

## QUANTITATIVE ANALYSIS OF JATROPHA GROWTH: MICRONUTRIENT DELIVERY SYSTEM AND NPK COMBINED EFFECTS

### ANÁLISE QUANTITATIVA DE CRESCIMENTO DO PINHÃO MANSO: EFEITOS COMBINADOS DE UM SISTEMA DE FORNECIMENTO DE MICRONUTRIENTES E NPK

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**ABSTRACT:** Studies approaching jatropha (*Jatropha curcas* L.) growth through quantitative analysis parameters are limited, especially regarding the response to different fertilizer types and doses. In order to investigate the effects of a micronutrient delivery system (MDS) fertilizer, a full quantitative analysis of growth in jatropha young plants was performed, comparing this system effectiveness under different NPK doses. Plants were grown in 3.9 L pots containing local soil, with or without MDS (main plot), combined with NPK doses (0; 1.8; 4.7 and 7.4 g L<sup>-1</sup>) in subplots. Dose-response curves of quantitative analysis variables were generated for three periods of time (40, 80 and 120 days after sown) as a sub-subplot. Quantitative analysis of growth showed that most parameters evaluated in this study were improved by MDS application, resulting in benefits for jatropha initial development, regardless of NPK doses. Even without NPK supplementation or under the lowest dose evaluated (1.8 g L<sup>-1</sup>), MDS provided better growth of *J. curcas* plants, being usually equivalent to the highest doses of NPK (4.7 and 7.4 g L<sup>-1</sup>) without MDS. The effective response of jatropha young plants to MDS supplementation indicates that this kind of fertilizer played a relevant role in the species metabolism, resulting in faster growth and enhanced biomass allocation.

**KEYWORDS:** Bioavailable mineral nutrients; Micronutrient fertilizer; *Jatropha curcas* L.; Biomass allocation; Ecophysiology.

## INTRODUCTION

Growth, survival and reproduction are the three imperatives of any organism, and in plants, growth is particularly important because both survival and reproduction depend on plant size and therefore on growth rate (SHIPLEY, 2006). Poorter et al. (2012) emphasize that the relative amount of biomass present in the various organs, namely 'biomass allocation', is not fixed but may vary over time, across environments and among species. Therefore, a quantitative understanding of such patterns has many uses in agricultural practice and implementation, as these patterns, and the extent to which they vary among species, set limits on biomass production and utilization (REICH, 2002; OLIVEIRA et al., 2011; POORTER et al., 2012; BELTRÃO et al., 2016).

A quantitative analysis of plant growth is based on estimation of quantitative variables to provide a description and analysis of the biomass allocation to the different plant organs, which at any given time is a strong driver of the plant's capacity to take up carbon, water and nutrients for future use (EVANS, 1972; POORTER, 2012). Souza et al.

(2016) explain that among the estimates in a quantitative analysis of plant growth, it is possible to determine: the "absolute growth rate" as being the increase in plant phytomass in any one period; the "relative growth rate" as representing the amount of plant material produced by a given amount of existing material (g) over a time interval (days); and the "net assimilation rate" as an assessment of the leaf size, which is involved in the production of dry matter (BEADLE, 2014).

*Jatropha* (*Jatropha curcas* L.) is considered an important tropical biofuel plant. It is claimed that this species can be grown on soils with low nutrient content, but Negussie et al. (2016) have revealed in their recent study that yield is significantly low in non-fertilized trees. In contrast, for Lima et al. (2016), jatropha is a perennial species that requires expressive amounts of nutrients to produce satisfactorily. These authors observed that phosphate fertilization associated with organic fertilization significantly influenced plant height, number of branches, stem diameter, leaf area, number of seeds per plant and total mass of seeds.

Studies involving the use of micronutrients in jatropha are even more limited. However, studies

of micronutrient fertilization using micronutrient delivery system (MDS) fertilizers as a source of Cu and Zn could be beneficial for jatropha crop production. Copper is a vital component of electron transfer reactions mediated by cytochrome c oxidase and plastocyanin (YRUELA, 2005), whereas zinc increases the biosynthesis of chlorophyll and carotenoids (BROADLEY et al. 2007). Optimum Cu and Zn concentrations positively affect the development of the membrane system of chloroplasts and chlorophyll content (AHMAD et al., 2015).

