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Leaf Area Index, Dry Matter Accumulation and Allocation Trends in *Vicia faba* L. Affected by Inoculation with *Rhizobium* and *Pseudomonas*

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ABSTRACT

To investigate the effect of inoculation with *Rhizobium legominosarum* and *Pseudomonas fluorescens* on grain yield, leaf area index, and dry matter accumulation and allocation of *Vicia faba* L., an experiment was conducted as a split-plot in a randomized complete block design with four replications at Agricultural Research Station of Golestan Province, during the cropping seasons of 2015-2017. As the main-factor, *Rhizobium* was considered at two levels of with and without inoculation, while the sub-factor included *Pseudomonas* in density levels of 0, 9×10^3, 9×10^5, 9×10^7, and 9×10^9 cells or CFU mL^{-1} of inoculants. A logistic model by SAS, was used in order to estimate the changes in the leaf area index and the dry matter accumulation. Combined analysis of variance for the two years of study indicated that the climatic conditions influenced the bacteria effects. In the second year (less temperature and rainfall), the traits (maximum leaf area index, maximum dry weight and grain yield) indicated a significant reduction in comparison with the first year. *P. fluorescens* had a significant positive effect on grain yield in the two years. None of the bacteria had significant effect on maximum leaf area index. Results of the first year showed a positive effect of the inoculation of *Rhizobium* on maximum dry weight (8%) in comparison with the control. Co-inoculation of *Rhizobium* with *Peseudomonas* (9×10^5 CFU mL^{-1}) led to the greatest dry matter distribution coefficient for stems in podding stage. In the second year, *Peseudomonas* (9×10^9 CFU mL^{-1}) increased maximum dry weight (23%) in comparison with the control. Also, the density increase of *Peseudomonas* under co-inoculation with *Rhizobium* led to a significant reduction of the day to maximum LAI. In conclusion, co-inoculation *Rhizobium* with *Pseudomonas* can have a positive effect on the growth indices of faba bean.

Keywords: Co-inoculation, Dry matter distribution coefficients, Faba bean, Grain yield, Logistic model**.**

INTRODUCTION

Faba bean (*Vicia faba* L.) is a protein-rich legume that due to its high nutritional value is used for feed and food. Ecological benefits of faba bean, including the ability to fix atmospheric nitrogen and its positive effects on plant, encourage its use in crop rotation (Neugschwandtner *et al*., 2015).

The aim of modern agriculture is to achieve maximum rate of growth and yield through genetic breeding and environmental reforms. Quantitative analysis of growth is a suitable method for plant response to various environmental conditions in its life cycle. Since crop yields are affected by many factors in the growing season, the growth

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analysis is useful to explain crop yields differences (Sarmadnia and Kuchaki, 1995). Total dry matter and leaf area index are two important indices that are normally used to study plant physiology. Supply of plant nutrition is one of the most important strategies for increasing growth indices. In addition, use of chemical fertilizers to supply important nutrient elements such as nitrogen and phosphorus, apart from the increase in costs of production and transportation, may be followed by adverse environmental impacts. Application of organic fertilizers can prevent excessive use of chemical fertilizers (Hokmalipour and Seyedsharifi, 2014).

In this regard, application of Plant Growth-Promoting Rhizobacteria (PGPR) for seed inoculation is the most important strategy for sustainable management of agricultural ecosystems and increasing production. *Rhizobium* and *Pseudomonas* are useful soil-inhabiting bacteria that have been reported to increase yield by inoculation of seeds with these bacteria. In addition to biological fixation of nitrogen and dissolution of soil phosphorus, bacteria have effects on plant growth and development by producing growth hormones, particularly auxins, gibberellins and cytokinins, which significantly stimulate crop growth (Zahir and Frankenberger, 2004). Akhtar and Siddiqui (2008) reported that pea seed inoculation with *Rhizobium* significantly increased shoot dry weight and yield. Mahmood and Athar (2008) also obtained similar results on mung bean. Hokmalipour and Seyedsharifi (2014) reported that co-inoculation of barley seed with *Azospirillum lipoferum* and *Azotobacter chroococcum* resulted in a growth indices increase in all sampling stages compared to the control. *Rhizobium* inoculation in combination with PGPR such as KB133 led to a significant increase in seed yield of lentil (Biswas *et al*., 2012). An increase in yield and yield components can be attributed to the effective role of PGPR in nitrogen fixation and release in critical demand stages for nutrient elements, which increases the amount of nitrogen that can be consumed in sensitive stages of growth (Sharifi and Syiahkholaki, 2015).

This study mainly aimed to investigate the effect of the combined application of two bacteria in different populations, *Rhizobium leguminosarum* and *Peseudomonas fluorescens*, on the grain yield, leaf area index, dry matter accumulation and allocation trends of faba bean in two years.

