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# INFLUENCE OF EXOGENOUS SPRAYING CHITOSAN, ARGININE, AND GALANGAL EXTRACT ON FRUIT QUALITY OF HAYANI DATE PALM

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## ABSTRACT

The Hayani date palm is the most grown type in North Sinai, Egypt, and is widely sold in markets. However, its fruit quality is not good, and it is facing competition from imported cultivars like Barhi. As a result, it is important to improve the quality of Hayani fruits in order to keep them popular and maintain this local cultivar. In this study, the effects of spraying Hayani date palm fruits with chitosan, arginine, and galangal extract at 100 ppm, 10 ml/l, and 25 ml/l respectively either individually or mixed, on fruit yield and quality were investigated. Results indicated that fruit yield, yield components, and chemical and biochemical characteristics of Hayani dates fruits were improved when spraying arginine at 100 ppm. In general, the spray mixture of arginine at 100 ppm, chitosan at 10 ml/L, and galangal extract at 25 ml/L was mostly more effective than the individual application of each of them, as evidenced by the results of all parameters evaluated. This resulted in an increase in bunch weight, fruit weight per bunch, remaining number of fruits per bunch at harvest, flesh net weight, fruit dimensions, fruit volume, total soluble solids (TSS), DPPH scavenging activity, total phenolic content, and total vitamin C. Additionally, fruit color was darker red with this treatment application than the control treatment that shown as pale red color. Also, the mixture of arginine with chitosan and galangal extract treatment produced lower tannin content in the fruits.

## INTRODUCTION

Egypt leaded the world in date palm production with about 18% of global production in 2022. It produced 1.73 million tons from an estimated area of 58.6 thousand hectares **(FAOSTAT, 2023)**. Despite this, Egypt contributes about 3.15% of global date exports, with a revenue at around $70 million. According to the Food and Agriculture Organization, the lower quality of Egyptian dates than international standards is the main reasons for this.

Dates are a popular choice among local consumers due to their high nutritional value, wide cultivars, and low cost. Egypt has many cultivars such as Amhaat, Bent Eisha, Hayani, Zaghloul, Samani, etc. In North Sinai, the Hayani cultivar is the most widespread, followed by Amri, Hayani, Khedri, Kapoushi and Beid El Gamal which are spread in small areas **(Bekheet and El-Sharabasy, 2015; Rizk and El-Sharabasy, 2019).**

Generally, the Hayani cultivar is widely cultivated in the governorates of the coastal strip of the Mediterranean and Delta area. It requires high relative humidity and low heat requirements and is harvested early from mid-August until the end of September. It is considered one of the types of soft palm trees that are consumed at the end of the khalal stage, where the color of the fruits is dark red. The Hayani variety is also suitable for consumption at the beginning of the rutab stage, where it

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becomes blackish-brown in color. The productivity of the tree ranges between 90-180 kg, depending on the palm age, nutritional status, and the suitability of environmental factors. The most important morphological characteristics of the fruit are that the length is 5.50±0.50 cm, width is 2.50±0.20 cm, and weight is 21±4 g. The fruit shape is cylindrical with an obtuse top and truncated base. The fruits have a good flavor and a sweet, delicious taste **(Vij *et al.*, 2005; Rizk and El-Sharabasy, 2019)**.

The amino acids, organic compounds, and plant extract have been proven to have a positive effect on plant growth, fruit yield, and quality under poor conditions. Beside the amino acid, these compounds including such as antioxidants and important other elements, play an important role in enhancing plant growth, nutritional status, cell division, cell senescence, fruit set, and fruit quantity and quality in different fruit trees **(Rai, 2002; Abdel-Ghany 2020; Maury *et al.*, 2020; Sharif *et al.*, 2020; Yuniati *et al.*, 2022)**. Arginine is an amino acid that plays a crucial role in the physiological and developmental processes of plants. It is a vital regulator for various biochemical processes, serving as a precursor for polyamines, agmatine, proline, glutamate, and amino butyric acid. These molecules are essential messengers involved in plant growth, development, and productivity under poor environmental factors and also, improve fruit quality in a lot of fruit trees genus **(Kakkar *et al.*, 2000; Torrigiani *et al.*, 2004; Franco-Mora *et al.*, 2005 and Liu *et al.*, 2006)**. Meanwhile, Arginine increases enzymatic activity, T.S.S, and vitamin C in addition to reducing acidity **(Pakkish and Mohammadrezakhani, 2022)**.

Chitosan is a non-toxic substance that is derived from chitin, which is a naturally occurring polymer made up of N-acetylglucosamine. Specifically, chitosan is produced by removing the acetyl groups from chitin through a process called deacetylation **(Rinaudo, 2006; Davis, 2011)**. The molecular weight ranges from low (<150 kDa) to high (700-1200 kDa) molecular weight **(Verlee *et al.*, 2017)**. Chitosan has potential in agriculture benefits through helping plants grow and protect when applied. It can reduce stomatal opening by increasing ABA activity, improving enzyme activity, capacity to scavenge free radicals, capacity to antimicrobial, controlling algal growth, promoting slow release of fertilizers, and improving soil water **(Pandey *et al.*, 2018)**. In this line, treated Zaghloul date with chitosan 1% improved the fruit quality as fruit firmness, T.S.S (ºBrix), and total sugars, meanwhile lowest the tannins content compared with the untreated treatment **(Kamal *et al.*, 2014)**.

Moreover, plant extracts have a lot of benefits to improve plant growth and quality traits. Many previous studies reported recording a significant effect when spraying palm trees with some plant extracts such as garlic, onion, maringa, camphor and black cumin on Sakkoti Date Palms **(Zagzog and Saied, 2017)**, moringa leaf extract on two palm cultivars; Al-Khadrawi and Al-Buraim **(Abdulrahman *et al.*, 2023)**, cinnamon and turmeric on Sewi date Palm **(Elkady *et al.*, 2022)**, and garlic extract on Umm Al-Dahn date palm **(Auda *et al.*, 2023)**. This study focuses on the use of galangal as a promising new plant extract. The Galangal plant native to Southeast Asia; it is a ginger-like rhizome that belongs to *Alpinia* genus which was the largest family Zingiberaceae genera. It is including two species; *galanga* (namely greater galangal) and *officinarum* (namely lesser galangal). Uses as flavoring ingredients and spices in Southeast Asian countries in addition used in Chinese medicine as a remedy for gastrointestinal diseases like cramps and other treatments. Galangal is rich in essential oils, phenolic and flavonoid compounds, and diarylheptanoids which play important roles as antimicrobial, antioxidant, and insecticidal. Moreover, it

contains the flavonol compound galangin (flavonoid) that was also found in the propolis as well as cineole, camphor, methyl cinnamate, and guaiol **(Kress *et al.*, 2015; Aloud *et al.*, 2018; Zhou *et al.*, 2018)**. **Elkady *et al.* (2022)** investigated that spraying turmeric extract at 1000 ppm on Sewi date palm achieved high fruit yield and quality.

