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Management of postharvest green mold decay in common mandarin and Indian gooseberry with *Bacillus licheniformis* SR-14

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Abstract

Common mandarin and Indian gooseberry are extremely important fruits and used in several countries for their medicinal properties. *Bacillus licheniformis* SR-14 strain was tested *in vitro* for their antagonistic properties against *Penicillium digitatum*, the causal agent of postharvest green mold decay in common mandarin and Indian gooseberry. In dual culture bioassay as circular and semicircular patterns, the isolate quantitatively inhibits the growth of the pathogen by more than 72 and 57 %, respectively. *In vitro* plate assay detected that the strain produces hydrolytic enzymes like protease, chitinase and volatile compound. Treatment with the antagonist also resulted in the induction of defense enzymes like, phenylalanine ammonia lyase, β-1, 3-glucanase and higher phenol accumulation compared to control. *In vivo* application of the antagonist revealed that the maximum mean disease index reached to 1.16 and 1.41 in Indian gooseberry and mandarin, which showed around 65 and 63 % reduction in disease severity, respectively when compared to control. These experiments suggest that the strain could be considered as a promising mean for the control of mandarin and Indian gooseberry postharvest green mold decay and its use may be an effective method to improve the integrated disease management strategy.

Key words: Antagonist, biocontrol, *Citrus reticulata,* defense enzymes, *Emblica officinalis*

Introduction

Common mandarin (*Citrus reticulata* Blanco) is the chief commercial citrus cultivar in India (Singh, 2009). The importance of this fruit is based on the fact that it is a rich source of different vitamins, phosphorus, oil and citric acid (Bhardwaj *et al.,* 2010). Increased plasma vitamin c content and reduction of oxidative markers have been observed in humans drinking orange juice daily for two weeks (Sánchez-Moreno *et al.,* 2003). Reduction in plasma lipid peroxidation has also been observed with intake of orange juice (Johnston *et al.,* 2003). *Citrus* fruits are high in secondary metabolites, including flavonoids and coumarins, which are associated with a reduced risk of cancer, including gastric cancer, breast cancer, lung tumorigenesis, colonic tumorigenesis, hepatocarcinogenesis, hematopoietic malignancies, etc. (Hang *et al.,* 2009; Do-Hoon *et al.,* 2012; Asghar *et al.,* 2012; Ching-Shu *et al.,* 2013).

Indian gooseberry (*Emblica officinalis* Gaertn) is another important fruit of tropics and subtropics. It has played an important therapeutic role from time immemorial and is frequently recommended for its synergistic effects in both Ayurvedic and unani systems of medicine (Jain *et al.,* 1983). The fruit is a rich source of vitamin C and other nutrients like polyphenols, pectin, iron, calcium and phosphorus (Nath *et al.,* 1992; Singh *et al.,* 1993; Khopde *et al.,* 2001). Hepatoprotective activity of fruit extracts of Indian gooseberry have also been reported (Jose and Kuttan, 2000; Tasduq *et al.,* 2005; Reddy *et al.,* 2010). Extracts of Indian gooseberry possess antitumor activities (Yang *et al.,* 2009; Mahata *et al.,* 2013) and thus could be a potent source of drug for the treatment of various types of cancer.

Postharvest fungal pathogens are considered to be the main cause of losses of fresh fruits and vegetables at postharvest, distribution and consumption levels. Indian gooseberry suffers from a number of postharvest fungal diseases, commonly blue mold (Setty, 1959), fruit rot (Kulkarni and Sharma, 1971), black mold (Srivastava *et al.,* 1964) and green mold (Akhund *et al.,* 2010) caused by *P. islandicum, P. oxalicum Aspergillus niger* and *P. digitatum* respectively. Common mandarin as well has a very short postharvest storage life (Tietel *et al.,* 2011) and within this short time span a number of pathogens *P. italicum* (blue mold rot), *P. digitatum* (green mold decay), *Alternaria citri* (stem end rot), *A. niger* (black mold rot), *Colletotrichum gloeosporides* (anthracnose) causes severe postharvest losses (Chakraborty *et al.,* 2014). Among all, green mold causes serious economic loss during postharvest handling of both the fruits reducing the commercial life of harvested fruit (Oliveri *et al.,* 2007).

