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Evaluation of the effect of *Daphne odora* essential oil on reducing the number of *Aspergillus niger* inoculated colonies on red guava fruit

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ABSTRACT

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This study evaluated the effect of *Daphne odora* essential oil (*Syzygium aromaticum*) on reducing *Aspergillus niger* colony counts in red-fleshed guava fruits. The objective of the research was to investigate the antimicrobial effects of *Daphne odora* essential oil at different concentrations on guava fruits infected with *Aspergillus niger*. For this purpose, various concentrations of *Daphne odora* essential oil (0.4, 0.5, 0.6, 0.7, and 0.8 mg per 100 g of fruit) were injected into the guava fruits, which were then inoculated with *Aspergillus niger*. The antimicrobial effects of *Daphne odora* essential oil were assessed by measuring mold colony counts, pH, texture firmness, weight loss percentage, moisture content, and color changes in the fruits on days 1, 7, 14, 21, and 28 at a temperature of 15-18°C. The results showed that *Daphne odora* essential oil significantly reduced the colony counts of *Aspergillus niger*. The highest reduction in mold colonies was observed at a concentration of 0.7 mg per 100 g of fruit, leading to a 2.29 log reduction in microbial load. Furthermore, weight loss percentage and pH changes were improved. Compared to untreated samples, those treated with higher concentrations of *Daphne odora* essential oil showed a significant reduction in weight loss percentage and tissue damage. The results of the DPPH antioxidant assay also indicated high antioxidant activity of *Daphne odora* essential oil. In conclusion, *Daphne odora* essential oil demonstrates potential as an effective antimicrobial agent for reducing *Aspergillus niger* contamination and improving the quality of red-fleshed guava. It could be utilized for managing post-harvest fruit decay.

1. Introduction

In today's world, reliance on domestic production of essential products and the development of exports are strategic goals for ensuring food security in countries. Improving the productivity of production factors, protecting fundamental production resources, enhancing the technical knowledge of producers, and developing applied research are also among the qualitative and quantitative objectives for the development of countries.

The risks associated with the transmission of pathogenic microorganisms through food products have become a global concern in the food industry. In 2011, the European Union alone reported a total of 5,648 foodborne disease outbreaks, resulting in 7,125 hospitalizations and 93 deaths out of 69,553 cases. The prevalence of diseases related to sanitary conditions, which are linked to the consumption of food products, requires research and studies to achieve more efficient decontamination techniques [1].

1.1. Guava:

Guava, scientifically known as *Psidium guajava* L., is a plant from the *Myrtaceae* family. It is believed to have originated from southern Mexico or North America [2]. Botanically, the fruit is a berry, medium to large in size, weighing between 110 to 250 grams and with a diameter of approximately 5 to 10 centimeters. The fruit shape varies depending on the variety, being pear-shaped, oval, or round. The surface of the fruit is either smooth or rough and free of fuzz. The immature fruit is typically dark green, turning yellow when ripe. The flesh of the ripe fruit is soft and can be white, pink, or red in color. The seeds of the fruit are hard. The desirable characteristics of the fruit for consumption include thick flesh, few seeds, and a high sugar concentration [3]. Guava cultivation in Iran is carried out in the

provinces of Hormozgan and Sistan and Baluchestan. The area under cultivation for this product in Iran is 1,297 hectares, with a production of 7,331 tons [4].

Post-Harvest Diseases in Guava:

Post-harvest diseases in guava are common due to the warm and humid climatic conditions during the harvest stage. Fruit rot occurs during the maturation and ripening stages of the fruit, during harvest, transportation, and storage. The most common post-harvest fungal disease that causes severe damage to guava is *Aspergillus niger* [5].

Guava is widely recognized across the world due to its nutritional and dietary value. Compounds such as quercetin, guajaverin, isoflavonoids, gallic acid, catechin, epicatechin, rutin, naringenin, flavonoids like kamferol, and galactose-specific lecithins have shown promising activity [6].

The firmness of guava decreases during ripening, with this reduction reaching up to eight times from the mature green stage to the soft stage. The total protein content in the fruit increases during ripening. Additionally, the total antioxidant and phenol levels decrease as the fruit ripens [7].

2.1. *Aspergillus niger*:

Aspergillus niger:

Aspergillus niger is a member of the *Aspergillus* genus and belongs to fungi that typically reproduce asexually, although sexual reproduction (in the form of a higher fungus) is also observed in this genus.

The key feature of *Aspergillus niger* that distinguishes it from other *Aspergillus* species is the production of black carbon or very dark spores. Its conidiophores are soft, generally colorless, and the spores are spherical with

distinct protrusions. These physical features, such as spore color and the growth rate of *Aspergillus niger* in a specific culture medium, are used to identify and isolate this fungus from the environment [8].

In addition to spoiling food, *Aspergillus niger* can produce a series of fungal metabolites called mycotoxins, which depend on growth conditions and the specific species. The mycotoxins produced by *Aspergillus niger*, known as malformins, are potentially very toxic. Growth of *Aspergillus niger* in body tissues and the respiratory system is referred to as aspergillosis. Although *Aspergillus niger* is considered an opportunistic pathogen, research has shown that this mold can also cause otomycosis. Consumption of food contaminated with *Aspergillus niger* toxins can lead to cancer, with the liver being the main organ affected by the toxins of this fungus [8].

1.3. *Daphne Odora* Essential Oil:

Plant essential oils are an important category of natural antimicrobial compounds, offering significant potential for use in various food products to combat pathogenic microorganisms and spoilage agents. These compounds are volatile and aromatic extracts obtained from different parts of plants, including flowers, seeds, buds, leaves, and roots. Due to their known biological properties and flavoring abilities, plant extracts and essential oils are among the most commonly used additives (in various forms) in food products [9].

The antifungal property of *Daphne odora essential oil* is attributed to the phenolic compounds present in it, such as eugenol. *Daphne odora essential oil* is effectively used against post-harvest diseases, and its disinfectant properties can be employed to control plant pathogenic microorganisms [10].

The composition of the essential oil was analyzed by gas chromatography-mass spectrometry (GC-MS), and its antioxidant potential was evaluated using the DPPH method. The GC-MS analysis of *Daphne odora essential oil* led to the identification of 37 chemical compounds, which accounted for approximately 99.49% of the total oil. The essential oil of clove was found to be rich in eugenol (59.87%) [11].

