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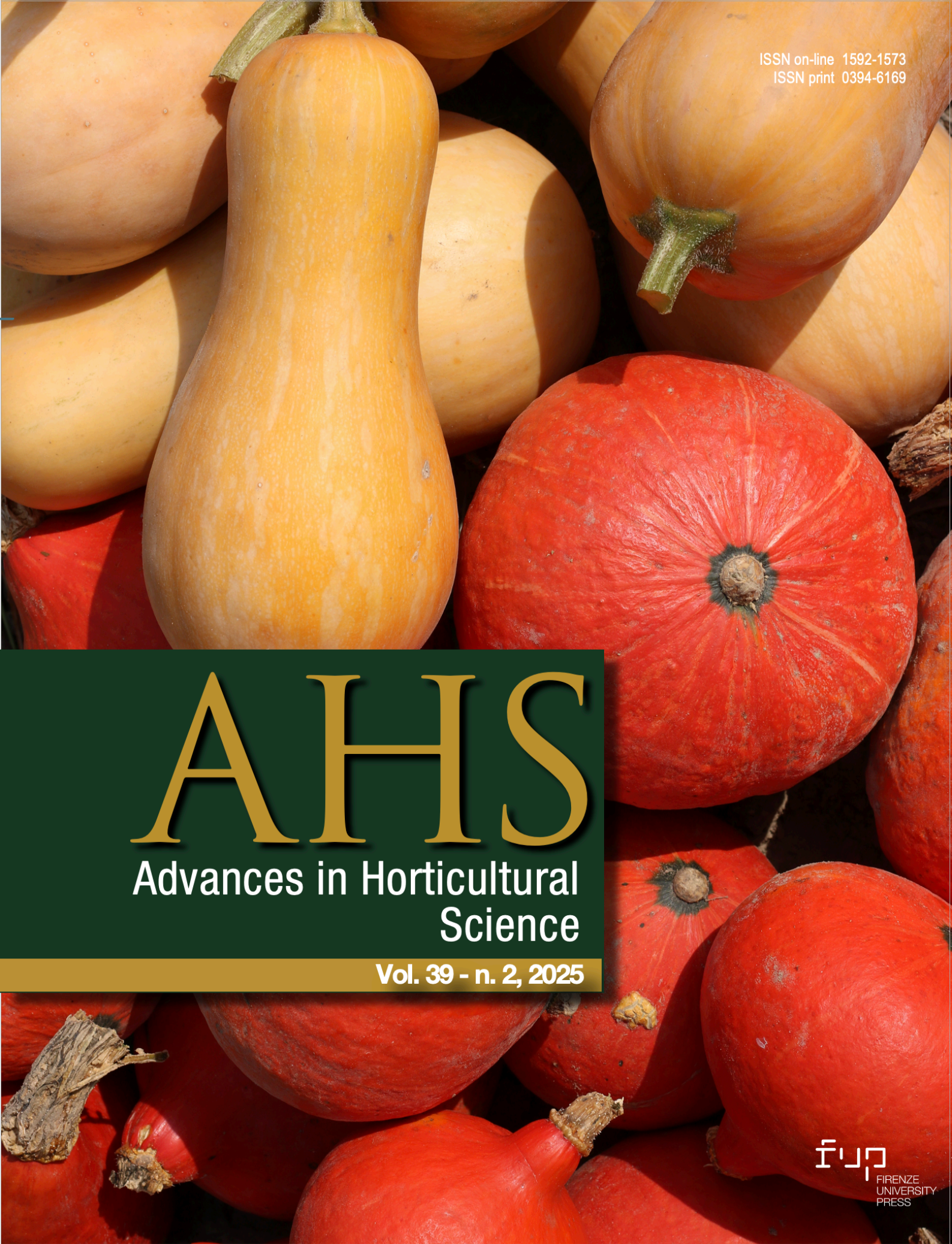

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Advances in Horticultural Science

Enhance the longevity and aesthetic appeal of *Anthurium andraeanum* cv. Fire cut leaves by using some natural components

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Key words: *Anthurium,* Arabic gum, chitosan, glossiness, leaf postharvest, rosehip oil.

Abstract: This experiment was conducted to examine the effects of rosehip oil (Rh oil), Arabic gum (AG), and chitosan (CS) on the vase life and quality of *Anthurium andraeanum* cv. Fire under laboratory conditions. The experimental layout followed a completely randomized design. Seven treatments were applied: tap water (control), Rh oil (2% or 4%), Rh oil (2% or 4%) + AG (5%), and Rh oil (2% or 4%) + AG (5%) + CS (500 ppm). The leaves were sprayed using a hand sprayer until runoff occurred. Results showed that the highest increases in vase life, final water uptake, chlorophyll a and b content, and the degree of leaf health and glossiness were obtained after the application of 4% Rh oil. Scanning electron micrographs illustrated that stomata were open in untreated leaves, moderately open after application of 2% Rh oil, slightly open after spraying with 4% Rh oil, and completely closed after the application of Rh oil (2% or 4%) + AG (5%).

1. Introduction

Anthurium andraeanum (*Flamingo flower*) is a very popular plant which grows up to 60 cm tall. It features rich green, elongated, heart-shaped leaves and waxy white or coral-colored spathes, which are used in wedding arrangements (Jack, 1985). Its attractive foliage and showy cut flowers make it highly valuable in the global flower market (Anand *et al.*, 2017). When the plant is not in flower, the leaves harmonize with those of other tropical plants suitable for shady spots (Jane and Graham, 1997). In recent years several varieties of Anthuriums have been introduced into the Egyptian market. Most of these varieties have large, glossy leaves, making them suitable for use as cut foliage. Unfortunately, *Anthuriums andraeanum* cv. Fire has large, pale green leaves, which reduce its economic value.

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To optimize the commercial worth of cut flowers, researchers have concentrated on enhancing their longevity and quality through flower preservation; nevertheless, chemical flower preservation has negative environmental consequences. (Moussa *et al.,* 2024). Natural ingredients, such as plant extracts and essential oils, are utilized instead of chemicals in cut flower preserving solutions due to their negative impact on human health, particularly those with silver components. Numerous researchers have explored the impact of natural materials (Sarhan *et al*., 2023). In this respect Hashemabadi *et al.* (2021) found that adding dill essential oil to *Dianthus caryophyllus* L. cv. Yellow Candy solution improved vase life and solution uptake compared to pure water. Bañuelos-Hernández *et al.* (2017) found that applying 1.0 and 1.5% chitosan coating to Heliconia bihai flower stems increased vase life by 10.3 and 7 days, respectively, compared to the control. Also, Creel (2006) reported that soaking flowers in 10% or 20% Acacia gum can extend the vase life of snapdragon.

The fruit of *Rosa canina* L. is known as rosehip (Rh) or rose haw. It is red to orange in color. It consists of approx. 30-35% seeds and 65-70% pericarp (Uggla and Nybom*,* 1998). Rosehip oil is rich in polyunsaturated fatty acids, stearic acid (48.11%), linoleic acid (35.38%), palmitoleic acid (33.78%) and eicosadienoic acid (30.57%) (Vasić *et al.,* 2020). Due to its antioxidant properties, rosehip oli is widely used in pharmaceutical industry (Franco *et al.,* 2007; Machmudah *et al.,* 2007).

Acacia Senegal and *Acacia Seyal* trees are the primary sources of Arabic gum (AG), a natural polysaccharide polymer used in the biological industry. Recently, Arabic gum has gained considerable attention as a postharvest edible coating due to its ability to preserve the quality and extend the shelf life of fresh products. It's excellent emulsifying, stabilizing, binding, and shelf-life-extending properties make it an efficient food additive (Tiamiyu *et al.,* 2023). The shelf life of various fruits and vegetables, including tomatoes (Ali *et al.,* 2010), sweet cherries (Mahafaudi and Hamdi, 2014), green chilies (Chitravathi *et al.,* 2014) and mangoes (Khaliq *et al.,* 2015), has been successfully extended by Arabic gum coating.

Chitosan (CS) is a naturally occurring polymer with several advantageous properties, including non-toxicity, biocompatibility, biodegradability, and the ability to form chelates. These characteristics, along with its versatility, make chitosan a valuable material for various applications (Lingait *et al*., 2024). It can be processed into hydrogels, nanoparticles, pastes, nanofibers, films, membranes, microgranules, sponges, etc. Additionally, chitosan can be modified by grafting, crosslinking, ion templating, or blending with other materials to provide a variety of specific properties for new and targeted applications (Kluczka, 2024). It's excellent film-forming ability makes chitosan an effective edible surface coating for fruits and vegetables.

This study investigate the effects of rosehip oil, Arabic gum, and chitosan on the vase life and visual appeal of *Anthurium andraeanum* cv. Fire cut leaves.

2. Materials and Methods

This experiment was carried out at Antoniades Gardens, Ornamental Plants Research and Landscape Gardening Department, Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt, in the years of 2022 and 2023.

Plant materials and treatments

On March 29, 2022, and March 31, 2023, during the first and second seasons, *Anthurium andraeanum* leaves were sourced from a well-known commercial nursery. The leaves were transported to the laboratory under dry conditions, and the petioles were re-cut to a length of 27 cm before treatment.

Preliminary experiment was done by using several concentrations of rosehip (Rh) oil, Arabic gum (AG), chitosan (CS) and their combinations. Seven treatments with the higher vase life and best appeal beside control were chosen to do this experiment. Treatments included:

i. Tap water (control);
ii. Rh oil (2%);
iii. Rh oil (4%);
iv. Rh (2%) + AG (5%);
v. Rh oil (4%) + AG (5%);
vi. Rh oil (2%) (5%) + CS (500 ppm);
vii. Rh oil (4%) + AG (5%) + CS (500 ppm).

All treatments included the addition of Tween 80 (2%). The *Anthurium* leaves were sprayed with the different treatments using a hand sprayer until runoff occurred. Afterward, the leaves were placed in glass jars containing 500 ml of tap water to complete their shelf-life period.

The leaves were maintained at the average

temperature of 19° to 23°, average humidity (59-65%) and 24 hours fluorescent light (about 550 -575 lux).

Data collection on postharvest characteristics

Vase life (VL) expressed in days. *Anthurium* leaf was excluded when about 20% of its surface was yellow. This stage was considered the end of the potential valuable longevity of the cut leaf.

Loss of leaf fresh weight percentage (LLFW). It was set at the end of vase life as expressed by the following formula:

$$\text{LLFW (\%)} = \frac{\text{(Initial leaf fresh weight - Final leaf fresh weight)}}{\text{(Initial leaf fresh weight)}} \times 100$$

Final water uptake (FWU) expressed in grams. Four evaporation control jars [jars which did not contain any leaves] were located between these containg leaves at different places. The evaporation of each jar was taken and the average of the four jars was calculated. It was calculated at the end of the experiment using the following formula:

$$\text{FWU (g)} = \quad \text{FWU (g)} = [\text{ASS} - \text{ASE}] - \text{ED}$$

Where ASS is the amount of solution at the start of the experiment, ASE is the amount of the solution remaining at the end of the experiment and ED is the average of evaporation data.

Leaf fresh weight/Leaf dry weight ratio (LWR). At the end of the experiment, the leaves were oven dried at 72°C for 48 hours for a constant weight to get the leaves dry weight. The fresh weight was then divided by the dry weight based on the following equation:

$$\text{LWR} = \frac{\text{Fresh weight per leaf (g)}}{\text{Dry weight per leaf (g)}} \times 100$$

The amount of transpired water from the leaf surface (TW) expressed in grams. After 2, 4 and 6 days from the beginning of experiment, the transpired water from the leaf surface was calculated as given by the formula below:

TW (g) = {WF + JW + WVS at (day n)} – {WF +JW+ WVS at day (n+1)})/(IFW)

Where WF is the weight of the leaf, JW is jar weight, WVS is the weight of vase solution, IFW is initial leaf fresh weight, n = 1, 3 and 5 days and n+1 is the next day.

where n = 1, 3 and 5 days and n+1 is the next day.

Relative fresh weight (RFW). Fresh weight of the leaves was set just before the immersion of the leaves into the solutions and recorded on the 2nd, 4th , 6th , 8th, 14th, 20th , 26th and 30th day from the beginning of the experiment. The fresh weight of each leaf was expressed relative to the initial weight to represent the water status of the leaf as follows:

$$\text{RFW} = \frac{\text{Wt}}{\text{W0}} \times 100$$

where (Wt) the weight of leaf (g) on the 2nd, 4th, 6th, 8th, 14th, 20th, 26th and 30th day from the beginning of the experiment, (W0) the initial fresh weight of the same leaf (g).

Vase Solution Uptake Rate (VSUR). It was measured according to the following formula:

$$\text{VSU rate} = \frac{\text{(St - 1) - St}}{\text{IFW of stem}} \times 100$$

where (St) is the weight of vase solution (g) after 2, 4, 6, 8 and 10 days from the beginning of experiment, (St-1) is weight of the vase solution (g) on the previous day and (IFW) is the initial fresh weight (g).

Chlorophyll content. Samples from each treatment were collected at the end of the vase life of the control treatment, and the amounts of chlorophyll a and b (mg/100 g fresh leaf weight) were determined according to Moran (1982).

Appearances and glossiness. On the 6th day from the experiment start. A jury of twenty members from different age groups evaluated the leaves visually for its glossiness and scored on a scale (not glossy, moderate glossy, glossy and high glossy) and its appearance and scored a scale (bad, good and very good).

Visualization of stomatal apparatus by Scanning Electron Microscope)SEM). At the end of the first experiment season, the treatments which led to the longest vase life were chosen to investigate its effect on stomata structure. *Anthurium* leaves were sprayed with these treatments beside tap water. After 24 hours of the treatment, small pieces of fresh specimens of *Anthurium* leaves were removed and fixed by immersing them immediately in 4F1G (Fixative, phosphate buffer solution) pH=7.4 at 4°C for 3 hours. Specimens were then post fixed in 2% Osmium tetroxide (OsO_4) in the buffer at 4°C for 2

hours. Samples were washed in the buffer and dehydrated at 4°C through a graded series of ethanol. Samples of *Anthurium* leaves were dried by means of critical point method , mounted using carbon paste on an Al- stub and coated with gold up to thickness in a sputter coating unit (JFC-1100E). Observation of stomata morphology in the coded specimens were performed in a JEOL JSM - 5300 scanning electron microscope operated between 15 and 20 Kev. The examination by electron microscope was done at the Electron Microscope Unit at the Faculty of Science, Alexandria University, Egypt (Tahmasebi *et al.,* 2015)

Experimental layout and statistical analysis

The experimental layout was a complete randomized design (CRD). It consisted of seven treatments with three replicates, and each replicate contained four cut leaves. The means of the different variables were compared using the "Least Significant Difference (LSD)" test at 5% level of probability. (Snedecor and Cochran, 1989).

3. Results

Post harvest parameters

The postharvest parameter results are summarized in Table 1. Vase life data show that all treatments outperformed the control. The most notable increase in vase life occurred with the application of 4% Rh oil in both seasons, with values of 33.22 days in 2022 and 33.28 days in 2023. In contrast, the lowest vase life was observed with the combination of 2% or 4% Rh oil + 5% AG + 500 ppm CS, which had a significance level value comparable to the control in both seasons.

The greatest reduction in LLWT percentage was achieved with 4% Rh oil, which resulted in 1.62% in 2022 and 1.88% in 2023. The highest increase in LFWT was found with the combination of 2% or 4% Rh oil + 5% AG + 500 ppm CS or 4% Rh oil + 5% AG, yielding similar significant results in both seasons.

The highest final water uptake was recorded with 2% or 4% Rh oil, with no significant difference between the two concentrations across both season. Additionally, the greatest increase in LWR was observed with 4% Rh oil, with values ranging between 5.36 in 2022 and 5.24 in 2023.

Table 2 revealed that during the first six days of the experiment the greatest significant increase in transpiration rate fluctuated between the control and application of either Rh oil (2%) + AG (5%) + CS (500 ppm) or Rh oil (4%) + AG (5%) + CS (500 ppm) in both experimental seasons. Two days after experiment started, in 2022 season, the application of Rh oil (4%) + AG (5%) + CS (500 ppm) resulted in the highest significant increase in transpiration rate, with a recorded value of 0.3391. In 2023 season, the highest significant increase in transpiration rate was obtained following control treatment, which recorded 0.3493, and Rh oil (4%) + AG (5%) + CS (500 ppm), which recorded 0.3411 with the same level of significance. Four days after the experiment began, the untreated control showed the largest significant rise, with results of 0.2469 and 0.3215 for 2022 and 2023 seasons , respectively. Furthermore, the application of Rh oil (4%) + AG (5%) + CS (500 ppm) in

Table 1 - Effect of foliar application of rosehip oil, Arabic gum, and chitosan on vase life, fresh weight loss, final water uptake, and fresh/dry weight ratio of *Anthurium* leaves during the 2022 and 2023 seasons

Treatment	VL (days)		LLFW (%)		FWU (g)		LWR (%)	
	2022	2023	2022	2023	2022	2023	2022	2023
Control	10.55	10.78	4.90	4.64	19.95	23.51	4.43	4.51
Rh oil (2%)	26.10	23.00	6.07	6.01	47.06	49.95	4.80	4.77
Rh oil (4%)	33.22	33.28	1.62	1.88	50.79	57.61	5.36	5.24
Rh oil (2%) + AG (5%)	17.87	17.83	7.22	6.27	23.01	28.46	4.81	4.46
Rh oil (4%) + AG (5%)	17.89	18.67	10.88	10.77	22.28	26.50	4.89	4.69
Rh oil (2%) + AG (5%) + CS (500 ppm)	13.11	14.17	11.56	10.74	25.08	25.03	4.26	4.46
Rh oil (4%) + AG (5%) + CS (500 ppm)	12.89	12.00	13.71	12.48	26.97	18.82	4.17	4.37
LSD at 0.05	5.66	5.38	3.23	2.14	12.15	14.53	0.18	0.06

LSD at 0.05 = Least Significant Difference test at 5% level of probability. Rh oil = Rosehip oil; AG = Arabic gum; CS = Chitosan. VL = vase life; LLFW = Loss of leaf fresh weight percentage; FWU = Final water uptake; LWR = leaf fresh weight/leaf dry weight ratio.

Table 2 - Effect of foliar spray with rosehip oil, Arabic gum, and chitosan on the amount of water transpired from the surface of Anthurium leaves during the 2022 and 2023 seasons, measured at 2, 4, and 6 days after the start of the experiment

Treatment	TW (g)					
	2022			2023		
	2 days	4 days	6 days	2 days	4 days	6 days
Control	0.3320	0.2469	0.1437	0.3493	0.3215	0.1570
Rh oil (2%)	0.2173	0.1482	0.1350	0.2478	0.1690	0.1447
Rh oil (4%)	0.1928	0.1638	0.1075	0.2316	0.1659	0.1343
Rh oil (2%) + AG (5%)	0.2574	0.1677	0.1401	0.2781	0.1694	0.1146
Rh oil (4%) + AG (5%)	0.1705	0.1287	0.1042	0.1616	0.1190	0.1194
Rh oil (2%) + AG (5%) + CS (500 ppm)	0.3228	0.2302	0.1736	0.3343	0.1852	0.1822
Rh oil (4%) + AG (5%) + CS (500 ppm)	0.3391	0.1830	0.1425	0.3411	0.1648	0.1482
LSD at 0.05	0.0029	0.0004	0.0003	0.0033	0.0007	0.0002

LSD at 0.05 = Least Significant Difference test at 5% level of probability. Rh oil = Rosehip oil; AG = Arabic gum; CS = Chitosan. TW = Amount of transpired water from the leaf surface.

both seasons produced the greatest significant rise six days after the experiment began, with values of 0.1736 in 2022 season and 0.18822 in 2023 season.

Fig. 1 - Effect of foliar application of rosehip oil, Arabic gum, and chitosan on the relative fresh weight (RFW%) of *Anthurium* cut leaves during the 2022 and 2023 seasons, measured at 2, 4, 6, 14, 20, 26, and 30 days after the start of the experiment.

For RFW, figure 1 showed that the untreated leaves had the greatest rise in RFW% on the 2nd day after the experiment began. While the largest drop in RFW was recorded following application of Rh oil (4%), AG (5%), and CS (500 ppm). Also, figure 1 revealed a minor decline in RFW% following all treatments, with the exception of Rh oil (4%) which increased slightly in the season of 2022, but for 2023, all treatments caused a slight decrease in RFW% on the 4th day after the experiment began. The drop in RFW% persisted marginally on the 6th day after start of the experiment for all treatments except Rh oil (4%) + AG (5%) + CS (500 ppm), which declined considerably in both seasons. On the 14th day of the experiment, the Rh oil (2%) + AG (5%) + CS (500 ppm) and Rh oil (4%) + AG (5%) + CS (500 ppm) treatments ended, and the RFW% continued to decline marginally following the use of Rh oil (2%) in both experimental seasons. On the 20th day, all treatments were discontinued with the exception of Rh oil 2% and 4%, and RFW% decreased gradually in 2022 season and 2023 season. On the 26th and 30th days after the trial began, the reduction in RFW% persisted somewhat.

Figure 2 shows that the control treatment had the greatest rise in VSUR on the 2nd day of the experiment, and this increase persisted on the 4th day of the experiment when compared to the other treatments. The VSUR of control treatments declined dramatically on the 6th and reached its lowest point on the 10th of the experiment in the 2022 and 2023 seasons. While the lowest VSUR value was observed on the 2nd day following the application of Rh oil (4%) treatment, the drop in this value over the first 10

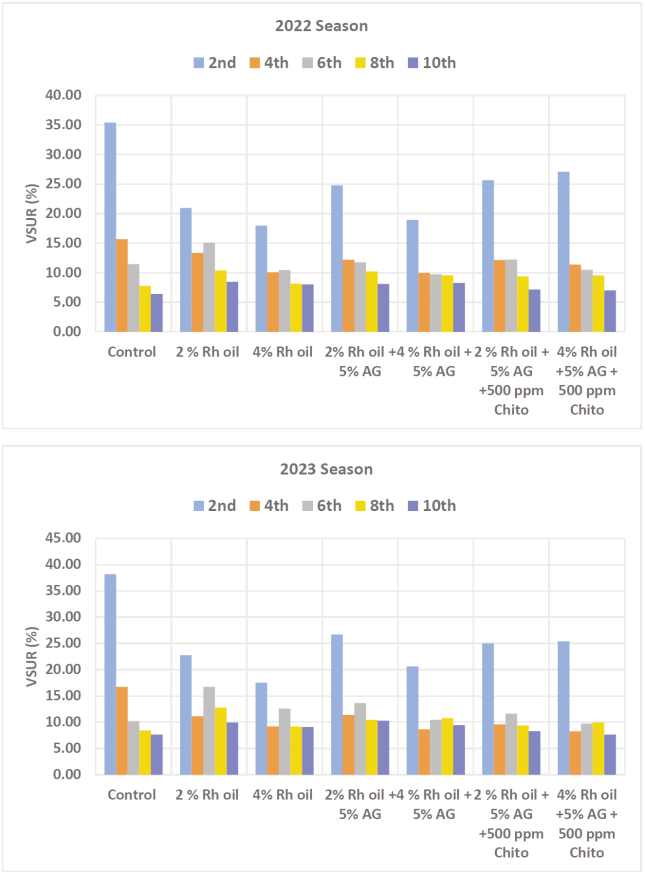

Fig. 2 - Effect of foliar application of rosehip oil, Arabic gum, and chitosan on vase solution uptake rate (VSUR) of *Anthurium* cut leaves during the 2022 and 2023 seasons, measured at 2, 4, 6, and 10 days after the start of the experiment.

days of the experiment persisted with a gradual rhythm in both experimental seasons.

Table 3 shows that the control treatment resulted in the lowest significant levels of leaf pigments. In contrast, the application of 4% Rh oil led to the most substantial increase in chlorophyll a and b levels in both seasons.

Leaves appearance and glossiness

Figure 3 shows that all jury members agreed the glossiness of *Anthurium* cut leaves was high when treated with Rh oil (2% or 4%) combined with AG (5%) and CS (500 ppm), as well as with 4% Rh oil alone. In contrast, untreated leaves were rated as not glossy. Additionally, figure 3 indicates that 60% of jury members rated the glossiness of leaves treated with 2% Rh oil alone as high.

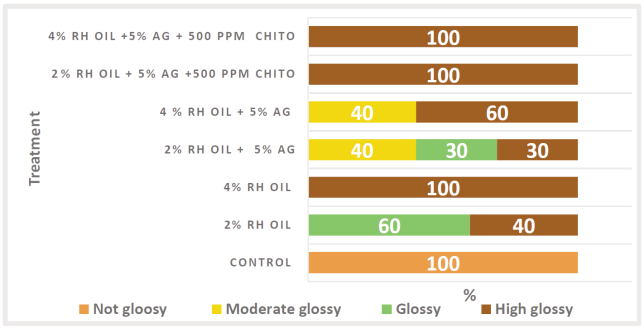

Fig. 3 - Percentage of jury evaluations for glossiness of *Anthurium* leaves following foliar application of rosehip oil, Arabic gum, and chitosan, compared to untreated (control) leaves.

Figure 4 shows that the overall opinion of the jury regarding the appearance quality of Anthurium cut leaves treated with Rh oil (2% or 4%) was very good. However, the application of Rh oil (2% or 4%) in combination with AG (5%) and CS (500 ppm) resulted in the development of brown patches on the leaves,

Table 3 - Effect of foliar spray with rosehip oil, Arabic gum, and chitosan on leaf pigment content in Anthurium leaves during the 2022 and 2023 seasons

Treatment	Chlorophyll (mg / 100 g FW)			
	a		b	
	2022	2023	2022	2023
Control	1.05	1.19	0.63	0.80
Rh oil (2%)	2.03	2.11	1.07	1.07
Rh oil (4%)	2.44	2.26	1.41	1.59
Rh oil (2%) + AG (5%)	2.17	1.96	1.20	1.02
Rh oil (4%) + AG (5%)	2.09	1.98	1.04	1.19
Rh oil (2%) + AG (5%) + CS (500 ppm)	1.78	2.10	0.91	1.04
Rh oil (4%) + AG (5%) + CS (500 ppm)	1.87	1.69	1.06	0.92
LSD at 0.05	0.10	0.11	0.03	0.04

LSD at 0.05 = Least Significant Difference test at 5% level of probability. Rh oil = Rosehip oil; AG = Arabic gum; CS = Chitosan. TW = Amount of transpired water from the leaf surface.

leading to a poor appearance rating for these treatments, as shown in Figures 4 and 5.

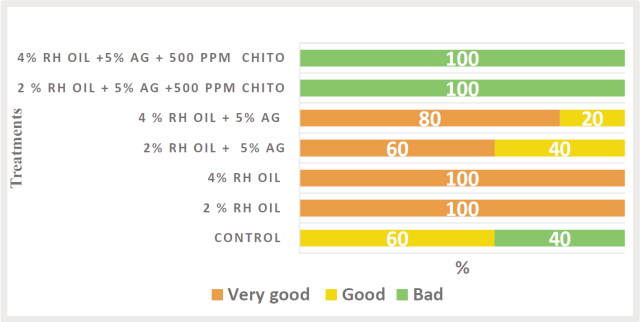

Fig. 4 - Jury evaluation percentages of *Anthurium* leaf appearance after foliar treatments with rosehip oil, Arabic gum, and chitosan, in comparison to control (untreated) leaves.

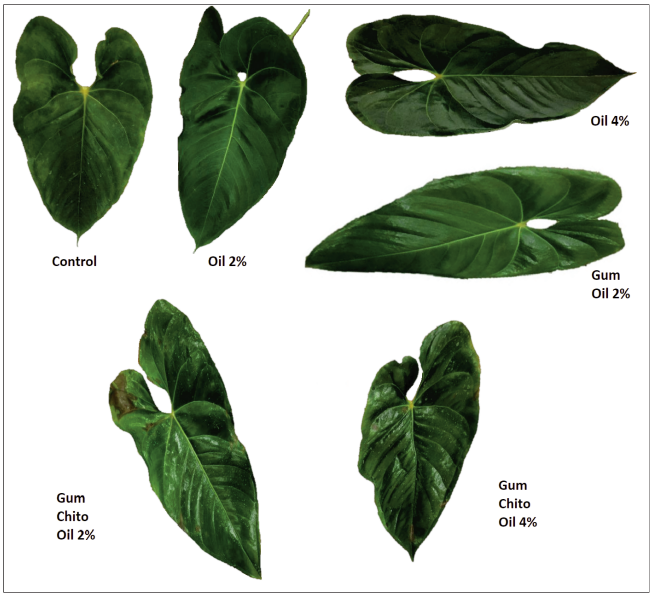

Fig. 5 - Effect of foliar application of rosehip oil, Arabic gum, and chitosan on the glossiness and appearance of *Anthurium* leaves on the 6th day after the start of the experiment, during the 2023 season.

Visualization of stomatal apparatus

Scanning electron micrographs of the abaxial surface of Anthurium leaves (Fig. 6) showed that fully open stomata were observed in untreated control leaves. Moderately open stomata were seen following application of 2% rosehip oil, while slightly open stomata appeared after spraying with 4% rosehip oil. In contrast, stomata were completely closed after treatment with rosehip oil at 2% or 4% combined with 5% Arabic gum.

Furthermore, close-up scanning electron

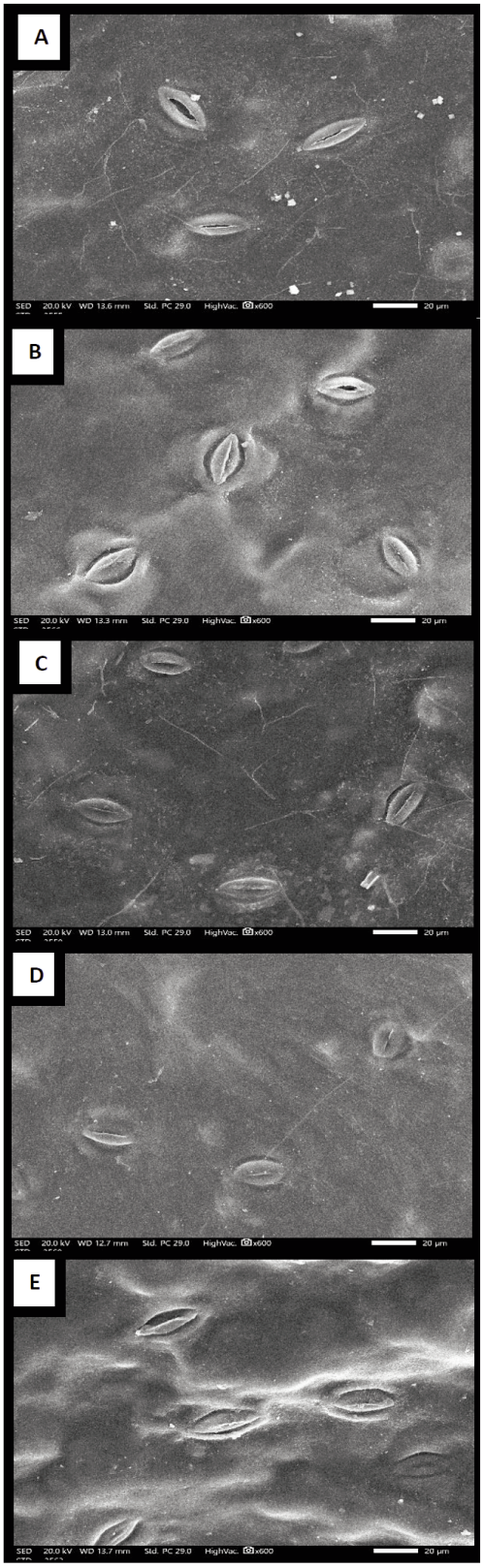

Fig. 6 - Scanning electron micrographs (SEM) of the abaxial surface of *Anthurium* leaves 24 hours after spraying with: (A) control; (B) 2% rosehip oil; (C) 4% rosehip oil; (D) 2% rosehip oil + 5% Arabic gum; (E) 4% rosehip oil + 5% Arabic gum. Micrographs were taken at 600× magnification; scale bar = 20 µm.

micrographs of individual stomata after different treatments (Fig. 7) revealed that the largest stomatal pore (width: 2.18 µm, height: 24.37 µm) was found in untreated leaves. A moderately sized pore (width: 1.438 µm, height: 12.87 µm) was recorded after treatment with 2% rosehip oil, while the smallest pore (width: 1.318 µm, height: 8.038 µm) was observed after treatment with 4% rosehip oil. Stomatal closure was evident following application of rosehip oil at 2% or 4% in combination with 5% Arabic gum.

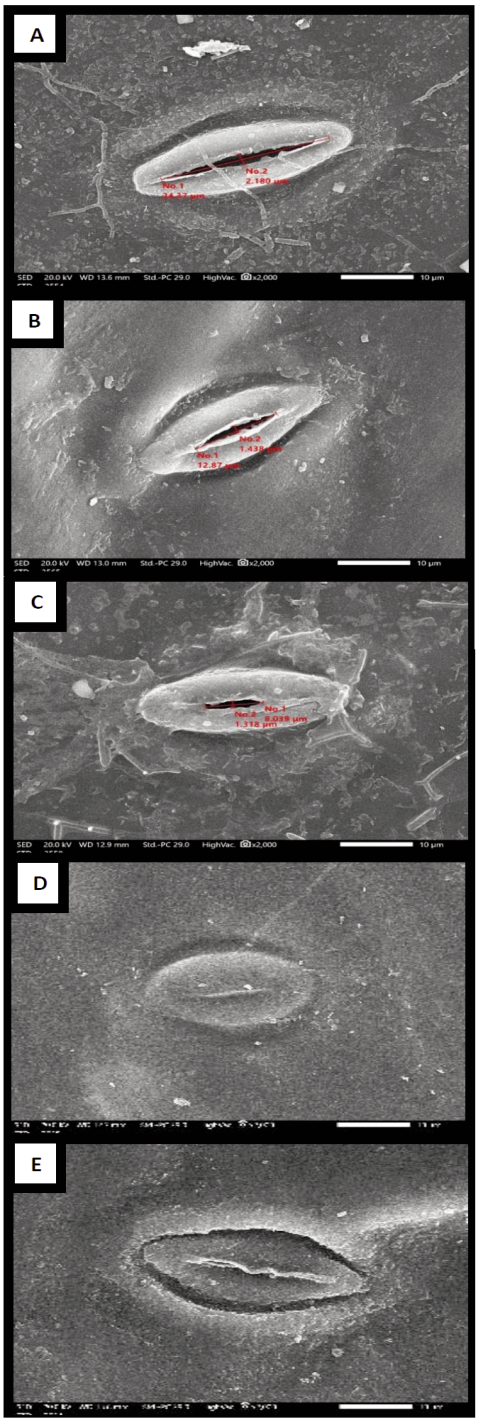

Fig. 7 - Scanning electron micrographs (SEM) of a single stoma on the abaxial surface of *Anthurium* leaves 24 hours after spraying with: (A) control; (B) 2% rosehip oil; (C) 4% rosehip oil; (D) 2% rosehip oil + 5% Arabic gum; (E) 4% rosehip oil + 5% Arabic gum. Micrographs were taken at 2000× magnification; scale bar = 10 µm.

4. Discussion and Conclusions

Stomata regulate the diffusive conductance of leaves, influencing both carbon assimilation and transpirational water loss. Their response is critical in maintaining the balance between water supply and atmospheric demand. Under low humidity conditions, for instance, reduced leaf water content triggers stomatal closure to limit transpiration and preserve internal water status (Thomas, 2005).

In the present study, foliar application of rosehip oil induced partial stomatal closure (Figs. 6 and 7), leading to decreased water loss through transpiration (Table 2). This effect contributed to improved water-use efficiency and leaf turgor maintenance, as reflected in the modest reduction in relative fresh weight during the experimental period (Fig. 1) and the gradual decline in vase solution uptake rate over the first 10 days (Fig. 2). The slight decrease in final fresh weight percentage (Table 1) may also be attributed to this stomatal behavior. These findings are consistent with previous reports identifying stomatal regulation as a key determinant of water loss in cut flowers (Fanourakis *et al.*, 2016; In *et al.*, 2016; Schroeder and Stimart, 2005). This stomatal response may be further explained by the chemical composition of rosehip oil, which contains a range of fatty acids - including myristic, palmitic, palmitoleic, stearic, oleic, linoleic, linolenic, and arachidic acids (Paladines *et al.*, 2014). Arachidic acid, in particular, has been associated with the endogenous synthesis of salicylic acid (SA), a signaling molecule known to induce stomatal closure, enhance membrane stability, and reduce lipid peroxidation (Coquoz *et al.*, 1995; Hakimeh, 2012).

During the first six days, high water loss through transpiration led to a strong drop in relative fresh weight (Fig. 1), reduced water uptake, shorter vase life, and faster chlorophyll breakdown (Table 1). In untreated leaves, this continuous water loss after detachment caused the flowers to lose freshness earlier and wilt faster. These results confirm that keeping good stomatal control after detachment is important to reduce water loss and maintain vase life

(Salunkhe *et al*., 1990).

Treatment with 4% rosehip oil markedly improved leaf longevity and freshness, likely by limiting early transpiration (Table 2), sustaining vase solution uptake over time (Fig. 2), and reducing fresh weight loss (Table 1). The increased chlorophyll a and b contents further supported better color retention and extended vase life, aligning with the findings of Rida (2019) on Aster New York.

Application of 4% rosehip oil as a foliar spray on *Anthurium* leaves is recommended, as it enhanced vase life, final water uptake, and chlorophyll a and b levels, while also promoting healthier and glossier leaf appearance.

References

ALI A., MAQBOOL M., RAMACHANDRAN S., ALDERSON P.G., 2010 - *Gum arabic as a novel edible coating for enhancing shelf-life and improving the postharvest quality of tomato (*Solanum Lycopersicum *L.) fruit*. - Postharvest Biol. Technol., 58(1): 42-47.

ANAND M., SANKARI A., ARULMOZHIYAN R., KAYALVIZHI K., 2017 - *Evaluation of different varieties of anthurium (*Anthurium andraeanum *Linden Ex André) for cut flower production under Shevaroys Hills.* - Electronic J. Plant Breeding, 8(3): 792-798.

BAÑUELOS-HERNÁNDEZ K.P., GARCIA-NAVA J.R., LEYVA-OVALLE O.R., PEÑA-VALDIVIA C.B., TREJO C., YBARRA-MONCADA M.C., 2017 - *Chitosan coating effect on vase life of flowering stems of* Heliconia bihai *(L.) L. cv. Halloween*. - Postharvest Biol. Technol., 132: 179-187.

CHITRAVATHI K., CHAUHAN O.P., RAJU P.S., 2014 - *Post-harvest shelf-life extension of green chilies (*Capsicum annuum *L.) using shellac-based edible surface coatings*. - Postharvest Biol. Technol., 92: 146-148.

COQUOZ L., BUCHALA A.J., MEUWLY PH, MÉTRAUX J.P., 1995 - *Arachidonic acid induces local but not systemic synthesis of salicylic acid and confers systemic resistance in potato plants to* Phytophthora infestans *and* Alternaria solani. - Am. Phytopath. Soc., 85(10): 1219-1224.

CREEL R., 2006 - *Effects of Acacia gum on post-harvest quality of cut flowers*. - Thesis, Graduate Faculty of Auburn University, Auburn, Alabama, USA.

FANOURAKIS D., BOURANIS D., GIDAY H., CARVALHO D.R.A., REZAEI NEJAD A., OTTOSEN C.O., 2016 - *Improving stomatal functioning at elevated growth air humidity: A review*. - J. Plant Physiol., 207: 51-60.

FRANCO D., SINEIRO J., PINELO M., NÚÑEZ M.J., 2007 - *Ethanolic extraction of* Rosa rubiginosa *soluble substances: oil solubility equilibria and kinetic studies*. - J. Food Eng., 79: 150-157.

HAKIMEH M., 2012 - *Salicylic acid and sodium nitroprusside improve postharvest life of chrysanthemums.* - Scientia Hortic., 145(3): 29-33.

HASHEMABADI D., ABEDINI ABOKSARI H., HEDAYAT RAD D., KAVIANI B., 2021 - *Herbal extracts and alcohol increase vase life of* Dianthus caryophyllus *L. cv 'Yellow Candy'*. - Revista Chapingo, Serie Horticultura, 27(3): 135-155.

IN B.-C., INAMOTO K., DOI M., PARK S.A., 2016 - *Using thermography to estimate leaf transpiration rates in cut roses for the development of vase life prediction models*. - Hortic. Environ. Biotechnol., 57: 53-60.

JACK K., 1985 - *The illustrated guide to flowering houseplants.* - Peerage Books, London, UK, pp. 76.

JANE C., GRAHAM C., 1997 - *Indoor plants: The essential guide to choosing and caring for houseplants*. - Chartwell Books, New York, NY, USA, pp. 240.

KHALIQ G., MOHAMMAD M.T.M., DING P., GHAZALI H.M., ALI A., 2015 - *Effect of gum arabic coating combined with calcium chloride on physico-chemical and qualitative properties of mango (*Mangifera indica *L.) fruit during low temperature storage*. - Scientia Hortic., 190: 187-194.

KLUCZKA J., 2024 - *Chitosan: Structural and chemical modification, properties, and application*. - Int. J. Mol. Sci., 25(1): 554.

LINGAIT D., RAHAGUDE R., GAHARWAR S.S., DAS R.S., VERMA M.G., SRIVASTAVA N., KUMAR A., MANDAVGANE S., 2024 - *A review on versatile applications of biomaterial/polycationic chitosan: An insight into the structure-property relationship*. - Int. J. Biol. Macromol., 257: 128676.

MACHMUDAH S., KAWAHITO Y., SASAKI M., GOTO M., 2007 - *Supercritical CO_2 extraction of rosehip seed oil: fatty acids composition and process optimization.* - J. Supercrit. Fluids, 41: 421-428.

