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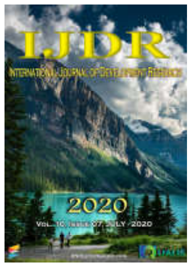

RESEARCH ARTICLE OPEN ACCESS

POSTHARVEST STORAGE OF ITALIAN TOMATOES USING *BACILLUS SUBTILIS*AND SALICYLIC ACID

*Darley Tiago Antunes, Marcio Roggia Zanuzo, Flávio Fernades Júnior, Santino Seabra Júnior, Marla Silva Diamante, Alessandro Bandeira Dalbianco and Maria Shirlyane Pereira do Nascimento

Postharvest storage of Italian tomatoes using *Bacillus subtilis* and Salicylic Acid

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Corresponding author: Darley Tiago Antunes

ABSTRACT

Tomato (Solanum lycoperscium) is one of the main vegetable crops and commonly grown around the world. The growing concern with food and environmental issues, as well as the rise of technologies with less environmental impact, makes it necessary to use bio-safe technological alternatives. Therefore, the objective of this study was to evaluate the application of Bacillus subtilis and Salicylic Acid in tomato post-harvest and their influence on the physical-chemical parameters of tomato fruits under cold storage. The work was conducted at the Biotechnology laboratory of the Federal University of Mato Grosso, Campus de Sinop, from 11/01/2019 to 11/22/2019. The parameters evaluated were: Percentage of mass loss, Total phenolic compounds, Lycopene, β-carotene, Total Soluble Solids, Determination of pH and Total Titratable Acidity. The application of Salicylic Acid and Bacillus subtilis did not promote significant changes in the values of Brix °, PH and ATT. Likewise, the AS and BS treatments did not influence the lycopene content. The carotenoid content was influenced by BS. The cultivar fascination was the one with the highest content of carotenoids and lycopene during storage.

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INTRODUCTION

Tomato *(Solanumlycoperscium)* is one of the leading olive crops and commonly cultivated worldwide. According to the FAO (Food and Agriculture, Organization),) approximately 45% of fresh food *in natura*is lost dueto infestation of pests and pathogens. In many cases, the control is carried out by the use of chemicals, but these can be harmful to human health and the environment (FAO, 2015; DROBY *et al*., 2016). The growing concern with food and environmental problems, as well as the rise of technologies with lower environmental impact, makes it necessary to use bio-safe technological alternatives in order to reduce post-harvest losses caused mainly by pathogens (Dimkpa *et al*., 2009). A possibility with biological emphasis is the use of bacteria, fungi and/or yeasts with physiological potential in growth, development and notthe protectivecharacter against pathogens. Among these microorganisms, growth-promoting bacteria (BPC's) have been standing out in this context, among them, *Bacillus subtilis*, *Streptomyceslydicus*, *Pseudomonas chlororaphis* and *Agrobacteriumradiobacter,* which are capable of establishing several physiological changes in the metabolism of the host plant, which can provide systemic resistance and prolonged shelf life without causing adverse effects on plants, humans or the environment (SARMA *et al*., 2012). BPC´s are beneficial non-pathogenic bacteria that can directly or indirectly promote resistance to diseases,and stress tolerance(SEIFI KALHOR *et al*., 2018)., e tolerância à estresse (They areto live freely in the rhizosphere, autonomously in the soil and in theplant phylosphere (Maksimov *et al*., 2015). One of the most widely used organismswithinBCP'sfor the development of biological plant protection products is *Bacillus subtilis,*besides being recognized as a safe microorganism for application inthe food industry (Maksimov *et al*., 2015). Several studies have shown the potential of active products of this bacteriumsuch as antibiotics, siderophores, lipopeptides, enzymes etc.Similarly, the genus *Bacillus* spp.sare able to modify phytomoniumbiosynthesis, act in the modulation of ethylene levels, positively influence nthe emission of volatile organic compounds andsystemic resistance/tolerance in the host, inaddition to the ability to positively influence resistance against fruit and vegetablepathogens in post-harvest, resulting in the extension of shelf life (Nagórska *et al*., 2007; García-

