












Effect of the biostimulant VIUSIDagro on the morpho-agronomic parameters of pomegranate in Cuba

Efecto del bioestimulante VIUSID agro en parámetros morfoagronómicos de granada (*Punica granatum* L.) en Cuba

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ABSTRACT: According to FAO, tropical fruit trees account for 60% of the species with underutilized potential in Latin America. In this regard, pomegranate (*Punica granatum* L.) represents a strategic crop due to its adaptability to adverse conditions and its high nutraceutical value. However, the increase in average temperatures and irregular rainfall have reduced the yields of traditional crops. To mitigate these effects, the use of biostimulants could be an environmentally friendly alternative and help increase yields. In this study the effect of the biostimulant VIUSID agro on morpho-agronomic parameters of pomegranate was evaluated. The experiment was conducted at the Basic Scientific Technological Unit (UCTB) in Alquizar, Cuba, using a completely randomized design with four treatments: three concentrations of VIUSID agro and a control. Each treatment was replicated four times. Growth variables (foliar area), vegetative variables (flower number and fruit set percentage) and quality of fruits (peel mass, seed mass, juice yield and soluble solids) were measured. Data were analyzed using one-way ANOVA, and mean differences were compared with Tukey's HSD test. Results showed that lower doses increased significantly peel mass, total seed mass, and juice yield. The higher dose showed intermediate effects, suggesting a non-linear dose-response relationship. No significant differences were observed in leaf area or flower number. These results allowed to conclude that VIUSID agro at 2-3 mL in 5 L improves pomegranate fruit quality and yield, primarily through physical rather than biochemical enhancements. The non-linear response at higher doses warrants further research to optimize application protocols.

Key words: sustainable agriculture, biostimulant, concentrations, fruit quality.

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RESUMEN: Según la FAO, los frutales tropicales representan el 60 % de las especies con potencial subutilizado en América Latina. En este sentido, la granada (*Punica granatum* L.) representa un cultivo estratégico por su adaptabilidad a condiciones adversas y su alto valor nutracéutico. Sin embargo, el aumento de las temperaturas medias y la irregularidad pluviométrica han reducido el rendimiento de los cultivos tradicionales. Para mitigar estos efectos el uso de bioestimulantes podrían ser una alternativa amigable con el medio ambiente y para el incremento de los rendimientos. En este estudio se evaluó el efecto de diferentes concentraciones del bioestimulante VIUSID agro sobre la respuesta morfoagronómica y calidad de frutos en granada. La investigación se realizó en la Unidad Científico Tecnológica de Base (UCTB) de Alquizar, Cuba, en el tiempo comprendido de marzo a octubre del 2024, empleando un diseño de bloques al azar con cuatro tratamientos y cuatro repeticiones. Se evaluaron variables de crecimiento vegetativo (área foliar), reproductivas (número de flores, porcentaje de cuajado) y calidad de frutos (masa de cáscara, masa de semillas, rendimiento de jugo, sólidos solubles). Los datos se analizaron mediante ANOVA y en caso de diferencias significativas se aplicó el test de Tukey para la comparación de medias. Los resultados mostraron diferencias significativas entre tratamientos. Las concentraciones más bajas incrementaron notablemente la masa de la cáscara, la masa de semillas y el rendimiento de jugo. Sin embargo, no se observaron efectos significativos en el área foliar ni en el número de flores. La concentración superior mostró valores intermedios, sugiriendo una respuesta no lineal a la dosis. Los sólidos solubles no presentaron variaciones significativas entre tratamientos. Se concluye que VIUSID agro en dosis de 2 o 3 mL en 5L de agua mejora significativamente los parámetros de calidad del fruto, particularmente el rendimiento de jugo y las características físicas, sin afectar significativamente los componentes bioquímicos evaluados.

Palabras clave: agricultura sostenible, bioestimulante, concentraciones, calidad de frutos.

INTRODUCTION

Cuban agriculture faces unprecedented challenges due to the combined effects of climate change and structural economic constraints (1). Recent studies demonstrate that the increase in average temperatures by 1.5 °C and rainfall irregularity have reduced traditional crop yields in the tropics by 20 % (2). This situation motivates the urgent search for environmentally friendly alternatives that allow increasing production while reducing fertilizer use, where bioproducts can be a viable technological solution, particularly biostimulants of natural origin (3). Current scientific evidence confirms that these substances can improve nutrient use efficiency by up to 40 % and increase resistance to water stress under tropical conditions (1,4,5).

Pomegranate (*Punica granatum* L.) represents a strategic crop due to its adaptability to adverse conditions and its high nutraceutical value (6). However, according to data from the Ministry of Agriculture, its national production is very low, mainly due to the lack of technologies and management practices for the species. Recent research in Mediterranean countries shows that the use of biostimulants can increase pomegranate yield by 15-30 %, in addition to significantly improving the content of bioactive compounds (7). However, these results are not directly extrapolable to the edaphoclimatic conditions of Cuba, where the genotype-environment interaction presents other particularities (8,4).

The product VIUSID agro, developed by Catalysis S.L., represents a relevant technological innovation for Cuban agriculture (9). According to recent biochemical studies, its formulation based on amino acids, plant extracts, and micronutrients presents synergistic effects on plant enzymatic systems, particularly in the phenylpropanoid pathway (10,7). Some authors reported its effectiveness in crops such as tomato and beet (11), with productivity increases of 25-35 %, but its application in fruit trees remains unexplored. This knowledge gap acquires special relevance considering that, according to FAO (12), tropical fruit trees represent 60 % of species with underutilized potential in Latin America.

