Role of antagonistic yeast *Candida tropicalis* YZ27 on postharvest life and quality of litchi cv. Bombai

V. Yeka Zhimo[1,*], Jayanta Saha[1], Balveer Singh[2] and Ivi Chakraborty[2]

[1]Department of Plant Pathology, and
[2]Department of Post Harvest Technology,
Bidhan Chandra Krishi Viswavidyalaya, Mohanpur,
Nadia 741 252, India

The efficacy of the antagonistic yeast (*Candida tropicalis* YZ27) as a biological control agent against postharvest natural decay and quality retention of litchi (cv. Bombai) was studied. Application of the yeast antagonist led to rapid colonization on the surface of the fruit and significantly reduced the natural decay incidence and severity following storage at ambient condition (28 ± 2°C, 78 ± 1% RH) for six days compared to control fruit. Application of *C. tropicalis* YZ27 prevented weight loss of fresh fruit (8.2%), pericarp browning (8%) and reduction of anthocyanin pigments (11%) of the pericarp over control (12.4%, 96.0% and 38.5% respectively in untreated fruit). Quality attributes like total soluble solids, titratable acidity and ascorbic acid content of litchi fruit were 17.18%, 0.53% and 7.78 mg/100 g in *C. tropicalis* YZ27 applied fruits compared to 11.95%, 0.30% and 6.16 mg/100 g respectively. The results indicate that *C. tropicalis* YZ27 has great potential for development of commercial biocontrol formulations as an alternative of SO_2 fumigation to control postharvest decay and quality retention of litchi fruit.

Keywords: Biocontrol agent, *Candida tropicalis*, fruit quality, litchi, postharvest life.

LITCHI (*Litchi chinensis* Sonn.), a member of the family Sapindaceae, is a tropical to subtropical fruit grown commercially worldwide[1]. The crop suffers from postharvest loss estimated to be as high as 50%, mainly due to decay caused by infection of microorganisms[2] during transportation and storage[3]. Fungi such as *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Colletotrichum* sp. and *Botryodiplodia* spp. cause decay during and after harvest through injuries during harvest or handling[4,5]. Commercially, litchi industry utilizes fumigation with SO_2 to tackle these problems[6]. However, consumer preferences, pesticide residue regulations and environmental concerns have threatened the use of chemicals to manage postharvest diseases in fruit and vegetables[7–11]. As such, new methodologies are necessary for overcoming postharvest storage problems in litchi cv. Bombai. Biological control using yeasts has emerged as a promising alternative to treatment with fungicides to control postharvest diseases in fruit and vegetables[12,13]. Their activity does not generally depend on the production of toxic metabolites. Also, they do not produce allergic spores or mycotoxins, are easily cultivated on simple media and can be produced on a large scale as commercial preparations[14]. Several yeasts have been identified as postharvest biocontrol agents against different diseases on different crops[15–23].

The present study was aimed to investigate the potential of the antagonistic yeast *Candida tropicalis* YZ27 for postharvest protection against natural decay caused by pathogenic fungi and quality retention of litchi fruit during storage.

C. tropicalis YZ27, previously identified as antagonistic to several postharvest pathogens[24] was used in this study. The culture was stored at 4°C on slants of yeast extract peptone dextrose agar (YEPD) medium (1% yeast extract, 2% peptone, 2% dextrose) supplemented with chloramphenicol (0.1%). Fresh liquid cultures of yeast were grown in 1 l Erlenmeyer flasks containing 250 ml of YEPD and incubated at 28°C for 48 h, after which the yeast cells were washed once with distilled sterilized water after removal of the growth medium by centrifugation at 3000 *g* for 10 min. Cell pellets were resuspended in distilled sterilized water and final cell concentration was adjusted according to requirements of the various experiments.

