Full Length Research Paper

Biologically-based strategies to reduce postharvest losses of tomato

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Accepted 6 May, 2011

The preharvest employment of *Bacillus subtilis* was highly effective in an augmentation of fruit set and improves general apperance of tomato at harvest. Cooling technology supported the poential of *B. subtilis*, in particularly prevention of postharvest losses of tomato fruits within marketing context. Furthermore, the resultant inhibition of color development and improvement of sensory traits of tomato fruits due to the preharvest application of our formulated *B. subtilis* with the help of cooling technology were indicative of general inhibition of senescence and ripening. This biologically-based strategy deserves further development and application under commercial requirements especially in developing countries like Egypt, where refrigeration is inadequate.

Key words: Biological control, general appearance, Bacillus subtilis.

INTRODUCTION

Tomato (Lycospersicon esculentum Mill) is one of the most important and widely distributed horticultural vegetable crops in the world as well as Egypt. Tomatoes are major contributors of the carotendiondes (especially lycopene), phenolics and vitamin C in daily diets (Causse et al., 2003). Moreover, results from the epidemiological studies have shown that tomato and its products may have a positive effect against various forms of cancer, especially prostate cancer and cardiovascular diseases (Ellinger et al., 2006). Worldwide postharvest tomato losses are as 30 to 40% (Kader, 1992) and even much higher in some developing countries such as Egypt due to the use of improper handling procedures and lack of methods to prevent decay and senescence (Prigojin et al., 2005). Currently, fungicides are used to control preharvest losses of tomato production (Baider and Cohen, 2003), but the use of prophylactic chemicals in these commodities (tomato fruits) is not allowed in different countries due to oncogenic risks (Unnikrishnan and Nath, 2002).

In Egypt, tomato fruits for the domestic market are not subjects to low temperature storage due to high costs. Consequently, gluts occur during harvest with shortfalls experienced afterwards. Crop grown for export do have low temperature storage facilities but quality and sensory traits of fruit losses still occur. The marketing of organic farming products has markedly expanded because of increased consumer demand for healthy food products, which are free of synthetic chemical residues and the resulting improvements in the production and distribution systems (Sylvander, 1993). However, since organic fruits are not treated with chemical fungicides, they suffer from relatively high rates of decay, which develops during storage and shelflife causing loss in the sensory traits of fruits (Yahia et al., 2004).

In recent years much research has focused on developing alternative non chemical strategies against postharvest diseases trigger softening and dramtic reduction in fruit firmness (Droby et al., 2009; Eshel et al., 2009). In this concept, our formulated *Bacillus subtilis* has been success to plant disease control (Abd_allah et al., 2003), improving the quality and sensory traits of plant yield (Abd_allah, 2005), support plant histology (Abd_allah et al., 2007) and enhancement of plant metabolism towards anabolism (Abd_allah and Ezzat, 2004;

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Abd_allah, 2005; Abd_allah and El-Didomany, 2007). Such success in our formulated biocontrol agent (*B. subtilis*) is still limited due to the production of stable and profitable food should be through combining different non-chemical (biological and physical) control methods (Eshel et al., 2000). Therefore, this study was designed to study the experimentation of formulated *B. subtilis* in concurrence with cooling regarding control postharvest depression in tomato fruit quality to better understanding for the metabolic as well physiological response of tomato fruit.

