Effect of Herbagreen Nano-Particles on Biochemical and Technological Parameters of Cereals (Wheat and Corn)

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Abstract

This research study intended to provide evidence and proof of the positive effects, that applying of the Bio-Fertilizer Herbagreen produced through nanotechnology has on the agriculture in Albania.

There are no doubts that nanotechnology is one of the most relevant innovations not only in agriculture, but also in other scientific fields such as biotechnology, medicine etc.

Herbagreen nano-particles are able to directly penetrate plants after being applied, thus becoming immediately part of the plants lifecycle and metabolism. This is due to the new nano- and micro-dimensions of the grinded particles after being treated through TMAC (Tribo-Mechanical Activation of Calcite). This technology does not interfere with the mineral initial composition. It simply grinds the mineral particles in nano-dimensions and by so doing increases sensibly their contact surface. This makes these particles "magically" influence the yield quantity and quality in the treated plants.

Based on the experiments performed during two consecutive years on the selected crops of corn and wheat, it was noticed that in addition to a yield increase in the plots treated with this nanotechnology, there was also an increase in the gluten values in wheat, while regarding the percentage values of proteins and lipids it was noted that the values of the treated plots with Herbagreen had the same values of the plots treated with the maximal dosage of chemical fertilizers. It was also concluded, that using of the Herbagreen fertilizers can avoid or reduce the soil and waters contamination caused by only traditional mineral fertilizers use. The financial costs for the treatment can be reduced as well.

Keywords: Lipids, proteins, nanotechnology, Herbagreen, nano-particles, TMAC

Introduction

Nanotechnology as a newly discovered field allows for advanced research with satisfying results in a variety of fields including agriculture (Brahic, 2005), (Jones, 2006), (Mehta, 2004). This article aims to highlight the positive effects of Herbagreen nanotechnology, which has given results in all kinds of plants and in some fruit trees as well as, viticulture (Artyszak, 2015), (Artyszak, 2014), (Kara, 2010).

Herbagreen is a 100% natural product (it contains Calcium Oxide CaO 35,9%, magnesium oxide MgO 1.9%, dioxide selenium SiO2 18.1%, phosphor P2O5 0.28%, potassium oxide K2O 0.1%, sulphur S 0.52% as well as a few other microelements in µm and nano granules), (Dumancic, 2010), (acting-herbagreen, 2008).

Around the world Herbagreen is being used intensively as an environmentally and plant friendly alternative combined or instead of other mineral fertilizers (Doran 2011), (NGTech, 2014). It allows us to limit the use of chemical fertilizers, decrease production costs, increase yield and the plants' biochemical parameters (Artyszak, 2014), (Trawczynski, 2013), (Ugrinovi, 2011). The technique for Herbagreen production is achieved through TMAC (Tribo-Mechanical Activation of Calcite), which is a method that has been perfected and patented by Dr. Tihomir Lelas. Calcites or different minerals (in our case limestone) are inserted in machinery called the activator. The limestone parts are thrown in there while colliding fast and on the opposite side in-between the two flexible wings, which compose the activator (with speed 3 times faster than the speed of sound), are broken and granulated into micro and nano dimensions. The pulverized limestone particles hold an active energy that is used in treating the plants, which react positively, readily and efficiently (Lelas, 1998), (acting-herbagreen, 2008). This technology does not change the mineral's composition; it only breaks it into nanoparticles with increased reactivity. Immediately after the application of this granulated powder in microscopic dimensions on the plants, we observe a difference in the leaf color as a start (which is very intensive, so much so that it is visible to the naked eye), the vitality and in the end in the yield and quality markers (Jones 2006), (Prifti,2015), (Prifti,2015), (Prifti,2014). This, as these particles immediately enter into the life processes of the plants, the most important being photosynthesis (SMART, 2016),(NGTech, 2014). The calcium carbonate particles, CaCO3 by penetrating through the leave stoma, are dissolved into calcium oxide (CaO) and carbon dioxide (CO2). Carbon dioxide is the main element or component in photosynthesis, which with the help of solar energy is converted into chemical energy potentially used by the plant. Glucose is the first organic component produced by photosynthesis. The intensity of photosynthesis is directly dependent on the concentration of carbon dioxide, thus by treating plants with Herbagreen we ensure the continuity of this component by harmonizing photosynthesis and all the progress of the primary and secondary mechanisms in the plant (Dumancic 2010), (Epstein 1999), (Fauteux 2005).

