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Effect of Some Fructoplane Antagonists and Postharvest Dip Treatments on Litchi Fruit Rots and Shelf Life

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Abstract: *Litchi (Litchi chinensis) fruits are extremely susceptible to postharvest fungal pathogens. Normally, fungicides are used to gain control over such postharvest pathogens. Carbendazim is popularly used for control of postharvest disease control in litchi. However, use of synthetic chemicals has been curtailed due to safety issues and biological control is increasingly explored as an alternative. Four isolates of Bacillus subtilis and two of antagonistic yeast were isolated from fructoplane of litchi. After preliminary screening, the best isolate was applied individually and in combination with chitosan (1.0%), potassium silicate (0.5%) and carbendazim (0.1%) to test the efficacy against postharvest pathogens of litchi. No incidence of fruit rot (decay) and browning was recorded in fruit treated with B. subtilis NRCL BS-1(1x10^8 cells/mL) up to 6 days stored at 32-36°C temperature and 65-76% relative humidity. Treatment with antagonists resulted in significantly lower fruit rot (0-10%) as well as lower percent disease index. Disease control obtained through application of B. subtilis, chitosan, and potassium silicate + chitosan were at par with that achieved through application of carbendazim. It is concluded that B. subtilis and chitosan merit as good alternatives to carbendazim to reduce fruit decay and improve shelf life of litchi fruit.*

Keywords: *Bacillus subtilis, fruit rots, litchi, yeast.*

INTRODUCTION

Litchi or Lychee (*Litchi chinensis* Sonn.) is extensively grown in China, India, Thailand, Vietnam and the rest of tropical Southeast Asia, the Indian Subcontinent [1], and more recently in South Africa, Brazil, the Caribbean, Queensland, California and Florida [2]. It is one of the important fruit crop of India which is cultivated in about 84,000 hectare area with production around 5.85 lakh metric tonnes and productivity about 7.0 metric tonnes/ha [3]. Postharvest loss in litchi has been estimated to be in the range of 35.3-43.8% [4], and much of this is due to rots caused by microorganisms. A wide range of fungi *viz.*, *Alternaria, Colletotrichum, Botryodiplodia, Aspergillus, Fusarium* and *Penicillium* sp. are reported to cause postharvest fruit rots if fruits are not handled properly. In Guangdong and Hainan (China) *C. gloeosporioides* was the main pathogen causing postharvest decay which came mainly from fruits with latent infection prior to harvest [5]. A mean pathological loss to a tune of 23.2% during 2012 and 17.9% during 2013 in the supply chain of litchi in India has been reported [4], highest being at retail level. The predominant fungal genera associated with fruit decay (rots) of litchi in India are *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Aspergillus flavus* and *Aspergillus niger* [4].

Biological control is one of the most promising alternatives to unpopular synthetic fungicides, and research on postharvest biocontrol has increased in recent decades [6]. Members of the genera *Bacillus* [7, 8], have been shown to be effective in the biological control of rots caused by fungi. Similarly, yeasts are promising antagonistic microorganisms

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because some species have the characteristics of an ideal biocontrol agent [9]. The fructoplane (fruit surface) is an excellent source of naturally occurring antagonists against postharvest fruit rots. Isolation of the antagonists can be improved by using fruit from unmanaged orchards [10, 11], where natural populations have not been disturbed by chemical usage, and the pool of potential antagonists is greater than in a chemically managed orchard [12]. The literature available on antagonists of fruit rot pathogens of litchi is however limited. With this background, an attempt was made to find effective antagonists particularly species of *Bacillus* and yeast to manage litchi fruit rots during 2014 and 2015.

The isolated antagonists were first tested by dip treatment in controlling fruit rots arising out of natural inoculums at ambient conditions. Then, they were further tested for their bioefficacy in controlling fruit rots and effect on shelf life of fruits. The best isolates along with other postharvest dip treatments *viz.,* defence activators (potassium silicate, chitosan and chitosan) and a standard fungicide (carbendazim), in single as well as in combination treatments were compared.

MATERIAL AND METHODS

Isolation of Potential Antagonists

Fruits of litchi cultivar 'Shahi' were obtained from experimental farm of National Research Centre on Litchi (NRCL), Muzaffarpur, where there has been no fungicidal spray. Three to five mature healthy litchi fruits were selected for isolation of antagonists *viz., Bacillus* sp. and yeast. Fruits were placed in separate 250 mL Erlenmeyer flasks containing 100 mL sterile distilled water plus 25% Ringer's Solution (NaCl-6.5 g, KCl-0.42 g. $CaCl_2$-0.25 g, $NaHCO_3$-0.2 g, distilled water -1000 mL) and shaken in a precision water bath at 120 rotations per minute (rpm) for one hour at 30°C. For the recovery of yeast isolates, the fruits were removed and the liquid suspension was used to make a serial dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. An aliquot of 0.1 mL from 10^{-4}, 10^{-5} and 10^{-6} dilutions were plated onto PDA (potato dextrose agar) plates amended with 0.15 gL^{-1} of Rose Bengal (RB). The resulting plates were incubated at 25°C for 3 days. Pure cultures of yeast isolates were made by sub-culturing from discrete colonies on the plates and were maintained on Yeast Malt Agar medium. For the isolation of *Bacillus* spp., similar serial dilutions were used but were heat treated at 80°C for 15 minutes in a water bath to eliminate non-spore-forming microbes. Aliquots of 0.1 mL were then poured onto Tryptone Soy Agar (TSA) (pancreatic digest of casein-15 g, soya peptone-5 g, NaCl-5 g, agar-15 g, distilled water- 1000 mL) plates; incubated at 28°C for 3 days. The representative colonies were arbitrarily selected and streaked onto fresh TSA plates to obtain single colonies.

Identification of Antagonists

Identification were based on morphological and biochemical characteristics, and conventional techniques according to Bergey's manual of determinative bacteriology [13] and consulting with published Keys [14]. The axenic cultures of all these were maintained in Plant Pathology laboratory at NRCL, Muzaffarpur.

