



# COMBINED APPLICATION OF MYCORRHIZAL ON FITOMAS-E IN PLANTS OF *Talipariti elatum* (Sw.) Fryxell (MAJAGUA)

## Aplicación combinada de micorriza y FitoMas-E en plantas de *Talipariti elatum* (Sw.) Fryxell (Majagua)

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**ABSTRACT.** The work was done with the objective of evaluating the combined application of mycorrhizal and FitoMas-E about the quality of the specie *Talipariti elatum* (Sw.) Fryxell (Majagua) developed in nursery until plantation on a typical Red Ferralitic soil. Four treatments were studied in nursery and plantation establishing a completely aleatory design and in the phase of nursery and in the phase of plantation an experimental design of randomized blocks and four replications was established too. The treatments were: T<sub>1</sub>- control, T<sub>2</sub>- FitoMas-E, T<sub>3</sub>- Mycorrhizal and T<sub>4</sub>- Mycorrhizal+FitoMas-E. It was used the strain *Glomus cubense* (INCAM-4) to reason 10 g planta<sup>-1</sup> direct form and FitoMas-E to reason 5 mL<sup>-1</sup> of water. It was obtained that the combined use of Mycorrhizal+FitoMas-E (T<sub>4</sub>) allows obtaining better results in the morphological stages as well as the morphological indexes in nursery and plantations: height, collar diameter root, leaf area, as well as morphological indexes (Aerial Part/Radical Part relation, Slenderness, Dickson Index and Vigor Index). In general, this work showed that the combined application of phytostimulant FitoMas-E with an efficient AMF improves crop development and better economic response.

**Key words:** majagua, nursery, plantation

**RESUMEN.** El trabajo se realizó con el objetivo de evaluar el efecto de la aplicación conjunta de micorriza y FitoMas-E sobre la calidad de la especie *Talipariti elatum* (Sw.) Fryxell (Majagua) desarrollada desde vivero hasta plantación sobre un suelo Ferralítico Rojo típico. Se estudiaron cuatro tratamientos tanto en vivero como en plantación, para su análisis, en la fase de vivero se estableció un diseño completamente aleatorio y en la fase de plantación un diseño experimental de bloques al azar con cuatro réplicas. Los tratamientos estudiados fueron: T<sub>1</sub>- Testigo, T<sub>2</sub>- FitoMas-E, T<sub>3</sub>- Micorriza y T<sub>4</sub>- Micorriza+FitoMas-E. Se aplicó la cepa *Glomus cubense* (INCAM-4) a razón de 10 g planta<sup>-1</sup> de forma directa y FitoMas-E a razón de 5 mL<sup>-1</sup> de agua. Se obtuvo como resultado que el empleo combinado de micorriza más FitoMas-E (T<sub>4</sub>) permite obtener los mayores incrementos en las variables morfológicas evaluadas, tanto en vivero como en plantación: altura, diámetro del cuello de la raíz, área foliar; así como los índices morfológicos (Relación Parte Aérea/Parte Radical, Esbeltez, Índice de Dickson e Índice de vigor). De forma general la aplicación conjunta del fitoestimulante FitoMas-E con una cepa eficiente de HMA incrementan el desarrollo y se obtiene una mejor respuesta económica.

**Palabras clave:** majagua, vivero, plantación

## INTRODUCTION

Nowadays, wood should be taken out of forests in shorter cutting periods than the ones used to, so any technique leading to reduce the stay of the wood tree in the field is welcomed, not only to meet the demand of the product, but also to reduce the pressure of the tree on the forest (1).

The Cuban Reforestation Program developed in the last 50 years, has allowed that forests cover at present 27,27 % of the national territory and for the year 2015 it should be extended to 29 % (2).

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Among the most used species in reforestation programs nationwide, and specifically in Guantánamo province till the year 2020<sup>A</sup>, *Talipariti elatum* (Sw.) Fryxell is prominent, it stands out in production systems by its high economic value and the multiple uses of the wood for its beauty and long durability, considered as precious wood (3).

In order to meet the consumption and production demand of this specie, it is a challenge to find propagation alternatives for this crop in Guantánamo

<sup>A</sup> Silot, A. Efecto de la aplicación de micorriza y FitoMas-E en los parámetros morfológicos y fisiológicos de la especie *Swietenia mahagoni* en vivero [Tesis de Ingeniería], Facultad Agroforestal de Montaña, Guantánamo, 2012, 57 p.

province to achieve a higher quality and survival of the seedlings, especially when 75 % of the province land surface is mountainous (4) and these areas are particularly devoted to forestry.

