Original Research Article

Management of postharvest anthracnose disease of papaya (*Carica papaya*) using antagonistic microorganisms

Abstract

The antagonistic potential of microbial agents isolated from different sources like compost, rhizosphere soil, healthy fruits surface were investigated against *Colletotrichum gloeosporiodes under in vitro and in vivo*. The results of*in vitro*evaluation of 22 isolates of antagonists against *Colletotrichum gloeosporiodes* by dual-culture test showed *Trichoderma harzianum* UHSF-15 (with 62.50% inhibition of radial growth), *Bacillus amyloliquifaciens* UHSB-BCA-15 isolated from banana rhizosphere soil (50.00% inhibition) and *Bacillus amyloliquifaciens*UHSB-BCA-16 isolated from kiwi fruit surface (47.85% inhibition) to be most effective.*In vivo* screening of five effective microbial agentsselected from *in vitro* studies revealed that, *Bacillus amyloliquifaciens* UHSB-BCA-15to be most effective with lowest per cent disease index of 13.89% followed by *Bacillus amyloliquifaciens*UHSB-BCA-16 with 16.67% and carbendazium at 0.1% with 20.83% at 9d after storage. The higher per cent disease index of 58.33% was recorded in untreated control fruits. In another experiment where *C.gloeosporoides*spore suspension (1x10^6 CFU mL^{-1}) was challenge inoculated on fruits, the fruits treated with biocontrol agent*Bacillus amyloliquifaciens* UHSB-BCA-15at concentrations of 1x10^8 CFU mL^{-1} for 5 minutes was found to be effective in reducing postharvest infection to a greater level with 33.33% disease index followed by *Bacillus amyloliquifaciens*UHSB-BCA-16 with50.00% disease index and carbendazium at 0.1% with 52.77% disease index compare to control (91.66% disease index) at 9d after storage.

Introduction

Papaya is an important climacteric fruit crop of the world, which ishighly nutritious with good medicinal value. The rapid ripening and softening of the fruit makes it highly susceptible to many postharvest diseasesduring transit, storage and market. Among the postharvest diseases, the fungal rots cause losses up to 40% of their market value (Abeywickrama*et al.,* 2012). Among the fungal diseases, anthracnose caused by *Colletotrichum gleosporoides* alone accounts for about 10.05% of total postharvest loss (Pramod *et al.,* 2007). The infections are usually initiated in the field at early stages of fruit development on the surface of the fruit but the pathogen remains quiescent until the fruit reaches climacteric phase (Ademe*et al.,* 2014). The initial symptoms of infection are water soaked, sunken spots at center of these spots, later turning black and then pink when the fungus produces spores(Rana *et al*., 2001).Currently the control of anthracnose, a major postharvest disease of papaya relies mainly on the use of synthetic fungicides (He *et al.,* 2003; Sansone *et al.,* 2005), but the use of chemicals may pose several adverse effect on consumers as the fruit is consumed within short period of time and may leave residue on fruits. Non chemical control methodslike physical and biological methods are of great importance. The physical treatment like hot water treatment may leads to fruit injury. Hence, control of the disease through use of microbial agents holds a great potential.

The biological control is a biological-based, non-toxic and environmentally safe approach for post-harvest disease control during the storage of harvested commodities. Biological control includes the use of wide range of antagonistic bacteria, fungi and other microorganisms on the surface of the fruits and vegetables and they have shown to protect fruits and vegetables against growth and infection of postharvest pathogens (Cota *et al.,*2008). Antagonists like *Bacillus subtilis, B. cereus, Pseudomonas aeruginosa, P. cepacia, P. fluorescens, P. putidaa, Streptomyces* spp., *Trichoderma harzianum*are being used as potential biocontrol agents against the post-harvest pathogens in many fruit crops (He *et al.,* 2003; Parthasarathy *et al.* 2017).

In the present investigation the antagonistic microorganisms were isolated from different sources like compost, rhizosphere soil and different fruits surfaces collected from different places and were evaluated for their efficacy under *in vitro*conditionby dual culture test. Five effective antagonistic organisms from *in vitro* evaluation were evaluated *in vivo* by treating the papaya fruits with suspensions of microbial agents against natural anthracnose infection and challenge inoculation with *Colletotrichum gloeosporiodes* spores. The fruits were observed for the per cent disease index.

