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Postharvest dip treatment of *Bacillus subtilis* for maintaining quality and shelf life of guava cv. Allahabad Safeda

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Abstract
In North India, guava fruits are harvested twice in a year yet maintaining the quality during storage is still a challenge due to its perishable nature. The use of chemicals for extending shelf life of guava should be avoided due to food safety concern since it is consumed along with skin. Recently bio-agents are being used as dip treatment for effective control of storage pathogens like *Colletotrichum* sp., *Pestalotiopsis* sp. and *Lasiodiplodia* sp. in fruits like apple, pear and strawberry. However, no such report is available for guava. In the present study *Bacillus subtilis* was used as bio-agent for enhancing the shelf life of guava. Guava fruits cv 'Allhabad Safeda' were subjected to post harvest dip treatments for 30 minutes with four strains of *Bacillus subtilis* viz. MTCC 7607, 7606, 7612, and 7605 @ 10^8cells/ml dip solution and stored at 18 ±2 ºC and 57±5 % R.H. The fruits were analyzed for physico-chemical parameters as well as microbial population at regular intervals. Among the treatments, fruits treated with *Bacillus subtilis* strain MTCC 7607 showed highest firmness after 10 days of storage. In general bio-agent treated fruits were glossy in appearance, better in quality and taste with uniform colour development and least disease incidence.Surface microbial analysis reflected the dominance of treated bacterial strain over other micro-organisms. Preliminary results indicate that *Bacillus subtilis* strain MTCC 7607 @ 10^8cells/ml could act as a potent bio-agent for shelf-life extension of guava to control postharvest diseases.

Keywords: Bio-agents, shelf-life, ascorbic acid, *Bacillus subtilis*, post-harvest

Introduction
Control of pathogens during post-harvest storage and handling has been a challenging task for which chemicals are generally used. Recently, bio-agents are considered as preferred tool to replace chemicals because of their safety on human health and the environment. There is a need to look for an effective bio-agent for control of post-harvest pathogens. Bio-agents actively produce antibiotics, whose action partially determines their effectiveness in controlling post harvest pathogens. Commercial products containing *Bacillus subtilis* (Serenade ad Rhio-plus) are being used for control of powdery mildew, late blight and brown rot on fruits Walton (2002) and Sharma (2009) [21]. The first report about *B. subtilis* affecting the physiological processes of postharvest of fruits is reduction in ethylene production in melons by Wang *et al*., (2010) [25]. Use of *Bacillus subtilis* as a biological antagonist has been reported from fruits such as apricot, avocado, cherry, citrus, litchi, strawberry, apple and pear Pussy and Wilson (1984) [16], Demoz and Korsten (2006) [4], Utkheda and Sholberg (1986) [23], Sing and Daverall (1984) [22], Jiang *et al*., (2001) [9], Zhao *et al*., (2007) [26] Qi *et al*., (2005) [17]. Lise and Katze (1992) [11] and Lise and Jager (1995) [12]. The objective of the study was to evaluate strains of *Bacillus subtilis* as potential bio-agent for maintaining the quality of guava fruits under ambient conditions.

Materials and methods
B. subtilis strains of cow dung origin were obtained from culture collection of Microbiology laboratory in the Division of Post-Harvest Management, ICAR-Central Institute for Subtropical Horticulture, Lucknow. The bacterial culture was maintained on nutrient agar slants. Four bacteria isolated from cow dung were used namely *Bacillus subtilis* (MTCC 7605), *Bacillus subtilis* (MTCC 7606), *Bacillus subtilis* (MTCC 7607), *Bacillus subtilis* (MTCC 7612) from Microbial Type Culture Collection **(**MTCC) IMTECH, Chandigarh. The anti-pathogenicity was tested against *Colletotrichum gloeosporioides, Botryodiplodia theobromae* and *Pestalotiapsidii* as per standard protocol by dual culture technique (Anjaiah

et al., 1998) [1]. *B.subtilis* strains were multiplied on nutrient agar plates were incubated at 30±2ºC for 3 days. The cells were collected with the help of cell scrapper and the final cell number was maintained @10^8cells/ml in distilled water.

Mature, hard green fruits of guava cv. 'Allhabad Safeda' were harvested along with stalk and two leaves during the morning hours from the orchards of ICAR-CISH farm and transported to the Post Harvest Laboratory. The uniform colour break stage and sound fruits were sorted, divided into five lots of equal weight and subjected to bio-agents dip treatments. The treatment included four strains of *Bacillus subtilis* MTCC7605, MTCC7606, MTCC7607 and MTCC7612 @10^8 cells /ml along with control (in distilled water) for 30 minutes. Subsequently the fruits were air dried, packed in brown paper bags and stored under ambient conditions (18 ±2ºC and 57±5 % R.H.).

