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RESPONSE OF ALFALFA FORAGE TO PHOSPHORUS, BORON AND MANGANESE BASED FERTILIZING SYSTEMS

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ABSTRACT

This study was conducted to explore the effect of phosphate solubilizing bacteria (PSB), boron (Borax and Boric acid) and manganese (manganese chelate and manganese sulfate) based fertilizers on forage production of alfalfa. Experiment was laid in a split plot factorial in the form of randomized complete block design with phosphorus bio-fertilizer rates (0 and 100 $g.ha^{-1}$) in main plots, while boron (0, Borax foliar application of 1.0 $lit.ha^{-1}$ and Boric acid soil application 10 kg. $ha^{-1)}$ and manganese fertilizer (0, manganese chelate foliar application 1.0 $lit.ha^{-1}$ and manganese sulfate soil application 10 kg. $ha^{-1)}$ were used in sub plots. The results showed that the highest dried forage yield was obtain by boron and manganese foliar application and bio-phosphorus utilization about 24.00 t/ha. Thus, triple application of PSB, Mn and B (100 $g.ha^{-1}$ as soil application and 1.0 $lit.ha^{-1}$ of each Mn and B solutions) could be recommended as the best treatment for improve forage yield of alfalfa (cv. Ranger) under the experiment environmental conditions.

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1 Introduction

Perennial crops such as Alfalfa (*Medicago sativa* L.) play an important role in sustainable and dynamic agriculture and human food production. With the attention given to sustainable agriculture in recent decades, there is renewed interest in legumes and cropping systems (Sweeney et al., 2011). Alfalfa is one of the most important forage crops in world and also in Iran. Due to its high yield potential, protein content and palatability, it is widely grown in almost all regions of Iran (Hosseinirad et al., 2013).

For sustainable production of Alfalfa, proper management of biomass yield and total dry matter, soil and foliar application of fertilizers management are required. Along with various fertilizing agents, production of alfalfa is also limited by the deficiency of Boron (Marschner, 1995; Dear & Wiear, 2004). According to Marschner (1995) boron is one of the micronutrient that required for the normal growth and development of plants and boron deficiency can be reduced the crop yield. Further, Dear & Wiear (2004) reported that Alfalfa needs more boron as compared to barley crop. Sugar transport, cell wall stability, photosynthesis, nucleic acid metabolism, respiration metabolisms and hormonal activity are the most important plant metabolisms in that boron play a major role (Welch, 1995, Marschner, 1995).

As good as boron, manganese is another necessary important element for the growth and developments of most crop plants (Zhengguo et al., 2009) and its deficiency retarded the crop production in many areas of the world (Eleyan et al., 2014). It is well reported that manganese played a key roles in photosynthesis, oxidation-reduction (redox) and metabolism of nitrogen in plants (Sadana et al., 2005; Inal & Gunes, 2008). Further, Shenker et al. (2004) reported that an optimal amount of Mn increased growth and chlorophyll content of tomato plants. The use of manganese sulfate fertilizer improves crop production and plant photosynthesis because Mn is an activator of many enzymes in plant.

In case of fertilizers, peoples are more concern only about the use of nitrogen fertilizers; very few farmers have knowledge about the use of phosphorus fertilizers. Application of phosphorus fertilizer is also essential for crops (Aulakh et al., 2003). Influence of phosphorus fertilization on agronomic performance of alfalfa has been well documented by various researchers (James et al., 1995; Li et al., 1998; Malhi et al., 2001; Reid et al., 2004).

Excess uses of chemical fertilizers are the major problems for modern agriculture system and these are responsible for water and soil contamination. Farmers are seeking for a viable alternative of chemical fertilizers. Phosphate solubilizing bacteria can be applied as safer sources of phosphorus instead of chemical phosphorus fertilizers. Phosphorus can be considered as a growth limiting nutrition due to its low availability to plants because its low solubility and fixation in soil (Soon, 1990; Thomazi et al 1990; Nahas, 2007). Phosphate solubilizing microorganisms have solved this problem to large extend by dissolving insoluble phosphate via production of inorganic or organic acids or by decrease of pH and bring about the dissolution of bound forms of phosphate, providing available phosphate that can be taken by the plants (Jana, 2007; Mohammadi & Sohrabi, 2012; Mardad et al., 2013). Among various phosphorus solubilizing microorganisms, phosphate solubilizes bacteria are very useful natural microbes that they can change the soil mineral phosphate to soluble phosphorus by production of organic acids and phosphatases acid (Rodriguez & Fraga, 1999). Pseudomonas as one of the most important plant growth-promoting bacteria (Singh et al., 2005; Malboobi et al., 2009) can make 70% of unavailable phosphorus, readily available for plant absorption (Chen, 2006; Kothamasi et al., 2006).

