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Research Article

Biocontrol Management of Postharvest *Penicillium* Rot Disease of Khasi Mandarin (*Citrus reticulata* Blanco) Oranges Using Native *B. subtilis* in Meghalaya, India

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ABSTRACT

Khasi mandarin is an important horticulture crop in the North-Eastern (NE) states of India particularly Meghalaya. Losses of the fruit due to the post-harvest disease Penicillium rot caused by Penicillium sp. have been reported in the state. Efficacy of native B. subtilis isolates was studied to minimise the loss of Khasi mandarin fruit due to Penicillium rot. Four B. subtilis isolates COB5Y1, Bs 167, Bs 197 and Bs 217 identified for potential antagonistic properties against Penicillium sp. under in-vitro were obtained from Plant Pathology Laboratory, School of Crop Protection, Central Agricultural University, Meghalaya, India were evaluated for their efficacy against Penicillium rot disease of Khasi mandarin oranges in-vivo. Three stages of application under field condition were employed i.e. application of B. subtilis at pre-harvest stage, at pre-harvest + post-harvest stage and at post-harvest stage of the fruit. It was observed that application of B. subtilis was most effective when applied at post-harvest stage by immersion of the fruit 24 h prior to inoculation with fungal suspension, with a record of 78.75% disease inhibition by isolate Bs 167 and 75% disease inhibition by isolate COB5Y1 when stored for upto 30 days at room temperature in cartoon boxes. B. subtilis was found to have no adverse effect on the fruit quality i.e. weight of the fruit, total soluble solids and acidity of the fruit. Viability test of B. subtilis isolates showed that storage of B. subtilis pellet suspension at 4°C was the best with most number of viable bacterial colonies even after 180 days.

Key words: *Native B. subtilis, Penicillium rot, Khasi mandarin, Meghalaya, In-vivo.*

INTRODUCTION

Mandarin is very important fruit crop in India next to banana. Amongst a variety of mandarin grown in India, Khasi mandarin is widely cultivated in the North-Eastern (NE) states of India *viz*. Meghalaya, Assam, Manipur, Tripura, Arunachal Pradesh and Sikkim[42]. Meghalaya and Assam have the maximum area and production of Khasi mandarin[41]. Mawsynram and Cherrapunji areas of Meghalaya which are the wettest places in the world produce excellent quality of Khasi mandarin of high commercial value[14].

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Khasi mandarin has the potential to go global with India signing a deal for agricultural and horticultural exports. The total export of orange from India is 33628 metric tons with the value of Rs 3529 lakh. Bangladesh is the biggest importer of Indian orange and share 89.6% of total export[2].

Although Meghalaya is one of the largest producers of orange in the country, due to the problem of postharvest diseases, there are considerable losses to the harvested fruits. The problem of postharvest disease *Penicillium* rot of Khasi mandarin has been one of major constraint for maximum production and export of the fruit from the state. Green mould caused by *P. digitatum* is the major postharvest disease of citrus and has been reported globally to causes serious losses annually[9]. In the North Eastern (NE) parts of India, upto 30–50% losses of citrus was reported to be due to *Penicillium* rot caused by *P. digitatum*[40]. In Meghalaya, 17.75-19.28% losses of Khasi mandarin was reported, due to *Penicillium* rot disease caused by *P. brevicompactum*[6].

B. subtilis is well known as one of the most active antagonist that has received attention and considered an interesting bacterium to investigate for inhibitory substances, since it produces a large number of peptide antibiotics. The antibacterial substances produced by *B. subtilis* has gain biological control potential against several phytopathogens. *B. subtilis* have shown promise for controlling a wide range of fungi that cause fruit decay and acts as an antagonist to plant pathogen growth through their production of antibiotics such as iturin, surfactin, fengycin, enzymes that degrade fungal structural polymers such as chitinase and β 1-3 glucanase, antifungal volatiles and endospores have made it resistant to extreme environmental[11,22,21,35,26].

The use of synthetic fungicides is of public concern due to the accumulation of chemical residues in the food chain and environmental, which is also the primary means to control postharvest diseases[10,4]. Carbendazim has been reported as a very effective fungicide against green mould of citrus and other postharvest rots and was widely used[47]. Global concern has arise which encourages shifting towards reduced use of fungicides on produce and seeking safer and eco-friendly alternatives for reducing the decay loss in the harvested commodities[29]. The present investigation has been conducted to study the efficacy of potential *B. subtilis* isolates against *Penicillium* rot pathogen of Khasi mandarin under storage condition to prolong the shelf life of the fruit.

MATERIAL AND METHODS

Evaluation of potential *B. subtilis* isolates against *Penicillium* rot of Khasi mandarin

Fruit preparation

Khasi mandarin oranges with similar colour, uniformity, size and maturity without wounds were selected. Fruits were washed with tap water and surface sterilized with 70% ethanol and air dried prior to wounding.

Antagonist preparation

Four potential *B. subtilis* isolates COB5Y1, Bs 167, Bs 197 and Bs 217 were obtained from Plant Pathology Laboratory, School of Crop Protection, Central Agricultural University, Meghalaya. Each was grown on nutrient agar (NA) media at 30°C for 24 h. A loopful of each culture was then transferred to a 250 ml conical flask containing 50 ml of nutrient broth (NB) and incubated on a rotary shaker (200 rpm) for 48 h at 30°C. The culture was then centrifuged for 15 min at 4000 rpm and pellets were re-suspended in sterile distilled water and centrifuged for a second time. Bacterial cell suspensions were adjusted to 10^8 cells/ml using a spectrophotometer optical density (OD) 0.5 at 550 Å wavelengths that equates to 10^8 cells/ml approximately[39].

