Asian Journal of Plant Sciences

ISSN 1682-3974
Effects of Nitrogen Fertilizing Systems and Harvest Frequencies on Forage Dry Matter Yield and Quality of Snail Medic (Medicago scutellata Var. Robinson)

A.H.A. Ahmadi, M.R. Chaichi and M. Mirzaazade
Department of Agronomy and Plant Breeding, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Abstract: The study was conducted to determine the effects of nitrogen fertilizing systems and harvest frequencies on DM (dry matter) yield and forage quality of snail medic, Medicago scutellata var. Robinson. Rhizobium meliloti inoculation and four levels of chemical nitrogen fertilizer (0, 25, 75 and 125 kg N ha⁻¹) were allocated to the main plots while four levels of harvest frequencies such as 1, 2 and 3 weeks intervals and control with only one harvest at 50% flowering were assigned to subplots. According to the results of this experiment, nitrogen fertilizing systems had a significant effect (p<0.05) on DM yield and CP (crude protein) concentration of snail medic and these traits increased with the increment in availability of nitrogen for plants. Harvest frequency increased the DM yield, CP and WSC (water soluble carbohydrate) concentrations, whereas decreased the ADF (acid detergent fiber) concentration. Interaction between harvest frequency and nitrogen fertilizer systems was significant (p<0.05) for DM yield and WSC concentration, hence treatment of 75 kg N ha⁻¹ with two-week harvest interval produced acceptable DM yield with proper quality in M. scutellata, which eliminated the necessity of Rhizobium inoculation and reduced the cost and environmental contamination compared to 125 kg N ha⁻¹.

Key words: Snail medic, Medicago scutellata, Rhizobium meliloti inoculation, nitrogen fertilizer, harvest frequency, forage quality

INTRODUCTION

Annual medic (Medicago spp.) pasture produces good quality forage suitable for grazing and is used extensively throughout dry land farming regions of the world (Walsh et al., 2001). On the other hand, the variability in the forage yield and forage quality of annual medicus illustrates the potential for selecting medicus to meet specific management practice and environments (Zhu et al., 1996). Medicus have quality potential similar to alfalfa which is influenced by the stage of maturity (Bauchan, 1998). M. scutellata grows more rapidly than alfalfa and has suitable adaptation to moderate climatic conditions. Snail medic is among those legumes with the greatest forage yield and quality (Zhu et al., 1996).

Plant phenologic stage is an important factor to determine the intensity of defoliation (Young et al., 1994). Time and frequency of harvest also must be regarded in forage harvesting (Turner et al., 2006). Many researchers have studied the effects of harvest system on annual medicus by grazing, but separating and determining the effects of harvest intensity and frequency from each other is a difficult task in this method (Roehor et al., 2004). So, with mechanical harvest it would be possible to separate these two effects from each other (Lawson et al., 1988). Soil nitrogen status has a significant influence on forage yield of annual medicus especially in ley-farming system. Annual medicus grow in pastoral soils usually produce more forage and nitrogen content than medicus which grow in soils under cultivation of cereals (Alston and Graham, 1982).

Zhu et al. (1998) investigated the effects of inoculation and nitrogen on herbage properties of annual medicus species and showed that nitrogen fertilizer could increase the herbage dry matter of spring-seeded M. scutellata only when inoculum was applied. They concluded that nitrogen deficiency limits the forage production in M. scutellata. Under mineral nitrate treatment, M. littoralis and M. polymorpha also produced the highest shoot dry matter yield (Farahani et al., 2004). Chaichi et al. (1996) reported that harvest or grazing frequency reduces the forage yield in M. truncatula. They also suggested that if frequency of harvest is properly managed for plants to have adequate time for regeneration and if they do not encounter environmental limitations such as water stress during growth, the biological yield will increase. Defoliation at early flowering in annual medicus enhances more branching and increases dry matter yield (Lowe and Bowdler, 1988).
Limited researches have mentioned the harvest management and nitrogen application on forage quality of medic. Muir et al. (2005) examined the forage and seed parameters of annual Medicago and Trifolium species in North-Central Texas as affected by harvest intensity and concluded that the forage nutritive value, was negatively affected if plants were allowed to accumulate forage throughout the growing season without some herbage removal. Snail medic had higher ADF concentration than red clover, due to its large fibrous seed pods (Zhu et al., 1996). Lawson et al. (2006) reported that more frequent defoliation resulted in soil reduction in the WSC (water soluble carbohydrate) and nitrogen concentrations in stolons of white clover. According to Aydin and Uzun (2005), nitrogen and phosphorus fertilization of rangelands affects yield, forage quality and the botanical composition; the economic optimum was found with the highest fertilizer doses providing 52 kg P ha$^{-1}$+180 kg N ha$^{-1}$ producing 4810 kg ha$^{-1}$ forage dry matter with a crude protein concentration of 124 g kg$^{-1}$.

