Türk Tarım ve Doğa Bilimleri Dergisi 9(2): 462–468, 2022
https://doi.org/10.30910/turkjans.1073218

TÜRK TARIM ve DOĞA BİLİMLERİ DERGİSİ

TURKISH JOURNAL of AGRICULTURAL and NATURAL SCIENCES

www.dergipark.gov.tr/turkjans

Research Article

Hibrit domates fidelerinin (*Lycopersicon esculentum* L.) vejetatif gelişimi için hümik maddelerin uygulaması

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Received: 16.02.2022 Received in revised: 28.03.2022 Accepted: 28.03.2022

Öz

Hümik ve fulvik asitler gibi hümik maddelerle ilişkili dayanıklı çeşitlerin ve mineral beslemenin kullanımının bitki gelişimini desteklediği, , bitkinin kök ve toprak üstü kısımlarının büyümesini desteklediği için bitkilerin vejetatif gelişimleri için uygun bir teknik olduğu bilinmektedir. Böylece daha iyi bir nihai üretkenlik sağlanmış olmaktadır. Bu çalışmanın amacı, serada üretilen Justyne® hibrit domateslerin vejetatif gelişiminde hümik maddelere dayalı Bio Humate® gübresinin etkilerini değerlendirmektir. Deneme; Brezilya’da Universidade Tuiuti do Paraná Üniversitesi'ndeki deneme seralarında uygulanmış olup tamamen tesadüf blokları deneme deseni ve 4 x 2 faktöriyel kullanılarak tasarlanıp, dört doz ticari ürün olarak satılan biyostimulant (0 (kontrol), 1.5, 2 ve 2.5 mL L^{-1} Bio Humate®) uygulanarak üç tekrarla yetiştirilen Justyne® domates hibritlerinde, ekimden sonra 42. ve 56. günlerde (DAT: Ekimden Sonraki Günler) örnekleme yapılarak gerçekleştirilmiştir. Uygulamalar 7 DAT'den başlayarak 14 günde bir uygulanmıştır. Bio Humate® gübresinin toprağa uygulanması, Justyne® hibrit domateslerin vejetatif gelişiminde değerlendirilen biyometrik ve biyokimyasal parametreler için 2 mL L^{-1} dozunda 56 DAT'de en düşük klorofil içeriği olarak ortaya çıkmasına rağmen daha iyi sonuçlar vermiştir.

Anahtar kelimeler: Domates, *Lycopersicon esculentum* L., Biyostimulant

Humic substances application fort he vegetative development of hybrid tomato seedlings (*Lycopersicon esculentum* L.)

Abstract

It is known that the use of resistant cultivars and mineral nutrition associated with humic substances, such as humic and fulvic acids, can favor plant development, promoting aerial parts and roots growth, being a viable technique for better vegetative development of plants in general, and, consequently, for better final productivity. The objective of this study was to assess the impact of humic substances fertilizer calls Bio Humate® in the Justyne® hybrid tomato seedlings development. The experiment was conducted in greenhouses of University of Tuiuti do Paraná/Brazil with a completely randomized experimental design, arranged in a 4 x 2 factorial with four doses of the commercial product’s application (0 (control), 1.5, 2, and 2.5 mL L^{-1} of Bio Humate®) on three repetitions Justyne® hybrid tomato seedlings that were subjected to samplings at 42nd and 56th days after transplanting (DAT)). The treatments were applied every 14 days, starting at 7 DAT at 42 and 56 DAT. Bio Humate® fertilizer treatment showed better results at 2 mL L^{-1} of doses for the biometric and biochemical parameters evaluated in the Justyne® hybrid tomato seedlings development, even though the chlorophyll content at 56 DAT is the lowest.

Key words: Tomato, *Lycopersicon esculentum* L., Biostimulant

Introduction

Tomato growing is a profitable business opportunity both worldwide and in Brazil. As per IBGE-Brazilian Institute of Geography and Statistics, around 62,000 hectares of tomato (Lycopersicon esculentum L) are grown in Brazil, including 4.5 million tons recorded in 2018. (IBGE, 2018).