Present study was conducted to explore the effect of simultaneous application of boron, manganese and phosphorus solubilizing bacteria on the nutritional composition and plant growth and development of alfalfa crop in Iran environmental condition.

2 Materials and Methods

2.1 Experimental Site and soil conditions

This experiment was conducted in 2013 at the research farm of Marv-Dasht in central areas of Fars province, Iran (29° 52' S, 52° 48' N). This site located at 1605 meters above sea level with average annual rainfall of 320 mm in 2011 and 2012. The maximum and minimum temperatures are 41 and 9 °C, respectively. Initially, field under cultivation of Alfalfa (First year of growing of perennial Alfalfa) were selected for study. Soil samples were collected from the 30 cm depth and analyzed for estimation of soil physicochemical properties according to the methods described by Ryan et al. (2001). The Soil chemical properties are presented in Table 1. The 10 year average annual precipitation ranged from 115-141 mm and the annual average temperature is 25-32^{o}C (Hosseinirad et al., 2013).

Table 1. Chemical and physical properties of the soil at the experimental site.

Depth (cm)	Soil texture	pH se	N (%)	P (ppm)	K (ppm)	B (ppm)	Mn ppm)	OC %	Ec (ms)
0-30	CL*	7.5	0.1	15	257.9	0.67	6.1	0.37	0.96

CL, Clay loam; OC, Organic Matter.

2.2 Experimental design

The treatments were Phosphate solubilizing bacteria (PSB), boron (Borax and Boric acid) and manganese (manganese chelate and manganese sulfate). Experiment was laid in a split plot factorial in the form of randomized complete block design that phosphorus bio-fertilizer rates (0 and 100 $g.ha^{-1}$) in main plots and boron (0, Borax foliar application of 1.0 $lit.ha^{-1}$ and Boric acid soil application 10 kg. $ha^{-1)}$ and manganese fertilizer (0, manganese chelate foliar application 1.0 $lit.ha^{-1}$ and manganese sulfate soil application 10 kg. $ha^{-1)}$ were in sub plots.

Phosphorus bio-fertilizer treatments was a combination of phosphorus solubilizing bacteria *Pantoea agglomerans* strains P5 and *Pseudomonas putida* strain P13 included $1*10^9$ CFU bacteria per 100 grams bio-fertilizer that registered as Barvar II®. For PSB treatments, 100 $g.ha^{-1}$ PSB was mixed in 400 liters of irrigation water and applied manually in soil as fertigation. Boric acid and manganese chelate were foliar application at 2 weeks after each cut stage and Borax and manganese sulfate were applied in soil after the first cut of alfalfa manually in spring.

The Alfalfa cultivar used in this study was *Medicago sativa* L. cv. Ranger. Total Eighteen plots were imposed in each blocks and a net plot area was 15 m2 (3.0m in 5.0m). The irrigation method was conducted by using classic sprinkler irrigation.

2.3 Sampling and measurements

Plants were harvested when 10% of fields crop have 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. After harvesting, the samples were dried in electrical oven (72 hours at 40°C) and then weighted for forage yield. Samples were sent to the laboratory for determination of nitrogen and protein content by method described by Nelson & Sommers (1973). Lateral branches of plants were counted manually for each plot.

2.4 Statistical analysis

The achieved data in experiment subjected to the analysis of variance method using SAS (version 9.1.3, 2004) software the means were compared based on Duncan multiple test at 5% level.