Fruits were withdrawn at regular intervals of 0,4,6,8 and 10 days from each treatment and cumulative physiological loss in weight (CPLW) percent was recorded. The firmness of fruits were measured with the help of penetrometre (McIntosh, USA, 8mm probe) and expressed as kg/cm^2. The fruits were thoroughly checked for diseases and number of spoilt fruits divided by the total number of fruits and expressed as percentage spoilage.

For chemical analysis, fruits withdrawn periodically from each treatment in three replications were cut into small pieces to be macerated into uniform pulp and analyzed for total soluble solids with help hand held digital refractometre (Atago, PAL 0-30%) and the reading was noted and adjusted with the correction factor at 20ºC temperature and expressed as degree Brix. The titratable acidity and ascorbic acid in fruit pulp was estimated by method as outlined by Ranganna (2000) [18]. Fruits were evaluated subjectively according to McDonald *et al*. (1998) measuring the rotted area in relation to the skin total area and expressed as percentage. Surface microbial counts were monitored at 2 days intervals as per the method of Collins and Lyne (1984) [2]. All the analysis was carried out in triplicates and the data recorded during the course of investigation were subjected to statistical analysis by SAS 9.3and CD at 0.05 level.

Results and discussion

Bacillus subtilis is well documented for its antimicrobial potential Krishna *et al*., (2011) [10]. Based on this antimicrobial property of *B. subtilis* was screened for its bio-control efficiency in petriplates versus *Colletotrichum gloeosporioides*, *Botryodiplodia theobromae* and *Pestalotiapsidii*. The results indicated that bacterial isolates had anti-pathogenic potential against the tested pathogens (Table 1 and fig 1).

Cumulative physiological loss in weight per cent (CPLW %) increased with the increase in storage period with least 10.05 per cent in*B. subtilis* strain MTCC 7607 followed by *B.subtilis* strain MTCC7606 (10.47) 10 daysof the storage period (Fig 2). In control fruits the CPLW percent was highest (15.37 %) on the 10 days of storage. Loss of weight during storage of fruits is one of the criteria used to study shelf life. It is due to evapo-transpiration by the fruit surface during storage. Weight loss throughout storage accelerates cell-breakdown and senescence. Wang *et al*. (2008) [24] has reported weight loss of watermelon during storage.

Firmness of the fruits expressed as Kg/cm^2 was found to decrease with the increase in storage period under ambient conditions with the onset of ripening and tissue softening. Significant difference was noticed in the firmness of the fruits among the different strains of *B. subtilis* (Fig.3.). The maximum firmness (11.87 Kg/cm^2) was noted in fruits treated with *B. subtilis* strain MTCC7607 followed by *B. subtilis* strain MTCC7606 (9.13 Kg/cm^2) and minimum firmness (6.20 Kg/cm^2) was exhibited in control fruits on the 6th day of storage thereafter there was a rapid decrease in the texture of fruits. This might be due to the interaction between the bio-agents on the surface of the fruits and storage period.

The total soluble solid content (TSS) of guava fruits variety 'Allahabad Safeda' increased and simultaneously the acidity percentage decreased with the increase in storage period. Among the treatments significant difference was studied for the total soluble solid contents. TSS was highest (13.33ºB) in control fruits on the 6th day of storage while it further decreased on the 8th and 10th day of storage (Fig.4.). Bio-agents treated fruit exhibited minimum TSS of 11.20ºBrix in strain MTCC7607 on the 6th day of storage. With the increase in storage period from 6th day to 8th day, the TSS increased from 11 to 13 ºBrix in *B. subtilis* strain MTCC7612. The acidity percentage also exhibited an opposite trend to that of TSS in control and *B. subtilis* treated fruits Fig.5. These findings are in accordance to the findings of Wang *et al*. (2010) [25] in TSS and acidity of watermelon.

Highest ascorbic acid content was observed on the 6th day of storage period in control fruits expressed as milligram per hundred grams of fruit pulp (248.57 mg/100g) and thereafter it decreased (Fig.6.). The fruits treated with different strains of *B. subtilis* had ascorbic acid content ranged between 200mg/100g up to 6th day of storage and decreased later on the 10th day of storage. Per cent CPLW is maximum in untreated fruits which are due to evaporation loss of moisture that might have concentrated the ascorbic acid content. These results are in harmony with the findings of Barakat *et al*., (2012) [14]. Colonization of *B. subtilis* on the fruit surface is an important and multifaceted process required for the competitive and exclusion of the storage pathogen. Antagonists must have high growth rates under favourable condition of humidity, temperature and nutrients Ippolito and Nigro (2000) [8] and Ippolito *et al*., (1997b) [7] and guava stored under ambient conditions were conducive for the colonization of bio-agents on the fruit surface.

