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Bio-organic Alternatives for Propagation of *Psidium Guajava* (L.) through Stem Cuttings

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ABSTRACT

The aim of this study was to use different stimulators for guajabo propagation using stem cuttings in semi-controlled conditions, on "La Nueva Esperanza" farm, run by "Renato Guitart" Strengthened Cooperative of Credits and Services, in the municipality of Camaguey, Cuba, between February and May, 2016. A chamber with zeolite substrate (1 - 3 mm diameter) was used for propagation, whereas irrigation was scheduled and localized. A completely randomized design was used for evaluation, with 6 treatments and 3 replicas. The number of plants with buds, plants with roots, leaves, and live plants achieved, were evaluated. Simple variance analysis (SSPS version 11.5.) was performed, and the means were compared according to the Duncan´s test (p:0.05). The bio-organic alternatives applied were efficient in stimulating the physiological mechanisms for plantlet growth. The best phenological behavior was observed in treatments with the natural liquid humus plus indoleacetic acid, the natural liquid humus plus phosphoric acid, and the fortified liquid humus.

KEY WORDS/: *Psidium guajava*, propagation, humus, phosphoric acid, indoleacetic acid, growth regulators

INTRODUCTION

The guava tree (*Psidium guajaba L.*) is a fruit plant originally from dry tropical regions, that grows on a wide variety of soils (Salazar *et al*., 2006). It is the most important cultivated species of the *Myrtaceae* family (Rai *et al*., 2007).

Knowledge of its genetic variability and the effects of the environment might help generate different breeding strategies (Thaipongy Booprakob, 2005). However, there are limitations to multiply the elite material or selections in adult plants, which causes severe problems associated to the application of gene transfer in the plants (Gómez Lim and Litz, 2004).

In guava, propagation may be done through stem cuttings, which is simple, economical and accessible to farmers and nursery personnel. The propagated plants are more uniform, taller, and healthier. They are obtained in a relatively short time, compared to other plant propagation techniques (González *et al*., 2001; Albany *et al*., 2004; Vílchez-Perozo *et al*., 2004). Bio

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regulators in agriculture are used to stimulate, control, and handle different plant parts and status, including roots, dormancy termination, flowering, fruit formation and growth, organ falling, plant size, and so on. Therefore, bioregulators cause changes to biological processes through metabolic effects (Oliva, 2006).

There are also different stem treatments to stimulate rooting of stem cuttings. Rooting is regulated by endogenous signals that keep the activity of root apical meristems, and they contribute with the generation of new lateral roots. Auxines play a key role in this, though other hormones help shape the overall root architecture (Jovanovic *et al.*, 2008).

The administration of auxines to species with poor root development is a useful practice to stimulate rooting; it accelerates root appearance, increases the number of rooted cuttings, and the number and quality of roots, and it also brings about more uniform growth and root development (Bacarín *et al.*, 1994).

Auxines are a group of phytohormones that regulate and cause growth by cell division or elongation; their role is also critical in embryonic and post-embryonic root development, as well as in gravitropism. They can be observed in aerial part of plants, or in the apexes of primary and secondary roots (Ljung *et al*., 2005). In all the species studied so far, inhibition of auxine transport has led to a quick drop in primary root growth (Blilou *et al*., 2005).

Today, the application of foreign conventional hormones and some chemical compounds is limited. This paper assesses the application of several bio-organic stimulators for guava reproduction, using stem cuttings under semi-controlled conditions, as a local replacement of synthetic hormones.

MATERIALS AND METHODS

Location and type of soil

This investigation took place between February and May 2016, on La Nueva Esperanza Farm, Renato Guitart CCS, on Circunvalación Sur, Camaguey city, Cuba. It is located on the 21º 20´43´´ north latitude, and 77º 52´ 50´´ west longitude, 95 meters above sea level, according to the cartographic chart of Camaguey (4680-III– c) at 1: 25 000 scale. The soil is predominantly brown, without typical carbonates (Soil Institute, 1975).

Several bio-organic alternatives were used in the study with the purpose of replacing imported goods that stimulate guava root development.

