



# GERMINATION OF *MORINGA OLEIFERA* L. WITH THE APPLICATION OF ECOMIC® AND TWO SYNTHETIC BIOSTIMULANTS UNDER CONTROLLED CONDITIONS

## GERMINACIÓN DE *MORINGA OLEIFERA* L. CON LA APLICACIÓN DE ECOMIC® Y DOS BIOESTIMULANTES SINTÉTICOS EN CONDICIONES CONTROLADAS

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Under controlled conditions, the effect of EcoMic® (*Glomus cubense*), TOMATICID and VIUSID® AGRO on the germination of *Moringa oleifera* Lam. was evaluated. The experiment was carried out in polyurethane foam trays, in a glass house. Four seeds were sown in each little well, which contained 120 g of substrate. Seeds were inoculated with the bioproducts, according to the recommendations of the manufacturer. A completely randomized design was applied, with 10 treatments and 10 repetitions. An ANOVA analysis was performed and differences among means were determined according to LSD of Fisher. In accumulated germination, 10 days after sowing, treatment 10 (EcoMic®, at the time of sowing, and VIUSID® AGRO, five days after sowing, 43.81%) was superior to the absolute control and the treatments 3 (EcoMic®), 4 (TOMATICID), 5 (VIUSID® AGRO) and 7 (EcoMic® VIUSID® AGRO) at the time of sowing. In partial germination, treatment 10 (30.71%) five days after sowing, as well as treatments 8 (EcoMic® + VIUSID® AGRO + TOMATICID, 45.52%) and 6 (EcoMic® + TOMATICID, 41.53%) at the time of sowing, were superior to the rest, except for 9 (EcoMic®, ST + TOMATICID) five days after sowing. In the total germination index, treatment 10 (14.46) surpassed the others, except for treatment 8 and the fertilized control. Germination speed index showed that treatment 10 (0.45) was superior to the others. It is concluded that the combination of EcoMic® and VIUSID® AGRO, five days after sowing, as well as that of EcoMic® + VIUSID® AGRO + TOMATICID and EcoMic® + TOMATICID, at the time of sowing, accelerated partial germination at 10 days after sowing with respect to the rest of treatments. The combination of EcoMic® and VIUSID® AGRO five days after sowing had a significant influence on the speed of germination and uniformity of seed vigor, which could constitute an advantage for the establishment of plantlets in the nursery. The effect of the bioproducts on germination capacity of seeds corresponded to the effect on their vigor and uniformity.

En condiciones controladas, se evaluó el efecto de EcoMic® (*Glomus cubense*), TOMATICID y VIUSID® AGRO en la germinación de *Moringa oleifera* Lam. El experimento se realizó en bandejas de espuma de poliuretano, en casa de cristal. Se sembraron cuatro semillas en cada pozuelo, que contenía 120 g de sustrato. Se inocularon las semillas con los bioproductos, según recomendaciones del fabricante. Se aplicó un diseño completamente aleatorizado, con 10 tratamientos y 10 repeticiones. Se realizó un análisis ANOVA y las diferencias entre medias se determinaron según LSD de Fisher. En la germinación acumulada, a los 10 después de la siembra, el tratamiento 10 (EcoMic®, al momento de la siembra y VIUSID® AGRO, a los cinco días después de la siembra, 43.81 %) fue superior al control absoluto y a los tratamientos 3 (EcoMic®), 4 (TOMATICID), 5 (VIUSID® AGRO) y 7 (EcoMic® VIUSID® AGRO) al momento de la siembra. En la germinación parcial, el tratamiento 10 (30.71 %) a los cinco días después de la siembra, así como los tratamientos 8 (EcoMic® + VIUSID® AGRO + TOMATICID, 45.52 %) y 6 (EcoMic® + TOMATICID, 41.53 %) al momento de la siembra, fueron superiores al resto, excepto al 9 (EcoMic®, MS + TOMATICID) cinco días después de la siembra. En el índice de germinación total, el tratamiento 10 (14.46) superó a los demás, excepto al 8 y al testigo fertilizado. El índice de velocidad de la germinación mostró que el 10 (0.45) fue superior a los demás. Se concluye que la combinación de EcoMic® y VIUSID® AGRO, cinco días después de la siembra, así como la de EcoMic® + VIUSID® AGRO + TOMATICID y EcoMic® + TOMATICID, al momento de la siembra, aceleraron la germinación parcial a los 10 días después de la siembra con respecto al resto de los tratamientos. La combinación EcoMic® (MS) y VIUSID® AGRO a los cinco días después de la siembra influyó notablemente en la velocidad de la germinación y uniformidad del vigor de las semillas, por lo que pudiera constituir una ventaja para el establecimiento de plántulas en vivero. El efecto de los bioproductos en la capacidad germinativa de las semillas se correspondió con el efecto sobre su vigor y uniformidad.

**Keywords:** *Glomus*, seeds, TOMATICID, VIUSID® AGRO

**Palabras clave:** *Glomus*, semillas, TOMATICID, VIUSID® AGRO

Received: April 01, 2024

Accepted: September 05, 2024

**Conflict of interests:** The authors declare that there is no conflict of interest

**CRediT authorship contribution statement:** C. J. Bécquer: Conceptualization, Investigation, Formal analysis, Methodology, Writing - original draft. Adelaida B. Fuentes: Investigation, Formal analysis, Methodology, Writing - original draft.



