



Enhancing the shelf-life of papaya (*var. Red lady*) through essential oils and probiotics by mitigating fruit rot.

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Abstract

A study to know the effect of essential oils and probiotics in enhancing the shelf life of papaya cv. Red Lady was conducted in two consecutive years (2023 and 2024). The study consisted of seven treatments with three replications, each with six fruits. Among the seven treatments, tulsi oil (2.5%) significantly, increased the shelf-life, exhibited lower total soluble sugars (TSS), reducing sugar and total sugar during 10 days of storage of papaya fruits, whereas, ginger oil (2%) lowered physiological loss in weight and exhibited higher fruit firmness. The performance of probiotics, viz., *Lactobacillus fermentum* AM1 and *L. plantarum* B1 in enhancing shelf life was not pronounced, when compared to the essential oils used in the study. Organoleptic evaluation has revealed that the fruits treated with ginger oil (2%) had better color, flavor, texture, and overall acceptance. Minimum fruit rot incidence was observed in fruits treated with ginger oil (2%) on 7th and 10th day of storage. Preharvest spray of papaya fruits with tulsi oil (2.5%) 15 days before breaker stage will help in enhancing the shelf life.

Keywords : Essential oil, Fruit rot, Papaya (Red Lady), Probiotics, Shelf-life

Introduction

Papaya (*Carica papaya* L.), which is a member of the Caricaceae family is a significant tropical fruit that is cultivated worldwide in tropical and subtropical regions. It is a delicious fruit packed with vitamins, vitamin C, vitamin B, cardenolides, tannins, alkaloids, carotenoids, anthraquinones, pro-vitamin A, saponins along with phenolic acids, minerals, flavonoids and dietary fiber. The ripe fruit possesses expectorant, diuretic, and carminative properties that aid digestion, and it has pharmacological effects such as antifertility, antiprotozoal, and anthelmintic activities. However, papaya is a highly perishable fruit which leads to post-harvest losses of approximately 30% of its yield. Major post-harvest challenges include its short shelf life, high respiration rate, water loss, attacks by diseases and insect pests, chilling injury and susceptibility to mechanical damage (Sivakumar and Wall, 2013). Fungal diseases alone are often responsible for post-harvest losses that can exceed 50% of total production, with papaya being prone to such diseases due to its climacteric nature. *Fusarium* rot, *Rhizopus* rot, anthracnose, *Penicillium* rot, *Aspergillus* rot and stem end rot were the six post-harvest diseases which are noticed when

the fruits skin yellowing is 25% (Wardlaw and Leonard, 1939; Alvarez and Nishijima, 1987). Fruit rot caused by *Colletotrichum gloeosporioides*, a fungus is the most commonly noticed disease during storage and in market chain (Siddiqui and Ali, 2014).

To manage the post-harvest fruit rot and to reduce reliance on fungicides, essential oils and natural antibacterial compounds offer promising alternatives. Plants such as moringa, ginger, cinnamon, garlic, tulsi, lemongrass, mint, and rosemary have been found to have antimicrobial properties. Additionally, various lactic acid bacteria species have been shown to produce antagonistic metabolites against spoilage microorganisms and vegetable and fruit diseases (Choi et al., 2018; Gajbhiye and Kapadnis, 2018). This study was initiated to explore these solutions further.

Materials and methods

Eight essential oils (100% pure) were purchased and given concentrations were prepared i.e., ginger oil (2 %), garlic oil (1%), cinnamon oil (0.4%), rosemary oil (0.4%), tulsi oil (2.5%), mint oil (0.2%), lemongrass oil (0.05%) and moringa

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oil (4%) were selected and purchased. *Colletotrichum gloeosporioides* were isolated from a papaya fruit and its pure culture was prepared. Poisoned food technique for antifungal activity of essential oil were carried out. Petri plates containing Potato Dextrose Agar media were treated with the selected essential oils. After this the pure culture isolate was inoculated to the petri plate consisting of PDA and essential oils. The percentage growth was recorded and four essential oils having high percentage of inhibition were selected for further study. Pure culture of probiotic strains i.e., *Lactobacillus plantarum* B1 and *Lactobacillus fermentum* AM1 were selected and dual culture method was carried out against *C. gloeosporioides*.

A 9-month-old papaya fruit orchard consisting of cultivar Red Lady was selected from local area. Further ten papaya plants exhibiting high productivity were selected. Four essential oils which showed high percentage of inhibition against *C. gloeosporioides* and two effective probiotic strains were selected for spraying. The desired concentration of essential oil solution as prepared by mixing with water. No surfactants were used, but soap was added as an emulsifier to the essential oil solution. The treatments for the foliar spray were as follows: T₁-cinnamon (0.4%), T₂-tulsi (2.5%), T₃-mint (0.2%), T₄-ginger (2%), T₅-*Lactobacillus plantarum* B1, T₆-*Lactobacillus fermentum* AM1) and T₇ - untreated check with four replications.

One week after the essential oil spray was taken up, all the selected trees in the field were inoculated with pure culture of *C. gloeosporioides*. One week after this the papaya fruits exhibiting maturity indices (breaker stage) were harvested. The fruits were wrapped in newspaper and placed in plastic crates. The fruits were brought to the post-harvest technology laboratory of Department of Horticulture at College of Agriculture, University of Agricultural Sciences, Raichur via truck covering a distance of 30 km. Then the fruits were arranged as per the treatment and replication at room temperature.

