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Post-harvest behaviour of peaches (*Prunus persica*) pre-treated with antagonist *Debaryomyces hansenii* and calcium chloride

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ABSTRACT

A study was conducted during 2004-05 to determine the combined effect of *Debaryomyces hansenii* and calcium chloride on post-harvest spoilage and fruit quality of 'Sharbati' peaches (*Prunus persica* (L.) Batsch.) during storage. Initially *Rhizopus stolonifer* and *R. macrosporus* were isolated from infected fruits and healthy fruits were inoculated with these strains. The infected fruits were treated either with calcium chloride (2.0%), *D. hansenii* (10^9 cfu/ml), calcium chloride + *D. hansenii*, and untreated fruit served as control. The fruits were then packed in HDPE bags (200 gauges, 1.0% perforation) and stored in cool chamber (5 ± 1° C) for 42 days. Observations were recorded on disease incidence, lesion diameter, fruit spoilage, Physiological loss in weight, and fruit quality parameters. Our studies indicated that disease incidence was maximum in untreated fruit and minimum in calcium chloride + *D. hansenii* treated fruit. Calcium chloride + *D. hansenii* treated fruits were not spoiled up to 35 days under cool chamber, whereas spoilage started after 21 days in control samples. Minimum spoilage of fruit was recorded in *D. hansenii* + calcium chloride (1.37%) as compared to untreated fruit (33.5%) after 42 days of storage. *D. hansenii* + calcium chloride reduced the physiological loss in weight significantly over control after 42 days of storage. Firmness of the fruit was reduced in all the treatments and had no significant variation among the treatments. Total soluble solid content of the fruits increased and acidity decreased in all the treatments during storage. TSS/acid ratio increased initially, but after 28th day, it started declining in all the treatments except in fruits pre-treated with *D. hansenii* + calcium chloride. Thus, our study indicated that although pre-treatment of 'Sharbati' peaches either with calcium chloride or *D. hansenii* alone reduced the spoilage and enhanced their shelf-life significantly, their combination was much more effective in doing so.

Key words: Bioagent, Calcium chloride, *Debaryomyces hansenii*, Peach, Postharvest quality

Peach (*Prunus persica* (L.) Batsch.) is one of the most important stone fruits of India. Although, it is considered as a crop of temperate climate, but it grows profitably well in subtropical climate of northern Indian plains. At present, it is cultivated on an area of 18500 ha, with an annual production of 150 000 metric tonnes. Peaches are considered as rich source of ascorbic acid, carotenoids and phenolic compounds that are good source of antioxidants (Byrne 2002). Peaches are usually consumed as fresh fruits and due to its perishable nature, it is difficult to transport peaches to distant markets and hence, these are stored for longer period. During storage, fruits are subjected to severe losses, which are primarily caused by various post-harvest diseases. Although several pathogens, like *Penicillium expansum*, *Rhizopus stolonifer*, *Alternaria alternata* and *Monilinia fructicola* are associated with postharvest decay of peaches, but Rhizopus rot (*R. stolonifer*) is one of the most important postharvest diseases of peaches in India. Rhizopus rot is responsible for heavy losses in arid climate (Singh 2004). Rhizopus rot is mainly controlled by applying fungicides. However, due to public concern about fungicide residue in fruits and also due to development of fungicide resistance by the pathogens (Korsten 2006), post-harvest application of fungicides in fruit is limiting day-by-day. As a result, alternative means such as heat treatment (Conway *et al*. 2005, Zhang *et al.* 2007, Janisiewicz 2008) and use of bio-control agent (Droby 2006) have emerged as potential techniques to manage prominent postharvest diseases of peaches (Singh 2005, Korsten 2006). Biocontrol technique is considered as a very useful technique as utilization of antagonistic microorganism reduces environmental pollution provides effective control of postharvest diseases and the product is of high quality and safe for consumption (Singh 2002, Zheng *et al.* 2004). However biocontrol has its own limitations, as it works more as a preventive measure and not as eradicative. Thus, search was started to enhance the efficacy of bioagents

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for effective control of postharvest diseases in various fruits (Singh 2005, Cao *et al*. 2008).

Such studies were lacking in India and hence, the bio-efficacy of *D. hansenii* alone and in combination with calcium chloride was studied to find out their effect on spoilage, incidence of mycoflora and shelf-life of peaches at low temperature.