Their production and quality can be affected from the soil preparation until the final consumer arrives. There are some aspects to take into account for the establishment of the crop as adapted cultivars, planting, soil pH, planting system, spacing, irrigation, weed control, nutritional contribution, phytosanitary control, weight loss, climatic factors and aspects harvest (Chitarrra and Chitarra, 1990).

Proper nutritional applications at different development stages of plant is an effective technique for increasing the production and quality of tomatoes. However, it is critical to consider the environmental impact that can be generated, depending on how cultivation is carried out, always looking for alternatives that minimize such harmful effects (Noordwijk and Cadisch, 2002; Brentrup et al., 2004).

Among the several available growing techniques of tomato farming for more environmental-friendly is the adoption of cultivars that are better appropriate to the climate and location, as well as more resistant to specific diseases and pests. Justyne® hybrid's long-lasting, early to mid-cycle, robust, and unpredictable growth provides an attractive alternative in this case. (TAKII, 2018).

Moreover, using less ecologically affected fertilizers, such as humic compounds, during agricultural activities might result in better agricultural productivity with better quality. According to Calvo et al. (2014), humic compounds can be labeled as "biostimulants" (even though there is no legal definition for the term in Brazil) of plants for their growth-promoting activity owing to enlarged roots and improved nutrient absorption. These compounds are formed of humic acids, fulvic acids, and humin, which are formed by biochemical conversions of organic matter constituents in the soil such as lignin, cellulose, hemicellulose, sugars, and amino acids (Primo et al., 2011).

Humic acid treatment in the soil is boosted photosynthesis (Baldotto et al., 2009; Ertani et al., 2011), respiration, protein synthesis (Nardi et al., 2002) and enzymatic activities (Nardi et al., 2007) in various cultivars. In the plenty of cultivars, foliar treatment of humic acid promoted increases in the content of sugar (Lima et al., 2011), carbohydrates (Aminifard et al., 2012), and starch (Canellas et al., 2002; Ertani et al., 2011; Nardi et al., 2007). Studies with humic substances in onion have shown all the aforementioned beneficial effects that these substances can promote (Bettoni et al., 2014, 2016 and 2017).

To the best of our knowledge, there are no reports on the use of fertilizers, which contain humic substances applied to the soil for hybrid tomato cultivation. Also, its application as biostimulants on tomato seedlings' adequate time period is not well described for improving vegetative development and future output with the best concentration of it. Therefore, the aim of this work was to assess the impact of humic acid based substances soil biostimulant, in the vegetative development of Justyne® hybrid tomato's grown in the greenhouse.

Material and Methods

The experiment was conducted in a greenhouse at the Tuiuti do Paraná University, located in the city of Curitiba, Paraná and its design was completely randomized with a two-factor factorial analyses (4 doses: 0 (control), 1.5, 2 and 2.5 mL L^{-1} of Bio Humate®) with 2 sampling stages (42 and 56 DAT)) treated to the commercial soil biostimulant of Bio Humate® to three replicates of Justyne® hybrid tomatoes during two sampling stages with application on every 14 days, starting at 7 DAT.

Humic acid based soil biostimulant was applied as Bio Humate® (MAP No SP-80819 10067-4) that contains (weight / weight with a density of 1.20 g mL^{-1}): 6.5% of total organic carbon; 0.15% N; 5.0% K_2O and 0.05% P_2O_5 including 36% of humic substances, derived from leonardite, being 24% of humic acids and 12% of fulvic acids.

When the seedlings have 4 definite full expanded leaves (approximately 25 days after planting), the plants were transplanted in to 5 L plastic pots containing a commercial substrate of Tropstrato HT Hortaliças including pine bark, vermiculite, PG mix 14.16.18, potassium nitrate, simple superphosphate and peat (Vida Verde Indústria e Comércio de Insumos Orgânicos Eireli) and irrigated at field capacity, without sticking to the leaves, in the amount of 150 mL per pot of the dose directly applied to the each seedlings root with a syringe, every 14 days, the first application being 7 days after transplantation (DAT), on April 12, 2019, with a total of four applications (7, 21, 35 and 49 DAT).