Material and methods

Isolation and identification of pathogen *Colletotrichum gloeosporiodes*

Six pathogen (*Colletotrichum gloeosporioides*) isolates wereisolated from anthracnose infected papaya (*Carica papaya*) fruitscollected from different places by standard serial dilution plating technique using potato dextrose agar (PDA) media(Boiled Potato extract from 200 g potato per liter; dextrose, 20 gL^{-1}; agar 20 gL^{-1}), supplemented with chloramphenicol (100 mg L^{-1}). The *C. gloeosporioides* spores and mycelia from the infected portion of the fruits were scraped using sterile scalpel and the pathogen sample was transferred to 9 ml of sterile water blank and serially diluted up to 10^{5} dilution in a Laminar Air Flow chamber and subsequently 1 ml aliquot from 10^{3}, 10^{4}and 10^{5} dilution were transferred on to sterile Petri dishesand2 0 to 25 ml of molten PDA media was poured and rotated in both clock-wise and anti-clockwise direction. The plates were then allowed to solidify for 30 minutes and incubated at 27 ± 1^{o}C for 5 d and observed periodically. After development of fungal colonies, pure colonies were transferred on to freshly prepared PDA plates. The pure culture of fungus was sub-cultured on PDA slants and preserved in refrigerator at 5^{o}C until used. The pathogenisolated from infected papaya fruits was identified using morphological features and by microscopic observations (Bartnett and Hunter, 1972) as *Colletotrichum gleosporioides.*The pathogen isolates were tested for their pathogenicity on healthy papaya fruits and the isolate with higher virulence index was selected for isolation of antagonistic microbial agents against the pathogen.

Isolation of biocontrol agents against *C.gloeosporiodes:*

Isolation of antagonistic microbes from compost and rhizosphere soil samples by employing poisoned food technique

For isolation of microbial agents, fivecompost samples and 35 rhizosphere soil samples from rhizosphere of healthy papaya plants in anthracnose affected field as well as from other fruit crops like, Mango, Banana and Pomegranate were collected. Ten grams of compost or rhizosphere soil was suspended in 100 ml of sterile 0.85% NaCl solution and kept in rotary shaker at 150 rpm for 30 min to dispense the spore chain or cells into water. The suspension was allowed to settle for 10 minutes and subsequently serially diluted up to 10^{-4}. From the serial dilutions (10^{-2}, 10^{-3} and 10^{-4} dilutions) one ml of aliquot was transferred to sterile Petri dishes and approximately 20 ml of cooled molten potato dextrose agar pre-seeded with spore suspension of pathogen (*C. gloeosporioides*at the rate of one ml of 10^{9} CFU mL^{-1}spore suspension per 50 ml of PDA media) was poured to these plates and allowed to solidify for one hour in Laminar Air Flow Chamber and later the plates were incubated at 27 ± 1°C for 5 d in BOD incubator (Make: Remi). After incubation period the plates were observed for fungal and bacterial colonies with a clear zone of inhibition around them. The colonies with inhibition zone were pure cultured on new media plates by quadrangle streaking method on PDA and Nutrient Agar media (NA) for fungal and bacterial isolates respectively. The pure cultured antagonist isolates were also maintained in PDA and NA slants for further screening against *C.gloeosporioides*.

Isolation of antagonistic microbes from healthy fruit surfaceby employing poisoned food technique

A thin layer of matured healthy fruit skin was scraped using sterile peeler and subsequently transferred to 100 ml water blanks. The blanks with fruit scrapes were agitated at 150 rpm for 30 min and subsequently subjected for serial dilution up to 10^{-4}. Same procedure was followed for 19 different fruits like Papaya, Banana, Pomegranate, Apple, Ber, Kiwi fruit and Citrus fruits like Lime, Sweet Orange and Mandarine collected from the farmers field as well as from the local fruit markets. The dilutions 10^{-2}, 10^{-3} and 10^{-4} were used for isolation of antagonistic microbial agents by employing poisoned food technique (2.2.1). The colonies with inhibition zone were pure cultured on Petri plate by multiple streaking methods on PDA, NA and Yeast Peptone Dextrose Agar (YPDA) media for fungal, bacterial and yeast isolates respectively. The pure cultures of antagonists were maintained in PDA, NA and YPDA slants for further screening against *C. gloeosporioides*.