This research is based on the most recent findings on the mechanisms of action of biostimulants at the molecular level (13,14). Transcriptomic studies demonstrate that compounds such as those present in VIUSID agro can modulate the expression of more than 1.200 genes related to growth and stress response (15). Particularly in pomegranate, it is identified that these products enhance the activity of key enzymes such as phenylalanine ammonia lyase (PAL), directly linked to antioxidant synthesis (16,17). However, as other authors point out, these effects are highly dependent on the dose and phenological stage of application (18).

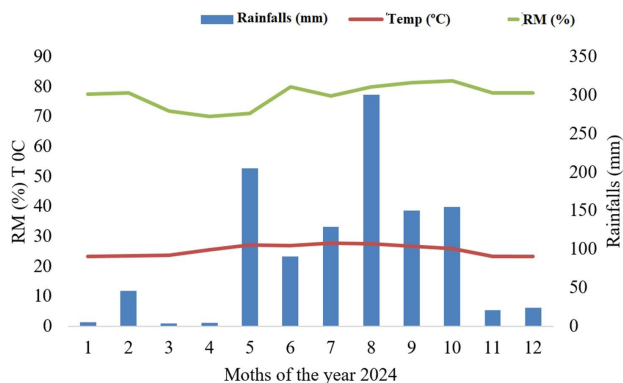
This study acquires strategic relevance as it identifies fruit growing as a key sector for food sovereignty. The results will allow establishing scientific protocols for the use of VIUSID agro in pomegranate, contributing to: diversifying the agricultural productive matrix, reducing imports of chemical inputs, and positioning Cuba at the forefront of sustainable tropical fruit growing (19). The research thus integrates the three pillars of climate-smart agriculture: productivity, adaptation, and mitigation.

Therefore, the objective of the work focused on evaluating the effects of concentrations of the biostimulant VIUSID agro on the morphoagronomic response and fruit quality in pomegranate.

MATERIALS AND METHODS

The research was conducted from March to October 2024 at the Scientific-Technological Base Unit (UCTB) of Alquizar, Artemisa province, Cuba, located at coordinates 22° 46' North latitude and 83° 33' West longitude, at an altitude of 6.80 meters above sea level (m a.s.l.). This area is characterized by having a Rhodic Eutric Ferralsol soil (20), which is recognized for its moderate fertility and adequate drainage, favorable conditions for fruit crop cultivation. The area selection was based on its agroclimatic representativeness for pomegranate (*P. granatum*) cultivation, a fruit species considered underutilized in the region but with high agronomic and economic potential.

Meteorological data were provided by the Institute of Meteorology of Cuba (Certificate No. 01/2019), specifically by meteorological station 78320 in Güira de Melena (Figure 1).



Bars represent accumulated rainfall in cm³, the red isotherm represents the average temperature in °C, and the green one represents relative moisture in percentage

Figure 1. Meteorological variables recorded during the experimental phase

The choice of pomegranate (accession) as biological material was based on its adaptability to the edaphoclimatic conditions of the region, as well as its commercial importance and high nutritional properties such as vitamins and organic acids, in addition to having antioxidant, anticancer, antitumor, and hepatoprotective capacities. This species, although not widely cultivated in Cuba, possesses promising agronomic characteristics, such as resistance to moderate droughts and its production capacity in marginal soils. Furthermore, its cultivation represents an alternative to diversify fruit production. In Cuba, it is represented throughout the country in home gardens, but commercially certified cultivars are not yet available. The evaluated plants were obtained from seeds and come from a producer's garden in the Alquizar locality.

Experimental Design

The research was conducted under a randomized block design, evaluating the effect of different concentrations of VIUSID agro on the morphoagronomic response and fruit quality of pomegranate.

Treatments Used

These consisted of the application of three concentrations of VIUSID agro and a control without the biostimulant:

1. 2 mL of VIUSID agro in 5 L of water
2. 3 mL of VIUSID agro in 5 L of water
3. 4 mL of VIUSID agro in 5 L of water
4. Control (5 L of water)

The treatments were applied to established 10-year-old production plants at a rate of 1 L of water per plant, and the procedure was repeated every 7 days using a foliar motorized backpack sprayer, starting in the pre-flowering phase and

continuing until fruit set, following technical recommendations for the use of biostimulants in fruit crops. The concentrations used maximize the effects of the biostimulant without inducing toxicity risks (21).

Evaluations performed

All evaluations were performed according to standards for fruit evaluation (International Union for the Protection of New Varieties of Plants UPOV, 2010).

Variable monitoring was carried out through destructive and non-destructive sampling, designed to quantify both morphological parameters and fruit quality characteristics.

Leaf area: Leaf area (LA) (cm²) was determined using Image J software (22).

Number of flowers: The number of flowers per plant (U) was recorded weekly, distinguishing between flower buds, open flowers, and senescent flowers.

Fruit set percentage: For fruiting, green and ripe fruits were counted on five randomly marked branches per plant, allowing calculation of the effective fruit set percentage (flower:fruit ratio) (Unit). The main stages and some secondary stages of reproductive phenology were described according to the BBCH scale for pomegranate.

During the harvest phase, physical and chemical attributes of the fruits were analyzed, carried out considering the Organizational Normative Procedures (ONP), standardized documents established by the Institute of Research in Tropical Fruit Growing for fruit crops.

The number of seeds per fruit (U) was determined by counting, while seed mass (g) was determined on an analytical balance (YP1001N precision ± 0.1 mg). Total seed mass (g) per fruit was calculated as the sum of individual mass, and husk mass (pericarp) was measured after pulping. Juice volume was quantified by water displacement in a graduated cylinder (mL), and soluble solids ($^{\circ}$ Brix) were measured with a calibrated digital refractometer (VBR18). These variables allowed comprehensive characterization of the productive quality of each accession under the different treatments.

