Original Research Article

Effect of antagonistic microorganisms on fruit quality and sensory properties of papaya (*Carica papaya*) var. Red Lady

Abstract

An experiment was conducted in the Department of Agricultural Microbiology and Post-Harvest Technology, COH, Bagalkot, Karnataka during the year 2017-2018 with intention of minimizing the post harvest diseases during storage by using ecofriendly approach that is use of biocontrol agents and their effects on fruit colour development and sensory parameters. A wide range of fungi, bacteria, yeast and actinomycetes can be used as antagonistic microorganisms against post-harvest pathogens in many horticulture produce. In the present experiment, five effective antagonistic microorganisms were assessed for their antifungal activity against *Colletotrichum gleosporiodies*, the causal agent of post-harvest Anthracnose disease of papaya fruits and fortheir effects on fruit development and sensory properties of papaya var. Red Lady. In the present experiment the papaya fruits treated with biocontrol agents and observed that, the papaya fruits treated with BCA_5-Isolate No.10(T_5) exhibited higher L*(56.53), a*(12.33), b*(43.60) colour values, higher carotene value (1.85 mg/100 g), total sugar content (11.6%) and good sensory scores for colour (8073), taste (8.6) and textue (9.03) at 9 days after storage under ambient storage condition followed by BCA-Isolate No.09(T_4) and carbendazium at 0.1% for 5 min (T_7). Treated on inoculated papaya fruits with *C. gloeosporioides*, T_5 (BCA_5 -Isolate No. 10) recorded higher L*(55.67), a*(11.61), b*(41.78) colour values, higher carotene value (1.42 mg/100 g), total sugar content (10.4%) and good sensory scores for colour (8.3), taste (8.13) and textue (8.73) at 9 days after storage under ambient storage condition followed by BCA_4 -Isolate No.9 (T_4) and carbendazium at 0.1% (T_7). These two isolates identified in this experiment can be effectively used alternative to chemicals against anthracnose which were effective in fruit colour development and sensory parameters.

Keywords: antagonistic microorganisms, sensory parameters, biocontrol agents, papaya fruits

Introduction

India ranks first in world papaya production and shares about 43.7% (Anon., 2016). Papaya (*Carica papaya* L.) is an important fruit crop, belonging to the family Caricaceae and is native to tropical America. From the nutritional point of view, papaya has calorific value ranging from 39 calories per 100 g. The ripe fruit contain 92.6% water, 7.5% carbohydrates, 1.0% protein, 0.5% fat, 4.5% dietary fiber and 0.1% p mineral matter (2.5% Ca, 1% P, 2.5% Mg, 0.5% Zn and 1.0% Fe) (Aravind *et al.,* 2013). It also contains many essential B-complex vitamins such as folic acid and pyridoxine (vitamin B-6). These vitamins are essential in the sense that body requires them from external sources to replenish and play a vital role in metabolism. Papaya has several medicinal values as it prevents atherosclerosis, diabetis and heart diseases. These nutritional values of papaya help to prevent the oxidation of cholesterol.

Papaya post-harvest losses are caused due to various factors such as mechanical damage, chilling injury, diseases and over ripeness. According to Ventura *et al.* (2004), postharvest rots may account for 100% of the total postharvest losses. Post-harvest diseases of papaya caused by fungi are responsible for causing losses to the tune of 45% of their market value (Abeywickrama*et al.,* 2012).In India, Anthracnose disease is prevalent throughout the country and has been reported to occur in Uttar Pradesh, Madhya Pradesh, Bihar, Karnataka and Kerala. However, anthracnose rot attains serious status during transit, storage and market, causing considerable economic losses (Rana, 2001). The initial symptoms are water soaked, sunken spots one-fourth to one inch in diameter on fruit. The centers of these spots later turn black and then pink when the fungus produces spores. The flesh beneath the spots becomes soft and watery, which spreads to the entire fruit, thereby reducing the fresh-market value and also quality.

Post harvest 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 post harvest diseases. Biological control is "the reduction in amount of inoculum or disease-producing activity of a pathogen accomplished by or through one or more organisms other than man" (Cook and Baker, 1983). In the present experiment papaya fruit treated with efficient biocontrol agents against post harvest Anthracnose disease, were evaluated for the fruit quality and sensory parameters at 9 days of storage under ambient condition.