Although many studies have been performed evaluating jatropha initial growth and development, most of them did not approach the plant growth through quantitative analysis parameters, especially regarding the response to different fertilizer types and doses. This type of investigation not only provides relevant insights on ecophysiological changes that occur in crops during their development (basic science) but also allows researchers and companies to use this information pool to recommend the most efficient fertilizer combinations for each crop (applied science). Therefore, the objective of the present study was to evaluate the effects of a micronutrient delivery system (MDS) fertilizer through a quantitative growth analysis of jatropha young plants, comparing this system effectiveness under different NPK doses.

## MATERIAL AND METHODS

### Location, plant material, and experimental conditions

Seeds originated from 12 jatropha accessions from India were selected for the trials. Both seeds and soil used in this study were collected from a jatropha collection field plot at University of Florida's Tropical Research and Educational Center (TREC) (25°50'N and 80°50'W, 3.8 m above sea level), in Homestead, FL, USA.

Soil analysis was performed by A&L Southern Agricultural Laboratories (Deerfield Beach, FL). The soil is classified as a Krome very gravelly loam, being very shallow, well-drained with limerock up to the soil surface, pH 7.4 to 8.4, low water holding capacity, 3% to 10% organic matter content, cation-exchange capacity 8.7–12.2 meq 100g<sup>-1</sup>, and low nutrient content (LI, 2001). Elements including Mg, Zn, Mn, and Fe, although present in the soil profile, are unavailable for plant uptake (CRANE et al., 2010).

The experiment was performed from October 2016 to January 2017 in a greenhouse (19.6

- 25.8° C; 31 - 86% average minimum/maximum temperature and air relative humidity, respectively), using 3.9 L plastic black pots filled with local soil. It consisted on the evaluation of the effects of a new commercial fertilizer (BAM-FX™ Bio-Available Minerals; Zero Gravity Solutions Inc, Boca Raton, FL, USA) that delivers balanced minerals into plant tissues (bioavailable mineral nutrients), known as MDS. According to the manufacturer, this is a highly positively charged cationic Zn<sup>2+</sup> and cationic Cu<sup>2+</sup> solution balanced together in a specific ratio, along with sulfate and ammonium. The laboratorial analysis carried out by Thornton Laboratories Testing & Inspection Services, Inc. (Tampa, FL) reported 2.03% Cu, 7.14% Zn and a redox potential of 484.4 mV. For treatments using the product, applications followed label recommendations; by soil drench (125 mL L<sup>-1</sup>) immediately before sown, and by foliar application (62.5 mL L<sup>-1</sup>) at 40 and 80 days after sown (DAS).

The NPK source included different doses of a commercial NPK fertilizer formulation 15-9-12 (Osmocote® Plus, Dublin, OH, USA) according to low, medium and high incorporation rates suggested on the product label, resulting in 1.8, 4.7 and 7.4 g L<sup>-1</sup> of container, respectively. The fertilizer manufacturer sheet provided a guaranteed analysis report including N 15%, P<sub>2</sub>O<sub>5</sub> 9%, K<sub>2</sub>O 12%, Mg 1.3%, S 5.9%, B 0.02%, Cu 0.05%, Fe 0.46%, Mn 0.06%, Mo 0.02% and Zn 0.05%.

### Experiment establishment design and characteristics evaluated

Six jatropha seeds were sown per pot at the beginning of this study. Germination and vigor of seedlings were evaluated during the first 10 DAS. Seedlings with poor vigor were removed to maintain the three most vigorous seedlings for each pot. Plants were irrigated using an automatic sprinkler irrigation system twice a day. Pesticide application was not necessary in this study.

Prior to each MDS foliar application (40 and 80 DAS), as well as in the final assessment of this study (120 DAS), one plant from each pot was randomly selected and removed to measure the growth characteristics and estimate growth variables. Leaf chlorophyll index was measured on three newly matured leaves with a SPAD 502 meter (Konica Minolta Sensing, Osaka, Japan). Plants were then harvested, all leaves were removed and the total leaf area per plant (LA) was determined with a leaf area meter (Li-Cor, Lincoln, NE; model Li-3000). Plants were uprooted carefully, and roots were washed with tap water to remove soil attached to the root system. Plant

tissues were then oven-dried at 80°C for two days, and leaf (LDW), stem (SDW) and root (RDW) dry weight were determined. We used this data to calculate the shoot (SDW), root (RDW), and plant dry weight (PDW).

For quantitative analysis of jatropha growth, the following parameters were used: root dry weight/shoot dry weight ratio (RDW/SDW), leaf area ratio (LAR), leaf weight ratio (LWR), specific leaf area (SLA), leaf area index (LAI), absolute growth rate (AGR), relative growth rate (RGR) and net assimilation rate (NAR).

