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Effect of IHPLUS®BF on seed germination of *Leucaena leucocephala* (Lam) de Witt cv. Cunningham

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Abstract

Objective: To evaluate the effect of inoculation with the biofertilizer IHPLUS®BF on seed germination of *Leucaena leucocephala* (Lam) de Witt cv. Cunningham.

Materials and Methods: The study was carried out at the Pastures and Forages Research Station Indio Hatuey, located in the Perico municipality, Matanzas province, Cuba. To study the germination response of seeds of *L. leucocephala* cv. Cunningham, an experiment was carried out with a completely randomized design with a 3 x 4 factorial arrangement. The treatments were formed by the combination of two factors: a) imbibition time and b) dilutions of IHPLUS®BF. Three different times (8, 10 and 28 h) were combined with four dilutions (2,5; 5; 10; 15 mL L^{-1}), and a control: seeds with thermal scarification treatment (water at 80 ºC for two minutes).

Results: Germination kinetics maintained a similar performance in all treatments. The best response was obtained with the combination of the thermal scarification method, with 28 hours of imbibition at 10 days and 96 % germination. Germination potential, speed index, average germination time and time to reach maximum germination performed best when the bioproduct was used with doses of 2,5, 5, 10 and 15 %, imbibed for 28 hours. These doses did not show significant differences among themselves and had higher germination efficiency and a minimum of six days to reach maximum germination.

Conclusions: The effectiveness of IHPLUS®BF during the germination process of *L. leucocephala* cv. Cunningham seeds was proven, being the best combination the use at 5 % for 28 hours with higher germination efficiency.

Keywords: biofertilizer, scarification, inoculation

Introduction

The inclusion of trees and shrubs in pastures is a viable alternative, due to their contribution in reducing erosion, improving soil fertility by providing atmospheric nitrogen and recycling nutrients, among other aspects. In addition, it has been proven that in systems where tree species are used, edible biomass and crude protein content of grasses increase, compared with those of improved grasses without fertilization (Crews *et al*., 2016).

According to Hernández-Hernández *et al*. (2020), the most commonly used tree species in silvopastoral systems is *Leucaena leucocephala* (Lam) de Witt cv. Cunningham. However, despite these advantages, its seeds are covered by a light layer of polysaccharides, galactose and mannose (Gutiérrez-de-Gotera *et al*., 2007), which impedes the passage of water and oxygen, reduces germination vigor and leads to germination percentages below 20 %, which limits its use (Sánchez-Paz and Ramírez-Villalobos, 2006).

To mitigate these limitations, different pre-germination treatments have been identified, such as scarification by manual removal of the testa with sandpaper, hydrothermal treatments, and immersion in water at room temperature or in sulfuric acid (Reino-Molina and Sánchez-Rendón, 2022).

The use of IHPLUS® BF, alone or in combination with the above-described scarification methods, could be a viable alternative. This bioproduct, produced at the Pastures and Forages Research Station Indio Hatuey (EEPFIH, for its initials in Spanish), based on microorganism technology, has been widely accepted by producers. It is certified for use as a biofertilizer and its application has beneficial effects, such as promoting germination, flowering, fruit development and plant reproduction (Díaz-Solares *et al*., 2020).

Microorganisms have traditionally been used to stimulate plant germination, growth and development, because they produce numerous bioactive compounds (López-Dávila *et al*., 2017). In Cuba,

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it has been used as a germination biostimulant in different crops (Tellez-Soria and Orberá-Ratón, 2018; Morocho and Leiva-Mora, 2019; Calero-Hurtado *et al*., 2019), an effect that can be related to the capacity of this bioproduct to excrete vitamins, organic acids, minerals, chelates and antioxidant substances that contribute to suppress the growth of phytopathogenic microorganisms and generate nutrients assimilable by plants, which stimulates their growth. However, there are no results of the application of IHPLUS®BF on *L. leucocephala* seeds. Based on this condition, the objective of this study was to evaluate the effect of inoculation with the biofertilizer IHPLUS® BF on the germination of seeds from *L. leucocephala* cv. Cunningham.

Materials and Methods

Location. The study was carried out at the Seed Laboratory of the EEPFIH, located at 22º 48" and 7" north latitude and 79º 32" and 2" west longitude, at 19 m.a.s.l., in Perico, Matanzas province, Cuba.

