

Growth and quality of mangosteen seedlings (*Garcinia mangostana* L.) in response to the application of humic acids

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Abstract- The time of formation of mangosteen seedlings propagated by seeds can be considered as the biggest difficulty in the seedling production system of this species. Considering that the use of humic acids as a growth promoter has been well documented in some species, the objective of this work was to evaluate the growth of mangosteen seedlings in response to the application of humic acid (HA) doses extracted from vermicompost (0; 2; 20; 40; 200 mg C L⁻¹). Growth rate was determined using the following variables: stem diameter, plant height, number of leaves, leaf area, stem dry matter, leaf dry matter, shoot dry matter, root dry matter, total dry matter and Dickson quality index. The dose 150 mg C L⁻¹ was the one that approached the maximum values and therefore should be recommended for the production of seedlings. The results at 240 days after planting (DAP) showed that the HA increased the growth of mangosteen seedlings, helping to reduce the time of seedling formation under conditions for permanent planting.

Index Terms: Quality index, vermicompost, humate.

Crescimento e qualidade de mudas de mangostanzeiro (*Garcinia mangostana* L.) em resposta à aplicação de ácidos húmicos

Resumo- O tempo de formação de mudas de mangostanzeiro propagadas por sementes pode ser encarado como o maior gargalo no sistema de produção de mudas dessa espécie. Considerando que o uso de ácidos húmicos como promotor de crescimento vem sendo bem documentado em algumas espécies, o objetivo deste trabalho foi avaliar o crescimento de mudas seminais de mangostanzeiro em resposta à aplicação de doses de ácidos húmicos (AH) extraídos de vermicomposto (0; 2; 20; 40; 200 mg C L⁻¹). A avaliação do crescimento foi realizada por meio das variáveis diâmetro do coleto, altura das plantas, número de folhas, área foliar, matéria seca do caule, matéria seca da folha, matéria seca da parte aérea, matéria seca da raiz, matéria seca total e Índice de qualidade de Dickson. A dose de 150 mg C L⁻¹ foi a que mais se aproximou dos valores máximos e portanto deve ser recomendada para a produção de mudas. Os resultados, aos 240 dias após o plantio (DAP), demonstraram que os AHs incrementam o crescimento de mudas de mangostanzeiro, auxiliando na redução do tempo de formação de mudas em condições para plantio definitivo.

Termos para indexação: Índice de qualidade, vermicomposto, humato.

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Received: August 31, 2018
Accepted: October 04, 2018

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Introduction

Mangosteen (*Garcinia mangostana* L.) is a fruit species belonging to the family Clusiaceas, originating in the Malay Archipelago. In Brazil, it is distributed and cultivated in the Amazon region and in the south of Bahia, regions where the climate is hot and humid, with rains well distributed during the year. Its fruits have excellent conditions for commercial exploitation, with quotations almost always exceeding fifty reais per kilogram (SACRAMENTO et al., 2007; CARVALHO, 2014).

Mangosteen may be propagated by grafting and cutting, however, as Moraes et al. (2007), the rooting index of cuttings is considered low. Because of the absence of genetic variability, seedling formation is still carried out using seeds in the main producing countries (SACRAMENTO et al., 2007).

The time of seedling formation is considered to be the main obstacle in the production system of mangosteen, which can stay up to two years in nursery. This long period is related to the way germination occurs, from which the seed is formed an adventitious root with few secondary roots and generally of insignificant size, constituting the definitive root system of the plant, which presents reduced capacity of absorption of water and nutrients (SACRAMENTO et al., 2009; CARVALHO, 2014).

In spite of the possibility of propagation by grafting, in practice no grafted plants are found in orchards of this fruit because of the slower growth in relation to seminal plants, besides problems of poor stem formation and decumbent branches, a fact that does not motivate the producers to use vegetative propagation in the formation of seedlings in commercial orchards (SACRAMENTO et al., 2007; CARVALHO, 2014).

The use of chemical stimulants, natural or not, together with the conventional techniques of seedling production, such as the use of humic substances, make it possible to obtain quality seedlings and reduce nursery time. In this context, the application of products derived from humic substances as growth promoters in cultivated plants has generated interest in the agricultural community (DOBBSS et al., 2010). Several studies have documented positive and promising results of the application of these substances on the growth of plant species (CANELLAS et al., 2002; BALDOTTO et al., 2009; CANELLAS and OLIVARES, 2014; OLIVARES et al., 2015; SILVA et al., 2015; BUSATO et al., 2016).