Fungal pathogen inoculums

Penicillium sp. was isolated from *Penicillium* rotted mandarin fruits and was grown on potato dextrose agar (PDA) media for 7-10 days at 26±1°C. Spores were harvested by flooding the surface of the media with sterile distilled water and gently agitating the plate to dislodge the spores. The spore concentration was adjusted to 10^6 spores/ml using a

spectrophotometer optical density (OD) 0.2 at 550 Å wavelengths that equates to 10^6 cells/ml approximately[34,39].

Pre-harvest treatment of the Khasi mandarin fruits with *B. subtilis*

Pre-harvest treatment was conducted in the farmer's field at Mawryngkneng village, East Khasi Hills District, Meghalaya. The selected fruits on the orange trees were wound inoculated uniformly by pricking using sterile dissecting needle (1) at the equator of fruits, (2) at the stem end of the fruit, (3) at the bottom end of the fruit and (4) at the equator + stem end + bottom end of the fruit. *B subtilis* suspension was sprayed on the selected fruits 24 h prior to inoculation with pathogen[39]. The 24 h *B. subtilis* treated fruits were then harvested and challenged with *Penicillium* sp. suspension. The treated fruits were stored in cartoon boxes at room temperature. Observations for disease incidence will be made at an interval of 7, 15 and 30 days. There were 4 fruits per replicate and each treatment was replicated four times. The control consisted of fruit sprayed with sterile distilled water instead of the bio-agent.

Pre harvest + Post-harvest treatment of the Khasi mandarin fruits with *B. subtilis*

Pre-harvest treatment was conducted at the farmer's field as described previously. After harvest of the fruits, post-harvest treatment was carried out. The fruits after harvest were immersed for 2 mins in an aqueous suspension of the antagonist *B. subtilis* and air dried for 24 h. The fruits were then challenged with *Penicillium* sp. suspension by dipping the fruits in the pathogen's suspension for 2 mins and then air dried. The treated fruits were then stored in cartoon boxes at room temperature and observation for disease incidence was made at an interval of 7, 15 and 30 days. There were 4 fruits per replicate and each treatment was replicated four times. The control consisted of fruit immersed in sterile distilled water instead of the bio-agent and then challenged with *Penicillium* sp.[34].

Post-harvest treatment of the Khasi mandarin fruits with *B. subtilis*

The test harvested fruits were wound inoculated uniformly by pricking using sterile dissecting needle (1) at the equator of fruits, (2) at the stem end of the fruit, (3) at the bottom end of the fruit and (4) at the equator + stem end + bottom end of the fruit. The fruits were then dipped in *B. subtilis* suspension for 2 mins, 24 h prior to inoculation with *Penicillium* sp. suspension. The treated fruits were stored in cartoon boxes at room and observation for disease incidence was made at an interval of 7, 15 and 30 days. There were 4 fruits per replicate and each treatment was replicated 4 times. The control consisted of fruit immersed in sterile distilled water instead of the bio-agent and then challenged with *Penicillium* sp.[34].

Treatments:

Pre-harvest-

$\mathbf{T_1}$ = Spray application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the equator of fruits only)

$\mathbf{T_2}$ = Spray application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the stem end of the fruit only)

$\mathbf{T_3}$ = Spray application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the bottom end of the fruit only)

$\mathbf{T_4}$ = Spray application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the equator + stem end + bottom end of the fruit)

$\mathbf{T_5}$ = Control

Pre harvest + Post-harvest-

$\mathbf{T_{1.1}}$ = Spray + dip application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the equator of fruits only)

$\mathbf{T_{2.1}}$ = Spray + dip application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the stem end of the fruit only)

$\mathbf{T_{3.1}}$ = Spray + dip application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the bottom end of the fruit only)

$\mathbf{T_{4.1}}$ = Spray + dip application of *B. subtilis* suspension 24 h prior to inoculation with

Penicillium sp. (wounds made at the equator + stem end + bottom end of the fruit)

$T_{5.1}$ = Control

Post-harvest-

$T_{1.2}$ = Dip application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the equator of fruits only)

$T_{2.2}$ = Dip application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the stem end of the fruit only)

$T_{3.2}$ = Dip application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the bottom end of the fruit only)

$T_{4.2}$ = Dip application of *B. subtilis* suspension 24 h prior to inoculation with *Penicillium* sp. (wounds made at the equator + stem end + bottom end of the fruit)

$T_{5.2}$ = Control

Disease incidence % = (number of infected fruits/ total no. of inoculated wounded fruits) x100[26].

Effects of antagonist on postharvest quality of the fruits

To evaluate the effect of the antagonist *B. subtilis* on postharvest quality of the Khasi mandarin fruits, the *B. subtilis* treated fruits were kept in cartoon boxes at room temperature for 15 days. Weight loss of the fruit, total soluble solids and titratable acidity of the fruits were recorded. A control fruit was treated with sterile distilled water[39].

Weight loss

The mass of the individual fruits was measured using a balance (0.001 g) (Metter toledo AB204-S) before treatment (A) and after storage (B) for 15 days. The mass loss was calculated as (A-B)/A.

Total soluble solids

Total soluble solids (TSS) were determined by measuring the refractive index of the juice diluted 1:1 using a hand refractometer "ATAGO N1" and the results were expressed as % Brix.

