DOI : 10.5958/0974-0112.2019.00079.3

Indian J. Hort. 76(3), September 2019: 502-507

Improved control on decay and postharvest quality deterioration of strawberry by microbial antagonists

Swarajya Laxmi Nayak, Shruti Sethi*, R.R. Sharma, Dinesh Singh, Surender Singh*****

Division of Food Science and Postharvest Technology, ICAR- Indian Agricultural Research Institute, New Delhi

ABSTRACT

Strawberry is a fruit of high commerce but it is prone to many postharvest diseases such as gray mold, *Rhizopus* rot, leather rot and *Aspergillus* rot. Although various fungicides have been recommended for the management of postharvest diseases of strawberry but due to carcinogenicity and acute residual toxicity, their use is discouraged. Therefore, the postharvest application of antagonistic microorganisms has emerged as an effective strategy to combat decay caused by fungi. Three micro-organisms viz., *Debaryomyces hansenii*, *Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were used at a concentration of 10^7 log cfu/ml to control the postharvest diseases of 'Winter Dawn' strawberry fruits. Treated fruits were stored under ambient (25±2 ºC and 50-55% RH) and low temperature (5±1 ºC and 90% RH) storage conditions. Results revealed that among those antagonists, *D. hansenii* was able to effectively control the postharvest diseases of strawberry and maintain the fruit quality. Minimum decay (20.42%) was observed in *Debaryomyces hansenii* treated fruit whereas maximum fruit decay was observed in water washed (control) strawberry fruit (26.36%) under ambient conditions. Under ambient storage conditions, the treated fruits sustained till three days whereas at low temperature conditions, the fruit remained acceptable for 10 days.The physio-chemical parameters were well maintained in treated strawberry fruits till the end of storage both under low as well as ambient storage conditions as compared to the control fruit.

Key words: *Fragaria* × *ananassa*, biocontrol agent, *Debaryomyces hansenii, Bacillus subtilis, Pseudomonas fluorescens.*

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is a non-climacteric fruit relished for its taste, attractive red colour and high nutritional value. It is an important source of antioxidants, anti-inflammatory phytonutrients and various other polyphenolic compounds (Khan *et al*., 8).The delicate and perishable nature of the fruit makes it labile to damage during handling and storage. Furthermore, it is prone to attack by pathogens resulting in many postharvest diseases resulting in significant postharvest losses (Davis, 3). Conventionally, sanitizing agents such as chlorine, hydrogen peroxide and synthetic fungicides have been applied to control the postharvest diseases of strawberry. However, they pose a threat to human life and the environment (El-Hadidi, 6). The synthetic chemicals impose carcinogenicity, teratogenicity and acute residual toxicity. Application of antagonistic microorganisms has emerged as an effective strategy to combat major postharvest fungal pathogens as they offer a cost effective, eco-friendly and permanent solution (Sharma *et al*., 12).Although the mechanism by which the biocontrol agents suppress the microorganism isnot clearly understood,but competition for nutrients and space, antibiosis, direct parasitism and induction of resistance in the host tissue are a few widely accepted explanations (Sharma *et al*., 12). Accordingly, several attempts have been made to use biocontrol agents in several fruits. For instance, *Bacillus subtilis* and *Trichoderma harzianum* control gray mould causing pathogen, *B. cinerea* effectively. Combined treatments of *B. amyloliquefaciens*, *Aureobasidium pullulans* and *Beauveria bassiana* have shown improved control of gray mould on strawberry fruit (Sylla *et al*., 15). Pratella and Mari (11) have reported postharvest application of *T. harzianum, T. viride*, *Gliocladium roseum* and *Paecilomyces variotii* to be better in controlling *Botrytis* rot in strawberries and *Alternaria* rot in lemons than pre-harvest applications. The present study was aimed to assess the efficacy of postharvest application of three antagonists on the strawberry fruits during storage.

MATERIALS AND METHODS

'Winter Dawn' strawberry fruits were harvested from a farmer field located in village Palla, Delhi, India at 70 % colour development stage in the early hours of the day. Sound fruits with uniform shape, size and colour were selected and transported to the laboratory at ICAR-Indian Agricultural Research Institute, New Delhi-110 012.