In addition to the considerable potential of humic substances to provide significant increases in plant growth, the low cost of obtaining these molecules should be considered important because they can be prepared from recyclable organic waste (MEIRELLES et al., 2017). Considering the importance of studies of humic acids (HA) in the formation of quality seedlings, it should be emphasized that the use should not occur in an empirical way, since the biological activity of HA is dependent on

the source, applied concentration and plant genotype (BALDOTTO and BALDOTTO, 2014).

In seedling production systems, it is becoming increasingly important to identify characteristics to evaluate the quality of a seedling before it is brought to the field. Dickson quality index (DQI) is a potential tool for estimating seedling quality because it includes relationships of morphological parameters such as height, diameter and dry biomass (SILVA et al., 2013).

The DQI is a good indicator in the analysis of the quality of seedlings, however, it presents the obstacle of being a methodology of destructive evaluation. In this context, defining the degree of correlation between morphological variables such as height and diameter of plants and DQI is important because these variables are easily measured and accepted by nurserymen because there is no need to destroy the plant.

The present study evaluated the growth of seedlings of mangosteen as a function of doses of HA and correlate to growth variables with DQI.

Material and methods

The experiment was carried out in a greenhouse on the campus of the State University of Santa Cruz (UESC), from May to November 2017, in the municipality of Ilhéus / BA. The experiment was conducted using the completely randomized design, in which the treatments were increasing doses of HA (humic acids) of 0; 2; 20; 40 and 200 mg C L⁻¹, with seven replicates.

The HA were extracted as described by Canellas et al. (2002), using NaOH 0.1 mol L⁻¹ (1:10, v: v) under N₂ atmosphere. After four hours of stirring, the material was centrifuged (3000 g, 20 min) and acidified to pH 1.5 with 6 mols L⁻¹ HCl. The material was centrifuged again and the supernatant discarded. HA were solubilized, precipitated two more times and treated for 16 h with diluted HCl: HF (1:20, v: v). After centrifugation, the HA were titrated to pH 7.0 with KOH 0.1 mol L⁻¹ and subjected to dialysis in membrane tubes with a limit of exclusion of 1000 Da until the value of the electrical conductivity of the water was equal to that of water distilled.

The humic acids used were extracted from vermicompost, presenting composition of 41% C and 3.6% of N. Solutions of HA at the tested concentrations were prepared and with the aid of micropipette was added 0.1 M NaOH to aid in resuspension of the particles. At the end the solutions were corrected to pH 7.

The mangosteen seeds were initially removed from the fruit, washed in running water and then placed to germinate in trays filled with the Carolina Soil® substrate (compound based on sphagnum peat, expanded vermiculite, dolomitic limestone, agricultural plaster and NPK fertilizer) sterilized. For planting of the experiment were selected seedlings with approximately 5 cm in height. At 30 days after germination, the germinated seeds were

transplanted into containers with a capacity of 5 dm³ of substrate, in which the doses (333.3 mL per application) of HA were added at 60, 120 and 180 days after planting (DAP).

Growth evaluations of the seedlings were carried out at 240 DAP. The following variables were evaluated: stem diameter (SD), plant height (H), number of leaves (NL), leaf area (LA), stem dry matter (SDM), leaf dry matter (LDM), shoot dry matter (SDDM), root dry matter (RDM), total dry matter (TDM) and Dickson quality index (DQI).

The values of H and SD were measured using a tape measure and digital caliper. LA data were obtained using a Li-3100 area meter (Licor, Nebraska, USA). The plants were then placed in a greenhouse at 65°C for 72 h and then weighed in an analytical balance with an accuracy of 0.01 g. The TDM was obtained by summing the SDDM and RDM values. To calculate the quality index of the seedlings, the formula (DICKSON et al., 1960) was used.

$$DQI = \frac{TDM}{\left(\frac{H}{SD}\right) + \left(\frac{SDDM}{RDM}\right)}$$

For statistical analysis, a Komolgorov-Smirnov test was performed to evaluate the normality of the data distribution. Afterwards, a variance analysis was performed, and when the significance test was verified by the F test, a regression analysis was performed as a function of the HA doses, and the equation with a significant effect was selected by the F test, at 5% probability and with a smaller sum of squares of the residue, that is, higher coefficient of determination (R²). Pearson's correlation analysis was performed for the variables that compose the DQI and the correlation coefficients verified by the Student's t-test at 5% of significance. All data were analyzed in software R (FERREIRA et al., 2011) and SigmaPlot 12.0 (Systat software, 2008).

