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Influence of Natural Extracts Dipping to Maintenance Fruit Quality and Shelf Life of Egyptian Guava

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ABSTRACT

Guava fruits are considered sensitive to low temperature and susceptible to infection by decay causing fungi making its transport and storability difficult. Use of plant extracts which having fungicidal and insecticidal properties is one of the alternative methods against fungi causing guava decline disease caused by *Phomopsis psidii*, *Lasiodiplodia theobroma* and *Epicoccum sp*p. Therefore, the present investigations were conducted during 2016 and 2017 seasons on guava fruits cv. Bassateen El Sabahia for maintaining quality and control disease development under cold and ambient conditions. The guava fruits were harvested at physiological maturity stage during the third week of January and treated with marigold flower extract, neem extract and mentha leaf extract at (10%) as dip treatment for 5 minutes. (Experiment 1).Treated fruits were packed in ventilated Corrugated Fibre Board (CFB) boxes and stored 28 days under cold storage at 6° C ± 2° and humidity 90-95% R.H. (Experiment 2). The fruits were stored for 6 days in ambient conditions at 15° C ± 2° with 65 – 70 % R.H. Under both of the storage conditions, all treatments exhibit potential to maintain the postharvest life of fruits by reducing linear growth and dry weight of the fungal spoilage as compared with untreated fruits. The lowest significant values of disease infection percentage were recorded by dipping fruits in neem leaf extract at 10% after 28 days of cold storage and 6 days at marketing in the two seasons. Among all extracts, neem in combination with (CFB) boxes effective in registered higher potential to retain the fruit quality for longer period under both conditions by reducing the weight loss, fruit spoilage and retaining the fruit firmness and quality attributes such as TSS, sugars, vitamin C and pectin. On the other hand, 10% Mentha leaf extract followed by marigold flower extract treatments proved to be highly effective in reducing spoilage under these treatments with respect to the untreated ones. Generally, the promising effects of these treatments showed that the winter season guava fruits can be stored up to 28th day under cold storage and up to 6th day under ambient condition without deteriorating the fruit quality.

Keywords: guava, marigold flower extract, neem extract, mentha leaf extract, post-harvest and marketing.

INTRODUCTION

Guava (*Psidium guajava* L.) is a productive and remunerative crop grown commercially in sub-tropical and tropical regions. Guava fruit is an excellent source of vitamin C (228.3 mg/100 g) (Thaipong *et al.,* 2006), containing about 17 % dry matter and 80 % moisture along with appreciable amount of minerals such as phosphorus, calcium, iron as well as vitamins ,niacin, pantothenic acid, thiamin and riboflavin (Seema, *et al*., 2015). In Egypt guava occupy about 38000 feddan, yielded about 314000 ton as annual fruit production with an exported range about 16.312.38 metric tons to many countries according to the statistics of Ministry of Agriculture and Land Reclamation (2013). In Egypt, by using some agricultural practices guava trees can produce fruits in winter season. Most of winter productions of guava fruits are exported to other countries, so improving productivity and fruit quality is important issue to gain more commercial advantages for growers. Besides, guava fruits are desired to local market and aboard in winter. Guava exports from Egypt are increased through air flight as the main transport system. The limiting factor for export is the high costs that reduce the profit level to the grower. Refrigeration appears to be a suitable way for sea transport. Guava fruits are highly perishable, susceptible to mechanical damage and chilling injury and require strategies to enhance postharvest shelf life for extended fresh fruit market period. Moreover, guava fruit is sensitive to susceptible infection by decay causing fungi making its transport and storability difficult.

The common postharvest fungi causing decay of guava fruits are *Phomopsis spp., Alternaria spp.*, *Epicoccum spp., Lasiodiplodia spp and Penicillium spp.* (Ammar, and El-Naggar, 2014). Inhibiting the growth of fungi has great significance in handling fruits, but in recent years, the use of synthetic fungicides has increased consumer concern and their use is becoming more restrictive due to carcinogenic effects, residual toxicity problems, environmental pollution, occurrence of microbial resistance (Marín *et al.,* 2003).

Use of plant extracts such as neem leaf, mentha leaf extracts and marigold flower extracts having fungicidal and insecticidal properties is one of the alternative methods. However, these extracts are residue free and safe from consumption point of view as compared to fungicides that are highly toxic to living beings. These extracts have a number of active ingredients that help in checking decay losses in fruits that are caused by the fungal infection (Anjum *et al.*, 2015). Corrugated Fibre Boxes (CFB) as packaging material have many advantages such as light weight, low cost, are easy to design, process and print and reduces mechanical damage. Also, they are easy to recycle, non-polluting and reusable (Bhuvaneswari *et al.*, 2017).

The present investigation aimed to study the suitability of use Corrugated Fibre Board (CFB) boxes and plant extracts such as marigold flower extract, neem extract and mentha leaf extract which having fungicidal and insecticidal properties as post-harvest treatments for prolonging the cold storage period and shelf life of guava fruits cv. Bassateen El Sabahia .