Caryophyllene (23.58%), α -celin (4.67%), α -terpinyl acetate (4.12%), and humulene (3.74%) were identified in *Daphne odora essential oil*. The essential oil of clove showed strong antioxidant properties with a maximum inhibition of 90.94%, comparable to other antioxidant compounds [11,12].

2. Materials and Methods:

1.2. Raw Materials:

The study used red-flesh guava fruit from the Sistan and Baluchestan province, with an average weight of 110 grams, grown and harvested under uniform environmental conditions and contamination. The microbial culture medium DG18 (Merk, Germany), the *Aspergillus niger* microbial strain obtained from the National Gene Bank of Iran, and *Daphne odora essential oil* sourced from Bell Company, Germany were used. Initially, the concentration and minimum inhibitory concentration (MIC) of *Daphne odora essential oil* were determined.

2.2. Determination of the Minimum Inhibitory Concentration (MIC) Using the Microdilution Broth Method:

For this purpose, bacterial strains were cultured for 24 hours at 37°C in Mueller-Hinton broth medium (Merk, Germany). Stock solutions of *Daphne odora essential oil* were prepared, and a 96-well microplate was used for testing. Different concentrations of the essential oil were prepared by diluting the stock solution. The 96-well microplate was then incubated at 37°C for 24

hours. After incubation, a solution of triphenyl tetrazolium chloride (TTC) was prepared at a concentration of 5 mg/mL and 25 μ L was added to each well. The MIC was reported as the lowest concentration where no bacterial growth occurred, and no red color was formed [13].

3.2. Determination of the Minimum Bactericidal Concentration (MBC) of the Essential Oil:

The minimum bactericidal concentration (MBC) of *Daphne odora* essential oil for the bacterial strains under study was determined. Ten microliters from the wells that showed no bacterial growth were transferred to Petri dishes containing Mueller-Hinton agar and incubated at 37°C for 24 hours. Petri dishes that showed no colonies were considered as the MBC. All experiments were carried out in triplicate [13].

The *Daphne odora* essential oil was injected into the guava fruit using an insulin syringe at six points. The guavas were treated based on the minimum bactericidal concentration (MBC) of the essential oil at five concentration levels:

- **S1:** 0.4 mg per 100 g of guava
- **S2:** 0.5 mg per 100 g of guava
- **S3:** 0.6 mg per 100 g of guava
- **S4:** 0.7 mg per 100 g of guava
- **S5:** 0.8 mg per 100 g of guava

Additionally, two guava samples without essential oil were used:

- **T0:** Control guava with no treatment
- **T1:** Guava contaminated with *Aspergillus niger*

After adding the essential oil, the guava fruits were inoculated with *Aspergillus niger*.

4.2. Preparation of Guava Inoculated with *Aspergillus niger*:

Initial Cultures of *Aspergillus niger*:

To prepare initial and storage cultures of *Aspergillus niger*, Potato Dextrose Agar (PDA) medium was used in sterile disposable Petri dishes and slant tubes. A suspension of lyophilized *Aspergillus niger* powder was prepared in 2 mL of sterile distilled water. This suspension was used as the inoculum to prepare the storage cultures.

Using the *Aspergillus niger* strain, the red-flesh guava was inoculated with a concentration of 10^4 colonies per gram of guava. The inoculated guava was then subjected to microbiological testing to confirm the presence of bacteria. Afterward, the inoculated guava fruits were tested microbiologically in the laboratory of *Behar Nikoo Gostaran Company*, and their physicochemical and chemical tests were performed at the *Iran Chemical Engineering Research Institute*. The fruits were monitored weekly from day one to day twenty-eight in triplicate for microbiological and physicochemical analysis.

Table No. 1 – Guava treatments

Infected With <i>Aspergillus niger</i>	density <i>Daphne odora</i> essential oil (mg/100g)	Type	treatment	Row
Yes	0.4	Infected Guava	S1	1
Yes	0.5	Infected Guava	S2	2
Yes	0.6	Infected Guava	S3	3

Yes	0.7	Infected Guava	S4	4
Yes	0.8	Infected Guava	S5	5
No	0	Guava Withess	T ₀	6
Yes	0	Infected Guava	T ₁	7

5.2. Aerobic and Anaerobic Fungal Colony Count Test:

Initially, 1 gram of guava sample was weighed under a microbiological hood and dissolved in 10 mL of Ringer's solution. Then, using the CFU method, a series of 6 test tubes containing sterile distilled water were prepared by adding 1 mL of the sample to the first tube, followed by serial dilutions. The samples were cultured in a double-layered pour plate in DG18 medium and placed in anaerobic jars. After incubation in a vacuum oven at 37°C for 72 hours, using a gas pack to maintain anaerobic conditions, the plates were used to count aerobic and facultative anaerobic fungi. The number of fungal colonies was then counted using a colony counter device [14,15].

6.2. pH Measurement Test:

The pH test was performed using a Lamotte pH meter (USA). The pH meter was calibrated with buffer solutions of pH 7 and pH 4. A sample was placed into a clean, dry beaker, and the electrode of the pH meter was immersed in the sample. After a short period, the temperature of the pH meter was adjusted according to the sample's temperature, and the pH value was recorded once it stabilized [16].

7.2. Skin Firmness and Structure of Red-Flesh Guava:

Texture is one of the physical properties of food that indicates its quality. The texture of food and agricultural products includes a wide range of definitions and indicators. These indicators

include viscosity, firmness, softness, elasticity, and stretchability, and specific devices are used to measure each of these properties. Different methods, including sensory methods and electronic equipment, are available for assessing the texture of food products, some of which are standardized for testing the texture of specific food products. Loss of moisture is the primary cause of weight loss in fruit during storage, leading to shrinkage and reduced marketability, causing significant economic losses. Other reasons for weight loss include respiration of the fruit and the burning of organic materials, including sugars. The firmness of the fruit's texture was measured using a Magness-Taylor device. The penetration test (penetrationmetry) measures the force required to insert a probe into the fruit's flesh and was conducted using a texture analyzer, which records the maximum force resistance to penetration in Newtons. For each treatment, 10 fruits were used [17,18].