Titratable acidity

Acidity was measured by titration of 5 mL of juice with 0.1 N NaOH and phenolphthalein as an indicator. Titratable acidity (TA) was calculated as a percentage citric acid by the formula %TA = [(mL NaOH) (N NaOH) (meq.wt.acid)/mL sample] x 100.

Viability test of *B. subtilis*

A loop full of fresh *B. subtilis* was inoculated in sterile distilled water in vials and stored as liquid formulation at room temperature, at 4°C and at -20°C. Samples of each formulation Bs 167, Bs 197, Bs 217 and COB5Y1 were taken at 60, 30 and 180 days and serially diluted in sterile distilled water and 0.1 mL volumes were plated on NA medium in Petri plates. Plates were incubated for 24 h at 30°C, after which *B. subtilis* colonies were counted[20]. Bacteria per ml of serially diluted sample were calculated using the following equation

$$\text{Bacterial population} = \frac{\text{Number of Colony}}{\text{Volume plated (ml) x total dilution used}}$$

Experimental design and data analysis

The field expenrimental designed followed was complete ramdomized block design (CRD). All data were subjected to analysis of variance (ANOVA) at 5% level of significance and differences between means were evaluated by the using MS-DOS Customized based programme[12].

RESULT AND DISCUSSION

Evaluation of potential *B. subtilis* isolate against *Penicillium* rot of Khasi mandarin

Khasi mandarin fruit were treated with *B. subtilis* 24 h prior to harvest for pre-harvest treatment to evaluate the antagonistic effect of the bio-agent against *Penicillium* sp. Application of the bio-agent at pre-harvest stage revealed that all the 4 *B. subtilis* isolates could not inhibit the pathogen *Penicillium* sp. completely. However, COB5Y1 and Bs 167 perform better than the other 2 isolates *i.e.* Bs 197 and Bs 217. COB5Y1 could inhibit the disease when stored for 7 days upto 28.75% (with 71.25% DI) and for 15 days only 17.5%

(with 82.5% DI) and could not inhibit the disease at all when stored for 30 days (with 100% DI). Bs 167 was found effective only upto 18.75% (with 81.25% DI) when stored for 7 days and for only 8.75% (with 88.75% disease incidence) when stored for 15 days and 100% DI when stored for 30 days (Table 1).

When the bio-agent was applied at pre-harvest + postharvest stage, it was observed that all the 4 *B. subtilis* isolates could not inhibit the pathogen *Penicillium* sp. completely. Similar result was observed as in pre-harvest application but with a higher disease inhibition per cent where, COB5Y1 and Bs 167 perform better than the other 2 isolates *i.e.* Bs 197 and Bs 217. COB5Y1 could inhibit the disease when stored for 7 days upto 43.75% (with 56.25% DI) and for 15 days only 33.75% (with 66.25% DI) and could not inhibit the disease at all when stored for 30 days (with 100% DI). Bs 167 was found effective upto 40% (with 60% DI) when stored for 7 days and for only 43.75% (with 56.25% DI) when stored for 15 days and 17.5% (with 82.50% DI) when stored for 30 days (Table 2).

Application of the bio-agent at post-harvest stage revealed the best result. Bs 167 and COB5Y1 performed better as compared to the other 2 isolates *i.e.* Bs 197 and Bs 217. Bs 167 could inhibit the disease when stored for 7 days upto 81.50% (with only 18.75% DI) and for 15 days upto 80% (with 20% DI) and could inhibit the disease even when stored for 30 days upto 78.75% (with 21.25% DI). COB5Y1 was found very effective as well upto 72.50% (with 27.50% DI) when stored for 7 days and for upto 67.50% (with 32.50% DI) when stored for 15 days and upto 75% (with 35% DI) when stored for 30 days. Bs 217 and Bs 197 could also inhibit the disease upto 75% (with 25% DI) and 56.25% (with 43.75% DI) respectively when stored for 7 days. When stored for 15 days both Bs 217 and Bs 197 could inhibit the disease upto 62.50% (with 37.50% DI) and 56.25% (with 43.75% DI) respectively. When the fruits were stored for 30 days the disease incidence recorded for Bs 217 and Bs 197 was 55% (with 45% DI) and 56.25% (with 43.75% DI) respectively (Table 3).

Mehrotra *et al*.[30] used bacterial bio-agent to apply as water suspension onto the wounds on the surface of citrus fruit prior to inoculation with pathogen and reported effective which showed reduce in disease incidence of *P. digitatum* and *P. italicum* decay of orange which supports the findings of the present investigation. Leibinger *et al*.[27] also found that suspension of *B. subtilis* with a concentration of 10^8 cfu/ml was successful against post-harvest pathogens *Penicillium* sp., *Botrytis cinerea* and *Pezicula malicorticis.* Antagonistic yeast concentration of 10^8 cfu/ml was reported to give higher protection against a concentration of 10^5 cfu/ml against *P. digitatum* and *P. italicum* of Clementine and Valencia citrus variety[25]. Application of the antagonist after harvest of the fruits 24 h prior to inoculation with fungal suspension was most successful which can be supported with the findings of Arrebola *et al*.[3].

