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Original

Organic and Mineral Biofertilization of *Tithonia diversifolia* (Hemsl.) A. Gray Seedlings in Nurseries

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ABSTRACT

Background: The current challenge consists of producing foods in resilient systems under climate change conditions, and protecting the environment. **Aim.** To evaluate organic and mineral biofertilization use for growth and development of *Tithonia diversifolia* seedlings in nursery conditions. **Materials and methods:** Two trials were performed: E-I, at the Renato Guitar Cooperative, and E-II, at the Indio Hatuey Experimental Station of Pastures and Forages (EEPFIH). A completely randomized experimental design was performed with 15 and 10 repetitions in experiments I and II, respectively. E-I included three treatments: T-1, on 100% unfertilized soil; T-2 on 50% soil + 50% biochar; T-3, 50% soil + 25% biochar + 25% sugar cane bagasse ashes. Biochar was made from the sickle bush (*D. cinerea*) and embedded in efficient microorganisms IHPLUS®BF. The physiological parameters of primary and secondary metabolisms were measured 30 days after, along with the morphological growth indicators at days 30 and 60. E-II included 5 treatments: T-1: Soil without fertilization; T-2: Combination of 50% soil-50% compost + *Rhizobium;* T-3: Combination of 50% soil-50% compost + *Trichoderma;* T-4: Combination of 50% soil-50% compost containing micronized dolomite+ *Rhizobium;* T-3: Combination of 50% soil-50% compost containing micronized dolomite dissolution + *Trichoderma.* The morphological indicators of growth were measured at 60 days, whereas the dry aerial biomass was measured at transplantation. **Results**: T-3 (50% soil + 25% IHPLUS®BF-enriched biochar + 25% ashes) was the best variant in trial I. In trial II, all the variants studied were better than the control. **Conclusion:** The results confirmed the effectiveness of such alternatives for the cultivation of *T. diversifolia* plants in nursing conditions.

Keywords: biochar, biofertilization, *T. diversifolia,* nurseries (Source: *AIMS)*

INTRODUCTION

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The current socioeconomic impact of scientific research and technological innovation in agriculture focuses on the environment and food sovereignty (Milera *et al*., 2020). In Cuba, agroecological practices have a circular character, which includes the utilization of biofertilizers such as beneficial microorganism-enriched biochar, compost, and natural minerals. Besides, recycling constitutes an opportunity to reuse and recycle residues and excess productions (Pentón *et al*., 2020a).

Biochar was preceded by an agroecological projection with a systemic approach, in which soil protection is a fundamental premise. It comprises arboreal species in crop and grazing land management and adds the study of the edaphic biota in research programs. It also substitutes chemical fertilizers with organic ones and fosters the creation of rows for humus production. Additionally, the program studies microorganisms that could be used to promote crop and animal development, seeking to use recycled residues (from plant and animal wastes, such as *Bombyx mori* worms). Finally, a rustic composting plant was installed (Pentón *et al*., 2020a).

Among the protein trees used for animal nutrition frequently are mulberry (*Morus alba* L.), tithonia (*Tithonia diversifolia* (Hemsl.) A. Gray), moringa (*Moringa oleifera* Lam.), leucena (*Leucaena leucocephala* (Lam.) de Wigth, which are also the top forage producing plants to populate cattle raising areas, with 30-40% wood residues (Pentón *et al*., 2020b). However, *T. diversifolia* is a herbaceous plant with large roots and a special ability to retrieve scarce nutrients from the soil, along with a broad range for adaptation and spreading in tropical areas. It tolerates soil acidity and fertility and can withstand trimming at soil level and burning, growing very fast at the expense of low intake and little handling. Besides, the plant also accumulates nitrogen in the leaves, like Leguminosae, and has high levels of phosphorous (Herrera and Ramírez, 2020; Ontivero, 2021).

Recommending new biofertilizers must result from a morphophysiological response during the first stages of growth, which would be conditioned by the properties of the substrate and the edaphological demands of the particular species. Accordingly, this paper aims to evaluate the effects of different biofertilization protocols using organic matter and minerals to promote growth and other features in *Tithonia diversifolia* seedlings in nurseries.

MATERIALS AND METHODS

The study was conducted in two different scenarios in the province of Matanzas, Cuba, in *Tithonia diversifolia* in the nursery, between April and June.

The propagules were collected from 120-day-old re-shooting plants without inflorescence. The branches were cut approximately 20 cm from the soil. Shoot diameter was 2.0-3.0 cm, and their length was 25-30 cm. One end of the shoots was planted 15 cm deep.

