# VARIATION IN CALCIUM AND PHOSPHORUS CONTENT OF *Medicago sativa* L.

**Mihai STAVARACHE[1], Costel SAMUIL[1], Doina TARCĂU[1], Constantin Iulian POPOVICI[1], Vasile VÎNTU[1*]**

e-mail: mihaistavarache@uaiasi.ro

**Abstract**

The research conducted during March-October 2011, on Ezăreni farm, studied the influence of innoculation, fertilization and plant growth stage at harvest on calcium (Ca), phosphorus (P) content and calcium/phosphorus ratio at alfalfa crop (*Medicago sativa* L.) in the second year after sowing, at first cut. Factor A - inoculation with two graduations ($a_1$ without seed inoculation, $a_2$ with seed inoculation); factor B - fertilization with four graduations ($b_1$ -unfertilized, $b_2$ - $N_{50}P_{50}$, $b_3$ -$N_{75}P_{50}$, $b_4$ -30 t/ha manure); factor C - plant growth stage at harvest with six graduations: early bud ($c_1$), mid bud ($c_2$), late bud ($c_3$); early bloom ($c_4$), 10% bloom ($c_5$) and full bloom ($c_6$). The results showed that each of the three studied factors had a different impact on the Ca and P content in the plants. Following analysis results of the influence of seed inoculation on the Ca and P content in alfalfa it was observed that this factor had not a significant influence. In fact, the differences were as small as 0.03 g·kg$^{-1}$ DM or there was no difference. Regarding P content in alfalfa plants, fertilization determined positive differences (0.27-0.35 g·kg$^{-1}$ DM) statistically assured in all studied variants and on Ca content, positive and negative differences, depending on the fertilizer type. The influence of harvest time on Ca content in the entire alfalfa plant, leaves or stems showed that as plants are aging, the concentration of this element increases very significant. Phosphorus content recorded a decrease with alfalfa aging.

**Key words**: inoculation, fertilization, harvesting growth stage, leaves, stems

Alfalfa (*Medicago sativa* L.) is one of the most valuable forage grown for its high yields and high forage quality that are obtained from it.

Calcium is a key element in plant metabolism, an element that cannot be replaced in the basic metabolic functions by any other cation. It is necessary to maintain the plasma cell component in an active and stable stage to maintain acid-base equilibrium by neutralizing organic acids that are in excess.

Calcium participate in the formation of cell walls with calcium acid pectate; interferes in the process of cell division; contributes to the development of the root system. Calcium plays an important role in the operation of detoxification of the body's other ions, Al, H, Fe, Mn, and some radicals resulting from the process of metabolism or taken from the nutritional environment. Calcium is the only mineral generally not toxic to plants, even if they accumulate in large quantities.

One of the major drawbacks of alfalfa is a small content of phosphorus, which is deficient from the point of view of the requirements of herbivores, especially in comparison with the content of calcium (ratio of calcium/phosphorus to meet the requirements of the animal body, must be between 2/1 3/1).

Along with nitrogen, phosphorus is one of the fundamental elements of plant nutrition, which consists of its participation in the composition of the substances involved in energy production (ATP, ADP, NAD) and molecular architecture of different nucleic acids.

In plants, phosphorus is found in an organic form (phytin, phosphatides, nucleic acids, phosphoric esters) and inorganic (alkali phosphates of calcium and magnesium) throughout the entire plant parts, especially in growing tissues, seeds, leaves, but also and substances crucial for life processes (phosphatides, fosfoprotide enzymes).

Applying appropriate cultivation technology, at alfalfa crop, helps to improve the phosphorus content, among technological elements, fertilization and harvesting age of plants plays an important role (Stancheva I. et al, 2008; Katić S. et al, 2009; Touil L. et al., 2009; Albu Aida et al, 2012; Sanz-Sáez Á. et al, 2012; Vîntu V. et al, 2012).

This research proposes to analyze the effect of inoculation, fertilization and harvesting growth

[1] University of Agricultural Sciences and Veterinary Medicine Iasi-Romania
* Coordinator

stage on the calcium content and phosphorus content of alflafa, in the conditions of NE Romania, as well to establish the correlation between harvesting growth stage and the calcium content and phosphorus content of leaves, stems and whole plants.

## MATERIALS AND METHODS

The research was conducted during March-October 2011, on Ezăreni farm, Iasi (47°05'-47°10' North latitude and 27°28'-27°33' East longitude). The soil from this region is a cambic chernozem with a pH between 6.68 to 7.01, medium supplied with humus (2.40%), total nitrogen (1.78%) and phosphorus (26.00 ppm) in the 0-30 cm horizon. Geographical area, including the farm Ezăreni, is characterized by a temperate climate with annual average temperature of 9.7°C and average annual precipitation of 518 mm.

The research was focused on the influence of innoculation, fertilization and plant growth stage at harvest onto the calcium (Ca) content, phosphorus (P) content and calcium/phosphorus ratio at the alfalfa crop (*Medicago sativa* L.) in the second year after sowing, first cut. The experimental design was a trifactorial split plot design 2x4x6, arranged as subdivided plots in three replicates. The plot harvested area was 10 sq. meters (2m x 5m).

The factors that were studied were: factor A - innoculation with two graduations ($a_1$ - without seed innoculation, $a_2$ with seed innoculation); factor B - fertilization with four graduations ($b_1$ - unfertilized, $b_2$ - $N_{50}P_{50}$, $b_3$ -$N_{75}P_{50}$, $b_4$ - 30 $t \cdot ha^{-1}$ manure); factor C - plant growth stage at harvest with six graduations early bud ($c_1$), mid bud ($c_2$), late bud ($c_3$); early bloom ($c_4$), 10% bloom ($c_5$) and full bloom ($c_6$) (Ball S.T., 1998; Mueller S.C. and Teuber L.R., 2007).