Gutiérrez *et al*., 2013; Arroyave-Toroa *et al*., 2017; Shafi & Tian, 2017;Lastochkina *et al*., 2019. Another product considered an alternative for thecontrol ofa maturation has been used, salicylic acid. This acid is a phytomonium regulating plant growth, as it provides a wide spectrum of metabolic and physiological reactions beneficial to the plant. It is a natural and safe phenolic compound, has great capacity to control losses in the post-harvest, mainly in horticultural crops, besides providing a control in the ripening process due to inhibition of ethylene biosynthesis (Asghari; Aghdam, 2010). The application of salicylic acid at a concentration of 14.48mMin tomato crop can prolong the shelf life of the fruit, without bringing risks to human consumption (BANDER *et al*., 2017).Other authors such as Mandal *et al*. (2018) have shown that the application of salicylic acid at a concentration of 0.4 mM via foliar?it can be an effective and viable alternative to prolong the shelf life of, tomatoes, corroborating the maintenance of the physicochemical qualities of the fruits during storage. Most studies have focused on the use of *Bacillus subtilis*and salicylic acid as inducers of resistance against pathogens in thepre-harvest, but its post-harvest application in tomato crop still lacks studies. Therefore, the objective of this study was to evaluate the application of *Bacillus subtilis* and Salicylic Acidin tomato postharvest and their influence on physicochemical parameters físico-químicos of tomato fruits under refrigerated storage.

MATERIALS AND METHODS

Plant material: The work was conducted in the Biotechnology Laboratory *Campus* of the Federal University of Mato Grosso, Sinop Campus, from 01/11/2019 to 11/22/2019. The tomato cultivars used in the assay were used two tomato cultivars in the assay, Fascínio® and Thaíse®,both of which were of determined growthhabit. The fruits were harvested in the *breaker stage* maturation phase, selected and standardized according to color,, uniformage, size and free of defects. They were then sanitized in sodium hypochlorite solution[100ppm] for 10 minutes.

Post-harvest treatment: *Bacillus subtilis* and salicylic acid: The tomatoes were separated into three groups, each of which received a postharvesttreatment, and among them, a control group. For the treatment with *Bacillus subtilis*, qst173 commercialstrain [2 x 10^{-8} CFU] were used as reference.A volume of 22 mL of the commercial product was diluted in 12 L ofdistilled water. .The salicylic acid solution was prepared at a concentration of 4 mM in 95% ethanol and diluted in 12L of distilled water. The control group consisted of distilled water destiladaonly.The tomato fruits were immersed for 15 minutes in the respective treatments, dried in the environment and then packed in low density polyethylene plastic packaging (EDP),perforated to allow gas exchange. In each package, 3 fruits were packed.Each package has been called an experimental unit. The samples were stored at ± 16 °C and ± 85% relative humidity for 7, 14 and 21 days. Each treatment was conducted with 3 repsandtições.Theanalyses consisted of physicochemical and biochemical analyses described as follows:

Percentage of mass loss: The mass loss was measured with data collection atthe time of fruit arrival inthelaboratory and before each round of analysis duringcthe period (7, 14 and 21 days after storage -DAA). The results were expressed as a percentage of mass loss in each analysis period during storage. The calculation was obtained by the following formula %WL = [(W1-W2)/W1] × (100), where %WL= percentage mass loss massa, W1= initial mass do fruito in (g), W2= finalomass offruit in (g) (ZHANG *et al*., 2002).

Total phenolic compounds: The analysis of total phenolic compounds was performed according to the Folin-Ciocalteaumethodology, described,by Singleton& Rossi (1995).The extraction consists of the use of 1 g grams of tomato crushed in methanol.Aliquots of the extract (100µL) were transferred to 5 mLballoons containing2/3 mL of distilled water. 0.5 mL of sodium carbonate was added at 25% and 0.5 mL of folin-ciocalteou reagent, completing the volume with distilled water. The samples remained at rest in an environment with light shelter for 1 hour, after that, the readings were performed in spectrophotometer with a wavelength of 760 nm,zeroing the absorbance of the device with white (2/3 mL of distilled water, 0.5 mL of sodium carbonate at 25% and 0.5 mL of the Folin-Ciocalteou reagent, completing the volume with distilled water).The results were expressed in mg of EAG g fruit-¹