MAHAFAUDI N., HAMDI S., 2014 - *Use of almond gum and gum arabic as novel edible coating to delay postharvest ripening and to maintain Sweet Cherry (*Prunus avium*) quality during storage*. - J. Food Process. Preserv., 39(6): 1499-1508.

MORAN R., 1982 - *Formula for determination of chlorophyll pigment extracted with N,N Diethylformamide.* - Plant Physiol., 69(6): 1376-1381.

MOUSSA M.M., MAZROU R.M., HASSAN F.A., 2024 - How sage and rosemary essential oils regulate postharvest senescence and extend the vase life of cut gladiolus spikes. - Horticulturae, 10(6): 638.

PALADINES D., VALERO D., VALVERDE J.M., DÍAZ-MULA H.M., SERRANO M., MARTÍNEZ-ROMERO D., 2014 - *The addition of rosehip oil improves the beneficial effect of* Aloe vera *gel on delaying ripening and maintaining postharvest quality of several stone fruit*. - Postharvest Biol. Technol., 92: 23-28.

RIDA M.F., 2019 - *Prolong the vase life of flower spike of Aster New York (*Symphyotrichum novi-belgii *L. cv.*

white Casablanca) by Diphenylamine . - Sci. J. Agric. Sci., (2): 10-19.

SALUNKHE D.K., BHAT N.R., DESAI B.B., 1990 - *Post-harvest biotechnology of flowers and ornamental plants*, Springer- Verlag, Berlin, Germany, pp. 192.

SARHAN A.M.Z., HEIKAL A.A.M., SAADAWY F.M., NOOR ELDEEN T.M., ABD ELKAREEM K.M., 2023 - *Effect of some chemical and natural preservative solutions on vase life, water relations and some chemical composition of* Dianthus caryophyllus *L. cut flowers*. - Adv. Hort. Sci., 37(3): 255-269.

SCHROEDER K.R., STIMART D.P., 2005 - *Comparison of stomatal density and postharvest transpiration between long-and short-lived cut flower genotypes of* Antirrhinum majus *L*. - J. Am. Soc. Hort. Sci., 130: 742-746.

SNEDECOR G.W., COCHRAN W., 1989 - *Statistical methods*. 8th edition. - Iowa State University Press, Blackwell Publishing, Ames, Iowa, USA, pp. 503.

TAHMASEBI P., JAVADPOUR F., SAHIMI M., 2015 - *Three-dimensional stochastic characterization of shale SEM images*. - Transport Porous Med., 110: 521-531.

THOMAS N.B., 2005 - *The control of stomata by water balance*. - New Phytologist, 168: 275-292.

TIAMIYU Q.O., ADEBAYO S.E., YUSUF A.A., 2023 - *Gum Arabic edible coating and its application in preservation of fresh fruits and vegetables*: A review. - Food Chem. Adv., 2: 1-8.

UGGLA M., NYBOM H., 1998 - *Domestication of a new crop in Sweden Dog Roses (Rosa sect. canina) for commercial rose hip production.* - Acta Horticulturae, 484: 147-153.

VASIĆ D., PAUNOVIĆ D., SPIROVIĆ TRIFUNOVIĆ B., MILADINOVIĆ J., VUJOŠEVIĆ L., DINOVIĆ D., POPOVIĆ-DORĐEVIĆ J., 2020 - *Fatty acid composition of rosehip seed oil*. - Acta Agricult. *Serb*., 25(49): 45-49.

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Lettuce postharvest quality: Affordable packaging and storage durations

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Abstract: The affordability of packaging materials and proper storage facilities to preserve the quality of lettuce (*Lactuca sativa* L.) has become a problematic issue for small-scale farmers in Malaysia, who have limited resources and rely on their crops for income. This study compares the post-harvest quality preservation of lettuce using plastic bags and newspapers as cost-effective packaging materials under storage durations of 0, 3, 6, 12, and 15 days. The lettuce quality parameters measured were chlorophyll, sucrose, weight loss, and overall visual quality (OVQ). The results showed newspapers had the highest mean weight loss (7.91 g), while plastic had the lowest one (5.81 g). However, chlorophyll content did not significantly differ between the two packaging types. Lettuce packaged in plastic bags had a significantly lower total soluble solids (TSS) content mean value (2.89%) compared to newspaper (3.89%). In addition, the plastic bag materials gave a better OVQ than that of the newspaper. Generally, the use of plastic bags as a packaging option for small-scale lettuce farmers in Malaysia is affordable and readily available.

1. Introduction

Lettuce (*Lactuca sativa* L.) is a widely consumed vegetable known for its high nutritional value, low calorie content, and potential health benefits. It is a valuable source of vitamins, minerals, and dietary fiber, which are essential for maintaining good health and preventing chronic diseases (Shatilov *et al*., 2019). The increasing demand for organic lettuce in Malaysia has led to more farmers adopting organic farming practices. This crop, along with tomatoes, brassicas, cabbage, and cucumbers, ranked in the top 5 among vegetables with the highest production in the country. FAOSTAT (2025) reported that lettuce production from Malaysian farmers was recorded at 101,680.39 metric tons in 2023. Such production gave a share of 8.99% of the total 1,130,287.58 metric tons' production of all 30 types of cash crops and vegetables in the country.

Being a perishable crop, lettuce requires proper post-harvest handling and storage conditions, such as optimal temperature and humidity levels,

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are critical to ensure the quality and freshness of lettuce. The optimal storage temperature ranges of 0ºC to 5°C is crucial to maintain the quality and freshness of lettuce (Gross *et al*., 2016). Farmers need to get the vegetables to the market or supplier as quickly as possible to maintain their freshness and quality. Once lettuce has lost its freshness, it cannot be sold, leading to further losses and waste (Ravula *et al*., 2020).

Unfortunately, many small-scale farmers in Malaysia still lack access to adequate storage facilities, and they often lack the equipment and know-how necessary to preserve their harvests, which can lead to large losses. When lettuce is stored improperly, it might wilt, become brown, and show indications of decomposition, which will make it unsellable. Inadequate storage practices can cause farmers to lose 20-50% of their harvest, resulting in waste and financial losses (Nkolisa *et al.*, 2018). Due to their limited resources and reliance on their crops for revenue, small-scale farmers are most affected by this issue.

Previous studies reported that polymeric films were commonly used as packaging materials for lettuce preservation. According to Lee and Chandra (2018), packaging films have shown beneficial effects in maintaining sensory, nutritional, and microbial qualities in many fresh or fresh-cut products. However, to the best of our knowledge, little is known about the benefits of plastic bags and newspapers, which have been identified as the most used packaging materials for preserving post-harvest vegetables among small-scale lettuce farmers in Malaysia. These materials were affordable and accessible for packaging options for preserving post-harvest vegetables among farmers. Therefore, this study compared newspaper and plastic bags as economical packaging materials for preserving the post-harvest quality of lettuce under varied storage durations. By systematically comparing these factors, this study offers novel insights into how different packaging materials influence key postharvest qualities such as weight loss, chlorophyll content, and total soluble solids (TSS), which sheds new light on their impact on the nutritional value and visual appeal of lettuce. Building on these findings, this study addresses a critical gap in understanding the role of effective and affordable packaging materials in maintaining lettuce quality during storage, with practical implications for small-scale lettuce farmers.

2. Materials and Methods

The lettuce was grown in a greenhouse at the Universiti Teknologi MARA (UiTM) farm, Jasin campus, Melaka, Malaysia, was harvested and transported to the postharvest laboratory within one h. Leaves that were overly mature, uneven, or abnormal, as well as those that were damaged or physically injured, were removed from the samples and discarded. The lettuce was chosen based on size, color, and absence of defects.

The plastic bag used in the experiment was made of low-density polyethylene (LDPE), measuring 15 cm (W) × 30 cm (L) + 4 cm (gusset). This type of plastic is specifically designed for packaging vegetables and fruits, and it is commonly used by the farmers for lettuce packaging. The plastic film has a thickness of 0.05 mm, with an oxygen transmission rate (OTR) of approximately 40,000 mL m^{-2} day^{-1} and a water vapor transmission rate (WVTR) of around 9 g m^{-2} day^{-1} under standard conditions.

The samples were then stored in a 5°C cooling room until the end of their shelf life. This temperature was selected as the optimal lettuce storage temperature range. This is in line with the FDA (2010), which advised that cut-leafy greens must be maintained at temperatures of 5°C or less during cold storage and display. In this study, the growth parameters of chlorophyll, sucrose, weight loss, and visual quality (OVQ) were measured.

Weight loss evaluation

The lettuce was weighed after being stored. Using an analytical balance (ELT602, Sartorius, Gottingen, Germany), the weight loss percentage was computed by subtracting the current sample weight from the initial sample weight (Waghmare and Annapure, 2015). The percentage of weight loss was computed using the formula in Equation (1):

$$\text{Weight loss (\%)} = (Wi - Wt)/Wi \times 100 \quad (1)$$

where *Wi* represents the initial sample weight (g) at day 0 and *Wt* represents the most recent sample weight (g) at day *t*.

Chlorophyll evaluation

The chlorophyll content of the lettuce leaves was measured using a SPAD 502 Plus Chlorophyll Meter (Minolta Camera Co., Osaka, Japan). The chlorophyll

content was measured by placing the meter's measuring head on the adaxial side of five randomly selected leaves from each treatment. The SPAD values were recorded, and the mean value for each treatment was calculated. The chlorophyll concentration was expressed in terms of SPAD units (SU).

Total soluble solids analysis

The total soluble solids (TSS) of the lettuce samples were determined using a Brix meter (Brand HUILEY, Model HT113ATC) in accordance with the instructions provided by the manufacturer. The sampled lettuces awas washed, cut into small pieces, and homogenized briefly. Before each measurement, the Brix meter was calibrated with distilled water. The measurement range of the Brix meter was 0-32%, and each sample was replicated three times. The measurements were conducted on days 0, 3, 6, 12, and 15 of storage.

Overall visual quality (OVQ)

The OVQ rating is a measure of the lettuce's condition during storage. It considers factors such as appearance, texture, and color to determine the overall quality of the lettuce. These variables can be affected by a variety of factors, such as lettuce storage duration, storage temperature, and the storage method employed. A panel comprised of three individuals from the university's farm was chosen to evaluate the OVQ rating. One man and two women, ages 20 to 23, were selected as the panelists.

Before the test, the panelists were trained to recognize and evaluate the lettuce's OVQ. This training is vital because it ensures that panel members can evaluate samples of lettuce consistently and accurately. The OVQ score considers the appearance of lettuce, including its color, texture, and overall freshness. OVQ was evaluated using a 9-point scale adapted from Wheeler *et al*. (2015): 9= excellent, 7= good, 5= acceptable, 3= poor, and 1= unusable. The overall visual quality was evaluated on the day of processing and every three days until the end of the shelf life during 5°C storage.

Factorial experiment in the IBM SPSS software was used to statistically analyze the effects of different packing materials and storage durations on mean weight loss, chlorophyll, and TSS content. The interaction between packaging materials and storage duration as factors towards the respective mean weight loss, chlorophyll, and TSS were evaluated.

An analysis of variance (ANOVA) was applied to test the mean significance between the storage duration and the quality of lettuce stored under the respective plastic and newspaper packaging. Tukey's range test was used to compare the differences between treatments.

3. Results and Discussion

Weight loss

As shown in Table 1, both packaging type and storage duration had a significant effect on weight loss. The packaging type showed a statistically significant difference with a p-value of 0.021, indicating that the choice of packaging material influenced the extent of weight loss. This is further supported by the data in Table 2, where lettuce stored in newspaper packaging experienced a higher mean weight loss (7.91 g) compared to plastic packaging (5.81 g), suggesting that newspaper is less effective in preserving the product and minimizing weight loss.

The p-value of less than 0.001 for the duration of

Table 1 - Analysis of variance of weight loss

Source of Variation	Degree of freedom	Sum of squares	Mean square	F value	Significant
Packaging	1	39.543	39.543	261.927 *	.021
Duration	5	398.222	79.644	6.107 **	<.001
Packaging x Duration	5	38.158	7.632	12.301	.349
Error	24	155.396	6.475		
Total	36	2327.250			

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.

Table 2 - Mean comparisons of the quality of lettuce with different packaging materials

Packaging material	Quality of lettuce		
	Weight loss (g)	Chlorophyll content (SU)	TSS content (%)
Control	3.15 c	13.23 a	4.72 a
Newspaper	7.91 a	12.80 a	3.89 b
Plastic	5.81 b	13.39 a	2.89 c

Means in the same column followed by the same letters are not significantly different at the 0.05 probability level.

storage indicated that the storage duration of lettuce had a significant effect on weight loss. This suggests that the longer the product was stored, the higher the weight loss would be. However, the p-value of 0.349 for the interaction between packaging type and storage duration suggested that there was no significant difference in the effect of storage duration on weight loss between plastic and newspaper packaging. This implies that both types of packaging were equally affected by storage duration; therefore, storage duration is an important factor to consider for both types of packaging.

Lettuce undergoes weight reduction during storage mostly because of transpiration and respiration. Respiration is the mechanism by which lettuce utilizes oxygen and emits carbon dioxide to produce energy, resulting in the decomposition of carbohydrates and the release of water. Furthermore, enzyme activity, subject to the influence of storage conditions, plays a role in these physiological alterations (Escalona *et al*., 2006; Yang *et al*., 2024; Nitu et *al*., 2025).

Overall, the study findings suggested that plastic packaging was more effective in preventing weight loss in lettuce compared to newspaper packaging. The lower weight loss in plastic packaging may be attributed to its better barrier properties, which can limit the exchange of gases, such as oxygen and carbon dioxide, between the product and its environment. These gases can promote respiration and accelerate the metabolic processes in lettuce, leading to weight loss (Semco, 2014). Moreover, plastic packaging can prevent moisture loss and maintain high humidity around the product, which can help reduce water loss and keep the product fresh for a longer period.

Chlorophyll content

The results in Table 3 show that the type of packaging material used had a significant effect on the SPAD value for chlorophyll, with a p-value of 0.021. This effect is further illustrated in Table 2, where the mean SPAD value for lettuce packaged in plastic (13.39 SU) was higher than that for lettuce packaged in newspaper (12.81 SU), indicating a higher concentration of chlorophyll in plastic-packaged lettuce. The duration of storage had a significant effect on the SPAD value for chlorophyll, with longer storage durations leading to lower chlorophyll content.

The findings of this study are consistent with previous research on the degradation of chlorophyll during storage. The interaction between packaging type and storage duration was not significant, suggesting that regardless of the type of packaging material used, the degradation of chlorophyll will

Table 3 - Analysis of variance of chlorophyll content

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	Significant
Packaging	1	39.543	3.121	5.054 *	.021
Duration	5	398.222	30.139	48.807 **	<.001
Packaging x duration	5	38.158	1.591	2.577	.349
Error	24	155.396	0.618		
Total	36	2327.250			

* significant at the 0.05 probability level.
** significant at the 0.01 probability level.

occur over time, resulting in a decrease in the SPAD value for chlorophyll in lettuce as stated by Mattos *et al*. (2013). While, in addition, Ferrante and Maggiore (2007) stated that as lettuce is a non-durable vegetable, the degradation of leaf pigments such as chlorophylls and carotenoids occurs during storage, leading to tissue browning. Degl'Innocenti et *al.* (2005) and Ferrante and Maggiore (2007) added that a common indicator of these processes is browning and discoloration resulting from damage to the leaf surface. The loss of color occurs due to the degradation of chlorophylls because of vegetable aging during storage.

In response to the above findings, the appropriate selection of packaging materials is crucial to maintaining the quality and freshness of lettuce during storage and transportation. The use of plastic packaging can result in higher SPAD values for chlorophyll in lettuce compared to newspaper packaging, but this effect may be limited over time due to the natural degradation of chlorophyll. Therefore, it is important to balance the need for optimal packaging materials with the limitations of prolonged storage durations.

Total soluble solids content

As shown in Table 4, both the type of packaging and the duration of storage had a statistically significant effect on the total soluble solids (TSS) content of lettuce ($p<0.001$). Table 2 further illustrates this effect, with lettuce stored in newspaper packaging exhibiting higher TSS levels (3.89%) than those stored in plastic packaging (2.89%). In addition, TSS levels increased progressively with longer storage durations, suggesting that extended storage promotes sucrose accumulation. The significant interaction between packaging type and storage duration indicates that the influence of storage time on TSS content differs depending on the packaging material used. Overall, these findings emphasize the importance of both packaging type and storage duration in determining the TSS content of lettuce

Packaging materials play a crucial role in maintaining the freshness and quality of lettuce by providing a barrier against moisture loss and physical damage during storage and transportation. The use of newspaper packaging may lead to higher sucrose levels in lettuce due to its absorbent nature, which can draw moisture from the vegetables. TSS content is a key component of the flavor and texture of fruits and vegetables, and excess moisture can lead to an increase in TSS production in lettuce. However, the absorbent nature of newspaper packaging can also increase the risk of moisture loss and spoilage in lettuce.

In contrast, plastic packaging can provide a more effective barrier against moisture loss and protect the lettuce from external factors that may affect its quality. This can lead to a lower concentration of sugars in the lettuce compared to newspaper packaging. It is important to use appropriate packaging materials that balance the need for moisture retention with the need for protection and safety. The findings of this study suggest that different packaging materials may have different effects on the TSS content levels of lettuce, and that storage duration is an important factor to consider when selecting the appropriate packaging.

Based on the comparison of mean quality values for lettuce packed with different materials (Table 5), it appears that the newspaper had the highest mean value (7.91 g) while the plastic had a lower mean value (5.81 g) for weight loss. Although there

Table 4 - Analysis of variance of TSS content

Source of variation	Degree of freedom	Sum of squares	Mean square	F value	Significant
Packaging	1	9.000	9.000	32.400**	<.001
Duration	5	21.222	4.244	15.280**	<.001
Packaging x duration	5	11.667	2.333	8.400**	<.001
Error	24	6.667	.278		
Total	36	462.000			

** significant at the 0.01 probability level.

is no specific research on the effect of packaging materials between paper and plastic on the quality and shelf life of lettuce, some studies have investigated the effect of packaging on the shelf life of other fruits and vegetables. A study on tomato storage conditions and packaging materials found that HDPE packaging material resulted in the least weight loss when stored in a refrigerator (Sualeh *et al*., 2016).

For the chlorophyll content, the mean value for the lettuce stored in plastic packaging is 13.39 SU, and for the lettuce stored in newspaper packaging, the mean value is 12.80 SU (Table 2), whereas there is no significant difference between these two packaging due to their falling into the same grouping. The chlorophyll content was not significantly different between the two types of packaging. This could be because chlorophyll is relatively stable and is not affected by moisture or air. However, it is important to note that the chlorophyll content can be affected by other factors, such as light exposure and temperature.

While for TSS content, the mean value for the lettuce packed with plastic has the lowest value, which is 2.89%, compared with the newspaper, whose mean value is 3.89% (Table 2). TSS corresponding to sucrose, which is a disaccharide composed of glucose and fructose and is commonly known as table sugar. The query suggests that the lower sucrose content in plastic packaging could be due to its hygroscopic nature, which leads to the absorption of moisture. Water vapor permeation through the package material occurs in three steps: water vapor adsorption, diffusion, and desorption from the package. This process is driven by transferring from high to low water activity or relative humidity (RH) (Sand, 2021). However, plastic packaging has lower moisture permeability, which could prevent the loss of moisture and the absorption of sucrose, resulting in lower levels of sucrose in plastic-packaged products.

Table 5 shows the weight loss and chlorophyll content of lettuce stored for 0 days in plastic and newspaper packaging are significant with the storage duration of 3, 6, 9, 12, and 15 days. This occurs due to the shock of the environment from a normal temperature to a cold temperature. However, there is no significant difference in TSS content between the storage durations of lettuce under plastic packaging. When the lettuce was stored under newspaper packaging, it was found that there were significant differences between the storage durations of 0, 3, 6, 9, 12, and 15 days.

Overall visual quality

The findings of this study, as presented in figure 1, indicate that the overall visual quality (OVQ) of lettuce stored at 5°C can be effectively maintained for up to 12 days with the use of plastic bags. This is evidenced by the consistency of OVQ scores of 7 or

Table 5 - Mean comparisons of the quality of lettuce with different storage durations under both packaging materials

Packaging	Quality of lettuce		
	Weight loss (g)	Chlorophyll content (SU)	TSS content (%)
Plastic			
Day 0	0.00 b	16.23 a	3.00 a
3	8.95 a	13.97 b	2.67 a
6	6.54 ab	13.77 b	2.67 a
9	5.93 ab	13.00 bc	2.67 a
12	5.04 ab	12.30 bc	3.00 a
15	8.44 ab	11.10 c	3.33 a
Newspaper			
Day 0	0.00 c	17.47 a	2.67 b
3	8.59 ab	13.77 b	3.33 b
6	7.78 b	13.10 bc	3.00 b
9	8.10 b	11.80 bc	3.00 b
12	9.93 ab	11.33 cd	5.00 a
15	13.07 a	9.37 d	6.33 a

Means in the same column followed by the same letters are not significantly different at the 0.05 probability level.

higher, which exceed the marketability cut-off score of 6. However, when stored in newspaper packing, the OVQ scores of lettuce samples deteriorated rapidly, falling below the marketability cut-off score after just 6 days.

The results suggest that the choice of packaging material is a critical factor in maintaining the quality

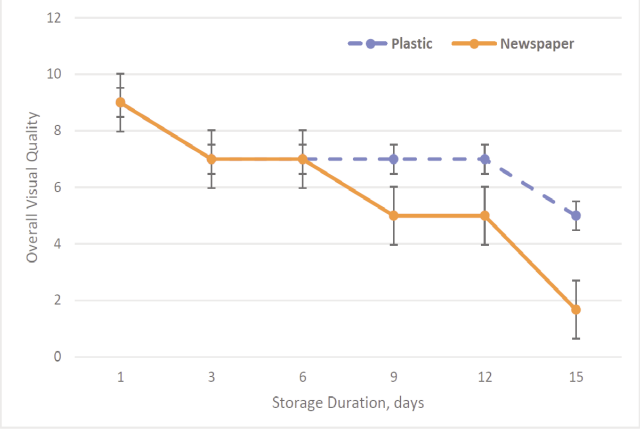

Fig. 1 - Overall visual quality of leaf lettuce packaged with plastic bags and newspapers during storage at 5°C for up to 15 days.

of lettuce during storage, with plastic bags proving more effective than newspaper in this regard. This may be attributed to plastic bags' superior ability to prevent moisture loss and inhibit microbial growth, which can contribute to the deterioration of lettuce quality and appearance. Additionally, the findings indicate that storage temperature plays a crucial role in preserving lettuce quality, with lower temperatures being more effective in achieving this objective.

The significant effect of storage duration on weight loss highlights the importance of proper storage conditions in preserving the quality and shelf-life of lettuce. Low temperatures, high humidity, and adequate ventilation are some of the essential storage conditions that can help slow down the metabolic processes and reduce weight loss in lettuce. The lack of significant interaction between packaging type and storage duration suggests that both types of packaging can benefit from proper storage conditions to minimize weight loss. Figure 2 shows the physical appearance of lettuce in 15 days using plastic in 5°C.

Overall, the study suggests that small-scale lettuce farmers in Malaysia may benefit from using plastic packaging to preserve the quality and freshness of their produce. However, the choice of packaging material should also consider factors such as cost and accessibility to ensure it is both effective and practical for small-scale operations. Additionally, to align with sustainable development goals, the environmental impact of using recycled materials such as newspapers should be an important consideration when selecting packaging options. It has been proven that newspaper as packaging material aligns with environmental sustainability goals due to its biodegradable nature and origin from renewable resources. Newspapers are also easily recycled or used for renewable energy, which helps reduce reliance on fossil resources.

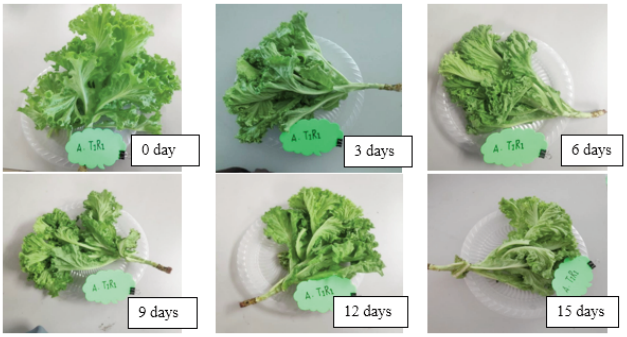

Fig. 2 - The physical appearance of lettuce in 15 days using plastic in 5°C.

4. Conclusions

The selection of appropriate packaging materials is crucial to maintaining the quality and freshness of lettuce during storage and transportation. The study found that plastic packaging was more effective in preventing weight loss and maintaining higher concentrations of chlorophyll in lettuce than newspaper packaging. However, newspaper packaging resulted in higher TSS levels compared to plastic packaging. The length of time the product was stored also had a significant effect on weight loss, chlorophyll degradation, and TSS levels. The study highlights the importance of balancing the need for optimal packaging materials with the limitations of prolonged storage durations.

Further studies are recommended to explore the use of alternative recycled packaging materials that are not only effective and environmentally friendly

but also affordable for small-scale farmers. This research could focus on identifying cost-effective and locally available options, such as biodegradable or compostable materials. Additionally, investigating the feasibility of a shared packaging system among small-scale farmers may offer a practical solution to reduce costs and enhance access to sustainable packaging options.

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References

DEGL'INNOCENTI E., GUIDI L., PARDOSSI A., TOGNONI F., 2005 - *Biochemical study of leaf browning in minimally processed leaves of lettuce (*Lactuca sativa *L. var.* acephala*).* - J. Agric. Food Chem., 53(26): 9980-9984.

ESCALONA V.H., VERLINDEN B.E., GEYSEN S., NICOLAÏ B.M., 2006 - *Changes in respiration of fresh-cut butterhead lettuce under controlled atmospheres using low and superatmospheric oxygen conditions with different carbon dioxide levels.* - Postharvest Bio. Tech., 39(1): 48-55.

FAOSTAT, 2025 - *Crops and livestock products (Malaysia). Crops and livestock products.* - https://www.fao.org/faostat/en/#data/QCL Accessed on 16 May 2025.

FDA, 2010 - *Program information manual retail food protection: Recommendations for the temperature control of cut leafy greens during storage and display in retail food establishments.* The United States Food and Drug Administration (FDA), USA, pp. 1-5.

FERRANTE A., MAGGIORE T., 2007 - *Chlorophyll a fluorescence measurement to evaluate storage time and temperature of Valeriana leafy vegetables*. - Postharvest Bio. Tech., 45(1): 73-80.

GROSS K.C., WANG C.Y., SALTVEIT M., 2016 - *The commercial storage of fruits, vegetables, and florist and nursery stocks*. - USDA Handbook, United States Dept. Agric., pp. 386-387.

LEE J.S., CHANDRA D., 2018 - *Effects of different packaging materials and methods on the physical, biochemical and sensory qualities of lettuce*. - J. Food Sci. Technol., 55(5): 1685-1694.

MATTOS L.M., MORETTI C.L., SILVA E.Y.Y., 2013 - *Effects of modified atmosphere packaging on quality attributes and physiological responses of fresh-cut crisp head lettuce*. - CYTA J. Food., 11(2013): 392-397.

NITU O.A., IVAN E.Ş., TRONAC A.S., ARSHAD A., 2025 - *Variety-specific lettuce responses to oxygen-enriched water and led light in a controlled greenhouse: A multivariate analysis across two cycles*. - Int. J. Plant Biol., 16(1): 29.

NKOLISA N., MAGWAZA L.S., WORKNEH T.S., CHIMPANGO A., 2018 - *Evaluating evaporative cooling system as an energy-free and cost-effective method for postharvest storage of tomatoes* (Solanum lycopersicum *L.) for smallholder farmers.* - Sci. Hort., 241(2018): 131-143.

RAVULA P., KASALA K., RAY S., 2020 - *Impact of COVID-19 on food security: Insights from Telangana, India.* - Agric. Econ. Res. Rev., 33(167): 167-168.

SAND C.K., 2021 - *Controlling moisture in foods using packaging.* - IFI. Institute of Food Technologists, Food Technology Magazine, September 1.

SEMCO, 2014 - *Post-harvest cooling and storing of lettuce*. - SEMCO/SEMCOLD LLC, Texas, USA, pp. 1-2.

SHATILOV M.V., RAZIN A.F., IVANOVA M.I., 2019 - *Analysis of the world lettuce market*. - IOP Conference Series: Earth and Environ. Sci., 395(1).

SUALEH A., DABA A., KIFLU S., MOHAMMED A., 2016 - *Effect of storage conditions and packing materials on shelf life of tomato.* - Food Sci. Qual. Manag., 56(2016): 60-67.

WAGHMARE R.B., ANNAPURE U.S., 2015 - *Integrated effect of sodium hypochlorite and modified atmosphere packaging on quality and shelf life of fresh-cut cilantro*. - Food Packaging Shelf Life, 3(2015): 62-69.

WHEELER L., KITINOJA L., BARRETT D., 2015 - *Use of insulated covers over product crates to reduce losses in amaranth during shipping delays*. - Agric., 5(4): 1204-1223.

YANG J., SONG J., LIU J., DONG X., ZHANG H., JEONG B.R., 2024 - *Prolonged post-harvest preservation in lettuce (*Lactuca sativa *L.) by reducing water loss rate and chlorophyll degradation regulated through lighting direction-induced morphophysiological improvements*. - Plants(Basel), 3(18): 2564.

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Impact of chitosan-aloe vera gel with coconut oil coating on postharvest quality and antioxidant of 'Gopalbhog' mango at ambient storage

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Key words: Antioxidant enzymes, coatings, mango, quality, storage.

Abstract: Mangoes are valuable fruits because of their nutritional value and commercial significance. It ripens rapidly but deteriorates in quality while stored. Therefore, natural and biodegradable materials must be used in post-harvest management to reduce crop losses. *Aloe vera* (AVG), chitosan (CTS), and coconut oil (CO) either alone or in combinations were tested on mango postharvest features during 12 days' storage at ambient conditions (27±2°C and 80-85% RH). At the end of storage, coatings (AVG+CTS) reduced weight loss (20.02, 37.88%) and decay (9.52, 18.46%) compared to controls while enhancing fruit quality, especially firmness (3.21, 1.44 Kg cm^{-2}), ascorbic acid (9.25, 5.89 mg 100 g^{-1}), TSS (10.77, 19.5°Brix), acidity (0.46, 0.41%) and pH. Furthermore, coated fruits' total phenol content and antioxidant activity were substantially higher than those of uncoated fruits. Control fruits exhibited the least activity of CAT and POD enzymes during storage, while coated fruits had the lowest PPO activity. The coated fruit peels discoloured less after storage than the control fruits. CO treatment had a deleterious effect on various measures, possibly due to its high concentration. These findings suggested that the CTS+AVG coating may be efficient at the right dose to retain bioactive components and mango (cv. Gopalbhog) fruit quality after harvest.

1. Introduction

Mango (*Mangifera indica* L.) is widely traded due to its versatility, taste, smell, and dietary content (Hossain, 2016). Its excellent nutritional value and abundance of vitamins and minerals make it extremely valuable (Athoo *et al.,* 2024). Bangladesh is the seventh-largest mango producer;

production currently occupies around 123,997.70 hectares, yielding over 1,482,937.04 MT which contributes 34.33% of the area and 26.21% of the production of total fruit crops in Bangladesh (Bangladesh Bureau of Statistics, 2024). In Bangladesh, there is a huge number of mango varieties; among these is Gopalbhog. This variety is an early well-liked type grown primarily in the north. The fruits produced by this variety are attractive, clean, juicy, fibreless, sweet and weigh 200-300g and pest-free thus, they demand a premium price in the market.

Mangoes are climacteric fruits that mature, soften, and ripen quickly, and are prone to mechanical damage that causes significant postharvest losses (Lawson *et al*., 2019). Due to their climacteric nature, mangoes cannot be stored at room temperature for extended periods, as they mature between 2 and 10 days after harvesting (Kumar *et al.,* 2023), with shelf life varying from a few days at ambient temperature to up to three weeks in cold storage at 13°C. Fruit maturation involves a series of metabolic activities, including loss of weight, increased respiration, structural polysaccharide changes, chlorophyll degradation, carotenoids biosynthesis, starch hydrolysis to sugars, and fruit ripening to acceptable quality (Khanum *et al.,* 2020). A number of factors contribute to post-harvest losses, including the use of improper harvesting, handling and transportation equipment, unsuitable packaging materials, temperature control, rough handling of fresh fruits, and inadequate road infrastructure (Kefas *et al.,* 2024). A variety of fungi attacks mango fruits once they reach maturity and are harvested from the tree. Postharvest losses of mangoes in Bangladesh are estimated to be around 35% (Alom *et al*., 2019). Many harvested mangoes never reach consumers due to these losses (Giovannoni *et al.*, 2017). Less shelf life and postharvest monitoring have hampered mango deliveries to distant markets. Therefore, maintaining mango fruit quality and extending post-harvest life requires effective strategies. Mangoes' postharvest life is artificially prolonged by chemicals that harm the environment and human health. Recently, several treatments that are nontoxic and non-harmful have been employed to enhance the postharvest quality of mangoes, including edible coatings (Liu *et al.,* 2020; Perez-Vazquez *et al.,* 2023; Aaqil *et al.,* 2024), essential oils, or nanoparticles (Kanwar *et al.,* 2024).

Applying chitosan exogenously improves antioxidant activity, maintains firmness, reduces transpiration rate, and enhances fruit quality overall (Wang *et al.,* 2021). Being a naturally occurring compound with antibacterial capabilities, it delays fruit deterioration by making mangos more durable and preventing microbial attacks (Parvin *et al.,* 2023). Chitosan can form layers and is safe and recyclable (Kumar *et al*., 2021). It could treat mango fruit postharvest diseases as an antibacterial agent (Shah *et al.,* 2020). As stated by Silva *et al.* (2017), chitosan has already extended the shelf life of lemon, papaya, and mango following harvest. According to Eshetu *et al.* (2019), chitosan, either by itself or in combination, lowers respiration, softening of tissue, loss of weight, disease, and more.

Aloe vera is an environmentally safe postharvest treatment that researchers are interested in. It is frequently added to edible coatings to enhance their antibacterial and moisturizing qualities. Aloe vera gel improves fruit preservation by increasing coating flexibility and barrier properties (Ahmed, 2024). Researchers have extensively studied it as an edible covering material to enhance food quality and safety. However, studies indicate that Aloe vera gel, either by itself or mixed with other ingredients in edible coatings, can minimise lipid oxidation, slow respiration, soften cell walls, promote weight loss, and prevent fruit decay. This effect prolongs the shelf life of mangoes (Amin *et al.*, 2021), table grapes (Ayyub *et al.*, 2024), apples (Kaur *et al.*, 2024), tomatoes (Tobing *et al*., 2023), and apricots (Farooq *et al*., 2023), while maintaining other quality characteristics.

Coconut oil, a tasty fruit coating, reduces respiration, transpiration, and ethylene production. It's rich in lauric acid. This acid may be endogenously converted to monolaurin, which has antimicrobial properties (Liberman *et al.,* 2006). In order to restrict respiration, transpiration, and microbial activity, coconut oil encircles stomata and lenticels (Bisen and Patel, 2012).

Hence, this study set out to analyze the impact of varying chitosan-aloe vera gel with coconut oil coating concentration on 'Gopalbhog' mango quality and shelf life at ambient storage. The aim of this study was to apply chitosan and *Aloe vera* gel as environmentally friendly preservation coatings for decreasing fruit softening, maintaining postharvest mango quality, and prolonging the commercial storage period.

2. Materials and Methods

Fruit material

Mature 'Gopalbhog' mangoes (peel turned yellow at the bottom and green at the top) from the center of the plant canopy were collected from an orchard near the HSTU (Lat. 25°38'11.6664'' N and Long. 88°38'10.9592'' E) in Bangladesh. We used local producers' harvest stages fruits, which were uniform in size and shape, had no damage and/or microbial infection, and were attractive in colour (green peel and yellow from bottom to top when mature). Fruits were transferred to the laboratory within 2 hours. 72 physiologically mature mangoes (4 fruits per replication) were cleaned with a sodium hypochlorite solution (1% v/v) and dried at room temperature before use.

When mango peels turned yellow at the bottom and green at the top, they were harvested.

Treatments and storage

The following six treatments were randomly assigned to six lots of fruits (12 fruits each lot): Control (distilled water), *Aloe vera* gel (AVG 1:1 v/v), chitosan solution (CTS, 1.5% w/v), Coconut oil (CO 1:1 v/v), AVG+CTS, and AVG+CO. Fruits were coated by brushing for five minutes, then air-dried for 2 hours at room temperature. Finally, treated fruits were stored at ambient conditions (27±2°C and 80-85% RH). Quality assessments were done every four days, from day 0 to day 12 of storage.

Aloe vera gel extraction

The picked *Aloe vera* leaves were peeled. The parenchyma, after being homogenised into a mucilaginous jelly, had to be filtered to get rid of fibrous materials (Song *et al*., 2013).

Chitosan solution preparation (CTS)

In 100 ml of 1% aqueous lactic acid (v/v), 1.5 grams of chitosan powder were dissolved in 1 ml of glycerin. To homogenize the solution, a magnetic stirrer was used for four hours at 25°C. The solution was filtered through three layers of muslin cloth.

Preparation of chitosan-aloe vera gel coating (CTS+AVG)

A magnetic stirrer was used to mix CTS and an AVG (1:1 v/v) at the ambient temperature for four hours.

Preparation of coconut oil-Aloe vera gel coating (CO+AVG)

In order to create a translucent liquid, CO and AVG (1:1 v/v) were pooled in a beaker and heated in a hot water bath.

Weight loss

Following the usual technique, the weight loss % was calculated:

Loss of weight (%)= [Initial fruit weight (g)- Fruit's weight on the observation day(g)]/[Initial fruit weight(g)] ×100

Fruit firmness, TSS and pH

Pressure testers measured fruit firmness in kg cm^{-2}. A 2 mm stainless-steel spherical probe entered the fruit sample. Three measures calculated the average stiffness. A digital refractometer calculated the TSS (°Brix). pH was measured with a Chinese digital pH meter (HI 2211 pH/ORP).

Vitamin C

Vitamin C was assessed as described by McHenry and Graham (1935) with slight modification. Briefly, Whatman No.1 filtered 5g of mango pulp with 5 ml of a 20% metaphosphoric acid solution. Five ml of filtrate was agitated with two drops of phenolphthalein solution in a small beaker and titrated with touching 2, 6-indophenol till pink.

Vitamin C (mg/100 g) = (Titrate × factor of dye (0.5) × prepared volume)/(Filtrate volume taken × weight of sample)

Titratable acidity (TA)

To test titratable acidity (citric acid %), 5 ml of the juice was titrated with NaOH (0.1 N) and phenolphthalein until it turned bright pink (pH = 8.0).

Color

We used a CR-2000 Japan chroma meter to quantify the color of the mango skin on two different sides of the fruit. The results were shown as L* values (positive means brightness, negative means darkness), a* values (negative means green, positive means red), and b* values (negative means blue, positive means yellow).

Decay incidence

The following equation is used to identify rotten fruits:

Decay (%) = (Number of decayed fruits)/(Number initial fruits) ×100

Total phenolic content (TPC)

Total phenolic compounds (TPC) were determined using the method described by Singleton and Rossi (1965) with a few adjustments. Methanol (10 ml) was used to extract and strain 1 g of fruit pulp. An aliquot (1 ml) was mixed with 0.5 ml of Folin-Ciocalteu reagents (Sigma Aldrich) and 7.5% (w/v) aqueous Na_2CO_3. Distilled water was added to bring the volume up to 10 ml. The samples were vortexed for 35 minutes at ambient temperature, then centrifuged for 10 minutes at 4000 rpm. Absorbance at 765 nm was checked using a UV 1800 Shaanxi, China spectrophotometer. The TPC was measured as mg of GAE (Gallic Acid Equivalent) per 100-gram fruit pulp.

Antioxidant activity by DPPH scavenging

Ten milliliters of methanol and a gram of fruit pulp were mixed to determine the antioxidant activity of the treatment. Whatman No. 1 filter paper was used to filter the mixture. After that, 0.1 ml of extract and 1.9 ml of DPPH solution (0.3 mM) were added to a Falcon tube, which was vortexed for half an hour. Trolox solutions (TE) were used to create the standard curve. A spectrophotometer (UV 1800 Shaanxi, China) was used to detect absorbance at 517 nm compared to a blank.

Assaying enzymes

The fruit pulp (0.2 g) was mashed in 3 ml phosphate buffer (100 mM, pH 7, and 4% polyvinylpolypropylene) and centrifuged at 12000 rpm for 15 minutes, aliquoted, and stored at 4°C for future use.

Polyphenol oxidase (PPO)

Enzyme extract (600 µl), 1200 µl phosphate buffer solution (100 mM, pH 7), and 600 µl catechol (100 mM,) were mixed together. Activity was validated as U g^{-1} FW after 2 minutes of 410 nm absorbance.

Catalase (CAT)

The mixture included K_2SO_4 buffers (700 µl, 50 mM, pH 7), H_2O_2 (100 µl), and 100 µl of EDTA (100 µl, 2.5 mM). A spectrophotometer recorded absorbance at 240 nm for two minutes. U g^{-1} FW referred to catalase activity.

Peroxidase (POD)

To observe the peroxidase activities, we used enzyme extract (100 µl), H_2O_2 (100 mM), guaiacol (20 mM), EDTA (2.5 mM), and phosphate buffer (600 µl, 100 mM, pH 7. Color development was measured at 470 nm absorbance for 2 minutes, and the results were shown as U g^{-1} FW.

Sensory evaluation

The coated fruits and control were evaluated for sensory attributes, including color, flavor, texture, sweetness, appearance, and overall expression, using a nine-point Hedonic Scale. 1 - Extreme dislike, 2 - Dislike very much, 3 - Moderate dislike, 4 - Slight dislike, 6 - Like slightly, 7 - Like moderate, 8 - Like very much, 9 - Like extremely (Salehin *et al.,* 2025).