MATERIALS AND METHODS

The experiment was carried out in two years (2015–2017) at a farm located at the Agricultural Research Station of Golestan Province (N 36° 54´, E 40° 25´, and 400 MSL) with 400 to 450 mm average annual rainfall. Soil samples of the field were randomly analyzed before starting the experiment (Table 1). The present study was performed as split-plot in Randomized Complete Block Design (RCBD) with four replications. *Rhizobium leguminosarum* bv. viciae, F40 strain in two levels (non-inoculation and inoculation with 10^8 bacteria per mL) was in the main-plots, and *Pseudomonas fluorescens* r187 strain in five density levels (0, 9×10^3, 9×10^5, 9×10^7 and 9×10^9 cell or CFU mL^{-1}) were in the sub-plots (Table 2). Bacteria was taken from Soil and Water Research Institute, Karaj, Iran. The field was cultivated by wheat in previous year, and all field preparation operations including plowing, disking, levelling, and uniform fertilizing by nitrogen (30 kg ha^{-1} as starter) were applied in the same way to all the plots. Each plot had six cultivation rows with four meters length (75 cm between the rows and plants spacing of 15 cm on the row) that led to a density of approximately 15 plants per square meter. The main-plots were separated by two meters distance from each other. Cultivation dates were November 9 for the first year and November 30 for the second year due to adverse climate conditions. An hour before planting *Vicia faba* L.cv. Barkat, seeds were inoculated with inoculant (10 mL of

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Table 1. Some physical and chemical properties of the soil in the experimental field**.**

Year	Depth (cm)	EC (dS m^{-1})	pH	O.C (%)	N (%)	K (mg kg^{-1})	P (mg kg^{-1})	%Clay	%Silt	%Sand	Soil texture
2015-16	0-30	1.1	7.4	1.5	0.15	362	9/0	16	60	24	Silt-loam
2016-17	0-30	1.0	7.7	1.4	0.14	333	11/4	20	66	14	Silt-loam

Table 2. Some properties of the bacteria used in the experiment (Soil and Water Research Institute, Karaj, Iran).

Bacteria	Acc-deaminase production	Phosphorus solubilizing activity	IAA production (mg L^{-1})	Siderophore production halo diameter/Colony diameter
P. fluorescens r187	+	+	5.8	0.5

inoculant kg^{-1} seeds), and cultivated at a depth of five cm after shade drying early in the morning. The maintenance operation consisted of weeding, pests and diseases control was performed similar to other farms in the region. Irrigation was not performed due to sufficient rainfall and its suitable distribution during the growing season.

The attributes include phonological stages: number of days to early flowering (R1), days to early podding (R3), days to grain formation (R5), days to physiological maturity (R7), and days to full maturity (R8). These were measured based on the Fehr and Caviness (1977). To measure leaf area index and dry weight, sampling was performed at seven times (Fourth-fifth trifoliolate emerging, branching, flowering, pod setting, grain forming, physiological maturity, and full maturity). Leaf, stem, and grain (pod+grain) separations of plants were dried at 70°C for 48 hours and weighed (with an accuracy of 0.01 grams). At each stage, five faba bean plants were used for leaf area index measurement using the Delta-T-DEVICES LTD model leaf scaling device, and then were extended to the predicted area. Grain yields per unit area were also measured.

In the present study, different nonlinear regression models were fitted to the data to analyze the accumulation of dry matter and leaf area index in relation to the time, and then logistic model was used because of better fitting to the data (Ghadirian *et al*., 2011). The logistic model (Equation 1) was used.

$$y = \frac{ae^{-a(x-b)(c)}}{\left(1+e^{-a(x-b)}\right)^2} \quad (1)$$

Where, y describes the trend of leaf area index changes during the plant growth period (x), *a* is constant coefficient and indicates the curve rotation rate, *b* is the time after planting when the maximum leaf area index occurs, and *c* is a constant coefficient. After fitting the model, the maximum leaf area index was determined by numerical method (Ghadirian *et al*., 2011).

Equation (2) was used to describe the trend of dry matter changes (W) in relation to time after the planting (x) where, W_{max} is the maximum amount of dry matter accumulation, *k* coefficient is the dry matter growth rate, and t_m is the time that crop growth rate is maximum, at which time the amount of dry material has reached half of its maximum value (Ghadirian *et al*., 2011).

$$w = \frac{w_{max}}{1+e^{-k(x-t_m)}} \quad (2)$$

The harvest index was calculated from Equation (3) (Jafarnodeh *et al*., 2017). $HI = (GY / BY)\times 100$ (3)

Where, *HI* is Harvest Index, *GY* is the Grain Yield (g m^{-2}), *BY* is Biomass Yield (dry weight of the entire plant including leaf, stem, pod, and grain, g m^{-2}).

Combined ANOVA was used for data analysis in the two years. ANOVA, comparison of means, and model fitting were performed by using SAS software and

PROC NLIN procedure for parameter estimating of each model by iterative optimization method (Soltani, 2007). The means were compared by LSD method and graphs were drawn in Excel software.

RESULTS AND DISCUSSION

Effect of Year on the Measured Traits

Combined ANOVA showed that the year had a significant effect ($P \leq 0.01$) on Grain Yield (GY), maximum LAI, the maximum dry weight and harvest index of faba bean (Table 4). In the second year, GY, LAI_{max} and W_{max} decreased significantly compared to the first year (Table 5, Figures 1, 2). According to the meteorological data (Table 3), the weather conditions were different in the two growing seasons in the location of the experiment. Air temperature and rainfall conditions in the second year (2016) delayed the planting. The growth season of faba bean was 199 days in the first year (9 November-28 May) and 187 days in the second year (30 November-6 June). During the growth period, the time interval between planting to emergence, the beginning of flowering, the beginning of the podding, the beginning of seed forming, the physiological ripening and the full ripening were recorded as 22, 112, 152, 162, 178, and 200 days in the first year and 40, 100, 140, 147, 167, and 187 days in

Table 3. Meteorological information at the experimental site in the two growing seasons of faba bean.

