

## **Influence of nitrogen fertilization on lettuce yields (*Lactuca sativa L.*) using the <sup>15</sup>N isotope label**

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**Abstract.** Nitrogen fertilization plays an important role in the growth of market gardening and the improvement of yields. Its efficiency of use is imperative for the preservation of the agricultural environment. An experiment is carried out over three consecutive years (2014/2015), (2015/2016) and (2016/2017), in a sub humid climate. The methodology adopted focuses on the variation of optimal nitrogen doses and their effects on the evolution of lettuce cultivation (*Lactuca sativa L.*), which has a socio-economic impact. The approach takes into account the isotopic marking technique, <sup>15</sup>N. The experimental device adopted is of the complete random block type, with four (04) levels: 0 (control), 60, 120 and 180 kg N ha<sup>-1</sup> with four (04) repetitions. These levels are used to diagnose the effect of different doses on biomass (dry matter) and yield. It has been shown those doses between 0 and 120 kg N ha<sup>-1</sup> increase significantly ( $p < 0.05$ ), yields and dry matter with values of 18.32, 45.49 to 57.93 t ha<sup>-1</sup> and 4.32, 5.52 to 9.77 t ha<sup>-1</sup>, respectively. The rate of 120 kg N ha<sup>-1</sup>, is shown statistically, as the efficient rate to cover the nitrogen needs of lettuce. This efficiency reaches 74.48%. Beyond that, nitrogen is not valorized by the crop. These results contribute to the realization of a technical reference system for lettuce cultivation, for an efficient use of nitrogen.

**Key words:** efficient rate, isotopic nitrogen <sup>15</sup>N, lettuce, nitrogen use efficiency.

### **INTRODUCTION**

Agricultural potential in Algeria is 20% focused in the north of the country, characterized by unfertile soils. These soils, represented by a pH greater than 8, have low nutrient content, low water retention capacity and low organic matter content, according to Mazoyer (1970) and Belaid (2016), this matter does not exceed 2%. This situation contributes to low crop yields. The technical itinerary that makes it possible to correct these deficiencies is fertilization.

As several authors have shown in their work on Algerian soils. In this context, the Halilat (2004) showed that the interaction of potassium (P) and nitrogen (N) fertilization significantly affects wheat grain yield in the Saharan zone. The maximum yield is 6.780 MT ha<sup>-1</sup> with the N<sub>250</sub>P<sub>180</sub> rate. Boukhalfa–Deraoui et al. (2011) showed that the

influence of phosphate fertilization on the behavior and yield of the common wheat crop grown under irrigation in arid areas increases the grain yield by 49.3% compared to the control. Haffaf et al. (2016); Saoudi et al. (2016), who conducted trials in the same semi-arid climate, respectively, on the production of durum wheat and barley seed, obtained maximum yields at similar rates. These yields reach the respective values of 33.82 and 33.25 q ha<sup>-1</sup>, i.e. gains of 11.52 and 9.76 q ha<sup>-1</sup>.

In Tunisia and Morocco, which have a similar climate, Marouani et al. (2013) have shown poor nitrogen use efficiency in seasonal potato cultivation. This inefficiency is linked to significant losses due to leaching. Kchaou et al. (2011) compared the nitrogen fertilizer value of sludge with that of urea, using isotope marking with <sup>15</sup>N, applied to forage sorghum. These authors found that sludge inputs caused significant increases in sorghum nitrogen yields and exports, comparable to those obtained in the presence of urea. They concluded that the actual nitrogen utilization coefficient of sludge nitrogen and urea offers values fluctuating between 25 and 32%. Ryan et al. (1997) showed that the effect of nitrogen fertilization on five durum wheat cultivars (Kyperounda, Marzak, Massa, Cocorit and Karim), conducted in rainy conditions, significantly influences biomass and yield.

According to FAO (2005), in Algeria, the use of fertilizers in agriculture is not controlled, despite the efforts made by farmers in charge of the cereal intensification program and potato farmers. Market gardening, which represents strategic crops in the country, is undergoing significant development. Their area of 320 100 ha in 2003, or 0.75% of the Utilized Agricultural Area (SAU), increased to 511 018 ha in 2015, or 1.18% of the SAU. Production increased from 49.08 million quintals to 124.69 million quintals, respectively, Ministry of Agriculture and Rural Development (MADR, 2015). Despite its importance in the country's economy, this sector suffers from a lack of a database and a scarcity of studies relating to fertilization in general and fertilizer use in particular.

