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CAMPUS RIO VERDE
DIRETORIA DE PESQUISA E PÓS - GRADUAÇÃO
PROGRAMA DE PÓS - GRADUAÇÃO EM CIÊNCIAS AGRÁRIAS – AGRONOMIA

SUBSTRATOS À BASE DE LODO SUÍNO E ADUBAÇÃO FOSFATADA NA PRODUÇÃO DE MUDAS DE *Guazuma ulmifolia* LAM. (MALVACEAE)

Autora: Patrícia Oliveira da Silva
Orientador: Prof. Dr. Leandro Carlos
Coorientador: Prof. Dr. José Milton Alves

Rio Verde - GO
Novembro – 2020

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BIOGRAFIA DA AUTORA

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LISTA DE SÍMBOLOS, SIGLAS, ABREVIAÇÕES E UNIDADES

Sigla/Símbolo	Significado	Unidade de medida
A	Taxa fotossintética	µmol CO_2 m-^{2} s -^{1}
AF	Área foliar	m^2
AFE	Área foliar específica	m^2 kg^{-1}
AcP	Acúmulo de fósforo	g $planta^{-1}$
Al^{3+}	Alumínio	$cmol_c$ dm^{-3}
Ca^{2+}	Cálcio	$cmol_c$ dm^{-3}
cac	Casca de arroz carbonizada	-
C*i*/C*a*	Razão entre concentração interna e externa de CO_2	-
cm	Centímetros	-
D	Diâmetro do coleto	mm
E	Taxa transpiratória	mmol (H_2O) m^{-2} s^{-1}
ETR	Taxa de transporte de elétrons	µmol m^{-2} s^{-1}
g	Gramas	-
gs	Condutância estomática	mol (H_2O) m^{-2} s^{-1}
H	Altura de planta	cm
HIV	Vírus da imunodeficiência humana	-
IQD	Índice de Qualidade de Dickson	-
K	Potássio	mg dm^{-3}
Kg	Quilograma	-
Ls	Lodo suíno	-
Le	Lodo de esgoto	-
m^2	Metros quadrados	-
MAP	Fosfato monoamônio	-

Mg^{2+}	Magnésio	$cmol_c\ dm^{-3}$
M.O	Matéria orgânica	dag kg^{-1}
MSC	Massa seca caulinar	g $planta^{-1}$
MSF	Massa seca foliar	g $planta^{-1}$
MSR	Massa seca radicular	g $planta^{-1}$
MST	Massa seca total	g $planta^{-1}$
NF	Número de folhas	-
P	Fósforo	mg dm^{-3}
pH	Potencial hidrogeniônico	-
RMC	Razão de massa caulinar	g g^{-1}
RMF	Razão de massa foliar	g g^{-1}
RMR	Razão de massa radicular	g g^{-1}
Ve	Vermiculita	-
V	Saturação de bases	%
%	Porcentagem	-

RESUMO

SILVA, PATRÍCIA OLIVEIRA DA. Instituto Federal Goiano – Campus Rio Verde – GO, novembro de 2020. **Substratos à base de lodo suíno e adubação fosfatada na produção de mudas de *Guazuma ulmifolia* Lam. (Malvaceae)**. Orientador: Prof. Dr. Leandro Carlos, Coorientador: Prof. Dr. José Milton Alves.

Guazuma ulmifolia Lam. (Malvaceae) apresenta elevado potencial para ser explorada pelo setor farmacêutico e alimentício, no entanto, pouco se sabe sobre as questões nutricionais em fase de muda. Com isso, este estudo teve o objetivo de avaliar se substratos à base de lodo suíno (Ls) e doses de fósforo (P) influenciam as características morfológicas e fisiológicas das mudas de *G. ulmifolia*. Para tanto, foram desenvolvidos dois experimentos. No experimento 1, as mudas foram cultivadas em tubetes de 50cm³, sob o delineamento de blocos ao acaso, em sete substratos (T1-50% de casca de arroz carbonizada (cac) + 50 % de vermiculita (ve); T2 - 20% de ls + 40% de cac e 40% de ve; T3 - 40% de ls e 30% de cac + 30% de ve; T4 - 60% de ls + 20% de cac + 20% de ve; T5 - 80% de ls + 10% de cac + 10% de ve; T6 - 100% de ls e T7 - 100% Bioplant®). Aos 200 dias após a semeadura, avaliou-se os índices Falker® de clorofilas e trocas gasosas, já a altura (H), diâmetro (D), número de folhas (NF), razão entre H e D, biomassa, razões alométricas, índice de qualidade de Dickson (IQD) e taxa de mortalidade, foram avaliados aos 210 dias após a semeadura. Os resultados desse experimento mostram que para a H, NF, trocas gasosas e biomassa da parte aérea o T4 e T5 promoveram os melhores resultados. Os maiores diâmetros foram proporcionados pelos T3, T4 e T5, enquanto os maiores índices de clorofilas e IQD pelos T4, T5 e T6. A maior taxa de mortalidade foi obtida no T7 (85%) seguida do T1 (37,5%). No experimento 2, as mudas foram cultivadas em vasos de 4 litros contendo LATOSSOLO VERMELHO Distrófico e submetidas a cinco doses de P (0, 100, 200, 300 e 400 mg dm^{-3}), sob delineamento inteiramente ao acaso. Após 115 dias de semeadura, avaliou-se as trocas gasosas e índice Falker® de clorofilas, já a H, D, NF, biomassa, razões alométricas, IQD, teor de P na parte aérea das mudas, foram avaliados aos 120 dias após a semeadura. Todas as variáveis estudadas foram influenciadas positivamente pelas doses de P, exceto a área foliar específica e as razões alométricas. Para todas as variáveis, a dose que promoveu os maiores valores foi a maior dose estudada (400 mg dm^{-3}), indicando que mudas de *G. ulmifolia* requerem altos níveis de P para terem seu comportamento fisiológico e morfológico potencializado. O teor de P se correlacionou fortemente e positivamente com todas as variáveis testadas. As variáveis de trocas gasosas se correlacionaram com as de crescimento, biomassa e IQD. Diante dos resultados encontrados para ambos os experimentos é possível afirmar que tanto substratos com lodo suíno, quanto doses fosfatadas são potencializadores do crescimento e desenvolvimento inicial de *G. ulmifolia*.

PALAVRAS-CHAVE: mutambo, fase inicial, medições fisiológicas e morfológicas.

ABSTRACT

SILVA, PATRÍCIA OLIVEIRA DA. Federal Institute Goiano – Campus Rio Verde – GO, november 2020. **Substrates based on swine wastewater and phosphate fertilizer in the production of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae).** Advisor: Prof. Dr. Leandro Carlos. Co-Advisor: Prof. Dr. José Milton Alves.

Guazuma ulmifolia Lam. (Malvaceae) has a high potential to be explored by the pharmaceutical and food sectors, however, little is known about the nutritional issues in the seedling phase. Thus, this study aimed to assess whether substrates based on swine wastewater (sw) and doses of phosphorus (P) influence the morphological and physiological characteristics of *G. ulmifolia* seedlings. For this, two experiments were carried out. In experiment 1, the seedlings were grown in 50 cm³ tubes, under a randomized block design, on seven substrates (T1-50% carbonized rice husk (crh) + 50% vermiculite (ve); T2 - 20% sw + 40% crh and 40% ve; T3 - 40% sw and 30% crh + 30% ve; T4 - 60% sw + 20% crh + 20% ve; T5 - 80 % sw + 10% crh + 10% ve; T6 - 100% sw and T7 - 100% Bioplant®). At 200 days after sowing, the Falker® indexes of chlorophylls and gas exchanges were evaluated, whereas height (H), diameter (D), number of leaves (NL), ratio between H and D, biomass, allometric ratios, Dickson's quality index (DQI) and mortality rate were taken 210 days after sowing. The results of this experiment show that for H, NL, gas exchange and shoot biomass, T4 and T5 promoted the best results. The largest diameters were provided by T3, T4 and T5, while the highest levels of chlorophyll and DQI by T4, T5 and T6. The highest mortality rate was obtained at T7 (85%) followed by T1 (37.5%). In experiment 2, the seedlings were grown in 4 liter pots containing Dystrophyc RED LATOSOL and subjected to five doses of P (0, 100, 200, 300 and 400 mg dm^{-3}), under a completely randomized design. After 115 days of sowing, gas exchange and Falker® index of chlorophylls were evaluated, whereas H, D, NL, biomass, allometric ratios, DQI, P content in the aerial part of the seedlings were evaluated at 120 days after sowing. All studied variables were positively influenced by P doses, except for the specific leaf area and allometric ratios. For all variables, the dose that promoted the highest values was the highest dose studied (400 mg dm^{-3}), indicating that *G. ulmifolia* seedlings require high P levels to have their physiological and morphological behavior enhanced. The P content was strongly and positively correlated with all tested variables. The gas exchange variables correlated with growth, biomass and DQI variables. In view of the results found for both experiments, it is possible to state that both substrates with swine sludge and phosphate doses are potentiators for growth and initial development of *G. ulmifolia*.

KEYWORDS: *mutamba*, initial phase, physiological and morphological measurements.

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1 INTRODUÇÃO GERAL

1.1 Importância da produção de mudas: uso de substratos e adubações

Diante das ações humanas como desmatamento, queimadas, liberação de gases do efeito estufa, queda dos níveis hídricos e temperaturas extremas, uma prática que está sendo impulsionada é a produção de mudas de espécies vegetais capazes de ajudar a reestabelecer o equilíbrio ambiental. Além disso, muitas destas espécies devem, de preferência, apresentar propriedades alimentícias, medicinais, madeireiras, ecológicas e também ornamentais (Silva et al. 2020). E, para que as mudas de tais espécies apresentem certa qualidade, muitos estudos vêm testando diferentes meios de produção. Em viveiros, por exemplo, tem sido comum a utilização de componentes orgânicos com o objetivo de melhorar os atributos químicos e físicos dos substratos (Delarmelina et al. 2014). Além do uso de substratos, vários outros estudos também têm testado doses de adubos minerais buscando encontrar a dose ideal e que proporcione o maior crescimento e desenvolvimento das mudas de diversas espécies.

Esses estudos são necessários, uma vez que a qualidade das mudas está entre os aspectos mais importantes para assegurar o sucesso das ações de revegetação, resgate de germoplasma e estabelecimento de florestas, quer sejam para fins comerciais ou conservacionistas (Silva Cruz et al. 2016). Além disso, a boa formação das mudas é essencial, porque contribui com o sucesso do plantio definitivo. O teor de nutrientes, principalmente, nitrogênio, fósforo e potássio, oriundos do solo ou substratos utilizados tem grande influência na qualidade das mudas produzidas e no desenvolvimento delas em campo (Tucci et al. 2009). No entanto, cada espécie apresenta um comportamento diferente sobre o mesmo substrato ou adubação mineral, as exigências nutricionais variam e isso impede que haja generalização, tanto no uso de substratos, quanto da adubação mineral.