Statistical analysis

Data was evaluated using a completely randomized design with three replications and two factorial designs. Statistical Tool for Agricultural Research (STAR, Version 2.0.1; IRRI, Laguna, Philippines) and R (version 3.4.2; R Core Team, 2017) with one-way ANOVA were used to analyse the data. LSD estimated mean value differences ($P<0.05$). PCA found likely associations between variables.

3. Results

Water loss influences fruit postharvest quality. Storage times and treatments significantly reduced fruit weight. Figure 1A shows that fruits lost weight as storage time increased. Control fruits lost the maximum weight (29.24%) during storage, while AVG+CTS dropped the least (20.02%). CTS, AVG, CO, and CO+AVG treated fruits lost 26.80%, 29.37%, 35.31%, and 26.07%, respectively.

Storage duration and treatments considerably influenced firmness. Throughout the storage period, the firmness gradually decreased regardless of treatment. For control and CTS+AVG, initially, the mango firmness values were 5.36 and 5.9 kg cm^{-2}, which dropped significantly at 12 days of storage, reaching values of 1.44 and 3.21 kg cm^{-2}, respectively (Fig. 1B).

Storage times and coatings significantly affected the mango fruit's mean total soluble solid (TSS) content. Figure 2A shows that fruit TSS increased significantly with increasing storage periods. The control had the maximum TSS concentration (19.50°Brix), while CTS+AVG and AVG-treated fruits had the lowest (10.77 and 11.0°Brix, respectively) at the end of the storage.

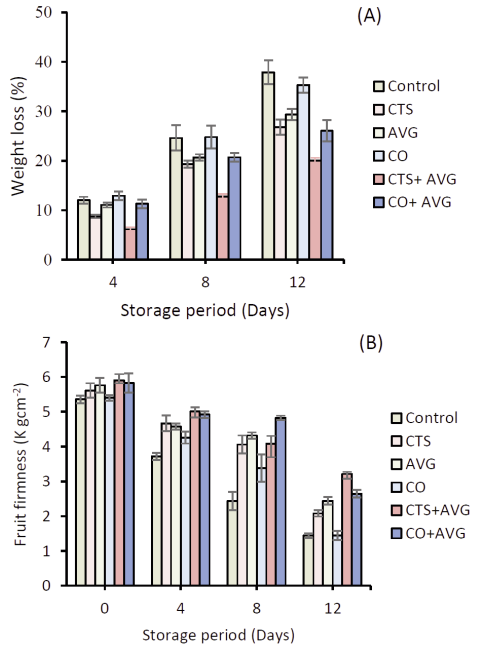

Fig. 1 - Mango weight loss (A) and fruit firmness (B) after 12 days of storage at 27±2°C and 80-85% relative humidity due to coatings and storage intervals. The vertical line shows the standard error of the means of three replicates. Control: Distilled water; CTS= Chitosan; AVG= Aloe vera gel; CO= Coconut oil; CTS+AVG= Chitosan+ Aloe vera gel; CO+AVG= Coconut oil+ Aloe vera gel.

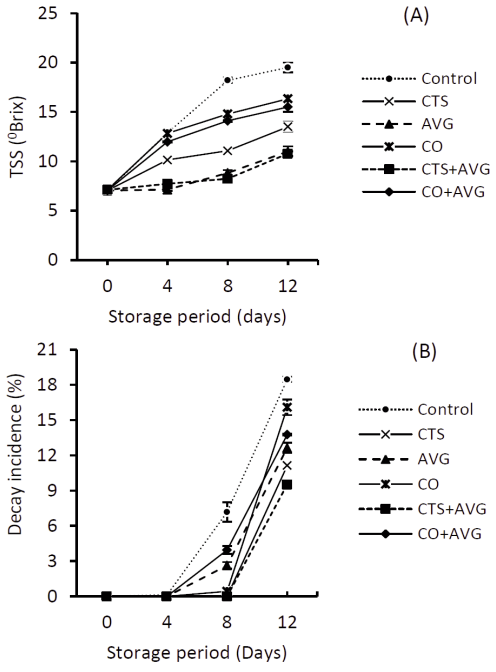

Fig. 2 - Mango TSS (A) and decay incidence (B) after 12 days of storage at 27±2°C and 80-85% relative humidity due to coatings and storage intervals. The vertical line shows the standard error of the means of three replicates. See Figure 1.

From figure 2B, it is observed that fruit degradation began after 4th day of storage in the control group, whereas treated fruits began to deteriorate on the 8th day of storage. Compared to the control fruit (18.46%), the mango fruits coated with CTS+AVG showed the least deterioration (9.52%) after 12 days of storage.

Color attracts customers' preferences. Coated treatments maintained color, whereas control fruits changed faster. Table 1 provides the color parameters L*, a*, and b*. All samples lost L* values during storage. Control and treated fruit differed greatly. The L* value of the control fruits was considerably lower (30.30) than that of the coated fruits during storage. Higher L* values (41.18) were recorded during storage for fruits covered with CTS+AVG.

After storage, control fruit exhibited higher a* values (-1.31) than coated fruits. The control and treated mangoes initially showed a rise in b* values, which decreased until the end of storage. However, the higher b* values (32.61) were found in CTS+AVG-treated fruits when compared to the control. Since fruit color affects product quality and fresh market value, customer approval is essential.

Mango fruit ascorbic acid content was significantly affected by storage and treatment. Due to physiological metabolism and ascorbic acid oxidation, treated and control mangoes lost vitamin C during storage (Fig. 3A). At 12 days, mean ascorbic acid was 7.56 mg 100 g^{-1}. CTS+AVG-treated fruits had a higher ascorbic acid level (9.25 mg 100g^{-1}), while control fruits had less (5.89 mg 100g^{-1}) after the storage compared to the other treatments.

Acidity decreased during storage. The mean acidity was 0.53% after 12 days storage. In figure 3B, the control had the least acidity (0.41%) while the CTS+AVG treatment had the highest (0.64%).

CTS+AVG-treated fruits exhibited a lower pH 3.39 while control showed higher P^H value 4.65 at the end of storage (Fig. 3C).

During storage, treated and control fruits significantly lost phenolic compounds. At 12 days, CTS+AVG had higher phenolic content (126.34 mg 100 g^{-1}), compared to the control (84.13 mg 100g^{-1}) (Fig. 4A).

Figure 4B shows that there was a significant decrease in antioxidant capacity in treated and control fruits after storage. At the conclusion of storage, CTS+AVG fruit samples showed the highest DPPH activity (293.51 µmol g^{-1}), followed by CTS

Table 1 - Mango peel color (L*, a, and b) after 12 days of storage at 27±2°C and 80-85% relative humidity due to coatings and storage intervals

Treatments	Storage periods (Days)				Mean (Treatments)
	0	4	8	12	
L*					
Control	43.09±3.29 def	37.53±0.32 hi	31.96±0.4 j	30.30±0.35 j	35.72 C
CTS	45.33±2.73 cde	43.35±0.32 def	40.69±0.11 fgh	37.35±0.41 i	41.68 B
AVG	50.40±0.55 ab	45.40±1.07 cde	42.77±0.20 def	40.96±0.47 fg	44.88 A
CO	47.80±0.17 bc	43.02±1.94 ef	40.86±0.20 fg	38.96±0.07 ghi	42.66 B
CTS+AVG	53.08±1.37 a	43.44±0.63 def	42.47±0.05 ef	41.18±0.11 fg	45.04 A
CO+AVG	46.28±1.38 cd	44.73±0.80 cde	43.62±0.01 def	40.82±0.26 fg	43.86 A
Mean (Storage periods)	47.66 A	42.92 B	40.59 C	38.26 D	
a*					
Control	-7.73±0.58 hij	-4.23±1.27 cde	-3.91±0.35 b	-1.31±0.06 ab	-2.07 A
CTS	-8.04±0.13 jk	-7.10±0.66 ghij	-5.47±0.07 efg	-3.40±0.06 cd	-6.00C D
AVG	-8.57±0.45 jk	-6.13±0.15 fgh	-5.08±0.10 def	-3.00±0.03 c	-5.70 C
CO	-7.93±1.25 ijk	-6.07±0.32 fgh	-4.08±0.68 b	-2.74±0.42 a	-3.15 B
CTS+AVG	-9.55±0.54 k	-6.71±0.06 fghi	-6.02±0.29 fgh	-4.09±0.33 cde	-6.59 D
CO+AVG	-88.84±0.31 jk	-7.76±0.37 hij	-4.22±1.48 b	-1.53±0.33 ab	-3.82 B
Mean (Storage periods)	-8.44 D	-6.33 C	-2.63 B	-0.81A	
b*					
Control	15.12±0.50 l	20.57±1.67 k	30.37±0.48 fcde	28.26±0.53 efghi	23.58 D
CTS	25.41±0.58 ij	27.09±0.73 ghij	30.91±1.40 cde	30.57±0.52 cdef	28.49 C
AVG	25.78±0.64 hij	27.58±1.02 fghi	30.90±0.13 cde	30.12±0.37 cdefg	28.59 BC
CO	28.82±2.12 efgh	32.25±0.78 cd	36.17±1.60 ab	33.13±0.58 bc	32.59 A
CTS+AVG	24.04±2.57 j	28.64±0.90 efgh	38.81±1.65 a	38.95±0.09 a	32.61 A
CO+AVG	29.00±0.72 efg	29.64±0.31 defg	30.69±0.38 cdef	29.77±0.29 defg	29.77 B
Mean (Storage periods)	24.69 D	27.63 C	32.98 A	31.80 B	

Values are means with three replicates ± SE. Means followed by different letters (s) indicate significant differences within the columns or rows. Control= Distilled water; CTS= Chitosan; AVG= Aloe vera gel; CO= Coconut oil; CTS+AVG= Chitosan+ Aloe vera gel; CO+AVG= Coconut oil + Aloe vera gel.

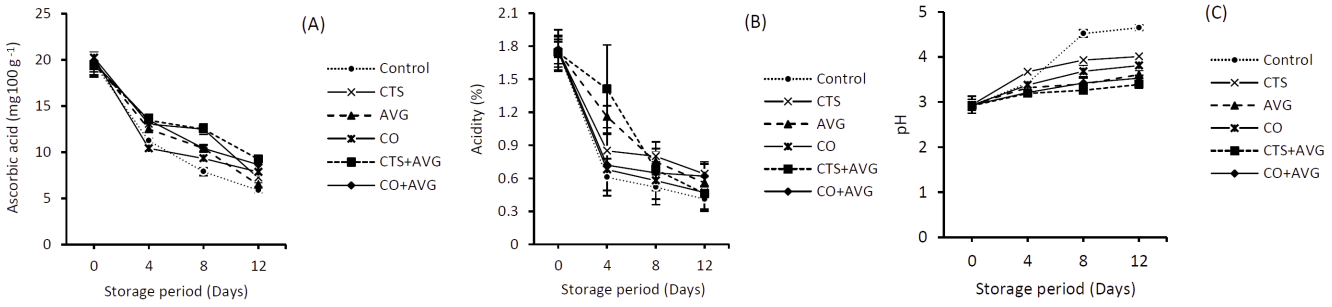

Fig. 3 - Mango ascorbic acid (A) acidity (B) and pH after 12 days of storage at 27±2°C and 80-85% relative humidity due to coatings and storage intervals. The vertical line shows the standard error of the means of three replicates. See Figure 1.

(244.29 µmol g^{-1}), AVG (276.12 µmol g^{-1}), CO (222.79 µmol g^{-1}), and CO+AVG (227.04 µmol g^{-1}). The control group had the lowest DPPH activity (182.65 µmol g^{-1}) compared to the coated groups, which may help the fruit generate antioxidant molecules and activate antioxidant defense enzymes.

Post-harvest treatments and storage durations significantly ($P< 0.05$) influenced mango fruit antioxidant enzymes. As demonstrated in figure 5A the control group had the highest polyphenol peroxidase (PPO) activity (7.17 U g^{-1}) after 12 days, while CTS+AVG-treated fruits had the lowest (3.95 U

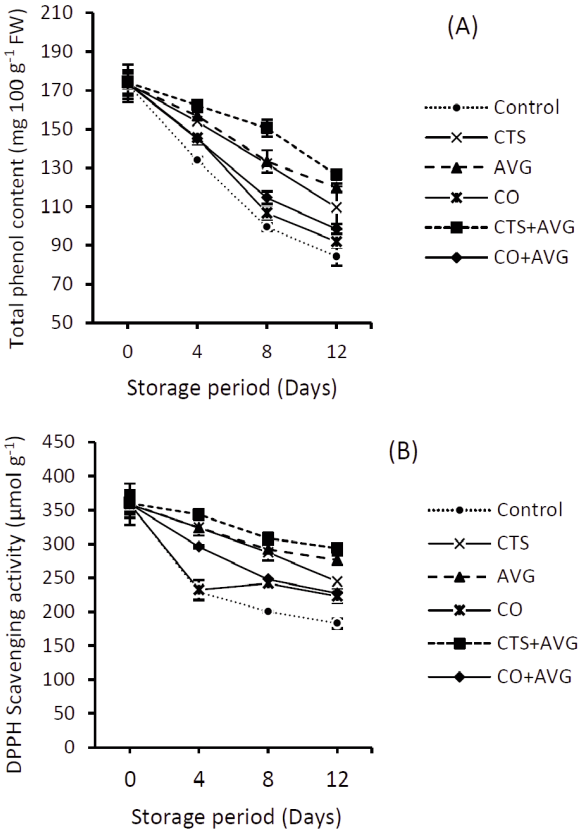

Fig. 4 - Mango total phenol content (A) and DPPH activity (B) after 12 days of storage at 27±2 °C and 80-85% relative humidity due to coatings and storage intervals. The vertical line shows the standard error of the means of three replicates. See Figure 1.

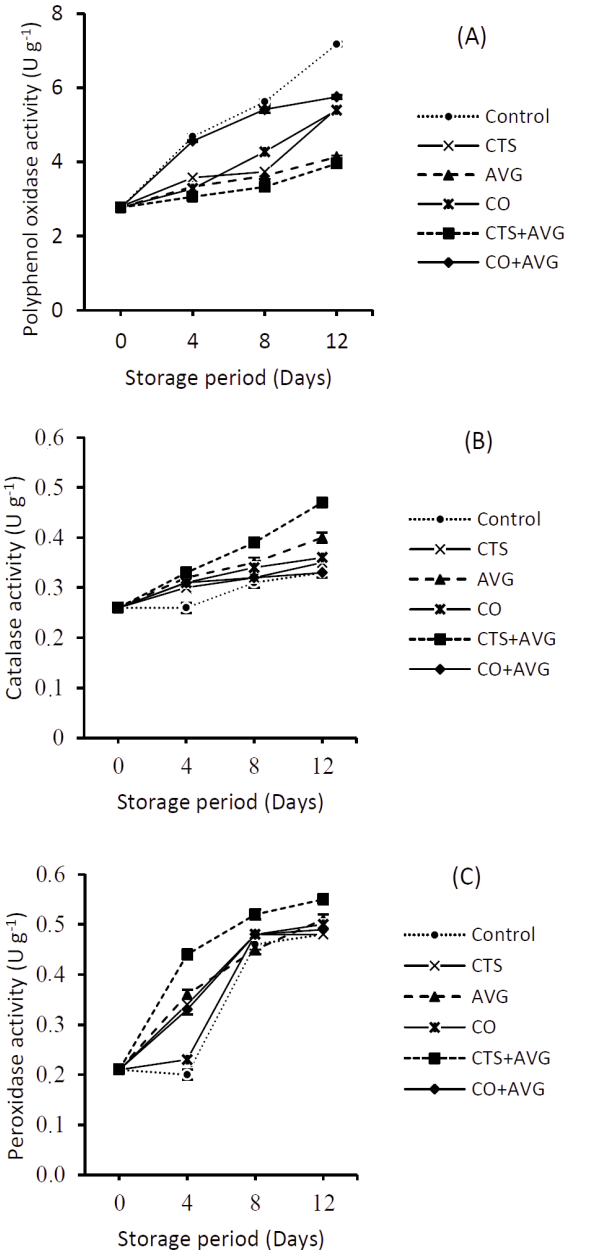

Fig. 5 - Mango PPO (A), CAT (B) and POD (C) activity after 12 days of storage at 27±2°C and 80-85% relative humidity due to coatings and storage intervals. The vertical line shows the standard error of the means of three replicates. See Figure 1.

g^{-1}). As shown in figure 5B treated fruit samples and control-maintained catalase activity (CAT) throughout storage. After 12 days of storing, the CTS+AVG group had higher CAT activity (0.47 U g^{-1}) than the control group (0.33 U g^{-1}). Figure 5C shows the maximum POD activity (0.55 U g^{-1}) in CTS+AVG-treated fruits after 12 days of storage. POD activity at 12 days of storage was in CTS (0.48 U g^{-1}), AVG (0.51 U g^{-1}), CO (0.50 U g^{-1}), and CO+AVG (0.49 U g^{-1}) while the control (0.48 U g^{-1}) exhibited lower POD activity.

Mango fruits treated with CTS + AVG had superior overall impression (7.71) compared to the control (7.01). CTS + AVG improved the mango fruit's flavor (7.97), sweetness (7.33), taste (7.63) and color (7.90) at 8 days of storage (Fig. 6).

Principal component analysis (PCA)

PCAs examined biochemical properties and antioxidant enzymes after postharvest treatment. Two PCAs explained 88.4% of PCA variation. PC1 (Dim 1) explained 80.1% of the dataset variation and PC2 (Dim 2) explained 8.3%. AA, TPC, DPPH, FF, L* values, POD, and CAT enzymes strongly connect with PC1, while TSS, WL, PPO enzyme, and a* values negatively correlate. PC2 was linked positively with b* values but negatively with TA and pH (Fig. 7).

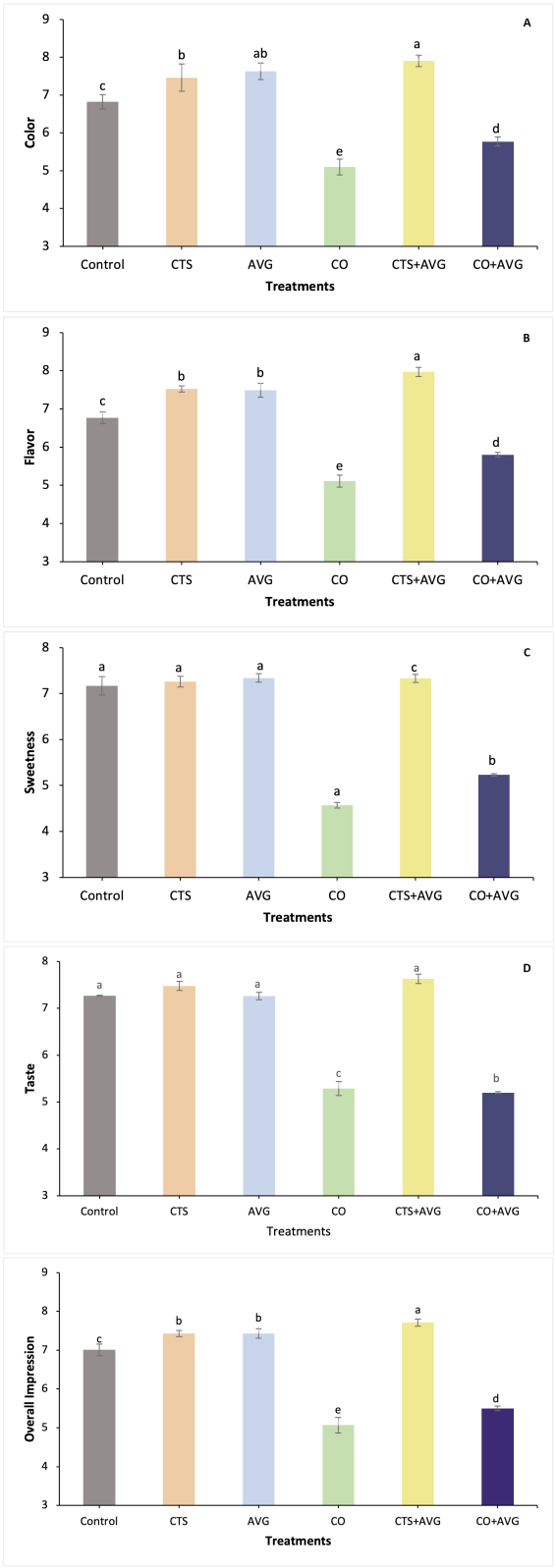

Fig. 6 - Mango color (A), flavor (B), sweetness (C), taste (D) and overall impression (E) at 8 days of storage at 27±2°C and 80-85% relative humidity due to coatings treatment. The vertical line shows the standard error of the means of three replicates. See Figure 1.

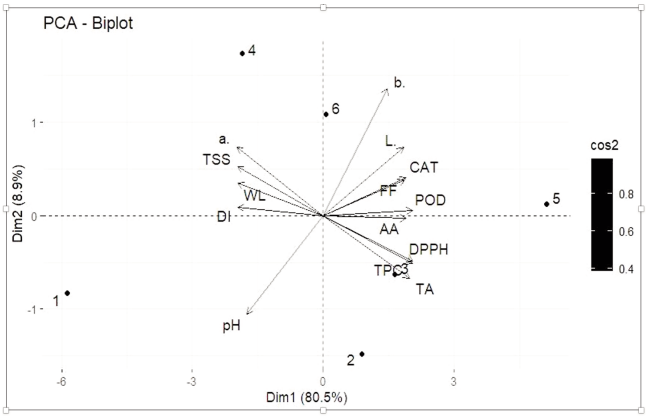

Fig. 7 - Principal component loading plot of physiochemical and antioxidant enzymes activities of mango fruit during storage.

4. Discussion and Conclusions

Mango ripening is associated with changes in color, texture, and flavor, resulting from modifications in the fruit's chemical composition. These include the conversion of starch to sugars, a reduction in acidity, and the generation of new volatile compounds (Taiti *et al.*, 2015). Fruit weight loss increases due to moisture evaporation and metabolic activity. Ncama *et al.* (2018) reported that water loss during postharvest storage leads to weight reduction, changes in texture and appearance, and shriveling. However, water loss can be reduced when fruit surfaces are covered with semipermeable edible coatings. Shah and Hashmi (2020) also discovered that CTS + AVG coatings reduce the weight of mango fruit.

CTS+AVG significantly maintained the firmness of mango fruits. According to Rukunuzzaman *et al.* (2025), mangoes treated with CTS + AVG decreased mango softening by reducing the breakdown of cell wall polymers, keeping the fruit firm. Our study also confirmed Rastegar and Atrash (2021) that CTS+AVG delayed mango fruit firmness.

Total soluble solids were significantly increased by moisture loss and the breakdown of carbohydrates into soluble sugars. However, edible coatings may limit respiration and exchange of gases in coated fruits by closing stomata, preventing an increased rate of TSS content (Chavan *et al.*, 2023). CTS+AVG-treated mangoes showed lower TSS during storage, as described by Yu *et al.* (2021).

The chitosan coating significantly decreased the frequency of disease-induced mango fruit deterioration while preserving fruit quality in agricultural commodities (Hasan *et al.,* 2020). Similar to this, AVG coating can prolong the shelf life of apricots by postponing microbial deterioration, whether it is applied alone or in conjunction with other treatments (Nourozi and Sayyari, 2020). In this study, the use of CTS either alone or in combination with AVG significantly decreased the incidence of mango fruit deterioration during storage.

Changes of color in climacteric fruits occur because of the alteration of chlorophyll to other pigments. Kaur *et al*. (2024) stated that CTS + AVG coatings slow down metabolic and pigment changes by regulating gas exchange in fruits while they are being stored. Due to the lower availability of oxygen and higher carbon dioxide in the internal microenvironment, it causes slower ripening (Paul *et al*., 2019).

The L* value decreased across all treatments, although it declined more slowly in treated fruits than in the controls. The lower a* values of the coated samples, which are more green than red throughout storage, may suggest a delay in mango fruit ripening. According to a previous study by Abdelshafy *et al.* (2023) and Begum *et al.* (2023), edible coatings can influence epidermal permeability, gas exchange, and oxidation. Seyed *et al.* (2021) found that mango fruits stored with *Aloe vera* gel and chitosan changed color less than control fruits.

Ascorbic acid, a powerful antioxidant, scavenges free radicals and reactive oxygen species during fruit ripening (Fenech *et al.,* 2019). The CTS + AVG coating in mango fruits might limit the permeability of oxygen and carbon dioxide on its surface and reduce the loss of ascorbic acid content. Our results were confirmed by Shah and Hashmi (2020), who reported higher levels of vitamin C in mango fruits treated with CTS+AVG.

Acidity presumably decreased due to widespread catabolisation of organic acids to sugars. A sudden reduction in TA indicated senescence in mango fruit (Shah and Hashmi, 2020). Seyed *et al.* (2021) found that *Aloe vera* gel with chitosan-treated mango fruits had the highest TA.

Fruit coated with CTS+AVG has the lowest pH. In line with our findings, Amin *et al.* (2021) discovered that CTS+ AVG reduces mango pH. According to Sogvar *et al.* (2016) who reported that strawberry's pH raised during storage in both control and *Aloe vera* gel-coated fruits; whereas control fruits had a higher pH value.

Retaining the fruit's nutritional quality (color, bitterness, astringency, acidity, and taste) during storage requires retaining phenolic chemicals, which decrease with ripening. Phenolic molecules, secondary plant metabolites, scavenge ROS to increase fruit antioxidants (Swallah *et al.,* 2020). An edible coating may reduce phenolic component oxidation in mangoes. Agreeing with Seyed *et al.* (2021), the current work determined that chitosan and Aloe vera gel coatings improve the phenolic retention of mangos during storage.

The treated fruits' antioxidant capacity, which is linked to total phenolic content, may boost the activity of DPPH scavenging. *Aloe vera* coatings-maintained antioxidant (DPPH) activity throughout storage (Khaliq *et al.,* 2019 a or b). Mango fruit treated with *Aloe vera* and chitosan during postharvest storage period showed improved DPPH scavenging activity, as reported by Begum *et al.* (2023).

The fruit's antioxidant system's genes for PPO, POD, and CAT increase throughout ripening, defending against ROS accumulation (Loay and El-Ezz*,* 2021, and Yu *et al.,* 2021). Most horticultural crops discolor when PPO oxidizes phenolic compounds, altering them to quinones. The coating reduced PPO activity, which may have activated defence-related enzymes to prevent browning of mango fruit and extend storage (Adiletta *et al.,* 2019). PPO and enzymatic browning may have been inhibited by fruit surface chitosan coating CO_2, O_2, and ethylene (Romanazzi *et al.,* 2018). These findings are consistent with previous findings in strawberry (Petriccione *et al.,* 2015), where chitosan coatings significantly reduced PPO activity and fruit discoloration. CAT activity reduces O_2 and H_2O_2. Shah and Hashmi (2020) discovered that mango fruit chitosan coating boosts CAT activity. POD, a fruit-specific oxyradical detoxifying enzyme, may reduce oxidative damage and a complex covering of chitosan and cinnamon oil increased POD activity in jujube, promoting storage disease resistance (Xing *et al.,* 2020).

According to these findings, using *Aloe vera* gel along with chitosan was the most effective way to mitigate decay symptoms and lessen the physicochemical alterations in mango fruit. Mangoes treated with AVG coatings, either separately or in combination, exhibited improved sensory quality,

according to Khaliq *et al*. (2019 a, b).

This study presented that chitosan and *Aloe vera* coatings may increase mango storage life by minimizing weight loss, reducing postharvest deterioration and retaining ascorbic acid, titratable acidity, firmness, and peel color throughout storage. Mango fruit with coatings had higher total phenol and antioxidant levels than the control and ripened more slowly. These coatings may boost CAT and POD antioxidant enzymes and lower PPO during storage. Given human health concerns, edible coatings like chitosan-aloe vera may improve mango storage quality. Natural edible coatings of *Aloe vera,* chitosan, and coconut oil may affect antioxidant enzyme activity. In order to make mango fruit appealing to consumers and enhance its storability with edible coatings, further research is needed.

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Reference

AAQIL M., PENG C., KAMAL A., NAWAZ T., GONG J., 2024 - *Recent approaches to the formulation, uses, and impact of edible coatings on fresh peach fruit*. - Foods, 13(2): 267.

ABDELSHAFY A.M., LUO Z., BELWAL T., BAN Z., LI L., 2023 - *A comprehensive review on preservation of shiitake mushroom* (Lentinus Edodes): *Techniques, research advances and influence on quality traits* [*review*]. - Food Rev. Int., 39(5): 2742-2775.

ADILETTA G.L., ZAMPELLA C., COLETTA M., PETRICCIONE, 2019 - *Chitosan coating to preserve the qualitative traits and improve antioxidant system in fresh figs (*Ficus carica L.*).* - Agriculture, 9(4): 84.

AHMED Z.F.R., 2024 - *Aloe vera/Chitosan-based edible film with enhanced antioxidant, antimicrobial, thermal, and barrier properties for sustainable food preservation*. - Polymers, 16(2): 242.

ALOM M.M., HOSSAIN M.M., SARKAR M.A.R., 2019 - *Post-harvest loss assessment of mango in selected areas of Bangladesh.* - J. Hort., 6(1): 1-6.

AMIN U., KHAN M.K.I., KHAN M.U., AKRAM M.E., PATEIRO M., LORENZO J.M., MAAN A.A., 2021 - *Improvement of the performance of Chitosan-Aloe vera coatings by adding beeswax on postharvest quality of Mango fruit*. - Foods, 10(10): 2240.

ATHOO T.O., YEGON D., OWINO W., KNOCHE M., 2024 - *Bagging prevents russeting and decreases postharvest water loss of mango fruit cv. 'Apple'.* - Postharvest Biol. Technol., 211: 112804.

AYYUB S., KHAN A.S., ANWAR R., ALI S., HASAN M.U., 2024 - *Aloe vera gel coating extends marketability and maintains quality by reducing rachis browning and preserving bioactive compounds of commercial table grape cultivars.* - Appl. Fruit Sci., 66: 1843-1853.

BANGLADESH BUREAU OF STATISTICS., 2024 - *Year book of agricultural statistics - 2023*. - BBS, Statistics and Informatics Division, Ministry of Planning, Government of the People's Republic of Bangladesh, pp. 212-213.

BEGUM L., AHMED M., RAHMAN M.A., RAHMAN M.H., AFRIN M.S., AKTER N., ISLAM M.T., 2023 - *Changes of postharvest nutritional quality and antioxidant enzymes in 'Haribhanga' mango by aloe vera gel with chitosan and coconut oil coating during ambient storage*. - J. Hort. Res., 31(2): 79-96.

BISEN S.K., PATEL N., 2012 - *Effect of skin coatings on prolonging shelf life of kagzi lime fruits (*Citrus aurantifolia Swingle). - J. Food Sci. Technol., 49: 753-759.

CHAVAN P., LATA K., KAUR T., JAMBRAK A.R., SHARMA S., ROY S., SINHMAR A., THORY R., SINGH G.P., AAYUSH K., ROUT A., 2023 - *Recent advances in the preservation of postharvest fruits using edible films and coatings: A comprehensive review*. - Food Chem., 135916.

ESHETU A., IBRAHIM A.M., FORSIDO S.F., KUYU C.G., 2019 - *Effect of beeswax and chitosan treatments on quality and shelf life of selected mango (*Mangifera indica *L.)* cultivars. - Heliyon, 5(1): 01116.

FAROOQ A., NIAZ B., SAEED F., AFZAAL M., ARMGHAN KHALID M., RAZA M.A., AL JBAWI E., 2023 - *Exploring the potential of aloe vera gel-based coating for shelf life extension and quality preservation of tomato*. - Int. J. Food Prop., 26(2): 2909-2923.

FENECH M., AMAYA I., VALPUESTA V., BOTELLA M.A., 2019 - *Vitamin C content in fruits: Biosynthesis and regulation.* - Front. Plant Sci., 9: 2006.

GIOVANNONI J., NGUYEN C., AMPOFO B., ZHONG S., FEI Z., 2017 - *The epigenome and transcriptional dynamics of fruit ripening.* - Annu. Rev. Plant Biol., 68: 61-84.

HASAN M.U., MALIK A.U., KHAN A.S., ANWAR R., MUHAMMAD L., AMJAD A., SHAH M.S., AMIN M., 2020 - *Impact of postharvest hot water treatment on two commercial mango cultivars of Pakistan under simulated air freight conditions for China.* - Pak. J. Agric. Sci., 57: 1381-1391.

HOSSAIN M.A., 2016 - *Changes in ripening associated biochemical and enzymatic characteristics of late season mango (*Mangifera indica L.*) of Bangladesh*. - J.

Sci. Res., 8(3): 387-398.

KANWAR P., RANA P., VATSALYA SWAROOP M., SANDEEP KUMAR N., 2024 - *Nano-technology enhanced edible coating application on climacteric and non-climacteric fruits: A review*. - Int. J. Adv. Biochem. Res., 8(6): 58-68.

KAUR N., SOMASUNDRAM C., RAZALI Z., AHMED Z.F.R., 2024 - *Sustainable Aloe vera/chitosan-based edible coatings reduce postharvest loss of stored fresh figs* (Ficus carica *L.*). - Front. Sustain. Food Syst., 8: 1459600.

KEFAS E.B., ALIYU B., TASHIWA Y.I., 2024 - *Postharvest losses, causes and mitigation in tomato transportation: a systematic review*. - J. Hort. Postharvest Res., 7(3): 223236.

KHALIQ G., MOHAMED M.T.M., GHAZALI H.M., DING P., ALI A., 2019 a - *Influence of gum arabic coating enriched with calcium chloride on physiological, biochemical and quality responses of mango (*Mangifera indica L.*) fruit stored under low temperature stress.* - Post. Biol. Technol., 111: 362-369.

KHALIQ G., RAMZAN M., BALOCH A.H., 2019 b - *Effect of Aloe vera gel coating enriched with* Fagonia indica *plant extract on physicochemical and antioxidant activity of sapodilla fruit during postharvest storag*e. - Food Chem., 286: 346-353.

KHANUM Z., TIZNADO-HERNÃ¡NDE M.E., ALI A., MUSHARRAF S.G., SHAKEEL M., KHAN I.A., 2020 - *Adaptation mechanism of mango fruit (*Mangifera indica *L. cv. Chaunsa White) to heat suggest modulation in several metabolic pathways.* - RSC Adv., 10(58): 35531-35544.

KUMAR N., PETKOSKA A.T., AL-HILIFI S.A., FAWOLE O.A., 2021 - *Effect of chitosan-pullulan composite edible coating functionalized with pomegranate peel extract on the shelf life of mango (*Mangifera indica*).* - Coatings, 11: 764-774.

KUMAR N., PRATIBHA, ASHUTOSH U., ANKA T.P., MAŁGORZATA G., KIELISZEK M., 2023 - *Extending the shelf life of mango (*Mangifera indica *L.) fruits by using edible coating based on xanthan gum and pomegranate peel extract.* - J. Food Meas. Charact., 17: 1300-1308.

LAWSON T., LYCETT G.W., ALI A., CHIN C.F., 2019 - *Characterization of Southeast Asia mangoes (*Mangifera indica L.*) according to their physicochemical attributes*. - Sci. Hort., 243: 189-196.

LIBERMAN S., ENIG M.G., PREUSS H.G., 2006 - *A review on monolaurin and lauric acid: Natural virucidal and bactericidal agents.* - Alter Complem. Therap., 12(6): 310-314.

LIU W., ZHANG M., BHANDARI B., 2020 - *A shelf life extension strategy for fruits and vegetables.* - Cri. Rev. Food Sci. Nutr., 60(10): 1706-1721.

LOAY A.A., EL-EZZ S.F.A., 2021 - *Performance of 'Flame seedless' grapevines grown on different rootstocks in response to soil salinity stress.* - Sci. Hort., 275: 109704.

MCHENRY E.W., GRAHAM M., 1935 - *Observations on the estimation of ascorbic acid by titration.* - Biochem. J., 29(9): 2013-2019.

NCAMA K., MAGWAZA L., MDITSHWA A., TESFAY S.Z., 2018 - *Plant-based edible coatings for managing postharvest quality of fresh horticultural produce: A review.* - Food Pack, Shelf Life, 16: 157-167.

NOUROZI F., SAYYARI M., 2020 - *Enrichment of* Aloe vera *gel with basil seed mucilage preserve bioactive compounds and postharvest quality of apricot fruits*. - Sci. Hort., 262: 109041.

PARVIN N., RAHMAN A., ROY J., RASHID M.H., PAUL N.C., MAHAMUD M.A., CHANDRA N., ASIF M., IMRAN S., SAKIL M.A., UDDIN F.M.J., MOLLA M.E., KHAN M.A., KABIR M.H., KADER M.A., 2023 - *Chitosan coating improves postharvest shelf-life of Mango (*Mangifera indica *L.*). - Horticulturae, 9(1): 64.

PAUL V., PANDEY R., MALIK S.K., 2019 - *Varietal variations in rate of ripening and respiration of mango* (Mangifera indica *L.) fruits: Anatomical substantiation*. - Plant Physiol. Rep., 24: 340-350.

PEREZ-VAZQUEZ A., BARCIELA P., CARPENA M., PRIETO M.A., 2023 - *Edible coatings as a natural packaging system to improve fruit and vegetable shelf life and quality*. - Foods, 12(19): 3570.

PETRICCIONE M., MASTROBUONI F., PASQUARIELLO M.S., ZAMPELLA L., NOBIS E., CAPRIOLO G., SCORTICHINI M., 2015 - *Effect of chitosan coating on the postharvest quality and antioxidant enzyme system response of strawberry fruit during cold storage.* - Foods, 4(4): 501-523.

RASTEGAR S., ATRASH S., 2021 - *Effect of alginate coating incorporated with Spirulina,* Aloe vera *and guar gum on physicochemical, respiration rate and color changes of mango fruits during cold storage.* - J. Food Meas. Charac., 15: 265-275.

ROMANAZZI G., FELIZIANI E., SIVAKUMAR D., 2018 - *Chitosan, a biopolymer with triple action on postharvest decay of fruit and vegetables: eliciting, antimicrobial and film-forming properties.* - Front. Micro., 9: 2745.

RUKUNUZZAMAN M., RAHMAN M.A., KHATUN M.A., BEGUM M.L., AKTER N., ISLAM M.T., 2025 - *Physio-biochemical and antioxidative enzymatic changes in ambient stored 'Misribhog' mango in response to chitosan and* Aloe vera *gel coatings*. - J. Hort. Postharvest Res., 8(3): 397-412.

SALEHIN S.S.A., KHATUN M.A., RUKUNUZZAMAN M., ISLAM M.M., 2025 - *Impact of tree bagging on physico-chemical qualities and economics of mango cv. BARI Aam 7*. - Future J. Hort., 1: 11-24.

SEYED R.H., RASTEGAR S., FARAMARZI S., 2021 - *Impact of edible coating derived from a combination of* Aloe vera *gel, chitosan and calcium chloride on maintain the*

quality of mango fruit at ambient temperature. - J. Food Meas. Charac., 15: 2932-2942.

SHAH S., HASHMI M.S., 2020 - *Chitosan-aloe vera gel coating delays postharvest decay of mango fruit.* - Hort. Environ. Biotechnol., 61(2): 279-289.

SILVA G.M.C., SILVA W.B., MEDEIROS D.B., SALVADOR A.R., CORDEIRO M.H.M., SILVA N.M., SANTANA D.B., MIZOBUTSI G.P., 2017 - *The chitosan affects severely the carbon metabolism in mango (*Mangifera indica *L. cv. Palmer) fruit during storage.* - Food Chem., 237: 372-378.

SINGLETON V.L., ROSSI J.A., 1965 - *Calorimetry of total phenolic with phosphomolybdic-phosphotungstic acid reagents.* - Am. J. Enol. Vitic., 16: 144-158.

SOGVAR O.B., SABA M.K., EMAMIFAR A., 2016 - Aloe vera *and ascorbic acid coatings maintain postharvest quality and reduce microbial load of strawberry fruit.* - Post. Biol. Technol., 114: 29-35.

SONG H.Y., JO W.S., SONG N.B., MIN S.C., SONG K.B., 2013 - *Quality change of apple slices coated with* Aloe vera *gel during storage.* - J. Food Sci., 78(6): 817-822.

SWALLAH M.S., SUN H., AFFOH R., FU H., YU H., 2020 - *Antioxidant potential overviews of secondary metabolites (Polyphenols) in fruits.* - Inter. J. Food Sci., 9081686.

TAITI C., COSTA C., MENESATTI P., CAPARROTTA S., BAZIHIZINA N., AZZARELLO E., PETRUCCI W.A., MASI E., GIORDANI E., 2015 - *Use of volatile organic compounds and physicochemical parameters for monitoring the post-harvest ripening of imported tropical fruits*. - Eur. Food Res. Technol., 241: 91-102.

TOBING O.L., MULYANINGSIH Y., AZIZ F.A., 2023 - *The effect of temperature and concentration edible coating of aloe vera gel (*Aloe Vera *L.) to the shelf life and sensory of tomatoes* (Solanum lycopersicum *L. cv Momotaro)*. - Indones. J. Appl. Res., 4(3): 264-276.

WANG Y., YAN Z., TANG W., ZHANG Q., LU B., LI Q., ZHANG G., 2021 - *Impact of chitosan, sucrose, glucose, and fructose on the postharvest decay, quality, enzyme activity, and defense-related gene expression of strawberries*. - Horticulturae, 7(12): 518.

XING Y., LIN H., CAO D., XU Q., HAN W., WANG R., CHE Z., LI X., 2020 - *Effect of chitosan coating with cinnamon oil on the quality and physiological attributes of China jujube fruits.* - Biomed Res., 10.

YU K., XU J., ZHOU L., ZOU L., LIU W., 2021 - *Effect of chitosan coatings with cinnamon essential oil on postharvest quality of mangoes.* - Foods, 10: 3003.