The post-harvest parameters such as firmness, physiological loss in weight, ascorbic acid, TSS, fruit color, reducing sugar, total sugar and shelf-life were recorded on 2nd, 4th, 6th, 8th and 10th day of storage at room temperature (28 °C to 32 °C) and 55 % to 65 % RH (Verma et al., 2024). Physiological loss in weight was expressed as per cent loss in weight. Firmness of papaya fruits was determined by using a digital fruit penetrometer. Hunter's lab colourimeter was used to measure the colour of treated and untreated fruits. Titrimetric method described by Ranganna et al. (1979) was adopted for estimation of ascorbic acid. The reducing sugar content was estimated by Nelson's Somogyi method. The total sugars

in the sample were estimated by Nelson's Somogyi method after inversion. Sensory evaluation was done on 6th day of storage when the fruit reached preferable stage for consumption. These were recorded based on a hedonic scale. The fruit characteristics like colour and appearance, taste and flavour, texture of pulp and overall acceptability were judged by nine point scale. Disease incidence of papaya fruits was recorded on 7th and 10th day as percent disease incidence. Completely Randomized Design was used in the experiment which was conducted in two consecutive years and the results were statistically analysed.

Experimental Results

The results reveal the mycelial growth of the test pathogen (Table.1 and Fig.1), which ranged from 0.00 mm in cinnamon oil (0.4 %), ginger oil (2 %), and tulsi oil (2.5 %) to 86.80 mm in moringa oil (4 %). The control has shown a mycelial growth of 87.60 per cent. Treatment-related variations in the percentage of growth inhibition were notable. Every treatment was statistically significant when compared with the control. Essential oils prevent the formation of fungus in several ways. They block enzymes essential for fungal growth and metabolism and increase the permeability of cell membranes, leading to leakage and cell death. Cinnamon oil (0.075%) and ginger oil (0.45%) were effective against anthracnose in mango (Sefu et al., 2015).

Table 1. Effect of essential oils on *in-vitro* inhibition of *Colletotrichum gloeosporioides*

Treatment (Essential oils)	Mycelial growth (mm) after 7 days of incubation*	Inhibition over control (%)
Cinnamon oil (0.4 %)	0.00	100.00
Ginger oil (2 %)	0.00	100.00
Rosemary oil (0.4 %)	86.00	1.86
Mint oil (0.2 %)	46.00	47.48
Lemongrass oil (0.05 %)	84.60	3.42
Garlic oil (1 %)	62.60	28.53
Tulsi oil (2.5 %)	0.00	100.00
Moringa oil (4 %)	86.80	1.25
Control	87.60	-
S. Em. ±	0.39	-
C.D. at 1%	1.58	-

*Mean of three replications

Table 2. Effect of probiotics on the inhibition of *Colletotrichum gloeosporioides*

Isolates	Mycelial growth (mm) after 7 days of incubation*	Inhibition over control (%)
<i>Lactobacillus fermentum</i> AM1	42.50	41.46
<i>Lactobacillus plantarum</i> B1	57.50	20.79
Control	72.60	-
S. Em. ±	0.67	-
C.D. at 1%	3.53	-