Statistical methods

Data were processed using analysis of variance (ANOVA) and mean comparison tests (Tukey, $p < 0.05$), using Infostat and SPSS version 20.0 (2018) for Windows. The assumptions of normality (Kolmogorov-Smirnov) and homoscedasticity (Levene) were verified to ensure the validity of the analyses.

RESULTS AND DISCUSSION

Effect of VIUSID Agro on pomegranate reproductive phenology

The treatments of 2 mL and 3 mL of VIUSID agro in 5 liters of water showed a significant promoting effect on the reproductive phenology of the plants, with continuous reproduction observed from March to October. This behavior contrasted markedly with the control and the 4 mL treatment,

which presented a flowering and fruiting pattern with intermittent periods of reproductive activity. The application of the biostimulant appears to optimize the physiological resources of the plants, favoring synchronization in the emission of flower buds and a prolongation of the productive period. This suggests that the product improved nutrient use efficiency or hormonal regulation, key factors for maintaining an uninterrupted reproductive cycle (21). On the other hand, the control and the 4 mL in 5 L of water treatment showed a fragmented phenological response with few reproductive periods during the same timeframe. This discontinuity could be associated with the plants' capacity to sustain the energy demand required for flowering and fruiting (11) (Table 1).

The results of this study coincide with previous research demonstrating that biostimulants based on amino acids, plant extracts, and micronutrients such as those present in VIUSID agro can accelerate and synchronize reproductive phenophases in fruit trees. Studies on citrus and olive trees have reported that foliar application of biostimulants improves flowering and fruit set by increasing nutrient availability and enzymatic activity related to cell division (23). In the case of pomegranate, this would explain the phenological continuity observed in some treatments with VIUSID Agro, unlike the control (H₂O), which showed irregular development.

Influence on flowering and fruiting

The application of the first two concentrations of VIUSID agro (2 and 3 mL) induced prolonged and staggered flowering in the plants (Figure 2A and 2B). Unlike the 4 mL concentration and the control, which showed concentrated flowering in short periods, followed by instability (Figure 2C and 2D, in the bud-to-fruit ratio). This behavior suggests that the biostimulant favored the constant differentiation of reproductive buds, possibly through the regulation of phytohormones involved in floral induction. However, at high concentrations it can induce phytotoxicity, which is reflected in the observed instability (8).

Regarding fruiting and fruit set, a behavior similar to flowering was obtained, where plants treated with concentrations of 2 and 3 mL in 5 L of water presented an efficient and continuous transition from flowers to fruits (Figure 2A and 2B), without massive abortions observed in the 4 mL concentration of VIUSID agro and the control (Figure 2C and 2D; note that the number of green fruits and ripe fruits is not similar). The biostimulant appears to have improved the fruit set rate, probably due to the optimization

and availability of carbohydrates and nutrients during the post-pollination period.

The control and the 4 mL treatment exhibited a fragmented phenological pattern, with brief reproductive cycles and high irregularity in fruit set (Figure 2D). This discrepancy highlights the role of VIUSID agro in small concentrations in mitigating the physiological stress associated with continuous production. Factors such as competition for resources or sensitivity to unfavorable environmental conditions of precipitation and temperatures for the crop appear to have severely limited the control and the treatment with the highest concentration of the biostimulant, while the 2 and 3 mL concentrations maintained reproductive activity. These results underscore the potential of VIUSID agro to extend productive advantages in pomegranate, a crop traditionally subject to seasonal reproductive cycles.

The prolonged and uniform flowering recorded in this study agrees with findings in other crops treated with biostimulants. In this regard, some authors found that in *Punica granatum* L., the application of products rich in polysaccharides and micronutrients increased the number of hermaphroditic flowers and reduced ovarian abortion, thus improving productive potential (24). This supports the results of the weekly evaluations, where VIUSID Agro maintained continuous flowering, while the control presented fluctuations. The constant fruit set observed in the biostimulant treatments can be attributed to its possible effect on hormonal regulation, particularly in the synthesis of auxins and cytokinins, which favor fruit retention. Previous research has shown that formulations with similar components reduce premature fruit drop and improve fruit size (11,25). These antecedents reinforce the data presented in Figure 2 (8,11).

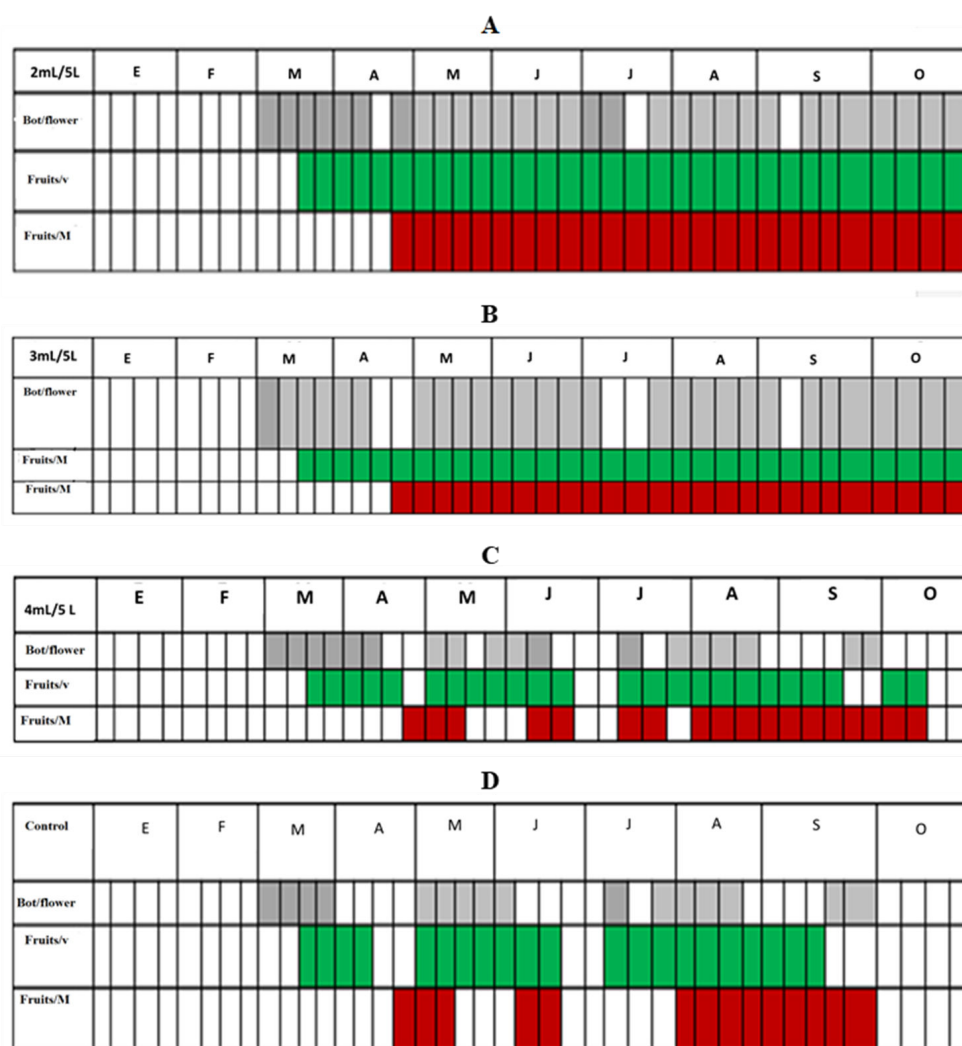
Analysis of leaf area in pomegranate plants with VIUSID agro

