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EFFECT OF EICHHORNIA CRASSIPES COMPOST ON THE QUALITY OF THEOBROMA CACAO NURSERY PLANTS

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Abstract. The study consisted of determining the effect of *E. crassipes* compost on the quality of *T. cacao* nursery plants, knowing the appropriate compost percentages for its addition as substrate and determining levels of lead (Pb), arsenic (As) and mercury (Hg) in *E. crassipes* plants, compost and *T. cacao* plants. A completely randomized design was used, with 5 treatments, 5 repetitions with 48 plants each, in an experimentation period of 4.5 months. Compost of *E. crassipes* was used in percentages of 10%, 20% and 40% mixed with soil in 90%, 80% and 60%. The treatments were identified as: T1 (T10: 90), T2 (T20: 80), T3 (T40: 60), a relative treatment T4 (TR, 100% soil and chemical fertilization) and a control treatment T5 (TT, 100 % land). The results of the ANOVA show that there is a statistically significant difference of the treatments in the quality of *T. cacao* plants for the diameter, Dickson's Quality Index (DQI) and total dry weight. The T1, T2, T3 and T4 were statistically superior to the control treatment T5 (TT). Plant height, slenderness index, and aerial dry weight-root dry weight ratio did not present statistically significant differences. The translocation of Pb to *T. cacao* plants was non-existent. The plants of T3 (T40: 60), showed chlorotic yellowing and symptoms of diseases in the 45 to 90 days of germination. It is recommended to use percentages of compost of *E. crassipes* not greater than 20%, for *T. cacao* plants in the nursery.

Keywords: *E. crassipes* compost, Cerrón Grande, quality of the *T. cacao* plants, heavy metals.

EFEECTO DEL COMPOST DE EICHHORNIA CRASSIPES EN LA CALIDAD DE PLANTAS EN VIVERO DE THEOBROMA CACAO

Resumen. El estudio consistió en determinar el efecto del compost de *E. crassipes* en la calidad de las plantas en vivero de *T. cacao*, conocer los porcentajes de compost apropiados para su adición como sustrato y determinar niveles de plomo (Pb), arsénico (As) y mercurio (Hg) en plantas de *E. crassipes*, compost y plantas de *T. cacao*. Se utilizó un diseño completamente al azar, con 5 tratamientos, 5 repeticiones con 48 plantas cada uno, en un período de experimentación de 4.5 meses. Se usó compost de *E. crassipes* en porcentajes de 10%, 20% y 40%

mezclados con tierra en 90%, 80% y 60%. Los tratamientos se identificaron como: T1 (T10:90), T2 (T20:80), T3 (T40:60), un tratamiento relativo T4 (TR, 100% tierra y fertilización química) y un tratamiento testigo T5 (TT, 100% tierra). Los resultados del ANOVA demuestran que existe diferencia estadística significativa de los tratamientos en la calidad de las plantas de *T. cacao* para el diámetro, Índice de Calidad de Dickson (ICD) y peso seco total. Los T1, T2, T3 y T4, fueron estadísticamente superiores al tratamiento testigo T5 (TT). La altura de planta, índice de esbeltez, y relación peso seco aéreo-peso seco radicular, no presentaron diferencia estadística significativa. La traslocación de Pb a plantas de *T. cacao* fue inexistente. Las plantas del T3 (T40:60), mostraron amarillamiento clorótico y síntomas de enfermedades en los 45 a los 90 días de germinadas. Se recomienda usar porcentajes de compost de *E. crassipes* no mayores al 20%, para plantas de *T. cacao* en vivero.

Palabras clave: *compost de E. crassipes*, Cerrón Grande, calidad de *Theobroma cacao*, metales pesados.

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Abstract. The study consisted of determining the effect of *E. crassipes* compost on the quality of *T. cacao* nursery plants, knowing the appropriate compost percentages for its addition as substrate and determining levels of lead (Pb), arsenic (As) and mercury (Hg) in *E. crassipes* plants, compost and *T. cacao* plants. A completely randomized design was used, with 5 treatments, 5 repetitions with 48 plants each, in an experimentation period of 4.5 months. Compost of *E. crassipes* was used in percentages of 10%, 20% and 40% mixed with soil in 90%, 80% and 60%. The treatments were identified as: T1 (T10: 90), T2 (T20: 80), T3 (T40: 60), a relative treatment T4 (TR, 100% soil and chemical fertilization) and a control treatment T5 (TT, 100 % land). The results of the ANOVA show that there is a statistically significant difference of the treatments in the quality of *T. cacao* plants for the diameter, Dickson's Quality Index (DQI) and total dry weight. The T1, T2, T3 and T4 were statistically superior to the control treatment T5 (TT). Plant height, slenderness index, and aerial dry weight-root dry weight ratio did not present statistically significant differences. The translocation of Pb to *T. cacao* plants was non-existent. The plants of T3 (T40: 60), showed chlorotic yellowing and symptoms of diseases in the 45 to 90 days of germination. It is recommended to use percentages of compost of *E. crassipes* not greater than 20%, for *T. cacao* plants in the nursery.

Keywords: *E. crassipes* compost, Cerrón Grande, quality of the *T. cacao* plants, heavy metals.