*Mean of three replications

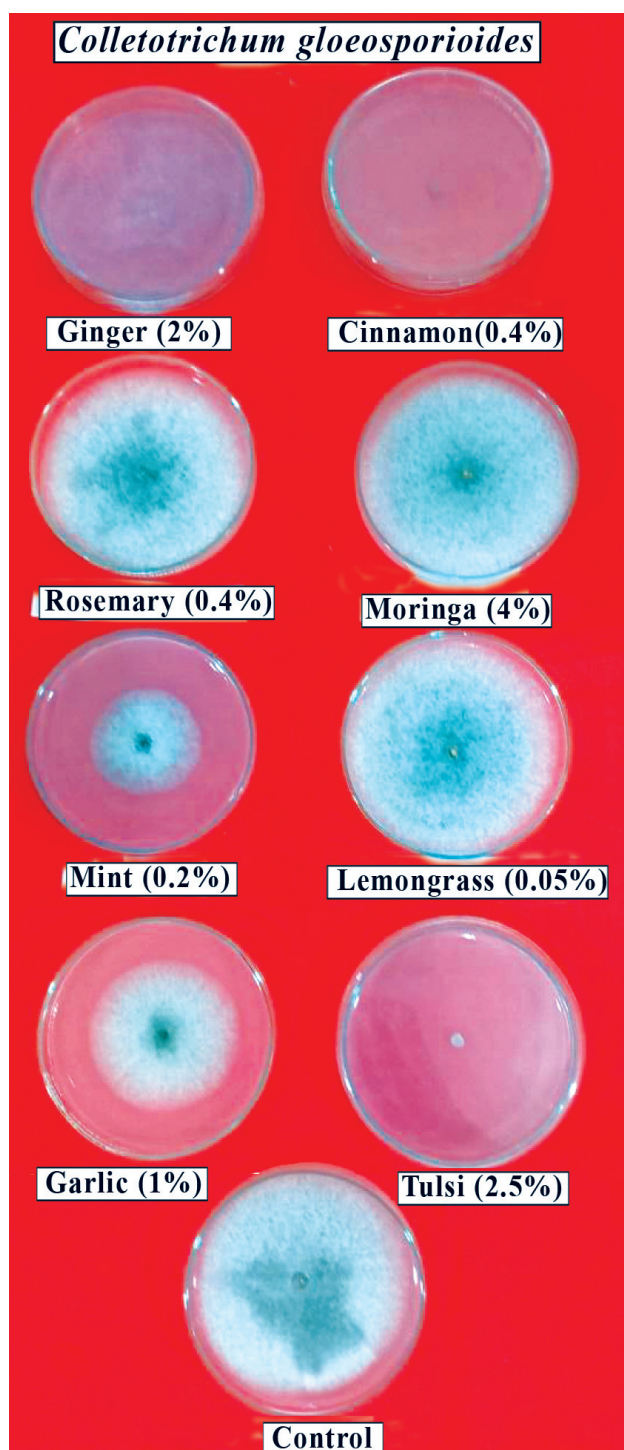


Figure 1. The inhibitory influence of essential oils on *Colletotrichum gloeosporioides* under in-vitro

By dual culture method the two isolates showed varying degrees of *C. gloeosporioides* growth inhibition (Table. 2 and Fig. 2). Among the isolates, *L. fermentum* AM1 exhibited the maximum inhibition (41.46 %) of mycelial growth and *L. plantarum* B1 isolate exhibited the minimum inhibition (20.79 %) of mycelial growth. Bacterial isolates *L. fermentum* AM1 and *L. plantarum* B1 have shown mycelial growth of 42.50 and 57.50 mm, respectively, whereas the control has

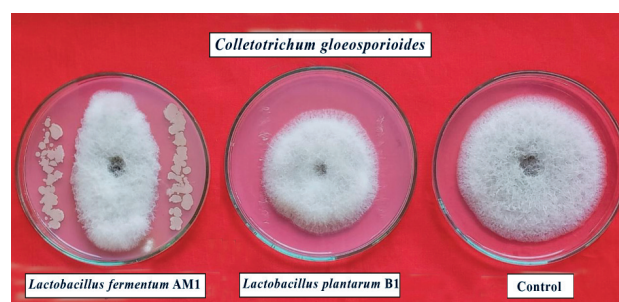


Figure 2. The inhibitory influence of probiotics on *Colletotrichum gloeosporioides* under in-vitro

the maximum mycelial growth of 72.60 mm. Riolo et al. (2023) reported the antifungal activity of *L. plantarum* against *C. gloeosporioides*.

Physiological loss in weight (PLW) of papaya fruit (Fig.3) showed a substantial variation during both the seasons and in pooled data. The PLW was minimum in the treatment consisting of ginger oil (2 %). PLW is expressed in percentage and was 2.82%, 5.81%, 7.88%, 11.22% and 15.10% in first season, 2.62%, 5.52%, 7.65%, 11.06% and 14.78% in second season and 2.72%, 5.66%, 7.76%, 11.14% and 14.94% in pooled data during 2nd, 4th, 6th, 8th and 10th day, respectively. Highest physiological loss in weight was recorded in untreated check. The probiotics treated fruits also exhibited higher physiological loss in weight. Results showed that untreated fruits experienced significant weight loss due to moisture loss from respiration and transpiration (Salunkhe and Desai, 1984), while treated fruits due to reduced respiration and moisture loss have reduced physiological loss in weight.

The influence of essential oils and probiotics in fruit firmness of papaya (Fig. 4) were found to be significant in 1st season, 2nd season and pooled data throughout the storage period. The treatment consisting of ginger oil (2 %) exhibited lower values during 2nd day (6.19 kg cm⁻², 6.25 kg cm⁻² and 6.22 kg cm⁻² during season one, season two and the combined data respectively), 4th day (5.89 kg cm⁻², 5.81 kg cm⁻² and 5.83 kg cm⁻² during season one, season two and the combined data respectively), 6th day (5.69 kg cm⁻², 5.78 kg cm⁻² and 5.75 kg cm⁻² during season one, season two and the combined data respectively), 8th day (5.45 kg cm⁻², 5.59 kg cm⁻² and 5.52 kg cm⁻² during season one, season two and the combined data respectively) and 10th day (5.15 kg cm⁻², 5.25 kg cm⁻² and 5.20 kg cm⁻² during season one, season two and the combined data respectively). Untreated check has exhibited the highest loss in firmness during the storage. Although all fruits lost firmness over time, those treated with essential oils and probiotics retained significantly more firmness than the untreated fruits, likely due to slower changes in pectin, starch, and cell wall components (Rao et al., 1971).

