Original Article

EFFECTIVENESS OF A MICRONUTRIENT DELIVERY SYSTEM FERTILIZER IN JATROPHA PLANTS IS RELATED TO ENHANCED PHOTOSYNTHESIS, GAS EXCHANGE AND BIOMASS ALLOCATION

EFICÁCIA DE UM SISTEMA DE FORNECIMENTO DE MICRONUTRIENTES EM PLANTAS DE JATROPHA ESTÁ RELACIONADO COM INCREMENTOS NA FOTOSSÍNTESE, TROCA GASOSA E ALOCAÇÃO DE BIOMASSA

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ABSTRACT: This study aimed at comparing the growth and physiological changes in *Jatropha curcas* L. (jatropha or physic nut) young plants fertilized or not with a commercial product based on a micronutrient delivery system (MDS), under different doses of NPK. Measurements of growth, chlorophyll content, and leaf gas exchange were performed in the greenhouse, where plants were arranged in a split-split plot design. Plants were grown for 120 days 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^{-1}) in subplots. Dose-response curves showed that most variables were positively responsive to NPK doses in plants growing without MDS, whereas slight responses or even opposite behavior was observed in MDS-fertilized plants. MDS application under low NPK doses resulted in higher biomass allocation in leaves and roots, increases in number of leaves and chlorophyll content, plant height, stem diameter, shoot and root dry weight, stomatal conductance, photosynthesis, leaf transpiration, and water use efficiency, as well as decreases in intercellular CO_2 in the leaf and vapor-pressure deficit. The enhanced biomass allocation, photosynthesis and gas exchange in MDS-supplemented plants indicates the relevant role played by this fertilizer in jatropha metabolism, resulting in more vigorous plants.

KEYWORDS: *Jatropha curcas* L.; Biofuel crops; Ecophysiology; Sustainable fertilizer management; Bioavailable mineral nutrients.

INTRODUCTION

The shortage of petroleum reserves and the abundant availability of plant and forest-based non-edible vegetable oils have increased their demand as substitutes of diesel (SHARMA; NARANG, 2015). In this context, jatropha or physic nut (*Jatropha curcas* L.) is a promising tropical biofuel plant and has been considered as a potential crop for marginal environments due to its ability to grow relatively well under harsh conditions, such as scarce rainfall and poor soil (SILVA et al., 2010). Nonetheless, recent studies have revealed that yield is significantly low in non-fertilized fields (LIMA et al., 2016; NEGUSSIE et al., 2016).

Studies with different fertilization strategies have indicated that jatropha requires significant amounts of nutrients to obtain satisfactory yields. Negussie et al. (2016) observed significant responses of jatropha to fertilization, especially with nitrogen, resulting in increased yield. In addition, Lima et al. (2016) reported that phosphate and organic fertilization together beneficially influenced plant height, number of branches, stem diameter, leaf area, number of seeds per plant, and total mass of jatropha seeds. In contrast, excessive amounts of mineral fertilizers in the soil, such as standard NPK products, may result in nonpoint source pollution, which is a worldwide issue mostly caused by surface runoff and interflow of nitrate and phosphate (CAO et al., 2017). A reduction of NPK fertilizer and consequently a potential reduction of nitrate runoff would allow growers to obtain better quality seedlings at lower costs and reduce the impact on the environment.

A fine balance between the use of marginal lands and obtaining satisfying yields in the field through adequate fertilization management is the major key guiding principle to develop an efficient renewable biofuel energy source (YONG et al., 2010). In this context, studies involving the use of micronutrients in jatropha are challenging and scarce in the literature, especially because the results can be highly variable depending on the particular micronutrient and amount supplemented. Specifically, for jatropha, increase in the concentrations of Fe, Mn, and Zn in the irrigation water up to 150 mg L^{-1} resulted in increased

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biomass (weight), photosynthetic pigments, and nutrient uptake. However, the increase in micronutrient concentrations above 150 mg L^{-1} showed negative effects in jatropha young plants (EL-KADER et al., 2012).