Growth indices were calculated according to the following formulas (EVANS, 1972; BEADLE, 2014; SOUZA et al. 2016):

$RDW/SDW$  (dimensionless) =  $RDW/SDW$  (ratio of root dry mass to shoot dry mass in grams).

$LAR$  ( $dm^2 g^{-1}$ ) =  $LA/PDW$ , where  $LA$  and  $PDW$  are leaf area and plant dry weight respectively.

$LWR$  ( $dm^2 g^{-1}$ ) =  $LDW/PDW$ , where  $LDW$  and  $PDW$  are leaf and plant dry weight respectively.

$SLA$  ( $dm^2 g^{-1}$ ) =  $LA/LDW$ , where  $LA$  and  $LDW$  are leaf area and leaf dry weight respectively.

$LAI$  (dimensionless) =  $RGR * (LA/PDW_f)$ , where  $RGR$  is the relative growth rate,  $LA$  and  $PDW_f$  are the leaf area and plant dry weight at the end of each harvest.

$AGR$  ( $g day^{-1}$ ) =  $(PDW_f - PDW_i)/(t_f - t_i)$ , where  $PDW_f$  and  $PDW_i$  are the plant dry weight at the start and the end of the experiment,  $t_f$  and  $t_i$  correspond to the final and initial period.

$RGR$  ( $g g^{-1} day^{-1}$ ) =  $(\ln PDW_f - \ln PDW_i)/(t_f - t_i)$ , where  $\ln PDW_f$  and  $\ln PDW_i$  are the natural logarithms for plant dry weight at the start and end, ( $t_i$  and  $t_f$  correspond to the period in days).

$NAR$  ( $g cm^{-2} day^{-1}$ ) =  $[(PDW_f - PDW_i)/(LAF - LA_i)] * [(\ln LAF - \ln LA_i)/(t_f - t_i)]$ , defined as the ratio expressed by the difference between the final and initial dry weight ( $PDW_f$  and  $PDW_i$ ) and final and initial leaf area ( $LAF - LA_i$ ), as well as the ratio of the difference between the natural logarithm of the final and initial leaf area ( $\ln LAF - \ln LA_i$ ) and of the period ( $t_f - t_i$ ).

### Statistical design and data analysis

Six replicates of three plants were used for each treatment. Treatments were arranged in a split-split plot design assessing different MDS and NPK combinations throughout time. The absence or presence of MDS was placed in main plot, and the NPK doses (0; 1.8; 4.7 and 7.4  $g L^{-1}$ ) in subplot. Dose-response curves of quantitative data were generated for three periods of time (40, 80 and 120 DAS) as a sub-subplot.

Dose-response relationships were assessed by adjustment of simple and multiple linear regression models used to predict growth responsiveness as a function of various MDS and NPK combinations. The fits of different regression models were compared through analysis of variance by F test at 5% probability. Among the regression models tested (linear and quadratic), the one with the highest coefficient of determination was defined for each set of variables, whose parameter estimators of the equation were significant to at least 5% probability. All calculations and graphs were performed using the Prism 7 analysis software (GraphPad Software, Inc.).

## RESULTS

### Biomass production and allocation

In jatropha, the deviations from the linear regression due to 'lack of fit' were not significant when compared with the within-sample variation (Figures 1, 2, 3 and 4). Therefore, with only two exceptions, the set of data evaluated in this study using linear regressions relating MDS and time with NPK were adequate.

Both shoot and root development improved with MDS application, even with no NPK supplementation in the soil (Figures 1A and 1B). At the end of the experiment (120 DAS), higher discrepancies were observed among the treatments, confirming that frequent MDS applications provided better results than high incorporation of NPK before sown. Furthermore, for this linear regression, there is a decrease in both SDW and RDW values inversely proportional to NPK dose increments, which infers deleterious effects of higher NPK doses and MDS application. This is probably because of the large amount of soluble salts incorporated into the soil. As expected, a similar behavior was observed for SDW/RDW, since these three variables were highly correlated to each other (Figure 1C). Therefore, MDS application not only provided a better initial growth of jatropha plants, but also enhanced the biomass allocation for roots, resulting in improved uptake of water and nutrients by the plants.

