

APPLICATION METHODS OF BIOSTIMULANTS AFFECT THE  
PRODUCTION AND POSTHARVEST CONSERVATION OF  
YELLOW MELON

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**Abstract**

Worldwide, Brazil holds the fifth position in melon fruits exportation, further expanding its products to provide for the growing demand. This expansion is the result of the development and application of new technologies, including the management of the use of biostimulants. However, for melon crops, the information in the literature on the use of biostimulants remains limited to the effects of different doses on fruit quality at the time of harvest. Therefore, this study aimed to evaluate the influence of different methods of pre-harvest application of two biostimulants on the production and postharvest conservation of fruits of yellow melon cv. Iracema. The treatments consisted of a combination of three factors: two plant biostimulants (Crop Set<sup>®</sup> and Spray Dunger<sup>®</sup>), two application methods of the products (fertigation and spraying), and five times of postharvest storage (0, 14, 21, 28 and 35 days). An additional control treatment corresponded to plants without biostimulant application. The fruits were evaluated for production and physicochemical attributes: average mass, yield, flesh firmness, titratable acidity, soluble solids content, SSC/TA ratio, pH, total soluble sugars, and weight loss. Fertigation is the recommended application method of biostimulants for yellow melon due to its effect on the increase of average mass, yield, flesh firmness, soluble solids content, and total soluble sugars of the fruits in relation to the spraying method.

**Keywords:** *Cucumis melo* L. Plant growth regulators. Postharvest quality. Storage conditions.

**1. Introduction**

Melon (*Cucumis melo* L.) is a vegetable belonging to the Cucurbitaceae family that is greatly appreciated and economically expressive, with a world production of 27,349,214 tonnes in 2018 (FAO 2020). Currently, melon is among the twenty Brazilian crops with the highest export volume, demonstrating its importance for agriculture of this country. The adoption of product's technologies has contributed to increasing the production of the fruits; however, to improve the yield and quality of fruits, it is important to test it before.

In Brazil, yellow melon is the group with the highest participation in the production and export of melons because of its highest postharvest conservation (Nunes et al. 2011). Fruits of these group are available for consumption when they reach a soluble solids content higher than 9° Brix (Lobo et al. 2019)

and have a shelf life greater than 30 days when stored at 10 °C (Tomaz et al. 2009). In order to extend the shelf life of the fruits to reach distant markets (Paiva et al. 2020), it is important to study their response to the adoption of new technologies during plant growth and development.

Between the crop management techniques for melon culture, which have been studied, are the use of plant biostimulants. Biostimulants are defined as mixtures of plant growth regulators and other compounds that are applied to plants to increase their nutritional efficiency, abiotic stress tolerance, and quality traits (Jardin 2015).

The efficiency and the advantages of biostimulants application have already been verified in research with different crop species including melon fruits (Aroucha et al. 2018; Vendruscolo et al. 2017; Torres Junior et al. 2017). These products improve the plant growth and development, stimulate cell division, and cell elongation, and improve the absorption of nutrients and water by plants (Calvo et al. 2014), and are a potential alternative to the melon growers.

Among plant biostimulants used by Brazilian growers in fruits and vegetables are Crop Set® and Dunger Spray®. Crop Set® is a vegetable biostimulant composed of *Yuccas chidigera* extracts with cytokinin-like action (Leão et al. 2005) and minerals (manganese, iron, and copper), as pointed out by the manufacturer (Improcrop-Kentucky-USA). Spray Dunger® is composed by auxin ( $\alpha$ -naphthaleneacetic acid), gibberellic acid (GA<sub>3</sub>), and minerals (nitrogen, potassium, and phosphorus), according to the manufacturer (Biolchim SPA-Italy).

For the application of biostimulants, spraying and fertigation are some of the application methods available. In foliar spraying, the absorption of nutrients present in the product occurs by diffusion through the leaf cuticle (Taiz and Zeiger 2014). On the other hand, in the fertigation system, diluted biostimulants in water are applied to infiltrate the soil, with root absorption predominating. This method, however, is less used than foliar application.