Therefore, this study aimed to enhance the fruit quality of Hayani date palms by spraying chitosan, arginine, and galangal extract individually or in combination treatment under sandy soil conditions.

# MATERIALS AND METHODS

## Plant Material

Nine healthy and same vigor Hayani date palms of 30 years old, grown scattered in sandy soil under a rainwater irrigation system and fertilized with organic manure yearly. The palms were selected randomly from the private orchard at Bir Al-Abd region, North Sinai, Egypt. The hand pollination was uniform, using the same pollen grain source. Moreover, the bunches were thinned to eight bunches.

## Experimental Treatments

This experiment included eight treatments (Table 1), which were applied four times into the kimri stage (once every 2 weeks) and two times into the khalal stage (once every 1 week). Hand sprayers were used to apply the treatment as a fine mist on fruits in all directions of the palm bunch. A plastic sheet was used to prevent inadvertent contamination of the other bunches. Using a one-way experimental design, the experiment followed a randomized complete block design (RCBD). Each treatment was applied to three replicates, with three palms per replicate. The palm included eight bunches divided into 8 treatments (each is one bunch). The palms were subjected to different spraying treatments as follows:

**Fig.1. Hayani date palm orchard at Bir Al-Abd region, North Sinai, Egypt**

**Table 1. List of experimental treatments and preparation method**

| Treatment | Preparation method |
|---|---|
| **Control** | Sprayed distilled water. |
| **Arginine** | Arginine® (PIOCHEM Co., India): Exactly 100 mg were dissolved in 1 L water (100 ppm). |
| **Galangal** | Stock solution extract: 250g ground of galangal roots were soaked 24h in 1L warm water (50°C) and then filtered for preparation (25 ml/L solution). |
| **Chitosan** | Chitosan® (Chitosan Egypt Co., Egypt): Exactly 10 ml were dissolved in 1 L water (10 ml/L). |
| **Arginine + Galangal** | Mix 500 ml Arginine solution (100 ppm) + 500 ml galangal extract solution (25 ml/L). |
| **Arginine + Chitosan** | Mix 500 ml Arginine solution (100 ppm) + 500 ml Chitosan solution (10 ml/L). |
| **Chitosan + Galangal** | 500 ml Chitosan solution (10 ml/L) + 500 ml galangal extract solution (25 ml/L). |
| **Arginine + Chitosan + Galangal** | Mix 333 ml Arginine solution (100 ppm) + 333 ml Chitosan solution (10 ml/L) + 333 ml galangal extract solution (25 ml/L). |

## Experimental Measurements

### Fruit yield and its components

Harvesting was carried out at the peak of the fruit full-color stage in the middle of September of both seasons of the study. The bunch per treatment was cut off and weight (kg), then fruit weight (kg bunch$^{-1}$), and the remaining number of fruits per bunch at harvest were calculated. In addition, 90 Hayani date palm fruits for each treatment divided into three replicates (every 30 fruits represents 3 bunches/ replicate) were randomly selected to determine fruit fresh weight (g), fruit dimensions (length, diameter, and shape index), fruit volume (cm$^3$), fruit flesh weight (g), flesh net weight (%), and fruit dry weight (g).

### Fruit chemical and biochemical traits

### Fruit skin color

Fruit skin color was determined by measuring the fruit pigments concentration (chlorophyll a, chlorophyll b, carotenoids, and anthocyanin content). Extract of chlorophyll a, b, and carotenoids was performed a 0.5 gram of fresh fruit peels gently crushed in 10 ml methanol then incubated in a water bath at 80$^o$C for 15 min. After cooling with tap water, centrifuge samples at 6000 rpm for 5 minutes and filtrate samples to get supernatant. The absorbance of photosynthetic pigments was assayed at wavelengths of 663, 645, and 450 nm using Jenway spectrophotometer for chlorophyll a, b, and carotenoids, respectively. The concentrations of pigments were calculated using the equation of **Rami (1982) and Lichtenthaler (1987)**.

Half-gram of fresh fruit peels was used to extract anthocyanin using 10 ml of ethanolic HCl (490 ml ethanol 95% + 10 ml concentrated HCl), incubated at 4oC for 24 h. After that, centrifuge samples at 6000 rpm for 5 minutes and filtrate samples to get supernatant. The absorbance was measured at 535 nm and calculated according to **Rabino *et al.* (1977)**.

**Total soluble solid, acidity and T.S.S/ acidity**

Total soluble solids (%) were measured using a hand refractometer (Model Generic, PR-32, China) in fruit juice. Total acidity (%) estimated according to **AOAC (2000)** in fruit juice by titrating method using 0.1N of NaOH and 2-3 points of ph.ph as an indicator and the results were calculated from the formula: Percent of titratable acidity= 0.1 N NaOH x ml of NaOH used x 0.067/ ml of Juice. T.S.S/Acidity ratio was calculated by dividing T.S.S % by acidity %, which that used as taste fruit quality and ripening index according to **Melgarejo *et al.*, (2014)**.

**Vitamin C**

The vitamin C content was determined according to **Pearson (1976)** in 5 ml fruit juice by titrimetric method using 2, 6 DCPIP pigment, and the results were calculated as mg/100 ml juice.

**Preparation of alcohol extract**

Twenty-five mg of the fresh pulp fruit was boiled in 10 ml of 70% ethyl alcohol on a water bath for 1 h. After boiling, the mixture was cooled down by tap water and then centrifuged at 3000 rpm for 15 minutes. The supernatant was collected and saved at 4$^{o}$C to sugars, tannins, DPPH scavenging activity, and total phenols estimation.

**Total sugars**

Total sugars were estimated by adding 4 ml of cold anthrone reagent to 1 ml of ethanolic extract and boiling for 10 min at 95°C **(Dubois *et at.*, 1956)**. The wavelength 620 nm was used to read total sugar absorbance. The Glucose standard curve was used to calculate the total sugar content in the samples as g of glucose equivalent/100 g of pulp fresh weight (y = 0.297x; R2 = 0.998).

**Total phenolic Content**

Folin–Ciocalteau reagent was used according to **Singleton and Rossi (1965)** to estimate the total phenolic content. A mixture between 0.1 ml sample, 1 mL of folin reagent at 10%, and 2 mL of $Na_2CO_3$ at 7.5% was gently shaken and left on the stand for 1 h at 25$^{o}$C±1. At 765 nm the absorbance of sample solution was measured. The gallic acid standard curve was used to calculate the total phenolic content in the samples as g of gallic acid equivalent/100 g of pulp fresh weight (y = 0.2635x; R2 = 0.991).