In the present study, a new fertilization strategy using a micronutrient delivery system (MDS) was evaluated for jatropha young plants. It consists of a highly positively charged zinc and copper solution balanced together in a specific ratio, along with approximately 25% sulfate and 0.2% ammonia. According to company information, the ionic minerals in this product are not bonded with salt. As a result, they are translocated to the entire plant after being introduced by drench or leaf treatments, being more fully absorbed, which prevents mineral salts that can cause run-off into streams and well water.

Therefore, plant growth and physiological parameters were examined, including biomass production and allocation in different plant organs, leaf chlorophyll content and leaf gas exchange. Hence, the main objective of this study was to evaluate the growth, chlorophyll content, photosynthesis, and gas exchange of jatropha young plants fertilized with MDS, combined with different doses of NPK.

MATERIAL AND METHODS

Location, plant material and experimental conditions

Seeds originated from 12 jatropha accessions from India were selected for the study. 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 $100g^{-1}$, 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 containers 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, known as MDS. 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, the product was applied following label recommendations; by soil drench (125 mL L^{-1}) immediately before sown, and by foliar application (62.5 mL L^{-1}) at 40 and 80 days after sown (DAS).

The NPK source was mixed with the soil before potting and included different doses of a commercial NPK fertilizer formulation 15-9-12 (Osmocote® Plus, Dublin, Ohio, USA) according to low, medium and high incorporation rates suggested on product label, resulting in 1.8, 4.7 and 7.4 g L^{-1} per container, respectively. The fertilizer manufacturer sheet provided a guaranteed analysis report including N 15%, P_2O_5 9%, K_2O 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, growth and physiological characteristics evaluations

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 and seedlings with poor vigor were removed to maintain the three most vigorous seedlings per 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 tagged for evaluations of growth, development and physiological characteristics. First, number of leaves per plant was counted, and measurements of plant height (PH) and stem diameter (SD) were performed. Leaf chlorophyll index was measured on three newly fully expanded leaves with a SPAD 502 meter (Konica Minolta Sensing, Osaka, Japan).

Leaf gas exchange was monitored with a portable infrared gas analyzer (Ciras-3, PP Systems, Amesbury, MA, USA) on the same three expanded leaves used for the chlorophyll index measurements, from 08:00 to 10:00 h in the morning (SILVA et al., 2010; RAJAONA et al., 2013). Leaf gas exchange measurements included intercellular CO_2 in the leaf (C_i), stomatal conductance (g_s), photosynthesis (A), vapor pressure deficit (VPD), leaf transpiration (E) and water use efficiency (WUF). Gas exchange parameters were recorded when stomatal conductance and intercellular CO_2 concentration were stable, usually between 5 and 10 min after leaf insertion into the infrared gas analyzer chamber (RAJAONA et al., 2013).

Thereafter, plants were harvested, and growth characteristics were evaluated at the Ornamental Horticulture and Tropical Plant Ecophysiology laboratories at TREC. All leaves were removed from each plant, 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. Leaves, stems and roots were then oven-dried at 80°C for two days and dry weight was determined. This data was used to estimate shoot (SDW) and root dry weight (RDW), as well as leaf (LMF), stem (SMF) and root mass fraction (RMF).

Experimental design and statistical 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 the main plot, and the NPK doses (0; 1.8; 4.7 and 7.4 g L^{-1}) in subplots.

Dose-response relationships were assessed by adjustment of simple and multiple linear regression models used to predict growth response 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 AND DISCUSSION

Growth parameters, leaf chlorophyll content and biomass allocation

For jatropha growth parameters, deviations from the linear regression due to 'lack of fit' were not significant when compared with the within-sample variation (Figures 1, 2 and 3). Thus, for the set of data that included jatropha seedling biomass production and allocation, fitting linear regressions that related to MDS and NPK were significantly appropriate for this study.