Materials and methods

Collection of pathogen and antagonistic microorganisms

The pathogen *Colletotrichum gleosporiodies* was isolated from infected papaya fruit showed typical symptoms of Anthracnose disease..

From 59 different fruit, soil and compost samples, 19 microbial agents exhibiting clear zone around their colonies towards *C. gleosporiodies* using poisoned food technique in agar plate were selected for further assay. 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.

Five microbial agents (fungi, bacteria and yeast) isolates are selected as the best antifungal agents towards *C. gleosporiodies* using the dual culture methods (data not sown) and which are collected from different sources (Table 1) are used in this study.

Preparation of liquid culture of microbial agents

The cultures of bacterial isolates were prepared by inoculating 2 loops of each culture to 250 ml of sterile *Nutrient Broth* (NB) and subsequent incubation at ??°C on a rotary shaker at 150 rpm for 48 h. Fungal and yeast cultures were grown on *Potato Dextrose Broth* (PDB) for 5 days in an BOD incubator at 25 ±1^{o}C in by inoculation of 5 mm disc of fungi.

Effect of antagonistic microbial agents on fruit quality and sensory properties of papaya (*Carica papaya*) var. Red Lady under ambient storage condition

The uniform size papaya fruits (*Carica papaya*) of Red Lady variety at optimum matured stage were collected and the fruits were dipped in the aqueous suspension of microbial agents

(1% v v^{-1}) for 5 min and allowed to dry for 10 to 15 min. The standard chemical, carbendazium treatment at 0.1% was used as a chemical control and hot water dipping treatment at 52^{o}C for 3 min was used as physical treatment. The untreated fruits served as untreated control. After 9days of storage at room temperature, the fruits were observed for the fruit colour development and sensory properties.

Effect of antagonistic microbial agents on fruit quality and sensory properties of papaya (*Carica papaya*) var. Red Lady inoculated with *C. gloeosporioides* under ambient storage condition

The spore suspension of the pathogen *C. gloeosporioides* was prepared by growing *C. gloeosporioides* on PDB at 25± 1°C for 7 days. 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% of sodium hypochlorite for 3 min and rinsed with distilled water for three times. The mature papaya fruits with similar size were inoculated by dipping onto conidial suspension of *C. gloeosporioides* (10^5 CFU mL^{-}1) for 5 min,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 1% v v^{-1} and allowed to dry for 10 to15 min. The treated fruits were kept in clean crates in separate sets according to treatments and incubated at ambient temperature (??°C) for 9?? days.. Each treatment was replicated thrice and six fruits were kept per replication.

Fruit surface colour (*L, *a**, *b**)**

Colour of the samples were measured using Colour Flex EZ colorimeter (Model: CFEZ 1919, Hunter associates laboratory. Inc., Reston) fitted with 45 mm diameter aperture. The instrument was calibrated using black and white tiles provided. Colour was expressed in *L** (lightness/darkness), *a** (redness/greenness) and *b** (yellowness/blueness) . Three measurements were performed per sample at different parts of the fruit surface and values of the samples were averaged.

Total sugars

The total sugar content of the papaya fruits was estimated by anthrone reagent method (Ranganna, 1986). Five ml of 2.5 N HCl was taken in a clean test tube and added with 100 mg of Papaya fruit sample. The samples were subjected to hot water bath (at 95˚C) for 3 h. After cooling it to room temperature, a pinch of sodium carbonate was added to neutralize the remaining acid in the sample. The sample was filtered using muslin cloth and the filtrate volume was made up to 100 ml using distilled water in a volumetric flask. From that, 0.2 ml filtrate was taken into a clean test tube and volume is made up to 1 ml with distilled water followed by addition of 4 ml Anthrone reagent and heated in a water bath at 50-60˚C for 5 min. Subsequently the sample was cooled to room temperature and the intensity of dark green colour developed was read using spectrophotometer at 630 nm. A graph was drawn by plotting concentration of the standard on the X-axis and absorbance on the Y-axis. From the graph, the amount of total sugar present in the sample was calculated. The values obtained were expressed as per cent (%).