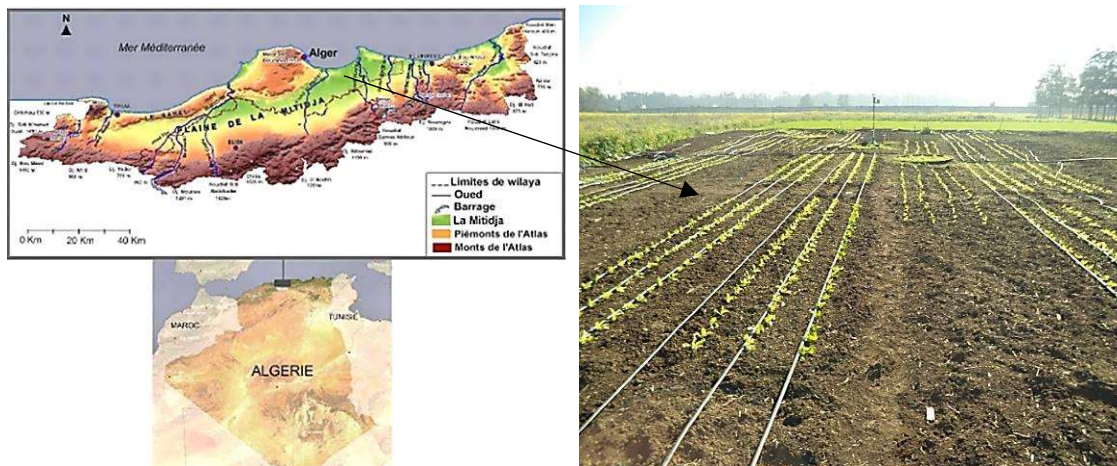
According to National Institute of Soil for Irrigation and Draining (INSID, 2009), fertilizers are applied in the absence of technical standards, neglecting the initial content of the soil; and consequently, inputs are often poorly fractionated resulting in waste, which is a source of soil and water pollution. In this perspective, this study uses the <sup>15</sup>N isotopic approach to assess nitrogen use efficiency. This new method, used by the IAEA (2001), highlights isotopic nitrogen <sup>15</sup>N, which represents the most commonly used stable isotope, in studies related to agriculture. It is the direct means of measuring nitrogen uptake by applied fattening, and the most reliable means of monitoring the flow and fate of nitrogen in the soil-plant system (Zapata, 1990; Bedard-Haughn et al., 2003).

To highlight the monitoring of this system, the plant material chosen is lettuce (*Lactuca sativa L.*), due to its short growing cycle. But also, because of the socio-economic impact that is beginning to dominate, at the national level. It is a source of wealth and income for producers. The average price of a kilo of lettuce is around € 0.49 (64 DZD), varying between a minimum price of € 0.22 (30 DZD) and a maximum price of € 0.89 (120 DZD). The objective of this study is essentially oriented towards the search for optimal nitrogen fertilizer doses in order to contribute to the production of technical standards for the efficient use of fertilizers.

## MATERIALS AND METHODS

### Experimental site

The experiment was carried out at the experimental station of the National Institute of Agronomic Research (INRA) of Mahdi Boualem (Fig. 1) located southwest of Algiers in the eastern part of Mitidja. The research station is located between 36°68' North of Latitude and 3°1' East of Longitude and at an altitude of 18 m.



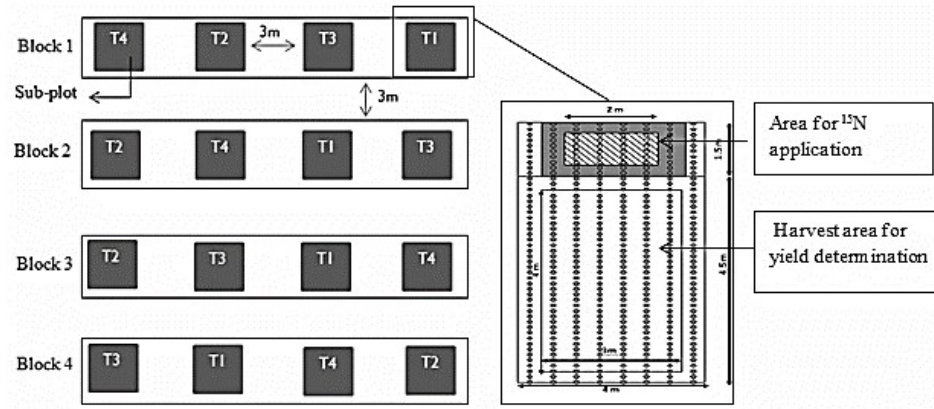
**Figure 1.** Location of the study area.

To give an overview of the climate that characterizes the area, the climate data used comes from the research station's automatic weather station. The measurements taken, at a daily time step, cover minimum and maximum temperatures ( $^{\circ}\text{C}$ ), precipitation (mm), wind speed ( $\text{m s}^{-1}$ ) at 2 m above the ground, solar radiation ( $\text{W m}^{-2}$ ) and relative humidity (%). These parameters were used to calculate the reference evapotranspiration using the FAO Penman–Monteith method (Allen et al., 1998).