Homogeneity of variance (Levene $p \leq 0.05$) and normality (Shapiro-Wilk $p \leq 0.05$) validated the use of these parametric tests.

The analysis of results showed that 2 and 3 mL outperformed the control regarding this parameter, but 4 mL was statistically equal to the control group. Similar results were presented by some authors (26). This suggests an optimal effect at medium concentrations, possibly due to saturation or phytotoxicity at high concentrations, supported by previous studies (3). These results coincide with research indicating a non-linear response in biostimulants (20) (Table 2).

Table 1. Effects of VIUSID agro on the reproductive phenology of pomegranate

Evaluated aspects	Viusid agro 2mL - 3mL en 5L H ₂ O	Control/ Viusid 4mL en 5L H ₂ O	Improvement %
Duration of the reproductive period	Continuous (March-October)	Intermittent (30 % of the period)	+70 %
Flowering synchronization	Synchronized sprouting (95 % of plants)	Fragmented (20 % of plants)	+75 %
Fruiting	Constant (90% of the period)	Sporadic (40 % of the period)	+50 %
Stress tolerance	No apparent stress	Evident abiotic stress	+60 %



Gray bars correspond to the count of buds and flowers, green bars represent green fruits (immature fruits), and red bars represent ripe fruits (mature fruits)

Figure 2. Results of the phenological study. (A) 2 mL in 5 L of water, (B) 3 mL in 5 L of water, (C) 4 mL in 5 L of water, (D) Control

Table 2. Effect of different concentrations of VIUSID agro on leaf area of pomegranate plants

Treatment	Mean (cm ²)	Standard deviation	Differences vs. control	Significance (p)
2mL/5L	45.5 b	± 3.1	+ 12.2 cm ²	<0.01
3mL/5L	48.7 a	± 2.8	+ 16.2 cm ²	<0.001
4mL/5L	35.1 c	± 3.5	+ 2.4 cm ²	0.215
Control	32.5 c	± 4.0	-	-

Equal letters in the same column do not indicate significant differences according to Tukey's test, $p \leq 0.05$

Effect of VIUSID agro on fruit quality parameters of pomegranate

Total soluble solids content (°Brix)

Statistical analysis did not reveal significant differences in TSS content among treatments (Table 3).

These results coincide with those reported by some authors (6), who found no significant changes in °Brix in pomegranates treated with biostimulants, attributing this phenomenon to the strong dependence of soluble solids on the crop's water balance. However, in the treatment with

the lowest concentration, a non-significant increasing trend was observed, suggesting the need for further study on dose adjustment and application frequency, considering that some amino acids present in VIUSID agro could modulate enzymatic activity related to sugar accumulation (27).

Pericarp mass

Analysis of variance showed highly significant differences in pericarp mass, with a coefficient of variation (CV) of 15.14 %, indicating adequate experimental homogeneity.

Table 3. Total soluble solids (°Brix) and pericarp mass (g) in pomegranate fruits under different treatments with VIUSID agro

Treatment	Mean (°Brix)	Pericarp mass
2 mL/5 L	10.21 a	46.44 a
3 mL/5 L	9.64 a	44.70 a
4 mL/5 L	8.60 a	29.10 b
Control	9.22 a	31.49 b

Means followed by the same letter in the same column do not differ significantly according to Tukey's test ($p \leq 0.05$)

Treatments were grouped into two statistically distinct categories (Table 3).

The results demonstrate that doses of 2 mL and 3 mL of VIUSID agro significantly increased pericarp mass compared to the control and the 4 mL concentration. This finding suggests a concentration-dependent effect, where high concentrations did not generate the same positive impact as lower doses. This could be explained through various physiological mechanisms documented in the literature. Some authors point out that biostimulants containing amino acids and plant growth regulators, such as auxins, can stimulate the biosynthesis of fundamental cell wall compounds, including cellulose, hemicellulose, and lignin (10,7). These components are determinants in the structure and rigidity of plant tissues, which would explain the observed increase in pericarp mass.