Experimental phases

In phase 1, the plant was in a plot of ground; phase 2 was developed in polyethylene bags in controlled conditions (land area covered by a net), at 30 ºC; the plantlets were irrigated through controlled microjets. Phase 3 took place in the open field, to encourage adaptation of the plant to the environmental conditions.

The experiment followed a completely randomized design with 6 treatments (Table 1), and 3 replicas in semi-controlled conditions, using 330 propagules/treatment.

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Table 1. Experimental design

Treatment	Product	Dosage and administration route
1 (CONTROL)	Fortified liquid humus from bagasse ash	120 ml per 600 ml water, foliar dipping
2	Natural fortified liquid humus from bagasse ash	120 ml per 600 ml water, foliar dipping
3	Improved liquid humus from bagasse ash	120 ml per 600 ml water, foliar dipping
4	Natural liquid humus with 2% zinc	120 ml per 600 ml water, foliar dipping
5	Natural liquid humus + phosphoric acid	120 ml per 600 ml water, foliar dipping
6	Natural liquid humus + indoleacetic acid	120 ml per 600 ml water, foliar dipping

The fortified liquid humus available in the experimental field was used as control.
Dipping lasted 10 seconds, as stem cutting oxidation is very quick.
Liquid humus from bagasse ash was fortified with phosphorine, azotobacter, and essential elements like nitrogen, phosphorous, potassium, calcium, magnesium, zinc, iron, copper and molybdenum. Imported conventional hormones Dip were also used. Phosphoric acid and indoleacetic acid
In phase 1, the experiment was made in a chamber for guava plantlet breeding, using stem cuttings, in a 22 m long x 1.20 m wide land bed, irrigated through micro-jets for 18 sec every 5 min. The well water supplied was evaluated as average, suitable for most crops.
In the first phase, the cuttings with two pairs of leaves (10 cm long), were planted 2 cm deep, using the semi-woody part cut at the base. The cuttings had been collected from plantations on the farm, previously washed or disinfected with copper solution before rooting treatment.
The indoleacetic acid was applied by dipping, 120 ml in 600 ml of water.
Zeolite from la Tasajera Mines, in Villa Clara province was used. The dust particles were 1-3 mm, with a high capacity for cation exchange and adequate properties. Thickness was 10 cm inside the chamber.
The main indicators of product efficiency were evaluated (cuttings with or without root callouses, cuttings with roots, the root system). Measurements were made 21 days after plantation, with 10 plants taken at random in each replica, and 3 observations, on average.
The cuttings used were collected from adjacent areas.
In phase 2, the plants with roots were removed from the chamber, and planted in 1.7 kg polyethylene bags, containing 75% compost, 20% soil, and 5% zeolite. The products were applied every seven days, early in the morning, before 10 am. Adaptation to climatic conditions lasted 15 days, until the plants were sold.
Statistical analysis
SSPS 11.5.1 was used for data assessment, with simple variance analysis. The Duncan´s multiple range test was applied for significance 5 %.

RESULTS AND DISCUSSION

The efficiency indicators of product application in phase 1 (Table 1), based on the number of plants with buds on days 20 and 40, had no significant differences in the treatments evaluated. It may have been caused by the nutritional effect provided by the products applied during the first stages of the plant, with an influence in their growth.

Table 2 Efficiency indicators with the application of products in phase 1

Treatment	Plants with buds. 20 days	Plants with buds. 40 days	Plants without buds 20 days	Plants without buds 40 days	Plants with roots 40 days
1	3.0	6.0	7.0	4.0 a	100 a
2	3.0	7.0	7.0	3.0 ab	98 b
3	4.0	7.0	6.0	3.0 ab	97 b
4	3.0	7.0	7.0	3.0 ab	102 a
5	2.0	7.0	8.0	3.0 ab	102 a
6	3.0	7.0	7.0	3.0 ab	102 a
Es_x	0.8233 NS	0.9169 NS	0.9888 NS	0.7865	0.9015