Efforts have been made to minimize the losses through developing a better understanding of the biology and aetiology of postharvest diseases, as well as by introducing adequate postharvest handling technologies and control strategies (Prusky *et al.,* 2013). While several approaches were suggested for managing postharvest decay, chemical control of postharvest diseases, is still the most widely used method. Increasing concerns, however, regarding residues of fungicides in the fruit, development of resistant biotypes of the pathogens, as well as risks associated with their continuous use have prompted the search for safe and effective alternative strategies. Many bacteria and yeasts have been proposed as biological control agents and they have been shown to inhibit postharvest decay of many fruits (Bull *et al.,* 1998,

Cirvilleri *et al.,* 2005; Scuderi *et al.,* 2009; Platania *et al.,* 2012). Their modes of action, that include antibiosis, competition and resistance induction, have been extensively studied. Some of the commercial bioformulations used for postharvest application are based on these mechanisms (Cirvilleri, 2008; Nunes, 2012; Liu *et al.,* 2013). In the present study, an attempt has been made for the postharvest management of common mandarin and Indian gooseberry against green mold (*P digitatum*) using a putative biocontrol agent *Bacillus licheniformis* SR-14.

Materials and methods

Organisms: The fungal pathogen *P. digitatum* (Pers.) Sacc was purchased from Agharkar Research Institute, Pune, India (FCCI 1862), subcultured and maintained on potato dextrose agar (PDA) medium and stored at 4°C for further study. The biocontrol agent was previously isolated by our laboratory researcher and identified as *B. licheniformis* strain SR-14 by microbial type culture collection (MTCC 8313), Chandigarh, India. The antagonist was subcultured and maintained on Nutrient Agar (NA) medium for subsequent use.

Dual culture bioassay: The antagonist *B. licheniformis* SR-14 from 24 h old culture (10^7 cells mL^{-1}) was streaked in the peptone (10 g L^{-1}) glucose (20 g L^{-1}) agar (20 g L^{-1}) (PGA) plate as circular / O and semicircular / U pattern. Then *P. digitatum* from three days old culture was subsequently inoculated at the center of O or U shaped region on the PGA plates (Skidmore and Dickinson, 1970). Inoculation only with the pathogen served as control. The plates in triplicate were incubated at 30 °C for 5 days and radial growth of the fungal colony (in mm) was measured at every 24 h intervals. Light microscopic (Labomed Lx 300) and scanning electron microscopic studies were also performed to detect physical and / or morphological changes of fungal mycelia.

Detection of** in vitro **antifungal compounds

i) Chitinase production: *B. licheniformis* SR-14 was inoculated on LB plate supplemented with 0.5 % colloidal chitin. Plates were incubated at 30 °C for three days. Development of clear halo zones around the bacterial colonies indicates enzymatic activity (Basha and Ulagnathan, 2002).

ii) Detection of hydrolytic enzymes: Production of hydrolytic enzyme was qualitatively assayed in minimal medium (Na_2HPO_4 33.9g/L, KH_2PO_4 15 g/L, NH_4Cl 5 g/L, NaCl 2.5 g/L) supplemented with gelatin, starch, pectin and carboxymethyl cellulose (CMC) for protease, amylase, pectinase and cellulase, respectively (1 %, w/v in each case). Plates were incubated for 48 h at 30 °C and formation of clear zone around bacterial colonies was read as positive (Gaur *et al.,* 2004).

iii) Production of hydrogen cyanide (HCN): Hydrogen cyanide (HCN) production was tested qualitatively according to the method of Wei *et al*. (1991). *Bacillus licheniformis* SR-14 was inoculated in TSA medium supplemented with amino acid glycine (4.4 g L^{-1} of medium). A strip of sterilized filter paper saturated with a solution containing picric acid 0.5 % (yellow) and sodium carbonate (2 %) was placed in the upper lid of the Petri dish. The Petri dishes (9 cm) were then sealed with parafilm and incubated at 30 °C for 4 days. A change of colour of the filter paper strip from yellow to light brown, brown or reddish brown was recorded as weak, moderate or strong cyanogenic potential, respectively.

