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Research article

Implementation of a nutritional system for the development of *Pinus cembroides* Zucc. in *Uruapan, Michoacán*

Implementación de un sistema nutricional para el desarrollo de *Pinus cembroides* Zucc. en Uruapan, Michoacán

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Abstract

Pinus cembroides is a pine tree of arid and semi-arid zones of Mexico, which belongs to the piñon pine group, which produces edible seeds for humans. In this work it is proposed that the application of organic amendments can promote the development of a pine plantation. The calculation of the amendment doses was made according to the specific nutrient requirements of *P. cembroides* plants, considering maximum doses for the treatments assessed under a randomized block design: 241 g of sheep manure, 241 g of *bocashi*, 48 mL of fish extract, 22 g of humic acids+26 mL of fish extract and 13 g of Nitrofoska perfect® per plant, applied every three months during one year, where five morphological variables were analyzed and a Slenderness index was determined. The effect of treatments and covariate were analyzed through an ANCOVA analysis of covariance, where significant differences between treatments were found for basal diameter (P=0.002), while the effect of the covariate was significant for basal diameter, plant height, number of shoots and Slenderness index (P<0.0001). An ANOVA analysis of variance was also performed for two additional variables, which indicated significant differences for the branch with the most outstanding length (P=0.02). It was concluded that amendments based on humic acids plus fish extract and *bocashi* supplemented with calcium sulfate were the most appropriate to promote the growth of *P. cembroides*.

Keywords: Humic acids, *bocashi*, amendments, fish extract, nut pine, forest plantations.

Resumen

Pinus cembroides es un pino de zonas áridas y semiáridas de México perteneciente al grupo de los piñoneros, los cuales producen semillas para consumo humano. En este trabajo se plantea que la aplicación de enmiendas orgánicas puede promover un mejor desarrollo de una plantación de piñoneros. El cálculo de las dosis de enmienda se hizo acorde con la necesidad de nutrientes específica para la especie, con base en las dosis máximas para los cinco tratamientos evaluados bajo un diseño de bloques al azar: 241 g de estiércol de borrego, 241 g de *bocashi,* 48 mL de extracto de pescado, 22 g de ácidos húmicos+26 mL de extracto de pescado y 13 g de *Nitrofoska perfect*® por planta, aplicadas cada tres meses durante un año. Se analizaron cinco variables morfológicas y se generó un Índice de Esbeltez. El efecto de los tratamientos y la covariable se analizaron a través de un análisis de covarianza ANCOVA, mediante el cual se determinaron diferencias significativas entre tratamientos para diámetro basal (P=0.002); el efecto de la covariable fue significativo para diámetro basal, altura de planta, número de brotes e Índice de Esbeltez (P<0.0001). Asimismo, se realizó un análisis de varianza ANOVA para dos variables adicionales, que indicó diferencias significativas para la rama de mayor longitud (P=0.02). Se concluye que las enmiendas a base de ácidos húmicos más extracto de pescado y *bocashi*, complementadas con sulfato de calcio son las más apropiadas para promover el crecimiento de *P. cembroides.*

Palabras clave: Ácidos húmicos, *bocashi*, enmiendas, extracto de pescado, piñonero, plantaciones forestales.

Introduction

The pinyon pine (*Pinus cembroides* Zucc.) is distributed from the Southwestern United States, Northern and Central Mexico to the state of *Veracruz* (Perry, 1991). This pine plays a role in the water cycle, contributes to reducing soil erosion, and provides habitat for wildlife (Comisión Nacional Forestal [Conafor], 2007). Its seed (pinyon) is considered a staple food for various species of birds and mammals. The species inhabits rugged, dry, stony landscapes with calcareous soils of low fertility and can withstand extreme droughts (Constante et al., 2009).

The species is slow-growing and has a high potential for adaptation to adverse conditions. It thrives in a wide range of ecological conditions, at altitudes between 1 500 and 2 800 m, with rainfall between 400 and 800 mm and average temperatures between 0 and 22 °C (Perry, 1991). Low temperatures are favorable for cone production (Conafor, 2007). It is also considered an optimal species for reforesting arid and semi-arid areas of Mexico (Perry, 1991; Zárate-Castrejón et al., 2021).

