The Future of Agriculture

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Efficiency of Postharvest Treatments with Citric Acid, Nicotinic Acid and Folic Acid on Stem End Rot of Lemon Caused by *Lasiodiplodia theobromae* During Cold Storage

Amira F. El-Wakil[1,*]; Sahar M. A. Eletreby[1]; El-khwaga, A.A.[3] and Thauria M. M. Abo El -wafa[2]

1- Fruit Handling Res. Dept., Hort. Res. Inst., Agric. Res. Cen., Giza, Egypt.
2- Post -harvest Dept., plant Pathology Research Institute, Agric. Res. Cen., Giza, Egypt.
3- Integrated Pest Management (IPM) Dept., plant Pathology Research Institute, Agric. Res. Cen., Giza, Egypt..

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https://doi.org/10.37229/fsa.fja.2025.06.15

[*]**Corresponding author:** amirafw@gmail.com

Future Science Association

Available online free at
www.futurejournals.org

Print ISSN: 2687-8151

Online ISSN: 2687-8216

Received: 3 May 2025
Accepted: 5 June 2025
Published: 15 June 2025

Abstract: Capability of citric acid, nicotinic acid and folic acid as postharvest treatments to induce resistance of lemon fruits against *Lasiodiplodia theobromae* was investigated *in vitro* and *in vivo*. Nicotinic acid and citric acid 3 g/L showed that efficacy in decreaseing fungal growth while folic acid at the same concentration showed no effect of mycelial growth *in vitro*. Stem end rot incidence on the lemon treated with nicotinic acid was significantly lower than the other treatments either naturally infected or artificially inoculated with *Lasiodiplodia theobromae*. Quality of treated lemon fruits with nicotinic acid and citric acid were maintained better than the control. The least values of weight loss (%) were obtained by nicotinic acid treatment of naturally infected and artificially inoculated lemons in both seasons in 2023 and 2024. Postharvest treatments with nicotinic acid or citric acid of naturally or artificially infected lemon fruits with *Lasiodiplodia theobromae* significantly delayed fruit deteriration during cold storage in comparison with other treatments and control. All postharvest treatments had a significant effect on delaying changes of fruit color values (L* and h°), and reduced the decreasing of fruit weight, hardness, TSS and acidity contents, especially those treated with nicotinic acid. Therefore, it could be recommended that treated lemon fruits by nicotinic acid is an effective method to improve its quality and storability during cold storage.

Key words: Lemon fruit, nicotinic acid, citric acid, Folic acid, cold storage.

1. Introduction

Lemon (*Citrus limon*) is considered one of the most important crops of family Rutacea, it is commercially cultivated in countries of the tropical and subtropical regions. Lemon is highly appreciated by consumers around the world because of their flavor and appearance **(Baldwin *et. al.*, 2014**). It has unrivalled flavor, acidity, a great of ascorbic acid and phenols as well as flavonoids. Although lemon is considered one of the non-climacteric fruit and produce low carbon dioxide and ethylene which led to a small physical and chemical changes during storage and transport (Kader, 2002). The main problem during storage is water evaporation through peel skin which leads to the increase of weight loss through vital processes such as respiration, transpiration and undesirable changes of peel colour from green to yellow (**Kaewsuksaeng *et. al.*, 2011**). The excessive water loss through lemon fruit skin encourages the production of ethylene which is related to the stimulation of chlorophyllase enzyme that increases the breakdown of chlorophyll which converts the peel colour from green to yellow. The quality of citrus fruits is determined by a combination of physical, chemical, and physiological traits, alongside nutrient concentration. High-quality limes are firm, green, and free from defects. Factors such as harvest timing, fruit variety, and environmental conditions also play a critical role in determining fruit quality **(Mukhim, *et al*., 2015 and Maftoonazad *et al.*, 2019).**

Post-harvest application of citric acid, nicotinic acid, and folic acid can maintain and improve citrus fruit quality by reducing decay, slowing degradation of organic acids, and enhancing firmness and nutritional content. Citric acid directly inhibits decay and helps maintain higher titratable acidity. Folic acid and nicotinic acid are vitamins that can improve the overall nutritional value and potentially contribute to longevity and flavor.

Folic acid (FA) is a water-soluble vitamin, also known as vitamin B9, which refers to tetrahydrofolate and its derivatives. Although the effect of folic acid on human health is known, its functions on plants have recently been discovered and little is known about the effect of exogenous folic treatment on post-harvest physiology (**Xu *et al*., 2021**). It has been reported that folic acid regulates gene expression through the riboswitch mechanism and also play a role in chlorophyll biosynthesis and oxidative stress tolerance (**Xu *et al*., 2021**).

Citric acid is often considered safe and, as an organic acid, can be used as a food additive (**Sommers, Fan, Handel, and Sokorai, 2003**). It can also prevent browning and fruit disease by reducing the respiration of postharvest fruits (**Pilizota and Sapers, 2004**) and is a good additive to improve the acidity and flavor of foods, thereby improving the quality of preservation and storage and preventing food spoilage.

Citric acid treatment can slow down the decrease in the soluble sugars and titratable acidity and is beneficial to maintain the fruit quality of Chinese jujube fruits during storage (**Zhao *et al*., 2009**). Thus, citric acid treatment is potentially an ideal fruit preservation approach.

