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Establishment of DRIS indices and foliar nutrient levels for corn plants fertilized with various nitrogen doses and source materials associated with marine alga *Lithothamnium*

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Abstract: The objective of this study was to evaluate leaf nutrient status and to establish the rules of the diagnosis and recommendation integrated system (DRIS) in corn for different nitrogen doses and source materials associated with *Lithothamnium*. The experiment was carried out from December 2010 to February 2012 in Uberlândia, Minas Gerais. The experimental design was randomized blocks with split-split plots. The main plot treatments were nitrogen source materials (immediate release and controlled-release urea), the subplot treatments were doses of 60, 100 and 120 kg of N ha^{-1} and control (without N application), and the sub-sub plots were treatments with or without the use of *Lithothamnium*. The dose of *Lithothamnium* corresponded to 20% of the dose of urea. Calculations to establish the diagnosis and recommendation integrated system (DRIS) were made during the experiment. In the high-yield population the treatments with the highest nitrogen dose provided the highest grain yield. The use of the controlled-release source ensured greater nutritional balance of plants. No effect of *Lithothamnium* on improving the efficiency of urea applied via topdressing in corn was observed. According to the DRIS indices, in the high-yield population the nutrients showed deficiencies in the following descending order: Mg>Fe>Zn>S>B>Mn>P>Cu>Ca>K>N and excess: B>S>Fe>Ca>K>Zn>Mn>Cu>Mg>P>N. The low-yield population followed the following order: Mg>Fe>Zn>Mn>Cu>N>B>P>S>Ca and excess: Zn>S>Cu>Mn>Ca>K> P>B>N>Mg>Fe.
Keywords: leaf analysis, nutritional balance indices, urea, *Zea mays.*

Introduction

Corn (*Zea mays* L.) has a greater economic importance due to the nutritional value of its grain. It plays an important role in the production of food for humans, feed for animals and it is used as a raw material in various industrial processes (Galvão et al., 2014; Souza et al., 2012). According to the data collected by Conab (National Supply Company) the 2014/15 harvest in Brazil yielded 85 million tons of grain, securing the country the third place in the world. Corn production from the main crop was 30,244,100 tons occupying an area of 6,156,100 hectares (Conab, 2015).

Several factors influence the achievement of high yields in corn. Among them an adequate supply of nutrients to plants via fertilization stands out. The nutritional requirements of any plant are determined by the amount of nutrients it uptakes during its cycle. It is therefore necessary that these nutrients are available in adequate quantities. It is known that nitrogen has the greatest effect on increasing grain yield of corn, as it plays an important role in plant metabolism, thereby affecting productivity (Soratto et al., 2011; Valderrama et al., 2011).

Nitrogen is a mineral nutrient which is required in the highest quantities by corn and it influences the grain yield the most. However, there are several processes in the soil which may cause N losses, such as chemical and biological reactions, losses by leaching, volatilization, denitrification and immobilization (Silva et al., 2005; Gava et al., 2006; Vitti et al., 2007).

Urea is the most popular nitrogen fertilizers in the world. However, its disadvantages are its highly hygroscopic nature and its high susceptibility to loss by volatilization, especially when it is applied on the soil surface in the no-till system (Martins et al., 2014). For these reasons, the management of nitrogen fertilization has been the subject of various research works aiming to increase its efficiency with consequent increases of crop productivity.

Controlled-release fertilizers aim to reduce the loss of nutrients and to adjust the timing of their release with the demand of plants (Cahill et al., 2010). Nitrogen fertilizers with controlled release are made of conventional urea granules coated with one or more layers of polymers or resins permeable to water, which regulate the process of release of the nutrients (Silva et al., 2012).

In addition to the controlled-release nitrogen fertilizers, other technologies can improve crop performance as the use of seaweed extracts. The use of algae extracts in agriculture has increased significantly in recent decades. Studies show that the use of algae helps to increase plant growth, sometimes with consequent increases in production (Carvalho, 2014). The *Lithothamnium* is a cosmopolitan calcareous marine alga, mainly composed of calcium carbonate and magnesium. Besides being a source of calcium and magnesium it also provides varying amounts of iron manganese, boron, nickel, copper, molybdenum and selenium. Furthermore, it contains iron, manganese, boron, nickel, copper, molybdenum and selenium (Melo&Neto, 2003).