Introduction

Water hyacinth (*E. crassipes*) is an aquatic plant that grows rapidly in water reservoirs (Alvarado, 2013). It is considered a perennial aquatic weed (Madsen and Robles, n.d.), and ranked among the 100 most harmful invasive alien species in the world (Lowe et al., 2004). According to the Food and Agriculture Organization of the United Nations (FAO, 1996), *E. crassipes* globally causes more serious and widespread problems than any other floating aquatic weed. Rzedowski and Rzedowski (2005) mention that *E. crassipes* spreads in such a way that it covers the mirrors of canals and other water reservoirs. Its abundance and volume in these bodies of water makes fishing and navigation impossible. According to Barrett and Forno (1982), the origin of *E. crassipes* seems to be the Brazilian Amazon with natural spread to other areas of the South American continent and introduced by human action to Central America and the Caribbean. The Aquatic Ecosystem Restoration Foundation (AERF, 2014), indicates that *E. crassipes* has been widely introduced in all tropical regions of the world, most recently in Lake Victoria, East Africa.

In El Salvador, the Cerrón Grande reservoir (with geographical coordinates 14° 03" N and 89° 04" W), constitutes the largest continental freshwater body (Ministry of Environment and Natural Resources [MARN], 2016), with a covered area of 135 km², built for the Cerrón Grande Hydroelectric Power Plant dam, with a nominal capacity of 135 MW, and an average

annual generation of 488 GWh (Hydroelectric Executive Commission of Río Lempa [CEL], n.d.). One of the preponderant problems in the Cerrón Grande reservoir is the presence and expansion of *E. crassipes*, which affects various economic sectors such as the fishing industry, tourism, and navigation (Alas, R. March 25, 2016). Also, it could affect the turbines for hydroelectric generation of the Cerrón Grande dam ("Endangered natural treasures, Cerrón Grande reservoir", 2016). MARN (2019) calculated a coverage of 3,000 hectares of *E. crassipes*, which is equivalent to 22.22% of the reservoir.

Sonter et al. (2018) mention that compost has recently gained acceptance for integrated solid waste and aquatic weed management, labeled as an environmentally friendly product and a sustainable solution for urban waste and aquatic weed management. In this regard, Mashavira et al. (2015), evaluated the effect of *E. crassipes* on tomato (*Lycopersicon esculentum*), on growth attributes, yield potential, and the accumulation of heavy metal levels of lead (Pb), copper (Cu), nickel (Ni), and zinc (Zn) in tomato fruit; while Enriquez (2013), studied the effect of compost based on *Eichhornia crassipes* on lettuce "*Lactuca sativa*," on the variables survival, yield in weight (kg), lettuce head diameter and concentration of the heavy metals, lead (Pb) and arsenic (As). As part of the control of *E. crassipes* in the Cerrón Grande reservoir, in the period from November 2019 to March 2020, 17.34 hectares were manually and mechanically harvested using *E. crassipes* harvesting barges (CEL, 2020). The Local Economic Development Agency and the Environmental Investment Fund of El Salvador (ADEL-FIAES, 2014) proposed making compost from *E. crassipes* plants extracted from the Cerrón Grande reservoir as one of the alternatives for its sustainable use and management.

In this context, this study was developed by preparing compost of *E. crassipes* from the Cerrón Grande reservoir, and its effect on the quality of cocoa plants (*Theobroma cacao*) in the nursery stage was evaluated. *T. cacao* is one of the species with high ecological and economic potential that CEL produces in the forest nurseries of its hydroelectric power plants to reforest the Lempa River basin. According to the Ministry of Agriculture and Livestock (MAG, 2018), due to the socio-environmental importance that this crop represents, in El Salvador, there is a "policy for the development of the cocoa chain." Therefore, the production of quality *T. cacao* plants in nurseries is essential for the success of the plantation.

According to Arteaga et al. (2003) the type of soil or substrate used in plant production is one of the factors that most influences the quality and cost of plant production in nurseries, so it is necessary to have alternatives to reduce costs, guaranteeing a certain plant quality. Prieto (2004), points out that an appropriate nursery process requires cultural practices related to substrates, containers, fertilizers, mycorrhizae, irrigation, prevention and control of pests and diseases.

Plant quality depends on the genetic characteristics of the germplasm and the techniques used for its reproduction (Prieto et al., 2009). The parameters height and diameter are considered estimators of plant performance after planting (Mexal and Landis, 1990) and can be easily quantified (Birchler et al., 1998). In addition, morphological attributes such as the slenderness index, which relates plant height and diameter, the ratio of the dry weight of the aerial and root parts, and the Dickson quality index, which is calculated by the ratio between the total dry weight of the plant and the sum of the slenderness index and the aerial part-root part ratio (Navarro et al. , 2006, Birchler et al., 1998, Dickson et al., 1960, cited by Mateo-Sánchez et al., 2011), are also used.