MATERIALS AND METHODS

Pathogen isolation and identification :

Phomopsis psidii, *Lasiodiplodia theobroma* and *Epicoccum sp*p. were isolated from naturally infected guava fruits cv. Bassateen El Sabahia. These isolates were the most aggressive in our collection and produced the largest lesions on inoculated fruits. These funguses were purified and maintained on Potato Dextrose Agar (PDA) and stored at 4°C, with periodic transfers through citrus fruits to maintain its aggressiveness. Guava fruits were ready for examination under a stereoscopic binocular microscope (6-50 x) for the presence of funguses and to study their habits characters. When necessary the compound microscope was used for

confirming the identification after having examined the morphology of conidia and conidiophores. Funguses presented on infested seeds were identified by means of comparison with the description sheets of Commonwealth Mycological Institute, Kew, Surrey, England (CMI), Danish Government Institute of Seed Pathology (DGISP) publications as well as publication of Singh *et al.,* 1991.

Effect of marigold flower extract, neem extract and mentha leaf extract on growth of fungi isolated from guava fruits cv. Bassateen El Sabahia and percentage of disease infection

a- Linear growth: Plant extracts were tested *in vitro* on the linear growth of the pathogenic fungi. Different concentrations were added to 10 mL of sterilized PDA before solidification and then poured in sterile Petri-dishes. After solidification, the plates were inoculated with fungal disc (5 mm) in the center of the plate and incubated at 27±1°C. Three plates for each treatment for each fungus were used as replicates; three plates were prepared to serve as control for each fungus. Linear growth was observed daily and diameter of fungal colonies were recorded when plates of any treatment were filled with the fungal growth.

b- Dry weight: One hundred mL of liquid PD medium in 250 mL Erlenmeyer flasks were amended with different concentrations of the tested compounds after autoclaving. Each flask was inoculated using two discs of 0.6 mm in diameter of fungal culture and then incubated at 20±2°C for 7 days. Control flasks contain no concentrations of these compounds. Three replicates were used for each concentration. At the end of incubation period, the mycelium was filtered off and washed several times with distilled water, then dried in an oven at 80°C for 48 h till constant weight (El-Morsy, 1993).

Field study:

The present investigations were conducted during 2016 and 2017 seasons on guava trees cv. Bassateen El Sabahia located in a private orchard at Cairo-Alexandria desert road, Egypt. The guava trees were 7-years-old grown in sandy soil, planted 4x5 meters apart under drip irrigation system. The selected trees almost were uniform and apparently diseases free. The guava fruits were harvested at physiological maturity stage when color changed from dark green to light green (Yusof *et al*., 1988) during the third week of January. The fruits equal in size and apparently insect and pathogen injury free transported directly to the Laboratory and infected fruits were sorted. Thereafter, samples of 15 fruits were taken in the beginning of the experiment to determine the initial fruits properties. Fruits were dipped for 5 minutes in various plant extracts and packed for storage as shown in Table (1).

Table 1. Treatments examined

NO	Treatments used
1	Control (dipping fruit with tap water) + Packed fruits in (CFB) boxes (5% ventilation)
2	Dipping fruits with marigold flower extract at 10%
3	Dipping fruits with marigold flower extract at 10% + Packed fruits in (CFB) boxes
4	Dipping fruits with neem extract at 10%
5	Dipping fruits with neem extract at 10% + Packed fruits in (CFB) boxes
6	Dipping fruits with menthe leaf extract at 10%
7	Dipping fruits with menthe leaf extract at 10% + Packed fruits in (CFB) boxes

Treatment solutions were prepared from leaf extracts of neem, mentha and flower of marigold at different concentrations on percentage weight basis as described by Gahukar (1996). Leaves and flowers were dried in shade and then ground to fine powder in an electric blender. The extract was prepared by adding 100 ml of distilled water to 100 g of leaf or flower powder and kept overnight. This gave 100% concentration of the plant extract (1:1 w/v). Aqueous solution of different treatments was prepared by diluting the extracts to 10% using distilled water which was passed through two layers of muslin cloth. The surfactant super film as a wetting agent was added at the rate of 40 cm/100 L water to all dipping solutions in order to obtain best penetration results.

Experiment 1:

For storage study, treated fruits were packed in ventilated Corrugated Fibre Board (CFB) boxes and stored 28 days at 6°C ±2 with 90 – 95 % R.H. and then the fruits were taken 14 days intervals to determine fruits characteristics.

Experiment 2:

For marketing study, fruits for each treatment were packed in ventilated Corrugated Fibre Board (CFB) boxes and held 6 days at room temperature conditions at 15°C ± 2 with 65 – 70 % R.H., and the fruits were taken at 3 and 6 days intervals to determine the following parameters:

Disease infection: It was calculated according to the next equation:

$$\text{Disease infection\%} = \frac{\text{No. of natural infected fruits}}{\text{No. of total fruits}} X100$$

Physical and chemical characteristics measured during storage period:

1- Loss in fruit weight: It was calculated according to the next equation:

$$\text{Loss in fruit weight \%} = \frac{\text{Initial weight- weight at sampling date}}{\text{Initial fruit weight}} x100$$

2– Decay: It was calculated according to the next equation:

$$\text{Decay \%} = \frac{\text{Weight of decayed fruits}}{\text{Initial fruit weight}} x100$$

3- Fruit firmness: It was measured on 10 fruits for each replicate by using a hand Effegi-Penetrometer supplemented with a plunger 8.1 mm. The average was estimated as lb inch-2 (AOAC, 2005).

4- Total soluble solids (TSS %): Total soluble solids in fruit juice were measured using a Carl-Zeiss hand refractometer (AOAC, 2005).