The leaf/shoot ratio was determined by separating the petiole, lamina, buds and flowers from the shoot, weighing them separately and calculating the ratio by weight. The forage yield was determined by weighing green plants harvested from an area of 10 sq m. The dry matter content was determined by drying plant samples at a temperature of 105 ºC for three hours. Leaf and shoot yields were calculated using the leaf/shoot ratio.

The calcium content was determined by atomic absorption spectrometry (SR ISO 6869/2004), with the Shimadzu AA-6300 atomic absorption spectrometer.

The phosphorus content was determined by the spectrophotometric method. The principle of the method consists in mineralization of the plant sample and treatment with the acid solution and vanado-molybdenum reagent solution, followed by the measurement of the absorbance of the yellow solution obtained, with the spectrophotometer (UV/VIS - Shimadzu UV 1240 mini) at a wavelength of 430 nm.

The biological material used was represented by Sandra alfalfa variety (F 660-94) registered in 2003 to I.N.C.D.A. Fundulea (Schitea Maria and Martura T., 2004). The innoculation of seeds was done 1 hour before seeding selected strains of *Rhizobium meliloti* Dangeard. The manure had the following chemical composition: N - 0.445%, $P_2O_5$-0,212% and $K_2O$-0,695%.

The results were statisticaly analyzed by the analyses of variance and limit differences. We also determined the correlation equations and the significance of the square regression between harvesting growth stage and the calcium contemt, phosphorus content and calcium/phosphorus ratio of whole plant, leaves and stems.

## RESULTS AND DISCUSSIONS

Analyzing the influence of the interaction between inoculation, fertilization and harvesting time on the calcium content of alfalfa plants it was observed that the variant $a_2b_4c_6$ inoculated, fertilized with 30 $t \cdot ha^{-1}$ manure, harvested at early bloom obtained the highest content of Ca in plants, 19.00%, and the lowest of 13.75% was recorded at he variant a1b3c1, without inoculation, fertilized with $N_{75}P_{50}$ fertilized, harvested in early bud stage. All differences were distinct and highly significant (positive or negative), except variants: $a_1b_2c_1$, $a_1b_2c2$, $a_2b_1c_1$ and $a_2b_2c_2$ where the differences did not reach statistical significance (*table 1*).

The interaction between seed inoculation, fertilizing and harvesting time on the phosphorus content of alfalfa plants showed that the variants $a_1b_3c_1$ without inoculation, fertilized with $N_{75}P_{50}$, harvested in early bud stage and variant $a_2b_3c_1$ inoculated, fertilized with $N_{75}P_{50}$, harvested in early bud have obtained the highest P content of 3.16 $g \cdot kg^{-1}$ DM and the lowest content of 2.04 $g \cdot kg^{-1}$ DM was determined at variant a1b1c6 without inoculation, fertilized, harvested at full bloom and variant $a_2b_1c_6$ inoculated, unfertilized, harvested at full bloom (*table 1*).

Interaction between inoculation, fertilization and harvesting time on the ratio Ca/P of alfalfa plants showed that the variant $a_2b_1c_6$ inoculated, fertilized, harvested at full bloom obtained the highest value of the ratio Ca/P of 9.04, and the lowest value of 4.35 was determined in variant $a_1b_3c_1$ without inoculation, fertilized with $N_{75}P_{50}$, harvested in early bud stage (*table 1*). The differences registered between control variant and other variants were between -13.5% and + 79.7%. With the exception of variants $a_1b_3c_1$ and $a_2b_1c_1$ the differences obtained were significant and in the case of treatments harvested in early bud stage, fertilizated, inoculated or not, differences were negative.

Results analyses showed that the interaction between inoculation, fertilization and harvesting time on the calcium content of alfalfa leaves, obtained differences between -0.1% and 14.0%.

Variant $a_2b_4c_6$ (inoculated, fertilized with 30 $t \cdot ha^{-1}$ manure, harvested at full bloom) obtained in

leaves, the highest content of Ca, of 30.92 g·kg$^{-1}$ DM and the lowest content of 27.08 g kg-1 DM was determined at variant a2b1c1 inoculated, fertilized, harvested at early bloom. Except variants $a_2b_1c_1$ and $a_1b_1c_2$ where differences compared to the control were not statistically assured, all the differences obtained in other versions were very significant (*table 2*).

Table 1

**Influence of interaction between inoculation, fertilization and growt stage at harvest on the alfalfa plants calcium and phosphorus content**