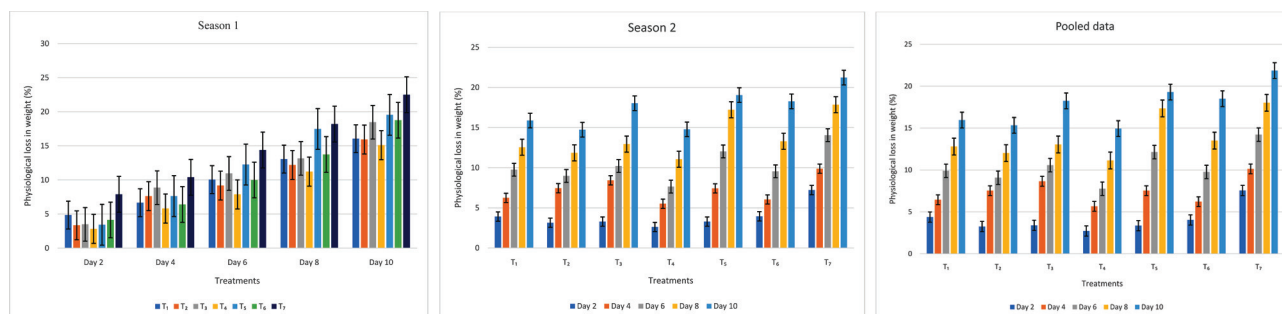


Figure 3. Effect of pre-harvest spray of essential oils and probiotics in physiological loss in weight of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

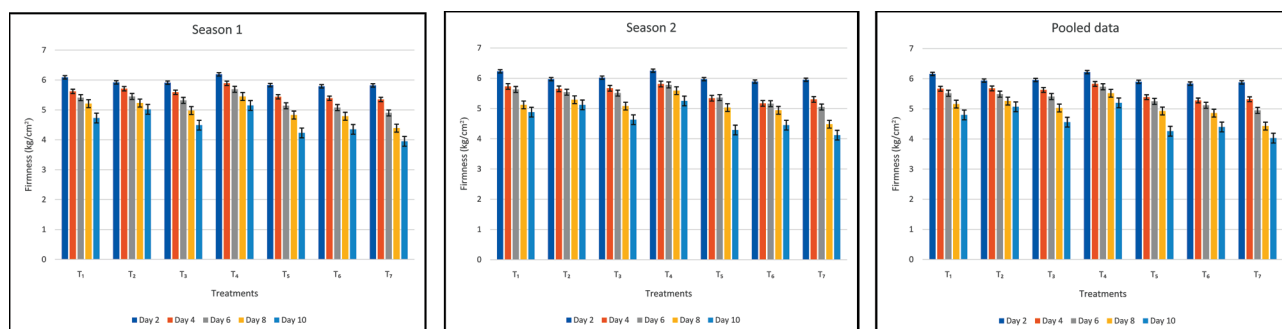


Figure 4. Effect of pre-harvest spray of essential oils and probiotics in firmness of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

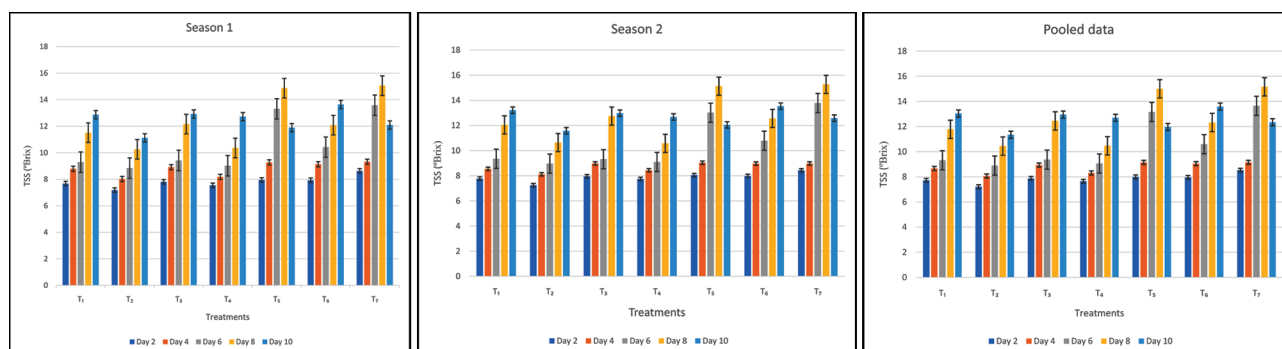


Figure 5. Effect of pre-harvest spray of essential oils and probiotics in total soluble solids (TSS) of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

The findings relating to TSS of papaya fruits were found to be significant. The Total Soluble Solids in papaya fruits exhibited significant variation in season one, season two and the combined data throughout the storage period (Fig.5). The treatment consisting of tulsi oil (2.5%) exhibited lower values during second day (7.190.Brix, 7.250.Brix and 7.220.Brix during season one, season two and the combined data respectively), fourth day (8.020.Brix, 8.120.Brix and 8.070.Brix during season one, season two and the combined data respectively), sixth day (8.840.Brix, 8.970.Brix and 8.900.Brix during season one, season two and the combined data respectively), eighth day (10.260.Brix, 10.640.Brix and 10.450.Brix during season one, season two and the combined data respectively) and tenth day (11.120.Brix, 11.580.Brix

and 11.350.Brix during season one, season two and the combined data respectively). Higher TSS was recorded in untreated check. As time progressed, the conversion of starch to sugars increased the total soluble solids (TSS). Once the complete hydrolysis of sugar happens there will be no more increase in TSS and a decline in TSS is expected because starch and other organic acids serve as the main substrate for respiration. The findings are consistent with Siddiqui et al. (1989) in ber, Gupta et al. (1987) in ber, and Jayachandran et al. (2005) in guava. Ascorbic acid of papaya fruit also revealed significant differences between the various treatments in ambient storage for 10 days. The ascorbic acid content of papaya fruits varied significantly between the seasons and in the combined data (Fig.6). Highest ascorbic