Litchi fruits (cv. Bombai) at 80–90% maturation based on fruit colour were harvested from Horticultural Research Station, Bidhan Chandra Krishi Viswavidyalaya, India. After harvest, fruits were transported to the laboratory and sorted for uniform size, colour stage and absence of mechanical damage. Fruits were surface-disinfected with 1.0% sodium hypochlorite solution for 2 min and air-dried at room temperature. One hundred and ten fruits per treatment per replication (ten fruits each for measuring the decay incidence and severity, pericarp browning and total fruit weight; 40 fruits each for measuring anthocyanin content, total soluble solids, titratable acidity and ascorbic acid (ten for each assay)) were dipped for 60 s into water suspension of yeast cells adjusted to a concentration to 1–4 × 10^8 CFU ml^{-1} and allowed to dry at room temperature for 30 min. Carbendazim (0.1%) was used as a standard fungicide to compare the efficacy and sterile distilled water (SDW) dipping was used as control. The treated fruits were packed in perforated and laminated corrugated fibre board (CFB) boxes and stored at ambient condition (28 ± 2°C, 78 ± 1% RH) in a ventilated chamber. The experiment was laid out in a completely randomized design with seven replications each.

Yeast-treated fruit samples from each of the seven replicates were collected every day over a period of six days and yeast populations were measured[25]. The yeast was recovered by removing five samples of fruit tissues including peel, with a cork borer (1 cm^2) and ground with a mortar and pestle in 10 ml of SDW. Then, 50 µl of serial tenfold dilutions were spread on YEPD agar plates

*For correspondence. (e-mail: vyzhimo@gmail.com)

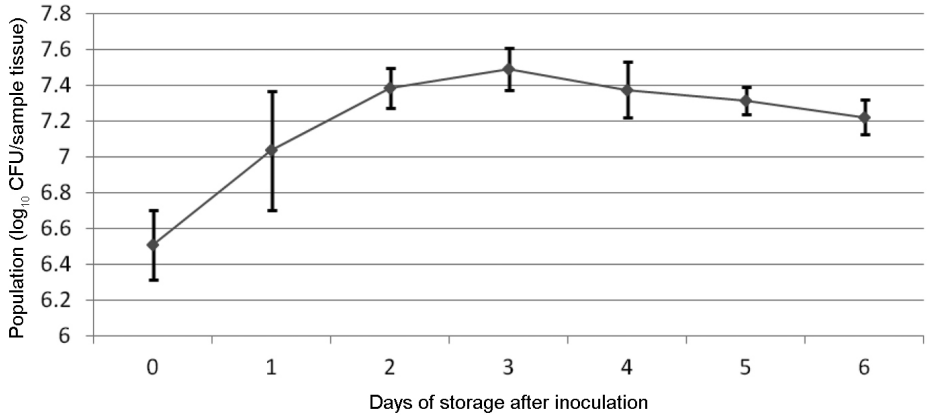

Figure 1. Population dynamics of *Candida tropicalis* YZ27 in litchi fruit tissues at ambient condition at different storage times after inoculation. Bars represent standard deviation of the means of five replications of each assay.

with chloramphenicol (0.1%) and carbendazim (0.1%) to inhibit growth of bacteria and filamentous fungi in the medium. Samples taken on the first day (1 h after treatment) served as time 0. Single colonies were counted after incubation for six days and expressed as the $\log_{10}$ CFU per sample of fruit tissues taken.

Postharvest disease incidence was expressed as percentage of fruit exhibiting a combined decay due to bacteria, yeast or fungi out of the total fruits in each treatment. Decay severity was calculated on a 1–5 scale, describing the severity of fungal decay (1, no decay; 2, 1–5 spots on the surface; 3, one-fourth of the fruit; 4, half of the fruit, and 5, three-fourths of the entire fruit area decayed)[26]. The organisms from decayed fruits were isolated as pure cultures on potato dextrose agar and identified using standard procedures[27]. The disease incidence and severity were assessed six days from the day of harvesting.