The success of pre-harvest application was reported to be dependent of the tolerance to environmental stress such as dry conditions, direct UV irradiation, high temperature, rapid climatic changes and nutrient availability[33]. The report could support the findings that pre-harvest spray of the bio-agent was not as successful as the postharvest application. The findings of Porat *et al*.[36] are also in line with the findings of the present investigation as they reported that application of the antagonist *B. subtilis* (10^8 cells/ml) as aqueous suspension by immersion at post-harvest stage showed successful control against the growth of *P. digitatum* and *P. italicum* on harvested citrus fruits. Auret reported that *B. licheniformis* (8251) and *B. subtilis* (8248) were very effective antagonists *in-vivo* against *P. digitatum* as compared to other *Bacillus* sp. *B. subtilis* strain GB07 achieved green mold control of Valencia upto 72.2 to 100% as reported by Zhang and Dou[49] which are in line with the findings of the present investigation. Similar finding was reported by Obagwu and Korsten[34] that *B. subtilis* applied by immersion of the harvested fruits in its suspension showed successful control over citrus green and blue molds caused by *P. digitatum* and *P.*

italicum even when the fruits were stored for 4 weeks.

Disease incidence with respect to point of inoculation

The wounds on the fruit was made at the equator of fruits, at the stem end of the fruit, at the bottom end of the fruit and at the equator + stem end + bottom end of the fruit to observed if there is any significant difference on the disease incidence. However, it was observed that the point of inoculation of the pathogen on the fruit did show significant difference on the disease incidence on the fruit. At all the 3 stages of application of the bio-agent *i.e*. at pre-harvest stage, at pre-harvest + post-harvest stage and at post-harvest stage of the fruit, it was observed that the DI% was highest in the control followed by treatment where, wounds was made at the equator + stem end + bottom end of the fruit, treatment where wound was made at the stem end of the fruit only and bottom end of the fruit only were at par when stored for 7, 15 and 30 days. The interaction between the bio-agent (A) and the point of inoculation (B) were found non-significant (NS) (Table 1, 2, 3). The disease initiates from the wounds made on the fruit irrespective of where the wound was made. Barkai-Goland[5] and Nunes *et al*.[33] supports the finding as they reported that postharvest pathogens are unable to penetrate directly through the cuticle but usually requires a wound through which conidia penetrates and initiates the disease from the point of inoculation.

B. subtilis isolates Bs 167 and COB5Y1 were found highly effective as compared to Bs197 and Bs 217 against *Penicillium* rot of Khasi mandarin when treated at post-harvest stage of the fruits which has been reported since the early 1980s that *B. subtilis* has potentiality as biological control agents against various fungal rot pathogen[43,38,44,19,16,24,28]. The in co-operation of the potential *B. subtilis* isolates with wax[38,37] or hot water treatment[34] or other biocontrol agent[15,45,48,31] can be further evaluated to obtain maximum disease inhibition as has found successful in several investigations done by renowned researches.

Effects of antagonist on postharvest quality of the fruits

The effect of the 4 potential *B. subtilis* isolates (COB5Y1, Bs 167, Bs 217, Bs 197) on the fruit quality parameters such as weight loss, total soluble solids and titratable acidity and the result showed that there was no significant effect on the quality of the fruit. There was slight decrease in the weight of the fruit, total soluble solids and acidity of the fruit but no significant effect by the treatment even after storage at room temperature for 15 days (Table 4). The findings can be supported with the reports of Sangwanich *et al*.[39] which observed that the quality parameters of fruits were retained following combination treatment with *B. subtilis* and incubation at 25°C for 7 days. Hangsing *et al*.[13] reported that the optimum fruit quality parameters of Khasi mandarin, of TSS is 7-14% Brix, acidity of the fruit 0.67-1.31% and weight of the fruit 10.31-109.90 gm. In the present investigation the TSS of the fruit ranges between 10-14% Brix, acidity 0.7-1.2% and barely 1% mass loss after treatment of the fruit with *B. subtilis* and storage for 15 days. The slight decrease in acidity, weight of the fruit and TSS can be associated with high rate of respiration, the transformation of pectic substances, starch, hemicelluloses or other polysaccharides in soluble sugar and also with dehydration of fruits during storage which were also noticed by Carrillo *et al*.[7], Zade *et al*.[46] in Nagpur mandarin, Deka *et al*.[8] in Khasi mandarin and Nath *et al*.[32] in Khasi mandarin.

Viability test of *B. subtilis*

The 4 potential *B. subtilis* isolates (COB5Y1, Bs 167, Bs 197, Bs 217) were tested for their viability when stored for 60, 120 and 180 days at 4°C, -20°C and at room temperature. It was observed that the viability of the isolates decreases with increase in the no. of storage days but they were all viable even after storage 180 days. With respect to storage temperature, storage at 4°C was found with most number of viable bacterial colonies even after 180 days. The viability of all the 4 *B. subtilis* isolates was greatly reduced when stored at room temperature. It was observed that storage at 4°C was the best amongst other temperature

even after 180 days. COB5Y1 and Bs 167 isolates showed constant significant viability at 5 % level of significance as compared to the other two test isolates (Bs 217 and Bs 197) when stored at 4°C, -20°C, room temperature for 60, 120 and even at 180 days (Table 5). Korsten and Cook[23] reported that freezing and long-term storage of *B. subtilis* cells reduces viable cells significantly with time which supports the findings of the present investigation. Storage of bacterial bio-control agent formulation as refrigerated liquid and as frozen pellets has been reported successful by Janisiewicz and Jeffers[18] and Abadias *et al.*[1]. Isnawati and Trimulyono[17] reported that *B. subtilis* was found viable when stored at 4°C upto 3 months. The reports above supports the finding that *B. subtilis* can be stored as pellet suspension at 4°C but decreases in viable cells with time.

