International Journal of Modern Agriculture, Volume 10, No.1, 2021
ISSN: 2305-7246

POST-HARVEST TREATMENT OF TOMATOES WITH LEAF POWDERS AT ROOM TEMPERATURE ENHANCES SHELF LIFE

Lalitha Pappu

Department of Microbiology, institute of Science, Gandhi Institute of Technology And Management, Visakhapatnam, India
Email: pappulalitha@gmail.com

Abstract

Preservation of tomato (*Solanum lycopersicum* L) at room temperature for prolonged periods is a challenge as tomato is a climacteric fruit. Of the existing methods of storage, only cold storage is effective. However, this is very expensive and not within the reach of farmer. The effect of shade dried leaf powders of several tropical plants on shelf life of tomatoes at room temperature was examined based on the hypothesis that leaves containing cytokinin would enhance the shelf life of climacteric fruits. The shade dried leaf powders of *Azadirachta indica* and *Peltophorum spp.* enhanced tomatoes shelf-life by 57 and 54.2% respectively. The enhanced shelf life of tomatoes was related to zeatin content of leaves. Increasing concentration of pure zeatin increased shelf- life. Total carbohydrate and protein contents remained unchanged but vitamin C content was reduced in both control and treated groups over time. Dried leaf powders can be used to by farmers to preserve tomato fruit at room temperature upto 60 days.

Key words: *Solanum lycopersicum*, climacteric fruit, cytokinin, Zeatin

Introduction

A major problem that needs the attention of agricultural scientists is the short shelf-life of tomatoes (*Solanum lycopersicum L*) at room temperature. Attempts to preserve tomato fruit have not been successful. Cold storage at 12°C is beneficial to extend shelf life but cold storage leads to loss of nutrients from the fruit (Ali et al, 2014).

Tomato fruit are climacteric and ripens faster after harvest due to autocatalytic production of ethylene (Alexander and Grierson, 2002). Advanced molecular biology techniques use RNAi technology to genetically engineer a variety of tomato which has increased shelf-life (Meli, 2010) but are susceptible to attack by specific pathogens and pests.

Enhancement of shelf life of tomatoes at room temperature can improve profit. Devising a simple method for enhancing tomato shelf- life at room temperature that is easy to implement, would be an important step in support of tomato growers.

Increased cell wall invertase activity in response to cytokinin was essential and sufficient for inhibition of senescence in leaves of Arabidopsis (Zwack, 2013). Exogenous application of benzylaminopurine and kinetin on bananas post harvest, increased shelf life of bananas (Nguemezi *et al.*, 2012). Cytokinins delay senescence in plants (Zwack, 2013), and are common to all plants. It may be that even though there are differences in habit and culture the basic activity of cytokinins would be similar throughout the plant kingdom. Irokanulo et al. (2015), reported that aqueous extract of *Moringa* leaf powder led to enhanced tomato shelf-life of tomatoes at room temperature and hypothesized this was due to high cytokinin content in *Moringa*.

Guyer (2014) reported that pathways of leaf and fruit senescence are common. A compound that delays leaf senescence may delay fruit senescence. This implies cytokinins can be used to delay senescence in climacteric fruit. Pure cytokinins are expensive. Leaves of different plant species may serve as a source of cytokinins.

Cytokinins are stable to heat and do not degrade easily. Hart et al (2016) reported cytokinins could withstand autoclaving temperature upto 3 cycles and yet be stable. A shade dried leaf powder rich in cytokinins may be used to enhance shelf life of tomatoes at room temperature. The concentration of cytokinins in plants is very low. This implies that they are capable of being active at low levels.

The study was undertaken to determine if cytokinin content in leaf samples could enhance shelf-life of tomatoes treated with shade dried leaf powders, or pure zeatin at room temperature.

Materials and methods

Sample collection and preparation

Leaves from 10 different species of plants were collected from various parts of Visakhapatnam (Table no 1). The leaves were washed with distilled water twice, blotted dry and placed in shade to dry at room temperature until dry. The leaves were ground to a fine powder using an electric blender.

