Slow-release and organic fertilizers on early growth of Rangpur lime

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ABSTRACT

Slow-release and organic fertilizers are promising alternatives to conventional fertilizers, as both reduce losses by leaching, volatilization and problems of toxicity and/or salinity to plants. The objective of this work was to evaluate the effect of different rates of the organic fertilizer Humato-Macota® compared with the slow-release fertilizer Osmocote® on the growth and nitrogen content in the dry matter of Rangpur lime. A field experiment was conducted in a factorial completely randomized design with an additional treatment (4 x 4 +1). The first factor consisted of four Humato-Macota® rates (0, 1, 2, and 3%) applied to the substrate; the second factor consisted of the same Humato-Macota® concentrations, but applied as fortnightly foliar sprays; the additional treatment consisted of application of 5 kgm^{-3} Osmocote® 18-05-09. Means of all growth characteristics (plant height, total dry matter, root/shoot ratio and leaf area) and the potential quantum yield of photosystem II (Fv/Fm) were higher when plants were fertilized with the slow-release fertilizer. The organic fertilizer applied alone did not meet the N requirement of Rangpur lime.

Key words: Fertilization, organic fertilizer, *Citrus limonia* L. Osbeck.

RESUMO

Uso de fertilizante de liberação lenta e orgânico no crescimento inicial de limoeiro 'cravo'

O uso de fertilizantes de liberação lenta e o de fertilizantes orgânicos são alternativas promissoras, uma vez que reduzem as perdas por lixiviação, volatilização e problemas como toxidez ou salinidade às plantas. Objetivou-se, neste trabalho, avaliar o efeito de diferentes doses de fertilizante orgânico (Humato-Macota®), comparando seu efeito com o do fertilizante de liberação lenta (Osmocote®), sobre o crescimento vegetativo e o teor de nitrogênio na massa de matéria seca de limoeiro ‘Cravo’. Foi adotado o delineamento inteiramente casualizado, em arranjo fatorial, com um tratamento adicional (4 x 4 +1), sendo o primeiro fator composto por quatro concentrações de Humato-Macota® (0; 1; 2; e 3%) aplicadas no substrato. Para composição do segundo fator, foram utilizadas as mesmas concentrações de Humato-Macota®, porém, via foliar, em pulverizações quinzenais. O tratamento adicional consistiu na aplicação de 5,0 kg m^{-3} de Osmocote,® da fórmula 18-05-09. Todas as características de crescimento avaliadas (altura, massa de matéria seca total, razão raiz/parte aérea e área foliar), bem como o rendimento quântico potencial do fotossistema II (Fv/Fm), tiveram valores médios maiores quando as plantas foram adubadas com o fertilizante de liberação lenta. O fertilizante orgânico aplicado isoladamente não supriu a necessidade de N do limoeiro ‘Cravo’.

Palavras-chave: Adubação, fertilizante orgânico, *Citrus limonia* L. Osbeck.

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INTRODUCTION

Citrus containerized nursery production is a technology that is still being refined (Pereira & Carvalho, 2006). The initial vigor of the rootstock is one of the seedling quality factors, as it reduces the time for rootstocks to reach the grafting point (Bernardi *et al.*, 2000).

Moreover, seedling growth in polytubes can be influenced by fertility of substrate, then supplementation with side dressing and/or foliar fertilization is usually required (Decarlos Neto *et al.* 2002; Scivittaro *et al.*, 2004).

Besides adequate fertilizer rates, split application is important, to avoid nutrient leaching, mainly N and K, due to continuous irrigation and the small size of containers (Prado *et al.*, 2008).

Studies on the effects of fertilization on growth of citrus rootstocks have shown that nitrogen, required in large amounts and used in key metabolic processes in plants, is a critical element to the process. Because of its high susceptibility to loss, nitrogen should be supplied gradually to plants, in split applications of soluble sources (Decarlos Neto *et al.*, 2002).