*Corresponding author's Email: docsethi@gmail.com
**Division of Plant Pathology, ICAR-IARI, New Delhi
***Division of Microbiology, Central University of Haryana

Potential strawberry postharvest pathogens, namely *Aspergillus niger* ITCC 6354, *Rhizophus stolonifer* ITCC 5100 and *Colletotrichum dematium* ITCC 5363 were obtained from Indian Type Culture Collection, Division of Plant Pathology, ICAR-IARI, New Delhi.

Microbial antagonists, namely, *Debaryomyces hansenii* MTCC1001*, Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 were procured form the Division of Plant Pathology, ICAR-IARI, New Delhi and applied to strawberry fruits at a concentration of 10^7 cfu/ml as dip treatment for 5 min. against the targeted pathogens.

The *in vitro* study was performed under aseptic laminar conditions with three replications per treatment. Pure culture of the isolated fungus was obtained by single spore isolation technique (Booth, 2) and maintained in PDA slants. The concentration of biocontrol agents was adjusted using a spectrophotometer to approximately 10^7cfu/ml ($OD_{600\,nm}$ = 0.5) and used as bacterial inoculums. Petri dishes (90 mm diameter) with potato dextrose agar (PDA) media were inoculated with a loopful of fungal mycelium with biocontrol agents at a concentration of 10^7 cfu/ml. A 2 cm distance was maintained between fungal mycelium (*A. niger*, *R. stolonifer* and *C. dematium*) and biocontrol agents (*D. hansenii, B. subtilis* and *P. fluorescens*) in petri dish. The petri dishes were incubated for 4 days at a temperature of 27º C. Observations were taken by measuring the extent of mycelial growth and the inhibitory action of biocontrol agents as evidenced by suppression of mycelial growth as under.

$$\text{Inhibition (\%)} = \frac{\text{Growth of fungus (mm) in control plate}}{\text{Growth of test fungus (mm) in treated plates}} \times 100$$

For the *in-vivo* study, the fruits were dipped in solution containing bioagents, namely *Debaryomyces hansenii* MTCC1001*, Bacillus subtilis* DTBS-5 and *Pseudomonas fluorescens* DTPF-3 at a concentration of 10^7 cfu/ml for 5 min. Two controls were taken for the experimental study; Control 1 (no water wash) wherein strawberry fruits without any treatment/ wash were taken and control 2: strawberry fruits given a water dip for 5 min. The fruit were air-dried, packed in plastic punnets and stored under ambient (25±2º C and 50-55% RH) and low temperature (5±1º C and 90% RH) storage conditions. Three replications for each treatment comprising of 50 fruits per replication were taken for the study and the physio-chemical analysis was done after regular intervals.

Total microbial count was determined by spread plate method. Serial dilution was done appropriately by using distilled water. A small volume (0.1µl) of the sample was pipetted and spread evenly with the help of a sterilized bent glass rod in the plates containing standard plate count agar (SPCA) and malt extract glucose yeast extract peptone agar (MGYP) as a selective media followed by incubation for 18- 24 h at 27 °C. The colonies obtained were multiplied by the appropriate dilution factor to determine the number of cfu/ml in the original sample. Entire microbial count study was done under aseptic laminar conditions.

Decay of the packed fruits during storage was assessed by calculating the percentage of visibly spoiled fruits in a punnet at every interval.

Physiological loss in weight of the treated and control strawberry fruits at each interval was determined by calculating the difference between the weight of the fruitsat the beginning of the experiment and the final weight of the fruits at the time of measurement (Kumar *et al*., 9)

Firmness (Newton) of the strawberries was determined by using a texture analyzer (model: TA+Di, Stable micro systems, UK) using a puncture probe with pre-test of 5 mm/second (Kumar *et al*., 9)

The experiment was conducted in a factorial completely randomised design with three replications, each replication having 50 fruits. Data analysis was performed using SAS 9.3 software and Duncan's Multiple Range Test (DMRT) was done to compare the treatments and the storage period.