Lime storage conditions are crucial for maintaining fruit quality. Typically, limes are stored at 10℃ and 85% RH. Using some post-harvest treatments such as nicotinic acid, citric acid and folic acid can delay fruit senescence, shrinkage from water evaporation and make a modified atmosphere around fruit skin. Additionally, these compounds stimulate biosynthetic pathways, leading to the production of protective substances, such as pathogenesis-related proteins and polyphenols (**Shi *et al*., 2018**).

The fungus *Lasiodiplodia theobromae* (Pat.) Griffon and Maubl. It is classified within the Ascomycetes, in the order Botryosphaeriales and family Botryosphaeriaceae **(Schoch, *et al*., 2006; Slippers, *et al*., 2013)**. It is a saprophyte and endophyte fungus and is considered a latent pathogen. **(Rubini, *et al*., 2005; Mohali, *et al*., 2005)**. The fungus causes regressive death of the branches, lesions on the stems, generates rubber and post-harvest fruit rots **(Sánchez, *et al.*, 1989; Herrera, *et al*., 1993)**.

Stem-end rot, caused by the fungi *Lasiodiplodia theobromae*, is widely prevalent and results in serious losses in humid growing areas such as lemon. Fungal spores enter calyx tissues or lodge beneath the calyx at the time of flowering and remain dormant until the fruits are harvested. Symptoms appear as water-soaked spots near the stem end of the fruit, which generally turn light to dark brown. The brownish decay proceeds down the rind of the fruit. In the case of *Lasiodiplodia*, the advancing margin of the rot progresses in lobes or a finger.

The objective of this research is to evaluate the impact of postharvest treatment on maintaining the quality of lemon fruit and decrease disease incidence of *Lasiodiplodia theobromae* during cold storage, thereby extending their shelf life. By understanding the effects of nicotinic acid, citric acid and folic acid. Thus, the aim of the present study is to enhance fruit integrity, reduceing post-harvest losses, and ensure consumer satisfaction.

2. Materials and Methods

2.1. Isolation and Identification of Pathogenic

Isolation and Identification of Pathogenic fungi, five 0.5 cm tissue sections were cut from each sample. These were disinfected with sodium hypochlorite at 1% by 3 min, washed with sterile distilled water, dried and sown separately in potato-dextrose-agar and incubated at 25°C. (**Cabrera, *et al*., 2012)**. The causal pathogen was then confirmed through Koch's postulates were performed to confirm the pathogenicity of each isolates. Sub-culturing was carried out at 7 day intervals and conidia from 7 day-old pure cultures were used for.

2.2. Pathogenicity test

The pathogenicity of the isolated pathogen was tested using Koch's postulates. The pathogenic culture with a diameter of 5 mm was inoculated on the PDA plate and cultured until the mycelium grew effectively. Thirty fresh and healthy lemon fruits were used in each treatment experiment. The surface was wiped with 75% alcohol for disinfection, washed with distilled water for alcohol, dried, and the center of the lemon fruits surface was punctured with a double punch. Pathogenic fungal cake was used inoculated to the center of the lemon fruit surface damage area, allowing the mycelium side to contact the wound area of the lemon fruits; additionally, inoculate non- pathogenic cake onto healthy lime lemon fruit as a control. Subsequently, the inoculated fruits were maintained at a constant temperature of 8◦C and a relative humidity of 95% for 7 days, followed by continuous observation and the recording of symptom development. Once symptoms manifested, the infected area of the fruit was collected and re isolated to complete Koch's postulate test and confirm whether the re-isolated fungus was the pathogen

2.3. Postharvest treatments

Postharvest treatments of lemon with citric acid, nicotinic acid and folic acid

Lemon fruits were purchased from commercial orchards in Behaira Governorate at the first week of December during the two successive seasons of 2023 and 2024. At maturity stage uniform and apparently healthy fruits, free from injuries and decay were harvested and immediately transported to the Fruit Handling Research Department, HRI. Lemon fruits were divided into two groups, one for the natural infection investigation and the other for artificial inoculation trials. The fruits allocated for artificial inoculation were surface sterilized by dipping in 70% alcohol for 2 min then washed thoroughly with sterilized distilled water. Surface sterilized fruits were left for air drying at room temperature. Fruits were punctured using a stainless-steel rod with 2-mm diameter and to 2-mm depth, as one puncture for fruit at the middle of one fruit side. Fruits were rinsed in *Lasiodiplodia theobromae*, spore suspension of 1 X 10^6 conidia /mL. The fruits divided into four treatments for each group as follows:

1. Control fruits were injured as above and treated with sterile distilled water containing Tween 20.
2. Fruits treated with citric acid as 0.3%
3. Fruits treated with nicotinic acid as 0.3%
4. Fruits treated with folic acid as 0.3%

All fruits were soaked for 2 min. Each treatment was represented in 3 replicates. All treatments were stored at 8 ± 1ºC and 90-95 % relative humidity (RH) in carton boxes. Changes in the physical and chemical properties of fruits were evaluated every two weeks from the beginning to the end of the storage period. After cold storage for 15 days or 60 days, dry rot disease incidence (%), fruit firmness and TSS were determined.

2.4. Disease assessment

- Disease incidence (%): The disease incidence was calculated for each replicate by relating decayed diseased fruits to the total number of fruits.