Month	2015-2016					2016-2017				
	Temp (C˚)			RH	Rainfall (mm)	Temp (C˚)			RH	Rainfall (mm)
	Min	Max	Mean			Min	Max	Mean		
November	-1.1	22.6	9.6	81	41.0	-5.4	25.7	7.4	77	26.2
December	0.1	28.5	10.1	78	45.3	-2.4	21.8	8.0	74	2.5
January	-1.8	24.5	7.9	81	76.5	-3.0	19.5	6.2	79	79.5
February	0.3	26.3	11.5	87	53.3	-4.2	26.2	10.6	76	18.5
March	3.0	28.8	14.6	79	76.6	1.7	29.6	14.1	80	40.2
April	6.4	37.2	20.9	78	36.5	9.8	38.9	20.3	76	37.1
May	14.1	37.9	25.0	66	62.6	12.4	38.2	25.1	60	2.2
Average	3.0	29.4	13.8	78.6	56.2	1.3	28.6	13.1	74.6	29.5

Table 4. Combined ANOVA for the coefficient of the logistic model for the leaf area index and dry matter accumulation of faba bean in the two study years. [a]

SOV	df	MS						
		LAI_{max}	b	W_{max}	tm	k	Grain yield	HI
Year	1	107.44**	321.60**	3446782.88**	541.84**	0.10**	1162760.21**	4336.26**
Block	6	0.78ns	55.58 *	77821.37 ns	103.66 **	0.002 ns	116980.13**	15.36 ns
Rhizobium (R)	1	0.82 ns	463.68 **	66453.15 ns	354.48 **	0.003 ns	23658.60**	0.38 ns
Year×R	1	0.52 ns	143.11 *	165592.70 ns	242.90**	0.009**	53864.41**	0.83 ns
R×Block	6	0.76	28.47	17493.23	128.41	0.003	7081.92 ns	13.35
Pseudomonas (P)	4	0.63 ns	73.00 *	248638.17**	209.74 **	0.005**	20935.16**	9.19 ns
R×P	4	0.96 *	46.40ns	8037.02 ns	66.23 ns	0.0003 ns	813.44 ns	27.27 ns
Year×P	4	0.20 ns	49.57 ns	45940.18 ns	151.68 **	0.001 ns	2989.07 ns	35.61*
Year×R×P	4	0.06 ns	48.79 ns	43698.28 ns	70.07 ns	0.002 ns	4014.98 ns	16.42 ns
Error	48	0.36	21.35	56351.11	27.58	0.001	3245.69	13.64
CV		12.39	2.94	13.64	3.58	19.79	8.98	7.13

[a] LAI_{max}: Maximum Leaf Area Index (was obtained by numerical solution); b: The time to the maximum leaf area index (day); Wmax: Maximum dry matter accumulation per unit area (g m^{-2}); t_m: Time to maximum growth rate; GY: Grain Yield and, HI: Harvest Index; ns, *, and **: Are not significant, significant at 5 and 1% levels of probability, respectively.

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Table 5. Means comparison for the effect of year on the coefficient of the logistic model.

Year	LAImax	b	W_{max}	tm	k	Grain yield (g m^{-2})	HI
(2015-2016)	6.05 [a]	159.34 [a]	1947.26[a]	144.14 [b]	0.12 [b]	754.90 [a]	44.39 [b]
(2016-2017)	3.73 [b]	155.33 [b]	1532.12[b]	149.35 [a]	0.19 [a]	513.78 [b]	59.12 [a]

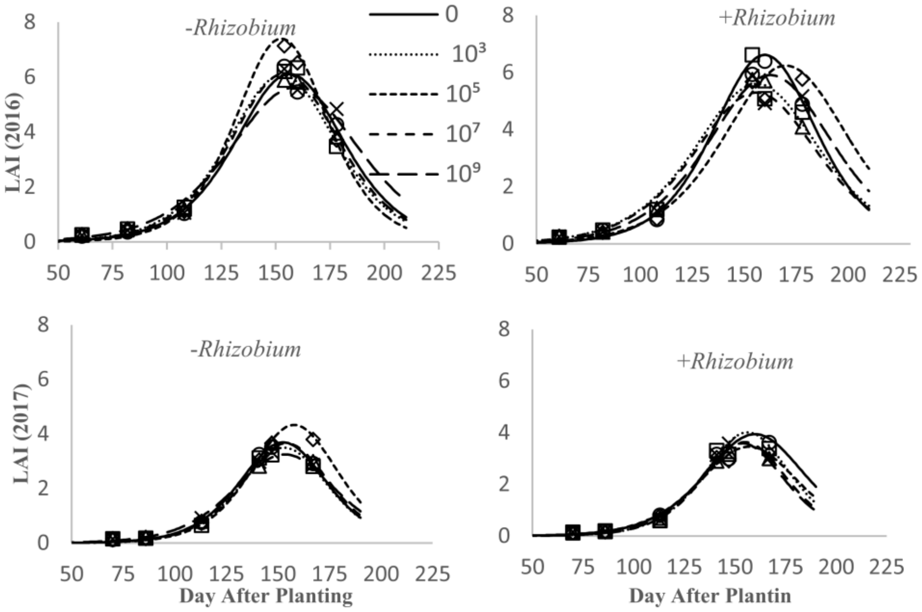

Figure 1. The trend of faba bean leaf area index changes during two years in different treatments (Application of *Pseudomonas* rates: 0, 9×10^3, 9×10^5, 9×10^7, 9×10^9 CFU mL^{-1}) in two conditions: - and + *Rhizobium*.

