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Exploring Responses of Berseem Clover Cultivars in Low Input Cultivation Management for Agricultural Sustainability

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ABSTRACT

Berseem clover (*Trifolium alexandrinum* L.) is one of the most important forage crops in Iran. In order to determine yield, yield components and qualitative characteristics of two cultivars of berseem clover in low input nitrogen farming in 2015 and 2016, two factor randomized complete block design with split plot combined analysis over time and four replications was used in Isfahan, Iran. The cultivars were Sacronomt and Multicut, and the nitrogen application levels were 10, 40 and 70 kg ha^{-1}. Herein, higher values of plant height, total fresh yield, total dry yield, nitrate, protein, organic matter and ash were seen in 2015, yet, higher phosphorous, potassium, Fe, Mn, Zn and Cu were evident in 2016. Moreover, Sacromont cultivar generated higher values of total fresh yield, total dry yield and Fe, while higher values of plant height, nitrate, protein, phosphorous, potassium, organic matter, ash, Mn, Zn and Cu were noted in Multicut. Increasing the nitrogen fertilizer application rate from 10 to 70 kg N ha^{-1} resulted in numerically increased plant height, total fresh and dry yield. Maximum nitrate, protein, potassium, organic matter, ash and Zn levels were obtained in an application of 70 kg N ha^{-1}. Over all, nitrate concentration in the crop increased step by step with the addition of nitrogen fertilizer. However, the highest values of phosphorous, Mn and Cu were achieved in an application of 40 kg N ha^{-1}. Nitrogen fertilizer management is, thus, one of the most important inputs in achieving high productivity and yield

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of berseem clover. The appropriate management of nitrogen is, therefore, important in cropping systems and a balance between applied levels is essential for normal plant growth and human consumption. All in all, both forage crop production and quality is vital for successful animal production in sustainable agriculture systems. On the basis of the results of this study, it could be concluded that application of 70 kg N ha^{-1} is good for both cultivars.

Keywords**:** Berseem clover, Cultivar, Nitrogen, Sustainable Agriculture, *Trifolium alexandrinum*

1. INTRODUCTION

One of the most important leguminous forages in both the middle east and the Mediterranean region is Berseem clover (*Trifolium alexandrinum L*.) (Di Santis et al., 2004; Vasilakoglou and Dhima, 2008; Etesami et al., 2013; Francchiolla et al., 2018).

Photo 1. *Trifolium alexandrinum* L.

Berseem clover is well-known as king of forages, because of its high production potential, easy cultivation, capacity to fix enormous amounts of atmospheric nitrogen and of course quick growth (Pecetti et al., 2012). Berseem clover is one the most important forage crops which had widely grown in Iran (Ranjbar, 2007; Soleymani et al., 2011). Nitrogen availability is one of the most important factors during plant growth and development (Soleymani and Shahrajabian, 2013; Abdollahi et al., 2018; Shahrajabian et al., 2018; Ogbaji et al., 2018; Yong et al., 2018). A high nitrogen content can lead to low efficiency of nitrogen use by animals with high losses in waste from animals fed grazed; while a high amount of nitrogen may benefit animal production response (Soleymani and Shahrajabian, 2011; Shahrajabian and Soleymani, 2017; Yong and Shahrajabian, 2017; Yong et al., 2017). Berseem clover has a high capacity for biological nitrogen fixation through mineralized root and nodules (Soleymani et al., 2011).

Rathod et al. (2002) revealed that application of 80 kg N ha^{-1} to be the optimum dose to increase green fodder as well as dry forage yield. Sharma and Sharma (2004) indicated that the application of nitrogen significantly increased green forage yield. Soleymani et al. (2011) was reported the significant effect of nitrogen on potassium, organic matter percentage, Fe and Mn content. Soleymani and Shahrajabian (2012) noticed that the total fresh yield, total dry yield and total protein of plant were significantly increased by application of nitrogen fertilizer.

Soleymai and Shahrajabian (2013) reported the meaningful impact of nitrogen on total fresh yield, total dry yield, protein, ash and nitrate of maize. Sing et al. (Singh et al., 2009) showed that the maximum ash content in popcorn, white corn, sweet corn and dent corn was 2.7%, 1.2%, 1.8% and 1.2%, respectively. Soleymani et al. (2011) showed that Sacromont had the highest contents of phosphorus, potassium and organic matter. The main purpose of this research was to evaluate the influence low nitrogen fertilizers on forage yield and quality parameters of two cultivars of berseem clover in semi-arid part of Iran.