| Indicator | | Ca content (g·kg$^{-1}$ DM) | P content (g·kg$^{-1}$ DM) | Ca/P ratio | Ca content (g·kg$^{-1}$ DM) | P content (g·kg$^{-1}$ DM) | Ca/P ratio |
|---|---|---|---|---|---|---|---|
| Variant | | $a_1$-without innoculation | | | $a_2$-with innoculation | | |
| $b_1$-$N_0P_0$ | $c_1$ | 14.24$^{Control}$ | 2.83$^{Control}$ | 5.03$^{Control}$ | 14.15 | 2.82 | 5.01 |
| | $c_2$ | 14.41** | 2.39°°° | 6.02*** | 15.00*** | 2.39°°° | 6.29*** |
| | $c_3$ | 15.81*** | 2.22°°° | 7.11*** | 15.51*** | 2.22°°° | 6.99*** |
| | $c_4$ | 17.32*** | 2.08°°° | 8.31*** | 16.88*** | 2.08°°° | 8.13*** |
| | $c_5$ | 17.52*** | 2.11°°° | 8.32*** | 17.34*** | 2.10°°° | 8.26*** |
| | $c_6$ | 18.25*** | 2.04°°° | 8.93*** | 18.43*** | 2.04°°° | 9.04*** |
| $b_2$-$N_{50}P_{50}$ | $c_1$ | 14.20 | 3.14*** | 4.52°°° | 13.84°°° | 3.11*** | 4.45°°° |
| | $c_2$ | 14.32 | 2.74°°° | 5.23*** | 14.51*** | 2.72°°° | 5.33*** |
| | $c_3$ | 15.57*** | 2.51°°° | 6.20*** | 15.25*** | 2.50°°° | 6.09*** |
| | $c_4$ | 17.11*** | 2.44°°° | 7.00*** | 16.81*** | 2.44°°° | 6.90*** |
| | $c_5$ | 17.40*** | 2.38°°° | 7.31*** | 17.45*** | 2.37°°° | 7.35*** |
| | $c_6$ | 18.24*** | 2.36°°° | 7.73*** | 18.47*** | 2.35°°° | 7.85*** |
| $b_3$-$N_{75}P_{50}$ | $c_1$ | 13.75°°° | 3.16*** | 4.35°°° | 13.84°°° | 3.16*** | 4.38°°° |
| | $c_2$ | 13.97°°° | 2.77°°° | 5.04 | 14.21 | 2.76°°° | 5.15*** |
| | $c_3$ | 14.88*** | 2.55°°° | 5.83*** | 14.54*** | 2.55°°° | 5.71*** |
| | $c_4$ | 16.54*** | 2.49°°° | 6.63*** | 16.30*** | 2.49°°° | 6.56*** |
| | $c_5$ | 17.55*** | 2.42°°° | 7.24*** | 17.57*** | 2.42°°° | 7.27*** |
| | $c_6$ | 18.16*** | 2.41°°° | 7.54 | 18.54*** | 2.40°°° | 7.73 |
| $b_4$- 30 Mg·ha$^{-1}$ manure | $c_1$ | 15.34*** | 3.14*** | 4.89°°° | 15.47*** | 3.14*** | 4.93°°° |
| | $c_2$ | 15.69*** | 2.70°°° | 5.62*** | 15.92*** | 2.70°°° | 5.90*** |
| | $c_3$ | 15.85*** | 2.46°°° | 6.44*** | 15.54*** | 2.45°°° | 6.34*** |
| | $c_4$ | 17.47*** | 2.39°°° | 7.30*** | 17.12*** | 2.39°°° | 7.17*** |
| | $c_5$ | 17.92*** | 2.33°°° | 7.70*** | 17.98*** | 2.32°°° | 7.75*** |
| | $c_6$ | 18.78*** | 2.31°°° | 8.14*** | 19.00 | 2.30°°° | 8.26*** |
| *LSD* | *5%* | *0.11* | *0.01* | *0.04* | *0.11* | *0.01* | *0.04* |
| | *1%* | *0.15* | *0.02* | *0.06* | *0.15* | *0.02* | *0.06* |
| | *0.1%* | *0.19* | *0.03* | *0.07* | *0.19* | *0.03* | *0.07* |

Regarding the influence of the interaction between inoculation, fertilization and harvesting time on the phosphorus content of alfalfa leaves it was observed that the variant $a_1b_3c_1$ without inoculation, fertilized with $N_{75}P_{50}$, harvested in early bud, obtained the highest content of leaves in P, of 4.00 g·kg$^{-1}$ DM and the lowest of 2.01 g·kg$^{-1}$ DM was recorded at variant $a_1b_1c_6$ without inoculation, fertilized, harvested at full bloom and variant $a_2b_1c_6$ inoculated, fertilized, harvested at full bloom. All differences between variants and control plot were negative and statistically assured except variants with mineral and organic fertilization ($b_2$, $b_3$, and $b_4$), harvested at early bud stage ($c_1$), where differences were positive (table 2).

Analyzing the influence of the interaction between inoculation, fertilization and harvesting time on the ratio Ca/P in alfalfa leaves it was observed that the differences obtained were between -1.8% and + 112.2%. The control variants $a_1b_2c_1$ (without inoculation, fertilized with $N_{50}P_{50}$, harvested in early bud stage) and $a_1b_3c_1$ (without inoculation, fertilized with $N_{75}P_{50}$, harvested in early bud stage) obtained in the leaves, the lowest value of the ratio Ca/P of 6.98 and highest of 15.09 was recorded in variant $a_2b_4c_6$, inoculated, fertilized with 30 t·ha$^{-1}$ manure, harvested at full bloom (*table 2*).

Analyzing the influence of the interaction between inoculation, fertilization and harvesting time on calcium content of alfalfa shoots it was observed that the differences obtained were between -0.01% and + 98.8%. Variant $a_2b_4c_6$ inoculated, fertilized with 30 t·ha$^{-1}$ manure, harvested at full bloom obtained the highest Ca content in plant shoots of 14.37 g·kg$^{-1}$ DM and the lowest Ca content in shoots, of 7.22 g kg-1 DM was obtained at variant $a_2b_1c_1$ inoculated, fertilized, harvested in early bud stage. All differences were very significant, except variants with or without inoculation, fertilized with $N_{50}P_{50}$ or without fertilization, harvested at early bud stage, where the differences were not significant (*table 3*).

