*Original Research Article*

# Effect of antagonistic microorganisms on extension of shelf-life and physiochemical properties of papaya (*Carica papaya*) var.Red Lady


## Abstract

A wide range of fungi, bacteria, yeast and actinomycetes can be used as antagonistic microorganisms against post-harvest pathogens in many horticulture produce. It is considered as non-toxic ecofriendly method to minimize post-harvest loss due to diseases during storage. In this contest an experiment was conducted in the Department of Agricultural Microbiology and Post-Harvest Technology, COH, Bagalkot, Karnataka during the year 2017-2018. Five effective antagonistic microorganisms, against post-harvest anthracnose disease causing organism *Colletotrichum gleosporiodies* were isolated and used to in papaya fruits with an objective to reduce the anthracnose disease infection and to extend shelf life of papaya fruits with retention of good physiochemical properties. In the present experiment the papaya fruits treated with biocontrol agents and observed that, the papaya fruits treated with $BCA_5$-Isolate No.10(T5) exhibited higher shelf life of 10 days with minimum physiological loss in weight (16.23%), higher firmness (2.16 N) and higher TSS ($14.10^o$ brix) and ascorbic acid (38.19 mg/100 g) at 9 days of storage followed by BCA-Isolate No.09(T4) and Carbendazium at 0.1 per cent for 5 min(T7). Similarly the effect of biocontrol agents on physiochemical properties of papaya fruits challenge inoculated with *C. gloeosporioides* showed that, fruits treated with $T_5$ ($BCA_5$ -Isolate No. 10) recorded higher shelf life of 9.67 days with lowest per cent physiological loss in weight (16.13%), higher firmness retention (2.22 N), higher TSS ($12.47^o$ brix), and ascorbic acid content (30.19 mg/100 g) at 9 days of storage followed by $BCA_4$ -Isolate No.9 ($T_4$) and Carbendazium at 0.1 per cent ($T_7$). These two isolates identified in this experiment can be effectively used alternative to chemicals against anthracnose.


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## Introduction:

Papaya, a very wholesome fruit, is high in nutritive and medicinal values and considered as source of the precursor of vitamin A after mango. It is highly perishable in nature. Post-harvest diseases of papaya caused by fungi are responsible for causing losses to the tune of 45 per cent of their market value (Abeywickrama *et al.,* 2012). In India, Anthracnose disease is prevalent throughout the country and anthracnose rot attains serious status during transit, storage and market, causing considerable economic losses (Rana, 2001). Papaya contains 86.6 per cent moisture, forming an ideal medium for the proliferation of many storage pathogens. Anthracnose of papaya caused by *Colletotrichum gleosporoides* is considered as one of the most devasting pathogen in post-harvest stage which decreases the acceptability and shelf life of the fruits during storage.

Postharvest diseases can be controlled by biological, physical and chemical methods. But the limitations of physical methods and risk of high levels of toxic residues by the use of chemicals, is one of the serious concerns since the fruits are consumed within short period after the harvest. Hence biological control is one of the better alternatives to overcome these problems in management of postharvest diseases. A wide range of fungi, bacteria, yeast and actinomycetes can be used as antagonistic microorganisms against post-harvest pathogens in many horticulture produce. It is considered as non-toxic ecofriendly method to control post-harvest diseases during storage. The present study studies on the effect of biocontrol agents treatment on shelf-life and physiochemical properties of papaya during storage.

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## Material and methods

### Collection of antagonistic microorganisms

Papaya anthracnose causal agent *Colletotrichum gleosporiodies* was isolated from infected papaya fruits collected from local market. The pathogen isolate was used for isolation of biocontrol agents from different sources by Poisoned food technique. From 59 different fruit, soil and compost samples, 19 biocontrol agents exhibiting clear zone of inhibition in poisoned food technique were identified for further evaluation against pathogen under *in vitro* by Dual culture technique. Among the antagonistic agents tested, *Trichoderma harzianum* collected from Biofertilizer Laboratory, Department of Agricultural Microbiology, College of Horticulture, Bagalkot was used as $T_1$, antagonistic bacteria isolated from the fruit surface of ber fruit (Isolate no.16 ), antagonistic bacteria isolated from compost sample (Isolate no.7), antagonistic yeast isolated from Kiwi fruit surface collected from local market (Isolate no.9) and Yeast isolated from banana rhizosphere soil (Isolate no.10) were used as $T_2$, $T_3$, $T_4$ and $T_5$ respectively.

**Preparation of liquid culture of microbial agents**

The overnight grown cultures of bacterial antagonists, grown on nutrient brot (NB) and the fungal isolates grown on potato dextrose broth (PDB) for 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 48h. 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.

**Effect of antagonistic microbial agents on physiochemical properties and shelf-life of Papaya (*Carica papaya*)**

The uniform size papaya fruits (*Carica papaya*) 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 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 physiochemical properties.