Preparation of Inoculums of Antagonists and Other Dip Treatments

Isolates of *Bacillus subtilis* were multiplied in tryptone soy broth while yeast isolates were multiplied in potato dextrose broth for 72 hours in an orbital shaker at 120 rpm 28 ± 1°C prior to treatments. For preparation of 1% chitosan solution, 45 g citric acid was dissolved in 3 L of DW, mixed thoroughly and then 30 g of chitosan was added to this solution followed by adjustment of pH to 3.0 and incubation overnight. Wherever combination treatment was there, fruits were first treated with chemicals and air dried for 5 min. followed by treatments with microorganism.

Postharvest Dip Treatments With Antagonists

Five isolates of *Bacillus subtilis*, two isolates of yeast, one isolate of *Trichoderma viride* (isolate NRCL T01) and their combination treatments were evaluated for preventive ability to control fruit decay pathogens. Fruits were kept at ambient conditions in the laboratory after treatments. Experiment was conducted in three replicates and each replication consisted of 30 fruits. There were three control treatments namely fruits dipped in tryptone soy broth, potato dextrose broth and distilled water.

Fruits were treated by dipping in the solution kept in pneumatic trough. The concentration of both *B. subtilis* and yeast isolates were 1×10^8 cell/mL. Observations were recorded up to 10^{th} days

Postharvest Dip Treatment of Antagonists and Other Chemicals

A total of 12 treatments *viz.*, *B. subtilis* (1×10^8 cell/mL), potassium silicate (0.5%), chitosan (1.0%), carbendazim (0.1%) and their combinations were taken during 2014, while one more defence activator, salicylic acid with three combination treatments were taken during 2015 making total treatments to 16. Thirty fruits in three replicates were taken for each treatment. Duration of dip treatments was 1 min. For control treatment, fruits were dipped in distilled water. Observations were recorded up to 10^{th} days.

Storage Conditions

After the treatments, fruits were stored in aerated polythene bags at ambient conditions in the laboratory *viz.*, 32 ± 2°C temperature and 65 ± 2% R.H. during 2014 and 36 ± 2°C temperature and 76 ± 6% R.H. during 2015.

Observations Recorded

Incidence of fruit rots (%), percent disease index (PDI), pathogen frequency *i.e.* incidence of different fruit rot pathogens expressed in percent), browning and biochemical parameters *viz.*, total soluble solids (TSS), titratable acidity (TA), anthocyanin of fruits were assessed. PDI was calculated by the formula: {PDI = ($n_1 \times 1 + n_2 \times 2 + n_3 \times 3$/ Total no. of fruits observed × Maximum grade used) × 100}, where n_1, n_2, and n_3 represent the total number of rotten/ decayed fruits falling under 1, 2, and 3 rating, respectively. Rating was based on proportion of fruit surface area rotten due to pathogen, where 1 = < 30%, 2 = 30-60% and 3 = > 60% surface area of fruits rotten. Assay of biochemical parameters were done on three randomly selected fruits per replicate per treatment. The TSS content of the juice was measured using an automatic digital refractometer and expressed as degrees Brix (°B). TA was determined by titration with 0.1 N NaOH in the presence of a phenolphthalein indicator and expressed as the percentage of malic acid, the predominant organic acid in litchi fruits. Pericarp browning was visually assessed based on the browning observed on the percent surface area of individual fruits and categorized as either low (< 50% browning) with acceptable marketability or high (> 50% browning) with limited marketability. The total anthocyanin content in litchi pericarp was estimated using the Ethanolic-HCl (85:15) method with some modifications. Pigment was extracted from 1 g fresh weight of pericarp tissue using the extraction solvent mixture. An Agilent Spectrophotometer (Cary 300) was used to measure the OD at 535 nm. The total anthocyanins content was expressed as mg/100 g. Sensory evaluation of litchi fruit was by a panel of five judges on the basis of external appearance of fruits, texture, taste, and flavour. A 9 point 'Hedonic Scale' (1: Extremely undesirable, 9: Extremely desirable) described by Resende [15] was used for inference.

Statistical Analysis

All experiments were arranged in completely randomized design, and each was comprised of three replications. Analysis of variance (ANOVA) using a completely randomized design (CRD) was conducted with SAS® 9.2 statistical software. The least significant differences (LSDs) between means at $p = 0.05$ and the standard error (SE) of means were computed.

RESULTS AND DISCUSSION

Isolation of Potential Antagonists from Fructoplane

Four isolates of *Bacillus subtilis* and two isolates of antagonistic yeast were isolated from healthy litchi fruit surface. The cells of *B. subtilis* were rod-shaped, Gram positive. The colony was circular, with ragged edges, cream or white in colour. The bacteria spread out from the centre, keeping the ragged circular shape of the colony. It was catalase-positive and did not hydrolyze casein. It formed endospores and was a facultative aerobe.

The colony of isolates of yeasts were white, creamy ovoidal. They showed a positive reaction for lipase activity. All yeast isolates showed budding as their mode of asexual reproduction. The yeast