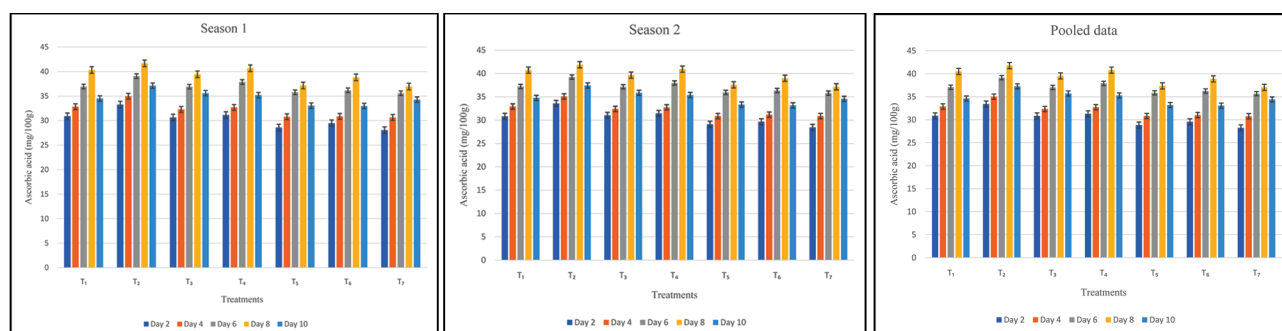


Figure 6. Effect of pre-harvest spray of essential oils and probiotics in ascorbic acid of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

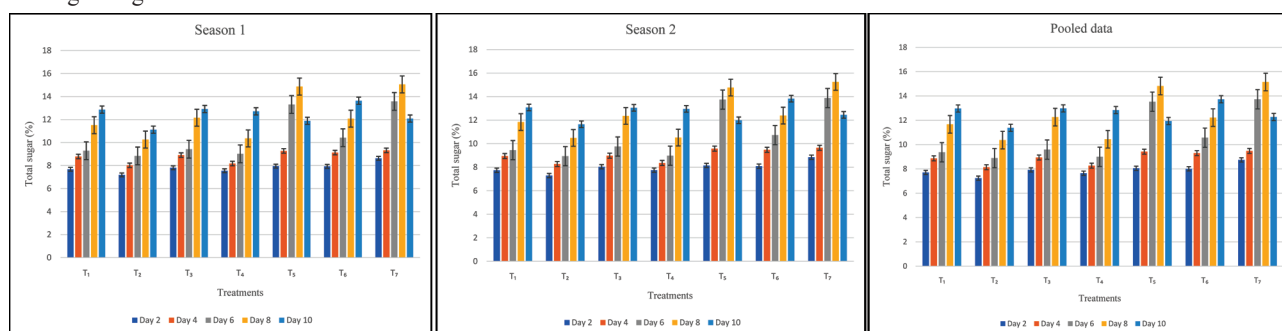


Figure 7. Effect of pre-harvest spray of essential oils and probiotics in total sugar of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

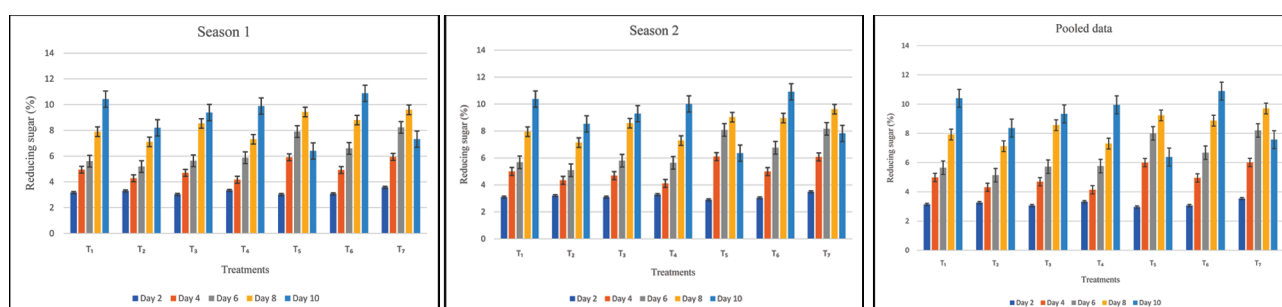


Figure 8. Effect of pre-harvest spray of essential oils and probiotics in reducing sugar of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

acid content was seen in the treatment consisting of tulsi oil (2.5%) i.e., 33.28 mg100 g⁻¹, 34.97 mg100 g⁻¹, 39.08 mg100 g⁻¹, 41.69 mg100 g⁻¹ and 37.12 mg100 g⁻¹ in first season, 33.58 mg100 g⁻¹, 35.09 mg100 g⁻¹, 39.24 mg100 g⁻¹, 41.88 mg100 g⁻¹ and 37.43 mg 100g⁻¹ in second season and 33.43 mg100 g⁻¹, 35.03 mg100 g⁻¹, 39.16 mg100 g⁻¹, 41.78 mg100 g⁻¹ and 37.27 mg 100g⁻¹ in pooled data during second, fourth, sixth, eighth and tenth day, respectively. Lowest ascorbic acid content was noticed in untreated check. The results clearly showed that, in all these treatments, ascorbic acid concentration of fruits increased as the storage duration increased. Rapid oxidation of organic acid in later stages of storage may be the cause of decrease in ascorbic acid concentration with successive prolongation of storage.