iv) Production of volatile substance: To investigate the inhibitory effect of the test bacterial isolates for the production of volatile compound 'inverted plate technique' was followed (Dennis and Webster, 1971). Strain SR-14 was grown in Petri dishes containing NA for 12 h. Agar discs (5 mm diameter) cut from a 4-days old culture of *P. digitatum* was placed at the centre of another Petri dish containing PDA. Then both plates inoculated with fungal pathogen and bacterial antagonist were placed in an inverted position and sealed together (mouth to mouth) with parafilm. Control plates containing *P. digitatum* were inverted over uninoculated NA plates. The plates were incubated at 30 °C for 5 days and colony diameter of the test pathogen was measured and compared with the control.

v) *In vivo* biocontrol activity of *B. licheniformis* SR-14: The effects of strain SR-14 on both the fruits were evaluated according to Platania *et al*. (2012) with some modifications. Fruits obtained from commercial orchards were carefully hand selected, surface-sterilized by dipping for 2 min in 2 % of sodium hypochlorite, rinsed with sterile distilled water (SDW) and air-dried. Fruits were wounded with a sterile needle to make 2-mm deep and 5-mm wide wounds on their peel at the equatorial region (three wounds for common mandarin and two for Indian gooseberry in each fruit). Wounds were inoculated with 20 µL of *B. licheniformis* SR-14 (1×10^9 CFU mL^{-1}). Fruits inoculated with Sterilized Distilled Water (SDW) served as control. After 72 h incubation at 30 °C, treated wounds were inoculated with 20 µL of conidial suspension of *P. digitatum* (1×10^6 CFU mL^{-1}). To evaluate the *in vivo* experiments four sets of treatments were designed; Set I- wounds treated with SDW (Control); Set II- treated with SDW and after 72 h, inoculated with conidial suspension of *P. digitatum* (1×10^6 CFU mL^{-1}); Set III- treated with *B. licheniformis* SR-14 (1×10^9 CFU mL^{-1}); and Set IV- treated with *B. licheniformis* SR-14 (1×10^9 CFU mL^{-1}) and after 72 h, inoculated with conidial suspension of *P. digitatum* (1×10^6 CFU mL^{-1}).

Fruits were stored at 30 °C in plastic trays at about 95 % relative humidity (RH). After 72 h of inoculation with the pathogen the disease severity was recorded at every 3 days' interval for upto 12 days in Indian gooseberry and 15 days in common mandarin. Disease severity was evaluated with an empirical scale 1-4 (1 = no visible symptoms; 2 = initial soft rot; 3 = presence of mycelium; 4 = sporulation). Thirty fruits were used for each treatment (three replicates each containing ten fruits) and the experiment was carried out twice.

Assay of the defense related proteins

Enzyme extraction: The fruit tissues taken from different sets after 12th and 15th day of incubation in Indian gooseberry and common mandarin respectively, were homogenised with liquid nitrogen. Five hundred milligrams of sample were extracted with 2 mL of different buffers containing 0.1 % polyvinyl pyrrolidone and 20 µL of 1 mM phenyl methane sulphonyl fluoride to assay different enzymes: 0.1M sodium acetate buffer (pH 5.0) for β-1, 3 glucanase; 0.1M sodium phosphate buffer (pH 7.0) for polyphenol oxidase (PPO); and 0.1M borate buffer (pH 8.7) for phenylalanine ammonia lyase (PAL). All the extraction procedures were conducted at 4° C. The homogenate was centrifuged at 12, 000 rpm for 20 min at 4° C. The supernatants were used for the enzymatic assay.

Polyphenol oxidase (PPO) assay: PPO activity was estimated as described by Mayer *et al*. (1965). About 200 µL of 0.01M catechol was added to the reaction mixture containing 200 µL of enzyme extract and 1.5 mL of 0.1M sodium phosphate buffer (pH 6.5). Enzyme activity was expressed as change in absorbance at 495 nm (Δ OD change) min^{-1} g^{-1} protein.