To give the soil characteristics, a soil profile was carried out over a depth of one meter, including three horizons, the results of which are presented in Table 1.

### Experimental protocol

The test is carried out in the field according to a complete randomized block experimental design, with four nitrogen levels, namely: T1 ( $0 \text{ kg N ha}^{-1}$ ), T2 ( $60 \text{ kg N ha}^{-1}$ ), T3 ( $120 \text{ kg N ha}^{-1}$ ) and T4 ( $180 \text{ kg N ha}^{-1}$ ) arranged in four blocks. Each block has four sub-plots (Fig. 2). Each micro-plot is 6 m long and 3 m wide, for a total area of  $18 \text{ m}^2$ , of which  $4.5 \text{ m}^2$  is used for  $^{15}\text{N}$ -labelled nitrogen.  $100 \text{ kg K ha}^{-1}$  and  $150 \text{ kg P ha}^{-1}$  of phosphate and potassium fertilizers were incorporated into the soil as bottom manure. This trial was repeated over the three years (2014/2015), (2015/2016) and (2016/2017). We used the  $^{15}\text{N}$  isotopic technique only in the 2014/2015 test because of its high cost. The quantities of nitrogen used are distributed throughout the crop development cycle, namely: 10% at 15 days after transplanting (DAT), 30% at 40 DAT, 40% at 60 DAT and 20% at 75 DAT.



**Figure 2.** Experimental device.

The crop taken into consideration is variety lettuce, stubborn from Nîmes, belonging to the lettuce to be applesauce class, which is eaten young, before it goes to seed. Lettuce seeds were sown in the honeycomb plates for 19 to 25 days in the nursery before being transplanted. The young lettuce plants were transplanted at the 3 to 4 leaf stage onto well ploughed soil in the field.

### Evaluation

Biomass is one of the essential parameters of research on the effectiveness of fertilizers, particularly in lettuce, where the growth of the aerial part is a determining factor in the agricultural value of this crop (Begoña et al., 2011). To evaluate this parameter, every ten days, six (6) plants per sub-plot were cut at ground level, field samples were taken back to the laboratory for drying in an oven for 48 hours at 70 °C.

To determine the isotopic composition of lettuce plants. The heads of the latter receiving  $^{15}\text{N}$  were divided into two parts (roots and leaves). Fresh weight was evaluated for all parts of the crop. The samples were dried at 70 °C for 24 hours, weighed to determine the dry weight, ground to a fine powder using a 0.3 mm sieve, then homogenized, for the determination of total nitrogen and excess  $^{15}\text{N}$ . Isotopic analysis of the samples from the lettuce culture was carried out at the National Center for Energy, Science and Nuclear Techniques (CNESTEN-Morocco).

The quantification of nitrogen from the fertilizer was measured using the isotopic dilution method from  $N_{dff}$  (nitrogen from the fertilizer), and the rate of nitrogen fertilizer applied, according to the following equations defined by the IAEA (2001):

$$\% N_{dff} = \frac{\text{atom } \%^{15}\text{N excess plant}}{\text{atom } \%^{15}\text{N excess fertilizer}} \cdot 100 \quad (1)$$

$$\% N_{dfs} = 100 - \% N_{dff} \quad (2)$$

$$\text{DM yield (kg ha}^{-1}\text{)} = \text{FW (kg)} \cdot \frac{10,000 \text{ (m}^2 \text{ ha}^{-1}\text{)}}{\text{area harvested (m}^2\text{)}} \cdot \frac{\text{SDW (kg)}}{\text{SFW (kg)}} \quad (3)$$

$$\text{N yield (kg ha}^{-1}\text{)} = \text{DM yield (kg ha}^{-1}\text{)} \cdot \frac{\% \text{N}}{100} \quad (4)$$

$$\text{Fertilizer N yield (kg ha}^{-1}\text{)} = \text{N yield (kg ha}^{-1}\text{)} \cdot \frac{\% N_{dff}}{100} \quad (5)$$

$$\% \text{ Fertilizer N utilization} = \frac{\text{Fertilizer N yield}}{\text{Rate of N application}} \cdot 100 \quad (6)$$

