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Alfalfa Response to Phosphorus Solubilizing Bacteria and Soil/Foliar Applied Boron and Manganese

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ABSTRACT: This experiment was conducted in south west region of Iran to study the effect of boron (B), manganese (Mn) and phosphorus solubilizing bacteria (P) on forage yield and yield components of three different ages of alfalfa plants (one-year, three-year and five-year cultivated plants). Field experiment was conducted in split plot factorial in the form of a randomized complete block design with three replications and three treatments: boron fertilizer in three levels (0, foliar application of 1 $L.ha^{-1}$ B and in soil application of 10 $kg.ha^{-1}$ B), manganese fertilizer in three levels (0, foliar application of 1 $L.ha^{-1}$ Mn and in soil application of 10 $kg.ha^{-1}$ Mn) as the sub plots, and phosphorus solubilizing bacteria (manure form) in two levels (0 and 100 g.ha-1) as the main plots. The highest forage yield and yield components were resulted from spraying boron and manganese along with phosphorus solubilizing bacteria application. Application of B, Mn and phosphorus solubilizing bacteria together increased forage yield in one-year old crops up to 24 $t.ha^{-1}$, three-year crops up to 36.11 $t.ha^{-1}$ and five-year crops up to 30.30 $t.ha^{-1}$. Lateral branches and protein in soil applied B and foliar applied Mn and phosphorus solubilizing bacteria was higher than Mn and phosphorus solubilizing bacteria applied alone or together. Results of this experiment indicated that the co-application of foliar boron, foliar manganese and phosphorus solubilizing bacteria in one-year, three-year and five-year alfalfas increased forage yield by 140%, 195.49% and 172.72%, respectively, compared with the control. Boron had positive effect on forage yield and yield components. Based on the results of this experiment, the co-application of B (spray), Mn (spray) and phosphate solubilizing bacteria is recommended for alfalfa forage production.

Keywords: integrated nutrient management, forage, *Medicago sativa* L., protein.

INTRODUCTION

Perennial crops such as alfalfa play important role in sustainable and productive agriculture and human food production. With the attention given to sustainable agriculture in recent decades, there is renewed interest in the use of legumes in cropping systems (Daniel *et al.*, 2011). Alfalfa (*Medicago sativa* L.) is the most commonly grown perennial crop for forage production in almost all regions of Iran. Because variation in yield of different ages of perennial crops due to senescence is an important challenge, this problem was studied in this experiment and treatments were applied on three different ages of alfalfa. A well planned fertilizing program is necessary for alfalfa forage production to ensure nutrients availability to plant to reach the highest yield.

Micronutrients are essential for normal physiological activity of plants and are as vital as the macronutrients (Du *et al.*, 2009). Some crops have higher boron requirement such as alfalfa, sunflower and rapeseed (Dear and Weir, 2004). The need for boron by legumes is higher than most of the other field crops (Murphy and Walsh, 1972). Boron role in plants is unique among the trace elements for normal crop growth (Tariq, 2007).

Although alfalfa is well adapted to a wide range of growing condition and soils; however, nutritional disorders caused by boron deficiency are common (Dell and Ltung, 1997; Shorrocks, 1997). Experiments indicated that boron fertilizer application increased alfalfa yield in each cuts (Turan *et al*., 2010). Some other researchers reported that manganese plays key roles in photosynthesis and reduction/oxidation and metabolism of nitrogen for plants (Sadana *et al*., 2005; Hopittke *et al*., 2007; Inal and Gunes, 2008).

Manganese is another necessary trace element for the growth of most crops (Zhengguo, 2009). Appling an optimal amount of Mn increased growth and chlorophyll content of tomato plants (Shenker *et al.*, 2004). The use of manganese sulfate fertilizer improves crop production and plant photosynthesis because Mn is an activator of many enzymes in plant.

Influence of phosphorus on alfalfa growth and yield is reported in many experiments (Sanderson and Jones, 1993; James *et al*., 1995). Alfalfa cultivation is known to minimize P losses due to the reduction of soil erosion of particulate phosphorus (insoluble phosphorus held on soil particles) (Anonymous, 2006).