Successful results in a forest plantation require good management throughout the entire production chain, which implies knowledge of the origin and quality of the germplasm, as well as proper management during the nursery stage to enhance its growth and development (Muñoz et al., 2015). Nutrition is perhaps the factor that can be improved by anthropogenic intervention through chemical soil analysis, seeking proper soil nutrient management and, if necessary, applying amendments that help improve its physical and chemical quality (Rojas, 2015).

However, before applying any type of fertilizer (organic and/or inorganic), it is essential to first determine the soil's physical and chemical characteristics to determine nutrient availability and deficiencies (Solis-Charcopa et al., 2017).

Although the nutrient content of organic fertilizers is low, they have a high value due to the benefits they provide to soil physical conditions (Organización de las Naciones Unidas para la Alimentación y la Agricultura [ONUAA], 2002).

In Mexico, a new form of food production is being implemented in small agricultural areas, where fruit crops are intercropped with timber trees, cornfields, or other sustainably managed crops. This results in a positive shift in the pursuit of generating economic resources, while also strengthening environmental services (Cano, 2024). To achieve this goal, it is necessary to study other alternatives that could be ideally adapted to this production concept (Secretaría del Bienestar, 2021).

In this sense, studies related to nutritional management with organic materials are necessary to define practices that seek to reverse the negative impact caused by agricultural activity based on obtaining maximum yields. Given that the use of amendments is a possible alternative, the objective of this study was to evaluate the application of different organic amendments on the growth and development of a *P. cembroides* plantation, as an alternative for the recovery of degraded soils and, in turn, as a source of income for commercial pine nut producers. It was hypothesized that the application of organic amendments increases the growth and development of field-established *P. cembroides*.

Materials and Methods

Study area

The research was carried out in the Forest Area of the *Presidente Juárez* Experimental Station of the Agrobiology Graduate School of the *Michoacana* University of *San Nicolás de Hidalgo* (*UMSNH*, for its acronym in Spanish) in the city of *Uruapan*, state of *Michoacán*, Mexico. The *Pinus cembroides* plantation was established in an area of 1 126.1 m^2, located between 19°22’28.7’’ and 19°22’34.8’’ N and -102°1’30.8’’ and -102°1’40.3’’ W (Figure 1).

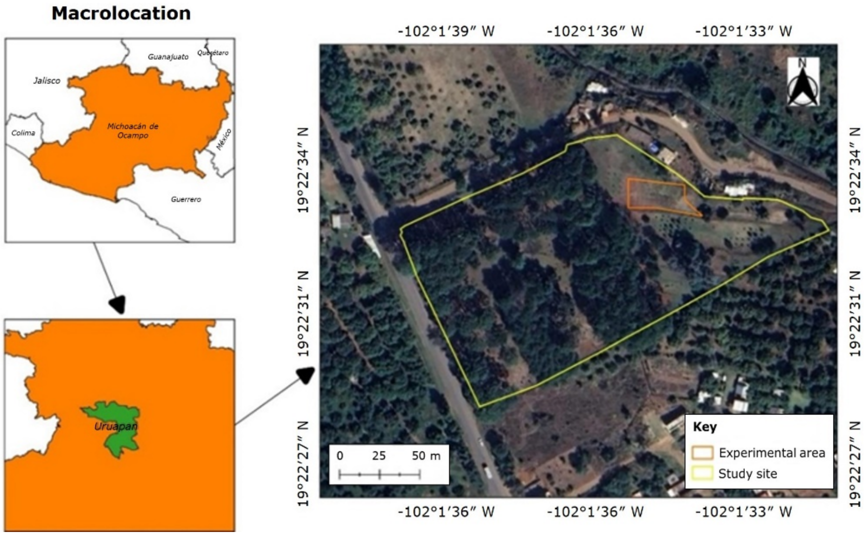


Figure 1. Geographic location of the *Pinus cembroides* Zucc. plantation in the Forest Area of the Experimental Station of the *Presidente Juárez* Graduate School of Agrobiology, *Uruapan*, *Michoacán*, Mexico.