Para a composição de substratos, são inúmeras as possibilidades de combinações. Uma vez que eles podem ser de origem orgânica, mineral (vermiculita, perlita e areia) ou sintética (lã de rocha, espuma fenólica e isopor), sendo que os orgânicos ainda podem ser originados de vegetais (casca de arroz, *pinus*, fibra de coco, turfa, torta de filtro) ou animais (esterco bovino ou de aves, lodo suíno, cama frango) (Ferraz et al. 2005). Entre os substratos, os à base de lodo suíno chama a atenção, pois nesse caso, podem ser uma alternativa viável, que além de dar uma destinação ao resíduo que seria descartado, e muitas vezes de forma inadequada, podem promover o crescimento e desenvolvimento adequado das mudas. Adicionalmente, apresenta elevada disponibilidade dos nutrientes requeridos para o crescimento adequado das plantas, como nitrogênio, fósforo, potássio entre outros (Miyazawa e Barbosa 2015). Dessa forma, se utilizado da maneira correta, o lodo suíno pode se tornar uma alternativa com viabilidade econômica e também ecológica.

Em relação à adubação mineral, a fosfatada é de extrema importância, pois o fósforo (P) faz parte dos processos metabólicos das plantas, como fotossíntese e respiração celular. Além disso, é constituinte de ácidos nucleicos, fosfolipídios, proteínas, ésteres de fosfato e moléculas de energia, como adenosina trifosfato (Dechen e Nachtigall 2007). O P também fornece energia para acionar vários processos celulares, por isso é primordial para o crescimento e desenvolvimento vegetal (Malhotra et al. 2018) e tem promovido resultados satisfatórios na produção de mudas de algumas espécies (Freitas et al. 2017, Carlos et al. 2018, Constantino et al. 2019). No entanto, assim como para os substratos, as plantas também apresentam exigências nutricionais diferentes quando se trata de adubação mineral, dessa forma, também se faz necessário testar doses para verificar qual delas promove o maior desempenho em cada espécie enquanto muda, de modo a evitar não só a toxidez, mas também desperdícios de insumos.

Mesmo sabendo da importância dos substratos e da adubação fosfatada na produção de mudas para muitas espécies, estudos dessa natureza são escassos. Para várias espécies florestais importantes, informações dessa natureza são extremamente necessárias, esse é o caso de *Guazuma ulmifolia* Lam.

1.2 Aspectos gerais de *Guazuma ulmifolia*

Guazuma ulmifolia é popularmente conhecida como mutamba (Brasil) ou guácimo (México) e representante da família botânica Malvaceae. A espécie ocorre do

México até a América do Sul, sendo que no Brasil ocorre em todos os domínios fitogeográficos (Colli-Silva 2019). De forma mais específica, pode ser encontrada em Floresta Estacional Decídua e Semidecídua, Floresta Ombrófila Densa de Várzea, Savana Florestada ou *lato sensu*, Caatinga, Ambiente fluvial, Pantanal Mato-Grossense, Brejo de altitude, Ecótono entre Savana e Restinga, e entre vários outros ambientes (Carvalho et al. 2007).

As sementes de *G. ulmifolia* possuem acentuada impermeabilidade à água, pela dormência do tipo tegumentar (exógena). Há vários métodos para a superação da dormência de suas sementes, porém, a imersão das mesmas em água quente por determinado tempo tem se mostrado ser o método mais eficiente (Nunes et al. 2006; Sobrinho et al. 2012; Silva et al. 2016). Uma vez superada a dormência, a germinação se inicia entre seis e 14 dias após a semeadura (Carvalho 2007). Em termos ecológicos, a espécie é considerada pioneira (Herrera-Peraza et al. 2016), heliófila (Carvalho 2007) com crescimento rápido (Campos-Filho e Sartorelli 2015; Silva et al. 2016). A produção de frutos inicia quando as plantas de *G. ulmifolia* atingem os cinco anos de idade (Carvalho 2007) e quando maduros são consumidos, principalmente, por aves e primatas, e torna a planta importante para programas de restauração ecológica (Almeida et al. 1998, Carvalho 2007, Calzavara et al. 2017). Além disso, as suas flores são melíferas, portanto, são de importância apícola (Pereira et al. 2019).

A espécie tem potencial para ser amplamente explorada pelo setor industrial, seja como alimento ou planta terapêutica (Pereira et al. 2019), uma vez que as folhas são usadas como fonte de proteína para ração animal em períodos de estiagem (Carvalho 2007; Campos-Filho e Sartorelli 2015; Mendoza et al. 2015) e os frutos apresentam alto teor de fibra alimentar e compostos fenólicos bioativos (Pereira et al. 2020). E, além de serem comestíveis *in natura*, podem ser usados para preparação de chá, licores e vinhos, extração de óleo para cosméticos, produção de farinha, sorvete e picolé. Os frutos também fornecem mucilagem que pode ser utilizada na confecção de bebidas e molhos (Carvalho 2007; Viana et al. 2011).

Dentre as características de *G. ulmifolia*, as que mais chamam atenção são as medicinais, entre os seus compostos ativos estão os flavonoides, saponinas, alcaloides, taninos, compostos fenólicos e esteroides, em diferentes partes da planta (Martins et al. 2019). De modo geral, o extrato aquoso obtido por decocção de casca de caule e folhas da mutamba mostraram ser antimicrobianos eficientes, antioxidantes e cardioprotetores (dos Santos et al. 2018). A planta ainda pode ser utilizada no combate aos parasitas

Leishmania brasiliensis, *L. infantum* e *Trypanossoma cruzi* (Calixto Júnior et al. 2016). E recentemente, tem sido indicada como tratamento alternativo contra o vírus do HIV no Brasil (Gouveia 2018) e Venezuela (Portadores... 2018; Singer 2018; Macedo 2019).

Apesar da importância da espécie, o comportamento silvicultural das mudas de *G. ulmifolia* é minimamente conhecido. O pouco conhecimento encontrado na literatura é proveniente dos trabalhos de Moraes Neto et al. (2003), Cruz et al. (2016) e Rocha et al. (2019). Moraes Neto et al. (2003) ao testarem doses e fontes de adubos na produção de mudas cultivadas em substrato composto por 60% de húmus de minhoca, 30% de casca de arroz carbonizada e 10% de terra de subsolos constataram que a adubação de base mais adubação de cobertura com sulfato de amônio e cloreto de potássio é o mais indicado. Cruz et al. (2016) ao estudarem diferentes substratos na produção de mudas de *G. ulmifolia* concluíram que entre os substratos testados, o tratamento composto por 80% de terra e 20% de esterno bovino foi o que promoveu o melhor crescimento das mudas. Já Rocha et al. (2019) afirmaram que o fornecimento elevado de nitrogênio, tendo como fonte o nitrato de amônio, nas mudas de *G. ulmifolia* cultivadas em substrato orgânico (de origem vegetal) evita a fotoinibição dinâmica em mudas e melhora sua qualidade morfofisiológica durante a aclimatação sob alta luz em viveiro. No entanto, essas informações não são bastantes para os produtores de mudas. Principalmente, para aqueles que dependem da espécie para uso de medicinal ou alimentação de animais.

Diante do exposto, são necessários novos estudos de natureza silviculturais, para que as mudas produzidas tenham qualidade suficientes para qualquer que seja sua destinação. E, ao constatar em outros estudos que tanto substratos quanto adubação fosfatada promoveram resultados positivos, este estudo trabalhou com a hipótese que as mudas de *G. ulmifolia* quando produzidas em substrato à base de lodo suíno, e doses de P apresentam melhor desempenho fisiológico e morfológico.

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2 OBJETIVOS

2.1 Geral

Avaliar se diferentes substratos à base de lodo suíno e doses crescentes de fósforo, de forma independente, promovem o crescimento de mudas de *Guazuma ulmifolia*, mediante alterações das características fisiológicas e morfológicas.

2.2 Específicos

-Testar se os substratos à base de lodo suíno podem ser utilizados na produção de mudas de *G. ulmifolia*; se as alterações promovidas pelos diferentes substratos são positivas, e em qual substrato as mudas apresentam melhor desempenho fisiológico e morfológico.

-Avaliar se o crescimento inicial de *G. ulmifolia* é influenciado por doses de fósforo; e se doses de fósforo promovem melhor desempenho fisiológico, morfológico e por fim, maior qualidade nas mudas em LATOSSOLO VERMELHO Distrófico.

3 CAPÍTULO I

Substrates based on swine wastewater in the production of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae)

(Normas de acordo com a revista Brazilian Journal of Botany)

Abstract

The use of swine wastewater (sw) as a substrate for seedling production has promoted satisfactory results. In this perspective, this study tested the hypothesis that seedlings of *Guazuma ulmifolia* Lam. grown in a substrate based on sw showed better physiological and morphological performance. The seedlings were cultivated in seven substrates: T1 – rice husk and vermiculite; T2 - 20% sw; T3 - 40% sw; T4 - 60% sw; T5 - 80% sw; T6 - 100% sw; and T7 - 100% Bioplant®. At 210 days after planting, the height (H), diameter (D), number of leaves (NF), as well as the H to D ratio, chlorophyll indices, gas exchange, biomass, allometric ratios, Dickson Quality Index (DQI), and mortality rate of the seedlings were evaluated. The data were analyzed by analysis of variance, and the means were compared by Tukey's test. For the H, NF, gas exchange, and shoot biomass, treatments T4 and T5 promoted the best results. Treatments T3, T4, and T5 provided the largest diameters, while T4, T5, and T6 provided the highest chlorophyll indices and DQI. The highest mortality rate was obtained with T7 (85%), followed by T1 (37.5%). The seedlings of *G. ulmifolia* showed better physiological and morphological performance in substrates with Ls than with T1 and T7, and treatments T4 and T5 are the most indicated for the production of seedlings of this species.

Keywords: Alternative substrates · Swine wastewater · Early growth · *Mutambo*

3.1 Introduction

Projects aimed at the recovery of degraded areas, commercial reforestation, conservation, or exploitation of forest species depend on the availability of seedlings with a certain quality level (Dionísio et al., 2017; Marques et al., 2018). The use of substrates that meet the nutritional demands of plants is among the factors that can provide higher quality (Martins et al., 2012; Siqueira et al., 2018). There are several possibilities of combinations and proportions for substrate formulation. However, residues deserve special attention, especially those of agro-industrial origin, such as swine wastewater, since large volumes of this product are generated and represent an environmental problem when its destination is not appropriate. Therefore, the use of this residue as a substrate, in addition to saving costs with seedling production, also ensures its appropriate destination, preventing the occurrence of environmental problems due to its accumulation (Caldeira et al., 2014; Nogueira et al., 2014; Krause et al., 2017).

If properly used, swine wastewater can boost seedling growth and development in several species as this residue is rich in nutrients required by plants (Miyazawa and Barbosa, 2015). Some studies have already verified that the use of swine farming residues promoted positive results in seedlings of forest species (Vieira et al., 2014; Rocha et al., 2017; Araújo et al., 2019; Santinato et al., 2019). On the other hand, swine wastewater can be harmful to some species, causing growth reduction and even the death of seedlings grown under high doses (Pereira et al., 2017). Therefore, knowledge about the use of this residue is extremely important. Since there is no information in this regard for several species, further studies are necessary to evaluate seedling performance and determine the ideal dose of swine wastewater that is not harmful to plant growth.

