Cultivos Tropicales, Vol. 45, No. 4, October-December 2024, e-ISSN: 1819-4087, p-ISSN: 0258-5936, https://ediciones.inca.edu.cu

Cu-ID: https://cu-id.com/2050/v45n4e02

Original article

Effect of biostimulants and an Arbuscular Mycorrhizal Fungus in the vegetative propagation of *Tithonia diversifolia* (Hemsl.) A. Gray

Efecto de bioestimulantes y hongos micorrízicos arbusculares en la propagación vegetativa de *Tithonia diversifolia* (Hemsl.) A. Gray

Jorge Corbera-Gorotiza[1*], Pedro José González-Cañizares[1], Reynerio Reyes-Rouseaux[2]

[1]Instituto Nacional de Ciencias Agrícolas (INCA). Carretera San José - Tapaste, km 3½, Gaveta Postal 1, San José de las Lajas, Mayabeque, Cuba. CP 32 700.

[2]Instituto de Investigaciones de Pastos y Forrajes. Avenida Independencia, km 8 ½ Boyeros, La Habana, Cuba.

ABSTRACT: In an experiment in bags, in the central area of the National Institute of Agricultural Sciences of Cuba, with the objective of evaluating the effect of different bioproducts on the growth and development of the tithonia crop [*Tithonia diversifolia* (Hemsl.) A. Gray], eight treatments were studied in a completely randomized design. Treatments made up of the simple inoculation of bioproducts and the combination of biostimulators with the arbuscular mycorrhizal fungi, plus a control without bioproducts. At 50 dap, the number, length and diameter of the branches, the number of leaves and their chlorophyll content, the nutritional status - N, P and K (aerial part and root), the fungal variables (frequency and colonization intensity) and dry mass (aerial part and root) were measured. The results showed a positive effect of bioproduct use, with different results between them and differences with the control, with a tendency to be higher the effects on the growth and development of tithonia when the combination of the arbuscular mycorrhizal fungi was used with the biostimulants, depending on the bioproducts evaluated and without very marked differences between said combinations. In general, the EcoMic® plus Bioenraíz® combination with the greatest effects on the variables evaluated stands out. The beneficial effects of the use of the different bioproducts and some of their combinations, could favor a sustainable production of this crop.

Keywords: Bioproducts, nursery, cuttings, growth, biomass production.

RESUMEN: Con el objetivo de evaluar el efecto de diferentes bioproductos en el crecimiento y desarrollo del cultivo de la tithonia [*Tithonia diversifolia* (Hemsl.) A. Gray], por su alto potencial en la alimentación bovina, se realizó un experimento en el área central del Instituto Nacional de Ciencias Agrícolas de Cuba; este se desarrolló en bolsas, con 8 tratamientos conformados por la inoculación simple de los bioproductos y la combinación de los bioestimulantes con el hongo micorrízico arbuscular, más un control sin bioproductos, utilizando un diseño completamente aleatorizado. Se midió, a los 50 días después de la plantación (ddp), el número, largo y diámetro de las ramas, el número de hojas y su contenido de clorofila, el estado nutricional - N, P y K (parte aérea y raíz), las variables fúngicas (frecuencia e intensidad de la colonización) y la masa seca (parte aérea y raíz). Los resultados mostraron un efecto positivo del empleo de los diferentes bioproductos, con resultados distintos entre ellos y diferencias con el control, con tendencia a ser superiores los efectos sobre el crecimiento y desarrollo de la tithonia cuando se utilizó la combinación del hongo micorrízico arbuscular con los bioestimulantes, en dependencia de los bioproductos evaluados, sin diferencias muy marcadas entre dichas combinaciones. Se destaca, de manera general, la combinación EcoMic® y Bioenraíz® con los mayores efectos sobre las variables evaluadas. Los efectos beneficiosos del empleo de los diferentes bioproductos y algunas de sus combinaciones, podría favorecer una producción sostenible de este cultivo.