Phenylalanine Ammonia Lyase (PAL) assay: PAL assay was performed according to the method of Dickerson *et al*. (1984) where the conversion of L-phenylalanine to trans cinnamic acid was monitered spectrophotometrically at 290 nm. About 0.4 mL of enzyme extract was incubated with 0.5 mL of 0.1M borate buffer (pH8) and 12 mM L-phenylalanine in the same buffer for 30 min at 30 °C. Enzyme activity was expressed as synthesis of trans cinnamic acid (n mol) min^{-1} g^{-1} protein.

β-1, 3 glucanase assay: β-1, 3 glucanase activity was estimated according to the method of Pan *et al*. (1991) with slight modifications. Crude enzyme extract of 50 µL was added to 50 µL of 1 % laminarin incubated at room temperature for 30 min. The reaction was stopped by adding 300 µL of dinitrosalicylic acid (DNS) and heated for 10 min in a boiling water bath. The resulting coloured solution was diluted with distilled water and the absorbance was recorded at 520 nm. The enzyme activity was expressed as µ mol glucose equivalent produced min^{-1} g^{-1} protein.

Total phenol estimation: Fruit samples (1 g) were homogenised in 10 mL of 80 % methanol and agitated for 15 min at 70 °C (Zieslin and Ben-Zaken, 1993). One millilitre of the methanolic extract was added to 5 mL of distilled water and 0.25 mL of 1 N Folin-Ciocalteau reagent, and the solution was incubated in the dark at 30 °C. Phenolic content was measured spectrophotometrically at 725 nm using gallic acid as standard. The amount of phenolics was expressed as µg gallic acid g^{-1} tissue.

Statistical Analysis: All data presented were the mean ± SE of three separate experiments conducted in triplicate. In all the experiments, the data obtained were subjected to analysis of variance (ANOVA) using SPSS software version 20 and the significance of difference between the treatments was determined using Duncan's Multiple Range Test ($P < 0.05$).

Results

Interaction of *B. licheniformis* SR-14 against *P. digitatum* in dual culture: In dual culture, significant growth inhibition of *P. digitatum* by *B. licheniformis* SR-14 was observed. Mycelial growth was restricted near bacterial growth and continued away from it. The growth inhibition of *P. digitatum* remained proportionate with an increased incubation period of up to 5 days. Quantitatively *B. licheniformis* SR-14 inhibited the growth of *P. digitatum* by 72.15 and 56.96 % in circular and semi-circular streaks after 120 h of incubation, respectively (Fig. 1). Light and Scanning electron microscopic examination of the mycelia at the interaction zone with the antagonist SR-14 showed signs of shrivelling, growth deformities, swelling, fragmentation, short branching, swollen branched conidiophore without conidia and lysis [Fig. 2 ii(a) to ii(c)]. The bacterium was found to provide biocontrol through direct confrontation on the fungal hyphae and killed them by probably producing cell wall degradative enzymes. Fig. 2 ii(c) shows highly perforated fungal hyphae with damaged walls.

Detection of *in vitro* antifungal compounds: The observed morphological abnormalities of hyphae in dual culture suggested that *B. licheniformis* SR-14 produces secondary metabolites with antifungal activity. *In vitro* assays detected that the antagonist produces chitinase and protease, as indicated by the formation of clear zone around colonies, when grown on chitin- and gelatin-amended media respectively (Table 1). Furthermore 'inverted plate technique' for the production of volatile compound showed significant growth reduction of *P. digitatum* after 72 h incubation in comparison to control (Table 1).

