

Evaluation of the Application of HeberNem-S® in open-field papaya cultivation

Evaluación de la aplicación de HeberNem-S® en el cultivo de papaya a cielo abierto

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ABSTRACT: The bionematicide and biofertilizer HeberNem-S® has been successfully used in protected and semi-protected crops, demonstrating its effectiveness in stimulating germination, growth, and flowering. However, its use in unprotected crops has not been sufficiently studied. This study represents the first evaluation of the effect of HeberNem-S® on vegetative growth and agricultural productivity in papaya cultivation under open-air conditions. The trial was conducted at the El Jibao farm in Camagüey, using seedlings of the Maradol Roja variety. A four-application schedule of HeberNem-S® was followed: at transplanted, and 40, 100, and 160 days later. In treated plants, morphophysiological indicators such as plant height and stem diameter increased significantly compared to the control group. Furthermore, flowering and fruiting occurred earlier in plants that received HeberNem-S® compared to the control group and compared to the variety reported for the same group. Agricultural production indicators also increased significantly: number of flowers and fruits per plant, length and diameter of technically ripe fruit, and average fruit weight, with an estimated yield of 87 kg/ha at the end of the crop, higher than the average reported in Cuba of 50 kg/ha. These results demonstrate the stimulating effect of HeberNem-S® on vegetative growth and agricultural productivity in papaya cultivation under open-air conditions.

Keywords: Sustainable Agriculture, Bionematicide, HeberNem-S®, Agricultural Productivity.

RESUMEN: El bionematicida y biofertilizante HeberNem-S® se ha empleado exitosamente en cultivos protegidos y semiprotegidos, demostrando su efectividad para la estimulación de la germinación, el crecimiento y la floración. Sin embargo, su uso en cultivos no protegidos no ha sido suficientemente estudiado. El presente trabajo representa la primera evaluación del efecto de HeberNem-S® sobre el crecimiento vegetativo y productividad agrícola en el cultivo de papaya en condiciones de cielo abierto. El ensayo se desarrolló en la finca El Jibao, Camagüey, con posturas de la variedad Maradol Roja. Se siguió un esquema de 4 aplicaciones de HeberNem-S®: al momento del trasplante, 40, 100 y 160 días después. En las plantas tratadas, respecto a la comparación con el control absoluto, aumentaron significativamente indicadores morfofisiológicos como: altura de planta y diámetro del tallo. Además, se produjo un adelanto de la floración y la fructificación en las plantas que recibieron HeberNem-S®, con respecto al grupo testigo y a lo reportado para la variedad. También se incrementaron significativamente indicadores agroproductivos: número de flores y frutos por planta, longitud y diámetro de los frutos con madurez técnica y peso promedio de los frutos, con un rendimiento estimado de 87 kg/ha al finalizar el cultivo, superior a la media reportada en Cuba de 50 kg/ha. Con estos resultados se demuestra el efecto estimulador de HeberNem-S® sobre el crecimiento vegetativo y productividad agrícola en el cultivo de papaya en condiciones de cielo abierto.

Palabras clave: Agricultura Sostenible, Bionematicida, HeberNem-S®, Productividad Agrícola.

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INTRODUCTION

The main challenge of modern agriculture is the production of high-quality, ecologically safe, and economically affordable food for a constantly growing global population (Marín, Mena, et al., 2013).

Modern agriculture requires high yields to meet human needs; the use of chemical pesticides to achieve this goal has yielded partially satisfactory results for several decades. The use of chemicals for pest control in agriculture has led to increasing ecosystem deterioration, in addition to causing food produced under this regime to be contaminated and harmful to consumers (Campos et al., 2003).

Currently, consumers are very interested in the origin of food products, how they were grown, and whether they are safe to eat, as well as their nutritional content, emphasizing their concern about possible contamination with agrochemicals, especially those for fresh consumption (Díaz et al., 2013).

Interest in biological control and microbial pesticides has increased in recent years due to the need to find alternatives to chemical products and to protect the environment. However, the success of using these types of products depends fundamentally on understanding their mechanisms of action and their interaction with other microorganisms in the plant rhizosphere (Marín et al., 2010).

In recent decades, there has been a growing trend in the use of native and non-native beneficial microorganisms, with the aim of improving crop yield and increasing production. In this context, numerous soil microorganisms with potential have been included in various integrated pest management practices and improved agricultural productivity (Marín, Mena, et al., 2013).

Currently, biofertilizers are considered components of integrated plant nutrition management and have been defined as substances containing live microorganisms that, once applied to seeds, plants, or soil, colonize the rhizosphere or interior of the plant and stimulate its development, thereby increasing nutrient availability and the health of the crop (Marín, Mena, et al., 2013).

At the Center for Genetic Engineering and Biotechnology (CIGB) in Camagüey, Cuba, the bacterium *Tsukamurella paurometabola* strain C-924 (currently known as *Brevibacterium celere* C-924) was obtained from a rhizospheric soil sample of plantain (*Musa* spp.). This species, which belongs to the *Actinomycetales* order, groups irregular, Gram-positive, non-motile bacilli with strictly aerobic metabolism. The strain obtained proved to be an effective control of plant-parasitic nematodes in different agricultural systems and constitutes the active ingredient of HeberNem[®], registered as a bionematicide and biofertilizer (Marín et al., 2013). Currently, the HeberNem product is produced in solid form (HeberNem-S[®]) with excellent bionematicidal properties.

