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Pre-harvest salicylic Acid Application and Different Packaging Material to Alleviate Chilling Injury of Sweet Basil (*Ocimum basilicum* L.) during Cold Storage

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Abstract: Fresh sweet basil has a very short storage period after harvesting. In this context we carried out to determine the effects of salicylic acid as pre-harvest treatment at different concentrations (0, 3, and 5mM), different packaging materials (low-density polyethylene and polypropylene) application combined with different temperatures (5^0 C, 10^0 C and ambient temperature) and stored for 0, 2, 4, 6, 8, and 10 days for chilling injury alleviation and prolonging the shelf life of sweet basil (*Ocimum basilicum*, L) fresh herb. This study conducted in the years 2021-2022. The results indicated that the lowest significant fresh weight loss % and the highest values of total chlorophyll, antioxidant activity, essential oil %, and its constituents were recorded for polypropylene bags. On the other hand, fresh herb stored at 10ºC gave the highest total chlorophyll, antioxidant activity and essential oil %. Moreover, 5 mM SA application was the most effective in alleviating chilling injury (CI) in the basil. At the end of storage (day 10), the CI score of 5 mM SA-treated sweet basil and stored at 10 ^{0} C or 5 ^{0} C in polypropylene bags was significantly lower than that of low-density polyethylene which it was lower than 2.5 (acceptable score). This may be due to the induction of defense a mechanism system by SA. So they may be considered polypropylene bags the most suitable packaging material to keep sweet basil fresh herb until 10 days under 5^0 C or 10ºC in both seasons.

Key words: Sweet basil, salicylic acid, pre-harvest, packaging.

1. Introduction

Sweet basil (*Ocimum basilicum* L.) is one of the most well-liked aromatic herbs, which is grown all over the world. **(Matthews *et al.*, 2018).** Strong antibacterial and antioxidant properties of basil essential oil have also been demonstrated, suggesting possible applications as a natural preservative. **(Takwa *et al.*, 2018).** Due to the growing demand for fresh, aromatic herbs, basil leaves are now an important competitor in the world market for food products. The transition toward healthier and better-tasting food preparations has led to a huge increase in demand for fresh basil over time. **(Curutchet *et al.*, 2014).**

Handling, storage, and transportation all have a significant impact on the shelf life, flavor, and ultimately the marketability of commercial herb output.

Harvested and processed basil is often shipped in refrigerated trucks with other herbs as most herbs are not chilling sensitive and low temperatures minimize disease and decay. While bulk shipment saves money by not requiring individual shipping arrangements, fresh-cut basil quality and marketability may be degraded. Growers can at least recognize when CI occurs before harvest or during cleaning, sorting, and grading at the farm, but they cannot control for injury that develops after the harvest leaves the farm. Unfavorable shipping or storage conditions result in the delivery of poor-quality produce, thus impacting future sales and markets and incurring a great economic loss**. (Brindisi *et al.*, 2021)**

Sweet basil being of tropical origin, is susceptible to chilling injury (CI) during low-temperature storage and transport. The most prevalent damage occurring in sweet basil exposed to temperatures below 12 ºC is brown spots of the interveinal areas of the leaf followed by the development of black necrosis and leaf abscission. Some other visual symptoms that might occur are loss of glossy appearance, wilting, and epinasty **(Begum *et al.*, 2023).**

Following harvest, a variety of factors, such as storage conditions and packaging material, may influence the speed which basil leaves deteriorate. Most fresh culinary herbs retain their quality best at cold temperatures **(Cantwell and Reid, 2002).** Since CI was recognized and reported in the literature, there has not been a release of commercial sweet basils that are chilling tolerant **(Dudai *et al.*, 2020).**

It has recently been demonstrated that salicylic acid, a plant growth regulator and safe chemical used to preserve postharvest quality, plays a significant role in the induction of bioactive substances such antioxidants and antioxidant enzymes. **(Supapvanich and Promyou, 2013).** Salicylic acid can reduce lipid peroxidation and get rid of ROS. In addition to stimulating plants' stress tolerance mechanism, salicylic acid-induced bioactive compounds have positive effects on human health. **(Supapvanich *et al.*, 2015).** On the other hand, little information regarding the effects of applying salicylic acid prior to harvest on the postharvest quality and bioactive components of sweet basil have been published. Salicylic acid improves fruit quality and storability, and has a toxicity effect on fungi during storage **(Barakat *et al*., 2015)**. Packaging modifies the composition of the air to give the best environment for preserving produce quality. A modified atmosphere formed by packing prolongs the storage life of produce by reducing respiration rate, enhancing moisture retention, and limiting physiological deterioration. **(Mangaraj *et al.*, 2009).**

So far, little research has been done to extend the storage life of fresh basil leaves. In order to formulate storage conditions for the enhancement of the shelf life of basil leaves, it is essential to understand the biochemical basis of quality maintenance of leaves during storage under different conditions.

Thus the purpose of this study was to investigate the methods that reduce damage to sweet basil caused by low temperatures during storage (Chilling injury), through salicylic acid as pre-harvest treatment and types of packaging material.

2. Materials and Methods

The present study aimed to investigate the effects of pre-harvest treatments with different concentrations of salicylic acid, packaging materials (low-density polyethylene and polypropylene) combined with different storage temperatures 5, 10 ^{0}C (90–95% humidity), and ambient temperature 28 ± 5^{0}C (60% humidity) on the storability and marketing visual quality of sweet basil fresh herb. The field experiment was carried out at Horticulture Research Institute and Food Technology Research Institute, ARC, Giza, during the two successive seasons 2021 and 2022.

Sweet basil seeds were obtained from the Farm of Medicinal and Aromatic Plants Research Department, El-Kanater El-khairia, El-Kalubia Governorate, Egypt, and sown on the 15th and 17th February 2021 and 2022, respectively, in seed trays. After 45 days from sowing, when the seedlings reached 20 cm height with 6-8 leaves, they were transplanted to the prepared plots in the experimental field. All plants received common agricultural practices, including fertilization, regular irrigation, and manual weed control, were done according to the recommendation of the Egyptian Ministry of Agriculture.

After two months of seedlings cultivation (during flowering stage), plants were sprayed with salicylic acid (SA) solution 0, 3, or 5 mM. Sweet basil was harvested after 24 h from SA spraying in the morning (7.30 am). Plants were harvested by cutting the herb 10 cm above the soil surface without bruising or injuring the leaves and stems on June 15th in the two seasons.

Treatments:

- Pre-harvest salicylic acid (SA): Control (without salicylic acid), Salicylic acid at 3mM and 5mM.
- Packaging Material: low-density Polyethylene (PE) and Polypropylene (PP)
- Storage temperature: 5 ^{o}C,10 ^{0}C and ambient temperature
- Storage time: 2, 4, 6, 8 and 10 days.

Two commercially available polymeric film bags were used as packaging materials. The investigated polymer materials were low-density polyethylene (LDPE) 20×30 cm of 40μm thickness and polypropylene (BOPP Biaxial oriented) 20×30 cm of 24 μm thickness.

The average oxygen transmission rates (OTR) of the PE and PP films are 4000 cc/ m^2/day and 1000 CC/m^2/day, respectively, while the water vapor transmission rate (WVTR) of films is LDPE 20 gm/m^2/day and 70 gm/m^2/day respectively.

Sweet basil plant packaging was heat sealed and stored; each package contained 50 gm as one replicate. The packages were divided into three groups; placed inside refrigerators and stored at two different temperatures 5 and 10 ^{0}C) at 95 % RH and ambient temperature of 28 ± 5^{0}C (60% humidity) for ten days.

Samples were taken at random from 3 replicates of each treatment and examined immediately after harvest as well as every 2 days intervals for the following properties:

1- Weight loss percentage was estimated according to the following equation:

$$\textbf{weight loss}\ \% = \frac{\text{initial weight of herb} - \text{weight of herb at sampling date}}{\text{initial weight of herbs}} \times 100$$

2- Headspace Gas Analysis

The levels of O_2 and CO_2 were determined before chemical, and physical analysis to verify packaging atmospheres for each group. Before opening bags, headspace gas analysis was measured by using an oxygen analyzer [Witt Oxybaby headspace gas analyzer (O_2/CO_2), model PCO_2 Plus/100, Gas Data Ltd.], Ethylene concentration was determined by using an Ethylene analyzer (F-950 three Gas Analyzer)

3- Chlorophyll content measurement:

Chlorophyll content was assayed according to the method of **Ferruzzi and Schwartz (2001).**

4-Antioxidant capacity assay and total phenolic content: Folin-Ciocalteu spectrophotometric method was selected to determine the total phenolic content as described by **Cefola *et al.* (2009).** The antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) spectrophotometric method as described by **Shan *et al.* (2005).**

5- Change of leave color during storage

The color of basil leaves was measured using a hand-held tristimulus reflectance colorimeter (model CR-400, Minolta Corp., Newburgh, NY, USA). The measurements were performed on leave surfaces before storage and also every sampling date during the storage period (0, 2, 4, 6, 8, and 10 days). CIE-L* (Lightness), a* (-greenness), and (Hue angle) H values according to **Shehata *et al.* (2020).**

6 - Chilling injury score measurement

The following scale was used to measure visual damage caused by chilling injury, or the browning of leaves **(Wongsheree *et al.*, 2009)**: (1) no damage; (2) a few dark spots; (3) less than 30% of the entire leaf area; (4) between 30% and 50% of the leaf area; and (5) more than 50% of the leaf area brown.

7 - Essential oil content was carried out according to the method of **ASTA (1985).**

8- Gas Liquid Chromatography (GLC) The volatile oil obtained from the fresh leaves was analyzed using Ds Chrome 6200 Gas Chromatography apparatus according to **(Adams, 2007).**

9- Statistical analysis: completely randomized design with three repetitions was used to automate the collected data in the factorial experiment. The factorial treatments were the combinations among four factors salicylic acid, package type, storage temperature, and storage time as described by **Gomez and Gomez (1984).** Statistical analysis was done by using the computer program MS-TATEC software version (4) using the L.S.D. test at 0.05.

The physical and chemical characteristics of the soil of the experimental field were determined according to **Jackson (1973)** and are shown in Table (1).

Table (1). Physical and chemical properties of the experimental soil

Physical properties of the experimental soil	
Clay%	38.51
Silt%	27.74
Fine sand%	31.42
Coarse sand%	2.33
Soil type	Clay sand
Chemical properties of the experimental soil	
pH	7.32
N_2 (ppm)	27.14
P_2O_5 (ppm)	102
K_2O (ppm)	166
Zn (ppm)	4.29
Fe (ppm)	1.89
B (ppm)	1.65
Mn (ppm)	0.52
Cu (ppm)	0.31

3. Results and Discussion

3.1. Fresh weight loss %

As the result shown in Table 2, the percentage of weight loss for the harvested and treated sweet basil increased considerably and consistently with prolonging the storage period. This may be due to respiration, transpiration and other senescence related metabolic process during storage). These results are in agreement with **(Masoud, 2011).**

Pre-harvest SA treatments had a significant effect on the loss of fresh weight during cold storage, all concentrations of SA reduced fresh weight loss compared to the control (without SA), SA at a rate of 5 mM gave the lowest values of weight loss with mean values 1.16%, and 1.27% in both seasons.

Herbs respiration, transpiration, and metabolic processes are mainly responsible for controlling weight loss. According to reports, SA closes stomata, which reduces herbs, and fruits weight loss and suppresses respiration rate. **(Zhang and Zhang, 2004).** The same as peach fruits of the cultivar "Delicia" that were treated with SA showed less weight loss than the control. **(Abbasi *et al.*, 2010)**. Also, **Supapvanich, *et al.*, (2015)** found that during storage, SA treatment reduced weight loss of lemon basil

Concerning the effect of packaging materials, data revealed that there were significant differences among the treatments during storage however, basil fresh herb packed in polypropylene had the lowest value of weight loss percentages 1.19 % and 1.40% as compared to those packed in polyethylene 1.58% and 1.79% during storage in the two seasons, respectively. This effect might be attributed to the low rate of respiration under polypropylene film since relative humidity was high inside the package, which diminished the weight loss during storage. Numerous studies have shown that film packaging can against water loss and can preserve the quality of perishable commodities **(Lee *et al.*, 2008).**

The polypropylene film used in this investigation did not have any holes, which prevented the sample from evaporating as normal. In contrast, the other package type (PE, polyethylene) demonstrated a considerable amount of water evaporation and higher evaporation. One of the main factors affecting the freshness and general quality of the products is maintaining the water content of fresh samples, particularly of leafy vegetables. Similar to **Lee (2008),** our results showed that using the right film to package sweet basil helps it stay fresher longer by minimizing water evaporation during storage and transportation before it reaches consumers.