Throughout the storage period the total sugar in papaya fruits exhibited significant variation in season one, season two and

the combined data (Fig.7)The treatment consisting of tulsi oil (2.5%) exhibited lower values during second day (7.19 %, 7.29 % and 7.24 % during season one, season two and the combined data respectively), fourth day (8.02 %, 8.27 % and 8.14 % during season one, season two and the combined data respectively), sixth day (8.84 %, 8.94 % and 8.89 % during season one, season two and the combined data respectively), eighth day (10.26 %, 10.49 % and 10.37 % during season one, season two and the combined data respectively) and tenth day (11.12 %, 11.65 % and 11.38 % during season one, season two and the combined data respectively). Total sugar was recorded higher for the untreated fruits. As the fruits ripen, the total sugar content increases; and then, it decreases. This might be due to the post-harvest hydrolysis of the starch reserve, which releases sugars.

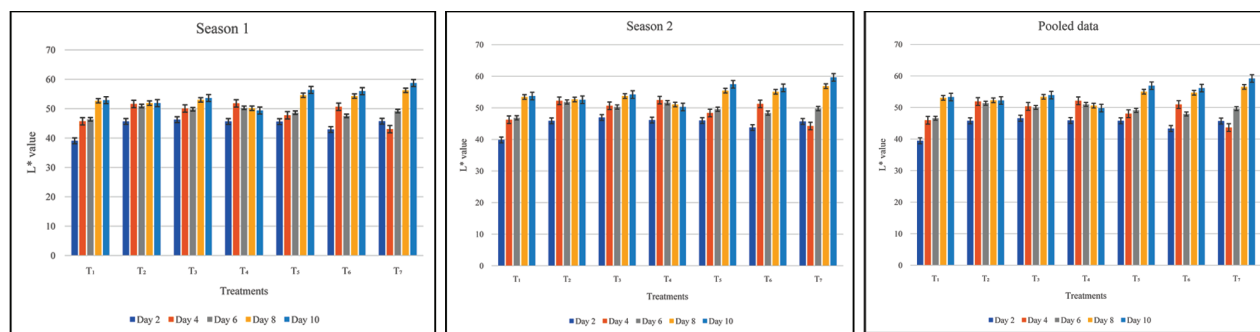


Figure 9. Effect of pre-harvest spray of essential oils and probiotics in L* value of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

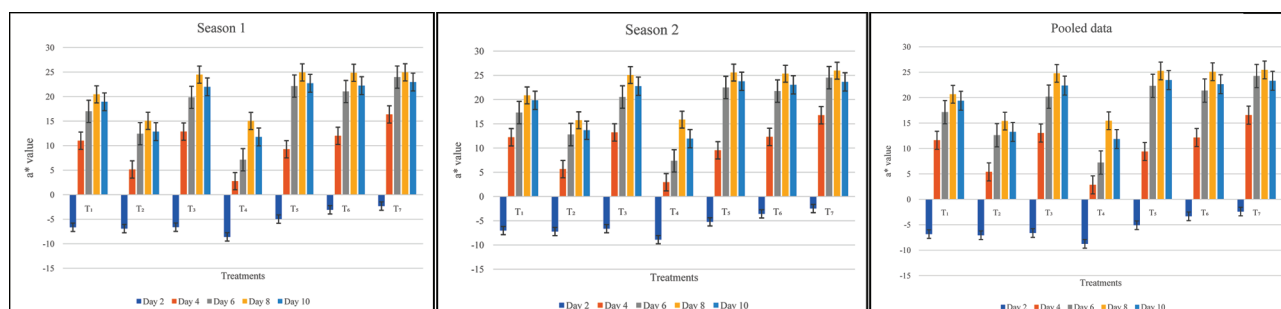


Figure 10. Effect of pre-harvest spray of essential oils and probiotics in a* value of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

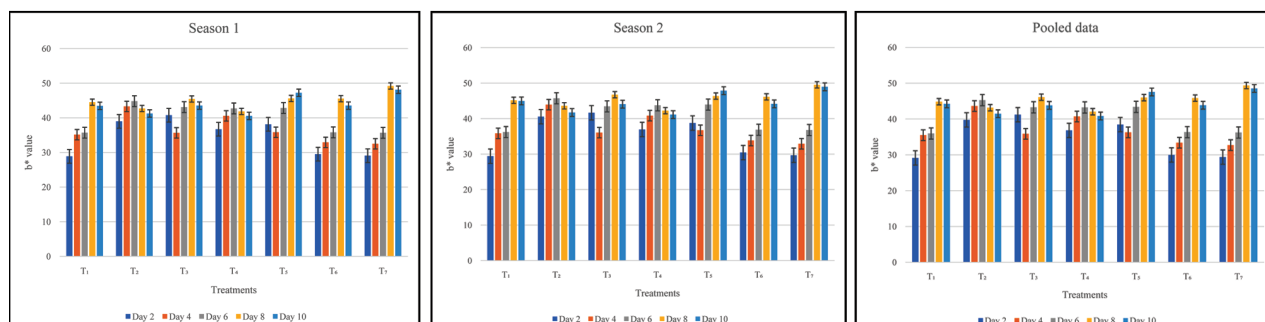


Figure 11. Effect of pre-harvest spray of essential oils and probiotics in b* value of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