HeberNem[®] is the first purely Cuban biological product that meets all the biological safety requirements established by regulatory authorities, having successfully passed 20 toxicological tests and 9 ecotoxicological tests. Its effectiveness has been demonstrated in several field

trials. To achieve its expansion and initial generalization throughout the country, its behavior within the agricultural ecosystem has also been studied, it has been evaluated in integrated pest management through trials covering behavior against bioregulatory fungi and bacteria and disease-causing agents, chemicals frequently used in protected crop systems, and arbuscular mycorrhizae.

The bacterium *B. celere* C-924 has been evaluated in several studies regarding its interaction with the mycorrhizal fungi *Glomus fasciculatum* and *Glomus clarum* on mycorrhizal colonization and leaf weight in lettuce (*Lactuca sativa* L.) (Marín et al., 2010); its interaction with *Rhizobium leguminosarum* biovar phaseoli CFH in bean crops by analyzing the phenological parameters of the plants (Marín, Mena, et al., 2013); its potential as a biological control agent, through in vitro tests in two culture media (Tryptone Soy Agar and Potato Dextrose Agar), of the following phytopathogenic fungi: *Alternaria longipes* (Ellis and Everh.), *Bipolaris oryzae* (Breda de Haan), *Colletotrichum gloeosporioides* (Penz.), *Fusarium oxysporum* f. sp. *cubense* (E.F. Sm.), *Sarocladium oryzae* (Sawada), *Pestalotia palmarum* (Cooke), *Pythium debaryanum* (R. Hesse), *Rhizopus stolonifer* (Ehrenb.) and *Thielaviopsis paradoxa* (De Seynes), which are fungal species that are causal agents of diseases in crops of economic interest (Marín, Wong, García, et al., 2013); and the determination of some of the plant growth promoting attributes of *B. celere* C-924, evaluating the effect of this bacteria on the growth of *Zea mays* L. in the absence of nematodes, concluding that *B. celere* C-924 is potentially applicable in the field not only as a nematicidal agent, but also as a plant growth promoter (Marín, Wong, Mena, et al., 2013).

Papaya (*Carica papaya* L.) is a widely consumed crop in Cuba and around the world, both directly and in prepared forms. Its fruit is highly valued for its nutritional value, given its high vitamin and mineral content, as well as for its digestive, laxative, antiparasitic, and wound-healing properties.

Cuba is one of the top producing countries in the Americas. The varieties planted in the country are primarily Maradol (Red and Yellow), INIVIT fb-2000 Dwarf, and INIVIT fb-4. Generally, the productive plants are replaced biannually, as trees older than this age have lower yields and reach larger sizes, which increases the cost of harvesting the fruit. This frequency leads to a high demand for seedlings to carry out field planting.

HeberNem[®] has been widely used in protected and semi-protected crops in the country, especially in short-cycle vegetables such as tomato, chard, radish, carrot, cucumber, among others. In this sense, a work is reported where an experiment was carried out to evaluate different doses of the bionematicide HeberNem[®] in the control of the nematode *Meloidogyne incognita* Chitwood and its participation in the growth and development of tomato (*Solanum lycopersicum*), obtaining as one of the results that, with HeberNem doses of 8 L/ha, 12 L/ha and 16 L/ha, the best results in the control of this nematode were achieved (Díaz et al., 2013).

However, to the authors' knowledge, the application of HeberNem-S® in field conditions or other long-cycle crops has not been sufficiently studied, hence the interest in searching for new niches and opportunities for the application of this product in the country. Based on this background, this study evaluated the effect of applying the HeberNem-S® powder on germination, vegetative development, and agricultural productivity in open-air papaya cultivation.

MATERIALS AND METHODS

Study location

The trial was conducted in collaboration with independent producer Alfredo Alba López on a private parcel located in the Puerto Príncipe neighborhood of Camagüey. Seeds of the Maradol Roja and INIVIT fb-1 cultivar Gigante Matancera varieties were used, sown manually at a rate of 2 to 3 seeds per bag. The substrate was provided by the supply company GELMA, while the producer received information from the project specialists regarding the application of the product, its properties and safety considerations.

HeberNem-S® protocol for papaya seedlings without seed treatment

A two-application schedule was followed: the first application coincided with seed sowing (day 0) and the product was reapplied 21 days later. The product was applied using the proposed concentration: 25 g of HeberNem-S® per 100 kg of growing medium. The average weight of the growing medium bags was estimated at 400 g/bag after averaging the results obtained from weighing a sample of 15 bags. The product was applied to the previously moistened growing medium using 10 L watering cans.

A partial measurement was taken 15 days after sowing, assessing: height (cm) from the base of the stem to the apical meristem and the number of germinated seeds/bag. At 21 days after planting, the height (cm) from the base of the stem to the apical meristem, the number of leaves, and the ratio of the length to width of the leaf blade of the last leaf were measured. 30 days after planting, the following variables were also evaluated: height (cm) from the base of the stem to the apical meristem, the number of leaves, the ratio of the length to width of the leaf blade of the last leaf, and the diameter of the plant stem. A sample n equivalent to the square root of the number of plants per treatment was evaluated. The results obtained were statistically analyzed using Statgraphics® Plus 19 software. The statistical means of the evaluated variables were compared using Student's t-test for independent samples, once the homogeneity of variance in each treatment was confirmed. Statistical differences were considered to exist for p -values ≤ 0.05 .

