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# Table of Contents

**Volume 7    Number 12**

**November 2017**

**Efficacy of Some Plant Extracts and Biodegradables Wrapping Materials as an Alternative  
to Fungicides in Increasing Shelf Life Citrus Fruits**

R. I. Khaleel.....621



# Open Journal of Ecology (OJE)

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# Efficacy of Some Plant Extracts and Biodegradables Wrapping Materials as an Alternative to Fungicides in Increasing Shelf Life Citrus Fruits

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## Abstract

Increasingly global there is an interest to develop a new method by replacing fungicides in packaging fresh fruit by using environment-friendly bio-pesticides, such as use of botanicals or biological control. Five ethanol extracts (concentrations 500 - 5000 ppm) of *Cerbera odollam* L. (Pong-pong), *Capsicum frutescence* L. (Chili), *Azadirachta indica* L. (Neem), *Cymbopogon nardus* L. (Lemon grass), *Zingiber officinale* L. (Ginger), were tested for their anti-fungal activity for citrus green mold, black and brown rot, compared with the control fungicide (Guazatine). Efficacy of the selected plant extract in cooperated with biodegradable materials wrappers (Polylactic acid, Pectin and Newspaper) was determined during storage at room temperature ( $\pm 25^{\circ}\text{C}$ ) to control fruit-pathogen decay under modified atmosphere packaging (MAP) to increase shelf life and improve the fruit quality. Crude extracts from pong-pong and chili showed fungal inhibition zone at 3000 ppm (c.100%), and increased shelf life and decreased weigh loss for fruits wrapped and stored at room temperature compared with untreated fruit.

## Keywords

Natural Antimicrobial, Plant Extracts, Bio-Pesticides, Biodegradable Materials

## 1. Introduction

The use of synthetic fungicides effects to the environment such as their residue effect can be harmful to human health, besides increased accumulation in water, soil, and fruit besides the elimination of the natural enemies of the involved pathogens [1]. The above scenario has led to the search for safe alternatives to

synthetic fungicides to control fungal post-harvest rot in citrus to decrease the loss and avoid the negative effects of using fungicides, with the current research focusing on the usage of botanicals. However, there is a growing global concern over the use of synthetic fungicides chemicals on food crops because of the continuous exposure of man to low levels of fungicides residue through his diet. In addition fungicides have an impact on the environment through accumulation in soil and water and effect on biological diversity [2]. These aspects have led to the implementation of more restrictive legislations regarding the maximum residue levels (MRL) of chemical residues in fruits exported particularly to world markets [3]. The application of fungicides often leads to new fungicide-resistant strains as in *P. digitatum* [4]. Keeping in view of these developments, the recent trend in disease management focuses on using natural plant products as all an alternative to fungicides. The natural plant products (botanicals) are generally safer as they degenerate fast thus avoiding residue effect [5]. The bioactive pathogen-inhibiting compounds may include compounds of flavonoids, glycosides, phenols and terpenoids [6]. This study focuses on fruit ripening and the problems related to quality loss during transportation, storage and marketing. The wrapping materials as they are biodegradable and eco-friendly could serve as an alternative to synthetic packaging such as plastic and aluminium foil. Wrapping for the present study is a form of active packaging based on the combination of modified atmosphere packaging used with natural antimicrobial compounds (plant extracts) in cooperation with biodegradable materials in order to improve post-harvest quality in the storage of freshly harvested citrus.

## 2. Materials and Methods

### 2.1. Preparing Fruits for the Wrapping

Fruits were wounded at a depth of 5.0 mm with a 1.25 mm diameter needle at the equator and inoculated by spraying with suspension of fungi. The treated fruit were left for an hour after spraying to stabilize the spores on the wound and wrapped with the 3 wrappers polylactic acid, pectin and newspaper. Fungicides and untreated fruits used as control. Each treatment and controls were tested in three replicates using Completely Randomized Design (CRD). Treated fruits were packed and stored in carton boxes and incubated at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  (RH 65% - 75%) in the laboratory. Evaluations of the fruit decay, weight loss and shelf life fruit were done after three weeks.

#### **Treatment Spray:**

- spray plant extract (5000 ppm)
- spray fungicides (Guazatin)
- Untreated fruits (Healthy Control)

#### **Wrap Process:**

- Polylactic acid wrap
- Pectin wrap
- New paper wrap

## 2.2. Fungal Decay

Fungal decay in stored fruit was evaluated after 21 days under storage conditions. The percentage of infection was determined using scale of 1 - 5 with Scale 1 indication no infection and Scale 5 indication high infection (Scale modified from [7]).