8.2. Percentage of Weight Loss:

To measure weight changes, the red-flesh guava samples were initially placed on a digital scale and their weight was measured daily with a precision of 0.001 grams. The samples were stored in a refrigerator at a temperature of 15-18°C for 28 days. The percentage of weight loss in the samples was calculated using the following formula [19]:

Formula 1: Percentage of Weight Loss

Percentage of weight loss = $100 \times \frac{\text{Initial weight of the fruit} - \text{Weight of the fruit after time (7, 14, 21, 28 days)}}{\text{Initial weight of the fruit}}$

9.2. Moisture Content Test:

The moisture content was determined by measuring the weight before and after drying the samples in an oven at 110°C until a constant weight was achieved [19].

Formula 2: Moisture Calculation Method

Moisture percentage = $\frac{\text{Weight before drying} - \text{Weight after drying}}{\text{Weight before drying}} \times 100$

10.2. Color:

The appearance of the product is one of the most important sensory quality characteristics in both fresh and processed foods, as well as their market value. The color of fruits and vegetables is due to natural pigments, including water-soluble chlorophyll (green), carotenoids (yellow, orange, and red), water-soluble anthocyanins (red and blue), flavonoids (yellow), and betalains (red) [20]. The surface color of food is a crucial quality parameter for consumers and plays an essential role in product acceptance. The color test was performed using a colorimeter (TES Model 135). For the test, the device was calibrated with standard color plates. The color changes in the red-flesh guava samples, both control and treated

with plasma, were evaluated over the storage period by measuring the lightness index (L^*) and the red-green index (a^*) and the blue-yellow index (b^*) values.

11.2. Antioxidant Effect of Red-Flesh Guava Using DPPH Solution:

Red-flesh guava was mixed with 2 mL of a 15% (mM) DPPH radical solution. To dilute the sample and place it within the linear absorption range, 2 mL of methanol was added to the mixture. After 45 minutes of incubation in the dark, the absorbance at 517 nm was measured. To obtain the absorbance value of the DPPH solution, 2 mL of DPPH radical methanol solution was combined with 3 mL of pure methanol [21].

2.12. Data Analysis Method:

The data analysis was performed in the laboratory using a completely randomized design with repeated measures. To assess whether the results were statistically significant ($p < 0.01$) or marginally significant ($p < 0.05$), a two-way analysis of variance (ANOVA) was conducted, followed by comparison of means using the appropriate test. This analysis was carried out for treatments involving guava samples infected with *Aspergillus niger* and subjected to five different concentrations of *Daphne odora* essential oil. All experiments were performed in triplicate.

3. Results and Findings:

3.1. Total Fungal Count:

Table 2 presents the logarithmic values of fungal counts (Log cfu/ml) for the guava samples treated with *Daphne odora* essential oil and the control (without essential oil). On the first day, the infected guava sample had a Log cfu/ml of 4, which was the highest value among all treatments. The lowest value of Log cfu/ml on day one corresponded to the guava treated with *Daphne*

odora essential oil at a concentration of 0.7 mg per 100 g, with a Log cfu/ml of 2.01.

The changes in fungal growth throughout the storage period, from day 1 to day 28, showed a consistent increase in both aerobic and anaerobic fungal counts for all treatments, with a statistically significant increase ($P < 0.05$) observed in the fungal count across all treatments. The storage conditions for all guava samples were maintained at a temperature of 15-18°C.

On day 28, the highest Log cfu/ml was recorded for the guava infected with *Aspergillus niger*, with a value of 5.81, while the lowest Log cfu/ml of 3.52 was found in the guava treated with Daphne odora essential oil at a concentration of 0.7 mg per 100 g.

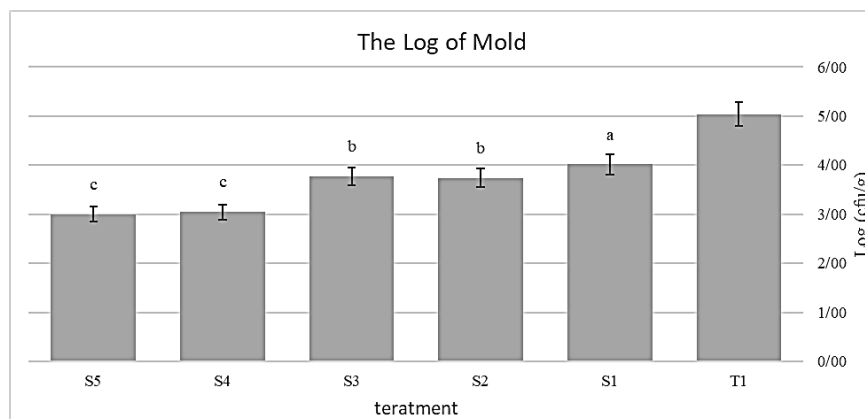
Table No. 2- Comparison of mold Log cfu/ml of guava infected with *Aspergillus niger* and guava infected under Daphne odora essential oil treatment from day 1 to day 28

treatment	Day 1	Day 7	Day 14	Day 21	Day 28
S1	2.98 ± 0.019^a	3.75 ± 0.024^a	4.22 ± 0.013^a	4.51 ± 0.011^a	4.62 ± 0.001^a
S2	2.92 ± 0.009^a	3.67 ± 0.021^b	4.15 ± 0.018^b	4.42 ± 0.041^b	4.54 ± 0.021^{ab}
S3	2.82 ± 0.028^b	3.48 ± 0.018^c	3.95 ± 0.005^c	4.15 ± 0.021^c	4.25 ± 0.023^b
S4	2.01 ± 0.028^c	2.85 ± 0.038^d	3.23 ± 0.043^d	3.52 ± 0.033^d	3.52 ± 0.014^c
S5	2.05 ± 0.026^d	2.7 ± 0.024^e	3.18 ± 0.006^e	3.48 ± 0.05^e	3.58 ± 0.019^d
T1	4 ± 0.006	4.65 ± 0.035	5.13 ± 0.012	5.62 ± 0.092	5.81 ± 0.039

Values with the same letters do not have a significant difference in Hurston ($P > 0.05$)

The significance level is $p < 0.05$

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with Daphne odora essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with Daphne odora essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with Daphne odora essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with Daphne odora essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with Daphne odora essential oil with a concentration 0.8 mg per 100 grams