**Effect of antagonistic microbial agents on physiochemical properties and shelf-life of Papaya (*Carica papaya*) challenge inoculated with *C. gloeosporioides***

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).

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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 $10^5$CFU $mL^{-1}$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 ($10^8$CFU $mL^{-1}$) 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.

## Oservations recorded:

The papaya fruits with different treatment were incubated at ambient temperature and observations on physiochemical properties and shelf-life of papaya fruits were recorded upto 9 d after storage. The testing methods adopted for physicochemical observations are described below.

**Physiological loss in weight**

The mass of papaya fruit was measured by balance before treatment (A) and at 9 d after storage (B), and the loss in weight was calculated as (A-B)/A.

**Total Soluble Solids ($^o$B)**

The juice extracted by squeezing the homogenized papaya pulp through muslin cloth was used to measure the TSS. It was determined by using ERMA hand refractometer, replicated three times.

**Ascorbic acid (mg/100 g)**

Ascorbic acid (Vitamin C) was estimated titrimetrically using 2,6-dichlorophenol indopheol dye as per the modified procedure of AOAC (Anon., 1984). One gram of sample was crushed with four per cent of Oxalic acid. This was filtered through a muslin cloth to get clear

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juice. Five ml of aliquot was titrated against 2, 6-dichlorophenol indophenols dye till the titration reaches pink colour end point, which persisted for at least 15 seconds. Ascorbic acid was estimated using the formula given below and expressed in mg per 100 g of edible part.

$$\text{Ascorbic acid (mg/100 g)} = \frac{\text{Ascorbic acid (mg) in standard x } TV_2 \text{ x Volume made up x 100}}{\text{Standard value x weight of sample x Aliquot taken x 1000}}$$

**Shelf life of the fruit (days)**

The shelf life or keeping quality of fruits was decided based on the appearance and edible stage of the fruits. When the fruits attained beyond the edible ripe stage, then those fruits were considered to have reached the end of their shelf life and shelf-life was expressed in days.

**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

**Effect of microbial agents treatment on physiochemical parameters of papaya fruits**

The papaya fruits treated with $T_5$ ($BCA_5$- isolate no.10) and $T_4$ ($BCA_4$- isolate no.9) exhibited minimum physiological loss in weight (16.23% and16.44%), higher firmness (2.16 N each), higher TSS (14.10% and 13.7%) and higher retention of ascorbic acid (381.90and 363.50 $mgkg^{-1}$) at 9 d of storage followed by $T_7$ (Chemical treatment with Carbendazium at 0.1%) (Table 1).The fruits treated with isolate no. 10 exhibited maximum shelf life of 10 d followed by isolate no. 9 (9 d) and Chemical (Carbendazium) treated fruits (8.67 d).

**Table 1: Effect of antagonistic microbial agents treatment on physiochemical parameters of papaya fruits variety Red Lady at 9d after storage under ambient condition**

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| Treatments | PLW (%) | Firmness (N) | TSS (%) | Ascorbic acid (mg kg$^{-1}$) | Shelf life (d) |
|---|---|---|---|---|---|
| **$T_1$ -** $BCA_1$ (isolate no. 22) | 17.00 | 1.46 | 13.47 | 355.90 | 8.33 |
| **$T_2$ -** $BCA_2$ (isolate no. 16) | 18.00 | 0.89 | 12.07 | 330.90 | 7.67 |
| **$T_3$ -** $BCA_3$ (isolate no. 7) | 17.00 | 1.17 | 12.98 | 355.20 | 8.33 |
| **$T_4$ -** $BCA_4$ (isolate no. 9) | 16.44 | 2.16 | 13.70 | 363.50 | 9.00 |
| **$T_5$ -** $BCA_5$ (isolate no. 10) | 16.23 | 2.16 | 14.10 | 381.90 | 10.00 |
| **$T_6$ -** Hot water dip | 17.75 | 0.92 | 12.83 | 347.30 | 8.00 |
| **$T_7$ -** Chemical treatment | 16.67 | 2.05 | 13.70 | 360.70 | 8.67 |
| **$T_8$ -** Untreated control | 20.22 | 0.65 | 11.90 | 330.70 | 7.67 |
| **Mean** | **17.49** | **1.43** | **13.09** | **353.20** | **8.46** |
| **SEm ±** | **0.31** | **0.03** | **0.10** | **3.00** | **0.32** |
| **CD (1%)** | **1.29** | **0.12** | **0.42** | **12.20** | **1.33** |

Note: BCA: Biocontrol agent; PLW: Physiological loss in weight TSS: Total soluble solids; Chemical: Carbendazium at 0.1 per cent