I-Trial: It was performed at the Renato Guitart Cooperative (CPA) in the municipality of Juan Gualberto Gomez, Matanzas, Cuba.

II-Trial: It took place at the Indio Hatuey Experimental Station of Pastures and Forages, Matanzas, Cuba.

Design and treatments A completely randomized experimental design was performed with 15 repetitions (I) and 10 repetitions (II).

I-Trial evaluated three treatments:

- T-1: 100% unfertilized soil.
- T-2: 50% + 50% IHPLUS®BF enriched biochar.
- T-3 50% soil + 25% IHPLUS®BF-enriched biochar + 25% ashes.

Indicators

Chlorophyll content (µg/cm²), flavonols and anthocyanins (relative absorbance), and nitrogen relative content (NBI®). Observations of the leaf face were made at 30 days. A leaf clip dualex sensor designed for abiotic stress studies was used (https://www.force-a.com/products/dualex).

The procedure consisted of using the probe to measure fluorescence from the leaf as a response to the laser, which is directly linked to the number of metabolites present (chlorophyll and flavonols-anthocyanins) expressed in micrograms per square centimeter (µg/cm²), and relative absorbance, respectively.

The index of anthocyanins was set by using the team variant known as Dualex Scientific+, which permits measuring the fluorescence of anthocyanins.

The NBI ® (Nitrogen Balance Index) corresponds to the chlorophyll/flavonols ratio (also nitrogen/carbon).

Stem length and diameter (cm), number of leaves per branch, and number of branches at 30 and 60 days. A millimeter ruler and gauge caliper were used.

II-Trial evaluated five treatments consisting of,

- T-1: Soil without fertilization.
- T-2: 50% soil ++ 50% IHPLUS®BF enriched compost + rhizobium.
- T-3: 50% soil ++ 50% IHPLUS®BF enriched compost + *Trichoderma*
- T-4: 50% soil ++ 50% micronized dolomite IHPLUS®BF enriched compost + rhizobium.
- T-5: 50% soil ++ 50% micronized dolomite IHPLUS®BF enriched compost + *Trichoderma*.

Indicators: number of shoots at 30 and 60 days, stem length and number of leaves at 60 days, and production of aerial biomass at the moment of transplantation (g). A millimeter ruler, gauge caliper, and a technical balance were used.

The dry aerial biomass of the seedlings was determined through a cut of the aerial fraction of the plants at the soil level, then it was dried in a stove at 70 ^oC for 72 hours, then the samples were weighed in a balance (ERN CXB 15K1). The results were expressed in grams (g).

Origin of substrate materials

Soil: The soil substrate used in the first trial was brown without carbonates; the second trial used red ferralitic soil.

IHPLUS®BF: This product is registered by the EEPFIH, and is derived from lactic fermentation in the presence of soil microorganisms captured in unperturbed sites. It embodies a series of different aerobic and anaerobic organisms, which are physiologically compatible and live in synergy.

Biochar: Biocharcoal was produced through pyrolysis of the sickle bush (*Dichrostachys cinerea* (L) Wigth & Arn) stems for two hours, in a ground-based rustic furnace. The Kon-Tiki (Schmidt *et al.,* 2014) technology was used. The biocharcoal was dipped in a 100% concentration of IHPLUS®BF for 24 hours.

Compost: It was made at the EEPFIH pilot plant for organomineral fertilizers, using cattle stools in aerobic conditions, natural vegetation, and rests of gardening material enriched with IHPLUS®BF (5L/ton of compost) during irrigation that took place every 15 days.

Micronized dolomite: It consisted of compost + 15% dolomite. The material was applied at a rate of 4g per bag.

Rhizobium: It was used as an aqueous solution (1:10 proportion), using 6.5mL inoculum: 58mL H_2O, at 1,62mL/bag.

Trichoderma: It was used at 1,5mL/bag (60mL H_2O with 2.1g Tricosove). The two fertilizers were sprayed on *T. diversifolia* propagules when planted.

The fertilizers matched the appropriate range of organic matter content for organic fertilizers. Moreover, the redox potential [Eh (pH7)] and pH were within the optimum range, the former between +350 and +450 mV, and the latter between 6.5 and 7.5.

Statistical analysis. InfoStat (Di Rienzo, 2008) was used for the statistical analysis. The theoretical assumptions of the analysis of variance were analyzed, along with variance homogeneity of Levene (1960), and error normality by Shapiro and Wilk (1965). The analysis of variance was performed according to a completely randomized design, and the mean comparison test was made.

