Title: Potential of forage biomass production of alfalfa (*Medicago sativa*) irrigated under urea without *Rhizobium meliloti* in arid area

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Abstract

In order to assess the forage potential of alfalfa grown under urea without *Rhizobium meliloti*, this 12-month study was conducted at the Faculty of Agronomy of Abdou Moumouni University in a completely randomized block design. Four treatments were carried out with different doses of urea in experimental plots measuring 3 m by 1 m: one dose of 30g/plot; one dose of 40g/plot; one dose of 50g/plot; and a final dose of 60g/plot, along with a control with seeds inoculated with Rhizobium meliloti.

Ten cuts were made over the 12 months, the forage production evolved in a sawtooth pattern and the results can be summarized as follows: 1909.30 kg DM/ha; 2460.43 kg DM/ha; 3182.86 kg DM/ha; 332 5.60 kg DM/ha; 2211.64 kg DM/ha respectively for dose 1; dose 2; dose 3; dose 4 and the control. Alfalfa showed leaf/stem ratios everywhere above 0.5 for all different doses of urea, which is synonymous with good physical quality of the forages obtained in terms of digestibility.

The results of this work show that the cultivation of alfalfa can be a solution to the crucial problem of livestock feeding in arid areas.

Keywords: alfalfa, forage potential, *Rhizobium meliloti,* urea, arid area

Introduction

In the Sahel countries, the quantitative and qualitative insufficiency of fodder resources is surely the real problem facing ruminant farming. In this region, fodder production is strongly correlated with the rainy season. In this area, natural pastures are the basis of ruminant feeding [1]. With yields rarely exceeding 600 kg DM/ha [2], this fodder can hardly cover the nutritional needs of the animals on its own [3]. This natural fodder accounts for about 90% of livestock feed [4], and is only used to maintain animals in the dry season [5].

Forage production in Niger depends on agro-ecological zones [6] and to this natural production, crop residues must be added. On the other hand, it should be noted today that grazing areas are occupied by urbanization and the agricultural front [7], but also by their invasion by so-called invasive plants that are not palatable [8], which also reduces the availability of fodder. One of the solutions to this situation is the cultivation of fodder. Among the forage plants, alfalfa (*Medicago sativa*) has proven to be one of the most popular. Adapted to various agro-climatic conditions [9], alfalfa is a very nutritious fodder because it is rich in protein [10], all associated with a good forage yield per hectare [11] but also ease of setting up the crop.

One of the difficulties in alfalfa forage production is the need to inoculate the seed with *Rhizobium meliloti* for its first establishment on soil, otherwise forage production will be stunted and unproductive [12]. It would therefore be useful to see the possibility of substituting the bacterium with fertilisers, particularly mineral fertilisers, in order to see the behaviour of the plant and its fodder potential without its *Rhizobium meliloti.*

This is why this study has set itself the objective of evaluating the fodder potential of alfalfa grown without *Rhizobium meliloti* but by substituting it with urea.

I. Materials and Methods

1.1. Experimental site

The site where this study was conducted is located at the experimental park of the Faculty of Agronomy of Abdou Moumouni University of Niamey, located at latitude 13° 30' North and longitude 2°08' East [13]. The climate is Sahelian with high temperatures (43°C) between April and June and low temperatures (15°C) between December and January [14]. A 2,000 m^2 plot of land was used as a field to conduct these experiments. This study was conducted over a twelve-month period from October 2021 to September 2022.

1.2. Soil characteristics

The physico-chemical characteristics of the soil of the experimental site show that the soil has a sandy fraction high at 77% with an average rate of fine particles which gives it a sandy loam texture. The soil is slightly acidic with a pH of around 6.26; with a very low content of organic carbon, total phosphorus and available nitrogen [15].

It is a land on which plant species (woody and non-woody) grow naturally, the majority of which are not palatable to domestic ruminants: *Sida cordifolia, Cleom viscosa, Euphorbia aegyptiacum, Sesamum alatum…*

1.3. Experimental plots

The land, which is fully fenced, was first ploughed deep with a tractor. After this work, the site was completely loosened in order to reduce the clods and give a fine and superficial texture more or less homogeneous to the soil.