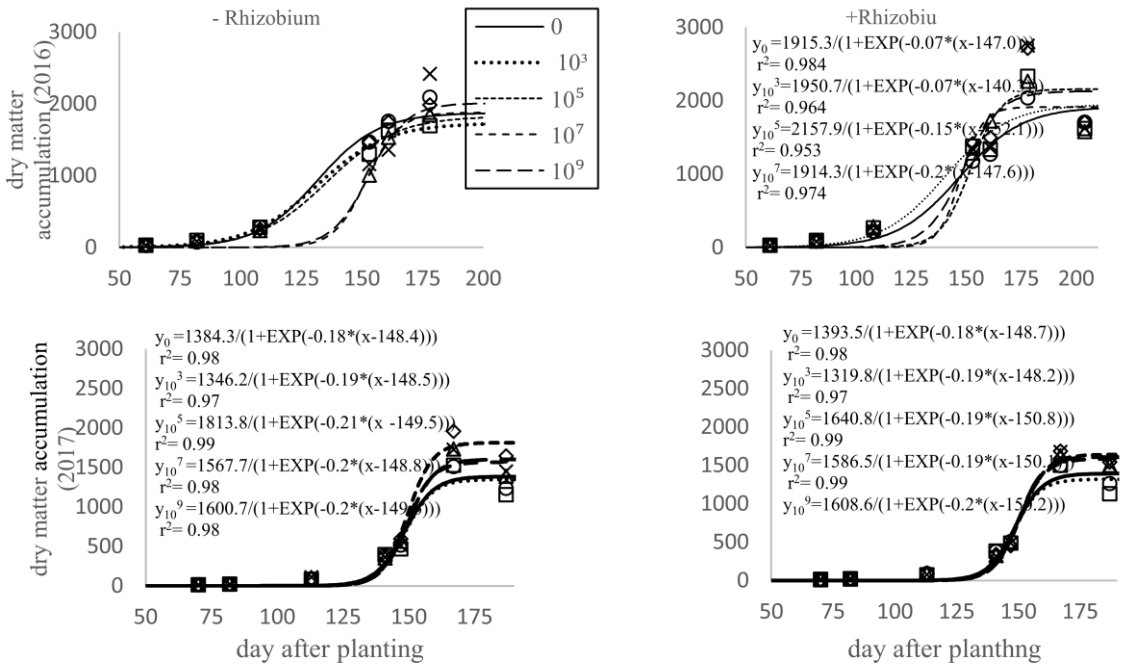

Figure 2. The trend of faba bean dry matter accumulation changes during the two years in different treatments (Application of *Pseudomonas* rates: 0, 9×10^3, 9×10^5, 9×10^7, 9×10^9 CFU mL^{-1}) under the two conditions: - and + *Rhizobium*. y= *Wmax*/{1+EXP[-*k**(x- *tm*)] }.

the second year, respectively. Hassanzadeh *et al*. (2013) reported that low temperature at the beginning of growth season prolongs the greening period of faba bean grain, on the other hand, with higher temperatures at the end of the growing season, the time required for different stages of phenology is reduced. Also, reduction in the number of days to ripening in delayed planting may be due to high temperature, which forces the plant to complete its life cycle faster and thus strongly reduces yield and yield components. According to the previous studies, weather conditions also affect the efficacy of bacteria in addition to the effect on the plant growth. Mahdavi *et al*. (2010) observed that low temperature of the root zone in the first stage reduced the nodule formation and nitrogen fixation of grass pea cultivars.

Zainali *et al*. (2012) stated that leaf production period in delayed cultivation was shortened, thus the LAI was reduced, similar to our results. Delay in planting (second year) reduced dry matter accumulation per unit area (Khalil *et al*., 2010). In the second year, the duration of vegetative and reproductive growth period decreased, but the reproductive period duration was less affected, so, more grains were produced per plant, which increased the HI (Nakhzari Moghaddam *et al*., 2014)

Effect of Bacteria on the Measured Traits

Grain Yield: The results of ANOVA in the two years showed that *P. fluorescens* had significant effect ($P\leq 0.05$) on grain yield (Table 6). Means comparison (Table 8) showed that the highest amount of grain yield was obtained when 9×10^9 CFU mL^{-1} of *P. fluorescens* were applied. Salehi and Aminpanah (2015) reported that pea plants seeds inoculated with *Pseudomonas* spp. produced higher grain yield than the un-inoculated ones, indicating a positive effect of phosphorus on flower formation and fruit set.