Collection of biocontrol agents:

The efficient and proven biocontrol agents against fungal plant pathogens *viz.*, *Trichoderma harzianum*UHSF-15, *Bacillus subtilis* UHSB-01 and *Pseudomonas fluorescens* UHSB-6 were collected from Biofertilizer Laboratory, Department of Agricultural Microbiology, College of Horticulture, Bagalkot for screening against *Colletotrichum* *gloeosporiodes.*

In vitro screening of antagonistic microbial isolates against *C. gloeosporiodes*

The microbial agents showing inhibition zones against pathogen in poisoned food technique were screened for their efficacy by dual culture method (Dennis and Webster, 1971) on PDA media. For dual culture test, approximately 25 ml of molten PDA was poured into Petri plates and allowed for solidification. Five millimetre diameter disc of pathogen was cut with the help of sterile cork borer and placed near the periphery of PDA plate. Similarly antagonistic fungal isolate was placed on the other periphery at an angle of 180^o, in case of bacterial and yeast antagonists, the antagonists were streaked at the centre of the plates. The plates inoculated with only pathogen was served as control. The plates were incubated at 27 ± 1^oC for seven days. Each treatment was replicated thrice. The extent of antagonistic activity by the antagonists was recorded on seventh day by measuring growth of the pathogen in dual culture plate and control plate. The per cent inhibition of pathogen (I) was calculated by using the formula suggested by Vincent (1927).

I = [100 X (Growth of the pathogen in control plate in millimetre - Growth of the pathogen in dual culture plate in millimetre)]/Growth of the pathogen in control plate in millimetre.

In vivo screening of microbial agents against *C.gloeosporiodes*

Selection of antagonistic microbial agents for *in vivo* studies

The five effective isolates of microbial agents from *in vitro* studies were selected and evaluated under *in vivo* for their efficacy against *C. gloeosporioides*against natural infection on fruits and against challenge inoculation with *C. gloeosporioides.*

Preparation of liquid culture of microbial agents

The overnight grown cultures of bacterial antagonists, which were grown on NBand the fungal and yeast isolates grown on PDBfor 5 d were used to prepare the aqueous suspension of microbial agents. The cultures of bacterial isolates were prepared by inoculating 2 loops of each culture to 250 ml of sterile nutrient broth and subsequent incubation on a rotary shaker at 150 rpm for 48 h. Similarly Fungal and yeast cultures were grown for 5 d in an BOD incubator at 25 ±1^{o}C in Potato Dextrose Broth (PDB) by inoculation of 5 mm disc of fungi and 2 loop full of yeastculture.

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In vivo screening of efficient microbial agents against natural anthracnose infection of papaya

The uniform size papaya fruits (*Caricapapaya*) of Red Lady variety at optimum matured stage were collected from the papaya fruit orchard maintained at College of Horticulture, Bagalkot, Karnataka, India and the fruits were dipped in the aqueous suspension of effective microbial agents (prepared by addition of 10 ml broth cultures of isolates containing 1x10^{8}CFUmL^{-1} in one liter of water) for 5 minutes and allowed to dry for 10-15 minutes. The treated fruits were kept in clean crates in separate sets according to treatments under ambient condition. Each treatment was replicated thrice, and six fruits were maintained per replication with completely randomized design. The standard chemical, carbendazium, treatment at 0.1 per cent for 5 minutes was used as a chemical control and hot water dipping treatment at 52^{o} C for 3 minutes was used as physical treatment.The untreated natural fruits served as untreated control. During the study the fruits were observed for the disease symptoms and the extent of infection was scored using 0-4 scale(0=No infection, 0.1-25.0% fruit surface infected=1, 25.1-50.0% fruit surface infected=2, 50.1-75.0% fruit surface infected=3 and 4=>75.0% fruit surface infected) (Pramod *et al.,* 2007).Per cent Disease Index (PDI) of postharvest disease of fruits was calculated by the formula given by Wheeler (1969). PDI= (Sum of all disease rating/ Total number of ratings) x (100/ Maximum disease grade).

Screening of antagonistic microbial agents against challenge inoculation of post harvest anthracnose disease pathogen (*C.gloeosporioides*) on papaya fruits under *in vivo*

Preparation of *C. gloeosporioides*inoculum

The Spore suspension of *C. gloeosporioides*was prepared by growing *C. gloeosporioides* on potato dextrose broth at 25± 1°C for 7 d.The culture suspension was then filtered through two layers of sterile muslin cloth. The concentration of conidia in the filtered suspension was diluted to 10^5 conidia ml^{-1} using sterile saline solution (0.9% NaCl) with the help of haemocytometer (Sariah, 1994).