The objectives of the research were to determine the effect of compost from *E. crassipes* plants extracted from the Cerrón Grande reservoir on the quality of *T. cacao* plants at the nursery stage, through the parameters of plant height, diameter, slenderness index, ratio of the dry weight of the aerial part, and dry weight of the root part (PSa/PSr), as well as the Dickson Quality Index (DQI); in addition, to determine the percentages of *E. crassipes* compost most appropriate to mix with soil for *T. cacao* production at the nursery stage and finally, to evaluate

the levels of heavy metals such as As, Pb, and Hg in fresh *E. crassipes* plants, in the compost obtained, and in the *T. cacao* plants to analyze the existence of translocation of these metals.

Method

The extraction of *E. crassipes* plants from the Cerrón Grande reservoir was carried out on December 06 and 07, 2018, in the Santa Teresa canton, municipality of Potonico, department of Chalatenango, at the location coordinates 13°57'48.10" N and 88°54'36.51" W, at a distance of 3.0 km in a northwest direction from the Cerrón Grande Hydroelectric Power Plant. For its extraction, quadrats of 1 m long x 1 m wide (1 m²) were made with PVC pipes of 1" diameter. The number of plants per m² recorded in the quadrats in each extraction was counted, they were left to drain for 3 minutes and then the plant mass of each quadrat was weighed. Next, parts of stems, leaves, and roots were taken from 100 fresh *E. crassipes* plants obtained from the quadrats by random sampling, for analysis of the heavy metals Pb, As, and Hg, and taken to the laboratory of the Salvadoran Foundation for Social and Economic Development (FUSADES). Pb and As were analyzed using the atomic absorption spectrophotometry method with a graphite furnace, and Hg was analyzed using the atomic absorption spectrophotometry method with a hydride generator. After extraction, the *E. crassipes* plants were transferred for composting to the site where the experimental design was implemented, located at coordinates 14° 05' 44.28" N and 89° 15' 20.08" W, in the sub-basin of the Jayuca River, in the village of La Cruz, canton Santa Rosa, municipality of Nueva Concepción, department of Chalatenango. Then, at the composting site, the *E. crassipes* plants were chopped in an agricultural machine used to chop grass. The chopped plant material was placed in the form of a mound 5.70 m long, 1.90 m wide, and 0.90 m high, on a 5 cm thick layer of unchopped *E. crassipes* plants to avoid contact with the soil, then the mound was covered with a plastic sheet. During the first six weeks, the mound was turned by hand to provide aeration twice a week, and then once a week for the following eleven weeks. During the last two weeks, the composted material was spread in a 30 cm thick layer, leaving it without a cover during the day, and at night it was placed again. At the end of this process, the *E. crassipes* compost was weighed and stored in sacks for the maturation process for 5 months before being used as a substrate for the production of *T. cacao* plants in the nursery stage, and a chemical analysis of Pb was performed. During the composting process, the temperature of the material and the relative humidity of the environment were measured daily. The composting period was from December 09, 2018 to April 21, 2019. The cultivation of *T. cacao* in the nursery stage was implemented from September 29, 2019 to February 23, 2020. Figure 1 shows the extraction of *E. crassipes* and composting.



Figure 1. A) Extraction of *E. crassipes* from the Cerrón Grande reservoir. B) Composting.

For the implementation of the study, a completely randomized experimental design was used with five treatments, five replications, and 48 plants per replication. The sample size of *T. cacao* plants for destructive analysis in the laboratory to determine the dry weight (g) was estimated with the formula:

$$n = \frac{t^2 * S^2}{e^2}$$

Where:

n: sample size.

S²: variance.

t² = Student's t at 95% confidence.

e² = error (5%). The error value is multiplied by the average, before being squared, thus increasing the number of samples to be processed.

The result showed a minimum of 121 plants to be sampled using the height variable; however, a total of 250 plants were sampled (50 for each treatment and 10 plants per replication), thus ensuring a representative sampling. Statistical analyses were performed with Statistix 8.1 software, student version. One-way analysis of variance (ANOVA), with a significance level of 0.05 was used. Significant differences in the means of the treatments were obtained with Tukey's statistical test.

In the test, compost made from *E. crassipes* from the Cerrón Grande reservoir was used in percentages of 10%, 20%, and 40%, mixed with soil in 90%, 80%, and 60% to complete 100% of the volume of the bags (8"x12") where the *T. cacao* seeds were planted. The 5 treatments were identified as follows: T₁ (10:90), T₂ (20:80), T₃ (40:60), a relative treatment T₄ (TR, 100% soil, and 3 chemical fertilizations with triple formula fertilizer 15 N-P-K, at 15, 45, and 90 days after germination, using two grams in the first fertilization and five grams in the second and third fertilization) and a control treatment T₅ (TT, 100% soil) without any additive.

Pest and disease control was carried out preventively. For soil insect control, Imidaclopid WG 0.8% was used, applied to the soil 15 days before planting *T. cacao* seeds. For foliar pest control, the insecticides Abamectin (1.8 EC) and Lambdacyhalothrin (2.5 EC) were used. Prevention of fungal diseases was done with Difenconazole, Azoxystrobin (32.5 SC) applied every 10 days. The *T. cacao* plants in the nursery were protected with shade using 73% saran mesh. They were arranged in 4 rows at a width of 0.50 m and 12 plants per row at a length of 1.56 m for each repetition. The width of the rows was 0.60 m x 0.65 m.