Lycopene andβ-carotene: Theconcentrations of lycopene and β-carotenewere determined according to Nagata and Yamashita (1992). Toperform theprocedure, 1 g of sample was weighed and the solvent acetone: hexane (4:6v/v) was added. It was then homogenized with the aid of aturrax.Thes samples beram kept at rest for a period of 1 hour at room temperatureunder light. The readings were performed inspectrophotometer (Thermo Scientific UV VIS 160 Evolution) in ondlengthsat453, 505, 645 and 663 nm.Theresultss wereexpressed in mg 100 g^{-1}f fresh sample, calculated by the following equations:

$$\text{Lycopene} = -0.0458A_{663}+0.204_{6445}+0.372A_{505}-0.0806A_{453}$$
$$\beta\text{-carotene} = 0.216A_{663}-1.22A_{645}-0.304A_{505}+0.452A_{453}$$

Total Soluble Solid: The content of total soluble solids was determined by manual refractometer ss(Atago nº1))and the resultsexpressedin ºBrix.

PH determination: The pH was determined with the use of pHmetro (Instrutherm®PH-2000)in tomato samples, and the result expressed in units.

Total Titratable Acidity: Acidity was measured according to AOAC (2016). Approximately 10g of the fruit were diluted in 90ml of distilled water. This volume was titrated with NaOH 0.1M up to pH 8.1. The fruits were crushed with the help of an industrial processor. juice extractor. The value was expressed as % citric acid.

Statistical Analysis: The experimental design was completely randomized (IHD) with a plot subdivided in time, with three replications. The data were tested for normality and later the means were compared by the Scott-Knott test ($p<0.05$) using the *software*SISVAR Version 5.7 (PT) (FERREIRA, 2019).

RESULTS AND DISCUSSION

The mass loss was significant for treatment, time and the interaction between treatment, season and cultivar. Regarding the treatments, Salicylic Acids (AS)) and *Bacillus subtilis*(BS)presented the lowest masslossbelow 3.5%, differing only from the control treatment (Figure1). When unfolding the interaction between both factors, it was found that only the S was influenced by time throughout storage (Figure 1). The fruits of the cultivar Fascínio treated with AS obtained a mass loss of 3.66; 0.90and 1.33% at 7, 14 and 21 DAA,respectively. For both cultivars analyzed here, the treatments with AS and BS the mass loss was always lower in relation to the control, showing that the application of AS and BS in the post-harvest can be an alternative in the maintenance of fruit mass for the sea cultivars (Figure 1). Similar results were observed by Mandal & Hazarika (2018) who working with doses ranging from 0,2-1,2 mM found that the increment of doses resulted in an average weight mass loss of 7.03% at 21 days of storage, while the control treatment was 14.65%. Kant *et al*. (2013) verified in tomatoes, in the cultivar Pusa Rohini that at a dose of 0.75 mM of AS the mass losses were on average 9.28%, while the control reached 34.01% over 22 days. This reduction in mass loss over storage time is associated with the action of AS in the control of ethylene production and inthe reduction of respiration (KANT *et al*. 2013).

Another'simportantparameter in tomato postharvest arethescontents ofTotal Soluble Solids es((SST),pH and Total Titratable Acidity (ATT). For thes variables pH and TA there was no statistical difference between the factors analyzed.However, for OSH there was a statistical difference for the interaction between treatment, cultivar and season. Regarding the treatment, it was observed that the application of salicylic acid in thepost-harvestinfluenced the higher SST content for the cultivar Fascínio in relation to the cultivar Thaíse, with a general average of 4.66 and 4.11ºBrix,, respectively (Table 1). Salicylic acid also promoted a higher SST content in fruits at 21 days after refrigerated storage as a function ofcultivars, with values of 5.00 and 4.00ºBrixfor the cultivarsFascinationand Thaisi, respectively (Table 1). In our study, the application of salicylic acid showed a slight increase in the parameters analyzed in relation to*Bacillus subtilis* and control, but there was no statistical difference between them. This shows that thecultivars studiedapresented apresentaram different behaviors to the application of AS. The possible explanation for this fact may be linked to the characteristics of waxes present in the cuticle of fruits and the fatty acids that composeit, which may interfere in their reactivity at the level of receptors in the membrane (ZHANG *et al*., 2013).. An analysis of the fatty acid profile could better explain this fact.