Mutambo (*Guazuma ulmifolia* Lam., Malvaceae) is among the species that demand this type of study, being highly recommended in reforestation programs (Calzavara et al., 2017) due to its rapid growth and for attracting the fauna. It also has medicinal importance, with the aqueous extract from its bark and leaves showing efficient antimicrobial, antioxidant, and cardioprotective properties (Dos Santos et al., 2018). The species has been used as an alternative treatment to HIV in Brazil and Venezuela (Gouveia, 2018; Portadores...2018; Singer, 2018; Macedo, 2019).

It is known that *mutambo* has the potential for wide exploitation by the industrial sector, either as food or as a therapeutic plant (Pereira et al., 2019). However, studies related to seedling production in substrates based on agro-industrial residues are still scarce. Considering that substrates based on swine wastewater promoted positive results in other species, the hypothesis was raised that *mutambo* seedlings grown in a substrate containing swine wastewater show better physiological and morphological performance. In order to test this hypothesis, this study aimed to evaluate the physiological and morphological characteristics of *mutambo* seedlings cultivated in different substrates, part of them containing swine wastewater in their composition.

3.2 Material and Methods

Cultivation conditions and experimental design - The present study was developed in the plant nursery of the Laboratory of Plant Tissue Culture, belonging to the Goiano Federal Institute - Campus Rio Verde, Goiás, Brazil, from March to October 2019. The climate of the region where the experiment was conducted is type Aw (Tropical), that is, markedly seasonal with a hot and rainy season (from October to March) and another cold and dry season (from April to September), with annual rainfall from 1,600 to 1,900 millimeters and mean annual temperature from 19 to 20°C (Alvares et al., 2018).

The seeds were collected from 10 parent plants located at the Federal University of Lavras, Minas Gerais, Brazil. Seed dormancy was overcome with hot water at 70°C for approximately 30 minutes or until the water temperature was reduced to 50°C (Nunes et al., 2006). The seeds were planted seven days after the formulation and addition of the substrates to the tubes, sowing three seeds per tube. After germination, thinning was performed by allowing only one plant per tube. The seedlings were cultivated in 50 cm^3 polypropylene tubes disinfected with 1% sodium hypochlorite before installing the experiment. Expanded polystyrene trays upheld by one-meter high benches were used to ensure a consistent water supply to the seedlings.

The experimental design was in randomized blocks, composed of seven treatments/substrates ($v\ v^{-1}$): T1 - 50% carbonized rice husk (crh) + 50 % vermiculite (ve); T2 - 20% sw + 40% crh and 40% ve; T3 - 40% sw and 30% crh + 30% ve; T4 - 60% sw + 20% crh + 20% ve; T5 - 80% sw + 10% crh + 10% ve; T6 - 100% sw; and T7 - 100% commercial substrate - Bioplant® (composed of *Pinus* bark, manure, sawdust, coconut fiber, vermiculite, rice husk, ash, agricultural gypsum, calcium carbonate, magnesium, magnesium thermophosphate, and fertilizer additives) and four replications, each composed of ten plants, with a total of 40 plants per treatment and 280 experimental units.

The previously dried sludge used in this study was collected during the finishing phase in a swine farm in the municipality of Rio Verde, Goiás. Five days after the collection, the sludge was sanitized by solarization with a transparent plastic sheet for 20 days. When each treatment was composed, samples were removed for the chemical characterization of each substrate, and the results are represented in Table 1.

Table 1 Chemical characterization of the substrates used for the production of *mutambo* seedlings (*Guazuma ulmifolia* Lam., Malvaceae). T1 - carbonized rice husk (crh) and vermiculite (ve); T2 - 20% of swine wastewater (sw); T3 - 40% sw; T4 - 60% sw; T5 - 80% sw; T6 - 100% sw; and T7 –Bioplant ® commercial substrate. Different letters differ significantly by Tukey's test at 5% probability.

Substrates	Al	Ca^{2+}	K	Mg^{2+}	P	pH	V
	$cmol_c\ dm^{-3}$		$mg\ dm^{-3}$	$cmol_c\ dm^{-3}$	$mg\ dm^{-3}$		%
T1	0.100b	0.50f	445.2b	4.3f	181.1g	7.7a	87.0a
T2	0.098b	0.04g	430.0c	5.4e	373.2f	7.6a	86.7b
T3	0.096b	1.58e	414.9d	6.7d	565.0e	7.5b	86.7b
T4	0.094c	2.12d	399.7e	7.8c	757.0d	7.4b	86.6c
T5	0.083d	2.66c	384.6d	8.9b	904.0c	7.3c	86.6c
T6	0.080d	3.20b	369.5g	10.2a	1.141a	7.2c	86.6c
T7	0.931a	6.90a	602.0a	3.6g	938.6b	5.2d	61.7d

Al^{3+}: aluminum, Ca^{2+}: calcium, K: potassium, M.O: organic matter, Mg^{2+}: magnesium, P: phosphorus, pH: potential of hydrogen, and V: base saturation.

The *mutambo* seedlings grew under full sunlight, with daily irrigation provided by an automatic micro-sprinkler system (4.3 liters/m²) four times a day, at 7:00 a.m., 11:30 a.m., 3:00 p.m., and 6:00 p.m.

Biometric measurements – At 210 days after sowing, plant height (H) and base diameter (D) were measured in all sampling units using a millimeter ruler and a digital caliper, respectively. The number of expanded leaves was also counted (NF). The height to diameter ratio was calculated after obtaining these individual measurements.

Photosynthetic pigment indices – At 200 days after sowing, the Falker® indices of chlorophyll a (Chl *a*) and b (Chl *b*) were determined in three sampling units from each replication per treatment. A ClorofiLOG chlorophyll meter was used for these analyses (Model CFL1030, Falker *Automação Agrícola* LLC, Porto Alegre, Brazil).

Subsequently, the chlorophyll *a* to *b* ratio (Chl *a*/ Chl *b*) and the total chlorophyll index (Chl *a* + Chl *b*) were calculated.

Physiological measurements - At 200 days after sowing, the gas exchange analyses were determined on a leaf of the second pair from the apex of the plant, in three sampling units per treatment. These analyses were performed using the same leaf in which the chlorophyll indices were measured. The variables evaluated were: photosynthetic rate [*A*, µmol (CO_2) m^{-2} s^{-1})], transpiration rate [*E*, mmol (H_2O) m^{-2} s^{-1}], stomatal conductance [g_s, mol (H_2O) m^{-2} s^{-1}], internal CO_2 concentration (Ci), external CO_2 concentration (Ca), ratio between the internal and external CO_2 concentration (Ci/Ca), ratio between photosynthesis and internal CO_2 concentration (*A*/Ci), and electron transport rate (ETR, µmol m^{-2} s^{-1}), measured with a portable infrared gas analyzer (model Li-6800xt, Li-Cor, Nebraska, USA) with constant irradiance (1,500 µmol m^{-2} s^{-1}) between 8:00 a.m. and 11 a.m.

Biomass determinations and allometric relationships – At 210 days after sowing, ten plants per treatment were removed from the tubes and washed. Subsequently, these plants were cut into leaves, stems, and roots and dried in a forced-air oven at 65ºC until constant weight. Finally, the leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM) were obtained with a digital balance. With these data, it was possible to calculate the total dry mass (TDM), which, in turn, was used to calculate the leaf mass ratio (LMR), the stem mass ratio (SMR), and the root mass ratio (RMR).

Dickson Quality Index (DQI) and Mortality Rate – Based on the growth and biomass data, it was possible to calculate the Dickson Quality Index (Dickson et al., 1960) of ten plants per treatment. At 210 days after sowing, the mortality rate was calculated according to the formula: number of dead plants divided by the total of plants in the treatment, multiplied by 100.

Statistical analysis – The data were subjected to analysis of variance (ANOVA) with the software SISVAR (Ferreira, 2014), and the means were compared by Tukey's test at 5% probability.

3.3 Results

Treatments 1, composed of 50% carbonized rice husk and 50% vermiculite, and 7, composed of 100% Bioplant®, resulted in *mutambo* seedlings with insufficient leaf area to perform the Falker® chlorophyll indices and gas exchange analyses; thus, Figures 1 and 2, respectively, do not depict the results of these analyses for such treatments.

Statistically, the treatments containing swine wastewater promoted a significant effect on the chlorophyll indices. Treatments 4, 5, and 6 promoted the highest indices of chlorophyll *a* and total in the *mutambo* seedlings, being statistically similar to each other (Fig. 1a and c). However, treatments 5 and 6 did not differ from treatments 2 and 3. The highest indices of chlorophyll *b* were recorded in treatments 3, 4, 5, and 6, which were statistically similar, but superior to treatment 2 (Fig. 1b). The chlorophyll *a* to *b* ratio did not differ across treatments (Fig. 1d).

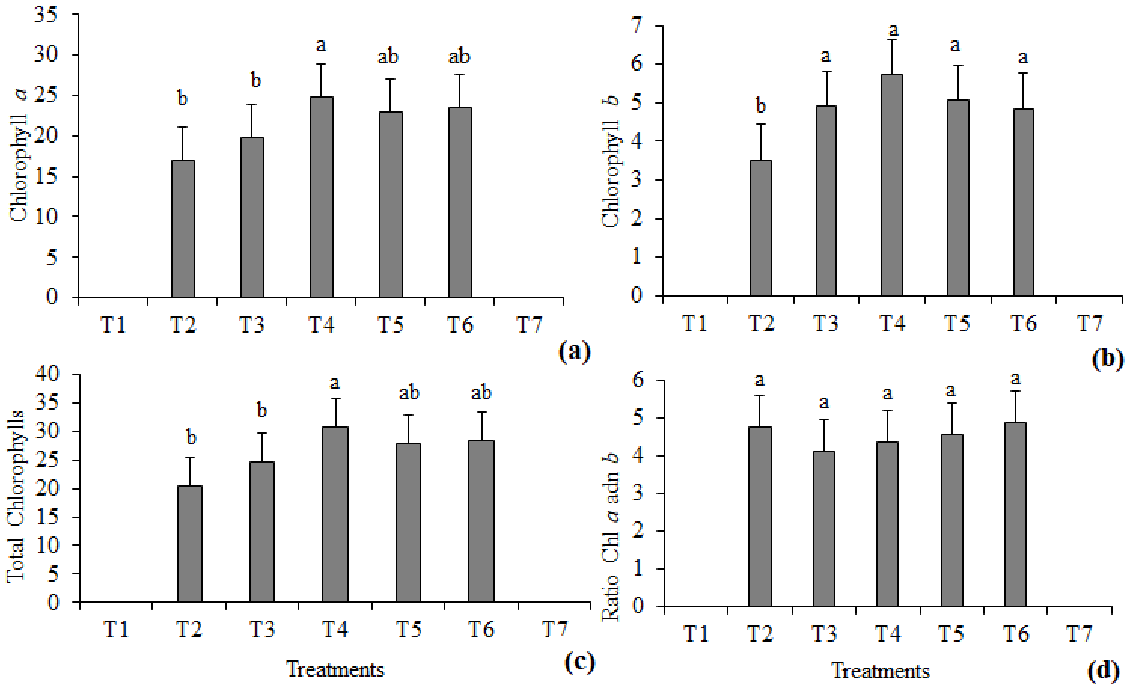

Fig. 1 Chlorophyll indices of *mutambo* seedlings (*Guazuma ulmifolia* Lam., *Malvaceae*) grown in different substrates at 200 days after sowing. Chlorophyll *a* index **(a)**, Chlorophyll *b* index **(b)**, Total chlorophylls **(c),** and Chlorophyll *a* to *b* ratio **(d)**. Mean values of standard deviation (±). Means with different letters differ significantly by Tukey's test at 5% probability.