LAI Changes Trend*:* In order to perform ANOVA and mean comparison, the parameters of the logistic model and LAI_{max} were calculated separately for each block (Tables 6 and 7). The results of ANOVA in the first year showed that none of the bacteria had significant effect on maximum LAI, but both *Rhizobium* and *Pseudomonas* bacteria had significant effect ($P\leq 0.05$) on days to the LAI_{max} (Table 6). *Rhizobium*, especially in combination with 9×10^5 and 9×10^9 *P. fluorescens* bacteria per mL, increased the time to the LAI_{max} (169.87 and 164.87 days after planting), compared to the control (156.85 days) (Table 7). It seems that under favorable climatic conditions of the first year (earlier planting), *Rhizobium* and *Pseudomonas* inoculation increased the time to LAI_{max} until seed forming stage. Possibly, hormones secretion by bacteria, especially auxin, and continuous nitrogen fixation and its absorption by the plant provide favorable conditions for increasing leaf area during reproductive period. As a result, absorption of sun radiation and, consequently, photosynthesis did not decrease during reproductive period, and, finally, more dry matter and yield were produced (Gan *et al*., 2011). Stewart *et al*. (2003) also stated that if the leaf area is maintained until the beginning of the grain formation, it would have a positive correlation with grain yield.

In the second year, similar to the first year, the results showed that the application of bacteria did not have a significant effect on the LAI_{max}, but the days to the LAI_{max} (b) was influenced by the interaction of these two bacteria ($P\leq 0.05$) (Table 6). *Rhizobium* bacterium significantly increased the time to reach the LAI_{max} (161 days) compared to the control (152.90), but its combined application with *P. fluorescens* significantly ($P\leq 0.05$) reduced its effect (Table 7). Due to the delayed cultivation in the second year and the limitation of vegetative growth, it seems that *P. fluorescens* ($\leq 9\times10^5$ CFU mL^{-1}), with a decrease in the days to reach the LAI_{max}, caused faster production of plant canopy and reaching the desired leaf area in

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Table 6. Analysis of variance for the coefficient of the logistic model for dry matter accumulation and leaf area index of faba bean in the two years.

S.O.V	df	MS^a											
		LAI_{max}		b		W_{max}		t_m		GY		HI	
		(2016)	(2017)	(2016)	(2017)	(2016)	(2017)	(2016)	(2017)	(2016)	(2017)	(2016)	(2017)
Block (B)	3	1.11^{ns}	0.45^{ns}	47.84^{ns}	63.3**	103948.95^{ns}	51693.78^{ns}	207.14^{ns}	0.17^{ns}	192858.50**	41101.77**	23.60^{ns}	7.11^{ns}
Rhizobium (R)	1	1.32^{ns}	0.02^{ns}	561.0*	45.80*	220923.63*	11122.22^{ns}	592.13^{ns}	5.26^{ns}	74459.64^{ns}	3063.37^{ns}	0.04^{ns}	1.17^{ns}
R×B	3	1.17	0.34	53.40	3.53	19423.14	15563.33	255.89	0.92	13111.38^{ns}	1052.46^{ns}	12.58	14.12
Peseudomunas (S)	4	0.69^{ns}	0.13^{ns}	105.07*	17.50^{ns}	85344.81^{ns}	209233.54**	356.43**	4.98*	17592.13*	6332.09*	35.07^{ns}	9.73^{ns}
R×S	4	0.55^{ns}	0.48^{ns}	67.65^{ns}	27.54*	41422.16^{ns}	10313.14^{ns}	134.51^{ns}	1.79^{ns}	3829.94^{ns}	998.48^{ns}	29.57^{ns}	14.11^{ns}
S×B	12	0.56^{ns}	0.29^{ns}	41.97^{ns}	13.79^{ns}	82767.80^{ns}	22312.73^{ns}	42.50^{ns}	1.74^{ns}	5076.9٩^{ns}	1429.27^{ns}	17.48^{ns}	8.11^{ns}
Error	12	0.44	0.18	21.92	7.71	101141.78	19182.14	64.98	1.09	4660.44	1819.04	19.68	9.30
CV		10.99	11.30	2.94	1.79	16.33	9.04	5.59	0.69	9.04	8.29	9.99	5.16

^{a} LAI_{max}: Maximum Leaf Area Index (was obtained by numerical solution); b: The time to the maximum leaf area index (day); Wmax: Maximum dry matter accumulation per unit area (g m^{-2}); t_m: Time to maximum growth rate; GY: Grain Yield and, HI: Harvest Index; ^{ns}, *, and **: Are not significant, significant at 5 and 1% levels of probability, respectively.