Jatropha leaf area increased in direct proportion to NPK doses, with the data adjusted to the linear regression model, showing that this variable was highly responsive to the NPK factor, regardless of MDS supplementation (Figure 1A). Although similar increments were observed for number of leaves (Figure 1B) and chlorophyll index (Figure 1C) from plants growing without MDS fertilizer applications, constant linear regressions applied for the other group of plants (treated with MDS), regardless of the NPK doses. Therefore, regardless of MDS fertilization, jatropha plants showed similar results for leaf area, being highly responsive to NPK incorporation in the soil, but MDS-supplemented plants presented significantly higher number of leaves and leaf chlorophyll content values compared with plants not treated with MDS. In this study, plants growing with frequent MDS applications did not show signs of leaf yellowing throughout the experiment, whereas plants not supplemented with MDS showed extensive leaf yellowing, especially under low NPK doses (Figure 1).

Plant height (PH) and SD of MDS-supplemented plants were not significantly affected by NPK doses, showing only small increases and decreases for both variables, respectively (Figures 2A and 2B). In contrast, the increase in NPK supply from 0 to 7.4 g L^{-1} resulted in increases for both variables in plants growing in the absence of MDS. Similar behavior was observed in this group of plants for shoot and root dry weight (Figures 2C and 2D). Both shoot and root development improved with MDS application, especially under no NPK supplementation, where higher discrepancies among the treatments were observed. Hence, this confirmed that frequent MDS applications provided better results than fertilization with high amounts of NPK. Furthermore, for the 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 (Figure 2).

Although NPK increases were responsible for directly proportional responses in LMF, plants fertilized with MDS showed higher mean values, varying between 7 and 13% (Figure 3A). Most of the weight of jatropha young plants corresponded to SMF, which was negatively affected by NPK doses in plants not treated with MDS (Figure 3B). Thus, for this group of plants, it could be inferred that the higher the NPK dose applied to the soil, the lower the allocation of biomass in stems for this species.

In contrast, NPK doses did not influence SMF and RMF significantly in MDS-fertilized plants, with mean values of approximately 75% and 15% for these variables, respectively (Figures 3B and 3C). Nonetheless, similar to LMF values, RMF of plants growing without MDS application was positively influenced by NPK fertilization (Figure 3).

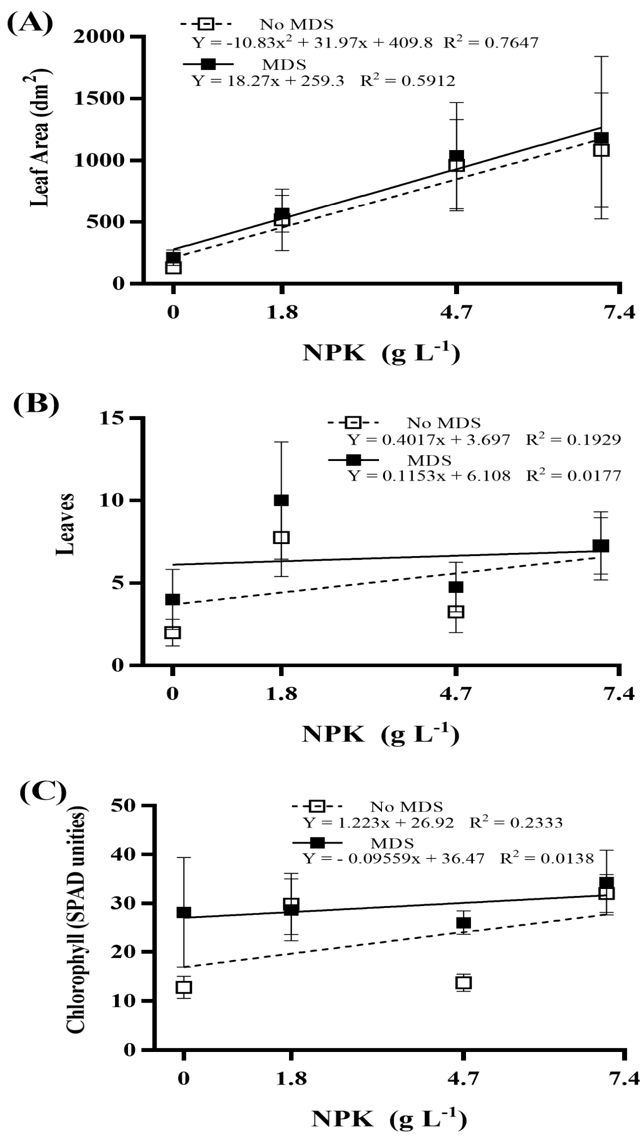

Figure 1. Leaf area (A), number of leaves (B) and chlorophyll content (C) of 120-day-old *Jatropha curcas* L. plants due to supplementation with different combinations of a micronutrient delivery system fertilizer (MDS) and NPK.