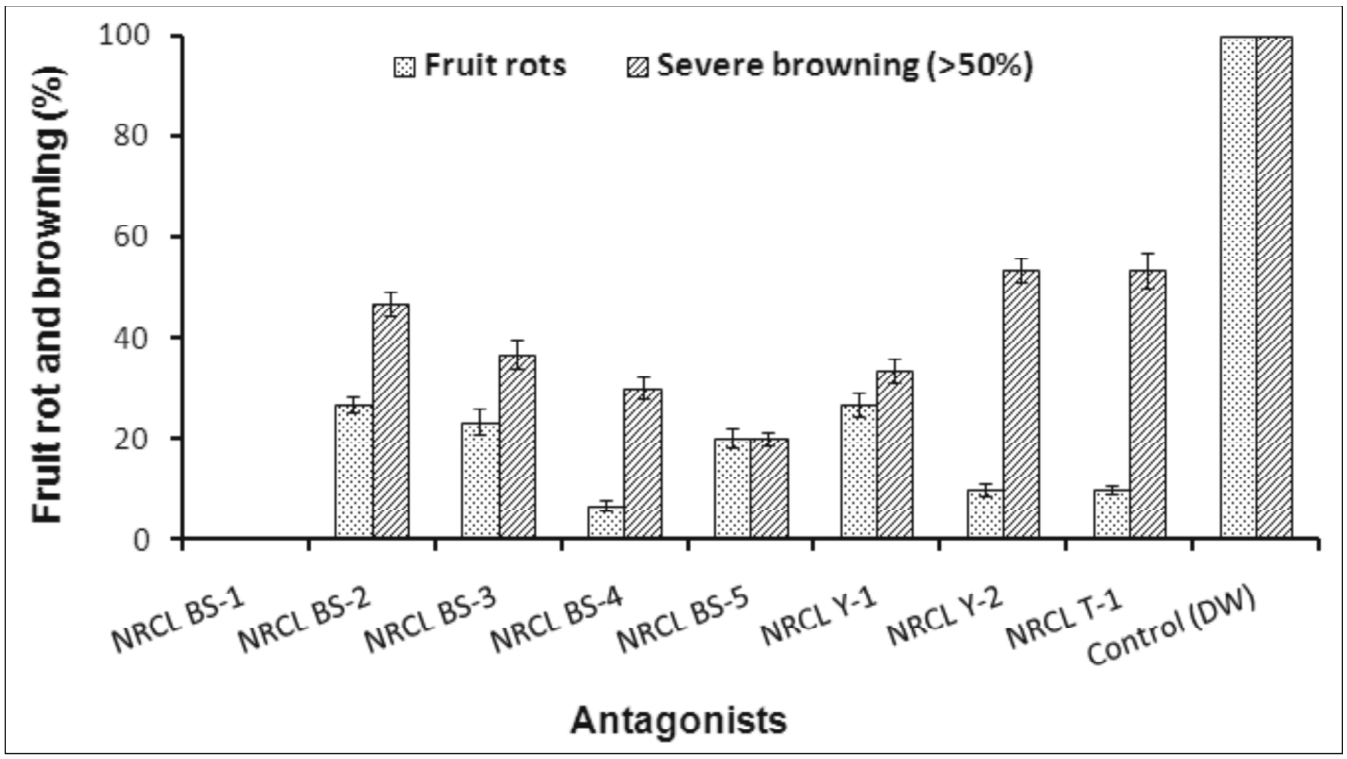

Figure 1: Effect of treatments with antagonists on fruit rots and browning of litchi fruits during 2014; data presented is cumulative values six-day after treatment

Table 1
Effect of antagonists on fruit rots, pathogen frequency and pericarp browning of litchi during 2014

Treatment	*Treatment detail*	*Fruit rot (%)*	*Pathogen frequency (%)*				*PDI*	*Fruit surface colonized by pathogens*			*Browning (%) #*	
			CG	*AA*	*AN*	*AF*		*< 30%*	*30-60%*	*> 60%*	*(< 50%)*	*(> 50%)*
T_1	NRCL BS-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	0.0
T_2	NRCL BS-2	26.7	87.5	12.5	0.0	0.0	79.2	25.0	12.5	62.5	0.0	46.7
T_3	NRCL BS-3	23.3	100.0	0.0	0.0	0.0	61.9	28.6	57.1	14.3	20.0	36.7
T_4	NRCL BS-4	6.7	100.0	0.0	0.0	0.0	66.7	50.0	0.0	50.0	26.7	30.0
T_5	NRCL BS-5	20.0	16.7	83.3	0.0	0.0	88.9	16.7	0.0	83.3	13.3	20.0
T_6	NRCL Y-1	26.7	37.5	50.0	12.5	0.0	75.0	12.5	50.0	37.5	20.0	33.3
T_7	NRCL Y-2	10.0	100.0	0.0	0.0	0.0	66.7	0.0	100.0	0.0	20.0	53.3
T_8	NRCL T-1	10.0	33.3	0.0	66.7	0.0	33.3	100.0	0.0	0.0	13.3	53.3
T_9	Control (TS broth)	100.0	18.5	58.2	10.0	13.3	92.0	4..0	10.0	86.0	0.0	100.0
T_{10}	Control (PD broth)	100.0	16.0	41.4	30.0	12.6	96.6	6.0	10.0	84.0	0.0	100.0
T_{11}	Control (DW)	100.0	23.3	33.3	30.0	13.4	83.3	10.0	20.0	70.0	0.0	100.0
LSD *(p = 0.05)*		3.88					6.63				3.13	5.78

CG = *Colletotrichum gloeosporioides*; AA=*Alternaria alternata*; AN= *Aspergillus niger*; AF= *Aspergillus flavus;* PDI= Percent disease index; #= All the fruits were rotten in control treatments. Data in the table represent cumulative value on 6th day after treatment

counts obtained in this study were relatively high (10^4 to 10^6 CFU/fruit). The high numbers of yeasts in the samples indicated that litchi fruit surfaces were a good habitat for yeasts. Yeasts which were found on the surfaces of litchi could have been deposited by vectors such as insects, dust and other particles in the air.

The aim was to isolate yeasts and *Bacillus* spp. antagonistic to fruit rots pathogens of litchi and to assess their potential for the biological control. This approach has been reported by Korsten [16] and Sivakumar [17]. However, it is the first study in which both yeasts and *Bacillus* have been isolated from litchi fructoplane in India and their antagonistic activity was evaluated against fruit rot pathogens and shelf life of litchi fruits.

Effect of Antagonists on Fruit Rots and Shelf Life of Litchi

During 2014, five *B. subtilis* and two yeast isolates and one Trichoderma isolate (NRCLT-01) were assessed for their efficacy in controlling fruit rot pathogens. The results indicated that though all the antagonists could inhibit pathogen development as well as they checked browning of fruits, among them *B. subtilis* isolate NRCL BS-1 was found the most effective antagonist since zero fruit rot incidence and browning was recorded as compared to control (100%) up to six days after treatment (Figure 1).