The total fruit weight (g) of ten fruits was recorded daily up to six days of storage using an electronic balance and expressed as per cent weight loss ((initial fresh weight of the fruit – weight of the fruit on the date of observation)/ initial fresh weight of the fruit) × 100). Pericarp browning incidence was expressed as percentage of fruit exhibiting browning symptoms out of the total fruits per treatment. The severity of browning was calculated visually thrice at two days interval from the date of harvesting as follows: 1, no symptoms of browning; 2, 1–2 brown spots; 3, some spots with browning; 4, 50% of the fruit surface with browning; 5, 75% of the entire fruit surface with browning[28]. Pericarp (10 g) from ten randomly selected fruits from each treatment was used to extract anthocyanin with methanolic HCl (0.15% HCl : 95% methanol, 15 : 85) for 4 h by pH differential method[29]. Absorbance of 1% anthocyanin solution was determined at 530 nm using a split beam UV/Vis spectrophotometer (OPTIZEN, 2120UV Plus, Mecasys Co, Ltd). The final concentration of total anthocyanin was expressed as mg/100 g pulp.

The fruit juice was extracted from ten randomly selected fruits from each treatment and total soluble solids (TSS) measured using a digital hand-held refractometer (Model RHB-32, Erma, Tokyo, Japan) and expressed as percentage. Next, the percentage (w/v) of titratable acidity (TA) was determined by titrating with 0.01 mol l^{-1} NaOH and calculated as citric acid equivalents using phenolphthalein as an indicator[30]. Ascorbic acid was estimated by titration of juice against 2,6-dicholorophenol-indophenol dye and expressed as mg/100 g pulp[31].

Eating quality was estimated three times at two days interval up to six days by a panel of five members on a 1–3 hedonic scale (1, poor taste, less juicy; 2, good taste, less juicy; 3, good taste, juicy fruit). The overall acceptability of the fruits was estimated on a 1–3 scale taking into consideration the overall parameters, including decay percentage, pericarp browning, colour, appearance and eating quality (1, poor; 2, fair; 3, good).

Experiments were arranged as completely randomized designs, each comprising seven replications. Data were tested for differences between the treatments by analysis of variance (ANOVA) and means were separated by Duncan's multiple range test with at $P = 0.05$ level of significance using the DSAAST software (ver. 1.101).

The initial yeast cell population recovered from the fruit tissues assayed increased progressively from 3.5×10^6 CFU ml^{-1}–1.3×10^7 CFU ml^{-1} at day 1 to 2.5×10^7 CFU ml^{-1} on day 2, and up to 3.1×10^7 CFU ml^{-1} on day 3, after which it gradually decreased to 2.4×10^6 CFU ml^{-1} on day 4 but maintained a concentration of 1.7×10^7 CFU ml^{-1} at day 6 (Figure 1).

Application of *C. tropicalis* YZ27 ($1–4 \times 10^8$ CFU ml^{-1}) reduced the natural decay caused by fungal pathogens significantly ($P = 0.05$) over different storage times at

ambient condition with 11.3% incidence at par with carbendazim (0.1%) compared with those of the control fruits which showed 78.7% incidence at the end of the experiment (Figure 2). The antagonist-treated fruits showed significant control of decay severity ($P = 0.05$) with a mean scale of 1.8 compared to 2.6 in carbendazim and 4.4 in control fruits after six days of storage (Figure 3). The fungal pathogens associated with decay of the fruits were isolated and identified as belonging to *Aspergillus*, *Rhizopus*, *Penicillium*, *Fusarium*, *Phomopsis*, *Alternaria*, *Trichoderma*, *Pestalotiopsis* and *Colletotrichum* species based on morphology of colonies, mycelia and spores characteristics. Mixed populations of postharvest fungi were observed in all treatments.