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## Introduction

*Moringa oleifera* Lam. (moringa, ben oil tree, drumstick tree, malunggay and miracle tree) is the best known of the 13 species identified within *Moringa* genus (Font Quer 1975). It stands out for its multiple uses and adaptation to different soil and climate conditions, making it an option for feeding livestock. This plant grows between one and two meters per year and reaches up to 12 m in height in the first three or four years (Pina *et al.* 2018). Generally, it grows well without applying high amounts of fertilizers. However, to improve growth and yield, organic or mineral fertilization is necessary (Guzmán-Albores *et al.* 2019). The need to shorten the nursery periods should be considered, because a phenological development, adequate for its transplantation to the field in 45 to 60 d (Oquendo 2014), is achieved with conventional methods.

Plana *et al.* (2016) pointed out that the use of arbuscular mycorrhizal fungi (AMF) leads to a symbiosis of the microorganism with the plant, which allows the transport of necessary nutrients for its metabolism and improves the chemical, physical and biological properties of soil. Mycorrhizal fungi are the main organisms associated with *Moringa* spp. (Knopf *et al.* 2013). According to Pita (2014), *Glomus fasciculatum* Tul. establishes mycorrhizal association with *M. oleifera*, which places this species among those that establish symbiosis with endomycorrhizas.

Various natural products are used to enhance the ecological management of agroecosystems, including biopesticides, phytostimulants and biostimulants (Núñez 2018). VIUSID® AGRO is one of the synthetic biostimulants applied to crops in Cuba, which contains nitrogen and free amino acids in its chemical composition, elements that play an essential role in the formation of proteins and facilitate their easy absorption into the plant (Simbaña 2011). Another available biostimulant is TOMATICID, a commercial product that contains 0.14 w/v of 4-chloro phenoxyacetic acid (4-CPA). This product acts as a plant growth regulator and allows very good fruiting in tomato plants, at the same time that it causes an advance in the ripening of fruits (Lami-Izquierdo *et al.* 2011). No references were found from other authors on the application of these biostimulants to improve the germination of moringa seeds.

The objective of this research was to evaluate the stimulating effect of EcoMic®, VIUSID® AGRO and TOMATICID on germination and initial development of *M. oleifera* for its future application in the nursery phase.

## Materials and Methods

**Experimental procedure.** The experiment was carried out in polyurethane foam trays, in a glass house, where seedlings grew under natural light conditions in a chamber of 2500 to 3500 lux, with temperatures of  $\pm 27$  °C. The relative humidity was 75/85 %. Four seeds were sown in each little well, containing 120 g of substrate. It was planted 1 cm deep. The start of germination was considered when the radicle had a minimum length of 2 mm. Seeds were soaked for 24 h prior to sowing, according to the methodology of Padilla *et al.* (2012).

**Substrate characteristics.** The used substrate was formed with 40 % organic matter and 40 % brown soil, without differentiation of carbonates and 20 % river sand. Table 1 shows its chemical characteristics.

**TOMATICID.** This product, which contains 0.14 w/v of 4-chloro phenoxyacetic acid (4-CPA), was applied at a concentration of 10 mL/L H<sub>2</sub>O, at a rate of 1 mL per seed at the time of sowing (TS) and after five days of sowing (DAS), depending on the treatment.

**VIUSID® AGRO.** This product was applied at a concentration of 1 mL/L H<sub>2</sub>O, at a rate of 1 mL per seed, at TS and at five DAS, depending on the treatment.

**Mycorrhizal inoculant.** The EcoMic® product was used, formulated from the arbuscular mycorrhizal fungal (AMF), INCAM4 strain (*Glomus cubense*). For its application, a certified solid inoculant was used, containing 25 spores/g of the substrate sold by the company LABIOFAM SA. For this purpose, the recommendations of the manufacturer were followed. It was applied at a rate of 5 g/little well at the time of sowing or at five DAS (according to the treatment), then it was irrigated (10 mLH<sub>2</sub>O/little well).

**Experimental design and statistical analysis.** A completely randomized design was applied, with 10 treatments (table 2) and 10 repetitions, for 10 little wells per treatment in total and 100 little wells in each experimental unit (three units, with 300 little wells). The units consisted of three blocks of polyurethane foam trays, with the same number of treatments and repetitions. Once the assumptions of normality and homogeneity of variance for the application of parametric tests were verified, an ANOVA analysis was performed. Differences between means were determined by the LSD of Fisher. Percentage data were transformed by  $2\arcsin\sqrt{P}$ . The statistical program StatGraphics Centurion XV (Anon 2007) was used.