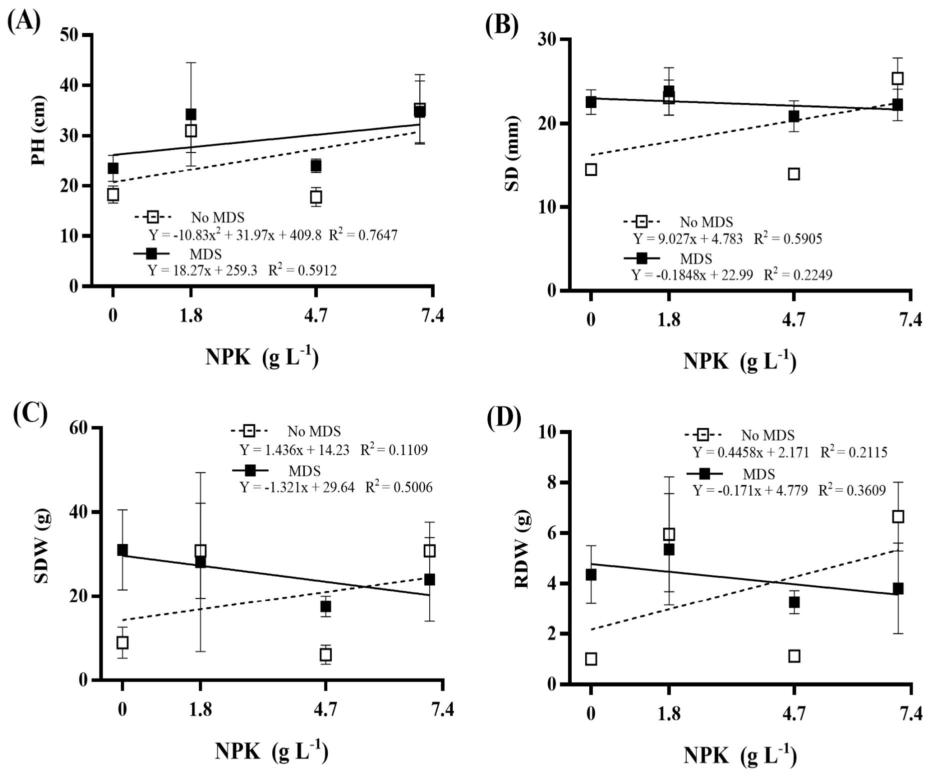

Figure 2. Plant height, PH (A); stem diameter, SD (B); shoot dry weight, SDW (C); and root dry weight, RDW (D) of 120-days-old *Jatropha curcas* L. plants due to supplementation with different combinations of a micronutrient delivery system fertilizer (MDS) and NPK.

Photosynthesis and leaf gas exchange

Gas exchange measurements on jatropha single leaves indicated that C_i was inversely proportional to NPK doses, regardless of MDS application (Figure 4A). Similar behavior was observed for g_s in plants growing without MDS (Figure 4B), whereas all other variables fitted linear regressions directly proportional to NPK increments, or quadratic regressions in which the data reached a maximum at mid values and then decreased (Figure 4).

The data for g_s, A and E variables showed similar behavior in MDS-supplemented plants, which fitted quadratic model regressions (Figures 4B, 4C and 4E). Similar to other variables previously mentioned, MDS fertilizer application resulted in higher values for these gas exchange parameters in plants growing in soil incorporated with NPK doses from 0 to 4.7 g L^{-1}. VPD values showed slight increases due to NPK dose increments, but the data for plants supplemented with MDS fitted a linear regression model, whereas the non-supplemented group was better represented by a quadratic regression model (Figure 4D). WUE in jatropha plants was benefitted by NPK supplementation, and it was directly proportional to NPK doses, although more pronounced responses could be observed in non-MDS linear regression models when compared to plants treated with MDS (Figure 4F). Nevertheless, better maintenance of high WUE values regardless of NPK fertilization was detected in MDS-treated plants, which showed better results for this parameter (Figure 4).