It is evident from the results presented in Figures 4 and 5 that pericarp browning of the fruits increased rapidly during storage with browning incidence of 8.0%, 50.7% and 96.0% and browning index of 2.4, 3.0 and 4.2 in control fruits after 2, 4 and 6 days of storage respectively, under ambient condition. Treatment with *C. tropicalis* YZ27 was significantly ($P < 0.05$) effective in reducing the pericarp browning of litchi superior over the standard fungicide carbendazim allowing an incidence of only in 6.7% of the treated fruits and an index measure of 1.4 at six days storage time under ambient condition. Total anthocyanin content of the fruits decreased significantly ($P = 0.05$), but in a more rapid manner in control fruits with 38.5% reduction compared to 11.0% reduction in yeast antagonist and 13.1% in fungicide-treated fruits after six days of storage (Figure 6). Litchi fruits treated with the yeast antagonist exhibited significant ($P = 0.05$) retention of fresh weight with 8.2% loss compared to 9.3% in carbendazim while control fruits recorded a rapid loss in fresh weight to the tune of 12.4% after six days of storage (Figure 7).

TSS, TA and ascorbic acid contents of litchi fruits decreased significantly gradually with increasing storage time, though the control fruits showed a greater decrease

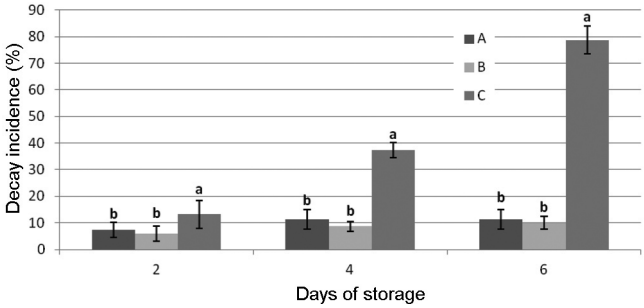

Figure 2. Effects of treatments A (*C. tropicalis* YZ27), B (carbendazim) and C (control) on natural decay incidence (percentage of fruits showing decay symptoms out of the total number of fruits) of litchi at 2, 4 and 6 days after storage at ambient temperature. Bars represent standard deviation of the means of seven replications of each treatment. Data in columns with different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

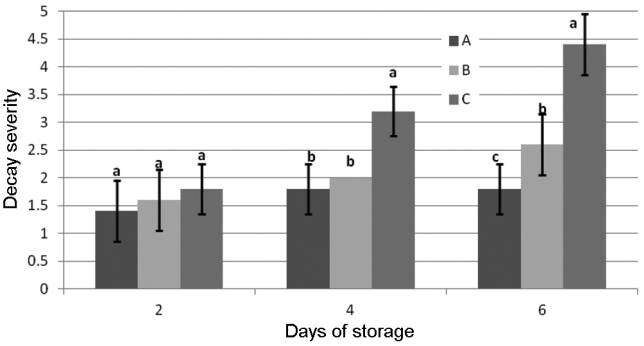

Figure 3. Effects of treatments A (*C. tropicalis* YZ27), B (carbendazim) and C (control) on natural decay severity (1, no decay; 2, 1–5 spots on the fruit surface; 3, one-fourth of the fruit; 4, half of the fruit and 5, three-fourths of entire fruit decayed) of litchi 2, 4 and 6 days after storage at ambient condition. Bars represent standard deviation of the means of seven replications of each treatment. Data in columns with different letters are significantly different according to Duncan's multiple range test at $P = 0.05$.

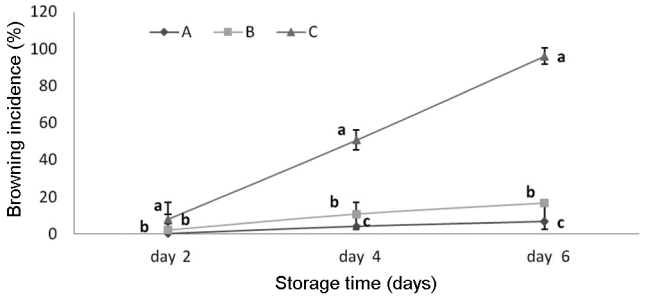

Figure 4. Browning incidence (%) recorded on litchi fruit inoculated with *C. tropicalis* YZ27 (A), carbendazim (B) and control (C) treatments at 2, 4 and 6 days of storage at ambient condition. Statistical analysis was performed for each series of data separately. Bars represent standard deviation of the means of seven replications of each treatment. Values with the same letter at each particular time of assay are not significantly different at $P = 0.05$ (Duncan's multiple range test).