The plots were designed to be 3m long and 1m wide. It is a randomized complete block device. Thus, three blocks have been designed. Each block has 5 X 3 plots corresponding to the different treatments of the experiment and the control. Each treatment was repeated three times per block and each block repeated 3 times, resulting in a total of nine replicates per treatment and 45 plots in total. The plots are spaced 0.5m apart while the blocks are spaced 1m apart.

1.4. Cultivation management

Sowing is done in a row at a rate of 10g of alfalfa seed per row (4 rows per plot, spaced 20cm apart) so 40g per plot. The seeds were mixed with a small amount of sand to ensure a fairly good distribution of the seeds along the entire line. As for the seeds of the control, they are seeds that have been inoculated with the bacterium *Rhizobium meliloti*. A dose of 6 kg of bottom manure, consisting of small ruminant manure, is applied before sowing.

At the end, after the cutting, which took place every thirty days, the weights of the plants, leaves and the completely stripped stem were determined using an electronic scale with a capacity of 400g. The fresh weight of the alfalfa was assessed by cutting the entire plot by weighing using a conventional scale. Then, the harvested forage is left in the open air for 24 hours. A 100g sample of the green fodder is taken for the determination of the dry matter in the oven.

1.5. Irrigation

The water is provided in such a way that the plants do not have any water stress. First, a deep watering is done twice a week followed by two superficial waterings. But, during the hot months of the year (April-May-June), the number of deep waterings was sometimes higher. The water source is a borehole powered by electrical energy.

1.6. Data collection and processing

The raw data collected in this way are entered in Excel for descriptive statistics and reported in SPSS for differential statistics. All data are expressed in conventional units averaged with standard deviation. Tukey's ANOVA test allowed for in-depth data statistics.

II. Results and discussion

2.1. Results

2.1.1. Alfalfa Growth from Emergence to First Cut

Plant growth began on the 20th day after emergence. The measurements made it possible to draw the growth curve given in Figure 1 below.

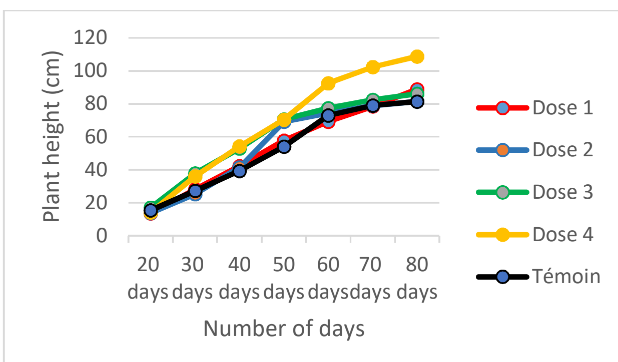


Figure 1: Growth rate of alfalfa from sowing to the first cut

The observation in Figure 1 above shows an almost linear growth of alfalfa for all the different doses and the control of the start of the measurement from the 20th day after sowing to the 60th day. From the 60th day onwards, this growth appeared linear for doses 1; 2 ; 3 and the control until the date of the first cut on the 80th day after sowing. It can be seen that there is no great difference between the first three doses and the control (81.3 cm; 81.44 cm; 86.10 c; 88.8 cm respectively for doses 1; 2; 3 and the control) while dose 4 stands out to reach a peak of 108.7 cm high on the day of the first cut.

2.1.2. Alfalfa forage production potential

Alfalfa, although grown without its seeds being inoculated with the specific bacterium, gave fairly substantial forage yields for all four treatments. Over the twelve months that the experiment lasted, ten cuts were made. Had it not been for the first cut that took place more than 80 days after sowing, there would have been more than ten cuts. The results obtained are given in Table I.