2. MATERIALS AND METHODS

An experiment was conducted at Mahoodabad Experimental Field in Isfahan in 2015 and 2016 (latitude 32º40′ N, longitude 51º48′ E, and 1570 m elevation), in order to determine yield, yield components and qualitative characteristics of two cultivars of berseem clover in low input nitrogen farming. Two Factors Randomized Complete Block design with split plot combined analysis over years with four replications was used. Cultivars were Sacronomt and Multicut, and the nitrogen levels included 10, 40 and 70 kg/ha. The nitrogen fertilizer was provided from urea source (46% pure N). Long-term average precipitation was 150 mm. A week before sowing, soil sample was taken at the depth of 0-30 cm, and soil texture was clay loam (Table 1). The soil preparation consisted of mouldboard ploughing (25 cm) followed by discing and smoothing with a land leveller. Ditches were designed separately for each replication. Each experimental plot had 5 rows with 6 m length.

The distance between rows of forage corn was 100 cm and cultivars of berseem clover were sown on the basis of 20 kg seeds per ha by skilful workers. The first irrigation was done right after seed plantation, and the second irrigation was done two weeks after first irrigation for better seedling establishment; other irrigation interval was 8 weeks. Because of soil richness in P and K, no phosphorus and potassium fertilizers were used in the research field. Weeds were controlled by hand weeding by skilful workers. Clover cultivars were harvested at 15% of flowering stage, and the second cut was used as green manure for sustainable agriculture. In this trial, rows number one and three and also 50 cm primer and edge lines were discarded from sampling, and lines number 2 and 4 were selected for final sampling.

The forage samples were dried at 60 ºC for 48 hours, then weighed and ground to pass a 1-mm screen prior to laboratory analysis. Forage samples were ground in a hammer mill with a 1 mm screen and then analyzed in triplicate for dry matter, protein, ash and nitrate. Nitrate content was also determined by spectrophotometer (410 nm) and salicylic acid method. The total protein content was calculated by amount of nitrogen with Micro-Kjeldahl method (nitrogen percentage × 6.25). Ash content determined by incinerating the samples in a muffle furnace at 550 ºC for 4 hours. All data were analyzed wit analysis of variance (ANOVA) in SAS statistical software. When ANOVA was used to identify treatment effects, Duncan's multiple range test (DMRT) was used to identify which treatments differed at 5% significance level.

Table 1. Soil analysis of experimental farm at 0-30 cm.

S (%)	K a.v.a (mg/kg)	P a.v.a (mg/kg)	N (%)	OC (%)	TNV (%)	pH	EC (ds/m^{-1})	SP	Soil texture
46	398	40.5	0.09	0.89	39	7.66	1.0	55	Clay loam

3. RESULTS AND DISCUSSION

Two years are significantly different for nitrate content. However, none of the experimental characteristics significantly influenced by year. The influence of cultivar was meaningful on plant height, nitrate and phosphorus. Nitrogen fertilizer also had significant effect on plant height, total fresh and dry yield, protein percentage, phosphorous and potassium. There were not any meaningful differences for ash, Fe, Mn, Zn and Cu between years. Although, cultivar has significant effect on ash and Mn, its impact on Fe, Zn and Zu was not meaningful. The influence of nitrogen fertilizer was significant on ash, Fe, Mn and Zn, but Cu was not significantly affected by nitrogen treatments.

The higher value of plant height (54.01 cm), total fresh yield (11.82 t ha^{-1}), total dry yield (1.36 t/ha), protein (17.09%) and organic matter (23.04%) was achieved in 2015, compare to 2016, which had no meaningful differences with each other. Although, the maximum phosphorus (0.32%) and potassium (2.58%) was related to 2016, there was not any meaningful difference between 2015 and 2016. The nitrate content was significantly higher in 2015 (201.30 ppm) compare to its value in 2016 (194.80 ppm) (Table 2). Although, the higher values of total fresh yield (11.69 t ha^{-1}), and total dry yield (1.36 t ha^{-1}) was observed in Sacromont, there was not any meaningful differences between two cultivars. Multicut had obtained the higher values of plant height (56.23 cm), nitrate content (246.70 ppm) and phosphorous (0.35%), which had meaningful differences with Sacromont.