Growth parameters, leaf chlorophyll content and biomass allocation

In this study, plants treated with MDS fertilizer presented not only a slightly higher leaf area (Figure 1A), but this treatment also enabled a relevant increase in number of leaves produced by plant (Figure 1B), which contained a significantly higher amount of chlorophyll (Figure 1C). Thus, MDS fertilization induced better development of source organs, such as leaves, which can be responsible for improvements in photosynthesis and gas exchange, especially during the young period of development, when the main source organs are leaves (GIRONDÉ et al., 2015).

Our results evidenced direct benefits of NPK fertilization on jatropha leaf production and expansion, mainly in plants grown with no MDS (Figure 1). Recent studies investigated NPK effects on this species and have also demonstrated effective response of jatropha plants to NPK fertilization (LIMA et al., 2016; NEGUSSIE et al., 2016). In contrast, chlorophyll index in MDS-treated plants slightly varied due to NPK doses, showing high SPAD values even with no incorporation of NPK into the soil (Figure 1C). Thus, the significant amount of Zn in MDS fertilizer formula (7.14%) may have contributed to the higher SPAD values in this treatment. Zn is an essential component of thousands of proteins in plants, and it is involved in the biosynthesis of chlorophyll, carotenoids and scores of metabolic reactions (BROADLEY et al., 2007). Cu is known to participate in numerous physiological processes and is an essential cofactor for important metalloproteins (YRUELA, 2005). A balanced concentration between Cu and Zn, as contained in MDS solution, positively affects the development of the membrane system of chloroplasts and chlorophyll content (AHMAD et al., 2015), supporting the results reported here. The MDS fertilizer played an important role on the enhancement of jatropha growth under poor soil conditions (0-1.8 g L^{-1} NPK), representing one of the major benefits of bioavailable mineral nutrient uptake by jatropha young plants (Figure 2). Therefore, as a primary source of Zn (7.14%) and Cu (2.03%) through soil drench and foliar applications in this study, it is likely that the finely balanced concentration of both elements in MDS enabled beneficial metabolism changes in jatropha young plants.

Although plants growing without MDS showed a better response to NPK fertilization, in MDS-fertilized plants both SDW and RDW mean values fitted linear regressions inversely proportional to increases in NPK, revealing that the combination of MDS and NPK may affect negatively jatropha growth (Figure 2). This may have occurred due to the large amount of soluble salts incorporated into the soil, added to the elements present in the MDS solution. It is well known that most of NPK commercial products also contain a certain amount of Zn and Cu, such as Cu 0.05% and Zn 0.05% in the fertilizer used in this study. Thus, MDS-supplemented plants may have experienced deleterious effects as a function of a negative interaction between high NPK doses and excessive Zn and Cu uptake. Although both elements are considered essential micronutrients for plant growth and development, they can be significantly toxic for many crops when available in high quantities (YRUELA, 2005; BROADLEY et al., 2007).

Our results indicate that MDS application not only provided a better initial growth of jatropha plants but also enhanced the biomass allocation for shoots and roots in poor soil conditions (no NPK fertilization), possibly as a result of improved uptake of water and nutrients by the plants (Figures 3A and 3C). This enhanced biomass allocation in shoots due to MDS fertilization agrees with the results found by Khurana-Kaul et al. (2010), who reported significant improvement in shoot bud induction when the concentration of $CuSO_4$ was increased to 10 times the normal level *in vitro*.

The negative influence of NPK doses on SMF indicates that excessive fertilization may be responsible for weak stem formation, and consequently, less vigorous seedlings formed, due to the inefficient support provided by stems (Figure 3B). These responses are usually related to the development of undesirable processes, such as etiolation. Jatropha seedlings may develop a robust pivoting root system and an etiolated aerial part because of environmental and phytotechnical factors, including high fertilization and density of sowing (RESENDE et al., 2012).