The objective of this experiment was to determine the effects of harvest management (harvesting interval) and application of different nitrogen fertilizing systems on dry matter yield and forage quality of snail medic (M. scutellata var. Robinson) and determine which treatment economically produce suitable forage yield and quality.

**MATERIALS AND METHODS**

**Location:** The experiment was conducted at the Agricultural Research Farm, University of Tehran, Karaj during 2006. The site is located at 35°25' N latitude, 71°25' E longitude with an altitude of 1321 m above sea level. Karaj is located about 30 km west of Tehran and has a semi-arid climate (275 mm annual precipitation). The soil of experimental site was clay loam with montmorillonite clay type, low in nitrogen (0.04-0.05%), low in organic matter (0.9 – 1%), phosphate concentration of 14.2 mg kg$^{-1}$ and alkaline in reaction with pH of 7.8 and EC = 0.44 dS m$^{-1}$.

**Experimental design and statistical analysis:** The experiment was carried out in randomized complete block design with split-plot design in four replications. Main plots included of Rhizobium meliloti inoculation (biologic fertilizer) and four levels of chemical nitrogen fertilizer (0, 25, 75 and 125 kg N ha$^{-1}$) whilst four levels of harvest frequencies (weekly-interval, two-week interval, three-week interval and control with only one harvest at 50% flowering) were assigned to the subplots. Data were statistically analyzed by using MSTATC statistical program and Duncan test was employed to classify mean values of different treatments when F-values were significant (p<0.05). Graph was generated by using MS-EXCEL software.

**Sowing and cultivation:** Seed of Medicago scutellata (var Robinson) was sown after scarification in subplots of 3×5 m dimensions on March 18, 2006. The soil temperature at sowing time was 8°C. Climatic conditions data during experimental period was as follow: 18.9°C mean temperature, 42% mean relative humidity, 57 mm precipitation. Each sub-plot consisted of 6 rows of snail medic sown on both sides of each ridge at a density of 128 plant m$^{-2}$. In the inoculated treatment, seeds were soaked in dilute sugar solution and then inoculated with bacteria powder of Rhizobium meliloti at 2 g kg$^{-1}$ seed ratio. Seeds were immediately sown after drying in shawok. Ammonium nitrate fertilizer containing 46% of nitrogen was applied as a nitrogen treatment. Each nitrogen treatment was applied in three stages including: sowing time, plant establishment (5 to 6 trifoliate leaves) and early flowering. In each stage, one thirds (33%) of each nitrogen fertilizer treatment was applied. All treatments were irrigated at weekly-interval. The plots were hand weeded in different vegetative stages and Malation pesticide was once applied against Aphids in April 28.