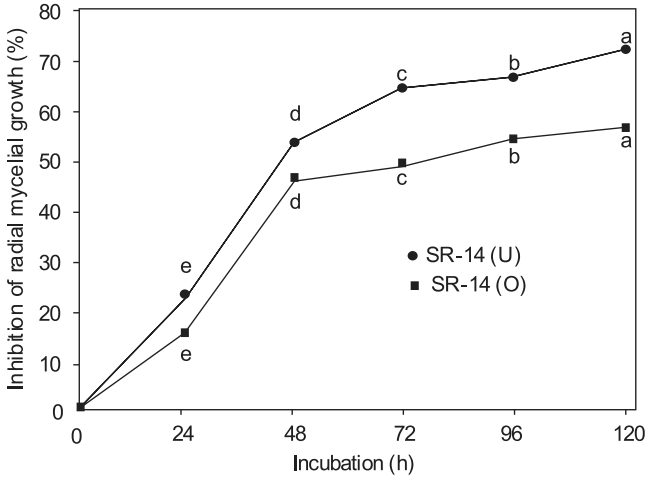

Fig. 1. Inhibition of *P. digitatum* by *B. licheniformis* SR-14 under dual plate culture using circular (O) and semicircular (U) method. Results are mean ± SE of three separate experiments, done in triplicate. Different letters indicate significant difference ($P < 0.05$) using Duncan's multiple range test.

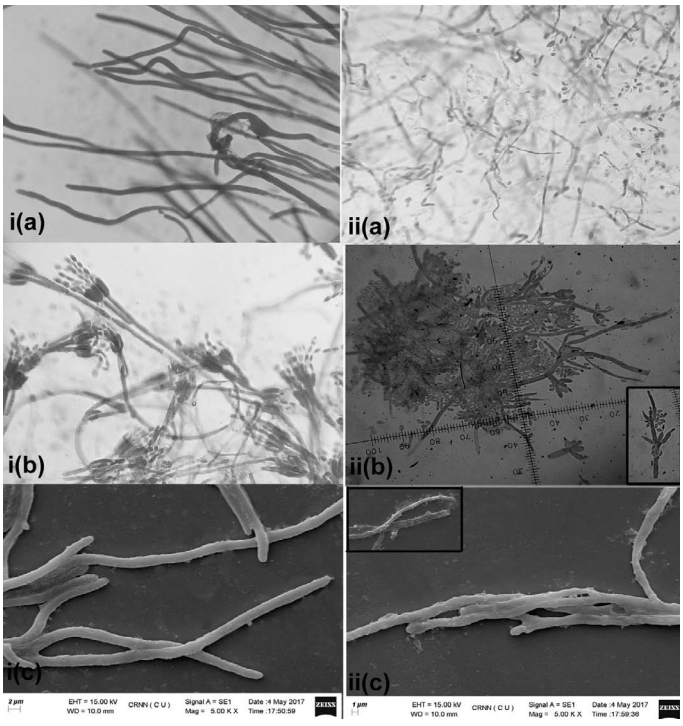

Fig. 2. Morphological analysis of mycelia and conidiophore with conidia by light (a and b) and scanning electron microscope (c). (i)- *P. digitatum* in the absence of antagonist *B. licheniformis* SR-14 and (ii)- in the presence of antagonist. i(a) and i(c)- healthy hyphal structure; ii(a)- deformed mycelia with shriveled hyphae and with short branching; i(b)- normal conidiophore with conidia; ii(b)- abnormal conidiophore with deformed phialides, ii(b inset)- branched abnormal conidiophore; ii(c)- SEM image of deformed short branched hyphae, ii(c inset)- Perforation in the hyphal wall.

Table 1. Secondary metabolites production and enzymatic activity of *B. licheniformis* SR-14

Different Metabolites	Rate of Production
Volatile substances	+++
Protease Activity	++
Chitinase Activity	+++
HCN	-
Amylase Activity	-
Pectinase Activity	-
Cellulase Activity	-