Estimation of cytokinin content of different leaf samples

Leaf samples (1g) were homogenized with 10mL of water at 35°C and gravity filtered through Whatman filter paper No. 1. The pH of the liquid was adjusted to 3 with 1N HCl. The liquid was extracted 3 times with dichloromethane. The aqueous layer and dichloromethane layer were collected separately. The pH of aqueous layer was adjusted to 8 with NaOH and extracted with n-butanol three times. The dichloromethane and butanol layers were evaporated to dryness at room temperature and residues dissolved in a minimum amount of butanol and mixed together. This sample was used for analysis by Liquid Chromatography- Mass Spectroscopy (LC-MS). Trans zeatin (20 mg L^{-1}) was used as the standard. The LC-MS solvent system was composed of :Water and 0.1 % formic acid and : methanol and 0.1.% formic acid. (Pan *et al*, 2010).

Shelf life studies with shade dried leaf powders

Fully ripe tomatoes were procured from local market from farmers and was ensured that these were of the same cultivar and of same age grown under identical conditions. The tomatoes were on an average 2.5inches in diameter and weighed 60g. The tomatoes were washed with distilled water, blotted dry and placed on slotted cardboards with 1 tomato per slot and exactly 1g of leaf powder was sprinkled per tomato. Untreated tomatoes served as control. Zeatin was procured from Sigma-Aldrich (Mumbai, India). The different concentrations of zeatin (5,10 and 15 mg L^{-1}) concentrations of zeatin were prepared in distilled water and used to treat tomatoes. One mL per tomato was applied evenly on each fruit placed on slotted cardboard. A conventional sprayer was used to spray. The tomatoes were observed for visible spoilage on a daily basis.

The tomatoes were observed for spoilage at 24 hour intervals and any sign of softness, or visible fungal growth was considered damage and the tomatoes were removed from the cardboard. Samples from spoiled regions were plated on nutrient agar and Sabouraud's agar plates and observed for growth. The cultures were identified by staining and microscopic examination.

Control and treated tomatoes were evaluated at one week interval till completion of experiment (8 weeks) for total sugar by anthrone method, total protein content by Lowry's method, and for vitamin C content estimated by redox titration.

All experiments were carried out triplicates in space and time. The data was subjected to one way ANOVA .

Results

Effect of zeatin on shelf life of tomatoes

There was a 100% spoilage in control tomatoes kept at room temperature by 27 days of treatment. The zeatin treated tomatoes exhibited longer shelf life. The increasing concentration of zeatin resulted in increased shelf life of tomatoes. The tomatoes treated with 5mg L^{-1} zeatin, the tomatoes were firm until day 63. The fruits were firm and there was no sign of visible damage or microbial spoilage in them. After 54 days the spoilage started and 100 % spoilage occurred day 63. A relatively few tomatoes treated with 10mg L^{-1} zeatin, got spoiled on day 18 and after 63 days fewer tomatoes were spoiled than for previous experiments. A lesser number of tomatoes treated with 15mg L^{-1} zeatin, were spoiled on day 18 and after 63 days, lesser number of tomatoes got spoiled than for previous experiments . For fruit treated with 10 and 15 mg L^{-1} all were spoiled on day 82 day and 90 respectively (data not shown because 63 by 63 days next crop will be ready and enhancement till this time is enough. Thereafter the reading were not taken at 3 day interval. Only the time taken for 100% spoilage was noted). Percent spoilage is presented through day 63 (fig 1). There was a reduction in total sugar content in control fruit from the start of experiment to the end of experiment; there was no difference in total sugar content in zeatin treated fruit. Protein content increased by 8.6, 18.1, 27.5, 29.3% in control and 5, 10, 15mg/L treated fruit respectively. There was no change in the lycopene content of control or treated fruit (average value was 1.2mg/100g of fruit). Vitamin C got reduced by 80% in control and treated fruit (Table no.2). Microorganisms isolated from spoiled tomatoes were *Pseudomonas, Streptococcus spp, Aspergillus niger* and *A. flavus*.