Results and discussion

The variables SD, H, NL and LA adjusted the regression with quadratic models and significance by the F test at 5% probability (Table 1). The same effect was observed for the variables SDM, LDM, SDDM, RDM, TDM and DQI (Table 2).

The response curves for the application of HA doses showed a quadratic effect for SD, H, NL and LA in mangosteen seedlings with 240 DAP (Figures 1A; 1B; 1C; 1D). Due to the quadratic behavior of these variables, it was possible to verify that doses of humic acids lower than 200 mg C L⁻¹ (Table 3) would provide greater SD (131.15 mg C L⁻¹), H (161.9 mg C L⁻¹), NL (148.69 mg C L⁻¹) e LA (148.52 mg C L⁻¹).

Effect of HA doses of vermicompost on pineapple seedlings in the acclimation phase were obtained by Baldotto et al. (2009), who verified quadratic increments for collection diameter and plant height. These authors argued that the biostimulating action of humic acids has been related to the degree of similarity with the auxins, and can promote plant growth in relatively small concentrations. Effects of HA action have been observed in plants, such as modifications in the aerial part and root system morphology, such as changes in plant physiology and biochemistry (SANTOS et al., 2018).

Baldotto et al. (2010) observed increases of 111 and 116% for leaf area and number of shoots respectively in 'Vitoria' pineapple plants treated with HA during acclimatization phase. In mangosteen seedlings the results observed for NL and LA also responded significantly to HA, with increases of 70 and 48% respectively. It was observed that plants treated with 200 mg C L⁻¹ of HA emitted lateral branches (plagiotropic) at 180 DAP, and plants treated with doses between 2, 20 and 40 mg C L⁻¹ of HA emitted plagiotropic branches at 210 DAP. The control plants did not emit lateral branches. This behavior is possibly related to the endogenous levels of plant hormones, especially the cytokinins, which combat apical dominance and promote axillary development (TAIZ and ZEIGER, 2017).

The effects of the use of HA isolated from vermicompost seem to be related to auxins, plant growth hormones. Canelas et al. (2002) detected in HA of high molecular weight the presence of indole-3-acetic acid molecules (IAA) while Quaggioti et al. (2004), detected IAA molecules in low molecular weight HA, and measured 27.75 nM IAA in 0.75 mg C L⁻¹ solution of HA.

When well managed, after two years of nursery, *Garcinia mangostana* L. seedlings reach an average height of 40 cm, being ready for final planting or to be used as a rootstock (SACRAMENTO et al., 2007; CARVALHO, 2014).

In the present study the seedlings treated with vermicompost HA presented larger heights in a shorter period of time, where \hat{Y}_{max} for ALT was 50 cm in response to 161.9 mg C L⁻¹ of HA (Table 1). It is expected that seedlings with collection diameter between 0.9 and 1.2 cm, in general, are suitable for permanent planting (CARVALHO, 2014). In this study the SD of mangosteen seedlings treated with HA at 240 DAP presented this pattern in shorter nursery time. In this way, the results can be attributed to the effect that HA exerts on the plants, since control plants (without HA application) had a mean 68% lower H and SD when compared to the adjusted optimal dose for these variables.

The HA isolates of vermicompost were able to stimulate the growth of the aerial part of the mangosteen, where the increments were curvilinear with quadratic rates for the variables SDM, LDM and SDDM (Figures 2A; 2B; 2C). It is still possible to highlight in Table 3 the dose-

response model showing the ideal dose of HA for SDM (130.8 mg C L⁻¹), LDM (154.1 mg C L⁻¹) and SDDM (144 mg C L⁻¹), where the adjusted regression equations can be used to estimate the maximum accumulation for SDM (10 g/ plant), LDM (19.9 g/ plant) e SDDM (29.9 g/ plant).