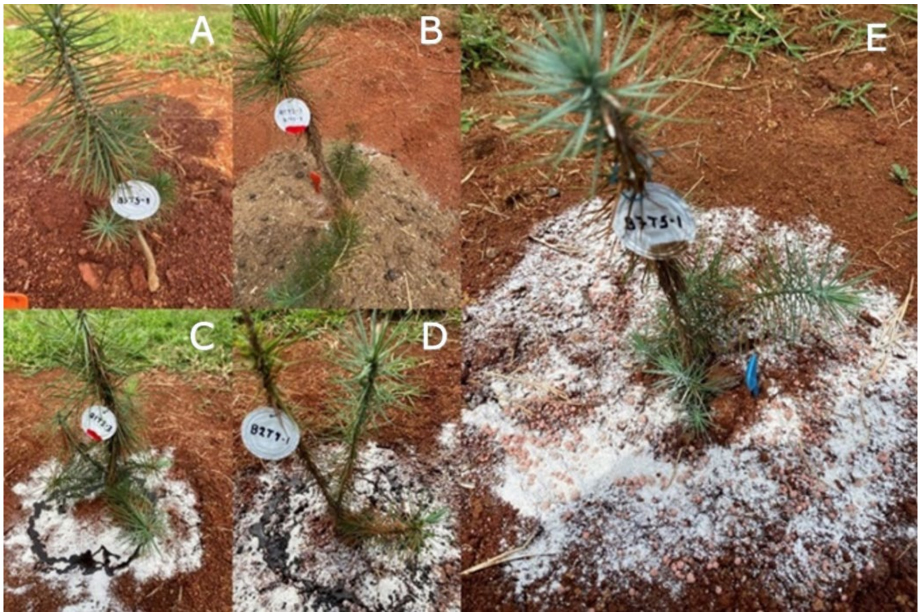
According to the meteorological records of the *Uruapan* climatic station for the period 1970-1999 (Instituto Municipal de Planeación Uruapan [Implan], 2021), the rainy season occurs during the months of May to October, with an accumulated annual average of 1 508 mm. The average annual maximum temperature was 29.7 °C, with an average annual temperature of 19.6 °C and an annual minimum temperature of 9.6 °C (Implan, 2021).

Soil Analysis

Two compound soil samples were taken from the experimental plot: the first in 2022, before the establishment of the *P. cembroides* plantation, and the second in 2023, one year after the treatments were applied. The procedure described in the Mexican Official Standard NOM-021-RECNAT-2000 (Secretaría de Medio Ambiente y Recursos Naturales [Semarnat], 2002) was followed. The samples were analyzed in the Fertilab® laboratory, where chemical parameters such as nitrogen, phosphorus, calcium, potassium, magnesium, sulfur, organic matter, pH, electrical conductivity, and Cation Exchange Capacity were determined. The amount of amendment for each treatment was calculated from the results of the initial soil analysis.

Treatment components

To define the treatments, the nutrient content of *bocashi* and sheep manure was also analyzed. Based on their content and the nutrient deficit in the soil, plus the addition of sheep manure, fish extract, and a combination of 46 % humic acids plus 54 % fish extract, five treatments were developed and applied: (A) *Bocashi*, (B) sheep manure, (C) Fish extract, (D) A combination of 46 % humic acids plus 54 % fish extract, and (E) Nitrofoska perfect® chemically synthesized fertilizer supplemented with calcium sulfate (*Agroblanca*®, Mexico) (Figure 2).



A = *Bocashi*; B = Sheep manure; C = Fish extract; D= Humic acids plus fish extract; E = Nitrofoska perfect® supplemented with calcium sulfate.

Figure 2. Application of treatments to the experimental unit (plant).

The amount of organic matter required in the soil is high (Table 1) to achieve this parameter at an average level, equivalent to 6.1 % according to NOM-021-RECNAT-2000 (Semarnat, 2002). Applying the required amounts of any of the materials to increase organic matter to an average level would imply an excess of nutrients that could cause an imbalance in the soil (Olivares-Campos et al., 2012).