Unequal letters differ for $p \leq 0.05$

Evaluation of plants without buds revealed that after 20 days there were no significant differences; however, the 40-day evaluation revealed statistical relevance. The largest number of plants without buds was observed in treatment 1, made of fortified liquid humus (4 plants). In terms of efficiency, this situation did not favor root appearance, with significant differences among the treatments. The other treatments had less plants without buds, which would induce root appearance. These results differed from Napoles (2013) in guava breeding using cuttings and bioorganic strategies; the number of plants was significant different. Treatments 4 (natural liquid humus, plus zinc); 5 (natural liquid humus, plus phosphoric acid); 6 (natural liquid humus, plus indoleacetic acid); and 1 (fortified liquid humus) had the best results in 102 plants in the first three treatments, and 100 plants in the fourth treatment. It opened new possibilities for natural liquid humus in combination with zinc, phosphoric acid, and indoleacetic acid, with the fortified liquid humus, but it did not coincide with Saborit (2013) working in similar conditions.

These results coincided with Saborit (2013), however, when he used fortified liquid humus in his treatments, but differed from Nápoles (2013) in similar experimental conditions. Similar results were achieved by Reynaldo (2005), cited by Martin (2010), using Pectimorf, an organic root stimulator, applied to ornamental plants and fruit trees, with positive results.

López and Montejo (2011) reported favorable effects on root shooting using bio-organic alternatives in tomato, including fortified liquid humus. Corrales *et al.* (2001), achieved 80% efficiency, with the use of biofertilizers in guava breeding, by means of cuttings.

The number of plants with roots in phase 2 (Table 3) had significant differences among the treatments. Treatments 4 (natural liquid humus, plus zinc); 5 (natural liquid humus, plus phosphoric acid); 6 (natural liquid humus, plus indoleacetic acid), and 1 (fortified liquid humus) were the best. The combinations of natural liquid humus with zinc, phosphoric acid and indoleacetic acid, were also favorable. Moreover, the fortified liquid humus can be used in identical conditions.

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Table 3. Number of plants with roots in phase 2

Treatment	70 days
1	101 a
2	97 b
3	97 b
4	102 a
5	105 a
6	105 a
Es_x	0.8045

Unequal letters differ for ≤0.05 %

The plant responded favorably to the best treatments, which brought benefits from the inclusion of zinc, phosphoric acid, AIA, and fortified liquid humus. The nutritional effects of these products also contribute to root growth stimulation in other fruit species.

Biostimulators promote lateral root development, even at very low concentrations, and they may be involved in stem and root system growth.

Divo de Cesar (2011) claimed that when hormones are applied in tropical areas, they are very susceptible, which may be explained by the fact that their stimulating activity is diminished when they act alone. Nevertheless, in combination with other organic products, they can increase their effects on plant growth, development, and rooting.

Similar results were accomplished by López and Montejo (2012), who observed a larger number of roots, after using such primers in garden greens.

This response was corroborated by Montejo *et al.* (2012) on suburban farms in the municipality of Camaguey, in fruit, using bio-organic products to improve nutrition, and achieved yields up to 10%.

The length of the root system (Table 4) was higher in the treatment (3.41 cm), forty days after cutting plantation, with a significant difference from the other treatments. It demonstrated the efficiency of foliage application.

Table 4 Root system for the two study phases (cm)

Treatment	40 days	70 days
1	2.38 ab	8.74 a
2	1.55 b	4.84 b
3	1.66 b	6.76 ab
4	2.17 ab	7.01 ab
5	2.61 ab	5.19 b
6	3.41 a	7.57 ab
Es_x	0.8794	0.9756

Unequal letters differ for p≤0.05%

The plant responded favorably via leaf estomes, transporting the humus dissolution, plus the phosphoric acid through the xylem and floema, into the rhizosphere to foster root emission. It also benefitted the appearance of foliage on the cuttings, and sprinkling, thanks to the great capacity of zeolite for cationic exchange. It can take over the elements provided by the product and release them slowly along the cycle. Regarding the bagged plants in the chamber, and more particularly, the length of roots, the best behavior was observed in treatment 1 (fortified liquid humus), with 8.47 cm, significantly different from the rest. This may have been caused by the effect of fortified liquid humus, whose composition includes cytochinine, giberlinem, auxines, minerals, and other important elements for proper root growth. Another reason may be the plant´s favorable response to the treatments using foliar applications.