A promising and practical alternative is the use of fertilizers that allow a gradual release of the nutrient during the seedling formation or organic fertilizers, based on fulvic and humic acids, to reduce losses by leaching and volatilization and avoid toxicity or salinity to plants (Khalaf & Koo, 1983; Shaviv, 200 1; Girardi & Mourão Filho, 2003). Slow-release fertilizers promote a more homogeneous nutrient distribution in the substrate. They also favor the synchronization between nutrient supply and the physiological demand of the plant, as the rate of nutrient release is directly proportional to temperature, with optimal values close to 21 ^{o}C, when plants are in full metabolic activity (Oertli, 1980; Perin *et al.*, 1999). In addition, these non-conventional fertilizers will reduce the need for additional fertilizations during the period of seedling formation (Compo, 2004).

The main disadvantage is their cost. Slow-release fertilizers are more expensive than soluble fertilizers and require rate adjustment in different production systems to optimize input use and ensure the economic production of rootstocks. Reliable information on rates and application modes for these products is still scarce in the literature, not meeting nursery growers needs (Girardi & Mourão Filho, 2003).

Furthermore, monitoring of N nutritional status in citrus rootstocks requires information on non-destructive methods to help the N diagnosis (Decarlos Neto *et al.*, 2002). Studies have shown that there is a positive correlation between plant growth characteristics and leaf chlorophyll content determined by spectrophotometry or using indirect meters (Shadchina & Dmitrieva, 1995; Neilsen *et al.*, 1995; Lopez-Cantarero *et al.* 1994; Minotti *et al.* 1994). These authors determined chlorophyll content in wheat by the method of Arnon (1949) and found that it can be used as a suitable characteristic for determination of N absorbed by plants.

Likewise, chlorophyll fluorescence has been used as a diagnostic tool for plant stress, including nutritional stress, with the advantage of being non-destructive and of rapid determination (Pestana *et al.*, 2001).

When plants are exposed to light, under biotic or abiotic stress, decreases in F_v/F_m ratio are often observed. Falqueto *et al.* (2008) found a high correlation between F_v/F_m and N content in rice cultivars.

The F_v/F_m ratio represents the efficiency of excitation energy capture by open PSII reaction center (P680) and electron transport to plastoquinone (PQH_2). The maximum level of fluorescence (F_m) indicates the full reduction of plastoquinone A (QA), whereas F_v is the variable fluorescence (= $F_m - F_o$) and indicates the magnitude of the initial fluorescence rise from F_o to F_m. In plants with a healthy photosynthetic apparatus, i.e., without stress, the F_v/F_m ratio varies between 0.750 and 0.850 (Bolhàr-Nordenkampf *et al.*, 1989), while a decrease in this ratio reflects the presence of photoinhibitory damage in the PSII reaction centers (Björkman & Demming, 1987).

Therefore, the aim of this study was to evaluate the initial growth of seedlings of Rangpur lime [*Citrus limonia* (L.) Osbeck] as affected by substrate fertilization with either slow release fertilizer (Osmocote®) or organic fertilizer (Humato-Macota®).

MATERIAL AND METHODS

A field experiment was carried out at the citrus orchard of the Universidade Federal de Viçosa (UFV), from 21/05 to 21/08 2007. Seed lots of Rangpur lime were obtained from the Citrus Collection of the Setor de Fruticultura - UFV.

The experiment was arranged in a factorial completely randomized design with an additional treatment (4 x 4 +1) and four replicates with 12 seedlings per plot. The factorial design consisted of four concentrations of organic fertilizer (Humato-Macota®) (0, 1, 2, and 3%) applied to the substrate at planting, and four concentrations (0, 1, 2, and 3%) applied as fortnightly foliar sprays after germination. The additional treatment consisted of application of 5 kg m^{-3} Osmocote® 18-05-09.