**Measurements:** The first harvest in each plot started after plant establishment in about 40 days after sowing by using a 1×1 m quadrat with 5 cm in height. Harvest treatments continued until 50% flowering in each plot. The sites of harvest samples were constant in all experimental plots all through the sampling period. Samples were dried by oven at 72°C for 48 h and then weighed. The number of harvests in each harvest frequency treatment was one for control at 50% flowering, 6 for weekly interval, 3 for two weekly intervals and 2 for three weekly intervals. Forage of these harvests in each treatment was accumulated to calculate the total DM yield at the end of the growing period. These samples then ground to pass through a 1 mm sieve. Near infrared reflectance spectroscopy (NIRS) was used to predict forage crude protein (CP) (Kjeldahl N × 6.25), water soluble carbohydrate (WSC) and acid detergent fiber (ADF) concentrations. Herbage samples for quality analyses were entered in a NIRS systems model 6500 (InfraSoft Int., Silver Spring, MD) scanning monochromator and reflectance spectra data were collected. Near infrared spectroscopy is used world-wide
for the routine analysis of many constituents in various tissues of many plant species. The reasons for near infrared spectroscopy being used as analytical method in this study include: minimal sample preparation is needed, analysis time is short, it is cost effective to analyze a single sample or large batches of samples, several constituents can be determined simultaneously, the results are usually more precise and can be more accurate than, as accurate as, or of acceptable accuracy, when compared with the method usually employed (Batten, 1998).

RESULTS

Dry matter yield: The statistical analysis of data indicated that, nitrogen fertilizing treatment and harvest frequency had significant effects (p<0.01) on DM yield. Interaction of nitrogen fertilizing systems with harvest frequency was also significant (p<0.05) (Table 1).

Harvest frequency had positive effect on DM yield whilst it increased by the increment of harvest frequency. Plants under two-week interval and control (one harvest at 50% flowering) produced the highest (5343 kg ha⁻¹) and lowest (3507 kg ha⁻¹) DM yield, respectively (Table 2). Plants under harvest treatment of weekly-interval produced higher DM yield than control (with one harvest at 50% flowering). However, DM yield produced in this treatment was significantly less than two and three-week harvest intervals (Fig. 1).

Dry forage yield increased by increment in soil nitrogen content either by inoculation or chemical N fertilizer. Chemical N treatment of 125 kg nitrogen ha⁻¹ with two harvest interval produced maximum DM yield (5548 kg ha⁻¹) while control (with one harvest at 50% flowering) had the minimum yield among all N fertilizing and harvest frequency treatments. On the other hand, there was no significant difference between 125 kg nitrogen ha⁻¹ and 75 kg nitrogen ha⁻¹ at two week harvest interval in respect to dry matter production. Inoculation had no significant effect on DM yield through all harvest treatments (Fig. 1).

Crude protein: Nitrogen fertilizing systems and harvest frequency had significant effect on forage CP concentration. Nitrogen fertilizer treatments increased the CP concentration of snail medic, compare to control (Table 1). 125 kg nitrogen ha⁻¹ had the higher and 25 kg nitrogen ha⁻¹ had the lower CP concentrations among nitrogen fertilizer treatments. On the other hand, the CP concentration of 125 kg nitrogen ha⁻¹ was not significantly higher than 75 kg nitrogen ha⁻¹. Also, medic produced less concentration of CP under inoculation treatment than control (Table 2).

Increase in harvest frequency raised the CP concentration of plans significantly; so weekly harvest interval with 24.7% DM had maximum CP concentration which was 41% more than control (one harvest at 50% flowering) (Table 2).

Water-soluble carbohydrate: Harvest frequency had significant effects (p<0.05) on WSC concentration. The water-soluble carbohydrate concentration of the plants was affected (p<0.05) by interaction of harvest frequency and nitrogen fertilizing system (Table 1). The only significant difference was between weekly harvest interval

| Table 1: Mean squares of nitrogen fertilizing systems and harvest frequency effects on dry matter (DM) yield, crude protein (CP), water soluble carbohydrate (WSC) and acid detergent fiber (ADF) concentrations of snail medic |
|------|--------|--------|--------|--------|--------|
| Replication & Error | DM | CP | WSC | ADF |
| Replication | 3 | 375.156* | 24.971** | 4.505** | 61.794* |
| Error | 12 | 1326.710 | 2.067 | 5.059 | 12.389 |
| Harvest frequency | 3 | 104.69913** | 463.002** | 7.609** | 345.801* |
| Nitrogen fertilizing systems & Harvest frequency | 12 | 1004.496* | 6.974** | 6.881* | 4.505* |
| Error | 45 | 475.952 | 3.738 | 2.824 | 8.656 |
| CV | 4.720 | 6.500 | 11.790 | 12.140 |