**Table 1.** Chemical characteristics of the substrate used in the experiment

| Sample          | Na      | K    | Ca   | Mg  | P, mg/kg | OM, % | pH, H <sub>2</sub> O |
|-----------------|---------|------|------|-----|----------|-------|----------------------|
|                 | cmol/kg |      |      |     |          |       |                      |
| Solid substrate | 0.15    | 0.47 | 10.9 | 2.0 | 258      | 5.29  | 7.0                  |

**Table 2.** Treatments established in the experiment

| Number | Treatments                                        |
|--------|---------------------------------------------------|
| 1      | Absolute control                                  |
| 2      | Chemically fertilized control                     |
| 3      | EcoMic® (TS)                                      |
| 4      | TOMATICID 10 mL/L (TS)                            |
| 5      | VIUSID® AGRO 1mL/L (TS)                           |
| 6      | EcoMic® (TS) + TOMATICID (TS)                     |
| 7      | EcoMic® (TS) + VIUSID® AGRO (TS)                  |
| 8      | EcoMic® (TS) + VIUSID® AGRO (TS) + TOMATICID (TS) |
| 9      | EcoMic® (TS) + TOMATICID (five DAS)               |
| 10     | EcoMic® (TS) + VIUSID® AGRO (five DAS)            |

**Chemical fertilization.** The fertilized control consisted of an application of 23.84 mg of  $\text{NH}_4\text{NO}_3$ /little well, equivalent to 150 kgN/ha.

Cumulative and total germination percentage (PGA, %), at five DAS, 10 DAS, 15 DAS and 20 DAS according to the formula  $\text{PG} = \text{NSG}/\text{NSS} \times 100$  (Ede *et al.* 2015)

Where:

NSG: number of germinated seeds

NSS: number of sown seeds

Partial germination percentage (PGP, %) at five DAS, 10 DAS, 15 DAS and 20 DAS according to the formula  $\text{PGP} = \text{NSGti}/\text{NSS} \times 100$  (Ede *et al.* 2015)

Where:

NSGti: number of seeds germinated in ti (time in which the seeds were sprout).

NSS: number of sown seeds

Index of phase germination (IG), according to the formula  $\text{IG} = (\text{ni} \cdot \text{ti})/\text{N}$  (Islam *et al.* 2012)

Where:

ti: days elapsed since sowing

ni: number of seeds germinated in ti

N: total seeds

Total germination index (IG), according to the formula Scott *et al.* (1984):

$$\text{IG} = \sum (\text{ni} \cdot \text{ti})/\text{N}$$

Where:

ti: days elapsed since sowing (sum of time periods)

ni: number of germinated seeds in ti (sum of germinated seeds in each period)

N: total seeds

Germination speed index (IVG), according to the formula of Terry *et al.* (2014):

$$\text{IVG} = \sum (\text{ni}/\text{ti})$$

Where:

ni: number of seeds germinated in you ti

ti: time elapsed since sowing

Inoculation efficiency index (IEI, %) based on the final cumulative germination percentage (PG), according to the formula of Santillana *et al.* (2012):

$$\text{IEI} = [(TI - CA)/CA \times 100]$$

Where:

TI: PG of inoculated treatment

CA: PG of absolute control

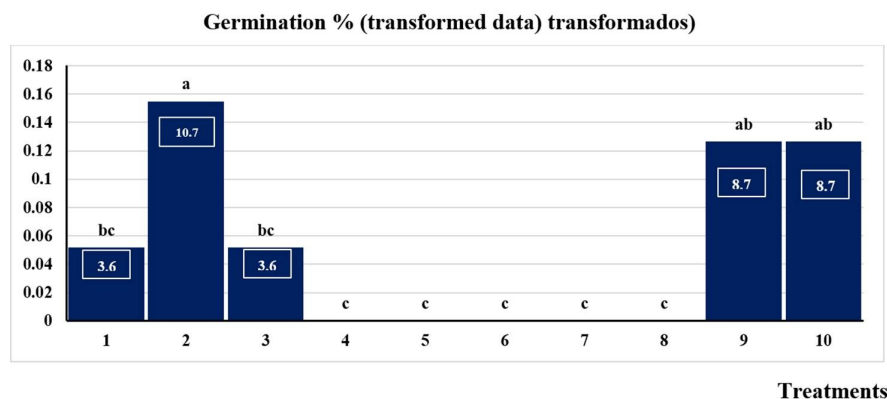
## Results and Discussion

In the five DAS phase (figure 1), the fertilized treatment was superior to the absolute control and the inoculated treatments, although it shared letters with treatment 3

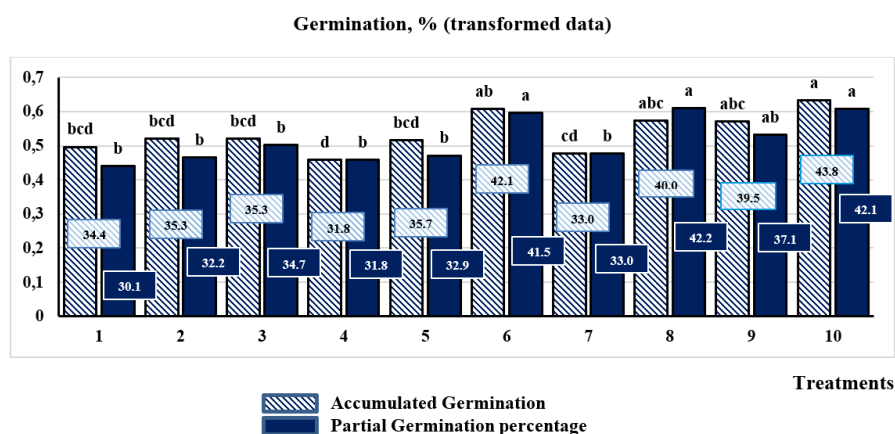
In this phase, most of the inoculated treatments did not present germination, except those where EcoMic® (sowing) and each biostimulant were applied at five DAS. There were no significant differences among the germinated treatments, neither with the fertilized control nor with the negative control. It is interesting to note that the absolute control, as well as the fertilized control and three of the inoculated treatments, presented germination in this phase. Meanwhile, the rest of the inoculated treatments did not germinate. This could be associated with an initial inhibition of germination due to the effect of abscisic acid (ABA) (Posada *et al.* 2021), caused by the presence of AMF in some treatments. However, this possibility has not yet been defined because the changes in hormonal levels in plants when inoculated seem to depend on the type of AMF and the plant species (Bernardo *et al.* 2020). Noguera-Talavera *et al.* (2018) state that moringa germination begins on the third day with the highest number of germinations between 9 and 11 d. These results also contradict Cadillo (2022), who obtained, under nursery conditions, emergence percentages of 61 to 85 % at seven DAS for *M. oleifera* seeds, treated with different synthetic biostimulants.