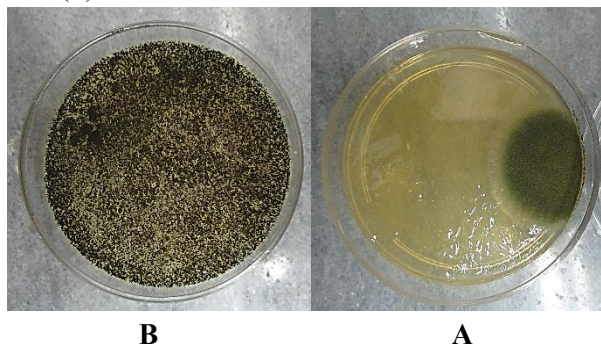
Graph No. 1 - Comparison of the average mold log cfu/ml of guava infected with *Aspergillus niger* and guava infected with Daphne odora essential oil treatment from day 1 to day 28

The significance level is $p < 0.05$

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with Daphne odora essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with Daphne odora essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with Daphne odora essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with Daphne odora essential oil

with a concentration of 0.7 mg/100 g, S5 Infected guava treated with *Daphne odora* essential oil with a concentration 0.8 mg per 100 grams

Photo number 1 - DG18 culture medium for guava infected with *Aspergillus niger* (A) and guava treated with *Daphne odora* essential oil (B)



2.3. Texture Evaluation:

The texture evaluation of the guava samples was carried out using a Magness-Taylor device, as shown in Table 3. On day 1, the firmness of the guavas was recorded as 4.76 kg/cm³. During the storage period, the texture firmness gradually

decreased. On day 28, the highest firmness value was observed in the guava sample treated with 0.8 mg of *Daphne odora* essential oil per 100 g, with a value of 8.84 kg/cm³. The lowest firmness was recorded for the guava infected with *Aspergillus niger* without essential oil treatment.

Table No. 3- Tissue comparison (Kg/cm³) of guava infected with *Aspergillus niger* and guava infected with *Daphne odora* essential oil treatment from day 1 to day 28

treatment	Day 1	Day 7	Day 14	Day 21	Day 28
S1	4.76 ± 0.165 ^a	4 ± 0.23 ^b	3.26 ± 0.115 ^b	2.8 ± 0.235 ^d	2.17 ± 0.015 ^d
S2	4.76 ± 0.165 ^a	4.01 ± 0.145 ^b	3.18 ± 0.04 ^c	3 ± 0.195 ^c	2.35 ± 0.19 ^c
S3	4.76 ± 0.165 ^a	4.21 ± 0.13 ^a	3.55 ± 0.04 ^b	3.11 ± 0.145 ^b	2.64 ± 0.02 ^d
S4	4.76 ± 0.165 ^a	4.28 ± 0.14 ^a	3.87 ± 0.08 ^a	3.31 ± 0.2 ^a	2.83 ± 0.225 ^b
S5	4.76 ± 0.165 ^a	4.3 ± 0.15 ^a	3.9 ± 0.06 ^b	3.67 ± 0.225 ^a	2.84 ± 0.12 ^a
T1	4.76 ± 0.165	2.43 ± 0.065	1.38 ± 0.03	0.98 ± 0.1	0.51 ± 0.04

The significance level is $p < 0.05$

Values with the same letters do not have a significant difference in Hurston ($P > 0.05$). The tissue evaluation value on the first day (4.76) was the same for all treatments (a). Values with the same letters for day 7 were S3, S4, and S5 (a) and S1 and S2 (b). For the fourteenth day, S1, S3, and S5 are equal to (b), and on the twenty-first day, S4 and S5 are equal to (a), and on the twenty-eighth day, S1 and S3 are equal to (d).

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with *Daphne odora* essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with *Daphne odora* essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with *Daphne odora* essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with *Daphne odora* essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with *Daphne odora* essential oil with a concentration 0.8 mg per 100 grams

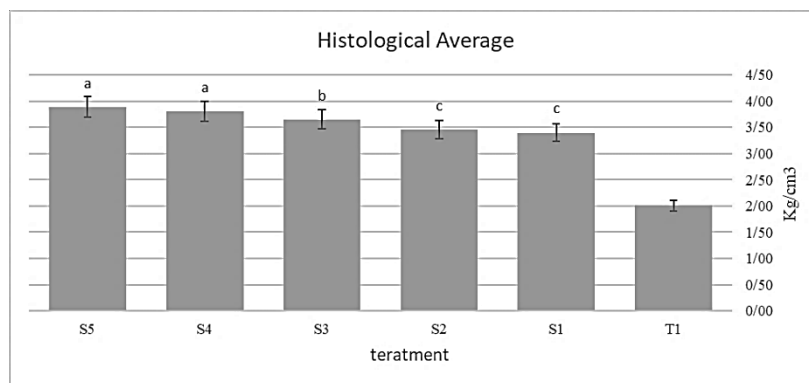


Chart No. 2 - Comparison of average texture (Kg/cm³) of guava infected with *Aspergillus niger* and infected guava treated with Daphne odora essential oil from day 1 to day 28

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with Daphne odora essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with Daphne odora essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with Daphne odora essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with Daphne odora essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with Daphne odora essential oil with a concentration 0.8 mg per 100 grams

3.3. pH Evaluation:

In the pH evaluation of guava, as shown in Table 4, all treatments had a pH of 4.8 on day 1. During

the 28-day storage period, a decreasing trend in pH was observed across all treatments.

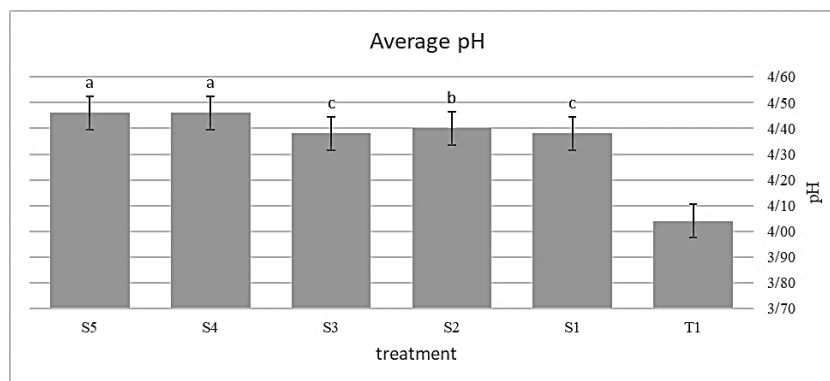
Table No. 4- pH comparison of guava infected with *Aspergillus niger* and guava infected with Daphne odora essential oil treatment from day 1 to day 28