Materials and Methods

The experiments in this study were carried out in the soils of the Experimental Station of the Agricultural University of Tirana (EDE Valias). According FAO classification they are fluvial soils. It was performed for two consecutive years on wheat and corn according to the randomized block scheme with 5 variants, four replications each, (Prifti,2015), (Prifti,2015), (Prifti,2014). Variants are described below:

1. Control (1 variant, no treatment, the actual soil fertility).
2. Classic basic fertilizer applied dose (Nitrogen, phosphor, potassium) 3kv/ha DAP (diammonium phosphate), 2kv/ha potassium sulfate, and 4 kv/ha urea.
3. 30% less than the classic dose of fertilizer meaning 90 kg/ha DAP, 60 kg/ha potassium sulfate and 120 kg/ha nitrogen fertilizer)
4. 30% less than the classic dose of fertilizer and leaf treatment with Herbagreen
5. 30% less than the classic dose of fertilizer and two leaf treatments with Herbagreen (nanotechnology).

Proteins were measured via the classic method with the Kjeldahl apparatus. The Kjeldahl method consists of three steps: The sample is first digested in strong sulfuric acid in the presence of a catalyst, which helps in conversion of the amine nitrogen to ammonium ions.

1. The ammonium ions are then converted into ammonia gas, heated and distilled. The ammonia gas is led into a trapping solution where it dissolves and becomes an ammonium ion once again,
2. Finally the amount of the ammonia that has been trapped is determined by titration with a standard solution, and a calculation made.

Method description

Determination is based on the mineralization of the grinded sample and the distillation of the mineralized nitrogen and its definition in a 0.1 N HCl solution.

Procedure

1 g of granulated sample was weighed on the technical scale and was then put in the mineralization-distillation dish where 20 ml of sulphuric acid

d- 1.84 g/cm^3 and 5 g catalyzing mixture are added, and is transferred to the mineralizing unit. The mineralizing temperature was set to 400^{0}C and after this temperature was reached the mineralization process continues for one hour and 30 minutes. The mineralizing dish was removed from the mineralizing apparatus and after cooling the solution was poured into messkolben 250 ml, it was then filled with water to the mark and mixed. 25 ml of solution is pipetted into the distilling dish and one drop of phenolphthalein 2% , 25 ml sodium hydroxide 30% were added. The distilling dish was put into the distillator. In an Erlenmeyer flask (300ml) 100 ml boric acid 1% with mixed indicator is added. The Erlenmeyer is placed into the tube at the end of the condensator and the apparatus was locked for the distillation for 15 minutes. Following the distillation the Erlenmeyer with the distillators was removed from the apparatus and the solution titrated with 0,1 N HCl solution until color change is observed.

Calculations

P % = (Vx0.1x14x 6.25x 250x100)/1000x1x25=Vx8.75

P-proteins,V- ml of 0.1 N HCl solution used

For the fats:

Method description

Determination is based on the extraction of the homogenized sample with diethyl etherin the Sechelt apparatus, the extract distillation and weighing of the fat extracted.

According to the Solxhet's procedure, oil and fat from solid material are extracted by repeated washing (percolation) with an organic solvent, usually hexane or petroleum ether, under reflux in a special glassware.

In this method the sample was dried, ground into small particles and placed in a porous cellulose thimble. The thimble was placed in an extraction chamber, which was suspended above a flask containing the solvent and below a condenser. The flask was heated and the solvent evaporates and moved up into the condenser where it was converted into a liquid that trickles into the extraction containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and trickles back down into the boiling flask. At the end of extraction after closing a stopcock between the funnel and the extraction chamber. The solvent in the flask is then evaporated and the mass of the remaining lipids measured. The percentage of lipid in the initial sample can be calculated.

Procedure

1-2 g of sample was weighed on the technical scale and was then placed on the prepared paper cartridge which was placed in the extraction unit.

