Short communication

Effect of the application of microorganisms on the growth of *Morus alba* Linn under semi-controlled conditions

Ana Gladys Gallardo-López[1] *https://orcid.org/0000-0002-7568-8845*, Marisol Lafargue-Savón[1] *https://orcid.org/0000-0001-6801-5397*, Ana Luisa Carter-Veranes[1] *https://orcid.org/0000-0001-5480-7318*, Nancy Noa-Lobaina[1] *https://orcid.org/0000-0001-8335-2068*, María Gallardo-López[1] *https://orcid.org/0000-0002-7292-2313,* Irliadis Urgelles-Cardoza[1] *https://orcid.org/0000-0002-3387-2943* y Lisette Labadié-Pérez[1] *https://orcid.org/0000-0003-4006-8977*

[1]*Centro de Desarrollo de la Montaña. Limonar de Monte Ruz, El Salvador, Guantánamo. CP 9950, Cuba.*
E-mail: anagladys@cdm.gtmo.inf.cu, marisol@cdm.gtmo.inf.cu, luisa@cdm.gtmo.inf.cu, nancy@cdm.gtmo.inf.cu, maría@cdm.gtmo.inf.cu, irliadis@cdm.gtmo.inf.cu, sofia@cdm.gtmo.inf.cu

Abstract

Objective: To evaluate the application of microorganisms with native strains on the growth of *Morus alba* Linn under semi-controlled conditions.

Materials and Methods: The work was carried out at the Mountain Development Center, El Salvador municipality, Guantánamo province. Stakes of *M. alba*, 30 cm long with five buds, were used. Fersialytic soil and organic matter (sheep manure) were used as substrate in a 3:1 ratio. Four concentrations of the microorganism with native strains were applied: 5, 10, 15 and 20 mL/L, which constituted the treatments. A control was used, with water only. A complete randomized experimental design was applied, with 20 plants per treatment. Measurements were branch number, root number, leaf number, branch length, stem diameter and root length. The data were processed from a simple classification analysis of variance.

Results: There were no significant differences for the number of branches and number of leaves. Regarding the number of roots, the best treatment was T2, which differed significantly from the others. Treatments T2 and T3 tended to have the highest numerical values. As for branch length and root length, there were significant differences among treatments. No differences were found in stem diameter. The highest numerical values in all indicators were obtained with the application of 5 and 10 mL/L of the bioproduct on *M. alba* seedlings.

Conclusions: With the application of 5 and 10 mL/L of microorganisms with native strains, the performance of branch length, number of roots and root length of *M. alba* seedlings was favored.

Keywords: foliar application, bacterium, technology

Introduction

Efficient microorganisms (EM) constitute a set of beneficial bacteria with multiple novel applications, in environmental, animal husbandry and agricultural areas. The use of these resources constitutes a natural alternative, capable of promoting and stimulating plant development (Escobar-Oña *et al*., 2017).

In Japan, a microbial inoculant was established, based on effective microorganisms, which has proven to enhance soil condition, crop development and production, which captured the interest of the world. It was necessary to move from an agriculture based on the use of agrochemicals to a sustainable agriculture through the use of efficacious and affordable methods to achieve success. The EM technology is a potential tool, which offers benefits to the farmer, in terms of achieving economically, environmentally and socially sustainable productivity systems (Higa and Parr, 2018).

EMs comprise a wide variety of microbes: lactic acid bacteria, photosynthetic bacteria, yeasts, actinomycetes and fungi. From an agricultural point of view, EMs stimulate seed germination, benefit flowering, yield and fruit development, and allow for more fruitful plant reproduction. Likewise, it has been proven that they enhance the physical system of soils, increase their chemical fertility and eradicate various phytopathogenic compounds, promoters of diseases in many crops (Morocho and Leiva-Mora, 2019).