Manure-based phosphate solubilizing bacteria increase P solubility in soil and consequently its availability to plant roots by dissolving insoluble phosphate (Jana, 2007; Rasipour and Aliasghar Zadeh, 2007). Application of phosphorus fertilizer in different experiments increased yield of sorghum (Daniel, 2011), the number of wheat panicles in hectare (Sweeney *et al*., 2000) and improved alfalfa yield (Hosseinirad *et al*., 2013).

There are few reports about the effect of boron on crop plants in Iran; however, interest in the international researches on this element was the motivation for this research. Our objective in this experiment was to study the effect of boron, manganese and phosphorus solubilizing bacteria on the forage yield of alfalfa and its protein content.

MATERIALS AND METHODS

This experiment was conducted in 2013 at the research farm of Marvdasht in central areas of Fars province, Iran (29° 52' S, 52° 48' N). At first, fields under cultivation of one-year, three-year and five-year old alfalfas were selected. Soil sample analysis was done in the early spring. Soil Samples were collected from 30 cm depth for one-year and three-year alfalfa and from 60 cm for five-year alfalfa. Soil properties and fertilizer application history are detailed in Table 1.

Table 1: Physico-chemical properties of the soil at the experimental sites.

Alfalfa Field	Depth	Soil texture	pH	N (%)	P (ppm)	K (ppm)	B (ppm)	Mn (ppm)	EC (ms)
One-year	0-30	Clay loam	7.8	0.1	15	257.9	0.67	6.1	0.96
Three-year	0-30	Clay	8.06	0.18	17.5	295.2	0.93	9.3	0.82
Five-year	30-60	Clay loam	7.82	0.2	16.05	308.5	0.67	8.4	1.18

A. Experimental design

The experiment was conducted in split plot factorial in the form of a randomized complete block design with three replications and the following treatments:

Phosphorus solubilizing bacteria in the main plots: in two levels including a bacteria free control and application of phosphorus solubilizing bacteria at the rate of 100 g.ha^{-1}.

Boron in the sub plots: in three levels including a B free control, foliar application of boric acid at the rate of 1 L.ha^{-1} and in soil application of borax at the rate of 10 kg.ha^{-1}.

Manganese in the sub plots: in three levels including a Mn free control, foliar application of manganese sulfate at the rate of 1 L.ha^{-1} and in soil application of manganese sulfate at the rate of 10 kg.ha^{-1}.

According to the treatments in three farms, there were 162 experimental plots. The alfalfa cultivar used in this study was *Medicago sativa* L. cv. Rangar.

B. Field operations

Plots were fertilized at the end of each cutting harvest. The irrigation was conducted using sprinkler method. Boron is more easily leached away from soil compared with other trace elements, and because of poor mobility boron, it must be continuously taken up by plants throughout the growing season (Dear and Weir, 2004; Oullette, 1958; Kubota *et al*., 1948).

C. Sampling and measurements

Harvest was conducted when the fields were at 10% flowering. To avoid the marginal effect, the middle rows of each plot were randomly harvested. Samples were cut 4.5 cm above the soil surface. Under fertilization and irrigation, alfalfa forage yield would improve when cutting is conducted closer to the soil surface, lower that 5 cm (Shen, 2013). After harvest, samples were dried in 68°C oven for 48 h, and were then weighted to obtain yield. Samples were sent to the laboratory for determination of protein content. Lateral branches of plants were randomly counted in each plot.

D. Statistical analysis

The data of yield, lateral branches and protein content of each treatment were calculated and statistically analyzed and compared by the F test. SAS software was used for statistical analysis and means were compared using Tuky's protected LSD test at P≤0.05.

RESULTS AND DISCUSSION

Alfalfa forage yield was affected by the three-fold interaction of B × Mn × phosphorus solubilizing bacteria (Table 2). By applying boron fertilizer in both forms (soil/foliar), forage yield changes followed a particular trend. Yield was increased with added B, especially with Mn fertilization and phosphate solubilizing bacteria (PSB). Results indicated that in one-year, three-year and five-year alfalfas the highest forage yield was 24, 36.11 and 30.3 t/ha, respectively, which was related to the three-fold interaction of B (foliar) × Mn (foliar) × PSB, and the lowest forage yield was 10, 12.22 and 11.11 t/ha, respectively, which was achieved in the control (Table 4).