The mature papaya fruits brought from the farmers field maintained at Bagalkot, Karnataka were surface sterilized with 0.5 per cent of Sodium hypochlorite for 3 minutes and rinsed with distilled water for three times. Subsequently the fruits were inoculated with conidial suspension of *C.gloeosporioides* containing 10^5CFU $mL^{-1}$for 5 minutes and later taken out and placed in a clean dry tray and allowed to dry. After three hours of pathogen inoculation, the fruits were treated by spraying with aqueous suspension of efficient microbial agents (10^8CFU mL^{-1}) at the rate of 10 ml per liter of water and allowed to dry for 10-15 minutes. The treated fruits were kept in clean crates in separate sets according to treatments and incubated in clean area in Laboratory for observation. Each treatment was replicated thrice and six fruits were kept per replication. During the study the fruits were observed for the disease symptoms and the extent of infection was scored using 0-4 scale (Pramod *et al.,* 2007) as mentioned above (2.4.3). Per cent disease index (PDI) was calculated using formula mentioned in previous section (2.4.3) and per cent disease decrease over control were calculated with the formula, Percent disease decrease over control = (Per cent infection in control – Per cent infection in treatment)/ Per cent infection in control.

Identification of efficient antagonistic microbial agents:

The most efficient biocontrol isolates identified in this study were identified by 16S rRNA sequencing (Oliveira *et al.*, 2004).

Statistical analysis

The data obtained in this experiment were subjected to statistical analysis by ANOVA for completely randomized design (CRD analysis). Statistical analysis was performed using Web Agri Stat Package (WASP) Version 2 (Jangam and Thali, 2010). The level of significance used in F and t test was p=0.01. Critical difference and SE.m ± values were calculated whenever F-test was found significant.

Results and Discussion

Identification of *Colletotrichum gloeosporoides*

The ten days old cultures of *Colletotrichum gloeosporoides*produced white mycelium on potato dextrose agar (PDA) media which later turned into grey, dark grey, black with regular margins. The mycelium of pathogen was hyaline, septate and branched. Sporulation was abundant with maximum fruiting bodies at center of the plate as against mycelium growth towards periphery**.** On reverse side of culture plates,pathogen colony depicted grayish to dark grey concentric rings with sector formation.The size of the conidia varied from 9-20 X 3-7.5 µm when observed under the microscope. Similarly, conidia shape was found oblong or cylindrical shape with rounded ends having hyaline, aseptate with one to two oil globules. The similar morphological and microscopic characters of *C.gloeosporoides* were observed by Archana (2014) and Jayalaxmi (2010).

Isolation and *in vitro* evaluation of microbial agents against *Colletotrichum gloeosporioides*:

A total of 19 microbial agentsshowing clear zone of inhibition (Fig. 1) against *C.gloeosporiodes*in poisoned food technique were isolated from 59 samples used. Among the 22 microbial agentsevaluated against *C.gloeosporoides*under*in vitro*, *Trichoderma harzianum*UHSF-15 was found to be the most effective with 62.50% inhibition of radial growth followed by isolateno. 10 with 50%, which was on par with isolateno.9 (47.85%), isolateno. 7 (47.14%) and isolateno.16 (46.43%). The minimum inhibition of radial growth of *C.gloeosporioides*was recorded in isolateno. 4 (12.4%) (Table 1, Fig. 2.).

The higher antagonistic potential of *T.harzianum* against *C. gloeosporioides* was reported by Patel and Joshi (2001); Ashoka *et al.* (2005); Prabhakar *et al.* (2008). The antagonistic activity of *Trichoderma* spp. Against *C. gloeosporioides* might be attributed to competition for space and nutrition, in addition todirect parasitism action by coiling and drawing the nutrition from pathogen mycelia (Raheja and Thakore, 2002) and by production of volatile antibiotics like dermadin and trichodermin against pathogen (Godtfredsen and Vagedal, 1965).