## 2.3. Weight Loss

The percentage of weight loss was calculated by determining the progressive reduction in fruit weight during storage after a period of 21 days in relation to the original fresh weight at the beginning of storage [8].

$$WL(\%) = WL \text{ before storage} - WL \text{ after storage} / WL \text{ before storage} \times 100 \quad (1)$$

## 2.4. Statistical Analysis

Data collected and analysed using analysis of variance (ANOVA). Significant differences between mean values were determined using Duncan's Multiple Range test ( $P \leq 0.05$ ). Following ANOVA statistical analysis, which were performed using SPSS version 19-2012 (SPSS Inc., Chicago, USA).

## 3. Results and Discussion

### 3.1. Fruit Decay

ANOVA statistical analysis (**Table 1**) showed packaging fruits with sprayplant extracts and wrap fruit using (Polylactic acid, Pectin and Newspaper) decreased fungal decay for stored fruit and recorded averages better than the untreated fruit in controlling infection of Green mold, Black rot and Fusarium rot. Generally, Polylactic acid plus Pong-pong, Chili and Neem were better than pectin and newspaper in reducing fungal infection that recorded 0% fruit decay. While mean percentages of the fungal decay for pectin and Newspaper were 2.22%, 3.7%, 4.44% and 2.22%, 3.7%, 3.7% sequentially with same plant.

Result current study using Polylastic acid, Pectin and Newspaper in cooperation with plant extracts recorded significant in fruit decay compared with result [9] and [10] which application of wrapping polyolefin film and Salicylic acid plus Calcium Chloride to reduce percentage of the decay fruits in Pomegranate (*Punica granatum* L.) and Kiwifruit (*Actinidia deliciosa*). Fruits stored using Polylactic acid, Pectin and Newspaper may be help to reduce levels of  $O_2$  and  $CO_2$  (reduce rate respiration) during improve modified atmosphere packaging (MAP), which could induce decreased microorganism proliferation with good permeability on the produce. This result is in agreement with studies carried out by [11] and [12], who used plant derivatives to improve atmosphere packaging (MAP) during storage fruit after harvest. Also, natural products (bioactive compounds) of the plant derivatives such as eugenol, phenolics and glycosides enhanced antifungal activity during prolonged [13] and [14].

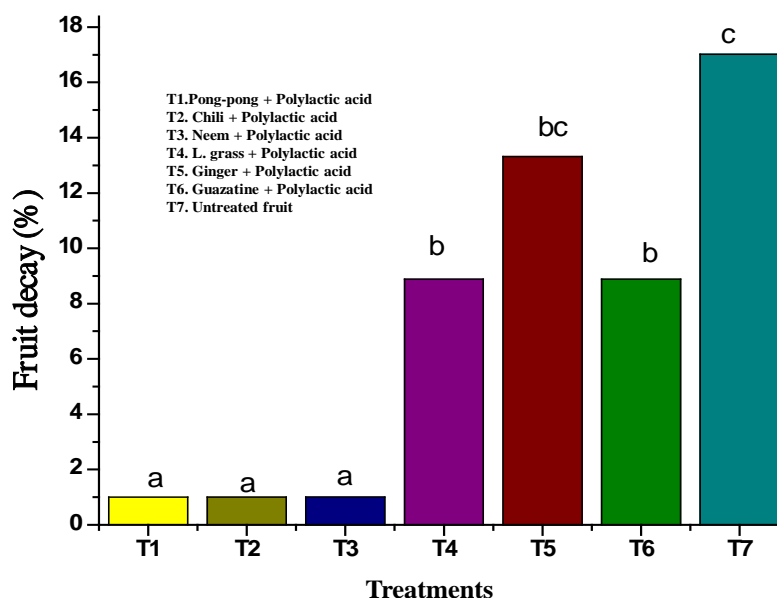
**Table 1.** Effect of spraying plant extracts at concentration 5000 ppm with wrap fruit using three type of the biodegradable materials (Polylactic acid (PLA), Pectin and newspaper) on fungal decay (%) on the orange surface at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and RH 65% - 75% for 21 days.