Palabras clave: Bioproductos, vivero, esquejes, crecimiento, producción de biomasa.

*Author for correspondence: jcorbera@inca.edu.cu

Received: 25/09/2023

Accepted: 18/09/2024

Conflict of interest: The authors declare that they have no conflict of interest.

Author contributions: Conceptualization- Jorge Corbera-Gorotiza, Pedro José González Cañizares. **Research-** Jorge Corbera-Gorotiza, Pedro José González Cañizares, Reynerio Reyes-Rouseaux. **Methodology-** Jorge Corbera-Gorotiza, Pedro José González Cañizares, Reynerio Reyes-Rouseaux. **Supervision-** Jorge Corbera-Gorotiza, Pedro José González Cañizares. **Writing of the initial draft, Writing and final editing and data curation-** Jorge Corbera-Gorotiza.

INTRODUCTION

An alternative to mitigate the situation that arises with the quality of the pastures that make up the diets of cattle is the creation of fodder banks. These constitute a viable nutritional strategy in the supplementation of ruminants in the tropics, due to the nutritional benefits they provide with a notable decrease in the use of external inputs (1).

For the conformation of these fodder banks, a group of grasses can be used that provide high concentrations of standing biomass or proteinaceous plants that fulfill the same function, with *Tithonia diversifolia* (Hemsl.) A. Gray, standing out among the latter, due to its agronomic characteristics. Gray, due to its agronomic and bromatological characteristics, in addition to its high ecological and productive plasticity, which makes it one of the most accepted protein plants to be supplied to cattle herds in Cuba and other regions of the world (2).

Tithonia diversifolia (Hemsl.), commonly known as buttercup, is a forage plant that can be established through sexual and vegetative seed; its nutritional composition shows high levels of protein and soluble carbohydrates, low content of neutral detergent fiber (NDF) and adequate levels of minerals; its establishment and use as green manure can improve soil fertility conditions to increase pasture production and yield. These characteristics give it a high potential in cattle feed, either as a resource for browsing, processed into meal or silage, to reduce production costs in livestock farms (3). It is a multifunctional species with tolerance to various soil conditions, including low fertility, acidity and low phosphorus levels (4). Above all, due to the abundant biomass offered by this plant, of which dry mass (DM) values are related from 14.4 to 30.6 t DM ha^{-1}, defined by the variety (5).

Its propagation can be done through sexual seed and vegetative seed, being this last one the most used method, although developed studies allude to a level of loss of cuttings due to the humidity present in the soil, together with other factors of direct and indirect type that intervene in its establishment (6). From this, it is necessary to deepen in studies of alternatives that make possible the reduction of these losses, when agamic seed is used for the propagation of this plant.

Among the alternatives for sustainable management in crop nutrition are bioproducts of biological origin with beneficial effects such as biofertilizers and biostimulants that allow the development of a profitable and ecological agriculture (7).

Biofertilizers are any bacterial or fungal inoculant applied to plants with the objective of increasing the availability and utilization of nutrients (8). One of the microorganisms used as biofertilizers are strains of arbuscular mycorrhizal fungi, the result of evolution that has allowed plants to adapt to stress conditions, both abiotic and biotic, through a greater capacity to absorb nutrients and water, better tolerate salinity conditions and high concentrations of heavy metals, improve soil structure, increase biological activity in the rhizosphere of plants and participate in the cycles of N, C and other elements, as well as reduce the damage caused by different phytopathogens, among other ecoservices. Therefore, mycorrhizae increase the resilience of the system (9).

On the other hand, biostimulants refer to a very broad concept, since they are substances and/or microorganisms whose function is to stimulate natural processes that improve the absorption and assimilation of nutrients, treat abiotic stress or improve some of their agronomic characteristics (10). They are organic substances used to enhance plant growth and development and allow greater resistance to biotic and abiotic stress conditions. Their composition may include auxins, gibberellins, cytokinins, abscisic acid, jasmonic acid or another phytohormone (11).