'+++' Stronger Production; '++' Moderate production '-' No production

***In vivo* biocontrol activity of *B. licheniformis* SR-14 against *P. digitatum*:** *Bacillus licheniformis* SR-14 strain was *in vivo* tested, on common mandarin and Indian gooseberry. Strain SR-14 considerably reduced disease incidence compared to the control. Disease severity was increased along with incubation period in the Set I (pathogen treated) whereas a significant protection was found in Set IV (challenged inoculation) (Table 2 and 3; Fig. 3 and 4). After 12th day of incubation in Indian gooseberry and 15th day in mandarin the mean disease severity in Set I reached 3.29 and 3.81 respectively (Table 2 and 3). Scanning electron microscopy of the fruit surface showed profusely branched mycelia indicating severe infection of host tissue by the green mold (Fig. 3B, B1 and 4B, B1). On the contrary, the mean disease severity in Set IV at 12th (Indian gooseberry) and 15th (mandarin) day of incubation reached only 1.16 and 1.41 indicating 65 % and 63 % reduction in disease severity respectively. SEM study of the treated fruits illustrated that SR-14 strongly inhibits mycelia formation and if mycelia developed it causes perforation in the fungal cell wall leading to the death of the fungus (Fig. 3D, D1 and 4D, D1). Moreover, it was also recorded from the SEM study that bacterial strain strongly colonizes at the wound sites in Set III *i.e.*, when fruits were treated with only antagonist (Fig. 3C, C1 and 4C, C1).

Figs. of Set II (Fig. 3B, B1 and 4B, B1) illustrated profuse growth of green mold at wound site and severe damage to the tissue. On the other hand, Set IV illustration (Fig 3D, D1 and 4D, D1) exhibited restricted growth of mycelium with the perforation in the hyphal wall and healthy appearance of the fruits. Furthermore, Figs. of Set III (Fig. 3C and 4C) showed a strong colonization of the antagonist at the wound site. These observations suggest that the biocontrol agent SR-14 have a potential antagonistic activity against *P. digitatum* a notorious postharvest pathogen.

Table 2. Efficacy of *B. licheniformis* SR-14 for the control of post-harvest green mold pathogen in Indian gooseberry

Treatments	3 days	6 days	9 days	12 days
Set I	1.00±0.01^{f}	1.01 ±0.01^{f}	1.03±0.02	1.35±0.05^{d}
Set II	1.04±0.03	1.52 ± 0.02^{e}	2.33±0.06^{b}	3.29±0.12^{a}
Set III	1.00±0.06^{f}	1.01 ± 0.05^{f}	1.01±0.01^{f}	1.01±0.01^{f}
Set IV	1.00±0.01^{f}	1.03 ± 0.03^{f}	1.07±0.06^{f}	1.16±0.032^{e}

Disease severity was rated on a 1-4 scale. Results are mean ± SE of three separate experiments, done in triplicate. Different letters within the row indicate significant difference ($P < 0.05$) using Duncan's multiple range test. Same letter within the row denotes no significant difference between the groups.

Table 3. Efficacy of *B. licheniformis* SR-14 for the control of post-harvest green mold pathogen in common mandarin

Treatments	3 days	6 days	9 days	12 days	15 days
Set I	1.01±0.01^{k}	1.01±0.01^{k}	1.04±0.03^{ijk}	1.26±0.09^{ef}	1.29±0.06^{e}
Set II	1.02±0.03^{jk}	1.15±0.03^{gh}	1.85±0.05^{e}	3.26±0.13^{b}	3.81±0.19^{a}
Set III	1.00±0.01^{k}	1.01±0.03^{k}	1.06±0.02^{hijk}	1.1±0.01^{hij}	1.11±0.02^{hi}
Set IV	1.05±0.03^{ijk}	1.2±0.05^{fg}	1.25±0.03^{ef}	1.31±0.02^{e}	1.41±0.03^{d}

Disease severity was rated on a 1-4 scale. Results are mean ± SE of three separate experiments, done in triplicate. Different letters within the row indicate significant difference ($P < 0.05$) using Duncan's multiple range test. Same letter within the row denotes no significant difference between the groups.

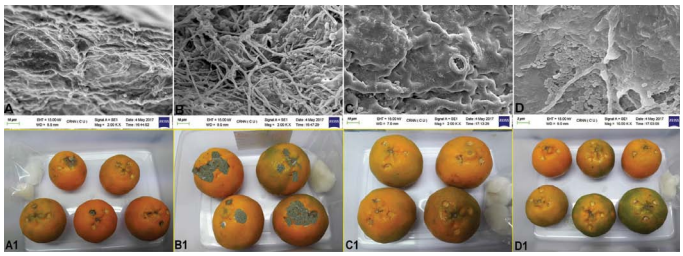

Fig. 3. Scanning electron microscopy of the tissue sample (A-D) and digital photograph (A1-D1) of the common mandarin 15th day after inoculation. Set I- (A, A1). control; Set II- B. showing profusely branched mycelia, B1. severe infection on fruits; Set III- C. showing wound site colonization; Set IV- D. showing mycophagy action of antagonist SR-14.