**DPPH scavenging activity**

According to **Kim *et al.* (2002)**, 1 ml of ethanolic sample mixed with 2 ml of DPPH solution was left in the dark at 25$^{o}$C±1 for 10 min and read at 517 nm. With the same previous steps, the ethanolic sample will be replaced with distilled water to measure the control absorbance. The DPPH scavenging activity of fruit pulp extract solution was estimated according to the formula: Inhibition (%) = 1 – [(control ABS– sample ABS)/ (control ABS)] x 100.

**Tannins:**

About 0.25 ml ethanolic sample in a test tube was added 1.5 ml vanillin solution in methanol (4%) and leave 2 min on the stand. After that, 0.750 ml of HCL (2%) in methanol was added and incubated at 30$^{o}$C±1 for 15 min, and absorbance read at 550 nm **(Yamiko *et al.*, 2003)**. At 500 nm the absorbance of the sample solution was measured. The catechin in vanillin standard curve was used to calculate the total tannin content in the samples as mg of catechin equivalent/g of pulp fresh weight (y = 0.0037x; R2 = 0.987).

## Statistical Analyses

The statistical analyses were carried out using Co-STAT software V.8 packages. Data were statistically analyzed by the one-factor analysis of variance (ANOVA), meanwhile, the multiple ranges test (DMRT) was used to determine the variance at a 5% probability level **(Duncan, 1955)**.

# RESULTS

## Fruit Yield and its Components

The impact of foliar application of arginine, chitosan, and galangal at different concentrations, either individually or in combination, on the fruit yield and components of Hayani CV fruits are shown in Table 2. The results showed that all treatments significantly increased the yield per tree in the 2019 and 2020 seasons compared to the control treatment, which only used water. The mixed solution of arginine, chitosan, and galangal at 100 ppm, 10 ml/L, and 25 ml/L concentration, respectively, resulted in a greater increase in bunch weight than the control treatment and galangal at 500 ppm. The triple treatment resulted in a 28% increase in bunch weight during the 2019 season and a 37% increase during the 2020 season, compared to the individual treatments and the control. The lowest bunch weight was recorded in the control treatment (12.91 and 11.59 Kg) and galangal treatment (12.99 and 12.44 Kg) in both seasons.

It was observed that a mixed solution containing arginine, chitosan, and galangal resulted in the highest fruit weight per bunch (12.00 and 12.08 kg bunch$^{-1}$) during the 1$^{st}$ and 2$^{nd}$ seasons. On the other hand, the control treatment and the galangal treatment had the lowest fruit weight per bunch (6.05, 6.59, 6.53, and 6.23 kg bunch$^{-1}$), in both seasons respectively.

The galangal treatment at 25 ml/L concentration continued to give the lowest values for the fruit yield and its components, as they recorded a lower remaining number of fruits per bunch at harvest (594 and 555) in both seasons. In contrast, the highest values (819 and 820) were observed with arginine, chitosan, and galangal mixture solution treatment. In addition, it was observed that the weight of fresh fruit increased significantly when treated with a combination solution of arginine (100 ppm) and chitosan (10 ml/L). The fruit weight was recorded as 15.29 and 16.22 g fruit$^{-1}$ in both seasons, which was higher compared to the untreated fruits with a weight of 10.13 and 10.08 g fruit$^{-1}$. The dual treatment resulted in an estimated increase of approximately 34% and 38% in both seasons compared to the control treatment.

Data presented in Table 3 demonstrated that studied treatments significantly increased fruit flesh weight. In this respect and based on data in Table 3, the highest fruit flesh weight was obtained from spraying with Arginine at 100 ppm +Chitosan at 10 ml/L (13.11 and 13.79 g fruit$^{-1}$ in 1$^{st}$ and 2$^{nd}$ seasons, respectively) followed by the mixture solution of arginine, chitosan, and galangal (12.93 and 13.01 g fruit$^{-1}$ in 1$^{st}$ and 2$^{nd}$ seasons, respectively). At the same time, the lowest values were detected for fruit flesh weight with untreated fruits (8.66 and 8.42 g fruit$^{-1}$) and galangal at 25 ml/L in 1$^{st}$ and 2$^{nd}$ seasons, respectively.

Regarding flesh net weight, spraying with a homogeneous solution between arginine, chitosan, and galangal at 100 ppm, 10 ml/L, and 25 ml/L, respectively, increased the flesh net weight (88.09 and 88.38 %) during both seasons. In contrast, the other tested concentrations decrease the flesh's net weight during both seasons.

Also, data in Table 4 investigated the effect of the arginine, chitosan, and galangal individual or mixed on the dry weight of Hayani date palm fruit during the 2019 and 2020 seasons. The highest fruit dry weight was obtained from spraying interaction treatment between arginine at 100 ppm + chitosan at 10 ml/L (5.73 and 5.89 g fruit$^{-1}$ in 1$^{st}$ and 2$^{nd}$ seasons, respectively) followed by the mixture solution of arginine, chitosan, and galangal (5.59 and 5.88 g fruit$^{-1}$ in 1$^{st}$ and 2$^{nd}$ seasons, respectively). In contrast, the lowest values were detected for fruit dry weight with untreated fruits (2.83 and 2.85 g fruit$^{-1}$) and galangal at 25 ml/L in 1$^{st}$ and 2$^{nd}$ seasons, respectively.

**Table 2. The effect of spraying with arginine, chitosan, and galangal individual or mixed on the bunch weight, fruit weight/ bunch, remaining number of fruits per bunch, and fruit fresh weight of Hayani date palm during the 2019 and 2020 seasons.**

| Treatment | Bunch weight (Kg) | | Fruit weight/ bunch (kg bunch$^{-1}$) | | Remaining number of fruits/bunch | | Fruit fresh weight (g fruit$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|
| | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 |
| T1 | 12.91 d | 11.59 e | 6.05 d | 6.59 e | 598 c | 656 cd | 10.13 d | 10.08 d |
| T2 | 14.85 c | 14.63 c | 8.96 c | 8.64 cd | 662 bc | 609 de | 13.61 ab | 14.19 b |
| T3 | 13.41 d | 13.21 d | 8.52 c | 8.14 d | 691 bc | 654 cd | 12.37 bc | 12.47 c |
| T4 | 16.32 b | 16.23 b | 10.83 b | 10.97 b | 708 abc | 676 bcd | 15.29 a | 16.22 a |
| T5 | 12.99 d | 12.44 de | 6.53 d | 6.23 e | 594 c | 555 e | 11.02 cd | 11.22 cd |
| T6 | 15.72 bc | 15.81 b | 8.41 c | 9.44 c | 687 bc | 755 ab | 12.38 bc | 12.62 c |
| T7 | 15.67 bc | 15.92 b | 8.91 c | 8.63 cd | 743 ab | 707 bc | 11.99 bc | 12.24 c |
| T8 | 17.94 a | 18.22 a | 12.00 a | 12.08 a | 819 a | 820 a | 14.67 a | 14.72 b |

* Means marked by the same letter are not significantly different at 5% level

* T1: Control (Spray water), T2: Arginine (100 ppm), T3:Arginine (100 ppm)+Galangal (25 ml/L), T4: Arginine (100 ppm) + Chitosan (10 ml/L), T5: Galangal (25 ml/L), T6: Chitosan (10 ml/L), T7: Chitosan (10 ml/L) + Galangal (25 ml/L), T8: Arginine (100 ppm)+ Chitosan (10 ml/L)+ Galangal (25 ml/L).