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Effect of partial-extreme root restriction and nutrient solution concentration on the performance of hydroponically grown tomato

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no conflict of interests.

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Abstract: Tomato is a valuable agricultural commodity widely used across Africa with the potential to contribute to food and nutritional security. However, its yield, quality, and profitability are hindered by several challenges. The study evaluated the impact of partial-extreme root restriction and no root restriction on the performance of Jaguar tomato cultivar in two different nutrient solution concentrations: standard (2.4 dS m^{-1}) and half concentration (1.2 dS m^{-1}). The cultivation spanned three months using a recirculating hydroponic system arranged in a 2 x 2 factorial in a randomized complete block design with three replications. Data were collected on physio-morphological responses, yield, fruit quality, and water uptake. Plant growth, leaf gas exchange, yield, fruit quality, total water uptake, and root growth were significantly influenced by the nutrient solution concentration with root restriction. Particularly, plant growth, photosynthesis, total water use (52-62%), and yield were significantly reduced but fruit quality was improved by 25% compared to previous findings in Ghana. Conversely, the standard nutrient solution concentration without root restriction recorded the highest yield of 32.4 kg $m^{-2}y^{-1}$. These findings can serve as a manipulative hydroponic tool to increase tomato productivity and resource-use efficiency, especially in regions with limited water availability.

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1. Introduction

Tomato (*Solanum lycopersicum* L.) is a globally important crop, which is widely grown using various cultivation systems in different countries. The crop is cultivated for its edible fruits, which are used as a vegetable, for medicinal purposes, among others (Quinet *et al.*, 2019). The current target for growing tomatoes among industrialized countries is to meet medicinal and nutritional needs.

One of the most efficient but cost-effective cultivation system as adopted in some industrialized countries including Japan is the 'low node-order pinching at high-density planting' (LN&HD) (Watanabe, 2006; Takahashi *et al.,* 2012; Kinoshita *et al.*, 2014). This system adopts a low substrate volume at high-density cultivation plus pinching (topping) between the first and the fourth truss. With the low substrate volume, the plants are subjected to root restriction.

Vegetable production using root restriction is becoming popular especially where there is a need to adapt to adverse growing conditions, such as space constraints, limited water availability, and extreme temperatures (Shi *et al.,* 2008; Yamaura *et al.*, 2020). Root restriction is a cultivation strategy that involves deliberately confining plant roots within smaller container sizes with low substrate volume, thereby limiting their natural expansion. This technique influences root architecture and physiological processes, subsequently influencing overall plant growth, development, and resource allocation.

Root restriction affects the physiology of grown plants (Peterson and Krizek, 1992; Salisu *et al.*, 2018). The findings of Shi *et al.* (2008), Mugnai and Al-Debei (2011), and Campany *et al.* (2017) revealed that root restriction impairs the photosynthetic process due to a reduction in stomatal conductance. A reduction in photosynthesis in root-restricted plants might also be attributed to the physiological downregulation of photosynthetic activities due to high carbohydrate accumulation in the shoots of the plants (Pezeshki and Santo, 1998). However, other authors have indicated no significant differences in photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, and transpiration between root-restricted and control plants (Kharkina *et al.,* 1999; Zakaria *et al.*, 2020). An earlier study by Hieke *et al.* (2002) showed that there was no inhibition of photosynthesis once there was new plant shoot regrowth.

Root restriction has also been reported to reduce plant growth (Ismail and Noor, 1966; Mugnai and Al-Debei, 2011; Ayarna *et al.*, 2021). Bihmidine *et al.* (2013) revealed that photosynthates were rather translocated into the stems of root-restricted pepper plants when the reproductive sinks were limited, leading to a yield reduction of 23%. Root restriction has also been reported to reduce fruit yield in tomato (Saito *et al.*, 2008).

Root restriction reduces water uptake (Saito *et al.*, 2008), leading to a subsequent reduction in transpiration (Bar-Tal *et al.*, 1994). Using root restriction can increase the sugar content of tomato (Li *et al.,* 2022). Root restriction has been reported to increase total sugar content due to reduced water uptake (Zakaria *et al.*, 2020). However, Saito *et al.* (2008) reported that root restriction did not affect the sugar content of tomato but reduced its water uptake.

Conventional root restriction confines the root system within the grow pot throughout the plant's growth cycle, limiting any further root expansion. In contrast, partial-extreme root restriction (characterized by a very low substrate volume, such as 0.25 L) utilizes a small (such as 0.25 L capacity) grow pot with an open base, initially imposing spatial confinement before allowing root extension beyond the restricted volume. After an initial phase of extreme root restriction in the small pot, this approach is expected to promote continuous root proliferation and growth, enhancing resource use efficiency and overall plant performance. Partial root restriction in tomato has been reported by Ayarna *et al.* (2021), who revealed that partially root-restricted plants produced more fine young roots, which were more efficient in the uptake of water and nutrients, subsequently increasing tomato yield.

In hydroponic cultivation systems, nutrients are supplied as a nutrient solution for plant uptake and utilization. Hoagland (1929) and Schwarz *et al.* (2002) emphasized that nutrient solution formulation should be synchronized with the cultivation system as well as the associated crop. Many nutrient solution formulations with appropriate concentrations have been developed to provide adequate nutrients for plant use (Jones, 1982; Sakamoto and Suzuki, 2020). However, improper or disproportionate formulation of nutrient solutions can adversely affect crop yield at any growth stage.

Many growers have attempted to use high amounts of fertilizer to achieve higher yields, but this practice has resulted in poor performance, with reduced yield and fruit quality (Zhang *et al.,* 2017). Nutrient solutions in hydroponic systems have low buffer capacity (Agius *et al.,* 2022), which can negatively impact plant growth. To prevent this, Lu *et al.* (2022) emphasized the need for judicious nutrient solution management. When the nutrient solution concentration (NSC) is relatively low (1.5 dS m^{-1}) in unrestricted root conditions, nutrient availability is inadequate, reducing fruit quality (Cliff *et al.,* 2012; Beesigamukama *et al.,* 2020) and causing low nutrient stress, which hampers plant growth, photosynthesis, and stomatal conductance (Beesigamukama *et al.,* 2020; Lu *et al.,* 2022). Conversely, while a high NSC (4.5 dS m^{-1}) leads to excess nutrient availability, causing stress and weakening plant growth (Anjum *et al.,* 2011; Rosadi *et al.,* 2014), it can also improve fruit quality by enhancing photosynthetic rate, transpiration rate, and stomatal conductance (Wang, 2017; Yang *et al.,* 2017). Meanwhile, tomato yield is not adversely affected at moderate NSC levels ranging from 1.5 to 2.4 dS m^{-1} (Veit-Köhler *et al.*, 1999).

Depending on the grower's objectives, nutrient solutions are maintained at 1.2 dS m^{-1} or higher. The Enshi nutrient solution recipe has been formulated for the cultivation of all vegetable crops at a standard concentration of 2.4 dS m^{-1}. A half-concentration of the Enshi recipe (1.2 dS m^{-1}) has been employed for the cultivation of root-restricted tomatoes in Japan with success. In general, extreme root restriction and higher nutrient solution concentration (NSC) are strategies specifically aimed at enhancing tomato fruit quality, albeit at the expense of yield.

Ghana consistently records low tomato yields and a low sugar content of 3.5-5.6% Brix (Nkansah *et al.,* 2003), making it crucial for implementing effective strategies to improve tomato yield and sugar content in the country. There are relatively few studies or reports on the effects of nutrient solution concentration on tomatoes grown under extreme but partially restricted-root conditions. The objective of this study was to evaluate the effect of partial-extreme root restriction and nutrient solution concentration on the performance of tomato, with the expectation that this approach would enhance the yield and fruit quality of tomato.

2. Materials and Methods

Experimental materials and procedures

The study was conducted between February and April 2024 at the University of Ghana's Forest and Horticultural Crops Research Centre at Kade, Ghana (43VX+GGG), in a greenhouse. Jaguar, a tropical tomato cultivar (Technisem Savanna Seed Company Limited-France) was used for the study. The greenhouse daily ambient temperature and humidity were recorded using thermorecorder-TR-72wb (T&D Holdings, Inc., Tokyo, Japan).

Two factors, namely root restriction of tomato and varied nutrient solutions of standard and halve concentrations, were evaluated. The tomato plants were subjected to partial-extreme root restriction (Fig. 1) as the main treatment, which was compared to the control treatment, with no root restriction. The adoption of extreme root restriction with a 0.20 L substrate volume in this study followed the method of Zhang *et al.* (2015), who subjected tomato plants to extreme root restriction using a 0.25 L extreme-low substrate volume in D-trays, geared toward improving fruit quality. However, the pattern of partial-extreme root restriction was after Ayarna *et al.* (2021). The cultivation of plants was carried out in

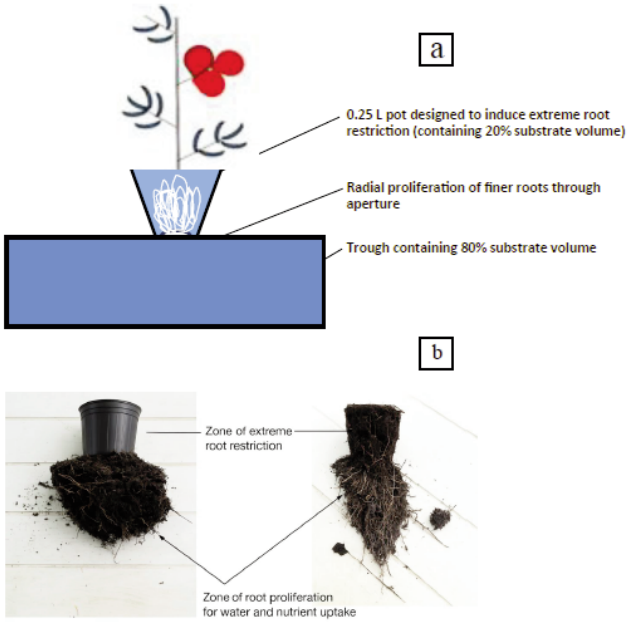

Fig. 1 - (a) Schematic representation of partial-extreme root restriction in hydroponic tomato cultivation. (b) Zones of extreme but partial root restriction and root proliferation.

Table 1 - Characterization of the adopted nutrient recipe in terms of macro- and micronutrients concentration

Nutrients	Standard nutrient solution concentration (2.4 dS m^{-1})
Macro-nutrients	mM
NH_4-N	1.3
NO_3-N	16
PO_4-P	1.3
K	8.0
Ca	4.0
Mg	2.0
SO_4-S	2.0
Micro-nutrients	ppm
Fe	3.0
Mn	0.5
Cu	0.02
Zn	0.05
Mo	0.01
B	0.5

a recirculating hydroponic system, using cocopeat as the substrate. A substrate volume of 1.0 L was used per plant in all treatments. In the root restriction treatment, the substrate was segmented into 0.20 L (pot) plus 0.80 L (trough). In the 0.20 L pot, the plants were subjected to an initial extreme root restriction; after which finer young roots were expected to proliferate into the 0.80 L trough for further water and nutrient absorption. The control treatment (unrestricted plant) was grown directly in the trough, containing 1.0 L of cocopeat.

A nutrient solution with a concentration of 2.4 dS m^{-1} was prepared following the Enshi recipe (Hori, 1966) as shown in Table 1. This concentration was then halved through dilution to 1.2 dS m^{-1}. The nutrient solutions (1.2 and 2.4 dS m^{-1}) were maintained within a pH of 5.5-6.5 and were delivered to the root zone of each plant using a drip system, in accordance with the adopted treatments.

Tomato seeds from the evaluated cultivar were sown in cell trays filled with cocopeat as the sowing medium. The seeds were then watered; and placed in a dark chamber under greenhouse conditions until they germinated. The germinated seedlings were supplied daily with a nutrient solution concentration of 0.5 dS m^{-1} using the Nutrient Film Technique until the third week, when they were ready for transplanting.

Twenty seedlings were transplanted into each treatment on the third week after seed germination at a spacing of 0.2 by 1.2 m. The set-up used an automated irrigation system to supply the tomato plants with nutrient solutions (1.2 or 2.4 dS m^{-1}) for 24 minutes daily, following treatment conditions from transplanting to harvest. After anthesis, 1.0 mL L^{-1} 4-Chlorophenoxyacetic acid was sprayed on the flowers every other day to enhance fruit set. Plants in each treatment were pinched at the last three leaves above the third truss to terminate further growth. Fifteen plants were tagged for data collection in each treatment.

Data collection and experimental design

Data were collected on the following parameters: morphological and physiological responses, yield, and water use efficiency. Morphological responses which were collected at 74 days after transplanting included: plant height, girth (measured below the third truss), and number of leaves per plant.

Physiological parameters were measured between 12:30 p.m. and 1:30 p.m. on the second and sixth weeks after transplanting. These included photosynthetic rate (Pr), transpiration (Tr), stomatal conductance (Gs), and the intercellular CO_2 concentration (Ci) using the LI-6400 Portable Photosynthesis System (LI-COR, Lincoln, Nebraska, USA). The leaves immediately below the first and third trusses were measured for photosynthetic parameters in the second and sixth weeks after transplanting, respectively.

All mature ripe fruits were harvested and counted to determine total and average fruit weights. The average fruit weight was determined as the ratio of the total weight to the total number of harvested fruits. Total sugar content (brix %) of the blended tomato juice was determined using the Atago™ pocket refractometer.

Plant water uptake was measured as the difference between total volume of nutrient solution supplied and the volume of nutrient solution left in the reservoir 14 h after daily irrigation. The total water used was measured as the total amount of nutrient solution absorbed per plant in the cultivation period (74 d). Water use efficiency was determined as the fruit yield per total water used per plant. After harvest, the fresh roots were cautiously extracted, wiped with a soft face towel and weighed. Additionally, portions of the root, which proliferated beyond the zone of extreme root restriction were

collected and weight as the portion involved with water and nutrient uptake.

The experiment was laid out in a 2 x 2 factorial in a randomized complete block designed with three replications. Data collected were analyzed with the SISVAR version 5.6 (Ferreira, 2008) while the Tukey's honestly significant difference (HSD 0.05) was used to separate the means at $p<0.05$. Grouped graphs were constructed using GraphPad Prism version 8.0 for Windows, GraphPad Software, San Diego, California USA.

3. Results

Greenhouse ambient temperature and humidity

The greenhouse ambient humidity and temperature recorded during the study are shown in figures 2 a and b.

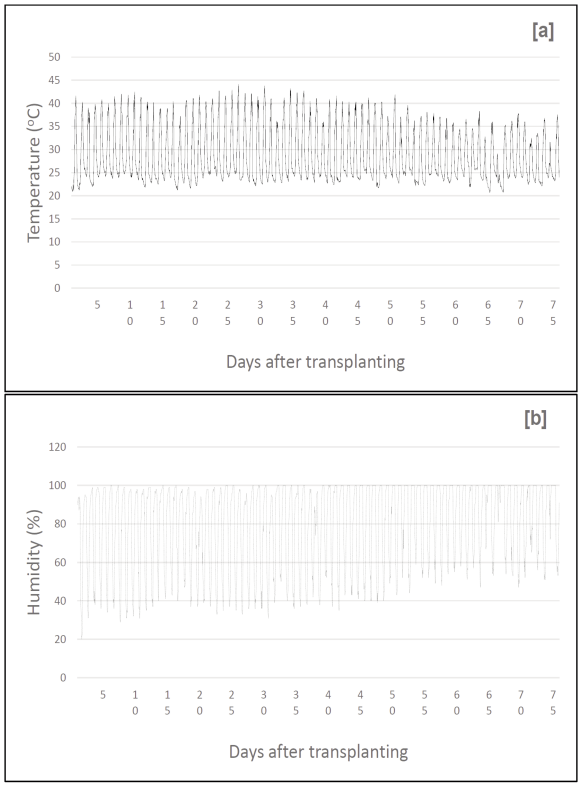

Fig. 2 - Greenhouse ambient humidity (a) and temperature (b) during the experiment.

Plant morphological and physiological responses

Partial-extreme root restriction (R) and nutrient solution concentration (NSC) significantly $p<0.05$ affected the growth of tomato (Table 2). Plant height, girth, and leaf number decreased with root restriction compared to the unrestricted roots. Plant height and leaf number were significantly higher with the standard NSC compared to the half concentration.

According to figure 3, the photosynthetic rate (Pr) of the Jaguar tomato was not affected by root restriction at both nutrient concentrations, even though the stomatal conductance (Gs), transpiration (Tr) and intercellular CO_2 concentration (Ci) decreased significantly ($p<0.05$) at the vegetative phase (second week after transplanting) in the restricted treatment. Compared to the control, root restriction reduced Pr, Gs, Tr and Ci significantly at the reproductive phase (sixth week after transplanting) as shown in figure 4.

The NSC had a significant impact on the physiological parameters of tomato during both the vegetative and reproductive growth stages (Figs. 3 and 4). The interaction effect of root restriction and NSC significantly affected the physiological response of tomato at the two stages of growth. The standard nutrient solution concentration with root restriction markedly reduced the Pr, Ci, and Gs compared to the unrestricted roots during the reproductive phase of growth (Fig. 3). In contrast, during the vegetative stage, root restriction under half-strength NSC increased Pr, while Ci, Gs, and Tr decreased relative to the no-restriction treatment (Fig. 3).

Table 2 - Morphological response of tomato to partial-extreme root restriction and nutrient solution concentration at 74 days after transplanting

Nutrient solution concentration	Root restriction	Plant height (cm)	Stem girth (mm)	Leaf number
Standard	Restricted	116.3 ± 0.88 aA	8.7 ± 0.07 Ab	11.7 ± 2.19 bB
	Unrestricted	117.3 ± 5.04 aB	11.7 ± 0.03 aA	20.0 ± 1.15 aA
Half concentration	Restricted	102.7 ± 2.30 bB	10.3 ± 0.07 aA	14.3 ± 0.33 bA
	Unrestricted	161.0 ± 6.81 aA	10.0 ± 0.06 aA	17.0 ± 0.41 aB
p-values		<0.01	0.045	0.048

Small letters compare means within root restriction, while capital letters compare means within nutrient solution concentration (NSC). Values in the same column or row followed by the same letters indicate no significant difference according to Tukey HSD ($p<0.05$).

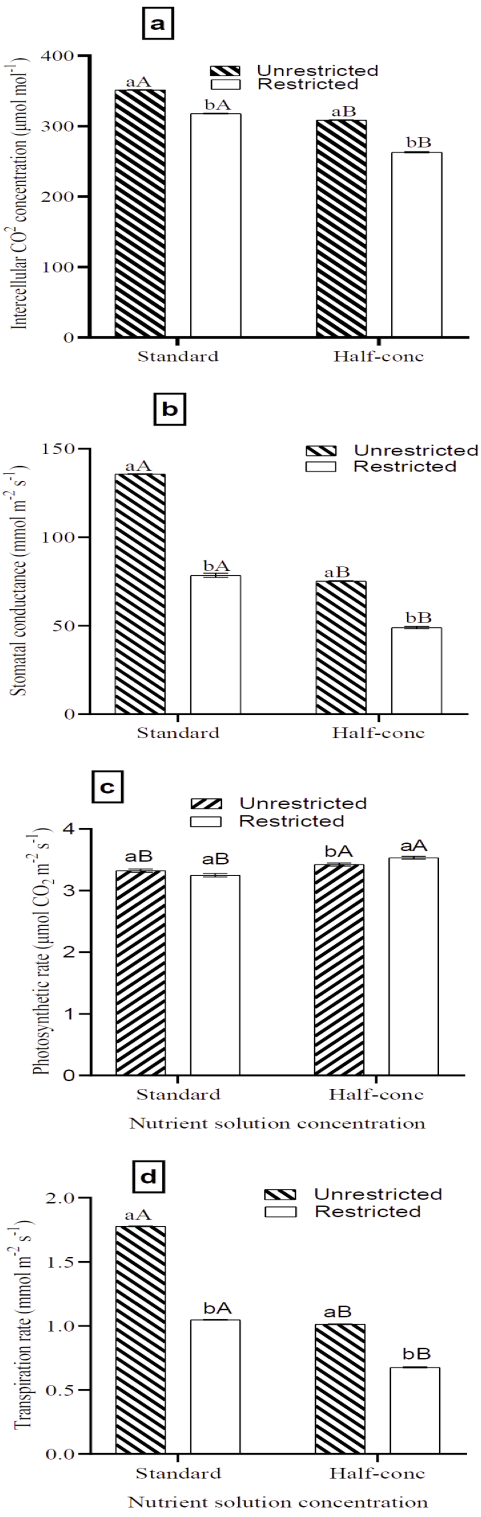

Fig. 3 - Physiological response of tomato to NSC and root restriction 2 weeks after transplanting. Lowercase letters compare means within NSC, while uppercase letters compare means among NSC And levels of R.

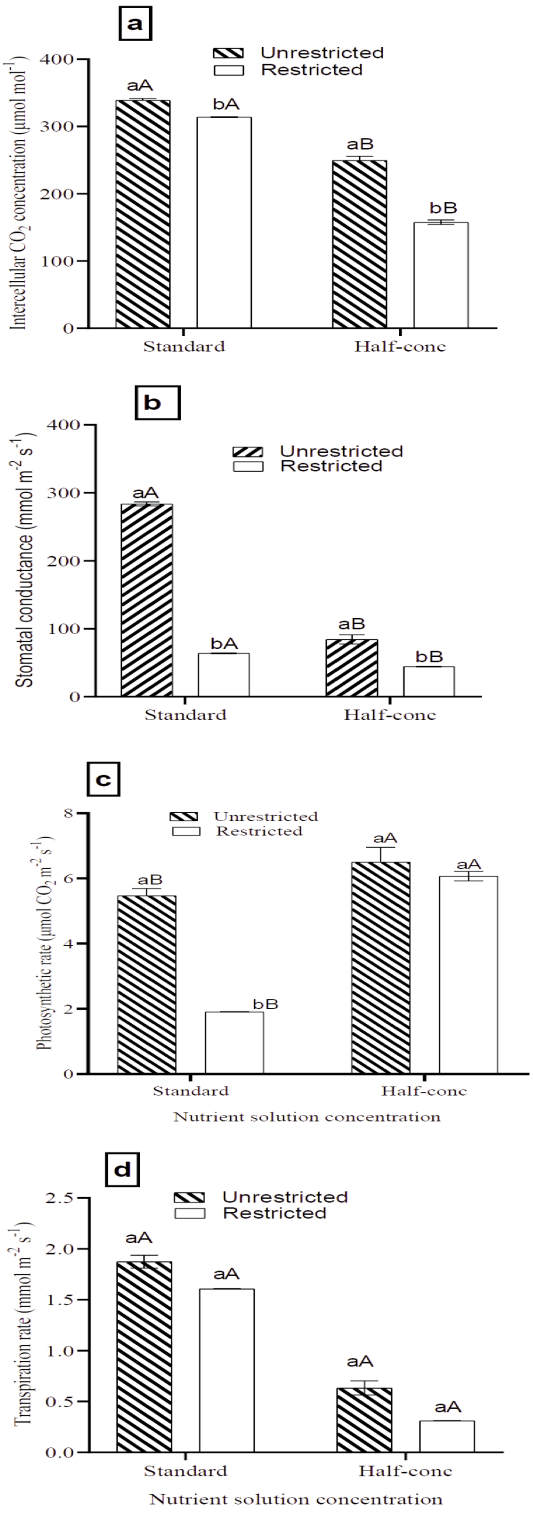

Fig. 4 - Physiological response of tomato to NSC and root restriction 6 weeks after transplanting. Lowercase letters compare means within NSC, while uppercase letters compare means among NSC and levels of R.

Yield and sugar content

Root restriction and the NSC did not significantly ($p<0.05$) affect the number of fruits produced per plant as shown in Table 3. The yield, average fruit weight, and sugar content (brix%) were markedly affected by root restriction and the NSC. Partial

Table 3 - Influence of root restriction and nutrient solution concentration on tomato yield, yield components, and sugar content

Parameter	Nutrient solution concentration	Restricted (Mean ± SE)	Unrestricted (Mean ± SE)	p-value
Fruit number per plant	Standard	11.3 ± 0.5 aA	13.0 ± 0.6 aA	0.1963
	Half-conc	13.3 ± 0.9 aA	12.9 ± 0.129 aA	
Yield (kg m^{-2})	Standard	3.88 ± 0.3 bB	8.08 ± 0.22 aA	<0.01
	Half-conc	7.32 ± 0.19 aA	7.08 ± 0.13 aB	
Average fruit weight (g)	Standard	76.2 ± 0.30 bB	149.6 ± 0.22 aA	0.0001
	Half-conc	140.6 ± 0.19 aA	130.9 ± 0.12 bB	
Brix (%)	Standard	6.97 ± 0.09 aA	4.57 ± 0.03 bB	<0.001
	Half-conc	4.43 ± 0.2 aB	4.2 ± 0.06 aB	

Small letters compare means within root restriction, while capital letters compare means within NSC and levels of root restriction. Values in the same column or row followed by the same letters indicate no significant difference according to Tukey HSD ($p<0.05$).

extreme root restriction with the standard concentration of nutrient solution significantly reduced the yield and the average fruit weight of the cultivar compared to the other treatments. Unrestricted roots grown in the standard NSC had the highest yield of 8.1 kg m^{-2} with a low sugar content of 4.6%. Conversely, extreme partially restricted roots grown in the same NSC recorded the highest sugar content of 6.9% but with the lowest yield of 3.63 kg m^{-2}.

Water uptake trend and water use efficiency, and root growth characteristics

Figure 5 illustrates that the trend of water uptake among the treatments were similar between day 1 and 28 but diverged on the 30th day after transplanting. Water uptake in the restricted roots cultivated in the standard concentration was generally lower than the other treatments throughout the cultivation period. However, peak of water uptake in the other treatments was observed between the 33rd and 56th day after transplanting.

The total water use (TWU) and its efficiency were significantly ($p<0.05$) affected by root restriction as shown in Table 4. Total water use was markedly reduced in restricted roots compared to unrestricted roots, leading to a higher water use efficiency in the former. The amount of water used was influenced significantly by the concentration of nutrient solution. Standard NSC reduced water uptake than the half concentration.

Root growth (fresh weight) was markedly affected by root restriction and NSC. Root growth was markedly reduced by root restriction compared to the unrestricted. The standard NSC had a significant reducing effect on root growth than the half concentration. Root proliferation (zone of root growth beyond the zone of extreme partial root restriction) was reduced with the standard (high) NSC.

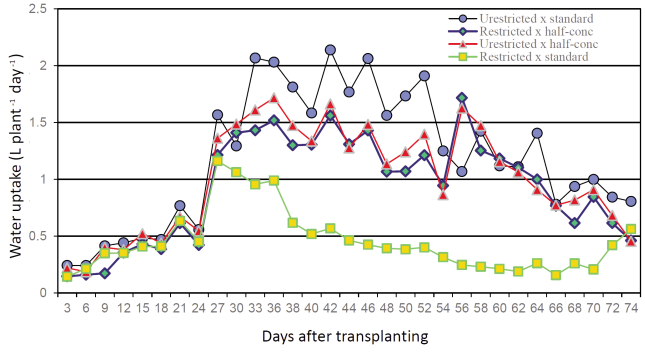

Fig. 5 - Influence of partial root restriction and nutrient solution concentration on the trend of tomato daily water uptake.

4. Discussion and Conclusions

Plant root restriction is a cultivation technique in horticulture where roots are confined within a limited space and this subsequently limits the plant growth potential. Numerous reports indicate that this technique generally results in improved fruit quality (sugar accumulation, anthocyanin enrichment) despite the reduction in photosynthesis (Wu *et al.* 2018; Zakaria *et al.,* 2020). The adoption of high nutrient solution concentrations in soilless culture has been used singly as efforts to increase

Table 4 - Influence of root restriction and nutrient solution concentration (NSC) on water uptake and water use efficiency

Nutrient solution concentration	Restricted		Unrestricted
Total water uptake (l plant $^{-1}$)			
Standard	13.9 ± 0.058 bB		36.93 ± 0.14 aA
Half concentration	28.9 ± 0.46 aB		31.0 ± 0.48 bA
p-value		<0.001	
Water use efficiency (g fresh fruit weight L^{-1})			
Standard	259.2 ± 0.058 aA		218.8 ± 0.145 aA
Half concentration	253.3 ± 0.463 aA		228.5 ± 0.481 aA
p-value		0.563	
Root fresh weight (g plant $^{-1}$)			
Standard	31.67 ± 1.67 bB		62.67 ± 1.15 bA
Half concentration	56.00 ± 1.73 aB		70.00 ± 1.45 aA
p-value		0.002	
Proliferation root mass (% fresh weight plant^{-1})			
Standard	5.00 ± 1.15 b (16.1%)		
Half concentration	12.67 ± 0.33 a (22.6%)		
p-value	0.034		

Small letters compare means within root restriction, while capital letters compare means within NSC and levels of root restriction. Values in the same column followed by the same letters indicate no significant difference according to Tukey HSD ($p<0.05$).

fruit quality but usage coupled with root restriction remains unclear hence this study tried to obtain plausible explanations to the mechanisms that influences plant growth, yield, fruit quality and water uptake under these two factors i.e., root restriction and nutrient solution concentration.

Physio-morphological responses to plant root-restrictions and NSC

In this study, plant root-restriction reduced the growth of tomato, which has been confirmed in the finding of Kasai *et al.* (2012). The work of Zakaria *et al.* (2020) on chili pepper reported a 14% reduction in plant height due to root restriction. However, the findings of this work showed that partial-extreme root restriction of tomato reduced plant height by 21% compared to the control. Although these comparisons are from two different crops, the trends of the impact of root restriction are similar. The differences in absolute percentage change might be due to the extent of root mass reduction because of the initial extreme restriction imposed on tomato roots. Tomato plants subjected to root restriction under the standard concentration also showed reduced plant growth in terms of height, girth, and leaf number. A high NSC is known to impair water uptake (Ding *et al.,* 2018), and in partial extreme root-restricted conditions this might have influenced the growth reduction.

The tomato plants with extreme partially restricted roots showed decreased leaf gas exchange compared to the control plants six weeks after transplanting. An impairment in photosynthetic rate due to a reduction in stomatal conductance in root-restricted plants has been confirmed in the works of Shi *et al.* (2008), Mugnai and Al-Debei (2011), and Campany *et al.* (2017). Nevertheless, the outcomes of this study differed from Zakaria *et al.* (2020) and Santos *et al.* (2022) findings, which indicated that leaf gas exchange of chili pepper and jenipapo was not significantly affected by root restriction.

The standard nutrient solution concentration, with or without root restriction, induced a reduction in the photosynthetic rate of tomato compared to the half-strength, despite an increase in the Tr, Gs, and Ci. This finding could be attributed to downregulation of photosynthetic rate due to water stress, particularly in the partially extreme root-restricted plants, which demonstrated sink limitation resulting from a decrease in root mass. The findings

of Beesigamukama *et al.* (2020) suggested that a low NSC induces nutrient stress hence, photosynthetic rate is significantly reduced. The findings of this study, however, showed that the photosynthetic rate of tomato could be reduced by 41% when grown in the standard nutrient solution concentration of 2.4 dS m^{-1}.

Furthermore, the findings of the study revealed that partial extreme root-restricted tomato plants, which were grown in the standard nutrient solution concentration showed a 65-70% reduction (downregulation) in the photosynthetic rate at the generative phase. In other studies, Lu *et al.* (2022) reported that a NSC of 1.5 dS m^{-1} could induce nutrient stress, reducing the rate of photosynthesis of cherry tomato, but they reiterate that the rate of photosynthesis can only be maintained at a concentration of 3.0-5.0 dS m^{-1}. On the other hand, this present study found that a NSC of 1.2 dS m^{-1} was sufficient to provide necessary nutrients to plants without causing any nutrient stress, as plants in the partially extreme restricted root treatment did not show signs of nutrient deficiency. Additionally, when the 1.2 dS m^{-1} NSC is doubled, the cultivar turned out with a divergent response when the roots were restricted.

Yield, fruit quality, and water uptake under plant root-restriction and NSC

The tomato yield decreased significantly by 35.4% due to partial extreme root restriction. This restriction inhibited root growth, leading to a diminished sink capacity and ultimately inducing a downregulation of photosynthesis. Bihmidine *et al.* (2013) found that pepper experienced a 23% decrease in yield due to root restriction. Other studies (Saito, *et al.*, 2008; Ayarna *et al.*, 2021) have also reported yield reductions in tomatoes due to root restriction. Partial extreme root restriction, in this study, increased the fruit quality (sugar content) of tomato by 52%, which is a 25% improvement over values previously recorded in Ghana (Nkansah *et al.*, 2003). The findings of Li *et al.* (2022) also reported that root restriction increased the sugar content of tomatoes.

Tomato fruits from the standard NSC had higher sugar content without affecting yield. This finding aligns with previous studies by Veit-köhler *et al.* (1999) and Wang (2017). Findings from this study revealed that partial-extreme root restriction with the standard NSC increased the sugar content of tomato while the yield was markedly reduced. These two technical hydroponic tools could be employed to increase the sugar content of tomatoes, especially, the cherry type. Furthermore, the synergistic effect of root restriction and standard nutrient solution concentration generally reduced water uptake in the tomato cultivar. The reduction in water uptake was markedly lower at the generative phase of growth. Osmotic stress in the root environment most probably accounts for the remarkable reduced water uptake in the tomato plants that were subjected to root restriction in the standard NSC. After the initial extreme root restriction, subsequent root proliferation produced a smaller root mass with a higher absorptive surface area per unit due to the presence of finer, younger roots. However, these roots remain generally disadvantaged by a reduced overall absorptive capacity compared to unrestricted roots. Under these conditions, an NSC of 2.4 dS m^{-1} was sufficient to induce water stress, leading to reduced water uptake. This observation is consistent with the findings of Saito *et al.* (2008) and Liu *et al.* (2023), who reported that root restriction under high NSC conditions enhanced tomato fruit quality but reduced fruit size due to water stress. Partial-extreme root restriction of tomato reduced total water uptake by 37% compared to the control plants. Water use was more efficient in the partially extreme root-restricted plants however, the yield was negatively affected because of a reduced photosynthetic rate with low dry matter production. The findings of Ismael and Dalia (1995) and Bar-Tal *et al.* (1994) confirmed that water uptake reduces with root restriction in tomato. A high NSC significantly decreased the uptake of water, and yield of tomato when roots are extremely confined (restricted).

In an environment with extreme partial root restriction, the standard NSC, which denotes a higher nutrient solution concentration, induced a significant reduction in root growth. This suggests that the concentration of the nutrient solution has a notable impact on root growth in such conditions. In the environment with extreme root restriction, our observations indicate that 16.1% portion of root mass was present in the standard concentration of the nutrient solution, while 22.6% was evident in the half concentration. Under extreme partial root-restricted conditions, only 16-22% of the root mass was found to be most probably actively involved in

water and nutrient uptake. Reduced root growth indicates a decrease in the plant sink structure (capacity), which influenced the downregulation of photosynthesis and yield reduction.

Under normal growth conditions, slight changes in NSC may not have an adverse effect on tomato performance, unless the associated roots are restricted. When roots are extremely but partially restricted in their growth under the same nutrient solution concentration, there is a trade-off between sugar content and yield. In the low node order pinching at high density planting, partial-extreme root restriction and NSC are effective manipulative hydroponic tools for comparatively increasing the yield and fruit quality of tomato while conserving water. In general, the productivity of tomatoes could be improved at a cost-effective level since the cultivation system involves the use of low substrate volume. This growing system can allow four cultivation cycles of tomato per year. The sugar content of tomatoes grown in Ghana could be improved by 25% with a corresponding yield of 14.5 kg m^{-2} y^{-1}. Cherry tomatoes could also be grown in these conditions to improve fruit quality while significantly reducing water use. Additionally, geographical areas with limited water resources could benefit from the use of this tomato cultivation system. While maintaining the fruit quality within the reported range, the yield of tomato could be increased to 32.4 kg m^{-2} y^{-1} when the standard NSC without root restriction is adopted under greenhouse conditions.

References

AGIUS C., VON TUCHER S., ROZHON W., 2022 - *The effect of salinity on fruit quality and yield of herry tomatoes*. - Horticulturae, 8: 59.

ANJUM S.A., XIE X., WANG L.C., SALEEM M.F., MAN C., LEI W., 2011 - *Morphological, physiological and biochemical responses of plants to drought stress.* - Afr. J. Agric. Res., 6: 2026-2032.

AYARNA A.W., TSUKAGOSHI S., ODURO NKANSAH G., 2021 - *Effect of root restriction on the performance of three-truss cultivated tomato in the low-node pinching order at high-density cultivation system*. - Horticulturae, 7: 60.

BAR-TAL A., FEIGIN A., RYLSKI I., PRESSMAN E., 1994 - *Effects of root pruning and N-NO_3 solution concentration on nutrient uptake and transpiration of tomato plants*. - Sci. Hort., 58: 77-90.

BEESIGAMUKAMA D., MOCHOGE B., KORIR N.K., FIABOE K.K., NAKIMBUGWE D., KHAMIS F.M., SUBRAMANIAN S., DUBOIS T., MUSYOKA M.W., EKESI S., 2020 - *Exploring black soldier fly frass as novel fertilizer for improved growth, yield, and nitrogen use efficiency of maize under field conditions.* - Front. Plant Sci., 11: 574592.

BIHMIDINE S., HUNTER III C.T., JOHNS C.E., KOCH K.E., BRAUN D.M., 2013 - *Regulation of assimilate import into sink organs: update on molecular drivers of sink strength.* - Front. Plant Sci., 4: 177.

CAMPANY C.E., MEDLYN B.E., DUURSMA R.A., 2017 - *Reduced growth due to belowground sink limitation is not fully explained by reduced photosynthesis*. - Tree Physiol., 37: 1042-1054.

CLIFF M.A., LI J.B., TOIVONEN P.M., EHRET D.L., 2012 - *Effects of nutrient solution electrical conductivity on the compositional and sensory characteristics of greenhouse tomato fruit*. - Postharvest Biol Technol., 74: 132-140.

DING X., JIANG Y., ZHAO H., GUO D., HE L., LIU F., ZHOU Q., NANDWANI D., HUI D., YU J., 2018 - *Electrical conductivity of nutrient solution influenced photosynthesis, quality, and antioxidant enzyme activity of pakchoi* (Brassica campestris *L.* ssp. Chinensis) in a hydroponic system. - PLOS One, 13(8): e0202090.

FERREIRA D., 2008 - *SISVAR: a program for statistical analysis and teaching*. - Rev. Symp., 6: 36-41.

HIEKE S., MENZEL C., LÜDDERS P., 2002 - *Shoot development, chlorophyll, gas exchange and carbohydrates in lychee seedlings* (Litchi chinensis). - Tree Physiol., 22: 947-953.

HOAGLAND D.R., 1929 - *The absorption of ions by plants*. - Soil Sci., 16: 225-246.

HORI H., 1966 - *Gravel culture of vegetable and ornamental crops* (In Japanese). Yoken-do, Tokyo.

ISMAIL M.R., DALIA S., 1995 - *Growth, physiological processes and yield of tomatoes grown in different root zone volumes using sand culture.* - Pertanika J. Trop. Agric. Sci., 18: 141-147.

ISMAIL M.R., NOOR K.M., 1996 - *Growth, water relations and physiological processes of starfruit plants under root growth restriction*. - Sci. Hort., 66: 51-55.

JONES J.B. Jr., 1982 - *Hydroponics: its history and use in plant nutrition studies.* - J. Plant. Nutr., 5: 1003-1030.

KASAI M., KOIDE K., ICHIKAWA Y., 2012 - *Effect of pot size on various characteristics related to photosynthetic matter production in soybean plants*. - Int. J. Agron., 751731, pp. 1-7.

KHARKINA T.G., OTTOSEN C.O., ROSENQVIST E., 1999 - *Effect of root restriction on growth physiology of cucumber plants*. - Physiol. Plant., 105: 434-441.

KINOSHITA T., YANO T., SUGIURA M., NAGASAKI Y., 2014 - *Effects of controlled-release fertilizer on leaf area index*

and fruit yield in high-density soilless tomato culture using low node-order pinching. - PLOS One, 9: e113074.

LI D., LIU B., WANG Z., LI X., SUN S., MA C., WANG L., WANG S., 2022 - *Sugar accumulation may be regulated by a transcriptional cascade of ABA-VvGRIP55-VvMYB15-VvSWEET15 in grape berries under root restriction*. - Plant Sci., 322: 111288.

LIU D., CHEN J., HAO Y., YANG X., CHEN R., ZHANG Y., 2023 - *Effects of extreme root restriction on the nutritional and flavor quality, and sucrose metabolism of tomato* (Solanum lycopersicum L.). - Horticulturae, 9: 813.

LU T., YU H., WANG T., ZHANG T., SHI C., JIANG W., 2022 - *Influence of the electrical conductivity of the nutrient solution in different phenological stages on the growth and yield of cherry tomato.* - Hortic., 8: 378.

MUGNAI S., AL-DEBEI H., 2011 - *Growth reduction in root-restricted tomato plants is linked to photosynthetic impairment and starch accumulation in the leaves*. - Adv. Hort. Sci., 25(2): 99-105.

NKANSAH G., OWUSU E., BONSU K., DENNIS E., 2003 - *Effect of mulch types on growth, yield and fruit quality of tomato (*Lycopersicon esculentum *Mill.)*. - Ghana J. Hort., 2: 55-64.

PETERSON T.A., KRIZEK D., 1992 - *A flow-through hydroponic system for the study of root restriction*. - J. Plant. Nutr., 15: 893-911.

PEZESHKI S., SANTOS M., 1998 - *Relationships among rhizosphere oxygen deficiency, root restriction, photosynthesis, and growth in baldcypress* (Taxodium distichum L.) *seedlings.* - Photosynthetica, 35: 381-390.