Physiological loss in weight of basil leaves increased significantly during storage under all conditions. However, loss was quite lower at 5 ^{0}C (1.18% and 1.25 in both seasons) as compared to basil herb stored at 10 ^{0}C (1.42% and 1.47%) or under ambient conditions (1.99% and 2.23%), which similar results were noticed in both seasons and could be attributed to the slowdown of physiological process such as respiration and transpiration that occur at low temperatures. These results agreed with **(Masoud, 2011).**

The interaction between pre-harvest treatments, packaging materials, storage temperatures, and storage period was significant in the two seasons. After 10 days of storage, sweet basil plants treated with 5mM of SA and packed in a polypropylene bag and stored at 5 or 10 ^{o}C had the lowest value of weight loss percentage 0.92% and 1.19% in the first season and 1.34% and 1.63% in the second season respectively, while the highest weight loss percentages 3.81% and 4.01% were obtained from plant packed in polyethylene bag and stored under ambient conditions after 4 days in two seasons.

3.2. Gas composition inside the packages

Data in Figs (1 and 2) showed that in the two seasons; there was a significant decrease in O_2 % and an increase of CO_2% during the storage period as a result of its respiration. Similar results were obtained by **Julio and Cantwell, (1997)** who attributed that to the consumption of O_2 and production of CO_2 from parsley plants during their respiration process.

Concerning the effect of storage temperature, the data revealed that, the highest mean values of O_2 (17.52% and 17.41%) and lowest mean values of CO_2 during storage had been measured inside the packages stored at 5 ^{o}C. These results were similar to that reported by **Julio and Cantwell, (1997).** In contrast, decreased values of oxygen and increased values of carbon dioxide during storage had been measured inside the packages stored at 10°C and ambient temperature.

Oxygen concentration decreased whereas carbon dioxide concentration increased in all types of bags Fig. (1 and 2). The PE bag, which has the highest oxygen transmission rates, showed the lowest atmosphere modification. Oxygen and carbon dioxide concentration in the package continued to change until the equilibrium point occurred. The atmospheric compositions at the equilibrium point in the PE bag found oxygen concentration with mean of 16.07% and16.31% in the first and the second seasons, respectively. Carbon dioxide concentrations were found at 2.94% and 2.71% in the first and the second seasons, respectively. This is due to the high permeability to oxygen and carbon dioxide and low permeability to water vapor, which causes condensation of water vapor and damage to the stored basil plants. On the other hand, The PP bag which has the highest oxygen transmission rates, showed the lowest atmosphere modification. On the other hand, PP bags were found oxygen concentrations with mean values of 16.63% and 17% in the first and the second seasons, respectively. While carbon dioxide concentrations were found at 2.66%, and 2.33%, in the first and the second seasons, respectively. This is due to the decreased permeability to oxygen and carbon dioxide and increased permeability to water vapor in polypropylene bags.

Table (2). Fresh weight loss % of sweet basil as affected by salicylic acid, packing type, and storage temperature during six storage times in the two seasons (2021 and 2022)

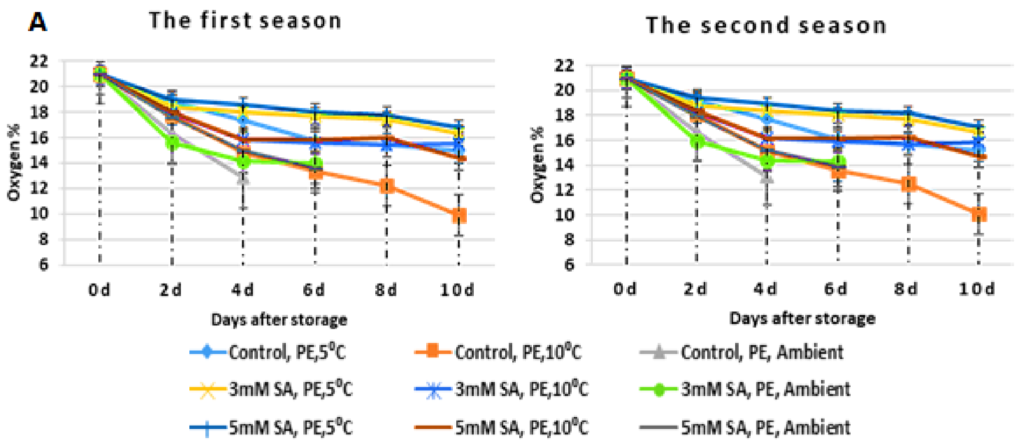
The First Season									
Pre-harvest	**Treatments**								
Salicylic Acid (SA)	**Packing Type (P)**	**Storage temp. (ST)**	**Storage time (T)**						**Mean**
			0	**2**	**4**	**6**	**8**	**10**	**SA**
SA0 Control	**PE**	**5**	0	1.1	1.21	2.33	3.11	3.23	
		10	0	1.28	1.45	2.82	3.68	4.11	
		Ambient	0	2.52	3.81	n. d.	n. d.	n. d.	**1.88**
	PP	**5**	0	0.82	0.93	2.08	2.77	3.12	
		10	0	0.9	0.96	2.43	3.4	3.86	
		Ambient	0	1.13	3.55	n. d.	n. d.	n. d.	
SA1 (3mM)	**PE**	**5**	0	1.03	1.16	1.24	2.35	2.85	
		10	0	1.17	1.26	1.35	2.86	3.33	
		Ambient	0	1.98	3.73	3.85	n. d.	n. d.	**1.55**
	PP	**5**	0	0.56	0.66	0.78	1.28	1.66	
		10	0	0.74	0.89	1.08	1.52	1.87	
		Ambient	0	0.92	3.43	3.54	n. d.	n. d.	
SA2 (5mM)	**PE**	**5**	0	0.54	0.8	1.08	1.17	1.31	
		10	0	0.63	0.96	1.23	1.66	1.81	
		Ambient	0	1.42	3.67	3.54	n. d.	n. d.	**1.16**
	PP	**5**	0	0.38	0.42	0.63	0.89	0.92	
		10	0	0.47	0.59	0.66	1.1	1.19	
		Ambient	0	0.9	2.87	3.14	n. d.	n. d.	
Mean (T)			**0.00**	**1.03**	**1.80**	**1.78**	**1.63**	**2.44**	
Mean (P)	**PE (Polyethylene)**		**1.58**						
	PP (Polypropylene)		**1.19**						
Mean (ST)		**5 ^{0}C**	**1.18**						
		10 ^{0}C	**1.42**						
		Ambient	**1.99**						
LSD $_{0.05}$			**(SA) = 0.145 (P) = 0.118 (ST) = 0.145 (T) = 0.205 SAxPxSTxTime = 0.470**						
The Second Season									
SA0 Control	**PE**	**5**	0	1.18	1.36	2.65	3.35	3.61	
		10	0	1.34	1.84	3.11	3.68	4.26	
		Ambient	0	2.85	4.01	n. d.	n. d.	n. d.	**2.01**
	PP	**5**	0	0.68	0.82	1.18	2.27	3.42	
		10	0	0.74	0.84	2.23	3.37	4.19	
		Ambient	0	2.51	3.78	n. d.	n. d.	n. d.	
SA1 (3mM)	**PE**	**5**	0	0.86	1.02	2.36	2.03	2.17	
		10	0	0.97	1.41	1.57	2.29	2.74	
		Ambient	0	2.34	3.66	4.32	n. d.	n. d.	**1.66**
	PP	**5**	0	0.47	0.58	1.04	1.87	2.35	
		10	0	0.61	0.78	1.17	1.87	2.48	
		Ambient	0	2.12	3.19	3.78	n. d.	n. d.	
SA2 (5mM)	**PE**	**5**	0	0.45	0.71	1.18	1.64	1.57	
		10	0	0.52	0.84	1.34	1.87	1.93	
		Ambient	0	2.21	3.09	3.47	n. d.	n. d.	**1.27**
	PP	**5**	0	0.32	0.37	0.69	1.28	1.34	
		10	0	0.39	0.43	0.72	1.35	1.63	
		Ambient	0	1.86	2.47	3.58	n. d.	n. d.	
Mean (T)			**0.00**	**1.25**	**1.73**	**2.15**	**2.24**	**2.64**	
Mean (P)	**PE (Polyethylene)**		**1.79**						
	PP (Polypropylene)		**1.40**						
Mean (ST)	**5 ^{0}C**		**1.25**						
	10 ^{0}C		**1.47**						
	Ambient		**2.23**						
LSD $_{0.05}$			**(SA) = 0.175 (P) = 0.143 (ST) = 0.175 (T) = 0.248 SAxPxSTxTime = 0.353**						

n. d. values not determined due to deterioration of basil, SA0= without salicylic acid (control), SA1=salicylic acid at rate 3mM, SA2=salicylic acid at rate 5mM, packing type (P) PE= low-density polyethylene, PP= polypropylene, Storage temperature (ST)=5^{0}C, 10^{0}C and Ambient temperature, Storage time (T)= days after storage, 0 time, 2,4,6,8 and 10 days.

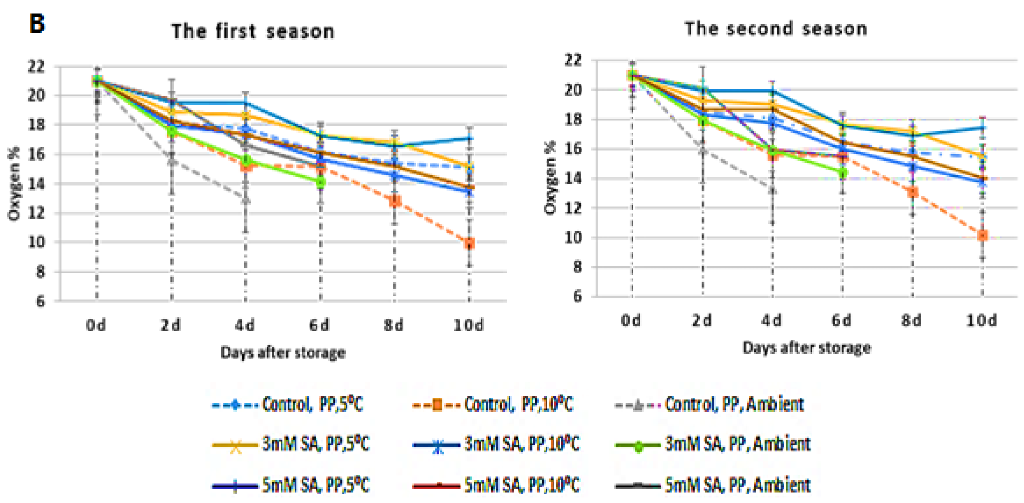
During storage, CO_2 was high in PE bags. Polyethylene (PE) films are permeable to O2 and CO2, which modifies the environment within the bag, in addition to serving as an effective barrier against water loss caused by the high humidity formed within the pack of non-perforated bags **(Kader, 1986).** In sealed PE bags, an excessive amount of humidity promoted the growth of pathogenic microorganisms.

Concerning the effect of pre-harvest treatments with salicylic acid, the data revealed that, the highest value of O_2 and lowest value of CO_2 during storage had been measured inside the packages. SA at rate of 5 mM gave the highest values of O_2 at 17.36% and 17.66% in both seasons. While, the same concentration of SA (5mM) gave the lowest values of CO_2 at 2.45% and 2.58% in the first and the second seasons, respectively, compared to control. These findings are consistent with **Koyuncu *et al.* (2019)** on pomegranate **and Sakaldaş *et al.* (2010)** on dill.

The interaction between pre-harvest treatment, packaging material, storage temperature, and storage period was significant. However, after 10 days of storage at 5 ºC and pre-harvest treatment with SA at a rate of 5 mM, the measured values of O_2 and CO_2 levels in the polypropylene bags were (16.21 % O_2 and 3.47 % CO_2) in the first season and (16.44 % O_2 and 3.61 % CO_2) in the second season. Followed by, after 10 days of storage at 10 ^{0}C and pre harvest treatments with SA at rate 5mM, the measured values of O_2 and CO_2 levels in the polypropylene bags were (15.07 % O_2 and 4.17 % CO_2) in the first season and (16.04 % O_2 and 3.13 % CO_2) in the second season, respectively.