The accumulated germination at 10 DAS (figure 2) showed that treatment 10 was superior ( $p < 0.05$ ) to absolute control and treatments 3, 4, 5 and 7, but had equal letters with respect to treatments 6, 8 and 9. Regarding partial germination, treatments 10, 8 and 6 were superior ( $p < 0.05$ ) to the rest, except for 9, with which they shared the same letters.

In the germination phase at 10 DAS, the effect of the inoculated treatments is better defined. In partial germination, the fertilized control was inferior to the treatments that stood out, a fact that indicates the superior effect of the bioproducts on germination in this phase. The common denominator for these treatments is the application of EcoMic®, which contains *Glomus cubense*. This genus of fungus is well known for its effect on increasing the germination of different species (Wasy *et al.* 2010). Ballina *et al.* (2017) reported the superior effect of AMF *Glomus intraradice* on the germination of *Senna racemosa* and *Bahinia forficata* (both belonging to the Fabaceae family).



**Figure 1.** Germination, % (transformed data by  $\sqrt{[0.5+x]}$ ) at five DAS of *Moringa oleifera*, inoculated with EcoMic®, TOMATICID and VIUSID® AGRO, simple or combined, in different moments. Percentage data is demonstrated with bars. Different letters indicate significant difference ( $p < 0.05$ ). Standard Error (S.E.): 0.011623



**Figure 2.** Germination, % (transformed data by  $\sqrt{[0.5+x]}$ ) at ten DAS of *Moringa oleifera*, inoculated with EcoMic®, TOMATICID and VIUSID® AGRO, simple or combined, in different moments. Percentage data is demonstrated with bars. Different letters indicate significant difference ( $p < 0.05$ ). Accumulated germination:  $p < 0.05$ , S.E.: 0.012744. Partial germination:  $p < 0.01$ , S.E.: 0.013359

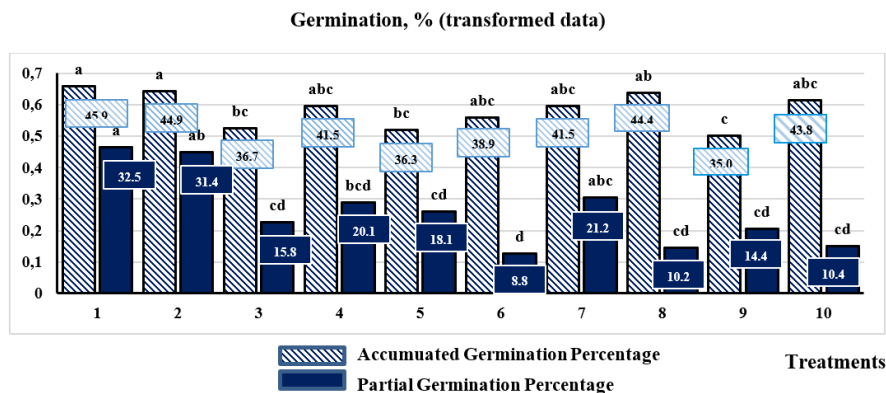
In the case of treatment 10, in addition to EcoMic®, VIUSID® AGRO was applied to the sowing at five DAS. This compound is known because of its high content of amino acids (alanine, glycine, glutamic acid, proline and arginine). This last amino acid constitutes 40% of proteins in seeds. Angelovici *et al.* (2011) pointed out that proteins in seeds are not only an important source of amino acids during germination, they are also essential for energy production, so the importance of this amino acid in the germination results of *M. oleifera* is not ruled out.

The application of TOMATICID did not show a significant effect as in the inoculation of EcoMic® with VIUSID® AGRO. However, in the values obtained with treatment 6 (EcoMic®, ST + TOMATICID, ST), as well as with treatment 9 (EcoMic®, ST + TOMATICID, five DAS), in partial and accumulated germination, it was observed that they share letters with the treatments that had the greatest effect, so the influence of the

4-chloro phenoxyacetic acid contained in TOMATICID is not rejected. According to Barberá (1989), many applications replace naphthaleneacetic acid and indoleacetic acid (important hormones for cell division), due to the occasional irregular results of these last two compounds.