Treatment and experimental design. To study the germination response of seeds from *L. leucocephala* cv. Cunningham to the application of different dilutions and imbibition times (table 1), an experiment was carried out with a completely randomized design, with a 3 x 4 factorial arrangement. The treatments consisted of combinations of two factors: a) imbibition time with three different times (8, 10 and 28 hours) and b) dilutions of IHPLUS® BF, with four dilutions (2,5; 5; 10; 15 mL L^{-1}), and a control with seeds with thermal scarification (water at 80 ºC for two minutes).

Table 1. Description of each treatment under study.

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

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

Experimental procedure. Freshly harvested seeds of *L. leucocephala* cv. Cunningham were used, which underwent standard germination and viability analysis with the application of the tetrazolium method (Suárez and Melgarejo, 2010). The embryos were classified according to their coloration: 1) alive with high vigor, totally stained intense red, 2) alive with low vigor, pale red color, and 3) non-viable, colorless. The result of germination and viability analysis was expressed as percentage of viable and non-viable embryos (Maldonado-Peralta *et al*., 2016).

All seeds before sowing were subject to thermal scarification, in water at 80 ºC, for two minutes (González and Mendoza, 1995). Subsequently, they were combined with the different treatments of IHPLUS®BF biofertilizer dilutions and imbibition times, according to their three-phase pattern of water absorption, at 25 ºC.

Determinations. The following germination indices were calculated, according to the methodology proposed by Bewley and Black (1994):

- *Germination potential* (G %): total germination value, expressed as a percentage.
- *Germination rate index* (GRI): It is obtained by dividing the number of germinated seeds by the number of days evaluated (from sowing to the last day of evaluation).

$$GRI = ni/ti$$

Where:
ni = number of seeds germinated from the first to the last.
ti = time in days (from the day of sowing to the end of the evaluation).

- *Average time to reach germination* (ATG): It is obtained from the multiplication of the time in days (starts from sowing) and the number of seeds that completed germination divided by the number of germinated seeds.

$$ATG = \sum(t \times n) / \sum n.$$

Where:
t = time in days.
n = number of seeds that completed germination.

- *Time to reach maximum germination* (TMax): contemplates the day when the number of germinated seeds did not increase further.

Statistical analysis. The data were processed by factorial analysis of variance and the means compared by Duncan's test for 5 % significance, after verifying that they complied with the normal distribution adjustment (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

The germination kinetics in each of the treatments is shown in figure 1. In general, a similar performance was observed in all treatments. In each dilution, the imbibition time at 28 hours showed the best response.

These kinetics show that the treatment that combined the thermal scarification method with 28 hours of imbibition, after 10 days, achieved 96 % germination. This performance is catalogued as normal and shows that, in all treatments, even in the control, the seeds did not show any type of innate dormancy after scarification. This result is related to that referred by Sánchez-Gómez *et al*. (2018), who propose that hot water softens the testa, at the same time that it presses the physical barrier of the macrosclereids, so that the intercellular spaces are connected. These treatments, therefore, allow or facilitate the contact of water with the embryo and stimulate its development. In addition, they eliminate and wash away inhibitors present in the seed coat, which favors germination (Flores-Romayna *et al*., 2020).

In addition, the additive effect of the bioproduct IHPLUS® BF is added with the entry into the seeds of growth regulating substances: auxins, cytokinins and gibberellins present in the biofertilizer through the imbibition process. These compounds stimulate cell division and cell elongation, processes that allow the growth of different plant structures (Iqbal and Hasnain, 2013).

Table 2 shows the performance of various indicators that allow to know in greater detail different characteristics of germination in seeds. As reported by Sobrevilla-Solís *et al*. (2013), these indicators include germination potential, germination rate index (GRI), maximum time (TMAX) and average time (ATG) to germination.

All the evaluated indicators (germination rate index, germination index, average germination time and time to reach maximum germination) presented better values in the treatments where the bioproduct was used with the four doses (2,5, 5, 5, 10 and 15 %), soaked for 28 hours, with no significant differences among them. There was higher germination efficiency and it took at least six days to reach maximum germination.