The flask weighed in advance and dried distillatory A_0. In the extractor, the extraction unit and one emptying and a half deciliter (reagent) were added, then connected to the extractor and condensator. It is then locked and the extraction was completed in at least 4-6 hours. When the extraction ended the apparatus was dismantled and deciliter is distilled to a very small amount. The flask was placed into the drying oven at 90^0C for 30 min. The flask with the extracted fat was cooled and weighed A_1

Calculation

Fat %= (A_1-A_0) x 100/m, A_1-the flusk weight with fat**,** A_0-empty flusk weight, m- sample weight

For wet gluten

Wet gluten in wheat flour is a visco-elastic substance made of gliadin and glutein, which is obtained by means of the specified method contained in this international standard. The gluten index is a measure of the gluten characteristics, which indicates whether the gluten is weak, normal or strong.

Procedure

In the porcelain scale 12.5 grams of wheat flour grinded in a milling mill with a 1 mm sieve was weighed. 7.5 ml of distilled water is added. The flour was mixed with the water using the glass rod in the beginning and then the left hand fingers. The dough is homogenized and made into spherical shape. The capsule was covered with crystal and left for 20-30 minutes, enough for the proteins to expand (dough to rise). Following this time the dough was washed with running water. In this process the starch left as a white liquid and wheat bran. This process lasts for 15-20 minutes. The dough was dried in hands until it starts to stick. At this point it was weighed immediately in a crystal. Then the gluten content was calculated using the following formula: $G(\%) = g * 100 \div p$where: G- % of glutein content, g -glutein weight in grams, p -flour weight in grams

The results of the experiments for proteins, fats and gluten for wheat; proteins and fats for corn are shown in the graphs below. The data belong to two consecutive years of experiment on wheat and corn plant.

These graphs represent the average of the replications per each one of the variants 1,2,3,4 and 5. Each variant had 4 replications. Based on the above mentioned methodology the parameters of each replication were analyzed and measured. Than it was done the average of the data for proteins, lipids in the corn and wheat and the gluten in wheat. The statistical elaboration of the data was done by using the ANOVA method and LTS –d test.

Results and discussion

According to the results on corn in 2013, the data in graphs show the average of proteins % per each variant. The data showed that Variant 5 (the one on which were used 30 % less of chemical fertilizers plus two Herbagreen treatments) had the highest values. Variant 5 was followed by Variant 2 (M=8.61,SD=.181), Variant 3 (M=8.46,SD=.111), Variant 4 (M=8.3,SD.182) and Variant 1.

Concerning to the lipids on corn in 2013, the data showed that variant 3 (the one on which were used 30 % less of chemical fertilizers) had the highest values (M=3.41, SD=.084) followed by variant 2 (M=3.3, SD =.106) Variant 3 (M=3.24,SD=.119), variant 5 (M=3.16, SD=.432), the Variant 4 represented the lowest lipids values (M=3.08, SD=.127)

Referred to % of proteins on corn (2014), the data showed that variant 4 (the one on which were used 30 % less of chemical fertilizers plus one Herbagreen leaf pulverization) had the highest values (M=8.96,SD=.029) followed by variant 3 (M=8.92, SD =.049) variant 5 (M=8.91,SD=.048), variant 2 (M=8.79,SD=.181). The variant 1 represented the lowest protein values (M=8.46, SD =.158)

Concerning to the % of lipids (on corn 2014), the data showed that variant 5 (the one on which were used 30 % less of chemical fertilizers plus two Herbagreen treatments) had the highest values (M=4.4,SD=.086), followed by variant 4 (M=4.2, SD =.070) Variant 2 (M= 4.12,SD=.069), variant 3 (M=4,SD=.070), the variant 1 represented the lowest values (M=3.96,SD =.167).

Results on wheat

The data on graphs show the average of proteins % per each variant. In 2014 the variant 3 (the one on which were used 30 % less of chemical fertilizers) had the highest values (M=15.39,SD=.063) followed by Variant 5 (M=15.30, SD =.182) variant 2 (M=15.24,SD=.043), Variant 4 (M=14.49,SD=.137), the variant 1 represented the lowest protein values (M=14.45, SD =.081)

About the average of lipids % per each variant, the data showed that Variant 2 (with maximal chemical fertilizer doses) had the highest values (M=2.77,SD=.057) followed by Variant 1 (M=2.6, SD =.091) variant 4 (M=2.16,SD=.048), variant 3 (M=2.14,SD=.055), the variant 5 represented the lowest lipids values (M=2.11, SD =.024)