5- Total titratable acidity (TA %): It was determined in fruit juice by titration using 0.1 N sodium hydroxide and calculated as citric acid (AOAC, 2005).

6- Total sugar %: The extract was prepared by taking 0.5 g of fresh pulp and extracting with 80% ethanol according to (Rangana, 1979).

7- Ascorbic acid (mg/100 g fresh weight): Ascorbic acid (vitamin C) was measured by the oxidation of ascorbic acid with 2, 6-dichlorophenol endophenol dye and the

results were expressed as mg/100 g fresh weight according to Ranganna, (1979).

8- Total pectin (g/100 g fresh weight): Levels of pectic substances in guava fruits were determined colorimetrically by carbazole sulfuricm acid method according to the method of Reitmier and Lore (1996). Results were expressed as g. anhydro galacturonic acid (A.G .A) per 100 gm on dry weight basis.

Statistical analysis: Data of both seasons were statistically analyzed as a randomized complete design by analysis of variance (ANOVA) technique procedure of CoStat v6.4 program. Differences among treatment means were compared by the Duncan's multiple range tests at $p<0.05$ level of probability in the two investigated seasons (Snedecor and Cochran, 1980).

RESULTS AND DISCUSSION

Influence of marigold flower extract, neem extract and mentha leaf extract on linear growth (cm), dry weight (g) of fungus isolated from guava fruits and disease infection % as postharvest treatments:

Results in Table (2) showed the effect of dipping fruits in natural extracts on linear growth and dry weight of Phomopsis psidii, Lasiodiplodia theobroma and Epicoccum spp. isolated from guava fruits cv. Bassateen El Sabahia. It was noticed that all treatments using (in vitro or in vivo) showed significant suppression of the radial growth and the biomass of guava decline fungi than the control. Moreover, the linear growth and dry weight of all pathogenic fungi were completely inhibited due to the treatment of neem extract at 10 % + (CFB) boxes.

Data in Table (3) presented the disease infection at cold storage and 6 days during marketing. The data revealed that, Phomopsis psidii was found to be the most dominant fungi on all samples as% frequency of isolated fungi. In this respect, as storage period advanced the disease infection decreased under cold storage and during marketing at room temperature. Thus, all the applied treatments reduced the disease infection than the control. Since, the percent of disease infection of the untreated fruits were 12.93 and 10.29 % after 28 days of cold storage and reached 15.28 and 13.18 % as marketing in the both seasons, respectively. Dipping fruits at neem extract 10 % + packed in (CFB) boxes gave the lowest significant value of disease infection percentage of Phomopsis psidii 0.00 % in the first and second seasons after 28 days of cold storage and 6 days of marketing in both seasons.

Table 2. Influence of applied treatments on linear growth (cm) and dry weight (g) of fungi isolated from guava fruits

Treatments	*Phomopsis psidii*		*Lasiodiplodia theobroma*		*Epicoccum spp.*	
	Linear Growth (cm)	Dry weight (g)	Linear Growth (cm)	Dry weight (g)	Linear Growth (cm)	Dry weight (g)
Control	9.62 a	1.70 a	9.61 a	1.80 a	9.63 a	1.77 a
Marigold flower extract at 10%	3.10 b	0.78 b	3.00 b	0.70 b	3.15 b	0.66 b
Marigold flower extract at 10% + (CFB) boxes	2.96 d	0.38e	2.90c	0.36 e	2.85 d	0.33 e
Neem extract at 10%	1.80 f	0.41d	1.87 e	0.40 c	1.90 f	0.39c
Neem extract at 10% + (CFB) boxes	0.00 g	0.00 d	0.00 f	0.00 g	0.00 g	0.00 g
Menthe leaf extract at 10%	2.98 c	0.44 c	2.99 b	0.30 f	2.91 c	0.31 f
Menthe leaf extract at 10% + (CFB) boxes	2.00 e	0.44c	2.07d	0.39 d	2.11 e	0.37 d

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

Table 3. Influence of applied treatments on disease infection percentage of guava fruits caused by blossom end rot (*Phomopsis psidii*) at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
Season 2016						
Control	0.00k	4.52e	12.93a	0.00 l	6.14c	15.28a
Marigold flower extract at 10%	0.00k	2.17i	4.60c	0.00 l	4.02h	5.17b
Marigold flower extract at 10% + (CFB) boxes	0.00k	0.00k	7.69b	0.00 l	2.52i	4.90e
Neem extract at 10%	0.00k	2.95h	4.10g	0.00 l	2.35j	4.98d
Neem extract at 10% + (CFB) boxes	0.00k	0.00k	0.00k	0.00 l	0.00 l	0.00 l
Menthe leaf extract at 10%	0.00k	2.14j	4.54d	0.00 l	2.30k	4.21g
Menthe leaf extract at 10% + (CFB) boxes	0.00k	0.00k	4.33f	0.00 l	0.00 l	4.73f
Season 2017						
Control + (CFB) boxes	0.00 l	3.96 g	10.29 a	0.00 j	5.29 d	13.18a
Marigold flower extract at 10%	0.00 l	2.08 k	4.34 d	0.00 j	2.03 i	4.52e
Marigold flower extract at 10% + (CFB) boxes	0.00 l	2.58 i	4.71 b	0.00 j	3.45g	7.90 c
Neem extract at 10%	0.00 l	2.96 h	4.10f	0.00 j	4.02 f	9.17 b
Neem extract at 10% + (CFB) boxes	0.00 l	0.00 l	0.00 l	0.00 j	0.00 j	0.00 j
Menthe leaf extract at 10%	0.00 l	2.15 j	4.54c	0.00 j	2.30 h	4.62e
Menthe leaf extract at 10%+ (CFB) boxes	0.00 l	0.00 l	4.13e	0.00 j	0.00 j	4.50e