QUINET M., ANGOSTO T., YUSTE-LISBONA F.J., BLANCHARD-GROS R., BIGOT S., MARTINEZ J.P., LUTTS S., 2019 - *Tomato fruit development and metabolism.* - Front. Plant Sci., 10: 1554.

ROSADI R.A.B., SENGE M., SUHANDY D., TUS A., 2014 - *The effect of EC levels of nutrient solution on the growth, yield, and quality of tomatoes* (Solanum lycopersicum) *under the hydroponic system*. - J. Agr. Eng. Biotechn., 2: 7-12.

SAITO T., FUKUDA N., IIKUBO T., INAI S., FUJII T., KONISHI C., EZURA H., 2008 - *Effects of root-volume restriction and salinity on the fruit yield and quality of processing tomato.* - J. Jpn. Soc. Hort. Sci., 77: 165-172.

SAKAMOTO M., SUZUKI T., 2020 - *Effect of nutrient solution concentration on the growth of hydroponic sweet potato.* - Agronomy, 10(11): 170810.

SALISU M.A., SULAIMAN Z., SAMAD M., KOLAPO O.K., 2018 - *Effect of various types and size of container on growth and root morphology of rubber* (Hevea Brasiliensis *Mull. Arg.)*. - IJSTR, 7: 21-27.

SANTOS C.S., DALMOLIN A.C., SCHILLING A.C., SANTOS M.S., CHAFFER B.S., MIELKE M.S., 2022 - *Root deformation affects mineral nutrition but not leaf gas exchange and growth of* Genipa americana *seedlings during the recovery phase after soil flooding.* - Braz. J. Biol., 28(82): e234018 82.

SCHWARZ D., KLÄRING H.P., 2001 - *Allometry to estimate leaf area of tomato.* - J. Plant. Nutr., 24: 1291-1309.

SCHWARZ D., KLÄRING H.P., VAN IERSEL M.W., INGRAM I.T., 2002 - *Growth and photosynthetic response of tomato to nutrient solution concentration at two light levels*. - J. Amer. Soc. Hort. Sci., 127: 984-990.

SHI K., DING X.T., DONG D.K., ZHOU Y.H., YU J.Q., 2008 - *Root restriction-induced limitation to photosynthesis in tomato (*Lycopersicon esculentum *Mill.) leaves.* - Sci. Hortic., 117: 197-202.

TAKAHASHI T., ISHIGAMI Y., GOTO E., NIIBORI K., GOTO K., 2012 - *Modeling the growth and yield of tomatoes cultivated with a low node-order pinching system at high plant density*. - Environ. Control Biol., 50: 53-61.

VEIT-KÖHLER U., KRUMBEIN A., KOSEGARTEN H., 1999 - *Effect of different water supply on plant growth and fruit quality of* Lycopersicon esculentum*.* - J. Plant Nutr. Soil Sci., 162*:* 583-588.

WANG P., 2017 - *Effects of nutrient solution concentration supply on growth, yield, and quality during growing stage*. - Northwest A&F University, Xianyang, China. (In Chinese).

WATANABE S., 2006 - *New growing system for tomato with low node-order pinching and high-density planting*. - Proc. Veg. Tea Sci., 3: 91-98.

WU Y., ZHOU J., WANG J., MEN X., LIU N., CHEN F., 2018 - *Effect of root restriction on quality and yield of watermelon cultivated in soilless facilities*. - Jiangsu Agric. Sci., 46: 113-115.

YAMAURA H., FUJITA S., IKEDA K., TAKANO N., KANNO K., MATSUO S., ZHAO T., NAKANO A., IWASAKI Y., 2020 - *Year-round production of tomato fruits by low node-order pinching and high-density planting in subtropical area in Japan.* - Acta Horticulturae, 1296: 457-462.

YANG P., CHEN R., LI J., WU Y., ZHENG Z., 2017 - *Effects of different irrigation limits and nutrient solution concentrations on tomato cultivated in substrate*. - Jiangsu Agric. Sci., 45: 126-128.

ZAKARIA N.I., ISMAIL M.R., AWANG Y., MEGAT WAHAB P.E., BERAHIM Z., 2020 - *Effect of root restriction on the growth, photosynthesis rate, and source and sink relationship of chilli (*Capsicum annuum *L.) grown in soilless culture*. - Biomed Res. Int., 2020.

ZHANG P., SENGE M., YOSHIYAMA K., ITO K., DAI Y., ZHANG F., 2015 - *Effects of low salinity stress on growth, yield, and water use efficiency of tomato under soilless cultivation*. - J. Irrigation, Drainage Rural Eng., 85: 15-21.

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Phytochemical evaluation of selected *Phalaenopsis* cultivars

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Abstract: The genus *Phalaenopsis* in family Orchidaceae has gained popularity in the global floriculture market for its value as potted plants and cut flowers. Hybridization plays a pivotal role in breeding *Phalaenopsis,* enabling the development of novel cultivars with desirable traits such as diverse floral pigmentation patterns, enhanced longevity and improved growth rate. The selection of suitable parental cultivars is a crucial determinant in the hybridization process, as it plays a key role in defining pigmentation patterns and enhancing desirable traits for the development of superior cultivars. This research investigated the phytochemical composition, including carotenoids, anthocyanins, flavonoids and phenolics contents of selected commercial *Phalaenopsis* cultivars. Using UV-vis spectrophotometry, the chlorophylls, flavonoids, carotenoids, anthocyanins and phenolics contents in the flowers, leaves and roots of six cultivars were analyzed quantitatively. The results revealed significant variations in phytochemical properties across the tested cultivars and plant organs. *Phalaenopsis* cvs. Taipei Gold Gold Star, Red Lip 1770, Golden Sands Canary, Sogo Yukidian V3 and Queen Beer Mantefon exhibited promising phytochemical profiles. Notably, cvs. Taipei Gold Gold Star, Golden Sands Canary and Queen Beer Mantefon were identified as ideal parental candidates for hybridization due to their potential to develop distinctive floral colorations and robust vegetative traits. These findings provide valuable insights into the floral coloration and the phytochemical richness of vegetative parts in *Phalaenopsis* cultivars. This knowledge can contribute to the development of innovative, high-quality cultivars with enhanced survival rates and greater consumer appeal.

1. Introduction

The family Orchidaceae, one of the largest families of flowering plants, ranks second only to the family Asteraceae in its diversity and ecological prominence. Comprising over 736 genera, Orchidaceae has established a significant presence in the global floriculture market, valued for its use in both potted plants and cut flowers (Chase *et al.*, 2015). Among its commercially significant genera, *Phalaenopsis* stands out for its refined,

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elegant aesthetic and extended longevity, captivating both growers and consumers (Hsiao *et al.*, 2011). Hybridization serves as the primary breeding approach for *Phalaenopsis*, with intergeneric and interspecific hybridizations being the most commonly employed methods to develop novel cultivars. To date, 35,129 *Phalaenopsis* hybrids have been registered with the Royal Horticultural Society, reflecting the immense popularity of the genus (Hsu *et al.*, 2018). Dynamic and evolving consumer preferences have significantly driven hybridization efforts, making *Phalaenopsis* a focal point in modern orchid breeding.

The breeding objectives for *Phalaenopsis* focus primarily on morphology, color, and scent, with color being a key factor in shaping initial consumer impressions (Hsu *et al.*, 2018). With the increasing popularity and demand for orchids, commercial cultivars are developed with specific characteristics that enhance their large-scale applicability. Key traits include resilience, which reduces the need for inputs such as water, fertilizers, and pesticides—an essential factor given rising production costs and the growing emphasis on sustainability. Modern floriculture, a resource-intensive sector that utilizes energy, water, fertilizers, and propagation materials, faces the challenge of meeting market demands while minimizing environmental impact (Darras, 2020; Cardoso and Vendrame, 2022; Cardoso *et al.*, 2023; Bhardwaj *et al.*, 2024).

Phytochemicals play a crucial role in plant growth and development. While primary metabolites support physiological functions, secondary metabolites contribute significantly to plant's defense mechanisms (Thacker and Ram, 2020; Wani *et al.,* 2022). In orchids, various phytochemicals have been identified, serving both physiological and commercial purposes. Notable genera with economic significance include *Dendrobium* (He *et al.,* 2023), *Phalaenopsis* (Ling and Subramanium, 2007; Minh *et al.,* 2016), *Vanda*, *Bulbophyllum* (Lalrosangpuii and Lalrokimi, 2021), and *Cymbidium* (Axiotis *et al.,* 2021). Understanding the phytochemical composition of these orchids is essential for developing innovative, sustainable, and high-value cultivars that align with market trends and consumer preferences.

The vibrant coloration of *Phalaenopsis* hybrids arises from a complex mechanism of pigment accumulation. Flower color is primarily influenced by pigments such as chlorophylls, carotenoids, anthocyanins, and betalains. While many flowers derive their color from a single source of pigment, *Phalaenopsis* exhibits a broader palette through the combination of pigments. Yellow to orange shades are mainly due to the accumulation of carotenoids, whereas blue to red shades are typically attributed to anthocyanins (Hsu *et al.*, 2018). Combinations of purple anthocyanins and yellow carotenoids can result in perceived colors such as brown, bronze, and red in flowers (Lightbourn *et al.*, 2008). Hence, novel colors can be produced through hybridization, facilitating the combination of different pigments (Voegelpoel, 1990). Moreover, differential coloration can generate various pigmentation patterns such as blotches, stripes along veins, and irregular markings. These striking patterns and colors increase the aesthetic value of *Phalaenopsis* cultivars, capturing the attention of consumers and expanding their market appeal. The quality of the flower depends on the vegetative growth rate of the plant, as vigorous growth often correlates with superior flower characteristics. Photosynthesis, a primary physiological process, drives plant growth by converting light energy into chemical energy, enabling the synthesis of carbohydrates. Chlorophyll, the key pigment in photosynthesis, plays an essential role in this process. The two main forms of chlorophyll-chlorophyll a and chlorophyll b-absorb solar energy, facilitate carbon dioxide fixation, and convert energy into carbohydrates, which are vital for plant growth and development. Consequently, higher chlorophyll content is directly associated with improved photosynthetic efficiency and plant vigor. In addition to chlorophyll, phenolic compounds also play a crucial role in plant physiology and aesthetics. These compounds, including flavonoids such as anthocyanins, contribute significantly to plant defense mechanisms and pigmentation. Phenolic compounds accumulate as an adaptive response to environmental stress, highlighting their role in plant resilience (Lattanzio *et al.*, 2012).

In *Phalaenopsis* cultivars with desirable traits, flower color intensity is a key selection criterion in tissue culture and breeding programs (Ling and Subramaniam, 2007). However, the phytochemical profiles of *Phalaenopsis* cultivars remain largely fragmented, limiting the ability to develop hybrids with targeted traits. This lack of comprehensive data highlights the need for in-depth phytochemical studies to support informed breeding decisions. Therefore, this study aims to evaluate the

phytochemical properties of selected *Phalaenopsis* cultivars, providing valuable insights for the development of high-value cultivars that align with market trends.

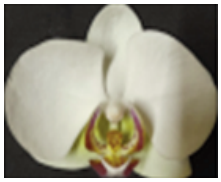
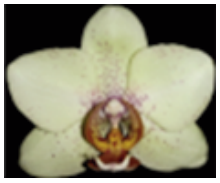
2. Materials and Methods

Plant materials

This study was conducted to evaluate phytochemical properties of selected *Phalaenopsis* cultivars.

Six commercially available *Phalaenopsis* cultivars with different color combinations were obtained from the growers. The details of the selected six commercial *Phalaenopsis* cultivars are presented in Table 1. The plants were maintained in the plant house of the Department of Plant and Molecular Biology, University of Kelaniya, Sri Lanka. The fresh, full-bloomed flowers, young leaves (the leaf nearest to the flower spike) and roots of each cultivar were collected in the morning and used for phytochemical analysis. The roots and leaves were cleaned with distilled water and wiped. All the samples were frozen at -80°C immediately after collection. The frozen samples were grounded using mortar and

Table 1 - List of selected commercial Phalaenopsis cultivars

No.	Name		Reference for the cultivar name	Abbreviation
1	*Phalaenopsis* cv. Red Lip 1770		Locally produced cultivar	RL1770
2	*Phalaenopsis* cv. Golden Sands Canary		(Lee *et al.,* 2020)	GSC
3	*Phalaenopsis* cv. Sogo Yukidian V3		(Lee *et al.,* 2020)	SYV3
4	*Phalaenopsis* cv. Queen Beer Mantefon		(Lee *et al.,* 2020)	QBM
5	*Phalaenopsis* cv. Taipei Gold Gold Star		(Lee *et al.,* 2020)	TGGS
6	*Phalaenopsis* cv. Brother Strips		(Lee *et al.,* 2020)	BS

pestle. The weights of each powdered plant material were measured and quantified to obtain a standard weight.

Determination of chlorophyll and carotenoid contents

Chlorophyll and carotenoid contents of leaves, roots and flowers were measured using the methods described by Nguyen *et al.* (2018) with slight modifications. Known volume of dried powder was mixed with a known volume of 80% acetone and kept at 4°C overnight. The mixture was centrifuged at 13,000 g for 5 min to obtain the supernatant. The supernatant was tested to determine the absorbance of chlorophyll *a*, chlorophyll *b* and carotenoids in 80% acetone at 664 nm, 647nm and 441 nm respectively using the UV- visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Concentrations of chlorophyll *a,* chlorophyll *b* and carotenoids were calculated using the following equations (Porra, 2002).

Chlorophyll a = (12.25 x Absorbance $_{664}$) - (2.55 x Absorbance $_{647}$) x volume of the supernatant (ml)/sample weight (g)

Chlorophyll b = (20.31 x Absorbance $_{647}$) – (4.91 x Absorbance $_{664}$) x volume of the supernatant (ml)/sample weight (g)

Carotenoids = (4.69 x Absorbance $_{441}$ x volume of the supernatant (ml)/sample weight (g)) – 0.267 (chlorophyll a ±b)

Total chlorophyll = Chlorophyll a ± Chlorophyll b

Determination of anthocyanin content

A known weight of dry powder of leaves, roots and flowers were mixed with a known volume of acidified methanol (99% methanol containing 1% HCl). The mixture was incubated for 24 hours at room temperature followed by centrifugation at 4°C and 3000 rpm for 5 minutes. The obtained supernatant was then subjected to measure the absorbance at 530 nm and 657 nm on the UV-visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Concentration of anthocyanins were calculated using the following equation.

Anthocyanin content = (Absorbance $_{530}$ - 0.33 × Absorbance $_{657}$ / 31.6) x volume of supernatant (ml) / sample weight (g)

Determination of total flavonoid content

The known weight of dry powder of leaves, roots and flowers were mixed with methanol and incubated for 24 hours at room temperature. The supernatant was obtained by centrifugation at 4°C and 3000 rpm for 5 minutes. Equal volumes of the supernatant were mixed with 2% Aluminum chloride. The mixture was stirred and kept for 15 minutes. The absorbance was measured at 430 nm using the UV-visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Quercetin was used as the reference standard. The flavonoid content of each plant part was expressed as milligrams of Quercetin equivalents of grams of dry weight (mg QE/g DW) using the following equation.

$C = C_1$ (Volume of the supernatant (ml))/(Dry weight (g))

Where C is total flavonoid content of the extract and C_1 the quercetin concentration (mg/ml).

Determination of total polyphenol content

The acidified methanol extracts were prepared using the same procedure done for the anthocyanin content analysis. 100 µl of the extract was mixed with 2 ml of 7.5 % Na_2CO_3 and allowed to equilibrate for 2 minutes. After 2 minutes diluted Folin-Ciocalteu reagent was added (1:10 v/v). The absorbance was measured at 765 nm using the UV- visible spectrophotometer (Thermo Fisher Scientific, Vantaa, Finland). Gallic acid was used as the reference standard. The polyphenol content of each plant part was expressed as milligrams of Gallic acid equivalents of grams of dry weight (mg GAE/g DW) using the following equation.

$C = C_1$ (Volume of the supernatant (ml))/(Dry weight (g))

Where C is the total polyphenol content of the extract and C_1 th gallic acid concentration (mg/ml).

Statistical analysis

All the experiments were conducted in triplicates (n=3). The data were expressed as mean ± SD (standard deviation). An analysis of variance (ANOVA) test was performed with Tukey's Honest Significant Difference test (HSD) at $p \leq 0.05$ using R software version 4.3.2.

3. Results

Chlorophyll content

Figure 1 illustrates the chlorophyll a, chlorophyll b, total chlorophyll (a + b), and the chlorophyll a/b ratio in extracts from different parts (leaves, roots, and flowers) of six *Phalaenopsis* cultivars. The analysis revealed that the concentrations of chlorophyll a and b were significantly higher in the

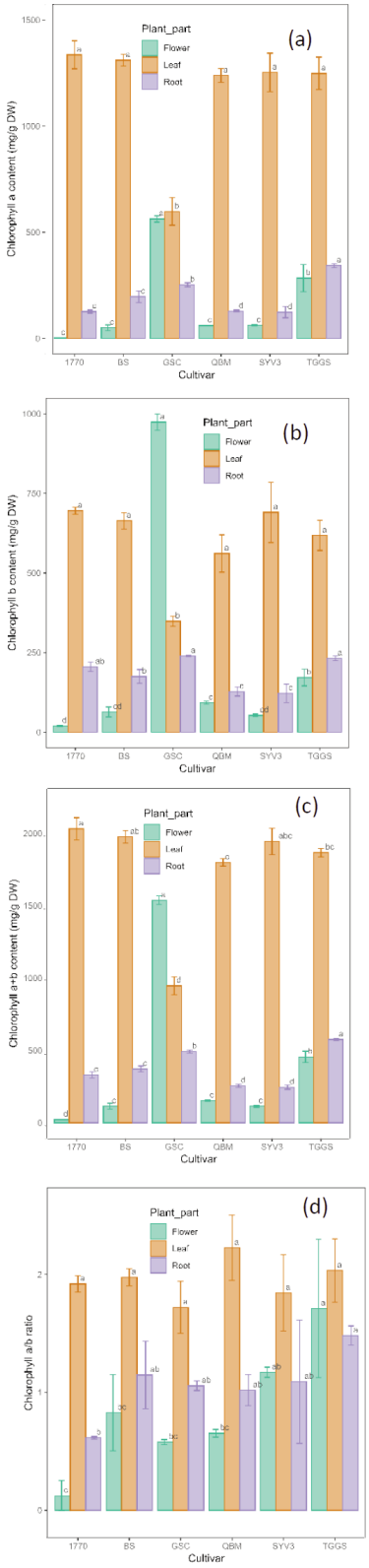


Fig. 1 - (a) Chlorophyll a, (b) chlorophyll b, (c) chlorophyll a+b contents and (d) chlorophyll ratio in extracts from different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3.

leaves compared to the roots and flowers across all tested cultivars. Similarly, the total chlorophyll (a + b) content in the leaves was higher than in the roots of the cultivars. Additionally, the leaves of all cultivars displayed a higher chlorophyll a/b ratio than that observed in the roots (Fig. 1). In this study, the leaves and roots of all tested cultivars, except for the roots of cv. Red Lip 1770 (0.62±0.01), had a chlorophyll a/b ratio higher than 1. Therefore, in most of the tested cultivars, the chlorophyll a/b ratio in leaf and root extracts was below 2.0, while in a few cases, it was approximately 2.0. These findings indicate the predominance of chlorophyll a over chlorophyll b in both leaves and roots, reflecting their physiological roles in photosynthesis. The analysis of chlorophyll content among the tested *Phalaenopsis* cultivars revealed significant variations in both leaf and root tissues.

The cv. Red Lip 1770 exhibited the highest levels of chlorophyll a (1335.81±65.94 mg g^{-1} DW) and chlorophyll b (695.80±11.00 mg g^{-1} DW) in the leaves, yielding the highest total chlorophyll content (chlorophyll a + b) of 2031.61±75.83 mg g^{-1} DW. Conversely, cv. Golden Sands Canary reported the lowest levels of chlorophyll a (598.02±65.01 mg g^{-1} DW) and chlorophyll b (348.59±16.20 mg g^{-1} DW), resulting in the lowest total chlorophyll content of 946.60±61.35 mg g^{-1} DW.

Chlorophyll content in root extracts varied significantly among cultivars. Cv. Taipei Gold Gold Star had the highest chlorophyll a content (343.53±8.96 mg g^{-1} DW), while cv. Golden Sands Canary exhibited the highest chlorophyll b content (239.67±1.99 mg g^{-1} DW). In contrast, cv. Sogo Yukidian V3 showed the lowest levels of both chlorophyll a (123.49±26.32 mg g^{-1} DW) and chlorophyll b (122.17 ± 28.63 mg g^{-1} DW).

Total chlorophyll content in roots followed a similar trend, with cv. Taipei Gold Gold Star achieving the highest value (575.53±5.11 mg g^{-1} DW), while cv. Sogo Yukidian V3 exhibited the lowest (245.66±14.44 mg g^{-1} DW).

Among all the examined *Phalaenopsis* flower samples, cv. Golden Sands Canary exhibited the highest chlorophyll *a* (563.16±14.80 mg g^{-1} DW) and chlorophyll *b* (973.66±25.26 mg g^{-1} DW) contents. As a result, its total chlorophyll (chlorophyll *a+b*) content was also the highest in *Phalaenopsis* cv. Golden Sands Canary (1536.82±29.49 mg g^{-1} DW). Conversely, cv. Red Lip 1770 had the lowest chlorophyll *a* (2.18±2.10 mg g^{-1} DW) and chlorophyll *b* (19.70±2.83 mg g^{-1} DW) contents, resulting in the

lowest total chlorophyll (*a*+*b*) content among all tested flowers. Additionally, all flower samples exhibited a chlorophyll *a*/*b* ratio of less than 1, except for cv. Taipei Gold Gold Star (1.70±0.56) and cv. Sogo Yukidian V3 (1.18±0.21).

Total flavonoid content

Figure 2 illustrates the total flavonoid content in extracts from various parts of *Phalaenopsis* cultivars, highlighting significant variation in flavonoid distribution within the same cultivar. Specifically, flower extracts contained significantly higher total flavonoid levels compared to other plant parts. Notably, the highest total flavonoid content was observed in cv. Brother Strips (0.42±0.0 mg QE g^{-1} DW) whereas cv. Queen Beer Mantefon exhibited the lowest (0.19±0.00 mg QE g^{-1} DW). On the other hand, root extracts, of cv. Queen Beer Mantefon contained significantly higher levels of flavonoids (0.114±0.04 mg QE.g^{-1} DW) compared to cv. Golden Sands Canary, which had the lowest value (0.057±0.01 mg QE g^{-1} DW).

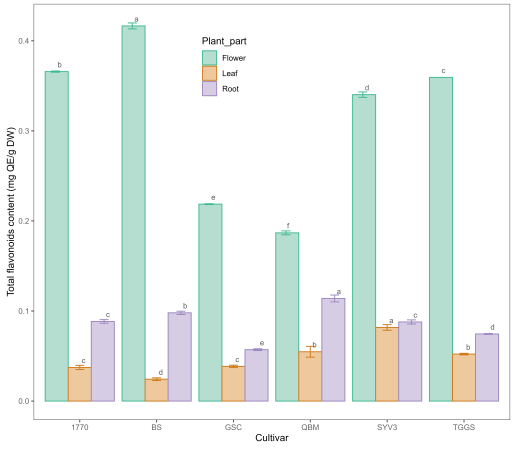


Fig. 2 - Total flavonoid contents in extracts from different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3; TGGS= *Phalaenopsis* cv. Taipei Gold Gold Star.

Interestingly, both cv. Sogo Yukidian V3 and cv. Red Lip 1770 reported equal total flavonoid levels in root extracts (0.088±0.02 mg QE g^{-1} DW). Moreover, leaf extracts of cv. Sogo Yukidian V3 exhibited the highest flavonoid content (0.082±0.00 mg QE·g^{-1} DW), while cv. Brother Strips had the lowest (0.024±0.00 mg QE·g^{-1} DW). These findings highlight the significant variation in flavonoid distribution across different plant organs of the same *Phalaenopsis* cultivar.

Total polyphenol content

Figure 3 depicts the total phenolics content in the leaves, flowers and roots of different *Phalaenopsis* cultivars, revealing substantial variation across plant organs within the same cultivar and among different cultivars. Flower sample from cv. Golden Sands Canary exhibited the highest phenolics content (10.58±0.109 mg.GAE g^{-1} DW) whereas those from cv. Sogo Yukidian V3 reported the lowest (1.60±0.01 mg.GAE g^{-1} DW). Leaf extracts from cv. Golden Sands Canary again showed the highest phenolics content (6.55±0.49 mg.GAE g^{-1} DW) whereas cv. Brother Strips had the lowest (1.75±0.01 mg.GAE g^{-1} DW). Similarly, root extracts from cv. Taipei Gold Gold Star were rich in phenolics (5.68±0.95 mg.GAE g^{-1} DW) whereas the lowest was *Phalaenopsis* cv. Brother Strips recorded the lowest amount (0.45±0.32 mg.GAE g^{-1} DW).

Anthocyanin and carotenoid contents

Anthocyanin and carotenoid contents in different plant parts of *Phalaenopsis* cultivars are shown in figures 4 (a) and 4 (b), respectively. Among the tested cultivars, the flower extracts of cv. Queen Beer Mantefon exhibited the highest anthocyanin content

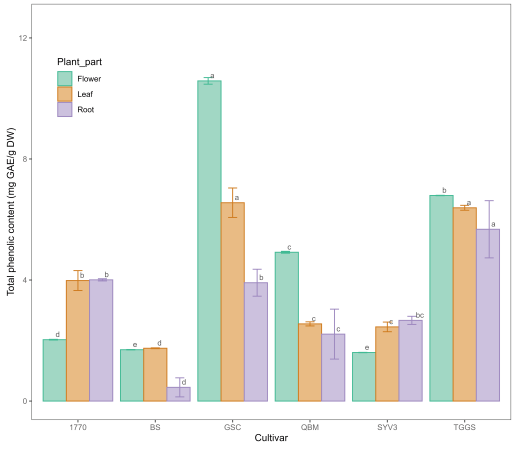


Fig. 3 - Total phenolic contents in different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3; TGGS= *Phalaenopsis* cv. Taipei Gold Gold Star.

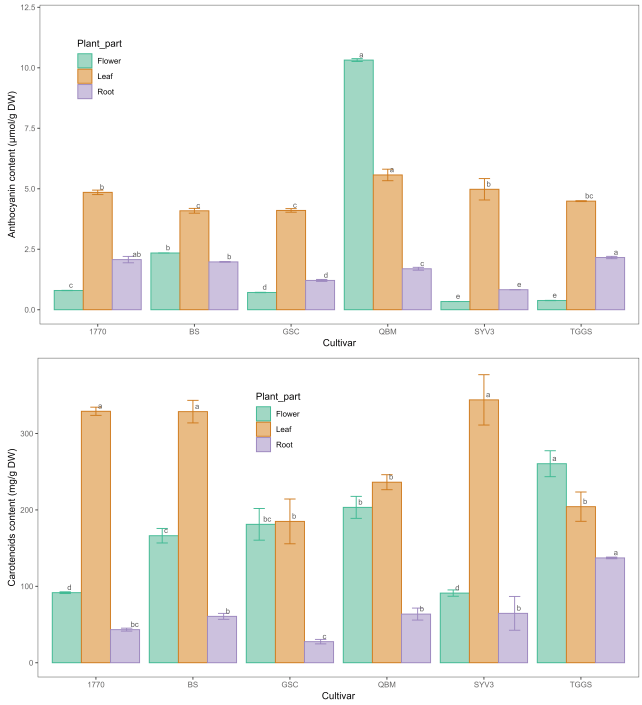


Fig. 4 - The anthocyanin contents (a) and the carotenoids contents (b) in different plant organs of the six *Phalaenopsis* cultivars tested. BS= *Phalaenopsis* cv. Brother Strips; GSC= *Phalaenopsis* cv. Golden Sands Canary; QBM= *Phalaenopsis* cv. Queen Beer Mantefon; RL1770= *Phalaenopsis* cv. Red Lip 1770; SYV3= *Phalaenopsis* cv. Sogo Yukidian V3; TGGS= *Phalaenopsis* cv. Taipei Gold Gold Star.

(10.32±0.06 µmol g^{-1} DW), significantly surpassing all other cultivars. Interestingly, this cultivar had the lowest flavonoid content in flower extracts. In contrast, cv. Sogo Yukidian V3 showed the lowest anthocyanin content among the flower extracts (0.34±0.00 µmol g^{-1} DW). Although completely yellow cultivars such as Taipei Gold Gold Star showed no visible anthocyanin-related pigmentation, small amounts of anthocyanins were detected (0.38±0.01 µmol.g^{-1} DW). Conversely, this cultivar displayed the highest carotenoid content in its flower extracts (260.50±16.97 mg.g^{-1} DW), significantly exceeding those of the other tested cultivars. Flower extracts from cultivars with white tepals and colored labellum such as Red Lip 1770 and Sogo Yukidian V3, displayed lower contents of both anthocyanins and carotenoids. Furthermore, cv. Queen Beer Mantefon reported significantly higher content of both anthocyanins and carotenoids simultaneously, a pattern not observed in other cultivars. The yellow coloration of the flowers varied across cultivars, with Brother Strips displaying yellow only in the labellum, while Golden Sands Canary showing yellow pigmentation throughout the entire flower. Despite these color differences, there was considerable variation in both anthocyanin and carotenoid contents among the flower extracts of the tested cultivars. Root extracts generally displayed the lowest anthocyanin content among the tested plant organs.

The root extracts of cv. Taipei Gold Gold Star exhibited the highest anthocyanin content (2.16 ± 0.05 µmol/g DW), while cv. Sogo Yukidian V3 showed the lowest (0.83±0.01 µmol/g DW). In contrast, leaf extracts displayed more variability, with cv. Queen Beer Mantefon having the highest anthocyanin content (5.57±0.23 µmol/g DW), while cv. Brother Strips contained the lowest (4.09±0.10 µmol/g DW).

The carotenoid content was generally higher in leaf extracts compared to other plant organs, with the exception of cv. Taipei Gold Gold Star. In this cv, carotenoid levels were 204.27±19.19 mg.g^{-1} DW in leaves and 260.50±16.97 mg.g^{-1} DW in flowers. Root extracts consistently displayed lower carotenoid contents compared to other plant parts across all cultivars. Among leaf extracts, cv. Sogo Yukidian V3 exhibited the highest carotenoid content (344.05 ± 32.95 mg g^{-1} DW), whereas cv. Golden Sands Canary reported the lowest (184.99±29.29 mg.g^{-1} DW).

The highest carotenoid content was found in root extracts from cv. Taipei Gold Gold Star (137.19 ± 1.07 mg/g DW), while the lowest was in those from cv. Golden Sands Canary (27.61±2.92 mg/g DW). Overall, both anthocyanin and carotenoid levels exhibited significant variation across the different cultivars and plant organs.

4. Discussion and Conclusions

The genus *Phalaenopsis* within the family Orchidaceae holds significant prominence in the floriculture industry due to its prolonged blooming, captivating aesthetic, ease of cultivation, adaptability and its success in hybridization. Hybridization offers breeders a unique opportunity to develop hybrids with enhanced physiological traits and diverse flower coloration. However, achieving such advancements relies heavily on the selection of parental cultivars with desirable traits, making this step important for creating novel cultivars.

Breeding strategies, plant tissue culture, and

biotechnological advancements play a crucial role in developing novel traits and expanding the global commercialization of orchids (Tiwari *et al.,* 2024). Understanding the specific phytochemical properties of different plant parts, both floral and vegetative, in *Phalaenopsis* orchids provides valuable insights for informed cultivar selection, ultimately contributing to the development of resilient, high-quality, and commercially viable hybrids.

Comprehensive knowledge of anthocyanin and carotenoid contents in leaves, roots, and flowers provides insights into pigmentation, which is closely linked to flower coloration, as well as physiological responses such as light absorption and stress tolerance. These insights can enhance breeding efficiency and ensure targeted improvements in hybrid cultivars. This approach aligns with the findings of Nguyen *et al.* (2018), emphasizing the importance of exploring the phytochemical composition of orchids to unlock their full potential for breeding and cultivation. By leveraging such information, breeders can innovate more effectively and meet the growing demand for unique and visually appealing *Phalaenopsis* cultivars in the global market.

The chlorophyll content analysis of *Phalaenopsis* cultivars revealed that leaves exhibited significantly higher chlorophyll levels than roots, a pattern consistent with findings by Trelka *et al.* (2010). This is expected, as leaves serve as the primary site for photosynthesis in plants. Additionally, the chlorophyll *a*/*b* ratio was higher in leaves compared to roots, supporting observations by Martin *et al.* (2010), who reported that epiphytic orchids generally have higher chlorophyll *a*/*b* ratios in leaves than in roots. Notably, the chlorophyll *a*/*b* ratios in epiphytes are typically low, often around 2.0 or less.

In this study, the chlorophyll *a*/*b* ratios in leaves and roots of all tested cultivars, except the roots of Red Lip 1770 exceeded 1.0. While several ratios were less than 2.0, most were approximately 2.0, which aligns with the characteristic shade adaptation of epiphytic orchids. A reduced chlorophyll *a*/*b* ratio, as observed in previous orchid studies, suggests a functional adaptation to low-light environments, allowing these plants to efficiently capture and utilize available light.

The results obtained in this study further confirm the shade-adaptive traits of *Phalaenopsis* cultivars. Understanding these adaptations enhances the knowledge of their physiological responses and could support targeted breeding and cultivation strategies to optimize growth under various light conditions.

Higher chlorophylls content serves as a valuable indirect indicator of enhanced photosynthetic efficiency of the plant (Lin and Hsu, 2004). Among the tested cultivars, Red Lip 1770 exhibited the highest chlorophyll *a*, chlorophyll *b* and total chlorophyll levels in its leaf extracts. This suggests that Red Lip 1770 may have superior photosynthetic efficiency, particularly under shade conditions, making this trait critical during its vegetative phase. Interestingly, significant variations in chlorophyll content were observed in the roots. Notably, the cultivars with the highest chlorophyll content in their leaf extracts were not the same as those exhibiting the highest chlorophyll content in their roots. This divergence indicates a broader variability in chlorophyll distribution between leaves and roots across the tested cultivars. *Phalaenopsis* cv. Golden Sands Canary demonstrated the highest chlorophyll content in its flower extracts among the tested cultivars. These findings highlight the differential accumulation of chlorophylls among *Phalaenopsis* cultivars, which could be utilized for selecting superior parental lines in breeding programs.

However, flowers generally reported lower chlorophyll content compared to leaves and roots. This difference could be attributed to the degradation of chlorophyll or the reduced activity of chlorophyll-synthesizing enzymes in flowers. A similar phenomenon has been observed in carnations, where petals contain significant amounts of chlorophyll during early development, which declines as the flower matures (Nurcahyani *et al.,* 2021).

The presence of chlorophyll in flowers is essential for carbohydrate synthesis during their development, supporting energy requirements and metabolic processes. For *Phalaenopsis,* investigating chlorophyll content at different stages of flower development is essential to gain a deeper understanding of its role and dynamics. Such studies could provide insights into optimizing flowering conditions and improving overall plant health and productivity.

Significant variations in carotenoids and anthocyanin contents were observed in the flowers of studied *Phalaenopsis* cultivars. Cultivars with purple flowers such as Queen Beer Mantefon exhibited the highest levels of both anthocyanin and carotenoid contents indicating that these pigments contribute synergistically to the flower's coloration.

In contrast, yellow-flowered cultivars like Taipei Gold Gold Star contained anthocyanins in minimal quantities, with carotenoids serving as the dominant pigments. Notably, flowers with white petals and yellow labellum, such as Sogo Yukidian V3, displayed lower carotenoid content compared to entirely yellow flowers.

Interestingly, an inverse relationship was observed between flower and leaf pigment content with cultivars displaying low anthocyanin and carotenoid levels in their flowers while exhibiting higher levels in their leaves. This suggests a potential redistribution or differential regulation of pigment synthesis in different plant parts. Therefore, a wide variation in distribution of carotenoid and anthocyanin pigments was observed in different plant parts of the same cultivar. Furthermore, some flowers exhibited visible coloration that did not align with their measured pigment content, highlighting the complexity of pigment interactions. As reported by Narbona *et al.* (2021), variations in the type or ratio of pigments can influence flower color, while changes in pigment concentration primarily affect color intensity.

The results also underline the intricate patterns and color variations in *Phalaenopsis* flowers, which range from simple monochromatic tones to complex patterns. Such variability is typical of orchids and other ornamental species like irises and crowfoots. This study provides valuable insights into the pigment composition and color diversity in *Phalaenopsis* flowers, enhancing our understanding of their aesthetic and physiological characteristics.

Among the different plant parts analyzed, roots exhibited the highest flavonoid content, while flowers showed the lowest. Flavonoids, known for their multifunctionality, play important roles in various physiological and ecological processes. In epiphytic orchids, such as Phalaenopsis, the velamen radicum is vital for water and nutrient absorption, storage, and UV-B protection. Flavonoids are also integral to leaves and other epidermal tissues, providing photoprotection and antioxidant properties that safeguard plants from environmental stressors (Nguyen *et al.*, 2018). In flowers, flavonoids serve as UV-B protectants and as attractants for pollinators, facilitating successful pollination. Interestingly, cultivars with white petals, such as Red Lip 1770 and Sogo Yukidian V3, exhibited higher flavonoid content despite having minimal anthocyanin and carotenoid levels. This is consistent with findings that white petals, which reflect all wavelengths of visible light, often contain high concentrations of UV-absorbing flavonoids like flavones and flavonols (Narbona *et al.*, 2021). The significant variations in flavonoid content among cultivars may emphasize the potential of selecting cultivars with high flavonoid levels in specific plant parts as parental materials for breeding programs. A study on flowers, leaves, and stems of White Clover cultivars revealed the potential for selecting cultivars with targeted concentrations of flavonoids (Carlsen *et al.*, 2008). Hence, applying a similar selection approach in Phalaenopsis cultivars could enhance desirable traits, including stress tolerance and aesthetic appeal. Plant phenolics, a class of secondary metabolites, play a major role in mitigating oxidative stress by acting as strong antioxidant agents (Trelka *et al.*, 2010). By neutralizing reactive oxygen species (ROS), phenolics help minimize oxidative damage in plants, contributing to improved resilience and longevity. Additionally, phenolics are involved in delaying senescence, thereby enhancing the functional and aesthetic lifespan of plant organs, as highlighted by Cavaiuolo *et al.* (2013).

In this study, Golden Sands Canary exhibited the highest polyphenol content in both leaves and flowers, indicating its superior capacity to combat oxidative stress. Conversely, BS showed the lowest polyphenol levels in leaf and root extracts, suggesting a comparatively weaker antioxidant defense. These differences suggest that polyphenol content is a critical determinant of a plant's ability to endure oxidative stress, which, in turn, influences its storage life and performance as a potted plant or cut flower.

Selecting and breeding cultivars with high phenolics content could enhance stress tolerance and longevity in ornamental plants. Such efforts would not only improve the plants' defensive mechanisms but also potentially extend their market value and usability. Orchid varieties have high phenolics and flavonoids compounds and have higher restraint power of free radicals. Moreover, a relationship can be observed between phytochemicals and morphological traits (Ebrahimi *et al.,* 2020). The findings underscore the importance of incorporating antioxidant-related traits into breeding programs to produce robust and resilient *Phalaenopsis* cultivars with superior oxidative stress management capacities.

Breeding efforts for *Phalaenopsis* have largely

focused on producing white, pink, and red hybrids, leading to market saturation. Orchid breeders are now shifting their focus toward developing flowers with unique pigmentation patterns distributed across various regions of the flower (Hsu *et al.,* 2018). The findings of this study highlighted the potential of leveraging cultivars with differential pigment accumulation to achieve this goal. For instance, combining anthocyanin- and carotenoid-rich cultivars such as *Phalaenopsis* cv. Queen Beer Mantefon with white cultivars like Red Lip 1770 and Sogo Yukidian V3 could result in novel hybrids with diverse and striking pigmentation patterns. The creation of novel flower coloration patterns is critical for maintaining consumer interest in *Phalaenopsis* orchids, as aesthetic appeal remains a key factor driving market demand. Among the tested cultivars, Queen Beer Mantefon stood out for its high concentrations of both anthocyanin and carotenoids, while *Phalaenopsis* cv. Brother Strips and Golden Sands Canary reported the highest flavonoid and polyphenol contents, respectively. These traits indicate potential resilience and extended storage life, adding further value to these cultivars as breeding materials. Additionally, the study revealed significant variation in chlorophyll, flavonoid, and polyphenol content across the vegetative parts of different cultivars. These traits are equally important for breeding robust plants with enhanced stress tolerance, longevity, and overall vigor.

In conclusion, this study provides insights into floral pigmentation and phytochemical composition of vegetative organs, facilitating the identification and selection of desirable traits for *Phalaenopsis* breeding programs. By selecting parental materials based on pigment profiles and vegetative characteristics, breeders can develop new cultivars with enhanced aesthetic appeal and improved physiological performance. Such advancements will not only enhance the ornamental value of *Phalaenopsis* orchids but also strengthen their adaptability and commercial potential. Despite these findings, the relationship between phytochemicals and the physiological traits in commercial *Phalaenopsis* cultivars remains unexplored. Among the studied commercial cultivars, Taipei Gold Gold Star, Golden Sands Canary and Queen Beer Mantefon can be recommended as potential parental candidates for hybridization due to their distinctive floral colorations and robust vegetative traits for adaptability.

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References

AXIOTIS E., ANGELIS A., ANTONIADI L., PETRAKIS E.A., SKALTSOUNIS L.A., 2021 - *Phytochemical analysis and dermo-cosmetic evaluation of* Cymbidium *sp. (Orchidaceae) cultivation by-products.* - Antioxidants, 11(1): 101.