MATERIALS AND METHODS

Experimental soil and seeds

The study was carried out in greenhouse of tomato production areas in Ismalia, Egypt throughout the growing season of 2007. The soil used throughout this study was a sandy loam with the following properties; organic carbon 0.62; total soluble salts 0.30%, moisture holding capacity 14.8% and pH 7.5. Seeds of tomato (L. esculentum Mill var. Brillant) provided with blue seed Co, Nasr City Cairo, Egypt. The seeds were germinated in the main nursery, then transplanted into a greenhouse (528 m² with capacity of 1/1200 seedlings). Before the transplantation, the root system of each tomato seedling was dipped in a cell suspension of B. subtilis for one hour (Abd Allah, 1995). The formulation of biocontrol agent, B. subtiltis was carried out according to Abd_allah (1995) and used in a rate of 10 g/l chlorine free water (1 g of formulated B. subtilis contain 3.6 x 109 cfu). Control seedlings (without treatement of formulated B. subtilis) were used as reference. Planting (greenhouse) received dropping-irrigation and fertilizers as usual. Formulated B. subtilis (10 g/l) were sprinkled on the seedlings (60 L of formulated B. subtilis solution/greenhouse) at early morning (one chance/week) periodical until end of the experiment (fruiting stage). During the growing season, light pink tomatoes were picked from each treatment. The formulated B. subtilis as well as non-treated control one and then fruit quality was evaluated as viable quality (VQ). Fruit set ratio was calculated as a percentage of fruit number (diameter must be 10 mm) to flower number (including buds just before anthesis).

Storage experiment

Immediately after the harvest, tomato fruits were sorted to eliminate unwanted fruits. The fruits selected to be uniform in size (150± 5 g), light pink color and without any defects. The selected fruits were placed in a double layer in closed cardboard boxes (50 x 30 x 15 cm) with apertures for aeration (Such cardboard boxes are designed for exportation. The boxes were stored either at 20 °C for 15 days (Simulation of local marketing) or at 12 °C for 10 days plus 5 days at 20 °C (Simulation of sea transport and marketing in USA and EU). The storage experiment was carried out in triplicates for each treatment (40 kg, four boxes).

Quality evaluation

General appearance

Visual fruit quality was scored on three point's scale 1 to 3 in which quality is taken as under (1) poor visual appearance; (2) good and acceptable appearance; (3) excellent appearance. A test panel

consisting of aroma, colour, firmness, rots development on calyx flesh, defect, dry calyx and dirty tomato fruits.

Eating quality

Assessment of eating quality was conducted on individual tomato fruits using objective measures. Tomato fruits were chosen and homogenized following by centrifugation with $8000 \times g$ for 20 min at 5%. The resulting supernatants were filtered using cheesecloth and then frozen at -20% until use for analysis.

Firmness

The firmness of tomato fruits was evaluated according to the scale of Kader et al. (1973) with the following accounts: 5, very hard; 4, hard; 3, moderately firm; 2, slightly firm; 1, soft.

Fresh weight loss

Weight loss was determined by weighting tomato fruits at the beginning of the experiment and reweights it again at the end. It was expressed as the loss of initial fresh weight and expressed as the percentage.

Acidity (total titrable acidity)

For each treatment, the supernatant (10 g equivalent) was diluted with 50 ml of distilled water. The solutions were titrated against 0.1 N NaOH (phenolphthalein used as indicator) to end point of pH 8.2 (citric acid used as reference) and acidity expressed as percentage of citric acid (Wills and Ku, 2002).

Total soluble solids

Total soluble solid was evaluated as percent by a hand reractometer using drops of tomato fruits juice that previously extracted.

RESULTS AND DISCUSSION

Currently, the usage of chemical agents remains the major method for choice by far for managing postharvest losses and few postharvest biocontrol products that are commercially available have limited use, mostly in r ich markets (Droby et al., 2009). Biosave (Pseudomonas syringe Van Hall) and Shemer (Metschnikowia fructicola Kurtzman & Droby) have been registered commercially for postharvest loss control (Kurtzman and Droby, 2001). We found that the preharvest application of our formulated B. subtilis was highly effective (significantly) in an augmentation of fruit set of tomato by 24.8% compared with non-treated control one (Table 1). Plant growth regulators such as auxins and gibberellins are known to affect parthenocarby (Chowdhury et al., 2007) and flowering (Khan and Chaudhry, 2006); therefore, synthesized auxins and gibberellins are often too used for promotion of fruit set in some fruity vegetables production including tomato (Sasaki et al., 2005). The production of plant

Table 1. Effect of preharvest treatment of tomato with formulated *B. subtilis* on fruit set (%) and general appearance (GA) of tomato fruits.