Anthracnose and stem-end-rot in guava variety Allahabad Safeda was found maximum14.85 and 19.67 per cent in control fruits followed by *B. subtilis* strain MTCC7606 i.e., 6.26 and 12.63 % on the 8th and 10th day of storage respectively (Fig.7.) There was no spoilage observed throughout the storage period of 10 days in *B. subtilis strains* of MTCC7607, MTCC7612 and MTCC7605. *Bacillus subtilis* produces iturin, a powerful antifungal peptide Gueldner *et al*., (1988) [6], as well as gramicidin S Edwards and Seddon, (2001) [5]. *Bacillus* species are the most common types of bacteria and epiphytic and endophytic species of *Bacillus* have the potential to reduce pathogenicity Omur *et al*. (2008) [15]. Results are similar to the findings of Lise and Katze (1992) [11] in control of avocado post harvest diseases and mode of action of *Bacillus subtilis* in avocado post-harvest pathogen Lise and Jager (1995) [12]. Spadero (2004) [3] elucidated the biological control of postharvest fruit diseases thereby enhancing the shelf life of fruits.

The surface microbial load indicated the dominance of bio-agents in treated fruits compared to control where other yeasts, molds and bacteria dominated (Table.2.) Reduction in surface microbes was recorded over the progress of storage period which indicates the surface contaminating microbes might have reduced due to the formation of biofilm by the

bio-agent. Bio-control efficiency of the bio-agent could be activated to the ability of *B. subtilis* to produce acyl-homoserine lactones (HLS) an enzyme which inactivates N-acyl-homoserine (AHL) activity by hydrolyzing the lactone bond of AHL Morikava (2006) [13]. Due to production of iturin, a powerful antifungal peptide Gueldner *et al*., (1988) [6], as well as gramicidin S by *B. subtilis* Edwards and Seddon, (2001) [5]. The influence of competition for nutrition, possible production of antibacterial and antifungal compounds and interaction between the fruit surface and the bio-agent as reported by Castoria *et al*., (2001) [19], Samir, *et al*., (2009) [20] and Sharma *et al*., (2009) [21].

The present study shows that *Bacillus subtilis* strain MTCC7605, MTCC7607 and MTCC7612were effective in controlling post harvest diseases of guava viz. anthracnose and end rot. In addition, there is presence of strong bio-control capacity to enhance shelf life of guava. In the present investigation *Bacillus subtilis* strain MTCC7605, MTCC7607 and MTCC7612 for enhancing shelf life of guava cv. Allahabad Safeda as a bio-control organism have a potential for control of postharvest pathogen during storage.

Table 1: Anti-pathogenicity of *B. subtilis* strains for causal pathogens in guava fruits.

Causal pathogen	Effective bio-agent
Phytophthora parasitica var. *nicotinae*	*Bacillus subtilis* (MTCC 7605), *Bacillus subtilis* (MTCC 7606).
Pestalotiopsis sp.	*Bacillus subtilis* (MTCC 7607); *Bacillus subtilis* (MTCC 7606); *Bacillussubtilis* (MTCC 7612); *Bacillus subtilis* (MTCC 7605).
Lasiodiplodia sp.	*Bacillus subtilis* (MTCC 7607); *Bacillus subtilis* (MTCC 7606); *Bacillussubtilis* (MTCC 7612); *Bacillus subtilis* (MTCC 7605).
Colletotrichum sp.	*Bacillus subtilis* (MTCC 7607); *Bacillus subtilis* (MTCC 7606); *Bacillussubtilis* (MTCC 7612); *Bacillus subtilis* (MTCC 7605).

Table 2: Surface microbial counts in guava cv. Allahabad Safeda treated with *B. subtilis* strains during storage.