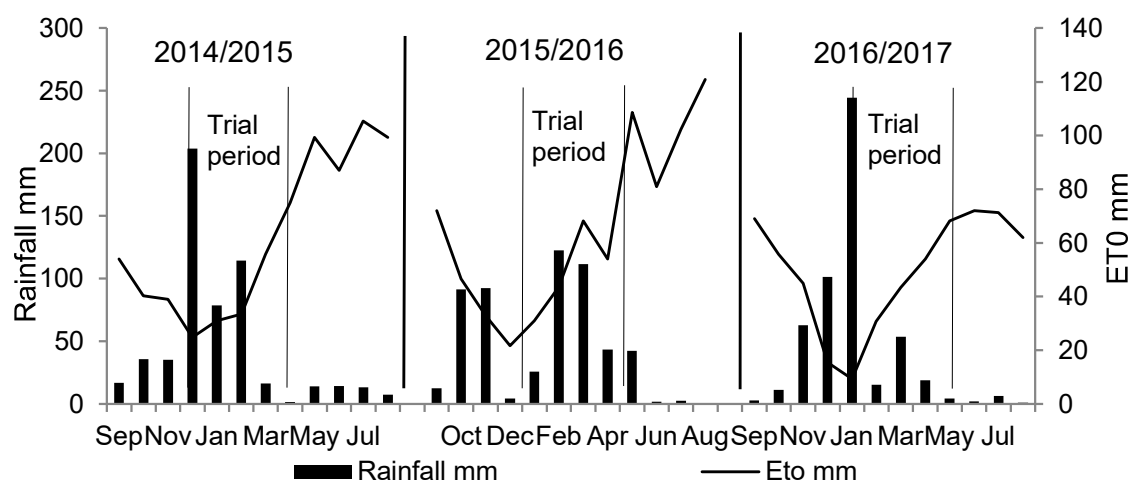
where Ndff – Fraction of N in the plant derived from the <sup>15</sup>N labeled fertilizer; Ndfs – Nitrogen derived from soil; FW – Fresh weight per area harvested; SDW – Subsample dry weight; SFW – Subsample fresh weight; DM – Dry matter Yield.

**Statistical analysis:** The obtained results were statistically evaluated by the analysis of variance (one-way ANOVA) method. The effects of the different treatments applied are estimated using the statistical software SPSS 25. The comparison of the averages was made by the LSD (Least Significant Difference) test at the threshold  $\alpha = 5\%$ .

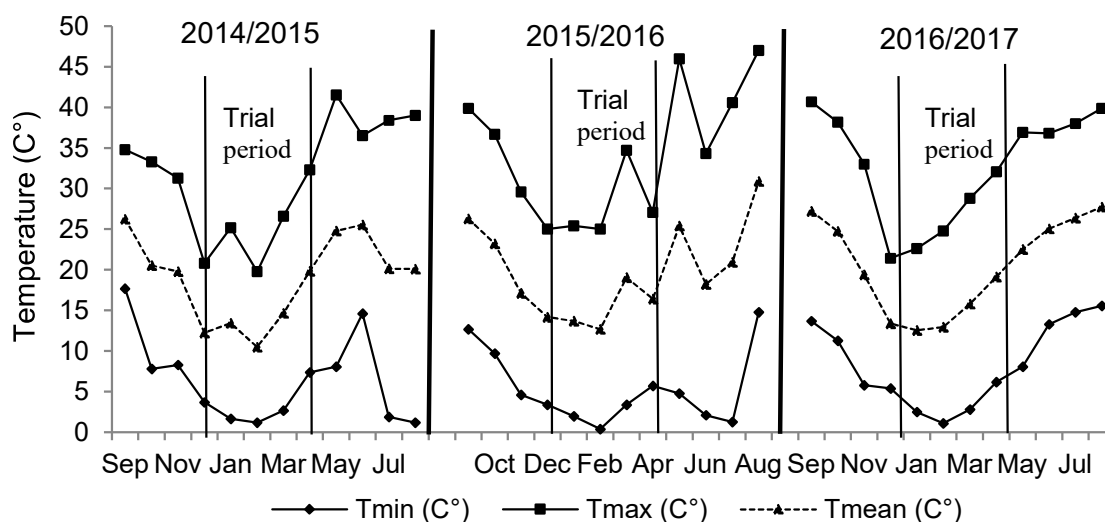
## RESULTS AND DISCUSSION

### Analysis of climate data

The variations in precipitation and ET<sub>0</sub> are shown in Fig. 3, which illustrates the distribution of rainfall during the three years of experience 2014/2015, 2015/2016 and 2016/2017. The cumulative rainfall received between September and August is in the order of 552, 551 and 525 mm respectively. Those corresponding to the experimental seasons (January to April) are close to the averages of 211.4, 303.4 and 332.6 mm. The corresponding potential annual evapotranspiration is in the order of 744.3, 782.6 and 596.3 mm. The ones corresponding to the growing seasons are, respectively, 195.4, 196.5 and 137.5 mm. Despite high rainfall amounts during the second year's experimental season, ET<sub>0</sub> remains the highest compared to the other two years of experimentation. This increase is due to the high average temperatures during the corresponding months. Fig. 4 shows the monthly average temperature fluctuations over the three agricultural years. They are between 0.4 and 47 °C.



