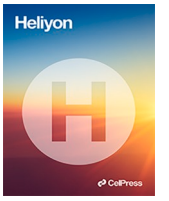
Heliyon 10 (2024) e28758

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Mitigating postharvest quantitative and qualitative losses in mango fruits through the application of biocontrol agents: An in-vivo and in-vitro assessment

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ARTICLE INFO

Keywords:
Mango
Postharvest loss
Biocontrol agents
Postharvest pathogen

ABSTRACT

Mango is a commercial fruit crop of India that suffers huge postharvest losses every year. The application of biocontrol agents (BCAs) bears a vast potential for managing the same, which is yet to be exploited to its fullest extent. Hence, studies were conducted for BCAs application of *Debaryomyces hansenii*, *Bacillus subtilis* and *Pseudomonas fluorescens* strains on mango fruit under *in-vitro*, *in-vivo* conditions to know the efficacy of these BCAs on the postharvest pathogen, shelf life and quality retention of mango fruit. The 'poisoned food technique' was attempted for *in-vitro* studies. For the *in-vivo* studies, fruit of the commercial cultivar 'Amrapali' were un-inoculated and pre-inoculated with major postharvest pathogens (anthracnose: Colletotrichum *gloeosporioides* and stem-end rot: *Botryodiplodia theobromae*) were treated with BCA, followed by ambient storage at (24 ± 4 °C, 75 ± 5 % RH). From the results, it has been observed that under *in vitro* studies, BCA *Debaryomyces hansenii* (Strain: KP006) and *Bacillus subtilis* (Strain: BJ0011) at the treatment level 10^8 CFU mL^{-1} while, the *Pseudomonas fluorescens* at 10^9 CFU mL^{-1} (Strain: BE0001) were significantly effective for pathogen inhibition. However, under the *in vivo* studies, the BCA *Debaryomyces hansenii* (Strain: KP006) at 10^8 CFU mL^{-1} treatment level was found to significantly reduce the pathogen's decay incidence while positively influencing the shelf life and biochemical (quality) attributes. This treatment increased the storage life of mango fruit by more than three days over control fruit. Therefore, BCA *Debaryomyces hansenii* (Strain: KP006) at 10^8 CFU mL^{-1} can be used to control the postharvest pathological loss of mango fruit without affecting its internal quality.

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https://doi.org/10.1016/j.heliyon.2024.e28758
Received 6 August 2023; Received in revised form 28 February 2024; Accepted 24 March 2024
Available online 28 March 2024

1. Introduction

Mango (*Mangifera indica* L.) the 'King of fruit' possesses special table decoration features, including delicious taste, high nutrition, and exceptional flavor, making it one of India's most economically important fruit of India.("Export Regulations," n.d.) Due to its climacteric nature, mango fruit are highly active physiologically and biologically even after harvest and prone to severe postharvest losses [1,2]. The high perishable nature and susceptibility of mangoes to postharvest diseases hinders their availability and trade and export. A nationwide survey reported that more than 25–30 % of mangoes are lost during handling and storage [2]. Various factors are associated with postharvest losses in mango, of which the pathogens and quality loss are of major concern [3].

Although several postharvest diseases have been reported in mango fruit but anthracnose, (causing agent *Colletotrichum gloeosporioides* Penz and Sacc) and stem-end rot (caused by Botryodiplodia theobromae Pat.) are the major diseases responsible for postharvest crop loss [4–6]. The development of control methods for these postharvest diseases is a top priority for mango researchers. Several techniques have been advised to control these infections, including pre-harvest sprays of mancozeb or prochloraz (0.02 %) and postharvest dips with other fungicides [7]. Although fungicides bring satisfactory control of both diseases, their residue on fruit is a major concern among traders, exporters, and consumers [8,9]. Thus, the use of synthetic chemicals is being increasingly restricted due to their adverse influence on humans and the environment [10,11]. Among the non-chemical approaches hot water dips for 15 min (49–55 °C), vapor heat or forced-air dry heat given for the control of fruit fly or stone weevil also bring some control to these diseases [7].

Among the eco and health-safe approaches in recent times, the use of biocontrol agents (BCAs) is gaining popularity [12–15]. Recent studies on applications of BCAs include *Pseudomonas synxantha* for postharvest treatment of stone fruit to prevent spoilage by *Monilinia* species; *Trichoderma pseudokoningii and Rhizopus nigricans* for pre-harvest treatment of kiwifruit trees; *Bacillus velezensis* for postharvest treatment of mangoes; *Bacillus halotolerans* against grey mold in strawberry fruit, and so on [4,16]. Now several commercial BCAs such as Aspire, Bio-Save 100 and 110 and Yield Plus *etc.,* have been developed which have slowly replaced the use of synthetic fungicides in crop such as apple, citrus etc. [7,11,17,18]. Though applications of these in mango are yet to be explored.

There are some important concerns in mango fruit industry such as quality and safety trade requirement at national and international market, increasing demand of organic consignments, MRL export criteria, consumer preference to eco and health friendly postharvest treatments *etc* which encourages researchers to derive the eco and health friendly novel technologies for postharvest management of mango fruit [8]. Therefore, keeping this in view a study was attempted to envisage the use of postharvest application of BCA on mango fruit to control the occurrence of major pathogen while observing its effect on shelf life and quality retention during storage.

2. Materials and methods

2.1. Site of experiment

The study was carried out at the 'Division of Food Science and Postharvest Technology', 'Division of Plant Pathology' and 'The Indian Type Culture Collection (ITCC)' at ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi-110012. The fruit of the commercial variety 'Amrapali' were procured from the departmental orchard of the 'Division of Fruits and Horticultural Technology', ICAR-IARI, New Delhi for isolation of pathogens and for *in vivo* experiment. The fruits were harvested at appropriate harvesting indices of specific gravity of more than one ($\geq$1.0) [19].