In the 15 DAS phase (figure 3), the accumulated germination showed that absolute control, as well as the fertilized treatment, were superior ( $p < 0.05$ ) to treatments 3, 5 and 9, although they shared the same letters with the rest. In partial germination, the absolute control was superior ( $p < 0.001$ ) to the other treatments, except the fertilized one and 7, which had statistical similarity.

The 15 DAS phase showed germination marked by lower values of the inoculated treatments compared to the absolute control and the fertilized control in partial germination, except in treatment 7. Also, in this phase, the fertilized control and the negative control are equal in their values. Regarding cumulative germination, although the values of



**Figure 3.** Germination, % (transformed data by  $\sqrt{[0.5+x]}$ ) at 15 DAS of *Moringa oleifera*, inoculated with EcoMic®, TOMATICID and VIUSID® AGRO, simple or combined, in different moments. Percentage data is demonstrated with bars. Different letters indicate significant difference. Accumulated germination:  $p < 0.05$ , S.E.: 0.014110. Partial germination:  $p < 0.01$ , S.E.: 0.020638

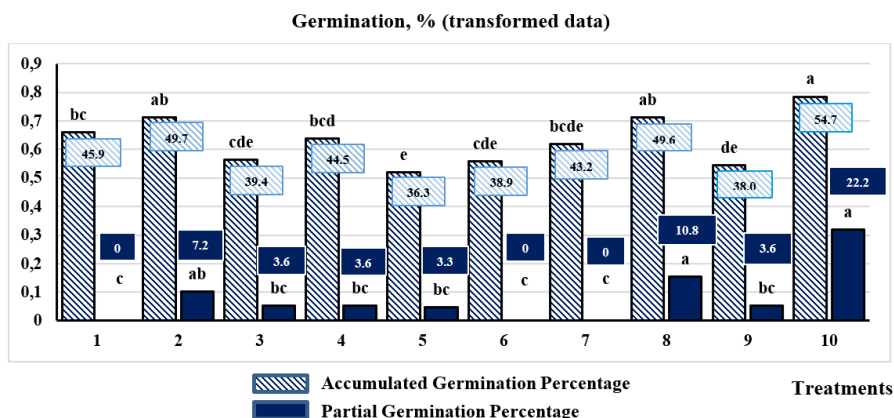
inoculated treatments are mostly equal to the fertilized control, they also do so with the absolute control. These results contradict what Noda and Castañeda (2012) reported, who obtained the highest percentage of germination at 15 and 16 DAS in *Jatropha curcas* seeds (*Euphorbiaceae* family), when inoculating them with EcoMic®. However, Serbelló *et al.* (2014) demonstrated the inhibitory effect of *Glomus fasciculatum* on seeds of *Carica papaya* L. (*Caricaceae* family) in the first days after sowing, although this effect decreases until reaching a germination percentage similar to the rest of the treatments after 28 d. This result should be further studied in other experiments, due to its physiological basis.

There are no references to the effect of TOMATICID and VIUSID® AGRO in this phase of the variable under study. Inferior values of the treatments inoculated with said bioproducts, as well as the statistical similarity of the fertilized control with the control, could be attributed to insufficient mobilization of the compounds from the seed reserve in this phase.

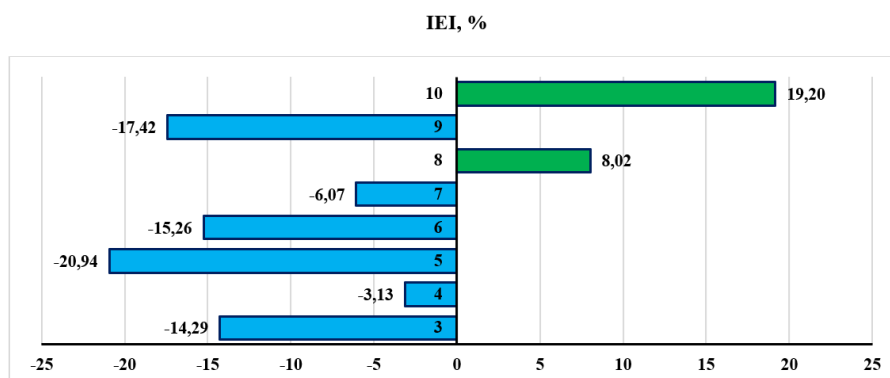
At 20 DAS (figure 4), in the accumulated germination, treatment 10 was superior ( $p < 0.001$ ) to the rest, except for the fertilized control and treatment 8. In the partial, it was also found that treatment 10 and 8 were superior ( $p < 0.001$ ) to the rest, except for the fertilized control, with which they shared the same letters.

With respect to the inoculation efficiency index based on the final accumulated germination (IEI, %) in the 20 DAS phase (figure 5), treatments 10 and 8 presented positive values in their IEI, while it was negative in the rest.