Treatment		Fruit set (%)	General appearance (GA)
Non-treated control		69.2	2.35
Formulated B. subtilis		92.1	2.74
LSD at:	0.05	12.4	0.27
	0.01	20.3	0.38

Table 2. Effect of preharvest treatment of tomato with formulated *B. subtilis* and storage under different temperatures (℃) on general appearance (GA) of tomato fruits.

		General appearance (GA) after storage at different temperatures (°C)			
Treatment		Storage at 20 °C for 15 day (like local marketing)	Storage at 12℃ for 10 day + 20℃ for 5 day (like transport marketing)		
Non-treated of	control	1.7	2.3		
Formulated E	3. subtilis	2.5	3.4		
LSD at:	0.05	0.30	0.26		
	0.01	0.55	0.47		

growth hormones via B. subtilis has been demonstrated previously (Arkhipova et al., 2005). Consequently, it has similar suggested mechanism to exogenous application of plant growth hormones. Mechanisms other than production of growth stimulatory by which B. subtilis might increase fruit set including production of substance that interacts with the plant's own growth regulatory system and enhancement plant nutrition (Loper and Schroth, 1986). Moreover, the application of *B. subtilis* induced systemic physiological changes in plant metabolism which result in greater improvement towards anabolism (Abd Allah and Ezzat, 2004; Abd Allah et al., 2006) and quality of plant yield (Abd_Allah, 2005) via control of plant pathogenic fungi (Abd_Allah, 2007; Abd Allah et al., 2003); induction of systematic resistance and promotion of endogenous plant hormones such as gibberellins (Kloepper et al., 2004) and enhancement of plant vigor index through stimulate beneficial rhizobacteria in the rhizosphere of host plant, hence, enhance plant nutrition (Abd Allah and El-Didamony, 2007).

A pre-harvest treatment of *B. subtilis* protects crop fruits against decay and improves its quality (Leibinger et al., 1997). In the same connection, the formulated *B. subtilis* ameliorated the general appearance of tomato fruits (Table 1) compared with non-treated control one. Suppression of plant pathogens (Abd_Allah, 2005) increases the chance to produce healthy, stable and profitable food with minimum defects. Some mechanisms was suggested to be the major role in the biocontrol activity of many antagonists such as nutrient competition (Janisiewicz and Korsten, 2002), direct interaction (Schotsmans et al., 2008), production of cell wall

hydrolytic enzymes (Bar-Shimon et al., 2004), induction of systematic resistance (Droby et al., 2002) and production of antimicrobial substances (El-Khallal, 2007). Information on the mechanisms of action for most of the antagonists investigated is still incomplete because of the difficulties associated with the study of complex interactions between a host, a pathogen and an antagonist, as well as other resident microorganisms (Droby et al., 2009). Although, biocontrol has achieved some remarkable success, it is well recognized that at least for the time being, if it is to be part of large-scale agriculture, it should be combined with other control practices (such as cooling), leading integrated pest management (IPM). It was found that cooling was highly effective to control the decrease in general appearance of stored tomato fruits compared with non-cooled one (Table 2). A similar observation was made by Prigojin et al. (2005). Postharvest life functions can not be stopped, but they can be showed down by controlling the storage environment. Low temperatures found to be inhibitor for synthesis of MACC [1-(malonylamino) cyclopropane-1carboxylic acid) which appear to be the basic process that regulated ACC [1-aminocyclopropane-1-carboxylic acid] hence, ethylene production (Larrigaudière et al., 2009). The reason for such an inhibition in cold remains to be explained, it is probably due to a direct effect of low temperature on ACC synthase activity according to Arrhenius model (Khan and Singh, 2007). In addition, the results have no significant difference between the general appearance of tomato fruits at zero harvest time (Table 1) and those cooled (Table 2), both were in the absence of B. subtilis treatment. This underscores the point that postharvest treatment (cooling) cannot improve quality;

Table 3. Effect of preharvest treatment of tomato wi	h formulated <i>B. subtilis</i>	and storage under	different temperatures (°C) on
sensory traits of tomato fruits.			