Despite the benefits of biostimulants on the fruit quality, studies on these products only inform their effect on the quality of fruits at harvest, when applied with spraying method in different doses. Thus, an investigation about the methods of application of a biostimulant or its influence on the postharvest conservation of the fruits are necessary to improve the effectiveness of these products.

In this context, this study aimed to evaluate the influence of different methods of pre-harvest application of two biostimulants on the production and postharvest conservation of yellow melon cv. Iracema.

## 2. Material and Methods

The experiment was carried out at Jardim farm, located in Mossoró, Rio Grande do Norte state, Brazil (4° 39'39" S, 37° 23'13" W; at an altitude of 51 m above sea level). The climate condition of this region is BSw<sub>h</sub>' (Köppen), which corresponds to hot and dry climate, with fairly irregular rainfall (annual average of 673.9 mm), temperature of 27 °C, and relative humidity of 68.9%.

The experiment was set up in a completely randomized split-plot (2 x 2 x 5) design with an additional treatment, with four replications of two fruits per experimental unit. The plots consisted of two plant biostimulants (Crop Set® e Spray Dunger®), two application methods of the products (fertigation and spraying), and five postharvest storage times (0, 14, 21, 28, and 35 days after harvest). An additional control treatment corresponded to plants without biostimulant application. Fruits of yellow melon (*Cucumis melo* L. var *inodorus* Naud.) cv. Iracema were used in the experiment.

Both commercial products were applied on two different days. Crop Set® was applied at 15 and 28 days after transplanting (DAT) and Spray Dunger® at 18 and 30 DAT. The spray was applied with a 20 L backpack sprayer, and fertigation through the addition of the product in irrigation lines. In each application day, Crop Set® and Spray Dunger® were sprayed using 1041 mL ha<sup>-1</sup> and 2 kg ha<sup>-1</sup>, respectively; and in the fertigation application, we used 400 mL ha<sup>-1</sup> and 1.1 kg ha<sup>-1</sup>, respectively. Days of application and doses were based on the manufacturer's recommendations.

The harvest was done at 65 DAT, at the maturity stage. Part of the fruits was characterized previously by sampling four replicates of two fruits per experimental unit and the other parts were stored in a cold room at 10 ± 2 °C and RH 80 ± 2%, for 14, 21, 28, and 35 days.

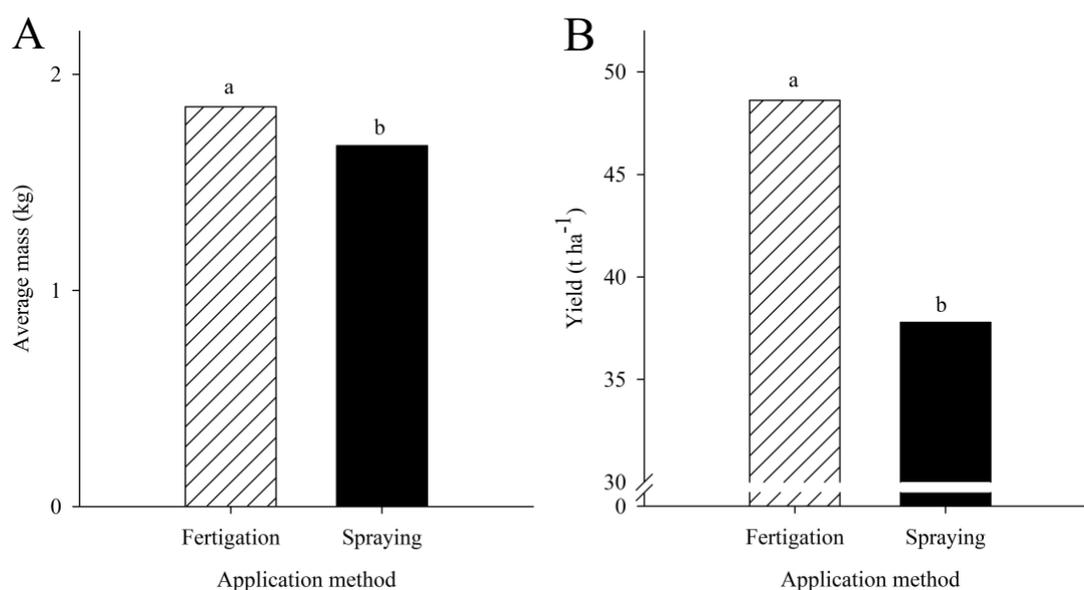
After harvest (day zero), the fruits were evaluated for production attributes: average mass (kg) and yield ( $\text{t ha}^{-1}$ ). After harvest and each storage time, the fruits were evaluated for quality attributes: flesh firmness, determined using a manual penetrometer (model FT 327, McCormick, USA), with values expressed in N; titratable acidity (TA), evaluated by titrimetry and expressed in g of citric acid  $100 \text{ g}^{-1}$  of pulp sample; soluble solids content (SSC), determined with a refractometer (model PR-100 Palette, AtagoCo. Ltd., Japan), with results in  $^{\circ}$  Brix; SSC/TA ratio; pH; total soluble sugars (TSS), determined by the Antrona method (Yemn and Willis 1954), expressed in %; and weight loss, determined by the difference between the mass of fruits after harvest and after each storage time, expressed in %.