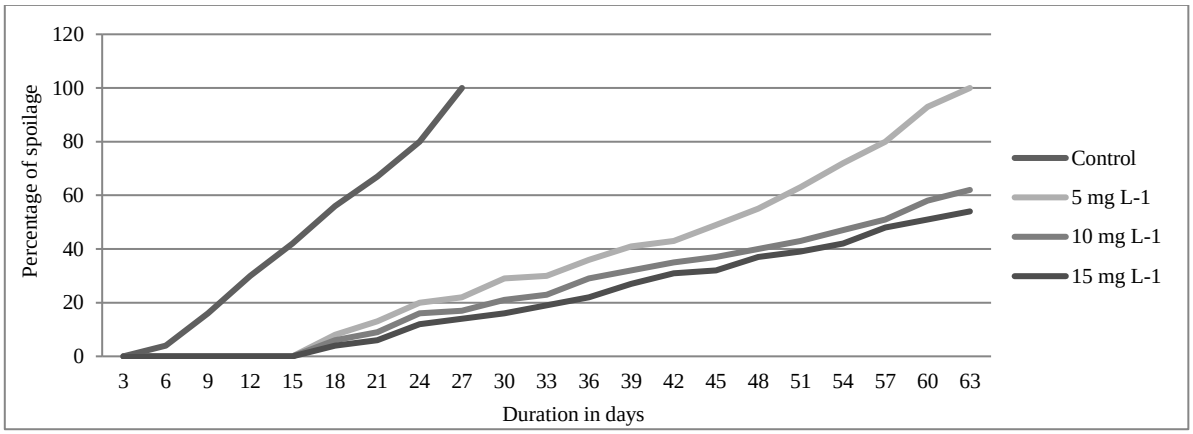

Fig.1. Effect of zeatin on shelf-life of tomatoes.

Zeatin content in different leaf samples

The zeatin content of leaf samples was estimated by analyzing the LC-MS by protocol suggested by Pan *et al* in 2010. Highest content of zeatin was in *Azadirachta indica* followed by *Peltophorum*, *Piper betel*, *Eucalyptus* and *Pongamia*, respectively (Table no. 1). There was no difference in zeatin values in dry and fresh samples (data not shown). Zeatin was absent in *Litchi chinensis, Lantana camara*, *Acacia auriculiformis, Psidium guajava* and *mangifera indica.*

Table 1. Zeatin content in leaf samples collected

S.No	Name of leaf sample	Zeatin content in mg/g
1	*Litchi chinensis sonn.*	0
2	*Azadirachta indica L*	0.26
3	*Peltophorum spp*	0.20
4	*Lantana camara L*	0
5	*Eucalyptus spp*	0.04
6	*Piper betle L*	0.05
7	*Pongamia pinnata L*	0.03
8	*Acacia auriculiformis*	0
9	*Psidium guajava L*	0
10	*Mangifera indica L*	0

Shelf life studies with shade dried leaf powders

The shelf life of tomatoes increased in all treated samples compared to the control. The maximum shelf life was for tomatoes treated with *Azadiractta indica* (64 days) followed by *Peltophroum* (59 days) and *Piper* betel (56 days). When treated with *Mangifera indica* and *Lantana camara*) the shelf life was similar to the control. The data is represented in fig. 2