When the *T. cacao* plants reached 4 months and 15 days after germination, a destructive analysis of the plants, stem, leaves, and roots was carried out to measure the dry weight (g). Following the methodology of Fernandez, et al. (2010), the plants in the center of each replicate were sampled to avoid edge effect. Each plant was measured for diameter at the base of the stem (mm) with a vernier and height (cm) with a tape measure. The soil was separated from the plants using water to leave the root bare. The root was cut and the stems with leaves and roots were placed separately in plastic bags, identified for each treatment and each repetition, obtaining 25 samples of stems with leaves and 25 samples of roots. They were put to dry for 72 hours through the oven method at 50 °C. Once dried, the weight of the stem with leaves, root dry weight, and total dry weight were measured. This test was carried out at the Agricultural Chemistry Laboratory of the National Center for Agricultural and Forestry Technology "Enrique Álvarez Córdova" (CENTA), located in San Andrés, La Libertad, El Salvador. Taking as a reference the methodology of Fonseca et al. (2002), described by Piña and Arboleda (2010), the slenderness index (SI) was estimated with the data obtained from the laboratory, according to equation 1, and the Dickson Quality Index (DQI), with equation 2, as detailed below:

$$SI = \frac{\text{Height of the aerial part (cm)}}{\text{Stem diameter (mm)}} \quad \text{Eq. 1.}$$

$$DQI = \frac{\text{Total dry weight (g)}}{\frac{\text{Height of the aerial part (cm)}}{\text{Stem diameter (mm)}} + \frac{\text{Aerial dry weight (g)}}{\text{Root dry weight (g)}}} \quad \text{Eq. 2.}$$

According to Prieto et al. (2009) the slenderness index is an indicator of the plant's resistance to wind desiccation, its survival and growth in dry sites. Its value should be less than six; higher values indicate that the plant has a thinner stem in relation to its size. The Dickson Quality Index (DQI) groups variables related to plant quality. The higher the index value, the better the plant quality. Biomass correlates with plant survival and growth in the field. The ratio of aerial part to root part was calculated as the quotient between the dry weight of the aerial part in grams and the dry weight of the root in grams (PSa/PSr). Ratios above 2.5 indicate disproportion and the existence of an insufficient root system to provide energy to the aerial part of the plant (Prieto et al., 2009). In addition, *T. cacao* plants grown at T₃ (T40:60) were sampled to evaluate if there was translocation of Pb from the substrate to the nursery plants.

Due to the fact that during the cultivation process of *T. cacao* plants it was observed that in the 5 replicates of T₃ (T40:60) and at a non-significant level in T₂ (T20:80) some *T. cacao* plants first presented a progressive leaf discoloration turning chlorotic yellow and then showed symptoms of disease in the foliage, it was decided to perform a foliar analysis on plants of the T₃ treatment (T40:60) taken from the 5 replicates. The analysis was carried out at the FUSADES laboratory, San Salvador, El Salvador. On the other hand, in the Plant Parasitology laboratory of CENTA, an analysis of the presence of pathogens in *E. crassipes* compost was carried out in T₅ (TT) and T₃ (T40:60) in order to determine the pathogens that caused the disease. The Potato Dextrose Agar (PDA) method was used as culture medium, under strict asepsis in a laminar flow chamber with ultraviolet light. Soil and substrate were homogenized and 10g were taken. Dilutions of 1/10,000 and 1/100,000 were prepared and 1 ml of each dilution was taken and placed in glass petri dishes sealed with parafilm tape containing PDA culture medium. The petri dishes were placed in an incubator at 22°C for 5 to 8 days, until fungal and bacterial growth was observed. For the identification of the microorganisms present, a sample of each of the fungi and bacteria grown in the petri dish was taken with a deception needle through a stereoscopic microscope and placed on a slide containing a drop of sterile water, covered with a coverslip and placed in a compound microscope where each of the fungal genera present in the samples was identified (R.F. de Serrano, personal communication, May 24, 2021).

Results

The overall mean plant height of *T. cacao* plants was 42.92 cm and a coefficient of variation of 13.35. T₁ reached the highest mean with 46.34 cm, followed by T₄ with 44.46 cm, T₂ with 43.37 cm, and T₃ with 41.78 cm. The control or T₅ registered the lowest mean with 38.65 cm (Table 1). The analysis of variance showed no statistical difference between the means at a significance level of 0.05 (Table 2).

Table 1
Results of Tukey's test for statistical differences of means.

Treatment	Variables studied		
	Height (cm)	Diameter (mm)	Slenderness index
T1 (T10:90)	46.34±4.28 _a	5.00±0.32 _{ab}	9.62±0.71 _a
T2 (T20:80)	43.37±4.39 _a	4.97±0.35 _{ab}	8.98±0.43 _a
T3 (T40:60)	41.78±8.98 _a	5.07±0.50 _a	8.39±1.22 _a
T4 (TR)	44.46±3.72 _a	4.71±0.42 _{ab}	9.82±0.92 _a
T5 (TT)	38.65±5.64 _a	4.18±0.59 _b	9.45±0.85 _a

Note: Means with different letters within a column are significantly different. ($p < 0.05$), according to Tukey's test.