In agriculture, microorganisms are indispensable to preserve soil fertility and promote healthy and strong crops. Since a few years ago, microorganisms applied in agriculture have gained greater relevance, as the favorable results of their application as

Received: January 23, 2023
Accepted: July 21, 2023

How to cite a paper: Gallardo-López, Ana Gladys; Lafargue-Savón, Marisol; Carter-Veranes, Ana Luisa; Noa-Lobaina, Nancy; Gallardo-López, María; Urgelles-Cardoza, Irliadis & Lisette Labadié-Pérez. Effect of the application of microorganisms on the growth of *Morus alba* Linn under semi-controlled conditions. *Pastures and Forages.* 46:e16, 2023

substitutes for other kinds of fertilizers have been proven(Soriano-Pons, 2019).

In Cuba, this technology was initiated by the Experimental Station of Pastures and Forages Indio Hatuey, Matanzas province. At present, it is used in several regions of the country by farmers and producers in the treatment of digestive diseases in animals. It is also used as a probiotic in the control of odors in production facilities, in the treatment of waste, in addition to its use as a biofertilizer and as a biological controller (Blanco *et al*., 2016). EMs are also used as biostimulators of the growth of different forage plants, such as *Morus alba* Linn (mulberry), which is a perennial ligneous plant, of low to medium size, with a much higher nutrient content than alfalfa. This forage plant is a great producer of edible biomass and is considered an interesting alternative in ruminant feeding to supplement low-quality forage diets (Martín-Martín *et al*., 2017). In addition, it shows high protein and energy content (Mejía-Castillo, 2019).

In different animal husbandry systems, protein forage banks, such as *M. alba*, are necessary for maintenance and production, due to the contribution they represent in terms of protein, fats, minerals and fiber (Fonseca-López *et al*., 2019). The low productivity of livestock is directly related to the low availability of pasture and its low nutritive value, with poor biomass supply in the rainy season, which causes poor animal response (Herrera-Toscano and Carmenate-Figueredo, 2018). The objective of this research was to evaluate the application of microorganisms with native strains in the growth of *M. alba*, under semi-controlled conditions.

Materials and Methods

Location. The work was carried out at the Mountain Development Center, in El Salvador municipality, Guantánamo province, during the period from March to May, 2020. The facility is located at N 200 21'22'' and W 750 18' 59'', between parallels 189 and 191 of N latitude and meridians 657 and 659 of W longitude, in the Nipe-Sagua-Baracoa mountain range at 405 m.a.s.l.

Treatments and experimental design. The treatments were: T1 (control) with tap water, T2-5 mL/L, T3-10 mL/L, T4-15 mL/L and T5-20 mL/L, soaked for 30 min in a solution of microorganisms prepared with native strains. A complete randomized design was used with five treatments and four replicas, with 20 bags each.

Experimental procedure. Stakes 30 cm long, with five buds, planted in black polyethylene bags (13 x 28 cm long and 14,5 cm wide) were used in the research. The substrate was formed with Fersialytic soil (Hernández-Jiménez *et al*., 2015) and sheep manure was used as organic matter, in a 3:1 ratio.

The microorganisms were obtained from the artisanal plant of the Mountain Development Center, at a concentration above the order of 10^6 CFU/mL. Five microbial groups were formed: lactic acid bacteria (*Lactobacillus* sp., *Streptococcus* sp.), photosynthetic bacteria (*Rhodopseudomonas* sp., *Rhodobacter* sp.), yeasts (*Saccharomyces cerevisiae*), actinomycetes (*Actinomyces* sp., *Streptomyces* sp.) and fungi (*Aspergillus* sp., *Penicillium* sp). The product was applied by spraying the foliage of the plants every seven days, manually or with a 10-L backpack. Cultural attentions were carried out according to the technical instructions for the cultivation of *M. alba*.

Measurements. The morphological variables number of branches, number of roots, number of leaves, branch length, stem diameter and root length were evaluated. Five evaluations were made at 14, 21, 28, 35 and 42 days after product application and 15 per treatment were sampled.