The detailed data on efficacy of antagonists in controlling fruit rots, pathogen frequency and pericarp browning of litchi during 2014 is presented in table 1. The results indicated that the isolate NRCL BS-01 was the best in controlling fruit rots and other parameters as the values were zero and pericarp browning was also only 6.7% that is having acceptable marketability. The other antagonists *B. subtilis* and yeast also significantly reduced fruit rots (6.7-26.7%) and PDI (33.3-88.9%) as compared to different control treatments (TS broth, PD broth and DW) where fruit rots were 100.0% and PDI ranged from 83.3-96.6%. The data on pathogen frequency showed that among the four fruit rot pathogens the antagonists could very effectively control *A. alternata* and hence the other less dominant pathogen could get the chance to colonize the fruit surface. The colonized surface areas of fruits by the pathogens were low with antagonists' treatment over control. The application of *B. subtilis* or its extract to control of postharvest diseases of fruits and vegetables is relatively safe [18]. Fruit browning could be due to the damage of the structure of substrates and enzymes, leading to oxidation of phenolics [18].

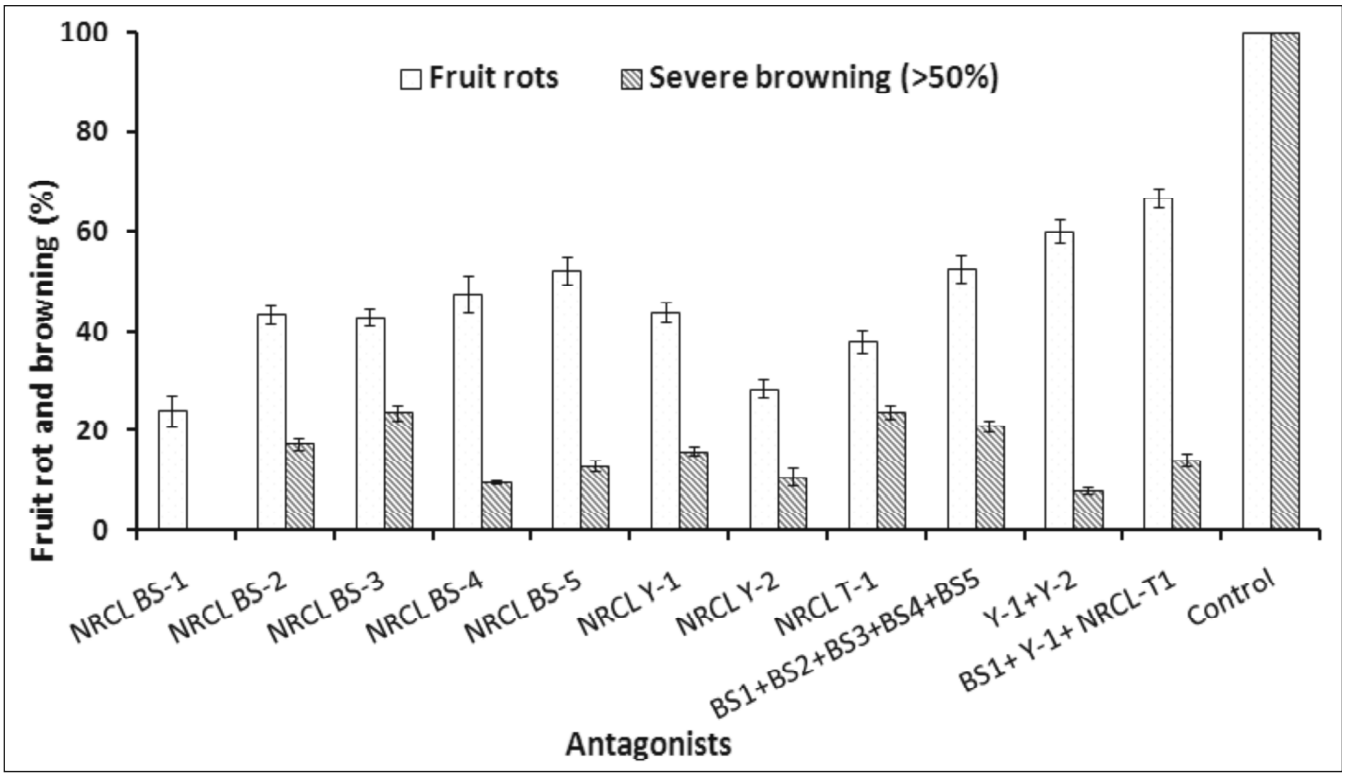

Figure 2: Effect of treatments with antagonists on fruit rots and browning of litchi fruits during 2015; data presented is cumulative values six-day after treatment

Table 2
Effect of antagonists on fruit rots, pathogen frequency and pericarp browning of litchi during 2015

Treatment	Treatment detail	Fruit rot (%)	Pathogen frequency (%)				PDI	Fruit surface colonized by pathogens			Browning (%) #	
			CG	AA	AN	AF		< 30%	30-60%	> 60%	< 50%	> 50%
T_1	NRCL BS-1	24.0	83.3	16.7	0.0	0.0	44.4	66.7	33.3	0.0	8.0	0.0
T_2	NRCL BS-2	43.5	30.0	70.0	0.0	0.0	53.3	50.0	40.0	10.0	13.0	17.4
T_3	NRCL BS-3	42.9	44.4	44.4	11.1	0.0	55.6	44.4	44.4	11.1	19.0	23.8
T_4	NRCL BS-4	47.6	30.0	60.0	10.0	0.0	46.7	60.0	40.0	0.0	28.6	9.5
T_5	NRCL BS-5	52.2	33.3	58.3	8.3	0.0	50.0	58.3	33.3	8.3	13.0	13.0
T_6	NRCL Y-1	44.0	27.3	54.5	9.1	9.1	57.6	45.5	36.4	18.2	16.0	16.0
T_7	NRCL Y-2	28.6	12.5	75.0	0.0	12.5	41.7	75.0	25.0	0.0	28.6	10.7
T_8	NRCL T-1	38.1	37.5	50.0	12.5	0.0	58.3	37.5	50.0	12.5	23.8	23.8
T_9	BS-1 + BS-2 + BS-3 + BS-4 + BS-5	52.6	50.0	50.0	0.0	0.0	53.3	50.0	40.0	10.0	5.3	21.1
T_{10}	Y-1 + Y-2	60.0	13.3	66.7	6.7	0.0	51.1	60.0	26.7	13.3	12.0	8.0
T_{11}	BS-1 + Y-1 + NRCL T-1	66.7	28.6	64.3	14.3	0.0	57.1	42.9	42.9	14.3	4.8	14.3
T_{12}	Control	100.0	29.4	58.8	11.8	0.0	66.7	29.4	41.2	29.4	0.0	100.0
LSD ($p = 0.05$)		6.47					6.13				2.99	3.18