This performance seems to be related to the water absorption curve in fresh seeds of *L. leucocephala* cv. Cunningham. Reino-Molina (2005), when determining the imbibition pattern of seeds in water, described that when fresh seeds of the above-mentioned cultivar were hydrated at an alternating temperature of

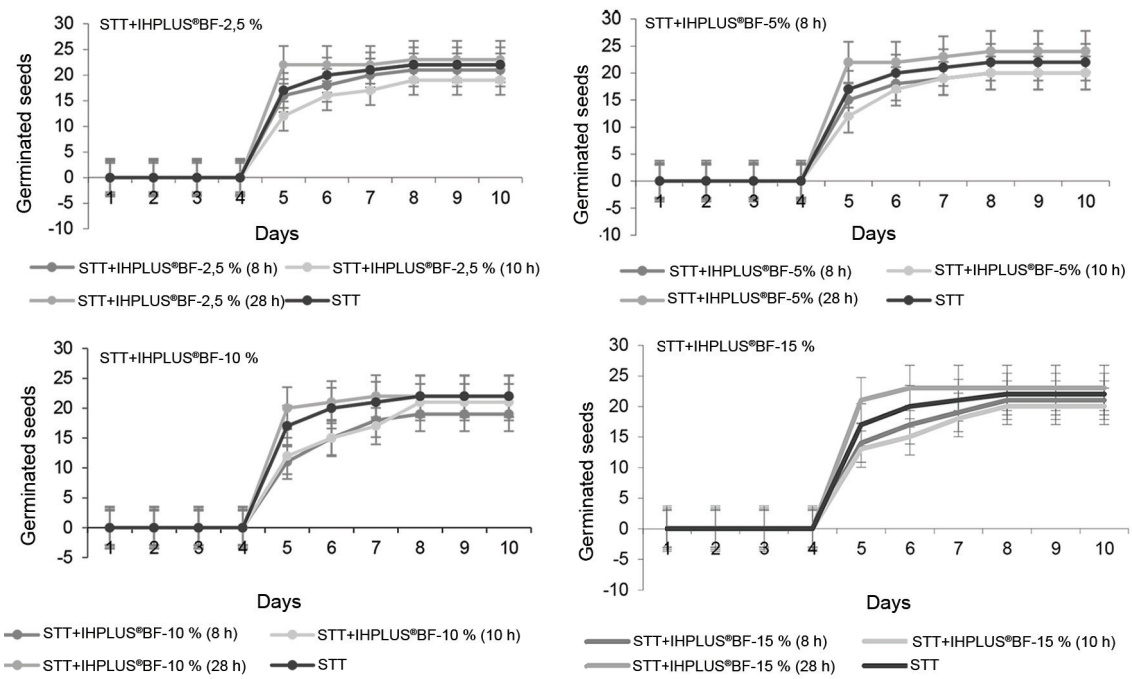

Figure 1. Germination kinetics of *L. leucocephala* cv. Cunningham seeds with different dilutions of IHPLUS® BF and imbibition time.

Table 2. Indicators related to seed germination of *L. leucocephala* cv. Cunningham.

Treatment	Germination potential, %	GRI, seeds days^{-1}	ATG, days	TMAX, days
STT+ IHPLUS® BF (2,5 %) 8 h	84,0^{bc}	2,1^{ab}	4,43^{bcd}	5,5
STT+ IHPLUS® BF (2,5 %) 10 h	76,0^{c}	2,0^{b}	4,39^{abcd}	6,8
STT+ IHPLUS® BF (2,5 %) 28 h	92,0^{ab}	2,3^{a}	4,65^{de}	6,5
STT+ IHPLUS® BF (5 %) 8 h	80,0^{bc}	2,1^{ab}	4,56^{cde}	6,5
STT+ IHPLUS® BF (5 %) 10 h	80,0^{bc}	2,1^{ab}	4,63^{de}	7,0
STT+ IHPLUS® BF (5 %) 28 h	96,0^{a}	2,5^{a}	4,62^{de}	7,0
STT+ IHPLUS® BF (10 %) 8 h	76,0^{c}	2,0^{b}	4,80^{e}	7,0
STT+ IHPLUS® BF (10 %) 10 h	84,0^{b}	2,1^{ab}	4,63^{de}	6,8
STT+ IHPLUS® BF (10 %) 28 h	88,0^{ab}	2,3^{a}	4,07^{a}	5,5
STT+ IHPLUS® BF (15 %) 8 h	84,0^{bc}	2,1^{ab}	4,17^{ab}	5,5
STT+ IHPLUS® BF (15 %) 10 h	80,0^{bc}	2,0^{ab}	4,24^{abc}	5,8
STT+ IHPLUS® BF (15 %) 28 h	92,0^{ab}	2,3^{a}	4,14^{ab}	6,0
Control STT	88,0^{b}	2,2^{ab}	44,48^{bcde}	6,3
P - value	0,0093	0,0093	0,0001	0,184
SE ±	3,82	0,10	0,11	0,45