Table 1 shows the chemical composition of the organic fertilizer. The additional treatment consisted of application of 5 kgm^{-3} of the slow-release fertilizer Osmocote® 18-05-09 to Plantmax substrate (Moisture: 50%; water holding

Table 1. Chemical composition of the organic fertilizer Humato-Macota®, isolated from California red worm castings, in mg L^{-1}

Humic acids	Fulvic acids	C	N	P	K	Ca	Mg	Fe	Cu	Zn	Mn
178.35	143.83	3.088	294	54.27	25	250.5	176.9	16.85	1.09	0.33	4.47

capacity: 150%; pH: 5.8 ± 0.5; electrical conductivity 1.2 ± 0.3 mS/cm) at sowing in 0.05 dm^3 poly tubes.

The organic fertilizer Humato-Macota®, at concentrations described above, was added to 100 mL of water and applied to the substrate of each plot at the time of sowing. Foliar sprays were carried out at 15, 30, 45, 60 and 75 days after germination.

The experiment was evaluated 90 days after germination. Initially, seedling height was measured and, later, chlorophyll content was measured with a SPAD-502® chlorophyll meter (Minolta, Japan). For this evaluation, we considered the average of four SPAD readings, on the 3rd, 4th, 5th and 6th leaves from the apex of the plant in each plot.

The physiological status was evaluated using the parameters of chlorophyll *a* fluorescence (F_o, F_v, F_v/F_m) measured on the 5th leaf from the apex, previously adapted to the dark for 30 minutes. One measurement was recorded per plant, using a portable FIM 1500 fluorometer® (ADC, UK). Finally, the values obtained individually, per plant, were used to calculate an average per plot.

Then, plants were removed from the tubes, and substrate was washed from the roots. After drying, leaves were cut off and the seedlings were separated into aerial parts and root system at the base of the collar. The leaves were brought to the laboratory, where leaf area was determined by the destructive method, using the 2000 Area Meter®, expressed in cm^2.

After determination of leaf area, leaves and stems were dried in forced-air ovens to constant weight and dry matter was determined in grams (g). The same procedure was done for the roots. The dried material was ground in a Wiley mill to pass a 20-mesh sieve and samples were stored in paper bags. In these samples, total nitrogen content was determined by Kjeldahl method after sulfuric digestion (H_2SO_4 and H_2O_2) of plant tissue (Malavolta *et al.*, 1997), at the Laboratório de Nutrição Mineral de Plantas / UFV.

Data were examined by analysis of variance and regression analysis, a 5% probability level, using the Sistema de Análises Estatísticas e Genéticas (SAEG) software (UFV, 1997). The selection of the model in the regression analysis was based on the significance of the F test at 5% probability level, the biological significance and the coefficient of determination. Because there was no significant differences between means of the treatments that comprised the factorial, this group was contrasted with the additional treatment.

RESULTS AND DISCUSSION

Application of the organic fertilizer to substrate and supplementation via foliar sprays had no significant effect on seedling height, unless the concentrations of fertilizer in the substrate were combined with concentration of 2% applied as foliar sprays. The highest seedling height (average 10 cm) was recorded for the treatment with slow-release fertilizer (Table 2, Figure 1 A). These results agree with reports by Mendonça *et al.* (2007), who observed higher vigor in passion fruit seedlings treated with slowrelease fertilizer.

Total dry matter of seedlings was not influenced by the application of organic fertilizer, but was higher in plants fertilized with slow-release fertilizer (Table 2). The total dry matter of plants treated with slow-release fertilizer had means close to 0.80 g contrasting with 0.20 g in plants treated with organic fertilizer (Figure 1 B). The reason for that is the more ready availability of nutrients to plants by the mineral fertilizer (Osmocote®). The increased availability of nitrogen usually brings positive effects on the rate of carbon assimilation, since this nutrient is one of the main components of the photosynthetic system, and a consequent increase in total dry mass (Taiz & Zeiger, 2004).

The shoot/root dry matter ratio was 2.40 for the slow-release fertilizer, whereas for the organic fertilizer, it was higher (1. 11), regardless of the concentration and mode of application (Figure 1 C).

According to Marschner (1995), the nitrogen content may influence the shoot/root ratio. Under low nutrient availability, there is less growth of aerial parts and roots become long and unbranched. At adequate N levels, development and branching of the root system are normal. Under excess nutrient conditions, there is excessive branching, but the root system is reduced and development of the aerial part is stimulated.