Next to *T.harzianum*UHSF-15 the microbial agentsisolated from different sources like isolate no. 10 (50.00%) (isolated from the soil sample collected from Banana rhizosphere), isolate no. 9 (47.85%) (isolated from kiwi fruit collected from local fruit market), isolate no. 7 (47.14%)(isolated from compost sample) and isolateno. 16 (46.95%) (isolated from Ber fruits surface)werefound to reduce the radial growth of *C. gloeosporioides*to a significantly greater level. Similar results were noticed by Shi *et al.* (2010), where*Pseudomonas putida* isolated from pericarp of papaya fruit was found effective against anthracnose of papaya and reduced incidence up to 54%. The bacterial strain MJM5763, isolated from yam cultivation field has effectively inhibited the growth of *C.gloeosporioides* (65%), *Pestalotia*spp. (57.5%), *Fussarium oxysporum* (48%) and *Ralstonia solani* (40%) (Palaniyandi *et al.*,2011). Senthil *et al.*(2011) reported that *Bacillus subtilis* strains (EPC-16 and EPC-8), were effective in suppressing mycelia growth of grape pathogens *Penicillium expansum* and *Aspergillus carbonarius* under *in vitro* condition. Similarly, the two *Bacillus* strains TB09 and TB72 were reported to produce volatile organic compounds that effectively reduced the anthracnose incidence up to 94.28% and 87.06% respectively (Zheng *et al.*, 2013). Calvo *et al.* (2017) reported antifungal activity of bacteria strain BUZ-14 (*Bacillus amyloliquefaciens*) against many post-harvest pathogens of orange, apple, grape and stone fruits like *Botrytis cinerea*, *Moniliniafruticola*, *Monilinialaxa*, *Pencillimdigitatum*, *Pencilliumexpansum* and *P. italicum*, which produces many bioactive compounds *viz*, iturin, fengycin, bacilysin, bacillomycin and difficidin for their antifungal activity.

Table 1: *In vitro* evaluation of microbial agents against *C.gloeosporoides*by dual culture test

Microbial agents	**Radial growth of *C. gloeosporoides* (cm)**	**Inhibition over control (%)**
Isolate no. 1	4.20	40.00 (6.33)
Isolate no. 2	5.55	20.71 (4.53)
Isolate no. 3	5.45	22.14 (4.70)
Isolate no. 4	6.15	12.14 (3.49)
Isolate no. 5	4.00	42.85 (6.55)
Isolate no. 6	5.60	18.90 (4.35)
Isolate no. 7	3.70	47.14 (6.85)
Isolate no. 8	3.95	42.07 (6.48)
Isolate no. 9	3.65	47.85 (6.92)
Isolate no. 10	3.50	50.00 (7.07)
Isolate no. 11	4.00	42.86 (6.49)
Isolate no. 12	4.00	42.86 (6.55)
Isolate no. 13	4.10	41.43 (6.45)
Isolate no. 14	4.50	35.71 (6.00)
Isolate no. 15	5.25	24.95 (5.00)
Isolate no. 16	3.75	46.95 (6.89)
Isolate no. 17	5.20	25.50 (5.05)
Isolate no. 18	4.35	37.50 (6.12)
Isolate no. 19	3.85	44.35 (6.77)
*Pseudomonas fluorescens*UHSB-06	4.20	39.95 (6.33)
Bacillus subtilis UHSB-01	4.85	30.71 (5.53)
*Trichoderma harzianum*UHSF-15	2.65	62.50 (7.90)
Mean	**4.38**	**37.23**
SE m ±	**0.32**	**0.10**
CD (5 %)	**1.14**	**0.37**

Note: The values in the parenthesis are square root transformed value and the statistical analysis was done for transformed value.

Isolate No. 10 Isolate No. 9 Control

Fig. 1. The biocontrol agents inhibiting the growth of *C. gloeosporoides* in poisoned food technique

Trichoderma harzianum UHSF-15 Isolate No. 7 Isolate No. 9

Isolate No. 10 Isolate No. 16 Control

Fig. 2. Effectiveisolatesofbiocontrol agents inhibiting *C. gloeosporoides* in dual-culture test

In vivo evaluation of microbial agents against *Colletotrichum gloeosporioides*

Evaluation of microbial agents against natural incidence of anthracnose on papaya fruits under *in vivo*

The lowest per cent disease index was recorded in fruits treated with T_5 (BCA_5 -isolate no. 10) with 13.89% followed by T_4 (BCA_4-isolate no. 9) with 16.67% and T_7 (Chemical treatment with carbendazium at 0.1%) with 20.83% during storage of papaya fruits (Table 2; Fig.3). The per cent disease index in untreated control fruits was 58.33%. The decreased disease incidence in microbial agents treated treatments might be attributed to the antagonistic mechanism exhibited by the isolates of microbial agents as described by Sharma *et al.* (2009) *viz.*, competition for the nutrients and space, direct parasitism, production of antibiotics or production of volatile organic compounds against pathogen.

