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Efecto de la inoculación de las semillas de Leucaena leucocephala (Lam) de Witt cv. Cunningham con IHPLUS ® BF en la etapa de vivero

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Scientific Paper

Effect of inoculation of seeds from *Leucaena leucocephala* (Lam) de Witt cv. Cunningham with IHPLUS® BF at the nursery stage

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Abstract

Objective: To evaluate the effect of inoculation of seeds from *Leucaena leucocephala* (Lam) de Witt cv. Cunningham with the biofertilizer IHPLUS® BF on seedling growth, development and vigor during the nursery stage.

Materials and Methods: The study was conducted in polyethylene bags with a substrate of soil and organic matter. Five seeds/bag and 10 bags/treatment were used in a complete randomized design. The treatments were formed by the combination of two factors: A) imbibition time and B) dilutions of IHPLUS® BF). Three different times were combined: A) 8, 10 and 28 hours with four dilutions, B) 2,5; 5; 10 and 15 ml L^{-1}) and a control with seeds with heat treatment, thermal scarification and water at 80 ºC for two minutes. Emergence and days to emergence, plant height, stem length, stem diameter, number of leaves and root length were evaluated in each treatment. The quality index classification was used to evaluate the morphological attributes of *L. leucocephala* seedlings.

Results: The combination of seeds with thermal scarification and 28 hours of imbibition in 2,5% IHPLUS® BF resulted in 100 % seedling emergence/day, as well as higher emergence rate. There were significant differences among treatments in plant height, stem length and stem diameter ($p \leq 0,05$).The combination of the thermal scarification method with the bioproduct IHPLUS® BF (2,5 %) showed better results, with values of 116,4 cm, 83 cm and 6,26 mm in height, length and stem diameter, respectively.

Conclusions: In freshly harvested seeds of *L. leucocephala* cv. Cunningham, the combined use of the thermal scarification method, with 28 hours of imbibition in a solution of IHPLUS® BF at a concentration of 2,5 favored the germination and seedling emergence processes in nursery. This allowed an additive effect that was evidenced in the stimulation of growth and vegetative development of this plant.

Keywords: growth, emergence, seedlings

Introduction

Leucaena leucocephala (Lam) de Witt has shown an outstanding performance in the development of animal husbandry in Cuba and other countries, due to its high protein content and good yield, in addition to its high capacity to fix atmospheric nitrogen. Fixations above 500 kg N/ha/year are reported (Hernández-Hernández *et al*., 2020).

Seedling emergence is the phenological event that most influences the success of a plantation. It represents the moment when a seedling becomes independent of the non-renewable seed reserves originally produced by its parents, and is when photosynthetic autotrophism begins.The time of emergence often determines whether a plant competes successfully with other species (González-Valdivia *et al*., 2020). In addition, it is determined by multiple and complex interactions among environmental conditions, soil, and intrinsic characteristics of the seed and seedling (Flores-Romayna *et al*., 2020).

The application of beneficial microorganisms to seeds is an efficient mechanism for the placement of microbial inoculants in the soil, where they will be well positioned to germinate and colonize seedling roots, protecting them against pests and diseases (Tanya-Morocho and Leiva-Mora, 2019).

Based on the above-explained facts, the objective of this study was to evaluate the effect of inoculation of seeds from *L. leucocephala* cv. Cunningham with the biofertilizer IHPLUS®BF on seedling growth, development and vigor during the nursery stage.

Materials and Methods

Location. The study was carried out at the Pastures and Forages Research Station Indio Hatuey (EEPFIH, for its initials in Spanish), located at 22º

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48' and 7'' North latitude and 79º 32' and 2'' West longitude, at 19 masl., in the Perico municipality, Matanzas province, Cuba.

Treatment and experimental design. The treatments (table 1) were formed by the combination of two factors: A) imbibition time which was combined with three different times 8, 10 and 28 hours and B) dilutions of IHPLUS® BF, with four dilutions (2,5; 5; 10 and 15 mL L^{-1}) and a control: seeds with thermal treatment, thermal scarification (water at 80 ºC for two minutes). A complete randomized design with factorial arrangement was used and 10 replicas per treatment.

Table 1. Description of each treatment under study.