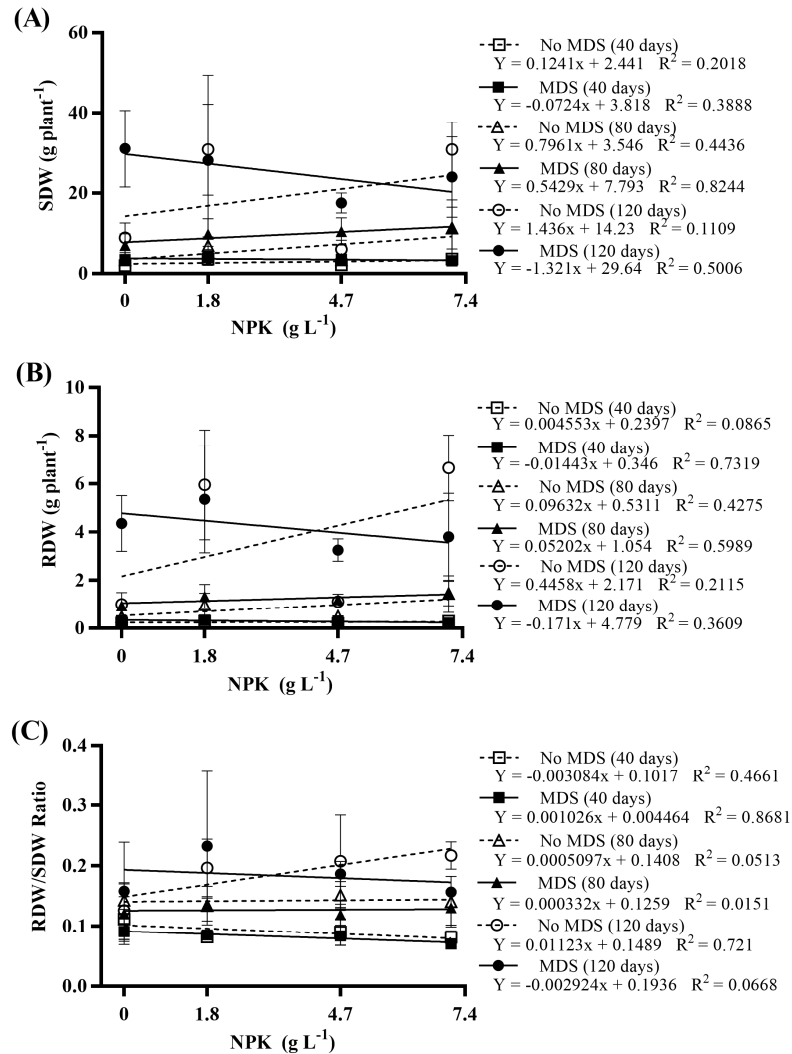
### Leaf area, chlorophyll, and source-sink relations

Jatropha leaf area increased in direct proportion to NPK doses, with the data adjusted to linear regression model, except at 40 days with no MDS fertilizer application, which was better adjusted to a quadratic regression model (Figure 2A). Although we observed similar increments for chlorophyll index (SPAD values) in leaves from

plants growing without MDS fertilizer applications, there were practically constant linear regressions for the other group of plants (treated with MDS) regardless of the NPK doses (Figure 2B).

Most values obtained for LAR and LWR were similar to each other and did not differ among plants treated and not treated with MDS fertilizer,

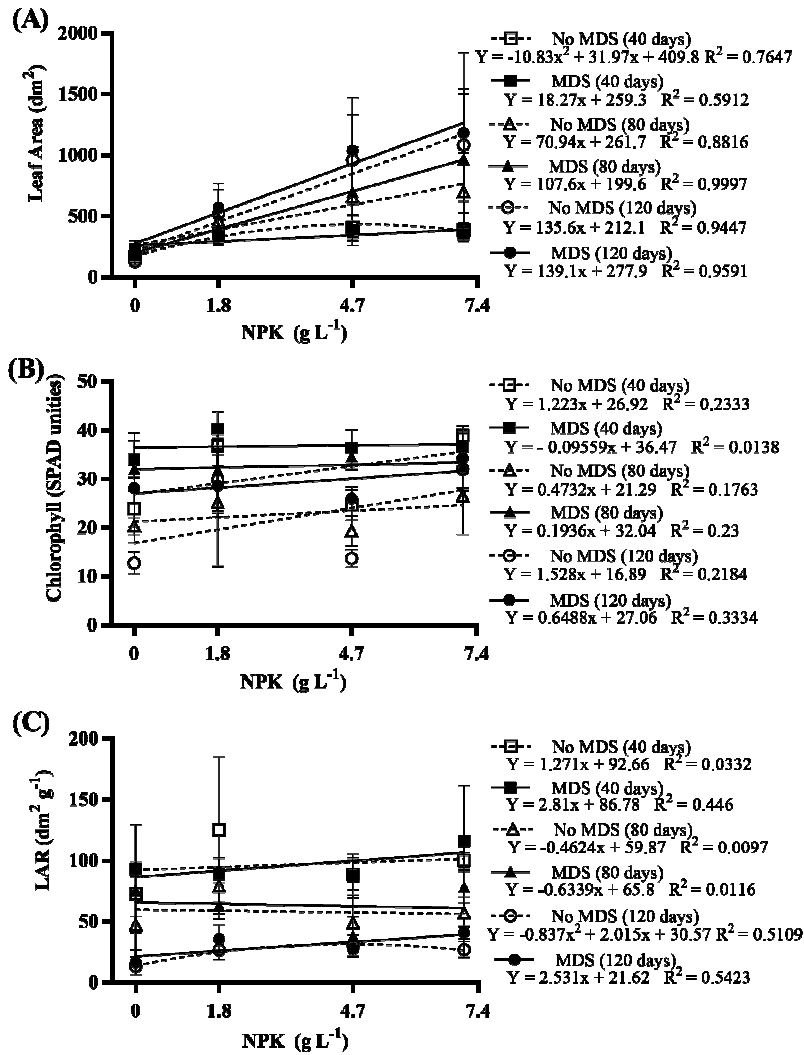
with most of them better adjusted to linear regression models (Figures 2C and 3A). These variables showed low or no variation as a function of NPK doses, with more noticeable increment observed for LWR in plants not treated with MDS at 120 DAS (Figure 3A).