2.2. Pathogen isolation and identification from mango fruit samples

The pathogens such as *Colletotrichum gloeosporioides* and Botryodiplodia *theobromae* were isolated from 'Amrapali' mango fruit and were cultured (one to two weeks) on potato dextrose agar (PDA) at temperature 25 °C [20]. Conidia of *C. gloeosporioides* and Botryodiplodia *theobromae* were harvested by adding 5 mL sterile, de-ionized water treated with 0.05 % Triton X-100 to the placed Petri dishes. Colonies were rubbed using a sterile glass rod followed by conidia suspension passing through two cheese-cloth layers. Dilution was made to the suspension to an absorbance of 0.1 at 425 nm, which was measured with spectrophotometer, comprising about 1×10^6 conidia mL^{-1} [20]. PDA slants were used for continuous re-isolations to maintain the pathogenicity of the inoculums. Microscopic observations then identified the fungal isolate as per the taxonomic key and description documented in form of catalogue www.iari.res.in/files/ITCC_Catalogue_1936-2016-16092016.pdf of ITCC, New Delhi, India.

2.3. Procurement and preparation of biocontrol agents (BCAs)

Three BCAs viz., *Debaryomyces hansenii*- KP006, *Bacillus subtilis*- BJ0011, *Pseudomonas fluorescens*- BE0001 were procured from the Division of Plant Pathology, ICAR-IARI, New Delhi. Using a bacteriological loop, pure cultures of each BCA were streaked in a Petri dish containing potato dextrose agar medium. Plates were incubated at 27 °C for 4 d, after which BCAs were harvested, suspended in distilled water, and adjusted to an absorbance of 1 at 600 nm, resulting in a concentration of 10^9 CFU mL^{-1}. The suspensions were then diluted by serial dilution method to prepare the subsequent concentrations of 10^8 CFU mL^{-1}, 10^7 CFU mL^{-1} and 10^6 CFU mL^{-1} for *in vitro* antifungal assay of BCAs.

2.4. In vitro studies

2.4.1. Antifungal assay of biocontrol agents (BCAs)

The effect of the selected BCAs on the growth of pathogens in terms of inhibition percentage after 10 d of incubation on potato dextrose agar media at 25 °C (mean of six replicates) was studied at four different concentrations *viz.*, 10^6, 10^7, 10^8 and 10^9 CFU mL^{-1}, which consisted of 12 treatments (Table S1).

2.4.2. Estimation of inhibition percentage

The percentage of inhibition was determined using a modified version of the method described by Jhalegar [21]. A 5 mm diameter agar disc or disc from a pure culture of *C. gloeosporioides* and Botryodiplodia *theobromae* was deposited on PDA plates containing varying quantities (10^6, 10^7, 10^8, and 10^9 CFU mL^{-1}) of BCAs using the dual culture approach (*D. hansenii*, *B. subtilis*, *P. fluorescens*). The petridish without smeared BCAs served as the control. Petri plates were incubated for 10 d at 25 °C, during this the daily radial growth was recorded until the pathogen touched the edge of the control plate.

2.5. In vivo studies

2.5.1. In vivo antifungal assay of biocontrol agents (BCAs)

To test the effectiveness of biocontrol agents (BCAs) in preventing fungal infections in mature 'Amrapali' mango fruit, different concentrations (ranging from 106 to 108 conidia mL−1) of *Pseudomonas fluorescens* (Strain: BE0001), *Bacillus subtilis* (Strain: BJ0011), and *Debaryomyces hansenii* (Strain: KP006) were applied to the fruit for 5 min as a postharvest dip treatment [21]. The fruit was then stored under ambient conditions (24 ± 4 °C, 75 ± 5 % RH) and subjected to an *in vivo* antifungal assay. To perform the assay, the fruit was first washed, rinsed, and air-dried at 25–28 °C. Then, the fruit was pre-inoculated with two fungal pathogens, *Colletotrichum gloeosporioides* and Botryodiplodia *theobromae*, using a steel rod dipped in the inoculum suspension and making a single puncture on the fruit. Un-inoculated fruit was used as a control [20]. The BCAs were then applied to the fruit by immersing/dipping the air-dried fruit in the solutions of respective BCAs for 5 min at 25 °C. After dipping, the fruit was air-dried again and kept at ambient conditions for observation at 3-day intervals. The experiment was conducted for different observation periods to assess the effectiveness of the different concentrations of BCAs in preventing fungal infections in the fruit [21,22].

2.5.2. Percent fruit decay, decay area and lesion diameter on fruit surface

Based on symptoms/damage of a particular disease, percent fruit decay was estimated using following formula.

Percent fruit decay = (number of fruit decayed / total number of fruit) x 100

The decay area on fruit surface was recorded as per the method of Corkidi et al. [23] by recording the fruit surface area (cm^2) affected by a specific pathogen Corkidi et al. [23] Similarly, the lesion diameter was recorded as per the method followed by Sivakumar et al. [24] and expressed in diameter lesion of pathogenic infection [14]. The BCA treated fruit were compared with control fruit which were not treated with any BCA followed by storage at ambient conditions.

2.5.3. Storage life of fruit

The storage life of mango fruit was calculated by using Physiological Loss in Weight (PLW) standard, where the fruit exhibiting more than 10 % PLW loss were considered as 'shelf life completed fruit' [25]. The shelf life of fruit was represented in days.

2.6. Identification of pathogens

The pathogen affected fruit were analyzed and their identification was carried out with the help of compound microscope based on morphological characteristics of the pathogen according to appropriate taxonomic key and description (Id 10.625.16) at the 'Division of Plant Pathology' ICAR-IARI and 'Indian Type Culture Collection Facility', ICAR-IARI, lab.