GP-germination potential, GRI-germination rate index, ATG-average germination time, Tmax- time to reach maximum germination. a, b, c, d and e: Means with unequal letters in the same column differ significantly for $p \leq 0,05$.

25/30 ºC, they followed a three-phase pattern of water absorption, as occurs in the generality of seeds of all crops. The aforementioned author stated that the end of phase I is reached at 8 hours; phase II is a long process of water absorption, which until 25 hours constitutes a stationary segment of the curve, and phase III constitutes the last stage of imbibition that represents the emergence of the radicle (visible germination), which was reached after 30 hours of hydration of these seeds.

Seed imbibition time depends on numerous factors: species, seed quality (health, size and weight). It is also related to internal factors, such as maturity, and extrinsic factors, like humidity, temperature, oxygen supply, presence or absence of light and seed dormancy. All these influence the germination percentage, as referred by Valdez-Yopla (2017).

There are different reports regarding imbibition time. González-Fuente (2017), in a similar study on seeds of *Sorghum bicolor* L. (Moench) cv. UDG-110, treated with IHPLUS® BF, found that the highest values were obtained with the variant 6 % - four hours of imbibition with percentage higher than 80 %. Carrillo-Sosa *et al*. (2017), in a study where they evaluated the effectiveness of the bioproduct LEBAME (obtained by the Cuban Research Institute of Sugarcane Derivatives ICIDCA) found no significant differences among the three imbibition times (15, 30 and 60 minutes). Therefore, from the practical point of view, the imbibition of tomato seeds in LEBAME for 15 minutes (less time), was pointed out as the best proposal.

The results of this research also coincide with those obtained by different authors, who studied the effect of different biopreparations based on microorganisms isolated from the rhizosphere on the germination process of various species (Quintero *et al*., 2018).

Conclusions

The effectiveness of this bioproduct on different indicators related to the germination process of *L. leucocephala* cv. Cunningham seeds was proven. The best combination was the use of this bioproduct at 5 % with higher germination efficiency (six days as minimum to reach its maximum germination).

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Conflict of interest

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

Authors' contributions

- Saray Sánchez-Cárdenas. Study design, data processing and manuscript writing.

- Joisel Lázaro-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.

Bibliographic references

Bewley, J. D. & Black, M. *Seeds. Physiology of development and germination*. New York: Plenum Press, 1994.

Calero-Hurtado, A.; Quintero-Rodríguez, Elieni; Pérez-Díaz, Yanery; Olivera-Viciedo, D.; Peña-Calzada, Kolima & Jiménez-Hernãndez, Janet. Efecto entre microorganismos eficientes y Fitomas-e en el incremento agroproductivo del frijol. *Rev. Bio. Agro*. 17 (1):25-33, 2019. DOI: https://doi.org/10.18684/bsaa.v17n1.1201.

Carrillo-Sosa, Yudines; Terry-Alfonso, Elein; Díaz-de-Villegas, María E.; Ruiz-Padrón, Josefa & Delgado, Grizel. Efecto del LEBAME en la germinación de semillas de tomate (*Solanum lycopersicum* L.). *Cultivos Tropicales*. 38 (3):30-35. http://scielo.sld.cu/scielo.php?script=sci_abstract&pid=S0258-59362017000300004, 2017

Crews, T. E.; Blesh, Jennifer; Culman, S. W.; Hayes, R. C.; Jensen, E. S.; Mack, Michelle C. *et al*. Going where no grains have gone before: From early to mid-succession. *Agric.. Ecosyst. Environ*. 223:223-238, 2016. DOI: https://doi.org/10.1016/j.agee.2016.03.012.