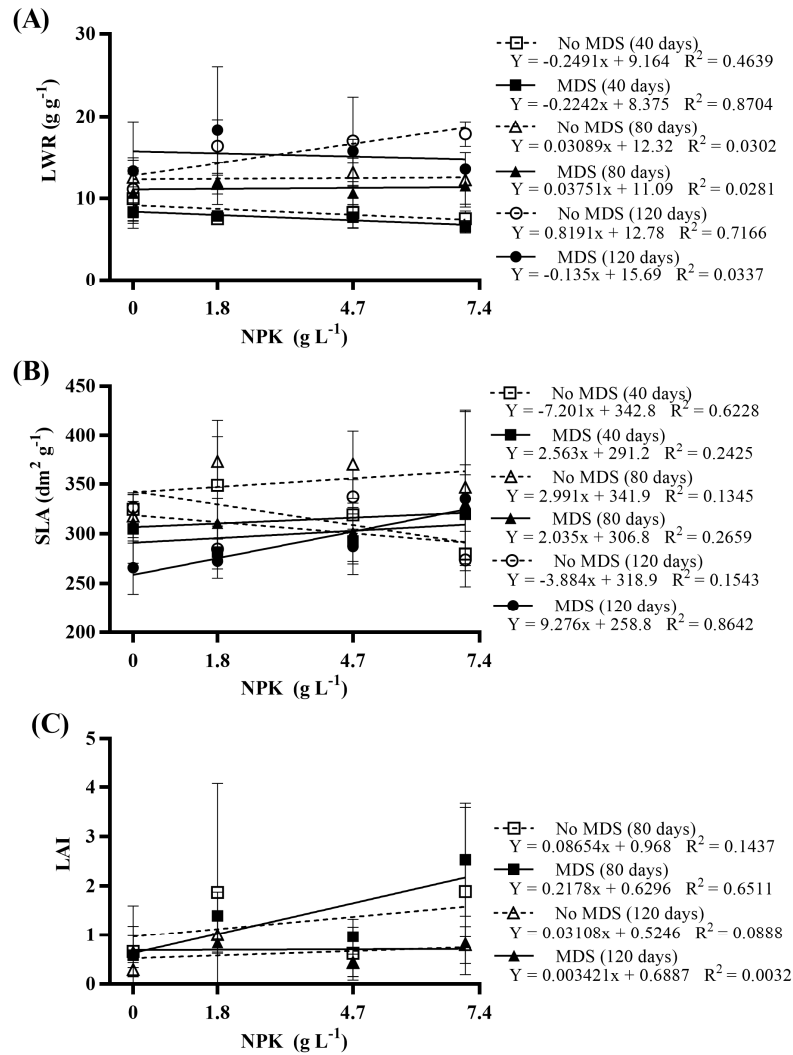
**Figure 1.** Shoot dry weight, SDW (A); root dry weight, RDW (B); and root dry weight/shoot dry weight ratio, RDW/SDW (C) in 40, 80 and 120 day-old *Jatropha curcas* L. plants due to fertilization with a micronutrient delivery system (MDS) and different doses of NPK.