Regarding the interaction between the three factors studied (inoculation, fertilization and

harvesting time) on the phosphorus content of alfalfa shoots it was observed that the differences obtained were between -10.0% and 20.3%. Variant $a_1b_3c_1$ without inoculation, fertilized with $N_{75}P_{50}$, harvested in early bud stage obtained the highest P content in shoots of 2.78 g·kg$^{-1}$ DM and the lowest of 2.08 g·kg$^{-1}$ DM was obtained at variant $a_2b_1c_6$ inoculated, fertilized, harvested at the end of bloom (*table 3*).

Table 2

**Influence of interaction between inoculation, fertilization and growt stage at harvest on the alfalfa leaves calcium and phosphorus content**

| Indicator | | Ca content (g·kg$^{-1}$ DM) | P content (g·kg$^{-1}$ DM) | Ca/P | Ca content (g·kg$^{-1}$ DM) | P content (g·kg$^{-1}$ DM) | Ca/P |
|---|---|---|---|---|---|---|---|
| **Variant** | | **$a_1$-without innoculation** | | | **$a_2$-with innoculation** | | |
| **$b_1$-$N_0P_0$** | **$c_1$** | 27.12$^{Control}$ | 3.81$^{Control}$ | 7.11$^{Control}$ | 27.08 | 3.79 | 7.12 |
| | **$c_2$** | 27.27 | 3.00°°° | 9.08*** | 27.62*** | 2.99°°° | 9.22*** |
| | **$c_3$** | 28.08*** | 2.45°°° | 11.45*** | 28.05*** | 2.45°°° | 11.47*** |
| | **$c_4$** | 28.74*** | 2.27°°° | 12.66*** | 28.19*** | 2.26°°° | 12.46*** |
| | **$c_5$** | 29.10*** | 2.10°°° | 13.85*** | 29.09*** | 2.09°°° | 13.89*** |
| | **$c_6$** | 29.20*** | 2.01°°° | 14.51*** | 29.99*** | 2.01°°° | 14.94*** |
| **$b_2$-$N_{50}P_{50}$** | **$c_1$** | 27.67*** | 3.96*** | 6.98°° | 27.63*** | 3.95*** | 6.99°° |
| | **$c_2$** | 27.82*** | 3.12°°° | 8.92*** | 27.86*** | 3.11°°° | 8.95*** |
| | **$c_3$** | 28.30*** | 2.55°°° | 11.10*** | 27.92*** | 2.54°°° | 10.99*** |
| | **$c_4$** | 29.32*** | 2.36°°° | 12.43*** | 28.76*** | 2.35°°° | 12.23*** |
| | **$c_5$** | 29.69*** | 2.18°°° | 13.60*** | 29.68*** | 2.18°°° | 13.64*** |
| | **$c_6$** | 29.79*** | 2.09°°° | 14.25*** | 30.59*** | 2.09°°° | 14.67*** |
| **$b_3$-$N_{75}P_{50}$** | **$c_1$** | 27.91*** | 4.00*** | 6.98°° | 27.87*** | 3.99*** | 6.99°° |
| | **$c_2$** | 28.06*** | 3.15°°° | 8.91*** | 27.78*** | 3.14°°° | 8.85*** |
| | **$c_3$** | 28.21*** | 2.57°°° | 10.96*** | 28.49*** | 2.57°°° | 11.10*** |
| | **$c_4$** | 29.58*** | 2.38°°° | 12.42*** | 29.02*** | 2.37°°° | 12.22*** |
| | **$c_5$** | 29.95*** | 2.20°°° | 13.59*** | 29.94*** | 2.20°°° | 13.63*** |
| | **$c_6$** | 30.06*** | 2.11°°° | 14.24*** | 30.86*** | 2.11°°° | 14.66*** |
| **$b_4$- 30 Mg·ha$^{-1}$ manure** | **$c_1$** | 27.97*** | 3.89*** | 7.18 | 27.93*** | 3.88*** | 7.19 |
| | **$c_2$** | 28.12*** | 3.07°°° | 9.17*** | 27.84*** | 3.06°°° | 9.10*** |
| | **$c_3$** | 28.93*** | 2.50°°° | 11.55*** | 28.21*** | 2.50°°° | 11.29*** |
| | **$c_4$** | 29.63*** | 2.32°°° | 12.79*** | 29.07*** | 2.31°°° | 12.58*** |
| | **$c_5$** | 30.01*** | 2.15°°° | 13.99*** | 30.00*** | 2.14°°° | 14.03*** |
| | **$c_6$** | 30.12*** | 2.06°°° | 14.65*** | 30.92*** | 2.05°°° | 15.09*** |
| *LSD* | *5%* | *0.24* | *0.02* | *0.09* | *0.24* | *0.02* | *0.09* |
| | *1%* | *0.32* | *0.03* | *0.12* | *0.32* | *0.03* | *0.12* |
| | *0.1%* | *0.42* | *0.04* | *0.16* | *0.42* | *0.04* | *0.16* |

Except variant $a_2b_1c_1$, differences between all other variants and control variant were statistically assured, but in the case of unfertilized variants were negative.

Data regarding the influence of interaction between inoculation, fertilization and harvesting time on the Ca/P ratio of alfalfa shoots showed that the differences were between -15.1% and +114.7%. Variant $a_1b_3c_1$, without inoculation, fertilized with $N_{75}P_{50}$, harvested in early bud stage and variant $a_2b_3c_1$ inoculated, fertilized with $N_{75}P_{50}$, harvested in early bud stage, obtained the lowest value of the ratio Ca/P in shoots of alfalfa, 2.65, and the highest value of 6.70 was obtained in the variant $a_2b_1c_6$ inoculated, fertilized, harvested at full bloom.