The shoot/root ratio is useful for studying the balance between organs, which is influenced by changes in the environment, since there is interdependence among organs, which can be altered by various factors such as temperature, water balance, nutrients and carbon. Changes in the morphology of the root system and its volume, forexample, can affect the uptake of water and nutrients, especially those with less mobility in the soil and that reach the roots by diffusion. Plants with well-developedroot systems usually have better conditions for the establishment in the field (Bernardi *et al.*, 2000).

Table 2. Summary of analysis of variance (Mean square) for plant height (ALT), leaf area (AF), total dry matter (MST), dry matter of aerial part (MSPA), root dry matter (MSR), root/shoot dry matter ratio (MS R/PA), initial fluorescence (Fo), potential quantum yield of photosystem II ($F_{v/Fm}$) and chlorophyll content (SPAD) of Rangpur lime seedlings fertilized with organic fertilizer and slow-release fertilizer

FV	ALT (cm)	AF (cm^2)	MST (g)	MSPA (g)	MSR (g)	MS R/PA (g)	F_0	F_v/F_m	SPAD
Substrate	0.486**	2.622^{ns}	0.001^{ns}	0.0006^{ns}	0.0002^{ns}	0.008^{ns}	1165.05^{ns}	0.0095^{ns}	3.1 1$_{n.s}$
Foliar	0.165^{ns}	1.871^{ns}	0.001^{ns}	0.0005^{ns}	0.0003^{ns}	0.006^{ns}	594.01^{ns}	0.0028$^{n.s}$	14.03^{ns}
Interaction	0.393**	1.003^{ns}	0.000^{ns}	0.0002^{ns}	0.0001^{ns}	0.015^{ns}	2808.35^{ns}	0.0037^{ns}	11.09^{ns}
CV %	6.96	19.67	14.22	16.71	13.56	14.7	22.98	8.7	14.15
Contrast	5.49**	21.49**	0.54**	0.42**	0.12**	-0.48**	52.08 ^{ns}	0.05^{ns}	33.52**
DMS	1.24	4.75	0.1	0.06	0.04	0.41	265.84	0.17	10.25