β-Carotene content

Five gram of sample was crushed repeatedly in 10-15 ml acetone, adding a few crystals of anhydrous sodium sulphate, with the help of pestle and mortar. The supernatant was decanted into a beaker and the process was repeated thrice. The combined supernatant was transferred to a separatory funnel and 10-15 ml of petroleum ether was added and mixed thoroughly. After formation of two separate layers the upper layer was collected and the volume was made up to 100 ml with petroleum ether in volumetric flask. The absorbance of each sample was recorded using spectrophotometer at 452 nm using petroleum ether as blank (Ranganna, 1986).

$$\beta \text{ carotene (mg/100 g)} = \frac{\text{O. D. sample x 13.9 x Volume made up of petroleum ether ? (ml?) x } 10^4}{\text{weight of sample (g or mg?) x 560 x 1000}}$$

Survey of sensory properties

Sensory evaluation of papaya fruit var. Red Lady was carried out by a semi-trained panel consisting of Teachers and Post-Graduate students of College of Horticulture, Bagalkot with the help of nine point hedonic rating scale (1 = dislike extremely, 2 = like only slightly, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6-like slightly, 7 like moderately, 8 like very much and 9 like extremely) for colour, texture and taste. The papaya fruits from different treatments along with untreated control were coded and served randomly to the panelist for sensory evaluation.

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

Table 1: Effect of antagonistic microbial agents treatment on fruit quality andsensory parameters of papaya (*Carica papaya*) var. Red Lady at 9 days after storage under ambient condition

	Fruit surface colour			Total sugar (%)	Carotene content (mg/100 g)	Survey of sensory parameters		
Treatments	*L**	*a**	*b**			Colour	Texture	Taste
T$_1$ - BCA$_1$ (isolate no. 22)	54.83	10.97	41.42	11.10	1.66	8.27	8.28	8.80
T$_2$ - BCA$_2$ (isolate no. 16)	53.72	9.00	40.83	9.53	1.44	8.18	7.94	8.60
T$_3$ - BCA$_3$ (isolate no. 7)	54.29	9.97	42.32	10.80	1.48	8.25	8.10	8.79
T$_4$ - BCA$_4$ (isolate no. 9)	56.52	11.77	42.95	11.58	1.83	8.57	8.41	8.83

T_5 - BCA_5 (isolate no. 10)	56.53	12.33	43.60	11.63	1.85	8.73	8.6	9.03
T_6 - Physical control treatment	53.77	9.33	41.12	10.27	1.54	8.20	8.03	8.63
T_7 - Chemical control treatment	56.47	11.50	42.63	11.43	1.73	8.43	8.36	8.80
T_8 - Untreated control	52.97	8.83	39.32	9.23	1.36	8.10	7.33	8.40
Mean	**54.89**	**10.46**	**41.90**	**10.70**	**1.61**	**8.35**	**8.13**	**8.71**
SEm ±	**NS**	**0.35**	**0.52**	**0.22**	**0.04**	**0.08**	**0.09**	**0.12**
CD (1%)	**NS**	**1.46**	**2.15**	**0.93**	**0.18**	**0.33**	**0.37**	**0.48**

Note: BCA: Biocontrol agent; Physical control treatment: Hot water dip at 52°C for 3 min;Chemical control treatment: Carbendazium at 0.1%.

The papaya fruits treated with T_5 (BCA_5- isolate no.10) and T_4 (BCA_4- isolate no.9) exhibited higher a* values (12.33 and 9.33), b* values (43.60 and 42.95) and carotene content (1.85 and 1.83), total sugars (11.63 and 11.58) and higher sensory scores for colour (8.73 and 8.57), texture (8.6 and 8.41) and Taste (9.03 and 8.83) (Table 1.) at 9 days after storage under ambient storage condition followed by Chemical (Carbendazium) treated fruits.