HeberNem-S® protocol for papaya seedlings

HeberNem-S® application to seeds

Pre-hydrated seeds of the Maradol Roja cultivar were split, discarding any that floated to the surface as they were considered non-viable. These were incubated for 8 hours in a HeberNem-S® solution at a concentration of 0.2 g/L. The required volume should be approximately three times the volume of seeds to be treated. In this case, 5 L of HeberNem-S® were prepared for approximately 1 kg of seeds. After incubating for 8 hours from morning, the solution was decanted, and the seeds were allowed to drain on a flat surface (sack) before being pre-germinated under low light conditions and moderate watering. The seeds germinated 100% between 7 and 10 days, at which point they were sown directly using a single seed per bag, for a total of approximately 10,000 bags. The seeds from the control treatment were pre-germinated under similar conditions and germinated 100%, but at a slower rate, mostly between 11 and 15 days.

Application of HeberNem-S® to the seedlings

A one-time application schedule was followed, coinciding with seed sowing (day 0). The product was applied using the proposed concentration: 25 g of HeberNem-S per 100 kg of substrate. The average weight of the substrate bags was estimated at 400 g/bag after averaging the results obtained from weighing a sample of 15 bags. The product was applied to the pre-moistened substrate using 10 L watering cans, and one 250 g bag was applied to 1,200 plants.

21 days after planting, height (cm) from the base of the stem to the apical meristem, number of leaves, and the ratio of the length to width of the leaf blade of the last leaf were measured. 27 days after planting, the following variables were also evaluated: height (cm) from the base of the stem to the apical meristem, number of leaves, and the ratio of the length to width of the leaf blade of the last leaf, as well as the diameter of the plant stem. A sample n equivalent to the square root of the number of plants per treatment was evaluated. The results were statistically analyzed using Statgraphics® Plus 19 software.

Application of HeberNem in open-field papaya cultivation

The trial was conducted in conjunction with an independent producer in areas of the El Jibao farm, located on the Camino a Maraguán, Camagüey municipality. The producer received training from project specialists on the product's application, its properties, and safety considerations for storage and handling, as well as advice and support throughout the crop's development. Plants of the Maradol Roja variety were planted. The seedlings used for transplanting had previously received applications of HeberNem-S®: seed imbibition prior to planting and applications on the day of planting and 21 days afterward.

The planting spacing used was 3 meters between rows and 3 meters between plants. A total of 20 rows of 40 plants each were planted. Of the 20 rows sown, 18 were treated with HeberNem-S® and the plants in the final two rows were left as negative controls, for a total of 720 plants treated with HeberNem-S and 40 plants as negative controls.

The seedlings were transplanted between May 17 and 23. HeberNem-S® applications were made coinciding with the last day of transplanting, considering this as the first application. The second, third, and fourth applications were made 40, 100, and 160 days after transplanting, respectively, coinciding with critical stages of vegetative and reproductive development in the crop's phenological cycle. Applications continued after 160 days, with one application every three months.

The applications were made with the soil previously moistened. The remaining time, the crop was kept dry, without the application of other chemical or biological products. 16-liter spray backpacks were used. Four 250-g bags of HeberNem-S® were resuspended in the volume of each backpack. The product was applied to 400 plants with each backpack, for a total of eight 250 g bags of HeberNem-S® for the 720 plants in the treatment.

Table 1 shows the crop evaluations. A sample *n* was evaluated, equivalent to the square root of the number of plants per treatment. The results were statistically analyzed using Statgraphics® Plus 19 software.

Table 1. Sampling days and variables to be measured

Sampling in days after transplantation (DAT)	Variables to evaluate
0	The data of the seedlings are taken.
22	Plant height (from the base of the stem to the apical meristem) and stem diameter.
38	Plant height (from the base of the stem to the apical meristem) and stem diameter.
78	Plant height (from the base of the stem to the apical meristem) and stem diameter.
150	Stem diameter, number of flowers, number of fruits and in the case of treated plants the weight of the first fruit.
210	Number of fruits, length and diameter of fruits, weight of fruits.

Source: Own elaboration

Table 2. Numerical values of the study variables evaluated

Day of sowing	Height (cm)	Length*Width (cm)	Number of leaves	Stem diameter (cm)	Sprouted seeds
Treatment with HeberNem-S®					
15	2.54 ± 0.49				2.33 ± 0.55
21	3.73 ± 0.87	5.74 ± 4.65	6.00 ± 1.95		
30	8.27 ± 0.90	24.87 ± 5.80	8.27 ± 0.69	3.76 ± 0.37	
Treatment without HeberNem-S®					
15	1.83 ± 0.35				1.47 ± 0.52
21	2.25 ± 0.47	1.95 ± 0.68	4.20 ± 0.56		
30	4.48 ± 1.09	7.23 ± 5.25	6.07 ± 0.96	2.92 ± 1.07	

Source: Own elaboration

RESULTS AND DISCUSSION

Evaluation of HeberNem-S® application in untreated papaya seedlings

All variables evaluated showed statistically significant differences in plants treated with HeberNem-S® compared to the control group. The increase in leaf number and the leaf blade length-to-width ratio in plants treated with HeberNem-S® can be interpreted as important variables for monitoring and predicting plant growth. Leaf area is an important variable in most agricultural and physiological studies related to plant growth, light capture, photosynthetic efficiency, respiration, transpiration, and response to irrigation and fertilization. Optimizing these physiological processes in treated plants allows for greater growth, vigor, and development compared to plants in the control treatment.