Treatment	Description
1	STT+ IHPLUS® BF (2,5 %) 8 horas
2	STT+ IHPLUS® BF (2,5 %) 10 horas
3	STT+ IHPLUS® BF (2,5 %) 28 horas
4	STT+ IHPLUS® BF (5 %) 8 horas
5	STT+ IHPLUS® BF (5 %) 10 horas
6	STT+ IHPLUS® BF (5 %) 28 horas
7	STT+ IHPLUS® BF (10 %) 8 horas
8	STT+ IHPLUS® BF (10 %) 10 horas
9	STT+ IHPLUS® BF (10 %) 28 horas
10	STT+ IHPLUS® BF (15 %) 8 horas
11	STT+ IHPLUS® BF (15 %) 10 horas
12	STT+ IHPLUS® BF (15 %) 28 horas
Control	STT

STT- Seeds treated with thermal scarification in water at 80 °C for two minutes IHPLUS® BF

Experimental procedure. Seeds from *L. leucocephala* cv. Cunningham, harvested in the basic seed area of EEPFIH in March, 2022, were used. They were dried in the legumes for 48 hours under sunlight, threshed and stored at room temperature in woven nylon sacks at 12 % humidity.

A nursery was formed with perforated black polyethylene bags of 1 kg, with a substrate with soil and organic matter (25 % compost-25 % humus and 50 % soil) and 5 seeds/bag were placed; after the seedling emerged, the weakest one was thinned to leave only one per bag (Alfaro and Martinez, 2008). Irrigation was carried out daily at field capacity. Ten bags/treatment were used, for a total of 160 bags.

Measurements. Emergence and days to emergence were evaluated in each treatment. At 75 days after planting, five plants were randomly selected and the following measurements were taken according to Payares-Díaz (2014): plant height, stem length, stem diameter, number of leaves and root length.

Mathematical analysis. To evaluate the morphological traits of *L. leucocephala* seedlings, the Dickson quality index classification (DQI), referred by Sáenz-Reyes *et al*. (2014), was used, whose classification is described in table 2.

Table 2. Classification of quality indices.

Index	Evaluation		
	Low	Medium	High
Dickson quality index (DQI),	< 2	0,2-0,4	>0,5

Data were processed by factorial analysis of variance and means were compared with Duncan's tenth (10) for 5 % significance, after verifying that they met the normal distribution fit (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test). Data processing was performed with the statistical package InfoEstat (Di-Rienzo *et al*., 2017).

Results and Discussion

Table 3 shows the results regarding seedling emergence. As can be seen, better response in terms of emergence and emergence rate was found in the combination of seeds with thermal scarification and with 28 hours of imbibition, since 100 % emergence of seedlings with higher emergence rate was achieved, expressed in emerged plants per day.

Several studies on *L. leucocephala* seed scarification have shown that chemical scarification exerts better results in the emergence percentage (Sánchez-Gómez *et al*., 2018). The most commonly used treatment is immersion in sulfuric acid at different concentrations in different lapses; however, this product could affect the physiological quality of the seed and prevent seedling emergence (Sánchez-Rendón *et al*., 2019).

Sánchez-Gómez *et al*. (2018), when comparing different scarification treatments on *L. leucocephala* seeds to evaluate their effect on germination energy and germination value, found that in seeds of this species scarification by immersion in water at 24 °C for 12 h, and in water at 80 °C for three minutes, favored total germination and increased the rate of this process. These authors also pointed out that pre-germination treatments on *L. leucocephala* seeds in nurseries increased germination by 91,5 %, when immersed for 10 minutes in hot water at 80 ºC.

Table 3. Percentage and emergence speed seedling of *L. leucocephala* cv. Cunningham.

Treatment	Seedling emergence, %	Emergence rate (emerged plants per day)
STT + IHPLUS® BF (2,5 %) 8 hours	80,0[b]	1,2
STT + IHPLUS® BF (2,5 %) 10 hours	76,7[bc]	1,1
STT + IHPLUS® BF (2,5 %) 28 hours	100,0[a]	1,5
STT + IHPLUS® BF (5 %) 8 hours	76,7[bc]	1,1
STT + IHPLUS® BF (5 %) 10 hours	76,7[bc]	1,1
STT + IHPLUS® BF (5 %) 28 hours	86,7[ab]	1,3
STT + IHPLUS® BF (10 %) 8 hours	76,7[bc]	1,1
STT + IHPLUS® BF (10 %) 10 hours	73,3[bc]	1,1
STT + IHPLUS® BF (10 %) 28 hours	63,3[c]	0,9
STT + IHPLUS® BF (15 %) 8 hours	66,7[c]	1,0
STT + IHPLUS® BF (15 %) 10 hours	76,7[bc]	1,15
STT + IHPLUS® BF (15 %) 28 hours	60,0[c]	0,90
STT	73,3[bc]	1,10
SE ±	0,270	0,060
P - value	0,02	0,001

They also considered that this treatment was very positive, since the seeds began to germinate on the fourth day and stabilized after 20 days.