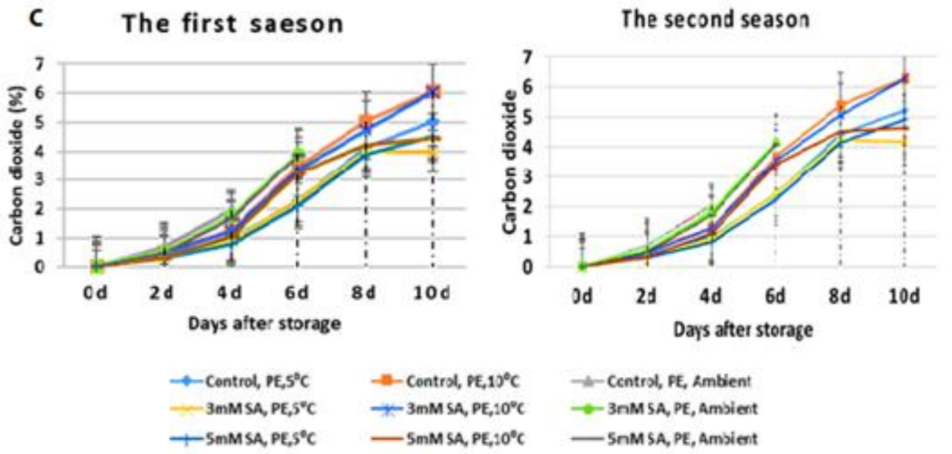


LSD $_{0.05}$ (SA) = 0. 40 (P) = 0.23 (ST) = 1.14 (T) = 1.02 SAxPxSTxTime = 1.55

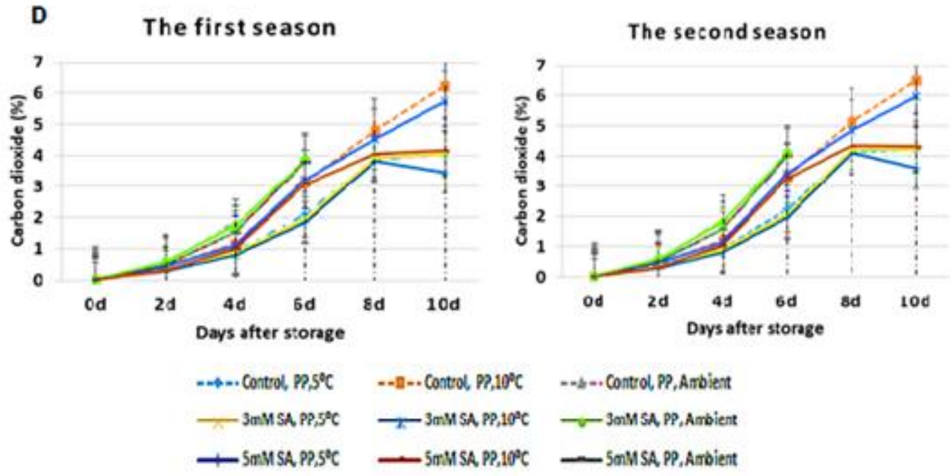


LSD $_{0.05}$ (SA) = 0. 40 (P) = 0.23 (ST) = 1.14 (T) = 1.02 SAxPxSTxTime = 1.55

Fig. 1. Oxygen percentage in fresh herb of sweet basil as affected by salicylic acid (0, 3, 5mM), packing type A: low-density polyethylene (PE), B: polypropylene (PP), storage temperature (5^{0}C, 10^{0}C, Ambient) during six storage times in the two seasons (2021 and 2022).



LSD $_{0.05}$ (SA) = 0.20 (P) = 0.11 (ST) = 0.14 (T) = 0.70 SAxPxSTxTime = 1.27



LSD $_{0.05}$ (SA) = 0.55 (P) = 0.11 (ST) = 0.14 (T) = 0.70 SAxPxSTxTime = 1.27

Fig. 2. Carbon dioxide percentage in the fresh herb of sweet basil as affected by salicylic acid (0, 3, 5mM), packing type C: low-density polyethylene (PE), D: polypropylene (PP), storage temperature (5^{0}C, 10^{0}C, Ambient) during six storage times in the two seasons (2021 and 2022).

3.3. Ethylene content (ppm)

Data in Fig. (3) showed that in the two seasons; there was a significant increase in ethylene during the storage period. Similar results had been obtained by **(Cantwell and Reid, 2002).**

Concerning the effect of storage temperature, the data revealed that, the highest value of ethylene during storage had been measured inside the packages stored at Ambient temperature, followed by the packages stored at 10 ^{0}C. while the lowest values of ethylene were measured at 5 ^{0}C. These results are similar, to **Martinez *et al.* (2005)**, only traces of ethylene were found right after cutting the leaves of iceberg lettuce, which did not create measurable amounts of ethylene during storage at 5°C. Moreover, **Spinardi and Ferrante (2012)** on lettuce, found that the ethylene content in stored bags at 4 ^{0}C lower than those stored at 10 ^{0}C. After five days of storage at 4 °C and 10 °C, the rate of ethylene production was approximately 15 times lower than it was at zero time. Because of the slower rates of biosynthesis during storage and the gradual leakage out of the package, the amount of ethylene accumulation in the bags has decreased.

Effect of package type on ethylene content, the presented data in Fig. (3) showed that PP packaging scored the lowest values of ethylene at 0.32 ppm in the first season, and 0.35 ppm in the second season. while, EP bags gave the highest values of ethylene 0.49 ppm in the first season and 0.50

ppm in the second season, respectively. This is due to the high permeability to low permeability of water vapor, which causes condensation of water vapor and damage to the stored basil plants. Too high humidity in sealed PE bags enhanced the growth of disease-causing microorganisms.

Concerning the effect of pre-harvest treatments with salicylic acid, the data revealed that, the lowest values of ethylene during storage had been measured inside the packages compared to control (without SA). SA at a rate of 5 mM gave the lowest values of ethylene at 0.35 and 0.37 ppm in both seasons.

The initial stage in the development of cold-induced injuries is membrane damage. Reactive oxygen species (ROS) are produced during this process, and antioxidant enzymes help to eliminate them from the membranes. When lipid molecules transition from a gel to a crystalline state, the metabolism is altered, leading to ion leakage, a decrease in mitochondrial activity, modifications in ethylene production and membrane-associated enzyme systems, as well as an accumulation of toxic metabolites. According to **Renhua *et al.* (2008)**, exogenous SA could therefore be beneficial in decreasing the rate of respiration and ethylene production. SA treatment prevented softening by suppressing ethylene biosynthesis, thus delaying the climacteric rise in ethylene **(Zhang and Zhang 2004).** The results revealed that only 1 mM SA ensured significantly higher firmness **(Zhang *et al.*, 2016).**

The interaction between pre-harvest treatment, packaging material, storage temperature, and storage period was significant. However, after 10 days of storage basil plants treated with SA at a rate of 5 mM, the measured values of ethylene concentration in the polypropylene bags were 0.41 and 0.43 ppm when storage at 5 ^{0}C or 0.48 and 0.50 ppm at 10 ^{0}C in both seasons. It is noted that there are no significant differences between 5 ^{0}C and 10 ^{0}C when treated plants with salicylic acid and packaged in polypropylene.

Change of leave color during storage

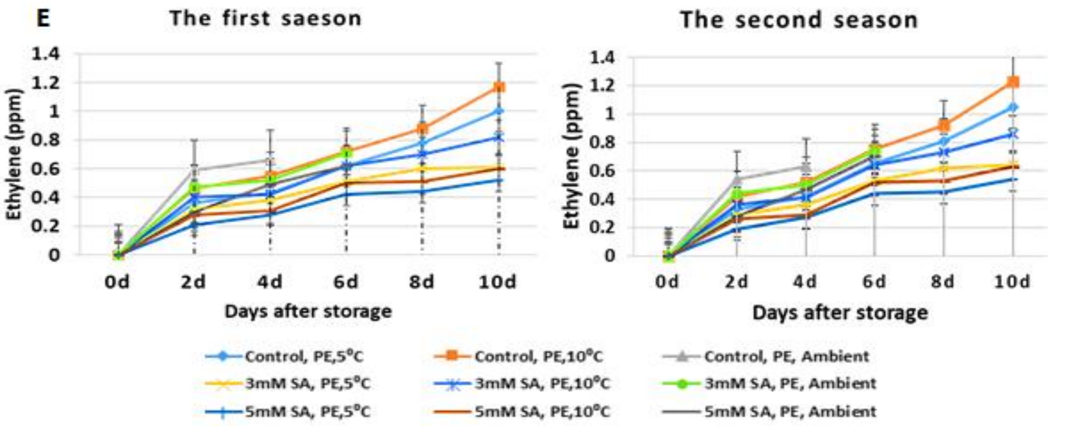
It is generally accepted that leafy vegetables' superficial color, particularly their greenness, greatly impacts their quality. As the results shown in Figs 4,5 and 6 The color degree of the homogenized samples (L, a value and hue angle) was measured; the L value is the measure for the lightness of the leaves, a is greenness, while the hue angle represents a coordinate in a standardized color space.

The lightness of basil fresh herb was affected by storage time. There was a decrease in L^*, a value, and hue angle value with increasing the storage period indicating darker color. These results agreed with **Suamuang *et al.*, (2016)** on holy basil plant.

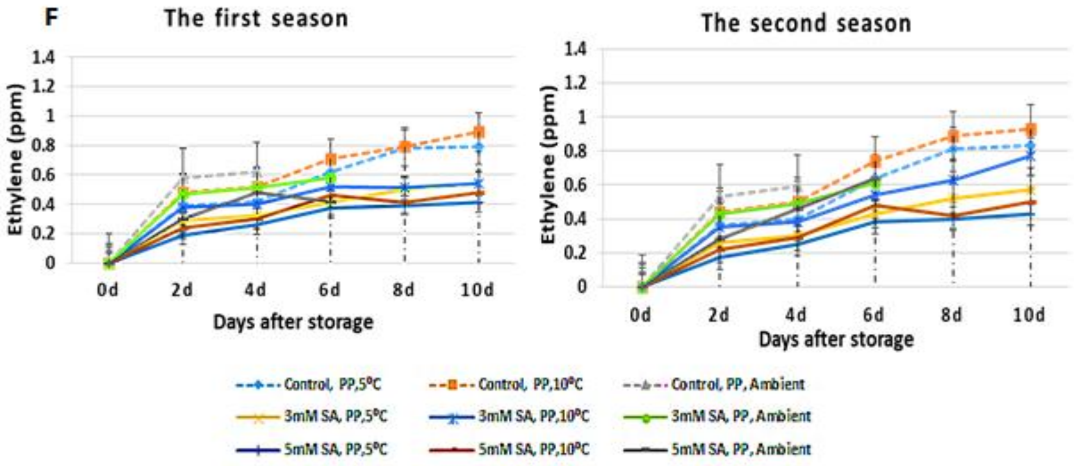
Concerning packaging material, the data revealed that there were significant differences between different packaging films. Sweet basil fresh herb packed in polypropylene bags gave the highest value of L, a, and hue angle values, resulting in lighter color and maintaining green color, while those packed with polyethylene gave the lowest values and resulted in darker color and green loss during storage. These results are true in the two seasons and agree with **(Lee, 2008).**

SA treatment kept the greenness value of sweet basil during cold storage whereas those of the control significantly decreased. Significant differences in lightness and hue value of both SA-treated and untreated sweet basil leaves were found over storage. Similarly, **Wei *et al.*, (2011)** found that SA treatment could maintain the superficial color of asparagus spears, due to delaying the deterioration in green color. The changes in chlorophyll content are generally related to the superficial color. The loss of total chlorophyll content is concomitant with the loss of greenness and the occurrence of yellowness in leafy vegetables **(Supapvanich *et al*., 2012).** SA at a rate of 5mM gave the highest values of lightness, greenness, and hue angle in both seasons.