As observed in the variables that compose the aerial part of the mangosteen seedlings the response to RDM at the doses of HA presented quadratic rates (Figure 2D). The increases can be explained by the similarity between the biological activity of HA and that exerted by growth hormones of endogenous auxin groups in plants and that promote increase in the number of lateral roots (CANELLAS et al., 2010). In studies with HA, Façanha et al. (2002) verified that HA isolated from vermicompost influenced the activation of plasma membrane H⁺-ATPase. These enzymes are capable of hydrolyzing ATP, and generate energy and electrochemical gradient that are closely involved with the increase of cellular wall plasticity by acidification of the apoplast, a process called acid growth theory (TAIZ and ZEIGER, 2017).

Many studies have revealed distinct responses from plants to HA application. For example, in maize plants treated with 50 mg C L⁻¹ of HA, a higher number of lateral roots was observed in comparison with the control plants (CANELLAS et al., 2008). In coffee seedlings the application of 40 mg C L⁻¹ of HA increased the induction and development of the root system (FAÇANHA et al., 2002). Similarly, in this study with mangosteen seedlings the HA exerted influence on the development of the root system, but the best performance in the accumulation of RDM occurred in higher concentrations (151.7 mg C L⁻¹) of HA applied. It is worth mentioning the importance of studies that make it possible to adjust a dose of HA that makes it possible to accelerate the development of slow growing plants, as is the case of mangosteen. Ayuso et al. (1996) reported a drop in yield of barley plants with the application of humic substances at the dose 100 mg C L⁻¹ and attributed this behavior to the formation of organometallic complexes of high molar mass that prevent the absorption of nutrients by plants

The addition of HA in the 200 mg C L⁻¹ mangosteen seedlings generated the highest DQI values of the seedlings

at 240 DAP (Figure 2F), with curvilinear increments of quadratic nature, where the adjusted optimal dose for this variable was 140.9 mg C L⁻¹ of HA (Table 3).

DQI has been used as a tool to evaluate the quality of seedlings in some fruit trees (CARLOS et al., 2014; OLIVEIRA et al., 2014; SOUZA et al., 2015; SODRÉ et al., 2016; PEREIRA et al., 2018). Generally, seedlings with higher DQI are more robust and with better distribution of biomass, which allows great capacity of development in the field, due to their high vigor (GOMES et al., 2002). The evaluation of the quality of the seedlings through the indices and growth variables can also serve as a parameter for the evaluation and identification of the maximum potential of seedling survival after field planting (SILVA et al., 2012).

In this study, the responses to the application of HA doses evidenced the “dose-dependent” effect of HA on the development of mangosteen seedlings. The results indicate the possibility of the use of HA extracted from vermicompost to accelerate the growth of mangosteen seedlings.

The significant Person correlation coefficients between growth variables and DQI ($p \leq 0.05$) are shown in Table 4. In the present study, RDM and DQI were highly correlated ($r = 0.99$; $p \leq 0.05$), being the variables with the highest correlation coefficients. This behavior is important because it allows estimating the survival and establishment of seedlings in the field (HERMANN, 1964).

The DQI is a tool with potential to evaluate the quality of seedlings of fruit species and Binotto et al. (2010) and Pereira et al. (2018) also observed a high correlation between RDM and DQI. However, in order to obtain this information about quality, there is a need to proceed with destructive methods of molting, where it is often unfeasible due to cost and/or time (BINOTTO, 2007). Thus, according to the data presented in this study, the high correlation coefficients for the morphological variables H ($r = 0.95$, $p \leq 0.05$) and SD ($r = 0.92$, $p \leq 0.05$) in mangosteen seedlings at 240 DAP suggest that these variables can be used as an alternative to DQI, and will be preferred by nurserymen because they do not need to destroy seedlings to measure quality before sending them to the field.

Table 1. Mean square of the analysis of variance of the regression of the quadratic model of the variables stem diameter (SD), plant height (H), number of leaves (NL) and leaf area (LA) of mangosteen seedlings in response to the application of humic acids extracted from vermicompost.

SV	DF	SD	H	NL	LA
Linear effect	1	15.25*	923.54*	237.23*	3467052.45*
Quadratic effect	1	5.56*	74.59*	32.16*	472730.49*
Regression deviations	2	0.62*	26.24*	0.49 ^{ns}	4841.91*
Residue	30	0.10	1.18	5.01	3756.40
VC (%)		4.1	2.7	8.35	5.2

* F Test ($p \leq 0.05$); Ns: Not significant ($p > 0.05$); SV: Source of variation; DF: Degree of freedom; VC: Variation coefficient.