There was a significant effect as a function of the treatments on the photosynthetic rate (*A*), stomatal conductance (*gs*), transpiration rate (*E*), ratio between photosynthesis and internal CO_2 concentration (*A/Ci*), and electron transport rate (ETR) of the *mutambo* seedlings. For all these variables, the results promoted by treatments 4 and 5 were superior to the remainder (Fig. 2a, b, c, d, e, f) but did not differ from each other. For *A*, *gs*, and *E*, treatments 2 and 3 did not differ from each other, only from treatment 6 (Fig. 2a, b, and c). Treatments 2, 3, and 6 did not differ from each other for the *A/Ci* and ETR variables (Fig. 2d and e).

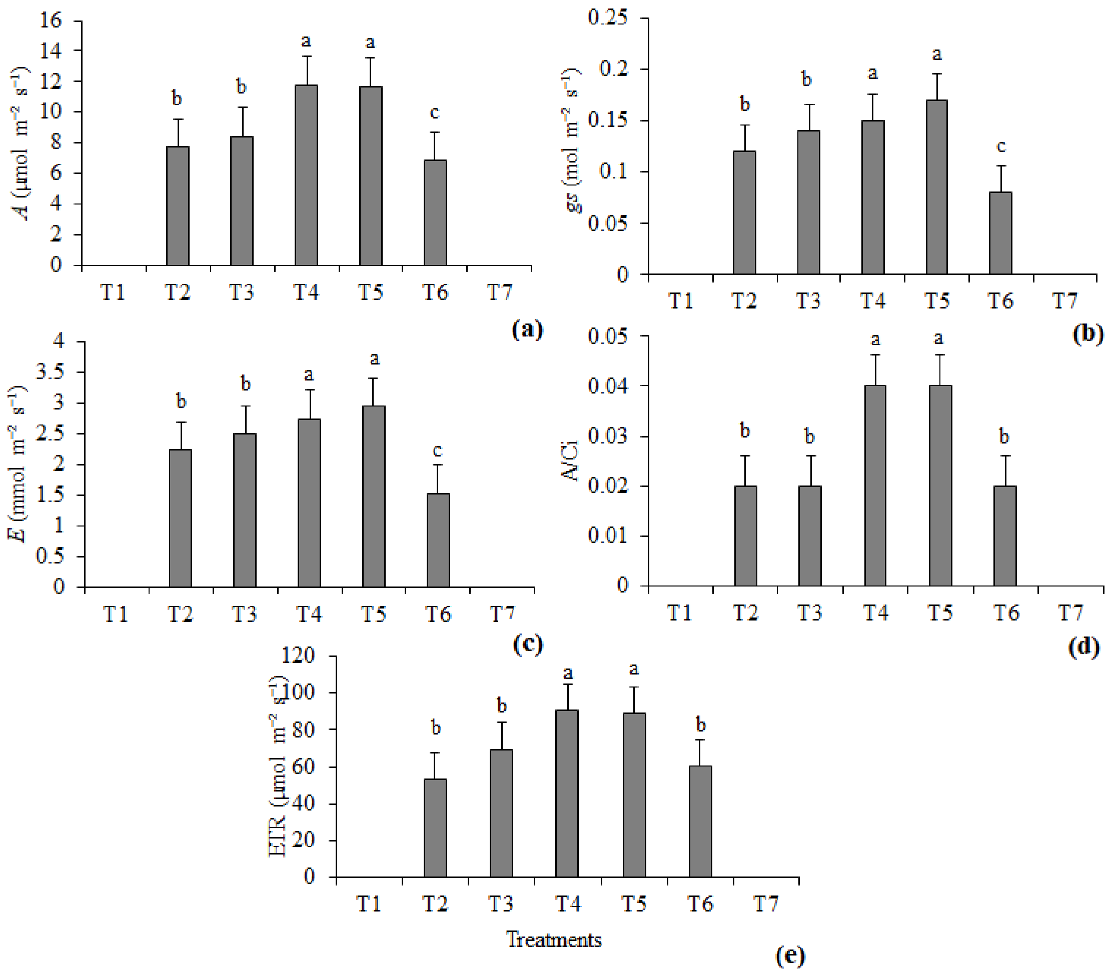

Fig. 2 Physiological variables of *mutambo* seedlings (*Guazuma ulmifolia* Lam., Malvaceae) grown in different substrates at 200 days after sowing. Photosynthetic rate **(a)**, Stomatal conductance **(b)**, Transpiration rate **(c)**, Ratio between photosynthesis and internal CO_2 concentration **(d)**, and Electron transport rate **(e)**. Mean values of standard deviation (±). Means with different letters differ significantly by Tukey's test at 5% probability.

For the biometric variables of height (H), base diameter (D), number of leaves (NF), and height to diameter ratio of *mutambo* seedlings, there was a significant effect across treatments ($p \leq 0.05$). The substrates that provided the highest H and NF values were substrates 4 and 5, which were statistically similar for both variables (Fig. 3a and c). Treatments 3, 4, and 5 provided the highest D values, with no statistical difference between the results (Fig. 3 b). The treatments were statistically similar for the height to diameter ratio, except for treatments 1 and 7 (Fig. 3 d), whose substrates promoted the lowest values for all biometric variables.

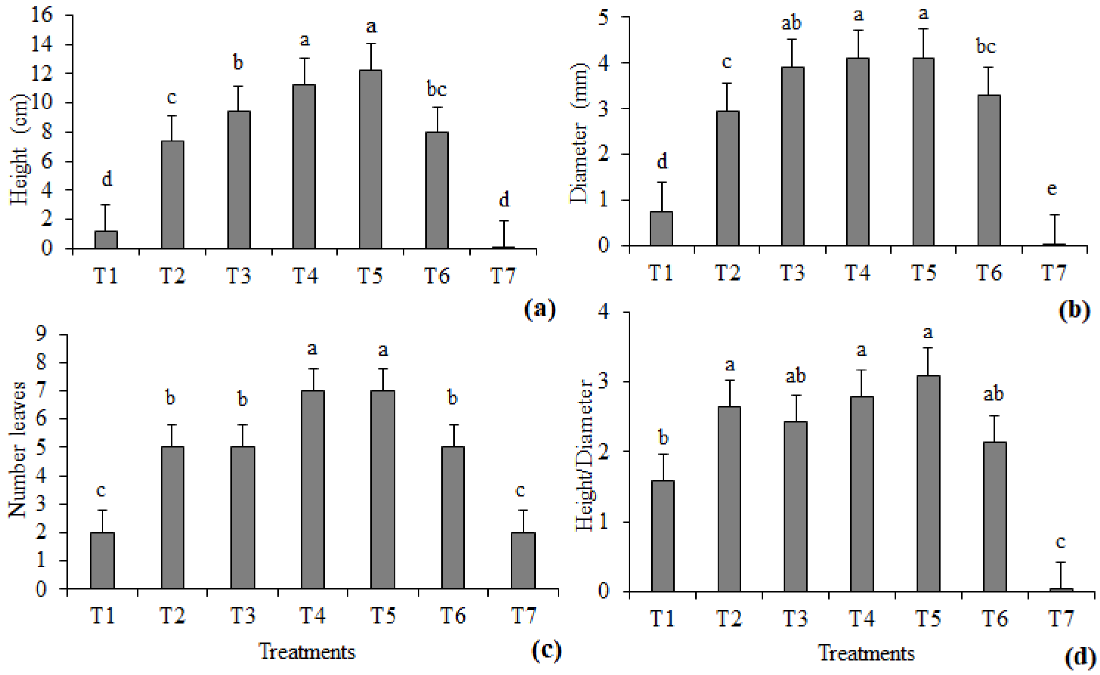

Fig. 3 Biometric variables of *mutambo* seedlings (*Guazuma ulmifolia* Lam., Malvaceae) grown in different substrates at 210 days after sowing. Mean values of standard deviation (±). Means with different letters differ significantly by Tukey's test at 5% probability.

The treatments promoted significant effects on the biomass variables and allometric relationships of the *mutambo* seedlings. For the leaf dry mass (LDM) and stem dry mass (SDM), the largest accumulations were obtained with treatments 4 and 5, which were similar to each other for both variables (Fig. 4a and c), while for the accumulation of root dry matter (RDM), the results promoted by treatments 2, 3, and 4 were superior to the remainder (Fig. 4e). Treatments 5 and 6 promoted seedlings with the highest values of leaf mass ratio (LMR), being similar to each other and different from the remainder (Fig. 4b). For the stem mass ratio (SMR) and root mass ratio (RMR), treatments 1 and 7 were statistically similar and stood out from the remainder as their seedlings showed the highest values for these variables (Fig. 4d and f).