2.5. Physiological Parameters

- Fresh weight losses percentage (FWL%): The fruits weighed before cold storage to obtain the initial weight. Then, it weighed after each period of cold storage. FWL calculated as a percentage of the initial weight according to the following equation: FWL%= $\frac{wi-ws}{wi} \times 100$

Where, Wi = Initial fruit weight, Ws = Fruit weight at each sampling period (**Hazali, *et al*., 2013; Ibrahim and Gad, 2015)**

- Fruit firmness: Firmness was determined using a metec firmness tester with 8-mm probe. Data of firmness were expressed as Ib/inch^2.

- Fruit color: Lightness and hue angle were estimated using Minolta Calorimeter (**Minolta Co. Ltd., Osaka, Japan)** as described by (**McGire, 1992).**

2.6. Chemical properties

- Total Soluble solids content (%): It was measured by a hand refractometer as Brix^o **(Hazali, *et al*., 2013)**.

- Titratable acidity percentage: Was determined by titrating 5 ml of the extracted juice against 0.1 N of NaOH using phenolphthalein as indicator. Titratable acidity was expressed as percentage of citric acid (g citric acid/100ml juice) according to **A.O.A.C., (2005).**

- Vitamin C (mg/ 100 ml juice): It was determined by titration against 2, 6 dichlorophenolendophenol soulution and using 2% oxalic acid solution as substrate described by (**Lucass, 1944).**

2.7. Statistical analysis

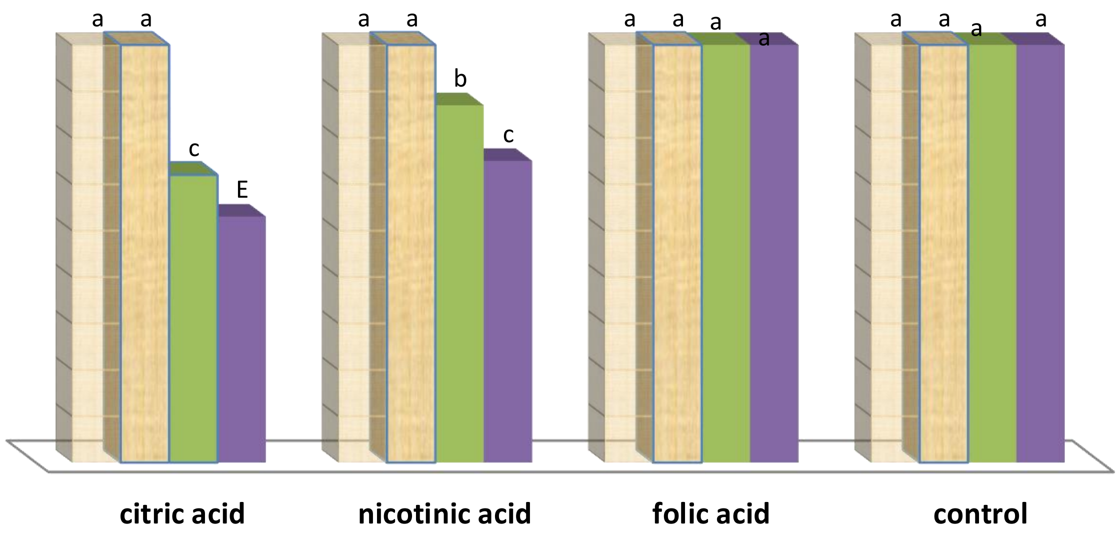
The data were arranged as a two factorial randomized complete design with three replicates. Except the disease incedince proberties (tables 1,2,3 and 4) which analysis as complete randomized experment only one factor, postharvest trearments. All data were subjected to statistical analysis according to the procedures reported by **Snedecor and Cochran (1993)** and means were compared by Duncan's Multiple range test at the 5% level of probability in the two seasons of experimentation.

3. Results

3.1. Efficiency of citric acid, nicotinic acid and folic acid on mycelial growth of *Lasidodiplodia theobrome*

The effect of citric acid, nicotinic acid and folic acid treatments on mycelial growth of *Lasidodiplodia theobrome* Fig. (1). All studied treatments of citric acid and nicotinic acid significantly decreased of linear growth of *Lasidodiplodia theobrome*. On the other side, all studied folic acid treatments had no effect on mycelial growth of *Lasidodiplodia theobrome*.

Fig.1.Effect of Citric, nicotinic and Folic acid on linear growth of *Lasidodiplodia theobrome*



3.2. Percentage of diseases incidence of lemon fruits rot

brown rots causing by *Lasidodiplodia theobrome* revealed that post-harvest treatments were significantly effective in controlling the disease incidence percentage of lemon fruit rots from natural and artificial infection with *Lasidodiplodia theobrome* over control (Table 1) Minimum mean fruit rots disease incidence (6.7and 16.7%) was recorded with the nicotinic acid treatment natural and artificial infection during season 2023, respectively. While the highest fruit disease incidence (33.3 and 100%) was recorded with untreated fruits (control) natural and artificial infection respectively. No fruit decay was observed in fruits treated with nicotinic acid until 30 days of storage.