**Table 3. The effects of spraying with arginine, chitosan, and galangal individual or mixed on the fruit flesh weight, flesh net weight, and fruit dry weight of Hayani date palm during the 2019 and 2020 seasons**

| Treatment | Fruit flesh weight (g fruit$^{-1}$) | | Flesh net weight (%) | | Fruit dry weight (g fruit$^{-1}$) | |
|---|---|---|---|---|---|---|
| | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 |
| T1 | 8.66 c | 8.42 d | 85.50 ab | 83.46 d | 2.83 f | 2.85 e |
| T2 | 11.77 ab | 12.30 b | 86.38 ab | 86.71 b | 5.16 b | 5.51 b |
| T3 | 10.50 bc | 10.62 c | 84.93 ab | 85.10 c | 4.33 d | 4.53 c |
| T4 | 13.11 a | 13.79 a | 85.76 ab | 85.06 c | 5.73 a | 5.89 a |
| T5 | 9.35 c | 9.62 cd | 84.65abc | 85.71 c | 3.82 e | 3.92 d |
| T6 | 10.27 bc | 10.86 c | 82.80 bc | 85.65 c | 4.55 c | 4.59 c |
| T7 | 9.71 c | 10.39 c | 80.98 c | 84.99 cd | 4.40 d | 4.43 cd |
| T8 | 12.93 a | 13.01 ab | 88.09 a | 88.38 a | 5.59 ab | 5.88 a |

* Means marked by the same letter are not significantly different at 5% level

* T1: Control (Spray water), T2: Arginine (100 ppm), T3:Arginine (100 ppm)+Galangal (25 ml/L), T4: Arginine (100 ppm)+Chitosan (10 ml/L), T5: Galangal (25 ml/L), T6: Chitosan (10 ml/L), T7: Chitosan (10 ml/L) + Galangal (25 ml/L), T8: Arginine (100 ppm)+ Chitosan (10 ml/L)+ Galangal (25 ml/L).

**Table 4. The effect of spraying with arginine, chitosan, and galangal individual or mixed on the fruit length, fruit diameter, fruit shape index, and fruit volume of Hayani date palm during the 2019 and 2020 seasons**

| Treatment | Fruit length (cm) | | Fruit diameter (cm) | | Fruit shape index (L/D) | | Fruit volume (mm) | |
|---|---|---|---|---|---|---|---|---|
| | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 | 2019 | 2020 |
| **T1** | 3.80 c | 3.74 c | 2.24 b | 2.16 c | 1.69 b | 1.72 cd | 10.00 d | 10.00 d |
| **T2** | 4.33 ab | 4.47 a | 2.48 a | 2.47 a | 1.74 ab | 1.80 ab | 14.00 ab | 14.33 ab |
| **T3** | 4.13 abc | 4.22 ab | 2.40 ab | 2.42 a | 1.72 ab | 1.74 c | 12.50 ab | 12.66abc |
| **T4** | 4.51 a | 4.45 a | 2.42 a | 2.53 a | 1.86 a | 1.75 bc | 14.33 a | 14.83 a |
| **T5** | 3.85 bc | 3.93 bc | 2.35 ab | 2.28 bc | 1.63 b | 1.72 cd | 10.50 cd | 10.83 cd |
| **T6** | 4.26 abc | 4.29 ab | 2.46 a | 2.39 ab | 1.73 ab | 1.79 abc | 14.00 ab | 13.33 ab |
| **T7** | 4.26 abc | 4.11 ab | 2.38 ab | 2.41 a | 1.79 ab | 1.70 e | 12.00 bc | 12.33 bc |
| **T8** | 4.27 abc | 4.40 a | 2.42 ab | 2.41 ab | 1.76 ab | 1.82 a | 14.50 a | 14.83 a |

* Means marked by the same letter are not significantly different at 5% level

* T1: Control (Spray water), T2: Arginine (100 ppm), T3:Arginine (100 ppm)+Galangal (25 ml/L), T4: Arginine (100 ppm)+Chitosan (10 ml/L), T5: Galangal (25 ml/L), T6: Chitosan (10 ml/L), T7: Chitosan (10 ml/L)+ Galangal (25 ml/L), T8: Arginine (100 ppm)+ Chitosan (10 ml/L)+ Galangal (25 ml/L).

Data illustrated in Table 4 showed a significant effect on the fruit dimensions and fruit size in response to treating the Hayani date palm fruit with arginine, chitosan, and galangal individually or mixed in compared with the control. Spraying arginine (100 ppm) with chitosan (10 ml/L) gave the highest values of fruit dimensions as fruit length (4.51 and 4.45 cm) in both seasons, fruit diameter (2.42 and 2.53 cm) in both seasons and fruit shape index recorded 1.86 in 2019 season while the complex of arginine, chitosan, and galangal solution recorded the highest value of fruit shape index (1.82) in 2020 season, followed by arginine treatment at 100 ppm (4.33 and 4.47) in fruit length, (2.48 and 2.47 cm) in fruit diameter and (2.47 and 1.74) in fruit shape index in 1$^{st}$ and 2$^{nd}$ seasons, respectively. While untreated date palm fruits recorded the lowest fruit dimensions, it was about (3.80 and 3.74 cm) in fruit length, (2.24 and 2.16 cm) in fruit diameter and (1.69 and 1.72) in fruit shape index in the 2019 and 2020 seasons, respectively.

Regarding fruit volume, during both seasons, the mixture solution of arginine, chitosan, and galangal solution and arginine (100 ppm) with chitosan (10 ml/L) treatments were more efficient than the other treatments. These treatments recorded the highest fruit volume values (14.50, 14.83, 14.33, and 14.83 mm) in the 2019 and 2020 seasons, respectively. Followed by arginine treatment at 100 ppm (14.00 and 14.33 mm) in 1$^{st}$ and 2$^{nd}$ seasons. However, fruit volume was significantly decreased with the control treatment, which recorded 10.00 and 10.00 mm in the 2019 and 2020 seasons.

## Fruit Chemical and Biochemical Traits

Fig. 2 illustrates the chlorophyll a, chlorophyll b, carotene, and anthocyanin content in date palm fruits during the harvest time of two seasons (2019 and 2020) in response to spraying with arginine, chitosan, and galangal individual or mixed. The results in Fig. 2 indicated that all applied treatments significantly enhance the chlorophyll a, chlorophyll b, carotene, and anthocyanin content in both seasons, than the untreated fruits (control).