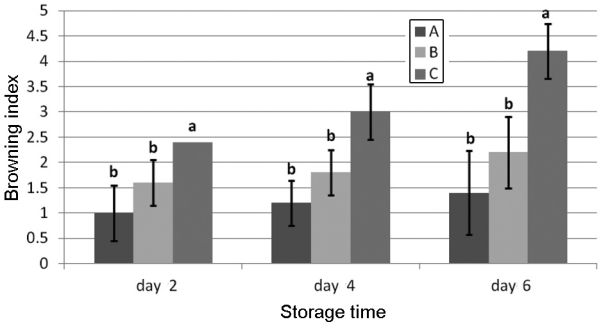

Figure 5. Browning index (1–5 scale) recorded on litchi fruit inoculated with *C. tropicalis* YZ27 (A), carbendazim (B) and control (C) treatments at 2, 4 and 6 days of storage at ambient condition. Statistical analysis was performed for each series of data separately. Bars represent the standard deviation of the means of seven replications of each treatment. Values with the same letter at each particular time of assay are not significantly different at $P = 0.05$ (Duncan's multiple range test).

Table 1. Effect of *Candida tropicalis* YZ27 treatment and different days of storage on quality attributes of litchi at ambient conditions (27.7 ± 1°C, 78 ± 1% RH)

Parameter	Treatment	Days of storage			
		0	2	4	6
Total soluble solids (%)	*C. tropicalis* YZ27	20.38 ± 0.83a	19.36 ± 0.79a	18.48 ± 0.46a	17.18 ± 0.84a
	Carbendazim	19.46 ± 0.98b	18.2 ± 0.69b	17.28 ± 1.15a	16.56 ± 0.62a
	Control	18.56 ± 0.60c	17.95 ± 0.60b	14.15 ± 1.20b	11.95 ± 0.55b
	LSD (0.05)	0.826	0.976	1.386	0.942
Titratable acidity (%)	*C. tropicalis* YZ27	0.72 ± 0.04a	0.67 ± 0.04a	0.61 ± 0.04a	0.53 ± 0.04a
	Carbendazim	0.58 ± 0.10b	0.54 ± 0.08b	0.51 ± 0.03b	0.45 ± 0.03b
	Control	0.67 ± 0.07ab	0.52 ± 0.06b	0.43 ± 0.04c	0.30 ± 0.02c
	LSD (0.05)	0.103	0.085	0.05	0.049
Ascorbic acid (mg/100 g pulp)	*C. tropicalis* YZ27	8.96 ± 0.42b	8.74 ± 0.41a	8.2 ± 0.29a	7.78 ± 0.36a
	Carbendazim	8.42 ± 0.33c	8.04 ± 0.36b	7.66 ± 0.30b	7.06 ± 0.24b
	Control	9.62 ± 0.36a	8.96 ± 0.36a	7.52 ± 0.36b	6.16 ± 0.21c
	LSD(0.05)	0.516	0.527	0.444	0.912

For each measurement, corresponding means ± SD followed by the same letter are not significantly different at $P = 0.05$ level of significance (Duncan's multiple range test).

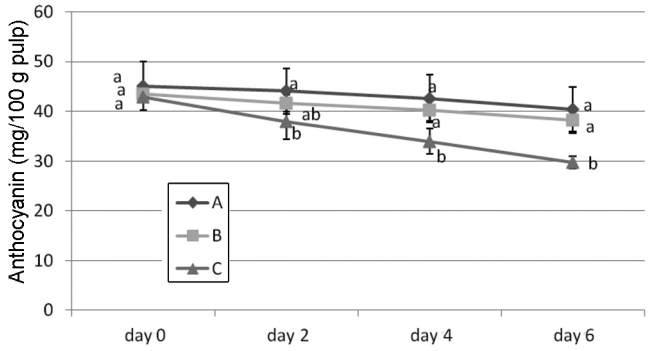

Figure 6. Effects of *C. tropicalis* YZ27 (A), carbendazim (B) and control (C) treatments on anthocyanin content (mg/100 g pulp) of litchi stored at ambient condition during different storage times. Statistical analysis was performed for each series of data separately. Bars represent standard deviation of the means of seven replications of each treatment. Values with the same letter at each particular time of assay are not significantly different at $P = 0.05$ (Duncan's multiple range test).