Table 1: Disease incidence (%) for pre harvest treatment at different point of inoculation of the Khasi mandarin fruits with *B. subtilis*

POI / Bioagent	7 DAI						15 DAI						30 DAI					
	T_1	T_2	T_3	T_4	T_5	Mean	T_1	T_2	T_3	T_4	T_5	Mean	T_1	T_2	T_3	T_4	T_5	Mean
COB 5Y1	56.25	62.50	68.75	75.00	93.75	**71.25**	81.25	68.75	81.25	87.50	93.75	**82.50**	100.00	100.00	100.00	100.00	100.00	100.00
Bs167	81.25	75.00	68.75	81.25	100.00	**81.25**	75.00	81.25	93.75	93.75	100.00	**88.75**	100.00	100.00	100.00	100.00	100.00	100.00
Bs 197	87.50	87.50	87.50	100.00	100.00	92.50	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Bs 217	93.75	100.00	100.00	100.00	100.00	98.75	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Mean	79.68	81.25	81.25	89.06	**98.44**	85.94	89.06	87.50	93.75	95.31	**98.44**	92.81	100.00	100.00	100.00	100.00	100.00	100.00
	Bioagents (A)		Point of inoculation (B)		AxB (Interaction)		Bioagents (A)		Point of inoculation (B)		AxB (Interaction)		Bioagents (A)		Point of inoculation (B)		AxB (Interaction)	
SEM (±)	3.15		2.82		6.30		2.82		2.52		5.64		-		-		-	
CD at 5 %	8.93		7.99		17.86 (NS)		7.99		7.15		15.98 (NS)		-		-		-	

N.B: T_1= Wounds at the equator of fruits, T_2= Wounds at the stem end of the fruit, T_3= Wounds at the bottom end of the fruit, T_4= Wounds at the equator + stem end + bottom end of the fruit, T_5= control no wound, **DAI**= days after inoculation **POI**= point of inoculation, **NS**= non-significant. Analysis was determined by two factors ANOVA at 5% level of significance

Table 2: Disease incidence (%) for pre+post harvest treatment at different point of inoculation of the Khasi mandarin fruits with *B. subtilis*

POI / Bioagent	7 DAI						15 DAI						30 DAI					
	$T_{1.1}$	$T_{2.1}$	$T_{3.1}$	$T_{4.1}$	$T_{5.1}$	Mean	$T_{1.1}$	$T_{2.1}$	$T_{3.1}$	$T_{4.1}$	$T_{5.1}$	Mean	$T_{1.1}$	$T_{2.1}$	$T_{3.1}$	$T_{4.1}$	$T_{5.1}$	Mean
COB 5Y1	37.50	43.75	43.75	81.25	93.75	**56.25**	81.25	43.75	43.75	68.75	93.75	**66.25**	100.00	100.00	100.00	100.00	100.00	**100.00**
Bs167	37.50	50.00	62.50	87.50	87.50	**60.00**	50.00	43.75	43.75	62.50	81.25	**56.25**	100.00	75.00	62.50	75.00	100.00	**82.50**
Bs 197	68.75	87.50	75.00	62.50	100.00	83.75	93.75	100.00	93.75	100.00	100.00	97.50	100.00	100.00	100.00	100.00	100.00	100.00
Bs 217	81.25	75.00	50.00	100.00	93.75	76.25	93.75	93.75	75.00	100.00	100.00	92.50	100.00	100.00	100.00	100.00	100.00	100.00
Mean	56.25	64.06	57.81	71.88	**93.75**	69.06	79.69	70.31	64.06	82.81	**93.75**	78.13	100.00	93.75	90.63	93.75	**100.00**	95.63
	Bioagents (A)		Point of inoculation (B)		AxB (Interaction)		Bioagents (A)		Point of inoculation (B)		AxB (Interaction)		Bioagents (A)		Point of inoculation (B)		AxB (Interaction)	
SEM (±)	5.37		4.80		10.73		4.81		4.30		9.62		2.42		2.17		4.84	
CD at 5 %	15.20		13.60		30.40 (NS)		13.62		12.18		27.24(NS)		6.86		6.13		13.71 (NS)	

N.B: $T_{1.1}$= Wounds at the equator of fruits, $T_{2.1}$= Wounds at the stem end of the fruit, $T_{3.1}$= Wounds at the bottom end of the fruit, $T_{4.1}$= Wounds at the equator + stem end + bottom end of the fruit, $T_{5.1}$= control no wound, **DAI**= days after inoculation, **POI**= point of inoculation, **NS**= non-significant. Analysis was determined by two factor ANOVA at 5% level of significance

Table 3: Disease incidence (%) for post-harvest treatment at different point of inoculation of the Khasi mandarin fruits with *B. subtilis*