3 Results and Discussion

3.1 Forage yield

Alfalfa forage yield was significantly influenced by the application of PSB, B and Mn fertilizers either individually or in combination. Results of individual application are represented in table 2. Results of study suggested that forage yield improved by adding B, Mn and Phosphorus solubilizing bacteria (PSB) especially when Mn was applied. At individual application, highest yield was obtained by using B as foliar application (20.01 $t.ha^{-1}$) and soil application B. While the lowest one was related to treatments without any fertilizer (10.40 $t.ha^{-1}$) and highest yield giving treatment represents 119.58 % increase in forage yield. Boron fertilizer applying in both forms (soil/foliar), made an increase in forage yield (Table 3). Like B, application of PSB also improved plant growth and it is not significantly differ that the foliar application of boron (Table 3). Also, result showed that the interaction of PSB1.BI (22.43 t.ha-1) and B1.Mn1 (21.61 t.ha-1) were suitable for producing higher forage yield and it shows superiority over the individual application but are not significantly different.

Using boron whether foliar or soil application without using of PSB didn't show notable effect on forage yield increasing but simultaneous application of boron and PSB resulted 110.06% in production increasing. In interaction, combined application of PSB1X B1 X Mn1 shows superiority (34 $t.ha^{-1}$) over the individual or combined application of any two fertilizers (Table 4), and also this treatment increased forage (103.31%) production in comparison to sample.

Table 2 Means comparison of PSB. B and Mn on studied traits in alfalfa.

SOV	Df	Forage yield	Lateral branches	Nitrogen of stem	Nitrogen of leaf	Protein of stem	Protein of leaf
R	2	0.035	25020	0.035	0.019	1.394	0.744
PSB	1	7.826**	4417237**	6.892**	9.902**	269.219**	386.826**
Error(PSB)	2	0.021	1114	0.005	0.001	1.107	1.055
B	2	15.354**	6989712**	7.265**	36.409**	283.818**	1422.248**
B.PSB	2	0.79**	163136**	0.813**	0.748**	31.781**	29.253**
Mn	2	1.358**	902826**	1.111**	1.11**	43.425**	43.386**
Mn.PSB	2	0.008ns	29007*	0.001ns	0.022ns	0.048ns	0.892ns
B.Mn	4	0.083**	11138ns	0.091**	0.026ns	3.584**	1.037ns
B.Mn.SB	4	0.039*	24659*	0.056*	0.054*	2.05*	1.88*
Error	32	0.013	9068	0.018	0.017	0.735	0.667
C.V.		3.46	7.68	5.37	3.67	5.37	3.67

*,**Significant at P=0.05 and P=0.01 levels, respectively. ns: Not Significant. CV: Coefficient Variation

Table 3 Means comparison of PSB. B and Mn for treats of alfalfa

treatments	Forage Yield $t.ha^{-1}$	Lateral Branches # ha^{-1}	Nitrogen of stem %	Nitrogen of leaf %	Protein of stem %	Protein of leaf %
PSB0	14.73 b	954.73 b	2.19 b	3.13 b	13.73 b	19.57 b
PSB1	18.54 a	1526.7 a	2.91 a	3.98 a	18.19 a	24.92 a
B0	11.37 c	530.86 c	1.84 b	1.92 b	11.51 c	12.03 b
B1	20.01 a	1697.53 a	3.06 a	4.50 a	19.14 a	28.18 a
B2	18.53 b	1493.83 b	2.75 ab	4.24 a	17.24 ab	26.53 ab
Mn0	15.19 b	1008.64 c	2.29 a	3.29 a	14.32 b	20.61 b
Mn1	17.93 a	1455.55 a	2.78 a	3.79 a	17.41 a	23.70 a
Mn2	16.76 ab	1258.02 ab	2.58 a	3.58 a	16.15 a	22.42 ab
PSB0 B0	10.68 d	353.09 f	1.73 f	1.73 d	10.81 f	10.83 d
PSB0 B1	17.59 b	1340.74 c	2.57 c	3.96 b	16.07 c	24.74 b
PSB0 B2	15.94 c	1170.37 d	2.29 d	3.71 b	14.31 d	23.16 c
PSB1 B0	12.07 d	708.64 e	1.95 e	2.12 c	12.21 e	13.24 d
PSB1 B1	22.43 a	2054.32 a	3.55 a	5.06 a	22.21 a	31.63 a
PSB1 B2	21.12 a	1817.28 b	3.22 b	4.79 ab	20.17 b	29.91 a
PSB0 Mn0	13.33 e	767.90 d	1.94 f	2.90 e	12.15 f	18.16 d
PSB0 Mn1	16.11 c	1155.56 c	2.42 d	3.36 cd	15.14 d	21.02 c
PSB0 Mn2	14.76 d	940.74 d	2.22 e	3.12 d	13.90 e	19.52 d
PSB1 Mn0	17.06 bc	1249.38 c	2.64 c	3.69 c	16.50 c	23.06 b
PSB1 Mn1	19.75 a	1755.56 a	3.15 a	4.22 a	19.68 a	26.39 a
PSB1 Mn2	18.81 b	1575.31 b	2.94 b	4.05 b	18.40 b	25.31 a
B0 Mn0	10.63 e	314.81 f	1.68 f	1.73 e	10.51 f	10.81 e
B0 Mn1	12.28 d	759.26 e	1.97 e	2.15 d	12.34 e	13.45 d
B0 Mn2	11.22 e	518.52 e	1.87 e	1.89 e	11.68 e	11.81 de
B1 Mn0	17.91 c	1414.81 c	2.65 cd	4.18 bc	16.56 cd	26.13 b
B1 Mn1	21.61 a	1925.93 a	3.41 a	4.78 a	21.28 a	29.89 a
B1 Mn2	20.52 a	1751.85 b	3.13 b	4.56 a	19.58 b	28.52 a
B2 Mn0	17.06 c	1296.30 d	2.55 d	3.98 c	15.91 d	24.89 c
B2 Mn1	19.91 b	1681.48 b	2.98 b	4.44 b	18.62 b	27.76 b
B2 Mn2	18.63 b	1503.70 c	2.75 c	4.30 b	17.19 c	26.93 b