2.7. Estimation of biochemical attributes

The biochemicals responsible for fruit quality were measured. The total phenol content is measured using the methodology followed by Singleton and Rossi [26] with slight modification [25]. Double-distilled water (2.5 mL) in a test tube was used to dilute the (0.5 mL) pulp and then it was incubated for 3 min after addition of 0.5 mL Folin-Ciocalteu reagent. Following incubation, 2 mL of 20% (w/v) Na_2CO_3 was added in sample tube and kept for 1 min for boiling in water bath. At 650 nm absorbance was recorded and using several gallic acid (GA) solutions standard curve was plotted. TPC were displayed as "μg GAE/g FW".

The total antioxidant activity of mango genotypes was estimated using DPPH (2,2-Diphenylpicrylhydrazyl) method [25]. After thoroughly mixing 0.1 mL extract sample with 3.9 mL of a 0.06 mM DPPH solution mixture was left for 30 min in dark and absorbed the absorbance at 517 nm. The (AOX) expressed as 'μmol TE/g'.

Both the BCAs treated and untreated fruit were analyzed and the data were recorded at 3 days intervals [26,27].

2.8. Experimental design and analysis of data

The experiments on *in vitro* and *in vivo* studies were laid out on factorial Completely Randomised Design (CRD). Six and three replications were taken for *in vitro* and *in vivo* studies respectively. The critical difference (CD) was calculated using 'Two-way ANOVA' through SAS 9.3 software and significant effects at $p < 0.05$ level were recorded to compare the results.

3. Results and discussion

3.1. In vitro antagonistic ability of BCAs against major pathogens of mango

The inhibition percentage was calculated by percent conversion of inhibition zone (cm^2) which is representative of the variability of antibiosis [28] and antifungal activity [29]. The increased inhibition percentage of the pathogen in growing media is an indication of its hindered growth as well [29]. The inhibition percentage of both the pathogens (C. *gloeosporioides* and Botryodiplodia *theobromae*) was significantly influenced by all the attempted BCAs (Table 1; Table S2). *D. hansenii* at 10^9 CFU mL^{-1} exhibited the maximum inhibition for both C. *gloeosporioides* (85.1 %) and Botryodiplodia *theobromae* (87.1 %), which might be due to the variability among BCAs and their concentration dependent inhibition for a specific pathogen. Among the attempted BCAs, *D. hansenii* and *B. subtilis* exhibited maximum inhibition percentage at their highest concentration (10^9 CFU mL^{-1}), but it was non-significant with 10^8 CFU mL^{-1} concentration (Table S2). Thus, 10^8 CFU mL^{-1} was recorded best for both the BCAs (*D. hansenii* and *B. subtilis*) and was considered to be attempted further in *in-vivo* condition. Huang et al. [30] and Reyes-Estebanez et al. [11] have also reported significant inhibitory activity of *Bacillus* species against C. *gloeosporioides*.

In a similar study, Luo et al. [6] also recommended 1×10^8 cells mL^{-1} concentration of *Debaromyces nepalensis* over other concentrations (1×10^7 and 1×10^9 cells mL^{-1}) for control of anthracnose in mango. Furthermore, *P. fluorescens* exhibited maximum inhibition at 10^9 CFU mL^{-1} concentration but the inhibition was significantly higher over all other attempted concentrations (Table 1). Reyes-Estebanez et al. [11] also reported higher biocontrol activity of *B. velezensis* at a very high spore concentration of

Table 1
Effect of different biocontrol agent (BCA's) on percentage inhibition of pathogens after 10 days of incubation on potato dextrose agar at 25 °C (mean of six replicates).

Inhibition percentage				
Pathogen	BCA's			
	Debaryomyces hansenii (CFU mL^{-1})			
	10^6	10^7	10^8	10^9
Colletotrichum gloeosporioides	70.0 ± 0.5a	80.0 ± 0.5b	84.0 ± 0.5c	85.1 ± 0.6c
Control	0.0a	0.0a	0.0a	0.0a
	Bacillus subtilis (CFU mL^{-1})			
	10^6	10^7	10^8	10^9
Colletotrichum gloeosporioides	67.1 ± 0.4a	70.2 ± 0.4b	76.7 ± 0.4c	78.0 ± 0.5d
Control	0.0a	0.0a	0.0a	0.0a
	Pseudomonas fluorescens (CFU mL^{-1})			
	10^6	10^7	10^8	10^9
Colletotrichum gloeosporioides	58.2 ± 0.3a	65.5 ± 0.3b	72.9 ± 0.4c	75.6 ± 0.4d
Control	0.0a	0.0a	0.0a	0.0a
Inhibition percentage				
Pathogen	BCA's			
	Debaryomyces hansenii (CFU mL^{-1})			
	10^6	10^7	10^8	10^9
Botryodiplodia theobromae	77.6 ± 0.5a	81.8 ± 0.5b	85.6 ± 0.6c	87.1 ± 0.6d
Control	0.0a	0.0a	0.0a	0.0a
	Bacillus subtilis (CFU mL^{-1})			
	10^6	10^7	10^8	10^9
Botryodiplodia theobromae	73.4 ± 0.4a	76.9 ± 0.4b	81.5 ± 0.5c	82.4 ± 0.6d
Control	0.0a	0.0a	0.0a	0.0a
	Pseudomonas fluorescens (CFU mL^{-1})			
	10^6	10^7	10^8	10^9
Botryodiplodia theobromae	66.4 ± 0.3a	69.3 ± 0.4b	75.8 ± 0.4c	78.4 ± 0.5d
Control	0.0a	0.0a	0.0a	0.0a

Mean values and intervals of Tukey's 95% according to the ANOVA test.
The superscript: The letter in the same column (a–d) refers to the significant differences ($p < 0.05$) between each treatment in the same row.