Unlike most variables analyzed in this study, SLA presented higher values for plants not treated with MDS, which values reduced at 40 and 120 DAS, and slightly increased at 80 DAS, due to NPK doses increment (Figure 3B). Nonetheless, we observed inverse behavior for LAI linear regression

models, with low values observed for all treatments under 0, 1.8 or 4.7 g L<sup>-1</sup> of NPK, however with significant increases in plants growing under MDS applications and 7.4 g L<sup>-1</sup> of NPK, mainly at 80 DAS (Figure 3C).



**Figure 2.** Leaf area (A); chlorophyll index (B); and leaf area ratio, LAR (C) in 40, 80 and 120 day-old *Jatropha curcas* L. plants due to fertilization with a micronutrient delivery system (MDS) and different doses of NPK.

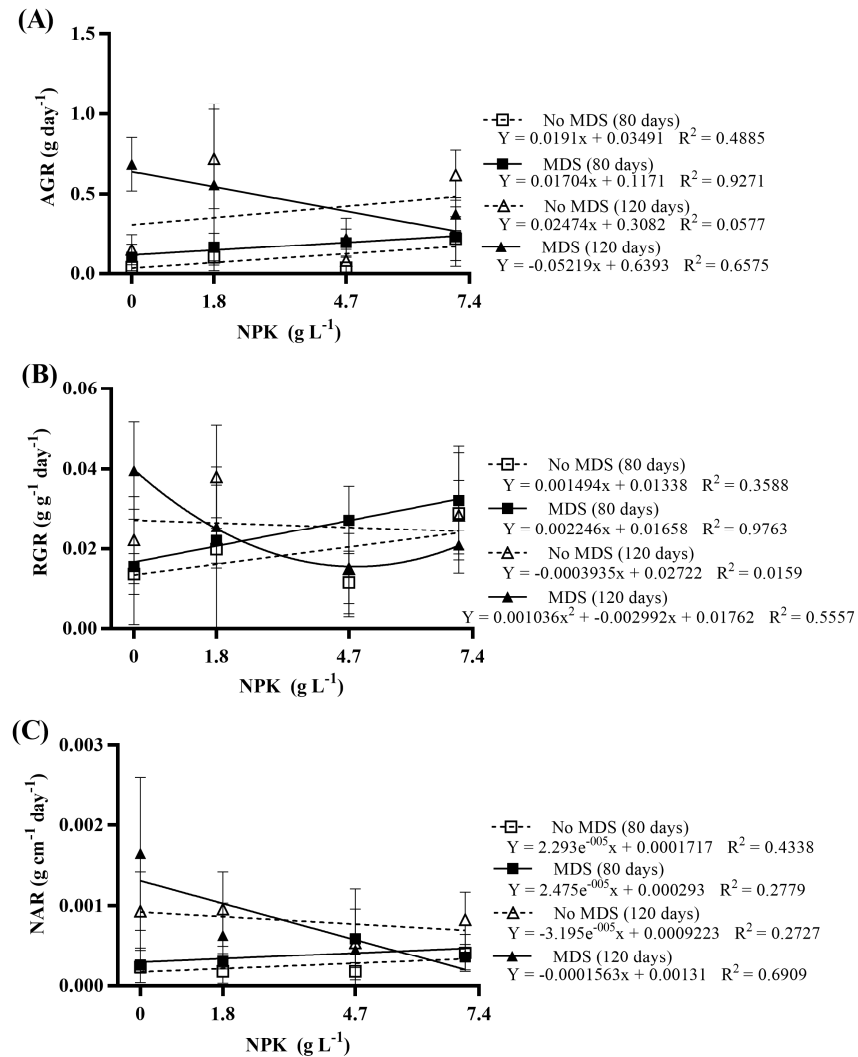


**Figure 3.** Leaf weight ratio, LWR (A); specific leaf area, SLR (B); and leaf area index, LAI (C) in 40, 80 and 120 day-old *Jatropha curcas* L. plants due to fertilization with a micronutrient delivery system (MDS) and different doses of NPK.

#### Quantitative growth analysis estimative

In the present study, AGR, RGR, and NAR variables presented similar behavior, with most data adjusted to linear regression models, except for RGR curve at 120 DAS in plants treated with MDS fertilizer, which the data fitted a quadratic model regression (Figure 4). Like other variables previously mentioned, MDS fertilizer application

resulted in higher values for these growth estimates in plants with no or low (1.8 g L<sup>-1</sup>) NPK supplementation. This was clearly emphasized on regressions of 120-day-old jatropha plants, in which these parameters were inversely proportional to NPK doses, whereas the other regression lines resulted in slightly increases due to higher NPK incorporation into the soil.