There were significant differences between different storage temperatures in L, a, and hue angle values. Sweet basil fresh herb stored at 10ºC gave a higher L value as compared to those stored at 5ºC and Ambient temperature in both seasons. Sweet basil plants stored at Ambient temperature and 5c gave the lowest values of hue angle as an important decreasing or intense yellowing compared to those stored at 10 ^{0}C, which maintained high hue angle values during storage. This result was similar to that found in basil (*Ocimum basilicum* L.) by **Hassan and Mahfouz (2010).**



LSD $_{0.05}$ (SA) = 0.07 (P) = 0.04 (ST) = 0.04 (T) = 0.06 SAxPxSTxTime = 0.12



LSD $_{0.05}$ (SA) = 0.05 (P) = 0.04 (ST) = 0.05 (T) = 0.07 SAxPxSTxTime = 0.10

Fig. 3. Ethylene concentration (ppm) as affected by salicylic acid (0, 3, 5mM), packing type E: low-density polyethylene (PE), F: polypropylene (PP), storage temperature (5^{0}C, 10^{0}C, Ambient) during six storage times in the two seasons (2021 and 2022).

The interaction between pre-harvest treatment, packaging material, storage temperature, and storage period was significant. However, after 10 days of storage sweet basil plants treated with SA at a rate of 5mM and packed in polypropylene bags, and stored at 10 or 5 ^{0}C gave the highest value of hue angle (maintaining green color), lightness, and greenness values while those untreated (control) and packed with polyethylene and stored at ambient temperature gave the lowest one resulted in green color loss. It is noted that the type of packaging (polypropylene) and pre-harvest treatment with salicylic acid at a rate of 5mM reduced the loss of green color and chlorophyll content at a storage temperature of 5c after 10 days of storage. These suggest that pre-harvest SA treatment was able to maintain the superficial color of sweet basil by retarding chlorophyll loss. These might involve the increase in photosynthesis rate and the number of chloroplasts by SA which described by **Radwan *et al.*, (2008).** Furthermore, it was reported by **Li *et al.*, (1992) and Kazemi *et al.*, (2011)** that the reduction of chlorophyll loss by SA application might due to the suppression of ACC synthase and ACC oxidase activities.

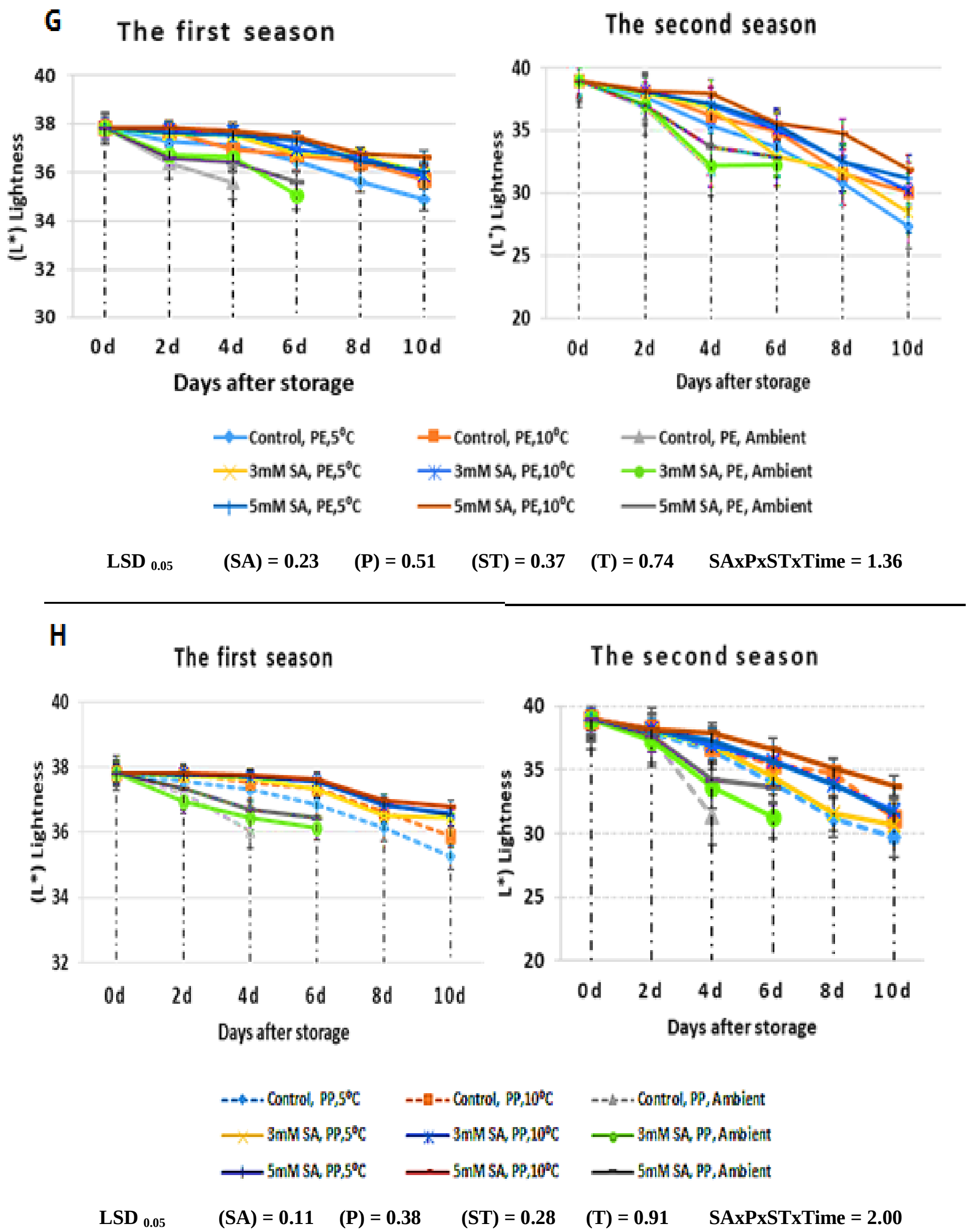
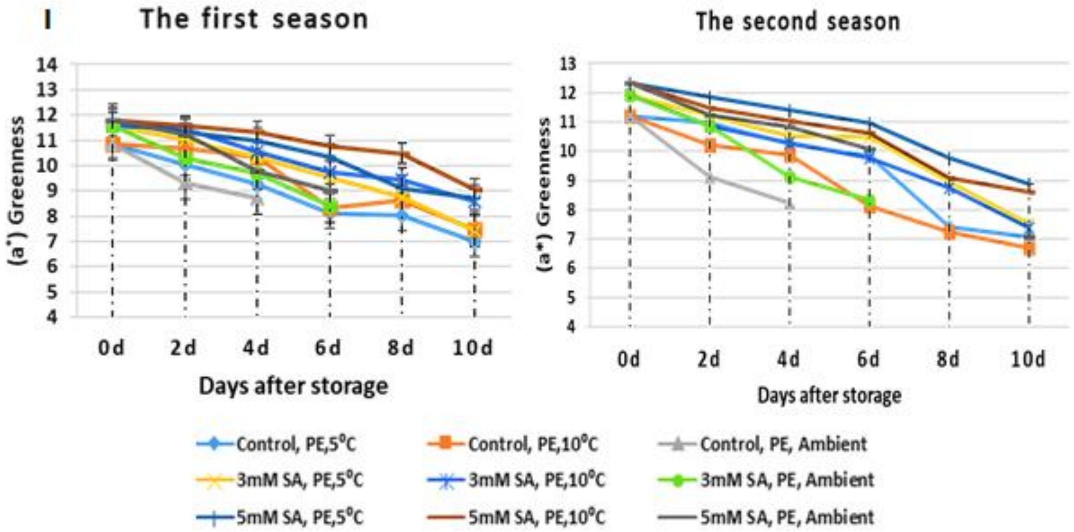


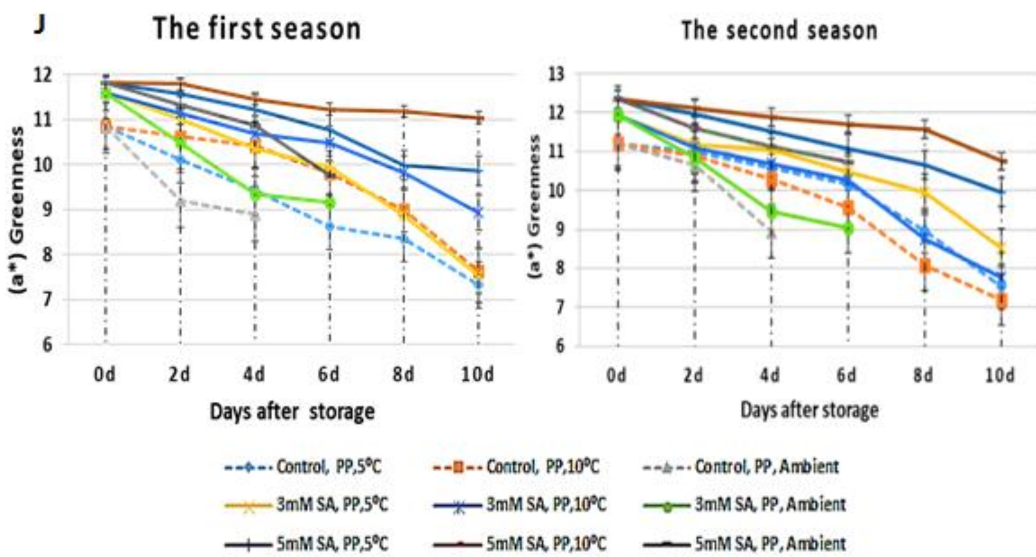
Fig. 4. Color degree (L*) Lightness in sweet basil as affected by salicylic acid (0, 3, 5mM), packing type G: low-density polyethylene (PE), H: polypropylene (PP), storage temperature (5^{0} C, 10^{0} C, Ambient) during six storage times in the two seasons (2021 and 2022).

I



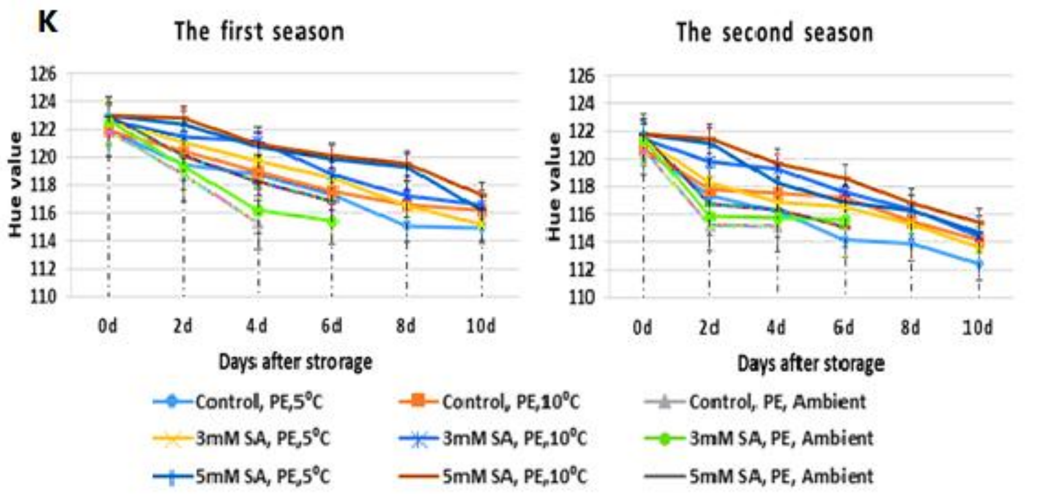
LSD $_{0.05}$ (SA) = 0.38 Packing (P) = 0.27 (ST) = 0.30 (T) = 0.61 SAxPxSTxTime = 1.66

J

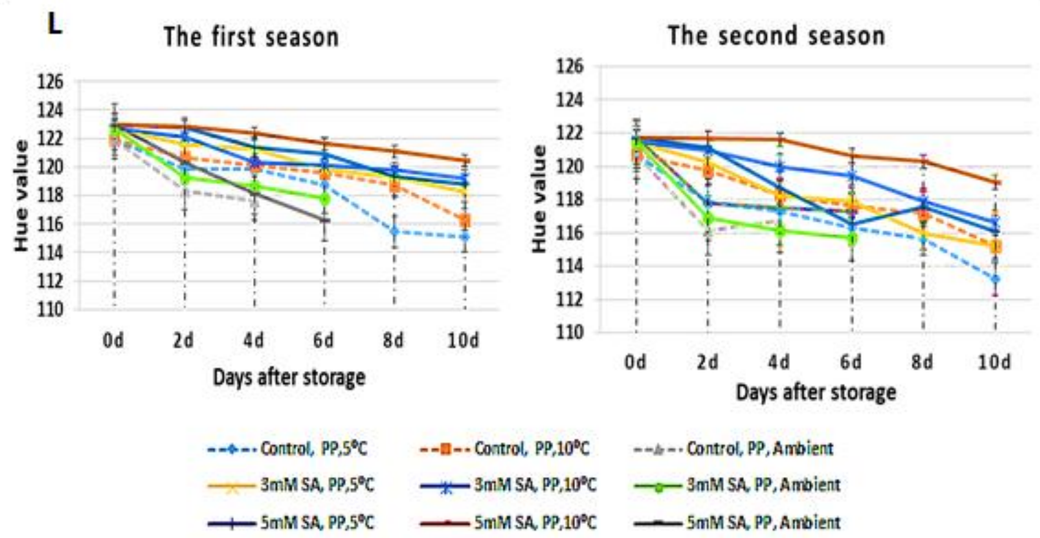


LSD $_{0.05}$ (SA) = 0.65 (P) = 0.48 (ST) = 0.57 (T) = 0. 35 SAxPxSTxTime = 1.24

Fig. 5. Color degree (a*) Greenness in sweet basil as affected by salicylic acid (0, 3, 5mM), packing type I: low-density polyethylene (PE), J: polypropylene (PP), storage temperature (5^0 C, 10^0 C, Ambient) during six storage times in the two seasons (2021 and 2022).