Table 2. Mean square of the analysis of variance of the regression of the quadratic model of the variables stem dry matter (SDM), leaf dry matter (LDM), shoot dry matter (SDDM), root dry matter (RDM), total dry matter (TDM) and Dickson quality index (DQI) of mangosteen seedlings in response to the application of humic acids extracted from vermicompost.

SV	DF	SDM	LDM	SDDM	RDM	TDM	DQI
Linear effect	1	35.33*	276.62*	509.65*	36.07*	816.89*	8.52*
Quadratic effect	1	13.17*	29.74*	82.49*	4.29*	124.398*	1.68*
Regression deviations	2	0.15*	1.28 ^{ns}	1.88*	0.48*	4.24 ^{ns}	0.11*
Residue	30	1.19	2.93	5.73	0.09	5.27	0.01
VC (%)		15.25	12.22	11.30	8.21	9.24	4.60

* F Test ($p \leq 0.05$); Ns: Not significant ($p \leq 0.05$); SV: Source of variation; DF: Degree of freedom; VC: Variation coefficient.

Table 3. Adjusted quadratic models, determination coefficient (R²), optimal dose and Y_{max} for the variables: SD (mm), H (cm), NL, LA (cm²), SDM (g), LDM (g), SDDM (g), RDM (g), TDM (g) and DQI of mangosteen seedlings at 240 DAP in response to the application of humic acids extracted from vermicompost.

Variable	Equation	R ²	Dose	Y _{max}
SD	$\hat{Y} = 6.7274 + 0.04213069x - 0.00016062x^2$	0.94	131.15	9.49
H	$\hat{Y} = 34.9425 + 0.19041608x - 0.00058808x^2$	0.95	161.90	50.36
NL	$\hat{Y} = 24.0568 + 0.11480947x - 0.00038619x^2$	0.99	148.69	32.58
LA	$\hat{Y} = 835.5078 + 13.90616088x - 0.04681637x^2$	0.99	148.52	1868.17
SDM	$\hat{Y} = 5.8571 + 0.06466146x - 0.00024707x^2$	0.99	130.85	10.09
LDM	$\hat{Y} = 11.1306 + 0.11449046x - 0.00037135x^2$	0.99	154.16	19.95
SDDM	$\hat{Y} = 16.9876 + 0.17915192x - 0.00061842x^2$	0.99	144.85	29.96
RDM	$\hat{Y} = 2.5964 + 0.04277551x - 0.00014099x^2$	0.98	151.70	5.84
TDM	$\hat{Y} = 19.5841 + 0.22192744x - 0.00075942x^2$	0.99	146.11	35.80
DQI	$\hat{Y} = 1.6606 + 0.02488341x - 0.00008825x^2$	0.98	140.98	3.41

SD – Stem diameter; H - Plant height; NL - Number of leaves; LA – Leaf area; SDM – Stem dry matter; LDM - Leaf dry matter; SDDM - Shoot dry matter; RDM - Root dry matter; TDM - Total dry matter; DQI - Dickson quality index.

Table 4. Pearson correlation coefficients between the Dickson Quality Score (DQI); stem diameter (SD); plant height (H), shoot dry mass (SDDM); root dry matter (RDM) and total dry matter (TDM) in mangosteen seedlings at 240 DAP.

	Average	STD	SD	H	SDDM	RDM	TDM	DQI
SD	7.59	0.85	1	----	----	----	----	----
H	39.98	5.65	0.89*	1	----	----	----	----
SDDM	21.18	4.75	0.82*	0.88*	1	----	----	----
RDM	3.65	1.13	0.85*	0.94*	0.81*	1	----	----
TDM	24.83	5.71	0.85*	0.93*	0.99*	0.87*	1	----
DQI	2.22	0.56	0.92*	0.95*	0.88*	0.99*	0.94*	1