POI / Bioagent	7 DAI						15 DAI						30 DAI					
	$T_{1.2}$	$T_{2.2}$	$T_{3.2}$	$T_{4.2}$	$T_{5.2}$	Mean	$T_{1.2}$	$T_{2.2}$	$T_{3.2}$	$T_{4.2}$	$T_{5.2}$	Mean	$T_{1.2}$	$T_{2.2}$	$T_{3.2}$	$T_{4.2}$	$T_{5.2}$	Mean
COB 5Y1	6.25	31.25	0.00	12.50	87.50	**27.50**	12.50	31.25	18.75	12.50	87.50	**32.50**	12.50	31.25	18.75	12.50	100.00	**35.00**
Bs167	0.00	0.00	0.00	6.25	87.50	**18.75**	0.00	0.00	0.00	0.00	100.00	**20.00**	0.00	0.00	0.00	6.25	100.0	**21.25**
Bs 197	0.00	62.50	0.00	68.70	87.50	43.75	0.00	62.50	0.00	68.75	87.50	43.75	0.00	62.50	0.00	68.75	87.50	43.75
Bs 217	6.25	0.00	12.50	18.75	87.50	25.00	18.75	6.25	43.75	31.25	87.50	37.50	18.75	6.25	62.50	37.50	100.00	45.00
Mean	3.13	23.44	3.13	26.56	**87.50**	28.75	7.81	25.00	15.63	28.13	**90.63**	33.44	7.81	25.00	20.31	31.25	**96.88**	36.25
	Bioagents (A)		**Point of inoculation (B)**		**AxB (Interaction)**		**Bioagents (A)**		**Point of inoculation (B)**		**AxB (Interaction)**		**Bioagents (A)**		**Point of inoculation (B)**		**AxB (Interaction)**	
SEM (±)	2.64		2.36		5.29		2.81		2.51		5.61		2.51		2.25		5.02	
CD at 5 %	7.49		6.70		14.97 (NS)		7.95		7.11		15.90 (NS)		7.11		6.36		14.22 (NS)	

N.B: $T_{1.2}$= Wounds at the equator of fruits, $T_{2.2}$= Wounds at the stem end of the fruit, $T_{3.2}$= Wounds at the bottom end of the fruit, $T_{4.2}$= Wounds at the equator + stem end + bottom end of the fruit, $T_{5.2}$= control no wound, **DAI**= days after inoculation, **POI**= point of inoculation, **NS**= non-significant. Analysis was determined by two factors ANOVA at 5% level of significance

Table 4: Effect of antagonist *B. subtilis* isolates on the postharvest qualities of the Khasi mandarin fruits

Treatments	Mass loss (%)	TSS (Brix %)	Titratable acidity (%)
COB5Y1	0.20±0.01^a	13.30±0.31^a	1.07±0.34^a
Bs 167	0.17±0.02^a	12.70±1.63^a	0.98±0.04^a
Bs 197	0.19±0.02^a	11.47±1.33^a	0.85±0.23^a
Bs 217	0.18±0.01^a	13.93±0.79^a	1.11±0.15^a
Control	0.19±0.02^a	12.13±0.78^a	0.64±1.28^a
CD at 5%	0.58	3.37	0.63

NB: Quality parameters of the Khasi mandarin fruit were obtained after 15 days at room temperature (~23°C). Mean values with in columns followed by the same letter are not significantly different where *P*=5% level of significance.

Table 5: Viability test of *B. subtilis* isolates

Isolates ↓	Viabilitity test (no. of colonies per ml x 10^{16})								
	60 days			120 days			180 days		
	4°C	-20°C	RT	4°C	-20°C	RT	4°C	-20°C	RT
COB5Y1	**1.932^a**	**11.837^a**	**00.849^a**	**1.763^a**	**00.720^a**	**00.671^a**	**1.015^{ab}**	**00.003^a**	**0.005^a**
Bs 167	**3.021^a**	**22.160^a**	**00.679^a**	**1.367^a**	**11.533^a**	**00.059^b**	**1.310^a**	**00.914^a**	**0.005^a**
Bs 197	**2.190^a**	**00.932^b**	**00.702^a**	**1.860^a**	**00.888^a**	**00.129^b**	**2.183^a**	**0.005^a**	**0.002^a**
Bs 217	**0.103^a**	**0.081^c**	**0.197^b**	**0.084^a**	**0.068^a**	**0.068^b**	**0.004^b**	**0.007^a**	**0.003^a**
SSEM(±)	**00.641**	**00.137**	**00.131**	**00.704**	**00.073**	**00.984**	**00.359**	**00.228**	**0.001**
CD at 5%	**2.089**	**0.446**	**0.427**	**2.293**	**0.237**	**0.321**	**1.170**	**0.745**	**0.003**

N.B: RT=room temperature. Each value is the mean number of CFU recorded for each isolate. Values within columns followed by the same letter are not significantly different, where *P* =5% level of significance.

CONCLUSION

Post-harvest application of *B. subtilis* as liquid formulation by immersing the fruits before storage and sending for sale to market was found as most effective with minimum *Penicillium* rot incidence even after 30 days. Storage of *B. subtilis* as liquid formulation at 4°C was found successful upto 180 days. The two potential *B. subtilis* isolates COB5Y1 and Bs 167 could serve as potential bio-agent

against post-harvest *Penicillium* rot of Khasi mandarin in Meghalaya which need further post-harvest evaluation. In co-operation of these potential *B. subtilis* isolates with wax or hot water treatment or other biocontrol agent can be further evaluated to obtain maximum disease inhibition as has found successful in several investigations.