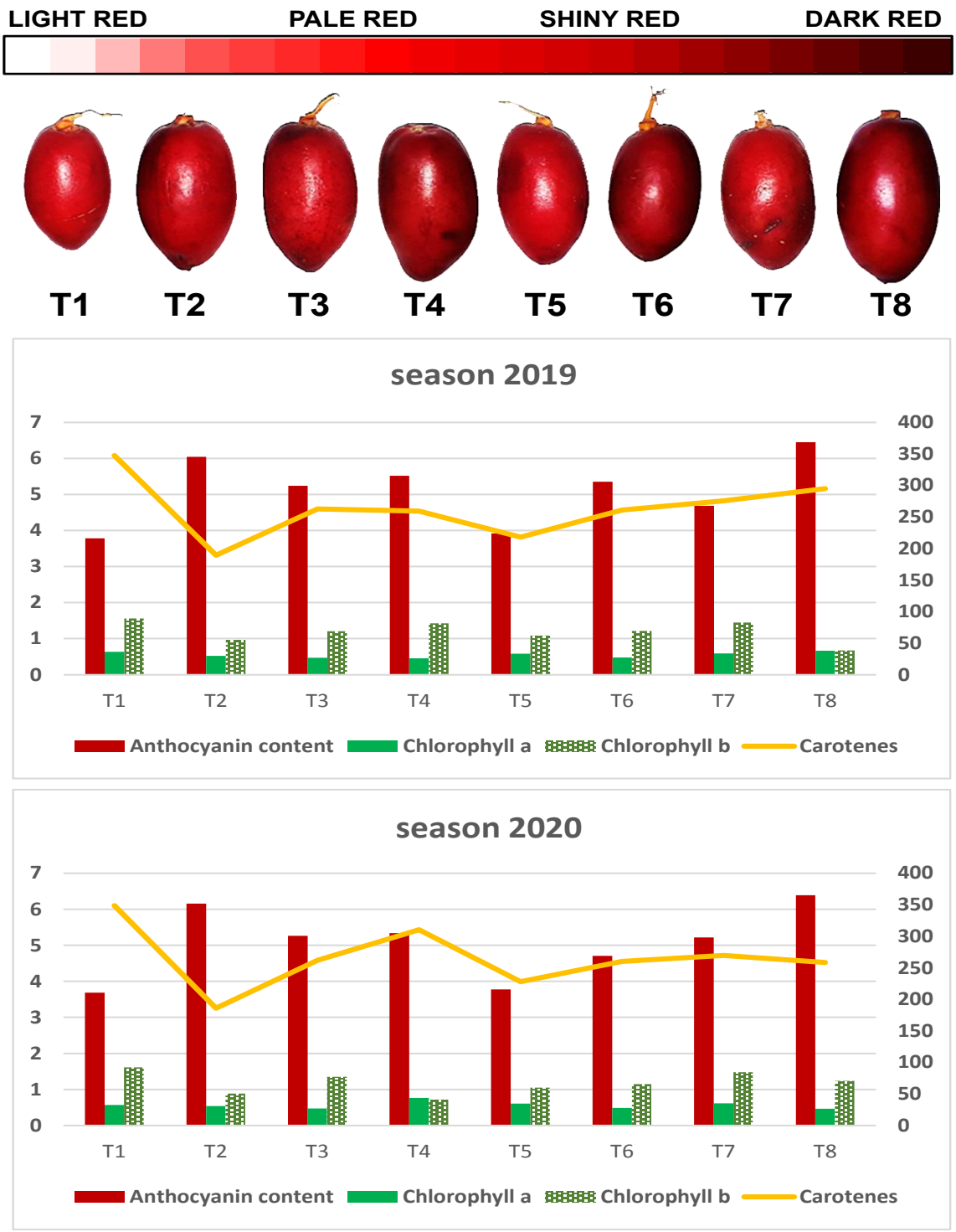


**Fig. 2. Effect of spraying with Arginine, Chitosan, and Galangal individually or mixed on the Hayani fruit color**

Notably, spraying fruits with a mixture of arginine, chitosan, and galangal mixed at concentrations of 100 ppm, 10 ml/L, and 25 ml/L respectively resulted in more colorful fruits compared to those that were not treated. The triple treatment recorded lower chlorophyll a (0.453 and 0.462 mg/g fruit peel FW), lower chlorophyll b (0.668 and 0.675 mg/g fruit peel FW), mild carotenes (295.07 and 258.69 mg/g fruit peel FW) and higher average of anthocyanin content (6.45 and 6.39 mg/100g fruit peel) in both seasons respectively. Whilst the highest chlorophyll a (0.668 and 0.762 mg/g fruit peel FW), chlorophyll b (1.557 and 1.616 mg/g fruit peel FW), carotenes (347.18 and 348.66 mg/g fruit peel FW) and lower average of anthocyanin content (3.78 and 3.69 mg/100g fruit peel) was observed for the untreated date palm fruits in 2019 and 2020 seasons respectively.

Fig. 3-a showed that total soluble solids (TSS) of Hayani date fruits significantly increased by spraying fruits with a mixture of arginine, chitosan, and galangal as mixed at concentrations of 100 ppm, 10 ml/L, and 25 ml/L respectively compared with control treatment in the two seasons. This represents an increase of 23.66% and 24% in both seasons, respectively, compared to an average control treatment of 22.03% and 22.16%.

Regarding the total acidity of date palm fruits, the results tabulated in Fig. 3-b showed that there was a significant difference in total acidity between different treatments including the untreated fruits and this was true in both seasons. Data indicated that the control (0.41 and 0.39%) gave the poor fruit quality the highest acidity ratio, while the lowest acidity ratio (0.13 and 0.12%) was recorded with chitosan treatment in both seasons, respectively. Additionally, the T.S.S/ Acidity ratio data in Fig. 3-c demonstrated a significant difference between treatments over the two seasons. Spraying chitosan alone at a concentration of 10 ml/L resulted in the highest T.S.S/Acidity ratio (174.82 and 177.86%) compared to other experimental treatments during both seasons, respectively. While the control treatment recorded the lowest ratio (53.61 and 56.57%).

Furthermore, there was a gradual increase in the total sugars when using arginine, chitosan, and galangal in combination compared to alone or without treatment (Fig. 3-d). The highest value was recorded with spraying mixture from arginine, chitosan, and galangal treatment (6.16 and 7.14 mg/g FW) meanwhile the control treatment gave the lowest ratio of total sugars (2.50 and 2.73 mg/g FW) in the first and second seasons, respectively.

The study also found that the use of arginine at 100 ppm in combination with chitosan at 500 ppm resulted in a significant decrease in tannins (0.35, 0.34, 0.34, 0.36, 0.29, and 0.29 mg/g FW) when spraying arginine and chitosan mix, chitosan and galangal mix, and a combination solution between arginine, chitosan, and galangal in both seasons, respectively (Fig. 3-e). The control treatment recorded the highest concentration of tannins as (0.56 and 0.62 mg/g FW).