BHARDWAJ R., KUMAR M., KAUSHAL N., KAMBOJ A.D., KRISHNAMOORTHI A., SINGH A., MOTLA R., 2024 - *From Lab to Bouquet: The biotechnological frontier in modern floriculture for sustainable and resilient flower farming.* - J. Adv. Biol. Biotechnol., 27(2): 119-137.

CARDOSO J.C., VENDRAME W.A., 2022 - *Innovation in propagation and cultivation of ornamental plants.* - Horticulturae, 8(3): 229.

CARDOSO J.C., VILCHERREZ-ATOCHE J.A., IIYAMA C.M., GERMANÀ M.A., VENDRAME W.A., 2023 - *Breeding of Orchids using conventional and biotechnological methods: advances and future prospects*, pp. 27-58. - In: TIWARI P., and J.T. CHENG (eds.) *Advances in orchid biology, Biotechnology and omics*. Springer Nature, Singapore, pp. 283.

CARLSEN S.C., MORTENSEN AG., OLESZEK W., PIACENTE S., STOCHMAL A., FOMSGAARD I.S., 2008 - *Variation in flavonoids in leaves, stems and flowers of white clover cultivars.* - Nat. Prod. Commun., 3(8): 1299-1306.

CAVAIUOLO M., FERRANTE A., CIRIELLO G., 2013 - *The role of polyphenols in delaying senescence and enhancing the functional and aesthetic lifespan of plant organs*. - J. Plant Biol., 68(4): 276-283.

CHASE M., CAMERON K., FREUDENSTEIN J., PRIDGEON A., SALAZAR G., VAN DEN BERG C., SCHUITEMAN A., 2015 - *An updated classification of Orchidaceae* - Bot. J. Linn. Soc*.,* 177(2): 151-174.

DARRAS A.I., 2020 - *Implementation of sustainable practices to ornamental plant cultivation worldwide: A critical review.* - Agronomy, 10(10): 1570.

EBRAHIMI A., ASADI A., MONFARED S.R., SAHEBI M., REZAEE S., KHALEDIAN Y., 2020 - *Evaluation of phenotypic diversity of the endangered orchid (*Orchis mascula*): Emphasizing on breeding, conservation and development.* - S. Afr. J. Bot., 132: 304-315.

HE T., FAN X., HUANG T., ZHANG Y., 2023 - *Phytochemical diversity of cultivated* Dendrobium nobile lindl. *in Southwestern China and its association with environmental conditions.* - Appl. Ecol. Environ. Res., 21(5): 3923-3942.

HSIAO Y., PAN Z., HSU C., YANG Y., HSU Y., CHUANG Y., SHIH H., CHEN W., TSAI W., CHEN H., 2011 - *Research on orchid biology and biotechnology*. - Plant Cell Physiol., 52(9): 1467-1486.

HSU C., CHEN H., CHEN W., 2018 - Phalaenopsis, pp. 567-625. - In J. HUYLENBROECK (ed.) *Ornamental crops*. Springer International Publishing, Cham, Switzerland, pp. 887.

LALROSANGPUII, LALROKIMI, 2021 - *A review on the phytochemical properties of five selected genera of orchids*. - Sci Vis., 21(2): 50-58.

LATTANZIO V., CARDINALI A., LINSALATA V., 2012 - *Plant Phenolics: A biochemical and physiological perspective*, pp. 1-39. – In: CHEYNIER V., P. SARNI-M, and S. QUIDEAU (eds.) *Recend advances in polyphenol research*. John Wiley & Sons, Ltd, Ames, IA, USA, pp. 364.

LEE Y., TSENG Y., LEE Y., CHUNG M., 2020 - *Chromosome constitution and nuclear DNA content of* Phalaenopsis *hybrid*s - Sci. Hortic*.,* 262:109089.

LIGHTBOURN G., GRIESBACH R., NOVOTNY J., CLEVIDENCE B., RAO D., STOMMEL J., 2008 - *Effects of anthocyanin and carotenoid combinations on foliage and immature fruit color of* Capsicum annuum L - J. Hered*.,* 99(2): 105-111.

LIN M., HSU B., 2004 - *Photosynthetic plasticity of* Phalaenopsis *in response to different light environments* - J. Plant Physiol*.,* 161(11): 1259-1268.

LING L., SUBRAMANIAM S., 2007 - *Biochemical analyses of* Phalaenopsis violacea *orchids.* - Asian J. Biochem., 2(4): 237-246.

MARTIN C., MAS E., LU C., ONG B., 2010 - *The photosynthetic pathway of the roots of twelve epiphytic orchids with CAM leaves*. - Photosynthetica*.,* 48(1): 42-50.

MINH T.N., KHANG D.T., TUYEN P.T., MINH L.T., ANH L.H., QUAN N.V., HA P.T.T., QUAN N.T., TOAN N.P., ELZAAWELY A.A., XUAN T.D., 2016 - *Phenolic compounds and antioxidant activity of* Phalaenopsis *orchid hybrids* - Antioxidants, 5(3): 31.

NARBONA E., DEL VALLE J.C., WHITTALL J.B., 2021 - *Painting the green canvas: how pigments produce flower colours*. - Biochemist, 43(3): 6-12.

NGUYEN H., KUAN-HUNG L., HUANG M., CHI-MING Y., TIN-HAN S., HSIUNG T., YEN-CHANG L., FUN-CHI T., 2018 - *Antioxidant activities of the methanol extracts of various parts of* Phalaenopsis *orchids with white, yellow, and purple flowers.* - Notul. Bot. Hort. Agrobot. Cluj-Napoca*,* 46(2): 457-465.

NURCAHYANI E., SHOLEKHAH., SUMARDI., QUDUS H., 2021 - *Analysis of total carbohydrate and chlorophyll content of the orchid plantlet [*Phalaenopsis amabilis (L.) Bl*.] resistant* Fusarium *wilt disease.* - J. Phys. Conf. Ser*.,* 1751: 1-6.

PORRA R., 2002 - *The chequered history of the development and use of simultaneous equations for the accurate determination of chlorophylls a and b*. - Photosynth. Res*.,* 73: 149-156.

THACKER H., RAM V., 2020 - *Role of phytochemicals in plants: A Review.* - Int. J. Res. Appl. Sci. Eng. Techn., 8(XII): 19-21.

TIWARI P., SHARMA A., BOSE S.K., PARK K.I., 2024 - *Advances in orchid biology: Biotechnological achievements, translational success, and commercial outcomes.* - Horticulturae, 10 (152).

TRELKA T., BREŚ W., JÓŹWIAK A., KOZŁOWSKA A., 2010 - Phalaenopsis *cultivation in different media. Part II. Nutrients and chlorophyll concentration in leaves and roots*. - Acta Sci. Pol. Hortorum Cultus, 9(3): 95-104.

VOEGELPOEL L., 1990 - *Flower color. An appreciation.* - Orchid Digest., 55: 82-87.

WANI T.A., BHAT I.A., GULERIA K., FAYAZ M., ANJU T., HARITHA K., KUMAR A., KALOO Z.A., 2022 - *Phytochemicals: Diversity, sources and their roles*, pp. 3-33. - In: SWAMY M.K., and A. KUMAR (eds.) *Phytochemical genomics: plant metabolomics and medicinal plant genomics*. Springer Nature, Singapore, pp. 698.

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Agro-morphological characterization of Indian garlic (*Allium sativum* L.) germplasm under mid hill of Northwest Himalaya

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Abstract: This study evaluated the genetic diversity of Indian long-day garlic genotypes based on agro-morphological and biochemical traits. A significant variation was observed across 23 traits, indicating high genetic diversity. Key traits such as bulb weight and 20-clove weight, leaf thickness, and clove showed substantial variability and antioxidant properties highlighted the potential for developing high-quality garlic varieties. Hierarchical cluster analysis grouped 94 genotypes into four clusters based on key traits, emphasizing their utility for breeding programs. Genotypes VGS-55 and VGS-43 excelled in growth traits, while VGS-51 and VGS-49 demonstrated superior biochemical content. Principal component analysis (PCA) revealed that the first two components accounted for 40.73% of the total variability, with yield-promoting traits dominating PC1 and biochemical traits influencing PC3. Trait association studies indicated strong positive correlations between bulb yield and traits like bulb weight (r=0.97 *) and equatorial diameter (r=0.73 ***), whereas no significant association was observed between bulb yield and biochemical traits. These findings underscore the immense genetic potential within Indian garlic germplasm for breeding programs targeting higher yields and improved biochemical traits, catering to the increasing demand for both bulbs and fresh garlic leaves in India.**

1. Introduction

Genus *Allium* includes about 918 species distributed all over the world (The Plant list, 2013), of which only 30 to 36 have been distributed in the Indian plains and Himalayan region (Santapau and Henry, 1973; Karthikeyan *et al.,* 1989). Among this garlic (*Allium sativum* L.) is one of the oldest and strongest flavoured *Allium* species. Owing to its typical

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flavor, garlic is mostly used as a spice worldwide in the form of fresh or stored bulbs (Block, 2010). In many regions, people also enjoy fresh garlic leaves in salad (Koch and Lawson, 1996). Additionally, there has been a recent increase in the consumption of dehydrated garlic (Ogar *et al.,* 2021). As a result of its versatility and popularity, garlic has become the second most important species in the *Amaryllidaceae* family, following onions, for both culinary and medicinal uses (Kamenetsky and Rabinowitch, 2001). Garlic is also an excellent source of vitamins and minerals (selenium), flavonoids, antioxidants, lectins, several enzymes, and amino acids (Pizzorno and Murray, 2005). It contains about 33 sulphur compounds (Albrecht *et al.*, 2017), of which allicin (diallyl-dithiosulfinate) is an important constituent. It is formed when non-proteinogenic acid alliin (S-allyl cysteine sulfoxide) is exposed to enzyme alliinase upon tissue damage (Fesseha and Goa, 2019). Allicin is responsible for garlic's characteristic pungent odour and medicinal value. However, composition and concentration of these compounds mainly depend on cultivar types, place of origin, and growing environments (Baghalian *et al.,* 2005; Khar *et al.,* 2011). Garlic consumption helps improve health by enhancing immunity (Percival, 2016), reducing cholesterol and triglycerides (Yeh and Liu, 2001), lowering blood pressure levels (Ried *et al.,* 2013), curing skin allergies (Lee and Park, 2003), and reducing cancer risk (Sengupta *et al.,* 2004). The numerous health benefits associated with this versatile plant highlight its significance in supporting human well-being.

Despite its health benefits, there exists a notable demand-supply gap in garlic production, particularly in India. This gap arises from the limited production in tropical regions, where cultivation faces constraints such as suboptimal climatic conditions and lower yields compared to temperate countries. Garlic requires a cold period (vernalization) for proper bulb development. The mild winters in much of India are less effective for vernalization compared to the prolonged and colder winters in temperate countries. Additionally, garlic cultivation in India is largely concentrated in specific regions, with the Indian Himalayan region contributing minimally to the overall supply. This geographical limitation contrasts sharply with high-yielding temperate countries, where garlic production is more prevalent and efficient (Lawande *et al.,* 2009). India is second only to China in terms of area and production of garlic, but the national average productivity is only 5 t/ha (FAOSTAT, 2017) and ranks 74th in the world (FAOSTAT, 2010).

Garlic plants are sensitive to photoperiod and temperature and thrive in temperate conditions. Fertile flowers and true seed formation in garlic are observed only in its primary diversity centre (Hong *et al.,* 2000). Garlic flowers are hermaphrodites and entomophilous and are pollinated primarily by bees, butterflies and moths. Flowering is controlled by several genetic factors such as photoperiod and temperature (Kamenetsky *et al.,* 2004). However, in a country like India, which is close to the place of origin, garlic clones often form bulbils (aerial bulbs) instead of flowers (Kamenetsky and Rabinowitch, 2001). Therefore, it is essentially regenerated by cloves or bulbils in the region. Such asexual propagation methods are generally favourable for maintaining the true-to-type identity and uniformity of a variety or accession because there is no segregation of alleles. Despite its sexually sterile nature, garlic has differences in various characteristics such as: morphological (maturity, plant growth) characteristics (Panthee *et al.,* 2006; Wang *et al.,* 2014) and biochemical characteristics (Bhusal *et al.,* 2019; Chadha *et al.,* 2019; Barboza *et al.,* 2020; Benke *et al.,* 2021), reproductive (maturity, bolting behaviour) (Kamenetsky and Rabinowitch, 2001), and bulb characteristics (bulb shape, bulb size, bulb colour, storage life) (Bradley *et al.,* 1996; Wang *et al.,* 2014). This diversity is thought to be due to sexual reproduction in the wild plant (Maab and Klaas, 1995), phenotypic plasticity (Bradley *et al.,* 1996), and extensive somatic mutations (Ata, 2005), mainly due to their apomictic nature.

However, this diversity presents challenges, particularly when considering the adaptation of garlic varieties to different climatic conditions. Garlic from temperate climates does not grow well in tropical and subtropical areas, making it difficult to compare different varieties of garlic in similar climate conditions. Although numerous studies have been conducted on genetic variation in garlic germplasm at various levels, there remains a significant gap in research focused on evaluation, selection and development of long-day garlic varieties or strains specifically suitable for Indian conditions. Bridging this gap requires a strategic effort to expand garlic production into underutilized regions (i.e. Himalayan

states), thereby ensuring wider availability of this health-enhancing crop. This highlights the need for targeted research to address the unique climate challenges faced by garlic farmers in India. To delve deeper into these aspects, this study aims to assess the genetic diversity and population structure of 94 garlic germplasms in the mid-hill regions of Uttarakhand, with special emphasis on morphological and biochemical traits. This information serves as a starting point for improving breeding strategies and developing high-yielding, regionally adapted long day garlic varieties. Ultimately, this research aims to address the challenges of low productivity and promote garlic crop improvement in Indian temperate conditions.

2. Materials and Methods

To identify suitable garlic material for the long day climatic conditions in mid-Himalayan region, an experiment was conducted using a total of 94 garlic accessions collected from the Indian Himalayan region and distributed under the All-India Network Research Project (AINRP- Onion and Garlic) and was maintained at Institute Genebank. The current study was conducted over two consecutive years, during the Rabi seasons of 2020-21 and 2021-22, at the experimental farm of ICAR-Vivekananda Parvatiya Krishi Anusandhan Sansthan, located in Hawalbagh, Almora, Uttarakhand (latitude 29°35' N, longitude 79°39' E, and an elevation of 1250 meters above sea level). The region experiences a warm, temperate climate characterized by substantial but unpredictable rainfall, averaging 1132.5 mm annually. Summer lasts from late June to September, with an average yearly temperature of 23.5°C. The annual maximum temperature ranges between 20.0°C and 38.1°C, while the minimum temperature varies from 6.6°C to 25.2°C (Dev *et al.*, 2015). As per the Köppen Climate Classification, the area lies in the northern hemisphere and is classified under the "Cfa" subtype, indicating a humid subtropical climate.

Ninty-four garlic genotypes were cultivated in finely prepared soil with a planting depth of 20–30 cm. Prior to sowing, the experimental field was enriched with 20 t/ha of well-decomposed farmyard manure (FYM), which was thoroughly mixed into the soil. Consistent field management practices, including nutrient application, irrigation, and intercultural operations, were maintained throughout the cropping period. Nitrogen, Phosphorus, Potash, and Sulfur (NPKS) at 110:50:50:50 kg/ha were used as fertilizers. The first half of the N dose and the total doses of P and K were given at the time of transplantation, with the remaining N dose given 30 and 45 days later. Each genotype was planted in two rows, with row spacing of 15 cm and plant spacing of 10 cm, over a row length of 2 m. Cloves were directly sown in shallow line at a depth of 3-4 cm during the winter seasons of October 2020 and 2021. Watering was provided as required to prevent leaf wilting. The crop was harvested when the leaves reached senescence or necks fell off.

All morphological traits were recorded on five random plants in each replication. Data were recorded from five random plants in each replication for each 94 genotype to assess morphological, biochemical differences. These germplasms consist of varieties, landraces, improved materials, and cultivars (SM Table 1S). The observations were recorded on sixteen morphological and seven biochemical parameters. Morphological traits including number of leaves, fourth leaf width (mm), fourth leaf length (cm), pseudostem length (cm), pseudostem diameter (mm), plant height (cm), and neck thickness (mm) were recorded at 80 days of planting after completion of vegetative growth when the crop was in the field. While, other bulb traits viz. number of cloves per bulb, average weight of bulb (g), bulb polar diameter (mm), bulb equatorial diameter (mm), and weight of 50 cloves (g) were recorded after harvest at neck fall.

Clove samples were randomly taken from the harvested crop to quantify the above biochemical and antioxidant traits. Total soluble solids (TSS) were determined immediately after manual juice extraction from the macerated sample using a cotton cloth using a handheld digital refractometer model PAL-3 (ATAGO, Japan) and expressed in °Brix. The qualitative characteristics of the genotypes were recorded according to the descriptors of the Plant Varieties and Farmers' Rights Authority, Government of India. The biochemical properties, namely total soluble solids (TSS), 2,2'-azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH), total antioxidant activity (TAA), ferric reducing antioxidant power (FRAP), and Total polyphenols (TPP) were estimated in the quality laboratory of the Institute.

Chemicals and reagents

All of the solvents employed in the research were of analytical purity, whereas the water was HPLC pure. The solvents and reagents were procured from Merck-Sigma (Bangalore, India) and used without further purification. The extraction and analysis were carried out using UV-vis spectroscopy. Different biochemical observations were made on the harvested fruits. The experiments were done in triplicate, and the data are shown as mean values ± standard deviations.

Preparation of extracts

Methanolic extracts of garlic were used to measure antioxidant metabolites and activities. Fresh samples (2 g) were ground in a pestle and mortar in 20 ml of 80% methanol. The methanolic extracts of the samples were placed in an orbital shaker overnight (16 hours) to completely extract antioxidant metabolites. The methanolic extract was centrifugated at 5000 rpm for 10 minutes, and the supernatant was kept at 4°C for further analysis. Methanolic pure (80%) extract was used for the study. All assays were performed out in triplicate, and results are expressed as mean ± standard deviations.

Total polyphenolic content and antioxidant activities

Total polyphenolic content. The Folin-Ciocalteau reagent was used to calculate total phenolic compounds (Singleton and Rossi, 1965). The final solution was vigorously stirred in a vortex mixer after adding 0.9 mL of distilled water, 0.5 mL of Folin-Ciocalteau reagent and 2.5 mL of sodium carbonate solution to the freshly obtained extract. The absorbance was measured at 725 nm after keeping the reaction at 300°C for 40 min. The standard curve for gallic acid was prepared by taking a different concentration of gallic acid (10-100 µg).

Determination of 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH•). With some alterations, the DPPH test was carried out by detecting the reduction in absorbance of a methanolic DPPH solution at 515 nm in the presence of the extract (Brand-Williams *et al.,* 2005). The stock solution was made by dissolving 24 mg of DPPH in 100 mL methanol which was then stored at -20°C until needed, while, working solution was made by mixing 10 mL stock solution with 45 mL methanol to achieve an absorbance of 1.17±0.02 units at 515 nm. Garlic extracts (150 µL) were allowed to react for 24 hours in the dark with 2850 µL of DPPH working solution, and the absorbance was measured at 515 nm. The radical scavenging activity of DPPH• was calculated as a percentage of DPPH• discolouration using the equation:

$$\text{Radical scavenging (percent)} = (\text{A control} - \text{A sample}) / \text{A control} \times 100$$

Where, A sample is the absorbance of the solution recorded while adding extract/reference at a specific level. A control is the absorbance of the DPPH solution without adding extract.

Determination of scavenging effect on $ABTS^{\bullet+}$ radicals

The ABTS test was carried out by detecting the reduction in methanolic ABTS solution absorbance at 745 nm in the presence of the extract (Arnao *et al.,* 2001). The workable solution wa made by combining two stock solutions in equal amounts and allowing them to react for 12 hours at room temperature in the dark. The solution was then diluted to attain an absorbance of 0.9 0.02 units at 745 nm by combining 1 mL ABTS solution with 3 mL methanol. Garlic extracts (200 litres) were allowed to react for 30 minutes in the dark with 2000 liters of newly made ABTS solution, and absorbance was measured at 745 nm.

The percentage inhibition was calculated using the equation:

$$\text{Radical scavenging (percent)} = (\text{A control} - \text{A sample}) / \text{A control} \times 100$$

Where, A sample is the absorbance of the solution recorded when adding extract/reference at a specific level, and A control is the absorbance of the ABTS solution without extract.

Determination of total antioxidant activity

The total antioxidant activity of the methanolic extracts of both samples was determined using a phosphomolybdenum technique (Prieto *et al.,* 1999), which is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the creation of green phosphate / Mo (V) compounds. A sample extract of 0.3 mL was mixed with 2.7 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample was sealed and incubated for 90 minutes in a boiling water bath at 95°C. The absorbance was measured at 695 nm after the samples had cooled to room temperature. Total antioxidant activity was measured in trolox

equivalents (mM/g of extract).

Determination of ferric-reducing antioxidant power (FRAP)

The FRAP test was performed with few changes according to Benzie and Strain. 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 mL $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$ solution were produced as stock solutions. 25mL acetate buffer, 2.5mL TPTZ solution, and 2.5mL $FeCl_3.6H_2O$ solution were mixed and warmed to 37^0C before use to make a fresh working FRAP solution. Methanolic extracts of samples (150 L) were allowed to react with 2850 L of FRAP solution in the dark for 30 minutes, and the coloured product (ferrous tripyridyltriazine complex) was measured at 593 nm. The FRAP value was calculated by drawing a standard curve formed by adding ferrous sulfate heptahydrate (20-200 mM) to the FRAP reagent, with the results represented in mM equivalent to $FeSO_4.7H_2O$.

Statistical analysis

The experiment employed a randomized complete block design with two replications, using a mean value over two years for each trait in the diversity analyses. Using multivariate methods, such as principal component analysis, agro-morphological diversity was analysed (Jolliffe, 1986). R software was used to do multivariate cluster analysis based on PCA charts and Ward's technique (Ward, 1963). Biplot-PCA, Variables-PCA, and Individuals-PCA graphics of PCA visualisations were created using the R packages "devtools,""ggplot2," and "factoextra." Trait correlations were computed using the "metan" package in R, while Principal component scores and descriptive analysis were obtained via PAST04 software (Hammer *et al.,* 2001).

3. Results

Genetic diversity based on agro-morphological and biochemical traits

The results showed a huge variation among the characters of interest of Indian long-day garlic in terms of the twenty-three morphological and biochemical characters as given in supplementary materials Table 1S and Table 2S. Descriptive data analysis revealed that most traits, except for a few variables, exhibit high genetic diversity. The most variable traits were stem thickness, leaf thickness, bulb weight, bulb diameter, clove width, 20-clove weight and DPPH (% inhibition) with CV values greater than 35 percent. Among the 20 agronomic traits, ABTS (percent inhibition) (CV=9.33%) achieved the lowest CV value. Yield characteristics differed significantly between garlic genotypes, with bulb weight ranging from 6.50 to 35.13 g (CV= 34.60%) and 20 clove weight ranging from 9.0 to 68.0 g (CV= 27.08%). The number of cloves per bulb varied between 5.80 and 39.60. The polar diameter ranged from 2.17 to 4.10 cm (CV= 11.54%), while the equatorial diameter varied from 2.27 to 5.07 cm (CV= 15.73%).The average clove length was 26.90 mm, ranging from 16.33 to 41.50 mm, while clove width varied from 2.83 to 39.33 mm, with an average of 12.77 mm. Genotypes VGS-43 and VGS-103 were noted for their clove length and width. Leaf thickness ranged from 0.70 mm (VGS-98) to 1.85 mm (VGS-55), length from 13.00 cm (VGS-51) to 45.13 cm (VGS-96), and width from 1.15 cm (VGS-91) to 3.75 cm (VGS-43), with coefficients of variation of 14.08%, 17.49%, and 23.66%, respectively. Plant height varied from 14.80 cm (VGS-82) to 44.80 cm (VGS-55), and the number of leaves ranged from 4.20 (VGS-49) to 9.0 (VGS-95), with coefficients of variation of 19.03% and 17.49%, respectively. The increasing demand for fresh garlic leaves in India highlights the potential of these diverse genotypes for producing leafy garlic varieties. Additionally, there was considerable variation in quality metrics like TSS and total carbohydrates, with TSS being crucial for assessing bulb quality in the tested accessions.

Among 94 genotypes, 43 genotypes had TSS values in-between 16.16 and 43.90°Brix, with VGS-67 having the highest at 43.90°Brix, followed by VGS-19 at 43.60°Brix, and VGS-5-2 with the lowest at 16.16°Brix. Garlic cloves primarily consist of carbohydrates, making up 33.06% of the total carbohydrate content (USDA National Nutrient Database).The study found that carbohydrate content in the genotypes ranged from 14.39% (VGS-8) to 28.81% (VGS-11B), with an average of 21.42%. Important antioxidant properties suggested potential for developing healthier varieties. Total polyphenols varied between 0.30 and 1.25 mg GAE/g DW, with a standard deviation of 0.15 and a coefficient of variation of 29.90%. The DPPH inhibition ranged from 10.08% to 49.23%, with a standard deviation of 10.08 and a coefficient of variation of 49.23%.The free radical inhibition percentage against ABTS in garlic

accessions ranged from 31.65% to 83.42%, averaging 62.14%, indicating low genotypic variation. The total antioxidant activity varied between 12.71 and 66.43 mM Trolox equivalent/g DW, with a standard deviation of 6.43 and a coefficient of variation of 24.97%. The FRAP values ranged from 95.04 to 269.32 mM FeSO4 equivalent/g DW, with a standard deviation of 32.77 and a coefficient of variation of 21.26%. VGS-51 had the highest FRAP values among the genotypes tested (Table 1).

Hierarchical cluster analysis

Cluster analysis using Ward's minimum variance (Ward, 1963) identified four main groups among 94 genotypes, explaining 29.78%, 51.06%, 17.02%, and 2.12% of total germplasm, respectively (Fig. 1). Cluster 1, consisting of 28 genotypes, showed associations with higher clove numbers (e.g., VGS-32, VGS-33), PST (VGS-33), PD (Bhima Omkar, VGS-06, VGS-103), BW (VGS-89), and TY (VGS-89). All released varieties, except Swarna-9, are in this cluster. The second cluster, the largest with 48 genotypes, is characterized by maximum plant height (VGS-55), LT (VGS-55), and higher TSS (VGS-35, VGS-36, VGS-37, VGS-13 and VGS-14), TAA (VGS-11-B), and (VGS-11-B). This cluster is advantageous for selecting genotypes with enhanced growth and bulb quality. The third cluster included thirteen genotypes with

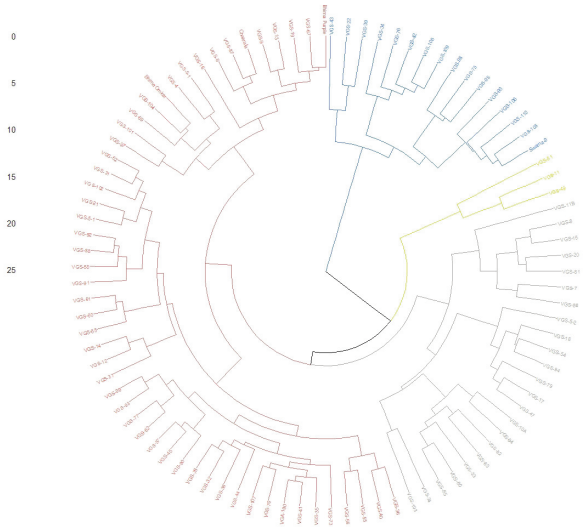

Fig. 1 - Dendrogram of cluster analysis of garlic germplasm based on morphological and biochemical traits (Ward Method).

Table 1 - Summary statistics morphological and biochemical characteristics of garlic accessions

Characteristics	Abbreviation	Min	Max	Mean	SE	Variance	SD	CV (%)
Plant height (cm)	PH	14.80	44.80	31.39	0.62	35.70	5.97	19.03
Leaf length (cm)	LL	13.00	45.13	32.01	0.58	31.35	5.60	17.49
Leaf width (cm)	LW	1.15	3.75	1.93	0.05	0.21	0.46	23.66
No. of leaves/plant	LN	4.20	9.00	5.65	0.09	0.74	0.86	15.27
Pseudostem thickness (mm)	PST	6.20	18.58	10.76	0.32	9.86	3.14	29.19
Leaf thickness (mm)	LT	0.70	1.85	1.33	0.02	0.03	0.19	14.08
Polar diameter (cm)	PD	2.17	4.10	3.25	0.04	0.14	0.37	11.54
Equatorial diameter (cm)	ED	2.27	5.07	3.62	0.06	0.32	0.57	15.73
20 clove weight (gm)	CW-20	9.00	68.00	25.41	1.18	131.02	11.45	45.04
Clove length (mm)	CL	16.33	41.50	26.90	0.40	14.94	3.87	14.37
Clove width (mm)	CW	2.83	39.53	12.77	0.49	22.85	4.78	37.44
No of clove layer/ bulb	CNL	1.00	3.00	2.38	0.04	0.19	0.43	18.23
No of clove/ bulb	CN	5.80	39.60	17.92	0.65	39.35	6.27	35.01
Bulb weight (gm)	BW	6.50	35.13	21.35	0.60	33.43	5.78	27.08
Total Yield (q /ha)	TY	32.50	175.67	105.67	3.09	899.52	29.99	28.38
Plant weight (g)	PW	8.00	52.67	23.99	0.87	71.52	8.46	35.25
TSS (°Brix)	TSS	16.16	43.90	38.27	0.46	19.77	4.45	11.62
Total Polyphenols (mg GAE/g)	TPP	0.30	1.25	0.51	0.02	0.02	0.15	29.90
DPPH (% Inhibition)	DPPH	10.08	49.23	27.49	1.01	96.64	9.83	35.76
ABTS (% Inhibition)	ABTS	31.65	83.42	62.14	0.60	33.62	5.80	9.33
TAA (mM Trolox equivalent/g DW)	TAA	12.71	66.43	25.74	0.66	41.32	6.43	24.97
FRAP Vale (mM $FeSo_4$ equivalent/g	FRAP	95.04	269.32	154.13	3.38	1074.06	32.77	21.26
Total carbohydrate (%)	TCA	14.39	28.81	21.42	0.35	11.76	3.43	16.01

distinctive leaf traits such as leaf length (VGS-96), leaf width (VGS-43), leaf number (VGS-95), and several yield-related traits such as bulb weight (VGS-96, VGS-105, Swarna-9) and total yield (VGS-39). In contrast, the fourth cluster contained only two genotypes, VGS-51 and VGS-49, which were characterized by their high biochemical content, including TPP, DPPH, ABTS, and FRAP.

Trait association study

The total bulb yield showed significant positive correlations with BW (0.97***), ED (0.73***), CW (0.24*), LN (0.39***), LL (0.29**), LW (0.24*), PST (0.48***), PW (0.66***), 20-CW (0.61***), and PD (0.25*). However, no association was found between bulb weight and various biochemical traits, including TAA, ABTS, total phenolics, and DPPH activities, suggesting that bulb weight has no influence on bulb quality traits. Furthermore, bulb weight, TSS, and bulb diameter had no relationship with plant height (Fig. 2). Conversely, TSS was negatively correlated with 20-clove weight (-33**), clove ABTS (-0.29**), and DPPH activity (-0.27**), while there was a non-significant negative correlation with clove phenol content (TPP), TAA, and FRAP values. Significant positive correlations were observed among DPPH (0.66**), TAA (0.39***), ABTS (0.55***), and FRAP (0.60***), indicating a complex gene action mechanism involving garlic quality parameters.

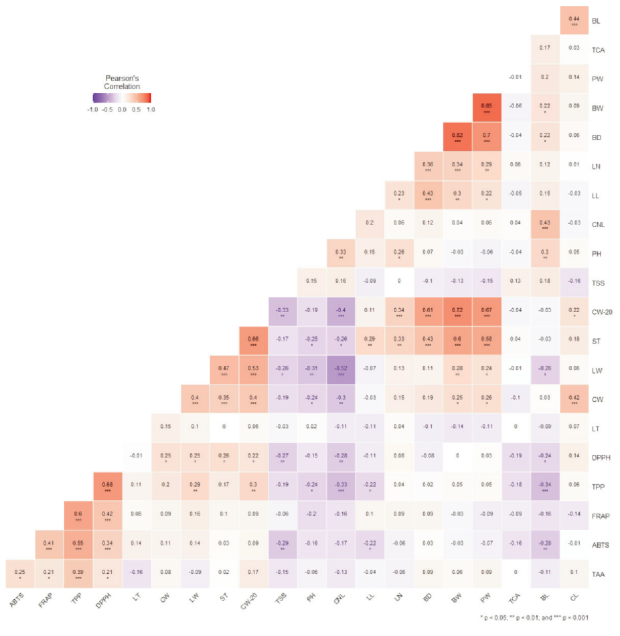

Fig. 2 - Pearson correlation matrix for morphological and biochemical traits in Indian long day garlic germplasm.

Principal component and Biplot analysis

Prior to performing Principal Component Analysis (PCA), Pearson correlation analysis was conducted to identify and remove highly correlated variables (BW and ED), as such variables can disproportionately influence the PCA results. The first principal component (PC1) accounts for 22.77% of the total variation, with the highest eigenvalue of 4.78. It includes features such as CW-20, PST, PW, TPP, DPPH and LW, which have positive contributions, while CN, CNL and TSS have negative effects (Table 2). The second principal component (PC2) has an eigenvalue of 3.22 and explains 15.31% of the variation. Positive contributions come from traits such as PD, TY, and PW, whereas most of the biochemical traits *i.e.,* TPP, DPPH, ABTS, TAA, and FRAP have negative contributions. The third principal component (PC3) explained 8.38% of the total variation, mainly due to biochemical features such as FRAP, DPPH (% inhibition), TPP, ABTS, TAA and a morphological trait *i.e.,* PH. The fourth principal component (PC4) accounted for 7.16% of the variation and was primarily influenced by clove length (CL), clove width (CW) and plant diameter (PD). Leaf thickness (LT) had the highest loadings in the fifth principal component (PC5), while total carbohydrates (TCA) and total soluble solids (TSS) dominated in the sixth principal component (PC6), highlighting their importance. Yield- promoting traits were mainly captured in PC1 and PC2, while PC3 focused on biochemical traits and highlighted their role in phenotypic variation. TPP, DPPH, ABTS, LW, and FRAP made the main positive contributions based on the squared cosine (Cos2), while CW-20, ST, BW, TY, and PW made notable negative contributions (Fig. 3). Genotypes VGS-10A, VGS-84, VGS-82, VGS-79, VGS-54, and VGS-70 had the highest square cosine values, while VGS-96, VGS-109, VGS-105, VGS-108, VGS-76, VGS-42, VGS-49, VGS-51, and Swarna-9 were the least contributions (Fig. 4). A biplot of PC1 and PC2 revealed distinct trait groups, including CNSS, TY, and various biochemical traits (TPP, DPPH, ABTS, TAA, FRAP). Genotypes VGS-96, VGS-43, VGS-34, VGS-51, VGS-49, VGS-44, VGS-12, VGS-14, VGS-97, VGS-104, and VGS-89 were in gaps, highlighting their uniqueness (Fig. 5).

Table 2 - Principal component loadings for morphological and biochemical traits of garlic

Traits	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
PH	-0.20	0.18	0.34	0.05	0.24	-0.16	-0.32	-0.14	-0.36	0.01
LL	-0.01	0.26	0.18	-0.28	-0.15	-0.23	0.49	0.29	-0.26	0.24
LW	0.32	0.03	-0.27	-0.12	0.20	0.02	0.01	-0.09	-0.06	-0.22
LN	0.09	0.27	0.25	-0.18	0.33	0.12	0.00	-0.06	-0.31	-0.06
PST	0.30	0.26	-0.05	-0.14	0.00	0.09	0.17	0.02	0.04	-0.02
LT	0.04	-0.06	-0.08	0.12	0.63	-0.12	-0.06	0.44	0.14	0.41
PD	-0.14	0.32	0.12	0.41	-0.04	0.12	0.22	0.03	0.17	-0.10
CW-20	0.36	0.25	-0.04	-0.04	-0.01	0.03	-0.20	0.01	0.03	0.11
CL	0.09	0.15	-0.01	0.68	0.00	-0.03	0.15	-0.01	-0.16	-0.03
CW	0.26	0.10	-0.11	0.33	0.16	-0.04	0.19	-0.14	0.01	0.18
CNL	-0.27	0.16	0.29	0.06	-0.11	-0.10	0.10	0.19	0.37	-0.20
CN	-0.33	0.07	0.25	-0.01	0.23	0.09	-0.09	-0.07	0.01	-0.06
TY	0.16	0.41	0.17	-0.11	0.04	0.00	-0.26	-0.06	0.16	-0.04
PW	0.21	0.37	0.04	-0.06	-0.19	0.02	-0.17	-0.01	0.37	0.04
TSS	-0.22	0.02	-0.01	-0.09	0.18	0.48	0.17	-0.48	0.27	0.37
TPP	0.29	-0.25	0.33	0.05	0.07	0.15	-0.03	-0.09	0.10	-0.02
DPPH	0.26	-0.17	0.28	0.09	0.05	0.00	0.16	-0.25	-0.18	-0.33
ABTS	0.18	-0.26	0.28	0.07	0.08	-0.07	-0.23	0.27	0.31	-0.16
TAA	0.13	-0.11	0.28	0.16	-0.44	0.22	-0.28	0.09	-0.24	0.52
FRAP	0.18	-0.21	0.37	-0.14	0.08	0.20	0.42	0.13	0.11	0.05
TCA	-0.07	0.09	-0.15	0.03	0.02	0.71	-0.08	0.48	-0.22	-0.27
Eigen value	4.78	3.22	1.76	1.50	1.32	1.12	0.98	0.90	0.81	0.75
Variance	22.77	15.31	8.38	7.16	6.26	5.35	4.67	4.29	3.85	3.57
Cumulative variance	22.77	38.08	46.47	53.63	59.89	65.24	69.91	74.20	78.05	81.63

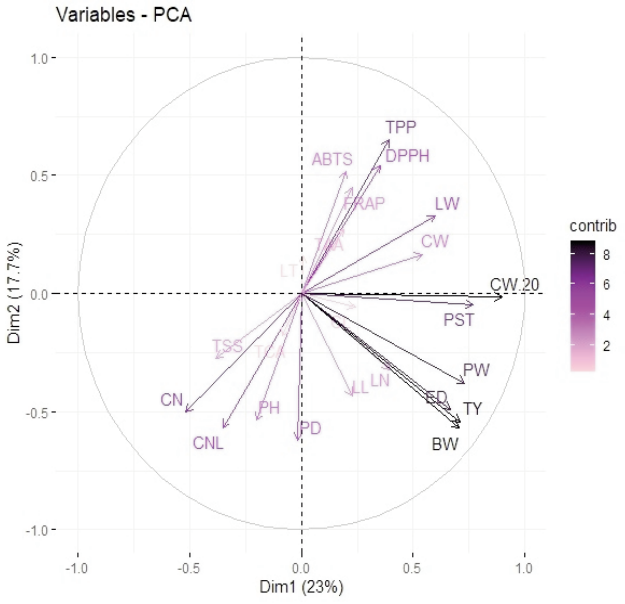

Fig. 3 - Contribution of different variables towards principal component analysis based on cos2 value.

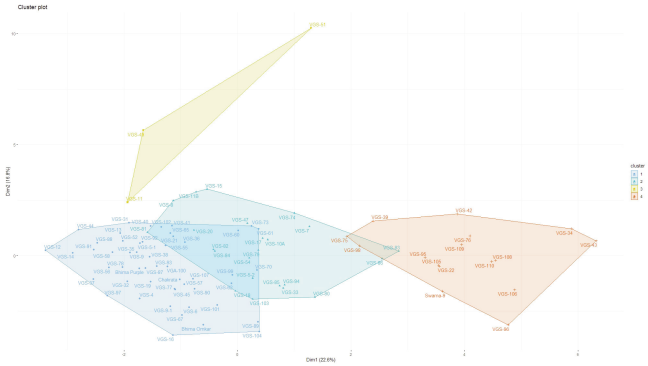

Fig. 4 - Contribution of different garlic accessions toward principal component analysis based on the cos2 value.

4. Discussion and Conclusions

The present study highlighted the significant genetic diversity among Indian long-day garlic

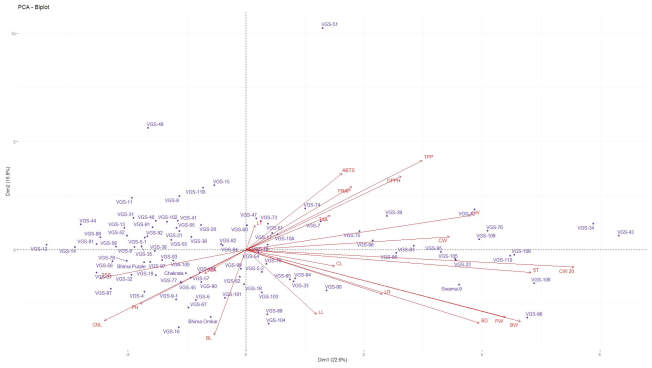

Fig. 5 - Segregation of 94 accessions of garlic according to morphological and biochemical traits determined by PC score and loadings.

genotypes based on agro morphological and biochemical traits. The wide range of mean and coefficient variations observed between traits highlights the potential for improving existing genotypes through clonal selection and paves the way for the development of new, tailored varieties suitable for high-altitude regions. Previously, Benke *et al.* (2020) reported similar genetic differences in garlic genotypes. Our results confirm the large variability found in morphological traits such as leaf width, stem thickness, bulb weight, 20-clove weight, clove width, and biochemical traits such as total phenolic content, DPPH inhibition, total antioxidant activity, and FRAP, and highlight their potential for selection and improvement. This is consistent with the findings of Bhusal *et al.*(2019); Narayan *et al.* (2019), who also found significant variability in yield-determining and biochemical traits in garlic genotypes and demonstrated the usefulness of such traits in the clonal selection highlighted programs. The variability of yield characteristics and biochemical properties offers opportunities for targeted selection. For example, features such as bulb equatorial diameter (CV = 56.54%) and clove length (CV = 34.82%), as reported by Ayed *et al.* (2019) indicate scope for clonal selection to develop new garlic varieties.