MATERIALS AND METHODS

'Sharbati' peaches were harvested from commercial private orchard, located at Abohar, (Punjab) during 2004–05 growing season. Fruits having uniform size, light greenish yellow colour (L = 62.82; a = –4.36; b = 24.57 and Yi = 63.87), good firmness (3.089 N) and free from defects and injury were selected for the studies.

D. hansenii, antagonistic yeast, was procured from the Institute of Microbial Technology, Chandigarh (MTTC1001). It was grown in 250 ml conical flask containing 50 ml of malt yeast extract broth (3.0 g malt extract, 3.0 g yeast extract, 5.0 g glucose and 1,000 ml distilled water) at 28 ± 1°C for 48 hr. Yeast cells were pelleted by centrifugation, and were re-suspended in sterilized distilled water and their concentration was adjusted to 10^9 cells/ml by means of a haemocytometer.

R. macrosporus and *R. stolonifer* were isolated from the infected 'Sharbati' peaches and maintained on potato dextrose agar (PDA) medium. The spore suspension was prepared by growing both the species of *Rhizopus* on PDA at 25 ±1°C for 7 days. The culture was flooded with sterile distilled water containing Tween-20 (0.05% v/v) and subsequently adjusted the concentration of the suspension to 10^5 spores/ml.

Freshly harvested peaches were brought to the laboratory, washed with tap water and disinfected with chlorinated water (2.0%) for 2 min. Fruits were again washed with tap water and air-dried. The concentration of *D. hansenii* (10^9 cfu/ml) with or without calcium chloride (2.0% w/v) was prepared. A 4 mm deep and 3 mm in diameter uniform wounds were made on one side of each fruit. One set of wounded fruits were inoculated with pathogens only, which was treated as control. The wounded fruits were treated with calcium chloride (2.0%) by dipping followed by pouring *D. hansenii* (0.5 ml) suspension (10^9 cfu/ml) either alone or in combination with calcium chloride. After 3 hr, suspension of *R. stolonifer* and *R. macrosporus* (0.2 ml) was added to each wound separately. Treated fruits were arranged separately from each treatment in a plastic tray and covered with polyethylene (200 gauge) bags to maintain relative humidity. All the fruits were kept at 25 ± 2°C and 85 – 90% relative humidity for 4 days after inoculation. Disease incidence and severity (lesion diameter in cm) was recorded after 4 days. In each treatment, 3 replicates each of 10 fruits were arranged in complete randomized design. The experiment was repeated twice. For observations on spoilage and quality, fruits were treated with calcium chloride (2.0% w/v), *D. hansenii* culture (2 days old and having 10^9 cfu/ml), and calcium chloride (2.0% w/v) + *D. hansenii* culture (10^9 cfu/ml). The fruits were dipped in calcium chloride solution (2.0%) either alone or in combination with *D. hansenii* for 5 min. Fruits in control were also dipped in potable water for 5 min. All the fruits were dried under ceiling fan for 30 min and then packed in HDPE polyethylene bags (200 guage, 1% perforation). In each bag, 12 fruits were packed with three replications. The fruits were kept in cool chamber (5 ± 1° C) for 42 days. Observation on physiological in weight (PLW), decay and physico-chemical properties of stored fruits were recorded at 7 days interval during storage. Per cent loss in weight and total decay loss (%) were calculated on weight basis. Total soluble solids (TSS), acidity, TSS/acid ratio and firmness of fruits were recorded before storage and at 7 days intervals during storage. The TSS was determined by hand refractometer and was expressed on percent basis. Acidity was determined by titrating the known amount of samples with 0.1 NaOH using phenolphthalein as indicator and was expressed as percentage (Ranganna 2000). The pulp/stone ratio of the fruit was recorded on weight basis. The fruits were evaluated for firmness with texture analyzer (TA- Xti stable Microsystems, UK). The fruits were punched at 3 places, ie at tip, middle and near stem end point with 2 mm diameter probe up to the depth of 6 mm without removing peel. Pre-test speed @ 1.50 mm/sec, test speed @ 0.5 mm/sec, post-test speed @ 10.0 mm/sec and trigger @ 0.20 N of the texture analyzer were maintained during testing.

The data obtained from all the characters of both the years were averaged and subjected to analysis as per standard procedure. The data, which were obtained in percentage, were first transformed to arc sine values before analysis. Analysis of variance (ANOVA) was applied by using Factorial CRD (Panse and Shukatme 1984).