- Number of branches: visual count (X- real average ranges of the $\overline{X}$).
- Root number: visual count (X- real average ranges of $\overline{X}$).
- Number of leaves per branch: visual count (X- real average ranges of $\overline{X}$)
- Branch length: measured with a graduated ruler from the basis to the tip of the branch.
- Stem diameter: measured with caliper (cm).
- Root length: measured with a graduated ruler (cm).

Statistical analysis. Statistical processing of the data was performed from a simple classification analysis of variance and means were compared using Duncan's multiple range test for $p < 0,05$. Branch length, stem diameter and root length were analyzed using the Kruskal Wallis nonparametric test and the Newman-Keuls test for $p < 0,05$, since these variables did not meet the test for normality and homogeneity of variance. The data on number of leaves, branches and roots were transformed by √x. Data analysis was performed using the statistical package Statgraphic. Plus version 5.1.

Results and Discussion

Table 1 shows the results of the effectiveness of microorganisms on the morphological variables of *M. alba* seedlings. There were no significant

Table 1. Effectiveness of microorganisms with native strains on morphological variables of *M. alba* seedlings.

Treatment	Number of branches		Number of roots		Number of leaves	
	X- real	Average ranges of the $\overline{X}$	X-real	Average ranges of the $\overline{X}$	X- real	Average ranges of the $\overline{X}$
T1-Control	1,5	1,1	15,2^{b}	3,71	11,6	3,25
T2-5 mL/L	2,0	1,3	27,4^{a}	5,16	13,8	3,65
T3-10 mL/L	1,8	1,3	16,8^{b}	4,03	12,6	3,48
T4-15 mL/L	1,4	1,1	14,6^{b}	3,69	10,6	3,22
T5-20 mL/L	1,5	1,1	14,1^{b}	3,54	10,4	3,07
SE ±	0,20	0,08	1,89	0,27	1,25	0,21

Means with unequal letters in the same column significantly differ $p \leq 0,05$

differences for the number of branches and number of leaves. As for the number of roots, the best treatment was T2, which differed statistically from the others. There was a tendency to show the highest numerical values in treatments T2 and T3.

Some researchers have worked with the cultivable portion of the *A. thaliana* microbiome in plants that retain the early flowering effect and have shown that microorganisms can modify multiple plant traits, including foliage development and flowering. According to Calero-Hurtado *et al.* (2018), with foliar application of efficient microorganisms combined with FitoMas-E®, leaf mass is increased. According to Martínez-González *et al.* (2017), with the application of several stimulants in two *Phaseolus vulgaris* L. (common bean) cultivars, the number of pods increased compared with the control treatment. Rodríguez-Núñez *et al.* (2019) referred that with the use of two foliar biostimulants and their combination with FitoMas-E®, the amount of efficient microorganisms was higher in the morphological performance of *Zea mays* L. (corn) plants with regards to the control, except in stem diameter.

Alejo-Aguiar and Mesa-Reinaldo (2019) noted that the application of efficient microorganisms in the *P. vulgaris* crop statistically outperformed the control in the evaluated morphoagronomic indicators.

Regarding branch length and root length (table 2), there were statistically significant differences among the evaluated treatments. With the application of 5 and 10 mL/L of efficient microorganisms, the highest numerical values were reached. This could be due to the joint action of the efficient microorganisms and the organic matter used. No significant differences were found among treatments in stem diameter.

The results of the effect of using this bioproduct, based on efficient microorganisms, are in correspondence with those obtained in the evaluation conducted by Calero-Hurtado *et al.* (2019), who applied efficient microorganisms on tomato seedlings, and achieved a superior response in stem diameter compared with the absence of EM. The results obtained by inoculating seeds and their subsequent foliar application stood out.