Furthermore, studies by Jabbes *et al.* (2012) and Baghalian *et al.* (2005) identified significant differences in plant height, number of cloves per bulb and bulb dimensions, consistent with the results of the current study. This variability forms the basis for improving both the agronomic and phytochemical properties of garlic. Present study also identified genotypes such as VGS-11B (high TAA and TCA content) and VGS-51 (high total phenolic content, DPPH, ABTS and FRAP activities) as candidates for developing healthier garlic varieties. Compared to existing commercial varieties, these genotypes showed significantly improved antioxidant and phenolic profiles, suggesting that they have the potential to meet growing consumer demand for nutrient-dense and health-promoting garlic products. These results echo Bhusal *et al.* (2019), who identified superior genotypes for antioxidant activity and suggested their use for health-oriented breeding programs. The analysis of the TSS (Total Soluble Solids) values in this study indicates a range of 16.16 to 43.90 °Brix, which relates these results to existing research and shows that they are slightly lower than those, observed in certain Indian genotypes from the western region (Bhusal *et al.,* 2019) and slightly higher than the values recorded for Egyptian garlic genotypes (Moustafa *et al.,* 2011).This variation highlights the potential for breeding programs aimed at growing garlic varieties with specific TSS characteristics. Such tailored varieties, particularly those with medium TSS and low pungency, can be particularly beneficial for improving post-harvest storage and processing, allowing growers to optimize quality and shelf life. Genotypes with high antioxidant activity and different TSS values are suitable for leaf purposes because TSS is not a crucial trait for selecting leaf genotypes. Our study found that VGS-55 (leaf thickness), VGS-43 (leaf width), VGS-95 (number of leaves) and VGS-96 (leaf length) are superior in leaf characteristics. Furthermore, Bhusal *et al.* (2019) identified PGS-105 as a genotype with higher antioxidant activity supporting improved health benefits in leaf production.

Despite the clonal propagation and inherent sterility of garlic, significant genetic variation has been observed, likely due to mutations, soma-clonal variations, or genetic transformation variations arising from sexual reproduction in the wild plant (Novak, 1990; Bradley *et al.,* 1996; Wang *et al.* 2014). These sources of variation are not unique to garlic and have also been reported in other clonally propagated crops such as potatoes (Zaag, 1987) and bananas (Ortiz and Vuylsteke, 1996). However, the extent and impact of such variations may vary depending on the genetic architecture and propagation method of each crop. The use of PCA and cluster analysis revealed four major groups

within the 94 genotypes, reflecting the diversity of morphological and biochemical traits. These clusters were dominated by the group of genotypes that were better in some traits. Fruit-related traits, for instance, were the most effective in differentiating across mango cultivars (Samsampour *et al.,* 2020). However, overlapping features were also recorded. Similarly, Benke *et al.* (2020) reported overlap in both qualitative and quantitative characteristics.

These findings align with Egea *et al.* (2017) and Wang *et al*. (2014), Oyetunde *et al*. (2021) on biochemical, color traits, and phenotypic diversity. Traits such as TPP, DPPH, and ABTS significantly influenced clustering patterns, emphasizing their role in defining genetic diversity, as seen in Chinese (Hassan *et al.,* 2015), Polish (Bozin *et al.,* 2008), and Serbian (Kim *et al.,* 2013) garlic genotypes.

Other studies also showed clustering based on various traits. For example, Bhusal *et al.* (2019) identified two clusters among 26 Indian garlic genotypes based on antioxidant and quality traits, Wang *et al.* (2014) clustered 212 garlic accessions into six groups based on morphological traits while Barboza *et al*. (2020) classified Argentinean genotypes into four groups using organo-sulfur and SSR markers. These studies highlight the value of clustering techniques in understanding genetic diversity and informing breeding programs. Furthermore, clustering patterns were unrelated to the geographic origins of the accessions, as noted by Benke *et al.* (2020). The clustering patterns and PCA results emphasize the importance of traits like TSS, antioxidant activity, and phenolic content in defining genetic diversity.

The high variability and significant correlations observed among yield and biochemical traits highlight the potential for using diverse genotypes in breeding programs. For example, strong correlations between bulb weight and clove dimensions could prioritize selection for these traits to enhance overall yield. Meanwhile, weak or non-significant correlations between yield and biochemical traits suggest that independent selection strategies might be necessary to balance productivity with quality improvements. This understanding enables breeders to design more focused and efficient strategies tailored to specific breeding goals. The total bulb yield showed strong positive correlations with traits like bulb weight, bulb diameter, and clove width, suggesting their importance in yield improvement. Conversely, the lack of association between bulb weight and biochemical traits indicates the need for independent selection strategies for yield and quality traits. Supporting this perspective, Jabbes *et al.* (2012), Imani and Shamili (2017) and Benke *et al.* (2021) found a strong positive correlation between marketable yield and yield-contributing traits in different garlic accessions. Panthee *et al.* (2006) reported comparable results in various Nepalese garlic accessions.

This study identifies the first two principal components (PCs) that encompass essential yield and biochemical traits, including bulb length, number of cloves, leaf count, plant weight, total soluble solids (TSS), clove length, total antioxidant activity (TAA), and total carotenoid content (TCA). Therefore, these PCs can aid in selecting genotypes with favorable horticultural and yield characteristics (Useche-Carrillo *et al.,* 2021). This collective evidence indicates that garlic's production potential is closely linked to its vegetative development, making these traits valuable for direct selection in garlic cultivation.

The study showed significant genetic diversity among 94 Indian garlic genotypes based on both agro-morphological and biochemical traits. Large differences were observed in traits such as bulb weight, clove size, leaf characteristics and biochemical parameters such as total polyphenols, antioxidant activity and total soluble solids (TSS). Notably, genotypes VGS-43 and VGS-103 had superior clove properties, while VGS-51 had the highest antioxidant properties. The cluster and principal component analyses identified distinct groups based on yield-promoting traits and biochemical content, providing a basis for selecting genotypes with specific desired traits. Yield-related traits such as bulb weight and 20-clove weight were positively correlated with several morphological traits but showed no association with biochemical traits such as total antioxidants, suggesting that these quality traits are independent of yield. The study highlights the potential of these different genotypes for breeding programs aimed at increasing both the yield and quality of garlic production, particularly improving antioxidant content and bulb quality. Future efforts should focus on integrating molecular markers into phenotypic assessments, which can help accelerate the development of high-yielding, nutrient-dense garlic varieties suitable for regional cultivation.

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References

ALBRECHT F., LEONTIEV R., JACOB C., SLUSARENKO A.J., 2017 - *An optimized facile procedure to synthesize and purify allicin*. - Molecules, 22: 770.

ARNAO M.B., CANO A., ACOSTA M., 2001 - *The hydrophilic and lipophilic contribution to total antioxidant activity*. - Food Chem., 73: 239-244.

ATA A.M., 2005 - *Constitutive heterochromatin diversification of two* Allium *species cultivated in Egypt*. - Proc. 7th Afr. Crop Sci. Soc. Conf., Kampala, Uganda, pp. 225-231.

AYED S., ENNOURI K., HANNACHI C., AYED S., 2019 - *Evaluation of genetic diversity in Tunisian garlic (*Allium sativum *L.) ecotypes*. - Genet. Resour. Crop Evol., 66: 995-1010.

BAGHALIAN K., ZIAI S.A., NAGHAVI M.R., BADI H.N., KHALICHI A., 2005 - *Evaluation of allicin content and botanical traits in Iranian garlic (*Allium sativum *L.) ecotypes*. - Sci. Hortic., 103: 155-166.

BARBOZA K., SALINAS M.C., ACUÑA C.V., BANNOUD F., BERETTA V., GARCÍA-LAMPASONA S., BURBA J.L., GALMARINI C.R., CAVAGNARO P.F., 2020 - *Assessment of genetic diversity and population structure in garlic (*Allium sativum *L.) germplasm collection varying in bulb content of pyruvate, phenolics, and solids*. - Sci. Hortic., 261: 108900.

BENKE A.P., KHAR A., MAHAJAN V., GUPTA A., SINGH M., 2020 - *Study on dispersion of genetic variation among Indian garlic ecotypes using agro-morphological traits*. - Indian J. Genet. Plant Breed., 80: 94-102.

BENKE A.P., KRISHNA R., MAHAJAN V., ANSARI W.A., GUPTA A.J., KHAR A., MANJUNATHA GOWDA D.C., 2021 - *Genetic diversity of Indian garlic core germplasm using agro-biochemical traits and SRAP markers*. - Saudi J. Biol. Sci., 28: 4833-4844.

BHUSAL R., ISLAM S., KHAR A., SINGH S., JAIN N., TOMAR B.S., 2019 - *Diversity analysis and trait association study for antioxidants and quality traits in landraces, farmers' varieties, and commercial varieties of Indian short-day garlic (*Allium sativum *L.)*. - Genet. Resour. Crop Evol., 66: 1843-1859.

BLOCK E., 2010 - *Garlic and other Alliums: The lore and the science.* - Royal Soc. Chem., Cambridge, UK, pp. 454.

BOZIN B., MIMICA-DUKIC N., SAMOJLIK I., GORAN A., IGIC R., 2008 - *Phenolics as antioxidants in garlic (*Allium sativum *L., Alliaceae)*. - Food Chem., 111: 925-929.

BRADLEY K.F., RIEGER M.A., COLLINS G.G., 1996 - *Classification of Australian garlic cultivars by DNA fingerprinting*. - Aust. J. Exp. Agric., 36: 613-618.

BRAND-WILLIAMS W., CUVELIER M.E., BERSET C., 1995 - *Use of free radical method to evaluate antioxidant activity*. - Food Sci. Technol., 28: 25-30.

CHADHA A., NAJLA MEZGHANI A., RHIMI A., DRIDI B.M., 2019 - *Morphological evaluation of Tunisian garlic (*Allium sativum *L.) landraces for growth and yield traits*. - J. Hortic. Postharvest Res., 2: 43-52.

DEV R., HEDAU N.K., PAL R.S., PASCHAPUR A., KANT L., 2025 Analysis of genetic diversity in caigua (Cyclanthera pedata (L.) Schrad.) in mid-hill of the Indian Himalayas. Plant Genet. Resour.: Characterisation Util., 23(4): 240-251

EGEA L.A., MÉRIDA-GARCÍA R., KILIAN A., HERNANDEZ P., DORADO G., 2017 - *Assessment of genetic diversity and structure of large garlic (*Allium sativum*) germplasm bank, by diversity arrays technology "genotyping-by-sequencing" platform (DArTseq)*. - Front Genet., 8: 98.

FAOSTAT, 2010 - *Global garlic production ranking*. - FAO, Food and Agriculture Organization of the United Nations. http://www.fao.org/faostat/en/

FAOSTAT, 2017 - *Garlic statistics*. - FAO, Food and Agriculture Organization of the United Nations. - http://www.fao.org/faostat/en/

FESSEHA H., GOA E., 2019 - *Therapeutic value of garlic (*Allium sativum*): A review*. - Adv. Food Technol. Nutr. Sci., 5: 107-117.

HAMMER Ø., HARPER D.A.T., RYAN P.D., 2001 - *PAST: Paleontological statistics software package for education and data analysis.* - Palaeontol. Electron., 4: 9.

HASSAN H.A.M., WANG H., LIU X., LI X., 2015 - *Impact of genetic factor and geographical location on allicin content of garlic (*Allium sativum*) germplasm from Egypt and China*. - Int. J. Agric. Biol., 17: 156-162.

HONG Y.S., HAM Y.A., CHOI J.H., KIM J., 2000 - *Effects of allyl sulfur compounds and garlic extract on the expression of Bcl-2, Bax, and p53 in non-small cell lung cancer cell lines*. - Exp. Mol. Med., 32: 127-134.

IMANI A., SHAMILI M., 2017 - *Phenology and pomology of almond's cultivars and genotypes using multivariate analysis.* - Adv. Hort. Sci., 32(1): 27-32.

JABBES N., ARNAULT I., AUGER J., AL MOHANDES DRIDI B., 2012 - *Agro-morphological markers and organo-sulphur compounds to assess diversity in Tunisian garlic landraces*. - Sci. Hortic., 148: 47-54.

JOLLIFFE I.T., 1986 - *Principal component analysis*. - Springer, New York, USA, pp. 271.

KAMENETSKY R., LONDON SHAFIR I., BAIZERMAN M., KHASSANOV F., KIK C., RABINOWITCH H.D., 2004 - *Garlic (*Allium sativum *L.) and its wild relatives from Central Asia: evaluation for fertility potential*. - Acta Horticulturae, 637: 83-91.

KAMENETSKY R., RABINOWITCH H.D., 2001 - *Floral development in bolting garlic*. - Sex. Plant Reprod., 13: 235-241.

KARTHIKEYAN S., JAIN S.K., NAYAR M.P., SANJAPPA S., 1989 - *Flora Indicae Enumeratio: Monocotyledonae*. - Flora Indica, 4. Botanical Survey of India, Howrah, Calcutta, India, pp. 435.

KHAR A., LAWANDE K.E., NEGI K.S., 2011 - *Microsatellite marker-based analysis of genetic diversity in short day tropical Indian onion and cross amplification in related* Allium *spp*. - Genet. Resour. Crop Evol., 58: 741-752.

KIM J.S., KANG O.J., GWEON O.C., 2013 - *Comparison of phenolic acids and flavonoids in black garlic at different thermal processing steps*. - J. Funct. Foods, 5: 80-86.

KOCH H.P., LAWSON L.D., 1996 - *Garlic: The science and therapeutic application of* Allium sativum *L. and related species*. - Williams & Wilkins, Philadelphia, USA, pp. 329.

LAWANDE K.E., KHAR A., MAHAJAN V., SRINIVAS P.S., SANKAR V., SINGH R.P., 2009 - *Onion and garlic research in India*. - J. Hort. Sci., 4: 91-119.

LEE J.W., PARK K.Y., 2003 - Allium *vegetables and cancer prevention*. - Am. J. Clin. Nutr., 78: 517S-520S.

MAAB H., KLAAS M., 1995 - *Interspecific hybridization between garlic (*Allium sativum*) and wild relatives*. - Euphytica, 84(3): 185-193.

MOUSTAFA Y., GADEL-HAK S.N.H., ABDEL NAEM G.F., ABDEL-WAHAB I.A., 2011 - *Studying different quantitative and qualitative traits of some white and colored bulb garlic genotypes grown under a drip irrigation system*. - Aust. J. Basic Appl. Sci., 5: 1415-1426.

NARAYAN R., SINGH D.B., KISHOR A., MER M.S., 2019 - *Studies on genetic variability, heritability and genetic advance in long day onion (*Allium cepa *L.) genotypes*. - Prog. Hortic., 51: 92-98.

NOVAK F.J., 1990 - Allium *tissue culture*, pp. 234-250. - In: RABINOWITCH H.D., and J.L. BREWSTER (eds.) *Onions and allied crops*. CRC Press, Boca Raton, FL, USA, pp. 332.

OGAR A., TIWARI B.K., CULLEN P.J., 2021 - *Dehydration of vegetables: Techniques and applications*. - Curr. Opin. Food Sci., 40: 1-7.

ORTIZ R., VUYLSTEKE D., 1996 - *Recent advances in* Musa *genetics, breeding and biotechnology*. - Plant Breeding Abstr., 66: 1355-1363.

OYETUNDE O.A., OLAYIWOLA M.O., OSHO B.T., 2021 - *Genetic diversity and trait profiles of some* Amaranthus *genotypes.* - Adv. Hort. Sci., 35(3): 277-284.

PANTHEE D.R., KC R.B., REGMI H.N., SUBEDI P.P., BHATTARAI S., DHAKAL J., 2006 - *Diversity analysis of garlic (*Allium sativum *L.) germplasms available in Nepal based on morphological characters*. - Genet. Resour. Crop Evol., 53: 205-212.

PERCIVAL S.S., 2016 - *Aged garlic extract modifies human immunity*. - J. Nutr., 146: 433S-436S.

PIZZORNO J.E. Jr., MURRAY M.T., 2005 - *USA textbook of natural medicine*, 3rd ed. Churchill Livingstone, London, UK, pp. 1944.

PRIETO P., PINEDA M., AGUILAR M., 1999 - *Spectrophotometric quantitation of antioxidant capacity through the formation of a phospho-molybdenum complex: specific application to the determination of vitamin E.* - Anal. Bioch., 269: 337-341.

RIED K., TOBEN C., FAKLER P., 2013 - *Effect of garlic on serum lipids: A meta-analysis*. - Nutr. Rev., 71: 282-299.

SAMSAMPOUR D., KAZEMZADEH-BENEH H., DAMIZADEH G.-R., MIRZAEI Z., 2020 - *Morphological and biochemical classification of Iranian Mango germplasm collection by multivariate analysis: Implications for breeding.* - Adv. Hort. Sci., 34(4): 381-395.

SANTAPAU H., HENRY A.N., 1973 - *A dictionary of the flowering plants in India*. - Pub. Inf. Directorate CSIR, New Delhi, India, pp. 198.

SENGUPTA A., GHOSH S., BHATTACHARJEE S., 2004 - *Dietary garlic and its role in cancer prevention*. - Asia Pac. J. Clin. Nutr., 13: 165-171.

SINGLETON V.L., ROSSI J.A. Jr., 1965 - *Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents*. - Am. J. Enol. Vitic., 16: 144-158.

THE PLANT LIST, 2013 - *World flora on line*. - https://wfoplantlist.org/

USECHE-CARRILLO N.V., BARRIENTOS-PRIEGO A.F., NÚÑEZ-COLÍN C.A., CAMPOS-ROJAS E., AYALA-ARREOLA J., 2021 - *Stem and leaf anatomical and physiological characteristics of 'Colín V-33' avocado seedlings.* - Adv. Hort. Sci., 35(2): 83-194.

WANG H., LI X., SHEN D., OIU Y., SONG J., 2014 - *Diversity evaluation of morphological traits and allicin content in garlic (*Allium sativum *L.) from China*. - Euphytica, 198: 243-254.

WARD J.H. Jr., 1963 - *Hierarchical grouping to optimize an objective function*. - J. Am. Stat. Assoc., 58: 236-244.

YEH Y.Y., LIU L., 2001 - *Cholesterol-lowering effects of garlic extracts and organosulfur compounds: Human and animal studies*. - J. Nutr., 131: 989S-993S.

ZAAG D.E., 1987 - *Potato storage and quality management. CIP Annual Report 1986-87*. - International Potato Center, Lima, Perú, pp. 210.

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Physiological tolerance of shallot varieties to airborne salinity in coastal sandy soils

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Key words: Resistant varieties, salinity tolerance index, seasonal shoreline wind.

Abstract: Shallot as a horticultural crop has various benefits and important uses as a provider of nutritional needs. Its uniqueness in aroma and flavor makes it commonly used as a seasoning so that it has a good economic value as an increase in farmers' income. Sandy land on the coast has the potential for shallot cultivation. The presence of wind that airborne salinity on coastal land requires the selection of tolerant varieties and knowledge of the level of airborne salinity concentration that shallot plants can tolerate. Experiments have been conducted from July to December 2023 in the screenhouse and horticultural agronomy lab, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto (7°24'27.7"S, 109°15'19.1"E). Treatments consisted of the use of shallot varieties Bali Karet (B_1) and Bima Brebes (B_2), with the application of several concentrations of airborne salinity consisting of 0, 6, 12, and 18 mS cm^{-1}. The Bali Karet variety excels in plant height and root dry weight morphologically. Physiologically, Bima Brebes has higher levels of chlorophyll a and stomatal density, while Bali Karet is superior in chlorophyll b. Harvest results show Bima Brebes produces more tubers, while Bali Karet produces higher fresh tuber weight per clump. Morphological parameters (plant height, root dry weight), physiology (chlorophyll a, chlorophyll b, stomatal aperture, stomatal density), and yield showed the highest value at the lowest air salinity concentration (0 mS cm^{-1}). Both varieties increased proline as a tolerance mechanism to 18 mS cm^{-1} air salinity. The best interaction occurred between Bali Karet and 0 mS cm^{-1} salinity on stomatal opening, and between Bima Brebes and 0 mS cm^{-1} salinity on stomatal density. Both varieties were classified as having moderate tolerance to 18 mS cm^{-1} salinity, but total chlorophyll was very sensitive to this salinity concentration.

1. Introduction

Horticultural crops play an important role in providing food nutrition

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as well as increasing farmers income. Horticultural development continues as technology advances. Horticultural products are an important source of valuable nutritional and nutraceutical compounds as nutrients needed by humans (Durazzo and Lucarini, 2022). Horticultural crops include fruit plants, medicinal plants, vegetable plants, plantation plants, spices, and ornamental plants, playing an important role in the economic development and prosperity of a country (Kour *et al*., 2022). The export of horticultural crop commodities provides a great opportunity globally in increasing the country's income and the welfare of farmers. One of the potential commodities in horticultural production activities is shallots.

Shallot (*Allium ascalonicum* L.) as a commodity type of horticulture with high economic potential for farmers' income. Shallot cultivation plays an important role in the national economy and globally. There is a high market demand for shallots domestically and internationally, increased production and technological development are required, contributing to food security. Shallot production reached 1.985 million tons in 2023, marking a 0.14% increase (2.87 thousand tons) compared to 2022. Household consumption of shallots in 2023 decreased by 4.07% (33.83 thousand tons), totaling 797.32 thousand tons compared to the previous year. The import value of shallot in 2023 reached US $1.82 million, increased of 21.94% (US $327.46 thousand) from 2022. The consumption needs of shallots by households in Indonesia have fluctuated in the last five years, respectively in 2019 by 750.63 thousand tons; 2020 by 729.82 thousand tons; 2021 by 790.63; 2022 by 831.14 thousand tons; and 2023 by 797.32 thousand tons (Badan Pusat Statistik, 2024).

Based on the high interest and potential, a strategy is needed to increase shallot productivity. As an archipelago, Indonesia has many islands spread across its territory. Indonesia as an archipelago consists of 17,504 islands, has a coastal area of 95,118 kilometers (Syamsuddin *et al*., 2019). The amount of sandy beach land is a potential in increasing agricultural land for farmers. Sand land is one of the potentials to overcome the problem of agricultural land conversion, as well as in horticultural development (Fikri, 2021). Seeing the increasingly limited cultivation land provides a highlight of the potential of coastal land as a feasible marginal land utilization effort. Extensification activities on coastal land can significantly increase the total shallot planting area, thereby increasing total production in an area. One of the efforts to meet shallot production needs is done with off season cultivation (Susanawati and Fauzan, 2019). Off season shallot cultivation can be done on coastal sand land (Fauzan, 2020). Different soil and climatic conditions make coastal land a challenge in conducting shallot cultivation activities. However, coastal sand land is easy to cultivate because of its loose texture so that it can save time and cost of land treatment and land is relatively safe from disease (Iriani, 2013).

Another major problem that needs to be considered in cultivation on coastal land is the presence of airborne salinity. A simple sensor exposure method with a wet sponge in coastal areas showed air salinity of 19.69 mS at 6 hours and 151.19 mS at 24 hours (Saparso *et al*., 2023). This shows that the air salinity in coastal areas is very high as indicated by the salt particles captured on the wet sponge. Evaporation that occurs in the sea around the coast causes salt particles to be carried into the atmosphere. Winds in coastal areas carry water vapor that has a certain level of salinity originating from the sea area. When carried inland on agricultural land, water vapor with a certain level of salinity can affect plants. Deposition of salt particles on the surface of leaves and other organs, allowing uptake by plants. Growth reduction due to high salinity results from a combination of osmotic stress causing water deficit and the impact of excess Na^+ and Cl^- ions on crucial biochemical processes (Munns and Tester, 2008). NaCl in high concentrations is toxic when accumulated in plant tissues. High concentrations of Na^+ disrupt the uptake of K^+ and Ca^{2+} nutrients, while high concentrations of Cl^- decrease photosynthetic capacity due to chlorophyll degradation (Tavakkoli *et al*., 2010). Salinity stress in plants influences numerous cellular mechanisms, such as disturbing cellular homeostasis, hindering photosynthesis, affecting mRNA processing, transcription, and protein synthesis, as well as disrupting energy metabolism, amino acid biosynthesis, and lipid metabolism (Hameed *et al*., 2021). Salinity stress can cause a reduction in photosynthesis efficiency, chlorophyll, total protein, biomass, stomatal closure and increasing the oxidative stress (Gupta and Huang, 2014).

Salinity stress in plants increases the production

of reactive oxygen species (ROS) through oxidative stress mechanisms. ROS are normal products of cell metabolism, but environmental stress increases their production excessively, damaging biomolecules and organelles. The role of ROS as signals or stressors is determined by the balance between their formation and elimination by the antioxidant system, and disruption of this balance leads to oxidative stress (Hasanuzzaman *et al*., 2021). Due to the presence of high salinity there is a water deficit and an increase in free radicals that damage cell structures, plants respond by synthesizing osmolytes such as proline and sugar. Proline has antioxidant activity, activates the detoxification system, contributes to cellular homeostasis by protecting redox balance, and serves as a protein precursor and energy source in the recovery process from stress (Mansour and Ali, 2017). Proline is able to minimize damage from ROS thereby reducing lipid peroxidation, which results in protection of the photosynthetic apparatus in various plant species (Ashraf and Foolad, 2007; Wani *et al.*, 2012).

Each crop variety has a different genetic makeup that determines its adaptability to environmental stress, such as salinity. Research shows that Allium species, including shallots, are plants that are quite sensitive to salinity stress (Kadayifci *et al*., 2005; Kiremit and Arslan, 2016). To investigate the effect of salinity on shallot, two different varieties were used: Bima Brebes and Bali Karet. Genetic differences in shallots of Bima Brebes and Bali Karet varieties cause differences in morphology, physiology, and yield in plants. Alavan *et al*. (2015), stated that different varieties affect the diversity of plant appearance, due to differences in plant traits (genetic) or environmental influences. The results of research by Karo and Manik (2020), showed that differences in shallot varieties had a significant effect on the number of flowers with the highest value being the Pancasona variety 2.93 stalks and the lowest Birma 0.07 stalks. According to Azmi *et al*. (2011), that several varieties planted on the same land have different bulb sizes for each variety.

To improve productivity on land with exposure to airborne salinity, it is necessary to select varieties that can adapt to salinity exposure. This selection of plant varieties is based on morphological, physiological and molecular markers (Soltabayeva *et al*., 2021). Currently, there is still no information and research on the impact of airborne salinity on shallots grown on the coast. Therefore, this study aims to determine the impact of airborne salinity on the morphology, physiology, and yield of shallot plants in two different varieties on coastal land.

2. Materials and Methods

Experimental design

Experiments have been conducted from July to December 2023 in the screenhouse and horticultural agronomy lab, Faculty of Agriculture, Jenderal Soedirman University, Purwokerto (7°24'27.7"S, 109°15'19.1"E). Screenhouse microclimate with daytime peaks of 33.17°C (36,7% RH) under solar radiation and nighttime lows of 27.03°C (55,74% RH) due to radiative cooling.

Experiment with factorial research with a two-factor completely randomized design (CRD) instrument. The first factor shallot varieties consisted of Karet Bali (B_1) and Bima Brebes (B_2), the second factor airborne salinity at a concentration of 0 mS cm^{-1} (A_0), 6 mS cm^{-1} (A_1), 12 mS cm^{-1} (A_2), and 18 mS cm^{-1} (A_3). There were 8 treatment combinations with 3 replications, there are 24 units, with 5 polybags each, making a total of 120 polybags.

Plant material

The shallot variety Bima Brebes originates from Brebes. The plant starts flowering in 50 days and can be harvested in 60 days. It reaches 34.5 cm in height and produces 7-12 bulbs per clump. The leaves are green, cylindrical, and 14-50 in number. Dry tuber production reaches 9.9 tons per hectare. This variety is quite resistant to tuber rot but susceptible to leaf tip rot. The tubers are oval and pink in color, suitable for lowlands (Annex to the Regulation of the Indonesian Minister of Agriculture Number: 594/Kpts/TP.240/8/1984 Dated: August 11, 1984).

The Bali Karet (Batu Ijo) variety of shallots originates from Batu, Malang. Plants start flowering in 45-50 days and are harvested in 55-60 days in the lowlands or 65-70 days in the highlands. It is between 45-60 cm tall and produces 2-6 bulbs per clump. The leaves are dark green, cylindrical, and number 45-50. The dry tuber production reaches 18.5 tons per hectare. The tubers are round and pink in color, and this variety is well adapted to areas with an altitude of 50-1000 meters above sea level (Annex to the Regulation of the Indonesian Minister of Agriculture Number: 366/Kpts/LB.240/6/2004 Dated: June 2, 2004).

Agronomic variables

Plant height (cm) was determined from the soil surface to the uppermost shoot. The roots were dried in an air-circulated oven at a constant temperature of 70°C until constant weight (72 hours). Root dry weight was then weighed using an analytical balance with an accuracy of 0.01 g and expressed in grams (g) per plant. Counting the number of tubers per clump was done at harvest time. Uniform and healthy sample plants were uprooted along with the tubers. After being cleared of soil, the clumps of tubers were manually separated from the remains of dried roots and leaves. Each bulb in a clump was counted manually, and the results were expressed as the number of bulbs per clump (bulbs per clump). Fresh bulb weight per clump was measured at harvest. Each whole clump was directly weighed using an analytical balance (accuracy 0.01 g). Measurement results were expressed in grams per clump (g).

Assessment of leaf greenness

Data on the greenness value of shallot leaves were observed in the late vegetative and late generative phases 34 and 47 days after planting, respectively. Leaf greenness value was determined with the SP3 leaf chlorophyll meter on the SPAD-502 plus device. Data on chlorophyll content in the leaves were taken randomly in the sample unit. The leaf greenness each leaf sample observed was then taken as the average value. The results of the average value of SPAD-502 plus as sample data are processed. Data collection in sunny weather to increase the accuracy of data collection.

Assessment of chlorophyll content

Chlorophyll concentration was determined using the modified International Rice Research Institute (IRRI) method (Alsuhendra, 2004). A total of 0.01 g of shallot leaves were weighed on a balance sheet, pulverized in a mortar with the addition of 10 ml of 80% acetone. Leaves that have been pulverized, filtered with filter paper. The shallot leaf extract was analyzed for chlorophyll content on a spectrophotometer, 663 and 645 nm wavelengths.

$$\text{Chl Content (mg L}^{-1}\text{)} = (20.2 \times A645) + (8.02 \times A663) \quad (1)$$

A663 = Absorbance at 663 nm wavelength
A645 = Absorbance at 645 nm wavelength

Assessment of stomatal characteristics

Stomatal opening was quantified by identifying epidermal impressions which were obtained from the abaxial leaf surface using clear nail polish. After application, transparent adhesive tape was pressed onto the coated section and carefully peeled to transfer the imprint. The tape-mounted impression was then affixed to a glass slide for stomatal aperture observation at 400× magnification. Imprints were examined under a compound light microscope equipped with a calibrated ocular micrometer. Stomatal opening width (µm) was measured as the maximum pore distance between guard cells.

Stomatal density was quantified by counting stomata within a defined microscopic field of view (area = 0.1589 mm² at 400× magnification). The density was calculated using the formula:

$$\text{Density} = \text{Number of stomata/Field of view area}$$

Proline content determination

Proline (µmol g^{-1} fresh weight) was determined based on the technique (Bates *et al.*, 1973), in 0.5 g fresh leaves that have been mashed given 10 mL of 3% 5-sulfosalicylic acid, then filtered. The filtrate was then given 2 mL ninhydrin (2,2-dihydroxyindane-1,3-dione) and 2 mL glacial acetic acid, put in a tube, for one hour heated at 100°C (212.0°F) with the addition of 4 mL toluene. The extract solution turned dark red indicating proline content, measured by Milton Roy 2D Spectrophotometer, wavelength 520 nm. The value on the spectrophotometer was calculated by the formula:

$$\text{Proline content (µmol g}^{-1}\text{ fresh weight)} = (64.3649 \times \text{absorbance}) + (-5.2987 \times 0.347) \quad (2)$$

64.3649 = The slope value of the standard curve, which indicates the increase in proline content (µmol g^{-1}) per unit increase in absorbance.
Absorbance = Spectrophotometric measurement value that is directly proportional to the concentration of proline in the sample.

Assessment of stress tolerance index (STI)

The stress tolerance index (STI) quantifies shallot yield under salinity stress relative to yield under normal conditions. This index was calculated using the formula established by Hooshmandi (2019):

$$STI = (Hp \times Hs) / (Hp)^2 \quad (3)$$

where STI is stress tolerance index, Hp= Yield of a genotype under non-stressed conditions, Hs the yield

of a genotype under stressed conditions, and Hp is Mean yield of all genotypes under non-stressed conditions.

Data analysis

Analysis of variance (ANOVA), was used in data analysis. Duncan's Multiple Range Test (DMRT) was then used on data significantly different at 5% standard error. Statistical data were processed using SPSS 26 supported by Microsoft Excel.

3. Results

The results show that salinity in several levels affects the morphological variables of shallots (Table 1, Fig. 1) . Plant height and root dry weight of shallots of Bali Karet varieties are 58.53 cm and 0.11 g plant^{-1} higher than Bima Brebes by 20.16% and 120%. While the leaf greenness of both varieties is not significantly different. Bali Karet variety is higher than Bima Brebes in all morphological parameters, indicating it is more tolerant to salinity stress. Airborne salinity treatment significantly reduces plant height, leaf greenness, and root dry weight variables with the highest values of 57.69 cm; 48.16;

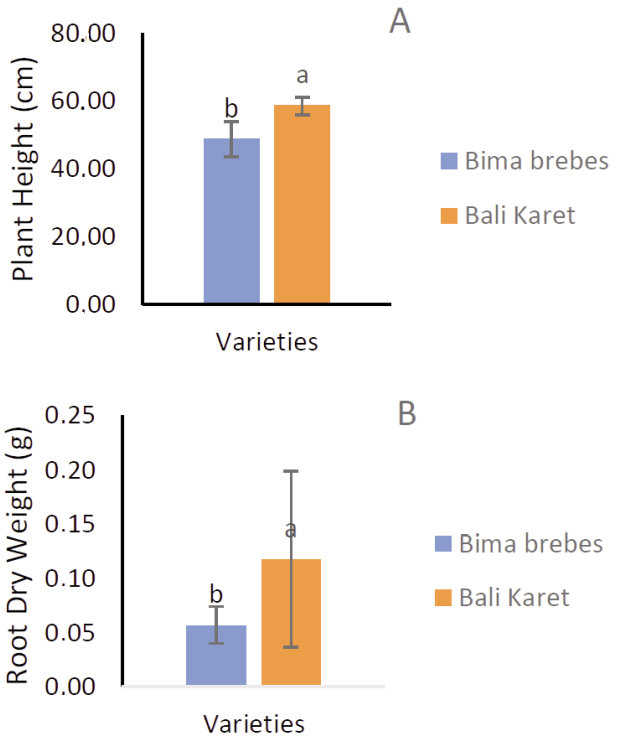

Fig. 1 - Effects of using different varieties (B1= Bali Karet, B2= Bima Brebes) on plant height (A) and root dry weight (B). Data are expressed as the mean of determination ± SD in 3 replicates.

Table 1 - Varietal effect and air salinity on shallot morphology

Treatment	Plant height (cm)	Leaf greenness	Root dry weight (g plant^{-1})
Varieties (B)			
Bali Karet (B_1)	58.53±2.55 a	43.67±6.24 a	0.11±0.06 a
Bima Brebes (B_2)	48.71±4.68 b	44.18±3.36 a	0.05±0.01 b
Airborne salinity (A)			
0 mS cm^{-1} (A_0)	57.69±5.18 a	48.16±2.43 a	0.12±0.08 a
6 mS cm^{-1} (A_1)	53.98±6.01 ab	45.00±4.17 ab	0.08±0.03 ab
12 mS cm^{-1} (A_2)	52.81±6.06 b	42.93±4.26 bc	0.08±0.04 ab
18 mS cm^{-1} (A_3)	49.99±6.49 b	39.57±4.71 c	0.06±0.03 b
Varieties (B) x Airborne salinity (A)			
B_1A_0	61.01±2.44 a	49.00±2.86 a	0.18±0.08 a
B_1A_1	59.30±1.60 ab	45.45±5.23 abc	0.10±0.03 b
B_1A_2	58.03±2.15 ab	42.53±5.96 bcd	0.10±0.04 b
B_1A_3	55.80±1.05 b	37.68±6.34 d	0.09±0.03 b
B_2A_0	54.37±5.30 b	47.34±2.35 ab	0.06±0.02 b
B_2A_1	48.68±1.76 c	44.56±3.93 abc	0.06±0.01 b
B_2A_2	47.60±2.34 c	43.34±3.06 bcd	0.06±0.02 b
B_2A_3	44.19±1.75 c	41.47±2.16 cd	0.05±0.00 b

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different ($p<0.05$).

and 0.12 g plant^{-1} at 0 mS cm^{-1} (A_0), respectively, with differences reaching 15.40%; 21.71%; and 100% at 18 mS cm^{-1} (A_3). The analysis of two shallot varieties at several levels of airborne salinity shows that Bali Karet and Bima Brebes varieties are slightly tolerant to airborne salinity and both have the same decreasing trend in morphology (plant height, leaf greenness, and root dry weight) as airborne salinity increases (Fig. 2); however, both varieties have different mechanisms to salinity stress.

In Table 2 and figure 3 can be observed that the shallot variety Bima Brebes has a value of 9.33 mg L^{-1} 13.69% greater than the value of chlorophyll a Bali Karet. In contrast, the Bali Karet variety has values of 6.85 mg L^{-1} and 16.19 mg L^{-1} respectively 86.14% and 11.58% greater than the chlorophyll b and total values of Bima Brebes. Physiological characteristics were significantly affected by the level of airborne salinity in chlorophyll a, b, and total variables (Fig. 4) with the highest values of 12.28 mg L^{-1}; 8.54 mg L^{-1}; and 20.83 mg L^{-1} at 0 mS cm^{-1} (A_0), these values were 57.44%; 288.18%; and 108.09% higher than the 18 mS cm^{-1} treatment (A_3). Considering the results of the two varieties under escalating airborne salinity, Bali Karet and Bima Brebes deploy contrasting chlorophyll strategies. Bali Karet boosts chlorophyll b to maximize light harvesting for growth, while Bima Brebes prioritizes chlorophyll a to protect

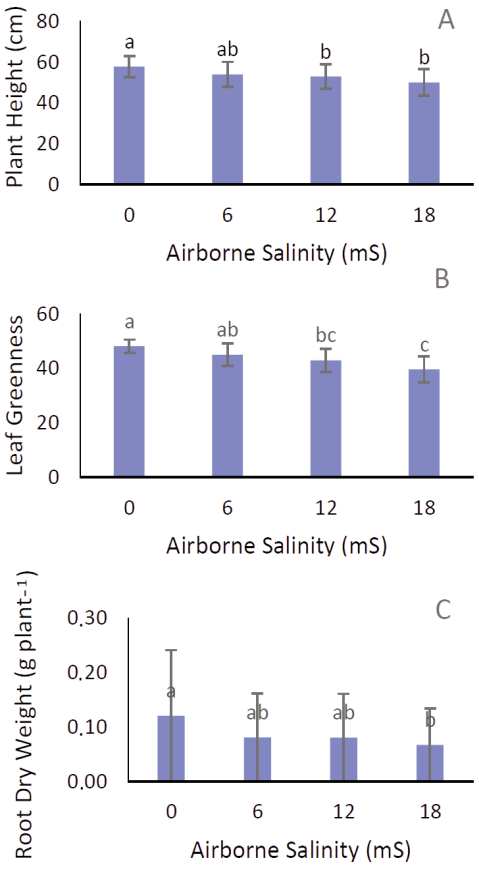

Fig. 2 - Effects of airborne salinity (0, 6, 12, and 18 mS) on plant height (A), leaf greenness (B), and root dry weight (C). Data are expressed as the mean of determination ± SD in 3 replicates.

Table 2 - Varietal effect and air salinity on shallot physiology (chlorophyll)

Treatments	Chlorophyll a (mg L-1)	Chlorophyll b (mg L^{-1})	Total chlorophyll (mg L^{-1})
Varieties (B)			
Bali Karet (B_1)	9.33±2.35 b	6.85±4.52 a	16.19±5.13 a
Bima Brebes (B_2)	10.81±2.61 a	3.68±2.58 b	14.51±4.40 a
Airborne salinity (A)			
0 mS cm^{-1} (A_0)	12.28±2.33 a	8.54±4.22 a	20.83±2.70 a
6 mS cm^{-1} (A_1)	10.88±1.66 ab	5.54±3.76 ab	16.43±2.56 b
12 mS cm^{-1} (A_2)	9.32±1.41 bc	4.78±3.63 ab	14.12±3.19 bc
18 mS cm^{-1} (A_3)	7.80±2.47 c	2.20±1.40 b	10.01±2.54 c
Varieties (B) x Airborne salinity (A)			
B_1A_0	10.88±2.49 bc	11.13±2.50 a	22.01±1.20 a
B_1A_1	10.25±1.88 bcd	7.04±5.30 b	17.29±3.49 bc
B_1A_2	8.71±1.65 bcd	6.17±5.19 b	14.89±4.63 cd
B_1A_3	7.50±2.71 d	3.07±1.19 bc	10.58±2.74 de
B_2A_0	13.68±1.22 a	5.97±4.27 b	19.65±3.56 ab
B_2A_1	11.52±1.47 ab	4.05±0.69 bc	15.57±1.44 bc
B_2A_2	9.94±1.07 bcd	3.40±0.54 bc	13.35±1.47 cde
B_2A_3	8.11±2.75 cd	1.34±1.11 c	9.45±2.76 e

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different ($p<0.05$).