The overall mean plant diameter was 4.78 mm and a coefficient of variation of 9.45. T₃ recorded the highest mean with 5.07 mm, followed by T₁ with 5.00 mm, and T₂ with 4.97 mm. Treatments T₄ and T₅ recorded the lowest means with 4.71 mm and 4.18 mm, respectively (Table 1). A statistical difference was found at a significance level of 0.05 between the means of the treatments (Table 2). Tukey's test shows that the means of treatments T₁, T₂, and T₄ are statistically equal, T₃ and T₅ are different, where T₃ is the best and T₅ of least quality.

In the slenderness index, lower values reflect better quality plants. The overall mean of the slenderness index, which is the result of dividing the height and diameter at the root collar, was 9.25 and a coefficient of variation of 9.40. T₄ with a mean of 9.82, T₁ with a mean of 9.62, and the control T₅ with a mean of 9.45 presented the highest slenderness index. While treatments T₂ and T₃ reached the lowest means, with 8.98 and 8.39 individually (Table 1). The analysis of variance showed that there was no statistical difference between the means at a significance level of 0.05 (Table 2).

Table 2
Analysis of variance to determine statistical differences in the variables studied.

Variable	Source of variation	GL	Sum of squares	Mean square	F	P
Height (cm)	Treatment	4	169.01	42.25	1.29	0.30NS
	Error	20	656.57	32.82		
	Total	24	825.58			
Diameter (mm)	Treatment	4	2.66	0.66	3.26	0.03*
	Error	20	4.09	0.20		
	Total	24	6.75			
Leanness Index	Treatment	4	6.52	1.63	2.15	0.11NS
	Error	20	15.14	0.75		
	Total	24	21.67			

Note: NS= Statistically non-significant and, *= Statistically significant.

Figure 2 shows the measurement of the height, diameter, and samples of *T. cacao* plants for dry weight analysis to determine the quality variables of the plants.



Figure 2. A) Height measurement of *T. cacao* plants. B) Diameter measurement.

The overall mean of the dry weight (g) aerial part-dry weight (g) root part (PSa/PSr) ratio was 10.11 and a coefficient of variation of 18.37. The treatments with the highest mean were T4, T1, and T5, which recorded a mean of 10.86, 10.26, and 10.10 g, respectively. Treatments T3 and T2 had the lowest means with 9.86 and 9.51 g, each (Table 3). Values above 2.5 indicate disproportion between the aerial part and the root part; therefore, according to these results, there is no balance among the relationship between these two variables. According to the analysis of variance, there is no statistical difference between the means at a significance level of 0.05 (Table 4).

Table 3

Results of Tukey's test for statistical differences of means.

Treatment	Variables studied		
	PSa/PSr (g)	PSt (g)	ICD
T1 (T10:90)	10.26±1.13 _a	91.20±13.36 _a	4.57±0.37 _a
T2 (T20:80)	9.51±2.00 _a	83.51±12.33 _{ab}	4.52±0.51 _{ab}
T3 (T40:60)	9.86±2.11 _a	83.91±12.98 _{ab}	4.63±0.65 _a
T4 (TR)	10.86±2.11 _a	84.73±13.31 _{ab}	4.15±0.90 _{ab}
T5 (TT)	10.10±1.74 _a	62.36±15.10 _b	3.22±0.86 _b

Note: Means with different letters within a column are significantly different ($p < 0.05$), according to Tukey's test.

The overall mean of the total dry weight variable was 81.14 g and a coefficient of variation of 16.58. Plant biomass is associated with plant survival and growth in the field. T₁ registered the best mean with 91.20 g. In second order are the treatments T₄, T₃, and T₂ that recorded means of 84.73 g, 83.91 g, and 83.51 g, individually. Lastly, there is T₅ with a mean of 62.36 g (Table 3). According to the analysis of variance, there is a significant statistical difference between the means of the treatments. According to Tukey's test, the means of treatments T₂, T₃, and T₄ are statistically equal, T₁ is statistically different from the other treatments, being the best, and T₅ is the worst with the lowest mean, so that the plants of T₁ with the best mean weight may have a higher percentage of survival in the plantation than those of T₅, which had the lowest mean weight.

The overall mean of the Dickson Quality Index was 4.22 and the coefficient of variation was 16.40. This index integrates the variables of plant quality, total plant dry weight (g), slenderness index (SI), and the ratio of aerial part dry weight - root dry weight (PSa/PSr); higher

values denote better quality plants, which may have more success in surviving in the sites where they are established in the field. T₃ registered the best mean with 4.64 DQI, followed by treatments T₁ with 4.57 DQI, and T₂ with 4.52 DQI. Fourth place was occupied by treatment T₄ with a mean of 4.16. The lowest mean was recorded by T₅ with 3.23 (Table 3). The results of the analysis of variance show that there is a significant statistical difference between the means of the treatments (Table 4). According to Tukey's test, the means of treatments T₂ and T₄ are statistically equal, as well as T₁ and T₃, with the best averages and T₅ with the lowest average.