On day 30, plants treated with HeberNem-S® are 1.84 times taller than untreated plants, as can be observed in table 2. These results coincide with those reported by (Gómez, 2019), where the application of a Cuban biofertilizer based on mycorrhizal fungi of the genus *Glomus*, commercially known as EcoMic®, was evaluated on the height of papaya crops (*Carica papaya* L.), finding that the application of this biofertilizer increased plant height at 90, 140, and 190 days after transplanting, for both treatments, that is, applying both EcoMic® alone and EcoMic® mixed with cattle manure, compared to the control without the application of the biofertilizer. Specifically, at 90 days, papaya plant height was 1.18 times and 1.14 times higher when treated with EcoMic® and cow manure and EcoMic® alone, respectively, compared to untreated plants (control). At 190 days, papaya plants were 1.09 times and 1.07 times taller when treated with EcoMic® plus cow manure and EcoMic® alone, respectively, compared to the control.

The plant height results from our study 30 days after applying HeberNem-S® are lower than those reported in (Cueto and Mesa, 2018), where the height of the papaya plant (Maradol Roja variety) at 28 days was 12.33 cm and 10.44 cm for treatments 2 and 3, respectively. In this case, treatment 2 consisted of the application of a biopreparation of efficient microorganisms (EM) with a weekly application

of 20 mL of a 20% EM dilution (200 mL/L), while treatment 3 consisted of a weekly application of 20 mL of a 24% EM dilution (240 mL/L).

The length-to-width ratio of the treated plants showed statistically significant differences compared to the untreated plants. In treated plants, the length-to-width ratio was 2.94 times greater at 21 days, and at 30 days it was 3.43 times greater, meaning that the leaf area of treated plants increased compared to the control treatment.

The number of leaves on treated plants was greater at 21 and 30 days. At 21 days, treated plants had 1.42 more leaves than untreated plants, and at 30 days, they had 1.36 more leaves than untreated plants. These results are consistent with those reported by (Mesa et al., 2015), where the number of leaves on papaya plants (Maradol Roja) at the time of transplanting for treatments 2, 3, and 4 with ME-50 was significantly higher than the control, although no statistically significant differences were detected between treatments. It is noteworthy that in this study, treatment 2 consisted of the addition of 1.5 L of an ME biopreparation (called ME-50) per 18 L of water, while treatments 3 and 4 added 2.0 L and 2.5 L of ME-50 per 18 L of water, respectively.

The stem diameter measured at 30 days was 1.28 times thicker than the diameter of untreated plants. These results are consistent with those reported by (Gómez, 2019), who evaluated the application of the biofertilizer EcoMic® alone and in combination with cow manure on the stem diameter of papaya plants at 90, 140, and 190 days of treatment. In this case, at 90 days of treatment, the stem diameter recorded during the application of EcoMic® alone and mixed with cow manure was 1.23 times higher and 1.31 times higher, respectively, compared to the diameter of plants not treated with this biofertilizer (control), while at 190 days, the diameter of papaya plants was 1.33 times higher and 1.39 times higher with respect to the application of EcoMic® alone and mixed with cow manure, respectively, compared to the control. Likewise, these results agree with those reported by (Mesa et al., 2015), where the diameter of the plants at 35 days after surfacing was 1.29, 1.40 and 1.27 times higher for treatments 2, 3 and 4, respectively, compared to the control sample. Furthermore, the number of germinated seeds was 1.58 times higher in treated plants. Quantitatively, it can be concluded that the parameters evaluated [plant height (Figure 1), length-to-width ratio of the last leaf, number of leaves, stem diameter, and germinated seeds] were higher in plants treated with HeberNem-S® than in those that did not.

The plant height results obtained in our study after 30 days are lower than those reported by (Mesa et al., 2015), where at 28 days the papaya plant heights were 9.24 cm, 9.38 cm, and 9.44 cm for treatments 2 (1.5 L of ME-50 per 18 L of water), 3 (2.0 L of ME-50 per 18 L of water), and 4 (2.5 L of ME-50 per 18 L of water), respectively.

Qualitatively, greater uniformity is observed among the plants that received HeberNem-S® compared to those of the control treatment, as shown below in figure 2.