One of the alternatives is the use of bioproducts, being one of them the biofertilizers produced with arbuscular mycorrhizal fungi (AMF), a symbiotic association between certain soil fungi and the roots of higher plants, where both symbionts are benefitted (5, 6); in addition to the biostimulant FitoMas-E to which stimulating properties of some plant physiological processes with antistress action<sup>B</sup>. However, there are few scientific reports published in Cuba on the FitoMas-E effect on Majagua combined with an efficient AMF isolate (7).

Taking the above-mentioned factors into account, this research looks forward to determine the effect of the combined application of mycorrhizae and FitoMas-E on the quality of the specie *Talipariti elatum* (Sw.) Fryxell developed in the nursery and in the field.

## MATERIALS AND METHODS

This research was conducted at the Basic Cooperative Production Unit "Eraldo Martínez Quiroga" pertaining to the Agricultural Coconut Enterprise of Baracoa municipality, Guantánamo province, in two stages: nursery and planting.

The nursery stage included the use of poly bags (15 cm x 20 cm), grouped in seedbeds of 1 m width by 4 m long; each bag received seeds of the *Talipariti elatum* (Sw.) Fryxell specie, produced at the seed unit of the Baracoa Comprehensive Forestry Enterprise and analyzed at the Agroforestry Research Institute of Baracoa.

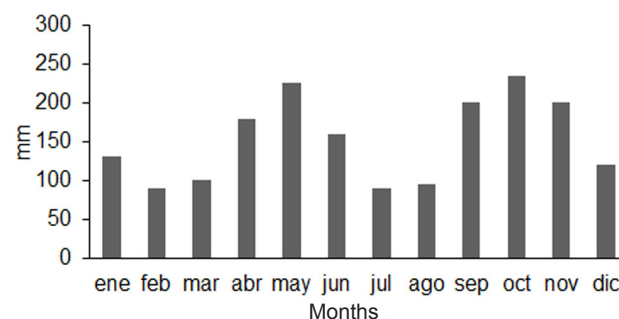
The planting stage was done by hand in the morning of the same day seedlings were taken out of the nursery, taking into account the agricultural Standard 4304-15:87. Planting forest seedlings. Process.

The soil used in both stages was typical red ferralitic (8), its chemical features are shown in Table I. In general, the soil can be grouped as to medium fertility and low exchangeable contents of K, Ca, Mg, as well as the Na content. Regarding the P<sub>2</sub>O<sub>5</sub> content, it is evaluated as low and the K<sub>2</sub>O as high.

**Table I. Characteristic of the soil used in the trial**

pH		Ca	Mg	K	Na	M.O	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O
H <sub>2</sub> O	CIK		cmol kg <sup>-1</sup>			%	mg 100 g <sup>-1</sup>	
7,2	6,5	2,7	1,40	0,23	0,57	2,75	0,80	10,83

Figure 1 shows the rainfall behavior as per the closest weather station to the trial site. In general, rainfall was above 100 mm the year around which is typical of the rainfall pattern for this region.



**Figure 1. Monthly rainfall behavior at the weather stations close to the experimental sites. Weather Station of Jamal, Baracoa, Guantánamo province**

Markers were used for seed sowing to achieve the same depth for all. They were then covered with soil, twice the size of the seed (9).

Plants received major cultural practices: irrigation, weeding, walkway hoeing, walkway raking, survival counting and seedling thinning (9).

Treatments were equally conceived, for the nursery and planting stages: for the nursery stage a fully randomized design was used and in the planting stage, a random block design with four replicates of 15 plants each was considered. They were structured as follows:

- T<sub>1</sub> - Without the application of bioproducts in the nursery and planting (Control)
- T<sub>2</sub> - FitoMas-E in nursery and planting.
- T<sub>3</sub> - Mycorrhizae in nursery and planting.
- T<sub>4</sub> - Mycorrhizae + FitoMas-E in nursery and planting.

These treatments included the application of 10 g of EcoMic® under the seed at sowing, using the specie *Glomus cubense*, INCAM-4 (10), from the National Institute of Agricultural Sciences (INCA), with a quality of 20 spores.gram<sup>-1</sup> of soil for a 50 % of root colonization.