Treatment		Sensory traits of tomato fruit				
	Storage condition	Weight loss (%)	Firmness unit	Total soluble sold (%)	Acidity (%)	Color
Non-treated Control	At 20 ℃ for 15 days (like local marketing)	4.42	3.51	4. 98	1.04	Dark red
	Storage at 12°C for 10 days + 20°C for 5 days (like transport marketing)	3.34	4.12	4. 85	0.92	Red
Formulated <i>B.</i> subtilis	At 20°C for 15 days (like local marketing)	2.87	4.08	4. 57	0.81	Light
	Storage at 12°C for 10 days + 20°C for 5 days (like transport marketing)	2.40	4.92	4. 28	0.72	Pink
LSD at:	0.05	0.47	0.56	0.25	0.11	
	0.01	0.79	0.75	0.76	0.27	

the best it can improve quality, the best it can hope to do is maintain it, so it must be (especially for export) combing between the biologically and non-chemically based strategies to reduce postharvest losses of tomato.

Our results (Table 2) indicated that the pre-harvest treatment of tomato with formulated B. subtilis caused highly significant increase in general appearance of tomato fruits either stored at 20 °C for 15 days (for local marketing) or at 12°C for 10 days + 20°C for 5 days (for transport and marketing in USA and EU) by percent of 32.0 and 32.3, respectively. Additionally, we observed that no visable difference in general apperance of tomato fruits stored at cooling system and preharvest treated with B. subtilis and stored at non-cooling system. Further suggested mechanism for mode of action of biocontrol agents is eliciting resistance responces in harvested fruits and vegetables (Droby et al., 1993; Wilson et al., 1994). In this context, Abd_Allah et al. (2006) reported that application of formulated B. subtilis elicited systemic resistance mechanisms in tomato via lipids metabolism.

Weight loss of tomato fruits was almost related to storage temperature (Znidarcic and Pozel, 2006). Such loss of tomato fruits is usually due to loss of water through transpiration, it can lead to wilting and shrivelling, which reduce both market value and consumer acceptability (Ball, 1997). In regard to this concept, our results (Table 3) showed that fruits stored at 12°C for 10 days + 20°C for 5 days (for transport and marketing in USA and EU) lost weight leisurely slower than those stored at 20°C for 15 days (for local marketing). In general, titrable acidity and total soluble solids of tomato fruits were increased with storge; however, firmness was decreased compared with those at the same picking time (Table 3). A simlar finding has been reported by Znidarcic and Pozel (2006). The resultant inhibition of color develop-

ment and improvement of sensory traits (firmness, total soluble solides and acidity) of tomato fruits due to the pre-harvest application of formulated *B. subtilis* was indicative of general inhibition of senescence and ripening (Table 3). A similar impression of *B. subtilis* was observed previously in citrus (Obagwu and Korsten, 2003), broccoli (Cui and Harling, 2006) and mango (Vivekananthan et al., 2006).

In conclusion, the quality of tomato fruits could be sustained for about two weeks (15 days) at ambient temperature after preharvest treatment with *B. subtilis*. This biologically-based strateiges deserves further development and application under commercial requirements especially in developing countries like Egypt where refrigeration is inadequate. We have here introduced a new paradigm for biocontrol research that many provide new apportunities for increasing the efficacy and consistency of biocontrol products.

ACKNOWLEDGMENT

Authors wish to thank College of Food and Agricultural Research Center and Deanship of Scientific Research, King Saud University Saudi Arabia for financially supporting this work.

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