Table 4

Analysis of variance to determine statistical differences in the variables studied

Variable	Source of variation	GL	Sum of squares	Mean square	F	P
Ratio of aerial part-root part (g)	Treatment	4	5.07	1.26	0.37	0.82NS
	Error	20	69.07	3.45		
	Total	24	74.15			
Total dry weight (g)	Treatment	4	2400.26	600.06	3.32	0.03*
	Error	20	3620.02	181.00		
	Total	24	6020.28			
Dickson Quality Index (DQI)	Treatment	4	6.86	1.71	3.58	0.02*
	Error	20	9.59	0.47		
	Total	24	16.45			

Note: NS= Statistically non-significant and, *= Statistically significant.

Regarding the heavy metals evaluated, Pb recorded an average of 3.54 ppm in fresh plants of *E. crassipes*. Hg and As were not detectable (Figure 3). For this reason, only Pb was analyzed in the *E. crassipes* compost, but it was no longer detectable (0.00 ppm). In spite of not detecting it in the compost samples analyzed, in order to fulfill the objective related to analyzing whether there is translocation of Pb, an analysis of this element was carried out in the *T. cacao* plants grown in treatment T₃, with a higher percentage of *E. crassipes* compost; according to the tests, it was not detectable either.

Due to the problems of progressive leaf discoloration turning chlorotic yellow and then symptoms of disease in *T. cacao* plants at T₃, a foliar analysis of nutrients was performed on the plants of that treatment. The results were compared with the reference levels used in the Agricultural Chemistry Laboratory of CENTA for the cultivation of *T. cacao*, based on Methods of Analysis of Soil Plants, Water, and Fertilizers. Fertilizer Development and Organization, 1999 (G. L. Enriquez, personal communication, 24 May 2021). N registered 3.18%, P 0.16%, K 1.32%, Ca 0.55%, Mg 0.20%, and S 0.24%. Fe presented 90.63 ppm, Cu 3.55 ppm, Mn 55.78 ppm, Zn 18.91 ppm, and B registered 67.52 ppm. N presented a higher level than recommended between 2.00% - 2.50%. The nutrients P, Mg, Cu, and Zn presented low levels, being the sufficient level 0.18% for P, 0.45% for Mg, between 8.0 ppm - 12.0 ppm for Cu, and between 20.0 - 100.0 ppm for Zn; while the nutrients K, Fe, Mn, and B presented sufficient levels: 1.30% - 2.2%, 60 - 200 ppm, 50 - 300 ppm, and 25 - 70 ppm, respectively (Table 5).

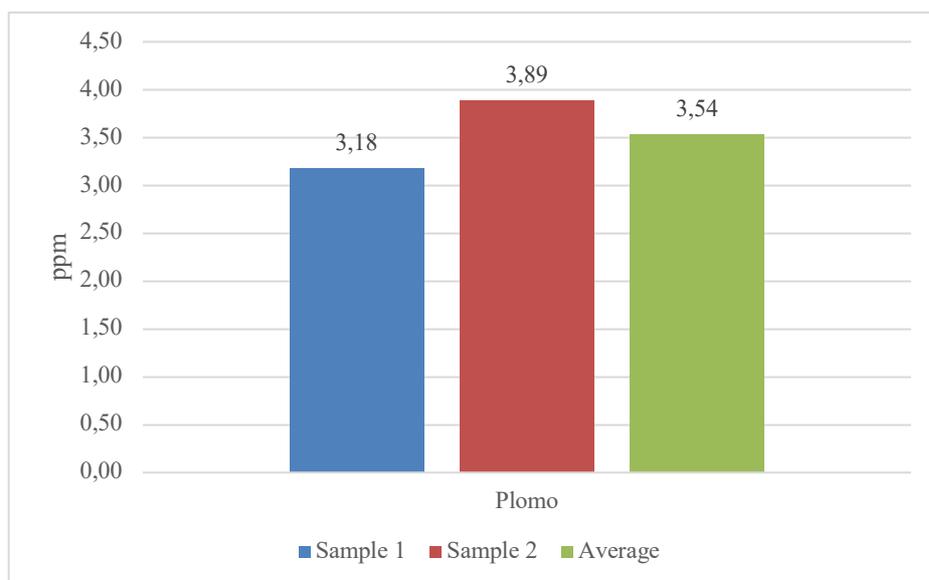


Figure 3. Results of Pb analysis in fresh *E. crassipes* plants.

Table 5

Foliar analysis of nutrients in T. cacao plants of the T₃ treatment (T40:60).

Element	Results	Unit	Low level	Sufficient level	High level
Total nitrogen	3.18	%	1.8 - 1.99	2.0 - 2.5	> 2.5
Phosphorus	0.16	%	0.13 - 0.18	> 0.18	
Potassium	1.32	%	1 - 1.29	1.3 - 2.2	> 2.2
Calcium	0.55	%	0.3 - 0.49	> 0.40	-
Magnesium	0.20	%	0.2 - 0.49	> 0.45	-
Sulfur	0.24	%	-	-	-
Iron	90.63	ppm	50 - 59	60 - 200	> 200
Copper	3.55	ppm	4 - 7	8 - 12	> 12
Manganese	55.78	ppm	22 - 49	50 - 300	> 300
Zinc	18.91	ppm	18 - 19	20 - 100	> 100
Boron	67.52	ppm	12 - 24	25 - 70	> 70

Note: Source: Results obtained at the FUSADES laboratory and reference level data used by the CENTA Agricultural Chemistry laboratory for cocoa cultivation, based on Methods of Analysis of Soil Plants, Water, and Fertilizers, Fertilizer Development and Organization, 1999 (G. L. Enriquez, personal communication, May 24, 2021).