Table 1: Plant vegetative parameters and forage yield of alfalfa

	Foot weight	Stem weight	Leaf weight	Number of tillers	Height	Dry matter	Ratio
Dose 1	17,85±8,55[a]	10,68±5,57[a]	7,21±3,30[a]	21,40±7,41[ab]	67,89±15,97[a]	2267,23±537,24[a]	0,59
Dose 2	17,59±1,88[a]	10,33±1,13[a]	7,26±0,84[a]	17,50±2,21[a]	67,46±19,13[a]	2460,43±418,75[b]	0,59
Dose 3	19,62±7,45[a]	11,21±4,47[a]	7,19±7,20[a]	24,50±7,07[b]	70,22±18,76[b]	3182,86±600,04[c]	0,56
Dose 4	19,58±6,16[a]	11,83±3,53[a]	7,46±2,88[a]	24,72±6,92[b]	74,81±21,51[b]	3356,80±805,48[c]	0,61
Control	17,93±5,86[a]	10,70±3,74[a]	7,24±2,16[a]	17,52±3,00[a]	65,83±3,92[a]	2678,24±590,98[b]	0,59

The elements accompanied in column of a power; of the same letters are statistically not different (P<0.05 according to the Tukey test); g = gram, cm = centimetre; kg DM/ha = kilogram of dry matter per hectare; F/T ratio = leaf to stem ratio. Dose 1 = 30g, Dose 2 = 40g, Dose 3 = 50g; Dose 4 = 60g.

The overall analysis of this table allows us to note two important facts: firstly, for all the plant variables studied (plant weight, stem weight, leaf weight, number of tillers per plant, cutting height), the control is always more or less equal to doses 1 and 2, whereas doses 3 and 4 are generally higher, and this, even for the yield of dry forage biomass produced, the very purpose of this study. But the leaf-to-stem ratio is not in the same order as the vegetative parameters. .

In terms of foot weights, the lowest value obtained is 16.84g for dose 1 while the highest value obtained is 19.69g for dose 4. But the statistically significant differences are only really visible between doses 1 and 4.

For the weight of the stem (this is the stem completely stripped of its leaves), there is a real statistical variation between doses 1; 4 ; the control and doses 2 and 3, which are not significantly different from each other.

The number of tillers showed during this study variable and statistically differences between the different treatments as well as the control. Everywhere, significant differences were observed except between doses 1 and the control with values of 20.63 and 21.40 tillers respectively.

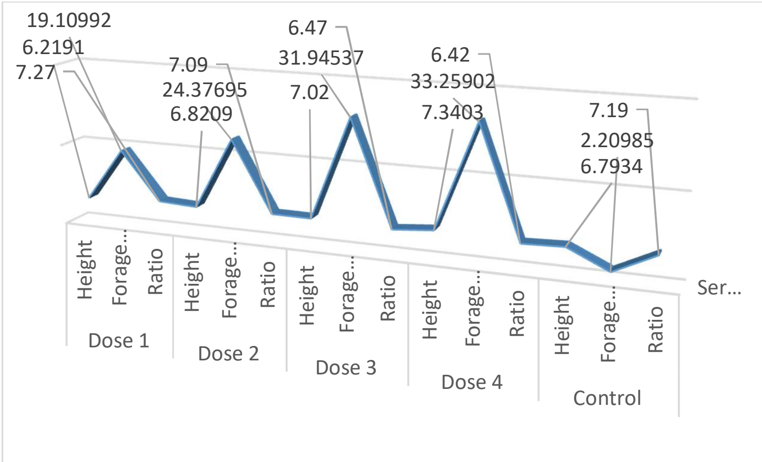
Concerning the height of alfalfa plants, relatively significant differences were observed between doses 1 ; 3 ; 4 and control.

The real statistically observable differences are observed in forage yield. The lowest yield obtained is that of dose 1 with 1909.30 kg DM/ha, while the largest obtained is with dose 4 with a forage production of 3325.60 kg DM/ha. Differences were observed between all treatments and the control outside of doses 3 and 4 which did not show statistically observable differences.

There was certainly no statistical test at the level of the leaf/stem ratio, as this is not the subject of a particularly important analysis. The objective of this ratio is to see the theoretical bulkiness potential of the forage produced, i.e. their ingestibility. The observation in the table shows ratios everywhere above 0.5 and between 0.56 and 0.61.

2.1.3. Analysis of forage quality as a function of plant height and leaf/stem ratio

The amount of forage biomass, as important as it is, is not enough on its own to define a good forage plant. A good quality forage plant must be productive, but palatable to animals and have large proportions of leaves.