Nursery emergence percentages were higher than those achieved under laboratory conditions. It seems that the environmental conditions to which the seeds were exposed in nursery conditions favored higher seedling emergence. This is an interesting result, since germination in the laboratory is generally higher than seedling emergence under nursery or field conditions. Sánchez-Gómez *et al.* (2018) obtained similar performance also when evaluating germination and emergence in seedlings of this legume under nursery conditions.

The performance of growth variables at 75 days is shown in table 4.

The results showed that there were significant differences among treatments in plant height, stem length and stem diameter, with the combination of the thermal scarification method with the bioproduct IHPLUS® BF (2,5 %) showing the best results, with values of 116,4 cm, 83,0 cm and 6,26 mm in height, length and stem diameter, respectively. No significant differences were found for the number of leaves and root length.

When evaluating the Dickson quality index (table 5), the highest values were obtained in the treatments where the thermal scarification method was combined, immersing the seed in water at 80 ºC for two minutes with the bioproduct IHPLUS® BF at different concentrations, despite the fact that the values of this index represented moderate quality for field conditions (Sáenz-Reyes *et al*., 2014).

The values obtained for this species exceed those reported by Cobas-López *et al*. (2020) in trees of the Fabaceae family, *Lysiloma latisiliquum* (L.) Benth, which reached Dickson quality index of 0,06 to 0,08. This showed that the use of this bioproduct influenced the quality of *L. leucocephala* seedlings, so it was considered a promising product for Cuban animal husbandry.

The differences among the treatments with application of IHPLUS® BF, and between these and the control, may be associated with the hormonal balance established inside the seeds, between the different growth regulators, endogenous as well as exogenous.

González-Fuentes (2017) found that the α-amylase activity when applying this bioproduct in *Sorghum* showed an increase in the aerial part of the seedlings treated with the biofertilizer IHPLUS® BF. Meanwhile, in the roots, the highest values were in the treatments with 2 % IHPLUS® BF. He also pointed out that the increase in the concentration of total soluble carbohydrates, reducing sugars and total soluble proteins in several of the treatments with the biofertilizer indicated a positive effect on the metabolism of the seedlings. According to these results,

Table 4. Performance of growth indicators according to the studied treatments.

Treatment	Plant height, cm	Stem length, cm	Stem diameter, mm	Number of leaves	Root length, cm
STT + IHPLUS® BF (2,5 %) 8 hours	109,8[abc]	73,0[ab]	6,2[a]	13,6	36,2
STT + IHPLUS® BF (2,5 %) 10 hours	116,4[a]	83,0[a]	6,2[a]	11,4	33,4
STT + IHPLUS® BF (2,5 %) 28 hours	108,8[abc]	77, 8[ab]	6,4[a]	11,6	31,0
STT + IHPLUS® BF (5 %) 8 hours	112,4[a]	76,8[ab]	5,8[abc]	12,2	35,6
STT + IHPLUS® BF (5 %) 10 hours	108,2[abc]	74,0[ab]	5,9[ab]	9,8	34,2
STT + IHPLUS® BF (5 %) 28 hours	101,2[abc]	68,4[bc]	5,4[abc]	10,8	32,8
STT + IHPLUS® BF (10 %) 8 hours	112,6[ab]	74,4[ab]	5,8[abc]	12,0	38,2
STT + IHPLUS® BF (10 %) 10 hours	105,0[abc]	72,0[ab]	5,3[abc]	11,0	33,0
STT + IHPLUS® BF (10 %) 28 hours	104,4[abc]	72,2[ab]	5,6[abc]	10,2	32,2
STT + IHPLUS® BF (15 %) 8 hours	95,6[bcd]	64,6[bc]	5,0[cd]	10,2	31,0
STT + IHPLUS® BF (15 %) 10 hours	91,4[cd]	61,2[bc]	4,9[d]	11,6	30,2
STT + IHPLUS® BF (15 %) 28 hours	104,0[abc]	73,6[ab]	5,8[abc]	11,2	30,4
Control	84,6[d]	54,6[c]	5,7[abc]	10,6	30
SE ±	6,02	4,96	0,32	0,75	3,46
P - value	0,02	0,01	0,04	0,04	0,91

a, b, c and d: Means with different letters in the same column significantly differ for p ≤ 0,05
STT- Seeds treated with thermal scarification in water at 80 ºC during two minutes IHPLUS® BF

Table 5. Dickson quality index (DQI).