* T-test ($p \leq 0.05$); Ns: not significant STD: standard deviation

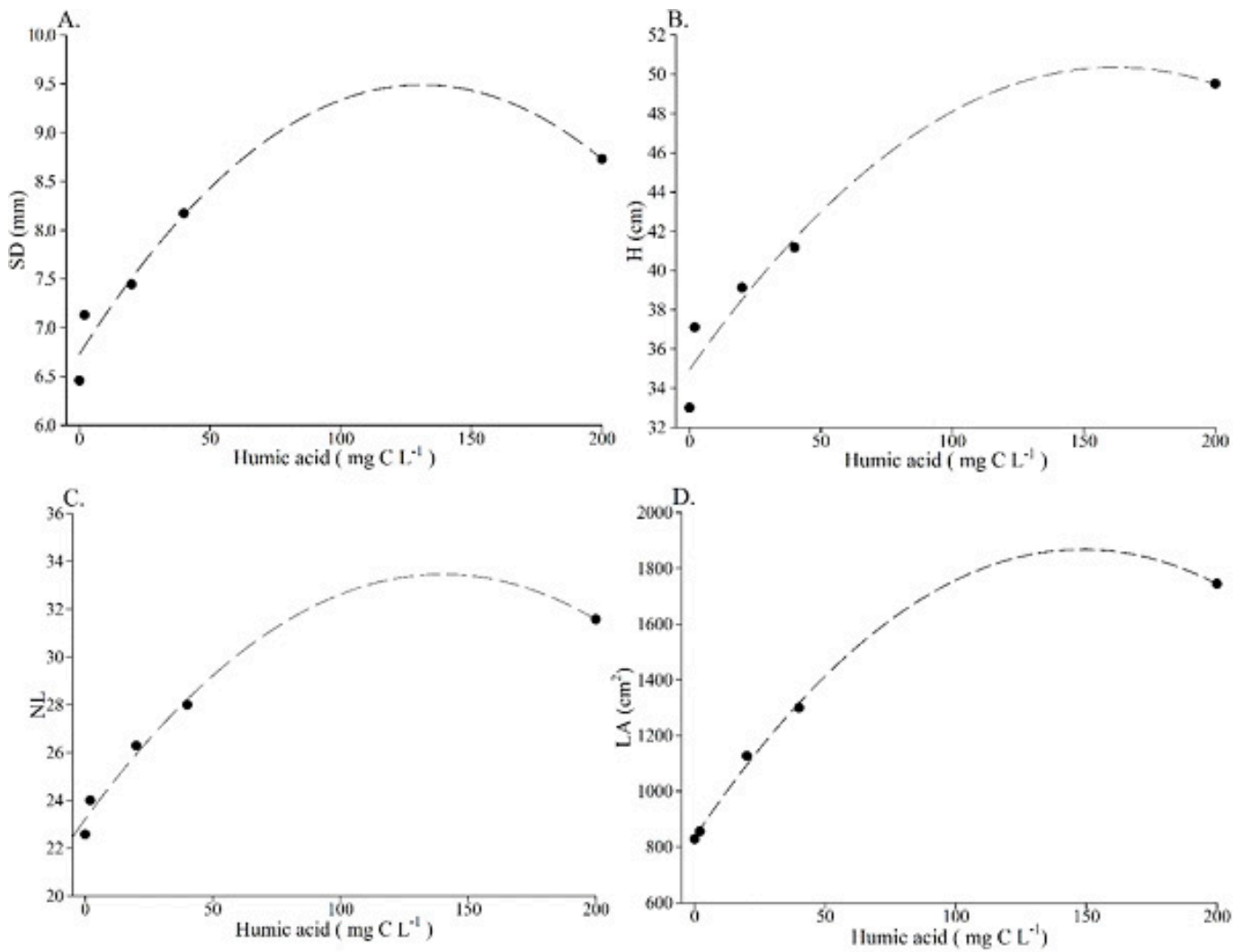


Figure 1. Quadratic regression curves for mangosteen seedlings submitted to humic acid doses (0; 2; 20; 40; 200 mg C L⁻¹). **A.** Stem diameter (SD), **B.** Plant height (H), **C.** Number of leaves (NL) **D.** Leaf area (LA).