** and * indicate significance of effects at 0.01 and 0.05 probability levels and ** stands for no significant effect, respectively.

| Table 2: Mean values of CP, WSC, ADF and DM concentrations (%) as affected by nitrogen fertilizing systems and harvest frequency |
|------|--------|--------|--------|--------|--------|
| Nitrogen fertilizing systems | Control (No inoculation and fertilizer) | Inoculation | 25 kg nitrogen ha⁻¹ | 75 kg nitrogen ha⁻¹ | 125 kg nitrogen ha⁻¹ |
| Control DM | 18.7bc | 17.9c | 20.3b | 23.0a | 23.6a |
| Inoculation DM | 5.7a | 5.7a | 5.9a | 6.0a | 6.1a |
| Control WSC | 35.2a | 35.0a | 34.3a | 33.9a | 33.5a |
| Inoculation WSC | 403.4a | 403.4a | 473.9b | 503.6ab | 527.6a |
| Harvest frequency | Control DM | 17.4d | 19.9b | 22.1b | 24.7a |
| Three-week interval DM | 5.7b | 5.8ab | 5.9ab | 6.1a | 23.8e |
| Two-week interval DM | 34.4a | 34.4a | 35.7d | 51.71b | 446.5c |

*Means of the same category followed by different letters are significantly different at 0.05% level of probability using Duncan test.
Fig. 1: Effect of nitrogen fertilizing systems and harvest frequency on dry matter yield of *M. scutellata*

Fig. 2: Effect of nitrogen fertilizing systems and harvest frequency on WSC concentration of *M. scutellata*

and control (one harvest at 50% flowering) treatments in respect to WSC concentration. Application of nitrogen fertilizer had positive, but insignificant, effect on WSC concentration (Table 2).

The effects of harvest frequency treatments on WSC concentration increased with application of nitrogen fertilizer (Fig. 2). According to Fig. 2, treatment of 125 kg nitrogen ha$^{-1}$ with weekly harvest interval produced highest WSC concentration, compare to other treatments, but only had significant difference in compare to treatment of no fertilizing with one harvest at 50% flowering.

**Acid detergent fiber:** ADF concentration was affected significantly ($p<0.05$) by harvest frequency (Table 1). Harvest frequency had negative effect on ADF concentration while it decreased by increment in harvest frequency. Plants under control (one harvest at 50% flowering) and weekly harvest interval produced the highest (34.4% DM) and lowest (23.8% DM) ADF concentrations, respectively (Table 2) - in other words, harvest frequency raised the ADF concentration about 30%. Plants under harvest treatment of weekly interval had no significant differences with ones under two week harvest interval.

**DISCUSSION**

Similar to findings of Ahmadi and Chaichi (2007), nitrogen fertilizer treatment increased the DM yield compared to control and 125 kg nitrogen ha$^{-1}$ produced the highest yield, among other N fertilizing systems. This is in support of findings by Alston and Graham (1982) who suggested that medics that grow in pasteurised soils usually produce more dry weight and nitrogen content than those that grow in soils under cereals cultivation because pastured soils have higher content of nitrogen. Total DM produced in weekly interval harvest treatment was significantly less than two and three week harvest intervals. Frequent and continuous harvesting and lack of enough time between harvests for plants to regenerate properly, could be the reason of reduction in DM yield in this treatment (Chaichi et al., 1996). Inoculation increased the DM yield but this increment was not significant through all harvest treatments. In fact, symbiotic N$_2$ fixation by *Rhizobium* inoculation could not supply enough nitrogen for *M. scutellata* (Ahmadi and Chaichi, 2007), probably because of its high requirement for nitrogen (Zhu et al., 1998) and therefore nitrogen deficiency limited the growth of snail medic, compare to chemical fertilizer treatments. This was previously shown in *M. littoralis* and *M. polymorpha* by Farahani et al. (2004).