RESULTS AND DISCUSSION

Table 1 shows evidence that rejects the normal distribution assumption (p<0.05) for the following variables: relative nitrogen, and stem length at 30 and 60 days, which required transformation according to Lnx. The other variables did not require transformation since they met the assumptions for the original variable.

Table 1. Statistical characteristics in trial I

Variable	n	Mean	SD	W*	P (single tail)
Chlorophyll at 30 days	30	22.13	3.65	0.93	0.1429
Flavonol at 30 days	30	1.62	0.17	0.97	0.7987
Anthocyanin at 30 days	30	0.28	0.02	0.95	0.5206
Relative nitrogen at 30 days	30	13.99	2.77	0.88	0.0070
Nitrogen Ln at 30 days	30	2.62	0.19	0.91	0.0656
Stem diameter at 30 days (cm)	45	1.23	0.38	0.96	0.3234
Stem length at 30 days (cm)	45	17.06	2.32	0.92	0.0132
Stem length Ln at 30 days (cm)	45	2.83	0.13	0.93	0.0502
Stem length at 60 days(cm)	45	28.01	13.24	0.88	0.0001
Stem length Ln at 60 days(cm)	45	3.21	0.49	0.93	0.0595

Table 2 shows the results of morphophysiological indicators of *T. diversifolia* in nursery, in trial I, the chlorophyll and nitrogen contents at 30 days were different in treatments T1 and T2 in comparison to T3 for p≤0.05.

Stem length, flavonol, and anthocyanin contents at 30 days showed no differences between the treatments. Stem diameter was significantly higher in T1, with no differences from T2.

The stem length study at 60 days (cm) showed differences between the unfertilized soil (T1) and the 50% soil + 50% IHPLUS®BF biochar enriched soil (t2), and the 50% soil + 25% IHPLUS®BF biochar enriched soil + 25% ashes (T3).

Table 2. Behavior of morphophysiological indicators of *T. diversifolia* in nursery

Treatments	T1	T2	T3	P≤0.05	SE±	*SE+ %*
Chlorophyll at 30 days	20.85b	21.10b	24.45a	0.0423	0.67	*3.03*
Flavonol at 30 days	1.67	1.65	1.52	0.1099	0.03	*1.85*
Anthocyanin at 30 days	0.29	0.28	0.27	0.2797	0.0044	*1.57*
Relative nitrogen at 30 days	2.53b (12.67)	2.56b (13.02)	2.77a (16.28)	0.0029	0.03	*1.14*
Stem diameter at 30 days (cm)	1.45a	1.21ab	1.03b	0.0068	0.06	*4.87*

Stem length at 30 days (cm)	2.79 (16.35)	2.86 (17.49)	2.85 (17.35)	0.3325	0.02	*0.71*
Stem length at 60 days (cm)	2.77c (17.80)	3.09b (22.64)	3.76a (43.60)	0.0001	0.07	*2.18*

(a,b,c) Original data means according to Ln X. a, b, c. Scripts with similar letters do not differ for P<0.05 (LSD Fisher).

In all the variables evaluated in trial II, the normal distribution assumption was accepted (p<0.05). However, it was necessary to change the number of leaves at 60 days into the square root of X (Table 3).

Table 3. Statistical characteristics in Trial II database

Variable	n	Mean	SD	W*	P (single tail)
Stem length at 60 days (cm)	50	21.56	6.12	0.94	0.1071
Number of leaves at 60 days	50	14.28	5.28	0.97	0.5034
The square root of the number of leaves at 60 days	50	3.71	0.73	0.97	0.4976
Dry aerial biomass at transplantation	50	33.60	3.46	0.95	0.1386

Table 4 shows the the morphophysiological behavior of *T. diversifolia* in the nursery, in trial II. The indicator stem length at day 60 and accumulated dry aerial biomass at transplantation showed differences in treatments T2, T3, T4, and T5, with p≤0.05, from T1.

The number of leaves did not differ between T1 and T3, and it was remarkably higher in T2, T4, and T5 (50% sol + 50% IHPLUS®BF enriched compost + *Rhizobium;* 50% soil + 50%IHPLUS®BF enriched compost + micronized dolomite + *Rhizobium*; and 50% soil +50% IHPLUS®BF enriched compost + micronized dolomite + *Trichoderma).*

Table 4. Behavior of morphophysiological indicators of *T. diversifolia* in the nursery

Indicators	Treatments					P≤0.05	SE±	*SE+ %*
	T1	T2	T3	T4	T5			
Stem length at 60 days (cm)	13.11b	24.62a	23.27a	23.21a	23.60a	0.0001	0.87	4.05
Number of leaves at 60 days	3.05c (9.60)	4.02a (16.60)	3.38bc (11.80)	3.90ab (15.40)	4.20a (18.00)	0.0004	0.10	2.69
Dry aerial biomass at transplantation (g)	28.59b	34.88a	34.64a	34.77a	35.11a	0.0001	0.49	1.46

(a,b,c) Transformed original data means according to Ln X. a, b, c. Scripts with similar letters do not differ for P<0.05 (LSD Fisher).