LSD $_{0.05}$ (SA) = 0.68 (P) = 0.15 (ST) = 0.35 (T) = 0.87 SAxPxSTxTime = 1.73



LSD $_{0.05}$ (SA) = 0.88 (P) = 0.53 (ST) = 0.70 (T) = 1.17 SAxPxSTxTime = 1.80

Fig. 6. Color degree (Hue value) in sweet basil as affected by salicylic acid (0, 3, 5mM), packing type K: low-density polyethylene (PE), L: polypropylene (PP), storage temperature (5^{0}C, 10^{0}C, Ambient) during six storage times in the two seasons (2021 and 2022).

3.4. Total chlorophyll content

Total chlorophyll content of sweet basil was decreased as the storage period was extended Fig. 7. The decrease of this content is probably due to the degradation of chlorophyll by chlorophyllase enzyme. Our results agreed with **Sakaldas and Kays (2010) and Masoud, (2011).**

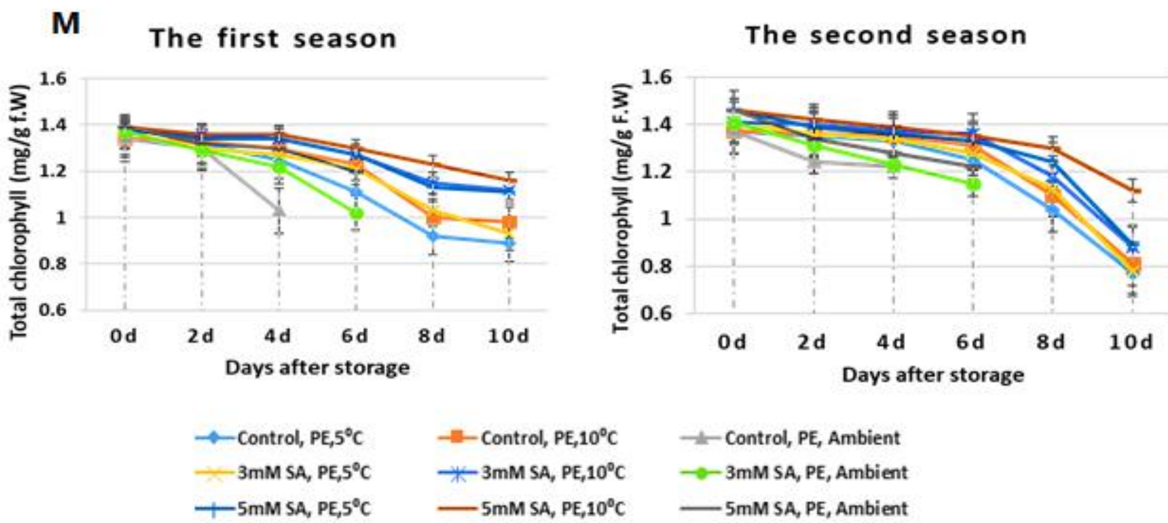
Barbosa *et al.*, (2016) indicated that there are two stages to the plants' degradation of chlorophyll. During the initial stage, the mass and the water level decreased in the leaves the pigments became more concentrated. The following shows a minor loss of green color or yellowing, which indicates a decline in quality over time in the medicinal and aromatic plants.

Regarding the effect of packaging materials, data showed that treatments during storage varied significantly from one another.; however sweet basil fresh herb packed in polypropylene seems to be the most effective in reducing the total chlorophyll loss; followed by polyethylene with significant differences between them. These results were true in the two seasons and agree with **Nand and Yadav (2014)** who concluded an increase in the value of total chlorophyll in PP (polypropylene) on *Anethum graveolens* L.

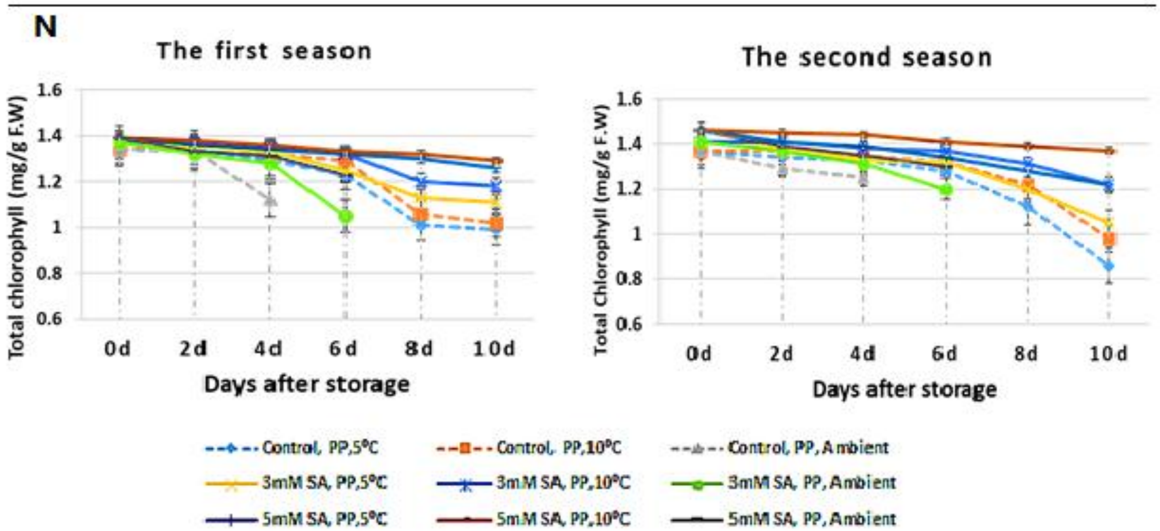
Regarding the effect of storage temperature, significant differences had been found in total chlorophyll content between different temperature degrees during storage. Regarding the effect of storage temperature, significant differences had been found in total chlorophyll content between different temperature degrees during storage, however, sweet basil plants stored at 10ºC gave the highest value of total chlorophyll content, followed by 5^{0}C. while those stored at an ambient temperature resulted in the lowest one. This result was similar to that found in basil (*Ocimum basilicum* L.) by **Hassan and Mahfouz (2010).,** who in a study on storage at 5 °C reported that the total chlorophyll content gradually decreased during storage.

The loss of total chlorophyll content is concomitant with the loss of greenness and the occurrence of yellowness in leafy vegetables **(Supapvanich *et al*., 2012).** In this investigation, the total chlorophyll content of the sweet basil was preserved by the SA application while it was significantly decreased in the untreated plants. (Fig.7.). SA at a rate of 5 mM gave the highest mean values of total chlorophyll content (1.27 and 1.31 mg/g) in both seasons. Similarly, the application of SA preserved the greenness and chlorophyll content of asparagus **(Wei *et al.*, 2011).** These suggest that pre-harvest SA treatment was able to maintain the superficial color of sweet basil by retarding chlorophyll loss. Also, **Radwan *et al.* (2008)** reported that an increase in the number of chloroplasts and the rate of photosynthesis by SA. Moreover**, Kazemi *et al.*, (2011)** found that The decrease of chlorophyll loss by SA application could due to the suppression of ACC synthase and ACC oxidase activities.

The interaction between pre-harvest treatment, packaging material, storage temperature, and storage period was significant. After 10 days of storage, sweet basil treated with SA at rate 5mM, packed in polypropylene bags and stored at 10 ^{0}C had the highest value of chlorophyll content (1.29 and 1.37 mg/g F.W) in both seasons, followed by sweet basil treated with SA at rate 5mM, packed in polypropylene bags and stored at 5 ^{0} C after 10 days. While the lowest one was found after 4 days of storage in those untreated with SA, packed in polyethylene and stored at ambient temperature.



LSD $_{0.05}$ (SA) = 0.04 (P) = 0.04 (ST) = 0.03 (T) = 0.06 SAxPxSTxTime = 0.11



LSD $_{0.05}$ (SA) = 0.03 (P) = 0.04 (ST) = 0.04 (T) = 0.07 SAxPxSTxTime = 0.10

Fig. 7. Total chlorophyll content mg/g in sweet basil as affected by salicylic acid (0, 3, 5mM), packing type M: low-density polyethylene (PE), N: polypropylene (PP), storage temperature (5^{o}C, 10^{o}C, Ambient) during six storage times in the two seasons (2021 and 2022).

3.5. Bioactive compounds

3.5.1. Total phenols and DPPH scavenging activity

Fresh herbs were frequently studied for their antioxidant properties, particularly those involving DPPH scavenging activity and total phenols, which are recognized as the physiologically active chemicals beneficial to human health. Total phenols and DPPH scavenging activity of basil fresh herb were affected by storage time as shown in Tables (3 and 4). There was a decrease in total phenols and DPPH scavenging activity with increasing the storage period. These results agreed with **Ghasemzadeh *et al.*, (2016)** on basil plant.

Total phenol and DPPH scavenging activity values of SA-treated Sweet basil were significantly higher than those of the control. SA at a rate of 5mM gave the highest values of total phenol (8.66 and 8.80 mg/g) and DPPH scavenging activity (76.26% and 74.48%) in both seasons. Recent research on fruits and vegetables has demonstrated that a variety of bioactive substances, including their concentration of flavonoids and phenolics **(Wei *et al.*, 2011)** and antioxidant enzymes **(Supapvanich *et al.*, 2012),** could be responsible for their antioxidant efficacy recorded (**Wei *et al.*, 2011).** In the similar vein, total phenols content in SA-treated rambutan fruit **(Supapvanich, 2015)** and peach fruit **(Razavi *et al.*, 2014)** were higher than those of the untreated fruits. These demonstrate that fresh produce is induced by SA to make physiologically active chemicals, which is a result of the defense mechanism being stimulated, as explained by **Supapvanich and Promyou (2013).** Also, **Erogul and Özsoydan (2020)** reported that preharvest SA treatment at rate 2mM increased antioxidant capacity and total phenols in peach compared to control

Concerning the effect of packaging materials, data showed that there were significant differences among treatments during storage; however sweet basil fresh herb packed in polypropylene gave the highest values of total phenols 8.13 and 8.09 mg/g and DPPH scavenging activity at 74.72% and 70.76% compared to polyethylene bags with significant differences between them. These results were true in the two seasons and agree with **(Sharma *et al.*, 2018)** on basil.

The decrease of total phenols and DPPH scavenging activity was significantly rapid under ambient conditions as compared to plants kept at 10 ^{0}C and 5 ^{0}C. however, sweet basil plants stored at 10ºC gave the highest value of total phenols 8.50 and 8.65 mg/g and DPPH scavenging activity at 76.75% and 73.34% in both seasons, followed by 5^{0}C. These results agreed with **Sharma *et al.*, (2018)** and **(Dos *et al.*, 2020)** who found fresh basil leaves stored for 7 days in the dark at 10°C showed higher phenolic content and antioxidant activity.