RESULTS AND DISCUSSION

In vitro studies revealed that the application of antagonistic yeast, *Debaryomyces hansenii* significantly controlled the pathogen, *Rhizophus stolonifer* followed by*Aspergillus niger* and *Colletotrichum dematium*, with a inhibition of 66%, 56% and ~50%, respectively (Fig. 1). Further, *Pseudomonas fluorescens* DTPF-3 significantly inhibited *R. stolonifer* by nearly 55%, followed by 53% and 51% inhibition for *A. niger* and *C. dematium*, respectively under *in vitro* conditions, till the 5th day of incubation at 27 ºC. Similarly, *Bacillus subtilis* DTBS-5 significantly inhibited the pathogen *R. stolonifer* by nearly 49%, *A. niger* by nearly 47% and *C. dematium* by nearly 42% under *in vitro* conditions. *Debaryomyces hansenii* was adjudged the best among the three biocontrol agents applied on 'Winter Dawn' strawberry fruits as it could significantly control all the three pathogens under *in vitro* conditions. However, it was most effective for controlling *R. stolonifer*, which caused soft rot in most of the fruits. The possible mechanism, through which biocontrol agents suppress the pathogenic fungi are attributed to direct parasitism, antibiosis (production of antibiotics) and competition for nutrient and space (Janisiewicz *et al*., 7; Sharma *et al*., 12).

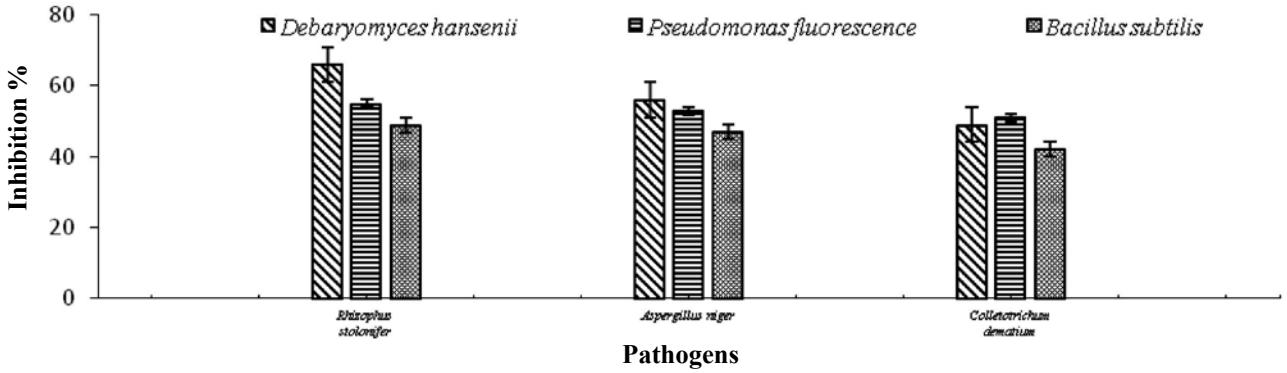

Fig. 1. *In vitro* efficacy of *Debaryomyces hansenii*, *Pseudomonas fluorescens* and *Bacillus subtilis* against different pathogens of strawberry.

It is evident from the Fig. 2 (a, b) that there was no bacterial growth on strawberry fruit whilst, maximum yeast count was exhibited by the yeast, *D. hansenii* treated fruits (4.19 log cfu/ml). Among the two controls used in the experiment, maximum yeast count (4.12 log cfu/ml) was observed in water dipped strawberries at the end of three days storage under ambient conditions (Fig. 2a). Fig. 2b represents the total microbial count of strawberry at the end of 10th day of storage under low temperature conditions. Maximum yeast count (3.83 log cfu/ml) was observed for *D. hansenii* treated fruits that varied non-significantly with those treated with *P. fluorescens* (3.60 log cfu/ml) or *Bacillus subtilis* (3.62 log cfu/ml). No bacterial contamination was observed in the inoculated plates and the strawberry fruits. The fact that strawberry is acidic in nature with a pH below 4.5, it may hinder the chances of bacterial contamination. Amongst different microbial antagonists, *D. hansenii* treated fruits also exhibited least decay *in vitro* conditions, perhaps because this yeast has the ability to multiply rapidly (Demirci, 4) and compete with the pathogen thus,inhibiting its growth effectively. We also observed a higher plate count for this microorganism due to its high growth rate. *P. flourescens* and *B. subtilis* also displayed significant reduction in total microbial count.