CG = *Colletotrichum gloeosporioides*; AA=*Alternaria alternata*; AN= *Aspergillus niger*; AF= *Aspergillus flavus;* PDI= Percent disease index; #= All the fruits were rotten in control treatment. Data in the table represent cumulative value on 6th day after treatment

During 2015, the treatments were same except three new combination treatments *viz.*, BS-1 + BS-2 + BS-3 + BS-4 + BS-5, Y-1 + Y-2 and BS-1+ Y-1+ NRCL T-1 (Figure 2 and Table 2). The results indicated that though all the antagonists could significantly inhibit pathogen development (fruit rots and PDI) as well as they checked browning of fruits, the parameters values were higher than in 2014. Among these antagonists, against *B. subtilis* isolate NRCL BS-1 was found the best. The combination treatments of antagonists were not found superior to individual treatments. The parameters values were comparatively higher than 2014 owing to higher temperature and humidity in ambient conditions (36 ± 2°C temperature and 76 ± 6% R.H.) during 2015.

Effect of Various Postharvest Dip Treatments on Fruit Rots of Litchi

Since *Bacillus subtilis* isolate NRCL BS-1 proved to be superior it was further tasted in combination with other postharvest treatment during 2014 and 2015 for their efficacy in controlling fruit rots and shelf life. The data on cumulative fruit rots during 2014 revealed that all the treatments were effective in controlling fruit rots up to 6th day. Fruits in control treatment started rotting on 3rd day (68.3%) and completely rotten on 4th day (Figure 3 and Figure 4).

The detailed cumulative data on progressive fruit rots and pathogen frequency up to 10th day of treatment during 2014 is presented in Table 3. The results indicated that out of 12 treatments, T_1 [*Bacillus subtilis*], T_3 [chitosan], T_5 [Pot. silicate + chitosan] and T_{11} [chitosan+carbendazim] were highly effective in controlling litchi fruit rots as it showed 0% fruit rotting till 7th day and were almost at par with carbendazim. Even up to 10th day significantly less rotting was observed in these treatments compared to control. A close perusal of the data on pathogen frequency showed that carbendazim is less effective against *A. alternata.* The data in Table 4 shows effect of treatments on quality parameters of fruits as assessed on 10th day of

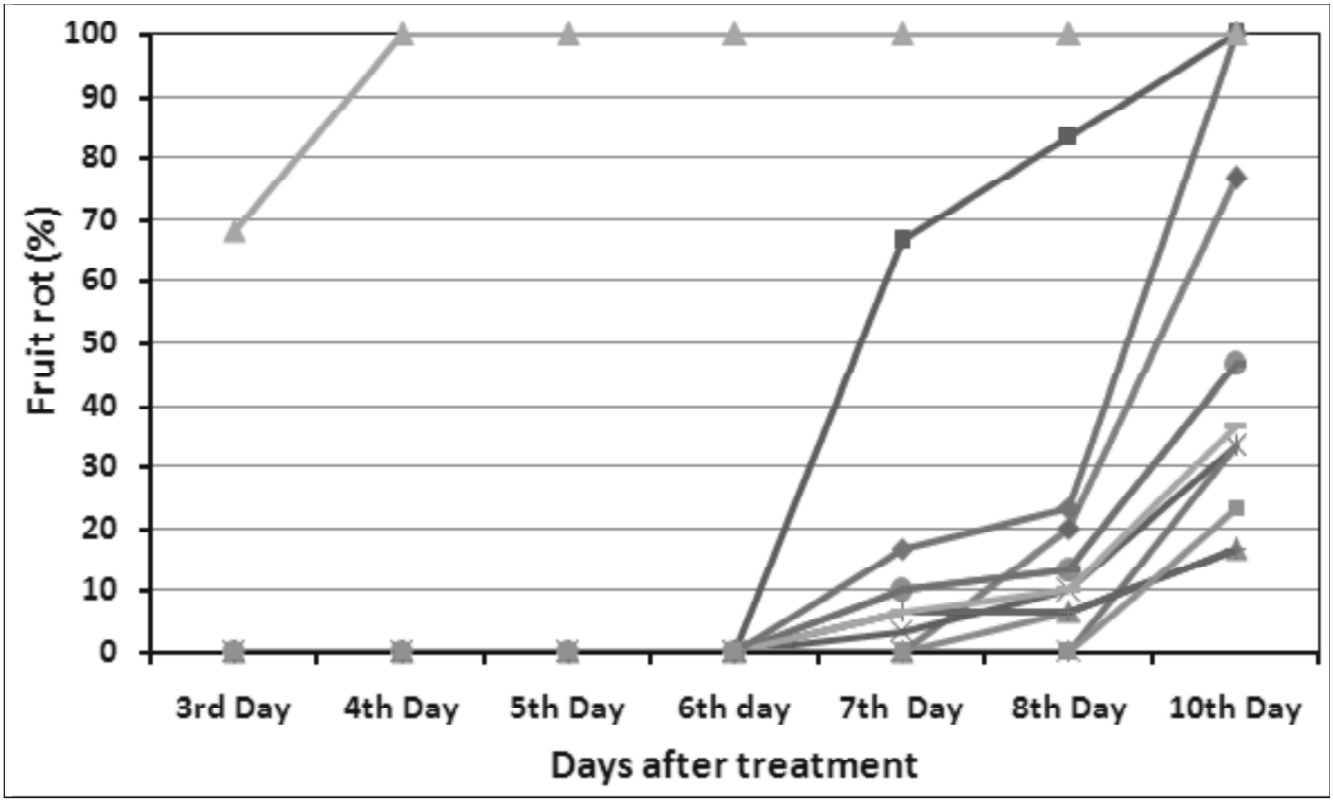

Figure 3: Effect of various postharvest dip treatments on litchi fruit rots during 2014