Díaz-Solares, Maykelis; Martín-Martín, G. J.; Miranda-Tortoló, Taymer; Fonte-Carballo, Leydi; Lamela-López, L.; Montejo-Sierra, I. L. *et al. Obtención y utilización de microorganismos nativos: el bioproducto IHPLUS®*. Matanzas, Cuba: EEPF Indio Hatuey. https://www.researchgate.net/publication/339916260_Obtencion_y_utilizacion_de_microorganismos_nativos_el_bioproducto_IHPLUS_R, 2020.

Di-Rienzo, J. A.; Balzarini, Mónica; Gonzalez, Laura; Casanoves, F.; Tablada, Margot & Robledo, C. W. *InfoStat versión 2017*. Argentina: Grupo InfoStat, FCA, Universidad Nacional de Córdoba. https://www.infostat.com.ar/index.php?mod=page&id=15, 2017.

Flores-Romayna, María A.; Ortega-Chávez, W. & Ortega-Mallqui, A. Evaluación de tratamientos pregerminativos en semillas de *Euterpe precatoria* Mart. (Huasaí) en la ciudad de Pucallpa, Perú. *CFORES*. 8 (1):88-103. http://cfores.upr.edu.cu/index.php/cfores/article/view/490, 2020.

González, Yolanda & Mendoza, F. Efecto del agua caliente en la germinación de *Leucaena leucocephala* cv. Cunningham. *Pastos y Forrajes*. 18 (1):59-65. https://payfo.ihatuey.cu/index.php?journal=pasto&page=article&op=view&path%5B%5D=1116&path%5B%5D=1633, 1995.

González-Fuentes, Yessika. *Efecto del IHplus® sobre el proceso de germinación de Sorghum bicolor L. (Moench)*. Tesis de diploma en opción al título de Ingeniero Agrónomo. Matanzas, Cuba: Facultad de Ciencias Agropecuarias, Universidad de Matanzas, 2017.

Gutiérrez-de-Gotera, Omaira; Añez-de-Servodio, Omaira; León-de-Pinto, Gladys; Abed-El-Kader, Dina & Molina, E. Análisis fisicoquímico y estructural del polisacárido de la goma de semilla de *Leucaena leucocephala*. *Ciencia*. 15 (4):481-487. https://produccioncientificaluz.org/index.php/ciencia/article/view/9807, 2007.

Hernández-Hernández, M.; López-Ortiz, Silvia; Jarillo-Rodríguez, J.; Ortega-Jiménez, E.; Pérez-Elizalde, S.; Díaz-Rivera, P. *et al*. Rendimiento y calidad nutritiva del forraje en un sistema silvopastoril intensivo con *Leucaena leucocephala* y *Megathyrsus maximus* cv. Tanzania. *Rev. mex. de cienc. pecuarias*. 11 (1):53-69, 2020. DOI: https://doi.org/10.22319/rmcp.v11i1.4565.

Iqbal, Atia & Hasnain, Shahida. Auxin producing *Pseudomonas* strains: biological candidates to modulate the growth of *Triticum aestivum* beneficially. *Am. J. Plant Sci*. 4 (9):1693-1700, 2013. DOI: https://doi.org/10.4236/ajps.2013.49206.

López-Dávila, E.; Gil-Unday, Zuleiqui; Henderson, Deborah; Calero-Hurtado, A. & Jiménez-Hernández, Janet. Uso de efluente de planta de biogás y microorganismos eficientes como biofertilizantes en plantas de cebolla (*Allium cepa* L., cv. 'Caribe-71'). *Cultivos Tropicales*. 38 (4):7-14. http://www.redalyc.org/articulo.oa?id=193254602001, 2017.

Maldonado-Peralta, M. A.; García-de-los-Santos, G.; García-Nava, J. R.; Ramírez-Herrera, C.; Hernández-Livera, A.; Valdez-Carrazco, J. M. *et al*. Seed viability and vigour of two nanche species (*Malpighia mexicana* and *Byrsonima crassifolia*). *Seed Sci. Technol*. 44 (1):1-9, 2016. DOI: http://doi.org/10.15258/sst.2016.44.1.03.