** significant at 1 % probability level. n.s = non significant

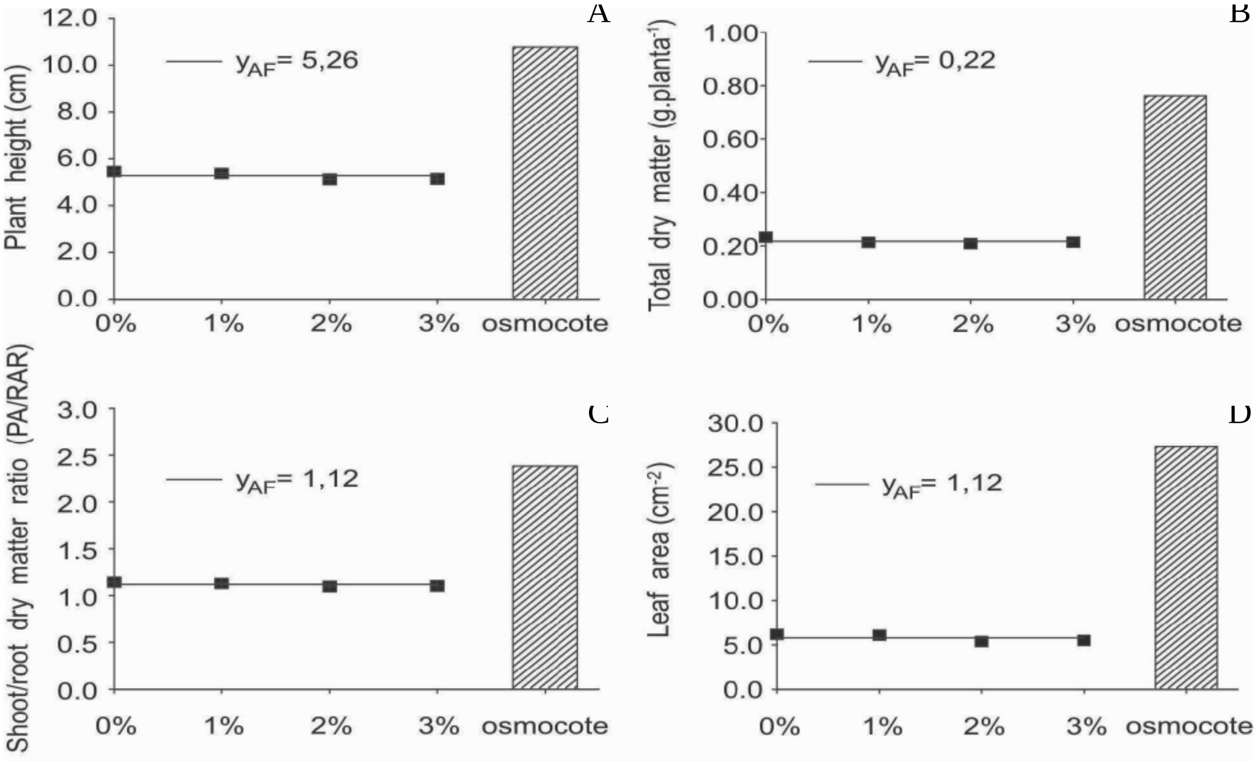

Figure 1. Plant height (A), total dry matter (B), root/shoot dry matter ratio (C), and leaf area (D) of Rangpur seedlings fertilized with the slow-release fertilizer Osmocote® (to the substrate) and the organic fertilizer Humato-Macota® (to the substrate and foliar supplementation).

Another factor to be considered is that in citrus, growth of roots and aerial parts occurs through alternating cycles. Bevington & Castle (1985) determined that, in young plants, even when temperature and soil moisture are not limiting factors, root growth is cyclical, alternating with the growth of the aerial part.

Leaf area was not affected by the use of the organic fertilizer, corresponding only to 22% of the treatment with Osmocote ® (Figure 1D). Leaf area is an important characteristic, because leaves are instrumental in capturing light energy and use it to convert the carbon atoms of CO_2 into organic carbon through photosynthesis.

The SPAD readings were also not significantly different among the treatments with the organic fertilizer, with means approximately 50% lower than those found for plants treated with the slow-release fertilizer. The lower SPAD readings in the treatments with the organic fertilizer, in comparison with the slow-release fertilizer (Figure 2A), indicate low nitrogen content in its composition.

Malavolta *et al.* (1997) reported that the leaf chlorophyll content indicates the level of plant nutrition, relative to N, and is not affected by its luxury consumption, because the plant does not produce chlorophyll more than it needs. Decarlos Neto *et al.* (2002) found that levels of leaf chlorophyll content in the citrus rootstocks Tangelo-Orlando, Rangpur, Volkamer, Cleopatra and Sunki had significant positive correlations with height, stem diameter, leaf area, dry matter of aerial parts and roots, and NO_3-N content in the aerial part.

Vale & Prado (2009) showed that SPAD readings provide fast and non-destructive estimates of chlorophyll content in leaves. These values correlated with nitrogen content and can be used as a diagnosis for the nutritional status of this nutrient in the plant. This is because between 50 and 70% of total N in leaf are associated with enzymes in chloroplasts.

The results obtained with the SPAD index correlated with the visual nitrogen deficiency symptoms (data not shown) in the treatments with organic fertilizer, regardless of the applied rates. There were no such symptoms in the treatments with the slow-release fertilizer. Because nitrogen is required in high quantities and influence major metabolic processes, the deficiency in these plants may be associated with low nitrogen content in the composition of the organic fertilizer. Vale *et al.* (2009) found that 1840 mg dm $^{-3}$ of nitrogen increased the content and accumulation of the nutrient in the dry matter, which wasa higher value than that found in the composition of the organic fertilizer (Table 1). Similar to the chlorophyll content, the photochemical quenching determined by the potential quantum yield of photosystem II (F_v/F_m) was lower in plants fertilized with the organic fertilizer (approximately 0.650), while plants fertilized with the slow-release fertilizer had a higher quantum yield (approximately0.700) (Figure 2C).