Table (1). Effect of postharvest treatments with Citric, nicotinic and Folic acid on lemon fruits disease incidence by *Lasidodiplodia theobrome* of natural and artificial infectied with *Lasidodiplodia theobrome* during cold storage at (8 ± 1°C and RH 90-95%) in 2023 season

Treatment	Disease incidence							
	Season 2023							
	Natural infection				Artificial infection			
	15Days	30 Days	45 Days	60 Days	15Days	30 Days	45 Days	60 Days
Citric acid	0.0	3.3	13.3	16.7	0.0	3.3	16.7	20
Nicotinic acid	0.0	0.0	3.3	6.7	0.0	0.0	6.7	16.7
Folic acid	0.0	6.7	16.7	20	0.0	6.7	20	33.3
Control	3.3	10	20	33.3	6.7	10	40	100
LSD at 0.5%	0.94	0.94	1.32	0.94	0.67	0.94	0.94	0.94

Brown rots causing by *Lasidodiplodia theobrome* revealed that post-harvest treatments with were significantly effective in controlling the disease incidence percentage of lemon fruit rots from natural and artificial infection with *Lasidodiplodia theobrome* over control (Table 2) Minimum mean fruit rots disease incidence (20 and 26.7%) recorded that nicotinic acid treatment natural and artificial infections during season 2024, respectively. While the highest lemon fruit disease incidence, were recorded that control.

Table (2). Effect of postharvest treatments with Citric, nicotinic and Folic acid on lemon fruits disease incidence by *Lasidodiplodia theobrome* of natural and artificial infectied with *Lasidodiplodia theobrome* during cold storage at (8 ± 1°C and RH 90-95%) in 2024 season

Treatment	Disease incidence							
	Season 2024							
	Natural infection				Artificial infection			
	15Days	30 Days	45 Days	60 Days	15Days	30 Days	45 Days	60 Days
Citric acid	3.3	6.7	20	26.7	0.0	10	20	33.3
Nicotinic acid	0.0	0.0	16.7	20	0.0	6.7	16.7	26.7
Folic acid	0.0	13.3	23.3	26.7	0.0	16.7	26.7	40
Control	6.7	16.7	26.7	33.3	13.3	20	50	100
LSD at 0.5%	0.94	1.33	1.15	1.15	0.67	0.94	1.48	0.94

Results in table (3) showed that the effect of citric acid, nicotinic acid and folic acid on fungi associated disease incidence percentage of lemon (*pencillium* spp.) of natural and artificial during cold storage conditions for 60 days in 2023 seasons.

The lowest values of disease incidence percentage recorded by nicotinic acid treatment, while untreated fruits exhibited the highest disease incidence percentage in the two seasons at in, natural and artificially infected.

Table (3). Effect of postharvest treatments with Citric, nicotinic and Folic acid on disease incidence by fungi other than *Lasidodiplodia theobrome* in lemon fruit of natural and artificial infected with *Lasidodiplodia theobrome* during cold storage at (8 ± 1°C and RH 90-95%) in 2023 season

Treatment	Disease incidence							
	Season 2023							
	Natural infection				Artificial infection			
	15Days	30 Days	45 Days	60 Days	15Days	30 Days	45 Days	60 Days
Citric acid	0.0	3.3	10	13.3	0.0	6.7	16.7	20
Nicotinic acid	0.0	0.0	3.3	6.7	0.0	3.3	10	16.7
Folic acid	3.3	6.7	13.3	20	6.7	13.3	20	33.7
Control	3.3	13.3	20	33.3	10	20	26.7	40
LSD at 0.5%	0.94	1.15	0.94	0.94	0.67	1.15	0.94	0.67

3.3. Fruit colour-represented as hº angle

Results in table (4) showed that the effect of citric acid, nicotinic acid and folic acid on fungi associated disease incidence percentage of lemon (*Pencillium* spp.) Of natural and artificial during cold storage conditions in 2024 season.

The lowest values of disease incidence percentage recorded by nicotinic acid treatment, while untreated fruits exhibited the highest disease incidence percentage in the two seasons at in, natural and artificially infected.

Table (4). Effect of postharvest treatments with citric, nicotinic and folic acid on disease incidence by fungi other than *Lasidodiplodia theobrome* in lemon fruit of natural and artificial infected with *Lasidodiplodia theobrome* during cold storage at (8 ± 1°C and RH 90-95%) in 2024 season

Treatment	Disease incidence							
	Season 2024							
	Natural infection				Artificial infection			
	15Days	30 Days	45 Days	60 Days	15Days	30 Days	45 Days	60 Days
Citric acid	0.0	6.7	13.3	16.7	0.0	10	20	23.3
Nicotinic acid	0.0	0.0	6.7	10	0.0	6.7	16.7	20
Folic acid	6.7	10	20	26.7	10	16.7	26.7	36.7
Control	10	16.7	26.7	40	13.3	26.7	33.3	50
LSD at 0.5%	0.67	0.94	0.94	0.94	0.67	1.15	1.15	0.94

3.4. Fruit weight loss percentage

Results in table (5) showed that the effect of citric acid, nicotinic acid and folic acid on lemon fruits weight loss percentage during cold storage conditions in 2023 /2024 seasons.

Fruit weight loss percentage increased gradually with the extending of the storage periods. All studied treatments significantly decreased weight loss percentage compared with control fruits in the two seasons of either natural or artificially infected with *Lasidodiplodia theobrome*. The lowest values of weight loss percentage recorded by nicotinic acid treatment, while untreated fruits either natural or artificially infected exhibited the highest weight loss percentage in the two seasons in this work.

As for the interaction, after 60 days of storage the lowest values (12.5 & 20.9 %) and (18.9& 23.9%) were recorded by nicotinic acid treatment in the natural and artificially infected fruits, respectively.

3.5. Total Soluble solid percentage (TSS %)

Data in table (6) indicated that total soluble solid contents of Lemon fruits gradually and significantly decreased with the increasing of cold storage periods during the two seasons in this work. Data also cleared that, total soluble solid contents of fruits treated with folic acid gave highest values in the two seasons compared with the other treatments of both naturally or artificially infections. On contrast, citric acid and untreated fruits showed the lowest percentage in the first and the second season respectively of natural infection.