To read this figure, please divide the forage yield in DM by 100; divide the height by 10 and multiply the ratio by 10.

Figure 2: Effect of alfalfa plant height and alfalfa forage yield on forage quality

The observation in Figure 2 clearly shows that, if the leaf-to-stem ratio is decisive in assessing the forage quality of alfalfa, the control would be the batch of alfalfa of better forage quality. Despite its average forage yield of 2,209.85 kg DM/ha, it appears with the highest leaf-to-stem ratio (0.791). As for the highest average forage yield, obtained with dose 4 (3325.90 kg DM/ha), it appears with the lowest leaf/stem ratio of the order of 0.624.

Similarly, the height of the plants, which greatly influenced the forage yield, had a negative effect on the leaf/stem ratio and therefore on the physical quality of the forages and this is visible in both cases: the control with an average stem height of 67.934 cm and dose 4 with an average height of 73.403 cm. Finally, the overall reading of the figure shows that the greater the forage yield and the height of the stems, the more the leaf/stem ratio decreases and the forage quality as well.

Table 2: Correlation between vegetative parameters of alfalfa plants

	Foot weight	Stem weight	Leaf weight	Number of tillers	Height	Dry matter
Foot weight	1	0,95**	0,80**	0,077*	0,075*	-0,013
Stem weight		1	0,66**	0,090**	0,062*	-0,018
Leaf weight			1	-0,025	0,023	-0,070*
Number of tiller				1	0,43**	0,47**
Height					1	0,38**
Dry matter						1

Dry matte = dry matter, data with two power stars are significant at the 5% threshold and those with a power star are significant at the 5% threshold.

The table shows strong positively significant correlations between the weight of a foot and the weight of the stem and that of the leaves at the 1% threshold (r= 0.95) and between the weight of the foot and the weight of the leaves (r = 0.80); as well as the correlation between the weight of a stem and the weight of the leaves of a stem (r= 0.66). In addition, there is an average but very significant correlation at the same threshold as above between the number of tillers and the dry matter yield (r = 0.47) and on the other hand between the height of the plants and the number of tillers (r = 0.43). On the other hand, the correlation is weaker but very significant at the 1% threshold between plant height and dry matter yield (r = 0.38).

A general model of an equation giving the share of each vegetative parameter to the dry matter yield is given by the following relationship in the following box:

$$\boxed{Y = 780{,}11 + 31{,}88\, X_1 - 46{,}90\, X_2 - 32{,}56 X_3 + 44{,}55 X_4 + 9{,}53 X_5 + 93{,}77 X_6}$$

With: Y = dry matter yield; X_1 *= weight of the plant's foot;* X_2 *= weight of the stem;* X_3 *= weight of the leaves;* X_4 *= number of tills;* X_5 *= height of the plant;* X_6 *= to the dose of urea.*

Each of the estimated parameters contributes significantly to the regression model with thresholds of 1% except for the weight of the feet, the weights of the leaves and the weight of the stems which have a threshold of 5%. This equation shows that the dose of urea has a significant effect ($P = 0.01$) in determining forage yield. However, it can be seen that the weight of the stem (X_2) and that of the leaves (X_3) have a negative and significant effect on the dry biomass yield ($P = 0.05$).

2.2. Discussion

The growth of alfalfa in this study is much accelerated. Apart from the first period which saw the plant establish over 20 days, significant heights were reached in only 30 days. These are much higher than the results of [17], who obtained a maximum height after cutting of 40 cm, whereas in this study, the lowest height obtained is 61.95 cm. In addition, the plant heights obtained in this study are more or less within the normal range of alfalfa size (30-80cm) for the most part [18] ; but the maximum height is slightly lower than that obtained by [19] with an average height of 75.14 cm. The heights of alfalfa grown without Rhizobium are broadly within the height range obtained by [20] ; [21] and [22] having obtained heights between 49 -74 cm respectively; 74-86 cm and 74.7-88 cm on alfalfa grown with the inoculum.

Alfalfa is indeed a plant that adapts to varied and diverse pedoclimatic conditions. This allows it to support a wide range of soils. Growing alfalfa on light soils has many advantages, including higher biomass production compared to heavy soils. Indeed, the plant has anatomical and physiological particularities that allow it to adapt and be found almost everywhere in all climates [23].