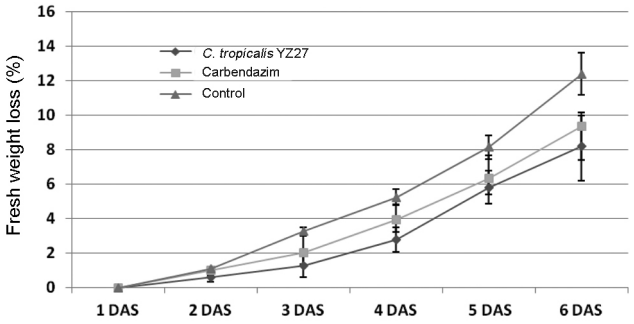

Figure 7. Percentage of fresh weight loss of litchi fruit during different storage times at ambient conditions upon treatments with *C. tropicalis* YZ27 (A), carbendazim (B) and control (C). Statistical analysis was performed for each series of data separately. Bars represent standard deviation of the means of seven replications of each treatment. Values with the same letter at each particular time of assay are not significantly different at $P = 0.05$ (Duncan's multiple range test).

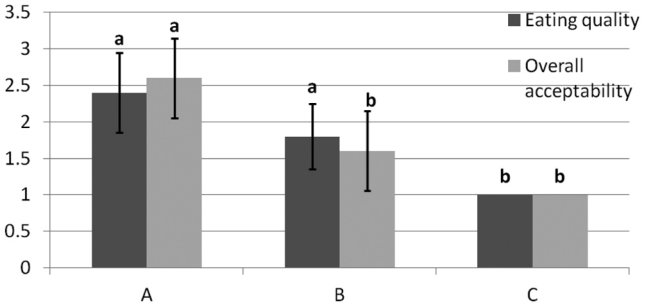

Figure 8. Effect of *C. tropicalis* YZ27 (A) on the eating quality and overall acceptability of litchi fruit after 6 days at ambient storage condition. B, Carbendazim-treated fruit; C, Control fruit. Bars represent standard deviation of the means of five member panels of each treatment. Means in each column followed by the same letter are not significantly different at $P = 0.05$ (Duncan's multiple range test).

in TSS and ascorbic acid contents (Table 1). After six days of storage, fruits treated with yeast antagonist YZ27-retained 17.18%, 0.53% and 7.78 mg/100 g weight of TSS, TA and ascorbic acid compared to 11.95%, 0.30% and 6.16 mg/100 g in control fruits respectively. The yeast YZ27-treated fruits also showed a statistically significant ($P = 0.05$) retention of TA and ascorbic acid compared to carbendazim-treated fruits, although no significant difference was observed in case of TSS content between the two treatments at six days of storage.

The eating quality and overall acceptability of yeast antagonist *C. tropicalis* YZ27-treated litchi fruits were higher than the control fruits and a significantly better ($P = 0.05$) overall acceptability of the yeast-treated fruits than fungicide treatment was also observed after six days of ambient storage condition.