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

Influence of marigold flower extract, neem extract and mentha leaf extract under cold storage and through marketing at room temperature on guava fruits characteristics:

1- Fruit loss in weight percentage:

Data in Table (4) presented the effect of cold storage and 6 days during marketing on loss in weight of guava fruits cv. Bassateen El Sabahia. Data cleared that, as storage period advanced the loss in fruit weight increased under cold storage and in the second experiment, at room temperature. Thus, loss in fruit weight decreased under all the dipping treatments compared to control. Since, the percent of loss in fruit weight of the untreated fruits were 9.53 and 9.23 % after 28 days of cold storage and it was 9.62 and 9.96 % in

the second experiment, after 6 days at marketing in both seasons, respectively. Dipping fruit at neem extract at 10% + packed in (CFB) boxes gave the lowest significant values of loss in weight percentage which ranged 4.14 and 6.58 % after 28 days of cold storage and it were 4.94 and 7.30 % after 6 days of marketing in both seasons, respectively.

Table 4. Influence of applied treatments on weight loss percentage of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
	Season 2016					
Control	0.00l	7.21c	9.53a	0.00k	7.92b	9.62a
Marigold flower extract at 10%	0.00l	5.95g	7.47b	0.00k	6.85e	7.94c
Marigold flower extract at 10%+ (CFB) boxes	0.00l	5.20h	7.20c	0.00k	6.60g	7.34d
Neem extract at 10%	0.00l	4.50j	6.58e	0.00k	6.16h	6.76f
Neem extract at 10%+ (CFB) boxes	0.00l	4.14k	6.25f	0.00k	5.91j	6.58g
Menthe leaf extract at 10%	0.00l	4.66i	6.96d	0.00k	6.23h	6.91e
Menthe leaf extract at 10%+ (CFB) boxes	0.00l	4.18k	6.33f	0.00k	6.05i	6.74f
	Season 2017					
Control + (CFB) boxes	0.00 l	7.88 b	9.23a	0.00 i	8.35b	9.96a
Marigold flower extract at 10%	0.00 l	6.22g	7.66c	0.00 i	6.55e	7.98c
Marigold flower extract at 10%+ (CFB) boxes	0.00 l	5.69h	7.33d	0.00 i	6.18f	7.80c
Neem extract at 10%	0.00 l	5.16ij	6.98ef	0.00 i	5.25h	7.38d
Neem extract at 10%+ (CFB) boxes	0.00 l	4.94k	6.87f	0.00 i l	5.06h	7.30d
Menthe leaf extract at 10%	0.00 l	5.21i	7.03e	0.00 i	5.49g	7.41d
Menthe leaf extract at 10%+ (CFB) boxes	0.00 l	5.06j	6.91f	0.00 i	5.10h	7.23d

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

2- Decay percentage:

All dipping treatments significantly decreased the percent of decayed fruits than the untreated fruits either after 28 days of cold storage or 6 days during marketing in the second experiment at room temperature in the both seasons as shown in Table (5). So, the percent of decayed fruits for the control were 36.80 and 38.26 % after 28 days of cold storage, but it reached about 37.73 and 38.33 % through marketing in the second experiment in both seasons. Furthermore, dipping Bassateen El Sabahia guava fruits at neem extract at 10% + packed in (CFB) boxes significantly inhibit any damaged fruits than all treatments used either after 28 days of cold storage or during marketing in both seasons, respectively.

Table 5. Influence of applied treatments on decay percentage of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
	Season 2016					
Control	0.00g	20.08c	36.80a	0.00g	11.48c	37.73a
Marigold flower extract at 10%	0.00g	12.03d	20.40b	0.00g	6.00d	12.07b
Marigold flower extract at 10%+ (CFB) boxes	0.00g	11.78d	19.85c	0.00g	5.64e	11.96b
Neem extract at 10%	0.00g	4.80f	11.35e	0.00g	0.00g	5.08f
Neem extract at 10%+ (CFB) boxes	0.00g	0.00g	0.00g	0.00g	0.00g	0.00g
Menthe leaf extract at 10%	0.00g	4.88f	11.80d	0.00g	5.03f	11.46c
Menthe leaf extract at 10%+ (CFB) boxes	0.00g	0.00g	4.91f	0.00g	0.00g	5.16f
	Season 2017					
Control + (CFB) boxes	0.00 h	25.73b	38.26a	0.00 g	12.31b	38.33a
Marigold flower extract at 10%	0.00 h	15.12d	20.28c	0.00 g	6.11d	12.25b
Marigold flower extract at 10%+ (CFB) boxes	0.00 h	12.99e	20.16c	0.00 g	5.42e	11.96c
Neem extract at 10%	0.00 h	5.13g	12.80ef	0.00 g	0.00g	5.10f
Neem extract at 10%+ (CFB) boxes	0.00 h	0.00 h	0.00 h	0.00 g	0.00g	0.00g
Menthe leaf extract at 10%	0.00 h	5.10g	12.58f	0.00 g	5.10f	12.24b
Menthe leaf extract at 10%+ (CFB) boxes	0.00 h	0.00 h	5.03g	0.00 g	0.00g	5.23ef