The number of tillers obtained with the use of urea on alfalfa varies between cuts and between different treatments. With a minimum of 17.50 and a maximum of 24.72 tillers per alfalfa plant for doses 2 and 4 respectively, these results are lower than those obtained by [24]. with a number of tillers per alfalfa plant between 29.9 and 33.17. It must be said that in the case of [25], the study conducted in eastern Turkey was in much more favourable ecological conditions for the plant. This would facilitate a better expression of his potential.

Alfalfa is a vigorous plant that can be harvested several times a year, resulting in high productivity in terms of forage yield [26]. In fact, in this study, ten cuts were made in twelve months of production and it would have been possible to do more if the cutting frequency was not defined for thirty days. The forage yields obtained in this study are lower than those obtained by [27] in Serbia, with a production of 16.6 t DM/ha and [28] with a minimum of 7.3 t DM/ha and a maximum of 15.5 t DM/ha, whereas in our case the maximum yield obtained is 5.35 t DM/ha, obtained with doses 4 to 4th cut.

Similarly, [21] had alfalfa forage yields of 7.87 t DM/ha by combining alfalfa with several grass species that have the effect of increasing above-ground biomass yield. It must be said that, although the plant adapts to a diversity of climates, western and temperate climates are more favorable to the flowering of the plant than tropical climates. But these forage yields are overall better than those of [18] with a total of 2.8 tons per hectare in three cuts, so the smallest yield is obtained in this study with dose 1 (2.26 t DM/ha) in a single cut. But it must be said that for the latter case, there was sowing in a pocket, whereas in our study, the sowing was done in rows. So the proportion of plants per plot is higher. The forage yields in this study are also better than those of [25] , who obtained 1.23 t DM/ha by also treating alfalfa with urea, but in this case, there is a harsher climate and this climate could be a factor that acted on alfalfa yield. This alfalfa forage production is much higher than that of several varieties of forage cowpea (*Vigna unguiculata*) TN256-87 (1,425 t/ha) and IN92E-26 (1,226 t/ha) which is widely used as a fodder crop to supplement animal feed [26].

It must also be said that with the forage yields resulting from this study, we are well above the natural fodder production in Niger. The minimum production obtained in this study is 2267.23 kg DM/ha, while natural fodder production, which averaged 1200 kg DM/ha about three decades ago, is now less than 1000 kg DM/ha. In addition to this drop in natural fodder production, we must also add the invasion of rangelands by unpalatable plants with a high rate of growth and colonisation of the environment, such as *Sida cordifolia, Calotropus procera, Cleom viscosa*, etc. which greatly reduce the forage potential. The maximum forage obtained, largely at the natural forage yield, shows that alfalfa, although not inoculated at a satisfactory production in our context [27].

The leaf/stem ratio, which is above 0.5 everywhere, is synonymous with good palatability of the alfalfa forage produced. This leaf/stem ratio is decisive, because the smaller it is, the more the plant's content of non-protein constituents increases and decreases the degradability of the plant's nitrogenous matter [28]. This proves once again the forage quality of alfalfa produced without *Rhizobium meliloti* because the ratio is quite high. Indeed, a ratio close to zero is synonymous with a decrease in the proportion of leaves to stems and the progressive lignification of the plant which affects the energy value, nitrogen value, mineral value and ingestibility of the plant [29].

Conclusion

This study showed the possibility of producing alfalfa for fodder without inoculating the seeds with Rhizobium meliloti. It showed that there is a possibility of substituting the bacterium with urea while having good forage yields. This substitution of the bacterium by urea did not impact the overall phenology of the plant because the vegetative cycle of the plant remained quite short with even a possibility of reducing it.

As alfalfa is a plant with multiple positive effects both for the soil and for its nutritional value, it would be complementary to see the effect of the use of urea on the plant on the quality of the soil, the nutritional value of the plant and its ability to make nodules.

ACKNOWLEDGMENTS

The autors thank the Regional Center of Excellency on Pastoral Production, Abdou Moumouni University

CONFLICT OF INTERESTS

None.

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