Amongst all BCAs, *D. hansenii* treated strawberry fruits had least fruit decay followed by *P. fluorescens*

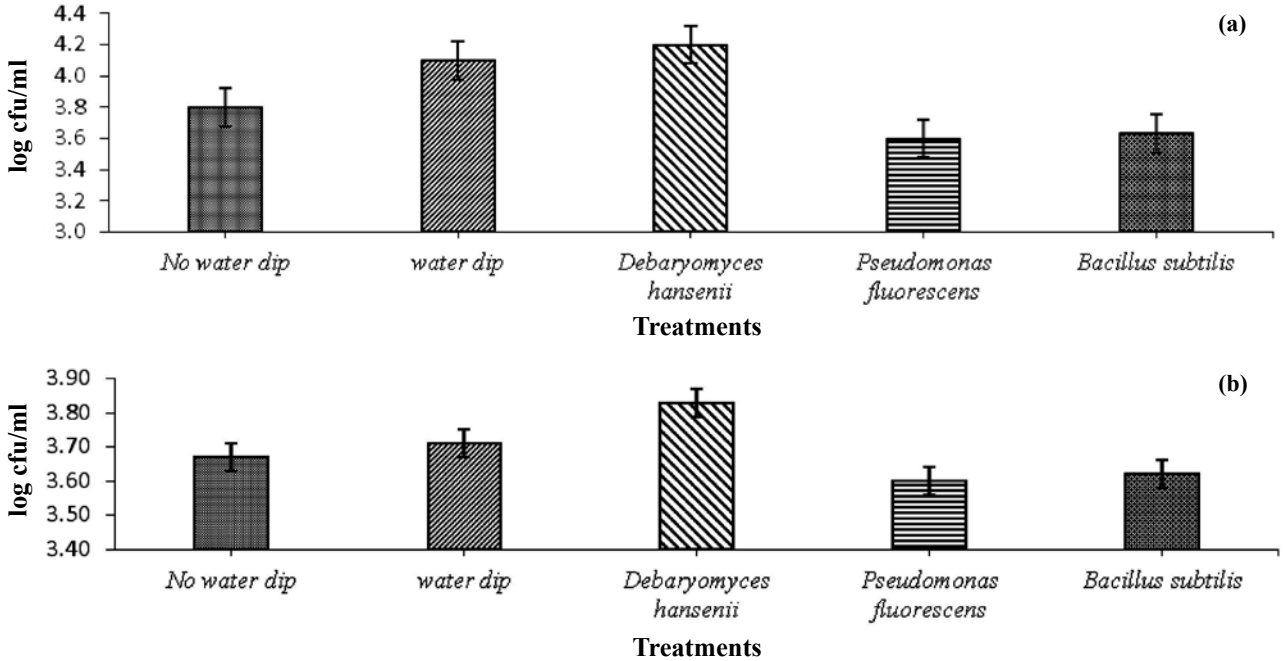

Fig. 2. Total microbial count (log cfu/ ml) in strawberry fruits cv. 'Winter Dawn' during a) ambient (25±2ºC and 50-55% RH) and b) low temperature (5±1ºC and 90% RH) storage.(n = 3, vertical bars represent standard deviation).

and *B. subtilis* treated fruits both under ambient as well as low temperature storage conditions till the end of storage. During the 3 days of storage under ambient conditions, minimum fruit decay (20.42%) was observed in *D. hansenii* treated fruits whereas maximum fruit decaywas observed in water washed strawberry fruits (26.36%) (Fig 3a). Under low temperature conditions also, *D. hansenii* controlled the decay till the 10th day of storage (Fig 3b) while maximum decay was recorded in fruits which were given water dip treatment. The differences in suppression of decay among the three biocontrol agents might be attributed to respective mode of action which may be the competition for space and nutrients, production of antibiotics and/or direct parasitism (Janisiewicz *et al*., 7; El-Ghaouth *et al*., 5; Sharma *et al*., 12). Other possible reason for low rate of decay could be the suppression of enzymatic activity of pathogens by production of lytic enzymes by microbial antagonists, which results in degradation of cell wall of the pathogens (Agrios, 1; Janisiewicz *et al*., 7).