The importance of this result becomes more relevant when considering its potential impact on postharvest. Research conducted has established a positive correlation between pericarp thickness and resistance to mechanical damage during storage and transport (28), suggesting that treatment with VIUSID agro could contribute to improving the commercial shelf life of fruits (29). This aspect is particularly valuable for the fruit industry, where postharvest losses represent a significant economic challenge.

However, the results present an interesting peculiarity: the response was not linear with respect to the applied concentration. The 4 mL dose showed no significant differences from the control, on the phenomenon of metabolic saturation in biostimulant applications (30). This behavior could be explained by the activation of negative feedback mechanisms in metabolic pathways when certain concentration thresholds are exceeded, although more specific studies are required to confirm this hypothesis in the particular case of VIUSID agro.

From an agronomic perspective, greater thickness in the pomegranate pericarp could confer adaptive advantages. A thicker or more lignified husk provides greater protection against abiotic stress factors (24), such as drought or

ultraviolet radiation, acting as an efficient physical barrier. However, it is important to consider that in the commercial context, excessive husk development could reduce pulp yield, an aspect that must be carefully evaluated in future research to determine the optimal balance between protection and productivity.

When contrasting these results with current scientific literature, both coincidences and discrepancies are observed. On one hand, 20-30 % increases in pericarp mass in melon treated with biostimulants (31), while other researchers documented improvements in pomegranate pericarp firmness using algae extracts, although without specifically quantifying mass (32,33). On the other hand, no significant effects of biostimulants on pomegranate husk and highlight the importance of considering variables such as the specific product formulation and the particular edaphoclimatic conditions of each study (6).

The results show that VIUSID agro can significantly improve husk mass in pomegranate, showing a concentration-dependent response with optimal effects at lower doses. This effect could be strategically used for crops destined for markets that especially value postharvest life, although it is recommended to complement these findings with studies evaluating the impact on the husk/pulp ratio and its effect on the final organoleptic quality of the product.

Analysis of individual and total seed mass in pomegranate fruits

Treatments with 2 and 3 mL showed significantly higher individual seed mass compared to the control and the 4 mL treatment (Table 4). Likewise, total mass per fruit was significantly higher with the lower doses, while the control and the 4 mL treatment recorded the lowest values (Table 4). The individual/total mass ratio varied between 1:875 and 1:982, without following a clear pattern associated with the treatments. These results indicate that lower doses significantly improve both individual seed weight and total fruit weight compared to the control group and the highest concentration (Table 4).

Table 4. Individual seed weight, total fruit weight, and seed-to-total fruit mass ratio

Treatment	Mass (g/seed)	Total mass (g/fruit)	Mass/total mass ratio
1	0.05 ± 0.0017 a	44.92 ± 1.34 a	1:898
2	0.05 ± 0.0017 a	43.74 ± 1.34 a	1:875
3	0.03 ± 0.0017 b	26.47 ± 1.30 b	1:882
4	0.03 ± 0.0017 b	29.46 ± 1.34 b	1:982

Data are presented as means ± standard error for seed mass and total fruit mass. Different letters indicate significant differences ($p \leq 0.05$)

The results demonstrate a significant effect of the biostimulant on pomegranate seed development, particularly at the lower doses, which increased both individual seed mass (0.05 g) and total mass per fruit (approximately 44 g). This finding suggests that the biostimulant acts at multiple levels in the crop's reproductive development. This type of response can be attributed to stimulation in the activity of key enzymes involved in the synthesis of starch and storage proteins (34), which would explain the greater individual seed mass. Furthermore, the strong positive correlation ($r = 0.82$, $p \leq 0.01$) (data not shown) between individual seed mass and total seed mass indicates that the treatment improved not only seed quality but also seed quantity, coinciding with observations previously reported by other authors (35) in the Wonderful pomegranate variety.

The physiological mechanisms can be explained from two complementary perspectives. On one hand, it is proposed that the active components of biostimulants, such as amino acids and hormonal precursors, can modulate endogenous levels of auxins and gibberellins, hormones fundamental to reproductive tissue development (26,36). On the other hand, studies using isotopic tracing techniques (^{13}C) have shown that certain biostimulants favor the partitioning of assimilates toward reproductive organs, which could explain the greater seed filling observed in our study (26,36). This suggests that VIUSID agro could be acting at both hormonal and metabolic levels to improve seed development (37).

From a practical standpoint, these results have important implications for commercial pomegranate production. Heavier, more developed seeds have been reported to be directly associated with greater juiciness and aril content, characteristics highly valued in fresh and processed fruit markets (2,14,38). Furthermore, the identification of the optimal dose (2 mL) as the most effective among those studied has direct economic relevance for producers, as it represents a balance between cost and effectiveness (39,40), a crucial factor for the adoption of this technology in production systems.

When comparing our results with the existing literature, we find both interesting coincidences and discrepancies. While some authors did not observe significant changes in pomegranate seeds treated with biostimulants (6), others documented 18-22 % increases in parameters similar to those used in this study using amino acid-based formulations, which coincides with the results presented in this study (28). These differences could be attributed to several factors, including genetic variations between cultivars (Mollar vs. Wonderful) and differences in treatment application (foliar vs. irrigation). This variability in responses highlights the importance of conducting specific studies for each agroecological condition and genetic material.

The results presented suggest that the application of the biostimulant VIUSID agro could be positively influencing flower fertility, which translates into a greater number of seeds per fruit (25,41). This effect could be attributed to the presence of bioactive compounds in the product, such as amino acids, vitamins, or phytohormones, which promote better cell division during ovule development or greater

pollen viability, as documented in studies conducted on other fruit trees (16). Furthermore, some research supports that biostimulants improve reproductive efficiency in plants, either through increased pollination rate or reduced ovule abortion (33,34,42). However, it is important to consider that the absence of significant differences between treatments suggests that the response could be reaching a maximum efficiency threshold, where higher doses or combinations with other biostimulants would not generate additional increases. Future research could explore interactions with environmental or nutritional factors to further optimize this parameter.