This performance is due to the beneficial action of microorganisms in the soil-plant system, which contribute to accelerate the viability of nutrients for plants and ensure greater growth and development (Rashid *et al.*, 2016).

Table 2. Effectiveness of efficient microorganisms on morphological indicators of *M. alba*.

Treatment	Branch length, cm	Stem diameter, cm	Root length, cm
T1-Control	21,36^{abc}	0,39	12,8^{d}
T2-5 mL/L	24,5^{ab}	0,42	18,3^{a}
T3-10 mL/L	24,8^{a}	0,38	15,2^{b}
T4-15 mL/L	19,6^{bc}	0,34	13,8^{bc}
T5-20 mL/L	18,8^{c}	0,36	13,1^{c}
SE ±	0,979	0,0198	0,454

Means with unequal letters in the same column significantly differ $p \leq 0,05$

Conclusions

The application of 5 and 10 mL/L of microorganisms with native strains favored the performance of branch length, number of roots and root length of *M. alba* seedlings.

Acknowledgments

The authors thank the national project P 131 LH 003-068, Strengthening the health of Pelibuey sheep to stimulate food production in mountainous localities of the eastern region, for funding this research. This project belongs to the Mountain Development Center.

Conflict of interests

The authors declare that there is no conflict of interests.

Authors' contributions

- Ana Gladys Gallardo-López. Designed the research, worked on the set-up and evaluation of the experiment and writing of the manuscript.
- María Gallardo-López. Performed the crop evaluations during the duration of the experiment and participated in the agro-technical work and agricultural activities.
- Marisol Lafarge-Savón. Prepared the experimental database, participated in the statistical processing and elaboration of the database.
- Ana Luisa Carter-Veranes. Participated in the evaluations of the experiment.
- Nancy Noa-Lobaina. Participated in the interpretation of the results and revision of the manuscript.
- Irliadis Urgelles-Cardoza. Collaborated in the revision of the scientific article and in the interpretation of the results.
- Lisette Labadié-Pérez. Participated in the search for information for the preparation of the manuscript.

Bibliographic references

Alejo-Aguiar, L. O. & Mesa-Reinaldo, J. R. Efecto de un biopreparado de microorganismos eficientes sobre el cultivo del frijol común (*Phaseolus vulgaris*, L.) en un suelo Pardo sialítico mullido, sin carbonatos. *Rev. Cient. Agroeco*. 7 (2):111-118, https://aes.ucf.edu.cu/index.php/aes/article/view/299, 2019.

Blanco, D.; Suárez, J.; Donis, F. & González, O. Biodigestores y microorganismos nativos. En: F. Funes Aguilar y L. L. Vázquez Moreno, eds. *Avances de la Agroecología en Cuba*. Matanzas, Cuba: EEPF Indio Hatuey, 2016.

Calero-Hurtado, A.; Quintero-Rodríguez, Elieni; Olivera-Viciedo, D.; Pérez-Díaz, Yanery; Castro-Lizazo, I.; Jiménez, Janet *et al*. Respuesta de dos cultivares de frijol común a la aplicación foliar de microorganismos eficientes. *Cultivos Tropicales*. 39 (3):5-10, http://scielo.sld.cu/pdf/ctr/v39n3/ctr01318.pdf, 2018.

Calero-Hurtado, A.; Quintero-Rodríguez, Elieni; Pérez-Díaz, Yanery; Olivera-Viciedo, D.; Peña, Kolima; Castro-Lizazo, I. *et al*. Evaluación de microorganismos eficientes en la producción de plántulas de tomate (*Solanum lycopersicum* L.). *Rev. Cienc. Agr*. 36 (1):67-78. https://dialnet.unirioja.es/servlet/articulo?codigo=7059962, 2019.