Similar results were achieved by Saborit (2013) and Nápoles (2013), using bio-organic products, based on fortified liquid humus for guava plantlet breeding from cuttings. It may also be favored by the use of zeolite, and the proper contents applied in controlled conditions.

The presence of active leaves was stable among treatments (Table 5), which may have been caused by the effect of the composition of the products, with a good photosynthetic activity, and the assimilation of nutrients that promote efficient leaf development. Eventually, the leaves will age and fall, but foliage is recovered and increased, because they were well fed (López and Montejo, 2012).

Table 5 Number of leaves in phases 1 and 2

Treatment	40 days	70 days
1	3.0	4.0
2	3.0	4.0
3	3.0	4.0
4	3.0	4.0
5	3.0	4.0
6	3.0	4.0
Es_x	0.1702 NS	0.1752 NS

Unequal letters differ for ≤0.05 %

Plant survival to 80 days in phase 2 (Table 6) was the best in treatments 6 (natural liquid humus, plus indoleacetic acid); 5 (natural liquid humus, plus phosphoric acid); and 1 (fortified liquid humus), with 104, 102, and 103 plants, respectively. A positive effect of liquid humus plus zinc, phosphoric acid, and indoleacetic acid was observed. In phase 2, after 80 days, a significant difference was observed, coinciding with identical treatments to the previous phase, with 104, and 103 plants, which may have been caused by the combined effects of these products on plant metabolism. Nápoles (2013) and Saborit (2013) also had similar results for reproduction of guava plantlets in semi controlled conditions, using stem cuttings.

Table 6 Number of live plants

Treatment	60 days	80 days
1	103 a	102 a
2	92 b	100 a
3	94 b	99 ab
4	96 b	94 b
5	104 a	102 a
6	105 a	104 a
ES_X	0.9575	0.8755

Unequal letters differ for p≤0.05%

Plant survival in phase 3 (Table 7) had significant differences among the treatments, with the best results for 6 (natural liquid humus, plus indoleacetic acid); 5 (natural liquid humus, plus phosphoric acid); and 1 (fortified liquid humus). The combined effects of bioorganic products with the root stimulators produced positive plant development through different mechanisms, which was evidenced in the treatments.

Table 7 Number of live plants

Treatment	90 days
1	102 a
2	94 b
3	95 b
4	95 b
5	100 a
6	101 a
ESx	0.8755

Unequal letters differ for p≤0.05%

These results coincided with Nápoles (2013) and Saborit (2013), in studies of reproduction of guava plantlets through stem cuttings, using bioorganic products in similar experimental conditions.

The best economic response was observed in treatment 1 (fortified liquid humus), against treatment 2 (natural liquid humus) (Table 8).

Table 8 Economic assessment

Indicators		Treatment 2 (CUP)	Treatment 1 (CUP)
Salary		400.00	400.00
Bags		9.40	10.20
Compost		8.60	9.20
Energy		100.00	100.00
Products		5.00	12.00
Subtotal expenses		523.00	531.40
Unexpected expenses		52.30	53.14
Total expenses		575.30	584.54
Production value		762.00	916.00
Benefits	CUP	186.70	331.46
Benefit vs. treatment 2	CUP		144.76

Plant survival was essential for estimation of the production value over expenses, which accounted for a $ 331.46 benefit, better than treatment 2, with $ 186.70. Accordingly, treatment 1 can be applied in production conditions.

CONCLUSIONS

Evaluation of some phenological indicators of guava plantlets obtained from stem cuttings in phases 1 and 2, using bio-organic strategies was positive. The best values were observed in treatments 1 (fortified liquid humus); 5 (natural liquid humus, plus phosphoric acid); and 6 (natural liquid humus, plus indoleacetic acid).

Treatments 1, 5, and 6 showed positive survival values after the application of bioorganic alternatives in guava.

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