Mean comparison showed that application of phosphorus solubilizing bacteria in one-year alfalfa increased forage yield by 26.12%, the number of lateral branches by 59.95%, stem protein content by 32.55% and leaf protein content by 27.38%, compared with the control.

Application of PSB in three-year alfalfa increased forage yield, the number of lateral branches, stem protein content and leaf protein content by 32.42%, 36.83%, 18.08% and 8.60%, respectively, compared with the control. This treatment also increased forage yield, the number of lateral branches, stem protein content and leaf protein content of the five-year alfalfa by 30.47% 33.41% 11.07% and 12.66%, respectively, compared with the control (Table 3). Applying PSB increased forage yield in three different ages of alfalfa (Table 3).

Table 2: Analysis of variance of the effect of treatments on the measured traits.

S.O.V.	D.F.	Yield		Lateral branches		Stem Protein content		Leaf Protein content	
K	2	720.5576	**	1495.642	**	1285.97	**	1476.039	**
REP(K)	6	6.525979		10.78889		3.849167		4.444944	
PSB	1	1124.381	**	1337.02	**	461.3068	**	514.303	**
K*PSB	2	27.88484	**	6.281728	**	27.36219	**	33.95843	**
REP(K*PSB	6	0.025507		0.492099		0.254768		0.216404	
B	2	1931.226	**	2544.643	**	636.6261	**	1582.1	**
K*B	4	41.31783	**	24.67963	**	23.2744	**	174.1549	**
PSB*B	2	95.70259	**	45.0084	**	37.56892	**	22.73896	**
K*PSB*B	4	2.159353	**	0.625062	ns	9.365542	**	9.274075	**
Mn	2	193.4476	**	286.0941	**	83.58014	**	82.32216	**
K*Mn	4	5.529204	**	1.500741	*	2.153265	**	1.390746	**
PSB*Mn	2	0.965615	*	3.527654	**	0.245997	ns	1.980592	**
K*PSB*Mn	4	0.128984	ns	0.189877	ns	0.387809	ns	0.163963	ns
B*Mn	4	6.627344	**	3.909259	**	3.625882	**	1.257643	**
K*B*Mn	8	0.346636	ns	0.299259	ns	1.244491	**	1.421129	**
PSB*B*Mn	4	0.8331	*	5.117654	**	1.49861	**	0.256524	ns
K*PSB*B*Mn	8	0.237556	ns	0.110988	ns	0.821769	*	0.806739	**
Error	96	0.33147		0.54966		0.346372		0.262336	
CV (%)		2.84		4.31		3.28		1.92	

** = $P< 0.01$ * = $P< 0.05$ ns = non-significant, K, PSB, B and Mn refer to three years of alfalfa, phosphorus solubilizing bacteria, boron and manganese.

Table 3: Influence of phosphorus solubilizing bacteria the measured traits.

PBS	Yield		Lateral Branches		Stem Protein content		Leaf Protein content	
One-year alfalfa								
0	14.70	B	8.59	B	13.73	B	19.57	B
1	18.54	A	13.74	A	18.20	A	24.93	A
Three-year alfalfa								
0	20.60	B	17.62	B	21.56	B	30.93	B
1	27.28	A	24.11	A	25.46	A	33.59	A
Five-year alfalfa								
0	17.49	B	16.76	B	13.73	B	23.76	B
1	22.82	A	22.36	A	15.25	A	26.77	A

Means in a column followed by the same letter are not significantly different at P≤0.05.

Malboobi *et al.* (2009) reported that Pseudomonas, which is of the most important plant growth promoting rhizobacteria, can make 70% of unavailable P readily available for plant absorption. In this experiment, the maximum yield was obtained when B was applied in any form along with Mn and PSB. Moreover, individual application of manganese and phosphorus also increased the yield. This procedure is similar to the one, three and five-year old alfalfas (Tables 4, 5 and 6). The significant response to B, with Mn fertilization and PSB application may be partially because dicotyledons crops such as alfalfa have higher need for boron than monocotyledons (Tariq and Mott, 2007).