The *Lithothamnium* has the ability to absorb minerals from the environment and turn them into chemical compounds which are easily absorbed by plants. *Lithothamnium* is also attributed to increase: (i) pH, (ii) the availability of essential nutrients, (iii) biological activity and (iv) cation exchange capacity (CEC) of the soil, thus promoting favorable conditions for the availability and uptake of nutrients by plants (Mendonca et al., 2006; Moreira et al., 2011).

The diagnosis of the nutritional status of plants, such as critical levels of nutrients in plants, mainly in leaves, depends on the reference values for the crops. These values are generally established in calibration experiments under controlled environments, totally different from field conditions, what limits the identification of nutritional status. Calibration experiments do not considered variables such as: crops, climate, fertilization, soil types, among others, which influence the demand for nutrients by plants (Bhargava&Chadha, 1988). Thus, a practical and efficient method which complements the data obtained during the analysis of soil for fertilization management is the characterization of the nutritional status of plants based on their yield. The values are obtained in a particular location and serve as reference values (Beaufils, 1973).

Unlike other methods, the DRIS (Diagnosis and Recommendation Integrated System) is an integrated procedure which identifies in the plant the sufficiency of each nutrient in relation to others, instead of considering only the critical concentration of each specific nutrient (Samra&Arora, 1997). The DRIS evaluates the nutritional status of the plants considering the balance among nutrients. Thereby, a nutritionally balanced crop can respond with high yield, which does not happen in conditions of deficiency or nutritional imbalances (Guindani et al., 2009). Using the DRIS one can identify where the production is limited by nutritional imbalance, even when the content of any of the nutrients is below its critical level (Baldock& Schulte, 1996).

The DRIS method is based on the calculation of an index for each nutrient. It compares the ratio of a specific nutrient to each of the other nutrients in the sample under diagnosis with the ratio involving the same nutrient in a crop of high yield (Pinto et al., 2009).

In this context, the objective of this study was to: (i) evaluate the efficiency of *Lithothamnium* in corn using various nitrogen doses and sources, and (ii) to obtain the nutritional status of the high and low-yield populations using the rules of the diagnosis and recommendation integrated system (DRIS).

Methods

The experiment was carried out in Uberlândia, Minas Gerais, at the Federal Institute of TriânguloMineiro, from December 2010 to February 2011. The experimental area is located at the geographic coordinates of 18º46'12'' south latitude and 48º17'17'' west longitude.

Soil sampling in the experimental area was conducted at a depth of 20 cm for the chemical (Table 1) and physical characterization. The soil was classified as clayey red latosol (121 g kg^{-1} of coarse sand, 69 g kg^{-1} of fine sand, 24 g kg^{-1} of silt and 806 g kg^{-1} of clay).

Table 1.Chemical characterization of the soil on the experimental area, 0-20cm depth.

P	K	SO_4^-	Al	Ca	Mg	H+Al	SB	T	V	OM
--------mg dm^{-3}-------			-----------------$cmol_c$ dm^{-3}-------					--------%------		g kg^{-1}
9.5	34	4	0.4	0.4	0.1	4.30	0.59	4.89	12	26

pH H_2O	B	Cu	Fe	Mn	Zn
	--mg dm^{-3}--				
5.2	0.12	1.2	66	1.2	0.4

P, K = (HCl 0.05 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1}) avaliable P (Mehlich-1 extrator); Ca, Mg, Al, (KCl 1 mol L^{-1}); H+Al = (Buffer – SMP at pH 7.5)pH H_2O (1:2.5); SB = Sum of bases; T = CEC at pH 7.0; V = Base saturation; O.M= Organic matter O.M. = Colorimetric method (Embrapa, 2011).