In this last phase of germination, it was observed that the greatest effect of bioproducts occurred in the treatments where EcoMic® was applied at sowing and VIUSID® AGRO at five DAS. This was followed by the application of the three bioproducts at the same time, during sowing (figure 4). Note that these treatments, which presented a higher IEI (figure 5), included EcoMic® combined with VIUSID® AGRO. The treatment in which EcoMic® (ST) + VIUSID® AGRO (five DAS) was applied was considered to have high efficiency. Hernández (2016) assured that



**Figure 4.** Germination, % (transformed data by  $\sqrt{[0.5+x]}$ ) at 20 DAS of *Moringa oleifera*, inoculated with EcoMic®, TOMATICID and VIUSID® AGRO, simple or combined, in different moments. Percentage data is demonstrated within bars. Different letters indicate significant difference. Accumulated germination:  $p < 0.05$ , S.E.: 0.014102. Partial germination:  $p < 0.0001$ , S.E.: 0.013491



**Figure 5.** Inoculation efficiency index (%) in *Moringa oleifera* with EcoMic®, TOMATICID and VIUSID® AGRO based on the final accumulated germination

the application of the biostimulant Biozyme TF (hormonal complex of gibberellin, auxin and cytokinin-zeatin) had a superior effect on the final germination of *M. oleifera* seeds.

The application of EcoMic®, as well as VIUSID® AGRO, showed the high efficiency of these bioproducts. The effect of EcoMic® on this variable coincides with Noda and Castañeda (2012), who considered that the use of EcoMic® as seed inoculum could be a satisfactory practice to obtain good emergence percentages, which would represent a greater number of seedlings in relatively short periods of time. Reyes-Pérez *et al.* (2021) also found that the percentage of highest emergence in *Cucumis sativus* seeds (*Cucurbitaceae* family) was shown with the application of *Glomus cubense*. However, Bover-Felices *et al.* (2017) did not obtain superior results in the emergence of seeds of *Leucaena leucocephala* (*Fabaceae* family), when inoculating them with *G. cubense*. Knopf *et al.* (2013) observed that *M. oleifera* seeds, sown in soils with a high population of AMF, had better germination than seeds sown in soils with a low population of these fungi.

The high zinc content in VIUSID®AGRO favors the formation of new tissues and, specifically, germination (Catalysis 2016). It is noteworthy that, although the application of EcoMic® did not favor seed germination in other treatments in this last phase, the combination of this biofertilizer at the time of sowing with VIUSID® AGRO at five DAS showed a synergistic effect superior to the other combinations. However, the presence of TOMATICID had no marked influence on this variable.

It should be stated that low germination percentages were obtained, even in the best treatments. This can be related to the quality of the seed used, which could have lost its viability due to the storage time to which it was subjected. Ramírez (2019) indicated that storage time of moringa seeds may influence on low germination percentage, even after the inoculation with mycorrhizal fungi. Vital *et al.* (2018) pointed out that, when applying AMF to sunflower seeds (*Helianthus annuus*, *Asteraceae* family), the germination

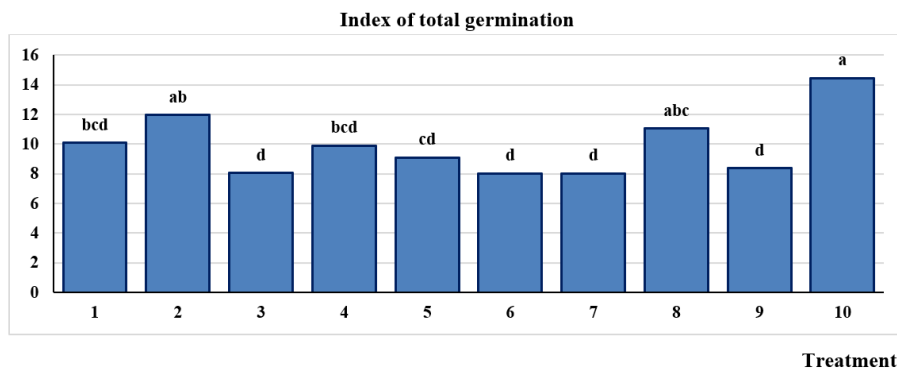
of these seeds depended on their genotype and storage time. Knopf *et al.* (2016) evaluated the use of mycorrhizal fungi in *Moringa* spp. and they found that there were low percentages of germination in five days (62.5 %, 33 %), as well as in 8 and 10 days (58 %), depending on the type of soil.

Figure 6 shows that treatment 10 surpassed ( $p < 0.001$ ) the rest, except for treatment 8 and the fertilized control, which has the same letters.

Total germination index provides a measure of germination time relative to germination capacity. This index affected this germination capacity and does not provide information about the distribution of germination events over time (González-Zertuche and Orozco-Segovia 1996), as does the phase germination index (Islam *et al.* 2012). However, it serves for having a general confirmation about how the germination capacity of the seeds behaves depending on the total germination time.

In this variable, it was observed that treatment 10 surpassed most of the treatments, although it presented statistical similarity with treatment 8 and the fertilized control. The presence of the EcoMic® biofertilizer in this treatment influenced, as well as other variables, on the germination capacity of *Moringa oleifera* seeds. It is known that the inoculation of plants with effective AMF species caused a marked increase in the 17 processes of nutrient absorption and translocation, whether by interception, mass flow or diffusion (Bennett and Cahill Jr 2016). In a study by Murali *et al.* (2023), it was recorded that the application of AMF in *M. oleifera*, for three consecutive years, had a greater effect on germination, compared to the inoculation of rhizospheric bacteria in the same period.