**Figure 4.** Absolute growth rate, AGR (A); relative growth rate, RGR (B); and net assimilation rate, NAR (C) in 40, 80 and 120 day-old *Jatropha curcas* L. plants due to fertilization with a micronutrient delivery system (MDS) and different doses of NPK.

## DISCUSSION

### Biomass production and allocation

Faster growth, higher biomass production, and better RDW/SDW ratio were observed in plants treated with MDS fertilizer, representing some of the major benefits of bioavailable mineral nutrients uptake by jatropha young plants. As a primary source of Zn and Cu through soil drench and foliar applications in the present study, MDS fertilizer showed an important role in enhancing jatropha growth. Zinc is described as an essential component of thousands of proteins in plants; is acquired from the soil solution primarily as  $Zn^{2+}$ , but also potentially complexed with organic ligands by roots, which feed the shoots via the xylem (BROADLEY et al., 2007). Copper is known to participate in

numerous physiological processes and is an essential cofactor for many metalloprotein (YRUELA, 2005).

Most of the commercial NPK products also contain a certain amount of Zn and Cu, such as Cu 0.05% and Zn 0.05% in the product evaluated in this study. Although both elements are considered essential micronutrients for plant development, they can be toxic when in excess (YRUELA, 2005; BROADLEY et al., 2007; BADONI et al., 2016). Therefore, MDS-supplemented plants may have had experienced deleterious effects of high NPK doses as a function of excessive uptake of Zn and Cu.

### Leaf area, chlorophyll, and source-sink relations

The higher LA values observed in response to NPK incorporation evidenced direct benefits on

jatropha leaf production and expansion (Figure 2A). Similarly, recent studies in the literature have investigated NPK effects on jatropa, from early development through final yield, which have demonstrated effective response of this species to NPK fertilization (LIMA et al., 2016; NEGUSSIE et al., 2016).

In contrast, chlorophyll content in MDS-treated plants is not affected by NPK doses, showing high SPAD values even with no incorporation of NPK into the soil (Figure 2B). Therefore, the significant amount of Zn in MDS fertilizer formula (7.14%) may have contributed for the higher SPAD values, since this element is involved in the biosynthesis of chlorophyll, carotenoids and in scores of metabolic reactions (SUBBA et al., 2014).

The similarities verified for LAR and LWR among plants treated or not treated with MDS fertilizer and indifference to NPK increment in the soil demonstrated the low response of these variables to both factors evaluated in this study (Figures 2C and 3A). When analyzing LAR for most regression models fitted to the data, a lower gap between treatments including the presence and absence of MDS was observed, i.e., a similar leaf area for the same production of plant dry weight. For LWR, a slightly difference in behavior was observed at 120 DAS in plants supplemented with NPK (7.4 g L<sup>-1</sup>) only, requiring a greater leaf area to produce the same amount of photoassimilates (lesser efficiency of the photosynthetic apparatus), and therefore resulting in less accumulation of photoassimilates in the plant (SHIPLEY, 2006; SOUZA et al., 2016).

In this study, increasing NPK supply resulted in a slight increase in total leaf area for a given unit of plant biomass, i.e., LAR (Figure 2C). This resulted from increases in the area of light capture per biomass invested in leaves, i.e., SLA (FRESCHE et al., 2015), as we usually observed at 80 DAS in jatropa plants growing without MDS (Figure 3B). This may be related to low efficiency in the partition of photoassimilates in this group of plants compared to those that were treated with MDS, as the latter presented an enhanced source-sink metabolism, and consequently, improved biomass allocation to the different plant organs (Figures 1 and 2).

It is also known that jatropa is an evergreen tree, which under natural conditions could be considered as an advantage trait, especially under abiotic stresses, such as low fertility or moisture in the soil. Thus, Poorter et al. (2015) believe that the SLA of evergreen woody species is about 2-3 times

lower than that of deciduous species, and all else being equal, this lower SLA would have to be compensated by a 2-3 times higher leaf mass fraction to reach a similar LAI. Apparently, this LAI compensation was observed in plants supplemented with MDS and higher doses of NPK in detriment of SLA values, which showed effective response to both factors evaluated (Figure 3C).