Several management approaches have been reported as alternatives to the existing sulphur fumigation, to minimize postharvest decay in litchi, like the use of

antioxidants[32], nitric oxide[33], salicyclic acid, chitosan and their combinations[34], and application of bacterial biological control agent *Bacillus subtilis*[2,26]. The use of antagonistic yeasts in biological control programmes has advantages over other methods[35,36]. In the present study the antagonistic yeast *C. tropicalis* YZ27 reduced disease incidence as well as severity remarkably well. Pericarp in litchi is characterized by the presence of tubercles exhibiting a rough surface texture[37] and during storage, the fungal spores germinate and proliferate into the pericarp through micro-cracks appearing during fruit development, injuries during packing and transportation, and subsequently colonize the fruits surface[37]. *C. tropicalis* grew rapidly on the surface of the treated fruits indicating they could adapt and acclimatize to the environment of the surface of litchi fruits and occupy the living space quick. The result suggests that competition between the organisms for space and nutrition may be the main mechanism by which this yeast acts as an antagonist, as demonstrated in other antagonistic yeasts too[12,38]. The loss in fresh weight in fruits after harvest occurs mainly due to rapid loss of moisture from the pericarp eventually leading to further injuries in the form of micro-cracks in the pericarps which progressively proliferate inwards to the fruit mesocarp[33]. These micro-cracks lead to further moisture loss, pathogen attack and browning of the pericarp[39]. Application of the yeast antagonist significantly minimized weight loss of litchi fruits compared to control, probably by rapid colonization on the micro-cracks and preventing moisture loss. Litchi fruits are generally harvested after maturation they are non-climacteric in nature[40]. After harvest, the colour of the pericarp turns from red to brown within 48 h due to degradation of anthocyanin pigments involving enzymes along with oxidation of phenolic compounds which reduce the commercial value of the fruit[41]. In this study, the loss in anthocyanin content of litchi fruit pericarp during storage was substantially reduced by the application of *C. tropicalis* YZ27, resulting in lesser browning incidence and browning. Antagonistic yeast treatment also maintained higher levels of TSS, TA and ascorbic acid than control or fungicide-treated fruits. The delayed senescence process in yeast-treated fruits due to protection of the fruit surface by rapid yeast colonization maintaining the membrane integrity of the fruit pericarp may have delayed the respiration rate of stored fruits, thus reducing breakdown of sugars in the food matrix. Further, yeast colonization on the fruit surface may have reduced the permeability of oxygen by facilitating a physical barrier and thereby delaying the oxidative deterioration of ascorbic acid.

The results reported here showed that *C. tropicalis* YZ27 could control postharvest decay, reduce pericarp browning and improve quality parameters like fresh weight, TSS, TA and ascorbic acid content in litchi. Furthermore, studies should be focused on a combining the biocontrol agent with other control strategies, such as combination with safe compounds, waxing, atmosphere conditions, carrier and adhesive materials, to develop effective formulations for postharvest disease control in litchi cv. Bombai.

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Surface water quality in sacred groves of Garhwal Himalayan region, India

Purna Jana, Kaboli Wotsa, Sabyasachi Dasgupta and Nagendra P. Todaria*

Hemwati Nandan Bahuguna Garhwal University, Srinagar, Garhwal 246 174, India

We have studied the quality of surface water in three sacred groves of Garhwal (Uttarakhand, India). The water samples were collected in March–April 2013. Results showed that all water samples conformed to WHO standards for potability. Water of Hariyali Devi recorded the lowest dissolved oxygen and water of Tarkeshwar had the highest hardness. The quality water of Ravigaon was found to be the best among the waters of the three groves. Water from deodar-dominant forests recorded higher dissolved oxygen, total hardness, calcium and magnesium hardness. The overall drinking water quality of oak-dominated forests was found to be better.

Keywords: Deodar and oak forest types, sacred grove, water quality.

SINCE long, popular media and academic circles have given widespread recognition to the idea that indigenous people and some other small societies are exemplary conservationists[1,2]. Indigenous conservationism has often been attributed to spiritual respect and practical understanding of the natural world[2–5] and has given rise to sacred groves at some sites. A sacred grove is a grove or forest with a group of trees or patch of vegetation, which has been protected by local people through religious and cultural practices[6,7]. Sacred groves which have cultural or spiritual significance for the people living around them, have been protected by communities around the world for

*For correspondence. (e-mail: nagendratodaria@gmail.com)