All differences were statistically assured except variants $a_1b_3c_2$ (without inoculation, fertilized with $N_{75}P_{50}$, harvested in mid bud stage) and variant a2b1c1 (inoculated, fertilized, harvested in early bud stage), where differences obtained compared to the control were not statistically assured. In the case of variants harvested in early bud stage, fertilized, with or without seed inoculation differences were negative (*table 3*).

Regardless of the influence of many factors on the quality of alfalfa (cultivar, climatic conditions, fertilization, plant growth stage at harvest, etc.), regarding mineral content (Ca, P and Mg), the anatomy of the plant has a great influence. Thus, in the leaves of alfalfa is found a Ca content 300% and a P content 10% higher than in stems. As a consequence, the leaves stems is the indicator that greatly influences the content of alfalfa in these three elements.

Each of the three factors studied had a different impact on alfalfa Ca and P content or the ratio Ca/P. The inoculation factor, regardless of the anatomical part of the plant analyzed, between inoculated variants ($a_2$) and no inoculated variants ($a_1$) there was a insignificant difference of 0.00-0.03 g kg-1 DM (Ca and P content) or 0.00-0.03 units (Ca/P ratio), (*table 4*).

Table 3

**Influence of interaction between inoculation, fertilization and growth stage at harvest on the alfalfa stems calcium and phosphorus content**

| Indicator | | Ca content (g·kg$^{-1}$ DM) | P content (g·kg$^{-1}$ DM) | Ca/P | Ca content (g·kg$^{-1}$ DM) | P content (g·kg$^{-1}$ DM) | Ca/P |
|---|---|---|---|---|---|---|---|
| Variant | | $a_1$-without innoculation | | | $a_2$-with innoculation | | |
| $b_1$-$N_0P_0$ | $c_1$ | 7.23$^{Control}$ | 2.31$^{Control}$ | 3.12$^{Control}$ | 7.22 | 2.31 | 3.13 |
| | $c_2$ | 8.00*** | 2.18°°° | 3.67*** | 8.30*** | 2.17°°° | 3.82*** |
| | $c_3$ | 9.76*** | 2.12°°° | 4.61*** | 9.51*** | 2.11°°° | 4.50*** |
| | $c_4$ | 11.28*** | 2.11°°° | 5.34*** | 11.04*** | 2.11°°° | 5.24*** |
| | $c_5$ | 12.63*** | 2.09°°° | 6.05*** | 12.64*** | 2.09°°° | 6.07*** |
| | $c_6$ | 13.91*** | 2.09°°° | 6.66*** | 13.95*** | 2.08°°° | 6.70*** |
| $b_2$-$N_{50}P_{50}$ | $c_1$ | 7.29 | 2.72*** | 2.68°°° | 7.28 | 2.71*** | 2.68°°° |
| | $c_2$ | 8.07*** | 2.56*** | 3.15* | 8.38*** | 2.55*** | 3.28 |
| | $c_3$ | 9.85*** | 2.49*** | 3.95*** | 9.60*** | 2.48*** | 3.86 |
| | $c_4$ | 11.38*** | 2.48*** | 4.58*** | 11.14*** | 2.48*** | 4.49 |
| | $c_5$ | 12.74*** | 2.45*** | 5.19*** | 12.75*** | 2.45*** | 5.21 |
| | $c_6$ | 14.04*** | 2.46*** | 5.71*** | 14.08*** | 2.45*** | 5.75 |
| $b_3$-$N_{75}P_{50}$ | $c_1$ | 7.36*** | 2.78*** | 2.65°°° | 7.35*** | 2.77*** | 2.65°°° |
| | $c_2$ | 8.15*** | 2.62*** | 3.12 | 8.46*** | 2.61*** | 3.24*** |
| | $c_3$ | 9.95*** | 2.54*** | 3.91*** | 9.69*** | 2.54*** | 3.82*** |
| | $c_4$ | 11.50*** | 2.54*** | 4.53*** | 11.25*** | 2.53*** | 4.44*** |
| | $c_5$ | 12.87*** | 2.51*** | 5.13*** | 12.88*** | 2.50*** | 5.15*** |
| | $c_6$ | 14.18*** | 2.51*** | 5.65*** | 14.22*** | 2.50*** | 5.68*** |
| $b_4$- 30 Mg·ha$^{-1}$ manure | $c_1$ | 7.45*** | 2.66*** | 2.80°°° | 7.43*** | 2.65*** | 2.80°°° |
| | $c_2$ | 8.24*** | 2.51*** | 3.29*** | 8.55*** | 2.50*** | 3.42*** |
| | $c_3$ | 10.06*** | 2.44*** | 4.13*** | 9.80*** | 2.43*** | 4.03*** |
| | $c_4$ | 11.62*** | 2.43*** | 4.78*** | 11.37*** | 2.42*** | 4.69*** |
| | $c_5$ | 13.01*** | 2.40*** | 5.42*** | 13.02*** | 2.40*** | 5.44*** |
| | $c_6$ | 14.33*** | 2.40*** | 5.96*** | 14.37*** | 2.40*** | 6.00*** |
| *LSD* | *5%* | *0.07* | *0.01* | *0.03* | *0.07* | *0.01* | *0.03* |
| | *1%* | *0.09* | *0.02* | *0.04* | *0.09* | *0.02* | *0.04* |
| | *0.1%* | *0.11* | *0.03* | *0.05* | *0.11* | *0.03* | *0.05* |

Fertilization is one of the most important factors on which depends production, but most especially forage quality of alfalfa crop. In this case, mineral and organic fertilization showed a different impact on quality indicators. The Ca content of leaves and stems was higher than the unfertilized control variants (differences statistically assured), but because the leaves/stems ratio was higher in organic fertilization, the Ca content of alfalfa plants was very significantly higher in this variant. In the case of variants fertilized with mineral fertilizers, plant Ca content was lower than the control variant (*table 4*). The same trends were shown and the results of similar research conducted by: Lloveras J. et al, 2004; Ketterings Q.M. et al, 2008; Vasileva V., 2012.