treatment. Though there were no significant differences among treatments with regard to TSS, significant differences were observed with respect to acidity, anthocyanin content of fruits and organoleptic score. Acidity was significantly higher in fruits wherever chitosan treatment was given or after the treatments if fruit rotting was higher. There was very less reduction in anthocynin content (thereby colour of the fruits) with the treatment of *B. subtilis* (NRCL BS-1). The highest organoleptic score (8.50) was also with NRCL BS-1 which were statistically at par with chitosan, chitosan + NRCL BS-1 and chitosan + Pot. silicate treatment. The study thus provides two good alternatives to carbendazim *viz.*, *B. subtilis* and chitosan treatments for preventing fruit rots and for better shelf life of litchi. With chitosan, though there may be disliking of vegetarian people who would not prefer to have chitosan treated fruits as chitosan is being derived as a fish by-product.

During 2015 the same experiment of previous year was repeated and one more defence activator, salicylic acid with three combination treatments was evaluated. Results of experiment during 2015

Figure 4: Effect of postharvest dip treatments of fruits after six days stored at ambient conditions (Left: Control, Right: Treated with *Bacillus subtilis* isolate NRCL BS-1)

showed that out of 16 treatments, T_1 [*Bacillus subtilis*], T_{12} [*Bacillus subtilis* + salicylic acid] and T_8 [*Bacillus subtilis* + carbendazim] were highly effective in controlling litchi fruit rots as it showed 0% fruit rotting after 4th day, 5-10% rotting on 6th day and 25-40% rotting on 8th day after treatment as compared to 40.0% and 93.3% and 100.0% rotting after 4th, 6th and 8th day after treatment, respectively in the control (Figure 5).

The detailed data on effect of various postharvest dip treatments on fruit rots, percent

Table 3
Effect of postharvest dip treatments on fruit rots of litchi and pathogen frequency (cumulative basis) during 2014

		Observation on 7th day					*Observation on 8th day*					*Observation on 10th day*				
Treatment	*Treatment details*	*Fruit rot (%)*	*Pathogen frequency (%)*				*Fruit rot (%)*	*Pathogen frequency (%)*				*Fruit rot (%)*	*Pathogen frequency (%)*			
			CG	*AA*	*AN*	*AF*		*CG*	*AA*	*42.9*	*AF*		*CG*	*AA*	*AN*	*AF*
T_1	NRCL BS-1	0.0	0.0	0.0	0.0	0.0	20.0	100.0	0.0	31.2	0.0	76.7	52.2	43.5	4.3	0.0
T_2	Pot Sil.	66.7	95.0	0.0	5.0	0.0	83.3	96.0	0.0	32.4	0.0	100.0	83.3	13.3	3.3	0.0
T_3	Chit.	0.0	0.0	0.0	0.0	0.0	6.7	100.0	0.0	37.4	0.0	16.7	60.0	40.0	0.0	0.0
T_4	Carb.	3.3	100.0	0.0	0.0	0.0	10.0	33.3	66.7	37.1	0.0	33.3	70.0	30.0	0.0	0.0
T_5	Pot. Sil. + NRCL BS-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	33.4	0.0	33.3	40.0	50.0	10.0	0.0
T_6	Chitosan + NRCL BS-1	10.0	33.3	0.0	33.3	33.3	13.3	50.0	0.0	36.2	25.0	46.7	42.9	21.4	21.4	14.3
T_7	Carb. + NRCL BS-1	6.7	0.0	100.0	0.0	0.0	6.7	0.0	100.0	26.8	0.0	16.7	0.0	80.0	0.0	20.0
T_8	Pot. Sil.+ Chit.	10.0	33.3	0.0	33.3	33.3	13.3	50.0	0.0	35.6	25.0	46.7	35.7	35.7	14.3	14.3
T_9	Pot. Sil.+ Carb.	6.7	0.0	100.0	0.0	0.0	10.0	0.0	100.0	40.0	0.0	36.7	45.5	45.5	0.0	9.1
T_{10}	Chit. + Pot. Sil.	16.7	80.0	20.0	0.0	0.0	23.3	85.7	14.3	34.2	0.0	100.0	43.3	36.7	10.0	10.0
T_{11}	Chit.+ Carb.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	26.8	0.0	23.3	42.9	57.1	0.0	0.0
T_{12}	Control	100.0	23.3	33.3	30.0	13.3	100.0	23.3	33.3	30.0	13.3	100.0	23.3	33.3	30.0	13.3
LSD (p = 0.05)		3.18					2.37					2.24				

T_1 = *Bacillus subtilis* (1 × 10^8 cells/mL) T_2: Potassium silicate (0.5%); T_3 = Chitosan (1.0%); T_4 = Carbendazim (0.1%); T_5 = *Bacillus subtilis* (1 × 10^8 cells/mL) + Potassium silicate (0.5%); T_6 = *Bacillus subtilis* (1 × 10^8 cells/mL)+ Chitosan (1%); T_7 = *Bacillus subtilis* (1 × 10^8 cells/mL) + Carbendazim (0.1%); T_8 = Potassium silicate (0.5%) + Chitosan (1%); T_9 = Potassium silicate (0.5%) + Carbendazim (0.1%); T_{10} = Chitosan (1%) + Potassium silicate (0.5%), T_{11} = Chitosan (1%) + Carbendazim (0.1%); T_{12} = control; CG = *Colletotrichum gloeosporiodes*; AA = *Alternaria alternata*; AN= *Aspergillus niger*; AF = *Aspergillus flavus*

disease severity index (PDI) and pathogen frequency is presented in Table 5. Results showed that various dip treatments had not only significantly reduced the fruit rot incidence but also percent fruit area covered by the pathogen *i.e.* PDI was reduced. In absence of the biocontrol treatment, usually *Alternaria alternata* has been a dominating pathogen of fruit decay. The pathogen frequency data showed that treatment with *Bacillus subtilis* had more effectively controlled *A. alternata* and hence occurrence of other pathogens (frequency) were more, particularly *C. gloeosporioides*.