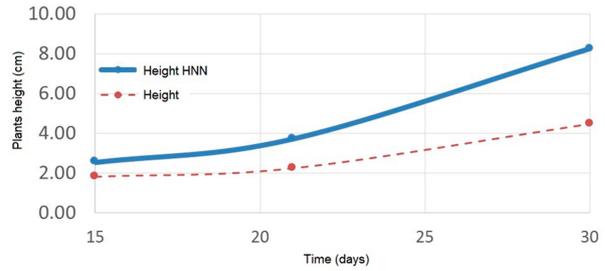


Figure 1. Graphical representation of plant height with and without HeberNem-S® application



Figure 2. Comparison of plants treated with and without treatment with HeberNem-S®. Left: Plants treated with HeberNem-S®. Right: Untreated plants 30 days after planting

The producer reports qualitatively, using the observation method, that the treated plants, due to their size and robustness, were ready for sale at the time of this evaluation 30 days after planting. The standard time for the development of seedlings is 45 days, so with the application of HeberNem-S® this cycle is shortened by 15 days, allowing for greater productive capacity with a consequent economic benefit for the producer.

Furthermore, greater leaf differentiation can be observed in treated plants than in control plants, with the start of basal leaf modification faster (Figures 3 and 4). It is likely that plants treated with HeberNem-S® increased their growth and accelerated their vegetative development stage, with an expected result of shortening and stimulating the flowering and fruiting stages under field conditions.

Evaluation of the application of HeberNem-S® to papaya seedlings by treating the seeds

The seeds had 100% germination, with germination rates varying between 7 and 10 days for seeds treated with HeberNem-S® and between 11 and 15 days for untreated seeds. Regarding plant height, measured on days 7, 21, and 27 after planting (DAP), it can be observed that plants treated with HeberNem-S® grew faster than those untreated with HeberNem-S®, and even faster than plants treated with HeberNem-S® but without treating the seeds, as can be seen in figure 5.



Figure 3. Comparison of plants treated and untreated with HeberNem-S[®]. Left: plants treated with HeberNem-S[®]. Right: untreated plant 30 days after planting



Figure 4. Plants treated with HeberNem-S[®] at 40 days after planting

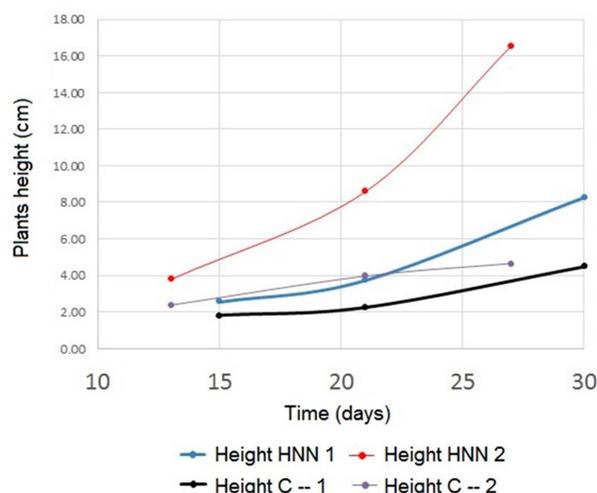


Figure 5. Growth kinetics of papaya seedlings

The height of treated plants at 27 days was 3.55 times greater than that of plants that received the control seed treatment and twice that of plants that received no seed treatment and were applied HeberNem-S[®] at two times. It was 3.69 times greater than that of plants that did not receive HeberNem-S[®] or seed treatment. The treatment that best increased plant growth rate was the one that included seed treatment with HeberNem-S[®] and received one application of HeberNem-S[®] on the day of planting.

Regarding the other parameters evaluated, the number of leaves was 2.09 times greater in treated plants. The length-to-width ratio of the last true leaf was 10.9 times greater in the treated group, and the stem diameter was 2.58 times greater in plants treated with HeberNem-S[®]. These parameters are very favorable for plant development, strength, and vitality because they result in an increase in plant surface area and a larger area for photosynthesis, while the increased stem diameter allows for greater transport of substances from the roots. These variables demonstrate that HeberNem-S[®] stimulates leaf growth and development in papaya seedlings (see table 3 for a summary of the parameters evaluated).

On day 27, 10 plants from each treatment were taken and root length, fresh plant weight and dry root weight were determined, the results being shown in table 4 and figure 6. In the case of root length, it was 4.41 times greater, the fresh plant weight was 9.89 times greater and the fresh root weight was 14.89 times greater, demonstrating that HeberNem-S[®] increased the rooting of papaya seedlings.

Table 3. Summary of the study variables evaluated

Days after planting (DAP)	With HeberNem-S [®]				Negative control			
	Height	Leaves	L*H	Diameter	Height	Leaves	L*H	Diameter
13	3.78	4.83			2.38	3.20		
21	8.58	9.12	25.73		3.968	4.52	2.68	
27	16.55	12.04	50.89	4.42	4.66	5.76	4.67	1.72

Source: Own elaboration.

Table 4. Data from the study carried out on the roots

Plant	With HeberNem-S®			Without treatment		
	Root length (cm)	Fresh plant weight (g)	Fresh root weight (g)	Root length (cm)	Fresh plant weight (g)	Fresh root weight (g)
1	33.4	6.943	1.502	2.3	0.955	0.115
2	17.2	6.481	1.414	2.8	0.389	0.11
3	10.9	8.661	1.787	3.1	1.289	0.185
4	97.3	7.881	3.466	19.1	0.284	0.063
5	20.7	5.886	0.72	9.7	1.609	0.212
6	29.8	13.061	2.666	3.0	1.263	0.083
7	21.0	11.188	2.761	14.1	1.399	0.188
8	26.3	6.086	0.867	3.4	0.286	0.114
9	9.5	4.303	0.676	2.5	0.365	0.123
10	30.0	9.768	4.685	7.1	0.28	0.187
Media	29.61	8.03	2.05	6.71	0.81	0.14

Source: Own elaboration.