Data were subjected to analysis of variance, and the means of the treatments with application of biostimulants were compared by the Tukey test ( $p \leq 0.05$ ). The control treatment was compared to the others using the Dunnett test ( $p \leq 0.05$ ). Regression analysis was applied for storage time. All analyses were carried out using statistical software Sisvar version 5.6 (Ferreira 2014).

### 3. Results and Discussion

For production attributes, the effect of the application method was observed on the average mass and yield of fruits (Figure 1). On the other hand, for quality attributes, there was a significant interaction effect between the three factors (plant biostimulant, application method, and storage time) on the flesh firmness (Table 1), TA (Table 2), pH (Table 3), and SSC/TA ratio (Table 4) of the fruits. The interaction between plant biostimulant and application method was significant on TSS (Figure 3A). The application method had an isolated effect on SSC (Figure 2A) and the storage time had an isolated effect on SSC (Figure 2B), TSS (Figure 3B), and weight loss (Figure 4).

Fertigation increased the average mass of fruits and yield in 9.7% (Figure 1A) and 22.3% (Figure 1B), respectively, when compared to the biostimulant application by spraying. This result is attributed to the higher radicular absorption of biostimulant by fertigation, that occurs via bulk flow, while in spraying it occurs by diffusion. Besides that, fruits from both treatments are adequate for the market, which allows for fruit weight varying from 0.9 to 2.7 kg (Calixto et al. 2019). Despite that fertigation showed the highest yield, in both application methods, the yield was superior to  $22 \text{ t ha}^{-1}$ , appointed by Figueiredo et al. (2017) as the average yield of yellow melons.



**Figure 1.** A – Average mass of yellow melon depending on application methods (fertigation and spraying); B – Yield of yellow melon depending on application methods (fertigation and spraying). Data represent mean values. Significant differences by Tukey test ( $p \leq 0.05$ ) are indicated by different letters.

The fruits from all the treatments decreased in flesh firmness throughout the storage time (Table 1). Although the pulp firmness of the control treatment did not differ from the firmness obtained with the application of biostimulants, the reduction in the firmness of the control fruits (52.7%) was greater than

those fertigated with Spray Dunger® (44.7%) and Crop Set® (46.3%). At 35 days, it was observed that the fruits from plants treated with Spray Dunger® by fertigation had higher flesh firmness than fruits from plants where Spray Dunger® was applied by spraying (Table 1).

Pre-harvest application of gibberellic acid, which is found in the formulation of Spray Dunger® in its GA<sub>3</sub> form, can increase the number of cells in the fruits and their cell wall/cell volume ratio, besides partially inhibiting the action of ethylene, which slows up the decrease in flesh firmness of fruits (Brackmann et al. 2002; Thompson 2014). Ouzounidou et al. (2008) also verified that gibberellic acid application caused a decrease in 37% on the respiration rate of the fruits, causing a reduction in the enzymatic activities related to flesh firmness. Thus, the shorter time of absorption of biostimulant when it is applied by fertigation provides higher modifications on the cell wall and consequently delays the loss of flesh firmness of fruits. In fertigation, the absorption of nutrients occurs by mass flow, a process known to be faster than diffusion, which is the form of absorption for the spraying method (Taiz and Zeiger 2014). Kohatsu et al. (2012) verified that the application of gibberellic acid reduced the loss of flesh firmness of cantaloupe melon fruits.