We used a simple hybrid of maize "impact" by Syngenta, planted in rows spaced 0.9 m apart, with an average population of 60,000 plants per hectare. Fertilization with a mixture of 08-28-16, formulated using urea (45% N), triple superphosphate (42% P_2O_5) and potassium chloride (58% K_2O), at a dose of 350 kg per hectare was done at planting.

The experimental design was randomized blocks with four replications on split-split plots. The main plot treatments were immediate release and controlled-release urea, the subplot treatments were doses of 60, 100 and 120 kg N ha^{-1} and control (without application), and the sub-sub plots were treatments with *Lithothamnium*, either incorporated or not, totaling 8 treatments, 32 plots and 64 subplots.

Each plot consisted of 16 lines 3.5m long. The subplots were composed of 8 lines each 3.5m long. The area of the plot was 6.75 m^2.

Topdressing was performed with immediate-release and controlled-release urea 35 days after planting, along with the application of *Lithothamnium*, whose dose corresponded to 20% of urea dose.

To evaluate the absorption of nutrients by corn, 15 corn leaves were removed in each plot. We considered as a diagnostic leaf the first leaf above the insertion of the female inflorescence at the R2 stage, characterized by flowering (Faquin, 2002).

To obtain the yield we removed the kernels from corn ears harvested from the two central rows of the plot. The ears were handpicked from each row, weighed and the data were used to estimate yield in bags per hectare.

The calculations to establish DRIS indices were based on high-yield populations (or reference population) and low-yield populations. The reference populations were those treatments whose yields were above 130 bags ha^{-1}.

Yield spreadsheets of the experiments, as well as the DRIS indices and the nutritional balance (NBI) were obtained using Excel software (Microsoft). The calculations were made using the method originally proposed by Beaufils (1973) (Equation 1, 2, 3 and 4)

If: $Y/X_\alpha < Y/X_n$

Then:

The $\int(X/Y_n) = [1-(Y/X_n/Y/X_\alpha)]x(100xk/CV)$ (1)

If $Y/X_\alpha = Y/X_n$

Then: $\int(X/Y) = 0(zero)$ (2)

If $Y/X_\alpha \geq Y/X_n$

$\int(X/Y_n) = [(Y/X_\alpha/Y/X_n)-]x(100xk/CV)$ (3)

Where: $\int(Y/X)$ = ratio of nutrient Y to X; Y/X_α = sample to nutrient ratio; Y/Xn = the standard to nutrient ratio; s= standard deviation of the ratio Y/Xn; CV= coefficient of variation (%) to the ratio Y/Xn; k=sensitivity constant.

$$I_y = \frac{\sum_{i=1} m\int(Y/X_i) - \sum_{j=1} m\int(X_i/Y)}{m+n} \quad (4)$$

Where: I_y: DRIS index for nutrient Y; Y: nutrient of the index; X: other nutrient; *m*: number of functions whose nutrient Y is the denominator; *n*: number of functions whose nutrient Y is in the numerator.

Using the DRIS formula we calculated relative indices for the nutrients, which were negative, positive or zero. The negative and positive indices indicate deficiency and excess, respectively, while values near zero indicate adequate levels. Having calculated the indices for each nutrient, we established nutritional balance index (NBI) according to the method originally proposed by Beaufils (1973).

$$\mathrm{NBI} = [\text{índice A}] + [\text{índice B}] + \mathrm{K} + [\text{índice N}]$$

Results and Discussion

Regarding the average foliar levels found in the populations of high and low yield, phosphorus (P), potassium (K), calcium (Ca), boron (B), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) are suitable for corn, according to Embrapa (2010) and Faquin (2002) (Table 2). However, according to the same authors, foliar nitrogen (N) and sulfur (S) are suitable only in high-yield population (Table 2).

Low levels of N and S found in corn leaves in the population of low yield are likely to have caused the low yield due to the interaction of these nutrients. According to Malavolta&Moraes (2007), the synergistic effect between nutrients directly influences the development of plants. Synergism occurs when an ion increases the absorption of other ions, resulting in a better plant development, which can be observed in this study.

For poaceae, as corn, the most required nutrient is nitrogen, whose absorption depends on the amount of sulfur available to plants. The balance between the amounts of nitrogen and sulfur in soil and plants is important because it reflects the nutritional status of the plant (Mattos&Monteiro, 2003).