The minimum weight loss was observed in *D.hansenii* treated fruits (2.27%), followed by *P. fluorescens* DTPF-3 treated fruits (2.37%) (Table 1). Both controls showed a higher loss in weight throughout storage period. Among the control samples, water washed strawberry fruits exhibited higher weight loss (5.7%) than the fruits which were not water washed (5.4%) under ambient conditions.

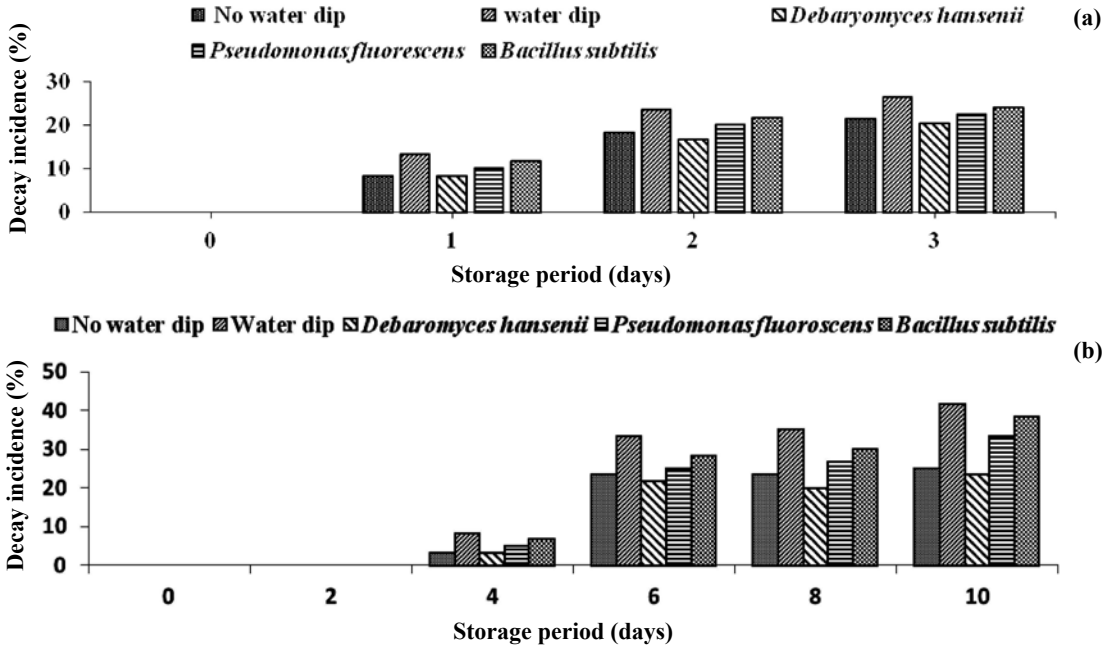

Fig. 3. Influence of biocontrol agents on strawberry fruit decay (%) during a) ambient (25±2ºC and 50-55% RH) and b) low temperature (5±1ºC and 90% RH) storage.(n = 3, vertical bars represent standard deviation).

Table 1. Influence of biocontrol agents on PLW (%) of strawberry fruits cv. 'Winter Dawn' under different conditions during storage.