<sup>B</sup> Montano, R. *FitoMas-E, bionutriente derivado de la industria azucarera. Composición, mecanismo de acción y evidencia experimental*, Instituto Cubano de Investigaciones de los Derivados de la Caña de Azúcar, La Habana, Cuba, 2008, 35 p.

FitoMas-E, from the Research Institute of Sugarcane Byproducts (ICIDCA), was sprinkled at the rate of 5 mL<sup>-1</sup> per liter of water to the foliage using a hand sprayer of 12 liters of capacity. The application of this product was made 5 days after seed germination in the nursery and 10 days after seedlings were transplanted to the field.

Cow manure, decomposed at 7:1 (seven parts of soil by one of organic matter), was applied as organic matter. This mixture was grounded and screened with a 2 mm screen at the start of the trials. Soil was placed at a cement platform, organic matter was then added to formulate the desired rates and then they were mixed several times until homogenization.

Out of each treatment in the nursery, 25 plants were evaluated, avoiding edge effects and complying with other author's criteria (3, 11). Ninety days after germination, morphological variables were evaluated: plant height (cm) by directly measuring it with a graduate rule, from the base of the stem to the tip of the plant; stem diameter (mm) measured from one centimeter above root crown using a slide gauge with precision of 0,05; foliar area (cm<sup>2</sup>) using the gravimetric method (12), from the linear dimensions of the leaves and according to the following formula  $AF (cm^2) = \text{length (cm)} \times \text{width (cm)} \times Fc$ , where Fc is the correction factor; Slenderness Index by dividing height and diameter (H/D); Aerial part relationship – Root part (RPA/RPR) dividing the aerial dry mass (stems, leaves) and the root dry mass; for the Vigour Index (IV), the following formula was used  $IV = \text{Log } \Sigma$  (of all morphological variables) and the Quality Dickson's Index (Qi) were determined by the following expression:  $QI = \text{PST} / (\text{Slenderness} + \text{RPA/RPR})$ .

In the planting stage, 15 plants with four replicates were evaluated. Samples were taken after 12 and 16 months after planting. Total plant height (m) and canopy height (m) were also evaluated by the visual method; stem diameter (cm) was measured with a slide gauge; Slenderness Index was calculated by dividing height between diameter (H/D) and the survival percentage was determined by the formula:  $\text{survival \%} = \text{Pv} / (\text{Pv} + \text{Pm}) \times 100$  where: Pv- living plants, Pm- dead plants.

The results were evaluated by the analysis of variance of souble classification and means were compared by Duncan's multiple range test for a significance of  $p \leq 0,05$ . The statistical package STATGRAPHICS Plus 5.1 was used for all statistical analyses (13).

## ECONOMIC EVALUATION

The economic evaluation took into account the technological guidelines with its cost chart<sup>c</sup>, including different economic components like materials and salary in: soil conditioning and preparation, organic matter transportation, AMF and FitoMas-E cost, mixture preparation, seedbeds, bags, silvicultural practices, etc., indirect costs and losses by damages during the productive process (10 %).

## RESULTS AND DISCUSSION

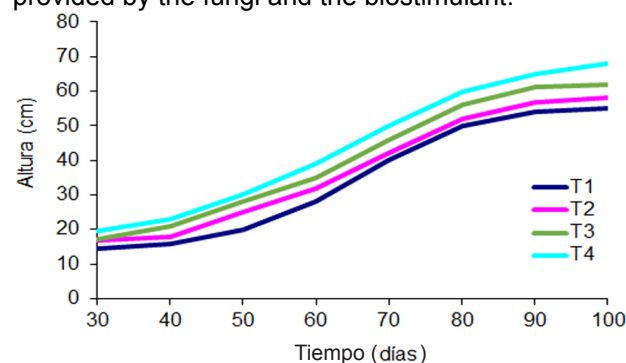
### GROWTH DYNAMICS DURING THE NURSERY STAGE.

Figure 2 shows the growth dynamics as to the height of *Talipariti elatum* plants according to the different studied treatments during the nursery stage.

There are three stages:

- ◆ Establishment stage, it lasts 40 days after planting.
- ◆ Fast-growth stage, with a duration of approximately 40 days after planting.
- ◆ Hardening stage, starts approximately 80 days after planting.

Plants reached similar increased heights with the four treatments, identifying the three stages in the development of forest seedlings, though those included in treatment four (Mycorrhizae + FitoMas-E) reached higher values, which coincides with the benefits provided by the fungi and the biostimulant.