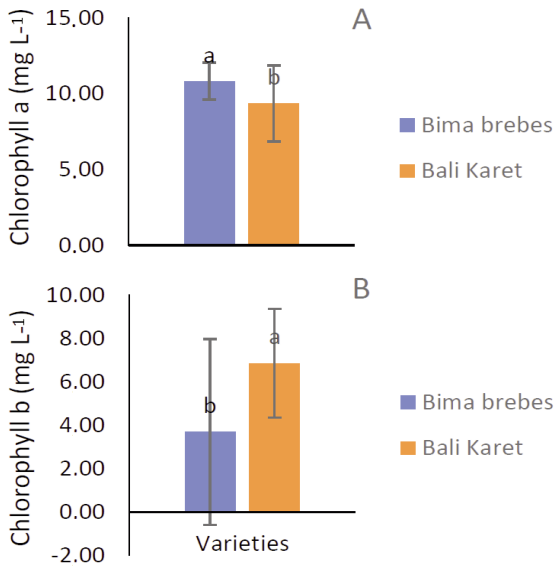

Fig. 3 - Effects of using different varieties (B1= Bali Karet, B2= Bima Brebes) on chlorophyll a (A) and chlorophyll b (B). Data are expressed as the mean of determination ± SD in 3 replicates.

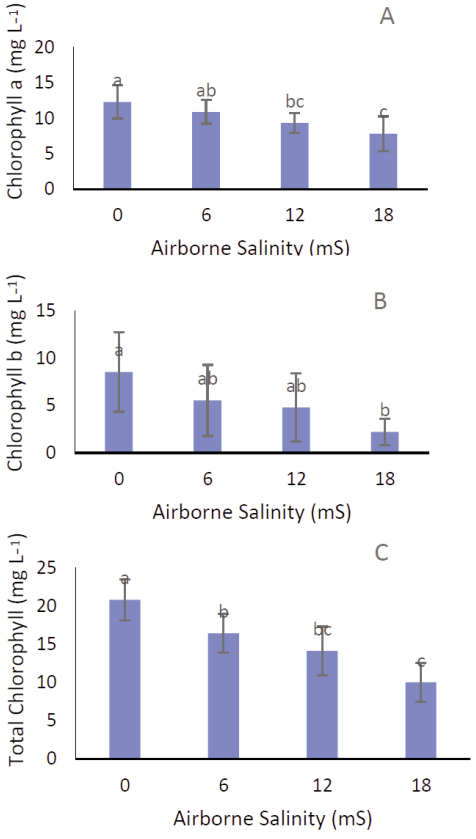

Fig. 4 - Effects of airborne salinity (0, 6, 12, and 18 mS) on chlorophyll a (A), chlorophyll b (B), total chlorophyll (C). Data are expressed as the mean of determination ± SD in 3 replicates.

photosynthetic reaction centers. This reflects a fundamental trade-off between photon capture (Bali Karet) and photochemical resilience (Bima Brebes), a divergence critical for variety-specific airborne salinity adaptation.

Table 3 shows that the use of different varieties on stomatal physiology impacts only density of stomata, with the variety of Bima Brebes having a stomatal density of 55.55 stomatal mm^{-2} greater 17.79% than the variety of Bali Karet. No significant differences are found on stomatal opening and proline content. Nevertheless, it can be noticed that Bali Karet variety has a value of 3.58 µm 4.68% greater than Bima Brebes on stomatal opening, and that Bali Karet variety has a value of 1.15 µmol g^{-1} fresh weight 19.01% lower than Bima Brebes on proline content. Several treatments at the airborne salinity level had an effect on decreasing stomatal opening and density and increasing proline. The highest value of stomal opening and stomatal density are 4.66 µm and 58.70 stomatal.mm^{-2} at 0 mS cm^{-1}, 100% and 36.61% surpassed the 18 mS cm^{-1} treatment. In opposite, the highest value on proline 2.24 µmol g^{-1} fresh weight fresh leaves at 18 mS cm^{-1} on proline up to 397.78% greater than the control (0 mS cm^{-1}). In the physiological characteristics, the interaction between the use of different varieties and the level of airborne salinity influenced considerably stomatal mechanism of stomatal opening and stomatal density with the highest values of 5.33 µm and 54.50 stomatal mm^{-2} (B_1A_0), respectively 128.76% and 52.96% surpassed B_1A_3 and B_2A_3 on stomatal opening and B_1A_3 on stomatal density (Fig. 5). Although there was no interaction on proline between the use of two shallot varieties and airborne salinity at several levels, it can be observed that the Bima Brebes variety accumulated higher proline than Bali Karet with the same increasing trend. This shows the type of adaptation of Bima Brebes on cellular adaptation, compared to Bali Karet which focuses on growth optimization (Fig. 6).

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different ($p<0.05$).

Table 4 shows that there is an influence of both varieties on yield characteristics, variable number of bulbs per clump Bima Brebes 6.66 pieces greater 55.61% than Bali Karet. In fresh bulb weight per clump on the contrary, Bali Karet has a value of 45.38

Table 3 - Varietal effect and air salinity on shallot physiology (stomatal and proline)

Treatment	Stomatal opening (µm)	Stomatal density (Stomatal mm^{-2})	Proline (µmol g^{-1} fresh weight)
Varieties (B)			
Bali Karet (B_1)	3.58±1.31 a	47.16±8.69 b	1.15±0.78 a
Bima Brebes (B_2)	3.42±0.90 a	55.55±6.48 a	1.42±0.92 a
Airborne salinity (A)			
0 mS cm^{-1} (A_0)	4.66± 0.82a	58.70±6.50 a	0.45±0.38 c
6 mS cm^{-1} (A_1)	4.16±0.41 a	52.41±8.59 ab	0.95±0.32 bc
12 mS cm^{-1} (A_2)	2.83±0.41 b	51.36±2.57 b	1.47±0.44 ab
18 mS cm^{-1} (A_3)	2.33±0.52 b	42.97±8.36 c	2.24±0.87 a
Varieties (B) x Airborne salinity (A)			
B_1A_0	5.33±0.58 a	54.50±3.63 abc	0.21±0.05 d
B_1A_1	4.00±0.00 abc	46.12±7.26 c	0.91±0.33 bcd
B_1A_2	2.66±0.58 cd	52.41±3.63 bc	1.53±0.30 abc
B_1A_3	2.33±0.58 d	35.63±3.63 d	1.94±0.76 ab
B_2A_0	4.00±0.00 abc	62.89±6.29 a	0.71±0.42 cd
B_2A_1	4.33±0.58 ab	58.70±3.63 ab	1.00±0.37 bcd
B_2A_2	3.00±0.00 bcd	50.31±0.00 bc	1.42±0.61 bc
B_2A_3	2.33±0.58 d	50.31±0.00 bc	2.55±1.03 a

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different ($p<0.05$).

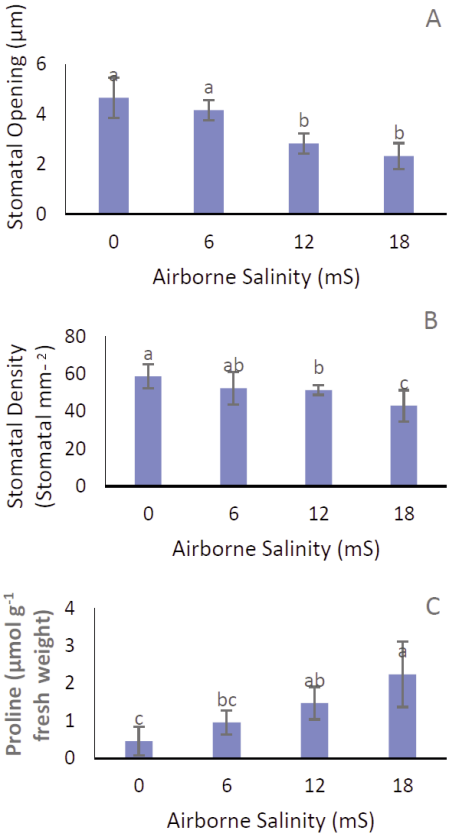

Fig. 5 - Effects of airborne salinity (0, 6, 12, and 18 mS) on stomatal opening (A), stomatal density (B), and proline (C). Data are expressed as the mean of determination ± SD in 3 replicates.

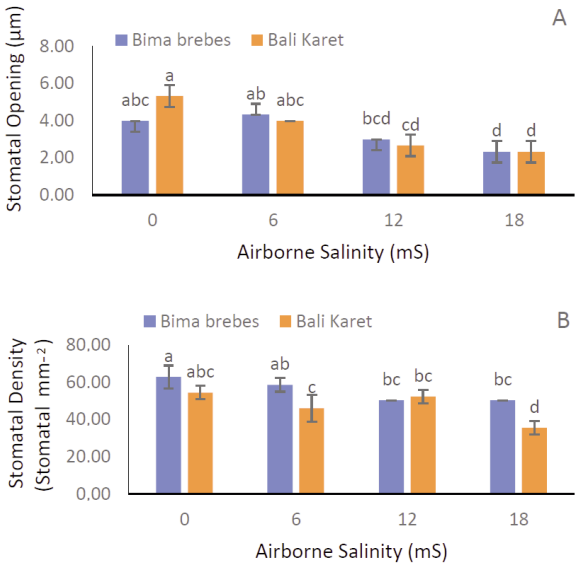

Fig. 6 - Interaction of different varieties (B1= Bali Karet, B2= Bima Brebes) with different levels of airborne salinity (0, 6, 12, and 18 mS) on stomatal opening (A) and stomatal density (B). Data are expressed as the mean of determination ± SD in 3 replicates.

g, 95.09% greater than Bima Brebes. The yield parameter in the airborne salinity treatment in the research conducted had no effect. The interaction of two shallot varieties at several levels of airborne salinity was not significant on yield. Bali Karet variety

Table 4 - Varietal effect and air salinity on shallot yield

Treatment	Number of bulbs per clump	Fresh bulb weight per clump (g)
Varieties (B)		
Bali Karet (B_1)	4.28±0.77 b	45.38±11.15 a
Bima Brebes (B_2)	6.66±1.23 a	23.26±10.46 b
Airborne salinity (A)		
0 mS cm^{-1} (A_0)	4.80±0.49 a	41.17±22.01 a
6 mS cm^{-1} (A_1)	5.90±1.52 a	32.78±14.57 a
12 mS cm^{-1} (A_2)	5.47±2.15 a	33.08±15.50 a
18 mS cm^{-1} (A_3)	5.73±1.85 a	30.27±9.15 a
Varieties (B) x Airborne salinity (A)		
B_1A_0	4.40±0.35 b	54.68±12.97 a
B_1A_1	4.67±0.42 b	43.85±12.53 abc
B_1A_2	3.73±1.21 b	45.49±10.19 ab
B_1A_3	4.33±0.92 b	37.51±5.36 abcd
B_2A_0	5.20±0.00 b	27.65±22.26 bcd
B_2A_1	7.13±1.01 a	21.71±2.34 cd
B_2A_2	7.20±1.06 a	20.66±5.88 d
B_2A_3	7.13±1.36 a	23.03±4.81 cd

Data are expressed as the mean of determination ± SD in 3 replicates. Means followed by the same letter in one column are not significantly different ($p<0.05$).

has an escape response shown in fresh bulb weight per clump which is higher than Bima Brebes, although Bima Brebes is higher in the number of bulbs per clump due to the defense response from airborne salinity stress (Fig. 7).

Table 5 show that shallot varieties Bali Karet and Bima Brebes were medium tolerant variety (mt) on 6, 12, and 18 mS cm^{-1} airborne salinity. This shows the ability of both varieties to tolerate salinity stress, but have different response mechanisms. The responses of the two varieties to physiology, morphology, and yield are shown in Tables 1-4.

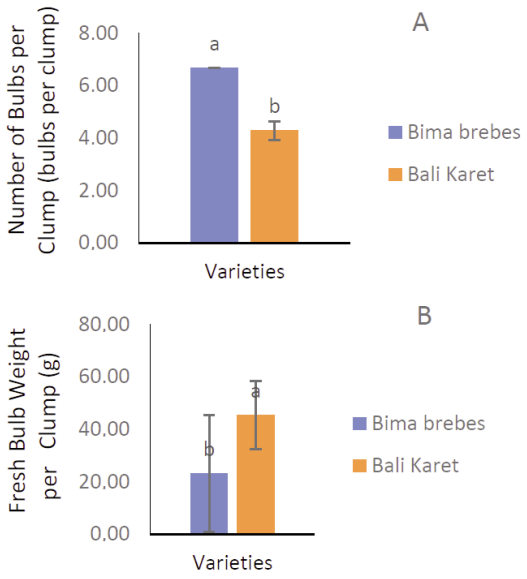

Fig. 7 - Effects of using different varieties (B1= Bali Karet, B2= Bima Brebes) on number of bulbs per clump (A) and fresh bulb weight per clump (B). Data are expressed as the mean of determination ± SD in 3 replicates.

4. Discussion and Conclusions

Salinity on certain levels can affect morphology, physiology and yield in plants. According to Shokat and Großkinsky (2019), salinity stress is one of the major problems in agriculture studied globally. Dry weight loss is one of the signs that plant growth is affected by salinity (Suharjo *et al*., 2021). The results in Table 1 show that the higher the airborne salinity, the lower the morphological variables in shallots. Salinity determines the ability of plants to grow because it can damage cells. High salinity levels affect water uptake by plants due to salt around the plant roots, which causes oxidative stress (Anwar *et al*., 2024), prolongs shoot emergence, slows leaf growth, reduces plant height, changes the form of tubers, and reduces their overall mass and size (Alam *et al*., 2023).

Table 5 - Varieties effect and airborne salinity on stress tolerance index

Variables	Varieties	Airborne salinity (mS cm^{-1})		
		6	12	18
Stress tolerance index	Bali Karet	0.802 (mt)	0.832 (mt)	0.686 (mt)
	Bima Brebes	0.785 (mt)	0.747 (mt)	0.833 (mt)

Stress Tolerance Index <0.5 sensitive variety (tt), 0.5-1.0 medium tolerant variety (mt). STI >1 tolerant variety (t) (Saparso *et al.*, 2024).

This research found that higher airborne salinity reduced morphological characteristics such as plant height, leaf greenness, and root dry weight. Those effects may be explained by the fact that saline environments generally have the same or even higher osmotic pressure than in plant cells, which can inhibit water from entering plant cells. Water flows from areas of low osmotic pressure to areas of higher osmotic pressure, causing plants in saline conditions to experience water stress. In *Allium cepa*, stress inhibits cell division so that the number of new cells is reduced and the meristem shrinks in size (Kielkowska, 2017). Osmotic pressure also affects the speed at which cells absorb nutrients (Zainuddin *et al*., 2017). Compared to the control, root fresh weight in pomegranate cultivars decreased by 46.3%, 57.4%, and 66% under 6, 9, and 12 dS m^{-1} salinity treatments, respectively, while root dry weight decreased by 45.4%, 52.5%, and 59% at the same salinity levels (Jadidi *et al*., 2020). Salinity stress significantly reduced pepper plant height (Badem and Söylemez, 2022), leaf greenness values were lower under higher NaCl stress (Rustikawati *et al*., 2023). According to research by Kul *et al*. (2021), water salinity caused 22.0% decrease in root fresh weight and 36.0% decrease in root dry weight of tomato compared to non-saline control and unamended control. Plants have evolved biochemical and molecular mechanisms, which work in sync as an integrated physiological response to soil salinity (Ruiz-Lozano *et al*., 2012). High salinity reduces crop production, subsequent growth, and cause physiological defects threatening global food security and prosperity (Balasubramaniam *et al*., 2023).

Furthermore, high salinity environments can damage plant membranes and chlorophyll in *Zea mays* and *Cyperus rotundus*, causing disturbances in nutrient absorption due to disturbed ion balance in plant roots (Pranasari *et al*., 2012). The accumulation of Na^+ and Cl^- in tissues disrupts enzyme function, photosynthesis, and cell division, especially in young leaves (Munns and Tester, 2008). Other responses in plants include selective buildup or exclusion of salt ions as maintenance on the photosynthesis process to reach adequate values for plant growth, changes in membrane structure, and phytohormone synthesis (Türkan and Demiral, 2009). The present study shows that as increasing airborne salinity from 0 to 18 mS cm^{-1} decreased total chlorophyll by 20.83, 16.43, 14.12, 10.01 mg L^{-1} recpectively. The highest value of stomal opening and stomatal density are 4.66 µm and 58.70 stomatal.mm^{-2} at 0 mS cm^{-1}, 100% and 36.61% surpassed the 18 mS cm^{-1} treatment. Accordingly, the results of research by Fakhri and Ekawati (2020), explained that different salinities had a significant effect on the chlorophyll a content in *Dunaliella* sp., an increase in salinity from 15 to 35 ppt caused a 32.65% decrease in chlorophyll a content with the highest concentration (11.27 mg L^{-1}) produced at 15 ppt salinity. Salinity inhibits the osmotic uptake of water, which negatively affects the carbon assimilation process, salinity decreases photosynthetic rate, transpiration, stomatal conductance and chlorophyll levels in plants, affecting the ability of plants to photosynthesize optimally (Ashraf and Ali, 2008). Salinity stress lowers the osmotic potential of the soil solution reducing the availability of water for plants and increasing the concentration of ions that are toxic to plants (Anugrah *et al*., 2022). Plants have mechanisms to deal with stress. Exposed to salinity stress on plants, stomatal will be closed to protect against water loss, leading to increased leaf temperature, salinity-induced stress resulting in stomatal regulation, with strategies to cope with ionic and osmotic pressures induced by NaCl (Orzechowska *et al*., 2021).

Accumulations of cytotoxic-dependent toxic ions such as Na^+ and Cl^- and formation of reactive oxygen species (ROS), can occur due to salinity stress disrupting plant development and growth through water stress (Isayenkov, 2012). Under conditions of oxidative stress, changes in cell metabolic processes occur, causing the production of ROS to increase excessively, damaging proteins, fats, nucleic acids, and can cause plant cell death (Ahmad *et al*., 2019). Plants activate antioxidants (SOD, CAT) and accumulate compatible solutes (proline, glycine betaine) for mitigation (Hasegawa *et al*., 2000). According to the research Saparso *et al*. (2023), higher proline content makes plants more tolerant of air salinity stress, proline content in the plant increases the higher the level of air salinity applied, where the highest proline content of corn plants treatment of 18 mS air salinity, which is 3.58 µmol g^{-1} and the lowest proline content in the treatment of 0 mS air salinity, which is 1.75 µmol g^{-1}. This is consistent with the results of this study, that increased exposure to airborne salinity increases proline levels. The proline functions as an osmolyte helping to maintain osmotic balance in plant cells, at high salinity water tends to escape from cells due to differences in ion concentration, the presence,

accumulation of proline so that plant cells can draw water in, prevent dehydration and maintain cell turgor. According to Khanna-Chopra *et al*. (2019), plants produce proline and accumulate in the cytosol, in response to stresses such as salinity, to modify the osmotic properties of the cytoplasm thereby increasing tolerance in plants. However, it is also known that proline can increase the resistance and growth ability of plants under stressful conditions, such as high salinity. Increase in proline under salinity stress as extra Nitrogen (N) and energy storage achieved through salinity-induced growth reduction for plant survival and growth under stress conditions (Kubala *et al*., 2015).

Table 3 and 5 indicate that the increase in proline due to exposure to 6 mS cm^{-1} to 18 mS cm^{-1} represents the ability of both varieties to maintain cell osmoregulators so as not to cause physiological and metabolic plant stress. According to Ayub *et al*. (2015), high proline in plants tolerant of environmental stress plays a role in regulating plant cell osmoregulators. The defense mechanism from cell damage due to ROS as free radicals, plants respond through the antioxidant defense system (Denaxa *et al*., 2020). Proline plays a very important role in reducing the negative effects of plant salinity stress by neutralizing free radicals formed due to increased ROS. Plants have enzymatic and non-enzymatic antioxidant defense systems, which play an important role in detoxifying ROS generated under stress conditions, it is known that proline acts as an enzyme protector and ROS antioxidant. (Khatun *et al*., 2020). According to Silva-Ortega *et al*. (2008), proline accumulates dominantly in leaves to maintain chlorophyll levels and cell turgor pressure, which is crucial for preserving photosynthetic productivity when facing salinity stress. Accumulation of proline in stressed plants occurs both through induction of proline bio-synthesizing gene expression (P5CR and P5CS) and by inhibition of genes associated with the degradation pathway.

Under osmotic stress conditions, proline synthesis is mediated by the enzymes encoded by the P5CS and P5CR genes in most plants (Furlan *et al.,* 2020).

Salinity has three effects on crop growth and yield in the form of ion unbalance, ionic and osmotic stress (Anshori *et al*., 2018). However, experiment results reported in this study showed that airborne salinity had no effect on shallot production. There was a 19.38% decrease in the number of bulbs per clump in the comparison of control and 18 mS cm^{-1} treatment, in addition to the fresh bulb weight per clump control was 26.48% greater than 18 mS cm^{-1} treatment, but both parameters were not significantly different. This is in line with research Saparso *et al.* (2024), in cauliflower and cabbage, unlike the physiological response, plants in the air salinity level treatment had no impact on yield. Salinity conditions affect plant nutrient uptake due to the presence of excess Na^+ and Cl^- ions that prevent the uptake of NO_3^-, Ca_2^+, and K^+ ions respectively (Kharisun *et al*., 2022), the decrease may be due to the fact that these elements are very important in the initiation of bulbs (Mardhiana *et al*., 2018). In research, some crops showed a decrease in yield due to salinity. Tomato yield decreased by 7.2% at 5 mS cm^{-1} salinity and increased at higher salinities (Zhang *et al*., 2016). Research on Onion Granex 33 variety against 6 NaCl concentrations showed that increasing NaCl concentration resulted in a decrease in the fresh weight of mature plant bulbs, even plants could not survive at 125 mM NaCl concentration (Ratnarajah and Gnanachelvam, 2021). According to research by Syamsiyah *et al*. (2020), high salinity levels did not significantly affect yield components such as growth, yield, number of tubers, fresh and dry tuber weight of local shallots of Brebes and Purbalingga which varieties are tolerant of salinity up to salinity levels of 3 mS cm^{-1}. Meanwhile, this study shows that exposure to airborne salinity at the highest level up to 18 mS cm^{-1} (A_3) does not significantly affect the yield of shallots Bima Brebes and Bali Karet on the variable number of bulbs per clump and fresh bulb weight per clump. In this study, it can be said that airborne salinity stress does not affect the yield of shallot varieties of Bima Brebes and Bali Karet because both varieties are tolerant and able to adapt to certain levels of salinity. This adaptability allows them to maintain physiological and morphological stability, resulting in consistent yields despite saline conditions. Bali Karet variety has an increased response on growth variables, while Bima Brebes has a response on physiological variables. The results of research by Hadianti and Damanhuri (2019), that the six varieties of shallots: Bima Brebes, Bauji, Super Philip, Tajuk, Katumi, and Trisula are tolerant of high salinity concentrations, at ppm 12,000 experiencing severe stress. Sidabariba and Sudjatmiko (2023), stated that with its advantages, the Bali Karet (Batu Ijo) variety can adapt well to its growing environment.

A schematic representation of the different

mechanisms performed by the two shallot varieties at increasing salinity levels is shown in figure 8.

This study shows that both varieties are medium tolerant (mt) or tolerant (t) to 18 mS cm^{-1} airborne salinity for most of the parameters, except that for total chlorophyll. More in detail, the following differences in the performances of the two varieties can be highlighted, where Bali Karet and Bima Brebes are tolerant to airborne salinity, but both have different response mechanisms to airborne salinity stress. The Bali Karet variety increases the ability to optimize growth, indicating escape type adaptation with a focus on short term productivity (higher plant height and root dry weight) and bulb weight; while the Bima Brebes variety reflects cellular defense based tolerance type adaptation (higher chlorophyll a and stomatal density) and number of bulbs. Of course different level of salinity determined different responses. Interestingly, both varieties of Bali Karet and Bima Brebes show a tolerance mechanism through increased osmoregulators with an increase in proline at the highest airborne salinity concentration (18 mS cm^{-1}).

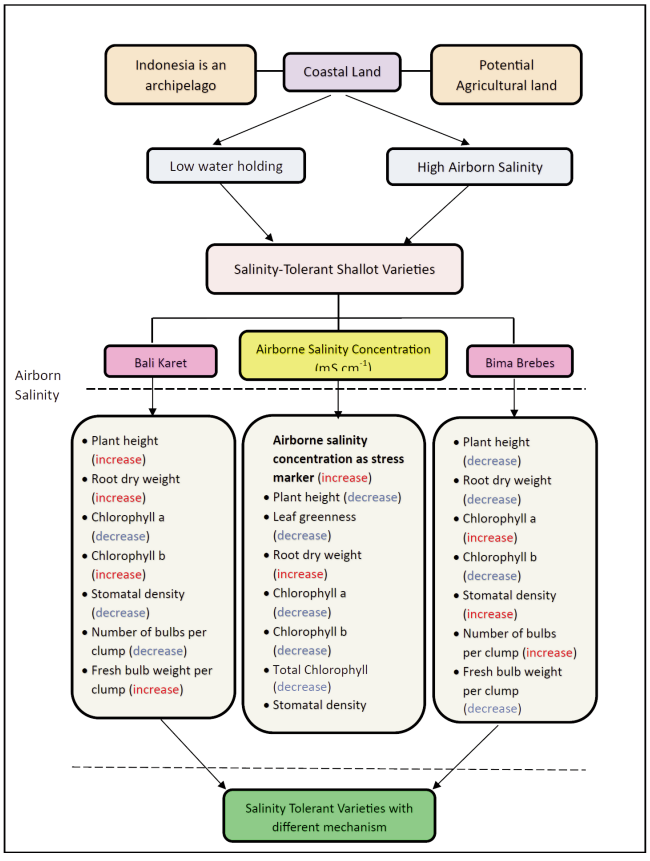

Fig. 8 - Effect of airborne salinity on morphology, physiology, and yield parameters in two shallot varieties.

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References

AHMAD R., HUSSAIN S., ANJUM M.A., KHALID M.F., SAQIB M., ZAKIR I., HASSAN A., FAHAD S., AHMAD S., 2019 - *Oxidative stress and antioxidant defense mechanisms in plants under salt stress*, pp. 191-205. - In: HASANUZZAMAN M., K.R. HAKEEM, K. NAHAR, and H.F. ALHARBY (eds.) *Plant abiotic stress tolerance: Agronomic, molecular and biotechnological approaches*. Springer, Cham, Switzerland, pp. 490.

ALAM M.A., RAHMAN M.A., RAHMAN M.M., HASAN M.M., NAHER S., FAHIM A.H.F., MOTTALIB A., ROY S., ISLAM R., MOZUMDER S.N., ALSUHAIBANI A.M., GABER A., HOSSAIN A., 2023 - *Performance valuation of onion (*Allium cepa *L.) genotypes under different levels of salinity for the development of cultivars suitable for saline regions*. - Front. Plant Sci., 14: 1154051.

ALAVAN A., HAYATI R., HAYATI E., 2015 - *Pengaruh pemupukan terhadap pertumbuhan beberapa varietas pad Gogo (*Oryza sativa *L.)*. - J. Floratek 10: 61-68.

ANSHORI M.F., PURWOKO B.S., DEWI I.S., ARDIE S.W., SUWARNO W.B., SAFITRI H., 2018 - *Determination of selection criteria for screening of rice genotypes for salinity tolerance*. - SABRAO J. Breed. Genet., 50(3): 279-294.

ANUGRAH D.E., SETIAWAN T.P., SASMITA R., AULIA E., AMININGSIH R., SARI V.N., HAJIJAH S.W., KENCANA Y.D., NUGRAHA E.D.S., SAFITRI I.K., PRATAMA J.S.A., SUHARJO U.K.J., FAHRURROZI F., 2022 - *Penggunaan indikator fisiologis untuk menentukan tingkat cekaman salinitas pada tanaman padi (*Oryza sativa *L.)*. - J. Agroqua: Media Informasi Agronomi Budidaya Perairan, 20(1): 50-65.

ANWAR N.H., KARYAWATI A.S., MAGHFOER M.D., KURNIAWAN A., 2024 - *Organic fertilizer alleviates salt stress in shallot by modulating plant physiological responses*. - J. Ecol. Engin., 25(7).

ASHRAF M., ALI Q., 2008 - *Relative membrane permeability and activities of some antioxidant enzymes as the key determinants of salt tolerance in canola (*Brassica napus *L.)*. - Environ. Exp. Bot., 63(1-3): 266-273.

ASHRAF M., FOOLAD M.R., 2007 - *Roles of glycine betaine and proline in improving abiotic stress resistance*. - Environ. Exp. Bot., 59: 206-216.

AYYUB C.M., ALI M., SHAHEEN M.R., QADRI R.W.K., KHAN I., JAHANGIR M.M., ABBASI K.Y., KAMAL S., ZAIN M., 2015 - *Enhancing the salt tolerance potential of*

*watermelon (*Citrullus lanatus*) by exogenous application of salicylic acid.* - Amer. J. Plant Sci., 6(19): 3267-3271.

AZMI C., HIDAYAT I.M., WIGUNA G., 2011 - *Pengaruh varietas dan ukuran umbi terhadap produktivitas bawang merah*. - J. Hortikultura, 21(3): 206-213.

BADAN PUSAT STATISTIK, 2024 - *Statistic of horticulture 2023* (Vol. 5). - Badan Pusat Statistik, Jakarta.

BADEM A., SÖYLEMEZ S., 2022 - *Effects of nitric oxide and silicon application on growth and productivity of pepper under salinity stress*. - J. King Saud Univ. - Sci., 34(6): 102189.

BALASUBRAMANIAM T., SHEN G., ESMAEILI N., ZHANG H., 2023 - *Plant's response mechanisms to salinity stress*. - Plants, 12(12): 1-22.

BATES L.S., WALDREN R.P.A., TEARE I.D., 1973 - *Rapid determination of free proline for water-stress studies*. - Plant Soil, 39: 205-207.

DENAXA N.K., DAMVAKARIS T., ROUSSOS P.A., 2020 - *Antioxidant defense system in young olive plants against drought stress and mitigation of adverse effects through external application of alleviating products*. - Scientia Hortic., 259: 108812.

DURAZZO A., LUCARINI M., 2022 - *Chemical properties, nutritional quality, and bioactive components of horticulture food*. - Horticulturae, 8(1): 3.

FAKHRI M., EKAWATI A.W., 2020 - *Pengaruh salinitas terhadap Pertumbuhan, Biomassa dan Klorofil-a* Dunaliella sp. - J. Fisheries Marine Res., 4(3): 393-398.

FAUZAN M., 2020 - *Pendapatan rumah tangga petani bawang merah lahan pasir pantai di Kabupaten Bantul.* - JAS, Jurnal Agri Sains, 4(1): 60-66.

FIKRI M.R.A., 2021 - *Faktor-Faktor yang Mempengaruhi Peranan Kelompok Tani dalam Penerapan Inovasi Teknologi Budidaya Cabai di Lahan Pasir Pantai Kabupaten Kulon Progo.* J. Agrimanex: Agribusiness, Rural Manag. Develop. Ext., 1(2): 20-27.

FURLAN A.L., BIANUCCI E., GIORDANO W., CASTRO S., BECKER D.F., 2020 - *Proline metabolic dynamics and implications in drought tolerance of peanut plants.* Plant Physiol. Biochem., 151, 566-578.

GUPTA B., HUANG B., 2014 - *Mechanism of salinity tolerance in plants: Physiological, biochemical, and molecular characterization*. - Int. J. Genomics, 2014: 701596.

HADIANTI F.N., DAMANHURI D., 2019 - *Toleransi Enam Varietas Tanaman Bawang Merah (*Allium ascalonicum *L.) pada Cekaman Salinitas*. - J. Produksi Tanaman, 7(12).

HAMEED A., AHMED M.Z., HUSSAIN T., AZIZ I., AHMAD N., GUL B., NIELSEN B.L., 2021 - *Effects of salinity stress on chloroplast structure and function*. - Cells, 10(8): 2023.

HASANUZZAMAN M., RAIHAN M.R.H., MASUD A.A.C., RAHMAN K., NOWROZ F., RAHMAN M., NAKAR K., FUJITA M., 2021 - *Regulation of reactive oxygen species and antioxidant defense in plants under salinity.* - Inter. J. Mol. Sci., 22(17): 9326.

HASEGAWA P.M., BRESSAN R.A., ZHU J.K., BOHNERT H.J., 2000 - *Plant cellular and molecular responses to high salinity*. - Ann. Rev. Plant Biol., 51(1): 463-499.

HOOSHMANDI B., 2019 - *Evaluation of tolerance to drought stress in wheat genotypes*. - Idesia, 37(2): 37-43.

IRIANI E., 2013 - *Prospek pengembangan inovasi teknologi bawang merah di lahan sub optimal (lahan pasir) dalam upaya peningkatan pendapatan petani*. - J. Litbang Provinsi Jawa Tengah, 11(2): 231-243.

ISAYENKOV S.V., 2012 - *Physiological and molecular aspects of salt stress in plants*. - Cytol. Genet., 46(5): 302-318.

JADIDI E., TATARI M., GHASEMNEZHAD M., SALEMI H.R., 2020 - *The salinity tolerance of pomegranate cultivars: Effects of salt stress on root and leaf mineral content*. - Adv. Hort. Sci., 34(3): 325-335.

KADAYIFCI A., TUYLU G., UCAR Y., CAKMAK B., 2005 - *Salt stress and* Allium *species*. - J. Plant Nutr., 28(10): 1865-1877.

KARO B.B., MANIK F., 2020 - *Observasi dan adaptasi 10 varietas bawang merah (allium cepa) di berastagi dataran tinggi basah*. - J. Agroteknosains, 4(2): 1-9.

KHANNA-CHOPRA R., SEMWAL V.K., LAKRA N., PAREEK A., 2019 - *Proline - A key regulator conferring plant tolerance to salinity and drought*, pp. 59-80. - In: HASANUZZAMAN M., M. FUJITA, H. OKU, and M.T. ISLAM (eds.) *Plant tolerance to environmental stress*. CRC Press, Boca Raton, FL, USA, pp. 488.

KHARISUN, SISNO, BUDIONO M.N., ROKHMINARSI, KURNIASIH K., 2022 - *The study of silica (Si) and salinity on the growth and yield of shallot plant (*Allium ascalonicum *L.) in an Entisol soil*. - Proceedings of the 2nd Int. Conf. for Smart Agriculture, Food, and Environment (ICSAFE 2021), Atlantis Press, Dordrecht, The Netherlands, pp. 18-31.

KHATUN M., MATSUSHIMA D., RHAMAN M.S., OKUMA E., NAKAMURA T., NAKAMURA Y., MUNEMASA S., MURATA Y., 2020 - *Exogenous proline enhances antioxidant enzyme activities but does not mitigate growth inhibition by selenate stress in tobacco BY-2 cells*. - Biosci., Biotechol., Biochem., 84(11): 2281-2292.

KIEŁKOWSKA A., 2017 - Allium cepa *root meristem cells under osmotic (sorbitol) and salt (NaCl) stress* in vitro. - Acta Bot. Croatica, 76(2): 146-153.

KIREMIT M.L., ARSLAN E., 2016 - *Effects of salt stress on shallot growth*. - European J. Hort. Sci., 81: 267-275.

KOUR D., KHAN S.S., KAUR T., KOUR H., SINGH G., YADAV A., YADAV A.N., 2022 - *Drought adaptive microbes as bioinoculants for the horticultural crops*. - Heliyon, 8(5): e09493.

KUBALA S., WOJTYLA Ł., QUINET M., LECHOWSKA K., LUTTS S., GARNCZARSKA M., 2015 - *Enhanced expression of the proline synthesis gene P5CSA in relation to seed osmopriming improvement of Brassica*

napus germination under salinity stress. - J. Plant Physiol., 183: 1-12.

KUL R., ARJUMEND T., EKINCI M., YILDIRIM E., TURAN M., ARGIN S., 2021 - *Biochar as an organic soil conditioner for mitigating salinity stress in tomato*. - Soil Sci. Plant Nutr., 67(6): 693-706.

MANSOUR M.M.F., ALI E.F., 2017 - *Evaluation of proline functions in saline conditions*. - Phytochem., 140: 52-68.

MARDHIANA F., SOEPARJONO S., DAN HANDOYO T., 2018) - *Pengaruh konsentrasi dan waktu aplikasi NaCl terhadap hasil dan mutu cabai merah (*Capsicum Annum *L.)*. - J. Appl. Agr. Sci., 2(1): 1-8.

MUNNS R., TESTER M., 2008 - *Mechanisms of salinity tolerance*. - Ann. Rev. Plant Biol., 59: 651-681.

ORZECHOWSKA A., TRTÍLEK M., TOKARZ K.M., SZYMAŃSKA R., NIEWIADOMSKA E., ROZPĄDEK P., WĄTOR K., 2021 - *Thermal analysis of stomatal response under salinity and high light*. - Inter. J. Mol. Sci., 22(9): 4663.

PRANASARI R., NURHIDAYATI T., PURWANI K., 2012 - *Persaingan Tanaman Jagung (Zea mays) dan Rumput Teki (*Cyperus rotundus*) pada Pengaruh Cekaman Garam (NaCl).* - J. Sains Seni ITS, 1 (1).

RATNARAJAH V., GNANACHELVAM N., 2021 - *Effect of abiotic stress on onion yield: A review*. - Adv. Technol., 1(1): 147-160.

RUIZ-LOZANO J.M., PORCEL R., AZCON C., AROCA R., 2012 - *Regulation by arbuscular mycorrhizae of the integrated physiological response to salinity in plants: New challenges in physiological and molecular studies*. - J. Exp. Bot., 63(11): 4033-4044.

RUSTIKAWATI R., HERISON C., SUTRAWATI M., UMROH D., 2023 - *Assessment of salinity tolerance on chili pepper genotypes*. - EDP Sciences, E3S Web Conferences, 373: 03023.

SAPARSO, FAOZI K., PUTRA F.P., 2024 - *Assessing the air salinity on agro-physiological response of* Brassica oleracea *var. capitata and* Brassica oleracea var. botrytis. - J. Appl. Nat. Sci., 16(1): 77-85.

SAPARSO, SUDARMAJI A., MUSTHAFA M.B., 2023 - *Physiological aspects of the growth of Corns (Bonanza 9-F1 and Bisi-18) to air salinity conditions on coastal area*. - *3rd Inter. Conf. Sustainable Agriculture for Rural Development, ICSARD 2022,* Atlantis Press, Dordrecht, The Netherlands, pp. 362-372.

SHOKAT S., GROßKINSKY D.K., 2019 - *Tackling salinity in sustainable agriculture-what developing countries may learn from approaches of the developed world*. - Sustainability, 11(17): 1-19.

SIDABARIBA C.J., SUDJATMIKO S., 2023 - *Pengaruh perlakuan pupuk organik terhadap pertumbuhan dan hasil tiga varietas bawang merah* (Allium cepa *var. aggregatum).* - Prosiding Seminar Nasional Pertanian Pesisir, 2(1): 150-164.

SILVA-ORTEGA C.O., OCHOA-ALFARO A.E., REYES-AGÜERO J.A., AGUADO-SANTACRUZ G.A., JIMÉNEZ-BREMONT J.F., 2008 - *Salt stress increases the expression of p5cs gene and induces proline accumulation in cactus pear*. - Plant Physiol. Biochem., 46(1): 82-92.

SOLTABAYEVA A., ONGALTAY A., OMONDI J.O., SRIVASTAVA S., 2021 - *Morphological, physiological and molecular markers for salt-stressed plants*. - Plants, 10(2): 243.

SUHARJO U.K.J., MARLIN M., PURNAMA D.S., 2021 - *Use of organic materials to reduce salinity stress in shallot plants*. - National Seminar in the Framework of the 45th Anniversary of UNS, 5(1): 430-437.

SUSANAWATI S., FAUZAN M., 2019 - *Risk of shallot supply chain: an analytical hierarchy process (AHP) model in Brebes Java, Indonesia*. - Int. J. Supply Chain Manag., 8(1): 124 -131.

SYAMSIYAH J., HERAWATI A., BINAFSIHI W., 2020 - *Study of levels water salinity on the growth of varieties of shallots (*Allium ascalonicum *L.) in Alfisols*. - IOP Conference Series: Earth and Environmental Science. IOP Publishing, 423(1): 012065.

SYAMSUDDIN N., SANTOSO N., DIATIN I., 2019 - *Inventarisasi Ekosistem Mangrove di Pesisir Randutatah, Kecamatan Paiton, Jawa Timur*. - J. Pengelolaan Sumberdaya Alam dan Lingkungan, 9(4): 893-903.

TAVAKKOLI E., RENGASAMY P., MCDONALD G.K., 2010 - *High concentrations of Na+ and Cl^- ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress*. - J. Exp. Bot., 61(15): 4449-4459.

TÜRKAN I., DEMIRAL T., 2009 - *Recent developments in understanding salinity tolerance*. - Environ. Exp. Bot., 67(1): 2-9.

WANI A.S., IRFAN M., HAYAT S., AHMAD A., 2012 - *Response of two mustard (*Brassica juncea *L.) cultivars differing in photosynthetic capacity subjected to proline*. - Protoplasma, 49: 75-87.

ZAINUDDIN M., HAMID N., MUDIARTI L., KURSISTYANTO N., ARYONO B., 2017 - *Pengaruh Media Hiposalin dan Hipersalin terhadap Respon Pertumbuhan dan Biopigmen Dunaliella salina.* - Jurnal Enggano, 2(1): 46-57.

ZHANG P., SENGE M., DAI Y., 2016 - *Effects of salinity stress on growth, yield, fruit quality, and water use efficiency of tomato under hydroponics system*. - Rev. Agric. Sci., 4: 46-55.