The presence of growth regulators in the composition of VIUSID® AGRO is another factor that must have had an important value in this variable. Catalysis (2018) stated that the *Ascomyces nodosum* algae, present in the formulation of this biostimulant, also contributes to growth inducers



**Figure 6.** Index of total germination of *Moringa oleifera* seed, inoculated with EcoMic®, TOMATICID and VIUSID® AGRO, simple or combined, in different moments. Different letters indicate significant difference ( $p < 0.001$ , S.E.: 0.282357)

to the formulation. This alga contains gibberellins and cytokinins, especially zeatin, which is highly biologically active, and both stimulate germination (Sandoval *et al.* 2018 and Borjas-Ventura *et al.* 2020).

Although there are no references on the effect of TOMATICID on this variable, as it is one of the components of the treatment in which EcoMic® (ST) + VIUSID® AGRO (ST) + TOMATICID (ST) was inoculated, it is inferred that it did not present a negative interaction with the rest of the bioproducts, despite the fact that no significant effect on the seed was demonstrated.

Figure 7 shows that at five DAS, treatment 10, as well as 9, were superior ( $p < 0.01$ ) to treatments 4, 6, 7 and 8. However, it showed letters equal to the fertilized control, and to the treatments 3 and absolute control. At 10 DAS, treatments 10, 6 and 8 showed superiority ( $p < 0.05$ ) with respect to the absolute control, fertilized control, treatment 5 and 7, although they shared the same letters with treatments 3, 4 and 9. At 15 DAS, treatment 10 decreased its values and was lower ( $p < 0.01$ ) than absolute control and fertilized control and shared the same letters with the rest. At 20 DAS, treatment 10 was superior ( $p < 0.05$ ) to the others.

The index of phase germination provides a measure of germination time in relation to germination capacity and is sensitive to changes in germination capacity (González-Zertuche and Orozco-Segovia 1996). This variable, in general, increased as days passed, which coincided with the reports of Mehta *et al.* (2014). However, from 15 DAS, there was a decrease in all treatments, except for the one in which EcoMic® was applied at sowing and VIUSID® AGRO at five DAS.

Considering that the index of phase germination is more sensitive to changes in germination capacity, it can be deduced that there was more intense germinative acceleration for treatments 10, 6 and 8, at 10 DAS. However, at 15 DAS, the absolute control showed letters equal to treatments 2, 4 and 5, and higher than treatments

10, 9, 8, 7, 6 and 3. This indicates that the bioproducts that induced germinative acceleration at 10 DAS did not exert a superior effect on the subsequent phase. In general, all treatments, except EcoMic® (ST) + VIUSID® AGRO (five DAS), decreased in their values after 15 DAS, indicating that there was a normal bell-shaped distribution pattern, which agrees with the results of Barraza *et al.* (2016) with higher values at 10 DAS in most treatments.

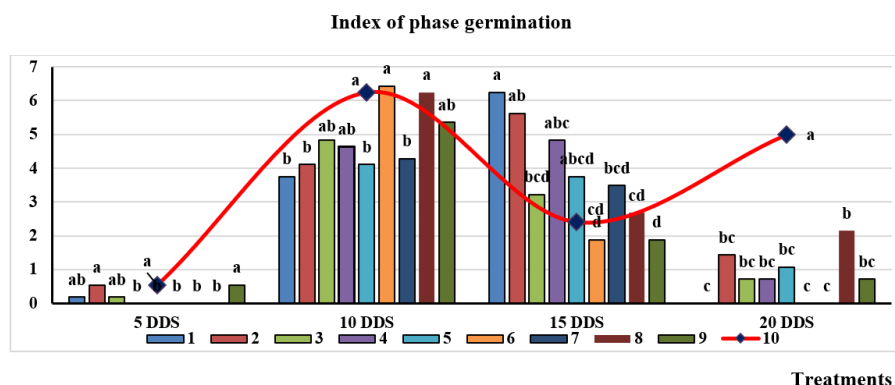
The application of EcoMic® (ST) + VIUSID® AGRO (five DAS) was superior to the rest of the treatments at 20 DAS and showed a higher index than that at 15 DAS. The above could be explained by the gibberellin content in VIUSID® AGRO, which can break seed dormancy and frequently replace the need for environmental stimuli, such as light and temperature. These phytohormones are important during the initial and final phases of germination (Weitbrecht *et al.* 2011).

VIUSID® AGRO has a molecular activation process, which allows it to accelerate and facilitate the process of enzymatic and metabolic reactions (Gavica and Gómez 2019). Regarding the effect of EcoMic®, it is known the ability of AMF to exude compounds that stimulate microbial activity in the ecological niche. Consequently, they all have influence on the germination process, since breaking seed dormancy favors the entire process (Rivera *et al.* 2011).

Germination speed index (figure 8) showed that the treatment in which EcoMic® was applied at sowing and VIUSID® AGRO at five DAS was superior ( $p < 0.0001$ ) to the rest of inoculated treatments, including absolute control and fertilized control. The latter was superior to the absolute control and treatments 3, 4, 5, 6 and 7, in addition to sharing letters equal to 8 and 9.

The treatment in which EcoMic® was applied at sowing and VIUSID® AGRO (five DDS) was the one that had superior effect on germination speed of *M. oleifera* seeds.