C. gloeosporioides in mangoes, supporting concentration-dependent action of BCAs. This behavior of BCAs for inhibition on pathogens might be due to higher competition of the BCAs for space, nutrition and also due to antagonistic activity of BCA [11,31]. Further, the observed variability among the BCAs for inhibition percentage is supported by various previous reports. For instance, Sugiprihatini et al. [28] revealed that among the twenty-one attempted BCA isolates, only three i.e. *Cryptococcus albidus, Pichia guilliermondii and Debaryomyces hansenii,* were found to most efficient antagonistic yeasts against *Botryodiplodia theobromae* pathogen of mango fruit. Similarly, Madbouly et al. [32] and Parisa et al. [33] reported different inhibitory activities of BCAs against the conidia of *Monilinia fructigena* from apples and *Penicillium digitatum* causing blue mold diseases in citrus, respectively.

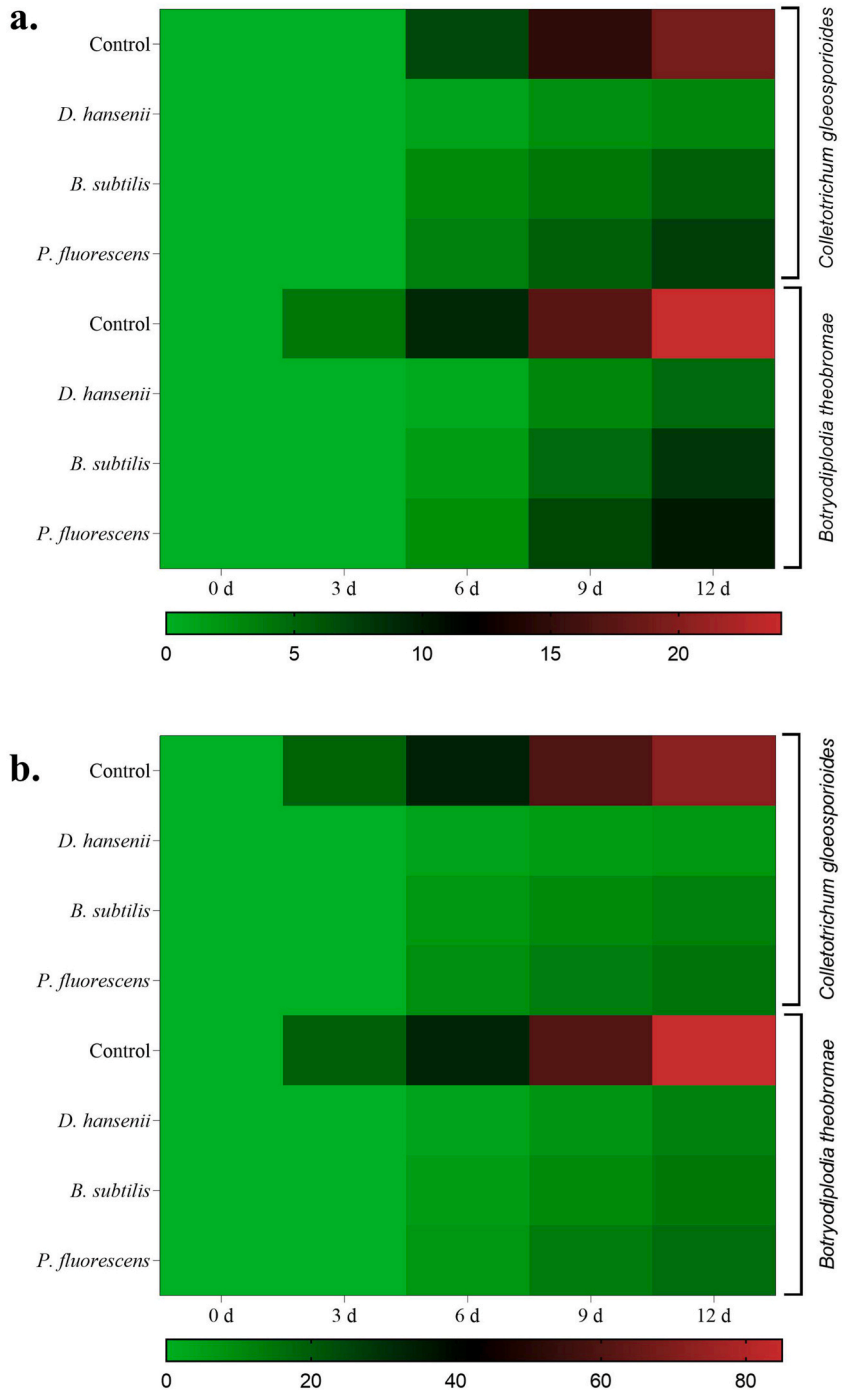

Fig. 1. (a) Lesion diameter (mm) and (b) decay diameter (mm) of 'Amrapali' mango fruit pre-inoculated with pathogens as affected by three different BCAs. Data are the means of 30 fruit across three replications at ambient storage conditions (25 ± 4 °C and 65 ± 5 % RH).

3.2. In vivo antagonistic ability of different BCAs against major postharvest pathogens of mango

Based on the best results in terms of inhibition percentage obtained under *in vitro* experiment, three BCAs treatments such as *D. hansenii* (10^8 CFU mL^{-1}), *B. subtilis* (10^8 CFU mL^{-1}) and *P. fluorescens* (10^9 CFU mL^{-1}) were attempted on un-inoculated and pre-inoculated (C. *gloeosporioides* and Botryodiplodia *theobromae*) fruit of Amrapali mango.