Reducing sugar in papaya fruit has exhibited significant differences between the various treatments in ambient storage for 10 days. Significantly in both the seasons (Fig.8) the reducing sugar content was lowest in the treatment consisting of tulsi oil (2.5%) i.e., 33.28 %, 34.97 %, 39.08 %, 41.69 % and 37.12 % in first season, 33.58 %, 35.09 %, 39.24 %, 41.88 % and 37.43 % in second season and 33.43 %, 35.03 %, 39.16 %, 41.78 % and 37.27 % in pooled data during second, fourth, sixth, eighth and tenth day, respectively. Highest value was recorded in untreated check. The creation of sugars through the hydrolysis of starch is the primary characteristic of the ripening process. The current analysis showed that, regardless of treatments, during storage, the fruit sugar level increased initially to a certain point and then decreased until the storage period ended. Veerannah and Selvaraj (1984) showed similar tendencies in decreasing total sugar content in papaya.

There is a significant difference between various treatments on L* value on ambient storage for 10 days. L* value of papaya fruits varied significantly between the seasons and in the combined data (Fig.9). The L* value was lowest in the treatment consisting of ginger oil (2 %) i.e., 45.65, 51.79, 50.29, 50.15 and 49.36 in first season, 46.12, 52.45, 51.64, 51.05 and 50.27 in second season and 45.88, 52.12, 50.96, 50.60 and 49.81 in pooled data during 2nd, 4th, 6th, 8th and 10th day, respectively. Maximum L* value was recorded in untreated check. When papaya fruits over ripe and spoil, the L* value might decrease due to the development of darker spots and microbial growth, which can make the surface appear less light. Ali *et al.* (2011) reported that in “Eksotika a!” papaya fruits, the L* value had shown an increase with the fruits coated with ginger oil (2 %). Mandal and Mualchin (2021), also studied the L* value of mango coated with ginger essential oil, which showed a similar trend as the above results.

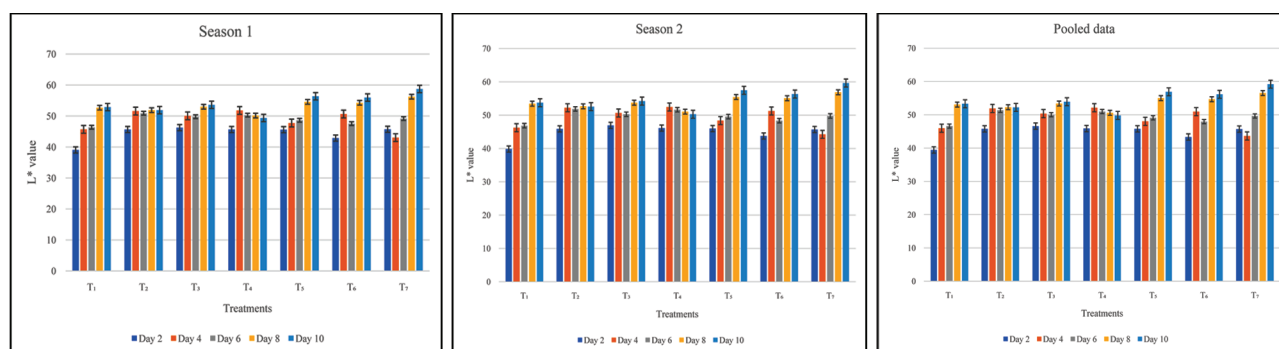


Figure 12. Effect of pre-harvest spray of essential oils and probiotics in sensory evaluation and shelf-life of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage

The findings relating to a^* value of papaya fruits after application of edible coatings were found significant. The a^* value in papaya fruits exhibited significant variation in season one, season two and the combined data throughout the storage period (Fig.10). The treatment consisting of ginger oil (2 %) exhibited lower values during second day (-8.6, -8.90 and -8.75 during season one, season two and the combined data respectively), fourth day (2.76, 2.95 and 2.85 during season one, season two and the combined data respectively), sixth day (7.13, 7.36 and 7.24 during season one, season two and the combined data respectively), eighth day (15.04, 15.86 and 15.45 during season one, season two and the combined data respectively) and tenth day (11.78, 11.93 and 11.85 during season one, season two and the combined data respectively). Higher a^* value was noticed in untreated fruits. The fruit's development of a yellowish colour during storage may be the result of enhanced carotenoids and anthocyanin pigment synthesis as well as the breakdown of the fruit's chlorophyll pigments (Wankier et al., 1970).