We did not find in the literature searched any study on the use of this organic fertilizer. The contrast of the organic fertilizer treatment groups with the additional treatment, the slow-release fertilizer, was significant for all characteristics. The efficiency of slow-release fertilizers in citrus has been reported in other studies (Jackson & Davies, 1984; Obreza, 1990; Zekri & Koo, 1992).

Total nitrogen levels in plants fertilized with the slow-release fertilizer (Osmocote®) were higher than in plants fertilized with the organic fertilizer (Humato-Macota®) (Figure 3), confirming the SPAD readings. Serrano *et al.* (2004) also reported increase in leaf N contents of Rangpur rootstocks with the incorporation of slow-release fertilizer. In the same way, Decarlos Neto (2000) evaluated the response of citrus rootstocks to different levels of nitrogen applied to the substrate and found mean total-N content similar to our study.

Increasing concentrations (from 0 to 3%) of organic fertilizer applied to the substrate, in combination with foliar supplementation, reduced total nitrogen (Figure 3).

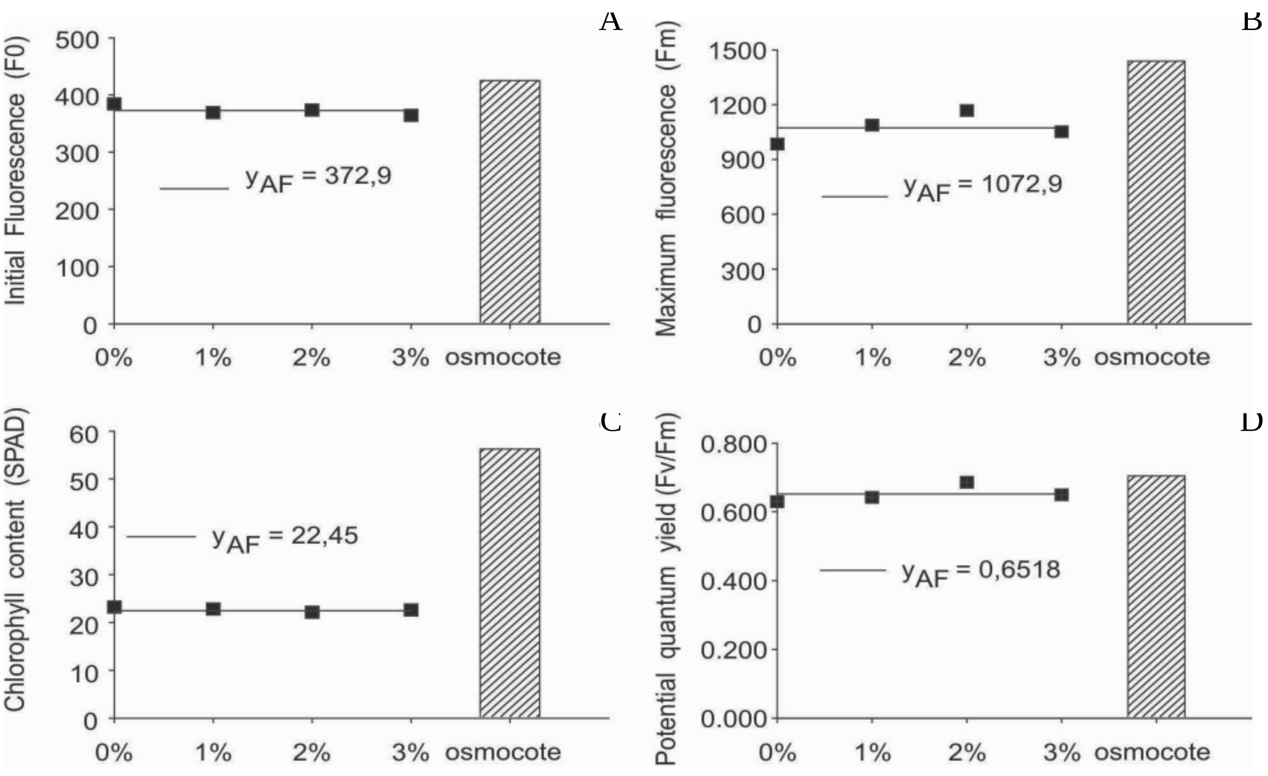

Figure 2. Initial fluorescence (F_o) (A), maximum fluorescence (F_m) (B), chlorophyll content (SPAD) (C), and potential quantum yield (F_v/F_m) (D) of Rangpur seedlings fertilized with the slow-release fertilizer Osmocote® (to the substrate) and the organic fertilizer Humato-Macota® (to the substrate and foliar supplementation).

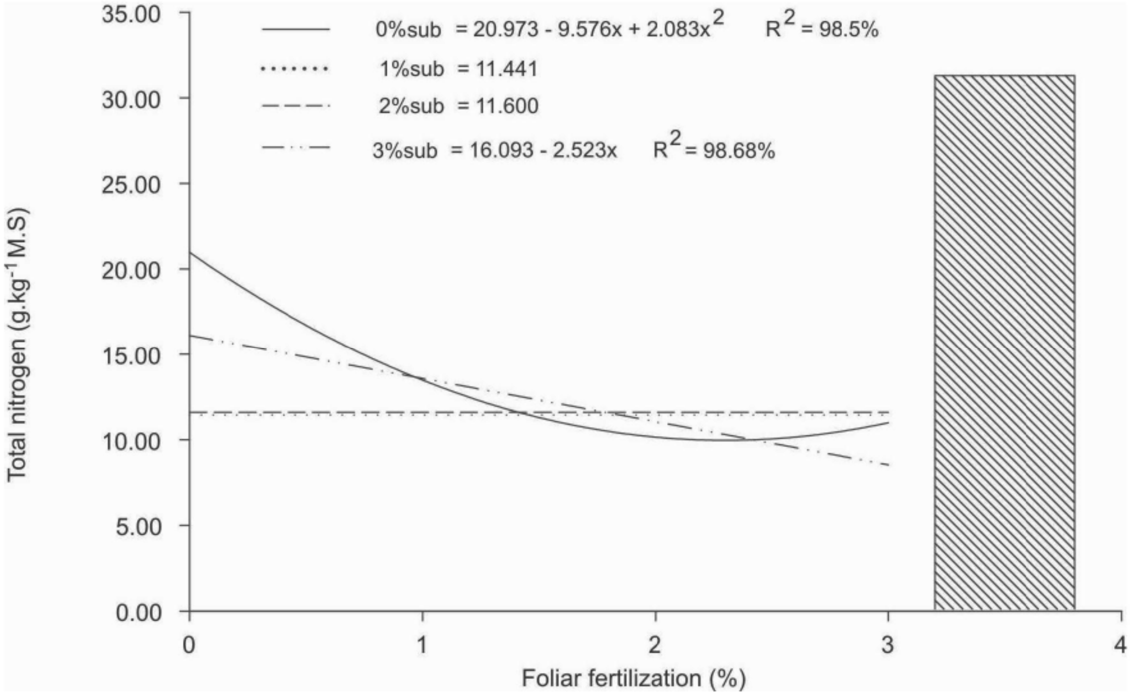

Figure 3. Total nitrogen content (g.kg-1 MS) in Rangpur seedlings fertilized with the slow-release fertilizer Osmocote® (to the substrate) and the organic fertilizer Humato-Macota® (to the substrate and foliar supplementation).

CONCLUSIONS

The organic fertilizer alone did not supply adequate nitrogen to Rangpur lime plants.

For all characteristics studied, the best vigor indices of Rangpur lime seedlings were obtained with slow-release fertilizer.

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