**Table 1.** Flesh firmness (N) of yellow melon depending on plant biostimulants (Crop Set® and Spray Dunger®), application methods (fertigation and spraying) and storage time.

Storage (days)	Control	Spray Dunger®		Crop Set®	
		Fertigation	Spraying	Fertigation	Spraying
0	23.76	23.94 <sup>Aaa</sup>	24.70 <sup>Aaa</sup>	23.32 <sup>Aaa</sup>	24.40 <sup>Aaa</sup>
14	17.65	15.50 <sup>Baa</sup>	16.71 <sup>Baa</sup>	16.19 <sup>Baa</sup>	17.21 <sup>Baa</sup>
21	15.88	14.29 <sup>Bab</sup>	16.02 <sup>Baa</sup>	16.29 <sup>Baa</sup>	13.86 <sup>Cbb</sup>
28	13.27	14.22 <sup>Baa</sup>	12.95 <sup>Ca</sup>	12.55 <sup>Ca</sup>	12.86 <sup>Ca</sup>
35	11.25	13.25 <sup>Baa</sup>	11.00 <sup>Cba</sup>	12.53 <sup>Ca</sup>	12.35 <sup>Ca</sup>

Means followed by the same letter do not differ by the Tukey test at  $p \leq 0.05$ . Uppercase letters compare the means within the column (storage days); bold lowercase letters compare application methods; and italic lowercase letters compare plant biostimulants. \*Means that differ significantly from the control treatment by the Dunnett test at  $p \leq 0.05$ .

At harvest (day zero), the fruits obtained from plants exposed to biostimulants, regardless of the form of application, showed higher titratable acidity (TA) than the control treatment, except for the fruits of plants fertigated with Crop Set®, which showed TA statistically equal to the control fruits. On the other hand, during storage, the acidity of the control fruits differed only from the spray treatment of Spray Dunger®. With the exception of Crop Set® applied by fertigation whose organic acid content reached a peak at 14 days, in the other treatments with biostimulant there was a decrease in TA during storage (Table 2). This decrease could be explained by the consumption of organic acids during the respiratory process of fruits, or its conversion into sugars (Tang et al. 2010). Morgado et al. (2015) also observed this effect in 'Louis' melons stored under four different temperatures (3, 6, 9, and 22 °C).

**Table 2.** TA (g of citric acid 100 g<sup>-1</sup> of pulp sample) of yellow melon depending on plant biostimulants (Crop Set® and Spray Dunger®), application methods (fertigation and spraying) and storage time.

Storage (days)	Control	Spray Dunger®		Crop Set®	
		Fertigation	Spraying	Fertigation	Spraying
0	0.095	0.175 <sup>Aaa+</sup>	0.164 <sup>Aaa+</sup>	0.089 <sup>Cbb</sup>	0.172 <sup>Aaa+</sup>
14	0.173	0.154 <sup>Abb</sup>	0.180 <sup>Aaa</sup>	0.182 <sup>Aaa</sup>	0.172 <sup>Aaa</sup>
21	0.124	0.126 <sup>Baa</sup>	0.114 <sup>Baa</sup>	0.124 <sup>Baa</sup>	0.128 <sup>Baa</sup>
28	0.110	0.126 <sup>Baa</sup>	0.125 <sup>Baa</sup>	0.110 <sup>BCaa</sup>	0.129 <sup>Baa</sup>
35	0.120	0.104 <sup>Baa</sup>	0.084 <sup>Cbb+</sup>	0.118 <sup>Baa</sup>	0.112 <sup>Baa</sup>

Means followed by the same letter do not differ by the Tukey test at  $p \leq 0.05$ . Uppercase letters compare the means within the column (storage days); bold lowercase letters compare application methods; and italic lowercase letters compare plant biostimulants. \*Means that differ significantly from the control treatment by the Dunnett test at  $p \leq 0.05$ .