Sulfur and nitrogen are structural components of amino acids such as cysteine, cystine and methionine. In addition to this, they are part of ferredoxin, an electron transferring molecule involved in the photosynthesis of plants (Mengel&Kirkby, 2001).

The foliar magnesium (Mg) found in corn plants in low and high-yield populations (Table 2) are not at adequate levels for this culture, according to Faquin (2002) and Embrapa (2010). The competition between nutrients may be synergistic when an ion assist another one, or antagonistic when an ion absorption is impaired by the presence of another ion. There is also a non-competitive inhibition when the ions do not compete for the same carrier site. An example of such interactions is the effect of K^{+} and Ca^{2+}cations which often induce Mg deficiency in plants (Silva &Trevisam, 2015). In this study, K and Ca levels are suitable for this crop, what may have caused the deficiency of Mg.

Table 2. Average foliar contents and high and low-yield populations of corn.

Yield	N	P	K	Ca	Mg	S	B	Cu	Fe	Mn	Zn
	-------------------------g kg^{-1}						---------------------mg kg^{-1}				
						Above 130 bags ha^{-1}					
149.63	29.42	3.12	24.20	2.84	1.45	1.57	4.69	13.76	100.58	88.23	28.52
						Below 130 bags ha^{-1}					
107.383	25.60	3.01	23.4	2.69	1.48	1.33	5.16	14.14	101.34	76.625	26.795

When the DRIS index for each nutrient is negative, it indicates that the nutrient is below the appropriate level, and when it is positive, it indicates that the nutrient is above the optimal level. Thus, a negative (-) DRIS parameters indicates deficiency, while a positive value (+) indicates excess of the nutrient in relation to the others, while a value closer to zero indicates that the nutrient is in equilibrium in the plant (Baldock& Schulte, 1996).

The relationship between nutritional balance and yield is evident. DRIS indices for the high yield group present lower values than the indices for the low yield group (Tables 3 and 4).

Table 3, which compares the high yield group with the low yield group, shows that N is closer to balance (close to zero) in the high yield group. Foliar N content was influenced by increasing nitrogen doses from the controlled-release fertilizer (Table 3). The treatments with the highest doses of N are in the high-yield group (Table 3). The highest grain yield was 161.46 bags ha^{-1}, obtained in the treatment with the highest N dose (120 kg ha^{-1} from the controlled-release urea showing that its use met the demand for N more effectively without the use of *Lithothamnium*). The importance of N in plants is great as it plays important roles in the metabolism of plants. It is a constituent of the molecules of proteins, coenzymes, nucleic acids, cytochromes, chlorophyll etc. It is also one of the most important nutrients responsible for increased production and it is the most absorbed nutrient by corn(Ferreira et al., 2001).

Nutrients in deficiency in the low-yield group were N, K, Fe and Mn, and a nutrient in excess was Zn. After N, K is the most required nutrient by corn, with 30% of its amount which is absorbed by the plants being exported to the grain (Rodrigues et al., 2014). The availability of N and K, and their proper proportion ratio in the soil solution are crucial for the growth and development of plants. The metabolism of nitrogen in plants requires adequate amounts of potassium in the cytoplasm (Xu et al., 2002), vital for the production of amino acids in the plants.

In the present study, it appears that as the levels of N increased, the levels of K increased as well. This behavior was reflected in the low-yield group, which showed lower levels of N and K. Panaullah et al. (2006) evaluating the absorption of potassium in the cultivation of rice and wheat in succession, found that nitrogen fertilization increased potassium uptake in wheat. According to these authors, the correct use of nitrogen fertilizer increased K uptake by 57%, with the supply of 120 and 80 kg ha^{-1} of N and K respectively. Although K is not a structural compound in plants, it plays an important role in many biochemical and physiological processes. Potassium can also influence the use of nitrogen by plants because the metabolism of nitrogen in plants requires adequate amounts of potassium in the cytoplasm (Xu et al., 2002).