Figure 6. Comparison of papaya seedlings' roots. Top: treated plants. Bottom: untreated plants

Evaluation of HeberNem-S® application on papaya under field conditions

One week after papaya transplantation, rainfall increased for two weeks, and 17 plants from the negative control (without HeberNem-S®) were lost, leaving only 23 plants as a control, for a transplant survival rate of 57.5%. Measurements taken 80 days after transplantation (DAT) showed that the height and stem diameter of plants treated with HeberNem-S® were 1.39 and 1.53 times greater, respectively. These values are associated with greater robustness and resistance to transplantation (Figure 7).

These height results are lower than those reported by (Cueto and Mesa, 2018), where plant height 27 days after transplanting papaya seedlings was 1.66 times and 1.42 times higher for treatments 2 and 3, respectively, compared to the control. It is worth noting that treatment 2 consisted of a weekly application of 60 mL of a 40% ME dilution (400 mL/L), while treatment 3 consisted of a weekly application of 60 mL of a 48% ME dilution (480 mL/L).

Furthermore, the diameter results of our study are lower than those reported by (Cueto and Mesa, 2018), where the diameter of the plants 27 days after transplanting were 1.95 times and 1.71 times greater for treatments 2 (400 mL/L of EM) and 3 (480 mL/L of EM), respectively, compared to the control samples.

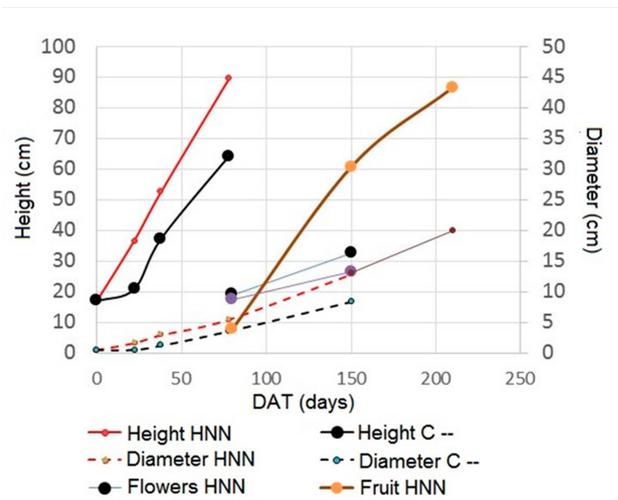


Figure 7. Agronomic variables evaluated in papaya cultivation under field conditions

According to (Peña et al., 2006), the diameter of the papaya plant is responsible for 80% of flowering and 50% of fruiting. Therefore, the results of our research should be considered taking into account the influence of this variable on increasing agricultural yields of papaya crops in plantations, through the production of robust and higher-quality seedlings.

On the other hand, flowering was also advanced, with the first flowers appearing before 80 days in plants that received HeberNem-S®, and after 120 days in the control group, the number of flowers was 1.32 times higher in the treated plants at 150 days. In the case of the appearance of the first fruits, an advance was also observed in the plants that received HeberNem-S®.

At 80 days, the plants to which HeberNem-S® was applied had an average of 3.93 fruits per plant, while the start of fruiting in the control group was around day 150, at which time, on average, the plants that had been applied HeberNem-S® had 2.31 times more fruits than the control group. These values for the fruit per plant indicator

are below the fruits per plant reported by (Gómez, 2019), which were 20.2 and 21.4 fruits per plant during the application of the EcoMic® biofertilizer alone and EcoMic® mixed with cattle manure, respectively, at 240 days after treatment, compared to the 10.2 fruits per plant obtained for the treatment without the EcoMic® biofertilizer.

In (Carpio et al., 2019), the fruits per plant were 14, 22, and 38 for the biological, organic, and chemical fertilizers, respectively, considering the Maradol papaya genotype, while for the Mulata genotype, the fruits per plant were 18, 31, and 30 for the biological, organic, and chemical fertilizers, respectively.

In this study, the treatments applied to the two papaya genotypes were as follows:

- Chemical: Urea (46% N) + 18-46-00 + KCl (66% K) at a dose of 60 + 230 + 100 g per plant.
- Organic: Natur-abono® (organic fertilizer), at a dose of 1.5 kg/plant.
- Biological: Bio Bravo® (*Glomus intraradises* + *Glomus fasciculatum* + *Trichoderma* spp. + *Azospirillum brasilense*), at a dose of 250 + 250 mL/ha (40,000 spores) + 250 mL/ha (1 x 1,011 spores) + 250 mL/ha (5 x 1,011 spores).