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REFERENCES

1. Abadias, M., Usall, J., Teixido, N. and Vinas, I., Liquid formulation of the post-harvest biocontrol agent Candida sake CPA-1 in isotonic solutions. *Phytopathol.* **93:** 436-442 (2003).
2. Anonymous, National Horticulture Board, Indian Horticulture Database. (2013).
3. Arrebola, E., Jacobs, R. and Korsten, L., Iturin A is the principal inhibitor in the biocontrol activity of *Bacillus amyloliquefaciens* PPCB004 against postharvest fungal pathogens. *J. Applied Microbiol.* **108(2010):** 386–395 (2009).
4. Arul, J., Emerging technologies for the control of postharvest diseases of fresh fruits and vegetables. In: Biological Control of Postharvest Diseases-Theory and Practice (Eds.), Wilson, C. L. and Wisniewski, M. E., CRC Press, Inc. Pp. 1–6 (1994).
5. Barkai-Goland, Postharvest diseases of fruits and vegetables. Development and Control, Elsevier, Amsterdam, Pp. 418 (2001).
6. Barman, K., Survey and management of post-harvest diseases of Khasi mandarin (*Citrus reticulata* Blanco) in Meghalaya. Thesis, College of Post Graduate Studies, School of Crop Protection, Umiam, Meghalaya (2010).
7. Carrillo, L. A., Cruz-Hernandez, A., Guevara-Lara, F. and Paredes- Lopez, O., Physico-chemical changes during ripening in storage of two varieties of prickly pear stored at 18 °C. *J. Food Sc. Technol*. **40:** 461–464 (2003).
8. Deka, B. C., Sharma, S. and Borah, S. C., Postharvest management practices for shelf life extension of Khasi mandarin. *Indian J. Hort.* **63:** 251–255 (2006).
9. Eckert, J. W. and Brown, G. E., Postharvest citrus diseases and their control. In: Fresh Citrus Fruits (Eds), Wardowski, F. W. S., Nagy and Grierson, W., New York: Van Nostrand Reinhold Company Inc. Pp. 315-360 (1986).
10. Eckert, J. W., Recent developments in the chemical control of postharvest disease. *Acta Hortic.* **269:** 477 (1990).
11. Fiddaman, P. J. and Rossall, S., The production of antifungal volatiles by *Bacillus subtilis*. *J. Appl. Bacteriol.* **74:** 119–126 (1993).
12. Gomez, A. K. and Gomez, A. A., Statistical procedures for agricultural research. *Int. Rice Research Institute Book*, John Willy and Sons, (1976).
13. Hangsing, H., Mathew, B. and Kalita, C. D., Performance of Khasi Mandarin in Garo Hills of Meghalaya. *Int. J. Sc., Environ, Technol.* **5(5):** 3213–3223 (2016).
14. Hazarika, K. T., Citrus genetic diversity of North-East India, their distribution Eco-geography and Eco-biology. *Genet. Resour. Crop Evol*. **2:** 2-5 (2012).
15. Hofstein, R., Droby, S., Chalutz, E., Wilson, C. L. and Friedlender, B., Scaling-up the production of an antagonist-from basic research to R and D. Biological Control of Postharvest Diseases of Fruits and Vegetables, Wrkshp. Proc., Shepherdstown, U.S. Dept. Agr.-Agr. Res. Serv. Publ. **92:** 188-201 (1991).
16. Huang, Y., Wild, B. L. and Morris, S. C., Postharvest biological control of

Penicillium digitatum decay on citrus fruit by *Bacillus pumulus*. *Ann. Appl. Biol*. **130:** 367-372 (1992).

17. Isnawati and Trimulyono, G., Temperature range and degree of acidity growth of isolate of indigenous bacteria on fermented feed "fermege". *J. of Physics*: *Conf. Series* **953:** 012209 (2018).
18. Janisiewicz, W. J. and Jeffers, S., Efficacy of commercial formulation of two biofungicides for control of blue mold and gray mold of apples in cold storage. *Crop protection* **16:** 629-633 (1997).
19. Janisiewicz, W. J. and Roitman, J., Biological control of blue-mold and gray-mold on apple and pear with *Pseudomonas cepacia*. *Phytopathol.* **78:** 1697-1700 (1988).
20. Jayaraj, J., Radhakrishnan, V. N., Kannan, R., Sakthivel, K., Suganya, D., Venkatesan, S. and Velazhahan, R., Development of new formulations of *Bacillus subtilis* for management of tomato damping-off caused by *Pythium aphanidermatum*. *Biocontrol Sc. and Technol.* **15(1):** 55-65 (2005).
21. Jiang, Y. M., Zhu, X. R. and Li, Y. B., Postharvest control of litchi fruit rot by *Bacillus subtilis*. *Lebensm. Wiss. U.-Technol.* **34:** 430–436 (2001).
22. Knox, O. G. G., Killham, K. and Leifert, C., Effects of increased nitrate availability on the control of plant pathogenic fungi by the soil bacterium *Bacillus subtilis*. *Appl. Soil Ecol*. **15:** 227–231 (2000).
23. Korsten, L. and Cook, N., Optimizing Culturing Conditions for *Bacillus subtilis*. *South African Avocado Growers' Association Yearbook.* **19:** 54-58 (1996).
24. Korsten, L., De Jager, E. S., Paul, I., Obagwu, J. and El-Ghaouth, A., Alternative control of citrus postharvest diseases. *Proc. Int. Soc. Citriculture*, in press (2000).
25. Lahlali, R., Serrhini, M. N. and Jijakli, M. H., Development of a Biological Control Method against Postharvest Diseases of citrus fruits. *Comm. Appl. Biol. Sci*. **70(3):** 47 (2005).
26. Leelasuphakul, W., Hemmaee, P. and Chuenchitt, S., Growth inhibitory properties of *Bacillus subtilis* strains and their metabolites against the green mold pathogen (*Penicillium digitatum* Sacc.) of citrus fruit. *Postharvest Biol. and Technol.* **48:** 113–121 (2008).
27. Leibinger, W., Breuker, B., Hahn, M. and Mendgen, K., Control of Postharvest Pathogens and Colonization of the Apple Surface by Antagonistic Microorganisms in the Field. *Phytopathol.* **87(11):** 1103-1110 (1997).
28. Long, C. A., Deng, B. X., Deng, X. X., Commercial testing of Kloeckera apiculata, isolate 34–9, for biological control of postharvest diseases of citrus fruit. *Annals of Microbiol.* **57(2):** 203–207 (2007).
29. Mari, M., Neri, F. and Bertolini, P., Novel Approaches to Prevent and Control Postharvest Diseases of Fruit. *Stewart Postharvest Rev*. **3(6):** Article 4 (2007).
30. Mehrotra, N. K., Sharma, N., Ghosh Nayek, R. and Nigam, M., Biological control of green and blue mould disease of citrus fruit by yeast. *Indian Phytopath*. **49(4):** 350-354 (1996).
31. Morsy, M. E., Abdel-Kawi, K. A. and Khalil, A. N. M., Efficiency of *Trichoderma viride* and *Bacillus subtilis* as Biocontrol Agents against *Fusarium solani* on Tomato Plants. *Egypt. J. Phytopathol.* **37(1):** 47-57 (2009).
32. Nath, A., Barman, K., Chandra, S. and Baiswar, P., Effect of plant extracts on quality of Khasi Mandarin (*Citrus reticulata* Blanco) fruits during ambient storage. *Food Bioprocess Technol*. **6:** 470–474 (2013).
33. Nunes, A. C., Manso, T. and Lima-Costa, E. M., Postharvest Biological Control of Citrus fruits. *Tree and Forestry Sc. And Biotechnol.* **3(2):** 116-126 (2009).
34. Obagwu, J. and Korsten, L., Integrated control of citrus green and blue molds using *Bacillus subtilis* in combination with sodium bicarbonate or hot water.