The majority of the secondary metabolites in sweet basil that are responsible for its fragrance, color, and pharmacological and antioxidant properties are likewise lost at lower storage temperatures **(Ghasemzadeh *et al.*, 2016).** Samples of basil kept for nine days at 4°C had substantially less antioxidants, polyphenols, and volatile aroma components than samples kept at 12°C. **(Fratianni *et al.*, 2017).** Sudden declines in basil volatile compounds have been observed even earlier after only 3 days at 5°C **(Rodeo and Mitcham, 2023).**

The interaction between pre-harvest treatment, packaging material, storage temperature, and storage period was significant. However, after 10 days of storage sweet basil plants treated with SA at a rate of 5mM and packed in polypropylene bags and stored at 10 ^{0}C and 5 ^{0}C gave the highest values of total phenol 7.85, 8.09 and 7.63, 7.97 mg/g and DPPH scavenging activity 70.97%, 74.26% and 71.40%, 74.85% in the first and the second seasons, respectively. While those untreated (control) and packed with polyethylene and stored at ambient temperature after 4 days gave the lowest values of total phenol and DPPH scavenging activity in both seasons.

Table (3). Total phenol (mg/g) in sweet basil as affected by salicylic acid, packing type, storage temperature during six storage times in the two seasons (2021-2022)

The First Season									
Pre-harvest	**Treatments**								
Salicylic Acid (SA)	**Packing Type (P)**	**Storage temp. (ST)**	**Storage time (T)**						**Mean SA**
			0	**2**	**4**	**6**	**8**	**10**	
SA0 Control	**PE**	**5**	8.59	8.40	8.02	7.75	6.45	6.13	
		10	8.59	8.49	8.19	7.84	7.28	6.79	
		Ambient	8.59	6.22	5.60	n.d.	n.d.	n.d.	**7.53**
	PP	**5**	8.59	8.34	8.34	7.94	7.56	6.88	
		10	8.59	8.55	8.42	8.11	7.36	7.37	
		Ambient	8.59	6.55	5.76	n.d.	n.d.	n.d.	
SA1 (3mM)	**PE**	**5**	9.01	8.38	8.21	7.90	7.87	7.29	
		10	9.01	8.97	8.83	8.41	8.21	7.54	
		Ambient	9.01	7.23	6.59	5.24	n.d.	n.d.	**7.92**
	PP	**5**	9.01	8.68	8.56	8.11	7.73	7.35	
		10	9.01	9.05	8.33	8.02	7.48	7.19	
		Ambient	9.01	8.34	6.91	5.72	n.d.	n.d.	
SA2 (5mM)	**PE**	**5**	10.10	9.17	8.93	8.82	7.51	7.48	
		10	10.10	9.84	9.58	9.22	8.44	7.71	
		Ambient	10.10	7.65	7.11	6.55	n.d.	n.d.	**8.66**
	PP	**5**	10.10	9.35	9.35	9.15	8.12	7.63	
		10	10.10	10.01	9.76	9.28	8.36	7.85	
		Ambient	10.10	8.21	7.43	6.71	n.d.	n.d.	
Mean (T)			**9.23**	**8.41**	**8.00**	**7.80**	**7.70**	**7.27**	
Mean (P)	**PE (Polyethylene)**		**7.95**						
	PP (Polypropylene)		**8.13**						
Mean (ST)	**5 ^{0}C**		**8.24**						
	10 ^{0}C		**8.50**						
	Ambient		**7.37**						
LSD $_{0.05}$			**(SA) = 0.083 (P) = 0.06 (ST) = 0.084 (T) = 0.118 SAxPxSTxTime = 0.23**						
The Second Season									
SA0 Control	**PE**	**5**	8.51	8.68	8.26	7.06	5.27	4.94	
		10	8.51	8.78	8.44	7.2	5.66	6.11	
		Ambient	8.51	6.08	4.71	n.d.	n.d.	n.d.	**7.18**
	PP	**5**	8.51	8.63	8.57	7.35	5.58	7.09	
		10	8.51	8.82	8.7	7.44	6.19	7.53	
		Ambient	8.51	6.42	4.8	n.d.	n.d.	n.d.	
SA1 (3mM)	**PE**	**5**	9.31	8.66	8.45	8.22	7.21	7.13	
		10	9.31	9.27	9.09	8.75	7.66	7.89	
		Ambient	9.31	6.62	5.08	4.51	n.d.	n.d.	**7.88**
	PP	**5**	9.31	8.97	8.61	8.32	8.35	7.91	
		10	9.31	9.36	9.2	8.92	8.72	8.13	
		Ambient	9.31	6.88	5.25	4.67	n.d.	n.d.	
SA2 (5mM)	**PE**	**5**	10.46	9.49	9.19	9.18	8.52	7.83	
		10	10.46	10.17	9.86	9.58	8.39	7.75	
		Ambient	10.46	7.45	6.66	5.86	n.d.	n.d.	**8.80**
	PP	**5**	10.46	9.67	9.63	8.52	8.55	7.97	
		10	10.46	10.35	10.05	9.65	9.12	8.09	
		Ambient	10.46	7.83	7.11	6.32	n.d.	n.d.	
Mean (T)			**9.43**	**8.45**	**7.87**	**7.60**	**7.44**	**7.36**	
Mean (P)	**PE (Polyethylene)**		**7.81**						
	PP (Polypropylene)		**8.09**						
Mean (ST)	**5 ^{0}C**		**8.29**						
	10 ^{0}C		**8.65**						
	Ambient		**6.91**						
LSD $_{0.05}$			**(SA) = 0.104 (P) = 0.085 (ST) = 0.104 (T) = 0.147 SAxPxSTxTime = 0.27**						

n. d. values not determined due to deterioration of basil, SA0= without salicylic acid (control), SA1=salicylic acid at rate 3mM, SA2=salicylic acid at rate 5mM, packing type (P) PE= low-density polyethylene, PP= polypropylene, Storage temperature (ST)=5^{0}C, 10^{0}C and Ambient temperature, Storage time (T)= days after storage, 0 time, 2,4,6,8 and 10 days.

Table (4). DPPH scavenging activity as affected by salicylic acid, packing type, storage temperature during six storage times in the two seasons (2021-2022)

The First Season									
Pre-harvest	**Treatments**								
Salicylic Acid (SA)	**Packing Type (P)**	**Storage temp. (ST)**	**Storage time (T)**						**Mean SA**
			0	**2**	**4**	**6**	**8**	**10**	
SA0 Control	**PE**	**5**	80.29	80.2	76.68	68.99	65.98	58.58	
		10	80.29	80.14	78.3	72.07	65.48	64.35	
		Ambient	80.29	66.28	51.09	n.d	n.d.	n.d.	**71.21**
	PP	**5**	80.29	80.45	77.31	75.09	69.85	63.89	
		10	80.29	80.33	79.36	78.49	64.64	63.38	
		Ambient	80.29	67.7	53.71	n.d.	n.d.	n.d.	
SA1 (3mM)	**PE**	**5**	82.59	81.14	78.92	71.02	67.21	64.17	
		10	82.59	81.7	81.36	74.98	69.43	67.23	
		Ambient	82.59	69.39	57.12	54.53	n.d.	n.d.	**73.42**
	PP	**5**	82.59	82.07	80.88	75.65	73.04	70.28	
		10	82.59	81.91	81.38	78.54	75.01	70.71	
		Ambient	82.59	72.47	61.95	56.74	n.d.	n.d.	
SA2 (5mM)	**PE**	**5**	83.41	81.94	79.34	75.57	71.71	66.69	
		10	83.41	82.11	82.01	79.4	73.48	67.65	
		Ambient	83.41	74.93	65.24	60.37	n.d.	n.d.	**76.26**
	PP	**5**	83.41	82.68	82.39	80.74	74.87	70.97	
		10	83.41	83.29	82.92	81.71	77.64	71.4	
		Ambient	83.41	75.61	69.69	62.76	n.d.	n.d.	
Mean (T)			**82.10**	**78.02**	**73.31**	**71.67**	**70.70**	**66.61**	
Mean (P)	**PE (Polyethylene)**		**72.54**						
	PP (Polypropylene)		**74.72**						
Mean (ST)	**5 ^{0}C**		**75.58**						
	10 ^{0}C		**76.75**						
	Ambient		**68.55**						
LSD $_{0.05}$			**(SA) = 0.23 (P) = 0.19 (ST) = 0.30 (T) = 0.32 SAxPxSTxTime = 0.56**						
The Second Season									
SA0 Control	**PE**	**5**	74.94	71.05	68.57	65.14	50.64	48.02	
		10	74.94	73.99	72.3	68.39	61.16	61.92	
		Ambient	74.94	60.2	47.11	n.d.	n.d.	n.d.	**65.23**
	PP	**5**	74.94	73.17	70.27	66.69	60.87	51.34	
		10	74.94	74.28	72.15	69.14	63.14	63.3	
		Ambient	74.94	63.58	50.68	n.d.	n.d.	n.d.	
SA1 (3mM)	**PE**	**5**	78.46	74.81	71.99	68.78	61.55	60.09	
		10	78.46	76.53	74.4	71.5	66.65	65.46	
		Ambient	78.46	67.04	55.24	50.25	n.d.	n.d.	**68.97**
	PP	**5**	78.46	77.54	75.41	70.18	63.7	63.22	
		10	78.46	77.69	75.88	73.12	70.21	68.38	
		Ambient	78.46	69.12	57.02	52.36	n.d.	n.d.	
SA2 (5mM)	**PE**	**5**	80.63	79.48	78.5	73.54	70.66	72.15	
		10	80.63	79.64	78.18	76.33	72.99	70.67	
		Ambient	80.63	70.59	61.45	54.39	n.d.	n.d.	**74.48**
	PP	**5**	80.63	80.11	78.82	77.92	74.82	74.26	
		10	80.63	80.51	80.33	79.85	76.13	74.85	
		Ambient	80.63	72.85	68.15	64.03	n.d.	n.d.	
Mean (T)			**78.01**	**73.45**	**68.69**	**67.60**	**66.04**	**64.47**	
Mean (P)	**PE (Polyethylene)**		**68.42**						
	PP (Polypropylene)		**70.76**						
Mean (ST)	**5 ^{0}C**		**70.59**						
	10 ^{0}C		**73.34**						
	Ambient		**64.83**						
LSD $_{0.05}$			**(SA) = 0.31 (P) = 0.26 (ST) = 0.38 (T) = 0.45 SAxPxSTxTime = 0.62**						

n. d. values not determined due to deterioration of basil, SA0= without salicylic acid (control), SA1=salicylic acid at rate 3mM, SA2=salicylic acid at rate 5mM, packing type (P) PE= low-density polyethylene, PP= polypropylene, Storage temperature (ST)=5^{0}C, 10^{0}C and Ambient temperature, Storage time (T)= days after storage, 0 time, 2,4,6,8 and 10 days

3.5.2. Chilling injury

Symptoms of chilling injury include leaf necrosis, which shows as brown spots on leaves (Fig. 10.) which due to the dysfunction of oil glands, wilting or loss of leaf turgidity and decay **(Brindisi *et al.*, 2021).**

As the result shown in Fig. 8. Day 2 marked the beginning of CI symptoms, which got worse for the course of the storage period. SA treatments were able to reduce CI of the basil plants (Fig.8.). Application of salicylic acid at rate 5 mM was the most effective in alleviating CI in the basil when compared to untreated plants and 3 mM of SA. This can due to the stimulation of a defense mechanism system by salicylic acid **(Supapvanich and Promyou, 2013)** which the cell membrane structure of oil glands in the basil leaves was also maintained. This clearly shown that SA treatment preserves membrane structure and enhances membrane properties, hence increasing plant resistance to chilling **(Luo, *et al.*, 2011).** Preharvest spraying with 5 mM SA in lemon basil clearly reduced the increase in EL and relieved CI. Furthermore, in comparison to the untreated basil, there was less malondialdehyde concentration, a secondary product from membrane oxidation **(Supapvanich, *et al.*, 2015).**

Concerning the effect of packaging materials, the results showed that there were significant differences among treatments during storage (Fig.8.); however sweet basil fresh herb packed in polypropylene gave the lowest values of CI symptoms followed by polyethylene with significant differences between them. These results agreed with **(Sharma *et al.*, 2018)** suggests that the suitable humidity level created by packaging material may have an effect in ameliorating chilling injury in basil.