Treatments	Ambient (25 ± 2°C and 50-55% RH)				Low temperature (5 ± 1°C and 90% RH)					
	Storage period (days)				Storage period (days)					
	0	1	2	3	0	2	4	6	8	10
Water wash	0^{g}	2.61^{de}	3.48^{c}	5.70^{a}	0^{o}	2.66^{kjl}	2.89^{j}	3.78^{gf}	4.84^{b}	5.77^{a}
No water wash	0^{g}	2.50^{e}	3.02^{dc}	5.42^{ba}	0^{o}	2.58^{kl}	2.78^{kjl}	3.62^{gh}	4.71^{cb}	5.48^{a}
Debaryomyces hansenii	0^{g}	1.60^{f}	2.35^{e}	5.13^{b}	0^{o}	1.79^{n}	2.16^{m}	2.87^{kj}	3.71^{g}	4.92^{b}
Pseudomonas fluorescens DTBS-5	0^{g}	1.71^{f}	2.52^{e}	5.24^{ba}	0^{o}	1.93^{nm}	2.55^{l}	3.26^{i}	4.07^{ef}	5.17^{ed}
Bacillus subtilis DTPF-3	0^{g}	1.82^{f}	2.81^{de}	5.39^{ba}	0^{o}	2.22^{m}	2.61^{kjl}	3.33^{ih}	4.46^{cd}	5.26^{ed}

*Means with same superscript are not significantly different

Table 2. Impact of biocontrol agents on firmness (N) of strawberry fruits cv. 'Winter Dawn' during storage under different conditions.

Treatments	Ambient (25 ± 2ºC and 50-55% RH)				Low temperature (5 ± 1ºC and 90% RH)					
	Storage period (days)				Storage period (days)					
	0	1	2	3	0	2	4	6	8	10
Water wash	1.30^{a}	1.28^{bdac}	1.22^{ebdhcf}	1.21^{gh}	1.30^{bac}	1.27^{ebdfcg}	1.25^{ehjfig}	1.22^{khjli}	1.19^{mln}	1.15^{n}
No water wash	1.29^{ba}	1.26^{ebdacf}	1.23^{edghf}	1.21^{h}	1.30^{bac}	1.28^{ebdfc}	1.25^{ehdfig}	1.23^{khjlig}	1.20^{kml}	1.17^{mn}
Debaryomyces hansenii	1.29^{ba}	1.27^{ebdac}	1.25^{ebdghcf}	1.23^{eghf}	1.32^{a}	1.31^{ba}	1.30^{bdac}	1.28^{ebdac}	1.26^{ehdfcg}	1.25^{ehjfig}
Pseudomonas fluorescens DTBS-5	1.27^{ebdac}	1.26^{ebdgcf}	1.24^{ebdghcf}	1.22^{ghf}	1.30^{bac}	1.29^{ebdac}	1.27^{ebdfcg}	1.25^{ehdfig}	1.23^{khjlig}	1.21^{kmjl}
Bacillus subtilis DTPF-3	1.28^{bac}	1.26^{ebdacf}	1.24^{edghcf}	1.21^{gh}	1.32^{a}	1.30^{bac}	1.28^{ebdac}	1.26^{ehdfcg}	1.24^{khjfig}	1.21^{kmjli}

*Means with same superscript are not significantly different.

Under low temperature storage conditions, the lowest PLW (2.57%) was observed in fruits treated with *D. hansenii* that was significantly higher than water washed control fruits (3.32%). Overall, *D. hansenii* treatment resulted in lower loss in weight of strawberry fruits as compared to other biocontrol agents and control fruits. The lower incidence of fruit decay and suppressed metabolic activity in microbial antagonist-treated fruits results in reduced weight loss as also reported earlier by Singh *et al.* (14) and Ladaniya (10).

Fruit firmness decreased in all the treatments with the progression of storage under both low and ambient storage condition. The highest fruit firmness was observed in *Debaryomyces hansenii* treated fruits (1.23 N) at the end of storage period, whereas the lowest firmness was found in water washed strawberry fruits on the 2ndday of storage (Table 2). Rest all other treatments showed nearly similar firmness values for the strawberry fruits (1.21 N). Under low temperature storage conditions also, firmness values showed a declining trend till 10thday of storage. Least loss of firmness (5.3%) was obtained in fruits treated with *Debaryomyces hansenii*. Similar effects of *Debaromyces hansenii* have been reported in peach earlier by Singh and Mondal (13). Treatment of BCA leads to suppression of the activity of cell wall degrading enzymes like pectin methyl esterase (PME) that further prevents softening of tissues (Zhang *et al*., 16).

