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A. Nath, K. Barman, S. Chandra & P. Baiswar

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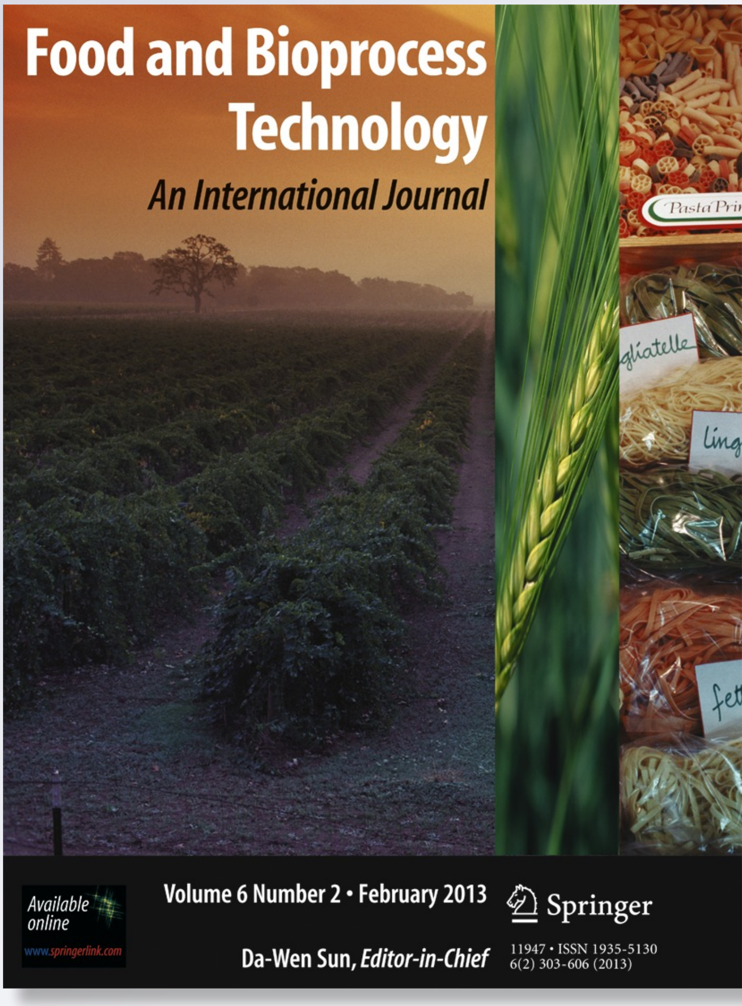

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ORIGINAL PAPER

Effect of Plant Extracts on Quality of *Khasi* Mandarin (*Citrus reticulata* Blanco) Fruits During Ambient Storage

A. Nath · K. Barman · S. Chandra · P. Baiswar

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Abstract *Khasi* mandarin (*Citrus reticulata* Blanco) fruits were treated with *Azadirachta indica*, *Ocimum sanctum*, *Aloe barbedensis*, carbendazim, *A. barbedensis* + *A. indica*, *A. barbedensis* + *O. sanctum*, *A. barbedensis* + carbendazim and control (water). Treated fruits were packed in corrugated fibre boxes and kept for a total period of 28 days at ambient storage (20±2 °C and 65±5% RH). Prominent pathogen associated with fruit rot was identified as *Penicillium brevicompactum*. The fruit quality during storage was evaluated in terms of disease severity (per cent disease index), inhibition of spore germination (%) of *P. brevicompactum*, decay loss (%), juice content retention (%), total soluble solids (%), acidity (%), ascorbic acid content (mg/100 g) and sensory scores. *O. sanctum*-treated fruits exhibited minimum decay loss (3.33%) up to a total period of 28 days which was even less than carbendazim (10%). *O. sanctum* was also highly effective in inhibiting spore germination of *P. brevicompactum* (96.5%). Overall acceptability score (7.33) was also high in *O. sanctum*-treated fruits as compared with other treatments at the end of the storage period.