Concerning the interaction, after 60 days of storage folic acid treatment exhibited the highest values of TSS % (7.1&8.4%) in natural infection and (7.3& 8.7%) in the artificial infection. While the lowest values were obtained by control fruits in both seasons.

Table (5). Effect of some postharvest treatments on Weight loss percentage of Lemon fruits either natural or infected with *Lasidodiplodia theobrome* during cold storage at 8 ± 1°C and RH 90-95% in 2023 and 2024 seasons

Treatments	Natural infection											
	First season						Second season					
	Storage period per Days						Storage period per Days					
	0	15	30	45	60	Mean (B)	0	15	30	45	60	Mean (B)
citric acid	0.0	8.3	12.3	14.6	17.3	10.5	0.0	9.0	19.3	26.4	31.9	17.3
nicotinic acid	0.0	4.5	7.2	9.5	12.5	6.7	0.0	9.0	11.1	16.9	20.9	11.6
folic acid	0.0	5.8	9.9	13.2	16.4	9.1	0.0	7.3	15.5	19.4	23.3	13.1
Control	0.0	15.8	22.1	24.0	26.7	17.7	0.0	16.7	19.3	26.9	34.7	19.5
Mean (A)	0.0	8.6	12.9	15.3	18.2		0.0	10.4	16.3	22.4	27.6	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	3.13		2.80		6.27		3.39		3.03		6.77	
	Artificial infection											
citric acid	0.0	10.7	19.3	25.1	31.8	17.4	0.0	12.3	16.9	25.5	32.2	17.4
nicotinic acid	0.0	6.9	10.3	14.2	18.9	10.1	0.0	9.2	14.4	21.0	23.9	13.7
folic acid	0.0	8.7	13.6	19.9	27.1	13.9	0.0	6.6	14.0	25.6	33.2	15.9
Control	0.0	14.2	22.9	30.6	36.2	20.8	0.0	13.5	23.0	29.5	34.8	20.2
Mean (A)	0.0	10.1	16.5	22.5	28.5		0.0	10.4	17.1	25.4	31.0	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	3.43		3.07		6.86		2.30		2.06		4.61	

Table (6). Effect of some postharvest treatments on Total soluble solids (TSS) %of Lemon fruits either natural or infected with *Lasidodiplodia theobrome* cold storage at 8 ± 1°C and RH 90-95% during 2023 and 2024 seasons

Treatments	Natural infection											
	First season						Second season					
	Storage period per Days						Storage period per Days					
	0	15	30	45	60	Mean (B)	0	15	30	45	60	Mean (B)
citric acid	8.4	7.9	6.9	6.8	6.6	7.3	10.3	9.2	8.9	8.3	7.8	8.9
nicotinic acid	8.4	8.0	7.5	7.2	6.8	7.6	10.3	9.5	9.1	8.6	8.0	9.1
folic acid	8.4	8.1	7.6	7.3	7.1	7.7	10.3	9.9	9.5	8.9	8.4	9.4
Control	8.4	8.1	7.4	7.2	6.2	7.5	10.3	9.0	8.7	8.1	7.5	8.7
Mean (A)	8.4	8.0	7.3	7.1	6.7		10.3	9.4	9.1	8.5	7.9	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	0.34		0.31		0.68		0.46		0.41		0.91	
	Artificial infection											
citric acid	8.4	7.5	7.1	6.7	6.5	7.3	10.3	9.1	8.7	8.3	7.8	8.8
nicotinic acid	8.4	7.9	7.4	7.0	6.8	7.5	10.3	9.5	9.0	8.8	8.1	9.1
folic acid	8.4	8.0	7.8	7.5	7.3	7.8	10.3	9.8	9.3	9.0	8.7	9.4
Control	8.4	7.2	6.9	6.5	6.0	7.0	10.3	9.0	8.5	8.1	7.4	8.7
Mean (A)	8.4	7.6	7.3	6.9	6.6		10.3	9.3	8.9	8.6	8.0	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	0.34		0.30		0.67		0.58		0.52		1.15	

3.6. Firmness (Ib/inch^2)

Data in table (7) indicate that Firmness of Lemon fruits gradually and significantly decreased with the advance in cold storage periods during the two seasons. Data also cleared that, firmness fruits treated with nicotinic acid gave the highest values in the two seasons compared with the other treatments of both naturally or artificially infections. On contrast, untreated fruits showed the lowest firmness in the both seasons of fruits either naturally or artificially infections.

Concerning the interaction, after 60 days of storage nicotinic acid treatment exhibited the highest values of firmness (26.1&20.3) in natural infection and (22.3&20.2**)** in the artificial infection. While the lowest values were obtained by control fruits in both seasons either fruits were natural or artificially infected with *Lasidodiplodia theobrome*.