About the application method of biostimulants, we observed a variation in the TA of fruits from plants treated with Spray Dunger®. Specifically, the TA was higher with the spraying method at 14 days of storage and lower at the last day of storage (Table 2). For Crop Set®, the effect of the application method was only

observed at harvest, when spraying increased the TA of fruits (Table 2). When comparing two products, Crop Set® decreased TA at day 0 when it was applied by fertigation, while Spray Dunger® decreased the fruit acidity at 14 and 35 days of storage when its application was made by fertigation and spraying, respectively (Table 2). The metabolism and morphogenesis of plants can be influenced by the biostimulant application (Hirose et al. 2008), altering the respiration of the fruits and its acidity.

Contrasting the effect of the application of the biostimulants, regardless of the application method, with the control treatment, slight differences were observed for the pH of the fruits (Table 3). Over storage, small variations were detected in the pH of fruits, but when comparing the harvest and the last day of storage, no differences were observed for all products and their application methods (Table 3). Due to the buffer capacity of some fruits, their pH stabilised even when the decrease of TA was high. Working with intact and fresh-cut melon fruits, Supapvanich et al. (2011) found constancy in the pH of fruits during storage.

The spraying method decreased the pH of fruits at 14 and 28 days when applying Crop Set® and Spray Dunger®, respectively (Table 3). This result contradicts those found by Pinto et al. (2008) that did not observe any effect of spraying biofertiliser on the pH of yellow melons. At 14 days of storage, for the spray application method, there was a difference between the biostimulants on the pH of the fruits, with the values obtained with Crop Set® slightly lower than those by Spray Dunger®. When evaluating the influence of Crop Set® application on the pH of melons of two cultivars, Aroucha et al. (2018) observed that the biostimulant increased the pH of 'Goldex' fruits, while 'Iracema' melons were not affected by this product.

**Table 3.** pH of yellow melon depending on plant biostimulants (Crop Set® and Spray Dunger®), application methods (fertigation and spraying) and storage time.

Storage (days)	Control	Spray Dunger®		Crop Set®	
		Fertigation	Spraying	Fertigation	Spraying
0	5.66	5.57 <sup>Aaa</sup>	5.58 <sup>Aaa</sup>	5.62 <sup>Aaa</sup>	5.56 <sup>Aaa+</sup>
14	5.47	5.50 <sup>Aaa</sup>	5.57 <sup>Aaa</sup>	5.60 <sup>Aaa</sup>	5.42 <sup>ABbb</sup>
21	5.52	5.53 <sup>Aaa</sup>	5.53 <sup>Aaa</sup>	5.50 <sup>ABaa</sup>	5.51 <sup>Aaa</sup>
28	5.40	5.44 <sup>Aaa</sup>	5.30 <sup>Bba</sup>	5.40 <sup>Baa</sup>	5.35 <sup>Baa</sup>
35	5.61	5.55 <sup>Aaa</sup>	5.48 <sup>Aaa+</sup>	5.55 <sup>Aaa</sup>	5.57 <sup>Aaa</sup>