These products contain physiologically active substances (auxins, gibberellins, cytokinins, amino acids, peptides and vitamins) that, when interacting with the plant, promote or trigger different metabolic events in function of stimulating crop growth, development and yield (12). The objective of their use is to obtain better quality crops and to provide solutions to some of the most common crop problems, such as lack of humidity, low light, temperature lags, etc., which can cause losses in their commercial value (10).

However, few studies have addressed the effect of the use of bioproducts on growth stimulation and forage production of *Tithonia diversifolia*. Taking into account this background, this study was conducted with the objective of evaluating the effect of biostimulants and an arbuscular mycorrhizal fungus on some indicators of plant growth and development during the propagation of tithonia cuttings in the nursery stage.

MATERIALS AND METHODS

The experiment was carried out in the greenhouse of the Department of Biofertilizers and Plant Nutrition of the National Institute of Agricultural Sciences (INCA), San José de las Lajas municipality, Mayabeque province, at 23º 08' North latitude and 82º11' West longitude.

It was carried out in a substrate composed of a mixture of Red Ferrallitic Leached Soil (13) and Organic Matter (cow dung) in a 3:1 ratio. Some of the components of the initial chemical fertility of the soil, organic matter and substrate are presented in Table 1.

Eight treatments were evaluated

- Control (no application of bioproducts, imbibition of the cuttings in water for 30 min).
- EcoMic® (inoculation at a dose of 10 g bag^{-1} in the planting hole)
- Pectimorf® (imbibition of cuttings in the product in liquid form for 30 min) (PL)
- Pectimorf® (imbibition of cuttings in the product in solid form for 30 min) (PS)
- Bioenraiz® (imbibition of the cuttings in the product for 30 min)
- EcoMic® + Pectimorf® (PL)
- EcoMic® + Pectimorf® (PS)
- EcoMic® + Bioenraiz® (PS)

Table 1. Components of initial chemical fertility of soil (0 - 20 cm), OM and substrate

Soil type	pH	P_2O_5	OM	cmol(+) kg^{-1}			
	H_2O	(mg $100g^{-1}$)	(%)	Na	K	Ca	Mg
F. R. Leachate	7.10	48.55	3.52	0.06	0.67	20.5	1.5
Cattle manure	6.40	984.70	26.39	trazas	0.71	2.0	0.9
Substrate 3:1	7.10	292.20	8.40	0.03	0.76	20.5	6.0

pH (H_2O): potentiometric method. Soil - solution ratio 1:2,5. OM (%): Walkley-Black.Assimilable P (mg $100g^{-1}$): Oniani (extraction with H_2SO_4, 0.1N).Assimilable K (cmol(+) kg^{-1}): Oniani (extraction with H_2SO_4, 0.1N).Exchangeable cations (cmol(+) kg^{-1}): Maslova (Ammonium acetate 1N, pH 7), determination by complexometry (Ca and Mg) and by flame photometry (Na and K)

Treatments were distributed in a completely randomized design, each in 10 bags (1 plant bag^{-1}) for a total of 80 plants (bags) in the experiment. 1.5 kg bags were used with substrate composed of a 3:1 soil-OM mix.

The planting of *Tithonia diversifolia* (Hemsl.) Gray was carried out with vegetative propagules (cuttings) of approximately 30 cm in length, taken from the upper and middle part of stems coming from a field cultivated with this species at the Research Institute of Pastures and Forages of Cuba.

For inoculation with the arbuscular mycorrhizal fungus (AMF), the commercial product EcoMic® (*Glomus cubense* species, INCAM-4 strain) was used, from the commercial mycorrhizal inoculum production plant, in solid support, of the Biofertilizers and Plant Nutrition Department of INCA, with a guaranteed minimum composition of 20 spores per gram of inoculant and 50 % of radical colonization.