Pathogens in E. crassipes compost at T₅ (TT) and T₃ (T40:60).

In the *E. crassipes* compost, four genera of fungi were found: *Aspergillus sp*, *Penicillium sp*, *Rhizopus sp*, *Sclerotium sp*; and one species of bacteria, which was not identified (Table 6). In T₅, two genera of fungi *Aspergillus sp* and *Fusarium sp*; and one bacterial species, which could not be identified, were recorded. On the one hand, in the substrate of T₃ (T40:60), seven genera of fungi *Aspergillus sp*, *Penicillium sp*, *Fusarium sp*, *Pythium sp*, *Cladosporium sp*, *Rosellinia sp*, and *Trichoderma sp* were found (Table 6). On the other hand, *Pythium sp* was found in the stem of the plants grown in T₃, and unidentified bacteria were present in the root and leaves. According to the results, the fungi that affected *T. cacao* plants in T₃ were *Fusarium*

sp, *Pythium sp*, and *Rosellinia sp*, the latter being the one with the highest incidence and the one that caused the most damage. In advanced stages, it manifested in the aerial parts causing yellowing, wilting, leaf drop, and dieback. This is consistent with that described by Phillips-Mora and Cerda (2011) and Alarcón et al. (2012), on the symptoms of the fungus *Rosellinea pepo* on *T. cacao* plants.

Table 6

Pathogens identified in E. crassipes compost, T₅ (TT), T₃ (T40:60), and in plants.

Fungal/bacterial genera	<i>E. crassipes</i> compost	T ₅ (TT)	T ₃ (T40:60) and plants	Observation
<i>Aspergillus sp</i>	x	x	x	Contaminant
<i>Penicillium sp</i>	x		x	Contaminant
<i>Fusarium sp</i>		x	x	
<i>Pythium sp</i>			x	
<i>Cladosporium sp</i>			x	
<i>Rosellinia sp</i>			x	Principal damage
<i>Trichoderma sp</i>			x	Antagonistic
<i>Rhizopus sp</i>	x			Contaminant
<i>Sclerotium sp</i>	x			
<i>Bacteria*</i>	x	x	x	

Note: Source: Results from CENTA parasitology laboratory.

The x indicates the presence of the pathogen. *without identifying its genus.

Discussion and conclusions

Total plant height (cm). Although the results of *T. cacao* plant height was superior in the percentages of *E. crassipes* compost substrate (T₁, T₂, and T₃), no significant difference was found compared to the control treatment T₅. Similar results were reported by González (2018), who found no statistical difference for the height of *T. cacao* clones in his study where he tested treatments of organic fertilizers, bokashi, and earthworm humus, at 15, 45 and 90 days after planting. Lliuya (2015) found a significant difference in the same variable in the treatments where he used guinea pig manure, compost, and chicken manure in the cultivation of *T. cacao* seedlings. Likewise, Ramírez et al. (2013) presented better results for the treatment with a mixture of soil, sand and guinea pig manure, where *T. cacao* plants reached 34 cm in height in the saran cover and 35 cm in the plastic cover after 120 days.

Diameter (mm). In the present investigation, the diameter means were statistically superior in the treatments with percentages of *E. crassipes* compost substrate (T₁, T₂, and T₃), with respect to the control treatment T₅, with T₃ being the best. These data coincide with those reported by Lliuya (2015), who found significant difference in the diameter of *T. cacao* plants at 120 days for the organic fertilizer treatments compost, chicken manure, and guinea pig manure. Ramirez et al. (2013) also present better results for the treatment with a mixture of soil, sand, and guinea pig manure, where *T. cacao* plants recorded 6.89 mm of thickness in the saran cover and 8.72 mm in the plastic cover at 120 days. The opposite case revealed by González (2018), where the diameter of plants of *T. cacao* clones showed no statistical difference in the treatments of organic fertilizers, bokashi, and earthworm humus, at 15, 45 and 90 days after planting.

Slenderness index. Lower values of this index indicate better quality plants. The means of treatments T₂ and T₃ presented the lowest values; in spite of this, there was no significant difference between treatments, including the control T₅. Similar results were found by Tut Si (2014), in his study with nursery plants of *Tabebuia donnell-smithii* (cortez blanco) produced in the substrates lombri-composite, sand, soil, and chicken manure, in different proportions and mixtures, where no significant difference was found among treatments.

Total dry weight (g). A significant difference was found in the treatments with percentages of *E. crassipes* compost T₂ and T₃ and the relative treatment T₄, obtaining similar effects among them; T₁ was superior to all the treatments, and T₅ was the one with the lowest weight. Mateo-Sánchez et al. (2011) found a significant difference ($p < 0.05$) for total dry weight in the production of *Cedrela odorata* (cedar) plants in nursery based on raw sawdust substrate mixed with peat moss-agrolite-vermiculite, registering the best result in plants grown with the 60% sawdust treatment, followed by the 70% sawdust treatment.