There was a significant increase in the DPPH scavenging activity (%) when the Hayani fruits were treated with arginine when mixed with galangal (92.27 and 92.22%), or mixed with chitosan (92.06 and 91.85%), or mix with chitosan, and galangal in combination (93.66 and 93.04%). The lowest ratio of DPPH scavenging activity (72.48 and 71.56%) was obtained from date palms sprayed with water only (control treatment) during the two experimental seasons (Fig. 3-f). Meanwhile, the control treatment (spraying water only) resulted in the minimum fruit Vit C. values of 5.63 and 5.93 (mg/100 ml juice) during the first and second seasons of 2019 and 2020, respectively. Moreover, the maximum fruit Vit C. values of 9.66 and 9.90 (mg/100 ml juice) during both seasons, respectively (Fig. 3-g).

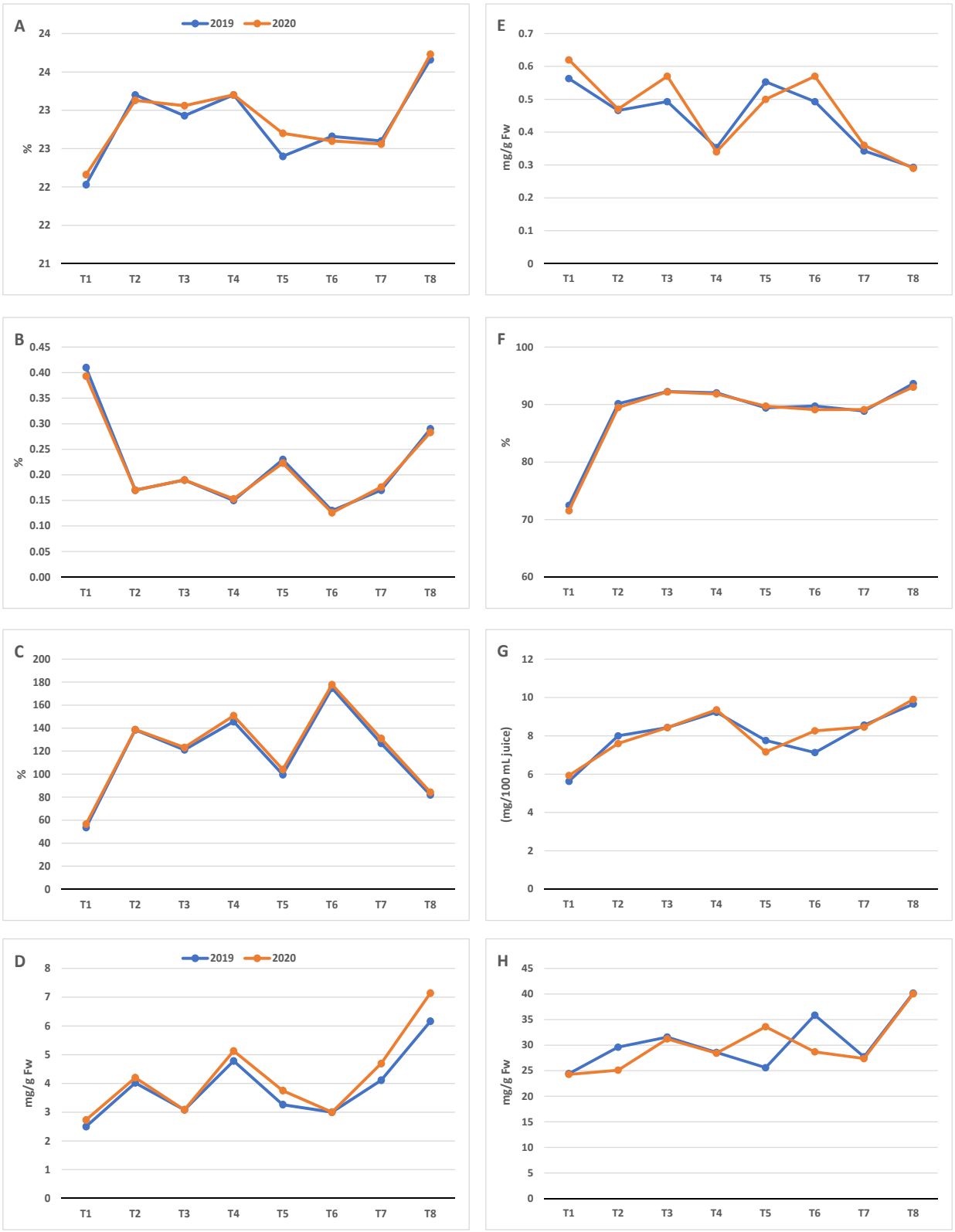


**Fig. 3. (A) Total soluble solid, (B) Acidity, (C) T.S.S/Acidity ratio, (D) Total sugars, (E) Tannins, (F) DPPH scavenging activity, (G) Total Vitamin C, (H) Total Phenolic Content**

Analysis of total phenolic content indicated that spraying all treatments except the control led to a significant increase in the concentration of phenol content during the 2019 and 2020 seasons (Fig. 3-h). In the 2019 season, the highest fruit phenolic content (40.17 mg/g FW) was achieved by the treatment of arginine when mixed with galangal and chitosan. Similarly, on the same line result noted in the 2020 season, the highly phenolic content (40.02 mg/g FW) was recorded after the spring solution of arginine when mixed with galangal and chitosan six times. While untreated fruits reached the minimum concentration (24.44 and 24.28 mg/g FW) in both seasons, respectively.

## DISCUSSION

Egypt is one of the major date-producing countries, but some local varieties have poor quality characteristics compared to international standards, which prevented Egypt from being among the top ten countries in exporting dates. The results of this study concluded that the spraying interaction treatments with arginine, chitosan, and galangal extract was superior in terms of improving bunch weight, fruit weight per bunch, remaining number of fruits per bunch, flesh net weight, fruit dimensions, fruit volume, color index, total soluble solids (TSS), DPPH scavenging activity, total phenolic content, and total vitamin C. These results are considered a positive indicator of the possibility of achieving applied agricultural solutions that can raise the quality of local date fruits.

Arginine acts as a precursor for polyamine synthesis such as putrescine, spermidine, and spermine which play a vital role in plant stress tolerances and regulate nitrogen absorbance, in addition it's very important in fruit setting and ripening stages **(Gupta *et al.*, 2013; Minocha *et al.*, 2014)**. This was reflected in recording the highest yield of Hayani date fruits, and the highest fresh weight and remaining number of fruits per bunch at harvest. Also, arginine converts in plant cells to nitric oxide, or proline amino acid, or into urea then ammonia through many metabolic pathways which are considered as most important messengers in all plant physio- and biochemical processes **(Yang and Gao, 2007; Winter *et al.*, 2015)**.