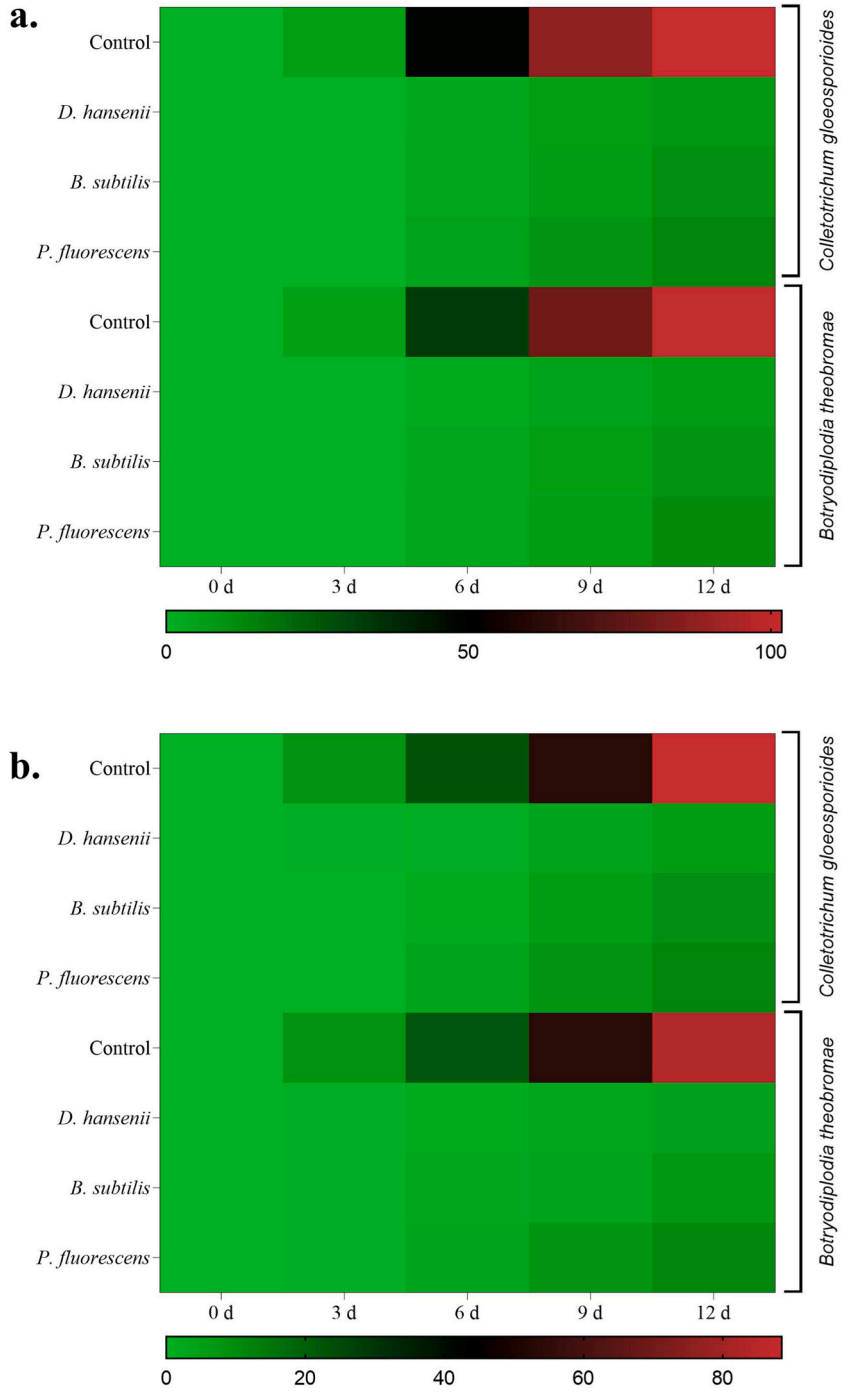

Fig. 2. Influence of BCA's on (a) percent fruit decay and (b) fruit decay area (%) of mango fruit cv. 'Amrapali' pre-inoculated with pathogens. Fruits were stored at ambient storage conditions (25 ± 4 °C and 65 ± 5 % RH). Data are the means of 30 fruits across three replications.

3.2.1. Lesion diameter

The diameter of lesions on the surface of the fruit might be caused by either direct or latent pathogen infection [34]. It can be used to determine the sensitivity of fruit to diseases [21]. Significant differences in lesion diameter (mm) were seen between the BCA treatment on control (non-inoculated) and/or fruit pre-inoculated with pathogens such as *C. gloeosporioides* and Botryodiplodia *theobromae* in the present investigation (Fig. 1a; Table S3). Lesion diameter of mango fruit increased with the progress in storage days, which might be due to increased host susceptibility with progress in storage period [6,35]. The highest lesion diameter of C. *gloeosporioides* (10.6 mm) and Botryodiplodia *theobromae* (13.4 mm) was exhibited by untreated mango fruit, while fruit treated with *D. hansenii* (10^8 CFU mL^{-1}) exhibited the lowest lesion diameter (1.5 mm and 1.9 mm, respectively) in both the pathogens pre-inoculated fruit, which might be due to higher antagonistic activity of *D. hansenii* against these pathogens (Table S4) by competition for space and nutrients between pathogen and BCA [36].

3.2.2. Decay diameter

Decay diameter is a transvers diameter of pathogen infection and is one of the measures to assess the infection process in post-harvest treated fruit by visual analysis [37]. The results of present study revealed significant differences for decay diameter among attempted BCAs treatments on untreated (control) and fruit pre-inoculated with pathogens, C. *gloeosporioides* and B. *theobromae* (Fig. 1b; Tables S3 and S5). Among the storage days, irrespective of BCA treatment, decay diameter of mango fruit increased (Table S3) with the progress in storage days which might be due to decreased defense mechanism of mango fruit with progress in storage period [6]. Our results corroborated with Madbouly, Elyousr, & Ismail [32] study on brown rot of apples. In context of BCA treatment, fruit treated with *D. hansenii* (10^8 CFU mL^{-1}) exhibited the lowest decay diameter of C. *gloeosporioides* (3.9 mm) and *B. theobromae* (5.3 mm). The highest decay diameter of C. *gloeosporioides* (45.2 mm) and *B. theobromae* (48.5 mm) was exhibited by untreated mango fruit (Tables S3 and S5). The lower decay diameter of BCA-treated fruit indicates higher antagonistic activity of BCA, *D. hansenii* [38] by which it succeeded in inhibiting the pathogen growth responsible for increment in decay diameter [37]. Further, the decay diameter varied in C. *gloeosporioides* and *B. theobromae* pre-inoculated fruit, this might be result of specificity of BCA-pathogen interaction, which is responsible for the differed bio-efficacy of *D. hansenii* [36].