Treatments	Storage period (days)				
Bacteria (cells/g)	**0 day**	**4 day**	**6 day**	**8 day**	**10 day**
Control	2.25×10^3	7.5×10^2	2.98×10^3	5.3×10^2	2.82×10^2
MTCC 7607	8.960×10^4	2.5×10^2	1.2×10^2	2.1×10^2	1.34×10^2
MTCC 7606	9.600×10^4	3.2×10^2	1.7×10^2	3.2×10^2	1.45×10^2
MTCC 7612	1.500×10^4	3.8×10^2	3.5×10^2	1.5×10^2	2.34×10^2
MTCC 7605	1.125×10^4	4.8×10^2	4.8×10^2	4.3×10^2	1.87×10^2
Yeast & mould count (cfu/g)	0 day	4 day	6 day	8 day	10 day
Control	24 x 10	5.5×10^2	2.6×10^2	4.23×10^2	5.34×10^3
MTCC 7607	2 x 10	7.8×10^2	6.9×10^2	3.23×10^2	2.15×10^3
MTCC 7606	3 x 10	8.9×10^2	2.6×10^2	4.26×10^2	3.31×10^3
MTCC 7612	0	5.2×10^2	4.6×10^2	5.34×10^2	7.03×10^3
MTCC 7605	14 x 10	4.2×10^2	8.9×10^2	3.24×10^2	5.00×10^3

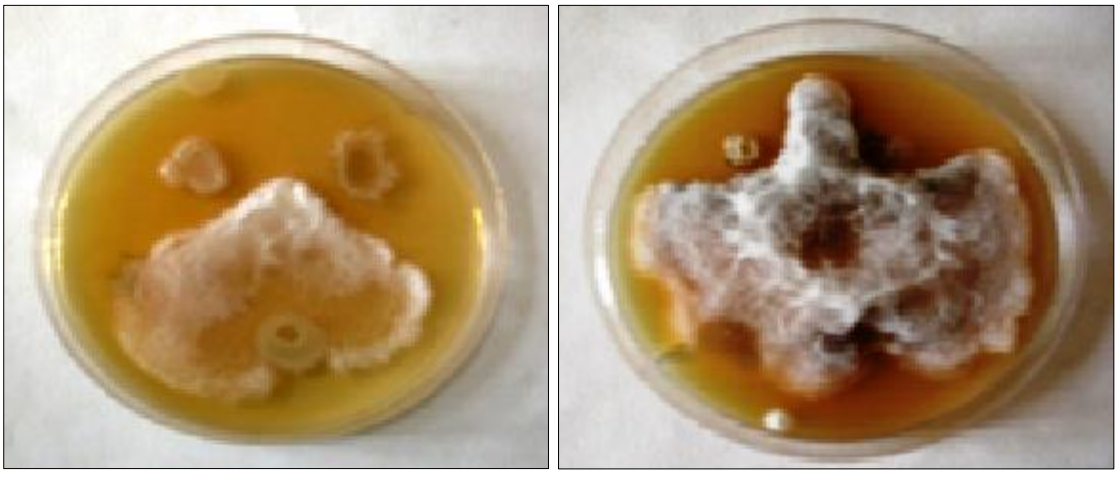

Fig 1: Anti-pathogenicity of *B. subtilis* strains for causal pathogens in guava fruits.

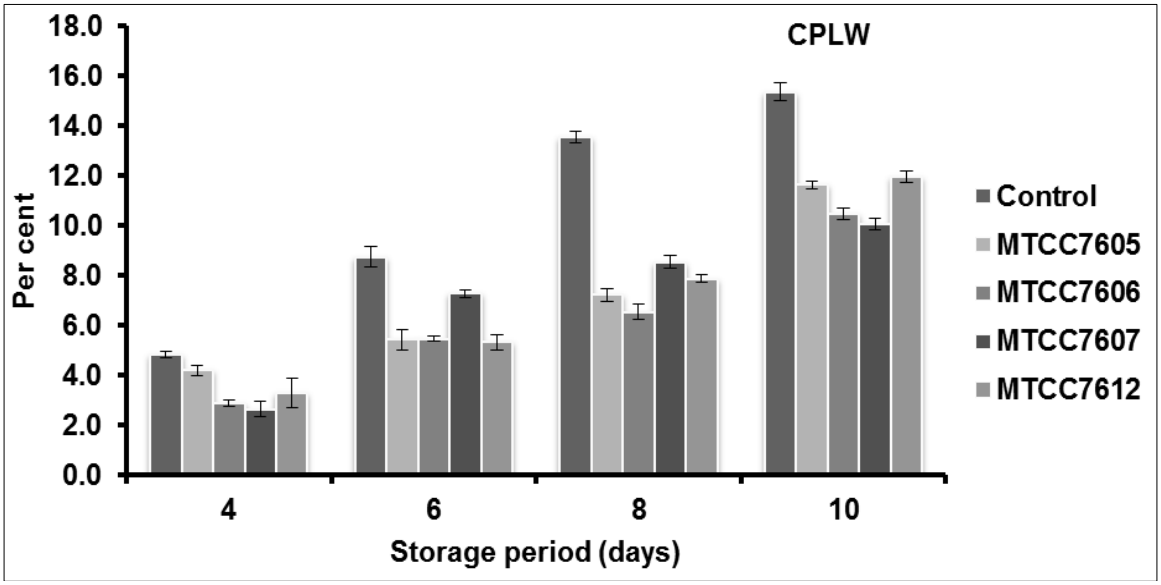

Fig 2: Cumulative physiological loss in weight per cent in guava cv. Allahabad Safeda treated with *B. subtilis* during storage.