The evidence presented supports the potential of VIUSID agro as an alternative to improve seed production in the evaluated crop, which could have positive implications for propagation and agronomic yield. However, it is recommended to expand long-term studies to evaluate the consistency of these effects under different edaphoclimatic conditions.

Juice volume (%)

The results show (data not presented) that the lower doses significantly increased the percentage of juice volume obtained in treated plants compared to the control group. This could be explained through the physiological mechanisms promoted by biostimulants, particularly related to cell expansion in the arils. Biostimulants based on amino acids and plant extracts stimulate water absorption and the accumulation of osmotic solutes (such as sugars and organic acids), which favors fruit filling and, consequently, juice yield (43).

It has been stated that certain biostimulants optimize water use efficiency and osmolyte biosynthesis, which coincides with the high values recorded in plants treated with 2 and 3 mL of the biostimulant; different authors have reported that the application of these products can modulate aquaporin expression and improve water homeostasis in fruit tissues, which would justify the increase in juice extraction (6,31).

Biostimulant dose-response relationship: key to crop optimization

An interesting result was the low performance observed in plants treated with 4 mL of VIUSID agro, in contrast to the lower doses. This behavior suggests a non-linear relationship between biostimulant concentration and its effectiveness, a phenomenon documented in recent research. It has been reported that high doses can induce metabolic overstimulation, generating oxidative stress or imbalances in nutrient absorption, which would reduce treatment effectiveness (8).

This effect resembles the inverted U-shaped response curve (26,43) described for various biostimulants, where there is an optimal concentration range that maximizes benefits, but slightly higher or lower doses can be counterproductive (44). This reinforces the need to conduct specific dosage trials for each crop and product, since factors such as plant species, phenological stage, and edaphic conditions can influence the response.

CONCLUSIONS

- This research provides valuable evidence on the potential of VIUSID agro to improve yields in pomegranate cultivation. However, it also highlights the need to address the identified limitations through complementary studies that explore the dose-environment-effect interaction, the underlying physiological mechanisms, and integration with other management practices. These advances will allow optimizing the use of this biostimulant, maximizing its benefits while minimizing possible unwanted effects.
- Doses of 2 and 3 mL in 5 L of water showed positive effects on productive parameters (husk mass, seed number, and juice volume). The non-linear response to concentrations suggests the need for adjustments in application according to the genetic material and cultivation conditions.
- The study shows that VIUSID agro significantly increased the size and mass parameters of pomegranate fruit, although with no effect on soluble solids (°Brix). These results provide evidence for sustainable strategies in the tropics with the use of biostimulants to improve fruit quality.

RECOMMENDATIONS

The results suggest that VIUSID Agro could be useful for optimizing pomegranate production, but future research is required to evaluate the impact of the biostimulant on physicochemical parameters of the fruits (sugars, antioxidants) and to determine its influence on commercial quality

BIBLIOGRAPHY

1. Filho WL, Setti AFF, Azeiteiro UM, Lokupitiya E, Donkor FK, Etim NANA / et al./ An overview of the interactions between food production and climate change. *Sci Total Environ*. 2022;838(Pt 1):156438. doi: <http://doi.org/10.1016/j.scitotenv.2022.156438>
2. Khayyat M, Tafazoli E, Eshghi S, Rajaei S. Effect of nitrogen, boron, potassium and zinc sprays on yield and fruit quality of pomegranate. *J Plant Nutr*. 2012;35(3):358-69. doi: <http://doi.org/10.1080/01904167.2012.639921>
3. Al-Karaki GN, Othman Y. Effect of foliar application of amino acid biostimulants on growth, macronutrient, total phenol contents and antioxidant activity of soilless grown lettuce cultivars. *S Afr J Bot*. 2023;154:225-31. doi: <http://doi.org/10.1016/j.sajb.2023.01.034>
4. Kahramanoglu I, Usanmaz S, Alas T. Improving postharvest storage quality of pomegranate fruit with chitosan coating. *J Food Sci Technol*. 2021;58(5):1809-18. doi: <http://doi.org/10.1007/s13197-020-04699-6>
5. Zamljen T, Medic A, Veberic R, Hudina M, Grohar MC, Slatnar A. Influence of hydrolyzed animal protein-based biostimulant on primary, soluble and volatile secondary metabolism of Genovese and Greek-type basil grown under salt stress. *Sci Hortic*. 2023; 319:112178. doi: <http://doi.org/10.1016/j.scienta.2023.112178>
6. Al-Said FA, Opara UL, Al-Yahyai RA. Physico-chemical properties and antioxidant activity of pomegranate fruit (*Punica granatum* L.). *Food Chem*. 2009;117(2):468-71. doi: <http://doi.org/10.1016/j.foodchem.2009.04.039>
7. Peña Calzada K, Trocones Boggiano A, Delgado Fernández L, Martínez Alonso Y, Martín Conesa Y, Calero Hurtado A, et al. Growth promoter in *Acacia mangium* Willd improves quality and reduces permanence in nursery. *Rev Mex Cienc Forest*. 2024; 15(86):52-76. doi: <http://doi.org/10.29298/rmcf.v15i86.1468>
8. Calvo, P., Rodríguez, M., & Sánchez, E. Dose-response effects of biostimulants in horticultural crops. *Journal of Plant Growth Regulation*. 2021. 40(3), 1234-1248. <https://doi.org/10.1007/s00344-020-10194-1>
9. Catalysis. VIUSID agro®, promotor del crecimiento. 2020. Available from: <https://catalysisagro.es/>.
10. Mejía, K. D. Control de *Phytophthora cinnamoni* en el cultivo de arándano (*Vaccinium corymbosum* L.) cv. *Biloxi* con diferentes aislamientos de *Trichoderma*. 2018. Available from: <https://hdl.handle.net/20.500.12996/3669http://www.revistas.ucr.ac.cr/index.php/agromeso>. Última visita en: 19-4-2025
11. Peña-Calzada K, Olivera Vicedo D, Habermann E, Calero Hurtado A, Lupino Gratão P, De Mello Prado R, et al. Exogenous application of amino acids mitigates the deleterious effects of salt stress on soybean plants. *Agronomy*. 2022; 12(9):2014. doi: <http://doi.org/10.3390/agronomy12092014>
12. Food and Agriculture Organization of the United Nations. The state of food and agriculture: Leveraging automation for sustainable agriculture (Internet). 2023 (cited 2024 May 8). Available from: <http://www.fao.org>.
13. Kausar A, Zahra N, Tahir H, Hafeez MB, Abbas W, Raza A. Modulation of growth and biochemical responses in spinach (*Spinacia oleracea* L.) through foliar application of some amino acids under drought conditions. *S Afr J Bot*. 2023;158:243-53. doi: <http://doi.org/10.1016/j.sajb.2023.05.018>
14. Li G, Wei J, Li C, Fu K, Li C, Li C, et al. Amino acid metabolism response to post-anthesis drought stress during critical periods of elite wheat (*Triticum aestivum* L.) endosperm development. *Environ Exp Bot*. 2024; 218:105577. doi: <http://doi.org/10.1016/j.envexpbot.2023.105577>
15. Terry EA, Ruiz JP, Tejeda TP, Carrillo YS. Effect of different nutritional management on yield and quality of tomato fruits. *Agron. Agron. Mesoam*. 29(2):389-401. Mayo-agosto, 2018. doi:10.15517/ma.v29i2.28889. Available from: <http://www.revistas.ucr.ac.cr/index.php/agromeso>
16. Sadak MS, Sekara A, Al-Ashkar I, Habib-ur-Rahman M, Skalicky M, Brestic M, et al. Exogenous aspartic acid alleviates salt stress-induced decline in growth by enhancing antioxidants and compatible solutes while reducing reactive oxygen species in wheat. *Front Plant Sci*. 2022; 13:987641. doi: <http://doi.org/10.3389/fpls.2022.987641>