Andthishypothesis may explain that the high content of SST in the cultivar Fascínio (4,13°Brix) influenced by the application of AS in the fruit,, may be related to the regulation of the activity of pectolytic enzymes,, because AS inhibits ethylene production and these enzymes are controlled by ethylene production (PECH *et al*., 2018).While, nthe cultivarThaíse the content of SST was lower when compared tocv.Fascínio (Tabela1). Similar results were observed by Almunqedhi *et al*. (2017), who when applying AS (14.48mM via foliar, in thepre-harvest of tomato (growthphaseofthe fruits), verified an increase in the content of °Brix in storage conditions atpré 25 ºC and shelf time of 16 days. In general, the fruits treated with salicylic acid, apparently, were firmer than the other treatments. Although it was not analyzed,, this factor can infer that the reduction of ethylene production, one of the key actions of as,, may have contributed to the improvement of fruit firmness since the control of ethylene in productionhurtsin reducing the action of pectinolytic enzymes that are dependent on ethylene (SUPAPVANICH S.., 2015). This effect has the ability to prolong the life of the fruit in post-harvest, by allowing the best organization of membranes and stiffness of the cell wall. Regarding lycopene content, there was a significant difference for the treatment factor,time,as well as for the interaction between time*versus*cultivar, and cultivar *versus* treatment.The fruits treated with AS obtained higher values than the fruits treated with BS and the control, with averages of 47.44;40.83 and 36.50 mg lycopene per 100 ml of fresh tomatoes,respectively (Table 2).. In the time factor,, the maximum accumulated lycopenecontent was verified in all treatments at 21 DAA.Mandal and Hazarita(2017), workingwith different doses of AS nto-post-harvest of tomatoes verified that the highest accumulations of lycopene were observed at 21 DAAwith an average of25 μg g^{-1}.

According to Pila *et al*. (2010) salicylic acid produces an effective effect in controlling weight loss, percentage of decay and other changes in the composition of tomato fruits, such as, pH, titratable acidity, total soluble solids, total sugars, total chlorophylls, carotenoids, lycopene in tomato stores. When analyzing the behavior of thecultivars withinthe treatments, it was verified that the application of AS influencedthecultivars studied. These results corroborate those verified by Zhera *et al*. (2016) in postharvest who observed lycopene increments in the 5th and 10th DAA at the dose of 1mM of AS..In our study, the cultivarFascínio was superior aThaíse with an average of 53.77 and 41.11mg $100ml^{-1}$ respectively (Table 2). Ingeneral,the cultivar Fascination was shown to be influenced by treatments with higher responses to AS, BS and CTRL,respectively. However, the cultivar Thaise was not responsive to any of the treatments. Based on these results,, it is verified that the effect of the application of the product is dependent on the genetic material. Possibly the thickness of the wall or even the response at the level of receptors positively interfere in the assimilation of the product. Mandal and Hazarika (2017) working with application of Salicylic Acid in tomatoes observed in their study that the concentration of lycopene in tomato fruitwas higher at 21 days of storage, reaching a concentration of 24μg g^{-1}.The same authors found that the dose of AS ranging from 1.0 to 1.2 mMwas able toreduce theloss ofmasa, the concentration ofsugars,accumulation of carotenoides and lycopene as a function of senescence retardation. Kant *et al*. (2013) observed that the application ofSalicylic Acidat a concentration of 0.75 mM increases shelf life by 7 dayswhen, compared tothe control. Result consistent with our work, where the highest accumulations are observed over the storage period, especially when the fruit is treated withSalicylic Acid (Table 2).Baninaiem *et al*. (2016) found in their study, areduction inthe loss of quality of tomato fruits when treated with AS at the concentration of 4mM during the storage period of 40 days at 10 °C, in which the fruits did not have high quality losses. One of the compounds of greatest interest in tomato fruit are carotenoids, which have several properties of great relevance to their consumption, mainly related to antioxidant activity.The highest concentration of carotenoids was observed at 14 DAA regardless of the treatment evaluated. Regarding the cultivars studied ins, in the treatment *Bacíllus subtilis*, the'Fascínio''presented the highest concentration of carotenoids whencomparedto 'Thaíse',with an average of',7,22 and 4,22mg $100ml^{-1}$,respectively.