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

3-Firmness (lb inch^{-2}):

It is clear from Table 6 that, as storage period advanced fruit firmness decreased under cold storage or during marketing at room temperature. Also, the data revealed that fruit firmness significantly reduced at all treatments used than the control at cold storage or marketing in the second experiment at room temperature through the two seasons. Thus, fruit firmness for the control treatment was 6.78 and 6.80 lb inch-^{2} after 28 days of cold storage, but it reached about 6.06 and 6.49 lb inch-^{2} through marketing in both seasons. In addition, application with neem extract at 10% + packed in (CFB) boxes gave a higher fruit firmness (9.03 and 9.01 lb inch-^{2}) after 28 days of cold storage while, reached 8.53 and 8.96 lb inch-^{2} from fruits held 6 days at room temperature in the second experiment of the two seasons, respectively.

4- Total soluble solids (TSS %):

Table 7 shows that total soluble solids in fruit juice of guava was gradually increased as storage period prolonged either after cold storage or during shelf life at room temperature. Since, all treatments gave significance higher values of TSS in fruit juice than the untreated fruits which ranged 11.10 and 10.33 % after 28 days of cold storage and it were 10.90 and 9.82 % after six days as marketing in both seasons under the study. Furthermore, treated fruits with neem extract at 10% + packed in (CFB) boxes showed a higher juice TSS (11.90 and 10.89%) after 28 days of cold storage while, reached 12.20 and 10.45% from fruits held 6 days at room temperature in the second experiment of the two seasons, respectively.

Table 6. Influence of applied treatments on firmness (lb inch^{-2}) of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
	Season 2016					
Control	10.20 a	8.04 f	6.78i	10.20 a	8.31 f	6.06 i
Marigold flower extract at 10%	10.20 a	8.90 de	7.34 h	10.20 a	9.03 d	7.70 h
Marigold flower extract at 10%+ (CFB) boxes	10.20 a	9.03 cd	7.50 g	10.20 a	9.03 d	7.75 h
Neem extract at 10%	10.20 a	9.83 b	8.82 e	10.20 a	9.56 bc	8.33 f
Neem extract at 10%+ (CFB) boxes	10.20 a	9.86 b	9.03 cd	10.20 a	9.70 b	8.53e
Menthe leaf extract at 10%	10.20 a	9.10 c	8.80 e	10.20 a	9.44 c	8.03 g
Menthe leaf extract at 10%+ (CFB) boxes	10.20 a	9.88 b	8.88 de	10.20 a	9.60 b	8.43 ef
	Season 2017					
Control + (CFB) boxes	11.20 a	8.95 d	6.80 g	11.20 a	9.24 f	6.49 k
Marigold flower extract at 10%	11.20 a	9.81 c	7.80 f	11.20 a	10.08 e	7.63 j
Marigold flower extract at 10%+ (CFB) boxes	11.20 a	10.03 c	7.90 f	11.20 a	10.37 d	7.71 j
Neem extract at 10%	11.20 a	10.61 b	8.86 de	11.20 a	10.55 cd	8.69 hi
Neem extract at 10%+ (CFB) boxes	11.20 a	10.76 b	9.01 d	11.20 a	10.80 b	8.96 g
Menthe leaf extract at 10%	11.20 a	10.60 b	8.76 de	11.20 a	10.39 d	8.60 i
Menthe leaf extract at 10%+ (CFB) boxes	11.20 a	10.66 b	8.68 e	11.20 a	10.66 bc	8.85 gh

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

Table 7. Influence of applied treatments on TSS % of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
	Season 2016					
Control	8.36i	9.23gh	11.10d	8.36i	8.93h	10.90d
Marigold flower extract at 10%	8.36i	9.16h	11.40c	8.36i	9.06gh	11.00d
Marigold flower extract at 10%+ (CFB) boxes	8.36i	9.43efg	11.36c	8.36i	9.16g	11.03d
Neem extract at 10%	8.36i	9.63e	11.73ab	8.36i	9.53f	11.73b
Neem extract at 10%+ (CFB) boxes	8.36i	9.50ef	11.90a	8.36i	9.80e	12.20a
Menthe leaf extract at 10%	8.36i	9.30fgh	11.33cd	8.36i	9.36f	11.33c
Menthe leaf extract at 10%+ (CFB) boxes	8.36i	9.13h	11.50bc	8.36i	9.53f	11.90b

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels.

	Season 2017					
Control + (CFB) boxes	7.03k	8.26j	10.33d	7.03j	8.11i	9.82d
Marigold flower extract at 10%	7.03k	8.40i	10.45c	7.03j	8.31h	9.86d
Marigold flower extract at 10%+ (CFB) boxes	7.03k	8.52h	10.61b	7.03j	8.41h	10.00c
Neem extract at 10%	7.03k	8.64fg	10.81a	7.03j	8.67fg	10.15b
Neem extract at 10% + (CFB) boxes	7.03k	8.78e	10.89a	7.03j	8.88e	10.45a
Menthe leaf extract at 10%	7.03k	8.58gh	10.68b	7.03j	8.59g	10.11bc
Menthe leaf extract at 10% + (CFB) boxes	7.03k	8.75ef	10.86a	7.03j	8.77ef	10.36a

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

5-Titratable acidity (TA %):

It is obvious from Table 8 that progress in storage period from harvest till 28 day at cold storage or during marketing at room temperature induced an increase in percent of total acidity in fruit juice. Since, the control fruits gave significance higher values of total acidity in fruit juice than the all treatments. Thus, the percent of total acidity in fruits juice for the control were 0.147 and 0.151 % after 28 days of cold storage, but it reached about 0.143 and 0.147 % through marketing in the second experiment in both seasons.