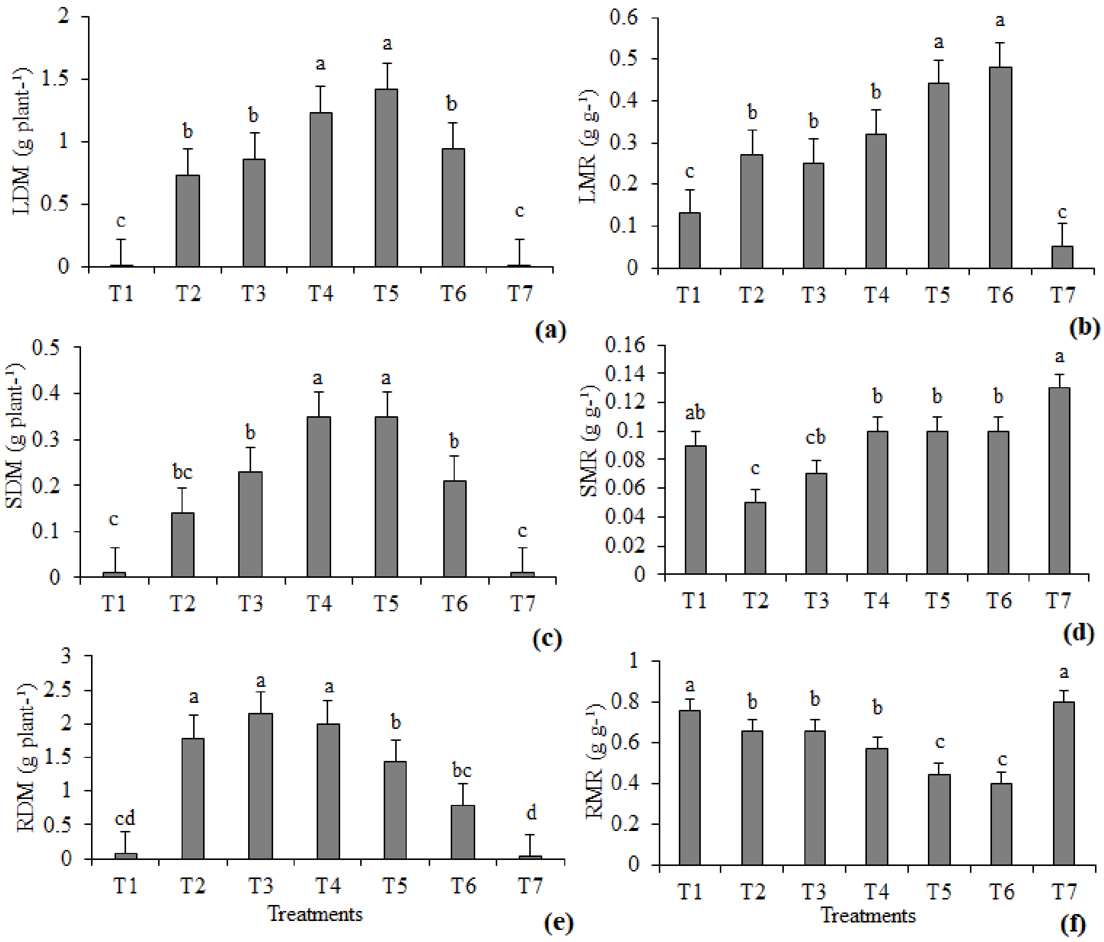

Fig. 4 Dry mass and allometric relationships of *mutambo* seedlings (*Guazuma ulmifolia* Lam., Malvaceae) grown in different substrates at 210 days after sowing. Leaf dry mass **(a)**, Leaf mass ratio **(b)**, Stem dry mass **(c)**, Stem mass ratio **(d)**, Root dry mass **(e),** and Root mass ratio **(f)**. Mean values of standard deviation (±). Means with different letters differ significantly by Tukey's test at 5% probability.

The treatments promoted significant differences in the Dickson Quality Index of the *mutambo* seedlings. The highest indices were found with treatments 4, 5, and 6, which did not differ from each other, while treatments 1 and 7 promoted the lowest indices (Fig. 5 a).

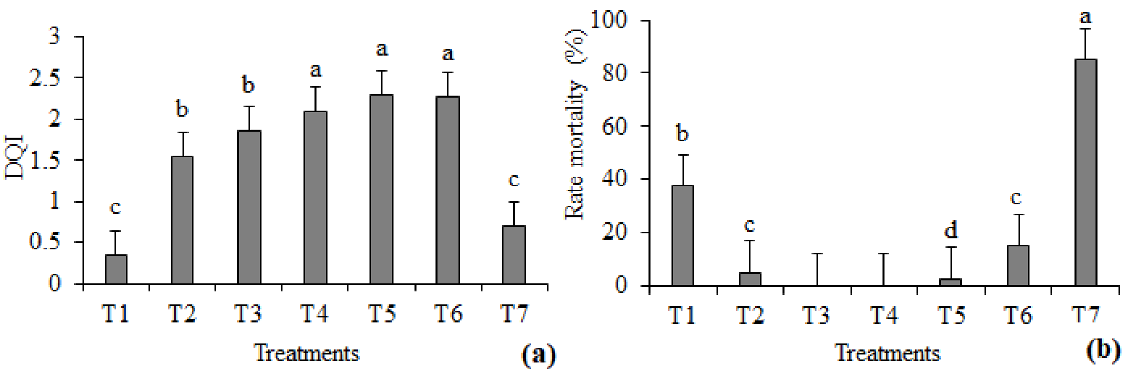

Fig. 5 Dickson Quality Index **(a)** and mortality rate **(b)** of *mutambo* seedlings (*Guazuma ulmifolia* Lam., Malvaceae) grown in different substrates at 210 days after sowing. Mean values of standard deviation (±). Means with different letters differ significantly by Tukey's test at 5% probability.

The treatments also promoted significant differences in the mortality rate of the seedlings. Treatments 1 and 7, in addition to promoting low-quality *mutambo* seedlings, were also the substrates in which the highest mortality rates were recorded (Fig. 5a and b). Treatment 7 promoted the highest mortality rate (85%), followed by treatments 1 (37.5), 6 (15%), 2 (5%), and 5 (2.5%). For treatments 3 and 4, the seedling mortality rate was zero (Fig. 5b).

3.4 Discussion

When well-nourished, seedlings usually show good physiological performance, resulting in greater growth and development. However, in this study, although the Bioplant® substrate (treatment 7) has provided higher nutrient contents (except for Mg and P) than the remaining treatments, it was not the substrate that promoted the greatest growth and development of *mutambo* seedlings. This behavior can be associated with the water conditions to which all substrates were subject, which did not favor treatment 7. Therefore, this treatment was the most harmful to the seedlings as it resulted in high mortality (85%) and reduced growth. One of the features of the Bioplant® substrate is its high water retention capacity, which may result in root rot in species that do not tolerate high moisture (Moura et al., 2019), since water accumulation results in low oxygenation for root development (Zorzeto et al., 2014), leading to plant death. High irrigation levels constitute another factor that can be attributed to this result, causing nutrient loss by leaching since it is a light and uncompacted product. The treatments containing swine wastewater are denser by nature, which may have benefited such substrates. Several other studies also verified inferior results for seedlings grown in Bioplant® compared with other substrates (Alves et al., 2012; Dutra et al., 2015; Borges et al., 2016; Lima et al., 2016). In this study, the commercial substrate Bioplant® also showed a very low or acid pH (5.2). According to Carvalho et al. (2007), the studied species prefers soils or substrates with pH above 5.5, and this was the only among the tested substrates with pH below this value.

For most variables analyzed, the treatments containing swine wastewater showed superior results. Among the substrates with swine wastewater, treatments T4, T5, and T6 stood out from the remainder. It is worth noting that, after the treatment with Bioplant®, these were the treatments that showed the highest nutritional contents, and T6 even showed Mg and P contents above those of the Bioplant® substrate (Table 1). T4 and T5 showed the highest investment in growth, number of leaves, and shoot biomass accumulation in the seedlings compared to the other treatments. Gonçalves et al. (2000) reported that the most appropriate substrates for seedling propagation are usually obtained by mixing from 70 to 80% of an organic component with 20 to 30% of a component used to increase macroporosity. In the present study, the proportions that constitute treatments 4 (60% sw + 40% crh and ve) and 5 (80% sw + 20% chr and ve) are very close to those reported by such researchers. Delarmelina et al. (2014) also obtained the best results for the growth of *Sesbania virgata* (Cav.) Pers. with the same proportions as treatments 4 and 5 of the present study, although with other constituents (60 to 80% of sewage sludge (sl) and 40 to 20% ve). Cabreira et al. (2017), studying three forest species, also verified that the 80% proportion of sewage sludge promoted the best results.

Based on the results achieved in this study, it can be said that the substrates with swine wastewater are indeed an alternative to produce *mutambo* seedlings. For the chlorophyll indices, in general, the best treatments were 4, 5, and 6; however, for these variables, all treatments containing swine wastewater showed superior results compared to the treatment composed of carbonized rice husk (T1) and the commercial substrate Bioplant® (T7). For all physiological variables evaluated in this study, treatments 4 and 5 were also the ones that promoted the best results. Among the physiological variables, stomatal conductance and transpiration are highlighted as they are essential regulatory mechanisms of the water vapor and carbon dioxide exchange between the leaf and the surrounding air, directly affecting plant growth (Iseki and Olaleye, 2019) and strongly relating to the photosynthetic rate (Wong et al., 1979). This strong relationship exists because the greater the stomatal opening, the higher the CO_2 diffusion into the substomatal chamber (Santos et al., 2013), which usually allows a higher photosynthetic rate. Therefore, the high values of stomatal conductance and transpiration obtained in the *mutambo* seedlings cultivated under treatments 4 and 5 led to greater CO_2 entry into the substomatal chamber and generated higher photosynthetic rates. With the increased photosynthetic rates compared to the remaining treatments, there was higher CO_2 fixation and greater investment in height, diameter, number of leaves, and biomass accumulation.

Taking into account the important variables when choosing the best treatment for seedling production, the height to diameter ratio stands out as higher values of this variable represent greater balance in seedling development (Souza et al., 2017). However, all treatments with swine wastewater were statistically similar. In this case, it is worth considering biomass accumulation, especially root biomass, for which the seedlings showed the best results with treatments 2, 3, and 4. If the interest lies in planting *mutambo* seedlings in the field, it is worth noting that the greater the development of the root system, the higher the water and nutrient uptake as this structure reaches greater depths into the soil profile (Salton and Tomazi, 2014). However, the number of leaves can also influence the choice of the ideal treatment as these structures constitute the main photosynthetic organs (Wright et al., 2004), and both the size and number of leaves are related to several biological processes, such as plant growth and survival (Tozer et al., 2015). In this way, in the present study, substrates 4 and 5 were the ones that provided the highest number of leaves and, therefore, showed the highest values of stomatal conductance, transpiration, and photosynthetic rate, which were also obtained with these treatments, along with the greater accumulation of stem and leaf dry mass.

Another variable that should be considered is the Dickson Quality Index, which determines seedling quality based on several morphological features, minimizing possible errors that may occur by using only one or two features (Vieira et al., 2019). The higher this index, the higher seedling quality, and so, substrates 4, 5, and 6 were the ones that promoted the highest quality *mutambo* seedlings since the highest indices were obtained with these treatments. This higher seedling quality is related to the nutritional characteristics of these substrates, which provided the seedlings with high contents of calcium, magnesium, phosphorus, and potassium (Table 1). Similar results to the present study were found for other species. Coelho et al. (2017) also obtained satisfactory results with seedlings of *Corymbia citriodora* ((Hook.) K.D. Hill & L.A.S. Johnson) due to the favorable nutritional conditions provided by the liquid swine wastewater used. Santinato et al. (2019), using different substrates to produce seedlings of *Coffea arabica* L., also verified that the treatments that contained swine organic compost showed higher concentrations of P, Ca^{2+}, and Mg^{2+}, which resulted in taller seedlings.

To conclude, the *mutambo* seedlings showed better physiological and morphological performance in substrates with swine wastewater than in the substrate composed of carbonized rice husk and vermiculite (T1) and the commercial substrate Bioplant® (T7). In view of these results, it can be said that the substrates with swine wastewater constitute an alternative to produce *mutambo* seedlings, and treatments 4 (60% Ls) and 5 (80% Ls) are the most indicated for the production of seedlings of this species.

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4 CAPÍTULO II

Physiological and morphological behavior of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) grown under phosphorus levels

(Normas de acordo com a revista Anais da Academia Brasileira de Ciências)

Abstract: Even as phosphorus fertilization benefits are continuously verified in the production of seedlings of forest species, there are species whose growth effects promoted by phosphorus are still unknown. Therefore, this study aimed to test whether increasing levels of phosphorus influence the physiological and morphological behavior and the quality of seedlings of *Guazuma ulmifolia* Lam. For that purpose, the seedlings were grown in a substrate produced from a Dystrophic RED LATOSOL (Oxisol) under a completely randomized design and subjected to five levels of phosphorus (0, 100, 200, 300, and 400 mg dm^{-3}). At 120 days, the biometric and physiological evaluations were performed, and the chlorophyll indices, biomass, allometric relationships, Dickson Quality Index, and the phosphorus content were determined. The data were subjected to analysis of variance by the F-test and subsequently to regression analysis and Pearson's linear correlation. Phosphorus fertilization promoted significant gains ($p \leq 0.05$) for all studied variables, except for the specific leaf area and allometric relationships. The phosphorus content was positively correlated with all tested variables. Based on the results obtained, it is concluded that the seedlings responded positively to phosphorus fertilization. Furthermore, phosphorus fertilization promoted better physiological and morphological performance and higher seedling quality.