In addition, the best source of organic nitrogen is amino acids like arginine, which makes up 50-90% of the nitrogen-free in fruits **(Oland, 1959)**. Therefore, spraying arginine on plants like Hayani date trees that grow in sandy soil promotes the synthesis of proteins, RNA, and DNA, as well as enhances fruit production **(Correa-Aragunde *et al.*, 2016)**. Moreover, arginine is contributing to providing nitrogen to the plant, along with fertilization, which enhances obtaining an abundant fruit yield **(Wang *et al.*, 2021**). From that, the results indicate that arginine, either individually or in mixed treatments causes a significant increase in fruit production, the remaining number of fruits per bunch at harvest, fresh dry weight, TSS, and total sugars. **Edwards (1986)** indicated that arginine is important to cell division; this explains the increased Hayani fruit length, width, and volume.

Some research has focused on the beneficial effects of spraying arginine on many horticultural plants. Among these studies, in the treated plants with arginine, the induction of carbohydrates was observed, which led to an increase in total soluble solids and total soluble sugar **(Petridis *et al.*, 2018)**. Also, **Mohseni *et al.* (2017)** found that treated Strawberry plants with 500μM arginine improved qualitative traits such as TSS, total sugar, anthocyanin, phenol content, and vitamin C.

On the same line, **Noroozlo *et al.* (2019) and Souri and Hatamian (2019)** demonstrated that, after the application of amino acids, fruits have been shown an increase in vitamin C concentration. In

Eggplant, spraying of arginine at 50 mg $L^{-1}$ gave the highest average of total fruit yield **(Al-Sultani and Al-Tufaili, 2020)**. In Apple, based on a study of **Chen *et al.* (2022)** alkaloids, anthocyanin, phenol, flavonoids, and protein as antioxidant metabolites were increased with the application of arginine. In Guava, arginine treatment contributed to improving the fruit yield, fruit firmness, total soluble solids content, vitamin C, and total sugar content **(Almutairi *et al.*, 2022)**.

In a related context, chitosan is described as a safe, non-toxic product that is widely used in agriculture due to its mechanism of action in promoting plant growth and increasing fruit yield and quality as well as protecting it from diseases **(Malerba and Cerana, 2016)**. This was confirmed by the results of this study. Hayani date fruit yield was improved, as well as fruit quality characteristics were increased such as total sugar content, phenols, antioxidant activity, and fruits color after spraying chitosan on fruits during the kimiri and khalal stages.

Chitosan plays an active role in accumulating hormones such as auxin and cytokinin related directly to increasing fruits physiological mechanisms of cell division and elongation through increased nutrient intake as well as activate synthesis enzymes of protein **(John *et al.*, 1997; Guan *et al.*, 2009; Dzung *et al.*, 2011)**. This was reflected in obtaining a high yield of Hayani date, the highest fresh and dry weight, the longest and widest fruit, and the largest in size. It contributes to accumulating ABA that makes safe water available in plant cells through regulating the movement of the stomatal and it increases water uptake **(Bittelli *et al.*, 2001; Amin *et al.*, 2007; Guan *et al.*, 2009; Mukarram *et al.*, 2023)**. Therefore, chitosan can reduce the severity of the impact of water shortages in relation to the yield index at harvest. Especially since the Hayani palm tree at the study site depends on rainwater for irrigation and the soil is sandy and well-drained.

Moreover, chitosan enhances the phenol synthesis pathway in plant cells **(Park *et al.*, 2019; Samari *et al.*, 2020)**. This may clear the increase in its concentration in the study results after applying the spray at 10 mg/l. Furthermore, chitosan has been capacity to activate antioxidant enzymes of reactive oxygen species **(Hameed *et al.*, 2013**; **Alkahtani *et al.*, 2020)**. Thus, increasing in the DPPH scavenging activity was recorded in Hayani fruits from 72.48 to 93.66% in the 2019 season and from 71.56 to 93.04% in the 2020 season.

In General, much research demonstrated that chitosan acts as a promoter to enhancing yield and increase fruit quality in horticultural crops. According to **Jasim *et al.* (2015)** the spraying chitosan at a concentration of 2% had a positive effect on date palm fruits, cvs. Barhi and Bream on various parameters such as fruit yield, fruit fresh weight, fruit volume, fruit length, fruit diameter, and juice acidity. Chitosan foliar application at 5 mL $L^{-1}$ promoted physical and chemical mango fruit traits **(Zagzog *et al.*, 2017)**. Chitosan as exogenous spraying at 800 ppm on Sakkoti date fruits four times recorded the best results of fruit yield and quality such as total soluble solids %, acidity % as malic acid, total sugars %, while reducing the total tannins % **(Al-Wasfy *et al.*, 2022)**. Zaghloul date fruits when treated with 1% chitosan induced the fruit quality **(Kamal *et al.*, 2014)**. Foliar chitosan treatment at 2 and 4g/L on Washington navel orange fruits were improving physical and chemical fruit properties such as increasing fruit weight, fruit shape index, TSS%, TSS/Acid, and V. C content **(Mohamed and Ahmed, 2019)**.

Based on the study results, it has been observed that spraying arginine and chitosan treatment is effective in improving fruit quality. However, the addition of galangal extract to this mixture has significantly enhanced the physical and chemical properties of the fruits. This proves that plant

extracts have numerous benefits in enhancing date palm yield and fruit quality traits (**Zagzog and Saied, 2017; Abdulrahman *et al.*, 2023; Auda *et al.*, 2023**). Galangal extract contains various essential organic compounds and is relatively inexpensive. As a result, farmers can easily acquire and use it to improve productivity and fruit quality.

Galangal is an herb that is rich in essential oils, phenolic and flavonoid compounds, and diarylheptanoids. These compounds have antioxidant properties that create an environment free of oxidation and the presence of free radicals (**Kress *et al.*, 2015; Aloud *et al.*, 2018; Zhou *et al.*, 2018)**. This helps the cells to perform their physiological and chemical processes necessary for growth and fruiting.

Despite the potential benefits, no research has been conducted on the effects of galangal on improving fruit quality. However, **Elkady *et al.* (2022)** conducted a study on Sewi date palm, investigating the effect of turmeric extract at 1000 ppm, which comes from the same plant family as galangal namely Zingiberaceae. They found that turmeric achieved a high yield and quality of Sewi date palm fruits, ranking second after treatments with dry yeast. Also, **Al-Zaqzouq and Saeed (2017)** used camphor and turmeric among 14 other plant extracts on Sakkoti date palms and they found that they had a significant effect on improving fruit yield and quality.