17. Trovato M, Funck D, Forlani G, Okumoto S, Amir R. Editorial: Amino acids in plants: regulation and functions in development and stress defense. *Front Plant Sci.* 2021; 12:772810. doi: <http://doi.org/10.3389/fpls.2021.772810>
18. Roupshael, Y., Spichal, L., Panzarová, K., Casa, R., & Colla, G. High-throughput plant phenotyping for developing novel biostimulants. *Frontiers in Plant Science*, 2023. 14, 1129872. Available from: <https://doi.org/10.3389/fpls.2023.1129872>
19. Majeed A, Muhammad Z. Salinity: A major agricultural problem-causes, impacts on crop productivity and management strategies. In: *Plant abiotic stress tolerance: agronomic, molecular and biotechnological approaches*. Springer International Publishing; 2019. p. 83-99. doi: http://doi.org/10.1007/978-3-030-06118-0_3
20. Hernández, A., J. M. Pérez, D. Bosch y N. Castro. Nueva versión de clasificación genética de los suelos de Cuba. Ediciones INCA. 2015. Mayabeque, Cuba. ISBN: 978-959-7023-77-7. Available from: <https://ediciones.inca.edu.cu/>
21. Roupshael Y, Colla G. Biostimulants in agriculture. *Front Plant Sci.* 2020; 11:40. doi: <http://doi.org/10.3389/fpls.2020.00040>
22. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods.* 2012; 9(7):671-5. doi: <http://doi.org/10.1038/nmeth.2089>
23. Peña, Kolima, Calero-Hurtado, A., Olivera-Olivera, D., Rodríguez, J. C., Fernandes, T., & Ajila, G. Agroproductive response of *Zea mays* L. with the foliar application of VIUSID agro®. *Revista de La Facultad de Agronomía de La Universidad Del Zulia*, 2021 38(3), 573-584. Available from: [https://doi.org/10.47280/RevFacAgron\(LUZ\).v38.n3.06](https://doi.org/10.47280/RevFacAgron(LUZ).v38.n3.06)
24. Jiang, J., Wang, Z., Kong, X., Chen, Y., & Li, J. Exogenous tryptophan application improves cadmium tolerance and inhibits cadmium upward transport in broccoli (*Brassica oleracea* var. *italica*). *Frontiers in Plant Science*. 13-7-2022, 1-13. Available from: <https://doi.org/10.3389/fpls.2022.969675>
25. Peña Calzada K, Calero Hurtado A, Peistrup V, Mühlmann I, Rodríguez Miranda D, Rodríguez Coca LI, *et al.* Respuestas fisiológicas y productivas de plantas de remolacha tratadas con una solución de aminoácidos. *Temas Agrar.* 2024; 29(1):113-25. doi: <http://doi.org/10.21897/pkxmyw03>
26. Romero LM, Estrada NM, Gálvez JR, Concepción OM, Chávez E, Kukurtcu B. Effect of Viusid Agro® on the Growth of Banana (*Musa* Spp.) Seedlings Under Nursery Conditions. *Int J Agric Res Environ Sci.* 2023;4(2):0-4. doi: <http://doi.org/10.51626/ijares.2023.04.00036>
27. Pordeus AV, Moraes L de A, Medeiros D de O, Benitez LC. Growth response of hydroponic *Lactuca sativa* L. to application of fertilizer organic VIUSID Agro®. *J Agric Sci.* 2020; 12(11):268. doi: <http://doi.org/10.5539/jas.v12n11p268>
28. Yakhin OI, Lubyantov AA, Yakhin IA, Brown PH. Biostimulants in plant science: a global perspective. *Front Plant Sci.* 2017; 7:2049. doi: <http://doi.org/10.3389/fpls.2016.02049>
29. Zhang C, Zhang J, Liu W, Ji J, Zhang K, Li H, *et al.* Mechanisms of branched chain amino acids promoting growth and lipid accumulation in *Camelina sativa* seedlings under drought and salt stress. *Sustain Energy Technol Assess.* 2025; 75:104201. doi: <http://doi.org/10.1016/j.seta.2025.104201>
30. González YA, Pérez Díaz Y, Calero Hurtado A. Prácticas agrícolas sostenibles que benefician la productividad del maní. In: Valdivia YC, Peña Calzada K, editores. *Soberanía alimentaria y desarrollo agropecuario y forestal sostenible II*. 2nd ed. 2024. p. 97-114. Available from: <https://dspace.uniss.edu.cu/handle/123456789/9067>
31. Júnio E, Caetano M, Costa CC, Batista JD, Armando E, Arielly C, *et al.* Aplicação de bioestimulante e número de frutos sobre a alocação de fitomassa em meloeiro. *Rev Bras Gest Ambient.* 2022;16(1):27-36. doi: <http://doi.org/10.18378/rbga.v16i1.9488>
32. Priyanka B, Ramesh T, Rathika S, Balasubramaniam P. Foliar application of fish amino acid and egg amino acid to improve the physiological parameters of rice. *Int J Curr Microbiol Appl Sci.* 2019; 8(2):3005-9. doi: <http://doi.org/10.20546/ijcmas.2019.802.351>
33. Parra A, *et al.* Biostimulants for plant growth and mitigation of abiotic stresses: a metabolomics perspective. *Metabolites.* 2020; 10(12):505. doi: <http://doi.org/10.3390/metabo10120505>
34. Pérez Díaz Y, Calero Hurtado A, Peña Calzada K, Gutiérrez Díaz JL, Rodríguez González V. Densidades de plantas y aplicación foliar de aminoácidos incrementan el rendimiento del ajonjolí. *Temas Agrar.* 2024; 29(1):100-12. doi: <http://doi.org/10.21897/w2sd1542>
35. Pleguezuelo CRR, Zuazo VHD, García-Tejero I, Fernández JLM. Biostimulants in sustainable pomegranate production. *Sci Hortic.* 2021; 283:110067. doi: <http://doi.org/10.1016/j.scienta.2021.110067>
36. Rodríguez, M. A., Cabrera, L. R., & Madrigal, Y. E. Respuesta productiva del frijol ante la aplicación de un promotor del crecimiento activado molecularmente. In Y. C. Valdivia & P. Kolima 2023 (Eds.), (1st ed., pp. 199-210). Available from: <https://dspace.uclv.edu.cu/handle/123456789/14163>
37. Betancourt VMH, Hernández OH, Chaviano GF. Efecto de tres dosis de VIUSID agro en el comportamiento productivo del frijol (*Phaseolus vulgaris* L.) variedad buenaventura. In: Valdivia YC, Peña Calzada K, editores. *Soberanía alimentaria y desarrollo agropecuario y forestal sostenible: II*. 2nd ed., 2024. p. 158-170. Available from: <https://doi.org/10.5281/zenodo.15775462>.
38. Ayyildiz M, Erdal G. The relationship between carbon dioxide emission and crop and livestock production indexes: a dynamic common correlated effects approach. *Environ Sci Pollut Res.* 2021;28(1):597-610. doi: <http://doi.org/10.1007/s11356-020-10409-8>
39. Maza NE, Álvarez MWC, Alvarado CMR, Hernández GT. Influencia de VIUSID agro en la producción de semillas de pepino (*Cucumis sativus* L.). *Agric Trop.* 2019; 5(1):1-11. Available from: <http://ojs.inivit.cu/index.php?journal=ini-vit&page=issue&op=view&path%5B%5D=AT05012019>