**Figure 3.** Precipitation, potential evapotranspiration (ET<sub>0</sub>) at monthly scale for the 2014/2015, 2015/2016 and 2016/2017 test years.



**Figure 4.** Monthly variations in maximum, minimum and average temperatures for the 2014/2015, 2015/2016 and 2016/2017 test years.

### Physical and chemical characteristics of the soil

The granulometric results indicate that the soil is characterized by a silty clay texture (Table 1). Chemical analysis results show that the soil is characterized by a basic pH (about 8). The organic matter content of the soil is low (less than 2%), which causes poor water and mineral retention.

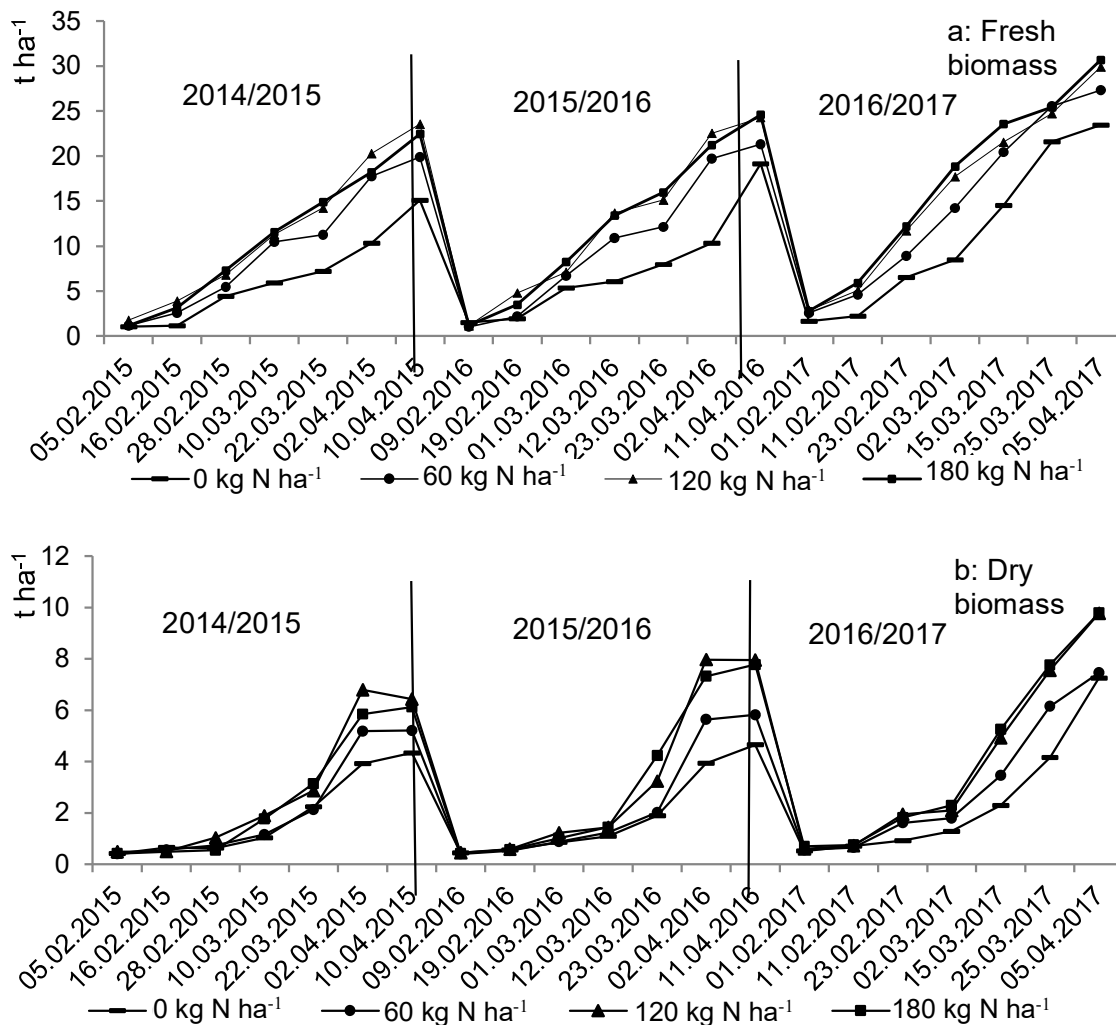
**Table 1.** Physical and chemical properties of the soil of experimental field