Evaluation of microbial agents against challenge inoculation of postharvest anthracnose disease pathogen (*C.gloeosporioides*) on papaya fruits under *in vivo*

Postharvest treatment of anthracnose pathogen (*C. gleosporoides*) inoculated fruits with biocontrol agentisolate no. 10 for 5 min was most effective in reducing postharvest infection with 33.33% disease index (PDI) followed by isolate no.9 with 50.00% and chemical treatment with carbendazium at 0.1 per cent with 52.77% disease index at 9 d after storage (Table 3; Fig.4). The higher per cent disease index of 91.66% was recorded in pathogen inoculated control fruits, which might be due to pathogen infection at earlier stage during fruit ripening or at climacteric phase. Reduced phenolic compounds during fruit ripening and susceptibility of fruit skin to chitinase enzyme which was secreted by pathogen (Chau and Alvarez, 1983) might be responsible for the disease development in the papaya fruit during storage.

Table 2: Effect of microbial agents treatment on incidence of anthracnose disease (*C.gloeosporoides*) in papaya at 9 d of storage under ambient condition

Treatments	**Per cent disease index (PDI)**	**Disease decrease over control (%)**
T_1 - BCA_1 (isolate no. 22)	25.00 (5.05)	57.14
T_2- BCA_2 (isolate no. 16)	33.33 (5.73)	42.85
T_3 - BCA_3 (isolate no. 7)	27.78 (5.30)	52.37
T_4- BCA_4 (isolate no. 9)	16.67 (4.09)	71.42
T_5 - BCA_5 (isolate no. 10)	13.89 (3.79)	76.19
T_6- Hot water dip	33.33 (5.73)	42.86
T_7- Chemical treatment	20.83 (4.56)	65.14
T_8- Untreated control	58.33 (7.64)	-
Mean	**28.65**	
SEm ±	**0.47**	
CD (1%)	**1.42**	

Note: The values in the parenthesis are squareroot transformed values and statistical analysis was carried out for transformed values.

BCA: Biocontrol agent; Chemical: Carbendazium at 0.1 per cent; Hot water dip (52^o C for 3 minutes)