In (Quiñones-Aguilar et al., 2014), the application of different organic substrates and arbuscular mycorrhizal fungi (AMF) on the growth of papaya (*Carica papaya* L.) plants of the Wax type was evaluated. The three organic substrates evaluated were: 1) soil (soil and sand, in a 2:1 v/v ratio); 2) filter cake (soil, sugarcane filter cake compost and sand, 2:2:1 v/v/v); and 3) pulp (soil, coffee pulp compost and sand, 2:2:1 v/v/v), while the AMF evaluated were: 1) *Glomus* sp. Zac-2; 2) *G. aggregatum* FS-39 and 3) uninoculated (SI). The results showed that the height of the papaya plant at 105 days after transplant was 53.80 cm using filter cake, 34.95 cm using pulp, and 31.62 cm applying soil. The stem diameter presented values of 17.23 cm using filter cake, 10.63 cm using pulp, and 9.60 cm applying soil at 105 days after transplant. Likewise, the plant height was 50.53 cm applying Zac-2 AMF; 45.38 cm using FS-39 AMF and 24.45 cm without inoculation, while the plant diameter had values of 15.72 cm using Zac-2 AMF; 13.83 cm applying FS-39 AMF and 7.92 cm without inoculation, all of them at 105 days after transplant. Finally, the dynamics of plant height and stem diameter were also analyzed due to the interaction between the organic matter source and AMF strain at 105 days after transplant. The highest plant height was 54.90 cm for the filter cake-Zac-2 AMF combination, while the lowest plant height was 8.60 cm for the soil-uninoculated combination. The highest stem diameter was 17.70 cm for the filter cake-Zac-2 AMF and filter cake-uninoculated combination, while the lowest stem diameter was 2.31 cm for the soil-uninoculated combination. It is worth noting that the experiments conducted by these authors were carried out in a tunnel greenhouse.

In our study, the fruits of the untreated group were not weighed because they were still too small to harvest at the time of evaluation. This behavior was maintained throughout the crop period, with the HeberNem-S® treatment producing an average of 49.4 fruits seven months after transplant and only 20 fruits (2.47 times lower) in the control treatment. The average fruit length and diameter seven months after transplant were 1.37 and 1.28 times greater in the HeberNem-S®-treated group.

In (Carpio et al., 2019), the fruit length and diameter for the Maradol genotype were 13.71 cm, 12.4 cm, and 12.58 cm for the application of biological, organic, and chemical fertilizers, respectively. Meanwhile, the fruit diameter for the Mulata genotype was 15.14 cm, 12.52 cm, and 14.46 cm for the application of biological, organic, and chemical fertilizers, respectively. Regarding the fruit length obtained in (Carpio et al., 2019), it was 21.37 cm, 20.03 cm, and 18.49 cm for the Maradol genotype after applying biological, organic, and chemical fertilizers, respectively. For the Mulata genotype, the fruit length was 24.54 cm, 22.31 cm, and 23.31 cm for the biological, organic, and chemical fertilizers, respectively.

The average fruit weight was similar, being 2.4 kg for the treated plants and 1.62 kg (1.48 times less) for the control plants.

These average fruit weight results are similar to those reported by (Gómez, 2019), where an average fruit weight of 2.41 kg and 2.33 kg was obtained for papaya plants treated with EcoMic® alone and EcoMic® mixed with cow manure, respectively, 240 days after treatment. It is worth noting that, in this study, the average fruit weight for papaya plants not treated with EcoMic® (control) was 1.64 kg.

The results above allow to estimate an agricultural yield in the treated plants exceeding 27 t ha⁻¹ for the first 12 months of cultivation. Considering the stability and uniformity shown by the crop, an annual production of 21.87 t ha⁻¹ for the control group and 49.40 t ha⁻¹ for the treated group can be estimated, assuming climatic stability and optimal crop health. This yield is substantially higher than the national average annual production in Cuba of 17.14 t ha⁻¹ reported by the ONEI in 2023.

In (Carpio et al., 2019), fruit weights for the Maradol genotype were 1,510 kg, 1,683 kg, and 1,706 kg for treatments with biological, organic, and chemical fertilizers, respectively, while for the Mulata genotype, fruit weights were 1,773 kg, 1,806 kg, and 1,906 kg for treatments with biological, organic, and chemical fertilizers, respectively.

The average results obtained for length, diameter, and weight (Figure 8) and size (Figure 9) of papaya fruits 210 days after transplantation are shown below.

In (Carpio et al., 2019), the effect of applying three types of fertilization (chemical, organic, and biological) on the growth and physical and chemical characteristics of two papaya genotypes (Maradol and Mulata) was evaluated. The results showed that the Maradol and Mulata genotypes showed a better growth response to the application of chemical fertilizer, due to shorter days to harvest

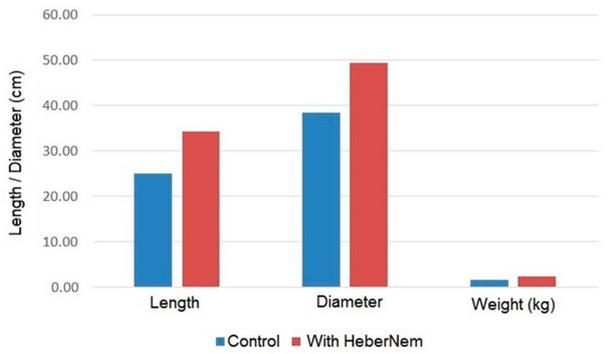


Figure 8. Evaluation of papaya fruits 210 days after transplantation



Figure 9. Evaluation of papaya fruit 210 days after transplanting. Above: without applying HeberNem-S®. Below: applying HeberNem-S®

and larger stem diameters compared to biological and organic fertilization. Furthermore, chemical fertilization in Maradol plants generated the highest number of fruits (38) and soluble solids content (11.49 °Brix), while in the Mulata genotype it promoted a better fruit weight (1,906 g) and yield (99.19 t/ha). It was concluded that biological inoculants favor an increase in fruit size.