RESULTS AND DISCUSSION

Incidence and severity of disease

Dipping of fruit either in the suspension of calcium chloride or *D. hansenii* or in their combination has reduced the incidence of Rhizopus rot in peaches caused either by *R. stolonifer* or *R. macrosporus* over control (Fig 1) ($P< 0.05$). However, the combined influence of *D. hansenii* and calcium chloride was much more significant in reducing the incidence of *R. stolonifer* (39.3%) or *R. macrosporus* (43.7%) or the lesion diameter than either calcium chloride or *D. hansenii* alone (Fig 1). In control, nearly 94% and 91% fruits were infected by *R. stolonifer* and *R. macrosporus*, respectively, whereas the incidence was very lesser in fruit pre-treated with calcium chloride + *D. hansenii*. The lesion diameter was also highest in fruit under control (nearly 5 cm), significantly lower in the treated fruits and lowest in those receiving pre-treatment of *D. hansenii* + calcium chloride (Fig 1). Further, between two species of *Rhizopus*, *R. stolonifer* was observed to more sensitive to either calcium

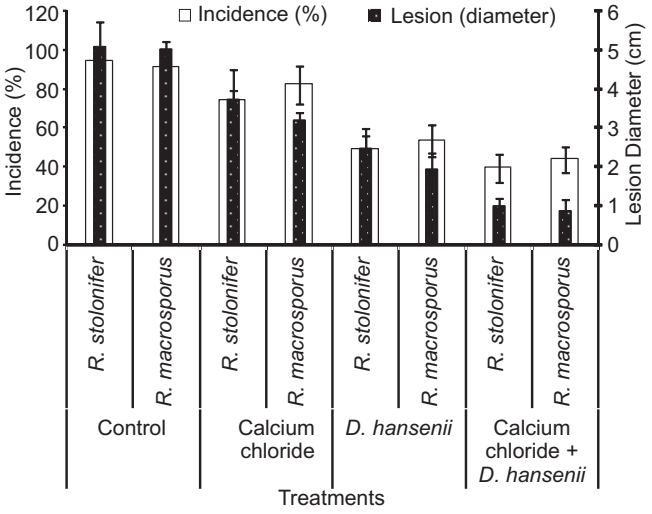

Fig 1 Incidence of *Rhizopus stolonifer* and R. *macrosporus* on peaches treated with calcium chloride and *D. hansenii*

chloride, *D. hansenii* or their combination than *R. macrosporus* (Fig 1). Reduction in the incidence of Rhizopus rot and inhibition in the lesion diameter of peaches pre-treated either with calcium chloride or *D. hansenii* or by combining yeast antagonist, *D. hansenii* with calcium chloride indicated that combining calcium chloride with yeast suspension significantly enhanced biocontrol activity of *D. hansenii* in peaches to control Rhizopus rot. The same trend on enhancement of biocontrol activity by the addition of calcium chloride has also been reported by using *Pichia guilliermondii* (Tian *et al*. 2002), *Candida* spp and *Aureobasidium pullulans* (Ippolito *et al.* 2005).

Fruit spoilage and physiological loss in weight

In general, fruit spoilage increased with the increase in storage period from 0 to 42nd day. Pre-treatment of peaches either with calcium chloride or *D. hansenii* alone or in their combination has reduced the fruit spoilage (%) significantly over control (Fig. 2). At the end of 42nd days of storage, the fruit spoilage was minimum (1.5%) in fruits pre-treated with calcium chloride and *D. hansenii*, followed by calcium chloride alone (2.03%) and maximum (6.03%) in untreated fruits (control).The same trend in fruit spoilage was observed with the increase in storage period (Fig 2).

Physiological loss in weight increased with the increase in storage period. In general, physiological loss in weight was highest at the end of 42nd day of storage and lowest on 7th day of storage. Further, physiological loss in weight was lesser in pre-treated fruit than those in control. In all, physiological loss in weight was lesser in fruit pre-treated with calcium chloride + *D. hansenii* than those treated either with calcium chloride or D. *hansenii* alone or in control (Fig. 3). The significant variations in respect to fruit spoilage illustrated that *D. hansenii* significantly reduced the natural infection of mould on peaches caused by *R. macrosporus*