For evaluations, the central plant of each repetition was considered. In 42 DAT (A1) and 56 DAT (A2), the following biometric variables were evaluated: number of leaves; root length; height of the aerial part, height of 1st node to 1st collar (height starting from 1st leaf until nodal roots); fresh weight (FW) of leaf, FW of stem, FW of aerial part and FW of root, root volume, leaf are, the dry weight (DW) of leaves, DW of stem, DW of aerial part and DW of root. The biochemical variables analyzed were: chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), chlorophyll a+b (Chl *a+b*) and carotenoids.

For the determination of the root length and height of the aerial part, a graduated ruler was used, with the root length obtained from the mean of the three largest roots per plant, and height of the aerial part, considered as the distance from the base to the apical part. Height of 1st node to 1st collar was measured in the mediated region of the plant, with a digital vernier. The root volume was determined by measuring the volume of water displaced by introducing them into test tubes with a known volume of water. The FW and DW were made by weighing the samples on an analytical balance. For the dry matter production determinations, the parts of the plant, separated and packed in paper bags, were located in a stove with air circulation at 80 ºC until reaching constant weight.

The determination of the leaf area (cm^2 plant^{-1}) was obtained by free image analysis software (ImageJ®), coupled to a scanner.

For biochemical variables, the most developed middle 3rd shoot at the time of evaluation was selected for measurements, with the 4th shoot at 42 DAT and the 6th shoot at 56 DAT. These leaves were cut into discs and weighed on a precision balance, being immediately frozen at -20 °C. Subsequently, 1 cm^2 leaf discs were taken, weighted in order to obtain FW of each discs and extracted in 5 mL of ethanol 95% in 80 °C hot bath. The absorbance from the extraction was measured at 665, 649 and 470 nm. The content of Chl *a*, Chl *b*, Chl *a+b* and carotenoids concentration were performed, according to the Lichtenthaler methodology (1987) as followings:

(I) Chl a = (13.7 x Δ_{665}) – (5.76 x Δ_{649})

(II) Chl b = (25.8 x Δ_{649}) – (7.6 x Δ_{665})

(III) Chl $a+b$ = (6.1 x Δ_{665}) – (20.04 x Δ_{649})

(IV) Carotenoids = [(1000 x Δ_{470}) – (2.13 x Chl a) – (97.64 x Chl b)]

Results and Discussion

The analysis of variance showed a significant influenced of the doses of the commercial product Bio Humate® for all the variables analysed.

Regression analysis (Figure 1) for biometric variables: number of leaves (Figure 1A), length (Figure 1D) and root volume (Figure 1B), as well as fresh leaf and root mass (Figure 1C) of plants in the function of the doses of Bio Humate®, indicated a quadratic tendency.