Table 2: Effect of antagonistic microbial agents treatment on frit quality and sensory parameters of papaya (*Carica papaya*) var. Red Lady inoculated with *Coletotrichum gleosporiodes* at 9 days after storage under ambient condition

Treatments	**Fruit surface colour**			**Total sugar (%)**	**Carotene content (mg/100 g)**	**Survey of sensory parameters**		
	L*	***a****	***b****			**Colour**	**Texture**	**Taste**
T_1 - BCA_1 (isolate no. 22)	52.28	10.30	39.58	9.96	1.12	8.23	7.65	8.57

T_2 - BCA$_2$ (isolate no. 16)	50.57	8.67	37.51	9.10	1.00	8.00	7.12	8.26
T_3 - BCA$_3$ (isolate no. 7)	51.49	9.30	39.31	9.27	1.06	8.08	7.38	8.41
T_4 - BCA$_4$ (isolate no. 9)	55.10	11.27	41.14	10.03	1.29	8.28	8.00	8.63
T_5 - BCA$_5$ (isolate no. 10)	55.67	11.61	41.78	10.40	1.42	8.30	8.13	8.73
T_6 - Hot water dip	51.31	9.17	38.83	8.80	1.08	8.00	7.29	8.31
T_7 - Chemical treatment	54.15	11.00	41.07	9.96	1.27	8.24	7.71	8.63
T_8 - Untreated control	50.33	8.83	35.98	8.60	0.99	7.20	7.07	7.00
Mean	**52.61**	**10.02**	**39.41**	**9.58**	**1.15**	**8.17**	**7.54**	**8.50**
SEm ±	**NS**	**0.26**	**0.42**	**0.14**	**0.032**	**0.06**	**0.14**	**0.10**
CD (1%)	**NS**	**1.08**	**1.73**	**0.57**	**0.13**	**0.23**	**0.59**	**0.40**

Note: BCA: Biocontrol agent; Physical control treatment: Hot water dip at 52°C for 3 min;Chemical control treatment: Carbendazium at 0.1%.

Challenge inoculated papaya fruits with *Colletotrichum gleosporiodes,* treated with T_5 (BCA$_5$- isolate no.10) and T_4 (BCA$_4$- isolate no.9) exhibited higher a* values (11.61and 11.27), b* values (41.78 and41.14) and carotene content (1.42 and 1.29), total sugars (10.40 and 10.03) and higher sensory scores for colour (8.30 and 8.5287), texture (8.13 and 8.00) and Taste (8.73 and 8.63) (Table 2.) at 9 days after storage under ambient storage condition followed by Chemical (Carbendazium) treated fruits.

The most efficient biocontrol isolates *viz.*, isolate no. 10 and isolate no. 9 observed in the present investigation were identified as *Bacillus amyloliquefaciens* (Both isolates).

There was a significant difference among all the treatments (Table 1. and Table 2.) with respect to total sugar of papaya fruits during storage. In the present investigation total sugar contents of papaya fruits increased up to 9th day of storage in all the treatments but showed decreased values in T_8 and T_2 on 9th day. The increase in total sugar during initial storage period might be due to faster catabolic processes taking place in the fruits, preparing it for the senescence. The reason for the increase in total sugar could be attributed to the hydrolysis of starch and other polysaccharides to soluble form of sugar (Yadav *et al.*, 2009). The decline in sugar content and at the later stages of storage in T_8 (Control fruits) and T_2 (BCA_2-Isolate No. 16) may be due to the fact that after complete hydrolysis of starch, there would no further increase in sugars was occurred and subsequently decline in the parameter is predictable as sugars along with organic acids are primary substrates for respiration. Similar findings have been reported by Arrebola *et al.* (2009), Luo *et al.* (2015), Sarhan *et al.* (2013) and Yadav *et al.* (2014). Delayed increase in the total sugar content in T_5 (BCA_5 -Isolate No. 10) followed by T_4 (BCA_4-Isolate No. 9) and T_7 (Chemical treatment with carbendazium at 0.5%) might be due to delayed metabolic transformation in soluble compounds and delay in ripening and senescence of fruits.