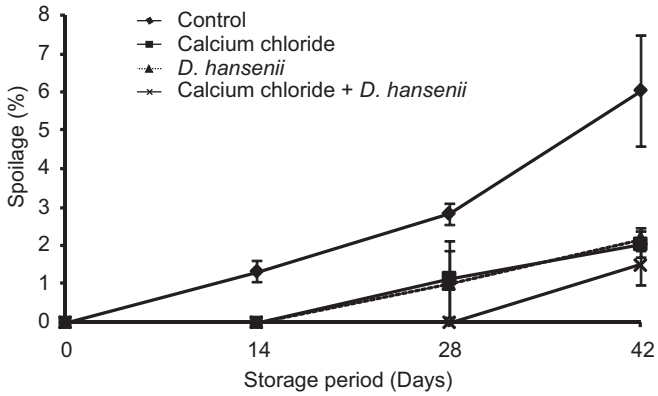

Fig 2 Spoilage (%) loss in peaches treated with calcium chloride and *D. hansenii* under cool chamber

and *R. stolonifer*. It has earlier been reported that initial inoculum's load of bioagent *D. hansenii* affected the growth of *R. macrosporus* (Singh 2004) and biocontrol effect is achieved when actively multiplying populations of yeast were inoculated in wounds. Postharvest calcium chloride treatments increased the shelf- life of pear fruits and reduced decay (Zhang *et al*. 2005). Calcium salt improved the efficacy of yeast biocontrol agent and controlled mould (Zheng *et al.* 2004). Further, it has been reported that calcium salts reduced the infection of grape wounds by *P. digitatum*, which may be due to their direct effect on host tissue, which make cell walls more resistant to enzymatic degradation or the inhibition of fungal pectinolytic enzymes (Zheng *et al.* 2004). The mechanism by which calcium enhances the biocontrol effect of yeast antagonists on fruit is still not known. Calcium (such as calcium chloride) conserved the qualities of fruits, prevented physiological disorders, reduced the rate of respiration, lessens the solubilization of pectic substance, maintaining the firmness and slows down the ripening process (Magee *et al*. 2002).

Fruit quality attributes

Total soluble solids content of peaches increased to 14°B after 28 days of storage and thereafter these decreased slightly

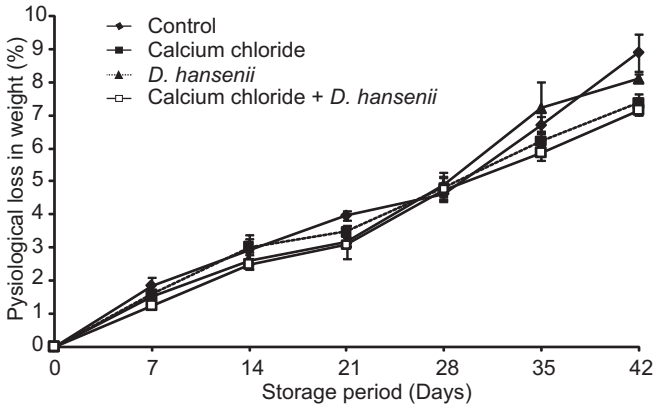

Fig 3 Physiological loss in weight of stored peach fruits treated with calcium chloride and *D. hansenii*

Table 1 Physico-chemical characteristics of peach fruits treated with *Debaryomyces hansenii* and calcium chloride after 42 days of storage.

Treatment	Physiochemical characteristics of peach fruits			
	TSS(° B)	Acidity (%)	TSS/acid ratio	Ascorbic acid (mg/100 ml juice)
Control	11.0	0.21	59.05	1.17
Calcium chloride (2.0% w/v)	11.9	0.25	56.21	2.28
D. hansenii (10^9 cfu/ml)	13.0	0.21	63.80	1.72
Calcium chloride (2.0 w/v) + *D. hansenii* (10^9 cfu/ml)	12.0	0.16	76.37	1.72

Initial value: TSS, 13.0 °B; Acidity, 0.55%; TSS/acid ratio, 23.65; Ascorbic acid, 3.7 mg/100 ml juice
$P = 0.05$: TSS: NS; acidity: NS; TSS/acid ratio: NS; ascorbic acid: NS

(13°B) in all the treatments (Table 1). In general, different treatments could not exert significant influence on TSS content of peaches. The non-significance between the treatments on physico-chemical characters of peach fruits may be due to variation in quality of individual fruits within the replication. Acidity of the fruit before treatment was 0.55% and reduction in the acidity was recorded during storage in all the treatments, which ranged from 0.16 to 0.25% after 42nd day of storage (Table 1). No significant variation ($P < 0.05$) in acidity of fruit was found within the treatment. TSS/acid ratio of the fruit increased during storage and no significant variation was recorded within the treatments. TSS/acid ratio increased (59.29–73.22) up to 28th day and thereafter, it decreased slightly but it was higher than the initial value (23.65). However, it increased in calcium chloride + *D. hansenii* treatment up to 42nd day of storage (76.86), which was higher than other treatments (Table 1). Ascorbic acid content reduced during storage and no treatment was effective to influence them significantly. However, at the end of 42nd day of storage, maximum ascorbic acid (2.28 mg/100 ml juice) was recorded in calcium chloride treated fruit.