Concerning to the % of gluten the data showed that variant 3 (the one on which were used 30 % less of chemical fertilizers) has the highest values (M=29.6, SD=.141) followed by variant 5 (M=28.8,SD=.037), variant 2 (M=27.8,SD=.071), variant 4 (M=27.6,SD=.414), variant 1 represented the lowest gluten values(M=20.2, SD =.081)

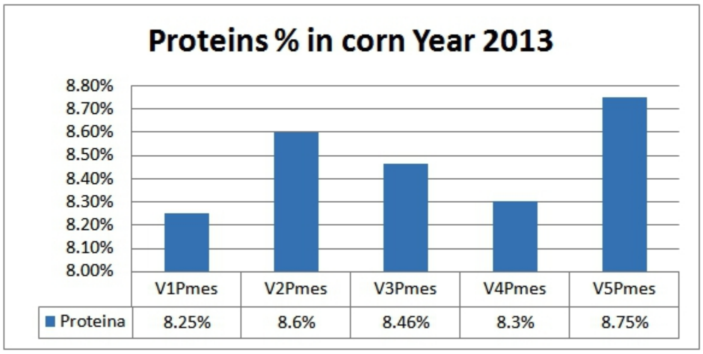
Wheat 2015

On the 2015 the data showed that variant 3 (the one on which were used 30 % less of chemical fertilizers) had the highest values (M=15.18, SD=.154) followed by variant 5 (M=15.11, SD =.088) variant 4 (M=14.07,SD=.0360), variant 2 (M=13.23,SD=.154), variant 1 represented the lowest values (M=12.185, SD =.162)

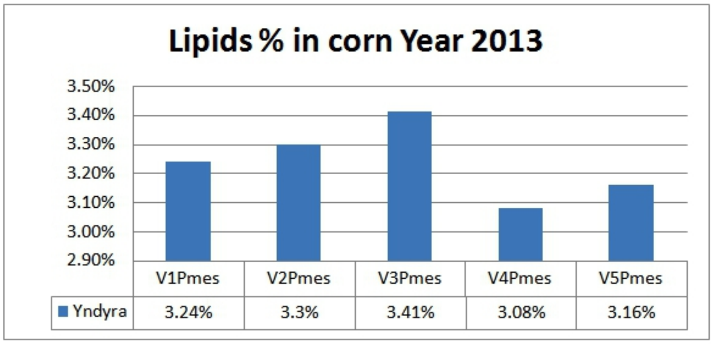
About the % of lipids per each variant, the data showed that variant 2 (with maximal chemical fertilizer doses) had the highest values (M=3.19, SD=.0139) followed by variant 3 (M=2.94, SD =.053) Variant 5 (M=2,81,SD=.086), variant 1 (M=2.43,SD=.402), variant 4 represented the lowest values (M=2.3, SD =.104)

Concerning to the % of gluten per each variant, the data showed that variant 5 (the one on which were used 30 % less of chemical fertilizers plus two Herbagreen bio fertilizer foliar treatments) had the highest values (M=33,SD=.102) followed by variant 3 (M=32,SD=.294), variant 4 (M=31.8,SD=.535), variant 2 (M=26.8,SD=.070), variant 1 represented the lowest values (M=22, SD =.102)

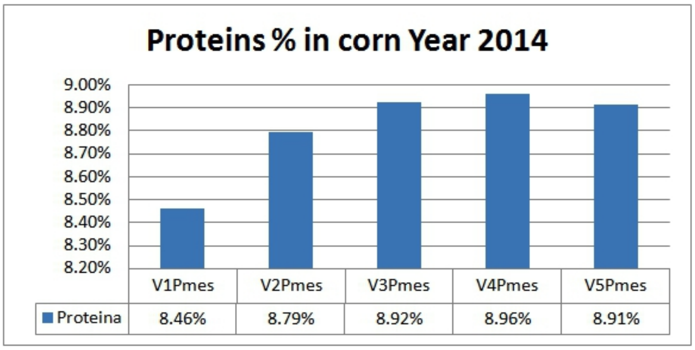
V1Pmes	8.25%
V2Pmes	8.6%
V3Pmes	8.46%
V4Pmes	8.3%
V5Pmes	8.75%