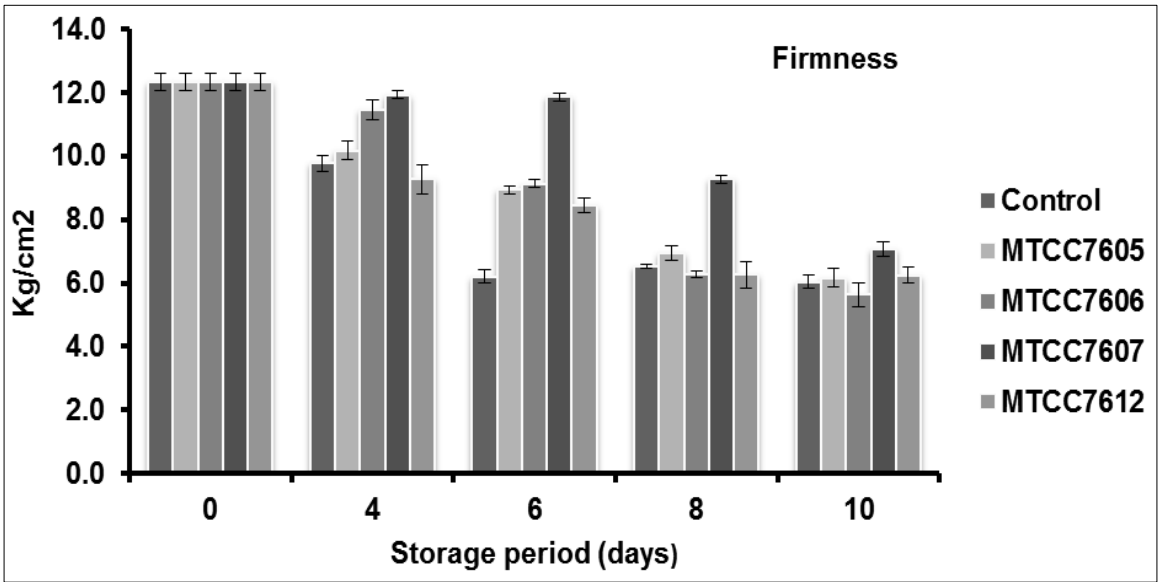

Fig 3: Firmness (Kg/cm^2) in guava cv. Allahabad Safeda treated with *B. subtilis* during storage.