Table (7). Effect of some postharvest treatments on Firmness of Lemon fruits either natural or infected with *Lasidodiplodia theobrome* cold storage at 8 ± 1°C and RH 90-95% during 2023 and 2024 seasons

	Natural infection											
Treatments	First season						Second season					
	Storage period per Days						Storage period per Days					
	0	15	30	45	60	Mean (B)	0	15	30	45	60	Mean (B)
citric acid	28.0	27.2	26.4	23.5	23.5	25.7	25.3	24.3	20.8	19.0	18.6	21.6
nicotinic acid	28.0	27.7	27.2	26.6	26.1	27.1	25.3	25.1	24.1	21.0	20.3	23.2
folic acid	28.0	27.3	26.9	25.6	25.4	26.7	25.3	24.7	21.7	19.5	19.0	22.0
control	28.0	26.9	24.4	22.7	22.4	24.9	25.3	23.7	20.3	18.5	17.7	21.1
Mean (A)	28.0	27.3	26.2	24.6	24.4		25.3	24.4	21.8	19.5	18.9	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	0.82		0.73		1.64		1.48		1.32		2.95	
	Artificial infection											
citric acid	28.0	26.8	25.9	20.1	19.0	23.9	25.3	21.7	19.5	17.9	17.4	20.4
nicotinic acid	28.0	27.7	26.9	23.4	22.3	25.7	25.3	24.8	23.2	20.9	20.2	22.9
folic acid	28.0	27.3	26.1	21.5	20.3	24.7	25.3	24.2	20.5	19.2	18.8	21.6
control	28.0	25.3	24.8	19.1	18.9	23.2	25.3	20.5	18.5	16.4	12.1	18.6
Mean (A)	28.0	26.8	25.9	21.0	20.1		25.3	22.8	20.4	18.6	17.1	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	1.13		1.01		2.25		1.50		1.35		3.01	

3.7. Vitamin C. Content (represented as mg/ 100 ml juice)

The results in table (8**)** showed that vitamin C content in lemon either non-infected or artificially infected with *Lasidodiplodia theobrome* gradually increased with the progress of the storage period during storage at 8±1°C with 90-95% RH for 60 Days. All studied treatments significantly increased fruit contents of vitamin C comparison with untreated fruits, more over there was a significant difference between all treatments in the two seasons. The high contents of vitamin C were obtained by nicotinic acid and citric acid treatments at natural infection first and second seasons, respectively and nicotinic acid at artificial infection in the both seasons. On the other hand, control fruit treatment exhibited the least values of vitamin C in the two seasons.

Concerning interaction between two study factors, after 60 days of storage fruit treated by nicotinic acid reported the highest values of vitamin C in the nature infection and nicotinic acid, citric acid at artificial infection in the first and second seasons, respectively.

Table (8). Effect of some postharvest treatments on Vitamin C. (mg/ 100 ml juice) of Lemon fruits either natural or infected with *Lasidodiplodia theobrome* cold storage at 8 ± 1°C and RH 90-95% during 2023 and 2024 seasons

Treatments	Natural infection											
	First season						Second season					
	Storage period per Days						Storage period per Days					
	0	15	30	45	60	Mean (B)	0	15	30	45	60	Mean (B)
citric acid	67.2	62.7	59.1	57.4	45.5	58.4	64.0	62.7	59.4	56.2	48.1	58.1
nicotinic acid	67.2	64.3	62.7	60.1	49.1	60.7	64.0	60.6	58.2	55.4	49.1	57.5
folic acid	67.2	60.7	58.3	56.0	48.7	58.2	64.0	58.7	49.3	47.7	42.8	52.5
Control	67.2	62.3	54.6	51.9	40.0	55.2	64.0	59.7	54.4	51.5	39.7	53.9
Mean (A)	67.2	62.5	58.7	56.3	45.8		64.0	60.4	55.3	52.7	44.9	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	2.36		2.11		4.71		2.69		2.40		5.37	
	Artificial infection											
citric acid	67.2	64.6	61.1	55.5	49.2	59.5	64.0	60.4	57.6	51.4	47.9	56.3
nicotinic acid	67.2	63.3	62.1	55.7	50.2	59.7	64.0	62.2	61.2	53.1	46.8	57.5
folic acid	67.2	63.3	60.3	53.0	46.9	58.1	64.0	59.5	56.5	50.5	43.9	54.9
Control	67.2	59.4	56.1	51.2	44.9	55.8	64.0	57.7	53.7	48.7	42.5	53.3
Mean (A)	67.2	62.7	59.9	53.9	47.8		64.0	60.0	57.3	50.9	45.3	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	2.29		2.05		4.59		1.98		1.77		3.95	

3.8. Total acidity contents of lemon fruits during storage

The data presented in table (9) showed the effect of acids applications on total acidity contents of lemon fruits during the storage period at 8 ± 1°C with 90-95% RH either fruits were naturally or artificially infected with *Lasidodiplodia theobrome*, acidity contents of lemon fruits significantly decreased with the increasing of storage period. On the other factor, data in last table cleared that, there was no significant differences between all studied postharvest treatments in the lemon fruits either naturally or artificially infected with *Lasidodiplodia theobrome* in the two seasons in this work.

As for interaction after 60 days of storage, citric acid and folic acid treatments resulted highest levels of titratable acidity in lemon fruits during the storage period compared to the control and nicotinic acid recorded the lowest value in the first and second seasons, respectively in the naturall infection .On the other side, fruits artificially infected with *Lasidodiplodia theobrome*, and treated with nicotinic acid recorded the lowest values of titratable acidity in lemon compared to the control recorded the highest values in the first and second seasons.