This is the first study in which both yeasts and *Bacillus* have been isolated from fructoplane of litchi in India and their antagonistic activity evaluated against fruit rot pathogens. This approach has also been reported by other workers [19, 16, 17] in litchi. The main characteristics of an ideal biocontrol agent were defined by Wilson and Wisniewski [20], and are related to biosafety, activity in a range of environments and against a variety of pathogens, and ease of management and use. *Bacillus subtilis* fits well on these criteria. Similarly, yeasts are genetically stable, do not need special nutrients to proliferate rapidly, are resistant to adverse environmental conditions, are effective against wide range of fruit pathogens, do not produce metabolites dangerous to human health and are not strongly affected by pesticides [9, 21]. Control of fruit rots by potassium silicate could be related to its fungistatic properties [22].

Table 4
Effect of postharvest dip treatments on quality parameters of fruits during 2014

		*Biochemical parameters**			
Treatment	*Treatment details*	*TSS (°B)*	*Acidity (%)*	*Anthocyanin (mg/100 g)*	*OR (1-9 scale)*
T_1	NRCL BS-1	18.70	0.40	36.1	8.50
T_2	Pot Sil.	17.55	0.42	21.2	6.50
T_3	Chit.	18.05	0.41	33.1	8.00
T_4	Carb.	18.05	0.33	32.4	7.38
T_5	Pot. Sil. + NRCL BS-1	18.30	0.32	25.1	7.25
T_6	Chitosan + NRCL BS-1	18.10	0.36	26.4	8.25
T_7	Carb.+ NRCL BS-1	18.40	0.32	24.2	6.50
T_8	Pot. Sil.+ Chit.	18.70	0.42	26.8	8.00
T_9	Pot. Sil.+ Carb.	17.90	0.36	25.6	7.25
T_{10}	Chit. + Pot. Sil.	18.10	0.44	31.0	7.88
T_{11}	Chit.+ Carb.	18.25	0.39	26.2	6.50
T_{12}	Control	#	#	#	4.13
LSD (p = 0.05)		NS	0.07	2.1	1.03

* Initial value of TSS = 19.2°B, Acidity = 0.40% and Anthocyanin = 37.3 mg/100 g fresh weight of pericarp tissue; OR = Organoleptic rating; # = All the fruits were rotten in control treatment; the data in table are mean value of three replication on 10th day after treatment.

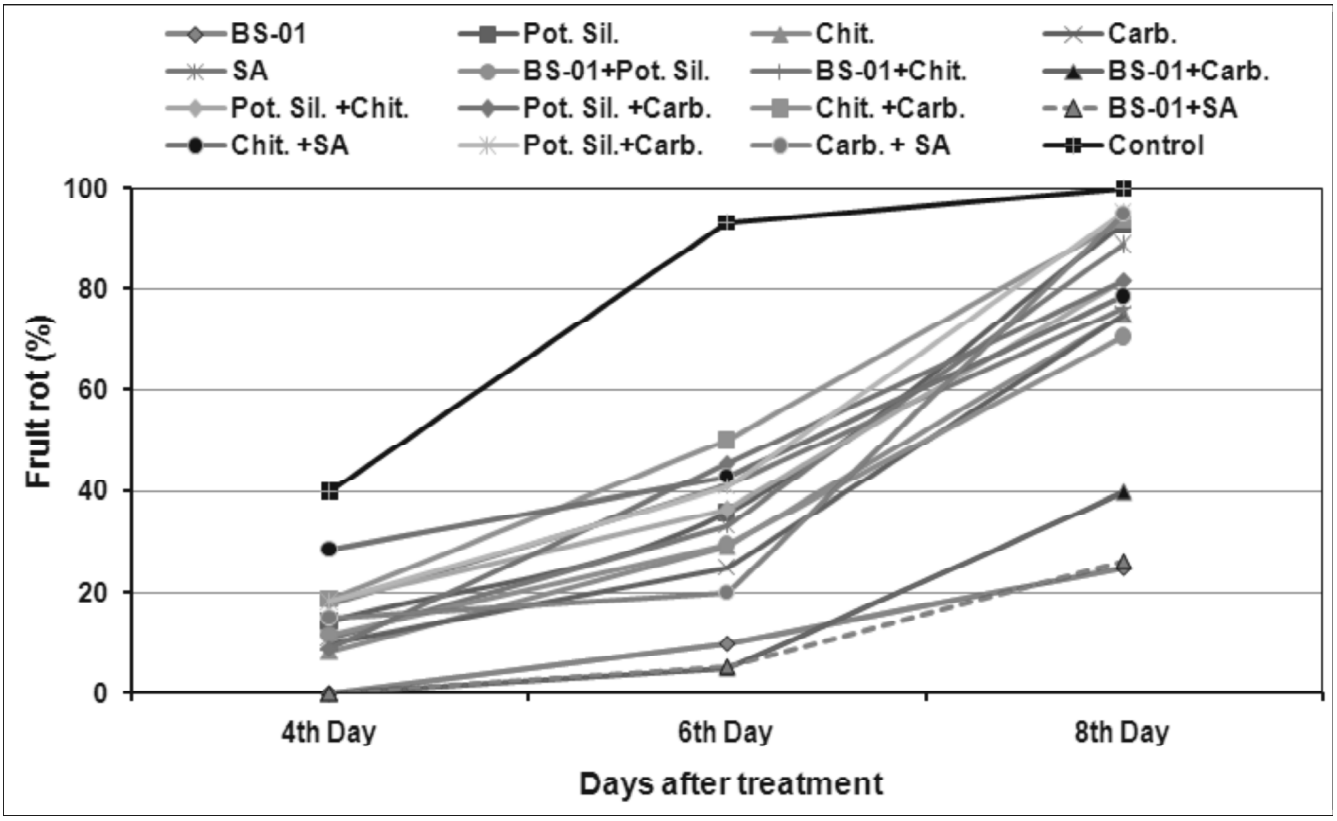

Figure 5: Effect of various postharvest dip treatments on litchi fruit rots during 2015