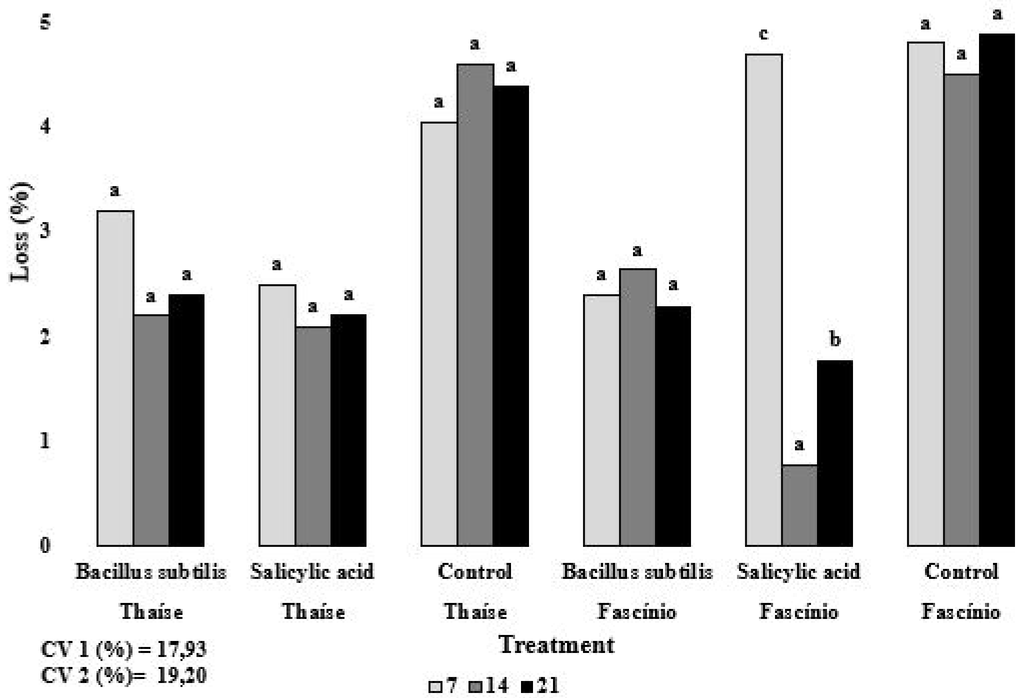

Figure 1. Loss of percentage mass of fruits of the cultivars Fascination and Thaíse as a function of the use of *Bacillus subtilis* and salicylic acid under refrigerated conditions

Table 1. Total Soluble Solids (SST), Hydrogen Potential (pH) and Total Titratable Acidity (ATT)content in tomato fruits of the cultivars Fascination and Thaíse as a function of the use of*Bacillus subtilis*and salicylic acid under refrigerated conditions

Treatments	Storage (Days)	Osh		Ph		Att	
		Fascination	Thaisis	Fascination	Thaisis	Fascination	Thaisis
Control	7	4.33 aA	4.66 aA	4.33 aA	4.33 aA	5.00 aA	4.00 aA
	14	4.00 aA	4.00 aA	5.33 aA	4.66 aA	4.00 aA	5.00 aA
	21	4.66 aA	4.00 aA	4.33 aA	4.00 aA	3.33 aA	2.66 a.M.
M. General		4.03 aA	4.10 a.M.	4.49 aA	3.69 bA	3.08 aA	3.40 aA
Bacíllus subtilis	7	4.66 aA	4.66 aA	4.00 aA	4.00 aA	5.00 aA	4.00 aA
	14	4.00 aA	4.00 aA	4.33 aA	4.66 aA	4.33 aA	4.66 aA
	21	4.33 aA	4.33 aA	4.00 aA	4.00 aA	3.33 aA	4.33 aA
M. General		4.16 aA	4.23 aA	4.33 aA	4.05 aA	3.57 aA	3.50 aA
Salicylic Acid	7	5.00 aA	4.33 aA	4.33 aA	4.33 aA	4.60 aA	3.66 aA
	14	4.00 aA	4.00 aA	4.33 aA	4.66 aA	4.00 aA	2.66 a.M.
	21	5.00 aA	4.00 aB	4.00 aA	4.00 aA	3.33 aA	4.00 aA
M. general		4.13 aA	3.93b	4.34 aA	3.80 aA	3.30 aA	2.32 bB
		CV $_1$ (%):3.85		CV $_1$ (%): 15.77		CV $_1$ (%): 19.98	
		CV $_2$ (%): 9.32		CV $_2$ (%):13.88		CV$_2$ (%): 19.82	