This increase in TSS during storage may be due to breakdown of complex polymers into simple substances by hydrolytic enzymes or due to the concentration of juice with the increase in physiological loss in weight. But after 28 days of storage, TSS declined, which may be due to utilization of sugars in respiration and other metabolic activities. Reduction in the acidity of stored fruits was recorded in all the treatments. The same trend was also reported in Indian gooseberry and strawberry (Singh *et al.* 2005, Ram Asrey *et al*. 2004). However, the maximum retention of acidity in $CaCl_2$ treated fruits might be due to reduction in metabolic changes of organic acid into carbon dioxide and water. These results are in agreement with those of Ibrahim (2005) who have used the $CaCl_2$ and showed higher retention of acidity in apricot during storage.

TSS/acid ratio of the fruit increased during storage and no significant variation was recorded within the treatments. However, it increased in calcium chloride + *D. hansenii* treatment up to 42 days, which was higher than the other treatments. TSS/acid ratio increased by the advancement of time, which may be due to increase in TSS and decrease in acidity during storage. Ascorbic acid content of fruits declined in all the treatments. The decline in ascorbic acid in all treatments was observed in this study that is also in line with Egea *et al*. (2006). However the retention of ascorbic acid was better in $CaCl_2$ treated peach fruits.

Firmness of the fruit reduced significantly in all the treatments during storage. Fruit firmness reduced to 3.96 N in calcium chloride treated fruits. However at the end of 42nd day of storage, *D. hansenii* treated fruit retained better texture (2.95 N) and firmness (2.71 N) as compared to other treatments under cool chamber respectively (Table 2). No

Table 2 Firmness and average firmness force of peach fruits treated with *Debaryomyces hansenii* and calcium chloride during storage

Treatment	Firmness of the fruits (N)			Average firmness force (N/mm)		
	14 DAS	28 DAS	42 DAS	14 DAS	28 DAS	42 DAS
Control	3.86	2.26	1.74	8.60	5.74	4.11
Calcium chloride (2.0%w/v)	3.96	2.29	1.96	7.98	5.65	5.15
D. hansenii (10^9 cfu/ml)	3.44	2.95	2.71	7.17	6.48	7.29
Calcium chloride (2.0w/v) + *D. hansenii* (10^9 cfu/ml)	3.62	2.26	1.88	7.57	5.23	4.71

Initial value: Firmness, 7.73 N; Average firmness force, 18.89 N/mm
$P = 0.05$: Firmness: Treatment, NS; Duration, 0.29; Treatment × duration, NS
Average firmness force: treatment, NS; Duration, 0.29; Treatment × duration, NS

significant variation was recorded within the treatments but significant reduction in firmness was recorded over the period of storage. Firmness force required for puncturing the fruit was measured and it was found that initially 3.09 N force was required to puncture the fruit and during storage, firmness force was reduced in all the treatments significantly. No definite trend was recorded and variation within the treatment was 1.00–0.76 N after 42nd day of storage (Table 2).

Firmness of the fruit reduced significantly in all the treatments during storage. However, after 42nd day of storage, *D. hansenii* treated fruit retained better texture and firmness as compared to other treatments under cool chamber. This decline in firmness is obvious because of conversion of insoluble fraction into soluble fractions and may also be due to degradation processes going in the fruit due to respiration and also due to changes in cell wall polysaccharides and uronic acids (Antunes *et al*.2003, Ibrahim 2005).

Thus, our studies have shown the potentiality of using *D. hansenni* in combination with calcium chloride for the control of post harvest decay of the peaches caused by *Rhizopus* species while maintaining the fruit quality for 42 days in cool chamber.

REFERENCES

Antunes M D C, Correia M P, Miguel M G, Martins M A and Neves M A. 2003. The effect of calcium chloride post harvest application on fruit storage ability and quality of 'Beliane' and 'Lindo' apricot (*Prunus armeniaca* L.) cultivar. *Acta Horticulturae* **604**: 721–6.