With LAI, a trend similar to the LA was observed regarding the regression model of the diverse treatments, with more evidence of improvement registered in MDS-treated plants. LAI is an important parameter in the growth analysis of a plant community, as it serves as an indicator of leaf ground cover (SHIPLEY, 2006; POORTER et al., 2012). Furthermore, this variable was highly responsive to NPK doses when plants were also supplemented to MDS, showing a synergistic effect of both fertilizers at 80 DAS, although it disappeared by the end of this study (120 DAS). Therefore, changes in the availability of nutrients, represented here for either MDS or NPK sources, triggered phenotypic changes in functional traits that determine the ability of jatropa plants to acquire and thereby mitigate the constraints imposed by the limiting resource (FRESCHE et al., 2015). This highlights an interesting behavior of this species, as LAI is one of the most frequently used parameters for the analysis of canopy structure and is an important structural characteristic of crop monitoring and crop productivity (BEHERA et al., 2010).

### Quantitative growth analysis estimates

The similarities among the estimates in this quantitative analysis of jatropa growth represented by AGR, RGR and NAR variables, confirm the beneficial effects of MDS supplementation already discussed in this article (Figure 4). Hence, this treatment involving a fertilizer source of Zn (7.14%) drives this crop to faster growth and efficient biomass allocation throughout time, even without NPK incorporated into the soil. Zn is a necessary cofactor for many biological reactions, known to limit oxidative degradation of auxin and is necessary to maintain membrane integrity (TSONEV; LIDON, 2012).

Although at 80 DAS this species was still positively responding to NPK increment, reversal behavior was seen in 120-day-old jatropa plants (Figure 4). These discrepancies certainly occur because AGR expresses the variation in plant growth (weight) for a given time, and the RGR is a measure of the speed with which a plant grows



compared to its original size (EVANS, 1972; POORTER et al., 2012; BEADLE, 2014).

Another important fact that must have affected the data observed in this study should be highlighted. Higher concentrations of Zn in the soil have direct effects on the growth and yield of plants (CHIBUIKE; OBIORA, 2014), and thus adversely affect agriculture (BADONI et al., 2016). Hussain and Maqsood (2010) emphasize that Zn concentration in soils lower than 125 ppm is considered optimum for the growth of plants. This certainly explains the drastic drop in NAR values of 120 day-old plants treated with MDS and high doses of NPK, as they would be subjected to excessive Zn, including Zn already present in the soil, Zn provided by NPK incorporation, and Zn provided by frequent MDS applications (Figure 4C).

## CONCLUSIONS

The effective response of jatropha young plants to MDS supplementation indicates that this

fertilizer played a relevant role in this species metabolism, resulting in faster growth and enhanced biomass allocation. Consequently, MDS could be a potential candidate to replace NPK fertilizers during initial crop development, not only for its higher efficiency, but also because of economic and ecological reasons.

Considering the reduction of NPK fertilizer and consequently potential reduction of nitrate runoff, the MDS fertilizer could be used as a standalone product or in conjunction with standard NPK fertilizers in lower doses.

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**RESUMO:** Estudos abordando o desenvolvimento do pinhão manso (*Jatropha curcas* L.) através de análises de parâmetros quantitativos são limitados, especialmente em relação à resposta a diferentes tipos e doses de fertilizantes. Para investigar os efeitos de um sistema de fornecimento de micronutrientes (MDS), realizou-se uma análise quantitativa completa do crescimento de plantas jovens de pinhão manso comparando a eficácia deste sistema sob diferentes doses de NPK. As plantas foram cultivadas por 120 dias em potes de 3,9 L contendo solo local, com ou sem MDS (parcela principal), combinado com doses de NPK (0; 1,8; 4,7 e 7,4 g L<sup>-1</sup>) nas subparcelas. Curvas dose-resposta das análises quantitativas das variáveis foram geradas para três períodos (40, 80 e 120 dias após semeadura), como uma sub-subparcela. As análises quantitativas de crescimento mostraram que a maioria dos parâmetros avaliados neste estudo foi melhorada pela aplicação do MDS, resultando em benefícios para o crescimento inicial do pinhão-manso, independentemente da dose de NPK. Mesmo sem suplementação de NPK ou sob a dose mais baixa avaliada (1,8 g L<sup>-1</sup>), o MDS proporcionou melhor crescimento do pinhão manso, sendo geralmente equivalente às maiores doses de NPK (4,7 e 7,4 g L<sup>-1</sup>) sem MDS. A resposta efetiva das plantas jovens do pinhão manso à suplementação com MDS indica que este tipo de fertilizante desempenhou um papel relevante no metabolismo desta espécie, resultando em um crescimento mais rápido e melhor alocação de biomassa.

**PALAVRAS-CHAVE:** Nutrientes minerais biodisponíveis; Fertilizante com micronutrientes; *Jatropha curcas* L.; Alocação de biomassa; Ecofisiologia.

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