6-Total sugar (%):

Data in Table 9 indicated that total sugar percent in fruit juice of guava fruits was gradually increased as storage period prolonged either after cold storage or during marketing at room temperature.

Since, the untreated fruits produced lower values of total sugar in the fruits than all treatments which ranged 7.01 and 6.53 % after 28days of cold storage and it were 6.51 and 6.25 % after six days as marketing in the second experiment during both seasons under the study. The data also disclose that, treated fruits with neem extract at 10% + packed in (CFB) boxes presented higher values of total sugars compare to the applied treatments averaged 7.73 and 7.20 % after 28 days of cold storage and it were 7.23 and 6.93 % after 6 days as marketing in both seasons, respectively.

7- Vitamin C mg/100 ml juice:

Data in Table (10) revealed that, vitamin C contents increased gradually during cold storage. Since, the higher pronounced values of vitamin C were found in fruits treated with neem extract at 10% + packed in (CFB) boxes, compare to the applied treatments averaged 131.83 and 127.13 mg/100 ml juice after 28 days of cold storage and it were 137.16 and 130.13 % after 6 days as marketing in both seasons, respectively.

8-Total pectin content (g /100 g fresh weight):

Data in Table (11) clear that, all applied treatments increased total pectin% than the untreated ones. However, total pectin% increased with the advance in storage period. Data showed that fruits treated with neem extract at 10% + packed in (CFB) boxes, gave higher pectin (1.21 and 1.39g/100 g f.w.) after 28days of cold storage and it were 1.10 and 1.28 g/100 g fresh weight) after 6 days as marketing during both seasons , respectively under the study. On the other hand, untreated fruits exhibited the least value in both seasons.

Table 8. Influence of applied treatments on acidity % of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
	Season 2016					
Control	0.105k	0.115i	0.147a	0.105e	0.103e	0.143a
Marigold flower extract at 10%	0.105k	0.120h	0.133ef	0.105e	0.108e	0.135b
Marigold flower extract at 10% + (CFB) boxes	0.105k	0.107jk	0.137cd	0.105e	0.120cd	0.134b
Neem extract at 10%	0.105k	0.120h	0.140bc	0.105e	0.117d	0.123cd
Neem extract at 10% + (CFB) boxes	0.105k	0.110j	0.130f	0.105e	0.103e	0.125c
Menthe leaf extract at 10%	0.105k	0.122gh	0.142b	0.105e	0.122cd	0.122cd
Menthe leaf extract at 10% + (CFB) boxes	0.105k	0.125g	0.134de	0.105e	0.109e	0.116d
	Season 2017					
Control + (CFB) boxes	0.097h	0.104g	0.151a	0.097j	0.108i	0.147a
Marigold flower extract at 10%	0.097h	0.118f	0.131cd	0.097j	0.124def	0.131c
Marigold flower extract at 10% + (CFB) boxes	0.097h	0.125e	0.128cde	0.097j	0.121efg	0.129cd
Neem extract at 10%	0.097h	0.123ef	0.124ef	0.097j	0.114h	0.119g
Neem extract at 10% + (CFB) boxes	0.097h	0.143b	0.134c	0.097j	0.136b	0.120fg
Menthe leaf extract at 10%	0.097h	0.122ef	0.132cd	0.097j	0.117gh	0.125de
Menthe leaf extract at 10% + (CFB) boxes	0.097h	0.123ef	0.126de	0.097j	0.131c	0.121efg

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

Table 9. Influence of applied treatments on total sugar % of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
	Season 2016					
Control	5.60g	5.80f	7.01c	5.60h	5.75g	6.51e
Marigold flower extract at 10%	5.60g	5.90ef	7.12c	5.60h	5.88f	6.61de
Marigold flower extract at 10% + (CFB) boxes	5.60g	6.01e	7.15c	5.60h	5.89f	6.70f
Neem extract at 10%	5.60g	6.00e	7.56b	5.60h	5.93f	7.03b
Neem extract at 10% + (CFB) boxes	5.60g	6.20d	7.73a	5.60h	6.00f	7.23a
Menthe leaf extract at 10%	5.60g	6.06de	7.40b	5.60h	5.87f	6.85c
Menthe leaf extract at 10% + (CFB) boxes	5.60g	6.06de	7.45b	5.60h	5.95f	7.11b
	Season 2017					
Control + (CFB) boxes	5.10k	5.70j	6.53f	5.10l	5.60k	6.25de
Marigold flower extract at 10%	5.10k	5.86i	6.76e	5.10l	5.66jk	6.36cd
Marigold flower extract at 10% + (CFB) boxes	5.10k	5.95hi	6.81de	5.10l	5.77ij	6.47c
Neem extract at 10%	5.10k	6.06gh	7.05bc	5.10l	5.85hi	6.71b
Neem extract at 10% + (CFB) boxes	5.10k	6.15g	7.20a	5.10l	6.13ef	6.93a
Menthe leaf extract at 10%	5.10k	6.03gh	6.93cd	5.10l	6.03fg	6.61b
Menthe leaf extract at 10%+ (CFB) boxes	5.10k	6.11g	7.14ab	5.10l	5.96gh	6.85a