The development of CI symptoms in sweet basil was affected by cold storage temperatures. Browning symptoms, as a manifestation of CI, were greater at 5°C compared to 10 ^{0}C or Ambient temperature. These results agreed with **Suamuang *et al.*, (2016)** on holy basil plant. It was also found that the severity of chilling injury symptoms was dependent on the period of exposure as well as on the specific storage temperature.

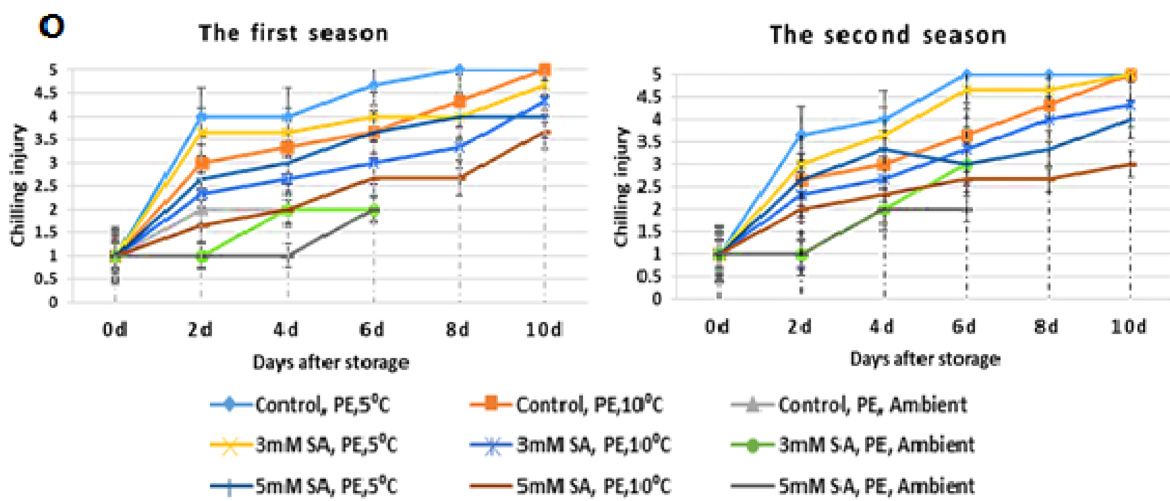
The interaction between pre-harvest treatment, packaging material, storage temperature and storage period was significant. However, after 10 days of storage sweet basil plants treated with SA at a rate of 5 mM and packed in polypropylene bags gave the lowest values of CI symptoms while those untreated (control) and packed with polyethylene and stored at 5 ^{0}C gave the highest values of CI symptom in both seasons. Fig (9). At the end of storage (day 10), the CI score of 5 mM SA-treated sweet basil and stored at 10 ^{0}C or 5 ^{0}C in polypropylene bags was significantly lower than that of others which it was lower than 2.5 (acceptable score).

3.6. Essential oil percentage

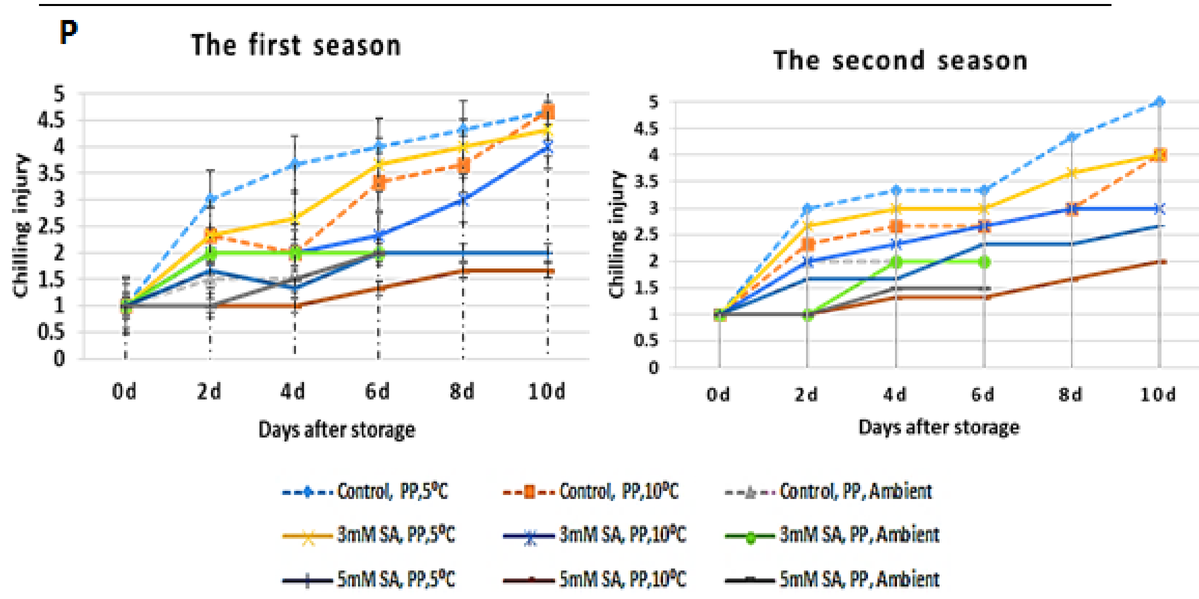
The mean values of sweet basil essential oil percentage of different treatments during storage time are shown in Table (5). Pre-harvest treatments with salicylic acid reduced loss of essential oil content during storage compared to the control (without SA). SA at a rate of 5 mM gave the maximum values of essential oil content 0.425% and 0.393 % in both seasons. These results agreed with **(Abdel-Hamid, 2020) on** Mint and Sage**.**

Regarding the effect of the storage period, results showed that essential oil decreased by increasing the storage period (Table, 5). These results are in line with **Rashed and Younis (2010)**, on oregano. Basil plants synthesize and store essential oil (EO) in superficial peltate glands and trichomes **(Gang *et al.*, 2001).** Fresh basil presented a linear decrease in the oil content during the storage period **(Silva *et al.*, 2005).** The impact of increased respiration rates on product temperature, organic matter decomposition, transpiration losses, decrease in inactive ingredients, and reduction in external quality traits could be the cause of this **(Bottcher *et al.*, 2001).**

Effect of packing on essential oil (%) of fresh herb during 2021 /2022 seasons, the presented data in Table (5), showed that PP packaging scored the highest essential oil content 0.401% and 0.371% than EP packaging 0.380% and 0.353% in both seasons. Similar results had been obtained by **El-Moghazy (2021)** on sweet basil.



LSD $_{0.05}$ (SA) = 0.17 (P) = 0.30 (ST) = 0.35 (T) = 0.26 SAxPxSTxTime = 0.74



LSD $_{0.05}$ (SA) = 0.21 (P) = 0.29 (ST) = 0.31 (T) = 0.25 SAxPxSTxTime = 0.62

Fig. 8. Chilling injury in sweet basil as affected by salicylic acid (0, 3, 5mM), packing type O: low-density polyethylene (PE), P: polypropylene (PP), storage temperature (5^{0}C, 10^{0}C, Ambient) during six storage times in the two seasons (2021 and 2022).

Control, PE, 0 time   Control, PE, 5 ⁰ C, 10   SA 5mM, PE, 5 ⁰ C, 10   SA 5mM, PE, 10 ⁰ C, 10

Control, PP, 0 time   Control, PP, 5 ⁰ C, 10   SA 5mM, PP, 5 ⁰ C, 10   SA 5mM, PP, 10 ⁰ C, 10

Fig. 9. Chilling injury in sweet basil as affected by salicylic acid (5mM), packing type: low-density polyethylene (PE), polypropylene (PP), storage temperature (5⁰C and 10⁰C) after 10 days of storage.

Chilling injury score measurement

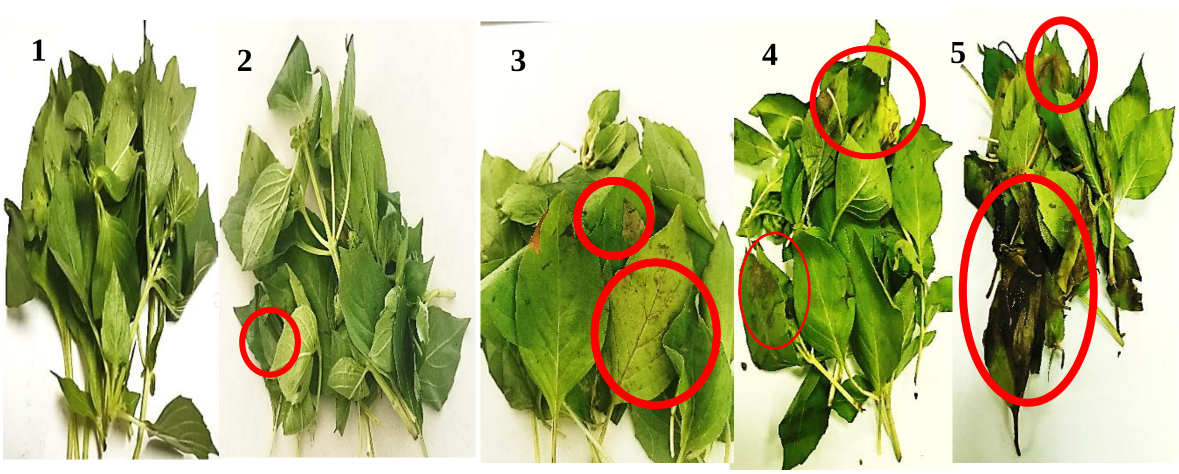


Fig. 10. Chilling injury (CI) score rating: (1) no damage, (2) several dark spots, (3) less than 30% of total leaf area brown, (4) 30–50% of leaf area brown, and (5) more than 50% of leaf area brown.

The data recorded on the essential oil percent (Table 5) showed that the effect of storage temperature, cleared significant differences in the essential oil percent between the different temperatures during the storage, however, sweet basil plants stored at 10 ^{o}C gave the highest values of essential oil content 0.407% and 0.385%, followed by 5 ^{0}C, while those stored at Ambient temperature resulted in the lowest values in both seasons. These results confirmed those of **Fratianni *et al.* (2017)**, who discovered that the majority of the secondary metabolites in basil that are responsible for its aroma, color, and pharmacological and antioxidant properties are likewise lost at lower storage temperatures. Samples of basil that were kept for nine days at 4°C had significantly lower antioxidants, polyphenols, and volatile aroma compounds than samples that were kept at 12°C. Basil volatile compound contents have been shown to suddenly drop even earlier, after three days at 5°C. **(Rodeo and Mitcham, 2023).**

The interaction between pre-harvest treatment, packaging material, storage temperature, and storage period was significant. However, after 10 days of storage sweet basil plants treated with SA at rate of 5 mM and packed in polypropylene bags and stored at 10 ^{0}C gave the highest values of essential oil content 0.401% and 0.383% while those untreated (control) and packed with polyethylene and stored at 5 ^{0}C after 10 days gave the lowest values of essential oil content 0.217% and 0.177% in both seasons.

The differences were not significant between 5 ^{0}C and 10 ^{0}C under the same storage and packaging type (PP) after 10 days of storage in both seasons. Application of salicylic acid at 5mM as pre-harvest treatment was the most effective in reduce CI in the basil which was stored at 5 ^{0}C and packed in polypropylene pages. Therefore, it reduced the loss of volatile oil content after 10 days of storage. This might be because SA induced a defense mechanism system **(Supapvanich and Promyou, 2013)**, which preserved the oil glands cell membrane structure in basil leaves.

3.7. G LC analysis of essential oil

Volatile oil compounds are responsible for the characteristic aroma and flavor of basil **(Chang *et al.*, 2007).** The basil essential oil composition as a result of different pre-harvest treatments, storage temperatures (^{o}C), and packaging materials during 10 days storage period are presented in Table (6) showed clearly that, application combinations had appreciable quantitative effects on essential oil constituents of sweet basil. six constituents were identified by GC in oil samples. The main components were found: α-pinene, myrcene, β-pinene, linalool, estragole (methyl chavicol) and eugenol. These compounds confer green, fresh, floral, and clove aroma and flavor to ‘sweet basil’ **(Patel *et al.*, 2021).** α-pinene, β-pinene, myrcene, linalool, and eugenol decreased with storage duration and were mostly affected by pre-harvest treatments, storage temperature, and packing materials.

When stored sweet basil in PE or PP bags, it was found that levels of these volatiles were generally lower at 5°C compared to 10°C after 4 days of storage. Also, plants stored at Ambient temperature gave the lowest levels of volatiles regardless of the type of package.