The higher potassium percentage (2.58%) and organic matter percentage (23.91%) was related to Multicut, which had no meaningful differences with Sacromont (Table 2). The higher values for plant height (55.75 cm), total fresh yield (12.09 t ha^{-1}), total dry yield (1.47 t ha^{-1}), nitrate content (242.60 ppm), protein percentage (17.14%), potassium (2.48%), and organic matter (23.92%) was related to application of 60 kg N ha^{-1}, followed by 40 and 10 kg N ha^{-1}. Although, there were not any meaningful differences between treatment for potassium and organic matter percentage, application of 70 kg N ha^{-1} had meaningful differences with other treatments for plant height, total fresh yield, total dry yield and nitrate content.

On the one hand, no meaningful difference between application of 40 kg N ha^{-1} and 70 kg N ha^{-1}; on the other hand, both treatments had significant differences with 10 kg N/ha. The maximum and minimum phosphorous percentage was obtained for 40 kg N/ha (0.32%), and 70 kg N/ha (0.29%), which had significant differences with each other. The phosphorous content in application of 10 kg N ha^{-1} was 0.31%, and it has just significant difference with application of 40 kg N ha^{-1} (Table 2).

Table 2. Mean comparison for experimental characteristics.

Treatment	Plant height (cm)	Total fresh yield (t ha^{-1})	Total dry yield (t ha^{-1})	Nitrate (ppm)	Protein (%)	Phosphorous (%)	Potassium (%)	Organic matter (%)
Year								
2015	54.01a	11.82a	1.36a	201.30a	17.09a	0.31a	2.32a	23.04a
2016	53.90a	11.48a	1.33a	194.80b	16.47a	0.32a	2.58a	22.91a
Cultivar								
Sacromont	51.69b	11.69a	1.36a	149.40b	16.26a	0.26b	2.31a	22.04a
Multicut	56.23a	11.60a	1.33a	246.70a	17.29a	0.35a	2.58a	23.91a
Nitrogen (kg/ha)								
10	52.24c	10.98c	1.24c	142.90c	16.08b	0.31b	2.39a	22.04a
40	53.89b	11.88b	1.35b	208.64b	17.11a	0.32a	2.47a	22.97a
70	55.75a	12.09a	1.47a	242.60a	17.14a	0.29b	2.48a	23.92a

Common letters within each column do not differ significantly.

Except ash percentage, the higher values of Fe (948.1 ppm), Mn (47.15 ppm), Zn (14.79 ppm), and Cu (15.15 pm) was obtained for 2016 compare those of related to 2015. However, ash percentage in 2015 (17.33%) was higher compare to its value in 2016 (17.04%).

There were not found any significant differences on experimental characteristics between 2015 and 2016 (Table 3). Multicut had obtained the higher values of ash percentage (17.94%), and Mn (51.06 ppm), which had meaningful differences with Sacromont.

Ash percentage and Mn content of Multicut was 17.94% and 51.06 ppm, respectively. Although, the higher value of Zn (15.17 ppm), and Cu (15.56 ppm) was related to Multicut, it had no significant differences with Sacromont.

Unlike other experimental characteristics, Fe content in Sacromont (938.4 ppm) was significantly higher than Multicut (910.2 ppm) (Table 3). The higher values of ash percentage (19.06%), and Zn (15.11 ppm) was obtained for application of 70 kg N ha^{-1}, followed by 40 kg N ha^{-1} and 10 kg N ha^{-1}, respectively. There were meaningful differences between 70 kg N ha^{-1} and 10 kg N ha^{-1} for ash percentage and Zn content.

The maximum and the minimum Fe content was achieved in usage of 10 kg N/ha (1077.2 ppm), and 40 kg N ha^{-1} (752 ppm), which had meaningful difference with each other. Fe content was 942.9 ppm for 70 kg N ha^{-1}, which had no significant differences with other treatments. The highest values of Mn (53.72 ppm), and Cu (15.17 ppm) was observed in application of 40 kg N/ha, and the minimum was related to usage 10 kg N/ha. Although, the significant difference was found between 70 kg N ha^{-1} and 10 kg N ha^{-1} in Zn content, application of 70 kg N ha^{-1} had no meaningful difference with 40 kg N ha^{-1}. There were not any significant differences in Cu between treatments (Table 3).