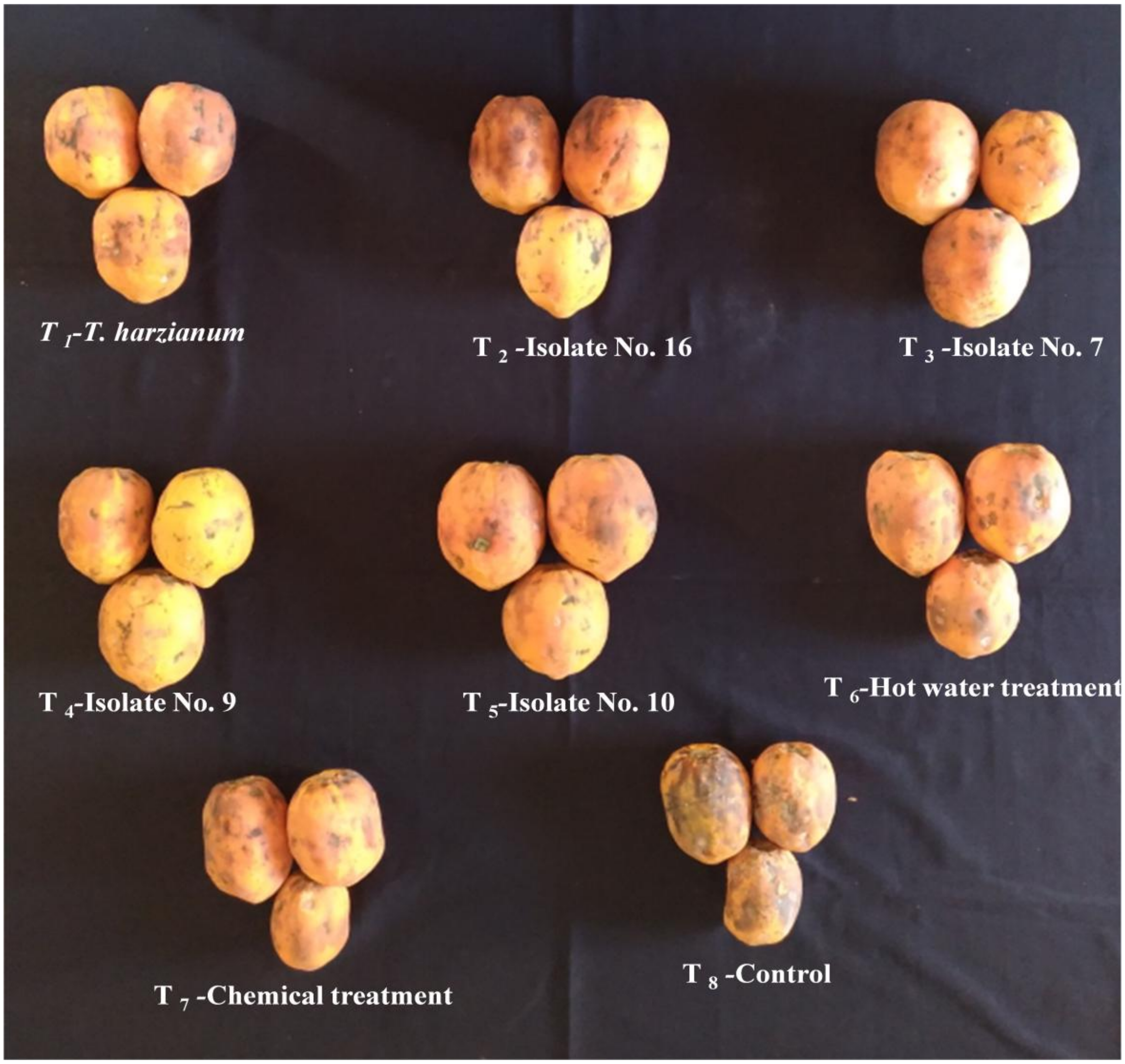

Fig. 3. Effect of biocontrol agents treatment on incidence of anthracnose disease (*C. gloeosporoides*) in papaya at 9 d of storage under ambient condition

Table 3: Effect of microbial agents treatment on incidence of anthracnose diseasein papaya fruits challenge inoculated with pathogen (*C.gloeosporoides*) at 9 d after storage under ambient condition

Treatments	**Per cent disease index (PDI)**	**Disease decreased over control (%)**
T_1 - BCA_1 (isolate no. 22)	58.33 (7.62)	36.36
T_2 - BCA_2 (isolate no. 16)	77.77 (8.80)	15.15
T_3 - BCA_3 (isolate no. 7)	58.33 (7.62)	36.36
T_4 - BCA_4 (isolate no. 9)	50.00 (7.05)	45.45
T_5 - BCA_5 (isolate no. 10)	33.33 (5.77)	63.64
T_6 - Hot water dip	63.88 (7.99)	30.31
T_7 - Chemical treatment	52.77 (7.24)	42.42
T_8 - Untreated control	91.66 (9.58)	-
Mean	**60.76**	
SEm ±	**0.29**	
CD (1%)	**0.87**	

Note: The values in the parenthesis are squareroot transformed values and statistical analysis was carried out for transformed values.

BCA: Biocontrol agent; Chemical: Carbendazium at 0.1 per cent; Hot water dip (52° C for 3 minutes)