After 10 days of storage sweet basil plants treated with SA at a rate of 5 mM and packed in polypropylene bags and stored at 10 ^{0}C gave the highest values of linalool 41.72%, eugenol 15.56% and estragole 1.79%. It is noted that pre-harvest treatment with salicylic acid at a rate of 5mM led to maintaining the percentage of volatile compounds in basil plants after 10 days of storage at a temperature of 5 ^{0}C and packaging in polypropylene (PP) bags, with mean values of linalool 40.28%, eugenol 15.09% and estragole 1.22%. While the lowest values of linalool 26.42%, eugenol 8.89%, and estragole 0.89% were recorded with untreated sweet basil which packed in polyethylene bags and stored at 5 ^{0}C after 10 days of storage.

Table (5). Essential oil content as affected by salicylic acid, packing type, storage temperature during six storage times in the two seasons (2021-2022)

The First Season									
Pre-harvest	**Treatments**								
Salicylic Acid (SA)	**Packing Type (P)**	**Storage temp. (ST)**	**Storage time (T)**						**Mean SA**
			0	**2**	**4**	**6**	**8**	**10**	
SA0 Control	**PE**	**5**	0.437	0.42	0.392	0.36	0.317	0.217	
		10	0.437	0.43	0.403	0.377	0.343	0.25	
		Ambient	0.437	0.304	0.267	n. d.	n. d.	n. d.	**0.362**
	PP	**5**	0.437	0.423	0.41	0.38	0.340	0.21	
		10	0.437	0.437	0.427	0.400	0.360	0.257	
		Ambient	0.437	0.327	0.294	n. d.	n. d.	n. d.	
SA1 (3mM)	**PE**	**5**	0.46	0.443	0.400	0.39	0.330	0.24	
		10	0.46	0.435	0.423	0.413	0.36	0.263	
		Ambient	0.46	0.346	0.323	0.303	n. d.	n. d.	**0.384**
	PP	**5**	0.46	0.44	0.442	0.427	0.363	0.253	
		10	0.46	0.457	0.44	0.42	0.38	0.287	
		Ambient	0.46	0.363	0.344	0.315	n. d.	n. d.	
SA2 (5mM)	**PE**	**5**	0.5	0.463	0.44	0.427	0.347	0.303	
		10	0.5	0.437	0.46	0.44	0.367	0.317	
		Ambient	0.5	0.413	0.337	0.313	n. d.	n. d.	**0.425**
	PP	**5**	0.5	0.503	0.463	0.447	0.413	0.390	
		10	0.5	0.497	0.48	0.47	0.433	0.401	
		Ambient	0.5	0.427	0.382	0.325	n. d.	n. d.	
Mean (T)			**0.466**	**0.420**	**0.396**	**0.388**	**0.363**	**0.282**	
Mean (P)	**PE (Polyethylene)**		**0.380**						
	PP (Polypropylene)		**0.401**						
Mean (ST)	**5 ^{0}C**		**0.394**						
	10 ^{0}C		**0.407**						
	Ambient		**0.369**						
LSD $_{0.05}$			**Salicylic acid (SA) = 0.005 Packing (P) = 0.004 Storage temperature (ST) = 0.005 Storage times (T) = 0.007 SAxPxSTxTime = 0.01**						
The Second Season									
SA0 Control	**PE**	**5**	0.421	0.401	0.343	0.323	0.266	0.177	
		10	0.421	0.38	0.364	0.348	0.288	0.205	
		Ambient	0.421	0.301	0.232	n. d.	n.d.	n.d.	**0.336**
	PP	**5**	0.421	0.389	0.37	0.342	0.305	0.242	
		10	0.421	0.401	0.386	0.368	0.322	0.28	
		Ambient	0.421	0.323	0.251	n.d.	n.d.	n.d.	
SA1 (3mM)	**PE**	**5**	0.446	0.39	0.371	0.349	0.276	0.249	
		10	0.446	0.404	0.382	0.371	0.321	0.305	
		Ambient	0.446	0.347	0.305	0.224			**0.356**
	PP	**5**	0.446	0.424	0.376	0.354	0.331	0.283	
		10	0.446	0.438	0.408	0.377	0.342	0.305	
		Ambient	0.446	0.363	0.281	0.246	n.d.	n.d.	
SA2 (5mM)	**PE**	**5**	0.483	0.441	0.408	0.382	0.304	0.28	
		10	0.483	0.468	0.426	0.404	0.369	0.329	
		Ambient	0.483	0.368	0.327	0.243	n.d.	n.d.	**0.393**
	PP	**5**	0.483	0.463	0.428	0.411	0.323	0.308	
		10	0.483	0.48	0.467	0.421	0.405	0.383	
		Ambient	0.483	0.39	0.308	0.283	n.d.	n.d.	
Mean (T)			**0.450**	**0.398**	**0.357**	**0.340**	**0.321**	**0.278**	
Mean (P)	**PE (Polyethylene)**		**0.353**						
	PP (Polypropylene)		**0.371**						
Mean (ST)	**5 ^{0}C**		**0.361**						
	10 ^{0}C		**0.385**						
	Ambient		**0.339**						
LSD $_{0.05}$			**Salicylic acid (SA) = 0.005 Packing (P) = 0.004 Storage temperature (ST) = 0.005 Storage times (T) = 0.007 SA×P×ST×Time = 0.04**						

n. d. values not determined due to deterioration of basil, SA0= without salicylic acid (control), SA1=salicylic acid at rate 3mM, SA2=salicylic acid at rate 5mM, packing type (P) PE= low-density polyethylene, PP= polypropylene, Storage temperature (ST)=5^{0}C, 10^{0}C and Ambient temperature, Storage time (T)= days after storage, 0 time, 2,4,6,8 and 10 days.

Since they are all monoterpenes, the other volatile substances discussed have the same biochemical route. Geranyl diphosphate (GDP) is the starting point for the synthesis of monoterpenes, which are created by the condensation of IPP and DMAPP, which are primarily synthesized in the plastid via the MEP pathway. Subsequently, GDP experiences many modifications from an extensive group of enzymes referred to as terpene synthases, resulting in the synthesis of distinct monoterpenes **(Dudareva *et al.*, 2004)**. On the other hand, eugenol is produced by the eugenol synthase enzyme by reducing a coniferyl alcohol ester. Eugenol is obtained from the amino acid phenylalanine through the phenylpropanoid the pathway. **(Koeduka *et al*., 2006).**

The loss of these volatile compounds during subsequent low-temperature storage is related to reduced expression levels of genes coding for important enzymes in the metabolic pathway. For instance, the expression of a linalool synthase gene coding for the enzyme responsible for the formation of linalool in a single-stage reaction from GDP was downregulated by chilling temperature in papaya fruit, resulting in impaired linalool production **(Gomes *et al.*, 2016).** A significant drop in volatile concentrations at 5°C in basil was related to a decrease in the transcript abundance of genes encoding for products and enzymes necessary for volatile production, which resulted in a lower quality of flavor. **(Zhang *et al.*, 2016).**

Similarly, after six days of storage at 5°C, basil leaves showed a decrease in estragole (methyl chavicol). Similar to eugenol, estragole is produced by adding a methyl group to chavicol by the phenylpropanoid pathway, starting with phenylalanine and being further converted by specific methyltransferase. **(Lewinsohn *et al.*, 2000).**

4. Conclusion

Chilling injury in basil can be appeared both by visible (browning and leaf discoloration) and invisible (loss of volatile compounds responsible for aroma and flavor) damage. When basil leaves are stored at temperatures well below the optimal temperature (12°C), chilling damage becomes more severe. It could be concluded that application of pre-harvest salicylic acid (SA) treatment at rate 5 mM was effectively able to delay CI (Chilling injury) symptom and prolong the shelf life of sweet basil stored in polypropylene bags at 5°C for 10 days.

Table (6). Volatiles components as affected by salicylic acid, packing type, storage temperature during six storage times in the first season

Components	α-pinene			myrcene			β-pinene			Linalool			Estragole			Eugenol		
	Storage times																	
Treatments	**0**	**4**	**10**	**0**	**4**	**10**	**0**	**4**	**10**	**0**	**4**	**10**	**0**	**4**	**10**	**0**	**4**	**10**
PE+Co+5 ^{0}C	3.44	3.31	2.11	3.67	3.21	2.57	2.61	2.11	1.98	44.83	35.11	26.42	2.03	1.21	0.89	18.38	12.24	8.89
PE+Co+10^{0}C	3.44	3.39	2.23	3.67	3.43	3.00	2.61	2.21	2.02	44.83	39.53	31.19	2.03	1.45	0.94	18.38	13.51	11.27
PE+Co+A	3.44	3.05	n.d.	3.67	3.17	n.d.	2.61	2.01	n.d.	44.83	22.89	n.d.	2.03	1.13	n.d.	18.38	12.76	n.d.
PP+Co+5 ^{0}C	3.44	3.34	2.45	3.67	3.56	2.69	2.61	2.37	2.01	44.83	35.32	28.17	2.03	1.24	0.93	18.38	13.03	9.37
PP+Co+10^{0}C	3.44	3.4	2.57	3.67	3.49	3.33	2.61	2.56	2.29	44.83	40.71	32.44	2.03	1.35	1.27	18.38	14.57	12.36
PP+Co+A	3.44	3.14	n.d.	3.67	3.23	n.d.	2.61	2.13	n.d.	44.83	29.84	n.d.	2.03	1.21	n.d.	18.38	13.32	n.d.
PE+SA1+5 ^{0}C	3.78	3.51	2.25	3.71	3.28	2.63	2.73	2.34	2.13	45.37	35.61	30.11	2.11	1.35	1.03	19.09	14.03	9.77
PE+SA1+10^{0}C	3.78	3.55	2.98	3.71	3.64	3.59	2.73	2.53	2.27	45.37	41.88	36.93	2.11	1.58	1.11	19.09	14.78	11.91
PE+SA1+A	3.78	3.23	n.d.	3.71	3.22	n.d.	2.73	2.19	n.d.	45.37	25.54	n.d.	2.11	1.29	n.d.	19.09	13.18	n.d.
PP+SA1+5 ^{0}C	3.78	3.58	2.55	3.71	3.53	3.09	2.73	2.48	2.21	45.37	36.78	29.32	2.11	1.44	1.15	19.09	14.24	10.61
PP+SA1+10^{0}C	3.78	3.69	3.24	3.71	3.68	3.45	2.73	2.61	2.33	45.37	42.15	38.74	2.11	1.78	1.32	19.09	16.32	12.44
PP+SA1+A	3.78	3.35	n.d.	3.71	3.34	n.d.	2.73	2.26	n.d.	45.37	30.21	n.d.	2.11	1.32	n.d.	19.09	13.58	n.d.
PE+SA2+5 ^{0}C	3.82	3.62	2.69	3.94	3.58	3.32	2.76	2.42	2.18	46.13	39.02	30.18	2.35	1.68	1.12	19.52	14.33	12.94
PE+SA2+10^{0}C	3.82	3.75	3.36	3.94	3.76	3.61	2.76	2.66	2.36	46.13	42.56	39.11	2.35	1.93	1.27	19.52	16.17	13.13
PE+SA2+A	3.82	3.43	n.d.	3.94	3.39	n.d.	2.76	2.27	n.d.	46.13	26.13	n.d.	2.35	1.33	n.d.	19.52	13.60	n.d.
PP+SA2+5 ^{0}C	3.82	3.65	2.59	3.94	3.66	3.49	2.76	2.61	2.54	46.13	41.61	40.28	2.35	1.75	1.22	19.52	15.45	15.09
PP+SA2+10^{0}C	3.82	3.77	3.49	3.94	3.84	3.71	2.76	2.72	2.58	46.13	43.86	41.72	2.35	2.24	1.79	19.52	18.43	15.56
PP+SA2+A	3.82	3.41	n.d.	3.94	3.41	n.d.	2.76	2.3	n.d.	46.13	32.45	n.d.	2.35	1.32	n.d.	19.52	13.66	n.d.

n. d. values not determined due to deterioration of basil, Co= without salicylic acid (control), SA1=salicylic acid at rate 3mM, SA2=salicylic acid at rate 5mM, packing type (P) PE= low-density polyethylene, PP= polypropylene, Storage temperature (ST)=5^{0}C, 10^{0}C and Ambient temperature, Storage time (T)= days after storage, 0 time, 4 and 10 days.

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