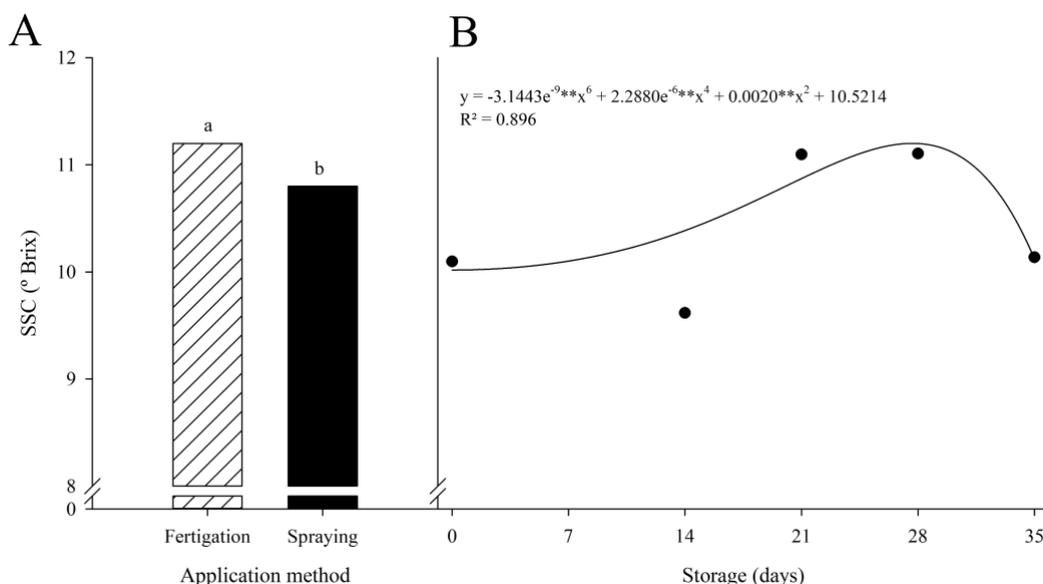
Means followed by the same letter do not differ by the Tukey test at  $p \leq 0.05$ . Uppercase letters compare the means within the column (storage days); bold lowercase letters compare application methods; and italic lowercase letters compare plant biostimulants. \*Means that differ significantly from the control treatment by the Dunnett test at  $p \leq 0.05$ .

The highest soluble solids content (SSC) was observed in fruits that received the application of biostimulants by fertigation (Figure 2A). This effect can be attributed to the higher balance on the supply of macro and micronutrients and hormones provided by fertigation, by the fact that, this application method results in higher and faster assimilation of the biostimulant by melon plants. When applied by fertigation, the commercial product Citogrower®, a cytokinin-based biostimulant, increased the SSC of hybrid melon fruits (Torres Junior et al. 2017). Vendruscolo et al. (2017) studied the effect of biostimulant Stimulate®, composed of cytokinin, gibberellic acid and auxin, and found positive effects of its application on the SSC of melon fruits.

In melon, the CPPU [1-(2-chloro-4-pyridyl)-3-phenylurea], a cytokinin-based product, is usually used for the production of parthenocarpic fruits. Its application can increase the fruit set and growth (Hayata et al. 2001), which can affect some metabolism processes of the fruits, including sugar accumulation. According to Huitrón et al. (2004) high CPPU concentrations can decrease the SSC of fruits, while Hayata et al. (2000) and Hayata et al. (2001) did not find an effect of CPPU application on the SSC of melons. Meanwhile, studies that evaluate the effect of gibberellic acids or auxins on the physicochemical quality of melons are scarce.

According to the adjusted regression model, the accumulation of soluble solids was slow at the beginning of storage, followed by a rapid accumulation until reaching a peak at 27.66 days with a value of 11.99° Brix. From that storage time, the SSC reduced to 10.65° Brix at 35 days (Figure 2). Besides this variation, the average SSC of fruits stayed above 10° Brix during storage, which is the recommended minimum value for melon fruits to be available for commercialization (Ferrante et al. 2008). The slight

increase on SSC of fruits over storage may be associated with the hydrolysis of the cell wall since melon fruits do not have reserves of starch to convert into sugars during the postharvest period (Li 2019).



**Figure 2.** A – SSC of yellow melon depending on application methods (fertigation and spraying); B – SSC of yellow melon depending on storage time. Data represent mean values. Significant differences by Tukey test ( $p \leq 0.05$ ) are indicated by different letters on the bar chart. \* and \*\* represent the significance of the parameters of the regression model at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

The SSC/TA ratio of the fruits varied over storage, with an increase comparing 0 and 35 days for all the treatments, except for the control treatment and Crop Set® applied by fertigation, which showed the inverse effect (Table 4). The increase on SSC/TA ratio of the fruits is attributed to the simultaneous effect of elevation of SSC on the reduction of TA during storage, being a parameter that indicates the flavour of the fruits. SSC/TA values at the harvest are similar to those found by Pereira et al. (2010) when evaluating yellow melons.

In the comparison of application methods, it was verified that the fertigation of Crop Set® was responsible for the highest SSC/TA values on harvest day (Table 4). In relation to the products, Crop Set® was superior to Spray Dunger® when applied by fertigation on harvest day, and inferior in the last day of storage, in both application methods (Table 4).