(n=25 plants per treatment)

**Figure 2. Graphic of the growth stages in the nursery stage for seedlings of *Talipariti elatum* (Sw.) Fryxell**

<sup>c</sup> Suros, E. *Determinación de costo tecnológico de las actividades forestales*, Universidad de Pinar del Río, Pinar del Río, Cuba, 2007, 65 p.

Other trials have reported similar growth dynamics indicative that after 90 days, seedlings start reducing growth, increase diameter and dry mass production, promoting a higher hardening (3).

The so much extended duration of the fast growth stage is very much related to the benefits provided by the fungus that uptakes a higher nutrients quantity from the soil and the water, allowing seedlings a higher growth and development (14). FitoMas-E also played an important role due to its phytostimulant nature and its effectiveness to favor bacterial growth (15).

When looking at Table II data, it was evident as a trend, that the combined use of Mycorrhizae and FitoMas-E, showed the best results as to height and diameter, followed by the treatment of mycorrhizae alone, though in the foliar area variable, there were not significant differences between treatments 2 and 3.

These results are due to the individual or combined action of the biostimulant components and the fungus action that facilitates nutrients uptake by plants (14).

An important aspect to point out is that in the absence of this combined treatment, both products can be individually applied and reach superior results to the application of soil mixed with organic matter (cow manure), even when manure is an important Nitrogen source with other minor elements necessary for plants growth (1).

The fact of finding a higher foliar surface in treatments including the biological alternatives, facilitates the grasp and fixation of light energy that makes possible the increased transportation of assimilable substances, otherwise, a faulty formation of the plants foliar surface, can lead to an important reduction of the assimilative processes, photosynthesis mainly (16).

The application of biological products makes possible for the plant be more tolerant to stressing factors like drought, ph unbalances, high salt contents, wind excess, among others (12).

Related to the above, there are results confirming other author's reports, in the sense that foliar area was higher when mycorrhizae combined with FitoMas-E was applied. The combination of mycorrhizae with FitoMas-E applied to plant development, is conditioned by the increased uptake of phosphorus through the symbiotic association existing among certain soil fungi and the roots of higher plants (17); it makes possible for the plants be more resistant to different adverse changes that might occur to the ecosystem.

Based on the previous statements, it is important for nurserymen involved in producing forest seedlings to recognize the importance of using mycorrhizae and FitoMas-E, since it allows the production of well-developed seedlings with less time and potential losses<sup>D</sup>.

Table III shows the behavior of morphological indexes, the best values were recorded with the combination of mycorrhizae+FitoMas-E) represented by treatment four.

As for the relation of the Aerial Part / Root Part (RPA/RPR), the best values were recorded in treatment four with the lowest means, by having values below the PA/RP relationship indicative of a higher capacity to overcome the critical rooting time. The interval of recommended values by different authors is very wide; it depends on multiple circumstances; values from 1.5 and 2 are suggested for this relationship because the lower the value, more favored water uptake will be compared to losses. This is a condition for dry areas indicative of a higher capability to overcome the critical rooting time (11).

Relative to Slenderness (H/D), Dickson's quality Index (QI) and vigor index, the best results showed up in the presence of both products (T4), with the highest means. It is equivalent that they have a higher mechanical resistance during planting or in the presence of strong winds, and that on one hand, total plant development is high and at the same time, aerial and root parts are balanced.

<sup>D</sup> Jordá, A. *Las ventajas del uso de Micorrizas en la producción de plantines forestales* [en línea], 2009, [Consultado: 5 de enero de 2013], Disponible en: <<http://la-pagina.de/micorrizalaj/>>.

**Table II. Effect of mycorrhizae and FitoMas-E on the growth of *Talipariti elatum* (Sw.) Fryxell. In the nursery**

Treatments	Height (cm)	Diameter (cm)	Foliar area (cm <sup>2</sup> )
1 Control	53,15 d	5,95 d	100,10 c
2 FitoMas-E	56,75 c	6,12 c	127,07 b
3 Mycorrhizae	61,10 b	6,35 b	112,45 b
4 Mycorrhizae + FitoMas-E	64,85 a	6,75 a	137,05 a
E.S	0,65*	0,006*	0,91*

\* Means with different letters in the same column differ among themselves according to Duncan's (p<0,05)

As to the vigor index, though there were not significant differences, the highest value was found for treatment 4 ( $T_4$ ), so the application of the alternatives under study not only favors growth and development, but also those strong plants able to withstand adverse conditions.