In one-year, three-year and five-year ages of alfalfa, the highest yield was increased by 140%, 195.50% and 172%, respectively, compared with the control. Lateral branches and protein content are important factors of alfalfa forage yield. Phosphorus solubilizing bacteria, boron and manganese application rates significantly affected the number of lateral branches (P<0.001). The highest number of lateral branches / plant was 21.01, 34.07 and 31 in one-year, three-year and five-year alfalfa, respectively, which was achieved in the three-fold interaction of B (foliar) × Mn (foliar) × PSB. In one-year, three-year and five-year alfalfas the lowest number of branches was 1.47, 7.40 and 6.87, respectively, which was related to the control (Table 4). Although three different ages of alfalfa did not differ in these results and due to environmental factors and ages, using boron, manganese and PSB together is preferred. Sengul (2002) reported that the number of stems per plant was consistent while plants aged. A factor to identify the quality of alfalfa is protein content.

Table 4: Influence of PSB × B × MN on one-year alfalfa.

PBS	B	MN	Yield		Lateral branches		Stem Protein content		Leaf Protein content	
0	0	0	10.00	R	1.47	O	9.38	K	10.03	P
0	0	1	11.56	O	5.67	L	12.07	I	13.87	N
0	0	2	10.11	Q	2.40	N	10.99	J	12.62	M
0	1	0	15.37	K	10.13	H	13.88	G	11.22	O
0	1	1	19.22	G	13.60	F	17.90	E	22.91	H
0	1	2	18.19	H	12.47	G	16.45	F	26.21	D
0	2	0	14.22	L	9.13	I	13.20	GH	25.09	E
0	2	1	17.55	I	11.93	G	15.47	F	21.95	I
0	2	2	16.04	J	10.53	H	14.27	G	24.25	F
1	0	0	10.85	P	4.20	M	11.64	IJ	23.27	G
1	0	1	13.00	M	8.00	J	12.62	HI	15.67	J
1	0	2	12.37	N	6.93	K	12.38	HI	14.30	K
1	1	0	20.45	E	15.33	E	19.24	CD	13.41	L
1	1	1	24.00	A	21.07	A	24.68	A	32.00	A
1	1	2	22.85	B	19.07	B	22.72	B	31.74	B
1	2	0	19.89	F	14.20	F	18.63	DE	27.84	E
1	2	1	22.26	C	18.33	C	21.77	B	31.29	BC
1	2	2	21.22	D	16.53	D	20.12	C	30.60	C

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$. PSB 0 and PSB 1 refer to control and soil application, B 0, B 1 and B 2 refer to control, foliar application and soil application, Mn 0, Mn 1 and Mn 2 refer to control, foliar application and soil application.

Table 5: Influence of PSB × B × MN on three-year alfalfa.

PBS	B	MN	Yield		Lateral branches		Stem Protein content		LEaf Protein content	
0	0	0	12.22	R	7.40	O	17.59	N	22.25	K
0	0	1	15.89	O	12.80	M	18.92	M	27.24	I
0	0	2	13.56	Q	9.40	N	17.79	N	25.50	J
0	1	0	21.82	K	19.80	I	21.95	J	33.21	F
0	1	1	27.55	G	24.67	F	25.82	G	34.88	CD
0	1	2	26.07	H	23.40	G	24.26	H	34.54	DE
0	2	0	20.04	L	18.20	J	21.31	K	32.77	F
0	2	1	25.07	I	22.20	H	23.87	H	34.40	DE
0	2	2	23.22	J	20.73	I	22.55	I	33.58	EF
1	0	0	14.74	P	11.80	M	18.42	M	26.19	J
1	0	1	18.11	M	15.53	K	20.02	L	30.69	G
1	0	2	17.00	N	14.07	L	19.57	L	29.02	H
1	1	0	30.15	E	26.40	E	26.98	E	35.45	BCD
1	1	1	36.11	A	34.07	A	30.33	A	36.96	A
1	1	2	34.67	B	31.47	B	29.72	B	36.71	A
1	2	0	28.60	F	25.47	EF	26.39	F	35.12	CD
1	2	1	33.74	C	30.20	C	29.12	C	36.33	AB
1	2	2	32.44	D	28.00	D	28.55	D	35.85	ABC