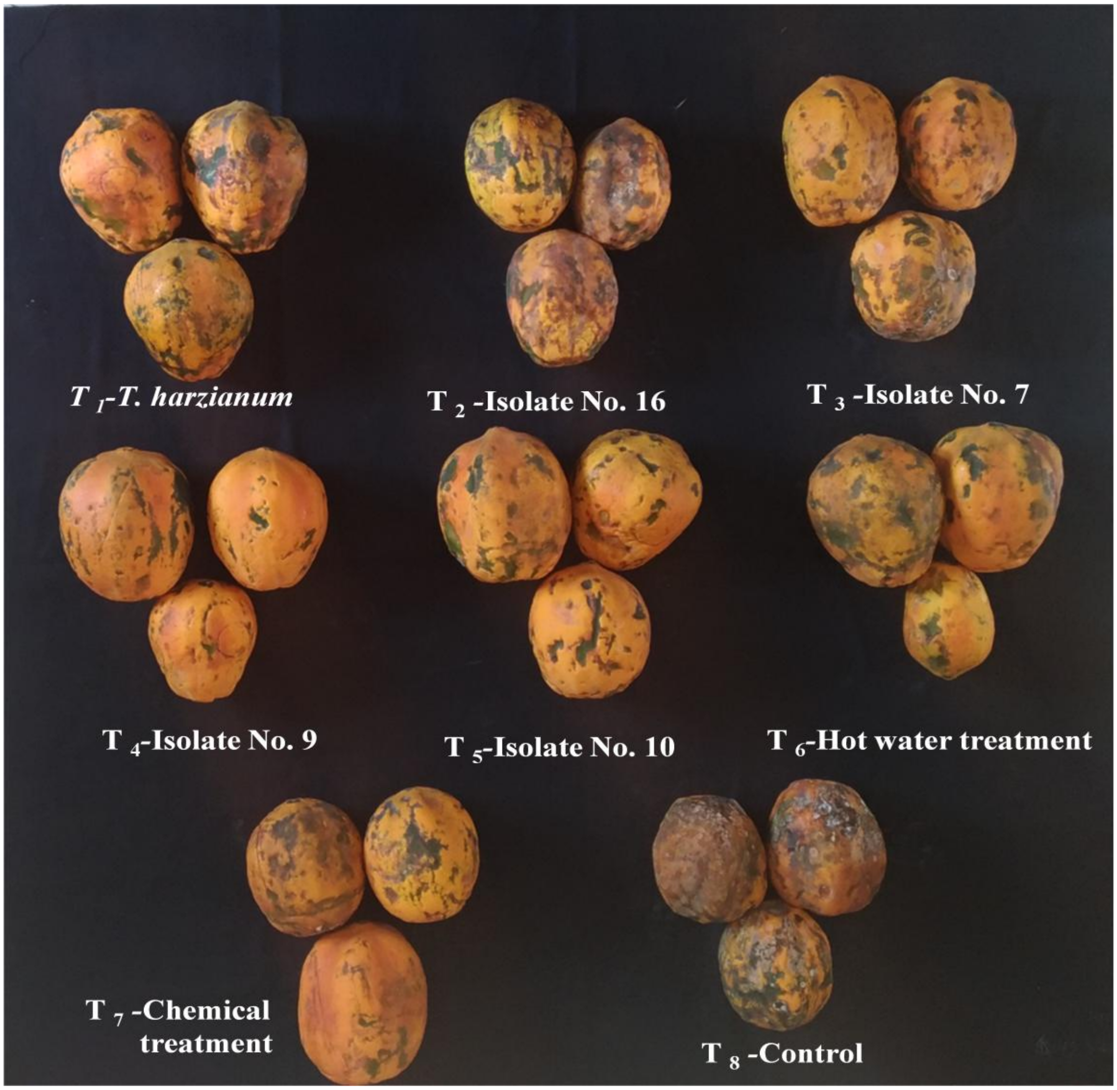

Fig. 4. Effect of biocontrol agents treatment on incidence of anthracnose disease in pathogen (*C.gloeosporioides*) challenge inoculated papaya at 9 d after storage under ambient condition

Identification of efficient microbial agents:

The most efficient biocontrol isolates *viz.,* isolate no. 10 and isolate no. 9 observed in the present investigation were identified as *Bacillus amyloliquifaciens* (Both isolates) and were given isolate no. UHSB-BCA-15 and UHSB-BCA-16 respectively.

In the present study the identified isolates were effective against anthracnose pathogen *C.gloeosporoides*by exhibiting some antagonistic mechanisms. Similary Kim and Chuan (2012) reported that *B. amyloliquefaciens* MET0908 secreted an extracellular β-1,3-glucanase, against *Colletotrichum lagenarium*, an anthracnose causing pathogen of watermelon which is a key enzymein the decomposition of fungalhyphal walls. Hu *et al.*(2010) reported that the antagonism of *Bacillus* is mainly due to the production of the antagonistic substances by its secondarymetabolism pathways.According to the reports of European Food Safety Authority (2008), some stains of *B. amyloliquefaciens*do not possess the genes encoding *Bacillus*enterotoxins or the key gene implicated in the synthesis of emetic toxins, or does not demonstrate phenotypic characteristic of toxin production. Hence these isolates can be used effectively to manage the postharvest anthracnose disease during storage with better fruit quality.

Conclusion:

The two isolates of antagonistic microorganisms *viz.,* Isolate No.10 (*Bacillus amyloliquifaciens*isolate no. UHSB-BCA-15) isolated from banana rhizosphere soil and Isolate No. 9 (*Bacillus amyloliquifaciens*Isolate no. UHSB-BCA-16)), isolated from kiwi fruit surface can be effectively used for postharvest treatment of papaya against Anthracnose caused by *Colletotrichum gleosporoides.*

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