Keywords *Citrus reticulate* · *Ocimum sanctum* · *Azadirachta indica* · *Aloe barbedensis* · *Penicillium brevicompactum*

Introduction

Citrus is one of the important fruit crops in India next to mango and banana. Among citrus, *Khasi* mandarin (*Citrus reticulata* Blanco) is one of the most popular and commercially grown mandarin cultivar in Meghalaya including other states of northeastern India. Khasi mandarin fruits are highly perishable in nature and have short shelf life of 5–7 days at ambient storage condition.

Khasi mandarin fruits are generally attacked by several plant pathogens. Fungal pathogens remain dormant during fruit growth, but severe damage is caused during ripening and storage, which ultimately contributes to significant losses to the growers and, in turn, to the national economy. Due to this, Khasi mandarin fruits cannot be stored at ambient temperature for longer duration. Khasi mandarin fruits also get damaged to an extent of 13.95% during marketing and transporting due to improper handling (Deka et al. 2004).

Uses of chemical fungicides are the main measure to reduce the incidence of postharvest diseases in various fruits and vegetables. However, the application of synthetic chemical fungicides to control postharvest diseases often results in chemical residues on fruits that may affect human health, and results in the development of resistant pathogens. On the other hand, the increasing demand for more 'natural' food has led to the research for alternative antimicrobial agents within the food industry (Guynot et al. 2005). There are many studies that showed antifungal effect of plant extracts against several species of fungi including both human and plant pathogenic types (Saks and Barkai-Golan 1995; Roller 2003). Many plant-based extracts, i.e. leaf extracts, have been reported to inhibit different plant diseases (Somda et al. 2007). Lazar et al. (2010) demonstrated the antifungal activity of essential oils against a range of postharvest pathogens including *Botrytis cinerea*, *Penicillium* spp., *Alternaria* spp., *Aspergillus* spp., *Monilinia* spp., *Geotrichum candidum*, *Colletotrichum* spp. and *Fusarium* spp. The inhibitory effects of essential oils/plant extracts on these fungal pathogens have been mostly attributed to the active ingredients viz. carvacrol, anethole, thymol, decanol, cinnamaldehyde, eugenol, citral, terpineol, cuminaldehyde, perillaldehyde, salicylaldehyde and benzaldehyde. The inhibitory effects of essential oils appear to be dependent

A. Nath (✉) · K. Barman · S. Chandra · P. Baiswar
ICAR Research Complex for NEH Region,
Umiam, Meghalaya 793103, India
e-mail: amitnath2005@gmail.com

on a number of variables such as target microorganism, host, concentration and method of applying the essential oils.

Azadirachta indica proved to be highly toxic to fungus *Fusarium oxysporum*, inhibiting the mycelial growth and spore germination completely at 100% concentration in the laboratory followed by *Ocimum bacillium* and *Lantana camera* (Bansal and Gupta 2000). *Aloe barbedensis* was reported to be used against core rot disease of kinnow fruits (Sharma et al. 2008).

In addition, natural antimicrobials are safer for the environment and consumers than chemical pesticides (Jayakumar et al. 2007). Thus, the study of natural antimicrobials is a promising area of research for maintaining the fruit quality by managing the postharvest diseases (Cantrell et al. 2005).

In view of these facts, a study was planned to evaluate different plant extracts against postharvest diseases of Khasi mandarin fruits for maintaining the quality during ambient storage. Survey and determination of prominent pathogen associated with fruit rot in this region were also conducted.