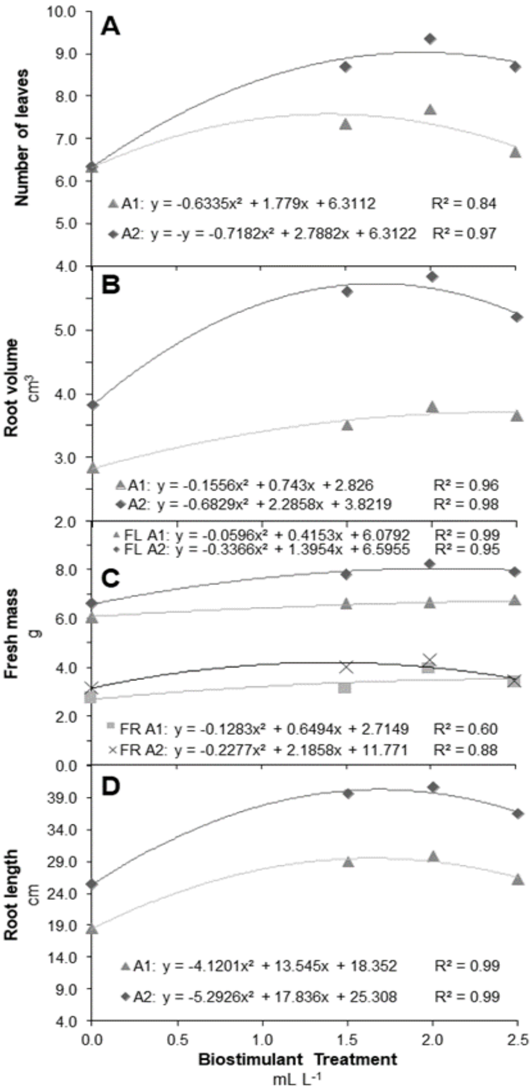

Figure 1. Number of leaves (A), root volume (B), fresh mass of leaves (FL) and roots (FR) (C) and root length (D) in Justyne® hybrid tomato seedlings treated with biostimulant of Humate® at 42 (A1) and 56 (A2) DAT

The highest number of leaves (Figure 1A) was found at a dose of 2.0 mL L^{-1} in both evaluations (A1 and A2), with 6.67 and 8.67 average number of leaves respectively. Root length (Figure 1D) showed better results at the dose of 2.0 mL L^{-1} in both evaluations, with 29.68 cm in A1 and 40.69 cm in A2, while the root volume (Figure 1B) expressed its best development at the dose of 2.0 mL L^{-1} in to the A1 and A2, 3.80 cm^3 (A1) and 5.83 cm^3 (A2), respectively.

The quadratic result observed in fresh leaf mass (Figure 1C) indicates that, up to the dose of

2.5 mL L^{-1} in A1 and 2.0 mL L^{-1} in A2, the application of the commercial product Bio Humate® implies an increase in the variable, with a maximum dose of 6.76 g and 8.26 g in A1 and A2, respectively. Similar results were found by Vieira et al. (2018), where the mean FW for the control was 39% lower than that observed for the highest tested dose, 6.0 mL L^{-1}. The fresh root mass obtained better results at the dose of 2.5 mL L^{-1} in both evaluations, with maximum doses in A1 of 3.37 g and A2 of 3.44 g.The observed increases in length, volume, and mass of fresh root observed relative to control may be explained by a likely similar action of growth hormones and cell division mechanisms, which increase the number of lateral roots that emerged, in addition to proliferation of root hair promoted by an action of auxin (Canellas and Olivares, 2014). Such an increase in the root system allows the plant to be able to explore a greater volume of soil and, consequently, improve its absorption of nutrients.

The same quadratic tendency was observed in the root and dry mass variables of the stem (Figure 2A). DW of root and DW of stem had better results at the dose of 2.0 mL L^{-1} in both A1 and A2.

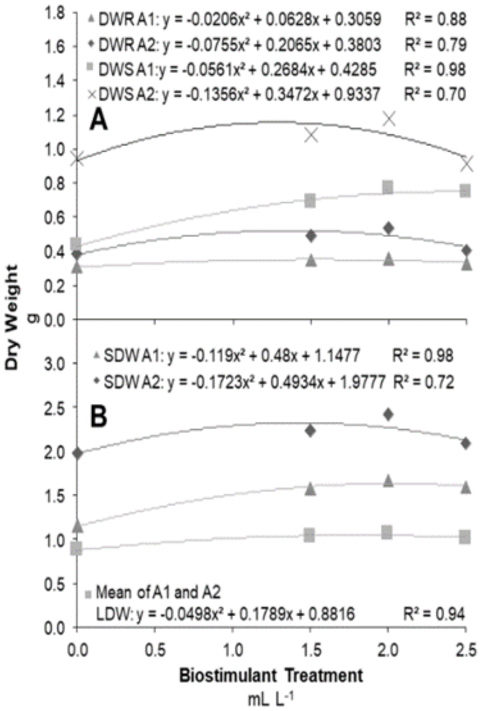

Figure 2. Dry weight of root (DWR; Dry weight of root) (A), stem (DWS; Dry weight of stem) (A), shoot (SDW; Shoot dry weight) (B) and leaves (LDW; Leaf dry weight) (B) in Justyne® hybrid tomato seedlings treated with biostimulant of Humate® at 42 (A1) and 56 (A2) DAT

The dry firing mass (Figure 2B) showed higher results in A1 and A2 at doses of 2.0 mL L^{-1}, respectively. For the variable dry mass of leaves, no significant interaction was observed between the doses and the evaluation time, only between the stations alone, and the dose of 2.0 ml L^{-1} presented the highest value (Figure 2B).