* Means followed by the same letters, lowercase in the column and uppercase in the row, do not differ significantly from each other by the Scott-Knott test ($p<0.05$). CV $_1$= treatment; CV$_2$= cultivar*time.

Table 2. Lycopene content (mg100 ml^{-1}) and β-carotene (mg100 ml^{-1}) in fruits of the cultivars Fascination and Thaíse as a function of the use of *Bacillus subtilis* and salicylic acid under refrigerated conditions

Treatment	Storage (Days)	Lycopene		Carotenoids	
		Fascination	Thaisis	Fascination	Thaisis
Control	7	27.00 bB	36.00 aA	3.66 bA	5.66 aA
	14	22.33 bC	45.33 aA	15,66toA	4.00 aB
	21	54.00 aA	34.33 bB	8,00 aA	3.00 aB
	M. General	34.44cA	38.55 aA	9.11 aA	3.55 toB
Bacíllus subtilis	7	21.66 bB	24.66b	5,66 aA	5.33 aA
	14	33.66 bB	45.00 aA	9.00 aA	5.33 aA
	21	62.66 aA	57.33 aA	7.00 aA	1.66 bB
	M. General	39.33bA	42.33aA	7.22 aA	4,22 toB
Salicylic Acid	7	55.00 aA	37.00 bA	19.33 aA	6.33 aA
	14	47.66 aA	24.00 bB	6.33 bA	11.00aB
	21	62.33 aA	58.66 aA	7.00 bA	6.33 aA
	M. General	53.77aA	41.11toB	10.88 aA	7.88 aA
CV 1 (%): 15.39					CV1(%): 119.68
CV 2 (%): 10.48					CV2(%): 71.83

Means followed by the same letras, lowercase in the column and uppercase in the row,donot differ significantly from each other by the Scott-Knott test ($p<0.05$).CV$_1$= treatment; CV$_2$= cultivar*time.

Whenunfolding the treatment as a function ofthe cultivar, it is observed in the control treatment that in 'Fascínio'' the carotenoid content was higher when compared to 'Thaíse',with misdayof 8.22 and 4.22 mg 100 ml^{-1} respectively. Similar to ctrl treatment, the application of BS was also higher for the cultivar Fascination in relation to cultivar Thaíse with an average of 9.11and3.55mg 100ml^{-1} respectively.In tomatoes treated with AS,the statistical difference was not observed regarding the carotenoid content between the cultivars studied (10.88 and7.88mg 100ml^{-1}, for 'Fascinio' and 'Thaíse', respectively) (Table 2).10,88 Kumar *et al*. (2017) working with different doses of AS (0.5-1.5mM) verified that at a dose of 0.75 mM,, over time,, there was an increase in the availability of carotenoids duringstorage, with maximum obtained at 12 DAA. Possibly the fact that there is no RESPONSE of AS in both cultivars may be related to the dose adopted, i.e., a higher concentration of salt in contact with the epidermis may have generated a disorganization in the membrane and may have reduced its influence on the control of carotenoid biosynthesis.Regarding the application of BS,, there was a reduction in the content of carotenoids when compared to the other treatments. Possibly the strain used may inhibit the action of ethylene corroborating for a reduction of synthesis and even degradation of carotenoid along maturation.

Conclusion

As a whole, the present work has been found that the postharvest treatment of tomato fruits with Salicylic Acid and *Bacillus subtilis*is an alternative that should be explored by the scientific community. The application of Salicylic Acid and *Bacillus subtilis*did not promote significant changes in brix°, PH and ATT values. Similarly, treatmentsAS and BS did not influence lycopene content.The carotenoid content was influenced by BS.Thecultivar fascination was the one with the highest content of carotenoids and lycopene during storage.

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