The following commercial products were used for the imbibition of the biostimulants: Pectimorf® in liquid form (PL) (galacturonide acid 58 - 61 %, 10 - 20 mg L^{-1}) and Pectimorf® in solid form (PS), as a fluid paste (solid - solution ratio 1: 1), from the Department of Plant Physiology and Biochemistry of INCA, Bioenraiz® (IAA, 230 mg L^{-1} for cuttings) from the Cuban Institute of Sugar Cane Derivatives Research (ICIDCA). For the control without bioproducts, imbibition was carried out in water, in order to homogenize with the other treatments.

At 50 days after planting (dap) the following variables were evaluated: number, length and diameter of branches, number of leaves and chlorophyll content in them, nutritional status - N, P and K (aerial part and root), dry mass (aerial part and root) and fungal variables were evaluated using the root staining technique (14) and the frequency of mycorrhizal colonization and colonization intensity, according to the methodologies described (15, 16).

The data, once normality and homogeneity of variance were verified, were processed by analysis of variance (IBM - SPSS Statistics 19 for Windows), where Duncan's multiple range test ($p < 0.05$) was used to discriminate the difference between means.

RESULTS AND DISCUSSION

The effect of treatments on the variables of growth, number, length and diameter of the branches is shown in Table 2, where significant differences are only observed for the length and diameter of the branches. All the treatments with the application of bioproducts were superior to the control, with no marked differences between the single applications and their combination with arbuscular mycorrhiza. It should be noted that among the combinations, only the EcoMic® + Bioenraiz® treatment stood out as showing differences with the single applications, although without differences with the other combinations.

The effect of the treatments on the number of leaves and their chlorophyll content (Table 3) showed similar results, in terms of the number of leaves, to those indicated for the previous variables, length and diameter of the branches; this was not the case for the chlorophyll content in leaves, which only showed significant differences with the control and not among the treatments with application of bioproducts, both in their simple forms and combined with AMF.

Table 2. Effect of treatments on the number, length and diameter of branches evaluated at 50 dap

Treatments	No. of branches	Length of branches	Diameter of branches
		(cm)	
Control	3.14	9.94 c	0.31 c
EcoMic ®	3.00	43.11 b	0.63 b
Pectimorf ® (PL)	3.43	39.46 b	0.60 b
Pectimorf ® (PS)	2.86	45.86 ab	0.69 ab
Bioenraiz ®	3.29	42.77 b	0.64 b
EcoMic ® + Pectimorf ® (PL)	3.43	45.60 ab	0.67 ab
EcoMic ® + Pectimorf ® (PS)	3.14	46.19 ab	0.67 ab
EcoMic ® + Bioenraiz ®	2.86	55.21 a	0.77 a
X	3.14	41.02	0.62
SE x	0.31 ns	4.94 *	0.05 *

Means with common letters in the same column do not differ significantly at $p < 0.05$

Table 3. Effect of treatments on the number of leaves and leaf chlorophyll (spad units) (50 dap)

Treatments	No. of leaves	Chlorophyll
Control	6.64 c	25.07 b
EcoMic ®	10.19 b	28.86 a
Pectimorf ® (PL)	10.00 b	29.33 a
Pectimorf ® (PS)	10.87 ab	29.76 a
Bioenraiz ®	10.61 b	29.53 a
EcoMic ® + Pectimorf ® (PL)	11.40 ab	30.94 a
EcoMic ® + Pectimorf ® (PS)	11.36 ab	31.41 a
EcoMic ® + Bioenraiz ®	12.34 a	31.99 a
X	10.43	29.61
SE x	0.73 *	1.47 *

Means with common letters in the same column do not differ significantly at $p < 0.05$

Similar results were found for stem diameter and number of leaves (17) with imbibition in Pectimorf® or inoculation with EcoMic® of seeds of *Leucaena leucocephala* cv. Cunningham, where the biological response of the plants was improved. Also, with the use of the biostimulant Bio Track- O^2 in Vanilla cuttings, at 0.75 mL L^{-1}, a favorable effect was found on the growth and development of plants, in terms of stem and leaf diameter, which could improve its propagation and establishment in the field (18). Another author, using biostimulants such as Ioduo and Biozyme TF, achieved increases in the leaf length of Janeiro grass (19).