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

Table 10. Influence of applied treatments on vitamin C mg/100 ml juice of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
	Season 2016					
Control	79.70 i	86.60h	109.60e	97.70l	87.86k	114.36e
Marigold flower extract at 10%	79.70 i	86.63h	114.36d	97.70l	88.46jk	128.00d
Marigold flower extract at 10% + (CFB) boxes	79.70 i	87.50h	114.70d	97.70l	89.13ij	129.66c
Neem extract at 10%	79.70 i	89.50g	128.96b	97.70l	89.66hi	135.13b
Neem extract at 10% + (CFB) boxes	79.70 i	90.73f	131.83a	97.70l	92.93f	137.16a
Menthe leaf extract at 10%	79.70 i	89.00g	115.86c	97.70l	90.33gh	136.33a
Menthe leaf extract at 10% + (CFB) boxes	79.70 i	89.70g	128.56b	97.70l	90.73g	13646a
	Season 2017					
Control + (CFB) boxes	75.80j	83.56i	103.10f	75.80h	84.43g	118.00d
Marigold flower extract at 10%	75.80j	84.86h	120.30e	75.80h	85.06fg	127.33c
Marigold flower extract at 10% + (CFB) boxes	75.80j	84.86h	120.83d	75.80h	85.66f	128.10b
Neem extract at 10%	75.80j	86.63g	125.23c	75.80h	86.66e	128.33b
Neem extract at 10% + (CFB) boxes	75.80j	86.80g	127.13a	75.80h	86.86e	130.13a
Menthe leaf extract at 10%	75.80j	86.40g	124.76c	75.80h	86.50e	128.00bc
Menthe leaf extract at 10% + (CFB) boxes	75.80j	86.80g	126.26b	75.80h	86.50e	129.63a

No significant differences between means followed by same letters by Duncan multiple range test at 0.05 levels.

DISCUSSION

Guava (*Psidium guajava* L.) is very important fruit crop of the subtropical as well as tropical regions in the world. It is usually known as 'Apple of Tropics'. Although, it is very rich nutritionally, still world trade of guava fruits is limited owing to its highly perishable nature, limited post-harvest life and susceptibility to chilling injury (Rai *et al*., 2010). Due to perishable nature, under ambient conditions the fruits of guava become overripe and mealy within few days, while, under cold storage, the shelf life can be extended up to four weeks at 6-8°C and 90-95% RH. Physiological loss in weight irrespective of treatments was increased during the storage period. The loss in weight of fresh

horticultural crops is mainly due to transpiration water loss (Zhu *et al*., 2008).

Considerable postharvest losses of fruit are brought by decay caused by fungal plant pathogens. Hence inhibiting the growth of fungi has great significance in postharvest fruit, but in recent years, the use of synthetic fungicides has increased consumer concern and their use is becoming more restrictive due to carcinogenic effects, residual toxicity problems, environmental pollution, occurrence of microbial resistance, and high inputs (Rial-Otero *et al*., 2005).

Table 11. Influence of applied treatments on Total pectin (g /100 g fresh weight) of guava fruits at cold storage and under market conditions during 2016 and 2017 seasons

Treatments	Cold storage (days)			Market condition (days)		
	0	14	28	0	3	6
Season 2016						
Control	0.78g	0.81efg	0.97c	0.78j	0.78j	0.93f
Marigold flower extract at 10%	0.78g	0.80fg	1.04b	0.78j	0.80ij	0.98e
Marigold flower extract at 10% + (CFB) boxes	0.78g	0.86de	1.05b	0.78j	0.83h	1.01de
Neem extract at 10%	0.78g	0.89d	1.20a	0.78j	0.86gh	1.06bc
Neem extract at 10% + (CFB) boxes	0.78g	0.89d	1.21a	0.78j	0.88g	1.10a
Menthe leaf extract at 10%	0.78g	0.86de	1.08b	0.78j	0.85gh	1.04cd
Menthe leaf extract at 10% + (CFB) boxes	0.78g	0.85def	1.06b	0.78j	0.83hi	1.09ab
Season 2017						
Control + (CFB) boxes	0.92k	0.95jk	1.08gh	0.92h	0.95gh	1.03e
Marigold flower extract at 10%	0.92k	0.97ij	1.15ef	0.92h	0.96gh	1.09cd
Marigold flower extract at 10% + (CFB) boxes	0.92k	1.01i	1.20d	0.92h	0.96gh	1.11cd
Neem extract at 10%	0.92k	1.11fg	1.32b	0.92h	0.98fg	1.25a
Neem extract at 10% + (CFB) boxes	0.92k	1.16de	1.39a	0.92h	1.08d	1.28a
Menthe leaf extract at 10%	0.92k	0.99ij	1.16def	0.92h	1.04e	1.12c
Menthe leaf extract at 10% + (CFB) boxes	0.92k	1.05h	1.25c	0.92h	1.01ef	1.19b

Means followed by the same letters are not significantly different by Duncan multiple range test at 0.05 levels.