**Table 4.** SSC/TA ratio of yellow melon depending on plant biostimulants (Crop Set® and Spray Dunger®), application methods (fertigation and spraying) and storage time.

Storage (days)	Control	Spray Dunger®		Crop Set®	
		Fertigation	Spraying	Fertigation	Spraying
0	114.4	60.0 <sup>Bbb</sup>	64.1 <sup>Cba</sup>	123.1 <sup>Aaa</sup>	63.5 <sup>BCba</sup>
14	59.4	67.8 <sup>Baa</sup>	60.2 <sup>Ca</sup>	55.8 <sup>Ca</sup>	54.0 <sup>Ca</sup>
21	95.8	99.7 <sup>Aaa</sup>	97.6 <sup>Baa</sup>	101.3 <sup>ABaa</sup>	86.8 <sup>ABaa</sup>
28	110.0	99.8 <sup>Aaa</sup>	94.2 <sup>Baa</sup>	106.4 <sup>Aaa</sup>	98.8 <sup>Aaa</sup>
35	85.7	109.2 <sup>Aaa</sup>	125.2 <sup>Aaa</sup>	81.3 <sup>Bab</sup>	94.4 <sup>Aab</sup>

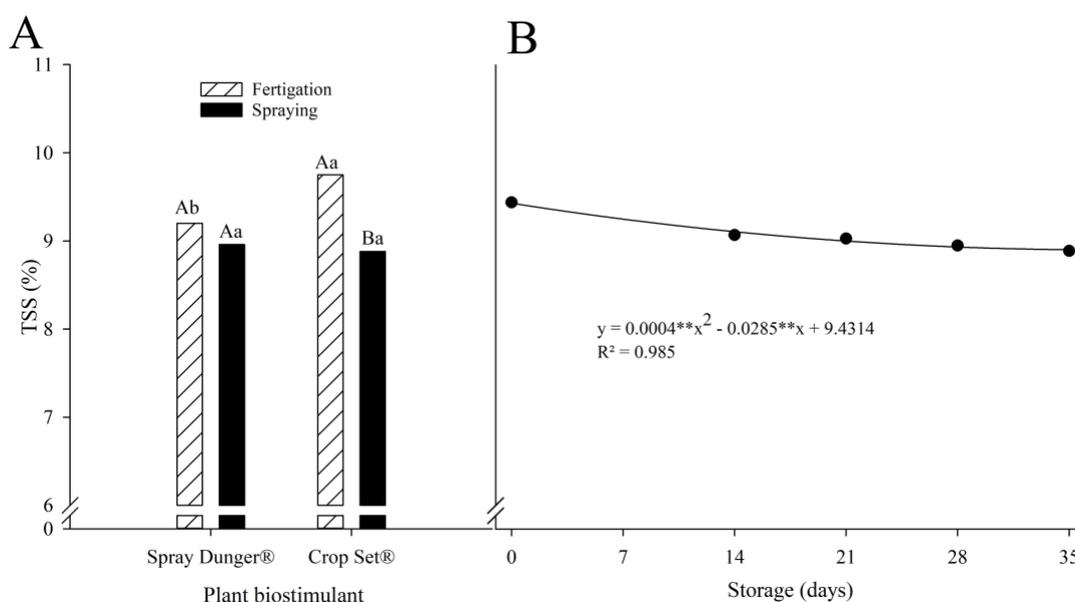
Means followed by the same letter do not differ by the Tukey test at  $p \leq 0.05$ . Uppercase letters compare the means within the column (storage days); bold lowercase letters compare application methods; and italic lowercase letters compare plant biostimulants. \*Means that differ significantly from the control treatment by the Dunnett test at  $p \leq 0.05$ .

Total soluble sugars (TSS) of the fruits of plants treated with Spray Danger® were not affected by the application method of the biostimulant. However, the application of Crop Set® via irrigation water increased TSS by 10% when compared to leaf application (Figure 3A). The application of plant growth regulators can alter the pattern of photoassimilates distribution inside the plant, which can also alter the accumulation of

carbohydrates (Calvo et al. 2014). Due to its faster absorption, fertigation increased the TSS of melon fruits that received Crop Set® application.