Materials and Methods

Materials and Treatment Combinations

Khasi mandarin fruits of uniform size and maturity were harvested on the same day from the orchard, Ri Bhoi District, Meghalaya, India, during the month of November, 2008 and 2009. Each year, a total of 900 fruits free from visual damage and diseases were considered for these studies. The fruits were initially washed with clean tap water and then dried off by keeping the fruits for 2 h under fan inside the laboratory. These fruits were treated with different treatments viz. leaf extracts of *A. indica* (50%), *Ocimum sanctum* (50%), *A. barbedensis* (50%), carbendazim (1,000 ppm), *A. barbedensis* + *A. indica* (1:1 *v/v* proportion), *A. barbedensis* + *O. sanctum* (1:1 *v/v* proportion), *A. barbedensis* + carbendazim (1:1 *v/v* proportion) and control (dipped in sterile distilled water). Each treatment was replicated 10 times, and each replication consisted of 10 fruits. In each treatment, fruits were dipped for 10 min as per the method suggested by Jadhao et al. (2007). Treated fruits were dried for 1 h inside the room followed by packing in corrugated fibre boxes. The packed fruits were stored up to a total period of 28 days at ambient condition (20±2 °C and 65±5% RH) for further studies.

Methods

Method of Plant Extracts Formulation

Plant extracts of *A. indica*, *O. sanctum* and *A. barbedensis* were prepared and evaluated by the method described by Gerard and Kuruchve (1994).

Fruit Quality Assessment

Fruit quality during storage was evaluated in terms of disease severity at 14 days of storage, while decay loss (%), juice (%), total soluble solids (TSS) (%), acidity (%) and ascorbic acid content (mg/100 g) were evaluated at an interval of 7 days up to a total period of 28 days. However, sensory evaluation was carried out at the beginning and at the end of storage period. In vitro studies on spore (*Penicillium brevicompactum*) germination inhibition (%) were also conducted using the method suggested by Jagtap and Nikam (2007).

Disease severity was recorded at 14 days of storage using a 0–5 standard scale, when fruits started showing typical disease symptoms. The per cent disease index (PDI) was calculated by using the following formula:

$$\mathrm{PDI} = \frac{\text{Sum of all numerical ratings}}{\text{Numbers of fruits} \times \text{Maximum score in scale}} \times 100.$$

TSS of fruit juice was determined by using a hand refractometer (ERMA, Japan) of 0–32% range, and acidity was estimated by the method of AOAC (1980). Ascorbic acid content was determined using 2,6-dichlorophenolindophenol dye method of Ranganna (1997). A 10 semi-trained panel of judges (non-smokers) evaluated the organoleptic fruit quality in terms of taste, texture, external appearance, flavour, colour and overall acceptability by using a 9-point hedonic scale (Amerine et al. 1965) where, 9 denoted 'extremely liked' and 1 indicated 'extremely disliked'.

Statistical Analysis

The data collected during experimentation were subjected to statistical analysis by Fisher's method of analysis of variance. The significance of variance within the data was determined by calculating the '*F*' value and by comparing it with the tabulated value of '*F*' at 5% level of probability as given by Snedecor and Cochran (1967). Further, the comparisons among the treatments were done by calculating the critical difference.

Results and Discussion

Pathogen Associated with Fruit Rot

Major markets in Meghalaya, India were surveyed for the collection of diseased fruits. Isolation and purification of the fungus from the diseased fruits were done on potato dextrose agar. Prominent pathogen associated with fruit rot of the Khasi mandarin was identified as *P. brevicompactum* (accession no. ITCC 6513, Indian Type Culture Collection, IARI, New Delhi, India). Pathogenicity was also confirmed by artificial inoculation. This is the first report for the occurrence of fungal pathogen viz. *P. brevicompactum* on citrus (Khasi mandarin) from India (Farr and Rossman 2009). However, Overy and Frisvad (2004) also reported that *P. brevicompactum* was associated with postharvest storage rot of *Zingiber officinale*. Singh and Thakur (2003) reported the association of different fungi, *P. digitatum* and *P. italicum* with citrus as the major pathogen during postharvest storage.

Disease Severity and Spore Germination Inhibition (%)

Effects of botanicals on the severity of disease (PDI) are presented in Table 1, which indicated that all the plant extracts significantly reduced disease severity in comparison to control. Treatments viz. *O. sanctum*, *A. barbedensis* + *O. sanctum* and *A. barbedensis* + carbendazim were equally effective in reducing the disease severity (1.33%) followed by *A. indica* (2.67%) on the 14th day of storage. Maximum disease severity (4.67%) was recorded in control fruits, while the fruits treated with carbendazim alone did not show any symptoms on the 14th day of storage. Carbendazim has also been reported as a very effective fungicide against green mould of citrus and other postharvest rots (Zade et al. 2003).