Photosynthesis and leaf gas exchange

It is noteworthy that an optimal growth condition for jatropha young plants was achieved in treatments involving MDS supplementation and 1.8 g L^{-1} NPK fertilization. These results seem to be correlated to the gas exchange parameters, as the most important variables involved in plant growth and biomass production (g_s, A and E) followed the same pattern (Figures 1B, 1C and 1E). Under the experimental conditions tested, the values for these parameters were adjusted to a quadratic regression model, reaching a maximum point when NPK doses were between 1.8 and 4.7 g L^{-1}. This demonstrated that excessive nutrient application had no further positive effect on jatropha growth, likely because the leaf N content reached their maximum physiological capacity in young plants, as similarly reported by Yong et al. (2010).

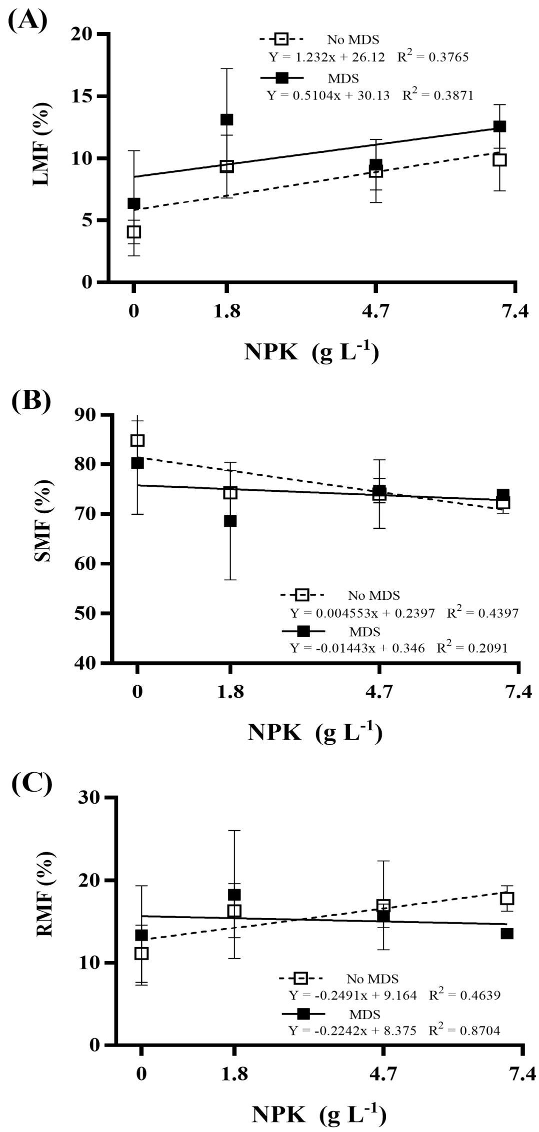

Figure 3. Leaf mass fraction, LMF (A), stem mass fraction, SMF (B) and root mass fraction, RMF (C) of 120-day-old *Jatropha curcas* L. plants due to supplementation with different combinations of a micronutrient delivery system fertilizer (MDS) and NPK.

Our results revealed that reductions of A in treatments without MDS were not determined by the lower g_s because this was not linked with lower C_i under higher NPK doses (Figure 4). In contrast with the hypothesis that these parameters would be linked to each other, slight increases in A mean values were observed proportionally to NPK doses in plants not treated with MDS. Therefore, limitations in C_i did not explain the variability in A among jatropha plants, regardless of MDS fertilization, indicating the presence of other limitations related to changes in several fluorescence parameters (MAXWELL; JOHNSON, 2000). In addition, not only abiotic factors exert control over leaf gas exchange, but also internal plant status parameters, which may affect carbon assimilation, such as N availability (LEBAUER; TRESEDER 2008; RAJAONA et al., 2013). Similar results to those found in this study were reported in sorghum (OLIVEIRA et al. 2011) and jatropha (PLOSCHUK et al., 2014), both evaluating photosynthesis responses to abiotic stresses during early vegetative stages.