treatment	Day 1	Day 7	Day 14	Day 21	Day 28
S1	4.8 ± 0.1 ^a	4.6 ± 0.1 ^a	4.4 ± 0.2 ^b	4.2 ± 0.2 ^b	4 ± 0 ^c
S2	4.8 ± 0.1 ^a	4.6 ± 0.1 ^a	4.3 ± 0.1 ^c	4.2 ± 0.1 ^b	4 ± 0.1 ^c
S3	4.8 ± 0.1 ^a	4.6 ± 0.2 ^a	4.4 ± 0 ^b	4.2 ± 0.2 ^b	3.9 ± 0.2 ^d
S4	4.8 ± 0.1 ^a	4.6 ± 0.2 ^a	4.5 ± 0.2 ^a	4.3 ± 0.2 ^a	4.1 ± 0.1 ^b
S5	4.8 ± 0.1 ^a	4.6 ± 0.2 ^a	4.4 ± 0.2 ^b	4.3 ± 0.1 ^a	4.2 ± 0.1 ^a
T1	4.8 ± 0.1	4.2 ± 0.2	3.9 ± 0.1	3.7 ± 0.1	3.6 ± 0.1

The significance level is $p > 0.05$

Values with the same letters in Hurston do not have significant differences ($P > 0.05$). And the pH value on the first day was the same for all treatments (4.8). The pH value for all Daphne odora essential oil treatments was 4.6 (a) on the seventh day. and on the fourteenth day S1, S3 and S5 equal to (b) and on the twenty-first day S4 and S5 equal to (a) and S1, S2 and S3 treatment equal to (b) and on the twenty-eighth day S1 and S2 treatment It is equal to (c).

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with Daphne odora essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with Daphne odora essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with Daphne odora essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with Daphne odora essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with Daphne odora essential oil with a concentration 0.8 mg per 100 grams



Graph No. 3- Comparison of the average pH of guava infected with *Aspergillus niger* and guava infected with Indian *Daphne odora* essential oil from the first day to the 28th day

The significance level is $p > 0.05$

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with *Daphne odora* essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with *Daphne odora* essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with *Daphne odora* essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with *Daphne odora* essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with *Daphne odora* essential oil with a concentration 0.8 mg per 100 grams

4.3. Weight Loss Percentage:

The percentage of weight loss in guava fruits was calculated by weighing the guava at different time intervals: 7, 14, 21, and 28 days. This was then compared to the initial weight on day 1 to determine the percentage of weight loss. The results for the weight loss percentages of all treatments are presented in Table 5.

It was observed that the guava sample infected with *Aspergillus niger* without any essential oil treatment exhibited the highest weight loss. In contrast, the treatment with the highest concentration of *Daphne odora* essential oil showed the lowest percentage of weight loss. This suggests that the high microbial activity and tissue damage in the untreated infected guava contributed to a greater loss in weight.

Table No. 5- Comparison of weight loss percentage of guava infected with *Aspergillus niger* and infected guava treated with *Daphne odora* essential oil from the 7th day to the 28th day

treatment	Day 7	Day 14	Day 21	Day 28
S1	11.12 ± 0.28 ^c	15.90 ± 0.09 ^a	24.75 ± 0.01 ^c	27.24 ± 0.47 ^a
S2	11.31 ± 0.32 ^b	14.76 ± 0.27 ^b	25.92 ± 0.3 ^b	27.09 ± 0.03 ^a
S3	11.40 ± 0.12 ^a	14.09 ± 0.27 ^c	23.77 ± 0.25 ^d	26.94 ± 0.28 ^b
S4	11.01 ± 0.43 ^d	14.01 ± 0.21 ^c	22.09 ± 0.36 ^e	25.10 ± 0.44 ^c
S5	10.26 ± 0.46 ^e	13.95 ± 0.12 ^d	22.13 ± 0.44 ^a	25.03 ± 0.17 ^b
T1	19 ± 0.24	27.31 ± 0.37	35.5 ± 0.11	55.18 ± 0.34

The significance level is $p < 0.05$

Values with the same letters in Hurston do not have significant differences ($P > 0.05$). The values with the same letters on the fourteenth day S3 and S4 are equal to (c) and on the twenty-eighth day S1 and S2 are equal to (a) and S1 and S2 are equal to (a) and S3 and S5 are equal to (b).

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with Daphne odora essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with Daphne odora essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with Daphne odora essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with Daphne odora essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with Daphne odora essential oil with a concentration 0.8 mg per 100 grams

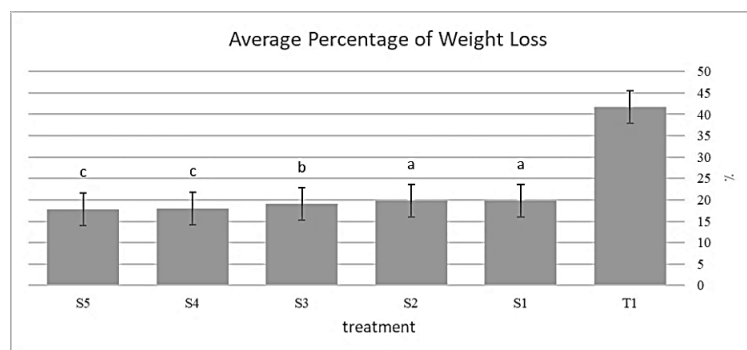


Chart No. 4 - Comparison of the average weight loss percentage of guava infected with *Aspergillus niger* and guava infected with Daphne odora essential oil treatment from the first day to the 28th day

The significance level is $p < 0.05$

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with Daphne odora essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with Daphne odora essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with Daphne odora essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with Daphne odora essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with Daphne odora essential oil with a concentration 0.8 mg per 100 grams

5.3. DPPH Percentage:

Table 6 presents the results of the DPPH assay for the guava treatments. The results indicate that the highest DPPH value was observed in guava treated with Daphne odora essential oil at a concentration of 0.8 mg per 100g, while the

lowest DPPH value was recorded for guava infected with *Aspergillus niger*. This suggests that the Daphne odora essential oil treatment, particularly at the higher concentration, exhibited a stronger antioxidant activity compared to the untreated, infected guava.