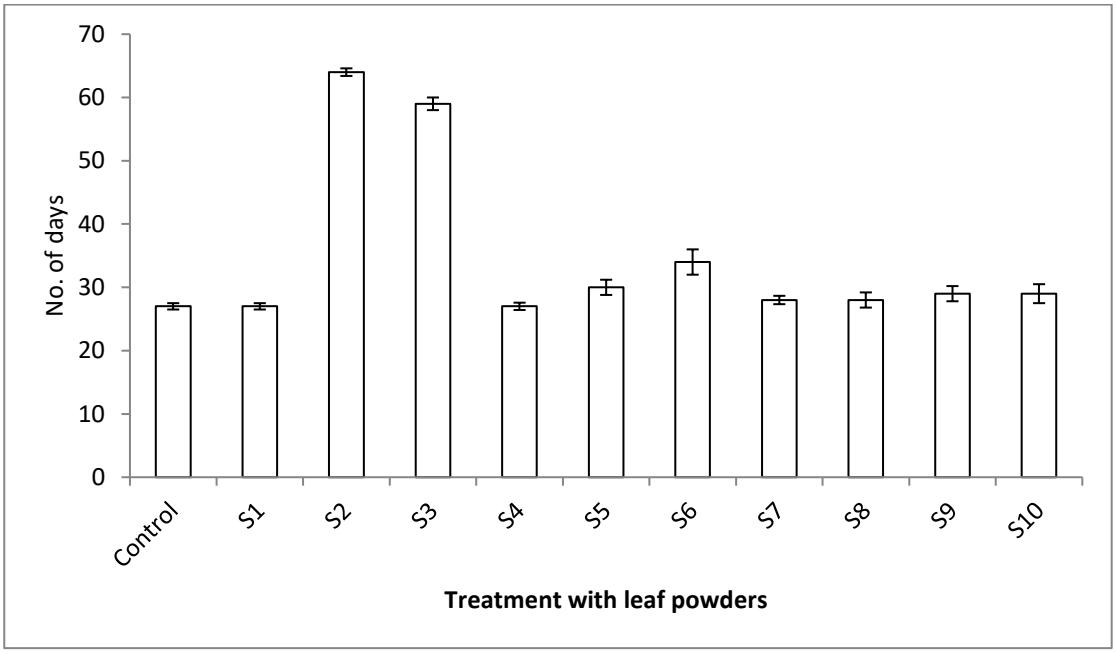

Fig 2. Shelf-life of tomatoes treated with leaf powders.

Values represent shelf-life in no. of days±SE

Nutritional evaluation

Total sugar and lycopene content remained same from the beginning to the end of the experiment in treated tomatoes but the vitamin C content reduced significantly in both control and treated tomatoes (Table 2). The sugar content decreased in control fruits (Fig.3). There was a 13.7% enhancement in protein content (Fig. 3) and an 81 % reduction in vitamin C content in treated tomatoes at the end of experiment when compared to fresh tomatoes (Fig.4.).

Table 2. Change in Total sugar, total protein, lycopene and Vitamin C content in control and zeatin treated tomatoes

Day	Zeatin	Total sugar (µg/g)	Protein(µg/g)	Vitamin C (mg/100g)
0	0	76a	116a	10.13a
	5	76a	116a	10.13a
	10	76a	116a	10.13a
	15	76a	116a	10.13a
63	0	50b	126b	2b
	5	78a	137c	2.1b
	10	76a	148d	2.13b
	15	79a	150d	2.01b

Data in the table analyzed with Least Squares Means and means separated with Least Significant Differences.
[a] values in columns followed by the same letter are not significantly different, P≤0.05.