Dickson Quality Index (DQI). The treatments with *E. crassipes* compost T₃ with a mean of 4.63 DQI, T₁ with 4.57 DQI, and T₂ with 4.52 DQI, presented the best means of Dickson quality index (DQI), compared to T₅, finding a significant statistical difference. Mateo-Sánchez et al. (2011) obtained a significant difference ($p < 0.01$) for the DQI in an experiment for the production of *Cedrela odorata* (cedar) plants in nursery based on raw sawdust substrate mixed with peat moss-agrolite-vermiculite. The best values were recorded for the treatments with 70%, 90%, and 60% sawdust. These data agree with those found in this study, where the treatments with a higher percentage of compost were superior to the control T₅.

Chemical analysis of heavy metals Pb, As, and Hg in E. crassipes plants prior to composting, Pb in compost, and in T. cacao plants. Pb recorded an average of 3.54 ppm in *E. crassipes* plants. Meanwhile, Hg, and As were not detectable; agreeing with those reported by ADEL-FIAES (2014), in samples of *E. crassipes* in 3 locations of the Cerrón Grande reservoir, which found between 2.41 and 3.0 ppm of Pb and also did not detect Hg and As. In turn, MARN (2012) in a leaf analysis sample of *E. crassipes* in the Metapán lagoon found 0.81 ppm of Pb, 2.26 ppm of As, and did not detect Hg. Coinciding in the presence of Pb, and the non-detection of Hg, in the plants of *E. crassipes* in both water bodies. According to NCh2880 (2004), the maximum level of trace elements in raw material for composting (mg/kg dry basis) for Hg is 10 mg/kg and for Pb 800 mg/kg. Therefore, the *E. crassipes* plants sampled in the Cerrón Grande reservoir meet the requirements for use as raw material for composting, with regard to Pb, which registered 3.54 ppm, and Hg, which was not detectable.

Foliar analysis and pathogens. According to the results of the foliar analysis and presence of pathogens, it is possible that when using a greater amount of *E. crassipes* compost, certain nutrients by excess or deficiency may cause a negative effect on plant development, as was observed in T₃ where 40% of *E. crassipes* compost was used, showing chlorotic yellowing of the leaves of *T. cacao* plants at the beginning of their development and then the presence of diseases. This could also be due to the presence of a greater number of pathogen species in this treatment, contrary to what was observed in the decrease of affected plants as less *E. crassipes* compost was used in treatments T₂ and T₁; therefore, it is recommended to use percentages of *E. crassipes* compost that do not exceed 20%. The symptoms of wilting or chlorosis that generally appear in cocoa nurseries are mainly due to fungal attack (Suárez, et al., cited by Pérez-Martínez, et al., 2017). On the other hand, it is common to find symptoms not caused by phytopathogens, but to nutritional deficiencies, excesses, or imbalances, manifested as chlorosis in whole plants, mottled chlorosis of the interveinal spaces of the leaves, deformation of the leaf lamina, decrease in leaf size, among others (Enriquez, 1985; Hardy, 1961, cited by Pérez-Martínez, et al., 2017), which agrees with the findings of this study.

A positive effect of the use of *E. crassipes* plant compost on the quality of *T. cacao* plants in the nursery stage was observed for diameter, total dry weight and Dickson Quality

Index (DQI). This last index is the most comprehensive of all, it was found that treatments T₁ and T₃ recorded the highest means, being statistically equal to each other and superior to the control treatment T₅, which obtained the lowest average and, therefore, plants of lower quality. In the variables height, slenderness index, aerial dry weight/root dry weight ratio, there was no statistical difference between the means of the treatments.

The treatment with the highest percentage of compost, T₃, obtained the highest mean with respect to the Dickson Quality Index. However, from 45 to 90 days of development of *T. cacao* plants in the nursery, plants were affected by chlorosis at the beginning and then by disease, possibly when using a greater amount of *E. crassipes* compost, certain nutrients caused by excess or deficiency caused this effect. It could also be due to the presence of more species of pathogens in this treatment, being the fungi *Fusarium sp*, *Pythium sp*, and *Rosellinia sp*, which most affected the plants; contrary to what was observed in the decrease of affected plants as less compost was used. Considering that T₃ presented symptoms probably due to the attack of phytopathogens, or due to nutritional imbalances, or both, it is recommended that the percentage of *E. crassipes* compost most appropriate to be used for the production of *T. cacao* in the nursery phase should not exceed 20%, according to this research.

According to the findings, no translocation of lead (Pb) to *T. cacao* plants was found. Likewise, *E. crassipes* from the samples in the Cerrón Grande reservoir complies with the requirements of the NCh2880 standard (2004) for use as raw material for composting, with respect to the trace elements Pb, which registered 3.54 ppm, and Hg and As, which were not detectable.

The results obtained from the research show that the use of *E. crassipes* compost from the Cerrón Grande reservoir has a positive effect on the quality of *T. cacao* plants in the nursery stage; and it was determined that the most appropriate percentages of *E. crassipes* compost to be mixed with soil for the production of *T. cacao* in the nursery stage should not exceed 20%. It was also determined that the heavy metal lead (Pb) recorded in the fresh plants of *E. crassipes* was not identified in the compost, nor in the *T. cacao* plants, so there was no translocation of this metal.

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