Use of plant extracts having fungicidal and insecticidal properties is one of the alternative methods. The treatment of fruits with extracts of neem, chinaberry, and marigold had a significant influence on specific gravity during the period of storage. The maintenance of highest specific gravity with neem extract could be attributed to its ability to check the growth of microbes that were responsible for increasing the metabolic rate of commodities and loss in weight through respiration Application of plant extracts reduced spoilage substantially but it increased as the storage period advanced (Suresh *et al*,. 2008). Reduction in spoilage due to rotting with neem extract may be attributed to the presence of the principle compound azadirachtin, which has the ability to check the growth of microbes that are responsible for causing spoilage (Malik *et al*., 2016). Reduction in spoilage due to rotting with neem extract may be attributed to the presence of the principle compound azadirachtin, which has the ability to check the growth of microbes that are responsible for causing spoilage (Okigbo *et al*., 2010). The problem of fruit growers and handlers may be solved by adopting packaging material like CFB boxes along which reduces mechanical damage to fruit during cold storage and marketing.

The fruit firmness is an important physical parameter to monitor the ripening progress. Cold storage condition exhibited slow rate of loss of fruit firmness as compared to ambient conditions. The retention of firmness which occurred during storage could be explained by retarded degradation of insoluble proto pectin to the more soluble pectic acid and pectin. During fruit ripening depolymerization or shortening of chain length of pectin substances occurs with an increase in pectin esterase and polygalactronase activities (Yaman and Bayoindirli, 2002). Increment in TSS was registered in guava fruits up to 28 days under cold and up to 6 days under ambient conditions. This increase in TSS in fruits might be due to reduction of the activities of various enzymes and by delaying the senescence, disorganization of cellular structure and checking of microbial activities (Kaur *et al*., 2017). The total sugars of the guava fruits increases till the end of the storage period. The increase in total sugars in all treatments under cold storage was probably due to dehydration, because fruits exhibited highest physiological losses in weight in most of the treatments resulting higher proportion of total sugars. Vitamin C content increased of guava fruits with progress in ripening might be due to the breakdown of starch to glucose which increases the biosynthesis of ascorbic acid (Seema, *et al*., 2015). Azadirchtin is considered as the most active principle substance in neem which has growth regulating, fungicidal and insecticidal properties. Also, it has minimal impact on non-target organisms and is compatible with other eco-friendly biocontrol agents. Retention of better firmness in Neem extract treated fruits can be attributed to the direct effect of azadirchtin, a principle active compound presented in neem formulations, on pectin molecules which are believed to regulate the calcium and pectin integrity, thereby lowering chances of its breakdown during storage (Chauhan *et al*., 2008).

CONCLUSION

Plant extracts are being used to manufacture natural or bio insecticides, which are environmental friendly and do not have any toxic effects on fruits. So these can be used as compare to synthetic chemical products. Among these extracts neem was best in retaining most of quality characteristics such as firmness, TSS, acidity, ascorbic acid and pectin content. Moreover, the extract of neem leaf alone or with packed in (CFB) boxes increase the shelf life of guava fruits by reducing the fungal and bacterial spoilage during storage.

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"تأثير المعاملة بالمستخلصات الطبيعية على جودة و إطالة عمر ثمار الجوافة المصرية"

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تم إجراء التجربة خلال موسمى 2016 -2017 على ثمار جوافة (منتخب الصباحية) المزروعة فى مزرعة خاصة بطريق مصر الإسكندرية الصحراوى، حيث أن ثمار الجوافه قابلة للإصابة بالعديد من الفطريات التى تجعلها عرضه للتلف أثناء التخزين البارد و التداول ، تم غمس الثمار فى المستخلصات الطبيعية التالية لمدة 5 ق (مستخلص زهرة القطيفة ، وخلاصة أوراق النيم ومستخلص أوراق النعناع بتركيز 10٪ لكل منها) ثم تم تعبئة بعض المعاملات فى صناديق من الألياف الممودجة (CFB) . تم إجراء تجربتين: التجربة (1) تم تخزين الثمار 28 يومًا تحت ظروف التخزين البارد عند 6 درجة مئوية ±2 ورطوبة 90-95٪ و تم أخذ عينات لإجراء التحليلات المختلفة كل أسبوعين . (التجربة 2) تم تخزين الثمار لمدة 6 أيام في جو الغرفة عند 15 درجة مئوية ±2 ورطوبة 65-70٪ . أدت جميع المعاملات للحفاظ على جودة الثمار و تقليل الإصابات الفطرية عن طريق خفض النمو الخطي والوزن الجاف للنمو الفطري بالمقارنة مع الثمار غير المعاملة. كما أظهرت المعاملة بمستخلص نبات النيم بتركيز 10٪ مع وضع الثمار فى صناديق CFB بعد 28 يومًا من التخزين البارد و 6 أيام في فى جو الغرفة في كلا الموسمين للحصول على أقل نسبة فقد فى وزن الثمار ومنع حدوث أى إصابات فطرية مقارنة بجميع المعاملات المستخدمة، كما حافظت على صلابة الثمار، محتواها من المواد الصلبة الذائبة، الحموضة، السكريات الكلية ، فيتامين C و البكتين . لذا يمكن تخزين ثمار الجوافة في فصل الشتاء حتى28 يوم تحت ظروف التخزين البارد و6أيام فى جو الغرفة دون حدوث تدهور فى جودة الثمار.