The results of in vitro studies of the inhibitory effect of different plant extracts on the germination of *P. brevicompactum* spores are presented in Table 1. Maximum inhibition (100%) was recorded in carbendazim followed by 96.5% and 86.1%, respectively, for *O. sanctum* and *A. barbedensis* + carbendazim, while there was no inhibition in control. *Ocimum* spp. was also reported to be effective for controlling postharvest diseases of citrus (Tripathi et al. 2004; Sharma et al. 2008). The inhibitory effect shown by *Ocimum* spp. was due to the presence of oil eugenol, thymol and phenol as described by Juntachote and Berghofer (2005). The plant extract of *A. indica* was also found to be effective for controlling postharvest disease of citrus (Sharma et al. 2008).

Decay Loss

The data pertaining to the per cent decay loss as affected by various treatments during storage are presented in Table 1. The decay loss (%) increased with the advancement of storage period irrespective of the treatments used. However, the minimum decay loss (3.3%) was recorded in fruits treated with *O. sanctum* followed by 10.0%, 13.3% and 13.3%, respectively, for carbendazim, *A. indica* and *A. barbedensis* + carbendazim at the end of storage. Maximum decay loss of 36.7% was recorded in control fruits. Jadhao et al. (2007) also reported similar results in Kagzi lime under ambient storage. The fruits treated with *O. sanctum* recorded the minimum decay loss which might be due to the presence of oil eugenol, thymol and phenol as described by Juntachote and Berghofer (2005) and Lazar et al. (2010).

Changes in Juice Content, Acidity, Total Soluble Solids and Ascorbic Acid Content

The juice content, acidity, TSS and ascorbic acid content of Khasi mandarin fruits before storage were found to be 47.4%, 0.78%, 11.0% and 36.1 mg/100 g, respectively. However, the fruits treated with *O. sanctum* showed the maximum retention of juice (39.3%), the minimum decrease in acidity (0.69%), the maximum TSS content (13.2%) and the maximum retention of ascorbic acid content (29.7 mg/100 g) as compared to control and other treatments at the end of storage. The

Table 1 Effect of different treatments on disease severity, inhibition of spore germination (%) and decay loss (%) of *Khasi* mandarin fruits due to *Penicillium brevicompactum* during ambient storage

Treatments	Percent disease index	Inhibition of spore germination (%)	Decay loss (%)			
			7 days	14 days	21 days	28 days
Azadirachta indica	2.67	70.4	0.0	10.0	13.3	13.3
Ocimum sanctum	1.33	96.5	0.0	0.0	0.0	3.3
Aloe barbedensis	3.33	21.1	0.0	10.0	16.7	23.3
Carbendazim	0.00	100.0	0.0	6.67	6.7	10.0
A. barbedensis + *A. indica*	3.33	60.2	0.0	10.0	16.7	20.0
A. barbedensis + *O. sanctum*	1.33	69.6	0.0	10.0	10.0	23.3
A. barbedensis + carbendazim	1.33	86.1	0.0	10.0	13.3	13.3
Control	4.67	0.00	0.0	13.33	16.7	36.7
SE(*d*)	0.22	0.09	NS	0.31	0.46	0.59
CD at 5%	0.46	0.20	NS	0.72	1.07	1.24