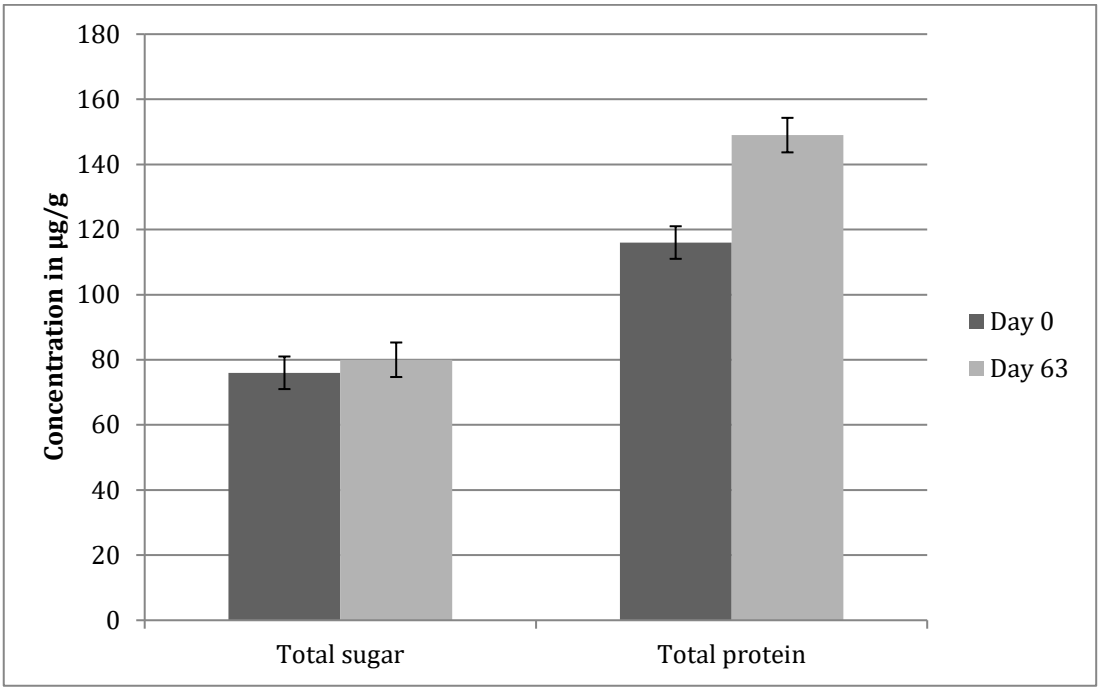

Fig. 3. Changes in total sugar and total protein in tomatoes treated with *Azadirachta indica* leaf powder

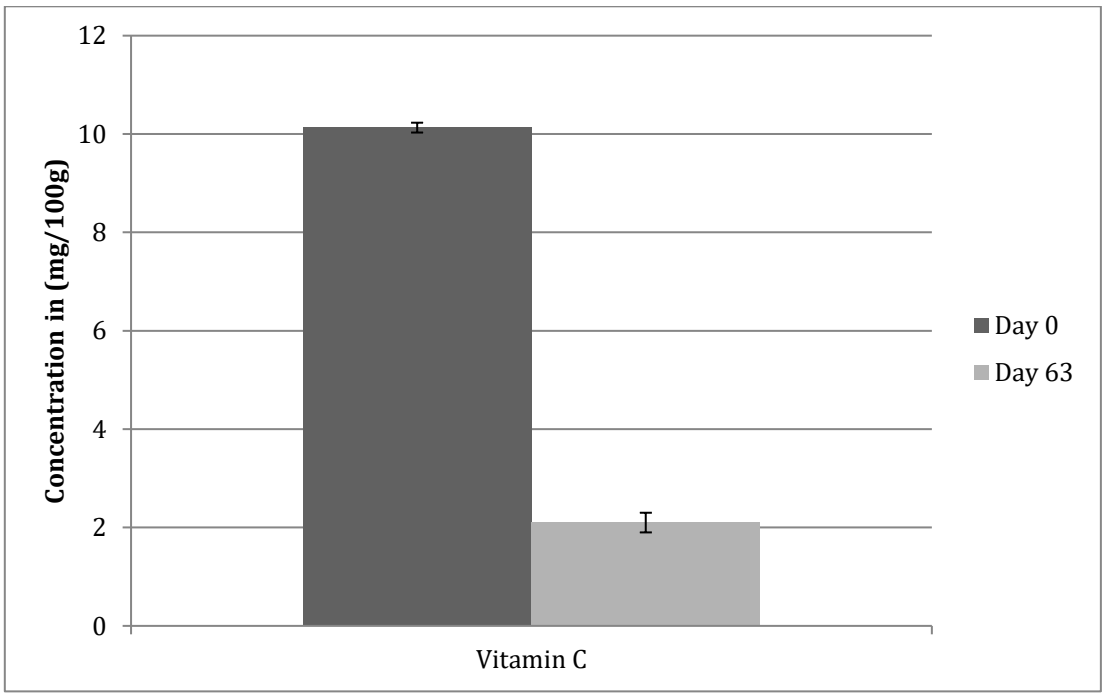

Fig. 4. Changes in Lycopene and Vitamin C content in Tomatoes treated with *Azadirachta indica* leaf powder

Discussion

Preservation of climacteric fruits for short duration by wrapping them in leaves was a traditional practice in Indian villages. However, its scientific basis has never been validated.