Keywords: responsive species, phosphorus, *mutambo*, growth promoter.

4.1 INTRODUCTION

The production of quality seedlings is related to a series of factors, among which fertilization is one of the most important since it is performed to promote plant nutrition: when appropriate and balanced, it provides higher plant growth and development (Natale et al., 2018). Among fertilization types, phosphorus fertilization is extremely important as it relates to vital plant processes, such as photosynthesis and respiration. Furthermore, phosphorus (P) is a constituent of nucleic acids, phospholipids, proteins, phosphate esters, and adenosine triphosphate (Dechen & Nachtigall, 2007). Therefore, P participates directly in several plant physiological processes and provides energy to trigger several cellular metabolic processes. Thus, this nutrient is essential in plant growth and development (Malhotra et al., 2018), especially in the early development stage.

P is one of the nutrients that deserve attention in seedling production, not only for the role it plays in plants but also for the low availability of this element in the soil under natural conditions (Silva et al., 2018). This occurs because the soils of tropical regions are naturally acid and highly weathered. Soils with these characteristics show high P adsorption capacity, which results in the low availability of this nutrient to plants (Guedes et al., 2016). Considering that this condition compromises the early plant growth, it is essential to understand how the P availability affects the morphological and physiological performance of seedlings of forest species.

In view of the previous considerations, several studies aimed to evaluate the effects of phosphorus fertilization on the production of seedlings of numerous species. The results obtained vary as the plants have distinct needs and also variable quality standards. However, in general, phosphorus fertilization has promoted positive effects on the early growth of many species (Santos et al., 2008; Freitas et al., 2017a; Andrade et al., 2018; Moreira et al., 2018; Santos et al., 2019). Although phosphorus fertilization benefits are widely publicized, there are still no studies of this nature for some species of food and medicinal importance, such as *Guazuma ulmifolia* Lam. (Malvaceae), commonly known in Brazil as *mutambo*.

G. ulmifolia is widely distributed throughout Mexico, Central, and South America, and in the Brazilian territory, it occurs in all phytogeographic domains (Colli-Silva, 2019). In medicinal terms, the bark extract is antimicrobial, antioxidant, and cardioprotective (Dos Santos et al., 2018). The plant also shows activity against parasites such as *Leishmania brasiliensis*, *L. infantum*, and *Trypanosoma cruzi* (Calixto Júnior et al., 2016). The tea or juice of the leaves of *G. ulmifolia* has been indicated by several doctors in Brazil and Venezuela as an alternative treatment for HIV patients (Gouveia, 2018, Portadores... 2018, Singer, 2018, Macedo, 2019). The fruits are consumed by birds and primates, highlighting their importance for reforestation programs (Calzavara et al., 2017), and can also be consumed by humans due to their high dietary fiber content and bioactive phenolic compounds (Pereira et al., 2020).

As seen above, the species has the potential for wide exploitation by the industrial sector, either as food or as a therapeutic plant (Pereira et al., 2019). However, silvicultural studies for this species are still scarce. Therefore, this study aimed to evaluate the growth and development of seedlings of *G. ulmifolia* produced with different levels of P to test the hypothesis that the seedlings of *G. ulmifolia,* when produced with phosphorus fertilization, show better physiological and morphological performance and, as a consequence, higher quality.

4.2 MATERIAL AND METHODS

Area, seedling cultivation conditions, and experimental design

The present study was conducted in a plant nursery of the Goiano Federal Institute - Campus Rio Verde, municipality of Rio Verde, Goiás, Brazil, under a completely randomized design composed of five treatments (0, 100, 200, 300, and 400 mg dm^{-3} of P) and four replications. The soil used as substrate was a Dystrophic RED LATOSOL (Oxisol) (Santos et al., 2018) collected at the 0.0 to 0.20 m depth layer, used for its low P content. Samples from this soil were collected and sent to the Solotech Cerrado LLC Laboratory for chemical characterization. As a result, the soil used in this study showed, in its natural condition: pH 5.1 ($CaCl_2$); 0.05 $cmol_c$ dm^{-3} aluminum (Al); 1.06 $cmol_c$ dm^{-3} calcium (Ca); 0.49 $cmol_c$ dm^{-3} magnesium (Mg); 2.6 mg dm^{-3} phosphorus (P); 128 mg dm^{-3} potassium (K); 41.3 g dm^{-3} of organic matter; 47% base saturation (V%); and 48, 40, and 12% of clay, sand, and silt, respectively. The extractors used for the chemical analyses were: Mehlich 1 for P and K extraction; KCl 1N for Ca, Mg, and Al extraction; and the colorimetric method for organic matter.

Preparation of the substrate and fertilization

Base saturation was increased to 60% (Raij, 1997) with the soil correctives Ca and Mg carbonate at a 4:1 ratio. Monoammonium phosphate (MAP) was used as a P source, and basic fertilization (mg dm^{-3}) was applied ten days before establishing the treatments. Basic fertilization (mg dm^{-3}) was performed 30 days after liming by providing 180 mg

dm^{-3} nitrogen, 150 mg dm^{-3} potassium, 40 mg dm^{-3} sulfur, 1.33 mg dm^{-3} copper, 0.81 mg dm^{-3} boron, and 4 mg dm^{-3} zinc, using as sources: urea and ammonium sulfate, potassium sulfate and potassium chloride, copper sulfate, boric acid, and zinc sulfate, respectively (Carlos et al., 2015). Since the MAP has both N and P in its constitution, a balancing was performed to match the amount of N in all P treatments.

Seed collection, preparation, and planting

The seeds of *Guazuma ulmifolia* were collected from ten parent plants located in the orchard of the Federal University of Lavras, municipality of Lavras, state of Minas Gerais. Seed dormancy was overcome with hot water at 70°C for approximately 30 minutes or until the water temperature was reduced to 50°C (Nunes et al., 2006). Sowing was performed in non-perforated 4-liter pots 45 days after acidity correction. Irrigation was performed daily, maintaining the substrate at 60% of field capacity by weighing the pots with the plants and directly irrigating the soil.

Morphological/biometric analyses

The biometric evaluations were performed 120 days after sowing by measuring the plant height (H) with a millimeter rule, while the base diameter (D) was obtained with a digital caliper. The number of fully expanded leaves (NF) was also counted.

Physiological/gas exchange analyses

At 115 days after sowing, the gas exchange analyses were performed using a portable infrared gas analyzer (Model Li-6800xt, Nebraska, USA), with constant irradiance (1,500μmol m^{-2} s^{-1}) between 8:00 a.m. and 11:00 a.m., by evaluating the following variables: photosynthetic rate [A, μmol (CO_2) m^{-2} s^{-1})], transpiration rate [E, mmol (H_2O) m^{-2} s^{-1}], stomatal conductance [g_s, mol (H_2O) m^{-2} s^{-1}], and electron transport rate (ETR, µmol m^{-2} s^{-1}).

Falker® Chlorophyll Indices

At 115 days after sowing, the photosynthetic pigments were analyzed by measuring the Falker® Chlorophyll Index (Chl *a* and Chl *b*) on the same leaf in which the gas exchange analyses were performed. A ClorofiLOG chlorophyll meter was used for this analysis (model CFL1030, Falker Automação Agrícola, Porto Alegre, Brazil). The total chlorophyll index was obtained with the individual chlorophyll data.

Biomass Analyses and Allometric Relationships

At 120 days after sowing, the seedlings were removed from the pots, cleaned, separated into leaves, stems, and roots, and dried in a forced-air oven at 65ºC until constant weight. Finally, the material was weighed in a digital balance, thus obtaining the biomass variables: foliar dry mass (MSF), stem dry mass (MSC), and root dry mass (MSR). The sum of these three variables resulted in the total dry mass (MST), after which the allometric relationships were also calculated: foliar mass ratio (MSF/MST, $g\ g^{-1}$), stem mass ratio (MSC/MST, $g\ g^{-1}$), and root mass ratio (MSR/MST, $g\ g^{-1}$).

Leaf area and specific leaf area

The leaf area (AF) was calculated with photographic records of the leaves from each experimental unit, obtained when the experiment was dismantled, using the software *Image J*® (*HIN*, *Bethesda*, *Maryland*, USA). With the AF and MSF data, it was possible to calculate the specific leaf area (AFE) through the formula: AF/MSF. Based on the growth and biomass data, it was also possible to calculate the Dickson Quality Index (IQD) (Dickson et al., 1960).

P content

The shoot P content of the seedlings (leaves and stems) was determined following the methodology by Embrapa (2009), and the reading was performed by spectrophotometry.

Statistical analyses

The data were subjected to analysis of variance by the F-test ($p \leq 0.05$) and to regression analysis to verify the adjustment of the data to the models. The choice of the model occurred according to the significance of each equation. Pearson’s linear correlation coefficient was calculated by involving the variables with each other. The analyses were performed in the statistical software programs Sisvar (Ferreira, 2014) and BioEstat (Ayres et al., 2007).

4.3 RESULTS

The supply of P levels promoted a significant increase ($p \leq 0.05$) in the shoot P content of the seedlings of *G. ulmifolia*. The 400 mg dm-³ level promoted the highest value among the tested levels, reaching 16 g kg^{-1} (Fig. 1).

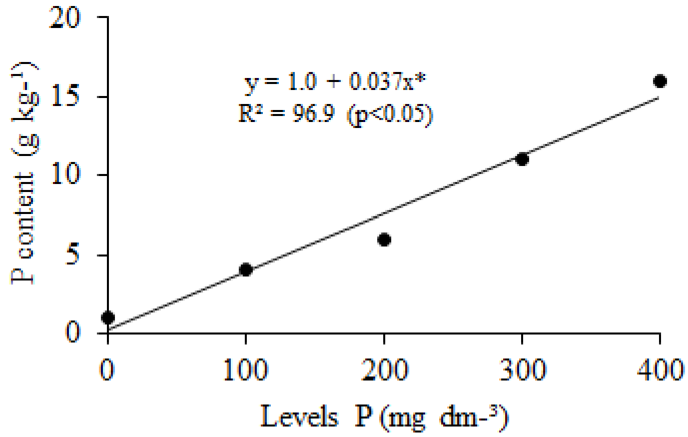

Figure 1. Phosphorus content of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing.

Phosphorus fertilization promoted a significant effect on the chlorophyll indices of the seedlings of *G. ulmifolia*. The data obtained fit the linear model, and the 400 mg dm-³ P level promoted the highest values for these variables, reaching the indices of 35 for chlorophyll *a*, 11 for chlorophyll *b*, and 46 for total chlorophylls (Fig. 2a, b, c, and d).