Table 1. Amount of organic matter required to raise organic matter to an average level of 6.1 % according to the deficit of this indicator.

Source of organic matter	Carbon content (%)	Amount (kg ha^{-1})
Bocashi	43.4	651 857.14
Sheep manure	83.2	340 031.25
Fish extract	41.4	683 347.83

Humic acids+fish	45.35	623 828

Every three months, the equivalent of 25 000 kg ha^{-1} of sheep and *bocashi* manure, 5 000 L ha^{-1} of fish extract, and 2 300 kg ha^{-1} plus 2 700 L ha^{-1} of a combination of humic acids and fish extract were applied, representing a fraction of the required soil organic matter in the area of interest. These amounts were considered maximum for these amendments and for gradual soil recovery (Table 2). All amendments were supplemented with calcium sulfate ($CaSO_4$) due to their varying calcium content.

Table 2. Amendment treatments applied in a *Pinus cembroides* Zucc. plantation, designed based on maximum application of organic amendments.

	Description	Amount per plant	Amount ha^{-1}
1.	*Bocashi*+$CaSO_4$	241 g+38 g	25 000+3 950 kg ha^{-1}
2.	Sheep manure+$CaSO_4$	241 g+44 g	25 000+4 592 kg ha^{-1}
3.	Fish extract+$CaSO_4$	48 mL+48 g	5 000 L ha^{-1}+5 000 kg ha^{-1}
4.	Humic acids+fish extract+$CaSO_4$	22 g+26 mL+48 g	2 300 kg ha^{-1}+2 700 L ha^{-1} +4 984 kg ha^{-1}
5.	Nitrofoska perfect®+$CaSO_4$	13 g+48 g	1 300+4 981 kg ha^{-1}

The treatments were applied for the first time on October 17th, 2022, and on three additional occasions: January 16th, April 17th and July 17th, 2023, in the area near the stem that covered the circumference of the area of interest (Figure 2).

The plantation was established on October 4th, 2022, with two-year-old plants, using a real-frame design (Ramírez et al., 2021), with plant spacing of 3 m, and on 35 cm wide by 35 cm deep scions. The seven blocks and their treatments were delimited randomly, with an identification card attached to each plant.

Experimental design and study variables

The experimental design was a randomized block design with seven replications, with five treatments plus a control. A total of 175 samples were planted, of which 49 were discarded due to the edge effect, resulting in 126 plants for the analyses. Each experimental unit consisted of three pines; this latter value was established to meet the assumptions of normal distribution and homoscedasticity of variances. Furthermore, the sample size of three trees per experimental unit, in the case of tree fertilization, is considered acceptable (Kiessling-Davison et al., 2007).

Data were collected every three months for one year (October 2022 to October 2023); the variables were: plant height (cm) with a graduated ruler (model 675, TKM® brand), basal diameter (mm) with a Vernier caliper (Analog model, Lion® brand) (Figure 3), with these the Slenderness index was determined, which represents the relationship between height (cm) and diameter (mm) (Gerding et al., 2006; Montes-Rivera et al., 2001), which is an indicator of resistance to desiccation, survival and potential growth of the plant in dry sites, whose value must be less than six (Prieto et al., 1999); the number of shoots (Venegas-González et al., 2016) (Figure 3), and on the last measurement date, the average crown diameter (cm) (Viveros-Viveros et al., 2005) and the longest branch (cm) (Sáenz-Romero et al., 1994) were obtained with a graduated ruler (model 675, TKM® brand).

A = Basal diameter (mm); B = Height (cm).

Figure 3. Data collection of morphological variables in *Pinus cembroides* trees.

Statistical analysis

After analyzing and checking the normality of the data using the Kolmogorov-Smirnov and Shapiro-Wilk tests, as well as the homogeneity of variances using the Levene test (Brown & Forsythe, 1974), an ANCOVA analysis of covariance was performed on the morphological variables of the experimental unit means, using the PROC GLM procedure in the SAS® statistical package version 9.4 (Statistical Analysis Systems [SAS], 2023). Plant height, basal diameter, Slenderness index, and number of shoots were used as covariates, and the Tukey test was performed when statistical significance was found (α=0.05).