Regression analysis of shoot height (Figure 3A), height of 1st node to 1st collar (Figure 3B), as well as leaf area (Figure 3C) and fresh stem and shoot masses (Figure 3D) indicated linear tendency, where the increases in the parameters respond to the increase in the doses of the product.

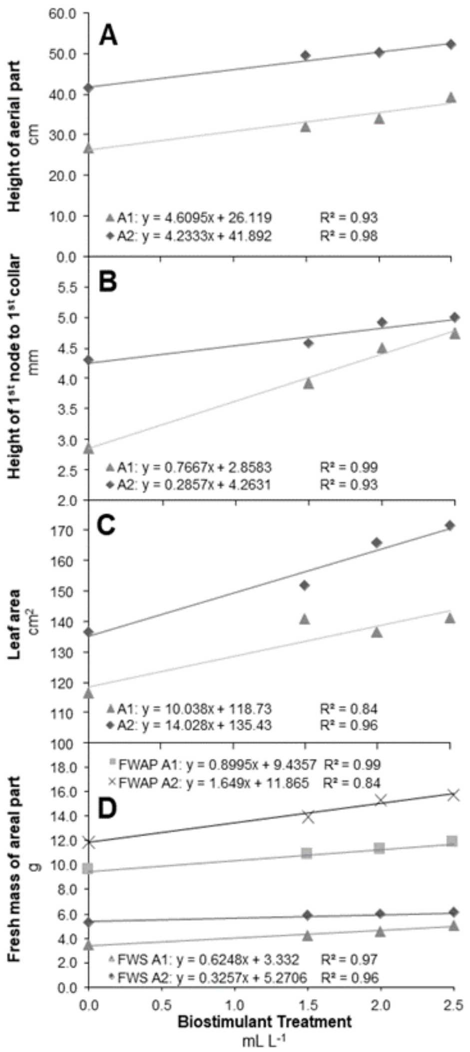

Figure 3. Height of aerial part (A), height of 1st node to 1st collar (B), leaf area (C) and fresh mass of areal part (FWAP; Fresh weight of aerial part, FWS; Fresh weight of stem) (D) in Justyne® hybrid tomato seedlings treated with biostimulant of Humate® at 42 (A1) and 56 (A2) DAT

These results demonstrate the positive effect of the product on the biometric parameters related to the aerial part of the plant, and for these variables the ideal recommended dose would be 2.5 mL of L^{-1}, and the maximum doses found for the variables are 39.27 cm (A1) and 52.10 cm (A2) for height of the aerial part (Figure 3A); 4.73 mm (A1) and 5.00 mm (A2) for height of 1st node to 1st collar (Figure 3B); 141.31 cm^2 (A1) and 171.43 cm^2 g (A2) for leaf area (Figure 3C) and 11.77 g (A1) and 15.70 g (A2) for FW of aerial part (Figure 3D).

Bernardes et al. (2011), found an increase in the dry mass and sprout height of tomato seedlings using the product Codahumus 20®, also based on humic substances. Rosa et al., (2009) observed the positive impact of the doses of humic substances on the dry mass of the part of the zone of bean plants. The results obtained by Vieira et al. (2018) also demonstrated a directly proportional increase in plant height concerning to the dose of humic substances applied to lettuce seedlings.
According to Hernandez et al. (2015), plants subjected to the application of humic substances present higher productivity responses related to the increase in chlorophyll content, ribulose activity 1-5 bisphosphate carbilase, stomach conductivity, accumulation of macro and micronutrients in the leaves and changes in carbohydrate metabolism. Corroborating their experiment, it is possible to observe increases in the content of Chl *a*, Chl *b*, Chl *a+b* and carotenoids (Figure 4A) at doses of 2.0 mL L^{-1} in both evaluations (A1 and A2).

