28	1.74	<b>1.51</b>
50% <sub>NP</sub> +R	1.90	2.27	<b>2.09</b>	1.75	2.25	<b>2.00</b>
50% <sub>NP</sub> +M	2.09	2.51	<b>2.30</b>	2.15	2.59	<b>2.37</b>
50% <sub>NP</sub> +RM	2.24	2.68	<b>2.46</b>	2.38	2.65	<b>2.52</b>
<b>Mean</b>	<b>1.97</b>	<b>2.40</b>		<b>1.99</b>	<b>2.37</b>	
L.S.D.	A=0.15	B=0.10	AB=0.14	A=0.10	B= 0.12	AB= 0.18
<b>Carotene (mg/g)</b>						
100% <sub>NP</sub>	0.33	0.42	<b>0.37</b>	0.29	0.32	<b>0.30</b>
50% <sub>NP</sub>	0.19	0.21	<b>0.20</b>	0.18	0.20	<b>0.19</b>
50% <sub>NP</sub> +R	0.22	0.28	<b>0.25</b>	0.21	0.24	<b>0.23</b>
50% <sub>NP</sub> +M	0.28	0.37	<b>0.33</b>	0.23	0.29	<b>0.26</b>
50% <sub>NP</sub> +RM	0.33	0.41	<b>0.37</b>	0.30	0.33	<b>0.32</b>
<b>Mean</b>	<b>0.27</b>	<b>0.34</b>		<b>0.24</b>	<b>0.27</b>	
L.S.D.	A=0.05	B=0.03	AB=0.04	A= 0.03	B= 0.03	AB= 0.04

**REFERENCES**

**Abd-Alla, M.H.; A.W.E. El-Enany; N.A. Nafady; D.M. Khalaf and F.M. Morsy. 2014.** Synergistic interaction of *Rhizobium leguminosarum* by *viciae* and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. Microbiol. Res., 169 (1): 49-58.

**Aboutalebian, M.A. and M. Malmir. 2017.** Soybean yield and yield components affected by the mycorrhiza and bradyrhizobium at different rates of starter nitrogen fertilizer. Semina: Ciências Agrárias, 38 (4): 2409-2418.

- Ahmed, Z.I.; M.S. Anjum and C.A. Rauf. 2006. Effect of rhizobium inoculation on growth and nodule formation of green gram. *Inter. J. Agric. Biolo. (Pakistan)*, 8 (2): 235 – 237.
- Arumugam, R.; S. Rajasekaran and S.M. Nagarajan. 2010. Response of Arbuscular mycorrhizal fungi and Rhizobium inoculation on growth and chlorophyll content of *Vigna unguiculata* (L) Walp Var. Pusa 151. *J. Appl. Sci. Environ. Manag.*, 14 (4): 113-115.
- Aryal, U.K.; H.L. Xu and M. Fujita. 2003. Rhizobia and AM fungal inoculation improve growth and nutrient uptake of bean plants under organic fertilization. *J. Sustain. Agric.*, 21(3): 27-39.
- Atzorn, R.; A. Crozier; C.T. Wheeler and G. Sandberg. 1988. Production of gibberellins and indole-3-acetic acid by *Rhizobium phaseoli* in relation to nodulation of *Phaseolus vulgaris* roots. *Planta*, 175(4): 532-538.
- Avis, T.J.; V. Gravel, H. Antoun and R.J. Tweddell. 2008. Multifaceted beneficial effects of rhizosphere microorganisms on plant health and productivity. *Soil Biol. Biochem.*, 40 (7): 1733-1740.
- Aysan, E. and S. Demir. 2009. Using arbuscular mycorrhizal fungi and *Rhizobium leguminosarum*, Biovar *phaseoli* against *Sclerotinia sclerotiorum* (Lib.) de bary in the common bean (*Phaseolus vulgaris* L.). *J. Pl. Pathol.*, 8: 74-78.
- Badr El-Din, S. and H. Moawad. 1988. Enhancement of nitrogen fixation in lentil, faba bean, and soybean by dual inoculation with Rhizobia and mycorrhizae. *Pl. Soil*, 108 (1): 117-123.
- Bai, B.; V.K. Suri; A. Kumar and A.K. Choudhary. 2016. Influence of dual inoculation of AM fungi and rhizobium on growth indices, production economics, and nutrient use efficiencies in garden pea (*Pisum sativum* L.). *Commun. Soil Sci. Pl. Anal.*, 47(8): 941-954.
- Barea, J.M. and C. Azcón-Aguilar. 1982. Production of plant growth-regulating substances by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *Appl. Environ. Microbiol.*, 43 (4): 810-813.
- Bhardwaj, D.; M.W. Ansari; R.K. Sahoo and N. Tuteja. 2014. Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microb. cell factories*, 13 (1): 66-76.
- Bhattacharjee, R. and U. Dey. 2014. Biofertilizer, a way towards organic agriculture: A review. *African J. Microbiol. Res.*, 8 (24): 2332-2343.
- Bhattacharjee, S. and G.D. Sharma. 2012. Effect of dual inoculation of Arbuscular Mycorrhiza and Rhizobium on the chlorophyll, nitrogen and phosphorus contents of pigeon pea (*Cajanus cajan* L.). *Adv. Micobiol.*, 2 (4): 561-654.
- Bhattacharjee, S. and G.D. Sharma. 2015. Effect of Arbuscular mycorrhizal fungi (AM fungi) and Rhizobium on the nutrient uptake of pigeon pea plant. *Inter. J.*, 3 (8): 833-836.