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$, PSB 0 and PSB 1 refer to control and soil application, B 0, B 1 and B 2 refer to control, foliar application and soil application, Mn 0, Mn 1 and Mn 2 refer to control, foliar application and soil application

Table 6: Influence of PSB × B × MN on five-year alfalfa.

PBS	B	MN	Yield		Lateral branches		Stem Protein content		Leaf Protein content	
0	0	0	11.11	Q	6.87	N	9.56	K	20.74	L
0	0	1	14.11	MN	12.40	L	12.62	HI	22.24	IJ
0	0	2	12.07	OP	9.40	M	11.04	J	21.13	KL
0	1	0	18.48	J	19.00	IJ	14.39	EFG	23.90	FG
0	1	1	23.00	FG	22.87	EFG	15.22	CDE	26.93	D
0	1	2	21.67	GH	21.80	FG	15.12	C.F	25.69	E
0	2	0	17.59	JK	17.60	J	13.81	FGH	23.37	GH
0	2	1	20.44	HI	21.07	GH	15.00	DEF	25.50	E
0	2	2	19.04	IJ	19.80	HI	14.63	DEF	24.36	F
1	0	0	13.19	NO	10.93	LM	12.10	IJ	21.82	JK
1	0	1	16.26	KL	15.07	K	13.22	GHI	23.05	H
1	0	2	15.26	LM	14.27	K	13.05	GHI	22.67	HI
1	1	0	24.92	DE	24.33	DE	15.58	B.E	27.47	D
1	1	1	30.30	A	31.00	A	18.84	A	30.51	A
1	1	2	28.15	B	28.80	B	16.68	B	30.12	AB
1	2	0	23.96	EF	23.40	EF	15.37	B.E	27.21	D
1	2	1	27.30	BC	27.53	BC	16.45	BC	29.41	B
1	2	2	26.08	CD	25.87	CD	16.00	BCD	28.65	C

Means in a column followed by the same letter are not significantly different at $P \leq 0.05$. PSB 0 and PSB 1 refer to control and soil application. B 0, B 1 and B 2 refer to control, foliar application and soil application. Mn 0, Mn 1 and Mn 2 refer to control, foliar application and soil application

Protein content was directly related to the dynamic of the dry matter accumulation (Lemaire *et al.,* 1994). Protein content in stem was significantly ($P<0.001$) affected by B × Mn × PSB interaction; however, protein content in leaves was not significantly affected. In one-year, three-year and five-year old alfalfas, the highest protein content in stem and leaves was increased as the result of treatments application. This increment could have various reasons. Turn *et al.* (2010) reported that boron application increased the content of N, P, K, Fe, Mg and Mn in alfalfa tissue, so one of the most important factors to determine the protein content in plant is nitrogen; increasing N content in plant by boron as the result of N application will consequently increase protein content. On the other hand, increasing the number of harvests in a season by cutting at five weeks stage improves the crud protein content (Hesterman *et al.,* 1993). In this experiment, the harvested was conducted every 33 days. Results of this experiment indicated that the co-application of B (foliar) × Mn (foliar) × PSB resulted in the highest stem protein content in one-year (24.68%), three-year (30.33%) and five-year (18.84%) alfalfas; the control treatment resulted in the lowest value of this trait (9.38%, 17.59% and 9.56%, respectively; Table 4). Application of B (foliar) × Mn (foliar) × PSB in one-year, three-year and five-year alfalfa increased leaf protein content by 219.04%, 66.11% and 47.10%, respectively, compared with the control (Table 4).

CONCLUSION

Application of boron, manganese and phosphorus solubilizing bacteria, together, significantly increased yield, lateral branches and protein content. Differences in the three fields may be attributed to different soil conditions and age of plants.

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