NS non significant, *SE* standard error, *CD* critical difference maximum retention of juice content in *O. sanctum*-treated fruits might be due to the presence of oil eugenol which acts as a protective moisture barrier during storage. These findings are in agreement with the findings of Borthakur et al. (2002) in Assam lemon (*Citrus limon* L.) fruits during storage. The maximum decrease in acidity in control fruits during storage might be due to high rate of respiration as compared to other treatments. Similar findings in the decrease in acidity during storage were also noticed by Zade et al. (2005) in Nagpur mandarin and Deka et al. (2006) in Khasi mandarin. Increase in TSS during storage might be associated with the transformation of pectic substances, starch, hemicelluloses or other polysaccharides in soluble sugar and also with dehydration of fruits (Carrillo et al. 2003). Similar results in the increase in TSS during storage period were found in Baramasi lemon (Borthakur and Kumar 2004). The reason for the maximum retention of ascorbic acid content in *O. sanctum*-treated fruits might be due to the presence of oil eugenol which acts as a protective oxygen barrier during storage and thereby retarding oxidation processes and ultimately lowering down the rate of conversion of ascorbic acid to dehydroascorbic acid. Jadhao et al. (2007) also reported similar findings in the decrease in ascorbic acid content during the storage of Kagzi lime.

Sensory Evaluation

The results obtained from the organoleptic scores with respect to taste, texture, appearance, flavour, colour and overall acceptability of fruits during storage are presented in Table 2. The decrease in organoleptic scores was recorded in all the treatments with the advancement of storage period. However, the highest taste score (7.4) was recorded in fruits treated with *O. sanctum* followed by *A. indica* (7.2), while the minimum taste score (5.7) was observed in control at the end of the storage period. Similarly, high organoleptic scores of 7.3, 7.5, 7.6, 7.6 and 7.3 were recorded for texture, appearance, flavour, colour and overall acceptability, respectively, at the end of storage. Though the fruits treated with *O. sanctum* had a peculiar smell during the initial storage period, the smell was not evident during the later stages of storage period, and no objectionable smell was detected by the judges during the sensory evaluation. The treated fruit pulp was also found to be free from any objectionable smell. Mahajan et al. (2006) and Aworh et al. (1991) also reported similar organoleptic scores in pear, oranges (*Citrus agege*) and grape fruits during storage.

Conclusions

Instead of using synthetic chemicals, plant extracts play an important role in maintaining the postharvest shelf life and the quality of mandarin fruits during storage. *O. sanctum* was the best treatment in reducing the decay loss, was also effective in the retention of ascorbic acid content and also gave high taste scores. The fruits treated with *O. sanctum* recorded minimum disease severity and maximum spore germination inhibition (96.5%) during ambient storage up to 14 days. Therefore, water extract of *O. sanctum* leaves may be considered as a safe and alternative method for the extension of postharvest shelf life of Khasi mandarin fruits. Due to its medicinal properties, *O. sanctum* treatment may also be preferred by farmers and consumers, as well.

Table 2 Effect of different treatments on sensory scores of Khasi mandarin fruits at the end of storage

Treatments	Final sensory scores (9-point hedonic scale) ±SD					
	Taste	Texture	Appearance	Flavour	Colour	Overall acceptability
Azadirachta indica (50%)	7.2±0.12	7.1±0.16	7.3±0.12	7.1±0.05	7.2±0.014	7.1±0.07
Ocimum sanctum (50%)	7.4±0.07	7.3±0.12	7.5±0.07	7.6±0.11	7.6±0.11	7.3±0.14
Aloe barbedensis (50%)	6.9±0.11	7.0±0.16	7.1±0.07	6.9±0.12	6.9±0.12	6.9±0.11
Carbendazim (1,000 ppm)	7.1±0.13	7.0±0.07	7.2±0.11	7.3±0.11	7.3±0.07	7.2±0.12
A. barbedensis + *A. indica*	7.0±0.11	6.9±0.20	7.1±0.07	7.1±0.12	7.1±0.07	6.9±0.07
A. barbedensis + *O. sanctum*	7.1±0.10	7.1±0.21	7.3±0.07	7.3±0.11	7.3±0.14	7.1±0.11
A. barbedensis + carbendazim	7.0±0.07	7.1±0.12	7.3±0.04	7.0±0.18	7.0±0.07	7.2±0.15
Control	5.7±0.16	5.9±0.11	5.9±0.11	5.6±0.18	5.8±0.09	5.5±0.12
SE(*d*)	0.13	0.11	0.12	0.16	0.19	0.13
CD at 5%	0.28	0.23	0.25	0.34	0.40	0.27

SE standard error, *CD* critical difference

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