It is clear that increase in the availability of nitrogen for medics, elevate their nitrogen and consequently their CP concentrations (Alston and Graham, 1982). Therefore, this could be the reason for CP increment with respect to more nitrogen fertilizer application. In this study we did not apply any nitrogen fertilizer as starter of root nodules action in inoculation plots; hence the soil nitrogen for optimum performance of nodules was not sufficient and decreased the N$_2$ fixation of nodules. When it is considered that soluble proteins account for around 80% of the plant nitrogen (Corre et al., 1996), the depression in N$_2$ fixation could be responsible for the decrease in CP concentration of inoculation treatment, compare to control that was not significant (Table 2). The increment in CP concentration by increment in harvest intervals is related to growth of new tissues following harvest that contains nitrogen and protein compounds (Lawson et al., 2000). This finding is consistent with that of Lawson et al. (1998) who showed that the nitrogen concentration of white clover did not reduce following defoliation.
In this study, starch concentration was not determined but according to Lawson et al. (1998), the small effects of defoliation frequency on the WSC concentration suggest that the plant buffer any changes in the WSC concentration by either the deposition or remobilization of starch. In contrast, Moran et al. (1953) found that more frequent defoliation reduced the WSC concentration in both roots and stolons of white clover.

The highest ADF concentration of control treatment in compare to other harvest treatments may have been due to large fibrous seed pods of smail medic (Zhu et al., 1996) because in this treatment plants were only one harvested at 50% flowering when producing pods. On the other hand, harvest frequencies induce the plants regrowth by producing new stems which increase the leaf-stem ratio and therefore decreased the ADF concentration.

The regression (Spearman) analysis showed positive correlation of DM yield with CP concentration (\(R = 0.69\)), ADF concentration (\(R = 0.71\)) and CP with WSC concentrations (\(R = 0.65\)). It also revealed a negative relationship between CP and ADF concentrations (\(R = -0.62\)).

Results of this experiment revealed that chemical N fertilizer treatment of 75 kg nitrogen ha\(^{-1}\) produces acceptable DM yield and CP concentration in *M. scutellata* and eliminates the necessity for *Rhizobium* inoculation and reduces the cost and environmental contamination of excessive fertilizer application compared to 125 kg nitrogen ha\(^{-1}\). Application of 75 kg nitrogen ha\(^{-1}\) along with two-week harvest interval can be recommended for suitable production of forage yield with acceptable quality (high CP and WSD and low ADF concentrations). This study has provided important evidence that proper harvesting or grazing management of medic along with availability of enough nitrogen for plants are critical issues to achieve favorable forage yield and quality in smail medic. Further research, however, is needed to determine how much cutting or grazing intensity (height of harvesting or grazing) this medic will tolerate and still produce sufficient forage yield with high quality, or which *Rhizobium* strains with what ratios are most suitable for an efficient inoculation.

REFERENCES


Medicago and Trifolium species in North-Central Texas as affected by harvest intensity. Agron. 
J., 97: 118-124.

Rochon, J.J., C.J. Doyle, J.M. Greef, A. Hopkins and 
G. Molle et al., 2004. Grazing legumes in Europe: A 
review of their status, management, benefits, research needs and future prospects. Grass Forage 

Turner, L.R., D.J. Donaghy, P.A. Lane and R.P. Rawnsley, 
2006. Effect of defoliation management, based on leaf 
stage, on perennial ryegrass (Lolium perenne L.), 
prairie grass (Bromus wildenowii Kunth) and 
cocksfoot (Dactylis glomerata L.) under dryland 
conditions. 1. Regeneration, tillering and water-
soluble carbohydrate conditions. 2. Nutritive value. 

Walsh, M.J., R.H. Delaney, R.W. Groose and J.M. Krall, 
2001. Performance of annual medic species 
(Medicago sp.) in Southeastern Wyoming. Agron. 
J., 93: 1249-1256.

Young, R.R., K.J. Mothorpe, H.I. Nicol and P.H. Croft, 
1994. Effects of sowing time and grazing on the dry 
matter yield, phonology, seed yield and hardseed 
levels of annual pasture legumes in western New 

and quality of six annual Medicago species in the 

Zhu, Y., C.C. Sheaffer, C.P. Vance, P.H. Graham, 
Inoculation and nitrogen affect herbage and 
symbiotic properties of annual Medicago species. 