Treatments	<i>Penicillium digitatum</i>	<i>Aspergillus niger</i>	<i>Fusarium sp</i>	Mean	**Infection
	Damage (%)				
Pong-pong + PLA	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	Clean
Chili + PLA	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	Clean
Neem + PLA	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	0.0 <sup>a</sup>	Clean
Lemon grass + PLA	8.88 <sup>b</sup>	8.80 <sup>b</sup>	8.88 <sup>b</sup>	8.88 <sup> b</sup>	Slight
Ginger+ PLA	13.32 <sup>b</sup>	13.32 <sup>bc</sup>	13.32 <sup>bc</sup>	13.32 <sup>bc</sup>	Slight
Guazatine + PLA	8.88 <sup>b</sup>	8.80 <sup>b</sup>	8.88 <sup>b</sup>	8.88 <sup>b</sup>	Slight
Untreated fruit	15.54 <sup>b</sup>	17.77 <sup>c</sup>	17.77 <sup>c</sup>	17.02 <sup>c</sup>	Moderate
Pong-pong + Pectin	2.20 <sup>a</sup>	2.22 <sup>a</sup>	2.20 <sup>a</sup>	2.22 <sup>a</sup>	Trace
Chili + Pectin	2.20 <sup>a</sup>	4.44 <sup>a</sup>	4.44 <sup>a</sup>	3.7 <sup>a</sup>	Trace
Neem + Pectin	2.20 <sup>a</sup>	4.44 <sup>a</sup>	6.66 <sup>ab</sup>	4.44 <sup>a</sup>	Trace
Lemon grass + Pectin	8.80 <sup>b</sup>	8.84 <sup>ab</sup>	8.88 <sup>ab</sup>	8.88 <sup>ab</sup>	Slight
Ginger + Pectin	11.10 <sup> bc</sup>	11.10 <sup>ab</sup>	13.32 <sup>ab</sup>	11.84 <sup>bc</sup>	Slight
Guazatine + Pectin	6.60 <sup>ab</sup>	4.44 <sup>a</sup>	6.66 <sup>ab</sup>	5.92 <sup>ab</sup>	Slight
Untreated fruit	17.77 <sup>c</sup>	15.5 <sup>b</sup>	17.77 <sup>b</sup>	16.29 <sup>c</sup>	Moderate
Pong-pong + Newspaper	2.22 <sup>a</sup>	2.22 <sup>a</sup>	2.22 <sup>a</sup>	2.22 <sup>a</sup>	Trace
Chili + Newspaper	2.22 <sup>a</sup>	2.22 <sup>a</sup>	4.44 <sup>ab</sup>	3.70 <sup>a</sup>	Trace
Neem + Newspaper	2.22 <sup>a</sup>	4.44 <sup>a</sup>	4.44 <sup>ab</sup>	3.70 <sup>a</sup>	Trace
Lemon grass + Newspaper	6.66 <sup>a</sup>	4.44 <sup>a</sup>	6.66 <sup>ab</sup>	6.66 <sup>a</sup>	Slight
Ginger + Newspaper	13.32 <sup>ab</sup>	6.66 <sup>a</sup>	13.33 <sup>ab</sup>	10.77 <sup>ab</sup>	Slight
Guazatine + Newspaper	6.66 <sup>a</sup>	4.44 <sup>a</sup>	6.66 <sup>ab</sup>	5.92 <sup>a</sup>	Slight
Untreated fruit	17.77 <sup>b</sup>	15.45 <sup>b</sup>	17.77 <sup>b</sup>	17.77 <sup>b</sup>	Moderate

\*\*Alphabets different in the same column show significant difference using Duncan's Multiple Range test ( $P \leq 0.05$ ) and average was calculated from three replicates.

Severity of decay was visually evaluated during a spray to crude plant extract, at  $28^{\circ}\text{C}/85\%$  RH. The degree of infection on fruit was rated using a scale of 1 to 5, where 1—Clean, (0%) infection; 2—Trace (1% - 5%) infection; 3—Slight (5% - 15%) infection; 4—Moderate (15% - 30%) infection; and 5—High (>30%) infection. (Scale modified from Tzortzakis, 2006). Results presented in **Figure 1** show different statistical analysis ( $P \leq 0.05$ ) between fruit treated using Polylactic acid and those treated with plant extracts at 5000 ppm. The treatment using Polylactic acid with Pong-pong, Chili and Neem improved the quality of fruits under storage, and recorded best values in reducing the percentage of fungal decay, thereby completely preventing the growth of fungi on the surface of fruit. These treatments significantly outperformed ( $P \leq 0.05$ ) other treatments and control.