The statistical model (Equation 1) employed was as follows:

$$Y_{ijkl} = \mu + T_i + B_j + Y\left(X_{ijk} - \bar{X}\right) + E_{ijkl} \qquad (1)$$

Where:

Y_{ijkl} = Response variable associated with treatment i, in the complete block j, replication k, and error l

μ = General mean

T_i = Effect of treatment i

B_j = Effect of block j

Y = Regression coefficient associated with the covariate

X_{ijk} = Value of the covariate for that experimental unit

$\bar{X}$ = Overall average of the covariate

E_{ijkl} = Experimental error associated with the measurement of treatment i, in block j, replication k

For the variables average crown diameter and longest branch, an ANOVA was performed on the means of the experimental units using the PROC GLM procedure in the SAS® statistical package version 9.4 (SAS, 2023). In cases where statistical significance was found, the Tukey test was performed (a=0.05).

The statistical model (Equation 2) used was as follows:

$$Yij_k = \mu + t_i + B_j + E_{ijk} \qquad (2)$$

Where:

Yij_k = Response variable associated with treatment *i*, in the complete block *j*

μ = General mean

T_i = Effect of treatment *i*

B_j = Effect of the complete block *j*

E_{ijk} = Experimental error associated with the measurement of treatment *i*, in replication *j*

Because the assessed did not meet the assumptions of normality or homoscedasticity of variance, other analysis options were explored with nonparametric data: (A) Ranges for each variable, (B) Ranges of total growth for each observation, and (C) the average total growth for each experimental unit. For the latter two, the initial data were subtracted from the final data to exclude the initial state of the pines as a covariate and a simple ANOVA was performed. All the options explored yielded the same results; the ANCOVA analysis of the experimental unit means presented in this research paper was selected because it outperformed the other options by obtaining the highest R^2 values and lower Coefficient of variation (*CV*) values. Furthermore, the assumptions of normality and homoscedasticity of the means were met, and the original data were analyzed instead of the ranges.

Results

Soil analysis

The results of the analysis indicated that the dominant soil type was Humic Andosol (Th), with high nutrient deficiencies due to the extraction of the first horizons (Table 3).

Table 3. Analysis of the chemical parameters and amount of organic matter available in the soil of the *Pinus cembroides* Zucc. plantation.

Parameter	Result	Interpretation
1. Organic matter	0.22 %	Very low
2. Nitrogen	3.75 ppm	Low
3. Phosphorus	0.94 ppm	Very low
4. Calcium	510 ppm	Low
5. Potassium	223 ppm	Medium
6. Magnesium	144 ppm	Low
7. Sulfur	29.4 ppm	Very low
8. pH	7.66	Moderately alkaline
9. Electrical Conductivity	0.07 dS n	Very low
10. Cation Exchange Capacity	4.33	Very low

Soil organic matter content

The results of the laboratory analysis conducted after one year of treatment application report an increase in the organic matter indicator for all treatments (Table 4). The highest value was obtained with the treatment based on humic acids+fish extract+$CaSO_4$, with 1.86 % organic matter content.

Table 4. Organic matter content present in the soil of a *Pinus cembroides* Zucc. plantation after one year of treatment with organic amendments.

Treatment	***OM* Content (%)**
1. *Bocashi*+$CaSO_4$	1.17
2. Sheep manure+$CaSO_4$	1.27
3. Fish extract+$CaSO_4$	0.66
4. Humic acids+fish extract+$CaSO_4$	1.86
5. Nitrofoska perfect®+$CaSO_4$	0.47
6. Control	0.64

OM = Percentage of organic matter.

Growth and development of *Pinus cembroides*

The analysis of covariance indicated highly significant differences between treatments for the basal diameter variable (F=5.03, P=0.002), while height (F=0.99, P=0.44), shoots (F=1.46, P=0.24), and Slenderness index (F=1.16, P=0.35) did not show such differences between treatments. The variables used as covariates were significant (P<0.05) and highly significant (P<0.01) for all four variables evaluated (Table 5).