Parameters	Horizon 1 (0–25 cm)	Horizon 2 (25–55 cm)	Horizon 3 (> 55 cm)
pH	7.87	7.77	7.8
Electric conductivity (dS m <sup>-1</sup> )	0.17	0.17	0.17
Bulk density (g cm <sup>-3</sup> )	1.28	1.29	1.29
Saturation humidity (Vol. %)	44.86	43.19	42.5
Field capacity (Vol. %)	33.52	34.50	34.29
Permanent Wilting point (Vol. %)	22.87	23.26	21.60
Total limestone %	0.73	0.48	0.80
Granulometry (%)			
Clay	42.81	48.50	51.89
Silt	48.35	44.67	44.20
Sand	7.98	7.77	7.80
Soil texture	Silty clay	Silty clay	Silty clay

### Effect of fertilization on above-ground biomass and yield

Fig. 5 shows the evolution of nitrogen doses at different phenological stages of the plant. This evolution is supported by the analysis of variance, which showed a very significant effect ( $p < 0.001$ ) of the fresh and dry above-ground biomass, in relation to the increase in nitrogen doses provided. A maximum of fresh and dry biomass is achieved at a rate of 120 kg N ha<sup>-1</sup>. Above this level, the increase in nitrogen rate is not significant. This result is consistent with that of Maurice et al. (1985) which showed that fertilization at high doses leads to a decrease in above-ground biomass. This is the case for the first year (2014/2015).

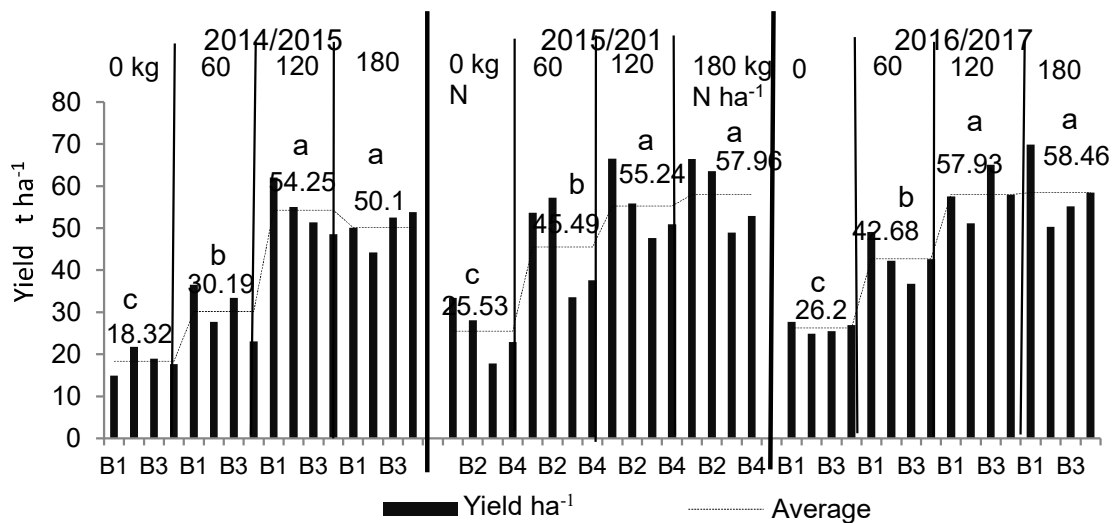




**Figure 5.** Effect of different fertilization levels on the evolution of fresh (a) and dry (b) above-ground biomass for the three growing seasons.

Fig. 6 shows lettuce yields as a function of the nitrogen rates applied. Indeed, the graph shows that, during the three experimental campaigns, the highest lettuce yields (57.93 and 58.46 t ha<sup>-1</sup>), are obtained by applying the 120 and 180 kg N ha<sup>-1</sup> doses. These doses are very highly significant ( $p < 0.001$ ) compared to those obtained (26.27 and 45.49 t ha<sup>-1</sup>) by providing minimum doses below 60 kg N ha<sup>-1</sup>.