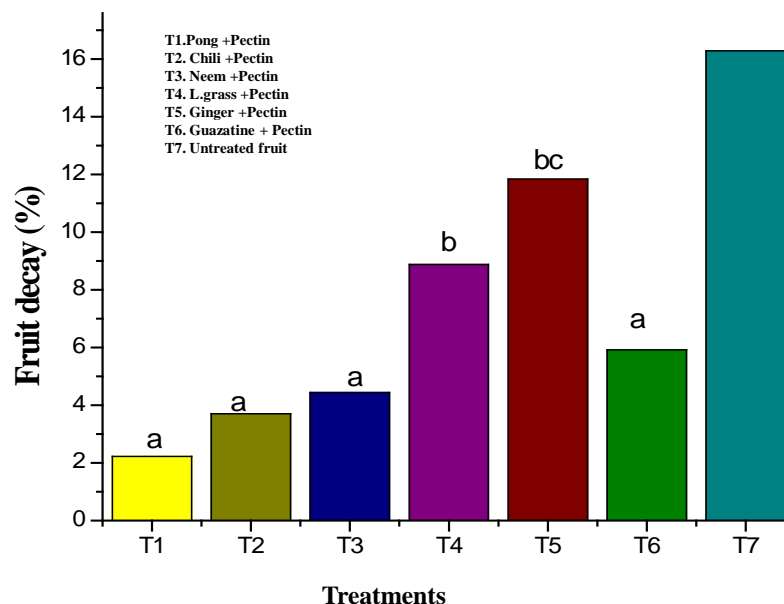




**Figure 1.** Observing the mean fruit decay of the fruit when wrapped with Polyactic acid wrapper and sprayed with different treatments and stored fruit in carton boxes at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and 65% - 75% RH for 21 days. \*\*Alphabets different in the same column show significant difference using Duncan's Multiple Range test ( $P \leq 0.05$ ) and average was calculated from three replicates.

Untreated fruit (control) gave a mean fruit decay of 17.02%, while lemon grass and Ginger recorded slight infection of 8.88% and 13.32% consecutively, but did not show any statistical difference with fungicide (Guazatine). The application of antimicrobial Polyactic acid (PLA) with spray plant extracts to improve the quality and shelf life of fruit has not yet been reported. However, a novel food-packaging concept has introduced a new generation of active biodegradable materials. This active packaging will restrict the growth of microorganisms and maintain food safety, even for non-sterile foods [15] and [16]. In a previous study [17] a bioactive thermoplastic film was successfully used to inhibition *Penicillium* sp, *Aspergillus niger* and *Sclerotinia sclerotiorum* growth. Polyvinyl alcohol (PVA, CGP and PVA) has been combined with natural polymers in many biomaterial applications to save foods and inhibition growth microbiology [18] and [19].

The effect of packaging fruit, after spraying plant extract on its surface and wrapping it using pectin tissue is shown in **Figure 2**. Statistical analysis ( $P \leq 0.05$ ) showed that wrapping the fruit using pectin tissue along with spraying Pong-pong, Chili and Neem extracts were the best treatments and showed significant decrease (Trace) in infection on surface fruit with 2.22%, 3.7% and 4.44%, respectively. Results showed light infection (5.92%) when using treatment from pectin with Guazatine. Also, Lemon grass and Ginger showed noticeable effect (slight) on the spread of infection in fruit stored, giving mean fruit decay values of 8.88% and 11.8%. The untreated fruit wrapped using pectin tissue showed moderate infection and demonstrated high values (16.29%) of fruit decay.