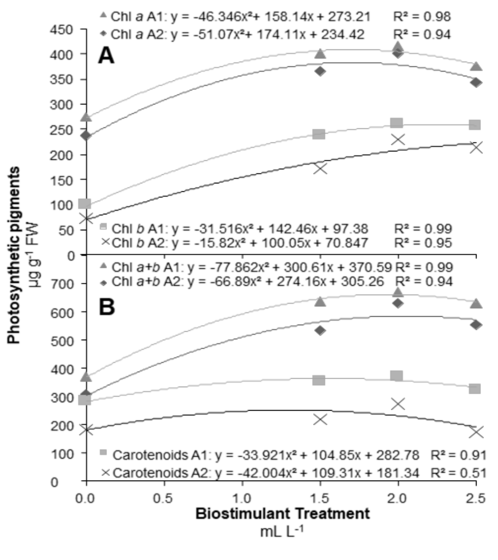

Figure 4. Photosynthetic pigments concentration of Chl *a* and Chl *b* (A), and Chl *a+b* and Carotenoids (B) in Justyne® hybrid tomato seedlings treated with biostimulant of Humate® at 42 (A1) and 56 (A2) DAT

Increases in the content of photosynthetic pigments in different cultivated plants have already been reported from the application of different organic materials (Ferrara and Brunetti, 2008; Fan et al., 2014). Rosa et al., (2009) observed increases in the Chl *a* / Chl *b* ratio after treating pineapple plants with humic acids. The application of solutions containing humic acid allows a more orderly stacking of the thylakoids , favouring the absorption of photons by chlorophylls, the transfer and conversion of light energy into a power reducer (NADPH) and energy (ATP), enhancing photosynthesis in higher vegetables (Fan et al., 2014). Furthermore, active compounds similar to plant hormones present in water soluble humic acid can increase acidification of the rhizospheric region, due to the activation of the enzyme H+-ATPase of the plasma membrane, increasing the absorption capacity of NH_4^+ and NO_3^- (Zandonadi et al., 2007; Canellas and Olivares, 2014). This would result in the synthesis of N^- containing molecules, such as chlorophylls.

Thus, it is possible to perceive that the use of the Bio Humate®, in the vegetative phase, of the Hybrid Tomato Justyne® promoted significant inputs in the biometric and biochemical parameters evaluated, 2 mL L^{-1} of the dose is the most sensitive for most of the analyses. For larger doses, in general, there was a visual phytotoxic tendency of the product such as yellowing leaf, necrosis, brownish.

Conclusions

The vegetative phases of the seedlings have a critical importance for the final product. In this period, seedlings are required an intensive care with additional soil treatment. While the seedlings were growth in the better conditions, the final products are more promising to reach the required yield and quality. Thus, the biostimulant applications in this phase has a crucial impact for nurseries who are producing the seedlings for farmers. The application via soil, of the Bio Humate® fertilizer, the base of humic substances, had a positive impact on the Justyne® hybrid tomato's seedlings development with interaction for all the biometric and biochemical parameters evaluated, except the mass fresh from the leaf. The parameters of height and fresh mass of stem, height of 1st node to 1st collar and fresh mass of the area, as well as the area of the leaf presented linear tendency as a function of the doses, having their values increased directly proportional to the increase in the doses applied. However, for all the other variables evaluated in this experiment, the regression analysis showed that the best dose for the Justyne® hybrid tomato's seedlings development, carried out in the greenhouse, is the dose of 2 mL L^{-1} of Bio Humate® fertilizer applied via soil.

Conflict of Interest Statement: The authors of the article declare that there is no conflict of interest.

Authors' Contribution: B.A.V.: investigation, formal analysis, and writing. M.M.B.: conceptualization, methodology, validation, resources, writing, reviewing and editing, visualization, supervision. conceptualization, methodology, validation, formal analysis, writing, reviewing and editing, visualization. T.K.: conceptualization, methodology, validation, writing, reviewing and editing. All the authors approved the paper for publication

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