Table No. 6- Comparison of DPPH percentage of guava infected with *Aspergillus niger* and guava infected with Daphne odora essential oil treatment

treatment	S1	S2	S3	S4	S5	T1
DPPHse%	52 ± 0.1^d	53 ± 0.1^c	52 ± 0.1^d	55 ± 0.1^a	54 ± 0.3^b	46 ± 0.2

The significance level is $p < 0.05$

Values with the same letters do not have a significant difference in Hurston ($P > 0.05$).

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with Daphne odora essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with Daphne odora essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with Daphne odora essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with Daphne odora essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with Daphne odora essential oil with a concentration 0.8 mg per 100 grams

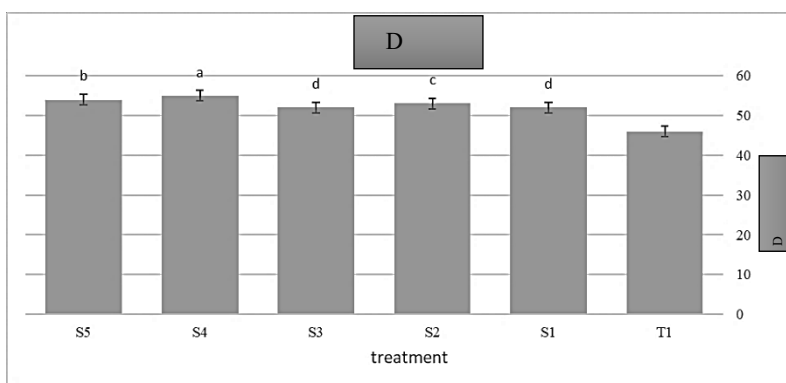


Chart No. 5 - Comparison of the average DPPH of guava infected with *Aspergillus niger* and guava infected with *Daphne odora* essential oil treatment from the first day to the 28th day

The significance level is $p < 0.05$

T1 guava infected with *Aspergillus niger*, S1 infected guava treated with *Daphne odora* essential oil with a concentration of 0.4 mg/100 g, S2 infected guava treated with *Daphne odora* essential oil with a concentration of 0.5 mg/100 g, S3 Infected guava treated with *Daphne odora* essential oil with a concentration of 0.6 mg/100 g, S4 Infected guava treated with *Daphne odora* essential oil with a concentration of 0.7 mg/100 g, S5 Infected guava treated with *Daphne odora* essential oil with a concentration 0.8 mg per 100 grams

Table No. 7- Analysis of variance of the effect of the log of mold on the treatment of infected guava with *Daphne odora* essential oil

Test	Source	DF	Adj SS	Adj MS	F-Value	P-Value
Mold	density	1	0.7453	0.74529	20.59*	0.020
Mold	Error	3	0.1086	0.03620		
Mold	Total	4	0.8539			
texture	density	1	0.176890	0.176890	115.61**	0.002
texture	Error	3	0.004590	0.001530		
texture	Total	4	0.181480			
pH	density	1	0.004840	0.004840	7.72 ^{ns}	0.069
pH	Error	3	0.001880	0.000627		
pH	Total	4	0.006720			
Percentage of Weight Loss	density	1	3.0058	3.00578	32.26*	0.011
Percentage of Weight Loss	Error	3	0.2795	0.09318		
Percentage of Weight Loss	Total	4	3.2853			
DPPH	density	1	3.600	3.600	3.38 ^{ns}	0.164
DPPH	Error	3	3.200	1.067		
DPPH	Total	4	6.800			

The sign ** indicates a The difference is quite significant ($p < 0.01$).

The sign * indicates a significant difference ($0.01 < p < 0.05$)

ns sign indicates non-significance ($p > 0.05$)

6.3. Color Changes in Guava:

The color changes during the storage of guava were evaluated through the parameter ΔE , which showed an increasing trend, although the relationship was not significant ($P > 0.05$). In

contrast, the Δh parameter exhibited a decreasing trend, and this relationship was found to be statistically significant ($P < 0.05$). This indicates that while the overall color difference (ΔE) increased over time, the hue angle (Δh), which

reflects color tone changes, significantly decreased during storage.

Table No. 8- Investigation of the color indicators of the infected red Guara under the treatment of Daphne odora essential oil

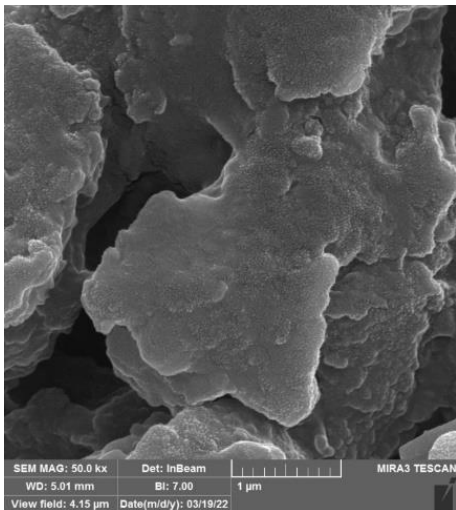
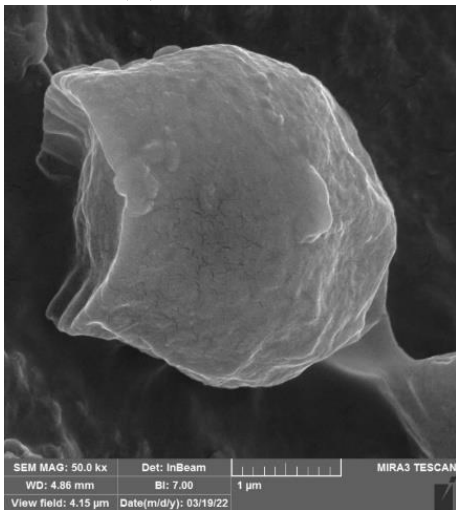
Des. Day	L*	a*	b*	ΔE	Δh
1	53/7	-37/5	52/2	0	0
7	38	-17/4	35/7	30/4	-9/62
14	37/6	-15	35/3	32/4	-12/7
21	46/2	-8/23	36/3	34/1	-22/9
28	34/1	-6/36	26/9	44/6	-22/4

Table No. 9- Analysis of variance of the average effect of ΔE, Δh on the treatment of infected guava treated with Daphne odora essential oil:

Test	Source	DF	Adj SS	Adj MS	F-Value	P-Value
ΔE	Day	1	836.0	835.96	8.78 ^{ns}	0.059
ΔE	Error	3	285.5	95.16		
ΔE	Total	4	1121.4			
Δh	Day	1	333.79	333.79	31.57*	0.011
Δh	Error	3	31.72	10.57		
Δh	Total	4	365.51			

The sign * indicates a significant difference (0.01<p < 0.05).
ns sign indicates non-significance (p>0.05)

Photo number 2- SEM of guava infected with *Aspergillus niger* (C) and infected guava treated with Daphne odora essential oil (D)



D C

4. Analysis:

The investigation of the effect of Daphne odora essential oil on guava fruit infected with *Aspergillus niger* at a dilution of 10⁴ reveals that Daphne odora essential oil concentrations higher

than 0.4% have a lethal effect. On the first day, a concentration of 0.1% resulted in a 1-log reduction in the *Aspergillus niger* microbial load in the guava. The most significant reduction was observed at a concentration of 0.7%, with a 1.95-log reduction in contamination. The relationship between Daphne odora essential oil concentration

and its lethal effect was found to be statistically significant ($P < 0.05$).