In this study, we observed that although, postharvest application of all the three antagonists had a significant effect on retention of strawberry fruit quality but *Debaryomyces hansenii* proved to be best in terms of its *in vitro* and *in vivo* efficacy. The antagonistic yeast, *Debaryomyces hansenii* significantly controlled decay and maintained postharvest quality of strawberry cv. 'Winter Dawn' up to 10 days at 5 ± 1 ºC storage and 3 days under ambient storage condition (25±2ºC).

ACKNOWLEDGEMENTS

We would like to thank Indian Type Culture Collection, Division of Plant Pathology, ICAR-IARI, New Delhi, for providing the pure cultures.

REFERENCES

1. Agrios, G. N. 2005. Plant Pathology, Academic Press, New York.
2. Booth, C. 1971. The Genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England, 237.
3. Davis, J. B. 2009. Strawberry success. Organic Gardening, **56**: 52–56.
4. Demirci, F. 2011. Effects of *Pseudomonas fluoresens* and *Candida famata* on blue mould of citrus caused by *Penicillum italicum*. *Australian J. Crop Sci* **5**: 344-49.
5. El-Ghaouth, A., Droby, S., Wilson, C. L., Wisniewski, M., Smilanick, J., and Korsten, L. 2004. Biological control of postharvest diseases of fruits and vegetables. In: Khachatourians, G.G. and Arora, D.K. (eds) *Applied Mycology and Biotechnology*: Agriculture and Food Production. Elsevier Science, Amsterdam, Netherlands, 11-27.
6. El-Hadidi, M. F. 1993. Studies on pesticides in fresh and processed apple fruits under certain developed pest control programs. Ph.D. Dissertation. Michigan State University. East Lanshing, MT.
7. Janisiewicz, W. J., Tworkoski, T. J. and Sharer, C. 2000. Characterizing the mechanism of biological control of postharvest diseases on

fruits with a simple method of study competition for nutrients. *Phytopath.* **90**: 1196-1200.

8. Khan, N., Syed, D.N., Ahmad, N., & Mukhtar, H. 2013. Fisetin: a dietary antioxidant for health promotion. *Antioxidants Redox Signaling,* **19**:151-62.
9. Kumar, P., Sethi, S., Sharma, R. R., Srivastav, M., Varghese, E. (2017) Effect of chitosan coating on postharvest life and quality of plum during storage at low temperature. *Sci. Hort.*, **226**: 104-09.
10. Ladaniya, M. S. 2011. Physico-chemical, respiratory and fungicide residue changes in wax coated mandarin fruit stored at chilling temperature with intermittent warming. *J. Food Sci. Technol.* **48**: 150-58.
11. Pratella, G. C. and Mari, M. 1993. Effectiveness of *Trichoderma, Gliocladium* and *Paecilomyces* in postharvest fruit Protection. *Postharvest Biol. Technol.* **3**: 49-56.
12. Sharma, R. R., Singh, D. and Singh, R. 2009. Biological control of postharvest diseases of fruits and vegetables by microbial antagonists: A review. *Biol. Control,* **50**: 205-21.
13. Singh, D. and Mandal, G. 2006. Improved control of *Rhizopus stolonifer* - induced storage rot of peach with hot water and antagonistic yeast, *Debaryomyces hansenii. Ind. Phytopath.* **59**: 168-73.
14. Singh, S., Singh, A.K. and Joshi, H.K. 2003. Storage behavior of Indian gooseberry (*Embilica officinalis*) cultivars in semi-arid ecosystem of Gujarat. *Indian J. Agric. Res.* **73**: 530-34.
15. Sylla, J., Alsanius, B.W., Kruger, E. and Wohanka, W. 2015. Control of *Botrytis cinerea* in strawberries by biological control agents applied as single or combined treatments. *European J. Plant Pathol*. **143**: 461-71.
16. Zhang, H., Wang, L., Longchuan, M., Dong, Y., Jiang, S., Xu, B. and Zheng, X. 2009. Biocontrol of major postharvest pathogens on postharvest quality parameters. *Biol. Control.* **220**: 79-85.

Received : April, 2018; Revised : August, 2019;
Accepted : August, 2019