- INFLUENCE OF MINERAL FERTILIZATION DEDUCTION..... 92**
- Bhattarai, N.; B. Baral; G. Shrestha and K.D. Yami. 2011.** Effect of mycorrhiza and rhizobium on *Phaseolus vulgaris*. *Sci. World*, 9 (9): 66-69.
- Black, C.A.; D.D. Evans and R.C. Dinauer. 1965.** Methods of soil analysis. Madison, WI: Amer. Soc. of Agron., 9: 653-708.
- Breeuwsma, A. and S. Silva. 1992.** Phosphorus fertilisation and environmental effects in the Netherlands and the Po region (Italy) (p. 39). Wageningen, Netherlands: DLO The Winand Staring Centre.
- Bulgarelli, R.G.; F.C.C. Marcos; R.V. Ribeiro and S.A.L. Andrade, 2017.** Mycorrhizae enhance nitrogen fixation and photosynthesis in phosphorus-starved soybean (*Glycine max* L. Merrill). *Environ. Exp. Bot.*, 140: 22-33.
- Carvajal-Muñoz, J.S. and C.E. Carmona-Garcia. 2012.** Benefits and limitations of biofertilization in agricultural practices. *Livestock Res. Rural Develop.*, 24 (3).
- Chapman, H.D. and Pratt, P.F., 1961.** Methods of Analysis for Soils, Plants and Waters. Priced Publication 4034. Division of Agriculture Sciences. University of California, Berkeley.
- Clark, R.B. and S.K. Zeto. 2000.** Mineral acquisition by arbuscular mycorrhizal plants. *J. Pl. Nutr.*, 23 (7): 867-902.
- Daniels-Hylton, K.D.M. and M.H. Ahmed. 1994.** Inoculation response in kidney bean (*Phaseolus vulgaris* L.) to vesicular-arbuscular mycorrhizal fungi and rhizobia in non-sterilized soil. *Biol. Fert. Soils*, 18(2): 95-98.
- Dar, G.H.; M.Y. Zargar and G.M. Beigh. 1997.** Biocontrol of fusarium root rot in the common bean (*Phaseolus vulgaris* L.) by using symbiotic *Glomus mosseae* and *Rhizobium leguminosarum*. *Microbial Ecol.*, 34 (1): 74-80.
- Dar, M.H.; R. Groach and N. Singh. 2015.** Effect of different biofertilizers under different levels of phosphorus on quality parameters of maize (*Zea mays* L.) and common bean (*Phaseolus vulgaris* L.) under intercropping system. *World J. Agric. Sci.*, 11(6): 363-370.
- Gardezi, A.K.; S.R. Marquez-Berber; B. Figueroa-Sandoval; U. Larqué-Saavedra and M. Escalona-Maurice. 2012.** Endomycorrhizal inoculation effect on beans (*Phaseolus vulgaris* L.), oat (*Avena sativa* L.), and wheat (*Triticum aestivum* L.) growth cultivated in two soil types under greenhouse conditions. *J. Systemics, Cybernetics and Informatics*, 10 (5): 68-72.
- Geneva, M.; G. Zehirov; E. Djonova; N. Kaloyanova; G. Georgiev and I. Stancheva. 2006.** The effect of inoculation of pea plants with mycorrhizal fungi and Rhizobium on nitrogen and phosphorus assimilation. *Pl. Soil Environ.*, 52 (10): 435-440.
- Govindarajulu, M.; P.E. Pfeffer; H. Jin; J. Abubaker; D.D. Douds; J.W. Allen; H. Bücking; P.J. Lammers and Y. Shachar-Hill. 2005.**  
*Fayoum J. Agric. Res. & Dev., Vol. 32, No.2, July, 2018*

- Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature*, 435 (7043): 819-823.
- Gunes, A.; A Inal.; M. Alpaslan; F. Eraslan; E.G. Bagci and N. Cicek. 2007.** Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (*Zea mays* L.) grown under salinity. *J. Pl. Physiol.*, 164 (6): 728-736.
- Hazarika, D.K.; K.K. Das; L.N. Dubey and A.K. Phookan. 2000.** Effect of Vesicular Arbuscular Mycorrhizal (VAM) fungi and Rhizobium on growth and yield of green gram [*Vigna radiata* (L.) Wilczek]. *J. Mycol. Pl. Pathol.*, 30 (3): 424-426.
- Ibijbijen, J.; S. Urquiaga; M. Ismaili; B.J.R. Alves and R.M. Boddey. 1996.** Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans (*Phaseolus vulgaris*). *New Phytol.*, 134: 353-360.
- Ju, X.T.; G.X. Xing; X.P. Chen; S.L. Zhang; L.J. Zhang; X.J. Liu; Z.L. Cui; B. Yin; P. Christie; Z.L. Zhu and F.S. Zhang. 2009.** Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proceedings of the National Academy of Sci.*, 106 (9): 3041-3046.
- Kucey, R.M.N. and R. Bonetti. 1988.** Effect of vesicular-arbuscular mycorrhizal fungi and captan on growth and N<sub>2</sub> fixation by Rhizobium-inoculated field beans. *Can. J. Soil Sci.*, 68(1): 143-149.
- Moradi, S.; J. Sheikhi and M. Zarei. 2013.** Effects of arbuscular mycorrhizal fungi and rhizobium on shoot and root growth of chickpea in a calcareous soil. *Inter. J. Agric.*, 3 (2): 381-385.
- Mortimer, P.E.; M.A. Pérez-Fernández and A.J. Valentine. 2008.** The role of arbuscular mycorrhizal colonization in the carbon and nutrient economy of the tripartite symbiosis with nodulated *Phaseolus vulgaris*. *Soil Biol. Biochem.*, 40 (5): 1019-1027.
- Nihorimbere, V.; M. Ongena; M. Smargiassi and P. Thonart. 2011.** Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnol. Agron. Soc. Environ.*, 15 (2): 327.
- Püschel, D.; M. Janoušková; A. Voříšková; H. Gryndlerová; M. Vosátka and J. Jansa. 2017.** Arbuscular mycorrhiza stimulates biological nitrogen fixation in two *Medicago* spp. through improved phosphorus acquisition. *Frontiers in pl. sci.*, 8: 1-12.
- Rajasekaran, S.; S.M. Nagarajan; K. Arumugam; R. Saravanamuthu and S. Balamurugan. 2006.** Effect of dual inoculation (Rhizobium and AM fungi) on chlorophyll content of *Arachishypogaea* L. CV. TMV-2. *Pl. Arch.*, 6 (2): 671-672.
- Safapour, M.; M. Ardakani; S. Khaghani; F. Rejali; K. Zargari; M. Changizi and M. Teimuri. 2011.** Response of yield and yield Components of three red bean (*Phaseolus vulgaris* L.) genotypes to co-inoculation

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with *Glomus intraradices* and *Rhizobium phaseoli*. Am. J. Agric. Environ. Sci., 11 (3): 398-405.