Table 4 shows the effects of the treatments on the N, P and K concentrations of the aerial and root biomass of the tithonia crop. For the aerial part, significant differences were observed, mainly for N and P, with a tendency to reach the highest values with the combinations of bioproducts. The best result was shown by the EcoMic® + Bioenraiz® combination for both elements. Increases in foliar N have been found in Triticales associated with Haba bean with the use of a biostimulant based on marine algae (20).

As for the element K, no clear responses were observed with the application of the bioproducts in any of their forms; values even tended to decrease with the combinations. In this sense, with the use of biofertilizers in this crop, increases in N concentrations have been achieved with respect to the non-inoculated treatment, with the greatest effects for the joint application of these, however, no significant differences were obtained for P and K (21).

The effect of bioproducts on the frequency and intensity of colonization (Table 5) showed no significant differences between the treatments with bioproducts, only these differed from the control.

Table 6 shows the effect of the bioproducts evaluated on the increase in yields of *T. diversifolia*, expressed in dry mass, showing that with the separate application of the bioproducts there was a significant increase in aerial dry mass, with Pectmorf® (PS) and Bioenraiz® bioproducts standing out. However, in general, the greatest effects were obtained with the combination of EcoMic® with the different biostimulants, with the highest values for the combination EcoMic® + Bioenraiz®.

The results coincide with those reported for this crop under field conditions with the combined use of biofertilizers, where increases in dry mass yields were achieved (21). Also, with those obtained for other crops such as Triticales associated with Haba bean with the use of biostimulant based on marine algae, where greater biomass and total forage were obtained (20).

For the root dry mass variable, a positive effect was observed for all the bioproducts used, with higher values than the control, although some did not differ significantly from the control. The results indicate that there was an increase in the root system, stimulated by the biostimulatory effects of the bioproducts evaluated. The application of solid and liquid Pectimorf® showed the highest values, which coincides with what was reported when using this bioproduct on guava cuttings (22).

Table 4. Effect of treatments on N, P and K concentrations (%) (50 dap)

Treatments	Aerial part			Root		
	N	P	K	N	P	K
Control	1.54 c	0.37 b	2.65 bc	1.08 c	0.45 ab	1.50 ab
EcoMic ®	1.99 bc	0.41 b	2.76 ab	1.38 a	0.49 a	1.57 a
Pectimorf ® (PL)	2.10 bc	0.40 b	2.90 a	1.25 abc	0.48 a	1.60 a
Pectimorf ® (PS)	1.67 c	0.43 ab	2.71 bc	1.13 bc	0.42 ab	1.36 bc
Bioenraiz ®	2.19 abc	0.45 ab	2.70 bc	1.32 ab	0.43 ab	1.40 bc
EcoMic ® + Pectimorf ® (PL)	1.93 bc	0.41 b	2.49 d	1.23 abc	0.42 ab	1.30 c
EcoMic ® + Pectimorf ® (PS)	2.45 ab	0.45 ab	2.59 cd	1.23 abc	0.42 ab	1.34 bc
EcoMic ® + Bioenraiz ®	2.81 a	0.54 a	2.57 cd	1.25 abc	0.37 b	1.41 bc
X	2.09	0.43	2.67	1.23	0.44	1.43
SE x	0.29 *	0.05 *	0.07 *	0.10 *	0.04 *	0.07 *

Means with common letters in the same column do not differ significantly at $p < 0.05$

Table 5. Effect of treatments on fungal variables (%) (50 dap)