Morocho, Mariuxi T. & Leiva-Mora, M. Microorganismos eficientes, propiedades funcionales y aplicaciones agrícolas. *Ctro. Agr*. 46 (2):93-103. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0253-57852019000200093&lng=es&tlng=es2019.

Quintero-Rodríguez, Elieni; Calero-Hurtado, A.; Pérez-Díaz, Yanery & Enríquez-Gómez, Lorettys. Efecto de diferentes bioestimulantes en el rendimiento del frijol común. *Ctro. Agr*. 45 (3):73-80. http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0253-57852018000300073&lng=pt&tlng=es, 2018.

Reino-Molina, J. Efectos de tratamientos de hidratación-deshidratación y de choque ácido sobre la germinación y emergencia en *Leucaena leucocephala*. Tesis presentada en opción al título de Master en Pastos y Forrajes. Matanzas, Cuba: EEPF Indio Hatuey, Universidad de Matanzas, 2005.

Reino-Molina, J. & Sánchez-Rendón, J. A. Efecto de la conservación al ambiente en semillas de *Leucaena leucocephala* cv. Cunningham sometidas a tratamientos hídricos. *Memorias de la Convención Producción Animal y Agrodesarrollo 2022*. Matanzas, Cuba: Instituto de Ciencia Animal, EEPF Indio Hatuey, 2022.

Sánchez-Gómez, A.; Rosendo-Ponce, A.; Vargas-Romero, J. M.; Rosales-Martínez, F.; Platas-Rosado, D. E. & Becerril-Pérez, C. M. Energía germinativa en guaje (*Leucaena leucocephala* cv. Cunningham) con diferentes métodos de escarificación de la semilla. *Agrociencia*. 52 (6):863-874. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S1405-31952018000600863&lng=es&nrm=iso, 2018.

SánchezPaz, Y. & Ramírez-Villalobos, Maribel. Tratamientos pregerminativos en semillas de *Leucaena leucocephala* (Lam.) de Wit. y *Prosopis juliflora* (Sw.) DC. *Rev. Fac. Agron*. 23 (3):257-272. http://ve.scielo.org/scielo.php?script=sci_arttext&pid=S0378-78182006000300001&lng=es&tlng=es, 2006.

Sobrevilla-Solís, J. A.; López-Herrera, Maritza; López-Escamilla, Ana L. & Romero-Bautista, Leticia. Evaluación de diferentes tratamientos pregerminativos y osmóticos en la germinación de semillas *Prosopis laevigata* (Humb. & Bonpl. ex Willd) M. C. Johnston. En: G. Pulido-Flores y S. Monks, eds. *Estudios científicos en el estado de Hidalgo y zonas aledañas*. Vol. II. Lincoln, EUA: Zea Books. https://digitalcommons.unl.edu/hidalgo/12/, 2013.

Suárez, D. & Melgarejo, Luz M. Biología y germinación de semillas. En: L. M. Melgarejo, ed. *Experimentos en Fisiología vegetal*. Bogotá: Universidad Nacional de Colombia. p. 13-24, 2010.

Tellez-Soria, Taniyurkis & Orberá-Ratón, Teresa. Efecto estimulador del crecimiento de dos biopreparados biotecnológicos en cultivos de remolacha (*Beta vulgaris* L.). *Rev. Cub. Quím*. 30 (3):483-494. https://www.redalyc.org/journal/4435/443557797008/html/, 2018.

Valdez-Yopla, M. H. *Caracterización morfológica y germinación de la semilla de valeriana (Valeriana pilosa Ruiz & Pav.).* Tesis para optar el título profesional de Ingeniero Agrónomo. Cajamarca, Perú: Escuela Académico Profesional de Agronomía, Facultad de Ciencias Agrarias, Universidad Nacional de Cajamarca. https://repositorio.unc.edu.pe/bitstream/handle/20.500.14074/1681/CARACTERIZACI%C3%93N%20MORFOL%C3%93GICA%20Y%20GERMINACI%C3%93N%20DE%20LA%20SEMILLA%20DE%20VALERIANA%20%28Valeriana%20pilosa%20Ruiz%20%26%20Pav.pdf?sequence=1&isAllowed=y, 2017.