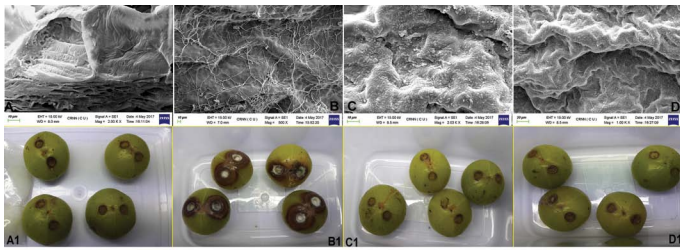

Fig. 4. Scanning electron microscopy of the tissue sample (A-D) and digital photograph (A1-D1) of the Indian gooseberry 12th day after inoculation. Set I- (A, A1). control; Set II- B. showing profusely branched mycelia, B1. severe infection on fruits; Set III- C. showing wound site colonization; Set IV- D. showing mycophagy action of

Effect of *B. licheniformis* SR-14 on defense enzymes: To understand the underlying mechanism behind this protective role of the antagonist, status of three important defense enzymes and total phenolics were estimated in all these sets after respective days of incubations (Table 4 and 5). Data from these table supports that the antagonist not only works through direct interaction with the pathogen, it can also evoke innate immunity in fruit tissue via increased production of all the studied defense enzymes and total phenolics content (Table 4 and 5).

Discussion

The present study demonstrated the efficacy of *B. licheniformis* SR-14 as a potent biocontrol agent for postharvest management of common mandarin and Indian gooseberry. Several antagonistic species of *Bacillus* were reported to be effective in reducing postharvest decay on fruit such as apple, banana, citrus and mango (Alvindia and Natsuaki, 2008; Huang *et al.,* 1992, Korsten *et al.,* 1992, Singh and Daverall, 1984). Habitat colonization by antagonists is prerequisite for biological control. Microorganisms that grow well on the host are usually better choice than noncolonisers (Andrews, 1992). Colonization at wound site by *B. licheniformis* SR-14 before pathogen inoculation was clearly seen under scanning electron microscope. Similar observation was also reported by Panebianco *et al*. (2015) who demonstrated that application of bacterial antagonist at the wound site showed elevated level of antagonistic activity and this is due to the fact that antagonist colonises the wound site prior to pathogen inoculation.

Table 4. Effect of exogenous treatment of *B. licheniformis* SR-14 alone or in co-culture with the pathogen on the production of defense enzymes and total phenol in common mandarin

Parameter	Set I	Set II	Set III	Set IV
PPO [(Δ OD change) min^{-1} g^{-1} protein]	27.13 ± 0.29^c	11.12 ± 0.18^d	42.08 ± 0.25^a	38.4 ± 0.33^b
β -1, 3-glucanase [μ mol glucose produced min^{-1} g^{-1} protein]	178.16 ±0.66^c	46.98 ± 0.49^d	317.32 ±0.4^a	297.68 ±0.43^b
PAL [n mol of transcinnamic acid min^{-1} g^{-1} protein]	0.08 ± 0.01^c	0.07 ± 0.01^c	0.19 ±0.01^a	0.14 ± 0.01^b
Total phenol [μg gallic acid g^{-1} tissue]	54.96 ±0.01^c	43.06 ±0.02^d	114.61 ±0.01^a	94.28 ±0.02^b

Results are mean ± SE of three separate experiments, done in triplicate. Different letters within the row indicate significant difference ($P < 0.05$) using Duncan's multiple range test. Same letter within the row denotes no significant difference between the groups. Set I- SDW (Control); Set II- SDW + *P. digitatum*; Set III- *B. licheniformis* SR-14; and Set IV- *B. licheniformis* SR-14 + *P. digitatum.*

Table 5. Effect of exogenous treatment of *B. licheniformis* SR-14 alone or in co-culture with the pathogen on the production of defense enzymes and total phenol in Indian gooseberry