Treatment	DQI	Evaluation
STT+ IHPLUS® BF (2,5 %) 8 hours	0,38	Medium
STT+ IHPLUS® BF (2,5 %) 10 hours	0,31	Medium
STT+ IHPLUS® BF (2,5 %) 28 hours	0,36	Medium
STT+ IHPLUS® BF (5 %) 8 hours	0,33	Medium
STT+ IHPLUS® BF (5 %) 10 hours	0,35	Medium
STT+ IHPLUS® BF (5 %) 28 hours	0,22	Medium
STT+ IHPLUS® BF (10 %) 8 hours	0,30	Medium
STT+ IHPLUS® BF (10 %) 10 hours	0,25	Medium
STT+ IHPLUS® BF (10 %) 28 hours	0,30	Medium
STT+ IHPLUS® BF (15 %) 8 hours	0,23	Medium
STT+ IHPLUS® BF (15 %) 10 hours	0,24	Medium
STT+ IH-Plus (15 %) 28 hours	0,27	Medium
STT	0,21	Medium

STT- Seeds treated with thermal scarification in water at 80 ºC for 2 minutes IHPLUS® BF

the above-cited author recommends the application of IHPLUS® BF for the germination processes of other crops and that it be incorporated among the biofertilizers used in Cuban agriculture.

The production of growth regulators by different soil microorganisms, which promote plant growth, has been corroborated by numerous investigations (Aung *et al*., 2018; Chaurasia *et al*., 2018). The main effect of this compound is to stimulate the growth of roots and stems through the constriction of new cells formed in the meristems. This effect depends on the concentration of the hormone and in some tissues it controls the cell division process (Tanya-Morocho and Leiva-Mora, 2019).

In general, the results showed that the use of microorganisms isolated from natural habitats, with the capacity to promote plant growth, is of great importance and relevance in today's agriculture. Table 6 shows the increases in each of the indicators with the combination of the thermal scarification method and the bioproduct IHPLUS® BF (2,5 %) during 28 hours. These results prove its effectiveness on seedling emergence and vigor, which is very interesting because of the great possibilities it offers for the establishment of this plant.

The microorganisms present in the bioproduct interact with the plant-soil ecosystem and contribute to crop growth and development in different ways, for example, by suppressing pathogens and agents that cause plant diseases, solubilizing minerals, conserving energy, maintaining the microbial balance of the soil, increasing photosynthetic efficiency and fixing biological nitrogen (Díaz-Solares *et al*., 2020).

In the complex economic context faced by Cuba, the use of effective bioproducts that stimulate the growth and development of plants of agricultural interest, such as *L. leucocephala*, is of vital importance, since it is the most widely used tree species in silvopastoral systems. This is because it improves the quality of the livestock diet and increases the amount of forage available in silvopastoral systems (Vite-Cristóbal *et al*., 2020).

Conclusions

In freshly harvested seeds of *L. leucocephala* cv. Cunningham, the combined use of the thermal scarification method, with 28 hours of imbibition in a solution of IHPLUS® BF, at a concentration of 2,5; allowed better germination and emergence of seedlings in the nursery, due to the additive effect that was recorded in the stimulation of growth and vegetative development of this plant.

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Conflict of interests

The authors declare that there are no conflicts of interests among them.

Authors' contributions

- Saray Sánchez-Cárdenas. Study design, data processing and manuscript writing.
- Joisel Vázquez-Martínez. Design and execution of the study and data interpretation.
- Dayara Domínguez-Ortega. Execution of the study and data interpretation.
- Ismaray Revueltas-Oramas. Execution of the study and data interpretation.
- Hilda Beatriz Wencomo-Cárdenas. Consulting and data interpretation.
- Amado Miguel Hernández-Mijangos. Execution of the study and data interpretation.
- Dayana Lascaiba-Espinosa. Execution of the study and data interpretation.

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Table 6. Effects of the treatments according to the dilutions of IHPLUS® BF.

Variable	Mean	Control	Increase compared with the control
Seedling emergence in nursery	100	73	27
Emergence rate index	1,5	1,1	0,4
Height	103,8	100,4	3,4
Stem length	77,3	63,4	13,9

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