It should be noted that in all variants of fertilization, the ratio Ca/P is significantly reduced due to the contribution of P by applying mineral fertilizers with nitrogen and phosphorus or manure, which is a complex fertilizer of the best quality. This result is beneficial to the animal organism, since the high ratio Ca/P of alfalfa being a shortcoming of alfalfa, and any improvement in this indicator is very important.

As development phenophases succed the content of Ca and P is increasingly more influenced by the content of stems in these indicators since the ratio leaf/stem changes in favor for plant stems (*table 4*). The results confirm the findings of other researchers (Schmierer J.L. et al, 2005; Lanyasunya T.P. et al, 2007; Katić S. et al, 2009).

The correlation between the plant growth stage at harvest and the content of alfalfa plants, leaves and stems in Ca and P is negative significant and distinctly significant (*figure 1*; *figure 2*).

While aging, the ratio Ca/P in leaves, stems and whole plant increases and the correlation between harvesting plant phenophase and the ratio Ca/P is distinctly significant (*figure 3*). This conclusion also fits the results of numerous studies by other researchers (Silva A.A. et al, 2005; Marković J. et al, 2007; Radović J. et al, 2009).

Table 4

**Influence of inoculation, fertilization and growt stage at harvest on the alfalfa plants, leaves and stems phosphorus content**

| Indicator | | Plants | | | Leaves | | | Stems | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Variant | | Ca content | P content | Ca/P ratio | Ca content | P content | Ca/P ratio | Ca content | P content | Ca/P ratio |
| | | (g·kg$^{-1}$ DM) | | | (g·kg$^{-1}$ DM) | | | (g·kg$^{-1}$ DM) | | |
| $a_1^{Control}$ | | 16.24$^{C}$ | 2.52$^{C}$ | 6.60$^{C}$ | 28.78$^{C}$ | 2.68$^{C}$ | 11.35$^{C}$ | 10.62$^{C}$ | 2.43$^{C}$ | 4.42$^{C}$ |
| $a_2$ | | 16.24 | 2.51 | 6.62 | 28.77 | 2.67 | 11.39 | 10.59 | 2.43 | 4.42 |
| *LSD* | *5%* | *0.14* | *0.04* | *0.07* | *0.39* | *0.05* | *0.20* | *0.07* | *0.03* | *0.05* |
| | *1%* | *0.24* | *0.07* | *0.12* | *0.65* | *0.08* | *0.34* | *0.12* | *0.05* | *0.08* |
| | *0.1%* | *0.44* | *0.12* | *0.22* | *1.22* | *0.14* | *0.63* | *0.22* | *0.10* | *0.14* |
| $b_1^{Control}$ | | 16.24$^{C}$ | 2.28$^{C}$ | 7.29$^{C}$ | 28.29$^{C}$ | 2.61$^{C}$ | 11.48$^{C}$ | 10.46$^{C}$ | 2.15$^{C}$ | 4.91$^{C}$ |
| $b_2$ | | 16.10° | 2.59*** | 6.33°°° | 28.75*** | 2.71*** | 11.23°° | 10.55** | 2.52*** | 4.21°°° |
| $b_3$ | | 15.82°°° | 2.63*** | 6.12°°° | 28.98*** | 2.73*** | 11.51°° | 10.65*** | 2.58*** | 4.16°°° |
| $b_4$ | | 16.80*** | 2.55*** | 6.70°°° | 29.06*** | 2.66** | 11.55 | 10.77*** | 2.47*** | 4.40°°° |
| *LSD* | *5%* | *0.11* | *0.03* | *0.03* | *0.20* | *0.03* | *0.13* | *0.05* | *0.02* | *0.03* |
| | *1%* | *0.17* | *0.05* | *0.05* | *0.30* | *0.05* | *0.20* | *0.08* | *0.04* | *0.05* |
| | *0.1%* | *0.27* | *0.07* | *0.08* | *0.48* | *0.08* | *0.33* | *0.13* | *0.06* | *0.07* |
| $c_1^{Control}$ | | 14.35$^{C}$ | 3.06$^{C}$ | 4.70$^{C}$ | 27.65$^{C}$ | 3.91$^{C}$ | 7.07$^{C}$ | 7.33$^{C}$ | 2.62$^{C}$ | 2.81$^{C}$ |
| $c_2$ | | 14.69** | 2.65°°° | 5.57*** | 27.69 | 3.08°°° | 9.03*** | 8.27*** | 2.46°°° | 3.37*** |
| $c_3$ | | 15.37*** | 2.43°°° | 6.34*** | 28.27** | 2.52°°° | 11.24*** | 9.78*** | 2.39°°° | 4.10*** |
| $c_4$ | | 16.94*** | 2.35°°° | 7.25*** | 29.04*** | 2.33°°° | 12.47*** | 11.32*** | 2.39°°° | 4.76*** |
| $c_5$ | | 17.59*** | 2.31°°° | 7.65*** | 29.69*** | 2.15°°° | 13.78*** | 12.82*** | 2.36°°° | 5.46*** |
| $c_6$ | | 18.48*** | 2.28°°° | 8.15*** | 30.19*** | 2.06°°° | 14.63*** | 14.13*** | 2.36°°° | 6.01*** |
| *LSD* | *5%* | *0.23* | *0.02* | *0.09* | *0.50* | *0.03* | *0.19* | *0.15* | *0.02* | *0.06* |
| | *1%* | *0.33* | *0.03* | *0.13* | *0.70* | *0.04* | *0.27* | *0.22* | *0.03* | *0.08* |
| | *0.1%* | *0.47* | *0.05* | *0.19* | *1.02* | *0.05* | *0.39* | *0.31* | *0.04* | *0.12* |