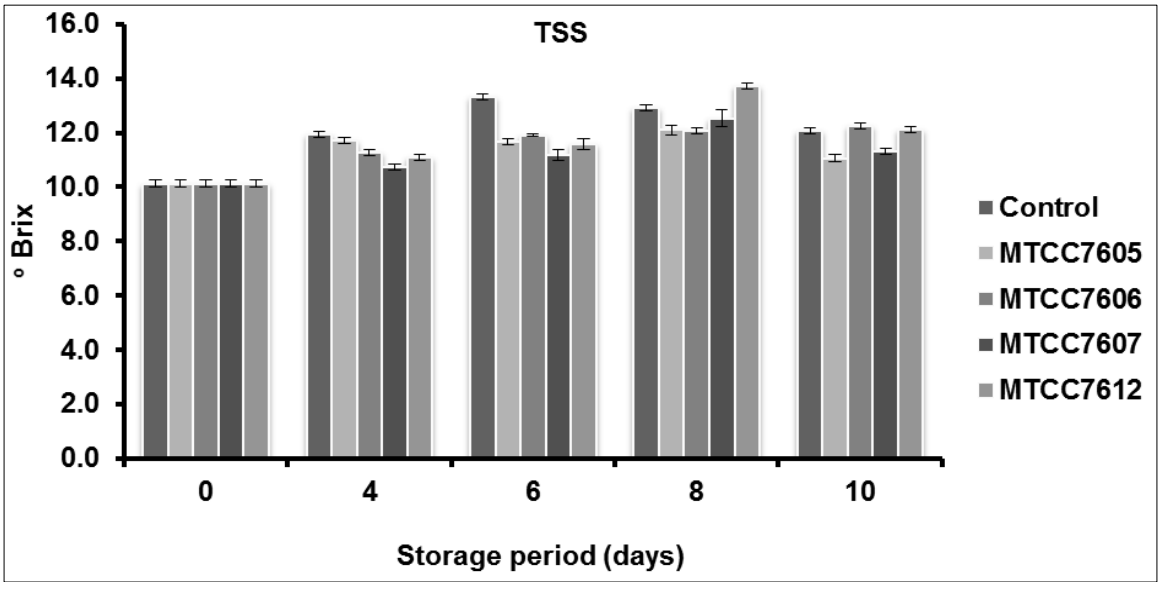

Fig 4: TSS (degree brix) in guava cv. Allahabad Safeda treated with *B. subtilis* during storage.

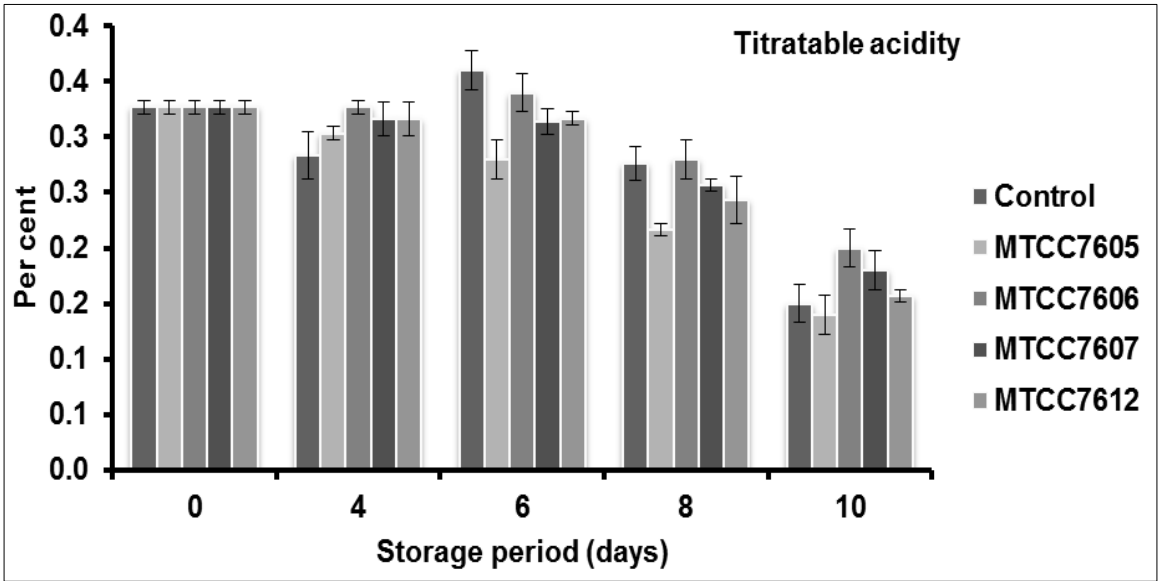

Fig 5: Tritratable acidity (per cent) in guava cv. Allahabad Safeda treated with *B. subtilis* strains during storage.