Means in same columns followed by the same letter were not significantly different at the 5% level; PSBO (Treatment without P biofertilizer); B0 (Treatment without Borax); Mn0 (Treatment without Mn); B1 (Foliar application of 1 lit/ha borax); B2 (Soil application of 10 kg/ha boric acid); Mn1 (Foliar application of Mn @ 1 lit/ha); Mn2 (Soil application of manganese sulfate @10 kg. ha).

These result were coincide with findings of Turan et al. (2010), who clarified that the highest yield of Alfalfa was obtained by using boron fertilizers. Similarly, Ahmed et al. (2010) reported that usage of bio-fertilizers which contains Azotobacter and Pseudomonas significantly have raised production of sorghum. Further, Kohno et al. (2008) reported a meaningfully increased in bean yield by using of manganese fertilizers.

3.2 Number of lateral branches

Alfalfa lateral branches per hectare are an effective agent in Alfalfa forage yield enhancing. It was significantly ($P<0.05$) influenced by three factors as well as interactions (table 2). The highest number of lateral branches was obtained from triple interaction of B (Foliar), Mn (Foliar) and PSB by 2340.74 branches ha^{-1} while the lowest one was obtained in control by 592.96 branches ha^{-1}) showing more 300 % increase in the number of lateral branches (Table 4). PSB and B rate significantly affected the Number of lateral branches ($P<0.001$). The best treatment in PSB and B interaction was recorded from PSB1B1 (Table 3). The Number of lateral branches in this interaction showing a colossal 481.81% increase compared to PSB0X B0 (Table 3). Also, PSB and Mn interaction effects was significantly affected the Number of lateral branches ($P<0.05$). The best treatment in PSB and Mn interaction was recorded from PSB1Mn1, so this treatment was caused 128.61% increase compared to P0Mn0 (Table 3). It can be concluded that PSB can increase the availability of phosphorus for producing high number lateral branches per hectare. The treatments containing PSB evidently performed better than the treatment without PSB application.

Application of phosphorus often caused re-growing of shoot parts of Alfalfa in absence drought stress condition (Jiang et al., 2009). The results identified that deficiency of boron in some plants such as Alfalfa, Red Clover, Turnip and some varieties of Cabbage will cause to reduction of production because they need higher amount of this element (Tariq & Mott, 2007; Sherrell & Toxopeus, 2012). Further, Reinbott & Blevins (2008) reported that application of boron and manganese increased number of branches in soybean crops which as a result made increasing in yield and yield component in this plant.