Treatments	Frequency of colonization	Intensity of colonization
Control	11.00 b	0.110 b
EcoMic ®	14.00 a	0.140 a
Pectimorf ® (PL)	13.00 a	0.130 a
Pectimorf ® (PS)	14.33 a	0.143 a
Bioenraiz ®	14.33 a	0.143 a
EcoMic ® + Pectimorf ® (PL)	13.67 a	0.137 a
EcoMic ® + Pectimorf ® (PS)	14.33 a	0.143 a
EcoMic ® + Bioenraiz ®	13.67 a	0.137 a
X	13.54	0.14
SE x	0.85 *	0.01 *

Means with common letters in the same column do not differ significantly at $p < 0.05$

Table 6. Effect of treatments on dry mass (g plant^{-1}) (50 dap)

Treatments	Aerial DM	Root DM
Control	2,23 d	0,84 b
EcoMic ®	8,27 bc	1,68 ab
Pectimorf ® (PL)	7,19 c	2,04 a
Pectimorf ® (PS)	10,54 a	2,42 a
Bioenraiz ®	9,67 ab	1,65 ab
EcoMic ® + Pectimorf ® (PL)	9,66 ab	1,86 a
EcoMic ® + Pectimorf ® (PS)	10,24 ab	1,61 ab
EcoMic ® + Bioenraiz ®	10,81 a	2,00 a
X	8,58	1,76
SE x	1,00 *	0,41 *

Means with common letters in the same column do not differ significantly at $p < 0.05$

CONCLUSIONS

- The use of the different bioproducts in their simple forms showed a positive effect on the variables evaluated, with differences between them depending on the bioproduct used and superior to the control.
- When the combination of AMF with biostimulants was used, the effects on the growth and development of Tithonia showed tendencies to be superior, depending on the combined bioproducts, without very marked differences between these combinations, highlighting, in general, the combination EcoMic® plus Bioroot® with the greatest effects on the variables evaluated.
- In general, beneficial effects of the use of the different bioproducts and some of their combinations were observed in this crop, which could favor its sustainable production.

ACKNOWLEDGE

Thanks are due to Dr. C. Alejandro B. Falcón Rodríguez, Researcher of the National Institute of Agricultural Sciences (INCA), for the donation of the Pectimorf® biostimulants (liquid and solid) and to M.Sc. Reynerio Reyes-Rouseaux for the delivery of the tithonia cuttings and Bioenraíz product ®.

BIBLIOGRAPHY

1. Cardona, J. L.; MahechaLedesma, L.; Angulo, J. Efecto sobre la fermentación in vitro de mezclas de *Tithonia diversifolia*, *Cenchrus clandestinum* y grasas poliinsaturadas. Agronomía Mesoamericana. 2017;28 (2):405-426. https://dx.doi.org/10.15517/ma.v28i2.25697.
2. Paniagua, L. D.; Arias, L. M.; Alpízar, A.; Castillo, M. Á.; Camacho, María I.; Padilla, J. E.; Campos, M.; Campos, M. Efecto de la densidad de siembra y edad de rebrote en la producción y composición bromatológica de *Tithonia diversifolia* (Hemsl.) A. Gray. Pastos y Forrajes. 2020;43(4):275-283. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942020000400275.
3. Londoño, C.; Mahecha, L.; Angulo, J. Desempeño agronómico y valor nutritivo de *Tithonia diversifolia* (Hemsl.) A. Gray para la alimentación de bovinos. Revista Colombiana de Ciencia Animal. 2019;11(1):28-41. DOI: http://doi.org/10.24188/recia,v11.n1.2019.693.
4. dos Santos Silva, A.M.; Santos, M.V.; da Silva, L.D.; dos Santos, J.B.; Ferreira, E.A.; Santos, L.D.T. Effects of irrigation and nitrogen fertilization rates on yield, agronomic efficiency and morphophysiology in *Tithonia diversifolia*. Agricultural Water Management. 2021;248: 106782. Available from: http://doi.org/10.1016/j.agwat.2021.106782.