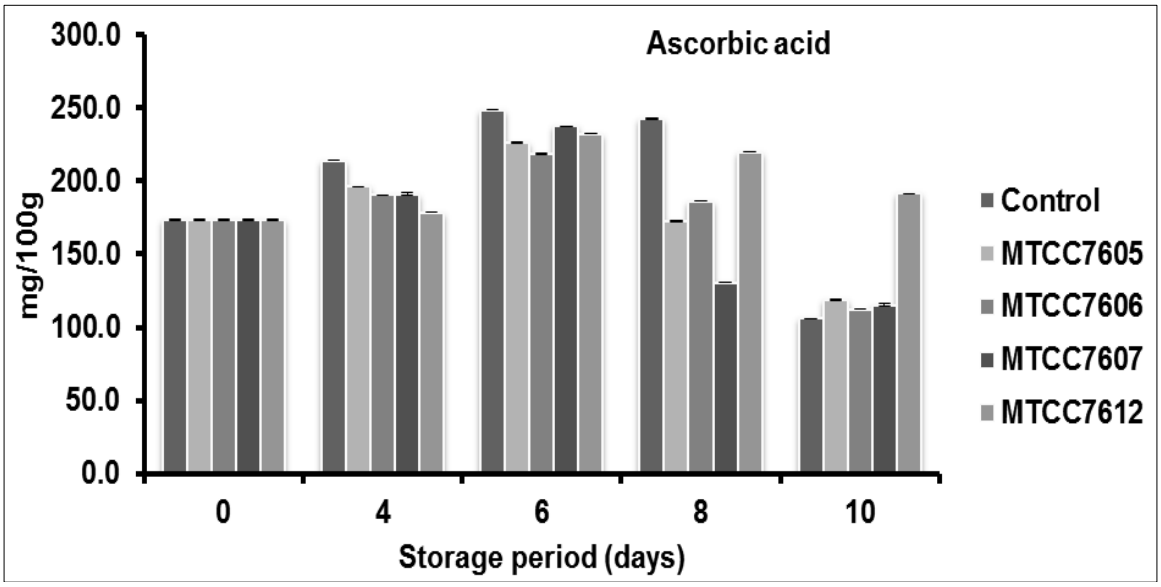

Fig 6: Ascorbic acid (mg/100g) in guava cv. Allahabad Safeda treated with *B. subtilis* strains during storage.

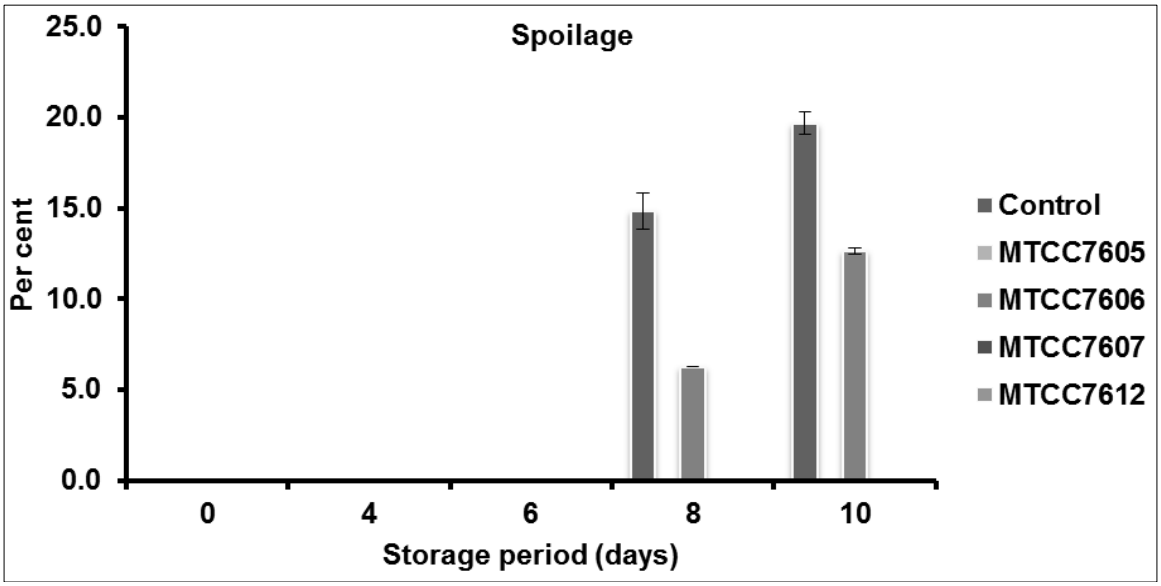

Fig 7: Spoilage (per cent) in guava cv. Allahabad Safeda treated with *B. subtilis* strains during storage.