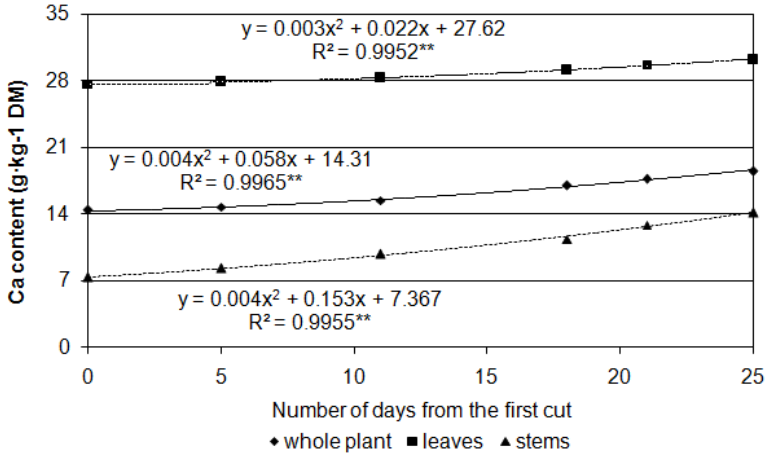


Figure 1 **Correlations between the growth stage at harvest and calcium content from leaves, stems and whole plant**

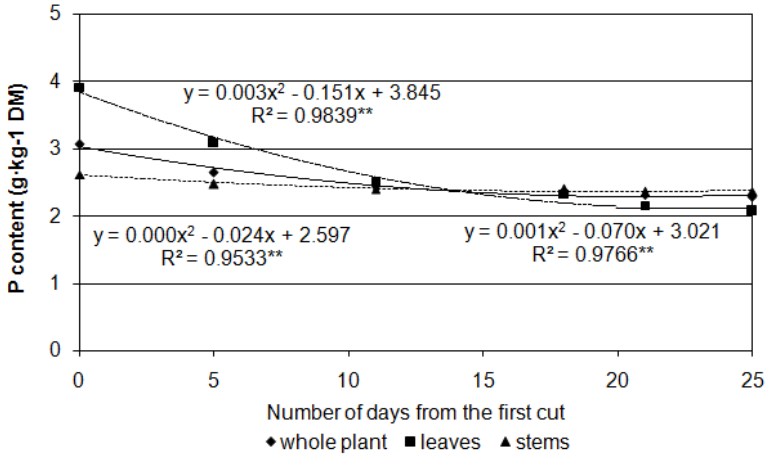


Figure 2 **Correlations between the growth stage at harvest and phosphorus content from leaves, stems and whole plant**

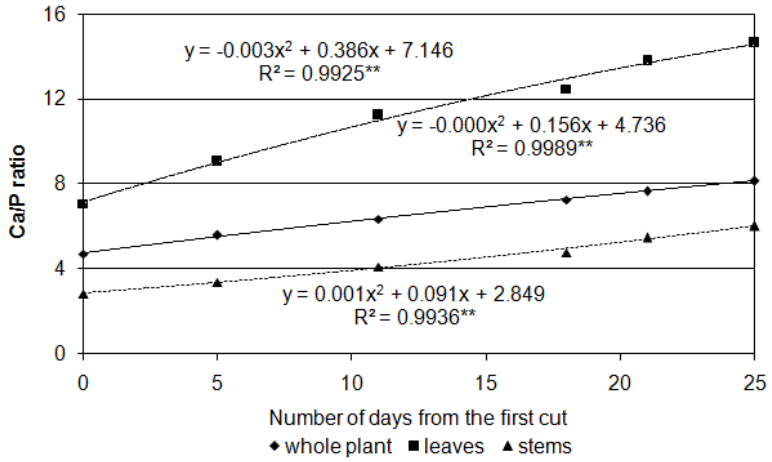


Figure 3 **Correlations between the growth stage at harvest and Ca/P ratio from leaves, stems and whole plant**

## CONCLUSION

Inoculation had no significant influence on alfalfa phosphorus content.

Calcium content in leaves and stems was higher than the unfertilized control (differences statistically assured), but because of the higher leaves percent in organic fertilizer variant, alfalfa plants Ca content was very significantly higher in this variant;

Fertilization determined the highest positive difference, statistically assured, due to phosphorus brought with the administration of mineral or organic fertilizers.