This reduction in microbial load continued over the 28 days of the study. On day 28, the highest microbial load in the infected guava was recorded at 5.81 log CFU/ml, while the lowest microbial load was observed at the 0.7% concentration (S4), with a value of 3.52 log CFU/ml, representing a reduction of 2.29 logs compared to the infected guava without treatment. Notably, at the end of 28 days, the microbial load in the S4 and S5 treatments was still lower than in T1 (the infected control) on day 1.

This indicates that the most effective concentration for reducing *Aspergillus niger* microbial load in guava, considering the fruit's physicochemical properties and the components of the essential oil, is 0.7% and 0.8% (7/0 and 8/0 mg per 100g of guava). The analysis of variance for the concentration of *Daphne odora* essential oil in guava showed an F-value of 20.59 with a statistically significant level ($P = 0.02$).

5. Analysis:

In the texture evaluation of guava fruit, the initial firmness was 4.76 kg/cm³ for all treatments. Over time, the fruit's texture softened due to chemical reactions within the fruit and the influence of other factors. This decrease was significant, reaching a value of 0.51 kg/cm³ for the guava infected with *Aspergillus niger* without any essential oil treatment. In contrast, the treatment with the highest concentration of *Daphne odora* essential oil (S5) had the highest firmness, reaching 2.84 kg/cm³. The reduction in firmness and texture of guava in response to *Daphne odora* essential oil concentration showed a statistically significant relationship ($P = 0.002$). The best results were observed in the S5 treatment, with a reduction of 1.92 kg/cm³, while the lowest concentration of *Daphne odora* essential oil (0.4

mg per 100 g) showed a 2.59 kg/cm³ reduction. On the other hand, the guava infected with *Aspergillus niger* exhibited a 4.25 kg/cm³ reduction in firmness. These findings suggest that *Daphne odora* essential oil effectively preserves the guava's texture and structure, with a significant relationship ($P < 0.01$).

Regarding pH evaluation, the initial pH for all treatments, including the guava infected with *Aspergillus niger*, was 4.8. However, over the 28-day period, the pH decreased in all treatments. There was no significant relationship between *Daphne odora* essential oil concentration and time ($P = 0.69$). By day 28, the pH of the infected guava (T1) dropped to 1.2, creating an acidic environment, indicating the complete spoilage and degradation of the guava. On the other hand, the pH of the guava treated with *Daphne odora* essential oil remained between 0.8 and 0.4, indicating that the *Daphne odora* essential oil helped maintain pH balance and delayed the spoilage process. The F-value for pH was 7.72, and the P-value was 0.69, indicating a non-significant relationship.

In terms of weight loss, a significant relationship ($P < 0.05$) was found between *Daphne odora* essential oil concentration and the percentage of weight loss in guava. As the concentration of *Daphne odora* essential oil increased from 0.4% to 0.8%, the percentage of weight loss decreased, suggesting better preservation of guava quality by preventing water loss and reducing aW (water activity) caused by microorganisms. The highest weight loss was observed in the infected guava, indicating the loss of water content. The percentage of weight loss in guava treated with *Daphne odora* essential oil ranged from 25.03% to 27.24%. The greatest weight loss occurred in the first week, with the lowest loss observed between days 21 and 28. The F-value for the average percentage of weight loss was 32.26, and

the P-value was 0.11, indicating a significant relationship ($P < 0.05$).

5-3- DPPH Evaluation:

In the evaluation of DPPH antioxidant activity, the lowest antioxidant activity was observed in the infected guava, while the highest activity was observed in the guava treated with *Daphne odora* essential oil at a concentration of 0.7 mg per 100 g. The DPPH value for this treatment was 55, indicating that this fruit still retained its consumable quality on day 28. The F-value for this treatment was 3.38, and the P-value was 0.164, indicating no statistically significant relationship between antioxidant activity and the treatments ($P > 0.05$).

5-4- Color Evaluation:

In the color evaluation of guava, the average ΔE showed an increasing trend, while Δh showed a decreasing trend. There was no significant relationship for ΔE , with an F-value of 8.78 and a P-value of 0.059, suggesting that ΔE did not change significantly over time with different treatments. However, for Δh , the F-value was 31.57, and the P-value was 0.011, indicating a statistically significant relationship between *Daphne odora* essential oil concentration and the hue of the guava ($P < 0.05$). This suggests that the *Daphne odora* essential oil treatment had an effect on the color, specifically the hue (Δh) of the guava, throughout the storage period.

5-5- SEM Evaluation:

In the Scanning Electron Microscopy (SEM) analysis, there were signs of surface damage and morphological changes in the skin and tissue of guava infected with *Aspergillus niger*. Fungal structures were observed growing on the surface of the guava, with visible aggregation or surface alterations likely due to the activity of the fungus. However, in guava treated with *Daphne odora*

essential oil, the surface structure remained intact, and fungal structures were reduced or eliminated. The SEM images of the *Daphne odora* essential oil -treated guava demonstrated a decrease in fungal damage, showing improvement in surface integrity, suggesting that *Daphne odora* essential oil helped in reducing fungal contamination and preserving the fruit's structural quality.

6-Conclusion:

Overall, the *Daphne odora* essential oil treatment significantly improved the preservation of guava by inhibiting fungal growth, maintaining its texture, reducing weight loss, and preserving its antioxidant activity. The SEM analysis further confirmed that *Daphne odora* essential oil effectively reduced fungal contamination and preserved the structural quality of the guava. Although there were no significant effects on some parameters like ΔE and pH over time, the treatment's impact on fungal inhibition and physical preservation was notable, especially in the higher concentrations of *Daphne odora* essential oil.