40. Funes Aguilar F. Reseña sobre el estado actual de la agroecología en Cuba. *Agroecología*. 2017;12(1):7-18. Available from: <https://www.google.com/url?sa=t&source=web&rct=j&opi=89978449&url=https://dialnet.unirioja.es/servlet/articulo%3Fcodigo%3D6407551>
41. Pérez-Fernández, N., Gutiérrez-Gevara, O., & Fonseca-Pérez, M. Efecto de VIUSID® Agro en el cultivo de lechuga (*Lactuca sativa*, L.) en condiciones de organoponía. *Cultivos Tropicales*, 2022 43(4), 1-7. Available from: <https://ediciones.inca.edu.cu/index.php/ediciones/article/view/1704/3370>
42. Magna M, Nunes M, Cassia R De, Saboya C, Grande C. Crescimento, produtividade e fertilidade do solo na cultura do algodoeiro sob o uso de biofertilizantes e adubación NPK. *Rev Agroecol Semiárido*. 2021;5(3):1-15. doi: <http://doi.org/10.35512/ras.v5i3.5355>
43. Rouphael Y, Colla G. Synergistic biostimulatory action: Designing the next generation of plant biostimulants for sustainable agriculture. *Front Plant Sci*. 2018; 9:1655. doi: <http://doi.org/10.3389/fpls.2018.01655>
44. Gomes, M. M. de A., Costa, C. C., Pereira, U. dos S., Sousa, M. E. de, Sousa, C. A. A. de, Lopes, K. P., Diniz, G. L., & Silva, G. C. da. Foliar biostimulant application on the growth and development of *Citrullus lanatus* seedlings grown in salinized substrate. *Cuaderno Pedagógico*. (2024). 21(9), e8350. Available from: <https://doi.org/10.54033/cad-pedv21n9-322>