3.2.3. Percent fruit decay (PFD)

Percent fruit decay is quantification of pathogen ability to cause fruit loss during storage [21]. The results revealed that fruit pre-inoculated with C. *gloeosporioides* and *B. theobromae* recorded an increasing PFD with the progress in storage days and was recorded to be the highest on day 12 of storage (Fig. 2a, Table S6), which might be due to increased host susceptibility towards pathogen infection [6,39]. Similar trend for increase in PFD on storage was reported. *D. hansenii* at 10^8 CFU mL-1, exhibited the lowest PFD for C. *gloeosporioides* (4.1 %), and *B. theobromae* (3.2 %) incidence. The reduced percent fruit decay incidence might be due to multiple actions such as biofilm adhering, competition for space and nutrients, parasitism to the pathogen and increased host resistance by induced biochemical and antioxidant enzymes of BCA [40,41]. The highest PFD for C. *gloeosporioides* (59.1 %), and *B. theobromae* (54.2

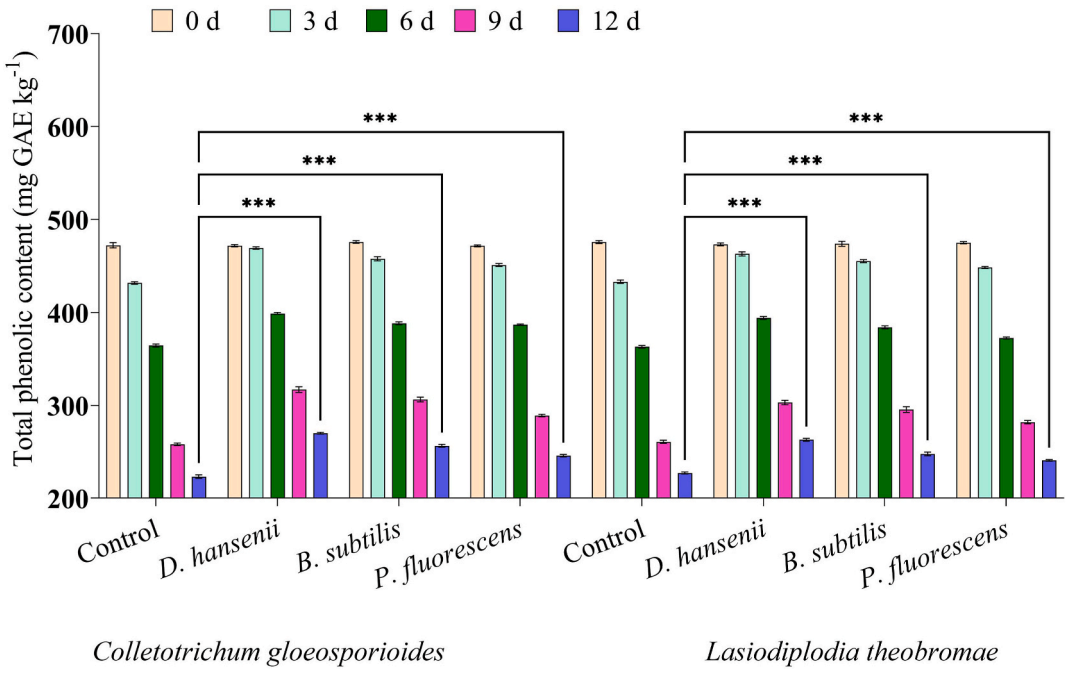

Fig. 3. Effect of BCA's on total phenolic content (mg GAE kg^{-1}) of mango fruit cv. 'Amrapali' pre-inoculated with pathogens. Fruits were stored at ambient storage conditions (25 ± 4 °C and 65 ± 5 % RH). Data are the means of 30 fruits across three replications.

%) was exhibited by control mango fruit (Fig. 2a–Table S7). Findings of this research support the Bautista-Rosales et al. [41] who reported that BCA can be used successfully to control C. *gloeosporioides* incidence of mango fruit and its use in integrated management of disease. Further, the mean of PFD varied in C. *gloeosporioides* and *B. theobromae* pre-inoculated fruit, which might be due to the specificity of BCA-pathogen interaction, responsible for the differed bio-efficacy of *D. hansenii* [32,36].

3.2.4. Fruit decay area percentage (FDA)

Decay area on fruit surface can be used as a measure for assessing the efficacy of applied postharvest treatment to reduce the pathogen attack [21,37]. The observations on impact of different BCA on fruit decay area of pathogens revealed that an increasing FDA was recorded with the progress in storage days (Tables S6 and S8), which might be due to reducing barrier property of fruit surface to combat the pathogen entry and subsequent establishment on fruit surface [6,39]. Fruit treated with *D. hansenii* at 10^8 CFU mL^{-1} concentration exhibited the lowest FDA for C. *gloeosporioides* (2.3 %) as well as *B. theobromae* (2.2 %), whereas, the highest FDA for both the pathogens was exhibited by control fruit (Fig. 2b; Tables S6 and S8). The lowest FDA percentage in *D. hansenii* at 10^8 CFU mL^{-1} concentration might be due to its ability to reduce the expansion and coverage of pathogen on fruit surface [37,43]. Contrarily, Grzegorczyk et al. [43] reported weaker wound site colonization of *D. hansenii* in peach fruit. Variation in FDA in C. *gloeosporioides* and *B. theobromae* pathogens might be due to the specificity of BCA-pathogen interaction, which is responsible for the differed bio-efficacy of *D. hansenii* [32,36].