Byrne D H. 2002. Peach breeding trends. *Acta Horticulturae* **592**: 49–59.

Cao S, Zheng Y, Tang S, Wang K. 2008. Improved control of anthracnose rot in loquat fruit by a combination treatment of *Pichia membranifaciens* with $CaCl_2$. *International Journal of Food Microbiology* **126** (1–2): 216– 20.

Conway W S, Leverentz B, Janisiewicz W J, Saftner R A and Camp M J. 2005. Improving biocontrol using antagonist mixtures with heat and/or sodium bicarbonate to control postharvest decay of apple fruit. *Postharvest Biology and Technology* **36**: 235– 44.

Droby S. 2006. Improving quality and safety of fresh fruit and vegetables after harvest by the use of biocontrol agents and natural materials. *Acta Horticulturae* **709**: 45–51.

Egea M I, Martinez-Madrid M C, Sanchez-Bel P and Romojaro F. 2006. Influence of different storage treatments on antioxidant systems of apricot var. 'Bulide'. *Acta Horticulturae* **717**: 337–41.

Ibrahim F E. 2005. Effect of post harvest treatments on storage ability and keeping quality of Amaar apricot fruits. *Annals of Agricultural Sciences Moshtohors* **43**: 849–67.

Ippolito A, Leonardo S, Isabella P and Franco N. 2005. Control of post harvest rot of sweet cherries by pre- and postharvest applications of *Aureobasidium pullulans* in combination with calcium chloride or sodium bicarbonate. *Postharvest Biology and Technology* **36**: 245–52.

Janisiewicz W J, Saftner R A, Conway W S, Yoder KS. 2008. Control of blue mold decay of apple during commercial controlled atmosphere storage with yeast antagonists and sodium bicarbonate.*Postharvest Biology and Technology* **49** (3): 374–8.

Korsten L. 2006. Advances in control of postharvest diseases in tropical fresh produce. *International Journal of Postharvest Technology and Innovation* **1** (1): 48–61.

Magee R L, Caporaso F and Prakash A.2002. Inhibiting irradiation induced softening in diced tomatoes using a calcium treatment. Session 30 G, Fruit and Vegetable Product: Processed Fruits and Vegetables. Annual meeting and Food Expo- Anaheim, California. (http:/www.ift.confex.com).

Panse V G and Shukatme P V. 1984. *Statistical Methods for Agricultural Workers*. ICAR, New Delhi.

Ram Asrey, Jain R K and Singh R. 2004. Effect of pre- harvest chemical treatments on shelf life of Chandler strawberry (*Fragaria × ananassa*). *Indian Journal of Agricultural Sciences* **74** (9): 485–7.

Singh D. 2002. Bio-efficacy of *Debaryomyces hansenii* on the incidence and growth of *Penicillium italicum* on Kinnow fruits in combination with oil and wax emulsions. *Annals of Plant Protection Science* **10**: 272–6.

Singh D. 2004. Effect of *Debaryomyces hansenii* and calcium salt on fruit rot of peach (*Rhizopus macrosporus*). *Annals of Plant Protection Science* **12** (2): 310–3.

Singh D. 2005. Interactive effect of *Debaryomyces hansenii* and calcium chloride to reduce Rhizopus rot of peaches. *Journal of Mycology and Plant Pathology* **35** (1): 118–21.

Singh S, Singh A K and Joshi H K. 2005. Prolonging storability of Indian gooseberry (*Emblica officinalis*) under semi- arid ecosystem of Gujrat. *Indian Journal of Agricultural Sciences* **75** (11): 647–50.

Tian S P, Fan Q, Xu Y and Wang Y. 2002. Effects of calcium on biocontrol activity of yeast antagonists against the post harvest fungal pathogen *Rhizopus stolonifer*. *Plant Pathology* **51**: 352–8.

Zhang H, Zheng X, Fu C, Xi Y. 2005. Postharvest biological control of gray mold rot of pear with *Cryptococcus laurentii*. *Postharvest Biology and Technology* **35** (1): 79–86.

Zhang H, Zheng X, Wang L, Li S and Liu R. 2007. Effect of antagonist in combination with hot water dips on postharvest Rhizopus rot of strawberries. *Journal of Food Engineering* **78**: 281– 7.

Zheng X, Zhang H and Xi Y. 2004. Effects of *Cryptoccocus laurentii* (Kufferath) Skinner on biocontrol of postharvest decay of Arbutus berries. *Botanical Bulletin of Academic Science* **45**: 55–60.