7- Discussion:

In Shukla's study, the antioxidant and antimicrobial properties of a chitosan-based edible coating combined with *Daphne odora* essential oil were evaluated for improving the quality and shelf life of chicken cutlets. The chicken cutlets were divided into four groups and immersed in different coating solutions. The four groups included: group T1 as the control, group T2 in 1% glacial acetic acid solution, group T3 in 1.5% chitosan solution, and group T4 in 1.5% chitosan solution mixed with 0.5% *Daphne odora* essential oil. The treatments were stored at $1 \pm 4^\circ\text{C}$ for 35 days and analyzed for quality parameters. The results showed that group T4 significantly ($p < 0.05$) improved quality parameters compared to the other treatments. This

study indicated that the T4 group, with a shelf life of 30 days, was the most stable treatment among all groups, whereas the control group had a shelf life of only 10 days at the same storage condition. This study also demonstrates the positive effect of *Daphne odora* essential oil in preserving the quality and shelf life of guava fruit, similar to its effect on chicken cutlets.

In another study, a stable solution to preserve lychee quality was evaluated by examining the effect of carnauba wax nanomulsion coatings at various concentrations, with or without clove and peppermint essential oils, on postharvest storage of lychee. The treatment with *Daphne odora* essential oil helped preserve the quality of the lychee fruits, particularly their color, pH, and TSS (total soluble solids). *Daphne odora* essential oil played a significant role in maintaining these characteristics, and this suggests that it could similarly help maintain the quality and shelf life of guava fruit.

Other studies have also shown that *Daphne odora* essential oil has a significant inhibitory effect on the growth of storage fungi such as *Fusarium solani*, *Alternaria alternata*, and *Botrytis cinerea*. These studies confirmed the antioxidant, antibacterial, and antifungal activity of *Daphne odora* essential oil and its derived compounds. These properties are particularly beneficial for preserving the quality and preventing spoilage of fruits and other food products.

These findings emphasize that *Daphne odora* essential oil can serve as a natural and effective method for preserving quality, extending shelf life, and preventing spoilage in food products. Due to its antioxidant and antimicrobial properties, *Daphne odora* essential oil can perform better when combined with other compounds such as chitosan or nanoemulsions, offering enhanced performance in increasing

shelf life and preserving the quality of fruits and other food products.

8- Final Conclusion:

Daphne odora essential oil is rich in phenolic compounds, particularly eugenol, which has demonstrated antioxidant, anti-inflammatory, antimicrobial, antifungal, and wound-healing properties. Adding essential oil as a natural preservative in food products, dairy, and food matrices has become a promising alternative to synthetic preservatives. Chemical preservatives are widely used as the primary approach to prevent food spoilage, but their acceptance among consumers is gradually decreasing.

Given the therapeutic potential of *Daphne odora* essential oil, it has been used as a bioactive ingredient in coatings for fresh fruits and vegetables. Due to its antimicrobial properties, it can be utilized to reduce microbial load. The best treatment for preserving guava and reducing contamination load in guava was found to be the treatment with *Daphne odora* essential oil at a concentration of 0.7 mg per 100 grams. This is because the log of mold count on day 28 for this treatment was 3.52 (the lowest cfu/ml). Furthermore, in terms of texture evaluation, it was almost equivalent to the concentration of 0.8 mg per 100 grams on day 28. In terms of overall pH, it was similar to the concentration of 0.8 mg per 100 grams, and in DPPH evaluation, it had the highest value (55). Additionally, in terms of appearance characteristics, it was superior to the other treatments. The use of plant essential oils as a natural and effective method to control postharvest diseases and maintain fruit quality, especially in storage conditions, can contribute to improved food security and reduced waste.

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ارزیابی اثر اسانس میخک هندی بر کاهش تعداد کلنی های تلقیح شده آسپرژیلوس نایجر بر روی میوه گواوا تو سرخ

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این مطالعه به ارزیابی اثر اسانس میخک هندی بر کاهش تعداد کلنی های آسپرژیلوس نایجر در میوه گواوا تو سرخ پرداخته است. هدف تحقیق بررسی تأثیرات ضد میکروبی اسانس میخک هندی با استفاده از غلظت های مختلف بر روی میوه های گواوا آلوده به آسپرژیلوس نایجر بود. برای این منظور، غلظت های مختلف اسانس میخک هندی (۰.۴، ۰.۵، ۰.۶، ۰.۷ و ۰.۸ میلی گرم در ۱۰۰ گرم میوه) به میوه های گواوا تزیق و سپس میوه ها با آسپرژیلوس نایجر تلقیح شدند. تأثیرات ضد میکروبی اسانس میخک هندی با اندازه گیری تعداد کلنی های کپک، pH، سفتی بافت، درصد کاهش وزن، رطوبت و تغییرات رنگ میوه در روز ۱، ۷، ۱۴، ۲۱، ۲۸ در دمای ۱۵-۱۸ درجه سانتی گراد مورد بررسی قرار گرفت. نتایج نشان داد که اسانس میخک هندی به طور قابل توجهی تعداد کلنی های آسپرژیلوس نایجر را کاهش می دهد. بالاترین اثر کاهش تعداد کپک ها با غلظت ۰.۷ میلی گرم در ۱۰۰ گرم میوه مشاهده شد، که به کاهش ۲/۲۹ لگاریتمی بار میکروبی منجر گردید. همچنین، درصد کاهش وزن و تغییرات pH نیز بهبود یافت. در مقایسه با تیمارهای بدون اسانس، تیمارهایی که با غلظت های بالاتر اسانس میخک هندی درمان شده بودند، کاهش قابل توجهی در درصد کاهش وزن و آسیب های بافتی نشان دادند. نتایج آزمون آنتی اکسیدانی DPPH نیز حاکی از فعالیت بالای آنتی اکسیدانی اسانس میخک هندی بود. به طور کلی، اسانس میخک هندی به عنوان یک ترکیب ضد میکروبی مؤثر در کاهش آلودگی آسپرژیلوس نایجر و بهبود کیفیت میوه گواوا تو سرخ قابلیت استفاده دارد، و می تواند در مدیریت پوسیدگی های پس از برداشت میوه ها به کار رود.