The results corroborated the effectiveness of the different fertilizer combinations in the two trials. The positive influence of the biofertilizers analyzed in this study may have been related to the richness of substances in the biochar and compost, both enriched with IHPLUS®BF microorganisms, dolomite (with high tenors of magnesium carbonate), biofertilizers *Rhizobium* and *Trichoderma*, which can fix nitrogen and produce vitamins, organic acids, chelates, and antioxidant substances, lead to quick decomposition of macromolecules, and stimulate plant growth at rates comparable to the one produced by inorganic fertilization. It was corroborated in trials by Pentón *et al*. (2021), in sorghum (*Sorghum bicolor* (L.) Moench), morera (*M. alba*), leucaena (*L. leucocephala*), habichuela [*Vigna unguiculata* (L.) Verdc.], and potato (*Solanun tuberosum* L.).

Rhizobacteria are growth promoters that increase the plant’ s vigor, stem growth, as well as root development. The presence of microorganisms in optimum amounts of organic matter improves the biostructure, favoring the addition of particles to the soil. Garro (2016) said that the inoculation of microorganisms increases the biodiversity of the microbial biota and improves the natural balance of soils, generating populations of microorganisms that suppress pathogens. They speed up the decomposition of organic matter by increasing microbial activity (Martínez *et al*., 2019). Moreover, because of the antioxidant effects they have, they can generate pest suppression mechanisms in plants by inducing systemic tolerance to phytopathogens and insect pests.

Leucaena and tithonia are known to have the capacity to be associated with combinations of beneficial microorganisms like phosphorus and *Azospirilum* solubilizing bacteria, adding more information about these species to improve highly impoverished soil fertility (Méndez *et al*., 2022).

When plant re-shooting and initial growth are higher than the ones observed in the controls, it suggests us that the organic arrangement used can be considered a plant nutrient or plant stimulant. This study indicated, for instance, the compatibility of species *Tithonia diversifolia* with the addition of IHPLUS®BF enriched biochar. This organic material can store between 0.75 and 1.75 mL IHPLUS®BF per embedded grain, with a slightly basic pH, near neutrality, and a redox potential nearing 400 mV, which is considered appropriate for plant growth (Pentón *et al*., 2022).

The high porosity and broad surface area of biochar facilitate the absorption of liquid, semi-liquid, and gaseous substances (Orésiga *et al.*, 2021), which can be observed for water retention, up to six-fold its weight (Schmidt *et al*., 2014). This feature is one of the many that explain its potential to improve the substrate’ s structure, reduce abiotic stress by moisture excess or water shortage, and become a nutrient hub.

Schmidt *et al*. (2014) conducted 21 field trials using fertilizers containing cow’ s urine-enriched biochar in the root of 13 different crops. The biochar-enriched substrates produced better yields than the controls without biochar and biochar-only. The authors attributed these results to the effect of biochar as an inducer of slow nutrient release with more balanced flows and a reduction of losses due to lixiviation or emission of gasses into the atmosphere.

Studies conducted in Cuba using biofertilizers based on biochars from different origins and enriching substances identified as promising materials obtained from sickle bush wood wastes using microorganisms IHPLUS®BF, MORERA with IHPLUS®BF microorganisms or urine, Leucaena with urine, and sugarcane bagasse with IHPLUS®BF or urine (Pentón *et al*., 2020b).

When mixed with the soil and a 1:2 proportion (biochar: soil), they enhanced the physical and chemical features of the rhizosphere and led to the conservation of optimum indicators of potassium and magnesium (Mg), particularly biochar, mulberry (*Morus alba*), calcium (Ca), and phosphorus (P), with a remarkable contribution of biochar from sugarcane bagasse (*Saccharum officinarum* L.); pH, organic matter, and an optimum redox potential Eh(pH7)[1], (Pentón *et al*., 2021; Fernández *et al.*, 2023).

In studies of cassava (*Manihot esculenta Crantz*), the application of biofertilizer at 700 g/m^2 [Biochar inoculated with an IHPLUS ® BF solution (1.5 v), plus cow' s urine (0.5 v), plus water (1 v), combined with compost], produced a significant increase in crop yields compared to chemical fertilization alone (Pentón *et al*., 2020a).