Significant difference was recorded in b^* value of papaya fruit during both the seasons and in pooled data (Fig.11). The b^* value was lowest in the treatment consisting of ginger oil (2 %) i.e., 36.72, 40.57, 42.72, 41.85 and 40.56 in first season, 36.94, 40.83, 43.75, 42.24 and 41.12 in second season and 36.83, 40.70, 43.23, 42.04 and 40.84 in pooled data during 2nd, 4th, 6th, 8th and 10th day, respectively. Fruits that were untreated showed the maximum b^* value. As the storage period increases and spoilage begins, there might be a stabilization or even a decrease in the b^* value due to browning, dark spots, and microbial growth, which can affect colour uniformity and intensity. An increase in the b^* value of mango treated with alginate and chitosan enriched with cinnamon essential oil has shown an increase in b^* value during its storage for 14 days (Li et al., 2019). Changes in the colour of mangoes treated with tulsi and mint essential oils were given by Mandal and Mualchin (2021) who showed a similar trend.

Shelf-life of papaya fruits (Fig.12) showed that the fruits treated with tulsi oil (2.5%) have the maximum value of 9.67, 9.39 and 9.47 days during first season, second season and pooled data respectively. Shelf-life was minimum in case of untreated fruits. The sensory evaluation of papaya fruits varied significantly in both the seasons. During ripening, the papaya fruits also showed slow changes in chemical parameters such as TSS, organic acids, reducing, and total sugars. Ginger oil contains gingerol and shagol and tulsi oil contains eugenol. These essential oils are volatile in nature. When these essential oils and also mint oil and cinnamon oil were sprayed on papaya fruits 15 days before their harvest, the volatile compounds in the oil might have got volatilized to varying degrees due to temperature variation in the field conditions. The rate of volatilization of volatile compounds could be lesser in ginger oil and tulsi oil. This could be the reason for ginger oil and tulsi oil performing

better when compared to other oils. In the current experiment, the probiotics were sprayed on the papaya fruits under field conditions, where temperature and relative humidity is not regulated. This could be the reasons for inefficiency of probiotics.

The sensory evaluation score was shown in Fig.12 which is highest for the treatment consisting of ginger oil (2 %) i.e., 7.32, 7.79, 7.83 and 7.76 in first season, 7.49, 7.94, 8.05 and 7.94 in second season and 7.40, 7.86, 7.94 and 7.85 in pooled data for colour, taste, texture and overall acceptability seven days after storage of papaya fruits. Lowest score was recorded in untreated check. Applying plant extract is therefore a strategy to reduce pathological loss while maintaining the external and internal quality features of fruits in an environmentally responsible manner.

Fig.13 shows the percent disease incidence in papaya fruits during storage. Minimum disease incidence was seen in fruits treated with ginger oil (2%) i.e., 5.30 and 19.75 in first season, on 7th and 10th day of storage. Essential oils possess antimicrobial properties. This features inherent in ginger oil

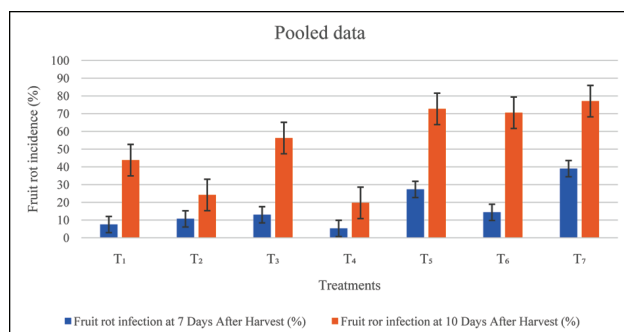


Figure 13. Effect of pre-harvest spray of essential oils and probiotics in fruit rot incidence of papaya (*Carica papaya* L.) fruits cv. Red Lady during storage



Figure 14. Tulsi oil (2.5%) sprayed (pre-harvest spray) fruits and untreated check at 7th day of storage



Figure 15. Ginger oil (2%) sprayed (pre-harvest spray) fruits and untreated check at 7th day of storage

might have reduced the percentage of disease incidence. Fig.14 and Fig.15 shows the comparative view on the percentage disease incidence between untreated fruits and fruits that are treated with tulsi oil (2.5%) and ginger oil (2%), respectively. Gum Arabic (10%) along with ginger oil (2%) has managed anthracnose in papaya (Ali et al., 2016), 10% gum arabic along with 0.4% cinnamon oil (0.4 %) (Maqbool et al., 2011), and chitosan (1 %) + mint essential oil (0.2 %) (Ayón et al., 2022).

Conclusion

The study found that essential oil treatments successfully maintained the quality of papaya fruits during storage, whereas probiotics did not exhibit any significant influence on the shelf life of papaya. Compared to untreated fruits, fruits treated with tulsi oil (2.5 %) has shown increased shelf-life, exhibited lower total soluble sugars (TSS), reducing sugar and total sugar. Ginger oil (2.0 %) exhibited lowered physiological loss in weight and exhibited higher fruit firmness. In addition to this, the ginger oil and tulsi oil treated papaya fruits exhibited a reduction in fruit rot, whereas

probiotics did not exhibit a reduction in fruit rot of papaya. As a result of this, it is concluded that there is an extension in shelf life of papaya fruits by the use of tulsi oil.

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