In all fertilization variants, the Ca/P ratio is significantly reduced due to the contribution of P by applying fertilizers with nitrogen and phosphorus or manure, which is a high quality complex fertilizer. This is a benefit for the animal body, the high Ca/P ratio is a weakness of alfalfa, and any improvement in this indicator is important;

With the advance of vegetation, calcium content in the entire plants, leaves and stems is growing. In this study, the correlation between growth stage at harvest and calcium content in entire plants, leaves and stems is positive;

Phosphorus content in entire plants, leaves and stems decreases, with the advance of vegetation. The correlation between growth stage at harvest and content of plant, leaves and stems of alfalfa in Ca and P is negative;

With the advance of vegetation, Ca/P ratio in leaves, stems and entire plant increases, the correlation between growth stage at harvest and the Ca/P ratio is significantly distinct.

## ACKNOWLEGMENTS

This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

## REFERENCES

**Albu Aida, Pop I.M., Radu-Rusu Cristina, 2012** - *Calcium (Ca) and phosphorus (P) concentration in dairy cow feed*. Lucrări Ştiinţifice - Seria Zootehnie, 57:70-74.

**Ball S.T., 1998.** *Alfalfa Growth Stages, New Mexico State University,* Guide A-330, availablel on-line at: http://lubbock.tamu.edu/.

**Crozier J.A., Allen V.G., Jack N.E., Fontenot J.P. and Cochran, 1997** - *Digetibility, apparent mineral absorption, and voluntary intake by horses fed alfalfa, tall fescue, and caucasian bluestem*. Journal of Animal Science, 75:1651-1658.

**Katić S., Milić D., Karagić Đ., Vasiljević S., Glamočić D., Jajić I., 2009** - *Variation of protein, cellulose and mineral contents of lucerne as influenced by cultivar and cut*. Biotechnology in Animal Husbandry, 25(5-6):1189-1195.

**Ketterings Q.M., Cherney J.H., Czymmek K.J., Frenay E., Klausner S.D., Chase L.E., Schukken Y.H., 2008** - *Manure Use for Alfalfa-Grass Production*. Department of Animal Science and Department of Crop and Soil Sciences College of Agriculture & Life Sciences, Cornell University, available on-line at: http://nmsp.cals.cornell.edu/publications/files/Manureandalfalfa.pdf.

**Lloveras J., Aran M., Villar P., Ballesta A., Arcaya A., Vilanova X., Delgado I. Munoz F., 2004** - *Effect of Swine Slurry on Alfalfa Production and on Tissue and Soil Nutrient Concentration*. Agronomy Journal, 96:986-991.

**Lanyasunya T.P., Mukisira E.A., Kariuki S.T., Ilatsia E.D., 2007** - *Effects of Commelina benghalensis, Vicia sativa and Medicago sativa used as protein supplements on performance of dorper sheep fed sorghum almum*. Tropical and Subtropical Agroecosystems, 7:211-216.

**Marković J., Radović J., Lugić Z., Sokolović D., 2007** - *The effect of development stage on chemical composition of alfalfa leaf and stem*. Biotechnology in Animal Husbandry, 23(5-6):383-388.

**Mauries M., 1994** - *La Luzerne Aujourd'hui*. France Agricole, Paris, France.

**Mueller S.C., Teuber L.R., 2007** - *Alfalfa Growth and Development*. In: Summers C.G.and Putnam D.H., (eds), Irrigated alfalfa management for Mediterranean and Desert zones, Chapter 3, University of California Agriculture and Natural Resources Publication 8289.

**Radović J., Sokolović D. şi Marković J., 2009** - *Alfalfa-most important perennial forage legume in animal husbandry*. Biotechnology in Animal Husbandry, 25(5-6):465-475

**Sanz-Sáez Á., Erice G., Aguirreolea J., Muñoz F., Sánchez-Diaz M., Irigoyen J.J., 2012** - *Alfalfa forage digestibility, quality and yield under future climate change scenarios vary with Sinorhizobium meliloti strain*. Journal of Plant Physiology, 169:782-788.

**Schitea Maria, Martura T., 2004** - *Sandra, un nou soi de lucernă creat la I.C.D.A. Fundulea*. Anual NARDI Fundulea, 71:181-190.

**Schmierer J.L., Mery R.D., Putnam D.H., 2005** - *Testing alfalfa for phosphorus and potassium nutrient deficiencies*. California Alfalfa and Forage Symposium, available on-line at: http://alfalfa.ucdavis.edu/+symposium/proceedings/ asdf/alf_symp/ 2005/05-201.pdf.

**Silva A.A., Varanda E.M., Primavesi Ana C.**, **2005** - *Effect of the inherent variation in the mineral concentration of alfalfa cultivars on aphid populations*. Bragantia, Campinas, 64(2):233-239

**Stancheva I., Geneva M., Djonova E., Kaloyanova N., Sichanova M., Boychinova M., Georgiev G., 2008** - *Response of alfalfa (Medicago sativa* L.*) growth at low accessible phosphorus source to the dual inoculation with mycorrhizal fungi and nitrogen fixing bacteria*. General and Applied Plant Physiology, 34(3-4):319-326.

**Touil L., Guesmi F., Farès K., Zagrouba C., Ferchichi A., 2009** - *Mineral composition and genetic variability of some mediterranean populations of the cultivated alfalafa (Medicago sativa* L.*) supported by morphological markers*. Asian Journal of Plant Sciences, 8:1-10.

**Vasileva V., 2012** - E*ffect of mineral nitrogen fertilization and water-deficiency stress on chemical composition of lucerne (Medicago sativa* L.*)*. Grassland Science in Europe, Grassland - a European Resource? 17:391-393.

**Vîntu V., Stavarache M., Samuil C., Muntianu I.C., 2012** - *Chemical composition dynamics of alfalfa (Medicago sativa* L.*) at different plant growth stages*, Grassland Science in Europe - Grassland a European Resource?, 17:394-396.