Zinc is an enzymatic activator of many metabolic processes such as the production of tryptophan - the precursor of auxins responsible for the growth of plant tissues (Mengel and Kirkby, 1987). The demand for zinc, by most crops, does not reach 1 kg ha^{-1}. In the plant, its adequate and toxic levels vary, ranging from 18-67 to 100-673 mg kg^{-1} respectively of dry matter of the shoots (Fageria, 2000). According to Table 2, Zn levels are suitable for corn according to Fageria (2000), even though presenting excess in low-yield group.

According to table 2, foliar Fe contents are considered adequate when compared with levels found in the literature also established with DRIS for this nutrient. However, its levels show deficiency in the population with low-yield (Table 4). The participation of Fe is essential for N assimilation by corn plants. It acts on the nitrite reductase which promotes the assimilation of N by the plants (Bredemeier&Mundstock, 2000). Most Fe deficiency in low-yield group may have been influenced by the assimilation of N, resulting in lower yields.

Regarding the nutritional indices in the high-yield corn, the nutrients in deficiency are in the following descending order: Mg>Fe>Zn>S>B>Mn>P>Cu>Ca>K>N, and in excess: B>S>Fe>Ca>K>Zn>Mn>Cu>Mg>P>N (Table 5). Magnesium, iron and zinc are deficient in high productivity corn. Regarding iron, it can be attributed to the soil used in this test. It is an Oxisol, a soil with high content of Fe due to the nature of iron oxides present in its source material (Reatto, 2016). The nutritional deficiency rates in the low-yield population are in the following descending order: Mg>Fe>Zn>Mn>Cu>N>B>P>S>Ca and in excess: Zn>S>Cu>Mn>Ca>K> P>B>N>Mg>Fe (Table 5). The low yield of the plants may be due to P deficiency, as it is the second most important macronutrient extracted by corn plants. Regarding excess, in general, there was none in relation to micronutrients.

Table 3. DRIS indices for macronutrients in high (> 130 bags ha^{-1}) and low (< 130 ha^{-1}) yield population of corn.

Doses			Yield	N	P	K	Ca	Mg	S	NB
N			bags ha^{-1}	DRIS indices for high-yield population						
120	Pol	Sem	161.46	-0.9	4.4	7.2	-1.0	5.1	-23.1	65.28
120	Pol	Litho	156.71	-3.8	2.4	-5.9	-13.8	-5.1	-1.9	74.71
120	Conv	Sem	154.8	3.5	-6.6	-4.6	-0.1	7.0	-0.2	57.82
60	Pol	Litho	154.74	-0.5	1.3	6.1	10.5	-12.1	5.5	75.28
90	Pol	Litho	139.81	0.8	4.4	-1.0	5.8	4.1	-0.7	45.34
90	Conv	Litho	130.26	1.7	-4.9	-0.5	-1.7	3.0	12.3	54.25
										62.11
				DRIS indices for low-yield population						
60	Conv	Sem	127.02	-0.1	-0.7	-27.4	12.6	-18.7	12.7	161.07
90	Pol	Sem	125.81	8.8	-1.6	-18.1	-0.5	-21.2	-0.6	84.10
90	Conv	Sem	123.84	4.2	6.4	-24.3	4.9	1.5	4.3	103.88
120	Conv	Litho	121.35	7.2	-12.5	9.2	0.4	2.0	-4.8	83.42
60	Conv	Litho	121.35	-6.2	-6.2	-15.4	7.5	10.1	7.6	116.38
0	Conv	Sem	95.95	-9.1	12.2	10.5	-4.2	-30.4	26.1	181.50
0	Conv	Litho	93.81	-12.0	1.8	-2.4	26.6	-58.5	18.0	212.52
60	Pol	Sem	93.23	-2.0	-4.0	5.1	5.6	-21.8	-0.8	100.86
0	Pol	Sem	86.69	-7.4	-3.5	6.5	-0.6	-9.4	-2.2	98.84
0	Pol	Litho	84.78	3.8	8.3	11.0	16.5	-36.3	11.7	129.54
										127.21

Pol (polymerizedurea). Conv (convencional ureia). Sem (sub-sub plot without the application of *Lithothamnium*). Litho (sub-sub plot with the application of *Lithothamnium*).