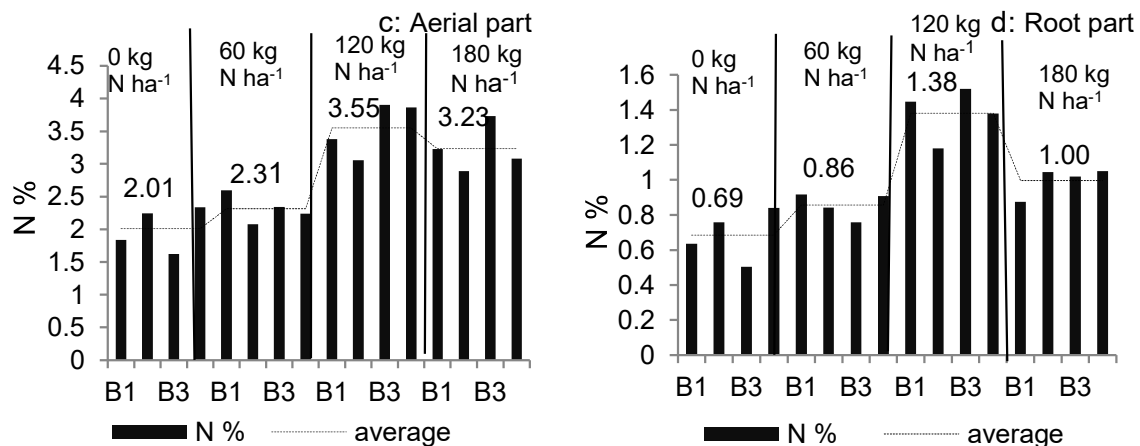
This result is consistent with Boroujerdnia et al. (2007) and Shahbazie (2005), who reported that increasing nitrogen levels from 0 to 120 kg of N ha<sup>-1</sup> has a positive effect on lettuce production. Nevertheless, in detail, the T4 treatment, from the 2014/2015 trial, shows a relatively lower yield of about 50.17 t ha<sup>-1</sup>, compared to that (54.25 t ha<sup>-1</sup>) of the T3 treatment, from the same year. The difference estimated at 3.08 t ha<sup>-1</sup>, can be explained by the toxicity of the plants or the non-attraction of nitrogen by the plants resulting from the consumption of excess nitrogen fertilizer, as highlighted by Tabatabaie & Malakoutie (1997). Lettuce's response to yields is considerably higher in 2016 and 2017 than in 2015. This result is related to higher rainfall amounts.



**Figure 6.** Effect of different fertilization levels on yield for the three growing seasons [a, b and c represent significant differences ( $p < 0.05$ )].

In this study, the average yield obtained by the 120 kg N ha<sup>-1</sup> dose during the three crop years fluctuated between 54.25 and 57.93 t ha<sup>-1</sup>, or an average of 5.5 kg m<sup>-2</sup>. Boroujerdnia et al. (2007) obtain a yield of about 7 kg m<sup>-2</sup> with a rate of 120 kg N ha<sup>-1</sup>. The difference can be explained by the choice of variety, soil fertility and precipitation. The results of this study suggest that the application of nitrogen in the form of urea at 120 kg N ha<sup>-1</sup> may be sufficient to cover the N requirements of the lettuce crop. These results are consistent with those obtained by Boroujerdnia et al. (2007), Regional Group of Expertise Nitrates (GREN, 2014), Awaad et al. (2016) and Gonzalez (2017).

### Effect of nitrogen fertilization on nitrogen content (%N) in dry matter



**Figure 7.** Effect of different fertilization levels on total nitrogen (N %) in lettuce leaves (c) and roots (d).

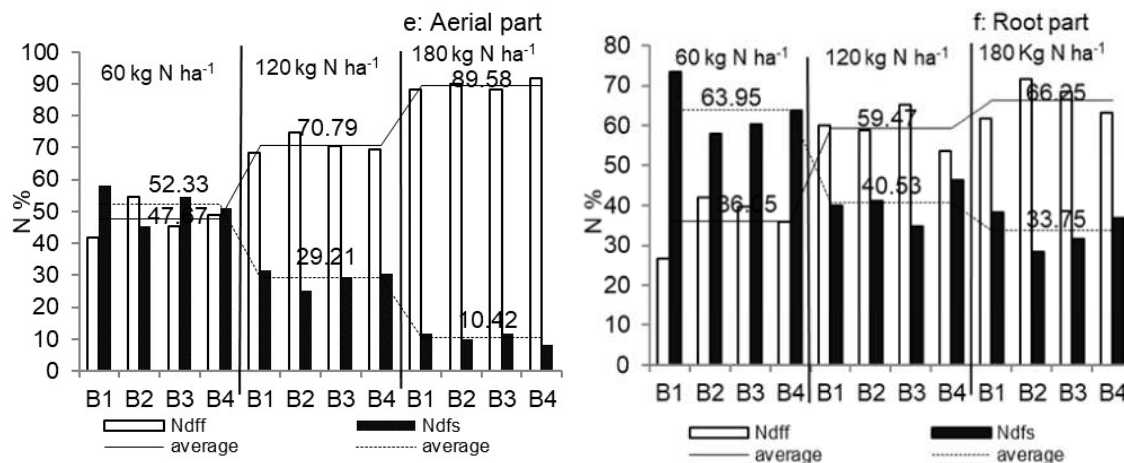


Fig. 7 shows a fluctuation in the assimilation of nitrogen doses from 0 to 120 kg N ha<sup>-1</sup> by leaves and roots in the different blocks. Statistically, these results show a very highly significant difference ( $p < 0.001$ ). The average nitrogen assimilation rate increases from 2.01 to 3.55% in the aerial part (c), it is from 0.69 to 1.38% in the root part (d). Above 120 kg N ha<sup>-1</sup>, the nitrogen content decreases in both the aerial and root parts. At a rate of 180 kg N ha<sup>-1</sup>, the results show that its use by the plant is ineffective. This result is consistent with that reported by Lawlor et al. (2001). A very close relationship ( $r = 0.86$ ) is observed between nitrogen doses and the content of this element in the whole plant (leaves + roots).