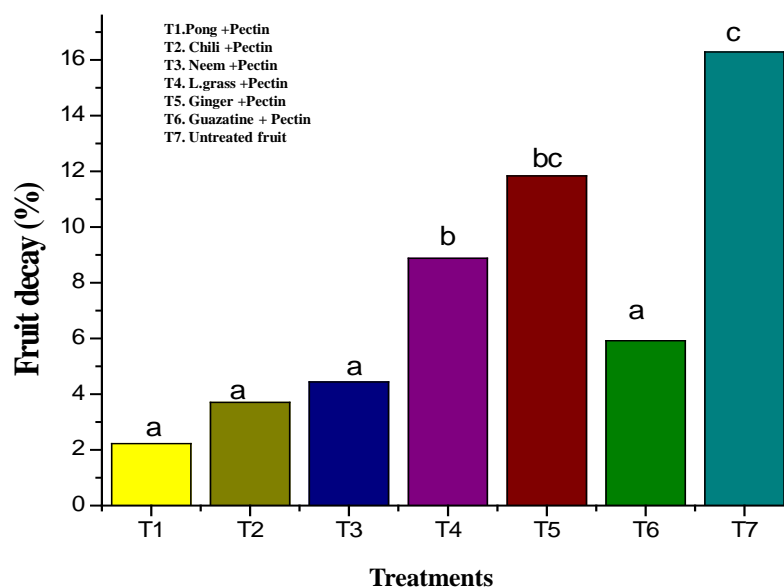
**Figure 2.** Observing the mean fruit decay of the fruit when wrapped with pectin wrapper and sprayed with different treatments and stored fruit in carton boxes at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and 65% - 75% RH for 21 days.

The post-harvest treatment of wrapping fruit using newspaper and the spraying of plant extracts demonstrated significant differences in fruit decay (**Figure 3**). Packaging fruits with News Paper and the spraying of extracts from Pong-pong, Chili and Neem decreased infection percentage (fruit decay). All treatments reduced fungi growth on fruits surface to Chili and Neem respectively. Minimal levels (Trace) and recorded values of 2.22%, 3.70% and 3.70% for Pong-pong. On the other hand, the maximum fruit decay shown in untreated fruits was 17.77%. Treating fruits with Lemon grass and Ginger were improved the quality of fruits by decreasing infection to 6.66% and 10.77% respectively causing the degree of infection to be slight.

The application of antimicrobial pectin and newspaper wrapping along with plant extracts in fruit decontamination has not yet been reported. However result current result come better of result [20], that evaluated Bio Save 10 LP and  $\text{SO}_2$  for their efficacy in controlling mycelia spread and sporulation of *Muscadine grape* during storage. The fruits were stored in emanating sheets, and removed bi-weekly up to 4-weeks ( $0^{\circ}\text{C} - 1^{\circ}\text{C}$ , 90% to 95% RH). It was found that treatment reduced molds, and improved overall fruit quality when compared to the untreated fruits. Furthermore, [21] reported that wrapping fruit with different types of the papers (white paper, brown paper, tissue paper and newspaper) inhibitions growth microbiology and improves post-harvest quality.

### 3.2. Weight Loss (%)

Data of ANOVA ( $P \leq 0.05$ ) as shown in **Table 2** points to weight loss in stored fruit. This is a clear variation from other treatments. The average weight loss was faster for untreated fruits compared to treated fruits. Generally, all the



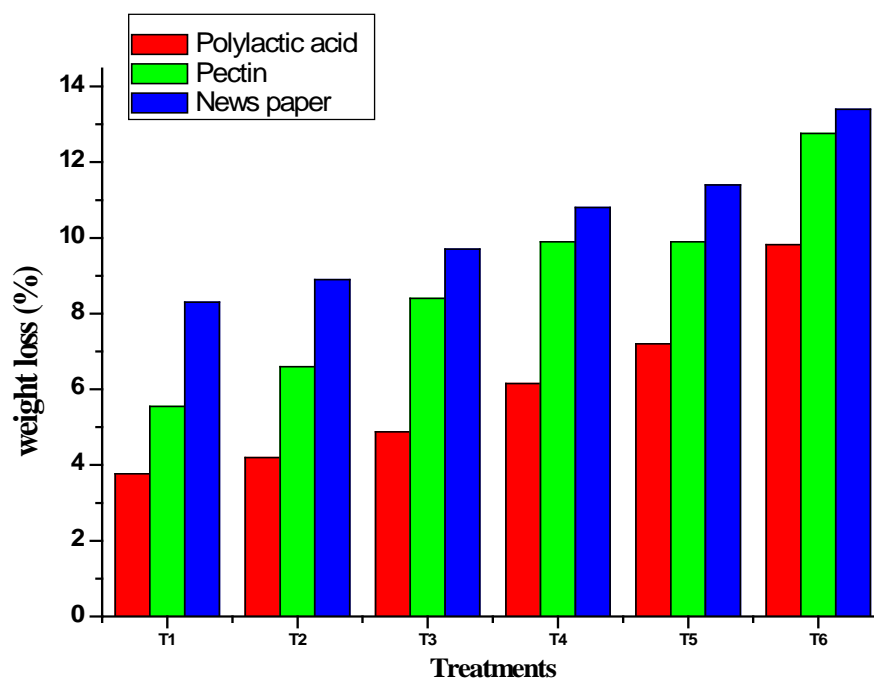
**Figure 3.** Observing the mean fruit decay of the fruit when wrapped with Newspaper wrapper and sprayed with different treatment and stored fruit in carton boxes at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and 65% - 75% RH for.