5. Guatusmal, C.; Escobar, L.D.; Meneses, D.H.; Cardona, J.L.; Castro, E. Production and quality of *Tithonia diversifolia* and *Sambucus nigra* high andean colombian tropic. Agronomy Mesoamerican, 2020;31(1):193–208. ISSN 2215-3608, doi: http://doi.org/10.15517/am.v31i1.36677.
6. González, D.; Ruiz, T. E.; Díaz, H. Sección del tallo y forma de plantación: su efecto en la producción de biomasa de *Tithonia diversifolia*. Revista Cubana de Ciencia Agrícola. 2013; 47(4):425-429. Available from: http://redalyc.org/articulo.oa?id=193029815017.
7. Tamayo, Y.; Juárez, P.; Capdevila, W.; Lescaille, J.; Terry, Elein. Bioproductos en el crecimiento y rendimiento de *Phaseolus vulgaris* L. var. Delicia 364. Terra Latinoamericana Número Especial 2020;38(3):667-678. DOI: https://doi.org/10.28940/terra.v38i3.672.
8. Demanet, R. Uso de bioestimulantes en el cultivo de Maíz para ensilaje y Remolacha forrajera. Empresas IANSA 10 de septiembre de 2020. Available from: https://praderasypasturas.com/documentos/115.-Bioestimulantes/2020.09.10-Charla_Aminoplus.pdf.
9. Rivera, R.; Fernández, F.; Ruíz, L.; González, P.J.; Rodríguez, Y.; Pérez, E.; *et al*. Manejo, integración y beneficios del biofertilizante micorrízico EcoMic® en la producción agrícola. Ediciones INCA, San José de las Lajas, Mayabeque, Cuba. 2020. 155 p. Available from: https://www.researchgate.net/publication/340223155_Manejo_integracion_y_beneficios_del_biofertilizante_micorrizico_EcoMicR_en_la_produccion_agricola.
10. Certis. Noticias y actualidad agrícola. ¿Qué es un Bioestimulante? ¿Cómo puede mejorar la calidad de tu cosecha? 06/04/2021. Revisado: 25 de agosto de 2022. Available from: https://www.certiseurope.es/news
11. Morales, C. G. Manual de manejo agronómico de la frutilla. Capítulo 6. Uso de bioestimulantes. Instituto de Desarrollo Agropecuario - Instituto de Investigaciones Agropecuarias. Boletín INIA / N° 17 INIA - INDAP, Santiago, Chile, 2017. ISSN 0717 – 4829. https://www.inia.cl/wp-content/uploads/ManualesdeProduccion/17%20Manual%20Frutilla.pdf
12. San Juan, Ana N.; Guevara, Yusmila; Gómez, Eulalia; Delgado, Grizel; Dopico, Daisy; Díaz de Villegas, María E.; Pérez, Marlyn; León, Vivian; Michelena, Georgina; Bell, A.; Estupiñan, S.; Montero L. LOS BIOPRODUCTOS. ResearchGate. Capítulo 17. 2020. https://www.researchgate.net/publication/346816607.
13. Hernández, A.; Pérez, J.; Bosch, D.; Castro, N. La clasificación de suelos de Cuba: énfasis en la versión 2015. Cultivos Tropicales. 2019;40(1): e15. https://ediciones.inca.edu.cu/index.php/ediciones/article/view/1504
14. Rodríguez, Y.; Arias, L.; Medina, A.; Mujica, Y.; Medina, L.R.; Fernández, K.; Mena, A. Alternativa de la técnica de tinción para determinar la colonización micorrízica. Cultivos Tropicales. 2015;36(2):18-21. Available from: https://ediciones.inca.edu.cu/index.php/ediciones/article/view/1706/3385