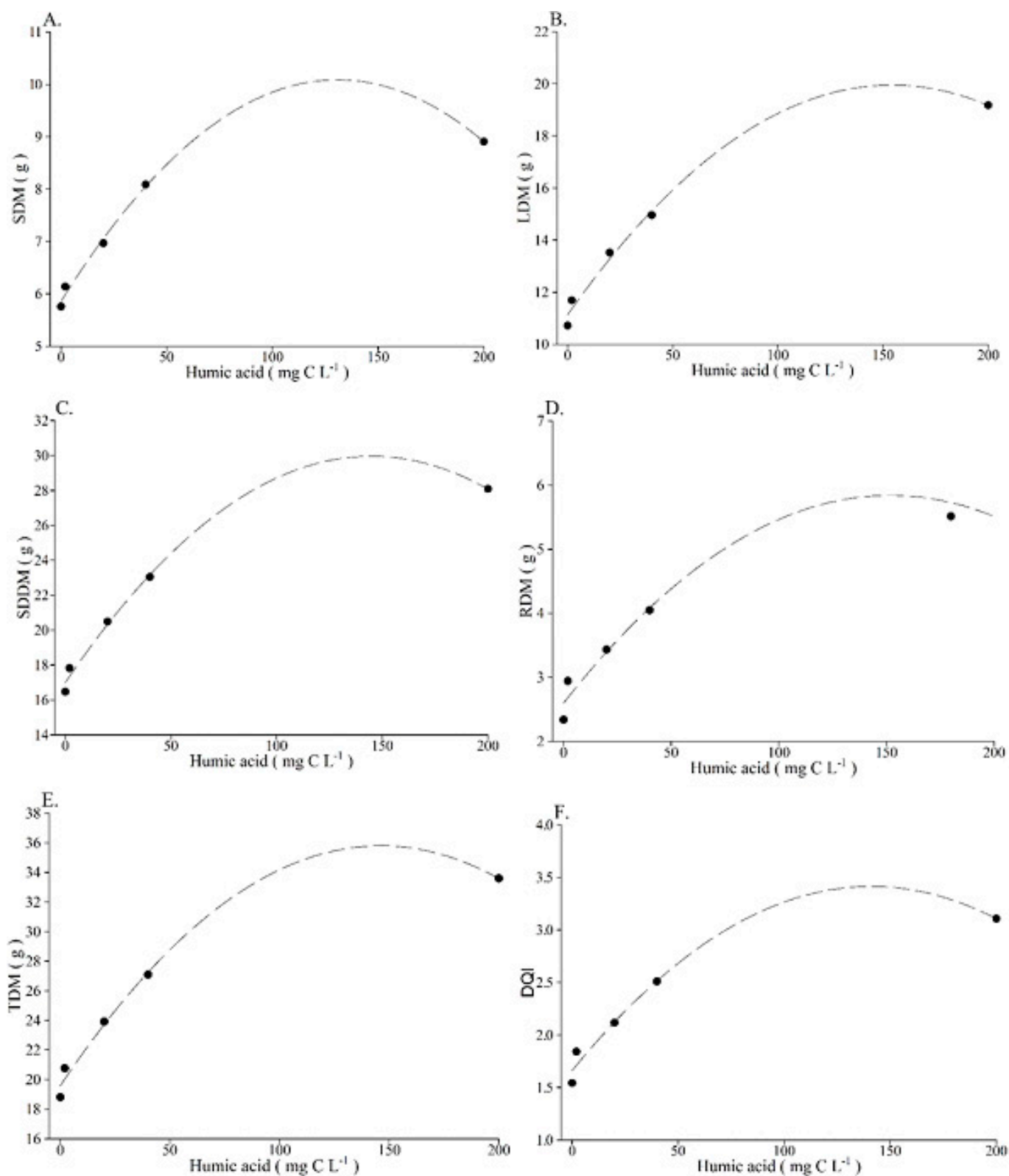


Figure 2. Quadratic regression curves for mangosteen seedlings submitted to humic acid doses (0; 2; 20; 40; 200 mg C L⁻¹). **A.** Stem dry matter (SDM), **B.** Leaf dry matter (LDM), **C.** Shoot dry matter (SDDM), **D.** Root dry matter (RDM), **E.** Total dry matter (TDM) **F.** Dickson quality index (DQI)

Conclusions

The HA had an effect as an efficient biostimulant for the growth and quality of mangosteen seedlings.

The dose 150 mg C L⁻¹ was the one that approached the maximum values and therefore should be recommended for the production of seedlings.

RDM was the variable best correlated with the DQI of the mangosteen seedlings.

The variables SD and H can serve as an alternative to DQI for evaluating the quality of mangosteen seedlings.

Acknowledgment

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

The authors are grateful to Fabio Lopes Olivares and Luciano Pasqualoto Canellas belonging to the Center for the Development of Biological Inputs for Agriculture (NUDIBA / UENF) for the material section and technical recommendations.

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