The concept of germination speed index is important because it is related to seed vigor, considering the number that germinate and the time required to achieve it (Meot-Duros and Magné 2008). In summary, this index expresses



**Figure 7.** Index of germination of *Moringa oleifera* seeds, inoculated with EcoMic®, TOMATICID and VIUSID® AGRO, simple or combined, in different moments. Growth line is demonstrated in treatment 10. Different letters indicate significant difference (five DAS:  $p < 0.01$ , S.E.: 0.046165. 10 DAS:  $p < 0.05$ , S.E.: 0.220090. 15 DAS:  $p < 0.01$ , S.E.: 0.306997. 20 DAS:  $p < 0.05$ , S.E.: 0.218673)

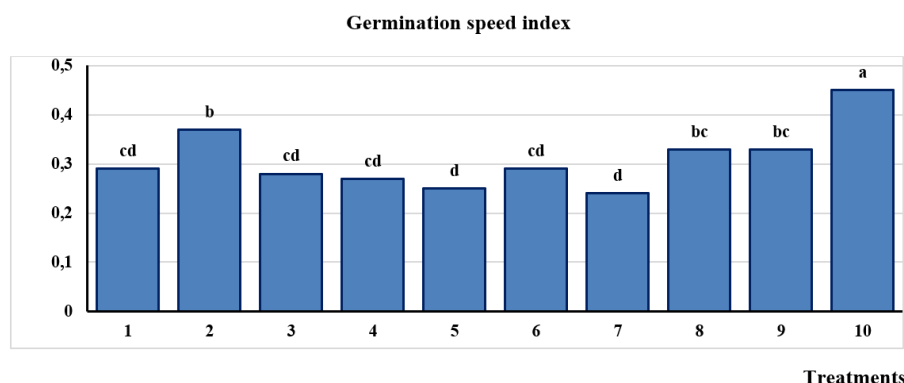
the speed in number of seeds germinated per day (Espindola *et al.* 2018). According to Venter (2000), under field (nursery) conditions, rapid germination is obviously an advantage for seedling establishment. Germination speed is, therefore, an expression of vigor. High vigor seeds germinate faster than low vigor seeds under any conditions. The influence of seed storage conditions on the germination speed of *M. oleifera* is also known (Silva *et al.* 2012).

In the variable that is discussed, it can be inferred that functionally the seeds were affected in terms of the speed with which the germination process occurred, according to the treatments. Among the bioproducts that were applied, the combination (in a fractional way) of EcoMic® and VIUSID® AGRO in treatment 10 markedly influenced, not only germination speed, but also the uniformity of seed vigor, which allowed that the latter could be expressed through a higher percentage of germination and at a higher speed compared to the other treatments. This could constitute an advantage for the establishment of seedlings in the nursery, since the seeds would germinate faster with the application of these bioproducts at different times. Peña *et al.* (2015) determined the effect of VIUSID® AGRO on

the germination of beans (*Phaseolus vulgaris* L., family: *Fabaceae*), as well as on the growth of seedlings under *in vitro* conditions. These authors concluded that immersion of bean seeds for three hours in a 0.02% VIUSID® AGRO solution favors germination speed and seedling development.

The results of this variable with the simple inoculation of EcoMic® contradict those found by Láinez (2021), who observed an increase in this variable compared to control, when inoculating AMF (different species) in *Cucumis melo* seeds (*Cucurbitaceae* family). It is possible that AMF do not have the same effect on the seeds of different plant species because they have different morphological, germination and dormancy characteristics. There is also the possibility that mycorrhizae cause a coating effect on seeds, which delays the breakdown of the testa by other soil microorganisms (Ballina *et al.* 2017).

The application of TOMATICID, despite having a positive influence on germination percentage when combined with the other bioproducts, did not have a superior effect on the speed of germination of the seeds.



**Figure 8.** Germination speed index of *Moringa oleifera* seeds, inoculated with EcoMic®, TOMATICID and VIUSID® AGRO, simple or combined, in different moments. Different letters indicate significant difference ( $p < 0.0001$ , S.E.: 0.008990)

This result decreases the importance of said bioproduct for stimulating germination in moringa.

It is concluded that the combination of EcoMic® (ST) and VIUSID® AGRO (five DAS), as well as the mixture of EcoMic® + VIUSID® AGRO + TOMATICID (ST) and EcoMic® (ST) + TOMATICID (ST) accelerated partial germination at 10 DAS regarding the rest of treatments. The combination of EcoMic® (ST) and VIUSID® AGRO (five DAS) significantly influenced the speed of germination and uniformity of seed vigor, which could constitute an advantage for the establishment of seedlings in the nursery. Results indicate that the effect of the bioproducts on the germination capacity of seeds was in line with the effect on their vigor and uniformity.

It is recommended to carry out tests to evaluate germination and initial development of seedlings in the nursery, with the application of the studied bioproducts and the combinations that had better effect in the present study.

### Acknowledgements

Thanks to G. Fernández MSc. student of Universidad de Sancti Spiritus for the participation in the experiment.

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**Germinación de Moringa Oleifera L. con la aplicación de ecomic<sup>®</sup> y dos bioestimulantes sintéticos en condiciones controladas**

*Cuban Journal of Agricultural Science*

vol. 58, e16, 2024

Ediciones ICA,

**ISSN:** 0864-0408

**ISSN-E:** 2079-3480