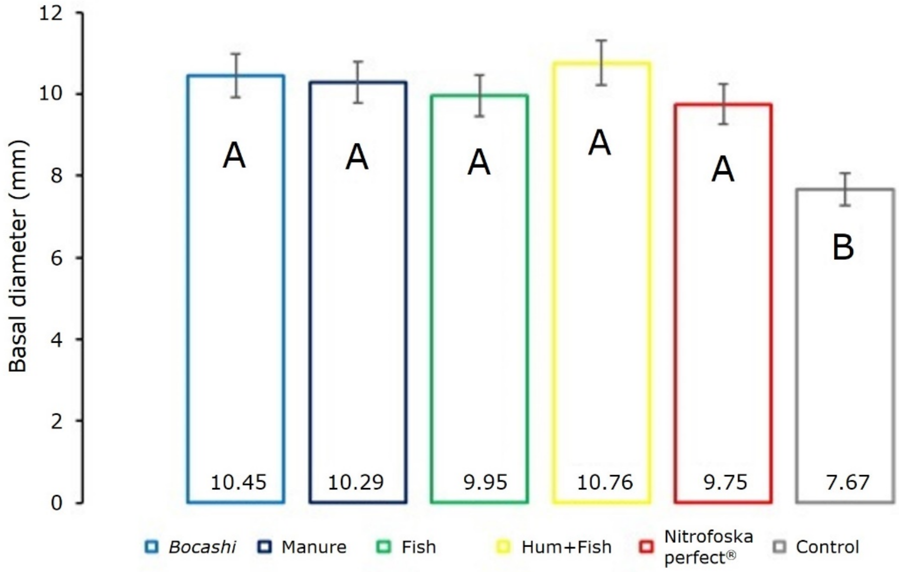
Table 5. Analysis of covariance (ANCOVA) between treatments and initial covariates of *Pinus cembroides* Zucc. from the Forest Area of the *Presidente Juárez* Graduate School of Agrobiology (*UMSNH*) Experimental Station.

Variable	**Mean squares**		***MSError***	***R²***	***CV***
	Treatments	**Covariate**			
BD	3.72**	24.06**	0.74	0.88	8.77
A	8.28 ns	165.7**	8.37	0.91	9.99
NB	14.48 ns	94.7**	9.95	0.71	22.42
IE	0.29 ns	1.9*	0.25	0.62	16.51

BD = Basal diameter (mm); *A* = Height (cm); *NB* = Number of shoots; *IE* = Slenderness index; *MSError* = Mean square error; R^2 = Coefficient of determination; *CV* = Coefficient of variation; *Significant (α=0.05); **Highly significant (α<0.01); ns = Non- significant.

The analysis of covariates showed that the initial physiological state of the pines had a greater influence than the application of the treatments on the final growth and development of *Pinus cembroides* in three of the four variables: height (F=19.79, P<0.0001), shoot number (F=9.52, P=0.005), and Slenderness index (F=7.63, P=0.01). For the basal diameter variable, using the initial basal diameter of the pines as a covariate, the analysis of covariance indicated highly significant differences between treatments (P=0.002), as well as for the covariate (F=35.51, P=0.0001).

According to the results of the Tukey test (α=0.05), all amendment treatments increased the basal diameter compared to the control, which allowed for the formation of a single statistical group (Figure 4). It is worth noting that the amendment based on humic acids plus fish extract showed a 40.3 % increase in basal diameter compared to the control, while the mineral amendment based on Nitrofoska perfect® obtained a 27.1 % increase in basal diameter with respect to that obtained by the control, which was the treatment with the lowest average basal diameter (Figure 4).



Bars with the same letters are statistically identical (Tukey, 0.05).

Figure 4. Organic amendments and their effect on the basal diameter of *Pinus cembroides* Zucc. in a plantation from the Forest Area of the *Presidente Juárez* Graduate School of Agrobiology (*UMSNH*) Experimental Station.