3.3 Nitrogen content in stem and leaf

Nitrogen is quantitatively most important nutrient that plants obtain from soil. Well-managed Alfalfa crop effectively absorb mineralized N from fertilizer and remove residual nitrate from the subsoil, irrigation water, and fix less N from the air. The value of legumes nitrogen fixing for improving production and higher yield can be achieved to balance soil elements by the application of bio-fertilizers (Kannaiyan, 2002). When two or more elements have a positive interaction with each other, synergistic effects occur. In this experiment, the synergistic relationship between the elements boron, manganese and phosphorus has been created so that these elements have been increased nitrogen uptake by Alfalfa. Triple interaction of B, Mn and PSB had significant effect ($P<0.05$) on nitrogen content in both stem (3.94%) and leaf (5.37%) tissues while the minimum was gained from control (without any fertilizer) (1.5% and 1.54%) showing a colossal 162.97 % and 248.62% increase in the N of stem and leaf, respectively (Table 4). At individual application, B is showing superiority over the other treatments while in combination of two, combined application of PSB and B is superior to all other treatments (Table 3). Dual interaction of B. PSB, the best result of leaf nitrogen content was pertained to B1.PSB1 (Table 3). Furthermore, interaction of B X PSB and B X Mn on nitrogen content in alfalfa stems were significant (Table 2), also, the interaction between boron and phosphorous increased 105.4% nitrogen content in stem rather than treatment B0PSB0 (Table 3). Some studies have recognized a positive correlation between nitrogen accumulation and utilization of phosphorous fertilizer (Waluyo et al., 2004; Turan et al., 2010). Therefore phosphorous not only have important role on forage production but also increased production with intensification of absorbing some elements such as nitrogen. Wicz (2000) studied 38 cultivars of Alfalfa and identified that more appropriate soil fertility and nitrogen concentration in plant tissues have led to better protein digestion and absorption by livestock.

3.4 Protein content in stem and leaf

Measurement of protein content as qualitative parameters in alfalfa forage is one of the most important ways to survey Alfalfa standards forage quality. Protein content can estimated through multiplying the nitrogen content by a coefficient conversion (Mariotti et al., 2008). Moreover, Protein content was directly correlated to the dry matter accumulation (Lemaire et al., 1994).

Table 4. Interaction effect of PSB. B. Mn for treats of alfalfa.

Treatment	Forage Yield $t.ha^{-1}$	Lateral Branches # ha^{-1}	Nitrogen of stem %	Nitrogen of leaf %	Protein of stem %	Protein of leaf %
PSB0 B0 Mn0	20.40 ^{f}	162.96 ^{g}	1.50 ^{g}	1.54 ^{g}	9.38 ^{h}	9.63 ^{h}
PSB0 B0 Mn1	21.55 ^{e}	629.63 ^{e}	1.93 ^{ef}	2.02 ^{f}	12.07 ^{g}	12.62 ^{g}
PSB0 B0 Mn2	20.07 ^{f}	266.67 ^{f}	1.758 ^{f}	1.64 ^{g}	10.98 ^{h}	10.22 ^{h}
PSB0 B1 Mn0	25.37 ^{d}	1125.93 ^{d}	2.22 ^{de}	3.67 ^{de}	13.88 ^{e}	22.91 ^{de}
PSB0 B1 Mn1	29.22 ^{c}	1511.11 ^{bc}	2.86 ^{c}	4.19 ^{c}	17.89 ^{c}	26.21 ^{c}
PSB0 B1 Mn2	28.18 ^{c}	1385.19 ^{c}	2.63 ^{d}	4.01 ^{cd}	16.45 ^{cd}	25.09 ^{cd}
PSB0 B2 Mn0	24.22 ^{d}	1014.82 ^{d}	2.11 ^{e}	3.51 ^{e}	13.20 ^{f}	21.95 ^{e}
PSB0 B2 Mn1	27.56 ^{c}	1325.93 ^{c}	2.47 ^{d}	3.88 ^{d}	15.47 ^{d}	24.25 ^{d}
PSB0 B2 Mn2	26.04 ^{d}	1170.37 ^{d}	2.28 ^{de}	3.72 ^{d}	14.27 ^{e}	23.27 ^{d}
PSB1 B0 Mn0	20.85 ^{f}	466.67 ^{f}	1.86 ^{f}	1.92 ^{g}	11.64 ^{g}	12.00 ^{g}
PSB1 B0 Mn1	23.00 ^{e}	888.89 ^{e}	2.02 ^{e}	2.28 ^{f}	12.62 ^{f}	14.29 ^{f}
PSB1 B0 Mn2	22.37 ^{e}	770.37 ^{e}	1.98 ^{e}	2.15 ^{f}	12.38 ^{fg}	13.41 ^{f}
PSB1 B1 Mn0	30.44 ^{b}	1703.7 ^{b}	3.08 ^{bc}	4.69 ^{b}	19.24 ^{bc}	29.36 ^{b}
PSB1 B1 Mn1	34.00 ^{a}	2340.74 ^{a}	3.95 ^{a}	5.37 ^{a}	24.68 ^{a}	33.58 ^{a}
PSB1 B1 Mn2	32.85 ^{a}	2118.52 ^{a}	3.63 ^{a}	5.11 ^{a}	22.72 ^{a}	31.95 ^{a}
PSB1 B2 Mn0	29.89 ^{bc}	1577.78 ^{b}	2.98 ^{c}	4.45 ^{bc}	18.63 ^{c}	27.84 ^{c}
PSB1 B2 Mn1	32.26 ^{a}	2037.04 ^{a}	3.48 ^{b}	5.01 ^{ab}	21.77 ^{b}	31.29 ^{a}
PSB1 B2 Mn2	31.22 ^{b}	1837.04 ^{b}	3.22 ^{b}	4.89 ^{b}	20.12 ^{b}	30.59 ^{b}