It is important to point out that the AMF used products from plant metabolism to perform its functions and at the same time, gave the plant back the increased uptake and translocation of nutrients for vital functions (18), likewise, FitoMas-E contains carbohydrates and aminoacids among its components that, once available to the plant, can bring about auxin synthesis and nutritive compounds, enough to reach an efficient plant response in terms of development (19).

Moreover, the application of efficient AMF isolates improves nutrients uptake by the plants and contribute to a rational use of fertilizers; the same is true for the vigor and sanitary status of the plants (20).

Table IV shows the analysis of the morphological parameters of the *Talipariti elatum* after 12 and 16 months of planting, where treatment four (mycorrhizae + FitoMas-E) was the most efficient one in both development stages, being proportional to the results attained in the nursery stage in which this treatment showed the best results.

Mycorrhizae in the forest world, provides a high quality product, with more survival possibilities, it also reports different benefits: increased vigor of the aerial part of the plant, reduction of germination failures, better adaptability and vigor (21).

The follow-up of inoculated species from the nursery, has shown that such seedlings are better adapted to the planting site since the AMF fungus works efficiently where the organic matter content is low making possible for the plants to intensify physiological processes with a better development of their morphological parameters.

Figure 3, related to the survival of the specie 16 months after planting, shows that treatment four (Mycorrhiza + FitoMas-E) had the best result with 99% of living plants.

These results coincide with the indications from the State Forestry Service and show that plants survival is an important parameter in the evaluation of species under experimental conditions, where the use of biological products in nurseries and in the field are of great importance<sup>E</sup>.

Survival values can be considered high even in the control treatment, which is above 85%, value established by the State Forestry Service to certify a plantation. This result can be related to climatic conditions at the studied area (Figure 1), where rainfall exceed 100 mm nearly the year around thus favoring plants adaptability to grow in humid places of semideciduous forests, rain forests and mountainous rain forests, these latter only represented in Baracoa (4). Moreover, the organic matter content of the soil was evaluated as medium (Table I), which favored the development of this specie.

<sup>E</sup> Linares, E. *Instructivo para determinar la supervivencia en plantaciones forestales*, [06], Ministerio de la Agricultura, La Habana, Cuba, 2005, 94 p.

**Table III. Effect of the mycorrhizae and FitoMas-E on the morphological index of *Talipariti elatum* (Sw.) Fryxell. At the end of the nursery stage**

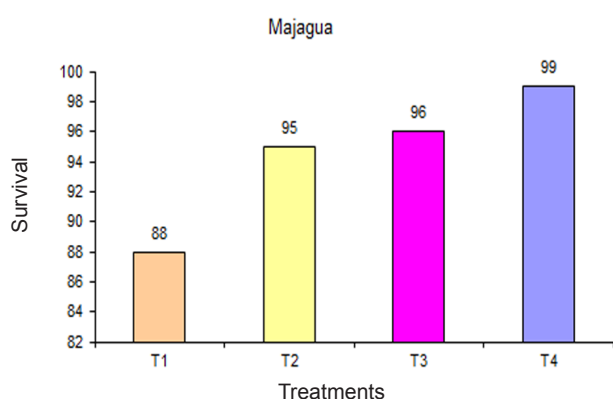
Treatments		RPA/RPR	H/D	QI	IV
1	Control	2,39 d	8,93 d	3,50 d	2,22
2	FitoMas-E	2,36 c	9,27 c	3,57 c	2,28
3	Mycorrhizae	2,15 b	9,38 b	3,62 b	2,30
4	Mycorrhizae + FitoMas-E	1,97 a	9,57 a	3,79 a	2,34
E.S		0,002*	0,003*	0,002*	-

\* Means with different letters in the same column differ among themselves according to Duncan's ( $p < 0,05$ )  
RPA/RPR: Relationship between the Aerial and Root Part; H/D: Slenderness index; QI: Dickson's index; IV: Vigor index

**Table IV. Effect of the mycorrhizae and FitoMas-E on the growth of *Talipariti elatum* (Sw.) Fryxell. Evaluated at 12 and 16 months after planting**