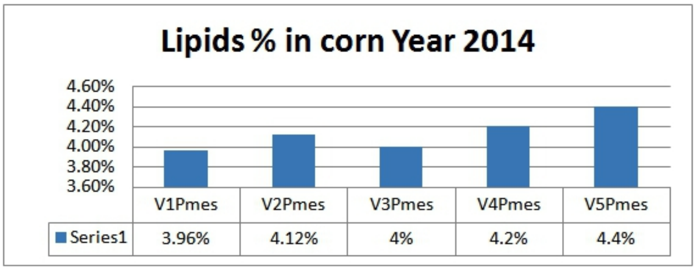
V1Pmes	3.24%
V2Pmes	3.3%
V3Pmes	3.41%
V4Pmes	3.08%
V5Pmes	3.16%



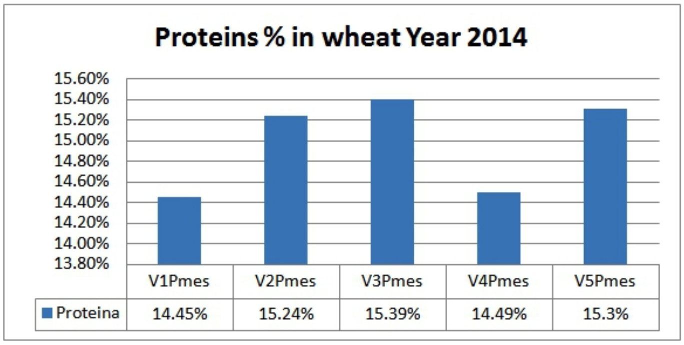
V1Pmes	8.46%
V2Pmes	8.79%
V3Pmes	8.92%
V4Pmes	8.96%
V5Pmes	8.91%



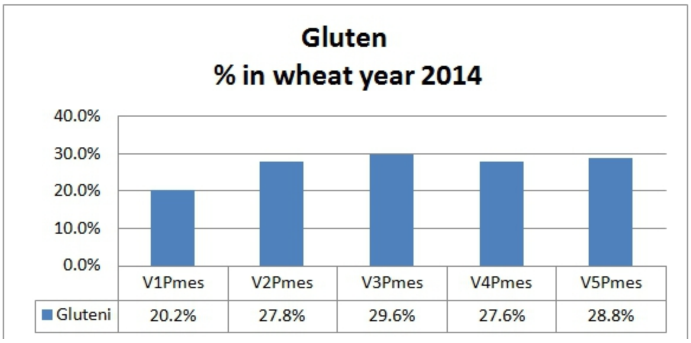
V1Pmes	3.96%
V2Pmes	4.12%
V3Pmes	4%
V4Pmes	4.2%
V5Pmes	4.4%



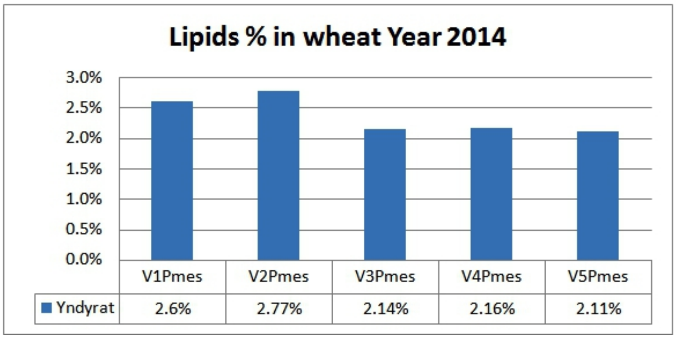
V1Pmes	14.45%
V2Pmes	15.24%
V3Pmes	15.39%
V4Pmes	14.49%
V5Pmes	15.3%



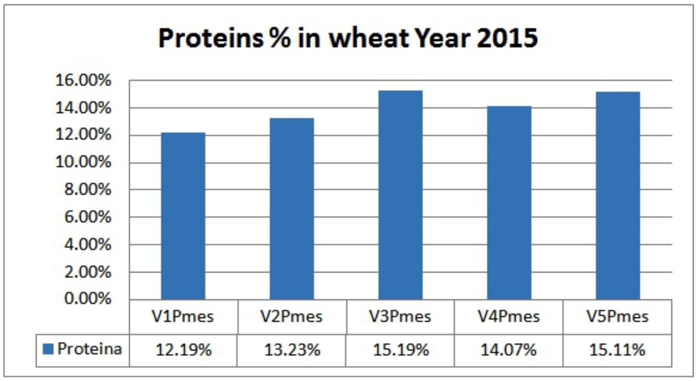
V1Pmes	20.2%
V2Pmes	27.8%
V3Pmes	29.6%
V4Pmes	27.6%
V5Pmes	28.8%