Certain hormones affect shelf life of climacteric fruits (Kumar et al, 2014). Most of those studies focused on the mechanism of fruit ripening before harvest. Shade dried leaves are not subjected to heat; hence loss of heat labile compounds does not occur. The cytokinins in the leaves exists in free and bound forms and in

bound form, cannot be lost with water during drying (Kieber, 2002). Increasing zeatin extended shelf life of tomatoes at room temperature. The Cytokinins are present in nanomol concentrations in plants and are potent regulators in plant physiology.

The shade dried leaf powders might serve as an inexpensive alternative to increase shelf-life of tomatoes at room temperature. The increase in shelf-life may be attributed to cytokinins in shade dried leaf powders, though the role of other compounds cannot be ruled out. An exclusion of other factors is not possible because cytokinin content in leaves varies with season. The leaves containing the highest amount of zeatin could bring about enhancement of shelf-life of tomatoes. The decrease in sugar content in control fruit indicates degradation of sugars over time; this was not observed in treated fruit. The increase in protein content may be attributed to synthesis of enzymes or proteins probably in response to exogenously applied cytokinins which many have inhibited further production of ethylene. This work needs to be done to examine molecular mechanisms underlying interaction of cytokinin in leaves influencing fruit ripening pathway in fruit post- harvest.

Cytokinins are known to delay senescence in leaves. The pathways of fruit ripening and leaf senescence have commonalities to a certain extent. Cytokinins inhibit the ethylene production enhancing the shelf life of tomatoes. The same principle might have operated when the tomatoes were treated with leaf powders. Use of powders can be adopted by farmers.

References

1. Aghofack-Nguemezi, J. and J. Manka`abiengwa. 2012. Effects of exogenously applied benzylaminopurine and kinetin on the ripening of banana (*Musa acuminata* Colla var. William) fruits. American Journal of Plant Physiology 7:154-163.
2. Alexander, L. and D. Grierson. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. Journal of Experimental Botany 53:2039-2055.
3. Guyer, L., S.S Hofstetter, B. Christ, B.S. Lira, M. Rossi, and S. Hörtensteiner. 2014. Different mechanisms are responsible for chlorophyll dephytylation during fruit ripening and leaf senescence in tomato. Plant Physiology 166(1):44-56.
4. Hart, D.S., A. Keightley, D. Sappington, P.T.M. Nguyen, C. Chritton, G.R. Seckinger, and K.C. Torres. 2016. Stability of adenine-based cytokinins in aqueous solution. *In vitro* Cellular & Developmental Biology 52:1-9.
5. Irokanulo, E.O., I.L. Egbezien, and S.O. Owa. 2015. Use of *Moringa oleifera* in preservation of fresh tomatoes. Journal of Agriculture and Veterinary Science 8(2):127-132.
6. Kieber JJ. Cytokinins. *The Arabidopsis Book / American Society of Plant Biologists*. 2002;1:e0063. doi:10.1199/tab.0063.
7. Rahul Kumar, Ashima Khurana and Arun K. Sharma, *Journal of Experimental Botany*, Volume 65, Issue 16, 1 August 2014, Pages,4561–4575,https://doi.org/10.1093/jxb/eru277
8. Meli, V.S, S. Ghosh, T.N. Prabha, N. Chakraborty, S. Chakraborty, and A. Datta. 2010. Enhancement of fruit shelf life by suppressing *N*-glycan processing enzymes. Proceedings of the National Academy of Sciences of the United States of America 107(6):2413-2418. (doi:10.1073/pnas.0909329107)
9. Pan X., R. Welti, and X. Wang. 2010. Quantitative analysis of major plant hormones in crude plant extracts by high-performance liquid chromatography-mass spectrometry. Nature Protocols 5(6):986-992
10. Vanitha, S.M., S.N.S. Chaurasia, P.M. Singh, S. Prakash, and S. Naik. 2013. Vegetable statistics. Technical Bulletin No. 51, Indian Institute of Vegetable Research, Varanasi, India.
11. Zwack, P.J. and A.M. Rashotte. 2013. Cytokinin inhibition of leaf senescence. Plant Signaling & Behavior 8(7):e24737.doi:10.4161/psb.24737.