The fruit colour transition from green to yellow colour in fruits is due to the chlorophyll degradation indicating an increased acceptability for consumption. The disappearance of green pigment chlorophyll is associated with the appearance of yellow pigment carotenoides (Montefiori *et al.*, 2009). The progression in peel colour development during ripening from dark green to orange red due to the degradation of chlorophyll structure was noticed during storage of papaya fruits. The main factor for this degradation might be due to changes in the pH of the fruit during storage (because of organic acid leakage from vacuoles) and chlorophyllase enzymes (Yang *et al.*, 2009). Significantly highest carotene content was recorded in T_5 (BCA_5 -Isolate No. 10) followed by T_4 (BCA_4-Isolate No. 9) and T_7 (Chemical treatment with carbendazium at 0.1%) at 9 days of storage (Table 1 and 2.) There will be an enhanced synthesis of carotenoids during the last phase of ripening process in papaya. The similar results were reported by Rohani and Zaipun (2001) in 'Ekssotika' papaya. The minimum carotene content was recorded in T_8 (control fruits) followed by T_2 (Isolate No. 16). There is significant difference were observed in *a** and *b** values of papaya fruits (Table 1.). During the studies on, the effect of biocontrol agents on challenge inoculated papaya fruits (Table 2.) revealed that, the treatments varied significantly among the treatments with respect to *L**, *a** and *b** values at 9 DAS (Table 2.). The maximum *L**, *a** and *b** value was observed for T_5 (BCA_5 -Isolate No. 10), T_4 (BCA_4-Isolate No. 9) and T_7 (Chemical treatment with carbendazium at 0.5%) at 9 DAS indicating delayed ripening of papaya fruits. The lower *L**, *a** and *b** values were observed for T_8 (control fruits) at 9 DAS, indicated complete turning of fruits into brownish at the end of storage. Similar results were observed in papaya (Singh and Varu, 2013).

Influence of biocontrol agents treatment were evaluated for their palatability by consumers by sensory scores for colour, texture and taste. During ripening, transition of chlorophyll into carotenoids (Kays 1991), biochemical conversions of starch into sugar (Martinez *et al.*, 1997), insoluble protopectin into pectin (Gerardi *et al.*, 2001) and loss of organic acid through oxidation (Campestre *et al.*, 2002) are responsible for the changes in these sensory parameters. At 9 DAS the maximum sensory scores for colour, texture and taste were recorded in T_5 (BCA_5 -Isolate No. 10) followed by T_4 (BCA_4-Isolate No. 9) and T_7 (Chemical treatment with carbendazium at 0.1%) due to climacteric peak. On the other hand the lower palatability rating was observed in T_8 (control fruits) and T_2 (BCA_2-Isolate No. 16) which might be attributed to the early onset of senescence of the tissue because of decreased firmness, dull appearance of the fruit skin and quality parameters. The similar results were reported by Arrebola *et al.* (2009) in peach fruits and Yadav *et al.* (2014) in papaya fruits during storage under ambient storage condition. At 9 DAS the fruits in T_8 (control) was found infected with anthracnose which might have reduced the synthesis of carotenoides (Table 2.)

The disappearance of green pigment chlorophyll is associated with the appearance of yellow pigment carotenoides (Montefiori *et al.*, 2009). In the present investigation significantly higher carotenoid content was recorded in T_5 (BCA_5 -Isolate No. 10) followed by T_4 (BCA_4-Isolate No. 9) and T_7 (chemical treatment with carbendazium at 0.1%) at 9 DAS and the lower carotene content was seen in T_8 (control fruits inoculated with *C. gleosporoides*) (Table 1 and Table 2.)

The highest score for colour, taste and texture of papaya fruits was recorded in T_5 (BCA_5 -Isolate No. 10) followed by T_4 (BCA_4-Isolate No. 9) and T_7 (Chemical treatment with carbendazium at 0.1%) at 9 DAS (Table 1. and Table 2.). The increased total sugar and carotene content in T_5 and T_4 might have increased the palatability of the papaya fruits at its climatric peak. Whereas the lowest scores for sensory evaluation were noticed in T_8 (Control papaya fruits challenge inoculated with pathogen) as they were infected by *Colletotrichum gleosporoides* at 9 DAS under ambient storage condition.

Conclusion

The two isolates of antagonistic microorganisms *viz.*, Isolate No. 10 (*Bacillus amyloliquefaciens*), isolated from banana rhizosphere soil and Isolate No. 9 (*Bacillus amyloliquefaciens*), isolated from kiwi fruit surface can be effectively used for postharvest treatment of papaya which enhances the fruit colour with good sensory parameters.

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