Postharvest Biol. and Technol. **28:** 187-194 (2003).

35. Pinchuk, I. V., Bressollier, P., Sorokulova, I. B., Verneuil, B. and Urdaci, M. C., Amicoumacin antibiotic production and genetic diversity of *Bacillus subtilis* strains isolated from different habitats. *Res. Microbiol*. **153:** 269–276 (2002).
36. Porat, R., Daus, A., Weiss, B., Cohen, L., Fallik, E. and Droby, S., Reduction of postharvest decay in organic citrus fruit by a short hot water brushing treatment. *Postharvest Biol. Technol.* **18:** 151-157 (2000).
37. Pusey, P. L., Wilson, C. L., Hotchkiss, M. W. and Franklin, J. D., Compatibility of *Bacillus subtilis* for Postharvest Control of Peach Brown Rot with Commercial Fruit Waxes, Dicloran, and Cold-Storage Conditions. *Plant Dis.* **70:** 587-590 (1986).
38. Pusey, P. L. and Wilson, C. L., Postharvest biological control of stone fruit brown rot by *Bacillus subtilis. Plant Dis*. **68:** 753-756 (1984).
39. Sangwanich, S., Sangchote, S. and Leelasuphakul, W., Biocontrol of Citrus green mould and postharvest quality parameters. *Int. Food Res. J.* **20(6):** 3381-3386 (2013).
40. Selvakumar, R., *Pseudomonas syringae* for management of post harvest rot in Khasi mandarin oranges. *Plant Dis*. **26(2):** 183-184 (2011).
41. Singh, K. A., Tombisana, M. N. G., Singh, K. B. and Mandal, N., Khasi mandarin: its importance, problems and prospects of cultivation in North-Eastern Himalayan region *Int. J. Agri., Environ. and Biotechnol*. **9(4):** 573-592 (2016).
42. Singh, S., Citrus industry in India. In: Citrus International Book Distributing Company (Eds.), Singh, S. and Naqvi, S. A. M. H., Lucknow, India. Pp. 3–41 (2001).
43. Singh, V. and Deverall, B. J., *Bacillus subtilis* as a control agent against fungal pathogens of citrus fruit. *Trans. Brit. Mycol. Soc*. **83:** 487-490 (1984).
44. Utkehede, R. S. and Sholberg, P. L., *In-vitro* inhibition of plant pathogens: *Bacillus subtilis* and *Enterobacter aerogenes in-vitro* control of two postharvest cherry diseases. *Can. J. Microbiol*. **32:** 963-967 (1986).
45. Wisniewski, E. M. and Wilson, L. C., Biological Control of Postharvest Diseases of Fruits and Vegetables: Recent Advances. *Hort. Sc.* **27(2):** 94-98 (1992).
46. Zade, V. R., Babhulkar, V. P., Paslawar, A. N. and Pardey, V. P., Extension of storage life of fungicidal waxol dip-treated Nagpur mandarin under ambient conditions. *Annals of Plant Physiol.* **19:** 150–153 (2005).
47. Zade, V. R., Damke, M. M. and Kadu, N. R., Studies on extension of shelf life of Nagpur mandarin under ambient conditions. *Annals of Plant Physiol.* **17:** 17–20 (2003).
48. Zamani, M., Tehrani, S. A., Ahmadzadeh, M., Behboodi, K. and Hosseininaveh, V., Biological control of *Penicillium digitatum* on oranges using *Pseudomonas* spp. either alone or in combination with hot sodium bicarbonate dipping. *Australasian Pl. Pathol*. **37:** 605–608 (2008).
49. Zhang, J. and Dou, H., Evaluation of *Bacillus subtilis* as potential biocontrol agent for postharvest green mold control on ‘valencia’ orange. *Proc. Fla. State Hort. Soc.* **115:** 60-64 (2002).