3.2.5. Total phenolic content (TPC)

The concentrations of phenolics in mango fruit act as antifungal metabolite against pathogens [34]. It was observed that TPC of pathogen pre-inoculated fruit, decreased with the progress in storage days (Fig. 3; Table S9). This might be due to effect of ripening and fruit softening process of mango on fruit phenolic content [22]. In context to BCA treatments, fruit treated with *D. hansenii* at 10^8 CFU mL^{-1}, exhibited the highest TPC in C. *gloeosporioides* (383.3 mg GAE kg^{-1}) and *B. theobromae* (378.1 mg GAE kg^{-1}) (Table S9 and S10) pre-inoculated fruit, which might be due to the delayed fruit ripening and lowered decay of mango fruit by *D. hansenii* at10^8 CFU mL^{-1} treated fruit. Further, the biosynthesis and retention of phenolics inhibit the occurrence of pathogens on mango fruit, as Zhang et al. [34] reported. Thus, the significant levels of phenols depicts the preventive approach towards pathogenic growth as these secondary metabolites serve as potent inhibitor in the matrix. Further, the variation among the overall mean of TPC in C. *gloeosporioides* and *B. theobromae* pre-inoculated fruit might be due to the specificity of BCA-pathogen interaction as reported by Corbaci and Ucar [36].

3.2.6. Total antioxidant activity (TAA)

Irrespective of treatments, the TAA of pre-inoculated fruit, treated with BCAs recorded for an increase in its content up to day 9 of storage and decline thereafter (Fig. 4; Table S11). This pattern of total antioxidant activity might be due to the synthesis of antioxidants with progression in storage up to day 9, followed by degradation thereafter during storage and ripening [44]. Similar results have been

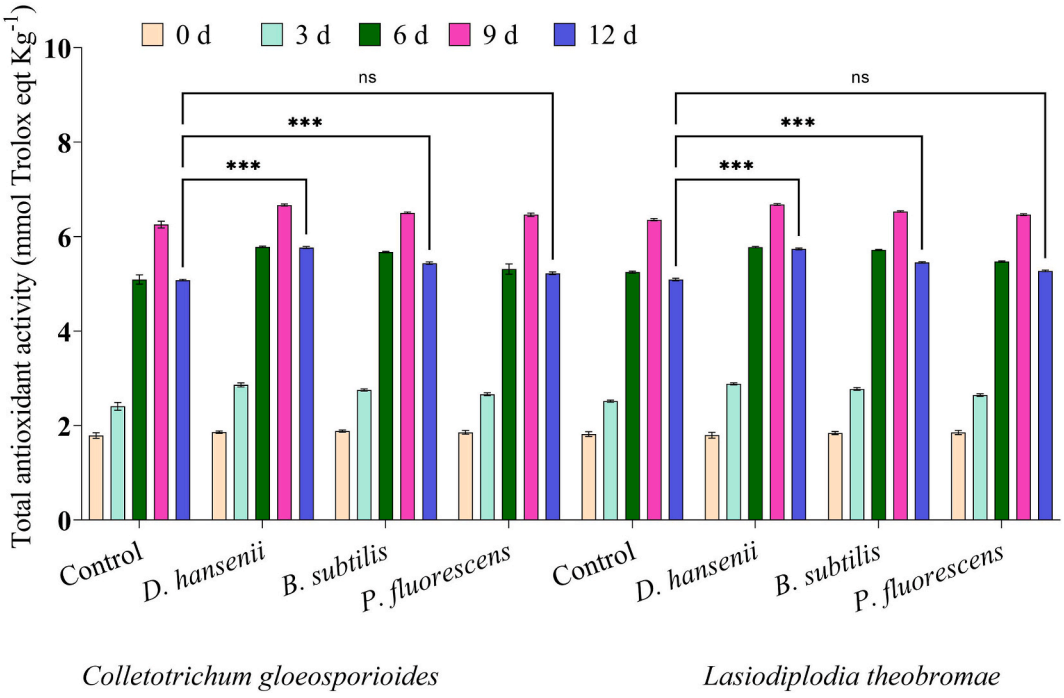

Fig. 4. Impact of BCA's on total antioxidant activity of mango fruit cv. 'Amrapali' pre-inoculated with pathogens. Fruits were stored at ambient storage conditions (25 ± 4 °C and 65 ± 5 % RH). Data are the means of 30 fruits across three replications.

reported by Wang et al. [9] in their study on preventing grey mold in strawberry. Among the treatments, fruit treated with *Debaryomyces hansenii* (Strain: KP006) at 10^8 CFU mL^{-1} concentration exhibited the highest TAA in fruit pre-inoculated with pathogens and control fruit recorded the least TAA (Tables S11 and S12). The higher antioxidant activity level in *D. hansenii* treated mango fruit might be due to the ability of BCAs to induce the biochemicals, which contribute towards pathogen inhibition as reported by Zhang et al. [34]. Furthermore, the differed mean values of TAA similar to that of TPC might be a similar indication of specificity of BCA-pathogen interaction [32].

3.3. Decay initiation (day), percent fruit decay and increase in storage life (day)

The results revealed that significant differences occurred for decay initiation (day), percent fruit decay and shelf life (day) among the treatments of BCAs (Table 2). Among the fruit subjected to pathogens, fruit treated with *D. hansenii,* at 10^8 CFU mL^{-1} concentration recorded for delayed occurrence of C. *gloeosporioides* (6.5 d) and *B. theobromae* (6.5 d), while in contrast, control fruit exhibited the earliest occurrence (3 d) in pathological decay by pathogens, C. *gloeosporioides* and *B. theobromae* (Table 2 and Table S13). Similarly, in context to percent fruit decay, fruit treated with *D. hansenii,* at 10^8 CFU mL^{-1} exhibited the best results, both in fruit naturally affected by C. *gloeosporioides* (3.26 %) and *B. theobromae,*(2.51 %) compared to the highest incidence of C. *gloeosporioides* (25.80 %) and *B. theobromae* (20.50 %) decay of un-inoculated fruit (Table 2). The lowered decay initiation and percent fruit decay by *D. hansenii* might have occurred due to its secondary metabolite production mechanism against the pathogens [41].