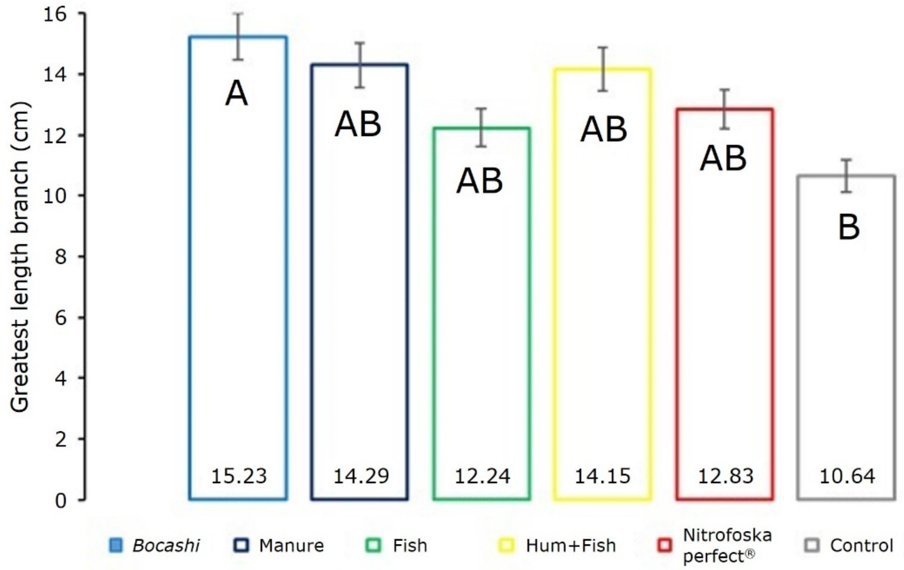
The analysis of variance indicated significant differences between treatments for the longest length branch (F=3.16, P=0.02), while no significant differences were found between treatments for average crown diameter (F=2.52, P=0.052) (Table 6).

Table 6. Analysis of variance (ANOVA) for the evaluation of *Pinus cembroides* Zucc. average crown diameter and longest branch length from the Forest Area of the *Presidente Juárez* Graduate School of Agrobiology (*UMSNH*) Experimental Station.

Variable	**Mean squares**	***MSError***	R^2	***CV***
Crown	10 ns	3.97	0.71	17.25
Branch	21.42*	6.78	0.67	19.5

Crown = Average crown diameter (cm); Branch = Longest length (cm); *MSError* = Mean square error; R^2 = Coefficient of determination; *CV* = Coefficient of variation; *Significant (*α*=0.05); ns = Non-significant.

From the results of the Tukey test (*α*=0.05), the *bocashi*-based amendment increased the longest length branch by 43 % compared to the control, which in turn allowed it to be formed into a single statistical group with the other treatments containing some type of amendment. The fish extract-based and Nitrofoska perfect® treatments had the lowest averages (Figure 5).



Bars with the same letters are statistically identical (Tukey, 0.05).

Figure 5. Organic amendments and their effect on the longest branch of a *Pinus cembroides* Zucc. plantation in the Forest Area of the *Presidente Juárez* Graduate School of Agrobiology (*UMSNH*) Experimental Station.

Discussion

The criteria used to develop the organic amendment treatments showed a significant positive effect on increasing the basal diameter and growth of the longest branch of *P. cembroides*. These criteria gradually increased the soil organic matter content from 0.22 to 1.86 %. Despite the increase in soil organic matter, it still remains very low according to the values published by NOM-021-RECNAT-2000 for agricultural soil (Semarnat, 2002).

However, it is considered that, with the application of the treatments, specifically the treatment of humic acids+fish extract+$CaSO_4$, an average level of 6.1 % organic matter content could be reached over a period of 3.6 years. This result is similar to that reported by Moraga (2021), who concluded that the use of organic amendments increases the soil organic matter content.

The significant effect observed in all treatments (except the control) on the increase in basal diameter in response to the application of all amendments reinforces the interpretation of the results of the soil chemical analysis and the amendments (Ruiz et al., 2005), because the treatments were calculated to optimize tree development based on soil nutrient deficits (Rodríguez et al., 2018), specifically by increasing soil organic matter content.