Table 3. Mean comparison for experimental characteristics.

Treatment	Ash (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
Year					
2015	17.33a	900.5a	46.59a	14.78a	14.52a
2016	17.04a	948.1a	47.15a	14.79a	15.15a
Cultivar					
Sacromont	16.44b	938.4a	42.67b	14.40a	14.12a
Multicut	17.94a	910.2a	51.06a	15.17a	15.56a
Nitrogen (kg/ha)					
10	15.50b	1077.2a	39.33c	13.83b	14.22a
40	17.00b	752.7b	53.72a	14.94a	15.17a
70	19.06a	942.9ab	47.56b	15.11a	15.11a

Common letters within each column do not differ significantly.

Appropriate nitrogen fertilizer management in crop production has important impact on both increase and stabilize the final yield in sustainable agriculture, and also will have obvious influence on environmental health. Furthermore, maintaining and improving forage production over time with marinating environmental quality is a challenge to agricultural researchers. Nitrogen is an essential element for plant growth and development; however, due to environmental pollution, high nitrate concentrations accumulate in edible parts of crops, particularly if excessive nitrogen fertilizer has been applied.

Berseem clover is an annual legume which has high productivity due to meaningful high growth rate. Consuming these crops may harm human health; thus, developing a suitable strategy for the agricultural application of nitrogen fertilizer is essential in sustainable agricultural production.

Soleymani et al. (2011) concluded that low input farming system is vital for reducing the risk of agrochemical pollution. The higher values of plant height, total fresh yield, total dry yield, nitrate, protein, organic matter and ash was related to 2015 compare to 2016, however, the higher phosphorous, potassium, Fe, Mn, Zn and Cu was obtained in 2016. On the one hand, Sacromont cultivar had obtained the higher values of total fresh yield, total dry yield and Fe.

On the other hand, the higher values of plant height, nitrate, protein, phosphorous, potassium, organic matter, ash, Mn, Zn and Cu was related to Multicut. Bruun et al. (2010) mentioned that the ash content is very important parameter for the evaluating of crop quality and cultivars. Forage yield and quality of berseem clover cultivars differ among application of different levels of nitrogen fertilizer (Sardana and Narwal, 1999).

The maximum plant height, total fresh yield, total dry yield, nitrate, protein, potassium, organic matter, ash and Zn was obtained in application of 70 kg N ha^{-1}. Balanced application of nitrogen fertilizer may lead to significant increase of agricultural yield (Soleymani and Shahrajabian, 2012a,b). While the highest values of phosphorous, Mn and Cu was achieved in application of 40 kg N ha^{-1}.

The maximum the minimum Fe content was related to 10 kg N ha^{-1}, and 40 kg N ha^{-1}, respectively. Excessive nitrogen fertilization could lead to negative consequences for not only the environment, but also may lead to decrease in low nitrogen use efficiency and nitrate accumulation in crops (Fontana and Nicola, 2009; Shahrajabian et al., 2011). Pieretii (2005) also revealed the importance of nutritive value of forages in agricultural systems. Hurtado et al. (2009) found that both the nutritive value and yield of berseem clover are influenced by changes in levels of nitrogen fertilizers. Sustainability of Iran agriculture may require change from predominant application of high chemical fertilizers, especially nitrogen fertilizers with considering low input farming and sustainable agriculture (Soleymani et al., 2012; Soleymani et al., 2013; Soleymani et al., 2016).

The fundamental parameter to optimize crop yield and production, and suitable management of nitrogen which may lead to balance in the nature is sufficient, adequate and appropriate nitrogen application in agricultural systems. Wang et al. (2007), and Soleymani and Shahrajabian (2012) also illustrated that quality of plant was dependent on growing conditions, especially nitrogen levels in agricultural fields. The appropriate management of nitrogen is important in cropping systems and a balance between applied levels is essential for normal plant growth and human consumption.

4. CONCLUSION

Nitrogen management as well as selecting appropriate cultivars are essential parameters for obtaining high forage quality products with high nutritive value of berseem clover in arid and semi-arid regions. The higher values for total fresh and dry yield achieved by Sacromont. According to the findings of this study, it could be concluded that application of 70 kg N ha^{-1} is good for both cultivars.

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