Table (9). Effect of some postharvest treatments on Titratable acidity % of Lemon fruits either natural or infected with *Lasidodiplodia theobrome* cold storage at 8 ± 1°C and RH 90-95% during 2023 and 2024 seasons

Treatments	Natural infection											
	First season						Second season					
	Storage period per Days						Storage period per Days					
	0	15	30	45	60	Mean (B)	0	15	30	45	60	Mean (B)
citric acid	5.7	5.5	5.1	4.6	4.0	5.0	6.5	6.1	5.8	5.1	3.8	5.4
nicotinic acid	5.7	5.4	4.8	4.0	3.6	4.7	6.5	6.1	5.6	4.6	4.4	5.4
folic acid	5.7	5.4	5.1	4.5	3.8	4.9	6.5	6.2	5.5	5.3	4.7	5.7
Control	5.7	5.3	4.6	4.2	3.5	4.7	6.5	6.2	5.3	5.2	4.1	5.5
Mean (A)	5.7	5.4	4.9	4.3	3.7		6.5	6.1	5.6	5.0	4.2	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	0.20		0.18		0.40		0.29		0.26		0.58	
	Artificial infection											
citric acid	5.7	5.3	4.8	4.27	3.5	4.7	6.5	6.0	5.2	4.5	3.8	5.2
nicotinic acid	5.7	4.8	4.4	3.90	3.6	4.5	6.5	5.5	5.0	4.6	3.8	5.1
folic acid	5.7	5.2	4.6	3.96	3.5	4.6	6.5	5.8	5.2	4.6	4.0	5.2
Control	5.7	5.2	4.7	4.21	3.5	4.7	6.5	6.0	5.4	4.6	3.8	5.3
Mean (A)	5.7	5.1	4.6	4.09	3.5		6.5	5.8	5.2	4.6	3.9	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	0.18		0.17		0.37		0.24		0.21		0.48	

3.9. Fruit Lightness (L*)

Results in table (10), show that lightness (L*) of fruits was gradually and significantly decreased towards at the end of the storage period either fruits were natural or artificial infected with the fungi.

On the other side, data also indicated that, there were significant difference between all treatments in the natural and artificial infections in the first seasons, while these differences were none significant during the second season. Control fruit treatment gave the highest values of lightness during the first season either infected or none infected with fungi, while those natural fruits treated with nicotinic acid gave the highest values during the second season. On contrast, those treated with citric acid exhibited the lowest values of L* during both seasons in this work.

Data also cleared that, there were a significant interaction among all treatments in this work either fruits were natural or artificial infected with the fungi in the two seasons in this work.

3.10. Fruit colour-represented as h° angle

Results in table (11) show that, Lemone fruit colour changed from green yellow or light yellow to yellow (hue angle values (h°) was decreased) with the increasing of cold storage periods. On the other hand, data also cleared that, all postharvest treatments studied in this experiment had no significant effect on fruit colour during cold storage.

As for interaction data cleared that, the only significant difference between all treatments in this study were returned to storage period effect either fruits were natural or artificial infected with the fungi.

Table (10). Effect of some postharvest treatments on Lightness of Lemon fruits either natural or infected with *Lasidodiplodia theobrome* cold storage at 8 ± 1°C and RH 90-95% during 2023 and 2024 seasons

Treatments	Natural infection											
	First season						Second season					
	Storage period per Days						Storage period per Days					
	0	15	30	45	60	Mean (B)	0	15	30	45	60	Mean (B)
citric acid	79.0	74.4	72.5	70.3	67.9	72.8	78.3	74.5	73.2	71.2	70.1	73.5
nicotinic acid	79.0	75.7	73.1	70.9	68.7	73.5	78.3	75.5	74.1	72.5	70.7	74.2
folic acid	79.0	75.0	72.5	70.8	69.5	73.4	78.3	74.8	73.4	71.5	69.6	73.5
Control	79.0	76.9	74.1	71.6	69.2	74.2	78.3	76.3	73.9	71.5	68.8	73.8
Mean (A)	79.0	75.5	73.1	70.9	68.8		78.3	75.3	73.7	71.7	69.8	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	1.18		1.05		2.35		1.16		1.04		2.32	
	Artificial infection											
citric acid	79.0	75.9	73.2	72.0	69.9	74.0	78.3	73.8	72.5	71.0	67.7	72.7
nicotinic acid	79.0	76.4	73.9	71.8	70.3	74.3	78.3	74.5	71.9	70.9	69.1	73.0
folic acid	79.0	76.9	74.4	72.9	70.5	74.7	78.3	74.2	72.8	71.2	68.1	72.9
Control	79.0	77.8	75.7	73.1	71.7	75.5	78.3	75.5	73.7	72.4	70.6	74.1
Mean (A)	79.0	76.8	74.3	72.4	70.6		78.3	74.5	72.7	71.4	68.9	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	1.23		1.10		2.46		1.30		1.16		2.60	

Table (11). Effect of some postharvest treatments on Hue angle of Lemon fruits either natural or infected with *Lasidodiplodia theobrome* cold storage at 8 ± 1°C and RH 90-95% during 2023 and 2024 seasons