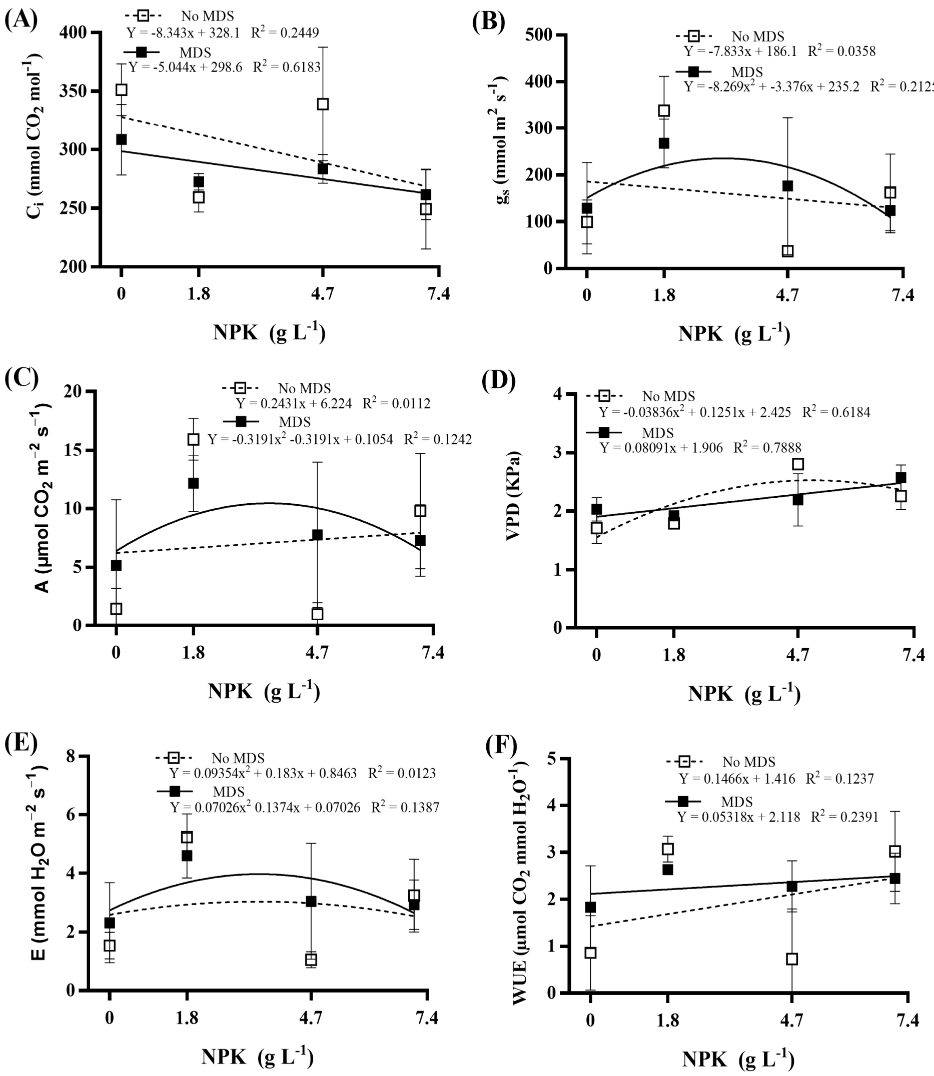

Figure 4. Intercellular CO_2 in the leaf, C_i (A); stomatal conductance, g_s (B); photosynthesis, A (C); vapor pressure deficit, VPD (D); leaf transpiration, E (E); and water use efficiency, WUF (F) of 120- day-old *Jatropha curcas* L. plants due to supplementation with different combinations of a micronutrient delivery system fertilizer (MDS) and NPK.

Considering fertilization effects, plants grown under no MDS fertilization had a lower A compared with those supplemented with MDS (Figure 4C), and this variation in leaf gas exchange was related to changes in the chlorophyll index value (SPAD), which presented similar results and steadily increased with NPK increase (Figure 2C). According to Rajaona et al. (2013), contrary to the analysis of carbon assimilation of crops from high-input agricultural systems, where abiotic stresses are minimized, many of the tropical and subtropical production systems are subject to a variety of stressors of which nutrients are among the most dominating factors. In fact, nutrient availability is considered one of the main abiotic drivers of carbon assimilation.