Parameter	Set I	Set II	Set III	Set IV
PPO	1.54 ± 0.06^c	0.949 ± 0.05^d	2.698 ± 0.32^a	2.215 ±0.26^b
β -1, 3-glucanase	349.52 ± 0.48^c	246.61 ± 0.49^d	570.61 ± 0.93^a	515.01 ± 0.77^b
PAL	0.14 ± 0.01^c	0.08 ± 0.01^d	0.37 ± 0.01^a	0.29 ± 0.01^b
Total phenol	234.12 ±0.02^c	92.91 ±0.01^d	337.01 ±0.41^a	316.01 ±0.02^b

Results are mean ± SE of three separate experiments, done in triplicate. Different letters within the row indicate significant difference ($P < 0.05$) using Duncan's multiple range test. Same letter within the row denotes no significant difference between the groups. Set I- SDW (Control); Set II- SDW + *P. digitatum*; Set III- *B. licheniformis* SR-14; and Set IV- *B. licheniformis* SR-14 + *P. digitatum.*

Activity expressed as:

PPO-Δ OD change) min^{-1} g^{-1} protein
β -1, 3-glucanase-μ mol glucose produced min^{-1} g^{-1} protein
PAL-n mol of transcinnamic acid min^{-1} g^{-1} protein
Total phenol-μg gallic acid g^{-1} tissue

Bacillus licheniformis SR-14 evaluated in this study showed a high level of antagonistic activity towards the tested pathogen. The performances of biocontrol agents *in vitro* and *in vivo* are frequently attributed to the production of antifungal compounds. An effort to characterize the antifungal activity of SR-14 in the present study revealed that the antagonist acted mainly by the release of secondary metabolites like volatile compound and lytic enzymes. These observations coincided with the findings of Ordentlich *et al*. (1988), Upadhyay and Jayswal (1992), Dowling and O'Gara (1994), Bano and Musarrat (2002), who demonstrated that the exposure of selected phytopathogenic fungi to lytic enzymes such as chitinase and protease could result in the degradation of the fungal cell wall. The defense enzymes PO and PPO play an important role in the biosynthesis of lignin and other oxidative phenols (Hammerschmidt *et al.,* 1982; Lamb and Dixon, 1997). PAL is the first enzyme of the phenylpropanoid pathway and is involved in the biosynthesis of phenolics, phytoalexins, and lignins (Pellegrini *et al.,* 1994). Therefore, the increase in PAL activity might have a contribution in improvement of plant defense. PR protein like β-1, 3-glucanase is a host-coded protein having direct action against fungal cell wall compounds like glucan. The present study demonstrated that reduction in disease severity against green mold pathogen by *B. licheniformis* SR-14 treatment was accompanied by increased activities of β-1, 3-glucanases. Phenols are involved in several physiological roles like phytoalexin accumulation, biosynthesis of lignin, and formation of structural barriers (Acharya *et al.,* 2011; Chandra *et al.,* 2014, Chakraborty *et al.,* 2016). The phenolic compounds can be oxidized by PPO and produce antimicrobial phenolic substances, such as quinines, which are more toxic to the pathogens than the former (Tian *et al.,* 2006). Higher accumulation of phenol produces greater resistance to pathogen attack (Acharya *et al.,* 2011, Chandra *et al.,* 2014, Chakraborty *et al.,* 2016). Our present study complements the findings of earlier worker by demonstrating considerable accumulation of phenolic compounds as compared to untreated control indicating greater resistance to pathogen.

Based on these results it can be concluded that *Bacillus licheniformis* SR-14 is an effective biocontrol agent that not only inhibited the growth of *P. digitatum* but also reduced the severity of disease and induced defense responses in mandarin and Indian gooseberry. The present paper endorses an integrated management approach of green mold decay by the use of a putative bacterial antagonist. However, more information regarding possible technology to extend the postharvest shelf life of fruits, postharvest handling under extreme weather conditions as well as an effective way of application of biocontrol agents are needed to be developed so that it would help the farmers to save these valued fruits and limit the use of hazardous fungicide.

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