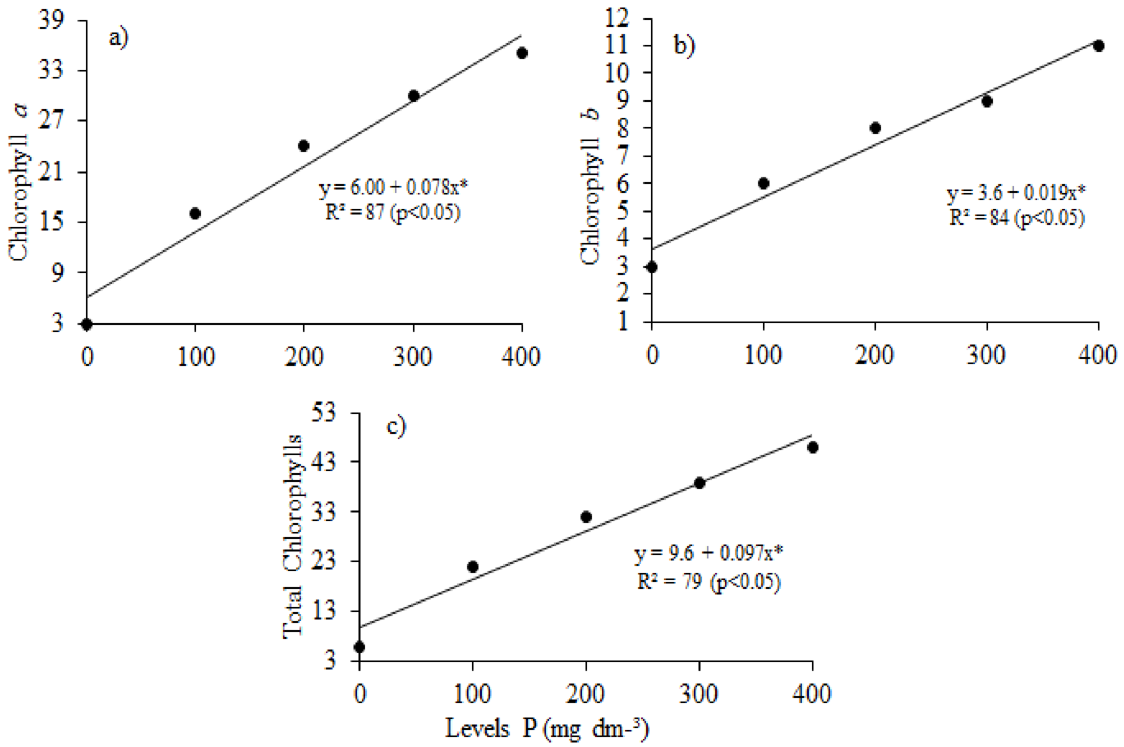

Figure 2. Falker® chlorophyll indices of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 115 days after sowing.

The P levels significantly influenced the physiological variables. The data obtained fit the linear model as the highest level tested was also the one that promoted the highest values for these variables, reaching 18.5 and 93.5 μmol m^{-2} s^{-1} for *A* and ETR (Fig. 3a and d), and 0.41 and 0.013 mol m^{-2} s^{-1} for *gs* and *E*, respectively (Fig. 3b and c).

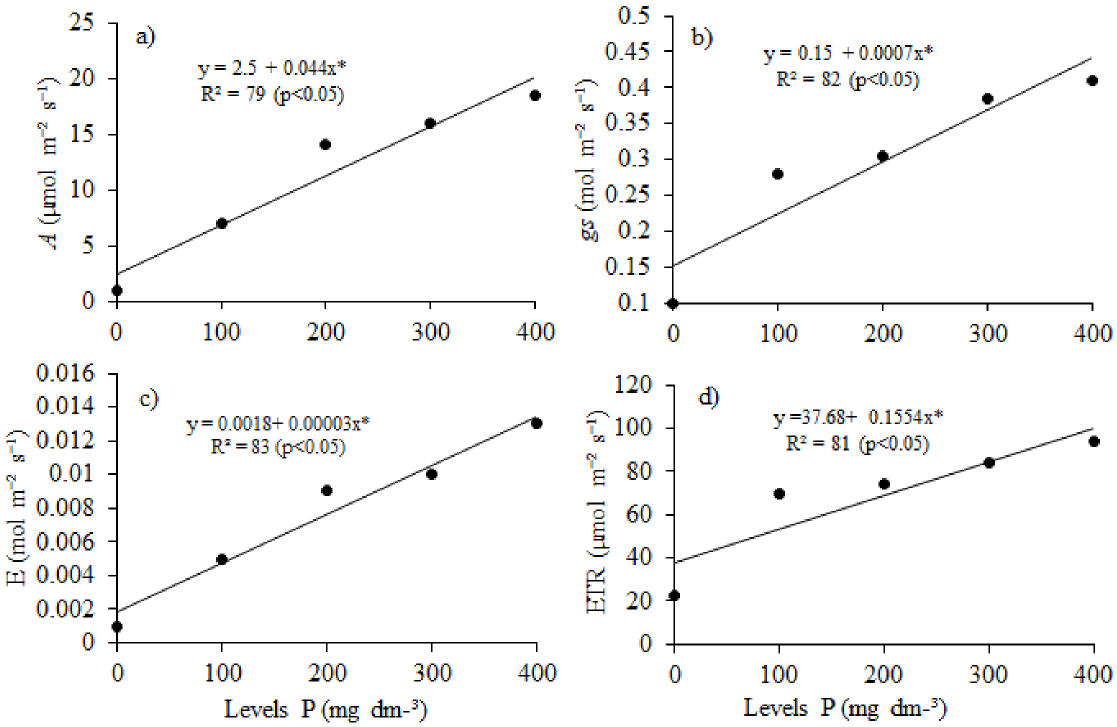

Figure 3. Physiological variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 115 days after sowing. a) Photosynthetic rate, b) Stomatal conductance, c) Transpiration rate, and d) Electron transport rate.

The biometric variables also showed significant gains with the phosphorus levels, except the AFE ($p \geq 0.05$). The highest P level tested, 400 mg dm-³, promoted the highest values, and the plants showed, on average, 50 cm height, 10 mm diameter, 22 leaves, and 800 cm² of leaf area (Fig. a, b, c, and d).

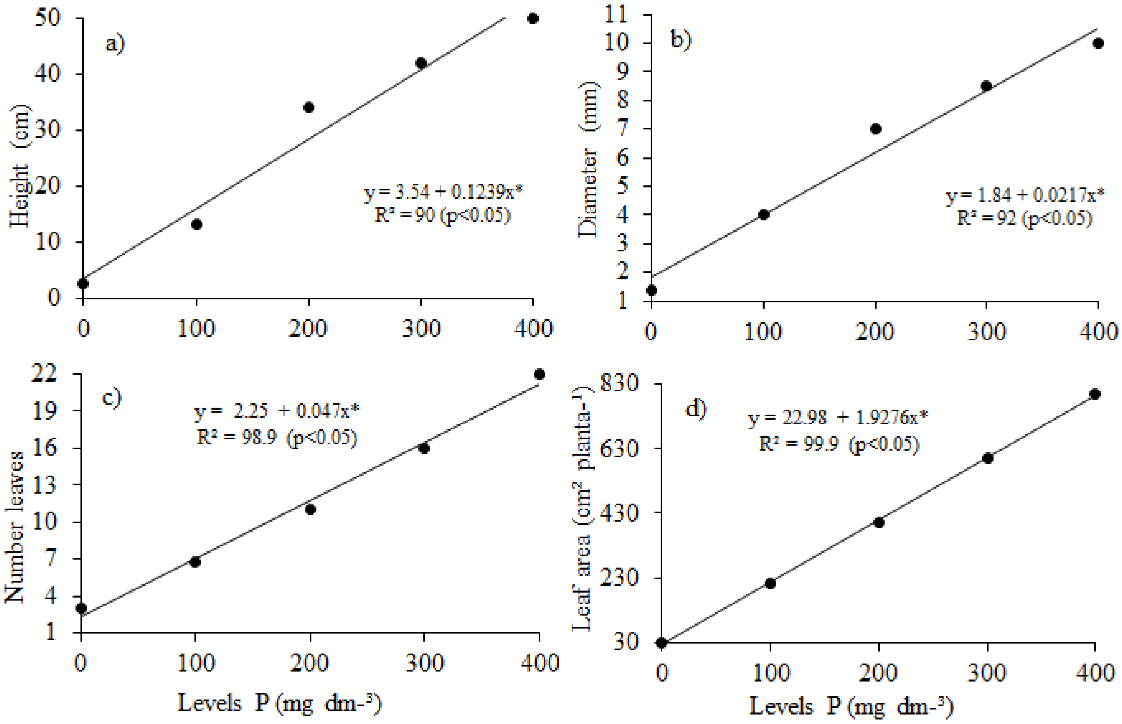

Figure 4. Biometric variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing. a) Height, b) Diameter, c) Number of leaves, and d) Leaf area.

The biomass variables were significantly affected by the P levels. For all biomass variables obtained, the highest values were found at the highest P level tested, 400 mg dm^{-3}. The seedlings showed, on average, 10.5 g of MSF, 5 g of MSC, 35 g of MSR, and 50.5 g of MST (Fig. 5a, b, c, and d). For the allometric relationships, the treatments promoted no differences.

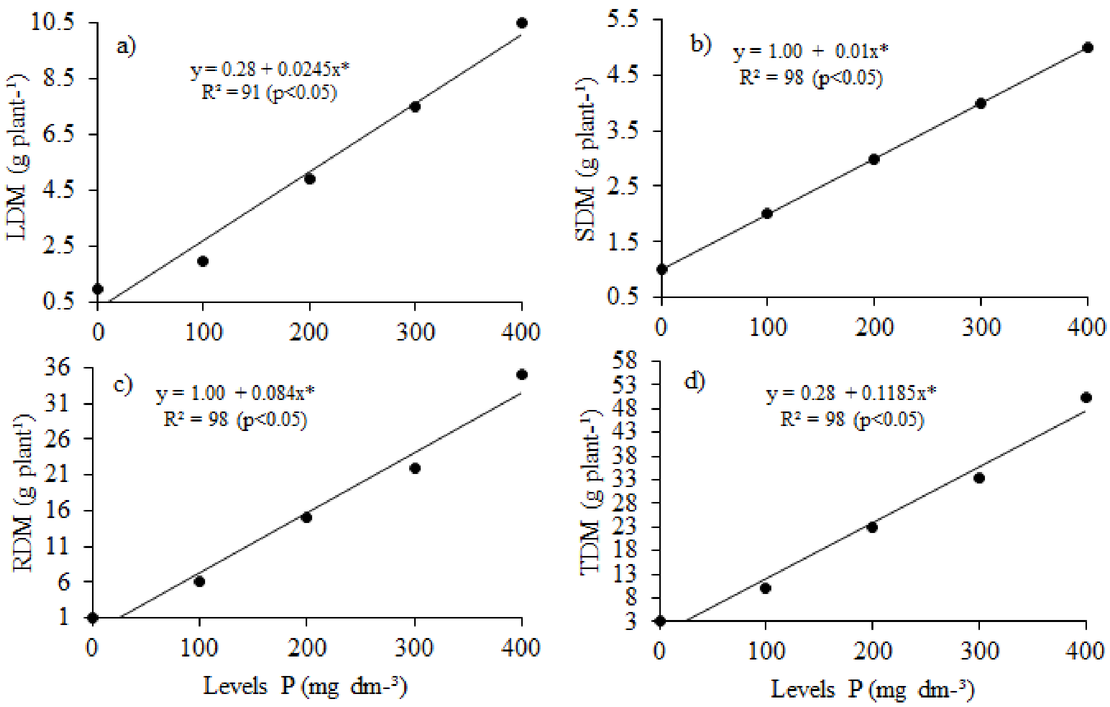

Figure 5. Biomass variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing. a) Leaf dry mass, b) Stem dry mass, c) Root dry mass, and d) Total dry mass.