Further, the data on storage life (days) revealed that it was recorded to be highest in *D. hansenii* treated un-inoculated fruit which might be due to delayed infestation both by C. *gloeosporioides* (3.5 d) and *B. theobromae* (4 d) during the storage period. In contrast, no increment in storage life was exhibited by control fruit which are naturally infested either by C. *gloeosporioides* and *B. theobromae* during storage period (Table 2). The increase in storage life of mango might be due to delayed pathogen attack and lowered decay incidence in case of *D. hansenii* treated fruit [42], which in turn is due to its ability to inhibit the pathogen [36]. The results of present study are in line with the findings of Mandal et al. [45] and Czarnecka et al. [46] who reported that *D. hansenii* increased the shelf life of peaches and apples, respectively, by reducing postharvest decay. Further, Rivas-Garcia et al. [31] had revealed the synergistic effect of *Debaryomyces hansenii* and *Stenotrophomonas rhizophila* in increasing the shelf life of musk melon. The differences with respect to mean of decay initiation (day), percent fruit decay and shelf life (day) in BCA treated fruit pre-inoculated with pathogens might be an indicative of specificity of *Debaryomyces hansenii*-pathogen interaction [32].

4. Conclusion

Under *in vitro* studies, the BCA, *Debaryomyces hansenii* (Strain: KP006) at 10^9 CFU mL^{-1} exhibited the maximum inhibition percentage of Colletotrichum *gloeosporioides* (87.1 %) and Botryodiplodia *theobromae* (85.1 %). Whereas, in context to individual BCA, *Debaryomyces hansenii* (Strain: KP006) and *Bacillus subtilis* (Strain: BJ0011) exhibited maximum efficacy at concentration 10^8 CFU mL^{-1}. However, *in vivo* studies revealed that *Debaryomyces hansenii* (Strain: KP006) at 10^8 CFU mL^{-1} was found best in significantly reducing the pathogens' postharvest incidence and extended shelf life by three days over untreated fruit. This treatment maintained postharvest quality by positively influencing all the studied decay, biochemicals (internal quality) and shelf-life attributes of mango fruit. Thus, it can be concluded that *Debaryomyces hansenii* (Strain: KP006) has a great potential to reduce the pathological decay and extend the shelf life of mango fruit. Further, it doesn't exert any adverse influence on the internal quality of fruit. The present research work will be very helpful for managing pathogens in the mango processing industries, where the incidence of postharvest losses due to pathogen infestation is higher.

Funding

This work was supported by grants from the IICAR ICAR-Indian Agricultural Research Institute, New Delhi.

Table 2
Effect of BCAs on initiation of decay (days), decayed fruit (%) and increase in storage life (d) of un-inoculated mango fruit cv. 'Amrapali' under natural infection of pathogens during ambient storage (25 ± 4 °C and 65 ± 5 % RH). Data are the mean of 30 fruit across three replications.

Biocontrol agent	*Colletotrichum gloeosporioides*			*Botryodiplodia theobromae*		
	Initiation of decay (days)	Percent fruit decayed	Increase in storage life (days)	Initiation of decay (days)	Percent fruit decayed	Increase in storage life (days)
Control	3.00 ± 0.5d	25.8 ± 2.5d	0.00 ± 0.0d	3.00 ± 0.5d	20.50 ± 3.5d	0.00 ± 0.0d
Debaryomyces hansenii (10^8 CFU mL^{-1})	6.50 ± 0.4a	3.26 ± 0.5a	3.50 ± 0.5a	6.50 ± 0.6a	2.51 ± 1.0a	4.00 ± 0.6a
Bacillus subtilis (10^8 CFU mL^{-1})	6.00 ± 0.5b	5.84 ± 0.5b	3.00 ± 0.3b	6.50 ± 0.5b	3.17 ± 1.0b	3.50 ± 0.5b
Pseudomonas fluorescens (10^9 CFU mL^{-1})	5.50 ± 0.5c	6.12 ± 0.5c	2.50 ± 0.3c	5.50 ± 0.5c	5.63 ± 1.5c	2.50 ± 0.5c

Mean values and intervals of Tukey's 95% according to the ANOVA test.
The superscript: The letter in the same column (a–d) refers to the significant differences ($p < 0.05$) between each treatment.

Ethics approval

Ethical approval is not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Code availability

Not applicable.

Data availability

Data included in this published article, its supplementary material and referenced in the article. No data associated with this study has been deposited into publicly available repository.

CRediT authorship contribution statement

Killi Prasad: Writing – original draft, Project administration, Methodology, Investigation, Conceptualization. **Ram Roshan Sharma:** Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ram Asrey:** Resources, Project administration, Methodology, Investigation. **Dinesh Singh:** Writing – review & editing, Validation, Visualization. **Milan Kumar Lal:** Writing – review & editing, Validation, Supervision, Project administration. **Jyoti Nishad:** Resources, Methodology, Formal analysis. **Rahul Kumar Tiwari:** Writing – original draft, Validation, Software, Resources. **Shruti Sethi:** Writing – original draft, Investigation, Formal analysis, Resources. **Manish Srivastav:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Ajay Arora:** Writing – review & editing, Validation, Supervision, Project administration. **Ravinder Kumar:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Authors would like to acknowledge the Directors of ICAR-Indian Agricultural Research Institute, New Delhi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e28758.

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