The increase in basal diameter is consistent with that published in other studies using chemical amendments, such as the one conducted by Vázquez-Cisneros et al. (2018) on *Pinus greggii* Engelm. *ex* Parl., using 7 and 14 g of controlled-release 12-24-12 N-P-K fertilizers; the result was a 9.17 mm increase in basal diameter. In a similar way, Madrid-Aispuro et al. (2020) described an increase in basal diameter when using different mixtures of peat moss, composted bark and pine sawdust plus 3 kg m^{-3} and 6 kg m^{-3} of Multicote 8® 18 % N+6 % P_2O_5+12 % K_2O in the production of *P.*

cembroides seedlings in a nursery, and Hernández et al. (2018) in *Pinus cooperi* C. E. Blanco achieved an increase in basal diameter with the application of more than 7 g of simple calcium superphosphate and with the interaction of this fertilizer and the application of 3.28 and 6.5 g of potassium sulfate. All these studies concluded that organic amendments or chemically synthesized fertilizers that provide nutrients promote an increase in pine stem diameter, regardless of whether they are organic or chemically synthesized (Luna, 2019). Furthermore, studies have shown that the application of organic amendments has a positive effect on soil recovery (organic matter content) and a negative effect if they are chemically synthesized (Navarro & Navarro, 2014).

The average branch length obtained with the *bocashi*-based amendment plus calcium sulfate was the highest compared to the results of the other treatments. This data is very important for *P. cembroides*, since it is considered a very slow-growing species (Perry, 1991; Zárate-Castrejón et al., 2021), but with enormous potential to adapt to adverse conditions, which are uncontrollable for humans, unlike soil fertility, which can be improved with anthropogenic intervention, as demonstrated in the present study.

Branch growth in response to the application of *bocashi*-based amendment coincides with that described by Jaramillo-López et al. (2015), who obtained pine trees 76.7 % taller in plantations used for *Pinus pseudostrobus* Lindl. reforestation in the *Crescencio Morales* community of the Monarch Butterfly Biosphere Reserve, previously fertilized with 25 % *bocashi*; that is, for every kilogram of soil, 250 g of *bocashi* were applied as an amendment in soil preparation.

Statistical differences have been found, with a 49.6 and 30.4 % increase in height in cacao (*Theobroma cacao* L.) plantations, with the respective use of 3 and 1.5 kg of *bocashi* (Condori et al., 2024), and an increase in plant height has been reported in a *Pinus greggii* plantation with the use of 7 and 14 g of a 12-24-12 controlled-release fertilizer formula (Vázquez-Cisneros et al., 2018).

The statistical differences in branch increase in *P. cembroides*, plant height in *P. pseudostrobus* (Jaramillo-López et al., 2015) and *T. cacao* (Condori et al., 2024) with the application of *bocashi*-based amendment differ in that *P. cembroides* is considered a slow-growing species, with 25 cm per year (Zárate-Castrejón et al., 2021), while *T. cacao* can reach 24.3 cm at 15 days in the nursery (Angulo et al., 2021) and *P. pseudostrobus* can grow up to 80 cm per year (Jaramillo-López et al., 2015), highlighting the importance of nutrition systems based on soil analysis and organic matter levels; this is consistent with Rojas's (2015) contributions to achieving greater development in species considered unattractive for establishment.

Conclusions

Treatments based on organic amendments increased the growth of *P. cembroides*, particularly in basal diameter and the extension of the longest branches. Amendments based on a combination of humic acids, fish extract, and *bocashi*, supplemented with calcium sulfate, are the most appropriate for promoting the growth of *P. cembroides*.

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Conflict of Interest

The authors declare no conflict of interest.

Contribution by author

Ramón Torres González: experimental design planning, experiment setup and maintenance, data collection and analysis, and manuscript writing; Patricia Delgado Valerio: experimental design and setup, data review and analysis, and manuscript review; Joel Pineda Pineda: soil analysis interpretation, treatment design, and manuscript review; Ulises Manzanilla Quiñones: experimental setup, data and manuscript review; Martha Elena Pedraza Santos: data and manuscript review; Cuauhtémoc Sáenz-Romero: experimental design, manuscript review.

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