**Table 2.** Effect of fruit wrappings and spray plant extracts on the physiological weight loss (%) of orange fruits during storage at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and 65 - 75 RH% for 21 days.

Plant extracts	Biodegradable materials (Wrapping)			Mean
	Polylactic acid	Pectin	News paper	
Pong-pong	3.77 <sup>a</sup>	5.55 <sup>a</sup>	8.30 <sup>a</sup>	5.87 <sup>a</sup>
Chili	4.2 <sup>b</sup>	6.60 <sup>b</sup>	8.9 <sup>b</sup>	6.56 <sup>b</sup>
Neem	4.88 <sup>c</sup>	8.4 <sup>c</sup>	9.7 <sup>c</sup>	7.66 <sup>c</sup>
Lemon grass	6.15 <sup>d</sup>	9.9 <sup>d</sup>	10.8 <sup>d</sup>	8.95 <sup>d</sup>
Ginger	7.2 <sup>e</sup>	9.9 <sup>d</sup>	11.4 <sup>e</sup>	9.5 <sup>e</sup>
Untreated	9.82 <sup>f</sup>	12.76 <sup>e</sup>	13.4 <sup>f</sup>	11.99 <sup>f</sup>

\*Data in columns (Biodegradable materials) and raw with different letters are statistically different according to Duncan's multiple range tests ( $P \leq 0.05$ ) and all treatments consisted of three replicates.

treatments improved the fruits quality including decreasing weight loss. Wrapping fruit with polylactic acid exceeded Pectin and Newspaper in reducing weight loss after 21 days of storage. It recorded values of 3.77%, 4.2%, 4.88%, 6.15% and 7.2% each for Pong-pong, Chili, Lemon grass and Ginger respectively. Conversely, fruits treated with the plant extracts and then packaged using Newspaper wraps recorded percentage of weight loss (8.30%, 8.9%, 9.7%, 10.8% and 11.4%). The reduction of weight loss may be due to improved storage conditions during modified atmosphere packaging (MAP), which increases relative humidity, thereby reducing respiration rates and fungal decay. Furthermore, plant extracts inhibition growth fungal on surface stored fruit by creating a thin film of extracts surrounding the fruit, which modifies the microclimate of the fruits.



**Figure 4.** Effects of combining Poly-lactic acid, Pectin and Newspaper with sprayed plant extracts on percentage weight loss (%) of stored samples at  $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and 65% - 75% for 21 days. T1. Pong-pong, T2. Chili, T3. Neem, T4. Lemon grass, T5. Ginger, T6. Untreated fruit.

These results are in agreement with previous studies [22] and [23] that used oil and plant extracts to decrease weight loss of fruit stored with different concentrations.

From ANOVA table at  $P \leq 0.05$  and **Figure 4** result showed that Pong-pong statistically outperformed the other plants in lowering weight loss by 5.87%. Likewise, extracts of Chili, Neem, Lemon grass and Ginger differed statistically (6.56%, 7.66%, 8.95% and 9.5%) while the untreated fruits had the highest value (11.99%). The current study recorded decrease in percentage of weight loss in stored fruit compared with findings of [24] [25] and [26], that used protective chemicals (polyethylene) to effectively control compositional changes by delaying the ripening process, leading to minimum quality loss above 3 weeks of storage at ambient temperature.

The increased shelf life of fruit could be due to the presence of physical barrier in gas diffusion through fruit stomata by which gas exchange takes place between internal tissues and external atmosphere. The lack of diffusion reduced respiratory rate and loss of water, thereby resulting in increased shelf life. These results support the works [11] and [27], which demonstrated the inhibition of fungal growth by plant extracts on fruits surface.

#### 4. Conclusion

Wrapping-treated citrus fruit with extracts of plant led to increasing shelf life and improving fruit quality under room condition ( $25 \pm 2$ ) storage. Modified

atmosphere packaging using biodegradable materials can be as a potential to replace commercial chemicals for citrus packaging with more future researches.

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