Table 7. The coefficients of the logistic model fitted to the leaf area index related to the interaction effect of *Rhizobium* and *Pseudomonas*.^{a}

Rhizobium	*Pseudomunas*	b		a±SE		c±SE		R^2	
		(2016)	(2017)	(2016)	(2017)	(2016)	(2017)	(2016)	(2017)
Without-inoculation	0	156.85^{cde}	152.90^{c}	0.06±0.006	0.07±0.003	407.8±38.66	211.1± 7.15	0.995	0.999
	10^3	151.95^{e}	153.30^{bc}	0.06±0.004	0.07±0.005	405.1±24.31	200.2± 12.14	0.998	0.998
	10^5	153.25^{e}	157.30^{ab}	0.07±0.003	0.07±0.002	423.0±17.68	247.7± 5.69	0.999	0.999
	10^7	155.35^{de}	154.17^{bc}	0.06±0.002	0.07±0.007	409.4±11.16	209.4± 15.38	0.999	0.997
	10^9	160.60^{bcd}	153.65^{bc}	0.05±0.007	0.06±0.002	444.0±52.90	216.9± 4.43	0.993	0.999
With-inoculation	0	162.30^{bc}	161.00^{a}	0.05±0.003	0.06±0.009	440.3± 19.07	263.1± 37.66	0.999	0.994
	10^3	157.52^{cde}	156.65^{abc}	0.05±0.009	0.07±0.011	448.2± 69.80	229.3± 29.45	0.993	0.993
	10^5	169.87^{a}	156.27^{abc}	0.05±0.005	0.07±0.007	489.2± 43.09	230.7± 24.99	0.998	0.993
	10^7	156.97^{cde}	154.47^{bc}	0.05±0.001	0.07±0.002	424.0± 8.42	207.9± 5.65	0.999	0.999
	10^9	164.87^{ab}	153.62^{bc}	0.05±0.005	0.07±0.004	517.8± 50.81	205.2± 8.23	0.998	0.999

^{a} (a and c) Are the constant coefficients of the model, b: Is the time to the maximum leaf area index (day) and, R^2 also shows the coefficient of determination. (a-e) Within each column, means followed by the same letter are not significantly different ($P< 0.05$).

Table 8. The coefficient of the logistic model to describe the accumulation of dry matter due to the effect of *Rhizobium* and *Pseudomonas***.**[a]

Treatment		Wmax		t_m		GY	
		(2016)	(2017)	(2016)	(2017)	(2016)	(2017)
Rhizobium	- Inoculation	1872.94[b]	1548.80[a]	140.30[a]	148.99[a]	711.75[a]	522.53[a]
	+ Inoculation	2021.58[a]	1515.45[a]	147.99[a]	149.71[a]	798.04[a]	505.03[a]
Pseudomonas	0	1884.5[a]	1404.55[c]	141.01[b]	148.72[b]	740.80[bc]	488.11[bc]
	10^3	1840.2[a]	1333.90[c]	136.82[b]	148.39[b]	690.78[c]	479.72[c]
	10^5	2006.0[a]	1734.73[a]	143.71[b]	150.27[a]	784.99[a]	526.30[ab]
	10^7	1908.9[a]	1579.30[b]	144.34[b]	149.47[ab]	744.15[ab]	531.24[ab]
	10^9	2096.8[a]	1608.13[ab]	154.85[a]	149.90[a]	813.77[a]	543.53[a]

[a] Wmax: Waximum dry matter accumulation per unit area (g m-2), t_m: Time to maximum growth rate, GY: Grain Yield. ***a-c*** For each bacteria, means followed by the same letter are not significantly different (P< 0.05).

less days. Karimi and Siddique (1991) stated that if the LAI reaches the desired level in less days, the maximum seed yield would be obtained, similar to our results.

Trend of Accumulative Dry Weight Change: Analysis of logistic parameters in the first year showed a significant difference (P≤ 0.05) between application and non-application of *Rhizobium* on the maximum of accumulative dry weight (Table 6). The results showed that the population of *P. fluorescens* had a significant effect (P≤ 0.01) on the t_m (time to maximum crop growth rate) (Table 6). Inoculation with *Rhizobium* increased the W_{max} by 8%, from 1,872.94 (non-inoculation) to 2,021.58 (inoculation) g m^{-2} (Table 8). Farnia *et al*. (2008) reported a positive correlation between nitrogen fixation and amount of dry matter produced by the soybean plant. According to the models, the highest amount of accumulative dry matter was obtained when *Rhizobium* and *Pseudomonas* (9×10^5, 9×10^9 CFU mL^{-1}) were applied.

The second year results showed that *P. fluorescens* population had significant effect (P≤ 0.01) on dry matter accumulation (Table 6). Population of 9×10^5 (bacteria per mL) and higher of *Pseudomonas* inoculum increased the W_{max} by 23% (1,734.73 g m^{-2}) compared to the control (Table 8). Yadegari *et al*. (2009) stated that *Pseudomonads* inoculation of red bean seeds increased the weight of dry matter, which is consistent with our results.

Distribution of Dry Matter Trend and Its Allocation Coefficients between Organs: The distribution of dry matter during the growing season for different plant organs including leaves, stems, and grain+pod has a sigmoid trend (Figure 3). In the first year, co-inoculation of *Rhizobium* and *Pseudomonas* bacteria in two populations, i.e. 9×10^5 and 9×10^9 CFU mL^{-1}, resulted in prolonged dry matter accumulation in the leaf and the stem, which continued until the physiological maturity of the plant (Figure 3), so, the plant had a good vegetative growth. Due to the prolongation of the accumulation process, the plant seems to benefit from the current photosynthesis for seed filling more than remobilization. Kheirizadeh Arogh *et al*. (2015) stated that the application of PGPRs improves the current photosynthesis of triticale by modulating the environmental stresses effect, which is consistent with our results. Tiwari *et al*. (2015) showed the role of *P. putida* in ameliorating drought stress on chickpea by modulating differential expression of genes involved in stress response, ethylene biosynthesis, salicylic acid and jasmonate signaling in chickpea.