Escobar-Oña, W. S.; Tafur-Recalde, V. L.; Pazmiño-Mayorga, J. A. & Vivas-Vivas, R. J. Respuesta del cultivo de fréjol caraota (*Phaseolus vulgaris* L.) a la aplicación foliar complementaria de tres bioestimulantes. *Dom. Cien*. 3 (3):556–571, 2017. DOI: https://doi.org/10.23857/dc.v3i3.492.

Fonseca-López, Dania; Salamanca-López, Anyela E.; Niño-Monroy, Laura E.; Rodríguez-Molano, C. E.; Hoyos-Concha, J. L.; Otero-Ramírez, I. D. *et al*. Caracterización nutricional y de producción de biomasa de *Sambucus peruviana*, *Sambucus nigra* y *Morus alba* en un banco forrajero. *Ciencia en Desarrollo*. 10 (2):23-32, 2019. DOI: https://doi.org/10.19053/01217488.v10.n2.2019.9098.

Hernández-Jiménez, A.; Pérez-Jiménez, J. M.; Bosch-Infante, D. & Castro-Speck, N. *Clasificación de los suelos de Cuba 2015*. Mayabeque, Cuba: Instituto Nacional de Ciencias Agrícolas, Instituto de Suelos, Ediciones INCA, 2015.

Herrera-Toscano, J. A. & Carmenate-Figueredo, O. Selección de recursos locales para la alimentación de ovinos en el municipio Las Tunas, Cuba. *Pastos y Forrajes*. 41 (3):176-182, http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=s0864-03942018000300003&lng=es&tlng=es, 2018.

Higa, T. & Parr, J. F. Microorganismos benéficos y efectivos para una agricultura y medio ambiente sostenible. Centro Internacional de Investigación de Agricultura Natural, Beltsville, USA. https://cdn.goconqr.com/uploads/media/pdf_media/42178589/ef577365-1bb5-4922-a61b-1e268f8784d6.pdf, 2018.

Martín-Martín, G. J.; Noda-Leyva, Yolai; Pentón-Fernández, Gertrudis; González-Ybarra, Niurca; Martínez-Peréz, Madeleidy; Díaz-Solares, Maykelis *et al*. *Morus alba* L. Una planta multipropósito para la producción animal en Cuba. *Anales de la ACC*. 7 (1):1-12, https://revistaccuba.sld.cu/index.php/revacc/article/view/466, 2017.

Martínez-González, Lisbel; Maqueira-López, L.; Nápoles-García, María C. & Núñez-Vázquez, Miriam. Efecto de bioestimulantes en el rendimiento de dos cultivares de frijol (*Phaseolus vulgaris* L.) Biofertilizados. *Cultivos Tropicales*. 38 (2):113-118, http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=s0258-59362017000200017&lng=es&tlng=es, 2017.

Mejía-Castillo, H. J. La morera (*Morus* sp.) como alternativa en sistemas silvopastoriles. *Rev. Iberoam. Bioecon. Cambio Clim.* 5 (9):1157–1163, 2019. DOI: https://doi.org/10.5377/ribcc.v5i9.7951.

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=es, 2019.

Rashid, M. I.; Mujawar, L. H.; Shahzad, T.; Almeelbi, T.; Ismail, I. M. I. & Oves, M. Bacteria and fungi can contribute to nutrients bioavailability and aggregate formation in degraded soils. *Microbiol. Res.* 183:26-41, 2016. DOI: https://doi.org/10.1016/j.micres.2015.11.007.

Rodríguez-Núñez, A.; García-González, M. T. & Fernández-Cancio, Y. Resultado del empleo de dos bioestimulantes foliares y su combinación en cultivo del maíz (*Zea mays* L.). *Infociencias*. 23 (1):47-58, http://www.infocienciass.cu/index.php/infociencia/article/view/311/1011, 2019.

Soriano-Pons, F. *Uso de microorganismos en la agricultura*. Valencia, España: Asociación Española de Fabricantes Agronutrientes. https://aefa-agronutrientes.org/uso-de-microorganismos-en-la-agricultura, 2019.