Similarly, (Vera et al., 2021) compared the effect of using four types of substrates for the production of papaya seedlings (*Carica papaya* Lim, Maradol Roja) under nursery-garden conditions. In this study, the highest germination percentage corresponded to Treatment 3 (50% black soil, 25% sand, and 25% compost) with an average of 94%, while the treatment with the greatest plant height was Treatment 5 (50% black soil, 20% sand, and 30% compost), with an average of 11.41 cm. Treatment 4 (50% black soil, 20% sand, and 30% humus) had the largest stem diameter, averaging 2.89 cm. Treatment 5 had the highest average total leaf diameter, at 8.03.

Likewise, (Escamilla et al., 2003) evaluated the effect of organic, inorganic (mineral), and foliar fertilization on various physiological variables of papaya (*Carica papaya* L.) cv. Maradol plants, such as leaf nutrient

concentration, yield, and fruit characteristics. In this study, organic fertilization consisted of the application of 4 kg of chicken manure per plant (6.4 t ha⁻¹) at the bottom of the strain, whose dimensions were 30x30x30 cm, prior to planting, while mineral fertilization consisted of the application of the 258-200-280 formula of N, P₂O₅ and K₂O to the soil. For its part, foliar fertilization consisted of spraying the foliar fertilizer Fertiquel Combi®, in doses of 3 g L⁻¹ of water plus calcium nitrate (3 g L⁻¹), at intervals of 15 days from the third month of plantation establishment until the beginning of the harvest. The results showed that mineral fertilization of ‘Maradol’ papaya plants increased plant height, stem diameter, number of fruits (total and those located in the lower and middle sections of the plant), and yield, while organic and foliar fertilization did not. Foliar fertilization increased the Mg content in the leaves of the Maradol papaya cultivar, and organic fertilization based on chicken manure increased the concentration of Ca and B. The application of mineral fertilization also increased B concentrations.

Other authors (Fundora-Sánchez et al., 2021) studied two methods of applying the bioproduct QuitoMax® to the growth and development of papaya (*Carica papaya* L.) var. Maradol seedlings under nursery-garden conditions. Plants were grown in a substrate composed of soil and cattle manure in a 1:1 (v:v) ratio. The nursery was maintained for 60 days, with manual irrigation three times a week. Every 10 days, the height and number of leaves produced were assessed, and after 60 days, the dry matter of the shoots and roots was quantified. The effect of the product was observed from 30 days after germination (DAG), with stimulation of height and number of leaves, regardless of the dose or time of application. The plants reached a maximum height of 7.70 to 7.90 cm, 10 leaves, 6.67 g of dry matter of the shoots, and 8.3 g of roots. Application of 0.1 g L⁻¹ of QuitoMax® by seed imbibition and foliar spray at 20 DAG was recommended.

In (Crespo, 2024), the effect of applying EcoMic® on the growth and yield of the papaya (*Carica papaya* L.) cultivar ‘Gigante Matancera’ was evaluated. In this study, the following growth variables were assessed: plant height, stem diameter, number of leaves, flowers, and fruits, as well as fruit quality variables: fruit weight, length, and diameter, Brix degrees, production (kg/plant), and yield (t ha⁻¹). The first evaluation was carried out eight days after planting, and then every 14 days. The result was that treatment with EcoMic® showed the best results in each of the growth variables evaluated. Fruit quality variables (weight, length, and diameter), as well as production (kg/plant) and yield (t.ha⁻¹), were higher with the application of EcoMic®, showing significant differences compared to the control. This demonstrates that the application of this biofertilizer has a positive effect on growth and development indicators in papaya plants, indicating that its application constitutes a viable option under current Cuban conditions.

Accordingly, (Cueto and Mesa, 2018) indicates that the application of biofertilizers in papaya crops helps plants increase their capacity to absorb water and nutrients from the soil, increasing their growth, height development, and leaf emission.

On the other hand, (Lozano and Santamaría, 2013) studied the application of biofertilizers in the production of Maradol variety papaya plants. They found that when the plants are established in the soil, root development and microbial colonization increase, resulting in improved plant height and leaf shedding. They concluded that the benefit of inoculation with beneficial microorganisms translates into improved development and fruit production in this plant.

Finally, (Alcántara et al., 2015) evaluated the influence of organic, biological, and chemical fertilization on the yield of two papaya genotypes (Maradol and Mulata). They indicated that microorganisms such as mycorrhizae make nutrients and water available to these plants so that they can be used more easily and naturally. They also improve the vigorous development of the plant, thus increasing its survival.

CONCLUSIONS

HeberNem-S® has a stimulating effect on germination and vegetative development in papaya seedlings under open-air conditions, as well as a stimulating effect on vegetative development and agricultural productivity in papaya cultivation under these conditions. It is recommended that the application of HeberNem-S® be extended to other papaya growing areas and yields be evaluated.

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