References

1. Anjaiah Koedam N, Nowak-Thompson B, Loper JE, Hofte M, Tambeng JT, Cornelis P. Involvement of phenazines and anthranilate in the antagonism of *Pseudomonas aeruginosa* PNA1 and Tn5 derivatives towards *fusarium* spp and pythium spp. Mol. Plant microbe Interact. 1998; 11:846-854.
2. Collins CH, Lyne PM. Microbiological Methods.5th Ed. Butterworth and Co. Publisher Ltd. London, 1984, 331-345.
3. Davide Spadero, Maria Lodorica Gullino. State of art and future prospects of the biological control of post harvest fruit diseases. International Journal of Food Microbiology. 2004; 91:185-194.
4. Demoz BT, Korsten L. *Bacillus subtilis* attachment, colonization, and survival on avocado flowers and its mode of action on stem-end rot pathogens. Biological Control. 2006; 37:68-74.
5. Edwards SG, Seddon B. Mode of antagonism of *Brevibacillus brevis* against *Botrytis cinerea in vitro*. J Appl. Microbiol. 2001; 91(4):652-659.
6. Gueldner RC, Reilly CC, Pusey RL, Costello CE, Arrendale RF, Cox RH *et al*. Isolation and identification of iturins as antifungal peptides in biological control of peach brown rot with *Bacillus subtilis*. J Agric. Food Chem. 1988; 36:366-370.
7. Ippolito A, Nigro F, Romanazzi G, Campanella V. Field application of *Aureobasidium pullulans* against Botrytis storage rot of strawberry. In: Bertolini, P., Sijmons, P.C., Guerzoni, M.E., Serra, F. (Eds.), Non-conventional Methods for the Control of Post-Harvest Disease and Microbiological Spoilage, Workshop Proceedings COST 914-COST 915. Bologna, Italy, 1997b, 127-133.
8. Ippolito A, Nigro F. Impact of preharvest application of biological control agents on post harvest disease of fresh fruits and vegetables. Crop Protection. 2000; 19:715-723.
9. Jiang YM, Zhu XR, Li YB. Postharvest control oflitchi fruit rot by *Bacillus subtilis.* Food Science and Technology. 2001; 34:430-436.
10. Krishna Radha E, Kumar Shamsher P, Kumar Veerendra B. Study on antioxidant activity and strain development of *Bacillus subtilis* (MTCC10619). Journal of Agricultural Technology. 2011; 7(6):1693-1703.
11. Lise Korsten, Jan Katze M. Post harvest Biological control of Avocado Post harvest Diseases. Pro. of Second World Avocado Congress, 1992, 473-477.
12. Lise Korsten, De Jager EE. Mode of action of *Bacillus subtilis* for control of Avocado postharvest pathogen. South African Avocado Association yearbook. 1995; 18:124-130.
13. Morikawa M. Beneficial biofilm formation by industrial bacteria *Bacillus subtilis* and related species. J Biosci. Bioeng. 2006; 101:1-8.
14. Barakat MR, Mohamed MAA, Essa MA, Zeinab Zaki A. Effect of Using Some Biological Post Harvest Treatments on Storability of Washington Navel Orange Fruits Compared with Imazalil Post Harvest Chemical Treatments. Journal of Horticultural Science & Ornamental Plants. 2012; 4(1):50-57.
15. Omur Baysal, Mikail Caliskan, Ozlem Yesilova. An inhibitory effect of a new *Bacillus subtilis* strain (E407) against *Fusarium oxysprum* f. sp. *Radicislyco persici*. Physiological and Molecular pathology. 2008; 73:25-32.
16. Pusey PL, Wilson CL. Postharvest biological control of stone fruit brown rot by *Bacillus subtilis*. Plant Disease. 1984; 68:753-756.
17. Qi DM, Hui M, Liang QM, Niu TG. Postharvest biological control of blue mold and black spot of applepear (*Pyrus bretschneideri* Rehd.) fruit by *Bacillus subtilis* H110. Chinese Journal of Applied and Environmental Biology. 2005; 11:171-174.
18. Ranganna S. Hand Book of analysis and quality control for fruits and vegetables products. Second ed. Tata Mc. Grate Hill Publication, Co. Ltd., New Delhi, 2000.
19. Castoria RF, De Curtis G, Lima L, Caperto S, Pacifico V, De Cicco. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogen of fruit: study on its mode of action. Post harvest Biology and Technology. 2001; 22:7-17
20. Samir Droby, Michael Wisniewski, Dumitra Macarisin, Charles Wilson. Twenty years of postharvest bio-control research: I it time for a new paradigm? Post harvest Biology and Technology. 2009; 52:137-145.
21. Sharma RR, Singh D, Singh R. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. Biological Control. 2009; 50:205-221
22. Singh V, Deverall BJ. *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. Transactions of the British Mycological Society. 1984; 83:487-490.
23. Utkhede RS, Sholberg PL. *In vitro* inhibition of plant pathogens by *Bacillus subtilis* and *Enterobacter aerogenes* and *in vivo* control of two postharvest cherry diseases. Canadian Journal of Microbiology. 1986; 32:963-967.
24. Wang YW, Hu WB, Xu L. Identification of the antagonistic *Bacillus* strain on melon fruit surface. Acta Phytopathologica Sinica. 2008; 38:317-324.
25. Wang Y, Xu Z, Zhu P, Liu Y, Zhang Z, Mastuda Y *et al*. Postharvest Biological Control Of Melon Pathogens Using *Bacillus subtilis* EXWB1. Journal of Plant Pathology. 2010; 92(3):645-652.
26. Zhao Y, Shao XF, Tu K, Chen JK. Inhibitory effect of *Bacillus subtilis* B10 on the diseases of postharvest strawberry. Journal of Fruit Science. 2007; 24:339-343.