### Nitrogen valorization by the crop

The contribution of N15-urea (Ndff) and N-soil (Ndfs) to the total amount of nitrogen absorbed by the aerial part (leaves) and the underground part (root system) is illustrated in Fig. 8. The latter, which shows how isotopic nitrogen is used, determines the real share of nitrogen assimilated by fertilizer inputs (Ndff) in favour of the real share and accurately evaluates the share of soil nitrogen (Ndfs) that contributes to the crop's diet, since, according to Fig. 8 (e and f), it is observed that at a rate of 60 kg N ha<sup>-1</sup>, the crop values the nitrogen contained in the soil better than the nitrogen supplied.

A better valuation of Ndff by the crop is carried out at a rate of 120 kg N ha<sup>-1</sup>, it is 70.79% for the leaves, and 59.47% for the roots. Ndfs decreased significantly with increasing nitrogen application rates. These observations are consistent with research by Zhaoming et al. (2016) on winter wheat and Kchaou et al. (2011) on sorghum.

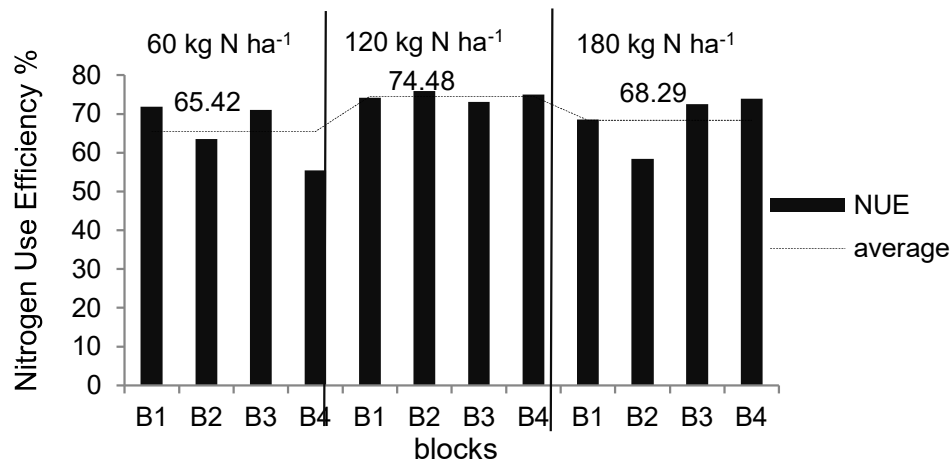


**Figure 8.** Nitrogen source in the aerial part (e) and root system (f) of lettuce.

### Nitrogen use efficiency (NUE)

Nitrogen use efficiency is an important indicator for nitrogen fertilizer application. In this context, Fig. 9 illustrates the variation in the percentage of nitrogen use efficiency as a function of defined thresholds. For doses ranging from 60, 120 to 180 kg N ha<sup>-1</sup>, the EUN varies, respectively, from 65.42, 74.48 to 68.38%. The percentage of nitrogen use efficiency (NUE), decreased from 74.48% to 68.38% by increasing the dose from 120 to 180 kg N ha<sup>-1</sup>. These results are similar to those reported by Khelil et al. (2005) and Kchaou et al. (2011). The 120 kg N ha<sup>-1</sup> dose allows the best efficiencies to be achieved.

That is 74.48% of the fertilizer applied is consumed by lettuce cultivation. The remaining 25.52% of N is either in the soil or lost through leaching. Lettuce is a short-cycle crop; it makes the best use of available nitrogen, as reported by Edith et al. (2017).



**Figure 9.** Variation in nitrogen use efficiency by lettuce, expressed as a percentage (%).

## CONCLUSIONS

To have a better understanding of the term fertilizer use efficiency, the use of isotopic techniques (<sup>15</sup>N) proved essential in order to evaluate the different sources of nitrogen assimilated by the lettuce crop. It appears that nitrogen fertilization allowed a good development of the crop, starting from the minimum rate of 60 kg N ha<sup>-1</sup>. The results obtained from this study showed that the nitrogen fertilizer rate had a significant influence on the evolution of the above-ground biomass and the yield of lettuce. The 120 kg N ha<sup>-1</sup> dose resulted in a significant yield of 54.25 t ha<sup>-1</sup>, compared to the other doses provided, with a nitrogen use efficiency of 74.48%. It is recommended as the efficient rate for the crop and those with the same morphological characteristics and that evolve under the same type of climate and soil.

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