Table 4. DRIS indices for micronutrients in high (> 130 bags ha^{-1}) and low (< 130 ha^{-1}) yield population of corn.

Doses			Productivity	B	Cu	Fe	Mn	Zn
N			bags ha^{-1}	DRIS indices for high-yield population				
120	Pol	Sem	161.46	-23.1	-3.4	-4.4	6.2	4.4
120	Pol	Litho	156.71	-1.9	-5.6	9.5	14.2	-1.2
120	Conv	Sem	154.8	-0.2	17.0	1.4	-4.2	-4.8
60	Pol	Litho	154.74	5.5	-7.7	2.2	-11.4	12.0
90	Pol	Litho	139.81	-0.7	-7.6	-7.2	6.0	1.5
90	Conv	Litho	130.26	12.3	7.8	-2.0	-6.7	-11.3
				DRIS indices for low-yield population				
60	Conv	Sem	127.02	-3.8	19.9	-21.0	-9.0	35.3
90	Pol	Sem	125.81	0.8	14.3	4.1	10.5	3.7
90	Conv	Sem	123.84	9.1	21.5	-12.1	-9.9	-5.7
120	Conv	Litho	121.35	15.2	5.1	2.6	-4.0	-20.4
60	Conv	Litho	121.35	8.2	-12.5	-13.1	-4.8	24.8
0	Conv	Sem	95.95	2.0	-4.1	-11.0	-32.0	39.9
0	Conv	Litho	93.81	9.1	8.9	-22.4	-10.9	41.8
60	Pol	Sem	93.23	-6.4	-4.4	-11.1	11.8	27.9
0	Pol	Sem	86.69	0.1	-13.8	-9.5	-3.1	42.8
0	Pol	Litho	84.78	7.9	0.4	-15.6	-12.8	5.1

Pol (polymerizedurea). Conv (convencional ureia). Sem (sub-sub plot without the application of *Lithothamnium*). Litho (sub-sub plot with the application of *Lithothamnium*).

Table 5.Indices of deficiency and excess of macro and micronutrients in the high and low-yield group of corn.due to the use of conventional urea and polymerized urea. with or without the application of *Lithothamnium*.

Order	Deficiency indices				Excess index			
	>130 bags ha^{-1}		<130 bags ha^{-1}		>130 bags ha^{-1}		<130 bags ha^{-1}	
1	Mg	-8.16	Mg	-28.0	B	12.40	Zn	27.66
2	Fe	-7.43	K	-17.50	S	8.90	S	13.40
3	Zn	-6.86	Fe	-16.54	Fe	8.80	Cu	11.68
4	S	-6.47	Zn	-13.05	Ca	8.15	Mn	11.15
5	B	-6.07	Mn	-10.81	K	6.65	Ca	10.58
6	Mn	-5.76	Cu	-8.70	Zn	6.30	K	8.46
7	P	-5.75	N	-6.13	Mn	5.96	P	7.17
8	Cu	-4.53	B	-5.10	Cu	4.36	B	6.55
9	Ca	-4.15	P	-4.75	Mg	3.84	N	6.00
10	K	-3.0	S	-2.10	P	3.12	Mg	4.53
11	N	-1.73	Ca	-1.76	N	2.15	Fe	3.35

Conclusions

In the high productivity population, corn treatments with the highest nitrogen doses ensured the highest grain yield.

The use of the controlled-release urea ensured greater nutritional balance in plants.

Lithothamnium did not improve the efficiency of urea applied via topdressing in corn.

According to DRIS in the high-yield population nutrients in deficiency were in the following descending order: Mg>Fe>Zn>S>B>Mn>P>Cu>Ca>K>N and in excess: B>S>Fe>Ca>K>Zn>Mn>Cu>Mg>P>N. As for the low-yield group, they followed the following order: Mg>Fe>Zn>Mn>Cu>N>B>P>S>Ca and in excess: Zn>S>Cu>Mn>Ca>K> P>B>N>Mg>Fe.

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