**Soares, B.L.; P.A.A. Ferreira; M. Rufini, F.A.D. Martins; D.P. Oliveira; R.P. Reis; M.J.B.D. Andrade and F.M.D.S. Moreira. 2016.** Agronomic and Economic Efficiency of Common-Bean Inoculation with Rhizobia and Mineral Nitrogen Fertilization. Rev. Bras. Ciênc. Solo, 40: e0150235. (DOI: 10.1590/18069657rbcs20150235)

**Tajini, F.; M. Trabelsi and J.J. Drevon. 2012.** Combined inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases phosphorus use efficiency for symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.). Saudi J. B. Sci., 19 (2): 157-163.

**Talaat, N.B. and A.M. Abdallah. 2008.** Response of Faba bean (*Vicia faba* L.) to dual inoculation with Rhizobium and VA Mycorrhiza under different levels of N and P fertilization. J. Appl. Sci. Res., 4 (9): 1092-1102.

**Temminghoff, E.E. and, V.J. Houba (eds.). 2004.** Plant analysis procedures (Vol. 179). Dordrecht: Kluwer Academic Publishers.

**Wang, X.; Q. Pan; F. Chen; X. Yan and H. Liao. 2011.** Effects of co-inoculation with arbuscular mycorrhizal fungi and rhizobia on soybean growth as related to root architecture and availability of N and P. Mycorrhiza, 21(3): 173-181.

تأثير خفض التسميد المعدني مع التلقيح المزدوج للمايكورايزا مع الرايزوبيم على صنفين من الفاصوليا تحت الظروف الحقلية

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من أجل دراسة تأثير التلقيح المزدوج لكل من المايكورايزا و الرايزوبيم (عقدين) على النمو، المحصول و التركيب الكيميائي علي نباتات الفاصوليا و الفاصوليا الحمراء تحت مستوى خفض قدره ٥٠% من الكميات الموصى بها من التسميد النتروجيني و الفسفوري تم إجراء تجربتين حقليتين لموسمي صيف ٢٠١٤-٢٠١٥. نتائج هذه التجربة أظهرت أن الفاصوليا الحمراء ذات أداء أفضل من الفاصوليا صنف بوليسا لكل الصفات المدروسة. النسبة للمعاملات المختبرة، وجد أنها أظهرت تأثير معنوي للصفات المدروسة بغض النظر عن الصنف المستخدم. أيضاً معاملة ٥٠% من النتروجين و الفوسفور قد أعطت أقل متوسطات للصفات المدروسة بصورة معنوية مقارنة بالمعاملات الأخرى، في كلا موسمي الدراسة. على الجانب الآخر نجد أن كل من ١٠٠% نتروجين و فسفور أو ٥٠% مع التلقيح المزدوج قد أعطيا أفضل تأثير معنوي و إيجابي على غالبية الصفات المدروسة مقارنة بباقي المعاملات، في كلا موسمي الدراسة بدون فرق معنوي بينهما ماعدا في صفة الوزن الخضري الطازج حيث أعطت المعاملة ١٠٠% نتروجين و فسفور متوسط أعلى من ٥٠% مع التلقيح المزدوج، في كلا موسمي الدراسة. التفاعل ما بين الأصناف و المعاملات وجد أنه معنوياً لكل الصفات تحت الدراسة مع تفوق معنوي لكل من ١٠٠% نتروجين و فسفور أو ٥٠% مع التلقيح المزدوج مع الفاصوليا الحمراء. يتضح من النتائج السابقة أنه يمكن لمنتجي الفاصوليا أن يخفضوا كميات التسميد المعدني لكل من النتروجين و الفوسفور بنسبة ٥٠% في حال استخدام التلقيح المزدوج لكل من الرايزوبيم (عقدين) و المايكورايزا مع حصولهم على نفس النمو و المحصول إضافة إلى خفض الأضرار البيئية المترتبة على الاستخدام الكثيف لتلك الأسمدة المعدنية.