Phosphorus fertilization promoted a significant effect on the IQD of the seedlings of *G. ulmifolia*. The equation that best fit was the linear model, and the highest P level tested, 400 mg dm^{-3}, promoted the highest index, 9 (Fig. 6).

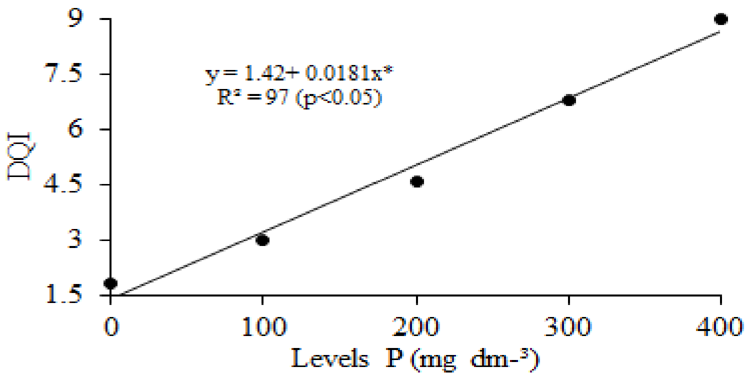

Figure 6. Dickson Quality Index of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) as a function of phosphorus levels at 120 days after sowing.

All significant correlations found were positive. The P level was strongly correlated with all studied variables. The photosynthetic rate correlated with the shoot P level of the seedlings as well as with the variables of growth, biomass accumulation, chlorophylls, and IQD. The IQD also showed to be highly correlated with the biometric variables and biomass. The ETR correlated with the chlorophylls *a* and *b* and with all physiological variables (Table I).

Table I. Pearson's linear correlation coefficient (r) between the variables of seedlings of *Guazuma ulmifolia* Lam. (Malvaceae) subjected to phosphorus levels. Height (H, cm), diameter (D, mm), number of leaves (NF), leaf area (LA, cm^2), leaf dry mass (LDM, g $plant^{-1}$), stem dry mass (SDM, g $plant^{-1}$), root dry mass (RDM, g $plant^{-1}$), Dickson Quality Index (DQI), Chlorophyll *a* (Chl *a*), Chlorophyll *b* (Chl *b*), photosynthetic rate (A, µmol (CO_2) m^{-2} s^{-1}), stomatal conductance (gs, mol (H_2O) m^{-2} s^{-1}), transpiration rate (E, mmol (H_2O) m^{-2} s^{-1}), electron transport rate (ETR, µmol m^{-2} s^{-1}), and shoot phosphorus content (P, g kg^{-1}).

	H	D	NF	LA	LDM	SDM	RDM	DQI	Chl *a*	Chl *b*	*A*	*gs*	E	ETR	P
H		0.99*	0.94*	0.96*	0.95*	0.85 ^{ns}	0.87^{ns}	0.85*	0.82^{ns}	0.87^{ns}	0.88*	0.90*	0.81*	0.83^{ns}	0.87*
D			0.93*	0.95*	0.93*	0.84 ^{ns}	0.84^{ns}	0.82*	0.86^{ns}	0.90*	0.82*	0.92*	0.87*	0.87^{ns}	0.91*
NF				0.99*	0.99*	0.97*	0.97*	0.96*	0.76^{ns}	0.85^{ns}	0.84*	0.90*	0.86*	0.81^{ns}	0.78*
LA					0.99*	0.95*	0.95*	0.94*	0.80^{ns}	0.88*	0.88*	0.93*	0.71*	0.85^{ns}	0.82*
LDM						0.96*	0.97*	0.97*	0.72^{ns}	0.81^{ns}	0.89*	0.87*	0.61^{ns}	0.77^{ns}	0.75*
SDM							0.99*	0.99*	0.64^{ns}	0.76^{ns}	0.84*	0.82^{ns}	0.51^{ns}	0.73^{ns}	0.86*
RDM								0.99*	0.61^{ns}	0.73^{ns}	0.80*	0.80^{ns}	0.63^{ns}	0.69^{ns}	0.84*
DQI									0.58^{ns}	0.71^{ns}	0.87*	0.78^{ns}	0.98*	0.66^{ns}	0.81*
Chl *a*										0.98*	0.99*	0.95*	0.95*	0.99*	0.99*
Chl *b*											0.98*	0.98*	0.99*	0.99*	0.97*
A												0.94*	0.91*	0.99*	0.97*
gs													0.90*	0.97*	0.94*
E														0.97*	0.95*
ETR															0.97*

Where: *significant at 5 % probability, ^{ns} not significant.

4.4 DISCUSSION

The results found in the present study show that the phosphorus levels promoted better physiological and morphological performance and higher quality of seedlings of *G. ulmifolia*. The species showed to be highly responsive to the application of P to the soil since a significant influence was verified for all studied variables, except for the specific leaf area and allometric relationships. Among the P levels tested, the 400 mg dm^{-3} level promoted the highest values for all variables. This high P requirement in the early stage is associated with the pioneer character of the species (Herrera-Peraza et al., 2016), and for showing a rapid growth (Silva et al., 2016). As a function of the improvement in soil fertility, there is greater investment in seedling growth as the seedlings require greater amounts of nutrients to meet the nutritional demand, allowing an increase in the biomass production potential of species with a marked early growth (Silva et al., 1997).

The greater P uptake by the seedlings allowed them to increase the Falker® indices of chlorophylls *a* and *b*, the number of leaves, and the leaf area. The higher indices of leaf photosynthetic pigments (chlorophylls) can be attributed to the increased P concentration in the tissues, which, in turn, accelerated the energetic metabolism and cell division (Marschner, 2012), also stimulating the increase in the leaf area. Chlorophylls are extremely important as they are responsible for light capture during photosynthesis, resulting in the excitement of the electrons used to boost the production of nicotinamide adenine dinucleotide phosphate (NADP) and chemical energy in the form of adenosine triphosphate (ATP) (Croft et al., 2017). The number of leaves and the leaf area are related to the ability to intercept solar radiation and boost CO_2 assimilation in order to accumulate dry matter (Crous et al., 2015; Huang et al., 2016). Berghetti et al. (2019) and Silva et al. (2020) found similar results to the present study, also verifying higher chlorophyll indices and increased leaf area in seedlings of *Cordia trichotoma* and *Eucalyptus urophylla x Eucalyptus grandis*, respectively, with the increase in the P levels.

With higher chlorophyll indices, number of leaves, leaf area, and shoot P concentration, the seedlings of *G. ulmifolia* were able to increase the efficiency of the photosynthetic apparatus, showing higher stomatal conductance, transpiration, electron transport rate, and higher photosynthetic rates. Berghetti et al. (2019 and 2020) also verified that the high concentrations of chlorophylls *a* and *b* and the increase in the leaf area as a function of the high P availability reflected higher photosynthetic rates. This increase in the photosynthetic process of the seedlings is also essential as plants with an adequate leaf P supply show an increase in CO_2 assimilation, carboxylation efficiency, and in the photochemical process, influencing the gain of biomass by plants (Warren, 2011). In this case, with the photosynthetic apparatus potentialized by the phosphorus levels, the seedlings of *G. ulmifolia* could specifically invest in height and diameter. Considering that P deficiency is one of the greatest limitations in the development of forest species (Zhu et al., 2018) since this nutrient plays a central role in plant growth, several studies have demonstrated that tropical tree species respond positively to the increase in the P levels, which is largely due to the low P levels in tropical soils (Fernandes et al., 2013).

The seedlings of *G. ulmifolia* also accumulated biomass as a function of the phosphorus levels. The results obtained in this study show that the seedlings showed greater vigor at higher P levels, indicating that the species requires high P levels during its early growth, such as occurs with *Senna macranthera* (DC. ex Collad.) H. S. Irwin & Barneby (Cruz et al., 2011), *Cassia grandis* L. f. (Andrade et al., 2018), and *Dalbergia nigra* ((Vell.) Fr. All. ex Benth (Gonçalves et al., 2014, Carlos et al., 2018). Biomass is one of the best features to determine seedling quality as it correlates with plant vigor (Cruz et al., 2011), and, even if destructive, it reflects the net photosynthesis value (Fernandes et al., 2019) and is also highly associated with the ability to survive in the field. Furthermore, the dry matter data (biomass) should be taken into account as they help reduce the erroneous classification of seedling quality, in the case of seedlings that are taller due to etiolation (Freitas et al., 2017a).

Another variable of the seedlings of *G. ulmifolia* that was positively influenced by the phosphorus levels was the Dickson Quality Index. This index determines seedling quality based on several morphological features, minimizing the possible errors that may occur by using only one or two features (Vieira et al., 2019). Therefore, with the results obtained, it is safe to affirm that the P levels increased the quality of the seedlings of *G. ulmifolia*. However, this was already foreseen as it also occurred for the variables needed to calculate this index, which agrees with the verified for other variables. This reinforces the high need for P that the seedlings require in the early stage in order to reach maximum growth, development, and vigor, with these being the most desirable features when the subject is seedling production.

Regarding the correlations found between the studied variables, values above 0.75 indicate that one variable allows inferring about the others (Freitas et al., 2017b); therefore, it is valid to say that the studied variables are strongly and positively interlinked as all significant correlations were positive. In physiological terms, the photosynthetic capacity is usually positively correlated with the P concentration, both in the soil and in the leaves (Bloomfield et al., 2014, Bahar et al., 2017), as leaf P is essential for the adjustment of net photosynthesis through the regulation of the main carbon metabolism intermediates (ATP, NADPH, and sugar phosphates, including ribulose 1-5 bisphosphate) (Bahar et al., 2017). In terms of growth, the correlations were high because the P levels lead to growth increments and a marked development in pioneer species, in addition to improving the physiological capacity of the seedlings, which reflects in their morphological behavior (Resende et al.,1999).

4.5 CONCLUSION

Based on the results obtained, it is concluded that the seedlings of *G. ulmifolia* responded positively to phosphorus fertilization. The P level of 400 mg dm^{-3} promoted seedlings with better physiological and morphological performance and higher quality.

4.6 ACKNOWLEDGMENTS

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5 CONCLUSÃO GERAL

Diante dos resultados encontrados para ambos os experimentos, pode-se afirmar que, tanto substratos com lodo suíno, quanto doses fosfatadas influenciaram positivamente o comportamento fisiológico e morfológicos das mudas de mutambo, sendo os melhores substratos para a produção de mudas *G. ulmifolia* o T4 e T5; já a melhor dose de P, foi a de 400 mg dm-^{3}.