The results of dry matter allocation coefficient from emergence to podding stage in bean showed that dry matter accumulation in leaf and stem increases linearly (Puri *et al*., 2013). The linear regression analysis of the treatments in the first year showed that the dry matter distribution coefficient varied from 0.18 to 0.25 for leaf and 0.75 to 0.82

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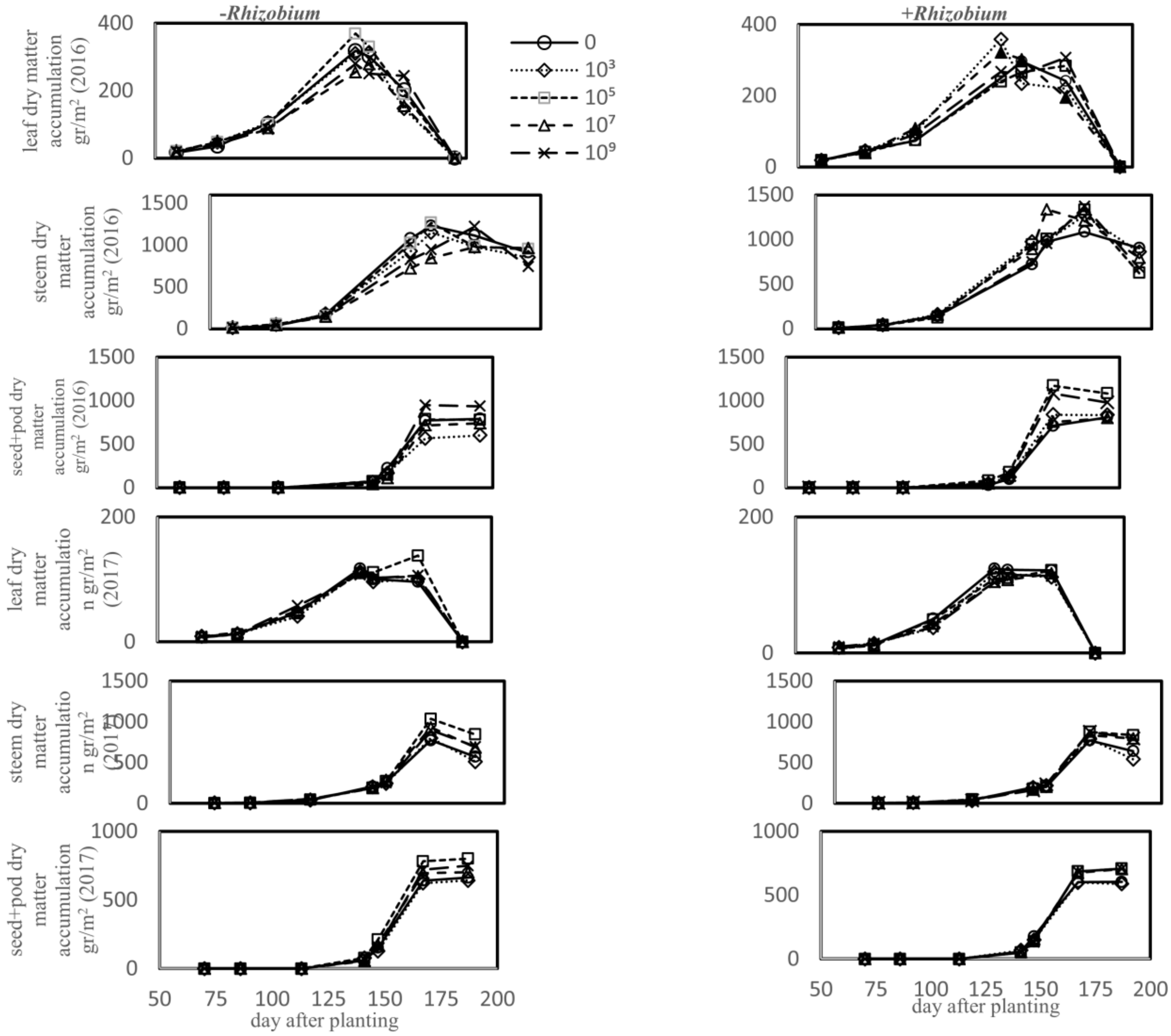

Figure 3. The trend of dry matter accumulation in the leaf, stem, and grain+pod of faba bean during the two study seasons (2015-2017).

for stem (Table 9). The co-inoculation of *Rhizobium* and *Pseudomonas* with the population of 9×10^5 CFU mL^{-1} had the highest stem allocation coefficient in the podding stage (Supplementary Table1). It can be concluded that this treatment induced a good vegetative growth before the reproductive stage.