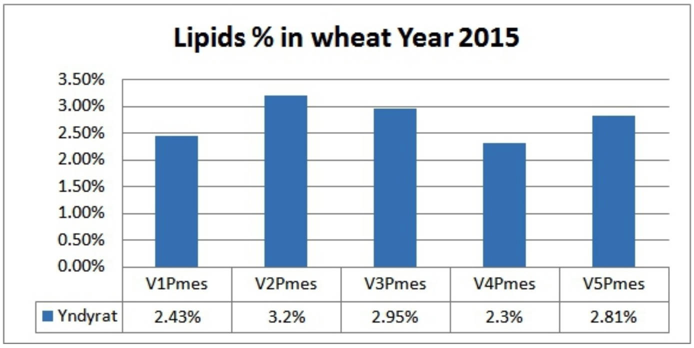
V1Pmes	2.6%
V2Pmes	2.77%
V3Pmes	2.14%
V4Pmes	2.16%
V5Pmes	2.11%



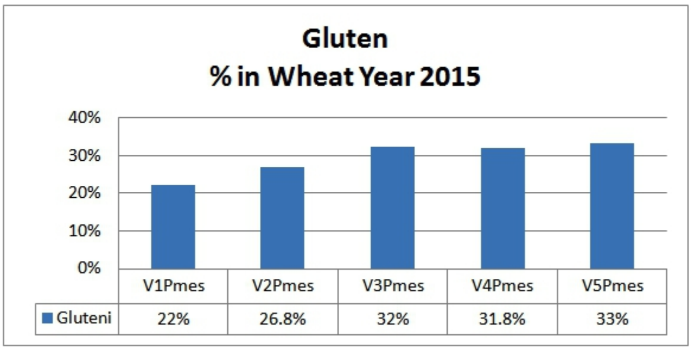
V1Pmes	12.19%
V2Pmes	13.23%
V3Pmes	15.19%
V4Pmes	14.07%
V5Pmes	15.11%



V1Pmes	2.43%
V2Pmes	3.2%
V3Pmes	2.95%
V4Pmes	2.3%
V5Pmes	2.81%



V1Pmes	22%
V2Pmes	26.8%
V3Pmes	32%
V4Pmes	31.8%
V5Pmes	33%



Conclusion

Concerning the corn results in 2013 the highest protein level was in the variant 5 with two Herbagreen sprayings at 8,75%, while the highest value for lipids 3,41% was in variant 3 with 120kg/ha ammonium nitrate, 90kg/ha DAP and 60kg/ha potassium sulphate.

Relating to the wheat results in 2014, the highest level of proteins were reached in variant 3 with 15,39%. The lipids content for wheat is an indicator of very little importance in the food industry. Referred to the study, it results that the highest level was registered in variant 2 with 2,77%. The use of Herbagreen does not influence this biochemical parameter.

The technological parameter gluten as a component of proteins is an important indicator in the bread production industry. According to the statistics of FAO organization the normal % of wet gluten must be not low of 20%. In 2014 the highest level was registered in the variant 3 with 29,6%. This was a relevant result because we were aiming to reach a satisfactory level of values with a decreased use of mineral fertilizers. This result was followed by the Variant 5 with two Herbagreen leaf fertilizer treatments. The same picture can be seen for 2015, where the level of wet gluten was higher for the variant with two treatments with Herbagreen fertilizer. Thus the difference in gluten between Variant 1 and the average of the variants with fertilizers was 8,9% so $(V_2 + V_3 + V_4 + V_5) /4 = 30,9\%$.

In 2014, the highest level of lipids for corn was reached in variant 5 with two Herbagreen leaf fertilizer treatments at 4,2% value.

This variant should be very favorable for the corn oil industry.

From the results could be concluded, that applying of the Herbagreen fertilizers could avoid or reduce the soil and waters contamination caused by only traditional mineral fertilizers use. The financial costs for the treatment could be reduced as well, but anyway it should require additional researches to strengthen the proved belief that by using Herbagreen Bio leaf fertilizer the Albanian agriculture could make a stable step towards sustained development.

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