Treatments	Ht (m)		Hc (m)		Dcr (cm)		H/D		
	12 months	16 months	12 months	16 months	12 months	16 months	12 months	16 months	
1	Testigo	0,88 d	1,18 d	0,30 d	0,55 c	1,25 d	1,05 d	0,70 c	1,12 d
2	FitoMas-E	1,05 c	1,39 c	0,37 c	0,76 b	1,40 c	1,18 c	0,75 b	1,18 c
3	Mycorrhiza	1,39 b	1,94 b	0,36 b	1,01 b	1,70 b	1,55 b	0,81 b	1,25 b
4	Mycorrhiza + FitoMas-E	1,58 a	2,27 a	0,55 a	1,44 a	1,75 a	1,70 a	0,90 a	1,34 a
E.S		0,103*	0,138*	0,067*	0,084*	0,131*	0,118*	0,057*	0,052*

\* Means with different letters in the same column differ among themselves according to Duncan's ( $p < 0,05$ )  
Ht: Total height; Hc: Canopy height; Dcr: root crown diameter; H/D: Slimness index



Treatments: T1- Without application (Control); T2 - FitoMas-E; T3 - Mycorrhizae; T4 - Mycorrhizae + FitoMas-E

**Figure 3. Survival percentage of the specie 16 months after planting**

All this shows once more, the importance of the combined application of biofertilizers and stimulants to produce positive effects on crops (22), so that positive significant changes in the propagation of this specie in Guantanamo province can take place in order to reach higher quality seedlings and survival and meet the increased current consumption and production demands.

The mycorrhizal effectiveness of the isolate used in the growth and development of *Talipariti elatum* plants could be proven, a factor related to the effectiveness of this isolate in the type of soil described above (20).

Other research works report that the main way to quantify mycorrhizal effectiveness is by evaluating the host plant response growth wise (23).

The above confirms other author's reports<sup>F</sup> (22, 24, 25) who have informed satisfactory results as to morphological variables and survival percentage when mycorrhizae and FitoMas-E were used with other perennial species.

<sup>F</sup> Rodríguez, Y. *Estrategia de diversificación de la producción en el sistema agroforestal de la empresa café y cacao «Yateras», Guantánamo* [Tesis de Doctorado], Universidad de Pinar del Río, Pinar del Río, Cuba, 2010, 149 p.

At present, biofertilizers and biostimulants are economically viable and accepted ecological ways to reduce the use of chemical fertilizers in agroecosystems and improve plants quality by using soil microorganisms (26).

## ECONOMIC EVALUATION

An important aspect of forestry is the economic evaluation (Table V), where expenses with and without applying donde Mycorrhizae and FitoMas-E are shown; the combined application of Mycorrhizae and FitoMas-E stands out since expenses significantly reduced in 2 568,09 pesos compared to the control.

This evaluation took into account the reduction of cultural practices derived from the combination of products, since plants reached a higher growth and development and therefore less manpower that influenced on costs reduction.

Treatments including the individual application of bioproducts, expenses were lower than the control, even when these expenses in raw materials were higher. It justifies the advantages these ammendments provide for the growth and development of this specie.

The economic results indicate that treating this type of soil with mycorrhizae, optimum quality seedlings can be produced, superior to those produced following the technical guideline in force (7:1), and considerably reducing the organic matter volumes used and nursery staying time. All this is equivalent to time saving and higher seedling quality.

## CONCLUSIONS

- ◆ There was a positive response of *Talipariti elatum* to the inoculation of AMF isolate combined with FitoMas-E.
- ◆ The best results for the morphological parameters were reached by combining mycorrhizae and FitoMas-E (T4), in the nursery and in the field. This result indicates the economic feasibility of using mycorrhizal inoculation and the biostimulant FitoMas-E.

**Table V. Behavior of the economic impact till the first maintenance**

Concepts	MU	T 1	T 2	T 3	T 4
Raw materials and other inputs	Pesos	3 090,50	3 160,50	3 110,50	3 400,50
Fuels	Pesos	200	200	200	150
Manpower expenses	Pesos	7 179,21	6 888,69	6 640,27	4 583,96
Total direct expenses	Pesos	10 469,71	10 249,19	9 950,77	8 134,46
Indirect expenses	Pesos	1 046,971	1 024,919	995,077	813,446
Total expenses	Pesos	11 516,68	11 274,11	10 945,85	8 947,91

Treatments: T<sub>1</sub>- Without application (Control); T<sub>2</sub> - FitoMas-E; T<sub>3</sub> - Mycorrhizae; T<sub>4</sub> - Mycorrhizae + FitoMas-E

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