In our study, increased growth and biomass might have been related to higher g_s, A, E and WUE, as well as lower VPD under middle doses of NPK (Figure 4). Thus, higher E values were not necessarily associated to reduced WUE, indicating that high transpiration rates may have resulted in better nutrient uptake and general gas exchange by MDS-supplemented plants instead of increases in water loss through the soil-plant-atmosphere continuum. In general, high transpiration rates may affect photosynthesis negatively due to the induction of stomatal closure. Although it was not the case in this study, such behavior can be observed with certain frequency in seedlings or young plants, especially under abiotic stress conditions (OLIVEIRA et al., 2011).

The similarities verified for VPD data among plants treated or not treated with MDS fertilizer and limited response to NPK increments in the soil demonstrated the low response of these variables to both factors evaluated in this study (Figure 4D). Additionally, there was a decline in g_s with VPD increase, as expected. A similar relationship between those variables was reported by Rajaona et al. (2013) in jatropha, who evaluated maximal stomatal conductance and prediction of g_s responses to variable VPD. The results in this study, therefore, demonstrate that the maintenance of relatively high g_s in plants supplemented with MDS and low NPK doses should have helped them to achieve better photosynthesis rates under slightly increased VPD values, resulting in faster growth and better biomass production.

Instantaneous WUE of plants grown without MDS supplementation increased with NPK increase, whereas opposite behavior was observed for linear regression models that fitted C_i and g_s variables in this study (Figure 4F). These results corroborate with the decrease expected with increasing g_s if A and g_s are not highly correlated (DAMOUR et al. 2010; RAJAONA et al., 2013), as observed for control plants (no MDS, no NPK fertilization). Thus, better water use efficiency caused by NPK fertilization seems to be one of the most important changes that provided better growth in jatropha plants growing without MDS.

CONCLUSIONS

The maintenance of high chlorophyll content and enhancement in photosynthesis and gas exchange observed in MDS-supplemented plants indicate the relevant role played by this fertilizer in jatropha metabolism, resulting in faster growth, better biomass allocation, and consequently, more vigorous plants.

Considering the potential reduction of nitrate runoff, this fertilizer could be used as a standalone product or in conjunction with standard NPK fertilizers in lower doses, not only for its higher efficiency, but also because of economic and ecological reasons. This could allow growers to obtain vigorous plants at lower costs and reduced impact on the environment.

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RESUMO: Este estudo teve como objetivo comparar o crescimento e as mudanças fisiológicas em plantas jovens de *Jatropha curcas* L. (pinhão manso), fertilizadas ou não, com um produto comercial baseado em um sistema de fornecimento de micronutrientes (MDS), sob diferentes doses de NPK. Medidas de crescimento, teor de clorofila e troca gasosa foliar foram realizados em casa de vegetação, onde as plantas foram arranjadas em um delineamento de blocos casualizados com parcela subdividida. 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^{-1}) nas subparcelas. Curvas dose-resposta mostraram que a maioria das variáveis responderam positivamente às doses de NPK em plantas crescendo sem MDS, enquanto respostas fracas ou mesmo comportamento oposto foi observado em plantas fertilizadas com MDS. A aplicação de MDS em doses baixas de NPK resultaram em maior alocação de biomassa nas folhas e raízes, aumento no número de folhas e teor de clorofila, altura da planta, diâmetro do caule, pesos secos da parte aérea e raiz, condutância estomatal, fotossíntese, transpiração foliar e eficiência no uso da água, bem como na redução do CO_2 intercelular na folha e déficit de pressão de vapor. O aumento na alocação de biomassa, fotossíntese e troca gasosa em plantas suplementadas com MDS indica o papel relevante deste fertilizante no metabolismo de pinhão manso, resultando em plantas mais vigorosas.

PALAVRAS-CHAVE: *Jatropha curcas* L.; Culturas de biocombustíveis; Ecofisiologia; Manejo sustentável de fertilizantes; Nutrientes minerais biodisponíveis.

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