Means in same columns followed by the same letter were not significantly different at the 5% level; PSBO (Treatment without P biofertilizer); B0 (Treatment without Borax); Mn0 (Treatment without Mn); B1 (Foliar application of 1 lit/ha borax); B2 (Soil application of 10 kg/ha boric acid); Mn1 (Foliar application of Mn @ 1 lit/ha); Mn2 (Soil application of manganese sulfate @10 kg. ha).

Interaction effects of B, Mn and PSB had signification effect on stem and leaf protein contents ($P<0.05$). Results revealed that factors which had significant differences in term of nitrogen content in leaf and stem were also had significant differences in term of leaf and stem protein this is the endorsement of the validity of this study which indicated coronation between amount of plant protein and amount of nitrogen in plant tissues.

The highest protein content of stem (24.67%) and leaf (33.58%) was recorded from B (Foliar), Mn (Foliar) and PSB combination (Table 4) while the lowest one are 9.38% and 9.63% was gained from control showing a colossal 162.95% and 248.6% increase in protein content of stem and leaf, respectively (Table 4). The highest protein content in alfalfa leaf was obtained by B and PSB interaction was concerned to B1PSB1 treatment (31.63%).

Results of current study revealed B1PSB1 raised 199.22% of leaf protein in comparison to B0PSB0 significantly. Interaction of B X PSB and BX Mn also had significant effect on protein content of alfalfa stem tissues. The highest stem protein was recorded in B and PSB interaction treatment in B1PSB1 level about 22.21% by means of increased 105.4% rather than B0PSB0 (Table 3). Furthermore, The highest interaction between B and Mn was obtained from B1Mn1 treatment by 22.28% and 102.47% growth in stem protein content in comparison to B0Mn0(Table 3).

Since no information was available about reaction of alfalfa ranger cultivar to studied fertilizers.

Results of study recognized the amount fertilizers include boron, manganese and phosphorus solubilizing bacteria which studied in this research was appropriate because not only the studied treatments didn't show any sign of toxic effects or nutrient deficiency but also they caused notable increasing in alfalfa production and other related characteristics. Also foliar application of boron and manganese was detected as a better method rather than soil application. It was decided that micronutrients elements to be used with foliar application and soil application and explore the effect of these fertilizers on the various growth and growth attributes with minimum toxicity.

Conclusion

Foliar application of boron, manganese and soil application of phosphorus solubilizing bacteria simultaneously, could be improved alfalfa forage yield significantly and substantially. These elements not only individually improved forage production quality and quantity but also interaction of these three showed superiority advanced in alfalfa forage yield. As an important outcome utilization of mentioned fertilizer management system is recommended for fill the gap between the alfalfa potential yield and average alfalfa forage production in the same condition.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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