Table 5
Effect of various postharvest dip treatments on fruit rots of litchi and pathogen frequency (cumulative basis) during 2015

	Observation on 4th day					*Observation on 6th day*		*Observation on 8th day*									
Treatment	*Fruit rot (%)*	*Pathogen frequency (%)*				*PDI (%)*	*Fruit rot (%)*	*Pathogen frequency (%)*				*PDI*	*Fruit (%) rot*	*Pathogen frequency*			*PDI*
		CG	*AA*	*AN*	*AF*			*CG*	*AA*	*AN*	*AF*			*CG*	*AA*	*AN*	*AF*	
T_1	0.0	0.0	0.0	0.0	0.0	0.0	10.0	100.0	0.0	0.0	0.0	50.0	25.0	80.0	0.0	0.0	20.0	46.7
T_2	14.3	50.0	50.0	0.0	0.0	50.0	35.7	40.0	60.0	0.0	0.0	46.7	92.9	30.8	61.5	7.7	0.0	61.5
T_3	8.3	0.0	100.0	0.0	0.0	33.3	29.2	28.6	57.1	14.3	0.0	57.1	75.0	22.2	66.7	11.1	0.0	55.6
T_4	10.0	0.0	50.0	50.0	0.0	33.3	25.0	40.0	40.0	20.0	0.0	33.3	75.0	33.3	46.7	13.3	6.7	48.9
T_5	11.1	0.0	50.0	0.0	50.0	66.7	33.3	16.7	50.0	0.0	33.3	61.1	88.9	25.0	56.3	0.0	12.5	54.2
T_6	11.8	50.0	50.0	0.0	0.0	33.3	29.4	40.0	40.0	20.0	0.0	60.0	70.6	41.7	33.3	16.7	8.3	63.9
T_7	17.6	33.3	66.7	0.0	0.0	44.4	41.2	42.9	57.1	0.0	0.0	57.1	76.5	38.5	46.2	7.7	7.7	61.5
T_8	0.0	0.0	0.0	0.0	0.0	0.0	5.0	0.0	100.0	0.0	0.0	33.3	40.0	37.5	50.0	25.0	0.0	66.7
T_9	18.2	50.0	50.0	0.0	0.0	50.0	36.4	50.0	50.0	0.0	0.0	58.3	81.8	55.6	44.4	0.0	0.0	66.7
T_{10}	9.1	100.0	0.0	0.0	0.0	33.3	45.5	40.0	40.0	0.0	20.0	66.7	81.8	44.4	44.4	0.0	11.1	55.6
T_{11}	18.8	66.7	33.3	0.0	0.0	33.3	50.0	50.0	50.0	0.0	0.0	62.5	93.8	40.0	46.7	6.7	6.7	53.3
T_{12}	0.0	0.0	0.0	0.0	0.0	0.0	5.3	0.0	100.0	0.0	0.0	33.3	26.3	40.0	40.0	20.0	0.0	53.3
T_{13}	28.6	50.0	25.0	25.0	0.0	41.7	42.9	66.7	16.7	16.7	0.0	50.0	78.6	45.5	27.3	18.2	9.1	54.5
T_{14}	18.2	50.0	50.0	0.0	0.0	50.0	40.9	44.4	55.6	0.0	0.0	55.6	95.5	42.9	47.6	9.5	0.0	54.0
T_{15}	15.0	33.3	33.3	0.0	33.3	66.7	20.0	50.0	25.0	0.0	25.0	58.3	95.0	47.4	31.6	10.5	10.5	50.9
T_{16}	40.0	33.3	66.7	0.0	0.0	72.2	93.3	28.6	57.1	7.1	7.1	78.6	100.0	26.7	60.0	6.7	6.7	80.0
LSD (p = 0.05)	2.88					5.86	4.32					5.82	5.87					4.68

T_1=*Bacillus subtilis* (1 × 10^8 cells/mL); T_2 = Potassium silicate (0.5%); T_3 = Chitosan (1%); T_4 = Carbendazim (0.1%); T_5 = Salicylic Acid 100 ppm; T_6 = *Bacillus subtilis* (1 × 10^8 cells/mL) + Potassium silicate (0.5%); T_7 = *Bacillus subtilis* (1 × 10^8 cells/mL) + Chitosan (1%); T_8 = *Bacillus subtilis* (1 × 10^8 cells/mL) + Carbendazim (0.1%); T_9 = Potassium silicate (0.5%) + Chitosan (1%); T_{10} = Potassium silicate (0.5%) + Carbendazim (0.1%); T_{11} = Chitosan (1%) + Carbendazim (0.1%); T_{12} = *Bacillus subtilis* + Salicylic acid; T_{13} = Chitosan + Salicylic acid; T_{14} = Pot. silicate + Salicylic acid; T_{15} = Carbendazim + Salicylic acid; T_{16} = Control; CG = *Colletotrichum gloeosporioides*; AA = *Alternaria alternata*; AN = *Aspergillus niger*; AF = *Aspergillus flavus;* PDI = Percent disease index

CONCLUSION

In the last few decades, research on the biological control of postharvest pathogens has increased exponentially. The application of fungicides to fruits after harvest to reduce fruit rots has been increasingly curtailed by the development of pathogen resistance to many key fungicides, the lack of replacement fungicides, negative public perception regarding the safety of pesticides and consequent restrictions on fungicide use. The result of our study conclusively proved the efficacy of novel isolate of *Bacillus subtilis,* NRCL BS-01 and chitosan in controlling fruit rots and enhancing shelf life which is at par with carbendazim treatment. The combined treatment of *B. subtilis* and yeast isolates however did not resulted in a synergistic effect. There is a need for further research on the integrated control of fruit rots (decay) of litchi. A suitable delivery system for bulk treatment of litchi fruits is required which is being developed at ICAR-NRCL, Muzaffarpur.

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