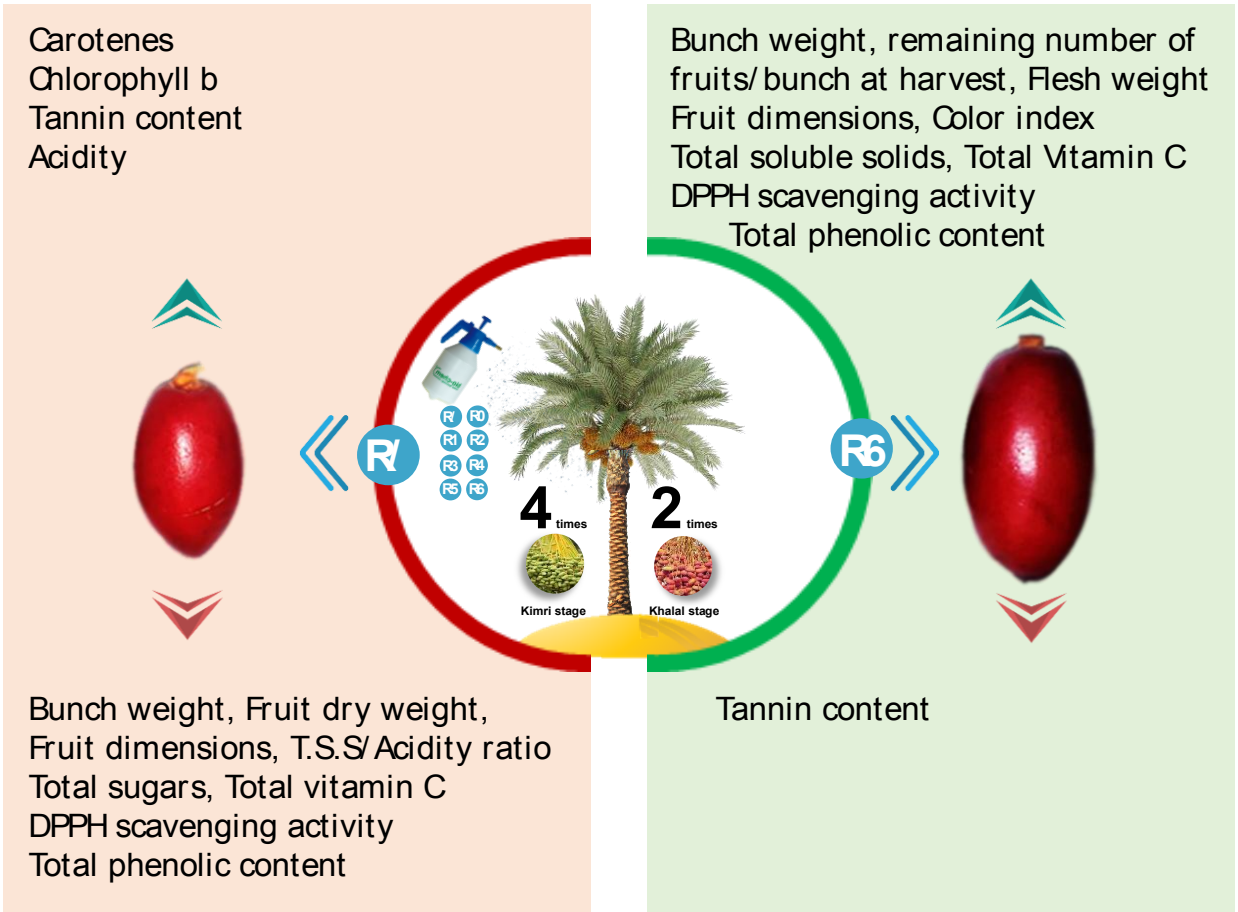


**Fig. 4. Schematic diagram illustrating the study conclusion after spraying with arginine, chitosan, and galangal extract on the quality of Hayani date fruits. Where, T1 = Control, and T2 = Arginine, chitosan, and galangal extract mix**

## Conclusion

Fruit yield, yield components, and chemical and biochemical characteristics of Hayani dates fruits were improved when spraying arginine at 100 ppm. This effect was further enhanced when chitosan at 10 ml/L and galangal extract at 25 ml/L were added to arginine solution and sprayed six times during the period of fruit growth and ripe under sandy soil conditions. This resulted in an increase in bunch weight, fruit weight per bunch, remaining number of fruits per bunch at harvest, flesh net weight, fruit dimensions, fruit volume, color index, total soluble solids (TSS), DPPH scavenging activity, total phenolic content, and total vitamin C. Additionally, the mixture of arginine with chitosan and galangal extract treatment produced lower tannin content in the fruits.

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**الملخص العربي**

# تأثير الرش الخارجي بالشيتوزان والأرجنين ومستخلص الخولنجان على جودة ثمار البلح الحياني

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نخيل البلح الحياني يعد الصنف الأكثر انتشارًا في شمال سيناء، مصر ويمتاز بشعبية كبيرة في الأسواق. إلا أن نوعية ثماره ليست جيدة، وتواجه منافسة من الأصناف المستوردة مثل صنف البرحي. ونتيجة لذلك، فإن تحسين جودة ثمار صنف الحياني بات أمرًا ملحًا من أجل الحفاظ على شعبيته بين المستهلكين والحفاظ على هذا الصنف المحلي من الاندثار. وفي هذا البحث تم دراسة تأثير رش ثمار نخيل البلح الحياني بالأرجينين والشيتوزان ومستخلص الخولنجان بتركيز 100 جزء في المليون و10 مل/لتر و25 مل/لتر على التوالي إما بصورة منفردة أو مختلطة، وذلك على المحصول وجودة الثمار. وأشارت النتائج إلى أن محصول الثمار ومكونات المحصول والخصائص الكيميائية والحيوية لثمار بلح الحياني قد تحسنت عند رش الأرجينين بتركيز 100 جزء في المليون. وبشكل عام، كان خليط الرش المكون من الأرجينين بتركيز 100 جزء في المليون، والشيتوزان بتركيز 10 مل/لتر ومستخلص الخولنجان بتركيز 25 مل/ لتر في الغالب أكثر فعالية من الرش المنفرد لكل منهم، وكان ذلك واضحًا لجميع الصفات التي تم تقييمها. حيث أدت تلك المعاملة إلى زيادة في وزن السباطة، ووزن الثمار، وعدد الثمار المتبقية عند الحصاد، والوزن الصافي للحم، وأبعاد الثمرة، وحجم الثمرة، والمواد الصلبة الذائبة الكلية، ونشاط مضادات الأكسدة، ومحتوى الفينول الكلي، وفيتامين سي. بالإضافة إلى ذلك، اكتسبت الثمار لون أحمر داكن بالمقارنة بمعاملة الكنترول التي ظهرت باللون الأحمر الفاتح. كذلك أدى خليط الأرجينين مع الشيتوزان ومستخلص الخولنجان إلى إنتاج محتوى أقل من التانيين في الثمار.

**الكلمات الاسترشادية:** الحياني، جودة الثمار، الأرجينين، الشيتوزان، مستخلص الخولنجان.

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