When comparing the two biostimulants, while the TSS did not differ between treatments with Spray Danger® and Crop Set® applied on leaves, in the application via fertigation, the Crop Set® yielded TSS values higher than that obtained on fruits of plants treated with Spray Danger® (Figure 3A). The cytokinin-based composition of Crop Set®, possibly, could be a characteristic that promotes a greater accumulation of sugars and consequently greater TSS in melon fruits due to the increase in cell size (Kano et al. 2005).

During storage, the fruits showed a slight decrease of 5.8% in their TSS (Figure 3B). This result can be attributed to the use of sugars on metabolic processes of the cells, beyond the fact that melon fruits do not have reserves of starch to convert into sugars over storage (Li 2019). Tomaz et al. (2009) observed a decrease of 13.2% on fruits of five yellow melon hybrids, higher than those found in this work.

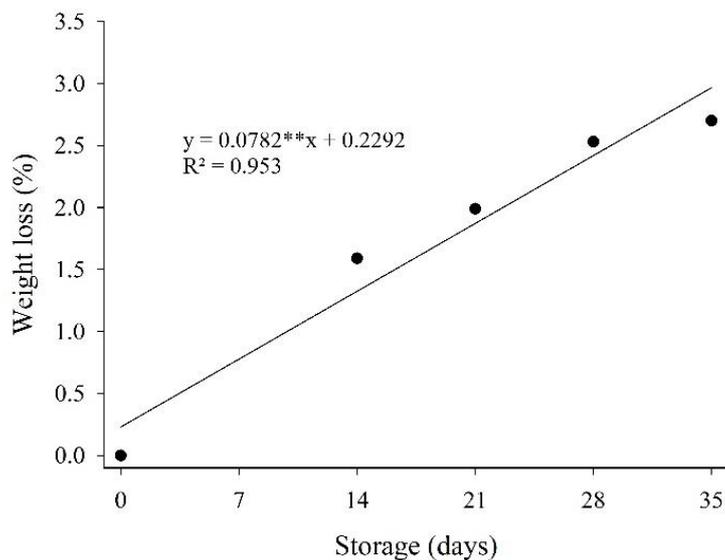


**Figure 3.** A – TSS (total soluble sugar) of yellow melon depending on plant biostimulants (Crop Set® and Spray Danger®); B – TSS of yellow melon depending on storage time. Data represent mean values.

Significant differences by Tukey test ( $p \leq 0.05$ ) are indicated by different letters on the bar chart.

Uppercase letters compare the means of application methods; lowercase letters compare plant biostimulants. \* and \*\* represent the significance of the parameters of the regression model at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

Regardless of the application method and type of biostimulant, the weight loss of the fruits increased linearly during storage at a rate of  $0.08\% \text{ day}^{-1}$ , reaching absolute values of 2.8% at 35 days. This weight loss value is considered low for storage conditions without the use of modified atmosphere. The weight loss, in this study, was lower than in yellow melon fruits of two cultivars, treated and untreated with Crop Set® reported by Aroucha et al. (2018). This physical parameter is very important from the economic point of view, since the commercialization of the fruits is determined according to its mass. The weight loss can still affect the external appearance of fruits, reducing its market value (Paiva et al. 2020).



**Figure 4.** Weight loss of yellow melon depending on storage time. \* and \*\* represent the significance of the parameters of the regression model at  $p \leq 0.05$  and  $p \leq 0.01$ , respectively.

#### 4. Conclusions

Fertigation is the recommended application method of biostimulants for yellow melon due to its effect on the increase of average mass, yield, flesh firmness, soluble solids content and total soluble sugars of the fruits in relation to spraying method.

**Authors' Contributions:** GÓES, G.B.: acquisition of data, analysis and interpretation of data; VILVERT, J.C.: analysis and interpretation of data, drafting the article; ARAÚJO, N.O.: analysis and interpretation of data, drafting the article; MEDEIROS, J.F.: conception and design; AROUCHA, E.M.M.: conception and design, critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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