Similarly, during the evaluation of efficient microbial agents on papaya fruits challenge inoculated with *C.gloeosporioides*, the fruits treated with $BCA_5$- isolate no. 10 ($T_5$) and $BCA_4$- isolate no. 9 ($T_4$) recorded lowest per cent physiological loss in weight (16.13 and 16.53% respectively), higher firmness retention (2.2 N each), higher TSS (12.47 and 12.27% respectively) and ascorbic acid content (301.90 and 301.70 mgkg$^{-1}$ respectively) at 9 d of storage (Table 2). The pathogen inoculated control fruits exhibited higher weight loss (21.55%), lower firmness (0.93 N), lower TSS (9.87%) and ascorbic acid content (242.10 mgkg$^{-1}$) at 9 d after storage. The fruits treated with isolate no. 10 exhibited maximum shelf life of 9.67 d followed by isolate no. 9 (8.67 d) and Chemical (Carbendazium) treated fruits (8.33 d), whereas the pathogen inoculated control fruits showed only 6.70 d.

The lower weight loss observed in T5 and T4 might be attributed to reduced physiological processes as the tissues respire and transpire at relatively slower rate resulting in reduced rate of moisture loss and other chemical ingredients. The higher retention of firmness was observed in microbial agents treated fruits which might be attributed to reduced pectin hydrolysis and modification of hemicellulose by the enzymes in papaya which might have ultimately lead to reduced concentration of soluble pectin in the cell wall (Paull *et al.*, 1999). The

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increase in TSS at the end of storage life could be attributed to the hydrolysis of starch and other polysaccharides to soluble form of sugar (Yadav *et al.*, 2009). The higher retention of ascorbic acid content might be due to reduced degradation through enzymatic oxidation of L-ascorbic acid to dehydro ascorbic acid during metabolic processes. The enhanced shelf life observed in treatments $T_5$, $T_4$ and $T_7$ might be due to minimum weight loss, retention of firmness and other quality parameters. The results of present investigation are in conformity with findings of Bharati (2006) and Yadav *et al.* (2014) who observed similar results in papaya. The similar pattern was observed with respect to physiochemical properties of microbial agents treated papaya fruits challenge inoculated with *C. gloeosporoides*. But the reduced biochemical parameters might be due to infection with *C. gloeosporoides* at early stages of the fruits during storage.

**Table 2: Effect of antagonistic microbial agents treatment on physiochemical parameters of *C.gloeosporioides* challenge inoculated papaya fruits variety Red Lady at 9d after storage under ambient condition**

| Treatments | PLW (%) | Firmness (N) | TSS (%) | Ascorbic acid ((mg kg$^{-1}$)) | Shelf life (d) |
|---|---|---|---|---|---|
| **$T_1$ -** $BCA_1$ (isolate no. 22) | 17.83 | 1.90 | 12.00 | 292.50 | 8.00 |
| **$T_2$ -** $BCA_2$ (isolate no. 16) | 19.47 | 1.55 | 11.67 | 265.00 | 7.67 |
| **$T_3$ -** $BCA_3$ (isolate no. 7) | 18.80 | 1.04 | 11.17 | 283.20 | 8.00 |
| **$T_4$ -** $BCA_4$ (isolate no. 9) | 16.53 | 2.21 | 12.27 | 301.70 | 8.67 |
| **$T_5$ -** $BCA_5$ (isolate no. 10) | 16.13 | 2.22 | 12.47 | 301.90 | 9.67 |
| **$T_6$ -** Hot water dip | 18.00 | 1.84 | 10.83 | 276.90 | 7.67 |
| **$T_7$ -** Chemical treatment | 17.50 | 2.04 | 12.03 | 295.40 | 8.33 |
| **$T_8$ -** Untreated control | 21.55 | 0.93 | 9.87 | 242.10 | 6.70 |
| **Mean** | **18.33** | **1.72** | **11.54** | **282.30** | **8.13** |
| **SEm ±** | **0.13** | **0.06** | **0.22** | **6.30** | **0.32** |
| **CD (1%)** | **0.54** | **0.24** | **0.91** | **26.10** | **1.33** |

Note: BCA: Biocontrol agent; PLW: Physiological loss in weight TSS: Total soluble solids; Chemical: Carbendazium at 0.1 per cent

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).

In the present study the identified isolates were effective against anthracnose pathogen *C.gloeosporoides* by exhibiting antagonistic mechanisms. Similary Kim and Chuan (2012)

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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 enzyme in the decomposition of fungal hyphal walls. Hu *etal.*(2010) reported that the antagonism of *Bacillus* is mainly due to the production of the antagonistic substances by its secondary metabolism 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 chacteristic 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*), isolated from banana rhizosphere soil and Isolate No. 9 (*Bacillus amyloliquifaciens*), isolated from kiwi fruit surface can be effectively used for postharvest treatment of papaya for enhancing the shelf life by retaining physiochemical properties.

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