15. Trouvelot, A.; Kough, J.; Gianinazzi-Pearson, V. Mesure du taux de Mycorrhization VA d´un Systeme Radiculaire. Recherche de Methodes d´Estimation Ayantune Fonctionnelle. En: Physiological and Genetical Aspects of Mycorrhizae (1:1985, 1 – 5 de Julio: Paris. p. 217–222). Proceedings of the 1st European Symposium on Mycorrhizae. Dijon, Francia. CNRS - INRA: 1986. 832 p. Available from: https://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=8758731
16. Herrera-Peraza RA, Furrazola E, Ferrer RL, Valle RF, Arias YT. Functional strategies of root hairs and arbuscular mycorrhizae in an evergreen tropical forest, Sierra del Rosario, Cuba. Revista CENIC. Ciencias Biológicas [Internet]. 2004;35(2):113–23. Available from: https://www.redalyc.org/pdf/1812/181226079010.pdf
17. Rizo, Maritza; Morales, D.; Sánchez, Tania; López, O.; Olivera, Yuseika; Benítez, M. A.; Ruz, F. Influencia del EcoMic® y el Pectimorf® en el establecimiento de *Leucaena leucocephala* (Lam.) de Wit. cv. Cunningham. Pastos y Forrajes. 2018;41(3):183-188. Estación Experimental de Pastos y Forrajes "Indio Hatuey", Cuba. Available from: http://www.redalyc.org/articulo.oa?id=269158218004.
18. Zarate, J.D. Efectos de la aplicación de Bioestimulantes en el pasto janeiro (*Erioclhoa polystachya*) en la zona de Babahoyo. Trabajo de Titulación de Ingeniera Agrónoma. Universidad Técnica de Babahoyo. Facultad de Ciencias Agropecuarias. Babahoyo - Los Ríos – Ecuador. 2020. 32 pp. Available from: http://dspace.utb.edu.ec/handle/49000/7980
19. Aquino-Zacarías, V.C; Azabache-Leyton, A.A; Gómez-Villanes, N.I; Jiménez-Dávalos, J; Pinedo-Taco, R. Efecto de un bioestimulante en el rendimiento de forraje de triticale (*x Triticosecale*) en siembra escalonada asociada con haba (*Vicia faba*). Tropical and Subtropical Agroecosystems. 2022;25(126):1-13. Available from: https://www.researchgate.net/publication/363287284_EFECTO_DE_UN_BIOESTIMULANTE_EN_EL_RENDIMIENTO_DE_FORRAJE_DE_TRITICALE_x_Triticosecale_EN_SIEMBRA_ESCALONADA_ASOCIADA_CON_HABA_Vicia_faba
20. Parismoreno, Laura; López, Heidy.; Litardo, Reina; Pérez, Iris. Efecto de bioestimulantes sobre el crecimiento de la *Vainilla tahitensis* en Daule, Ecuador. Ecociencia. 2021;8(6):25-38. Available from: https://doi.org/10.21855/ecociencia.86.570.
21. Méndez, S.; González, P. J.; Reyes, R.; Ramírez, J. F. Biofertilización con *Azospirillum brasilense* y *Rhizoglomus irregulare* en *Tithonia diversiforlia* (Hemsl.). Pastos y Forrajes. 2022;45:eE8. Available from: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0864-03942022000100008
22. Pérez J, Aranguren M, Luzbet R, Reynaldo IM, Rodríguez J. Aportes a la producción intensiva de plantas de guayabo (*Psidium guajava* L.) a partir de esquejes en los viveros comerciales. CitriFrut. 2013;30(2):11-16. Available from: https://scholar.google.es/scholar?hl=es&as_sdt=0%2C5&q=Aportes+a+la+producci%C3%B3n+intensiva+de+plantas+de+guayabo+%28Psidium+guajava+l.%29+a+partir+de+esquejes+en+los+viveros+comerciales.+CitriFrut.+2013%3B30%282%29%3A11-16&btnG=