Treatments	Natural infection											
	First season						Second season					
	Storage period per Days						Storage period per Days					
	0	15	30	45	60	Mean (B)	0	15	30	45	60	Mean (B)
citric acid	112.3	105.8	99.7	98.2	95.9	102.4	109.1	98.9	96.2	95.9	94.8	99.0
nicotinic acid	112.3	106.1	102.1	100.2	97.9	103.7	109.1	100.5	98.7	97.8	96.1	100.4
folic acid	112.3	105.2	98.2	96.4	94.0	101.2	109.1	101.6	97.4	96.0	95.1	99.8
Control	112.3	106.6	101.4	99.3	97.3	103.4	109.1	102.6	99.6	98.5	97.3	101.4
Mean (A)	112.3	105.9	100.4	98.5	96.3		109.1	100.9	98.0	97.0	95.8	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	3.70		N. S		7.40		3.19		N. S		6.38	
	Artificial infection											
citric acid	112.3	105.2	102.7	99.4	98.0	103.5	109.1	102.6	99.3	98.0	96.0	101.0
nicotinic acid	112.3	107.1	103.4	99.0	96.0	103.5	109.1	101.5	98.1	96.9	95.4	100.2
folic acid	112.3	105.5	101.9	98.1	96.5	102.9	109.1	100.6	97.9	96.7	94.5	99.8
Control	112.3	104.9	102.0	99.8	98.4	103.5	109.1	104.3	99.8	98.2	96.9	101.7
Mean (A)	112.3	105.7	102.5	99.1	97.2		109.1	102.2	98.8	97.5	95.7	
LSD	(A)		(B)		A*B		(A)		(B)		A*B	
	2.88		N. S		5.77		2.69		N. S		5.38	

4. DISCUSSION

Although stem end rot disease leads to significant fruit losses, the basic science of fruit, stem end rot disease is largely unknown. **(Galsurker *et. al*., 2018)** Nevertheless studies have shown that different treatments can decrease disease incidence of stem end rot by directly inhibiting the fungal growth (fig.1) or indirectly inducing fruits resistance (Table 1), or by indirectly changing the stem-end microbiome to a more diverse and less pathogenic community. Citric acid and nicotinic acid decreased linear growth of *Lasidodiplodia theobrome* up of 3 gram /liter tested concentrations. Mean while folic acid treated was evaluated applied treatments at all tested concentrations had no significant effect on mycelial growth of *Lasidodiplodia theobrome*. The use of nicotinic acid and citric acid to control stem end rots in fruits is a breakdown emerging area of interest, particularly in postharvest treatment, here's breakdown of how each component might contribute;

According to the results, all treatments demonstrated a reduction of weight loss compared to the control fruit during storage. After harvesting, the water content of the product gradually decreases due to transpiration and the respiration process (**Siboza *et al*., 2014**). This leads to wilting, loss of quality, and other undesirable changes in the product, ultimately reducing its economic value. The decrease in weight loss observed in fruits treated with nicotinic acid and citric acid can be attributed to their effect on maintaining membrane integrity at low temperatures, as reported in previous studies on pomegranate and blood orange (**Habibi *et al*., 2019 and Sayyari *et al*., 2011**)

Fruit firmness is an essential characteristic that not only affects customers' assessment of the quality of newly harvested lemons but also the determination of their ability to be preserved during postharvest operations

Total soluble solids (TSS) in fruits include sugars and a small percentage of amino acids, organic acids, vitamins, and minerals. TSS is a valuable quality parameter related to consumer acceptance and has a significant impact on fruit taste and is considered one of the chemical indicators (**Liu *et al*., 2020).** The soluble solids increase as the fruit ripens. As previously reported, postharvest treatments delayed TSS increases during cold storage, possibly due to delays in ripening and aging processes.

Ascorbic acid concentration decreased with the progress of the storage period. Similar finding was obtained by **Ghosh *et al*. (2015)** who observed that the increase of the storage period led to a decrease in ascorbic acid concentration in lemon fruits especially at the end of the storage period.

Vitamin C content of lemon fruit, all studied treatments significantly increased fruit contents of vitamin C comparison with untreated fruits, more over there was a significant difference between all treatments in the two seasons. The high contents of vitamin C were obtained by nicotinic acid and citric acid treatments at nature infection in the first and the second seasons, respectively and nicotinic acid at artificial infection in the both seasons. On the other hand, control fruit treatment exhibited the least values of vitamin C in the two seasons. Antioxidant compounds such as ascorbic acid are an important parameter in discussing the quality of fruits and vegetables (**Khorshidi, *et al*., 2011**). Antioxidant systems play an important role in quenching ROS and maintaining cellular redox homeostasis, thus modulating aging processes in plants (**Jimenez, *et al*., 2002).** The treated fruits had higher ascorbic acid than the control fruit. These compounds maintained a higher content of ascorbic acid probably due to the reduction of ascorbic acid oxidase activity and delayed ripening as well as the aging process (**Habibi *et al*., 2020**).

The higher acidity in fruits treated fruits might have been due to reduced hydrolysis of organic acids and subsequent accumulation of organic acids in the fruits which were oxidized at slower rate because of slower respiration rate. During storage, rate of respiration increases which consume organic acid and reduce the fruit acidity that affect the fruit flavor. With advancement of ripening processes, starch is converted into sugar as a result of hydrolysis, which is ultimately responsible for accelerated sugar level and reduction in acidity percent (**Baraiya *et al*., 2014)**.

Fruit colour-represented as hº angle value was decreased (highdensity of yellow color) with the advancement of cold storage period. This result agrees with that obtained by **Shaaban and Hussein (2017)** who observed that the increase of the storage period led to an increase in the density of yellow color of lemon fruits

The decrease of lightness (L*) value and the increase of yellow color density (hº) value through the storage period might be attributed to the production of ethylene which increases the activity of enzymes in fruits and led to the breakdown of chlorophyll and synthesis of carotenoids **(Wei *et al*., 2017)**

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