In the second year, *Pseudomonas fluorescens* in 9×10^5 CFU mL^{-1} and more populations increased the accumulation of dry matter in organs, especially stem and grain (grain+pod) (Figure 3). Linear models fitted to the coefficients of dry matter allocation showed that the dry matter distribution coefficient varied from 0.34 to 0.37 for leaf and from 0.63 to 0.66 for stem (Table 9). Application of *Pseudomonas* alone caused an increase in dry matter distribution coefficient for stem compared to the rest of treatments. *Pseudomonas fluorescens* is a living microorganism with the ability to dissolve insoluble phosphates into soluble forms in soil (Maliha *et al*., 2004). Increasing the amount of phosphorus caused the increase in height and dry weight of lentil plant (Singh and Singh, 2016). During vegetative growth, photosynthetic excess materials are mostly accumulated in the stem (Dordas and Sioulas, 2009).

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Supplementary Table 1. Dry matter allocation coefficients of leaf and stem from emergence to podding stage of faba bean in different treatments.

Year	Organ	-*Rhizobium*					+ *Rhizobium*				
		Pseudomonas					*Pseudomonas*				
		0	9×10^3	9×10^5	9×10^7	9×10^9	0	9×10^3	9×10^5	9×10^7	9×10^9
2015-2016	Leaf	0.21	0.23	0.25	0.24	0.23	0.24	0.26	0.18	0.25	0.24
	Stem	0.79	0.77	0.75	0.76	0.77	0.76	0.74	0.82	0.75	0.76
2016-2017	Leaf	0.34	0.33	0.35	0.36	0.34	0.37	0.36	0.36	0.37	0.37
	Stem	0.66	0.67	0.65	0.64	0.66	0.63	0.64	0.64	0.63	0.63

Pseudomonas bacterium seems to increase the accumulation of grain yield by increasing the allocation of dry matter to the stem (Figure 3).

CONCLUSIONS

Our results in the first year showed that *Rhizobium* and *Pseudomonas* bacteria prolonged the period of vegetative growth by increasing the number of days to reach LAI_{max}, thus providing the higher ability of photosynthesis to increase yield. In the second year, however, when the conditions of growth were undesirable, it seems that *Pseudomonas* bacterium improved the conditions and increased accumulation of dry matter and grain yield. *Pseudomonas* bacterium seems to be more effective than *Rhizobium* bacterium under the stress conditions (second year). Regardless of the effect of *Rhizobium* and *Pseudomonas* bacteria on growth indices, the statistical results of this research showed that during the two study years, different climatic conditions affected the efficiency level and the effects of bacterium. PGPRs seem to be using different strategies depending on different environmental conditions, therefore, further widespread investigations are needed.

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روند تغییرات شاخص سطح برگ، تجمع و تسهیم ماده خشک در باقلا (*Vicia faba* L.) در اثر تلقیح ریزوبیوم و سودوموناس

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چکیده

به منظور بررسی تأثیر کاربرد باکتری ریزوبیوم لگومینوساروم و سودوموناس فلورسنس بر شاخص سطح برگ، روند تجمع و تسهیم ماده خشک باقلا، آزمایشی در دو سال زراعی ۹۵-۱۳۹۴ و ۹۶-۱۳۹۵ در مزرعه‌ی ایستگاه تحقیقات کشاورزی استان گلستان، به صورت اسپیلت پلات در قالب طرح پایه بلوک‌های کامل تصادفی با چهار تکرار انجام شد. باکتری ریزوبیوم به‌عنوان عامل اصلی در دو سطح (عدم تلقیح و تلقیح) و باکتری سودوموناس در پنج سطح تراکم (صفر، $۹×۱۰^{۳}$، $۹×۱۰^{۵}$، $۹×۱۰^{۷}$، $۹×۱۰^{۹}$ باکتری زنده در هر میلی‌متر مایه تلقیح) به‌عنوان عامل فرعی در نظر گرفته شد. به‌منظور بررسی روند تغییرات شاخص سطح برگ و تجمع ماده خشک از مدل لجستیک استفاده گردید. نتایج تجزیه مرکب دو سال نشان داد که اثر باکتری‌ها، متأثر از شرایط اقلیمی دو سال است. در سال دوم صفات (حداکثر شاخص سطح برگ، حداکثر ماده خشک و عملکرد دانه) کاهش معنی‌داری نسبت به سال اول نشان دادند. باکتری سودوموناس ($۹×۱۰^{۹}$ CFU/ml) اثر مثبت معنی‌داری بر عملکرد دانه در دو سال داشت. هیچ یک از باکتری‌ها اثر معنی‌داری بر حداکثر شاخص سطح برگ نشان نداشتند. نتایج سال اول، اثر مثبت تلقیح ریزوبیوم را بر حداکثر ماده خشک (هشت درصد) نسبت به شاهد نشان داد و تلقیح دوگانه با سودوموناس ($۹×۱۰^{۵}$ CFU/ml) باعث افزایش ضریب تخصیص ماده خشک به ساقه در مرحله غلاف‌دهی گردید. در سال دوم، سودوموناس ($۹×۱۰^{۵}$ CFU/ml) منجر به افزایش ۲۳ درصدی حداکثر وزن خشک، نسبت به شاهد شد. همچنین در شرایط تلقیح دوگانه، افزایش تعداد باکتری سودوموناس، کاهش معنی‌دار در تعداد روز تا رسیدن به حداکثر شاخص برگ را سبب گردید. بطور کلی نتایج نشان داد که تلقیح ریزوبیوم با سودوموناس می‌تواند اثر مثبتی بر شاخص‌های رشد باقلا داشته‌باشد.