Schulz and Glaser (2012) observed a study of *Avena sativa* L growth for two periods in tropical conditions, on low fertility sandy soil, where the application of pure compost produced the best yields, followed by the compost + biochar combination. The addition of biochar to mineral fertilization increased plant growth significantly in comparison to fertilization alone. During the second period, compost and biochar increased the carbon content in the soil significantly. The addition of compost raised the cationic exchange capacity, biochar increased the saturation of bases, perhaps due to the presence of ashes. It did not reduce ammonium, nitrate, or phosphorus lixiviation, though it reduced nitrification. Overall, plant growth and fertility in the second year decreased as follows: compost > compost + biochar > chemical fertilization + biochar > chemical fertilization > control (unenriched soil).

The analysis of physiological indicators at 30 days indicated that the concentration of chlorophyll in the leaves, the nitrogen-relative content, flavonols, and anthocyanins were within logical values. It means that these values varied within the reports for arboreal species, such as *Gmelina arborea* Roxb, with 17.32-25.45 units *SPAD;* cherry *(Prunus avium* L.*)*, with 23.3-35.7 Dualet units; flavonols with 1.29-2.0; NBI with 18.4-23.4 units (Palma, 2021; Montenegro, 2020); and apple trees (*Malus domestica* Borkh), with 34.35-36.78 µg/cm² chlorophyll; 14.50-15.74 flavonol units (relative absorbance); NBI with 22.1-25.6 Dualet units (Valdebenito, 2020).

The biochar enriched using nutritious substances favored the physiological indicators determined by the nondestructive method of Dualet clip. It was demonstrated in nurseries on sugarcane biocharcoal substrate with water and mulberry substrate with IHPLUS®BF, with a higher correlation for mulberry to plant height with flavonols (0.85), and anthocyanin in the leaves (-0.82) in sorghum. The highest correlation was observed between the dry aerial biomass and chlorophyll (0.84).

The content of chlorophyll has a favorable relation with the photosynthetic rate, the relative content of nitrogen, crop yield, and crop productivity (Del Pozo *et al.*, 2016 and Barrantes Madrigal, 2018). Meanwhile, the relative nitrogen content is usually determined by $N\text{-}NO^3$ in the sap, and the levels of chlorophyll, as a direct estimation.

Today, reflectance and fluorescence are used in specific regions of the spectrum for the diagnostic of the state of nitrogen in the plants. The relative content of nitrogen NBI® is known to be directly related to the content of mass nitrogen, and it is less sensitive than chlorophyll to variations in the environmental variations (Cerovic *et al*., 2012). Rivacoba *et al*. (2014) demonstrated that the relative nitrogen content (NBI®) can detect the variations of total nitrogen in the leaves better under nitrogen fertilization deficiency.

The secondary metabolism of plants can also be detected using reflectance and fluorescence. Secondary metabolites, particularly phenolic compounds, are part of the molecular mechanisms of tolerance to nutrient and water shortages (Negrão *et al.*, 2017 and Gao *et al.*, 2018). They are powerful antioxidants and could contribute to restoring the cellular redox state (Sobia *et al.*, 2013; Jain *et al.*, 2015; Kendir and Köroğlu, 2015). Flavonoids constitute a group of polyphenols that include six major classes: chalcones, flavones, flavonols, flavanols, anthocyanins, and condensed tannins. Flavonols are mainly synthesized after exposure to light. Consequently, they are a good indicator of the history of plant-light interactions. Anthocyanins are closely related to the removal of reactive oxygen (RO), which is one way to lessen the impact of these reactive radicals on cellular components (Mervat and Dawood, 2014).

Polyphenols play an important defensive role when plants undergo oxidative stress derived from environmental factors, in terms of moisture deficit or excess, nutrient availability, or soil salinity. the concentrations of these antioxidants tend to vary from one site to another (Lattanzio, 2013), which increases their tenors as an antioxidant response triggered by stress. In that sense, Pérez *et al.* (2016) observed henequen plants (*Agave fourcroydes* cultivar ‘ Sac Ki’), which under water stress, reduced their chlorophyll content, while increasing soluble phenols, terpenes, flavonoids, and anthocyanins.

CONCLUSION

The results confirmed the effectiveness of such alternatives for the cultivation of *T. diversifolia* plants in the nursery.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: YLM, GMF, ABR, GPF, OBM, IPE; data analysis and interpretation: YLM, GMF, ABR, GPF, OBM, IPE; redaction of the manuscript: YLM, GMF, ABR, GPF, OBM, IPE.

CONFLICT OF INTEREST STATEMENT

The authors state there are no conflicts of interest whatsoever.